COST OF HORMESIS

THE COST OF HORMESIS

TRADE-OFFS IN AN ENERGETICALLY CONSTRAINED ENVIRONMENT: EMBRYONIC DEVELOPMENT OF THE LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS)*

By

CHARLES MITZ, B.SC., M.ENG.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the

Requirements for the Degree Doctor of Philosophy

McMaster University

DOCTOR OF PHILOSOPHY — McMaster University

(Medical Physics and Applied Radiation Sciences) — Hamilton, Ontario

TITLE: The Cost of Hormesis:

Trade-offs in an Energetically Constrained Environment: Embryonic Development in the Lake Whitefish (*Coregonus clupeaformis*)

AUTHOR: Charles Mitz

SUPERVISORS: Professors D. R. Boreham & J. Y. Wilson

NUMBER OF PAGES: xii, 162

Abstract

Exposure to low levels of ionizing radiation is known to trigger an adaptive response that includes immune stimulation and the up-regulation of long-lasting protective effects including improved detection and repair of DNA damage, enhanced growth, and longevity. While the hormetic aspects of the adaptive response clearly increase fitness in the presence of environmental stresses, they must decrease fitness under normal environmental conditions because the responsible biological mechanisms are not maintained in an upregulated state but rather require some form of sensitizing exposure. It has been suggested that stimulation of the adaptive response could be metabolically costly however no direct measurement of the metabolic cost of radiation stimulated growth has been previously attempted.

This thesis assessed whether radiation-stimulated growth in the lake whitefish (*Coregonus clupeaformis*) is accompanied by a trade-off in metabolic efficiency, or by a sustained increase in energetic demands. Exposure to a fractionated regime of ionizing radiation from a ^{137}Cs source was found to significantly stimulate growth in lake whitefish embryos compared to controls with a corresponding increase in yolk consumption. However, measurements of metabolic efficiency using a time series of unpreserved dry weights showed that the stimulated growth was unaccompanied by a reduction in metabolic efficiency.

An exponentially transformed mean temperature was used as a quantitative baseline to assess the vulnerability of developing whitefish embryos to phenological mismatch. Hatch timing was found to deviate from baseline predictions under asymmetrically variable thermal regimes. The deviations were attributed to the embryos growing to a more advanced stage of development at low temperatures. The term heterograde is proposed to describe the thermal dependency of hatching stage which may have evolved as a mechanism to synchronize the hatching of viable larvae to the increase of zooplankton density that accompanies spring break-up. A predictive model incorporating heterograde hatching yielded a significant improvement in predictive accuracy over previous models.

Energetic efficiency is of particular importance to the lake whitefish as it has one of the longest natural incubation periods of any freshwater fish, at some of the lowest incubation temperatures, and with eggs only 10% the size of most non-coregonine salmonids. The evolution of mechanisms to synchronize hatching to the break-up of winter ice cover highlights the vulnerability of the lake whitefish to phenological mismatch whether through accelerated development that causes embryos to hatch asynchronously with the break-up of winter ice cover, or through a sustained increase in energetic demands that increases their vulnerability to starvation. The absence of any measurable trade-off in metabolic efficiency points to the latter as a plausible general cost of hormesis.

Résumé

L'exposition à des niveaux faibles de radiation ionisante est connue pour déclencher une réponse adaptative qui inclut la stimulation immunitaire et l'augmentation des effets protecteurs à long terme incluant l'amélioration de la détection et la réparation de dégâts causés à l'ADN, la croissance renforcée et la longévité. Tandis que les aspects hormetique de la réponse adaptative augmentent clairement la santé physique en présence de stress environnementaux, ils doivent la diminuer dans des conditions environnementales normales parce que les mécanismes biologiques responsables ne sont pas maintenus dans un état amélioré, mais exigent plutôt une certaine forme d'exposition sensibilisante. Il a été suggéré que la stimulation de la réponse adaptative pourrait être métaboliquement coûteuse cependant aucune mesure directe du coût métabolique de la croissance stimulée de radiation a été précédemment essayée.

Cette thèse a évalué si la croissance stimulée de radiation du grand corégone (*Coregonus clupeaformis*) est accompagnée par un compromis dans l'efficacité métabolique ou par une augmentation durable de demandes énergiques. L'exposition à un régime fractionné de radiation ionisante d'une source de ^{137}Cs s'est avéré stimuler considérablement la croissance dans des embryons du grand corégone comparés aux contrôles avec une augmentation correspondante de la consommation de jaune. Cependant, les mesures d'efficacité métabolique utilisant une série de temps de poids secs non préservés ont montré que la croissance stimulée était non-accompagnée par une réduction de l'efficacité métabolique.

Une température moyenne exponentiellement transformée a été utilisée comme un point de référence quantitative pour évaluer la vulnérabilité du développement des embryons du grand corégone à la disparité phénologique. Le temps d'éclosion différait des prédictions de références sous des régimes thermiques asymétriquement variables. Les écarts ont été attribués aux embryons grandissant à dans un stade plus avancée de développement sous des températures basses. Le terme heterograde est proposé pour décrire la dépendance thermique dans l'étape d'éclosion qui peut avoir évolué comme un mécanisme pour synchroniser l'éclosion de larves viables pour l'augmentation de la densité de zooplancton qui accompagne le débâcle printanière Un modèle de prédiction incorporant l'éclosion heterograde a permis une amélioration significative dans l'exactitude des prédictions comparé aux modèles précédents.

L'efficacité énergique a une importance particulière pour le grand corégone comme il a une des périodes d'incubation naturelles les plus longues pour un poisson d'eau douce, avec des températures d'incubation les plus basses et avec des œufs seulement 10 % de la taille de la plupart des salmonidés non-coregonine. L'évolution de mécanismes pour synchroniser l'éclosion au démantèlement de couverture de glace hivernale met en évidence la vulnérabilité du grand corégone à la disparité phénologique et ce par le développement accéléré qui cause l'éclosion asynchrone des embryons avec le démantèlement de la couverture de glace d'hiver ou par une augmentation durable de demandes énergiques qui augmentent leur vulnérabilité à la famine. L'absence de compromis mesurable dans l'efficacité métabolique indique ce dernier comme un coût général plausible de hormesis.

Acknowledgements

Research is not a solitary activity but a collaborative effort where assistance, advice, and ideas are received and exchanged with advisors, committee members, research technicians, and fellow students. None of the work presented in this thesis would have been possible without the funding provided by Bruce Power Inc. and the Natural Sciences and Engineering Research Council of Canada (NSERC) or the assistance provided by the Ontario Ministry of Natural Resources and Forestry (MNRF) which donated fertilized lake whitefish embryos when gale force winds on Lake Huron jeopardized our ability to collect spawning whitefish, my work would have required at least an additional year to complete.

My co-advisors Doug Boreham and Joanna Wilson contributed encouragement, insights and advice in addition to helping me navigate the various procedural hurdles of Ph.D. studies as a part-time student. Jeroen Thompson, Colin Seymour, and Dave Rollo rounded out my committee and they have my gratitude for their guidance and support. Jeroen Thompson is especially deserving of recognition for the hours we spent discussing ideas, experiments, and implications, and for his encouraging me to give proper mathematical structure to various models.

I benefited greatly from advice and assistance from Lisa Stoa and my fellow students Chris Thome, Shayen Sreetharan, and Michael Lim as well as summer students Emily Hulley and Charlotte Mitz, and post-doctoral fellows Jennifer Lemon, John Eme and Casey Mueller. I would especially like to acknowledge Chris Thome who contributed to all the work presented in this thesis and with whom I shared the frantic but memorable experience of being ankle-deep in cascading water trying to seal valves and connectors on our rearing apparatus. Chris was simultaneously mentor, protégé, and friend. This work would have been neither as enjoyable nor as successful without him.

Most of all I want to acknowledge my fiancée and partner in research Mary Ellen Cybulski who encouraged me to undertake doctoral studies in my 40’s and stuck with me despite the juggling of impossible work demands and sacrifices to our personal life. Without her support and significant technical contributions to the research, this thesis would not have been possible. It has been a demanding, at times exhausting, but thoroughly enjoyable journey I’m fortunate to have experienced.

Contributions

The planning and experimental work presented in Chapters 3 to 6 was performed by the candidate under the supervision of Joanna Wilson and Douglas Boreham. Jeroen Thompson contributed to the theoretical work presented in Chapter 2. Assistance with experimental work for Chapters 3 and 4 was provided by Mary Ellen Cybulski, Lisa Stoa, and Emily Hulley, and for Chapter 5 by Mary Ellen Cybulski. Chris Thome contributed to each chapter. Model development, statistical analyses, and writing of the manuscripts were conducted by the candidate with guidance and suggestions from the co-authors.

Abbreviations and Acronyms

ANCOVA – analysis of covariance

ANOVA –analysis of variance

DFO – Department of Fisheries and Oceans

dpf – days post-fertilization

DW – dry weight

IGT – Incremental growth at temperature

IUCN - International Union for Conservation of Nature

LET – Linear Energy Transfer

MNRF – Ontario Ministry of Natural Resources and Forestry

OPG - Ontario Power Generation

Q_{10} – a measure of the rate change for a 10^{o}C change in temperature

RD – Relative Development

RMSE – root mean squared error

RTH – relative time to hatch

SD – Standard deviation

SL – Standard length

VEC - valued ecosystem component

WINGS -Whitefish Interactions with Nuclear Generating Stations

YCE - yolk conversion efficiency

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

Exposure to low levels of ionizing radiation is known to trigger an adaptive response that limits damage to DNA and stimulates its repair (Ludwików *et al.*, 2002; Feinendegen *et al.*, 2013), increases lifespan (Mitchel *et al.*, 2004; Mitchel, 2006), and provides protection against subsequent high dose exposures (Boreham and Mitchel, 1991; Azzam *et al.*, 1998; Tang and Loke, 2015). The benefits of this adaptive response in the presence of a stressor are clear, however, these benefits might also be presumed to increase fitness under normal environmental conditions either directly or as a contingency against future exposure to a relevant stressor. The fact that the adaptive response is not maintained in an upregulated state is an indication that the hormetic benefits are not cost-free but are associated with tradeoffs that decrease fitness under normal environmental conditions. The nature of this offsetting cost(s) is unknown although it has been suggested that stimulation of the adaptive response mechanisms could be metabolically costly (Forbes, 2001; Zhang *et al.*, 2008), or that the hormetic response could come at a sacrifice to other physiological processes (Saul *et al.*, 2013; Costantini *et al.*, 2014).

My research explored the hypothesis that low dose stimulation in the lake whitefish is accompanied by a trade-off in energetic efficiency. Such a trade-off could take the form of effects that cause an embryo to convert yolk nutrients less efficiently and therefore hatch with deficient energy reserves. Tradeoffs might also result from stimulated growth or accelerated development that cause embryos to hatch asynchronously with their normal food supply – a phenomenon known as phenological mismatch (Cushing, 1969; Genner *et al.*, 2009).

This thesis is structured in a "sandwich" format where each chapter (apart from this general introduction and concluding chapter) corresponds to a stand-alone paper. The earlier chapters in this thesis provide the theoretical foundation and methods that allowed the radiation questions to be addressed. They focus on the thermal scaling of growth and provide insights into energetic constraints affecting embryonic growth in the lake whitefish (*Coregonus clupeaformis)* that are essential to the assessment of phenological mismatch. Each is also relevant to an improved understanding of the ecological effects of cooling water discharge from nuclear generating stations.

1.1 Background – a Practical Rationale for the Work

Bruce Power currently operates the world's largest nuclear generating facility on the shores of Lake Huron under a long term lease from the Province of Ontario. Ontario Power Generation (OPG) operates two similarly large nuclear generating stations on Lake Ontario. Whitefish (both lake and round) have been identified as a valued ecosystem component (VEC) at these sites because of their known presence in the vicinity of the generating stations, their ecological importance, and their cultural and economic value as an important commercial and sport fish.

Any adverse effect to whitefish populations from increased temperatures in cooling water discharge would have significant economic as well as ecological consequences since electricity generating stations around the great lakes rely on lake water for cooling. These include 18 nuclear reactors in Canada and another 11 in the United States along with approximately 50 fossil fuel plants, all of which are located in the United States (Mills and Sharpe, 2010). Total water takings are high although the water is returned to the lake at a similar quality except for

temperature which may be significantly higher than ambient conditions. Should once-through cooling systems be found to have an unacceptable ecological impact, alternatives such as evaporative cooling systems or offshore diffusers (Golder, 2006) would greatly increase the capital cost of future generating facilities.

To better understand the potential ecological effects of cooling water discharges, OPG initiated a series of studies beginning in the 1970's which included the assessment of constant and fluctuating temperatures on embryonic growth and survival for a variety of fish species (Griffiths, 1978a and 1978b, 1979, 1980, and 1981), plume simulations (Gowans, 1982), and in situ incubation studies (Griffiths *et al.*, 1992). After 2001, Bruce Power joined with OPG in continuing regular annual monitoring work, and in the funding of external studies such as the Whitefish Interactions with Nuclear Generating Stations (WINGS) reports (Holmes *et al.*, 2002a and 2002b). Other research has examined the impacts of temperature shifts (Patrick *et al.*, 2013), embryological exposure to temperature on heat shock protein expression (Stefanovic, 2015) and embryological and larval phenotypes (Eme *et al.*, 2015; Mueller *et al.*, 2015). These studies along with earlier work (Price, 1940; Brooke, 1975; Berlin *et al.*, 1981; Luczynski, 1984; Luczynski and Kirklewska 1984) indicate that temperatures characteristic of once-through cooling water discharge are typically well below lethal thresholds for incubating lake whitefish. However our understanding of how embryonic development is affected by sublethal exposures remains limited. Such sublethal effects include the potential to alter growth/development rates which may cause embryos to hatch asynchronously with their normal food supply a phenomenon referred to as phenological mismatch (Yang and Rudolph,

2009). Other potential effects include stresses that affect the energetic efficiency of yolk absorption and may consequently affect energy reserves available to newly hatched larva during their transition to exogenous feeding. This is especially relevant if energy reserves are constrained by pre-hatching selective pressures favouring small egg size (Régnier *et al.*, 2012). Slight reductions in fitness resulting from sublethal stresses have the potential to affect competitive equilibria between interacting species that are differentially affected by temperature changes that otherwise remain within limits for viable development (e.g. Huey and Berrigan 2001).

1.2 The Lake Whitefish

The lake whitefish is a large bodied salmonid generally ranging from 30 to 60 cm in length and up to 5 kg in weight, with those from Lake Huron typically 45 to 65 cm in length and 1 to 3 kg in weight. The whitefish has a circumpolar distribution that includes the Great Lakes and deep water lakes and rivers throughout Canada excluding the arctic archipelago (Scott and Crossman, 1973; Holmes and Noakes, 2002a). It is considered conspecific with its Eurasian counterpart the European whitefish, *Coregonus lavaretus* (Bernatchez and Wilson, 1998; Crête-Lafrenière *et al.*, 2012).

Whitefish typically inhabit deep, cold, well-oxygenated waters and form part of an endogenous cold water species guild in the great lakes that includes the round whitefish (*Prospium cylindraecum)*, lake trout (S*alvelinus namaycush*), burbot (*Lota lota*), sculpin (*Myoxocephalus spp*.), and at least three cisco species dominated by *Coregonus artedii* in the nearshore and by *Coregonus hoyii* in the deep basins. A further four species of cisco were formerly present in

Lake Huron however the deepwater cisco (*Coregonus johannae*) and shortnose Cisco (*Coregonus reighardi*) are now considered extinct (IUCN Red List) while the blackfin cisco (*Coregonus nigripinnis*) and kiyi (*Coregonus kiyi*) are considered extirpated (Mandrak *et al.*, 2014). In contrast to the deep water cisco flock, the lake whitefish has rebounded from near commercial collapse in the late 1950's to a peak harvest of more than 8,000 tonnes during the early 2000s. Although the stock has since declined from the peak harvest, populations of the lake whitefish remain high (OMNR, 2013).

The lake whitefish is a benthic specialist feeding on a variety of zooplankton (*Cladocera, Copepoda*), aquatic insects (*Chironomidae* larvae and pupae, *Diporeia spp., Isopoda*, *Mysis relicta*, *Ephemeroptera* nymphs), and small clams and mussels (*Dreissena spp., Gastropoda, Sphaeriidae*) (Pothoven and Nalepa, 2006). The whitefish in turn forms an important prey species for lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*), burbot (*Lota lota*), walleye (*Sander vitreus*) and sea lamprey (*Petromyzon marinus*) (Scott and Crossman, 1973). Whitefish have been an important source of food historically (Prowse, 2009) and continue to be the most important commercial species in Lake Huron and other great lakes except Erie (Brenden *et al.*, 2010).

Spawning takes place in the fall when water temperatures drop below 10^{o}C (Scott and Crossman, 1973). Up to 50,000, 2 to 2.5 mm diameter spherical eggs are released into the water column and settle onto gravel or rock covered shoals between 2 and 6 m in depth (Hart, 1931, Scott and Crossman, 1973, Anras, 1999). Spawning takes place at night and may extend for a period of several weeks. In Lake Huron, spawning typically extends from mid to late

November. Toward the northern limit of their range, spawning is generally earlier although whitefish have been observed spawning in December in the Tartan Rapids of the Yellowknife River, NWT in water temperatures of less than 1^{o}C (P. Vecsei, personal communication). Eggs and embryos are preyed upon by crayfish (Mason and Evans, 2011) and a variety of fish species including yellow perch (*Perca flavescens*) and ciscoes (*Coregonus artedii*) (Hart, 1931), longnose suckers (*Catostomus catostomus*) (Nester and Poe, 1984) and mottled (*Cottus bairdi*) and slimy sculpins (*Cottus cognatus*) (Marsden and Harrison, 2014). Reshetnikov and Bogdanov (2011) report egg predation by aquatic insect larvae and it is likely that the invasive round goby (*Neogobius melanostomus*) is a whitefish egg predator (Chotkowski and Marsden, 1999).

Hatching takes place in spring, typically in April or May and coincides with the spring break-up of ice cover. Incubating embryos are thought to be especially sensitive to water temperature (Holmes and Noakes, 2002a; Golder, 2008) in part because the eggs are small compared to other salmonids, and their near-zero incubation temperatures which are amongst the lowest of any freshwater fish.

1.3 Thermal scaling of biological growth

In general, growth rates increase with increasing temperature from an initial minimum viable temperature following an exponential relationship to a temperature threshold beyond which the growth rate decreases (Sharpe and deMichele, 1977; Zonneveld and Kooijman, 1993). For a biological system governed by a hypothetical rate-controlling enzyme, the probability that a system will be in a state with the required enthalpy of activation is proportional to the

Boltzmann factor, and such reactions scale to temperature according to an exponential relationship known as the Arrhenius equation. Eyring (1935) provided a theoretical foundation for this exponential relationship based on reaction kinetics using a reference temperature T_{ref} (in K), for which the reaction rate is known. Eyrings approach was further modified (Sharpe and DeMichele, 1977) by incorporating a probabilistic treatment of enzyme inactivation kinetics to yield the Sharpe-Schoolfield equation. Both the Eyring and Sharpe-Schoolfield equations are based on an exponential relationship between growth and temperature for all (Eyring) or a portion (Sharpe-Schoolfield) of the thermal domain.

The thermal dependence of embryonic development in lake whitefish has been extensively documented under constant temperature conditions (Hart, 1931; Price, 1940; Bidgood, 1972; Brooke, 1975, Griffiths, 1979; Berlin *et al.*, 1977; Luczynski, 1984; Luczynski and Kirklewska 1984) and shown to follow an exponential relationship (Figure 1-1).

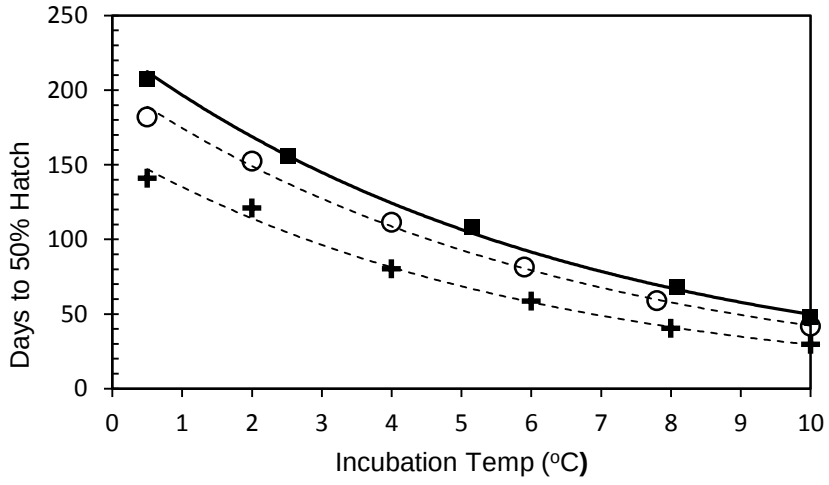


Figure 1-1: Days to median hatch (t_h) at constant incubation temperatures for different lake whitefish populations. (■ – data presented in this thesis *(*Lake Huron, t_h= 231exp-0.152T, R^2=0.998*);* ○- Brooke, 1975, Lake Michigan, t_h= 205exp-0.158T, R^2=0.998; + - Price, 1940, Lake Erie, t_h= 160exp-0.169T, R^2=0.997).

While the thermal scaling of growth is relatively well understood for constant temperatures, less research has been focused on the effect of variable thermal regimes on growth and hatch timing. Griffiths (1979), Patrick (2013), and Eme *et al.* (2015) investigated the effect of variable thermal regimes under laboratory conditions while Thome *et al.* (2016) carried out *in situ* incubation of lake whitefish near the Bruce Power Nuclear Generating Station on Lake Huron. Several whitefish-specific models for variable temperature development time have been proposed (Brooke, 1975; Griffiths, 1979; Thome *et al.*, 2016). The Brooke and Griffiths models take the form of a series of stage-wise or incremental polynomial equations while Thome *et al.* (2016) used an incremental exponential model. General models relating development time to variable thermal regimes include thermal sums such as the degree day (Neuheimer and Taggart, 2007; Chezik *et al.*, 2014). Environment Canada (OPG, 2014) has proposed the use of a constant degree day model to predict the hatching date for round whitefish despite its poor performance compared to incremental growth models (Griffiths, 1980).

All existing predictive models must be parameterized to specific populations/species. Some require the accurate determination of development stages (Brooke, 1975), making such parameterization especially difficult. The existing models also suffer from limitations in predictive accuracy when the thermal regimes are asymmetrically variable as generally encountered in nature, rather than cyclically fluctuating (Patrick *et al.*, 2013). Thermal sums are further affected by the asymptotic increase in development time at near zero temperatures, and all methods except the summation of growth increments (e.g. Alderdice and Velsen, 1978;

Thome *et al.*, 2016) are potentially affected by Jensen's inequality (Jensen, 1906; Ruel and Ayres, 1999; Martin and Huey, 2008; Ragland and Kingsolver, 2008) which causes actual growth rates under varying temperatures to exceed those that would be predicted by simply using the mean temperature. The inequality states that for a nonlinear function, $f(x)$, and a set of x values with a mean of $\bar{x}$, the average result of $f(x)$, $f(\bar{x})$ $f(x)$, is greater than the average of x, $\overline{f(x)}$ if $f(x)$ is an accelerating function and the variance of x is greater than zero. The greater the thermal variance, the greater the difference between observed growth under varying temperature incubation and that predicted using the mean temperature.

Chapter 3 presents a method to transform a variable thermal regime to an equivalent constant temperature, $\overline{T_e}$, yielding a theoretically equivalent biological growth capacity. The method accommodates Jensen's inequality and offers a continuous function that does not depend on identifying development stage. Provided that the thermal dependence of growth follows an exponential relationship and that the scaling of temperature-dependent growth is unchanged across the development period, then the method yields a constant temperature that mimics that of the variable regime. The method is mechanistic rather than empirical and dependent only on the validity of the bounding assumptions. For this reason, it offers a quantitative baseline that allows deviations from predicted behavior to be recognized and measured.

1.4 Thermal scaling of size-at-hatch

Hatchling size in the lake whitefish (*Coregonus clupeaformis*) is strongly temperature dependent with embryos incubated at low temperatures reaching a larger size than those

incubated at warmer temperatures (Brooke, 1975, Griffiths, 1979; Eme *et al*, 2015, and Mueller *et al.*, 2015). The mechanism(s) responsible for this apparent manifestation of the temperature-size rule remain unidentified.

Size-at-hatch involves two distinct but closely correlated processes: growth and development (sometimes referred to as differentiation/maturation). The former is simply the gain in dry mass with time while development is the progress toward recognizable morphological stages such as the development of organs, eye pigmentation, metamorphosis, or fin separation. If growth and development follow identical thermal scaling and hatching is a valid development stage, then embryos hatching at different temperatures should be identical in size apart from differences resulting from the temperature dependence of yolk conversion efficiency. If growth and development exhibit different thermal scaling, then the embryo size at a given development stage will vary as a function of temperature. Differential scaling has been postulated as a mechanism governing adult size in ectotherms (Silby and Atkinson, 1994; van der Have and Dejong, 1996; and Forster and Hirst, 2012) and the mechanism underlying TSR in general (Zuo *et al.*, 2012). However differential scaling of growth and development is not the only possible explanation for TSR. Larger hatchlings would also be expected if hatching is heterograde with embryos growing to a more advanced stage at colder temperatures (e.g. Klingenberg, 1998; Jordaan, 2002; Figure 1-2).

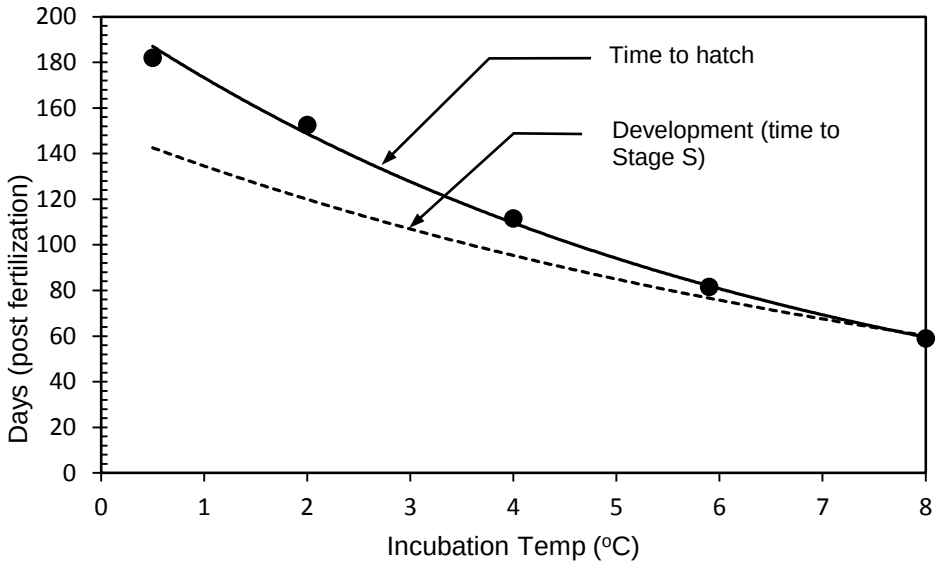


Figure 1-2: Graphical illustration of heterograde hatching. The solid line shows an exponential regression for mean hatching time for Lake Michigan whitefish from Brooke (1975) while the dashed line represents the time to reach a hypothetical development stage equivalent to stage at hatching for constant temperature incubation at 8°C. In this illustration, growth and differentiation follow an identical scaling relationship to temperature. In the differential scaling hypothesis, the dashed line would represent the time to grow to a specific size equivalent to the size at hatching for constant temperature incubation at 8°C

Chapter 4 examines the two size-at-hatch hypotheses using a variety of morphological indicators of absolute growth and development for embryos reared in a custom designed incubator system (Mitz *et al.*, 2014) under different constant and varying thermal regimes. Obtaining an accurate measurement of yolk conversion efficiency (YCE) presented a particular challenge as it progressively declines through development as the allocation of energy to maintenance increases. Conventional calculation methods for YCE may therefore reflect differences in development stage rather than actual differences in yolk conversion efficiency. To solve this problem, a method is proposed to allow YCE measurements made at different points in development to be normalized to a common baseline. The experimental results show that hatching in the lake whitefish is thermally heterograde, and that this is the dominant factor

affecting size-at-hatch although temperature-dependent differences in yolk conversion efficiency (YCE) also contributes. Heterograde hatching is suggested as a mechanism to synchronize the timing of hatching to the break-up of ice cover.

1.5 An improved hatch timing model

The confirmation of heterograde hatching provided the basis for the development of a predictive hatch timing model that extends the incremental exponential approach of Thome *et al.* (2016) by explicitly incorporating a thermal dependence of development differing from the thermal dependence of hatching. Chapter 5 presents this model which consists of a continuous monotonic component describing development stage, and a discontinuous non-monotonic component describing the temperature-dependence of stage-at-hatch. Comparison to experimental observations and hatch timing reported in published literature (e.g. Berlin *et al.*, 1977; Patrick *et al.*, 2013 and Mueller *et al.*, 2015) shows that the proposed model provides superior predictive accuracy compared to conventional time-domain models for ecologically relevant asymmetrically varying thermal regimes. The model also explains the phenomenon of thermally-induced mass hatching which is predicted, not as a form of stress response, but as the intersection of instantaneous temperature with the temperature-dependent stage of development at which hatching occurs.

1.6 Metabolic cost of radiation-stimulated growth

Hormesis is a reversed dose-response relationship in which stimulation occurs at low concentrations and inhibition at high concentrations. Such stimulation may include accelerated growth and greater stress resistance. Exposure to low levels of ionizing radiation is known to

trigger a series of biological responses that include immune stimulation and the up-regulation of long-lasting protective effects that limit damage to DNA and stimulate its repair (Feinendegen *et al.*, 2013). Experimental studies have shown that single low doses (priming exposures) of low LET radiation have induced protective effects against subsequent high dose exposures (Boreham and Mitchel, 1991; Azzam *et al.*, 1996; Broome *et al.*, 1999), increased cancer latency (Mitchel *et al.*, 2004), and reduced radiation-induced birth defects in mice (Norimura *et al.*, 1996; Boreham *et al.*, 2004). Other studies show that radiation-induced sensitization is protective against other forms of stress such as heat shock, and vice versa (Boreham and Mitchel, 1994). Calabrese and Baldwin (2003) argue that hormetic effects may represent the rule rather than the exception in nature and may arise from exposure to low levels of a wide variety of agents that are harmful at high concentrations.

This biological response (Tang and Loke, 2015) may also include radiation-stimulated growth which has been widely reported in plants (Breslavets, 1946) and was first recognized more than a century ago (Russell, 1915). Large scale experiments using ionizing radiation to enhance crop yields were even carried out in the USSR in the 1950's (Sax, 1963). Radiation enhanced growth has been less widely reported in animals although Simon et al. (2011) noted accelerated hatching in zebrafish (*Danio rerio*) and Van den Berghe *et al.* (2010) reported that low doses of radiation accelerated growth in tumours.

The mechanisms underlying growth stimulation are poorly understood. Calabrese (1999) has speculated that radiation may induce an overcompensation effect. Alternate mechanisms

include inactivation of inhibitory pathways (Miller and Miller, 1987) or even radiation as a direct input of energy (Dadachova and Casadevall, 2008).

Chapter 5 addresses the question of whether there exists a trade-off between radiation-stimulated growth and yolk conversion efficiency (YCE) in lake whitefish embryos. Eyed embryos (Stage 10 in the series of Sreetharan *et al.,* 2015) were exposed to fractionated doses of 662 keV gamma radiation ranging from 15 mGy to 8 Gy per fraction. A total of four fractions were delivered per treatment with a 14 day separation between dose fractions followed by constant temperature incubation at 2°C for a further 20 days. Embryos were subsequently dechorionated and imaged live before determination of YCE using unpreserved dry weight. The metabolic cost was found to be statistically indistinguishable from zero and provided independent confirmation of a parallel study using chronic radiation exposure.

1.7 Conclusions

Embryonic development in the lake whitefish occurs in an energetically constrained environment characterized by a small egg size and a lengthy incubation at low temperatures with hatch occurring synchronously with spring break-up. The limited energy reserves possessed by embryos of the lake whitefish (and related Coregonids) maintain relatively little margin for error. It follows then that environmental changes that affect the availability of energy or the efficiency of its use may give rise to biological impacts at levels well below those that cause direct mortality.

By combining the exponential mean with an accommodation of heterograde hatching, it is possible to model hatching as governed by the instantaneous temperature and stage of

differentiation rather than a monotonically increasing function of thermal history. The model provides a means (previously lacking) to predict hatching (fractional as well as median) under both cyclical and secularly variable thermal regimes. The model also provides a basis for the prediction of the size and yolk reserves of the hatching embryos which allows for a realistic assessment of the potential for phenological mismatch resulting from altered thermal regimes. The model explains the phenomenon of thermally-induced mass hatching not as a form of stress response, but as the intersection of instantaneous temperature with the stage of development at which hatching occurs.

Low dose exposure to ionizing radiation is shown to stimulate growth with no observable trade-off with metabolic efficiency. This result was the first to directly measure the metabolic cost of growth hormesis resulting from a fractionated exposure to ionizing radiation and demonstrates that any evolutionarily-relevant cost is more subtle than simply being metabolically costly. The absence of any trade-off in metabolic efficiency points to the phenological mismatch hypothesis as a plausible generalized cost applicable across different taxa and life stages.

1.8 References

Alderdice, D. F., & F. P. J. Velsen, 1978. Relation between temperature and incubation time for eggs of Chinook salmon (*Oncorhynchus tshawytscha*); *Journal of the Fisheries Research Board of Canada* 35: 69–75.

Anras, M.L.B., 1999. Movement and habitat use by lake whitefish during spawning in a boreal lake: integrating acoustic telemetry and geographic information systems; *Transactions of the American Fisheries Society* 128: 939-952.

Ash, G.R., 1975. The effects of thermal pollution on the egg survival of the lake whitefish (*Coregonus clupeaformis*, (Mitchill)); *Water Pollution Research in Canad*a 10: 9-16.

Azzam, E. I., S. M. de Toledo, G. P. Raaphorst and R. E. J. Mitchel, 1996. Low-Dose ionizing radiation decreases the frequency of Neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells; *Radiation Research*. 146, 369-373.

Berlin, W.H., L.T. Brooke and L.J. Stone, 1977. Verification of a model for predicting the effect of inconstant temperature on embryonic development of lake whitefish (*Coregonus clupeaformis);* Technical papers of the US Fish and Wildlife Service 92.

Bernatchez L, and C.C. Wilson, 1998. Comparative phylogeography of Nearctic and Palearctic fishes; Mol. Ecol. 7:431–452.

Bidgood, B. F. 1972. Divergent growth in lake whitefish populations from two eutrophic Alberta lakes; Ph.D. Thesis. University of Alberta, Edmonton, Alta. 106 p.

Boreham D.R. and R.E.J Mitchel, 1991. DNA lesions that signal the induction of radioresistance and DNA repair in yeast; *Radiation Research* 128:19-28.

Boreham D.R. and R.E.J Mitchel, 1994. Regulation of heat and radiation stress responses in yeast by HSP-104; *Radiation Research* 137:190-195.

Boreham, D.R., J.A. Dolling, S.R. Maves, S. Miller, D.P. Morrison and R.E.J. Mitchel, 1997. Heat-induced thermal tolerance and radiation resistance to apoptosis in human lymphocytes; *Biochemistry and Cell Biology*. 75 (4), 393-397.

Boreham D.R., J-A Dolling, C. Somers, J. Quinn and R. E. J. Mitchel, 2004. The adaptive response and protection against heritable mutations and fetal malformation; Proceedings of the 14th Pacific Basin Nuclear Conference, Honolulu, HI, Mar. 21-25.

Brenden, T. O., M. P. Ebener, T. M. Sutton, M. L. Jones, M. T. Arts, T. B. Johnson, M. A. Koops, G. M. Wright, and M. Faisal, 2010. Assessing the health of Lake Whitefish populations in the Laurentian Great Lakes: Lessons Learned and Research Recommendations; *Journal of Great Lakes Research* 36 (2010): 135-139.

Breslavets, L.B., 1946. Plants and X-rays. Moscow; Acad. Sci. USSR Press, (Translation American Institute of Biological Sciences 1960)

Brooke, L.T. 1975. Effect of different constant incubation temperatures on egg survival and embryonic development in lake whitefish (*Coregonus clupeaformis*); *Transactions of the American Fisheries Society*. 104 (3), 555-559.

Broome E.J, D. L. Brown and R.E.J. Mitchel, 1999. Dose responses for adaption to low doses of 60 Co γ rays and 3 H β particles in normal human fibroblasts; *Radiation Research* 158(2), 181-186.

Calabrese, E. J., 1999. Evidence that Hormesis represents an "Overcompensation" response to a Disruption in homeostasis; *Ecotoxicology and Environmental Safety* 42.2: 135-137.

Calabrese E.J. and L. A. Baldwin, 2003. Toxicology rethinks its central belief; *Nature* 421:691–692.

Chezik, K. A., N. P. Lester, P. A. Venturelli, and K. Tierney, 2014. Fish growth and Degree-Days I: Selecting a base temperature for an among-population study; *Canadian Journal of Fisheries and Aquatic Sciences* 71.9: 1303-1311.

Chotkowski, M. A., and J. E. Marsden, 1999. Round Goby and Mottled Sculpin predation on Lake Trout eggs and fry: Field predictions from laboratory experiments; *Journal of Great Lakes* Research 25.1: 26-35.

Costantini, D., P. Monaghan, and N. B. Metcalfe, 2014. Prior hormetic priming is costly under environmental mismatch; *Biology Letters* 10.2

Crête-Lafrenière A, L. K. Weir, and L. Bernatchez, 2012. Framing the Salmonidae family phylogenetic portrait: A more complete picture from increased taxon sampling. *PLoS ONE* 7(10)

Cushing, D. H., 1969. The regularity of the spawning season of some fishes; *Journal du Conseil pour l'Exploration de la* Mer 33:81–92.

Dadachova, E., and A. Casadevall, 2008. Ionizing Radiation: How fungi cope, adapt, and exploit with the help of Melanin; *Current Opinion in Microbiology* 11.6 (2008): 525-531.

Eme, J., C.A. Mueller, R.G. Manzon, C.M. Somers, D.R. Boreham, and J.Y. Wilson, 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings; *Comparative Biochemistry and Physiology* Part A: Molecular & Integrative Physiology. 179, 71-80.

Eyring, H., 1935. The activated complex in chemical reactions; *Journal of Chemical Physics*. 3 (2): 107–115.

Feinendegen, L. E., M. Pollycove, and R. D. Neumann, 2013. Hormesis by low dose radiation effects: Low-Dose Cancer Risk Modeling Must Recognize Up-Regulation of Protection; *Therapeutic Nuclear Medicine* Springer 2013 ISBN 978-3-540-36718-5

Forbes, V. E., 2000. Is hormesis an evolutionary expectation?; *Functional Ecology* 14.1 (2000): 12-24.

Forster, J., and A. G. Hirst, 2012. The temperature-size rule emerges from ontogenetic differences between growth and development rates; *Functional* Ecology Volume 26, Issue 2, 483–492.

Genner, M. J., N. C. Halliday, S. D. Simpson, A. J. Southward, S. J. Hawkins, and D. W. Sims, 2009. Temperature-driven phenological changes within a marine larval fish assemblage; *Journal of Plankton Research* 32.5 (2009): 699-708.

Golder Associates Ltd., 2006. Report on review of thermal mitigation technologies for Nuclear Generating Stations; submitted to Canadian Nuclear Safety Commission, Ottawa, CNSC Research Program Report RSP-0202

Golder Associates Ltd., 2008. EIS Studies: Aquatic environment Technical Support Document for the Bruce New Nuclear Power Plant Project Environmental Assessment.

Gowans, W.L., 1982. Pickering Nuclear Generating Station winter thermal plume study (1979-1980) Round Whitefish egg incubation simulations; Ontario Hydro Design and Development Report No. 81263, 41 p and App.

Griffiths, J.S., 1978a. Potential effects of unstable thermal discharge plumes on Yellow Perch eggs; Ontario Hydro Research Division Report No. 78-586-K.

Griffiths, J. S., 1978b. Potential effects of unstable thermal discharge plumes on Great Lakes Rainbow Smelt eggs; Ontario Hydro Research Division Report No. 78-343-K 58p.

Griffiths, J.S., 1979. Potential effects of unstable thermal discharges on incubation of Lake Whitefish eggs; Ontario Hydro Research Division Report No. 79-521-K.

Griffiths, J.S., 1980. Potential effects of unstable thermal discharges on incubation of Round Whitefish eggs; Ontario Hydro Research Division Report No. 80-140-K.

Griffiths, J.S., 1981. Potential effects of fluctuating thermal regimes on incubation of Walleye eggs; Ontario Hydro Research Division Report No. 81-77-K.

Griffiths J.S., J.D. Harcus & M.L Macinte, 1992. Field incubation of Round Whitefish eggs at Darlington NGS during 2-3 unit operation,1992-93; Report No A-NBP-95-39-CON

Hart, J.L. 1931. The spawning and early life history of the whitefish, Coregonus clupeaformis (Mitchill), in the Bay of Quinte, Ontario; *Contributions to Canadian Biology and Fisheries*. 6 (1), 165-214.

Holmes, J.A. and D.L.G. Noakes et al.,2002a. Whitefish Interactions with Nuclear Generating Stations: Lake Whitefish and Round Whitefish Biology: A review of ecological factors affecting growth, survival and reproduction; Prepared by Axelrod Institute for Ichthyology, University of Guelph for Ontario Power Generation, Chippewas of Nawash and Bruce Power, Inc. 235pp.(E-DOC 3451248 or 3451392).

Holmes, J.A. and D.L.G. Noakes et al.,2002b. Whitefish Interactions with Nuclear Generating Stations: Aquatic Effects of Nuclear Generating Stations on Fishes; Prepared by Axelrod Institute for Ichthyology, University of Guelph for Ontario Power Generation, Chippewas of Nawash and Bruce Power, Inc. 340pp.(E-DOC 3451221).

Huey, R. B., and D. Berrigan, 2001. Temperature, demography, and ectotherm fitness; *The American Naturalist* 158.2: 204-210.

IUCN (International Union for Conservation of Nature), 2016. http://www.iucn.org/about/work/programmes/species/our_work/the_iucn_red_list/ accessed March 9, 2016

Jensen, J.L., 1906. Sur les fonctions convexes et les inégualités entre les valeurs moyennes; *Acta Mathmatica*. 30, 175–19.

Jordaan, A., 2002. The effect of temperature on the development, growth and survival of Atlantic Cod (*Gadus morhua*) during early life-histories; Electronic Theses and Dissertations. Paper 141.

Klingenberg, C. P., 1998. Heterochrony and allometry: The analysis of evolutionary change in ontogeny; *Biological Reviews* 73.1 (1998): 79-123.

Luczynski, M. 1984. Improvement in the efficiency of stocking lakes with larvae of Coregonus albula L. by delaying hatching; *Aquaculture* 41: 99-111.

Luczynski, M. and A Kirklewska, 1984. Dependence of *Coregonus albula* embryogenesis rate on the incubation temperature; *Aquaculture*, vol. 42, no. 1, 43-55.

Ludwików G., X. Yun, R. A. Hoebe, N. A. P. Franken, F. Darroudi, J. Stap, C. H. van Oven, C. J. F. van Noorden and J. A. Aten, 2002. Induction of chromosome aberrations in unirradiated chromatin after partial irradiation of a cell nucleus. *International Journal of Radiation Biology*; 78, 239-247.

Mandrak, N.E., T.C. Pratt and S.M. Reid, 2014. Evaluating the current status of deepwater ciscoes (Coregonus spp.) in Canadian waters of Lake Huron, 2002-2012, with emphasis on Shortjaw Cisco (C. zenithicus); DFO v +12 p.

Marsden, J. E., and T. Harrison, 2014. Sculpin predation on Lake Trout eggs in interstices: Skull compression as a novel foraging mechanism; *Copeia* 2014.4: 654-658.

Martin, T. L. and R. B Huey, 2008. Why "Suboptimal" is optimal: Jensen's Inequality and ectotherm thermal preferences; *The American Naturalist* 171: E102-118.

Mason, K., and D. O. Evans, 2011. Predation of Lake Trout and Lake Whitefish embryos by Crayfish: Implications of shifts in crayfish dominance in Lake Simcoe; *Journal of Great Lakes Research* 37 (2011): 113-123.

Miller M.W and W.M. Miller, 1987. Radiation hormesis in plants; *Health Physics*, 52: 607-616.

Mills, P.C. and J.B. Sharpe. 2010. Estimated withdrawals and other elements of water use in the Great Lakes Basin of the United States in 2005; U.S. Geological Survey.

Mitchel R. E. J. Jackson and S. Carlisle, 2004. Upper dose thresholds for radiation-induced adaptive response against cancer in high-dose-exposed, cancer-prone, radiation-sensitive Trp53 heterozygous mice; *Radiation Research* 162:20-30.

Mitchel R. E. J., 2006. Low doses of radiation are protective in vitro and in vivo: Evolutionary Origins; *Dose-Response* 4(2):75–90, 2006

Mitz, C., C. Thome, M.E. Cybulski, L. Laframboise, C.M. Somers, R.G.Manzon, J.Y. Wilson and D.R. Boreham, 2014. A self-contained, controlled hatchery system for rearing lake whitefish embryos for experimental aquaculture; *North American Journal of Aquaculture*. 76 (3), 179-184.

Mueller, C.A., J. Eme, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype and cost of development in lake whitefish (Coregonus clupeaformis); *Journal of Comparative Physiology*. B, Biochemical, Systemic, and Environmental Physiology. 185 (3), 315-331.

Nalepa, T.F., S.A. Pothoven and D. L. Fanslow, 2009. Recent changes in benthic macroinvertebrate populations in Lake Huron and impact on the diet of lake whitefish (Coregonus clupeaformis); *Aquatic Ecosystem Health & Management*. 12 (1), 2-10.

Nester, R. T., and T. P. Poe, 1984. Predation on Lake Whitefish eggs by Longnose Suckers; *Journal of Great Lakes Research* 10.3 (1984): 327-328.

Neuheimer, A. B, and C. T. Taggart., 2007. The growing degree-day and fish size-at-age: The overlooked metric; *Canadian Journal of Fisheries and Aquatic Sciences* 64.2 (2007): 375-385.

Norimura, T., S. Nomoto, M. Katsuki, Y. Gondo and S. Kondo, 1996. P53-dependent apoptosis suppresses radiation–induced teratogenesis; *Nature Medicine*, 2(5), pp.577-580.

OMNR (Ontario Ministry of Natural Resources), 2013. Lake Huron commercial fishing summary for 2012; Ministry of Natural Resources (MNR), Upper Great Lakes Management Unit, Lake Huron, Report TR-LHA-2013-01

OPG (Ontario Power Generation), 2014. Reassessment of the impact of thermal plumes on round whitefish egg survival and hatch using the new COG model; File: N•REP-07250-0518222

Patrick, P.H., E. Chen, J. Parks, J. Powell, J. S. Poulton and C-L Fietsch, 2013. Effects of fixed and fluctuating temperature on hatch of Round Whitefish and Lake Whitefish eggs; *North American Journal of Fisheries Management* 33:1091-1099.

Pothoven, Steven A., and Thomas F. Nalepa, 2006. Feeding ecology of Lake Whitefish in Lake Huron; *Journal of Great Lakes Research* 32.3 : 489-501.

Price, J.W., 1940. Time-temperature relations in the incubation of the whitefish, Coregonus clupeaformis (Mitchill); *Journal of General Physiology*. 23 (4), 449-468.

Prowse, S.L., 2009. Much Ado about Netsinkers: An examination of pre-contact aboriginal netsinker manufacture and use patterns at five Woodland Period archaeological sites within Southern Ontario; *Ontario Archaeology*, 85-88.

Ragland, G.J. and J.G. Kingsolver, 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*; *Evolutionary Ecology Research* 10: 29-44.

Régnier, T., V. Bolliet, P. Gaudin, J. Labonne, 2012. Bigger is not always better: Egg size influences survival throughout incubation in Brown Trout (*Salmo trutta*); *Ecology of Freshwater Fish* 22.2 (2012): 169-177.

Reshetnikov, Y. S., and V. D. Bogdanov, 2011. Features of reproduction of whitefishes; *Journal of Ichthyology* 51.6 (2011): 432-456.

Ruel, J. J. and M. P. Ayres, 1999. Jensen's inequality predicts effects of environmental variation; *Trends in Evolution and Ecology* 14: 361-366.

Russell, E. J., 1915. The effect of radium on the growth of plants; *Nature* 96, 147-148.

Saul N, K. Pietsch, S.R. Stürzenbaum, R. Menzel and C.E. Steinberg, 2013. Hormesis and longevity with tannins: free of charge or cost-intensive?; *Chemosphere* 93:1005-1008.

Sax, K., 1963. The stimulation of plant growth by ionizing radiation; *Radiation Botany* Volume 3, Issue 3, 1963,179–186.

Scott, W.B. and E.J. Crossman, 1973. Freshwater fishes of Canada; Fish Research Board of Canada Bulletin 184.

Sharpe, P. J. H. and D. DeMichele. 1977. Reaction kinetics of poikilotherm development; *Journal of Theoretical Biology*, 64: 649-670.

Sibly R. M. and D. Atkinson, 1994 How rearing temperature affects optimal adult size in ectotherms; *Functional Ecology* 8, 486–493.

Simon, O., S. Massarin, F. Coppin, T.G. Hinton and R. Gilbin, 2011. Investigating the embryo/larval toxic and genotoxic effects of γ irradiation on zebrafish eggs; *Journal of Environmental Radioactivity*. 102 (11), 1039-1044.

Sreetharan, S., C. Thome, C. Mitz, J. Eme, C.A. Mueller, E.N. Hulley, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic development of lake whitefish (*Coregonus clupeaformis*): a staging series, analysis of growth and impacts of fixation; *Journal of Fish Biology* 87.3 (2015): 539-558.

Stefanovic, D.I., 2015. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish (*Coregonus clupeaformis*); M.Sc. thesis, University of Regina,

Thome, C., C. Mitz, C.M. Somers, R.G. Manzon, J.Y. Wilson, and D.R. Boreham, 2016. Incubation of Lake Whitefish (Coregonus clupeaformis) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development; *Canadian Journal of Fisheries and Aquatic Sciences, http://dx.doi.org/10.1139/cjfas-2015-0286*

Tang, F. R., and W. K. Loke, 2015. Molecular mechanisms of low dose ionizing radiation-induced Hormesis, Adaptive responses, radioresistance, bystander effects, and genomic instability; *International Journal of Radiation Biology* 91.1: 13-27.

Van den Berghe, E. P., and M. Sofia Vala I., L.R. Martins, N. Imaizumi, R.J. Nunes, J. Rino, and F. Kuonen, 2010. Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis; *PLoS ONE* 5(6): e11222.

van der Have, T. M. & G. de Jong, 1996. Adult size in ectotherms: temperature effects on growth and differentiation; *Journal of Theoretical Biology*. 138: 329–340.

Velsen, F. P. J., 1987. Temperature and incubation in Pacific Salmon and Rainbow Trout: Compilation of data on median hatching time, mortality and embryonic staging; *Canadian Data Report of Fisheries and Aquatic Sciences* No. 626.

Yang, L.H., and V. H. W. Rudolf, 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions; *Ecology Letters* 13.1, 1-10.

Zhang, Q., J. Pi, C. G. Woods, A. M. Jarabek, H. J. Clewell, and M. E. Andersen, 2008. Hormesis and adaptive cellular control systems; *Dose-Response* 6.2 (2008): 196-208.

Zonneveld, C. and S. A. L. M. Kooijman, 1993. Comparative kinetics of embryo development; *Bulletin of Mathematical Biology* 3:609-635.

Zuo, W., M.E. Moses, G. B. West, C. Hou, and J. H. Brown, 2012. A general model for effects of temperature on ectotherm ontogenetic growth and development: *Proceedings of the Royal Society* B (2012) 279, 1840–1846.

Chapter 2

A self-contained, controlled hatchery system for rearing Lake Whitefish embryos for experimental aquaculture

Charles Mitz[1], Chris Thome[1], Mary Ellen Cybulski, Lisa Stoa[1], Joanna Y. Wilson[3*], and Douglas R. Boreham[1,2,4]

Published in: North American Journal of Aquaculture

Mitz, C., C. Thome, M.E. Cybulski, L. Laframboise, C.M. Somers, R.G.Manzon, J.Y. Wilson and D.R. Boreham, 2014. A self-contained, controlled hatchery system for rearing lake whitefish embryos for experimental aquaculture; *North American Journal of Aquaculture.* 76 (3), 179-184.

Abstract

A self-contained, small-scale research hatchery was constructed in a modified chromatography refrigerator equipped with a filtered and UV-sterilized water recirculation system. Lake whitefish embryos were raised in conventional upwelling hatching jars, in dishes with a continuous slow "drip feed", and using a variety of static water incubation systems in petri dishes and multiwell plates. The optimal rearing density for petri dishes was found to be 50 embryos per dish, with weekly water changes. The highest survival in multiwell plates was seen in the 6 and 24 well sizes. Survival rates in most multiwell plates and petri dishes, as well as in the hatching jar incubators, was between 40 – 60%, which is in line with survival rates seen in commercial large scale rearing. Overall, these techniques permitted the rearing of large numbers of whitefish in separate batches and under controlled conditions, while greatly reducing space requirements and material costs. Our system is well suited for research and other situations requiring the temperature controlled rearing of embryos on a small scale.

* corresponding author

[1]*Department of Medical Physics and Applied Radiation Sciences, McMaster University*
[2]*Bruce Power, Tiverton, ON, Canada*
[3]*Department of Biology, McMaster University*
[4]*Northern Ontario School of Medicine*

2.1 Introduction

Considerable research and experimentation has been devoted to the development of fish rearing techniques for fisheries enhancement, restoration, and aquaculture. Many of the well-established rearing methods are designed to produce large numbers of similarly sized healthy fingerlings suitable for culture or stocking. Production of fish for research purposes encompasses a somewhat different suite of requirements, including the ability to conduct a large number of experimental trials under controlled temperature and water quality conditions. Similarly, it may be desirable to simultaneously raise multiple genetic lines of rare or endangered species in distinct batches. These requirements have spurred a number of innovative techniques including small scale incubation methods (Wedekind *et al.*, 2001, Wedekind and Müller, 2004, Barnes and Durben, 2008), in-situ incubators (Gunn and Keller, 1984, Manny *et al.*, 1989), and even apparatus for rearing single embryos (Bardega and Luczynski, 2007).

To test the impacts of multiple stressors (thermal, chemical, and radiological) on lake whitefish (*Coregonus clupeaformis*) embryos at different stages of embryogenesis, we required a closed and controlled hatchery system with a small foot print. Exposure to radioisotopes necessitated that embryos be housed in a commissioned radioisotope laboratory rather than an aquatics facility. To accommodate the chemical and radiological exposures, as well as the space constraints, we built a closed recirculating system that uses dechlorinated municipal water and is able to maintain low stable incubation temperatures.

2.2 Methods

Apparatus

To house developing embryos, we built two self-contained hatchery units (Figures 2-1 and 2-2). The basis of each unit was a 1.33 m^3 capacity VWR GDM-47 2 Door Chromatography Refrigerator with a Dixel XR02CX Digital Temperature Controller. The interior components of the system comprised a custom fabricated glass aquarium as a lower reservoir (130 L nominal capacity) with a single bulkhead fitting to magnetically coupled pumps mounted on the exterior of the refrigerator unit (combined capacity ca. 30 L/min). Two pumps were plumbed to provide redundancy in the event of a mechanical failure. Water from the lower reservoir was pumped through a media filter (i.e. carbon) and UV sanitization unit to an upper reservoir constructed of 75 mm diameter potable water grade PVC pipe with a vented overflow return to the lower reservoir to maintain constant and stable water pressures. Water from the upper reservoir was directed through two 50 mm diameter PVC manifolds supplying controlled amounts of water to individual drip fed Petri dish incubators and to a bank of seven upwelling hatching jar incubators (J32 Mini Hatching Jar, 300 mm high x 150 mm diameter; cap. maximum of 10,000 embryos per jar; Aquatic Eco-systems Incorporated) outletting to the lower reservoir. Temperature monitoring was provided using the factory installed temperature controller and Schlumberger DI 501 Mini-Diver loggers (accurate to 0.01°C) installed in the upper and lower reservoirs and connected to a remote computer using direct-read cables. Units were supplied with power by duplex outlets connected to the building emergency backup power system.

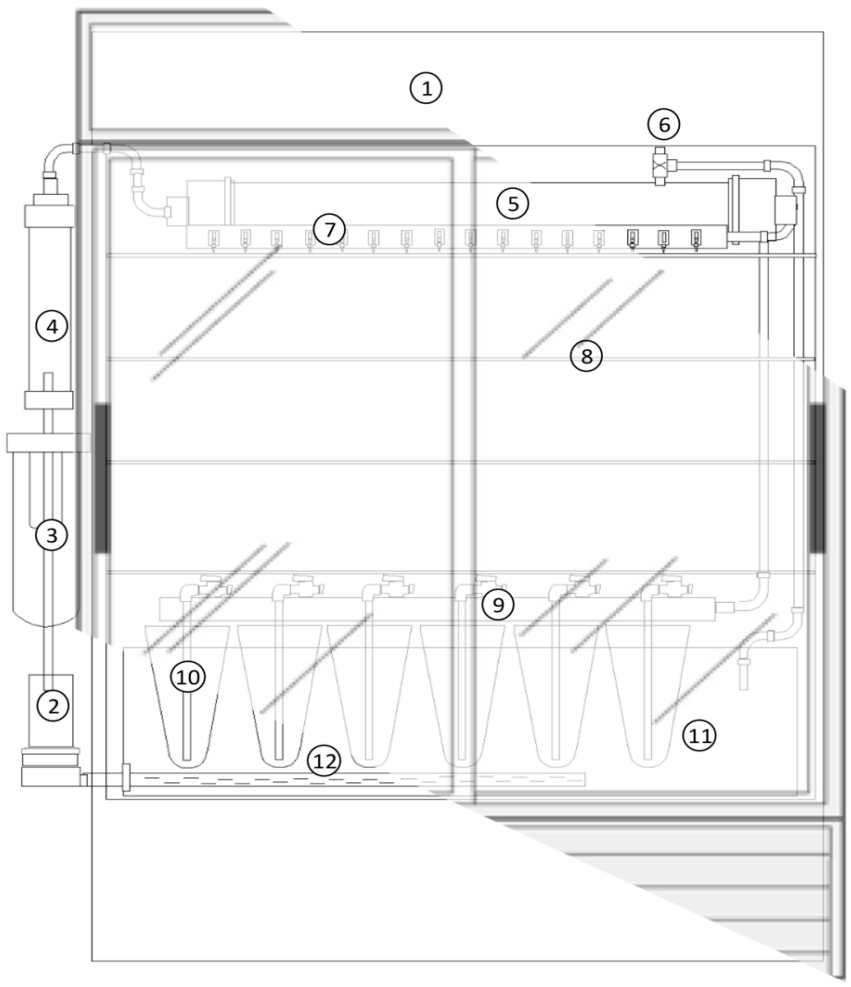


Figure 2-1. Embryo rearing apparatus. 1. VWR GDM-47 2 Door Chromatography Refrigerator; 2. 2 x Little Giant® PM3, 50 W magnetically coupled pumps; 3. 14-GCB2-10 Giant Carbon Block Filter; 4. 25 W EU25-U Emperor UV Sterilizer; 5. 75 mm diameter PVC reservoir; 6. Vented overflow return; 7. 50 mm dia. PVC header with approx 20 valved discharge ports; 8. Wire shelves for micro-incubators; 9. 50 mm dia. PVC header with eight valved ports; 10. Hatching jar incubators; 11. Lower reservoir; 12. Filtered intake.

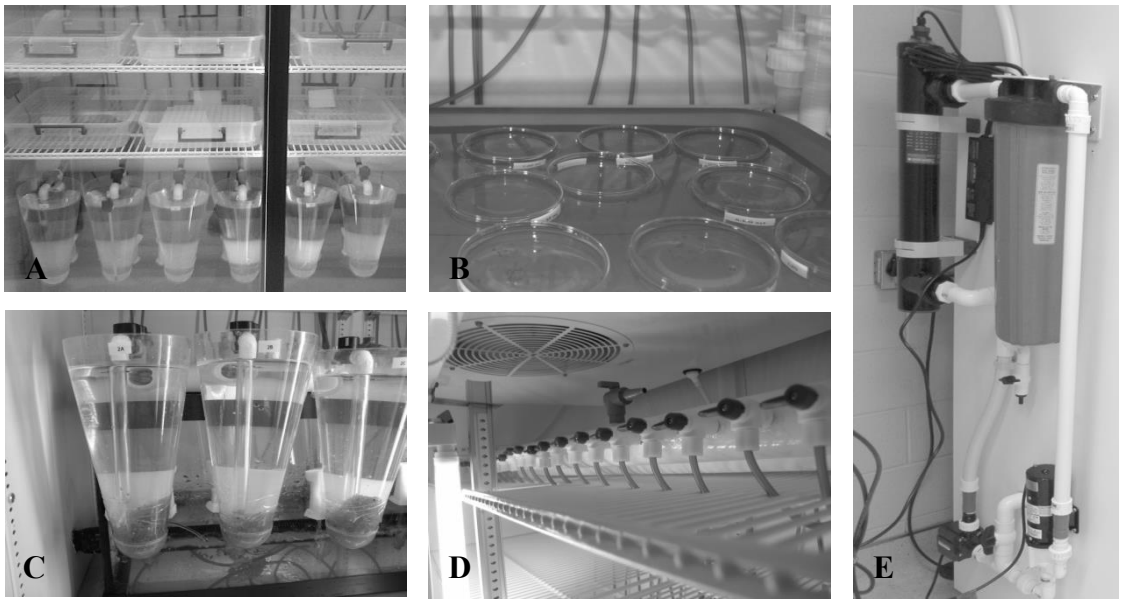


Figure 2-2. Detailed view of embryo incubator apparatus components. (A) Incubator trays and upwelling incubators (B) Static water Petri dish incubators on the shelves of the unit (C) Mini-hatch jar incubators (D) Vented manifold for the distribution of reservoir water to various incubators (E) Pumps and filters mounted on the exterior of the cooler units.

Flow to the hatching jars was controlled by a dual valve system driven by the head pressure from the elevated reservoir. Flow rates to each hatching jar could be controlled between 0.1 and 3 L/min. In addition to the hatching jar incubators, micro-incubators were placed on the cooler shelves. These included drip feed incubation, and static water incubation using petri dishes and multiwell plates. The smaller drip-feed lines were controlled via a single valve for a slow drip to a maximum of 150 ml/min. The constant pressure provided by the elevated reservoir gave stable flows over all ranges but the use of T-connector to divide the lines between two micro-incubators reduced flow stability as flow changed from one branch to another in an erratic manner. The latter could be remedied by placing a valve downstream of the T-connector for each micro-incubator.

Embryos

Lake whitefish embryos were produced via in vitro fertilization from gametes collected from Lake Simcoe (2011-2012) and Lake Huron (2012-2013). Lake Simcoe embryos were provided by the Ontario Ministry of Natural Resources at the eyed embryo stages. Lake Huron whitefish were gillnetted on November 15, 2012 between 3 and 6 m of ca. 7°C water south of Whitefish Island (44°42'37.74"N, 81°18'38.94"W). Eggs were stripped from 18 females, mixed with milt from 27 ripe males, allowed to harden, and then disinfected with Ovadine® (5 ml/L, Syndel Laboratories Limited) prior to being transported in 1.5 L plastic jars by truck to the rearing laboratory. All animal procedures were completed with approval from McMaster University's Animal Ethics Review Board.

Experimental design

Lake whitefish embryos were reared in both constant flow through hatching jar incubators as well as petri dish and multiwell plate static water incubators, from fertilization until hatch. Embryos measured approximately 3 mm in diameter, equating to 38 embryos per ml. In each refrigeration unit, 7 hatching jars were filled half full (5,000 embryos each, 130 ml) and maintained at a flow rate sufficient to impart a gentle rolling action to the eggs (typically about 1 L/min). Petri dishes (20 x 100 mm) and varying sized multiwell plates (6, 12, 24, 48 and 96 well) were used to examine the effects of embryo loading density. Petri dishes were loaded with Lake Simcoe embryos, between 20 and 180 per dish, to determine the percent viable hatch from the eyed stage. Dishes received either minimal water changes, regular weekly water changes or were fed with the continuous drip feed system. Multiwell plates were loaded with

Lake Huron embryos from fertilization, with a single embryo per well (Table 2.1). Significant differences in embryonic survival were analyzed using a t-test (SigmaPlot 11.0).

Table 2.1. Surface area and volumes for different still-water incubation methods.

Rearing dish	Embryo density	Surface area (cm^2 per embryo)	Volume (ml per embryo)	Wetted surface (cm^2 per embryo)
20 x 100 mm	25 per dish	3.14	6.28	8.17
20 x 100 mm	50 per dish	1.57	3.14	4.08
6 well plate	1 per well	9.60	15.5	35.35
12 well plate	1 per well	3.80	6.00	18.29
24 well plate	1 per well	2.00	3.50	12.88
48 well plate	1 per well	0.75	1.40	7.33
96 well plate	1 per well	0.32	0.37	3.42

Reservoir water was changed at a rate of approximately 10 L per day, or 8 % of total volume. In static water dishes, water was changed by gently spilling standing water from the petri dish and replacing with fresh water from the incubator reservoir, decanting again and replenishing water levels to about 1.5 cm depth (3/4 full). Water changes were carried out weekly and after removal of mortalities or hatched larvae. Water changes were less frequently carried out for the multiwell plates owing to their generally lower loading. Water was tested on a daily basis to monitor pH, ammonia, nitrite and nitrate levels (API freshwater test kit). In addition a full water analysis (AGAT Laboratories) was performed to test for anions, metals, conductivity, dissolved solids and alkalinity. Oxygen concentration was determined in the jars and dishes using an O_2 microelectrode (Microelectrodes Incorporated MI-730).

The embryos were reared at temperatures of between 2 and 6°C. The unit air temperature as well as the reservoir water temperature were recorded at 1 minute time intervals throughout

the development period to determine the temperature stability of circulating and static water incubation methods. Reservoir temperature was measured using built in Schlumberger DI 501 Mini-Diver loggers and air temperature was monitored using Onset HOBO Pendant (UA-002) loggers placed on the cooler shelves.

2.3 Results and discussion

Loading density

Survival in hatching jars was similar between both units, and was approximately 50% from fertilization to hatch. This is comparable to survival rates observed in similar lake whitefish studies (Price, 1940, Brooke, 1975) as well as the 30-50% survival from fertilization to hatch over the last three years at the White Lake Hatchery, which uses conventional intensive culture methods (J. Brumpton, Ontario Ministry of Natural Resources, personal communication, 2014). The majority of mortalities occurred early in development. Most embryos reaching the eyed stage survived until hatch, which is consistent for lake whitefish (Brooke, 1975). Rearing embryos in multiwell plates allowed us to observe development in single isolated embryos, without them being impacted by mortality or hatching events from neighboring embryos in the same dish. The 24 and 48 well plates were of most interest as they provided a large enough sample size per plate, while still allowing ample water volume per embryo. The 24 well plates showed a significantly higher survival rate at the eyed stage compared to the 48 well plates (Figure 2-3, $P = 0.043$). Embryos were also raised in 6, 12 and 96 well plates, with 6 showing the highest survival and 12 showing the lowest, although this was not analyzed statistically. The hatching rate was found to be suppressed in 96-well plates compared to plates having a

greater volume per well (data not shown). In petri dishes, constant flow drip feed had the greatest survival rates at higher loading densities. Static water dishes had survival rates greater than 80% when loaded with 50 or fewer eyed embryos with weekly water changes (Figure 2-4). Minimal water changes led to poor survival in dishes with greater than 30 embryos. Although Wedekind *et al.*, (2007) reported loading densities as high as 125 embryos (3.3 ml) per petri dish, we found the optimal loading density with weekly water changes to be 50 embryos (1.3 ml). Higher loading densities, similar to those reported by Wedekind *et al.*, (2007), required more frequent water exchange and were accompanied by a marked increase in hatch rate. A similar increase in hatching rate with high embryo loading density was reported by Barnes and Durben (2008) for rainbow trout (*Oncorhynchus mykiss*) and splake (*Salvelinus namaycush* X *Salvelinus fontinalis*).

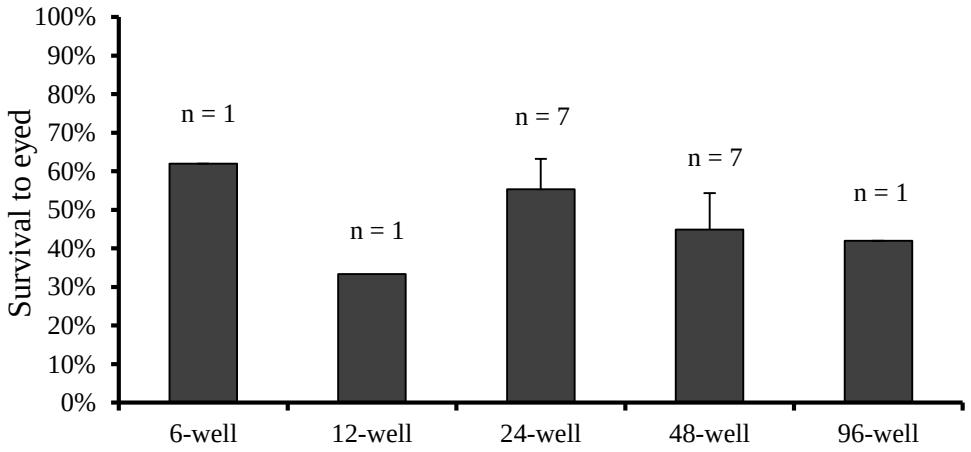


Figure 2-3. Percent lake whitefish embryo survival at eyed stage in various size multiwell plates. Embryos were produced from Lake Huron gametes and incbated in multiwell plates from fertilization until the eyed stage. Embryos in 24 well plates had significantly higher survival that in 48 well plates ($p = 0.043$). n = number of plates used, error bars represent SD.

The multiwell plates became visibly coated with a bacteria/fungi film after about 6 weeks. Multiwell plates generally have a higher surface area of wetted plastic surface per embryo than the petri dishes (Table 2.1). For this reason, water changes for the multiwell plates should include transferring the embryos to a new cleaned and disinfected multiwell plate rather than simply replacing the water.

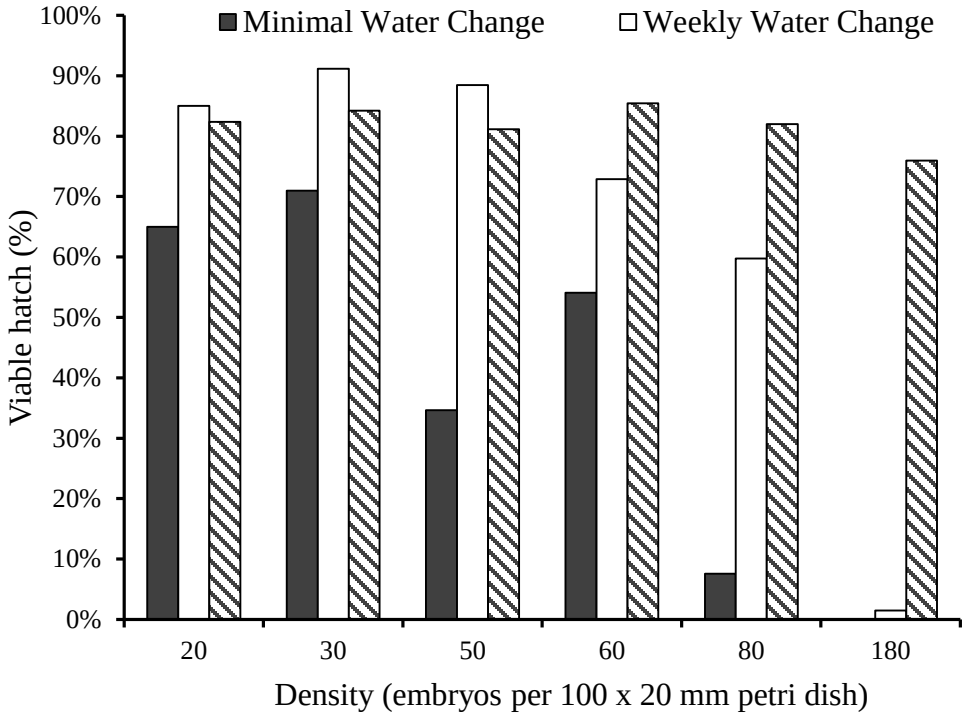


Figure 2-4. Percent viable hatch of lake whitefish embryos in 100 x 20 mm petri dishes with various loading densities. Dishes were loaded with Lake Simcoe embryos at the eyed stage. Dishes received either minimal water changes, weekly water changes or were fed with a constant water flow through a drip feed system at 150 ml/min. Bars represent the percent hatch from 1 dish (n=1).

Temperature stability

Refrigerators operate by the compression and subsequent evaporation of a refrigerant. Since the temperature of the evaporator is below 0°C, it is susceptible to ice accumulation, particularly in humid environments (Lawrence and Evans, 2008). Over time, the frost accumulation may become sufficient to cause the loss of cooling capacity. Our units were able maintain stable air temperatures as low as 2°C when used within their normal operational limits and with regular defrosting cycles of short duration. This defrost cycle resulted in low amplitude temperature oscillations in the circulating water as shown on Figure 2-5. The amplitude of these fluctuations, and the difference in temperature between the reservoirs and the cooler shelves can be modified by shorter defrost cycles, increased use of insulation, especially between the bottom of the cooler and the lower reservoir, and by increasing water volume in the reservoir. We found that a fluctuation of ± 0.2°C was acceptable for our purposes; still water incubation in petri dishes required the use of a water bath to dampen temperature fluctuations. The units were tested below their normal operational limit. Although we were able to maintain stable air temperatures lower than 1°C for several weeks, ice accumulation eventually caused cooling system failure and a spike in temperatures.

Water quality

Good water quality was achieved at loadings of up to 50,000 embryos (ca. 1.3 L) per incubator unit, except during the early stages of embryonic development and hatching, which caused elevated levels of ammonia. Although ammonia-induced mortalities were low, levels of 1 to 2 mg/L NH_3 occurred during hatching unless the water replacement rate was increased to 25%

per day or more. pH values remained stable throughout development between 8 – 8.5. Dissolved solids were measured between 400 – 450 mg/L and alkalinity (as $CaCO_3$) between 150 – 250 mg/L.

Oxygen levels ranged from 70 to 90% of saturation throughout embryogenesis. This level of oxygenation is sufficient to ensure no adverse effects (Silver *et al.*,1967, Czerkies *et al.*, 2002).

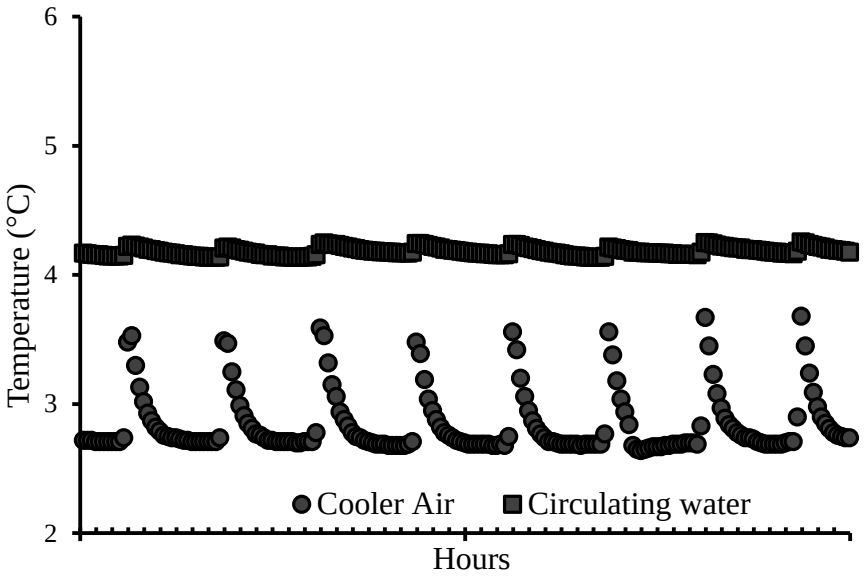


Figure 2-5. Temperature fluctuations in circulating reservoir water and refrigerator air due to defrost cycling.

2.4 Conclusion

We report on the design and construction of a cost-effective, self-contained hatchery system for lake whitefish, with reliable temperature control and a small foot print. The unit makes use of a closed water recirculation system with filtration and UV sterilization that allows for multiple configurations of flowing and static water incubation methods. Our system is

relatively inexpensive and easy to construct, and offers a novel approach for laboratories lacking conventional aquaculture facilities. This system was designed for rearing of lake whitefish embryos, however the technology is likely transferable to other cold water spawning fish in the same or other families. Higher rearing temperatures or larger embryos, characteristic of the non coregonid salmonids, would likely require a lower loading density compared to those described here.

2.5 Acknowledgements

This work was a component of a study funded by a grant from Bruce Power. The Ontario Ministry of Natural Resources White Lake hatchery is gratefully acknowledged for providing lake whitefish embryos for this research. Spawning adults were collected under a scientific fishing permit from the Ontario Ministry of Natural Resources to J.Y.Wilson.

2.6 References

Bardega, R. and M. Luczynski, 2007. Incubator for individual eggs of fish; *Environmental Biotechnology*. 3 (2), 49-51.

Barnes, M. E. and D.J. Durben, 2008. Petri dish incubation of eyed eggs from rainbow trout and splake; *North American Journal of Aquaculture*. 70 (4), 410-414.

Brooke, L.T. 1975. Effect of different constant incubation temperatures on egg survival and embryonic development in lake whitefish (*Coregonus clupeaformis*); *Transactions of the American Fisheries Society*. 104 (3), 555-559.

Czerkies, P., K. Kordalski, T. Golas, D. Krysinski and M. Luczynski, 2002. Oxygen requirements of whitefish and vendace (Coregonidae) embryos at final stages of their development; *Aquaculture*. 211 (1), 375-385.

Gunn, J.M. and W. Keller, 1984. Spawning site water chemistry and lake trout (*Salvelinus namaycush*) sac fry survival during spring snowmelt; *Canadian Journal of Fisheries and Aquatic Sciences*. 41 (2), 319-329.

Lawrence, J.M.W. and J.A. Evans, 2008. Refrigerant flow instability as a means to predict the need for defrosting the evaporator in a retail display freezer cabinet; *International Journal of Refrigeration*. 31 (1), 107-112.

Manny, B.A., D. J. Jude and R.L. Eshenroder, 1989. Field test of a bioassay procedure for assessing habitat quality on fish spawning grounds; *Transactions of the American Fisheries Society*. 118 (2), 175-182.

Price, J.W. 1940. Time-temperature relations in the incubation of the whitefish, *Coregonus clupeaformis* (Mitchill); *Journal of General Physiology*. 23 (4), 449-468.

Silver, S.J., C.E. Warren and P. Doudoroff, 1963. Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities; *Transactions of the American Fisheries Society*. 92 (4), 327-343.

Wedekind, C., R. Müller, A. Steffen and R. Eggler, 2001. A low-cost method of rearing multiple batches of fish; *Aquaculture*. 192 (1), 31-37.

Wedekind, C., and R. Müller, 2004. The experimental rearing of large salmonid eggs in petri dishes; *Functional Ecology*. 18 (1), 138-140.

Wedekind, C., G. Rudolfsen, A. Jacob, U. Davnah and R. Müller, 2007. The genetic consequences of hatchery-induced sperm competition in a salmonid; *Biological Conservation*. 137 (2), 180-188.

Chapter 3

A method to transform a variable thermal regime to a physiologically equivalent effective temperature

Charles Mitz[1*], Chris Thome[1,5], Jeroen Thompson[2], Richard Manzon[3], Joanna Wilson[4], and Douglas R. Boreham[1,2,5]

Manuscript in review: Journal of Thermal Biology

Abstract

We present a method to characterize variable thermal regimes in terms of an equivalent or effective temperature. Our method is based on a first order exponential transformation of a time series of temperatures to yield an exponentially-weighted mean temperature characteristic of the regime and independent of any particular species or end point. The resulting effective temperature, $\overline{T_e}$, is that which duplicates biological growth under a given variable thermal regime provided that such growth scales exponentially to temperature. The exponential mean allows growth under varying thermal regimes to be predicted using constant temperature models and offers a compact descriptor communicating the growth capacity of variable thermal regimes.

The method combines mathematical simplicity with translatability to different Q_{10} values without recourse to the underlining time series data. It also provides a quantitative baseline that improves on mean temperature by incorporating the effect of Jensen's inequality and it remains applicable at near zero temperatures where thermal sums lack accuracy.

* corresponding author

[1]*Department of Medical Physics and Applied Radiation Sciences, McMaster University*
[2]*Bruce Power, Tiverton, ON, Canada*
[3]*Department of Biology, University of Regina*
[4]*Department of Biology, McMaster University*
[5]*Northern Ontario School of Medicine*

3.1 Introduction

Numerous models have been proposed to describe the temperature dependence of biological growth under different constant temperature conditions. However, the ecological application of such models is hindered by the fact that most real-world thermal regimes are variable and intrinsically more complex owing to non-linearity in thermal scaling. The ability to accurately predict growth and development under conditions of varying temperatures is especially important when assessing subtle, ecosystem-level effects such as competitive equilibria and phenological mismatch (Visser and Holleman, 2001; Genner *et al.*, 2009) that may occur at temperatures below recognizable stress response thresholds. The ability to characterize temperature variations in natural thermal regimes is also desirable when considering the effects of climate change where increases in temperature variation may cause a greater impact than the changes in mean temperature (Vasseur *et al.*, 2014).

One of the earliest approaches to predicting growth under varying thermal regimes involved the method of thermal sums, widely known as the degree-day method. This method dates to the eighteenth century and has continued in use, with various modifications, to present (Neuheimer and Taggart, 2007). The degree day is the sum of the product of temperature and time. Commonly, complete development occurs for an approximately constant number of degree days.

The degree day method results in a relationship between temperature and growth that follows a power function whose slope is asymptotic to the Y-axis at zero degrees. A biological zero parameter (representing the temperature below which development ceases) is therefore

required to partially overcome this mathematical limitation, or to accommodate growth with a lower thermal bound at temperatures greater than zero. Figure 3-1 illustrates limitations of the degree day method compared to an exponential model when applied to a cold-developing species such as the lake whitefish (*Coregonus clupeaformis*). In addition to the problem of a biological zero, only a fraction of the thermal performance curve occurs at a near-constant number of degree days. Use of different values for biological zero can only partially overcome this fundamental limitation.

An alternative approach is the use of incremental growth-at-temperature (IGT) to predict total growth either as a continuous function of time steps (e.g. Alderdice and Velsen, 1978; Katsanevakis and Verriopoulos, 2006; Thome *et al.*, 2016) or through stage-wise growth models (e.g. Brooke, 1975; Berlin *et al.*, 1977) that apply different thermal scaling relationships to different development stages. In contrast to thermal sums, IGT adds increments of growth over discrete time steps $\Delta t_1, \Delta t_2, \Delta t_3 ....$ at temperatures $T_1, T_2, T_3 ....$ and thus allows for non-linear temperature dependence of biological growth. Known as Jensen's inequality (Ruel and Ayres, 1999; Martin and Huey, 2008; Ragland and Kingsolver, 2008), an accelerating thermal dependence causes growth rates under varying temperatures to exceed those that would be predicted by simply using the mean temperature. IGT is, to the best of our knowledge, the only existing method for modeling growth under varying thermal regimes that accounts for Jensen's inequality. Unfortunately IGT yields a summation of species-specific growth increments ill-suited for statistical analysis and unwieldy for the description of the thermal regime being considered.

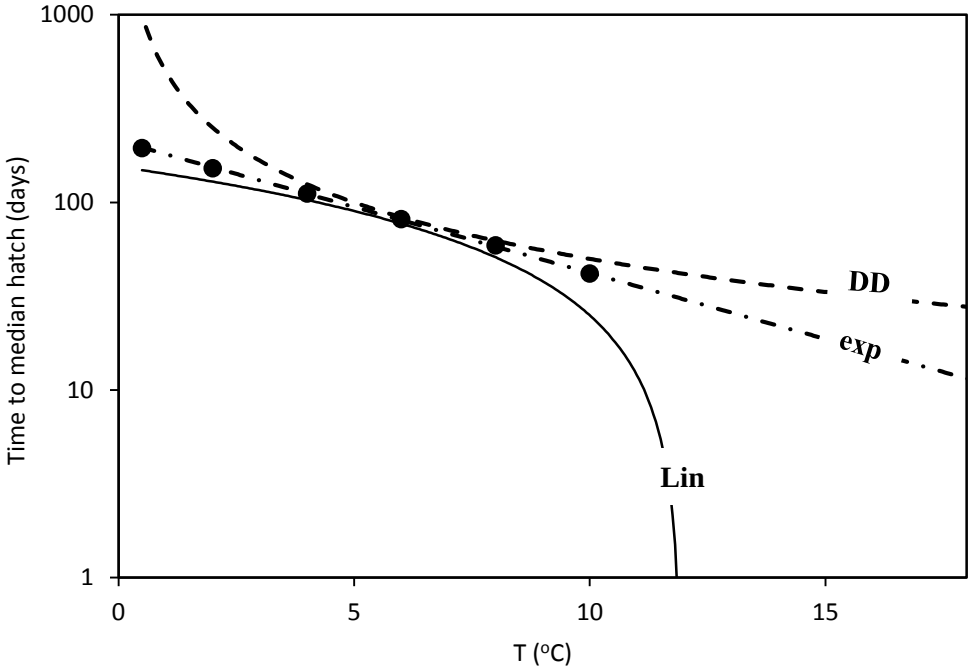


Figure 3-1. Observed days to median hatch for lake whitefish under constant incubation temperature (circles after Brooke 1975) compared to predicted curves based on **DD**: 500 degree days, **exp**: an exponential relationship given by $h_{50}=213e^{-0.16T}$ (regression using data from Brooke, 1975), and **Lin**: a linear relationship given by h_{50} =-13T+155. Application of a negative biological zero parameter would effectively shift Curve **DD** to the right but still result in a flatter slope than the observed temperature dependence of hatching.

Jensen's inequality may also be incorporated by transforming a variable thermal regime to an equivalent or effective temperature provided that the thermal dependence of growth follows an exponential relationship. The resulting method has advantages over thermal sums as a quantitative baseline and it may simultaneously facilitate the modelling of multiple species interactions while providing a compact, mathematically convenient, form that facilitates communication and statistical analysis of temperature-dependent phenomena.

3.2 Method Description and Development

Our approach may be summarized as follows:

1) Where biological growth to a consistent and definable end point (e.g. hatching) under varying temperatures may be described as the incremental sum of a continuous monotonic function of time-at-temperature $f(T)$, there exists an equivalent constant temperature, T_{eq}, such that $\sum_0^t f(T) = f(T_{eq})$

2) Where $f(T)$ is selected to be independent of species-specific parameters, it may be used to transform a varying thermal regime to an equivalent constant temperature, T_{eq} suitable for application across a range of taxa, biological processes, and end points.

Although $f(T)$ could be any continuous monotonically increasing equation, we have used a first order exponential relationship because of its mathematical simplicity and well-established mechanistic basis. Both the Eyring and Sharpe-Schoolfield equations are based on an exponential relationship between growth and temperature for all (Eyring) or a portion (Sharpe-Schoolfield) of the thermal domain (Figure 3-2). Where $f(T)$ is an exponential function of *T*, we show that T_{eq} takes the form of an exponentially transformed mean $\overline{T_e}$.

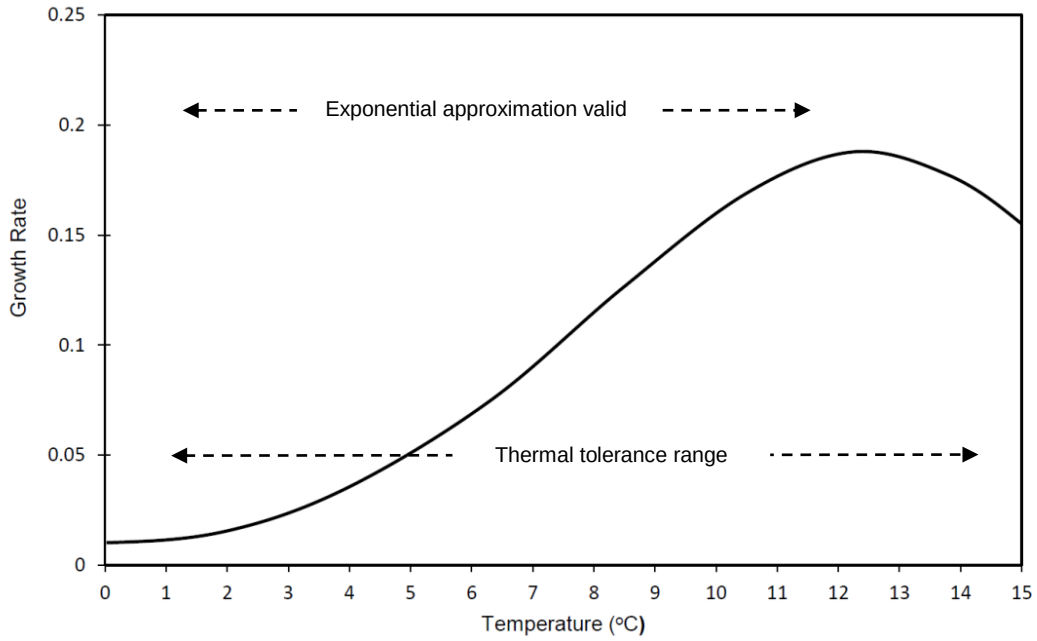


Figure 3-2: General shape of the growth-temperature relationship described by the Sharpe-Schoolfield equation (Sharpe & DeMichele, 1977). For much of the thermal tolerance range (0 to 15°C in the example shown), growth follows an exponential relationship as described in the Eyring equation.

Clarke and Johnston (1999) analyzed thermal dependence of resting metabolism for 69 species of teleost fish and found similar slopes despite significantly different rates of absolute growth. Similar conclusions were drawn by Gillooly *et al.* (2001) and Brown *et al.* (2004) who proposed a general model relating biological growth to size and temperature lending support to the hypothesis that the observed temperature dependency of growth in fish reflects biological processes common to life in general (Huey and Kingsolver, 2011; Clarke, 2004). Thus, the mechanistic basis for an exponential scaling of growth to temperature is supported by observations across different species and life stages.

When considering the time to grow to a definable end point, the simplest exponential relationship is:

$$t_h = \alpha e^{-\beta T} \qquad [1]$$

where the time, t_h, required to attain a biological end point (for example hatching), at constant temperature T, is inversely proportional to an exponential function defined by the exponent – β, and where α is a species-specific parameter defining the time to reach the specified end point at a temperature of zero degrees.

We may restate Equation 1 to eliminate the negative sign on β such that:

$$f(T) = \frac{1}{\alpha} e^{\beta T} \qquad [2]$$

The relationship between the equivalent constant temperature $\overline{T_e}$ and the variable values for T may be approximated numerically by:

$$\frac{1}{\alpha} e^{\beta \overline{T_e}} t_{sum} = \sum_0^{t_i} \frac{1}{\alpha} e^{\beta T} \qquad [3]$$

where t_{sum} is the total number of time increments. Dividing each side by $\frac{1}{\alpha}$ removes the species-specific parameter and allows for generalization in the form:

$$e^{\beta \overline{T_e}} t_{sum} = \sum_0^{t_i} e^{\beta T} = (e\beta^{T_0} \Delta t_0 + e\beta^{T_1} \Delta t_1 + ...e^{\beta T_n} \Delta t_n) \qquad [4]$$

This can be solved numerically by varying the value for $\overline{T_e}$ until the left and right sides of the equation are equal. Alternatively, we may use a numeric approximation of temperature dependent growth to determine the time, t_h, required to attain a hypothetical reference endpoint.

Since we have assumed that the fractional growth rate, $f(t)$, with variable temperature, $T(t)$, is the same as the fractional growth rate, $f(T)$ at the constant temperature T:

$$f(t) = 1/t_h = \frac{1}{\alpha} e^{\beta T(t)} \qquad [5]$$

The time-to-endpoint, t_h, is thus defined by the temperature as a function of time:

$$\int_0^{t_h} f(t)\,dt = \int_0^{t_h} \frac{1}{\alpha} e^{\beta T(t)}\,dt = 1 \qquad [6]$$

We seek an equivalent temperature, $\overline{T_e}$, such that the total growth at constant temperature $\overline{T_e}$, over time t_h is equal to the total growth over the same time with variable temperature $T(t)$:

$$\int_0^{t_h} \frac{1}{\alpha} e^{\beta \overline{T}_e}\,dt = \int_0^{t_h} \frac{1}{\alpha} e^{\beta T(t)}\,dt \qquad [7]$$

which has the solution:

$$\overline{T}_e = \frac{1}{\beta} \ln \frac{\alpha}{t_h} \qquad [8]$$

An excel spreadsheet to calculate $\overline{T_e}$ for a time series of temperature data is included as supplementary material.

The value used for β may also be compared to the Q_{10} descriptor (Van't Hoff, 1884). Q_{10} describes changes in growth rate, R, across a 10°C change in temperature

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}} \qquad [9]$$

Thus, our proposed β may be restated as $\beta = \frac{Ln Q_{10}}{10}$. [10]

3.3 Discussion

The exponentially transformed mean temperature $\overline{T_e}$ is a characteristic of the thermal regime and independent of any particular species or biological end point. This allows $\overline{T_e}$ to be applied to a variety of species-specific constant temperature growth models where such growth may be described as the incremental sum of a continuous function of time-at-temperature scaling according to the value of β used. Its applicability is not universal since the magnitude or rate of thermal fluctuations may be large enough to induce a stress response that will alter the exponential relationship between growth and temperature (van der Have and de Jong, 1996, Meeuwig *et al.*, 2004). The constant-temperature thermal equivalent $\overline{T_e}$ is valid for species and thermal ranges meeting the following criteria:

1) Growth to a consistent developmental end point under varying temperatures may be described as the incremental sum of a continuous function of time-at-temperature *T*.
2) Temperature dependence of growth follows an exponential relationship over the range of temperatures considered.
3) The scaling of temperature dependent growth is the same across the development period considered regardless of absolute changes due to size-dependent effects.

Provided that these conditions apply, calculated values for $\overline{T_e}$ may be applied to suitable species-specific constant temperature growth models. Derived values for $\overline{T_e}$ also offer a basis to assess deviations from the assumed exponential scaling relationship (i.e. where one or more of Conditions 1 to 3 are invalidated) and may serve as a quantitative baseline for statistical

analyses. For example, it is useful to determine how strongly an observed effect correlates to the characteristic being investigated (e.g. amplitude, frequency, or asymmetry of a variable thermal regime) or simply a result of the temperature. Thermal sums have limitations that affect their utility for this purpose and use of mean temperature neglects the effect of Jensen's inequality and may therefore understate the strength of a correlation. $\overline{T_e}$ provides an exponentially transformed mean that accommodates Jensen's inequality and is well suited for such applications.

Any error in the estimated value for β applies only to the difference between the mean temperature, T_μ and $\overline{T_e}$. The difference between T_μ and $\overline{T_e}$ is greatest under a bimodal temperature distribution where temperatures fluctuate between high and low end points. Under such a regime, the difference increases in proportion to the square of the thermal amplitude (Figure 3-3).

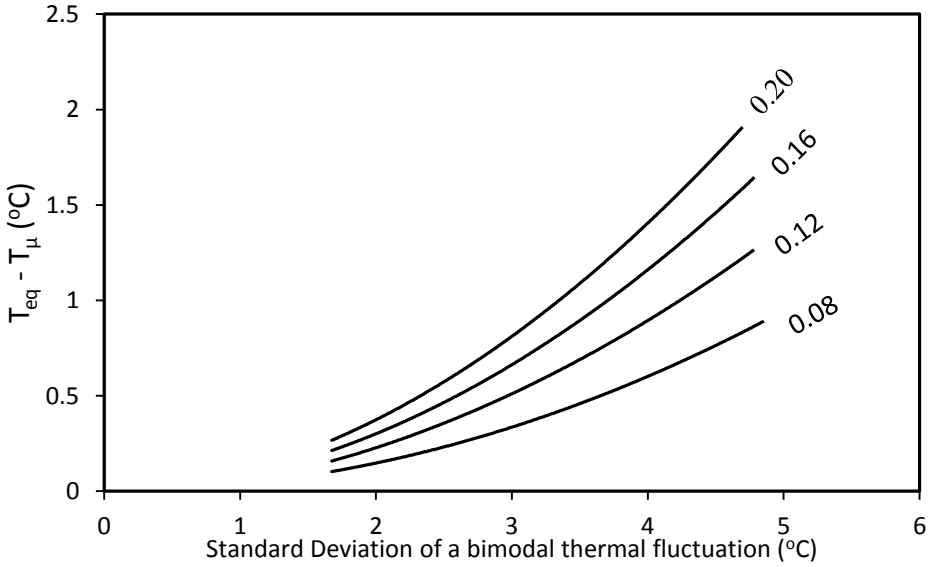


Figure 3-3. Difference between mean temperature (T_μ) and equivalent temperature ($\overline{T_e}$) for a bimodally varying thermal regime having different values of β (shown beside curves).

For a sinusoidally fluctuating thermal regime, the difference between T_μ and $\overline{T_e}$ is approximately half that for a bimodal regime of the same amplitude. The difference between T_μ and $\overline{T_e}$ increases linearly with β for all bimodal, sinusoidal, and random (Gaussian) temperature regimes for values of β within the ecologically relevant range.

With $\Delta T = \overline{T_e} - T_\mu$, we note that

$$\Delta T = \begin{cases} \frac{1}{\beta}\ln\cosh\beta A \approx \frac{A^2}{2}\beta - \frac{A^4}{12}\beta^3 & \text{bimodal} \\ \frac{1}{\beta}\ln I_0(\beta A) \approx \frac{A^2}{4}\beta - \frac{A^4}{64}\beta^3 & \text{sinusoidal} \\ \frac{\sigma^2}{2}\beta & \text{normal} \end{cases} \qquad [11]$$

Where A is the amplitude of a bimodal or sinusoidal temperature fluctuation, σ is standard deviation, and I_0 represents a modified Bessel function of the first kind.

Provided that the mean temperature and β (or Q_{10}) is reported, this near-linearity allows the resulting $\overline{T_e}$ to be adjusted for differing values for β without access to the underlying temperature data. For this reason, we suggest that the exponential mean be reported in the form $\overline{T_e}^{\beta}$ so an exponentially transformed mean using β =0.11 (i.e. Q_{10}=3.0) would be written as $\overline{T_e}^{0.11}$.

Because $\overline{T_e}$ is species-independent, the method may be used to characterize and compare different varying thermal regimes under conditions of competitive equilibria. In this instance, different thermal regimes may share identical mean temperatures but sustain different

biological growth rates on an ecosystem scale. Recent studies have shown that rising temperatures due to global warming may cause dramatic shifts in species ranges and interactions (Brooker *et al.*, 2007; Van der Putten *et al.*, 2010). In some cases, relative fitness is strongly influenced by comparably small differences in temperatures (Taniguchi *et al.*, 1998; Farrell, 2001). An increase in the amplitude of temperature fluctuations would advance hatching for species having a greater value for β relative to species whose temperature-dependent growth follows a lower value for β.

The need for improved thermal descriptors has been recognized (Steel *et al.*, 2012; Arismendi *et al.*, 2013) and use of $\overline{T_e}$ provides a basis to characterize variable thermal regimes whether these result from climate change, or more localized anthropogenic alteration of natural temperatures. The method offers both a tool for the assessment of growth effects of varying temperatures and, perhaps more importantly, a compact, and versatile metric for describing them.

3.4 Acknowledgements

We are grateful to Bruce Power for its support of this work, both financially and technically, and to grants from the Natural Sciences and Engineering Research Council of Canada (NSERC).

3.5 References

Alderdice, D. F. and F. P. J. Velsen, 1978. Relation between temperature and incubation time for eggs of Chinook salmon (*Oncorhynchus tshawytscha*); *Journal of the Fisheries Research Board of Canada* 35: 69–75.

Arismendi, I., S. L. Johnson, J. B. Dunham and R. Haggerty, 2013. Descriptors of natural thermal regimes in streams and their responsiveness to change in the Pacific Northwest of North America; *Freshwater Biology* 58: 880–894.

Berlin, W.H., L.T. Brooke and L.J. Stone, 1977. Verification of a model for predicting the effect of inconstant temperature on embryonic development of lake whitefish (*Coregonus clupeaformis*); Technical papers of the US Fish and Wildlife Service 92.

Brooke, L.T. 1975. Effect of different constant incubation temperatures on egg survival and embryonic development in lake whitefish (*Coregonus clupeaformis*); *Transactions of the American Fisheries Society*. 104 (3), 555-559.

Brooker, R. W., J.M.J. Travis, E.J. Clark, and C. Dytham, 2007. Modelling species' range shifts in a changing climate: The impacts of biotic interactions, dispersal distance and the rate of climate change; *Journal of Theoretical Biology* 245: 59–65.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West, 2004. Toward a metabolic theory of ecology; *Ecology* 85: 1771–1789.

Clarke, A. and N.M. Johnston, 1999. Scaling of metabolic rate with body mass and temperature in teleost fish; *Journal of Animal Ecology* 68: 893-905.

Clarke, A., 2004. Is there a universal temperature dependence of metabolism? *Functional Ecology* 18: 252-256.

Farrell, J. M., 2001. Reproductive success of sympatric northern pike and muskellunge in an upper St. Lawrence River Bay; *Transactions of the American Fisheries Society* 130: 796-808.

Genner, M. J., N. C. Halliday, S. D. Simpson, A. J. Southward, S. J. Hawkins, and D. W. Sims, 2009. Temperature-driven phenological changes within a marine larval fish assemblage; *Journal of Plankton Research* 32.5 (2009): 699-708.

Gillooly, J. F., J.H. Brown, G.B. West, V.M. Savage and E.L. Charnov, 2001. Effects of size and temperature on metabolic rate; *Science* 293: 2248–2251.

Huey, R. B. and J. G. Kingsolver, 2011. Variation in universal temperature dependence of biological rates; *Proceedings of the National Academy of Sciences* 108: 10377–10378.

Katsanevakis, S. and G. Verriopoulos, 2006. Modelling the effect of temperature on hatching and settlement patterns of meroplanktonic organisms: the case of the octopus; *Scientia Marina* 70(4) December 2006, 699-708.

Martin, T. L. and R. B. Huey, 2008. Why "Suboptimal" is optimal: Jensen's Inequality and ectotherm thermal preferences; *The American Naturalist* 171: E102-118.

Meeuwig, M.H., J.B. Dunham, J.P. Hayes and G.L. Vinyard, 2004. Effects of constant and cyclical thermal regimes on growth and feeding of juvenile cutthroat trout of variable sizes; *Ecology of Freshwater Fish* 13: 208-216.

Neuheimer, A. B, and C. T. Taggart., 2007. The growing degree-day and fish size-at-age: The overlooked metric; *Canadian Journal of Fisheries and Aquatic Sciences* 64.2 (2007): 375-385.

Ragland, G.J. and J.G. Kingsolver, 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii; Evolutionary Ecology Research* 10: 29-44.

Ruel, J. J. and M. P. Ayres, 1999. Jensen's inequality predicts effects of environmental variation; *Trends in Evolution and Ecology* 14: 361-366.

Sharpe, P. J. H. and D. DeMichele, 1977. Reaction kinetics of poikilotherm development; *Journal of Theoretical Biology*, 64: 649-670.

Steel, E. A., A. Tillotson, D. A. Larsen, A. H. Fullerton, K. P. Denton, and B. R. Beckman, 2012. Beyond the mean: The role of variability in predicting ecological effects of stream temperature on salmon; *Ecosphere* 3.11 (2012): art104

Taniguchi, Y., Rahel, F.J., Novinger, D.C. and K.G Gerow, 1998. Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients; *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1894-1901.

Thome, C., C. Mitz, C.M. Somers, R.G. Manzon, J.Y. Wilson, and D.R. Boreham, 2016. Incubation of Lake Whitefish (*Coregonus clupeaformis*) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development; *Canadian Journal of Fisheries and Aquatic Sciences, http://dx.doi.org/10.1139/cjfas-2015-0286.*

van der Have, T. M. and G. de Jong, 1996. Adult size in ectotherms: temperature effects on growth and differentiation; *Journal of Theoretical Biology*. 138: 329–340.

Van der Putten, W.H., M. Macel and E.Visser, 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels; *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 2025–2034.

Van't Hoff, J.H. 1884. Etudes de dynamique chimique. Muller, Amsterdam

Vasseur, D. A., J. P. DeLong, B. Gilbert, H. S. Greig, C. D. G. Harley, K. S. McCann, V. Savage, T. D. Tunney, and M. I. O'Connor, 2014. Increased temperature variation poses a greater risk to species than climate warming; *Proceedings of the Royal Society B: Biological Sciences* 281.1779 (2014)

Visser, M. E. and L. J. Holleman, 2001. Warmer springs disrupt the synchrony of oak and winter moth phenology; *Proceedings of the Royal Society B: Biological Sciences* 268: 289-294.

West, G.B., J.H. Brown and B.J. Enquist, 1999. The fourth dimension of life: Fractal geometry and allometric scaling of organisms; *Science* 284: 1677–1679.

Chapter 4

Thermal dependence of size-at-hatch in the lake whitefish (*Coregonus clupeaformis*)

Charles Mitz[1], Chris Thome[1,5], Mary Ellen Cybulski[1], Christopher M. Somers[3], Richard G. Manzon[3], Joanna Y. Wilson[4*], and Douglas R. Boreham[1,2,5]

Abstract

We examined hatch timing and morphological characteristics for whitefish embryos reared under different constant and varying temperatures to determine whether size-at-hatch reflects differences between the thermal scaling of growth and development, or whether hatching occurs at a more advanced stage of development at low temperatures. Our results show that lake whitefish hatch at different temperature-dependent development stages, and that this is the dominant factor affecting size-at-hatch although temperature-dependent differences in yolk conversion efficiency (YCE) also contribute. We propose the general term heterograde to describe this form of passively acting thermal dependence of development stage at hatching in order to differentiate it from phenomena in which hatching is triggered by an active response to environmental cues. Heterograde hatching is proposed as a possible mechanism to synchronize the timing of hatching to the break-up of winter ice cover.

*corresponding author

[1]*Department of Medical Physics and Applied Radiation Sciences, McMaster University*
[2]*Bruce Power, Tiverton, ON, Canada*
[3]*Department of Biology, University of Regina*
[4]*Department of Biology, McMaster University*
[5]*Northern Ontario School of Medicine*

4.1 Introduction

The size of newly hatched lake whitefish (*Coregonus clupeaformis*) embryos is strongly temperature-dependent with embryos incubated at low temperatures hatching at a larger size than those incubated at warmer temperatures (Hart, 1931; Price, 1940; Colby and Brooke, 1973; Brooke, 1975; Griffiths, 1979; Luczynski, 1984; Luczynski & Kirklewska 1984; Eme *et al.*, 2015, Mueller *et al.*, 2015 and Lee *et al.*, 2016). Understanding the dynamics and cause(s) of the temperature-dependent size-at-hatch in the lake whitefish would improve our ability to assess the potential for phenological mismatch (Cushing, 1969; Yang and Rudolph, 2009) resulting from anthropogenically altered thermal regimes that might cause whitefish embryos to hatch asynchronously to their normal food supply, or to hatch with insufficient yolk reserves to successfully transition to exogenous feeding. However, the mechanism(s) underlying hatching size remain poorly understood despite the whitefish being subject of intensive study due to its wide distribution and economic, cultural, and ecological importance.

Size-at-hatch is affected by two distinct but interrelated processes: growth and development. The former is simply the gain in dry mass with time while development is the progress toward recognizable morphological stages such as the development of organs, eye pigmentation, metamorphosis, or fin separation. If growth and development follow identical thermal scaling relationships with equal efficiency, then embryos should reach a given development stage at an identical size regardless of temperature. However, if growth and development exhibit differences in thermal scaling (i.e. if the Q_{10} for development differs from the Q_{10} for growth) then the embryo size at a given development stage will vary as a function of temperature. Scaling differences

between growth and development have been proposed as a mechanism governing adult size in ectotherms (Silby and Atkinson, 1994; van der Have and Dejong, 1996, and Forster and Hirst, 2012) and the mechanism underlying TSR in general (Zuo *et al.*, 2012). Alternatively, the larger size-at-hatch may result from temperature-dependent differences in growth efficiency such as that predicted by the Bertalanffy-Perrin model (Perrin,1995; Atkinson and Silby, 1997; Angilletta and Dunham, 2003), or it may simply reflect whitefish hatching at a more advanced stage of development at lower temperatures. This latter possibility is a form of developmental heterochrony analogous to the difference between a premature baby and a low birth-weight baby (Jordaan, 2002).

We propose the general term heterograde for hatching that occurs at different temperature-dependent development stages. Hatching that occurs at a fixed development stage, independent of temperature, may be termed isograde. The use of a specific descriptive term is useful to differentiate the thermal dependence of stage-at-hatch from comparable forms of heterochrony such as hypermorphosis (Fink, 1982) that act on an evolutionary time-scale, and from actively plastic phenomena in which hatching is triggered by active recognition and response to environmental cues (Martin *et al, 2011*; Warkentin, 2011). Both heterograde hatching and differential scaling between growth and development function passively without any form of active recognition and signalling in response to environmental cues. Plasticity in the timing of hatching and size-at-hatch in both cases is the consequence of non-plastic thermal scaling relationships that remain fixed across different environments. Figure 4-1 illustrates the heterograde hypothesis applied to the lake whitefish.

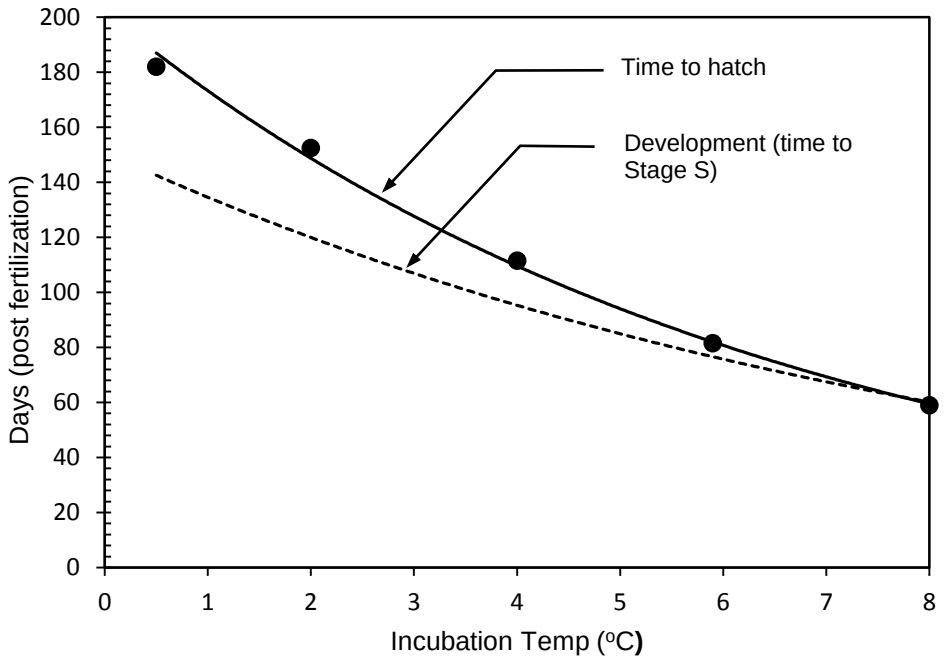


Figure 4-1: Graphical illustration of heterograde hatching. The solid line shows an exponential regression for mean hatching time (solid circles) for Lake Michigan whitefish from Brooke (1975) while the dashed line represents the time to reach a hypothetical development stage (Stage S) equivalent to stage at hatching for constant temperature incubation at 8°C. In this illustration, growth and development follow an identical scaling relationship to temperature. In the differential scaling hypothesis, the dashed line would represent the time to grow to a specific size equivalent to the size at hatching for constant temperature incubation at 8°C.

Because it is difficult to measure growth independently from development stage, it is necessary to test predictions that derive from the different size-at-hatch hypotheses such as the differences different hatch timing predicted when incubation temperatures vary asymmetrically. Asymmetrically varying regimes may incorporate a discrete shift in temperature or include progressively increasing or decreasing temperatures. The natural thermal regimes experienced by incubating whitefish embryos are asymmetrically variable with environmental uncertainty affecting both the timing of freeze-up and the date of spring break-up.

The distinction between the hatch timing predicted by the heterograde vs. differential scaling hypotheses may be clarified by considering embryonic growth in terms of relative development (RD) which, for constant temperature, is the cumulative incubation time divided by the time to reach a specific development stage. Relative time-to-hatch (RTH) is calculated in the same manner but the cumulative incubation time is divided by the time to hatch. For the differential scaling hypothesis, RD and RTH are identical when using hatching as a development stage. However, if the stage at hatching varies according to temperature (i.e. heterograde hatching) then RD and RTH will differ. In order to avoid confusion, this paper uses RD in a generic sense to refer to fractional development relative to the time of median hatch unless otherwise stated.

Under the differential scaling hypothesis, RD is continuous and monotonic (i.e. it must continually increase at a greater or lesser rate but can never reverse itself and decline) while heterograde hatching predicts an instantaneous change in RD accompanying a change in incubation temperature (Figure 4-2). If an upward increase in temperature occurs at a sufficiently advanced stage of development, the instantaneous change may surpass 100% RD and trigger immediate hatching. Conversely, a downward shift in incubation temperature would lead to an instantaneous decrease in RD and delay hatching or cause a hiatus in hatching that had already begun.

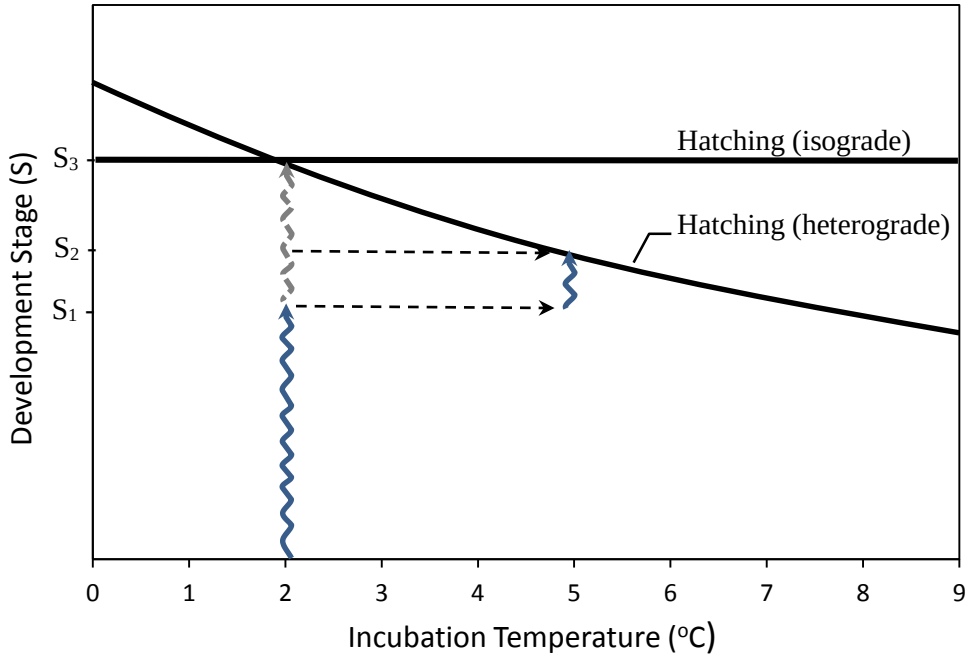


Figure 4-2: Effect of heterograde hatching on hatch timing under an asymmetrically variable thermal regime. Under isograde hatching, hatching occurs at developmental stage S_3 independent of the incubation temperature. However, under heterograde hatching, hatching will occur at developmental stages that are dependent on the incubation temperature. In this illustration, the development stage is shown for an embryo developing at 2°C to Development Stage S_1 (70% relative development under both the heterograde and isograde assumptions) when it is shifted to 5°C (broken line with arrows). Time is not shown but may be visualized as the Z-axis. As shown, the post-shift time to hatch will be overestimated under an isograde assumption (70% relative development under the isograde assumption vs. 80% relative development under the heterograde model). A shift from 2 to 5°C occurring at S_2 brings the embryos to 100% relative development under the heterograde model and is predicted to trigger mass hatching.

We specifically looked at three areas where differential scaling and heterograde hatching result in different predictions.

1) **Hatch Timing:** Embryos reared under progressively increasing thermal regimes are predicted to hatch earlier than the RD predicted using the mean incubation temperature if hatching is heterograde. Conversely, hatching would occur later than predicted for the

decreasing thermal regime. No difference is predicted if hatching is isograde (as is the case for differential scaling between growth and development).

2) **Morphological Indicators of Stage:** Morphological indicators of stage-at-hatch (e.g. fin indentation) are predicted to show a more advanced stage of development at low incubation temperatures (heterograde) vs. no significant difference (differential scaling).
3) **Post-shift Q_{10}:** Heterograde hatching is predicted to result in a post-shift (from a low base temperature to a higher post-shift temperature) Q_{10} that steadily increases as the temperature shift approaches the time of hatch while the differential scaling hypothesis predicts no significant change.

An assessment of yolk conversion efficiency (YCE) is also needed to determine the relative contribution of temperature-dependent differences in growth efficiency to size-at-hatch and to determine whether any scaling differences operate between growth and development or between anabolism and catabolism. Angilletta and Dunham (2003) show that the Bertalanffy-Perrin model (anabolism vs. catabolism) predicts decreasing efficiency with increasing temperatures while Zuo *et al*. (2012) show that differential scaling between growth and development results in an increasing efficiency of growth with increasing temperatures.

4.2 Methods and Materials

Egg collection and Incubation Procedures

We collected whitefish eggs from spawning Lake Huron fish gillnetted on November 21, 2013 in 3 to 6 m of ~7°C water south of Whitefish Island (44°42’37.74”N, 81°18’38.94”W). Eggs were stripped from multiple ripe females (mF) and fertilized with pooled milt from ripe males followed

by disinfection using Ovadine® (5 ml L^{-1}, Syndel Laboratories Ltd.). In 2014, additional eggs were obtained from the Ontario Ministry of Natural Resources and Forestry (MNRF) from a single female (sF) from Lake Simcoe fertilized by pooled milt from different males.

The fertilized eggs were transported in an ice bath to McMaster University where they were reared in refrigerated incubators using apparatus and methods described in Mitz *et al.* (2014). Incubation temperatures were held at nominal temperatures of 2, 5 and 8°C with an additional 0.5°C (nominal) temperature point obtained by incubating dishes on an unsaturated bed of crushed ice. Photoperiod was ambient with natural lighting through the laboratory windows and was consistent across all treatments. Data loggers (Schlumberger Mini-Diver and Onset Hobo) were placed at multiple locations within the incubator units and set to record water temperature every 5 minutes.

The embryos were reared in 24-well plates filled with dechlorinated municipal tap water. Embryos were moved to a clean multi-well plate during weekly water changes to prevent fungal contamination. Viable hatches were recorded and dead embryos removed daily. Embryo mortalities were recognized by the development of opacity on a significant portion of the egg or the disintegration of the chorion and embryo.

Hatched larvae were anesthetized using a solution of approximately 0.5% MS-222 (ethyl 3-aminobenzoate methanesulfonate, Sigma Aldrich) prior to being imaged live using an Axio Zoom V16 microscope (Carl Zeiss) equipped with a Canon EOS Rebel T1i camera. Dimensional measurements (Figure 4-3) were made to the nearest 1 μm using Zeiss Axiovison (Rel 4.9.1) digital image processing software. Following imaging, the embryos were placed intact or deyolked on

aluminum foil tares and dried to an asymptotic constant weight overnight at a temperature of 70°C in a VWR Brand Model 1500EM drying oven. Anhydrous silica gel was placed in the drying oven along with the tissue samples to maintain a low humidity during weighing (Mettler-Toledo XA105DU, ±0.01 mg). The dry weight of the larvae was estimated for intact embryos according to an experimentally-derived (see Results) relationship between standard length (SL) and dry weight of deyolked larvae. This value was subtracted from the total embryo dry weight to estimate the dry weight of the yolk. The relationship between embryo dry weight (including intact yolk) and somatic (i.e. yolk-free) dry weight was calculated using the weights of pooled embryos (10 yolked and 10 deyolked) taken at different times during development. Measurements were taken using unpreserved embryos to avoid possible confounding effects resulting from chemical preservation (Smith and Walker, 2003; Melo *et al.*, 2010; Sreetharan *et al.*, 2015).

Experimental Thermal Regimes

Embryos from the two populations (Huron and Simcoe) were incubated under a variety of constant and asymmetrically varying thermal regimes. Asymmetrically variable regimes included progressively increasing or decreasing regimes where initial development took place at either 2 or 8°C for approximately 1/3rd of expected RD, followed by transfer to progressively higher (→5→8°C) or lower (→5→2°C) temperatures for the remaining thirds of expected RD. Asymmetrically varying regimes also included initial incubation at 2°C for different lengths of time (81, 93, 106, 121, 138, and 151 days) before transfer to 5 or 8°C for their remaining development. The experimental thermal regimes are summarized on Table 4.1.

Table 4.1. Experimental Thermal Regimes

Regime	**Mean T (ºC)**	**Details**
mF Lake Huron 2013-2014 and 2014-2015		
Constant (0)	0.5±0.2	Fertilization → hatch
Constant (2)	2.5±0.87	Fertilization → hatch
Constant (5)	5.15±0.33	Fertilization → hatch
Constant (8)	8.1±0.28	Fertilization → hatch
Asymmetric (8→5→2°C)	3.4	17 d → 33d → hatch
Asymmetric (2→5→8°C)	4.2	45d → 33d → hatch
sF Lake Simcoe 2014-2015		
Constant (2)	2.0±0.145	Fertilization → hatch
Constant (5)	4.98±0.08	Fertilization → hatch
Constant (8)	7.93±0.17	Fertilization → hatch
Asymmetric (8→5→2°C)	3.3	17 d → 33d → hatch
Asymmetric (2→5→8°C)	4.1	45d → 33d → hatch
Asymmetric (2→5°C at different development times)	3.29, 3.05, 2.78, 2.67, 2.33, 2.19	(81, 93, 106, 121, 138, 151d) → hatch
Asymmetric (2→8°C at different development times)	3.26, 3.30, 2.98, 2.53, 2.25, 2.10	(81, 93, 106, 121, 138, 151d) → hatch

Temperatures are given as mean ± Standard Deviation. No standard deviations are given for the asymmetrically variable regimes although they experienced the same thermal variance as the constant temperature regimes for the time spent at the respective constant temperatures.

Morphological indicators of growth and development

We used dorsal fin indentation to augment existing staging tables (Price, 1940; Colby and Brooke, 1973; Sreetharan *et al.*, 2015) that lack readily identifiable morphological stage indicators late in development. Dorsal fin indentation increases progressively in lake whitefish with complete separation of the dorsal and adipose fins occurring three to five weeks post-hatch under natural thermal regimes in the Bay of Quinte, Canada (Hart, 1931). Fin definition has been successfully used as a means of continuous staging in post-hatching zebrafish (Parichy *et al.*,2009).

Our calculated fin indentation ratio and additional morphological measurements are shown on Figure 4-3. The area of the yolk sac and yolk oil globule were calculated from the measured parameters assuming an ellipsoidal shape.

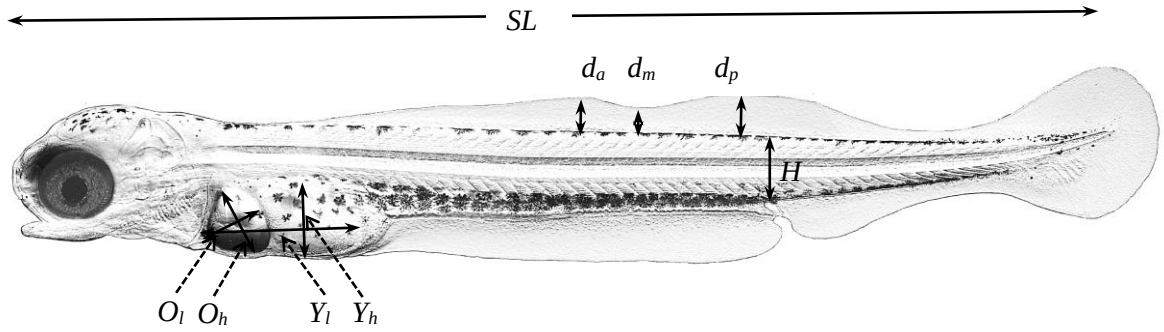


Figure 4-3: Morphological measurements included standard length (SL), body height measured immediately post-vent (H), yolk sac length (Y_l), yolk sac height (Y_h) oil globule length (O_l) and oil globule height (O_h). The area and volume of the yolk sac and yolk oil globule were calculated from the measured parameters assuming an ellipsoidal yolk shape. Fin indentation ratio $F = d_m/((d_a + d_p)/2)$ where d_m is the dorsal minimum, d_p is the posterior maximum, and d_a is the anterior maximum.

Thermal Dependence of Yolk Conversion Efficiency (YCE)

We estimated YCE using eggs from a single female (sF) in order to minimize confounding effects resulting from different initial egg size, or differential survival between eggs from different females. Initial egg size was determined on a dry weight basis from a sample of 90 individual eggs dried at 70^{o}C for 24 h and then weighed individually and in pools of 10 eggs each. The dry weight of the chorion was estimated by tearing the eggs and gently washing them with tap water until the chorions were free of yolk. The chorions were then examined under a microscope to ensure that they were complete (apart from the tear) and two pools of 40 intact chorions were dried overnight at 70^{o}C and weighed as described.

If hatching is heterograde, then it is necessary to normalize YCE across different stages of development owing to the increasing proportion of energy devoted to maintenance as body mass increases. YCE calculated conventionally by dividing the yolk free dry weight by the weight of yolk consumed (Rombough, 1988) may reflect differences in development rather than actual differences in yolk conversion efficiency.

We normalized YCE (Box 1) by relating somatic dry weight (W_S) and yolk dry weight (W_y) according to an empirically-derived hyperbolic function:

$$W_S = \frac{\varepsilon W_y}{(1 + a W_y)} \qquad [1]$$

where, ε is interpreted as a limiting thermodynamic efficiency [1] of yolk conversion (0.87) as the maintenance allocation approaches zero and a is experimentally determined and related to YCE. If we know W_S and W_y then we can substitute their values into Equation 1 to solve for a and use this value to normalize YCE to the point of theoretical yolk depletion.

Equation 1 cannot be used at the point of actual yolk depletion since embryos begin to lose dry mass before yolk consumption is complete (Blaxter and Hempel, 1963; Meyer *et al.*, 2012). We have assumed that the limiting efficiency of yolk conversion, ε is unaffected by temperature and therefore a constant. If ε is temperature dependent, then both a and ε will vary and normalized YCE will not be a function of a alone. This possibility does not meaningfully affect the normalization of YCE over the range of incubation temperatures and values for W_S considered in

[1] we have ignored any differences in the specific energy content of the yolk and somatic tissue so "efficiency" is simply a dry weight approximation

this paper but normalization over large differences in temperature and W_S should be treated with caution.

Box 1: Basis for normalization of YCE across development stages

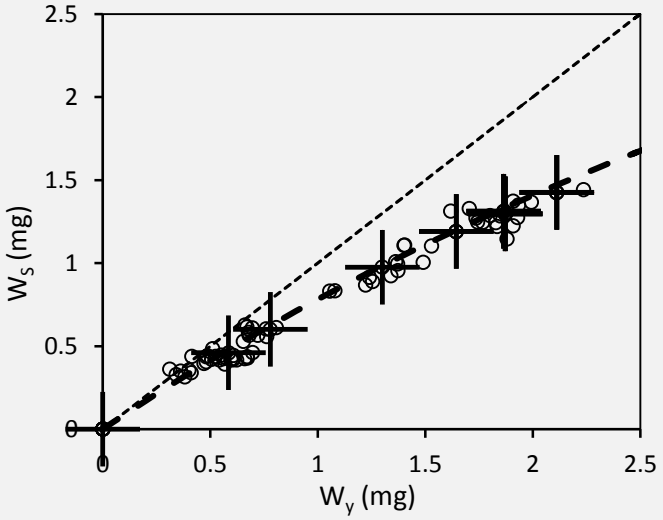
We can treat ontogenetic growth as a simplified energetic case where energy is partitioned between growth, $\left(\frac{dW_S}{dt}\right)$ and maintenance, $\left(\frac{dR_S}{dt}\right)$ according to:

$$\frac{dW_y}{dt} = \varepsilon^{-1}\frac{dW_S}{dt} + \frac{dR}{dt} \qquad [3]$$

and

$$\frac{dR}{dt} = f(W_s) \qquad [4]$$

where W_S is somatic (yolk-free) dry weight, W_y is the dry weight of yolk consumed at any point in time, and ε is a limiting efficiency of yolk conversion as the maintenance allocation approaches zero.



Observed relationship between somatic dry weight (W_S) and the dry weight of yolk consumed (W_y) for lake whitefish embryos incubated at a constant 5°C (heavy solid line). The light dashed line represents 100% conversion of yolk dry weight to somatic dry weight. Regression of the pooled data (+) using $W_s=\varepsilon W_y((1+aW_y)^{-1})$. yielded a near-perfect curve fit (R^2=0.999). Regression of the calculated points for individual embryos (○), resulted in a fit similar to that obtained for the pooled embryos (R^2=0.983, P<0.0001). Individual points closely matched the predicted value (RMSE 0.041 ± 0.012 mg) with no significant trend in the magnitude of error with development time (P=0.543).

Substituting [4] into [3] gives:

$$\frac{dW_y}{dt} = \varepsilon^{-1}\left(\frac{dW_S}{dt}\right) + f(W_s) \qquad [5]$$

If $\frac{dW_S}{dt}$ is a constant over a meaningful portion of embryonic development (Sreetharan *et al.,* 2015 and this study), then over that portion of development at any point in time:

$\frac{dW_y}{dt}$ is a function of W_S and W_S may be related to W_y in the form of a function, f where $\frac{dW_S}{dW_y}$ is equal to the instantaneous yolk conversion ratio (YCE). YCE may then be normalized to a consistent value for W_y.

Q_{10} following a shift in incubation temperature

Heterograde hatching may be detectable by changes in Q_{10} calculated following a shift in incubation temperature. Post-shift Q_{10} values should show no change if hatching is isograde (differential scaling), but the heterograde hypothesis would be expected to result in Q_{10} progressively increasing as the shifts approach the time of hatching.

Recall that Q_{10} describes changes in growth rate, *R*, across a 10°C change in temperature:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}} \qquad [6]$$

where R_1 and R_2 are the inverse of the time from the temperature shift to the timing of mean hatch. A one-time shift from a lower base temperature to higher incubation temperature occurs at a specific point in RD, which we denote f_0. We may quantify heterograde hatching using the term f_{het}, which we define as the difference in RD for a post-shift temperature, T_2, compared to T_1 (e.g. $f_{het} = 0.1$ means that median hatch t_h at T_2 occurs at an RD equivalent to 90% of t_h at T_1). For T_1, R_1 is proportional to $(1 - f_0)^{-1}$ or $\Delta f_0{}^{-1}$ but for T_2, R_2 is proportional to $(\Delta f_0 - f_{het})^{-1}$. If we substitute these values into Equation 6 we see that the post-shift Q_{10} remains unchanged where hatching is isograde ($f_{het} = 0$) but if $f_{het} > 0$ the post-shift Q_{10} increases with Δf_0 according to a hyperbolic function:

$$Post\text{-}shift\, Q_{10} = \frac{k \Delta f_0}{\Delta f_0 - f_{het}}^{\left(\frac{10}{T_2 - T_1} \right)} \qquad [7]$$

Where k is the pre-shift value for Q_{10}.

We determined post-shift Q_{10} values at six points in development (81, 93, 106, 121, 138, 151 dpf) beginning at the eyed stage (Stage 9 after Sreetharan *et al*., 2015) and continuing to pre-hatching for shifts from a base temperature of 2°C to 5 and 8°C respectively (Figure 4-4).

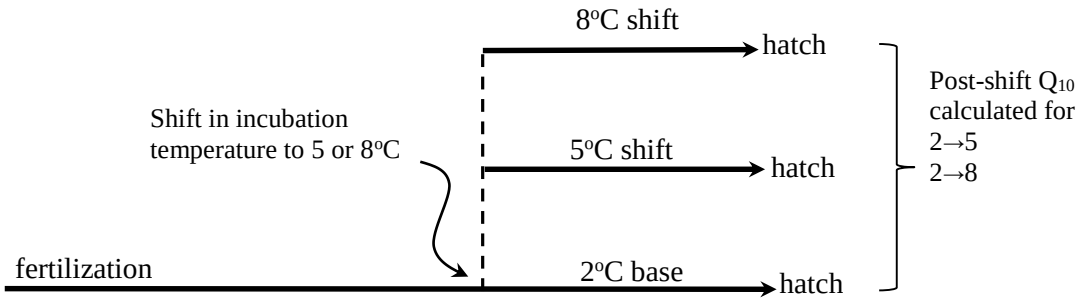


Figure 4-4: Temperature shifts used to determine implicit post-shift thermal scaling of time-to-hatch. If hatching is a valid developmental stage then temperature shifts from a base temperature to higher and lower temperatures will have little effect on the apparent post-shift value for Q_{10}. If hatching is heterograde then shifts in incubation temperature will accentuate the apparent thermal scaling and result in progressively higher implicit values for Q_{10} as development proceeds since the upward shift occurs closer to the hatching stage.

Statistical Analysis

Statistical analysis was conducted using Sigmaplot V11.0. Normalized YCE differences between temperature groups were analyzed using a one-way analysis of variance (ANOVA), while differences in dry weight vs SL for different temperatures were linearized using an exponential transformation followed by a one-way ANOVA. The time to 10, 50, and 90% hatch and the time to median hatch were determined after pooling the results of replicate dishes. Linear and non-linear regressions were solved by means of the least-squares method. Statistical differences between related curves were assessed by using a Johnson-Neyman ANCOVA with significance among pairwise slopes and elevations tested following a Bonferroni adjustment. Differences were considered statistically significant at $P \leq 0.05$.

4.3 Results

Morphological indicators of growth and development

Viable hatches were recorded at all temperatures, however survival was poor at 0.5°C (9% for mF Lake Huron and 0% for sF Lake Simcoe) and 8°C constant temperature treatments (9% for the mF Lake Huron and 7% for sF Lake Simcoe). Higher survival ranging from 18 to 58% was observed at other constant temperatures.

The time to median hatch (t_h) increased as an exponential function of constant temperature (T) for both Lake Huron $t_h = 231e^{-0.152T}$ (R^2 = 0.999, P < 0.001) and Lake Simcoe $t_h = 251e^{-0.163T}$ (R^2 = 0.999, P < 0.001) embryos. The duration of hatching (which we have measured as the difference between 10% and 90% hatching to avoid the confounding effect of outliers) was negatively associated with temperature ranging from 21 to 22 days at 2°C to 7 to 13 days at 8°C (Table 4.2). Larval standard length (SL) and dry weight at hatch was also affected by incubation temperature. At 2°C, the hatchlings were significantly longer (P<0.01) and heavier (P<0.01) than those reared at 5 and 8°C with the 2°C hatchlings more than 50% heavier than those hatching at 8°C. This difference was similar for both the sF-Simcoe and mF-Huron populations although standard deviations were greater with the mF-Huron trials. An inverse correlation was found between yolk area and hatching temperature ($F_{2,87}$ = 159.2, P<0.001) with the 8°C sF trial having a larger yolk area (5.5±0.174 mm^2) than the 5° and 2°C sF trials (3.5±0.16 and 1.5±0.088 mm^2 respectively). Oil globule area followed a similar relationship (1.20±0.061, 0.90±0.031 and 0.66±0.023 mm^2). Size-at-hatch varied with hatching rank (earlier vs. later hatching within a given incubation temperature) for the 5°C sF trial with early hatching larvae both shorter (P=0.10) and lighter

(P=0.12) than late hatching larvae. Larval height increased significantly with hatching rank for the 5°C sF trial ($F_{1,33}$ = 8.64, P=0.006). The 2°C sF embryos exhibited a significant change in SL with hatching rank ($F_{1,44}$ = 7.97, P=0.007) but no significant change in height was observed (P=0.29). The 8°C sF trial showed no significant difference in size between early and late hatching larvae for either length, dry weight, or height.

Yolk reserves varied with hatching rank as indicated in the analysis of yolk area for both the 5°C ($F_{1,33}$ = 41.6, P=<0.001) and 2°C ($F_{1,44}$ = 30.6, P=<0.001) sF trials (Figure 4-5B). Oil globule area also varied with hatching rank for the 5 and 2°C constant temperature incubations. No relationship was observed with hatching rank for either yolk or oil globule area for the 8°C trial for which hatching occurred over a short time interval. Total embryo dry weight at hatching varied with incubation temperature for the sF trials with mean weights of 1.822±0.0154, 1.930±0.0242, and 2.066±0.0175 obtained for 2, 5, and 8°C incubations respectively.

Dorsal fins were more deeply indented (i.e. fin definition ratio F decreased) for embryos hatching at low temperatures. F values ranged from 0.79±0.05 for the 8°C sF trial to 0.66±0.06 and 0.57±0.05 for the 5 and 2°C sF incubations respectively (Figure 4-5C). Fin indentation also increased with hatching rank within the 5°C sF trial ($F_{1,33}$ = 14.7, P=<0.001) but no significant difference between early and late-hatching embryos was observed for the 2 and 8°C sF trials. The relationship between the different morphological and dry weight measures and time for different constant incubation temperatures is shown on Figure 4-5 A to C. The same relationships for embryos reared under progressively increasing and decreasing temperatures is shown on Figure 4-5 D to F.

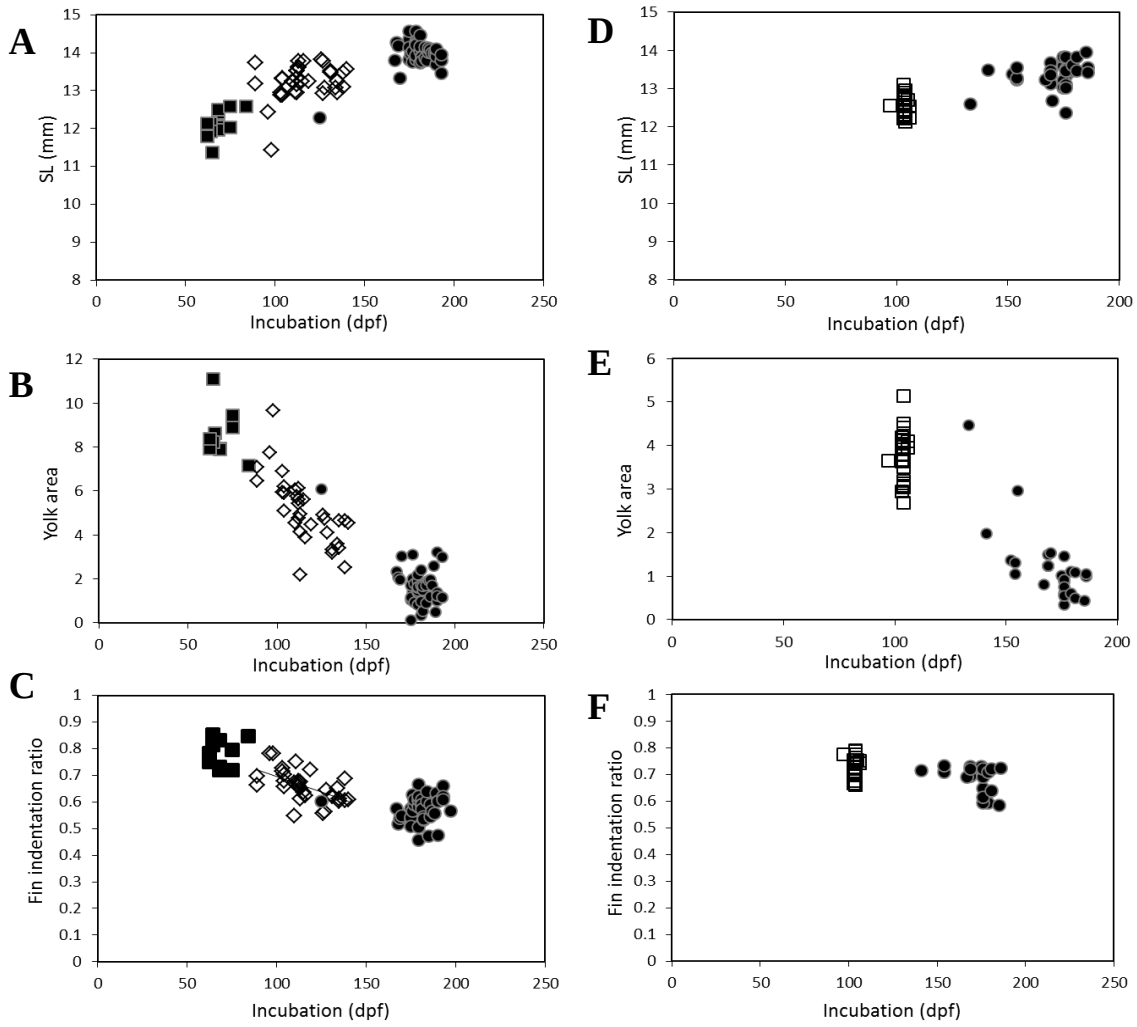


Figure 4-5: Graphical summary of measurements A – C: Summary of measurements of SL, yolk area, and fin indentation ratio for sF Lake Simcoe embryos hatching at different constant incubation temperatures (● – 2ºC, ◇- 5ºC, ■ - 8ºC). **D – F**: measurements of SL, yolk area, and fin indentation ratio for sF Lake Simcoe embryos hatching under progressively increasing (□) and progressively decreasing (●) thermal regimes. mF data for Lake Huron trials is not shown.

The time to median hatch differed markedly between the asymmetrically increasing thermal regimes (102 and 104 days) and the asymmetrically decreasing regimes for which hatching occurred nearly 70 days later (168 and 174) despite similar mean temperatures. The time to median hatch for the asymmetrically variable incubation regimes deviated from expectations based on the mean incubation temperature. Expressed in terms of predicted RD, the median hatch for increasing

thermal regimes occurred at 81 and 86% RD and at 119 and 117% RD for the decreasing thermal regimes for mF Lake Huron and sF Lake Simcoe respectively. These relationships persisted (92 - 95% and 132-133%) when using an exponentially weighted mean temperature (Chapter 3), which takes into account the effect of Jensen's inequality (Martin and Huey, 2008; Ragland and Kingsolver, 2008). The duration of hatching in the asymmetrically decreasing thermal regimes ranged from 21 to 28 days, while embryos reared under asymmetrically increasing regimes hatched in only 1 to 2 days

Embryos reared in an asymmetrically decreasing thermal regime hatched at 2^{o}C with a longer body (13.35±0.35 vs. 12.54±0.25 mm, $P<0.001$) and significantly greater fin indentation ratio (0.65±0.081 vs. 0.79±0.041, $P<0.001$) than those reared in an asymmetrically increasing thermal regime (Figure 4-5 D and F). Morphological indicators of stage such as fin indentation and body size under asymmetrically increasing or decreasing thermal regimes were similar to but not identical to those observed at their constant temperature equivalents. Fin indentation was slightly but significantly greater for the increasing thermal regime compared to the 8^{o}C constant regime and less for the decreasing regime than that observed for larvae incubated at 2^{o}C for their full development. Larvae hatching under an increasing thermal regime were significantly ($P<0.001$) longer (mean SL 12.539±0.0501) compared to the 8^{o}C constant temperature larvae (mean SL 12.12±0.103) perhaps reflecting a proportionately greater fraction of development spent at a more energetically efficient temperature. Similar relationships were apparent for the mF Lake Huron embryos.

Yolk reserves at hatch displayed an inverse relationship to temperature at the time of hatching with the larger embryos hatching with smaller yolks and smaller lipid globules. No significant relationship existed between yolk area and mean incubation temperature. A summary of hatch timing, SL, yolk and oil globule areas, fin indentation ratio and YCE is provided for different tested regimes on Table 4.2.

Table 4.2. Summary of time-to-hatch and morphological measurements for lake whitefish hatchlings reared under different constant and variable thermal regimes. Error represents standard error of the mean.

Regime (°C)	Live hatch (%)	h_{50}	h_{10}-h_{90}	SL (mm)	Yolk area (mm^2)	Oil globule area (mm^2)	Fin definition ratio, F	Yolk conversion efficiency	
								(nominal)	(normalized)
Lake Huron Embryos (mF)									
0.5	9	204	199-207	13.99 ±0.07(9)	1.88±0.08(9)	0.55±0.09(9)	0.52±0.02(9)	…	…
2	58	158	141-163	13.50±0.13(27)	2.71±0.14(27)	0.88±0.04(27)	0.61±0.01(27)	…	…
5	35	109	100-119	13.08±0.12(13)	8.15±0.55(11)	1.12±0.04(12)	0.68±0.02(12)	…	…
8	8	67	64-71	11.74±0.17 (6)	…	…	…	…	…
2→5→8	20	104	101-104	12.66±0.05(32)	8.79±0.11(30)	2.00±0.03(30)	0.73±0.07(30)	…	…
8→5→2	42	154	154-174	13.41±0.11(33)	4.15±0.39(32)	1.24±0.08(32)	0.66±0.02(32)	…	…
Lake Simcoe Embryos (sF)									
2	46	181	168-188	13.97±0.06(44)	3.04±0.17(44)	1.33±0.05(44)	0.57±0.01(44)	70.5±0.52(36)	69.2±0.49(36)
5	38	112	96-134	13.24±0.08(33)	6.99±0.32(33)	1.90±0.06(33)	0.66±0.01(33)	70.8±0.96(30)	67.5±0.85(30)
8	12	68	62-75	12.12±0.10(12)	11.02±0.35(12)	2.53±0.12(12)	0.79±0.02(10)	71.5±1.10(12)	65.8±0.38(12)*
2→5→8	18	104	101-104	12.54±0.05(25)	10.33±0.19(27)	2.25±0.03(27)	0.73±0.01(23)	…	…
8→5→2	37	170	153-179	13.35±0.06(33)	4.45±0.27(27)	1.52±0.08(27)	0.68±0.01(20)	…	…

* – conservative value derived using the standard length (SL) – dry weight relationship for the 2 and 5 degree groups which tended to have a higher condition factor. YCE would be lower for the 8-degree group if we applied a condition factor correction. h_x refers to fractional hatching with median hatching occurring at h_{50}. F is fin indentation ratio defined as the dorsal minimum divided by the average dorsal maxima.

Larval dry weight increased exponentially with increasing SL (Figure 4-6). No significant difference existed between the 2 and 5°C trials during late development when hatching was possible, however embryos reared at 8°C were significantly lighter for a given SL ($P<0.001$). No significant difference in the relationship between SL and dry weight was observed for the different variable thermal regimes (data not shown).

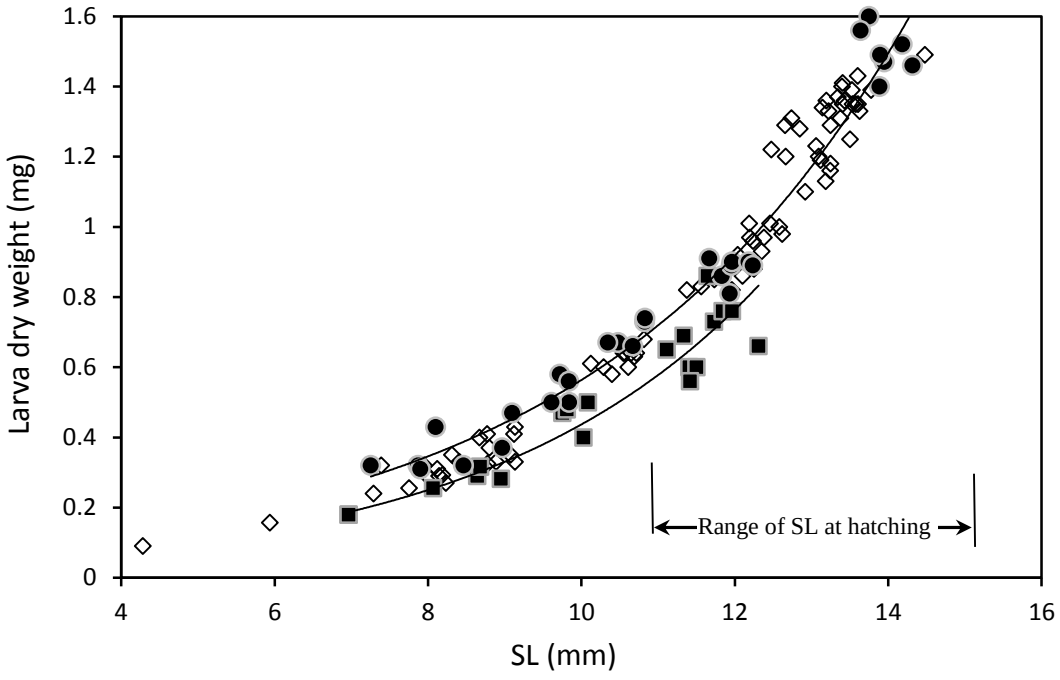


Figure 4-6: Relationship between unpreserved dry weight (DW) and standard length (SL) for constant temperature incubation (● – 2ºC, ◇- 5ºC, ■ - 8ºC) for Lake Simcoe sF embryos. Regression lines are given for the 2 and 5 ºC (DW = 0.0492exp0.244SL, R^2=0.97, P<0.001), and 8ºC trials (DW = 0.0268exp0.279SL, R^2=0.947, P<0.001).

Yolk free dry weight increased linearly with time for the 2 and 5ºC treatments. High mortality and short development at 8ºC prevented an estimation of pre-hatching dry weight increase. The relationship between somatic dry weight and time was found to be approximately linear for both the 5 and 2ºC trials over the portion of development for which reliable dry weight measurements can be obtained practical (i.e. a condition for YCE normalization as described in Box 1).

Thermal Dependence of Yolk Conversion Efficiency (YCE)

The dry weight of 124 eggs from Lake Huron females (mF) averaged 2.763±0.234 mg. The variance in initial egg weight was much lower for a 90 egg sample from the single female from

Lake Simcoe (sF) which had a mean dry weight of 2.735±0.081 mg. The dry weight of the sF eggs *ex* chorion was determined by subtracting the mean chorion dry weight (0.316±0.00424 mg) from the total egg dry weight.

We used nonlinear regression to evaluate the curve fit between somatic dry weight and yolk dry weight for pools of 10 yolked and 10 deyolked embryos for each point. Regressions using exponential, power and hyperbolic functions all yielded R^2 values of 0.999. We selected the hyperbolic function as it simplifies the separation of maintenance metabolism from energy allocated to anabolism.

Normalized YCE was significantly different for embryos reared under different constant temperatures ($F_{2,20}$=6.99, P <0.001). The yolk conversion ratio (dry weight basis) decreased with increasing incubation temperatures with the YCE for the 8^{o}C trials significantly lower than that for embryos reared at 5 and 2^{o}C (P<0.001). YCE was highest for the 2^{o}C embryos but the difference between normalized YCE for the 2 and 5 ^{o}C trials was non-significant (P=0.169). It is

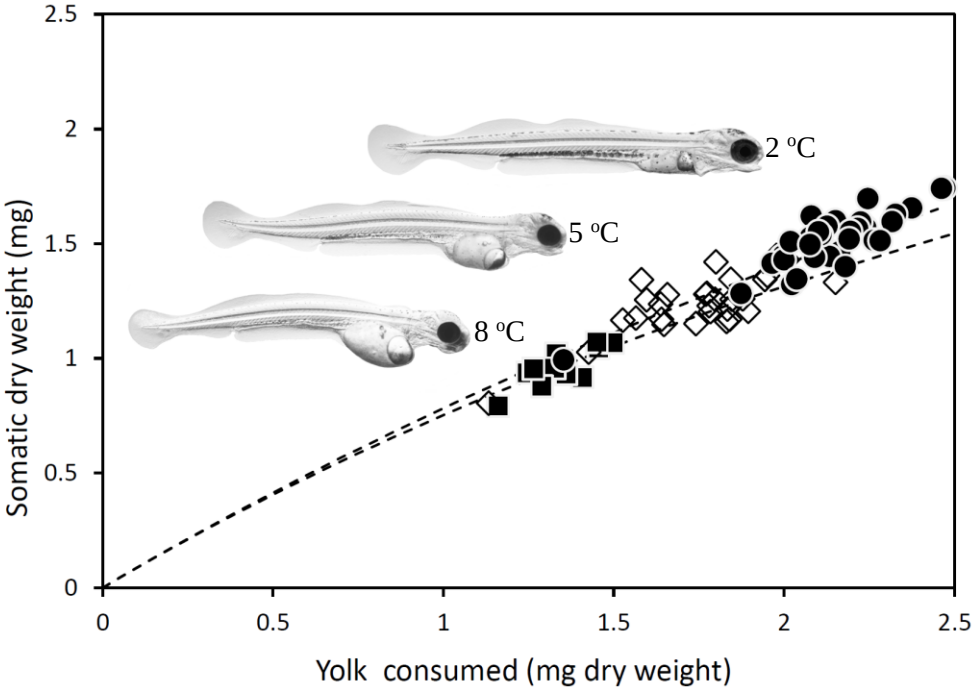


Figure 4-7: Observed conversion of yolk dry weight to somatic dry weight for embryos hatching at different constant incubation temperatures (● – 2ºC, ◇- 5ºC, ■ - 8ºC). The dashed lines represent different regression lines for the relationship $W_s=\varepsilon W_y((1+aW_y)^{-1})$. for 2 and 8ºC. A representative image of a hatchling for each incubation temperature is included.

noteworthy that the nominal YCE values show the reverse relationship with the 8ºC embryos having the highest nominal YCE although the differences are non-significant.

Q_{10} following a one-time increase in Incubation Temperature

Q_{10} values calculated post shift increased with decreasing interval between shift and hatching as shown on Figure 4-8.

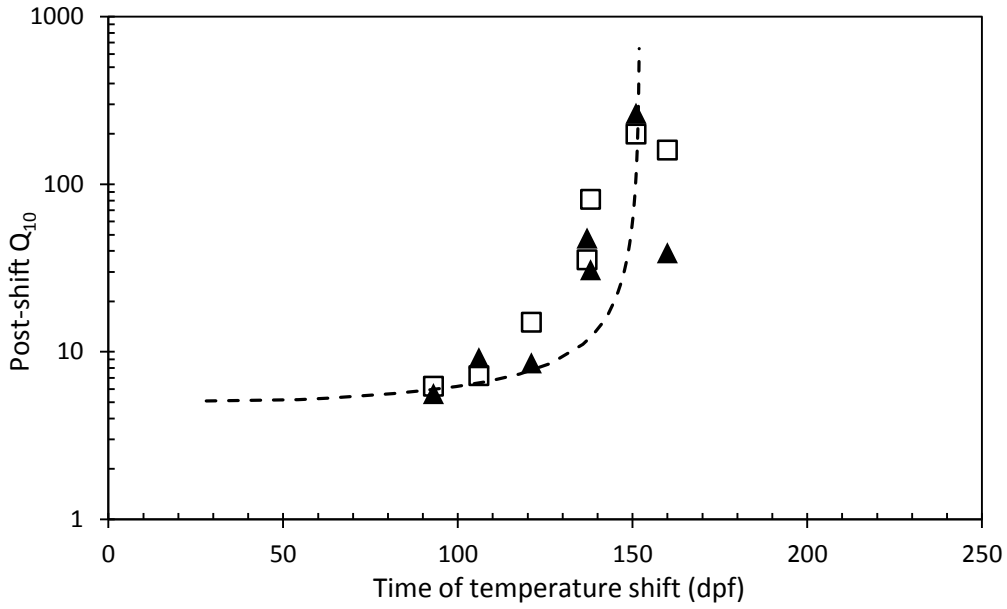


Figure 4-8: Calculated Q_{10} following a discrete temperature shift. Observed relationship between post-shift values for Q_{10} and the timing of the temperature shift. The dashed line shows the predicted hyperbolic relationship between post shift Q_{10} and the timing of the shift (□: 2→8ºC, ▲: 2→ 5ºC).

The increase in post shift Q_{10} to triple-digit values is sufficient to reject the null hypothesis of an unchanging Q_{10} that would result if hatching were isograde. The relationship between post-shift Q_{10} values and the shift timing appeared to follow a hyperbolic relationship approximating that predicted under the heterograde hypothesis. However, the data fit imperfectly to the derived hyperbolic model (R^2=0.36, P=0.04) and an exponential regression would yield a similar fit.

The reduction in incubation time (relative to the isograde expectation) following an increase in incubation temperature may be illustrated by plotting the percentage of calculated RD occurring pre and post-shift (Figure 4-9). This allows f_{het} to be determined from the graph.

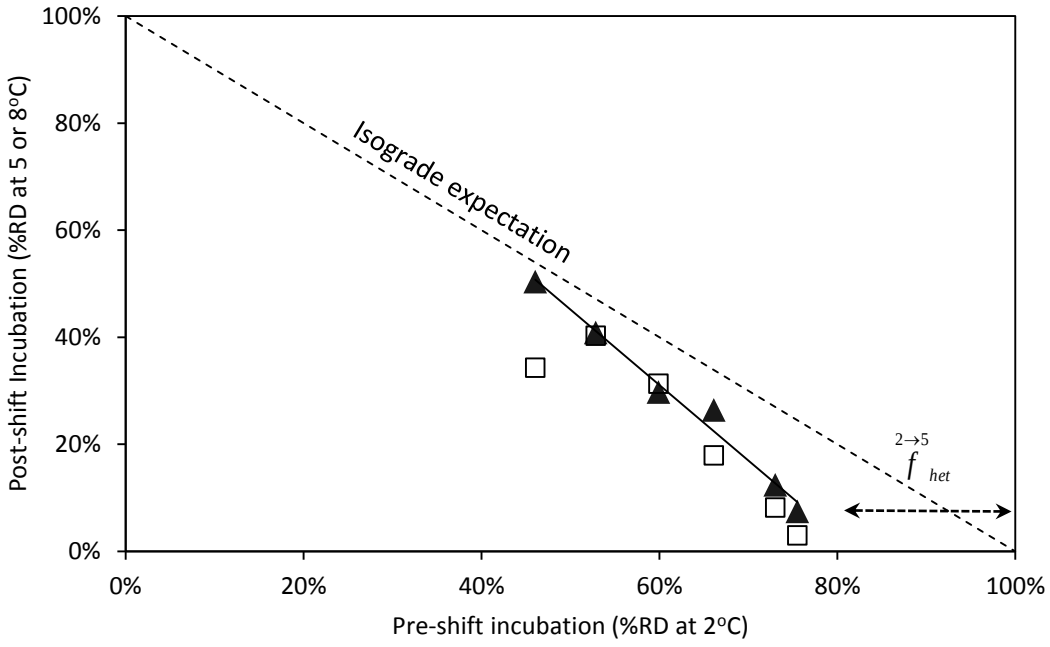


Figure 4-9: Relationship between calculated RD and the timing of the temperature shift (□: 2→8°C, ▲: 2→5°C). $f_{het}^{2\rightarrow5}$ is illustrated graphically. The solid line shows a linear regression for the 2→5°C data.

4.4 Discussion

We observed that lake whitefish embryos incubated at low temperatures hatch at larger size but with lower yolk reserves than those reared at higher temperatures. This is consistent with the findings of other researchers (Hart, 1931; Price, 1940; Colby and Brooke, 1973; Brooke, 1975; Griffiths, 1979; Luczynski, 1984; Luczynski & Kirklewska 1984; Eme *et al.*, 2015, Mueller *et al.*, 2015 and Lee *et al.*, 2016). Morphological indicators of development stage (i.e. fin indentation) suggest that the larger embryos hatched at a more advanced development stage than the smaller embryos reared in warmer temperatures. Embryos reared under an asymmetrically increasing regime hatched earlier and at smaller body size than expected under an isograde assumption, while

embryos reared under an asymmetrically decreasing regime hatcher later than predicted and at a larger body size (Figure 4-5 D to F). The calculation of post-shift Q_{10} values following an upward shift in temperature conformed to the heterograde prediction with values exceeding 100 compared to a base value of approximately 5. Thus, all three of our tests support the heterograde hypothesis that the larger size of low temperature hatchlings is the result of continued growth within the chorion to more advanced stages of development.

The magnitude of the heterograde effect may be quantified using a ratio, f_{het}, for the difference in hatching stage between the highest and lowest viable constant incubation temperatures for a species. We have taken this range to be between approximately 8 and 0°C for the lake whitefish based on this study and work by others (Price, 1940; Brooke, 1975; Griffiths, 1979).

The observed differences in hatch timing between asymmetrically increasing and decreasing regimes are consistent with $f_{het}^{0\rightarrow 8} \approx 0.33$ to 0.35 meaning that embryos at zero degrees will reach at temperature equivalent than that of hatching at 8°C at 65 to 67% of the 0°C RD. Alternatively, f_{het} may be normalized to a 10°C temperature difference using the thermal scaling (i.e. Q_{10}) of development and the thermal scaling for hatching under constant temperatures:

$$f_{het} = 1 - \left(\left(\frac{{}^{develop}Q_{10})}{{}^{hatch}Q_{10}} \right)^{\left(\frac{\Delta T}{10}\right)} \right) \qquad [8]$$

While we lack reliable direct measurements of the thermal scaling of pre-hatching growth rates, such scaling may be estimated using the temperature dependence of heart rate as a surrogate for growth. Eme *et al.* (2015) found that the thermal scaling of heart rate in later stages of development

(intermittent fin flutter or Colby and Brooke Stage 19) followed a relatively consistent Q_{10} of approximately 3. The thermal scaling of median hatch is tightly constrained to a narrow range of values (Q_{10} values between 4.6 and 5.2) depending on the population and experimental conditions. The difference between a Q_{10}=5 (hatching) and Q_{10}=3 (growth) is equivalent to $f_{het}^{0\rightarrow 8}$=0.27. A heterograde fraction of this magnitude is sufficient to account for all the observed differences in size-at-hatch based on the constant temperature growth rates we observed (Figure 4-7) thus supporting the heterograde hypothesis.

Temperature-dependent differences in the efficiency of yolk conversion were observed with the highest normalized YCE of 69.2±0.49% obtained for a constant 2°C incubation temperature and lower YCE of 67.5±0.85% and 65.8±0.38% determined for 5 and 8°C constant temperature incubation respectively. Observed efficiency differences are sufficient to account for only some 20 to 25% of the observed size difference at hatching between embryos reared under different constant temperatures. The observed thermal dependence of hatchling size cannot be accounted for by differences in growth efficiency alone.

We found no evidence that the thermal scaling of absolute growth was significantly different than the scaling of development based on measures such as the degree of fin indentation. The observed decrease in the efficiency of growth with increasing temperature was is consistent with the findings of Mueller *et al*., (2015) and is inconsistent with the predictions derived from differential scaling between growth and development (Zuo *et al*., 2012). A decrease in growth efficiency with increasing temperature is also consistent with predictions based on the von Bertalanffy-Perrin growth efficiency model. Angilletta and Dunham (2003) found that net growth efficiency

decreases with increasing temperature were present in only six of 20 species reviewed leading them to conclude that the von Bertalanffy-Perrin model does not apply to the majority of species.

Hatching is best understood as a life stage transition that may occur over a range of development (Spicer and Burggen, 2003; Schulte *et al.*, 2011; Touchon *et al.*, 2015) stages with the stage at hatch determined plastically in response to environmental signals or as a passive function of temperature. We have described heterograde hatching as non-plastic on the scale of individual ontogenetic development in order to differentiate the phenomenon from those involving recognition and transduction in response to environmental signals. In this context, the thermal dependence of stage-at-hatch may be seen as passively acting and analogous the thermal scaling of chemical reactions. A similar distinction was drawn by Schulte *et al.* (2011) although they used the term "passively plastic". We recognize that the interaction of a non-plastic thermal dependence of hatching stage will result in plasticity in the timing of hatching under variable temperature incubation.

The existence of heterograde hatching does not preclude the simultaneous existence of actively plastic hatching mechanisms or differences in thermal scaling between growth and development. Indeed the timing of hatching and size-at-hatch under real-world conditions is likely to reflect the interaction of multiple factors which may exert a greater or lesser influence under different environmental conditions. Czerkies *et al.* (2001) noted that hypoxia triggered precocious hatching in *Coregonus lavaretus* while similar hatching stimulation has been recorded in response to fungal infection (Wedekind, 2002) and predation (Wedekind and Müller, 2005). Thus, a plastic response

to environmental conditions clearly exists within the lake whitefish in parallel to a non-plastic thermal dependence of hatching stage.

Lake whitefish spawn over shallow cobble shoals in the fall (October and November) shortly before the formation of ice cover that remains until spring breakup (Hart, 1931; Scott and Crossman, 1973; Anras, 1999). Spawning typically extends for a period of several weeks. Thus, the natural thermal regime drops from temperatures of between 5 and 8^{o}C to near zero where the temperature remains until spring break up, the timing of which may vary significantly with latitude and from year to year (Duguay *et al.,* 2006). Following break-up, water temperatures rise rapidly due to warming and the mixing of deep isothermal waters with those of the shallow shoals where incubation takes place. Under these conditions, both early and late spawned embryos may reach a stage of development that would permit viable hatching under the higher temperatures that rapidly follow the breakup of ice cover (Weyhenmeyer *et al.,* 2004). Therefore hatching may occur nearly simultaneously for both early and late spawned embryos although the early spawners will be more developed with a larger body size and commensurately less yolk. This expectation is supported by observations by John and Hasler (1956) who reported little difference in the observed timing of hatch despite spawning dates that varied by as much as two weeks.

Synchronized hatching may confer a selective advantage to the whitefish since their eggs (and consequently yolk reserves) are small and larval survival is highly dependent on zooplankton density following hatching (Taylor and Freeberg, 1984; Reshetnikov and Bogdanov, 2011). Dostatni *et al.,* (1999) reported that the caloric quantity of zooplankton increased approximately 4-fold in the first three weeks following ice out with a peak in early May approximately 20-fold

higher. Embryos hatching asynchronously to this natural increase in food supply may therefore experience low survival either due to the paucity of prey in the case of early hatching, or to prey (primarily copepods and cladocerans) being too large for the restricted gape of late hatching larva.

Lake whitefish are known to experience thermally-induced mass hatching (Luczynski 1984; Dostatni *et al.*,1999; Hooper, 2006) in response to a small increase in temperature late in development. Heterograde hatching provides a mechanism to explain this phenomenon which superficially resembles environmentally cued hatching (Martin, 2011; Warkentin, 2011). However, in this case the rapid hatching is simply a mathematical consequence of the embryo development stage involving neither the recognition nor biochemical transduction of an environmental signal. An implication is that transient temperature peaks would be expected to have a very different effect on hatch timing depending on whether they are experienced early or late in development. Early in development, a transient temperature spike would have a relatively minor effect on hatch timing (consistent with observations in Lee *et al.*, 2016). Once hatching competence is reached a similar transient increase in incubation temperature is predicted to trigger mass hatching if the RD corresponding to the instantaneous temperature exceeds 100%.

The generality of heterograde hatching is not well understood as it has been reported for only a few species (Jordaan *et al.*, 2006; Steenfeldt *et al.*, 2010; Luczynski and Kolman, 1987). We speculate that the hatching of other fall spawning Coregonids will also prove to be heterograde. Luczynski and Kirklewska (1984) noted mass hatching was triggered by an increase in temperature when applied to late stage *C. albula* embryos, and it is possible that other fish species having a high developmental Q_{10} or exhibit mass hatching with an upward temperature shift may share a

thermal dependency in hatching stage. However, not all species (e.g. *Fundulus heteroclitus* which spawns year-round) experience mass hatching with increased temperature (diMichele and Taylor, 1980) suggesting that the generality of heterograde hatching may be limited.

We found that the temperature dependence of hatchling size in the lake whitefish is predominantly the consequence of differences in hatching stage. The term heterograde is proposed to describe this form of developmental heterochrony which we have identified as a mechanism to synchronize the timing of hatching to the break-up of winter ice cover. Heterograde hatching should be considered a potential confounding influence when predicting the timing of hatching under asymmetrically variable thermal regimes or when treating hatching as a development stage.

4.5 Acknowledgements

The authors wish to thank Lisa Stoa, Emily Hulley and Shayen Sreetharan for assistance with the experimental work and morphometric analysis. The Ontario Ministry of Natural Resources and Forestry is gratefully acknowledged for their assistance in providing whitefish embryos from Lake Simcoe. We are grateful to Bruce Power for its support of this work, both financially and technically, and for funding through a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development Grant.

4.6 References

Angilletta, M. J. and A. E. Dunham, 2003. The temperature-size rule in Ectotherms: Simple evolutionary explanations may not be general; *The American Naturalist*, Vol. 162, No. 3 2003, 332-342.

Anras, M.L.B., 1999. Movement and habitat use by lake whitefish during spawning in a boreal lake: integrating acoustic telemetry and geographic information systems; *Transactions of the American Fisheries Society* 128: 939-952.

Atkinson, D., and R. M. Sibly, 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle; *Trends in Ecology & Evolution* 12:235–239.

Berlin, W.H., L.T. Brooke and L.J. Stone, 1977. Verification of a model for predicting the effect of inconstant temperature on embryonic development of lake whitefish (*Coregonus clupeaformis*); *Technical papers of the US Fish and Wildlife Service* 92.

Blaxter, J. H. S., and G. Hempel, 1963. The influence of egg size on herring larvae (*Clupea harengus* L.); *ICES Journal of Marine Science* 28.2 (1963): 211-240.

Brooke, L.T. 1975. Effect of different constant incubation temperatures on egg survival and embryonic development in lake whitefish (*Coregonus clupeaformis*). *Transactions of the American Fisheries Society*. 104 (3), 555-559.

Colby, P.. J., and L. T. Brooke, 1973. Effects of temperature on embryonic development of Lake Herring (*Coregonus artedii*); *Journal of the Fisheries Research Board of Canada* 30.6 : 799-810.

Cushing, D. H., 1969. The regularity of the spawning season of some fishes; *Journal du Conseil pour l'Exploration de la Mer* 33:81–92.

Czerkies, P., P. Brzuzan, K. Kordalski, & M. Luczynski, 2001. Critical partial pressures of oxygen causing precocious hatching in *Coregonus lavaretus* and *C. albula* embryos; *Aquaculture* 196 (2001). 151–158.

DiMichele, L., Taylor, M.H., 1980. The environmental control of hatching in *Fundulus heteroclitus*; *Journal of Experimental Zoology* 214, 181–187.

Dostatni, D., A. Mamcarz, J., and P. Poczyczyński, 1999. Feeding of early- and late-hatched whitefish in illuminated cages; *Archives of Polish Fisheries*. Vol. 7, Fasc. 2 (1999) 281-295.

Duguay, C. R. , T. D. Prowse, R. Barrie R. Bonsal, R D. Brown, M. P. Lacroix and P. Ménard, 2006. Recent trends in Canadian lake ice cover; *Hydrological Processes*. 20, 781–801.

Eme, J., C.A. Mueller, R.G. Manzon, C.M. Somers, D.R. Boreham, and J.Y. Wilson, 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings. Comparative Biochemistry and Physiology Part A; *Molecular & Integrative Physiology*. 179, 71-80.

Fink, W. L., 1982. The conceptual relationship between ontogeny and phylogeny; *Paleobiology* Vol. 8, No. 3 (Summer, 1982), pp. 254-264.

Forster, J., and A. G. Hirst, 2012. The temperature-size rule emerges from ontogenetic differences between growth and development rates; *Functional Ecology* Volume 26, Issue 2, 483–492.

Griffiths, J.S. 1979. Potential effects of unstable thermal discharges on incubation of Lake Whitefish eggs; *Ontario Hydro Research Division Report No*. 79-521-K.

Griffiths, J.S. 1980. Potential effects of unstable thermal discharges on incubation of Round Whitefish eggs; *Ontario Hydro Research Division Report No*. 80-140-K.

Hart, J.L. 1931. The spawning and early life history of the whitefish, *Coregonus clupeaformis* (Mitchill), in the Bay of Quinte, Ontario; Cont*ributions to Canadian Biology and Fisheries* 6 (1), 165-214.

Hooper, G. W., 2006: Techniques used for the intensive culture of Lake Whitefish (*Coregonus clupeaformis*) in Ontario, Canada; *Special Publication Aquaculture Association of Canada* (11): 169-172.

Huey, R. B., and D. Berrigan, 2001. Temperature, demography, and ectotherm fitness; *The American Naturalist* 158.2: 204-210.

John, K. R., and A. D. Hasler, 1956. Observations on some factors affecting the hatching of eggs and the survival of young shallow-water Cisco, *Leucichthys Artedi* Lesueur, in Lake Mendota, Wisconsin; *Limnology and Oceanography*. 1.3 (1956): 176-194.

Jordaan, A., 2002. The effect of temperature on the development, growth and survival of Atlantic Cod (*Gadus morhua*) during early life-histories; Electronic Theses and Dissertations. Paper 141.

Jordaan, A., S. E. Hayhurst, and L. J. Kling, 2006. The influence of temperature on the stage at hatch of laboratory reared *Gadus morhua* and implications for comparisons of length and morphology; *Journal of Fish Biology* 68.1 (2006): 7-24.

Klingenberg, C. P., 1998. Heterochrony and allometry: The analysis of evolutionary change in ontogeny; *Biological Reviews* 73.1 (1998): 79-123.

Lee, A.H., J. Eme, Mueller, C.A., R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2016. The effects of increased constant incubation temperature and cumulative acute heat shock exposures on morphology and survival of Lake Whitefish (*Coregonus clupeaformis*) embryos; *Journal of Thermal Biology* 57 (2016) 11–20.

Luczynski, M. 1984. Improvement in the efficiency of stocking lakes with larvae of *Coregonus albula* L. by delaying hatching; *Aquaculture* 41: 99 111.

Luczynski, M., A Kirklewska, 1984. Dependence of *Coregonus albula* embryogenesis rate on the incubation temperature; *Aquaculture* , Vol. 42, no. 1, pp. 43-55.

Luczyński, M., and R. Kolman, 1987. Hatching of Coregonus albula and C. Lavaretus embryos at different stages of development; *Environmental Biology of Fishes* 19.4 (1987): 309-315.

Martin, K., K. Bailey, C. Moravek, and K. Carlson, 2011. Taking the plunge: California grunion embryos emerge rapidly with environmentally cued hatching; *Integrative and Comparative Biology* 51.1 (2011): 26-37.

Martin, T. L. and R. B Huey, 2008. Why "suboptimal" is optimal: Jensen's Inequality and ectotherm thermal preferences; *The American Naturalist* 171: E102-118.

Melo, M. T., C. Saturnino, J. N. S. Santos, R. M. Vasconcellos, A. G. Cruz-Filho and F. G. Araujo, 2010. Correction of the weight and length for juveniles Atherinella brasilliensis (*actinopterygii: atherinopsidae*) after fixation in formalin and preservation in ethanol; *Zoologia*. 27: 892-896.

Meyer, S., E. Caldarone, M. Chícharo, C. Clemmesen, A. Faria, C. Faulk, A. Folkvord, G. Holt, H. Høie, P. Kanstinger, A. Malzahn, D. Moran, C. Petereit, J. Støttrup and M. Peck, 2012. On the edge of death: Rates of decline and lower thresholds of biochemical condition in food-deprived fish larvae and juveniles; *Journal of Marine Systems* 93 (2012): 11-24.

Mitz, C., C. Thome, M.E. Cybulski, L. Laframboise, C.M. Somers, R.G.Manzon, J.Y. Wilson, D.R. Boreham, 2014. A self-contained, controlled hatchery system for rearing lake whitefish embryos for experimental aquaculture; *North American Journal of Aquaculture*. 76 (3), 179-184.

Mueller, C.A., J. Eme, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype and cost of development in lake whitefish (*Coregonus clupeaformis*); *Journal of Comparative Physiology*. B, Biochemical, Systemic, and Environmental Physiology. 185 (3), 315-331.

Muir, A. M., M. T Arts, M. A., Koops, T. B. Johnson, C. C. Krueger, T. M. Sutton and J. Rosenfeld, 2014. Reproductive life-history strategies in Lake Whitefish (*Coregonus clupeaformis*) from the Laurentian Great Lakes; *Canadian Journal of Fisheries and Aquatic Science* 71.8, 1256-1269.

Parichy, D. M., M. R. Elizondo, M. G. Mills, T. N. Gordon, & R. E. Engeszer, 2009. Normal table of post-embryonic Zebrafish development: Staging by externally visible anatomy of the living fish. Developmental Dynamics; *an Official Publication of the American Association of Anatomists*, 238(12), 2975–3015.

Perrin, N., 1995. About Berrigan and Charnov's life history puzzle; *Oikos* 73:137–139.

Price, J.W., 1940. Time-temperature relations in the incubation of the whitefish, *Coregonus clupeaformis* (Mitchill); *Journal of General Physiology*. 23 (4), 449-468.

Ragland, G.J. and J.G. Kingsolver, 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*; *Evolutionary Ecology Research* 10: 29-44.

Régnier, T., V. Bolliet, P. Gaudin, J. Labonne, 2012. Bigger is not always better: Egg size influences survival throughout incubation in Brown Trout (Salmo trutta); *Ecology of Freshwater Fish* 22.2 (2012): 169-177.

Reshetnikov, Y. S., and V. D. Bogdanov, 2011. Features of reproduction of whitefishes; *Journal of Ichthyology* 51.6 (2011): 432-456.

Rombough, P.J, 1988. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Canadian Journal of Zoology* 66:651–660.

Ruel, J. J. and M. P. Ayres, 1999. Jensen's inequality predicts effects of environmental variation; *Trends in Evolution and Ecology* 14: 361-366.

Scott, W.B. and E.J. Crossman, 1973. Freshwater fishes of Canada; *Fish Research Board of Canada Bulletin* 184.

Schulte, P. M., T. M. Healy, and N. A. Fangue, 2011. Thermal Performance Curves, Phenotypic Plasticity, and the time scales of temperature exposure; *Integrative and Comparative Biology* (51) No. 5, 691-702.

Sharpe, P. J. H. and D. DeMichele. 1977. Reaction kinetics of poikilotherm development; *Journal of Theoretical Biology*, 64: 649-670.

Sibly R. M. and D. Atkinson, 1994 How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* 8, 486–493.

Smith, B. B., & K. F. Walker, 2003. Shrinkage of 0+ carp (*Cyprinus carpio* L.) after preservation in ethanol; *Marine and Freshwater Research*. 54: 113-116.

Spicer, J. I., and W. W. Burggren, 2003. Development of physiological regulatory systems: Altering the timing of crucial events; *Zoology* 106.2 (2003): 91-99.

Sreetharan, S., C. Thome, C. Mitz, J. Eme, C.A. Mueller, E.N. Hulley, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic development of lake whitefish (*Coregonus clupeaformis*): a staging series, analysis of growth and impacts of fixation; *Journal of Fish Biology* 87.3 (2015): 539-558.

Steenfeldt S., I. Lund & E. Höglund, 2010. Is batch variability in hatching time related to size heterogeneity and cannibalism in pikeperch (*Sander lucioperca*)? *Aquaculture Research* 42, 727–732.

Taylor, W. W., and M. H. Freeberg. 1984. Effect of food abundance on larva1 Lake whitefish, *Coregonus clupeaformis* growth and survival; Fish. Biol. 25: 733-741.

Thome, C., C. Mitz, C.M. Somers, R.G. Manzon, J.Y. Wilson, and D.R. Boreham, 2016. Incubation of Lake Whitefish (*Coregonus clupeaformis*) embryos in cooling water discharge

and the impacts of fluctuating thermal regimes on development: *Canadian Journal of Fisheries and Aquatic Sciences, ttp://dx.doi.org/10.1139/cjfas-2015-0286.*

Touchon, J. C., M. W. McCoy, T. Landberg, J. R. Vonesh and K. M. Warkentin, 2015. Putting u/g in a new light: Plasticity in life history switch points reflects fine-scale adaptive responses; *Ecology* 96.8 (2015): 2192-2202.

van der Have, T. M. & G. de Jong, 1996. Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology*. 138: 329–340.

Warkentin, K. M., 2011. Environmentally cued hatching across taxa: Embryos respond to risk and opportunity; *Integrative and Comparative Biology* 51.1 (2011): 14-25.

Wedekind, C., 2002 Induced hatching to avoid infectious egg disease in whitefish; *Current Biology,* Vol. 12, 69–71.

Wedekind, C., and R. Müller. 2005. Risk-induced early hatching in Salmonids. *Ecology* 86:2525–2529.

Weyhenmeyer, G. A., M. Meili and D. M. Livingstone, 2004. Nonlinear temperature response of lake ice breakup; *Geophysical Research Letters* 31.7

Yang, L.H., and V. H. W. Rudolf, 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions; *Ecology Letters* 13.1, 1-10.

Zuo, W., M.E. Moses, G. B. West, C. Hou, and J. H. Brown, 2012. A general model for effects of temperature on ectotherm ontogenetic growth and development: *Proceedings of the Royal Society* B (2012) 279, 1840–1846.

A heterograde model for hatch timing in lake whitefish (*Coregonus clupeaformis*)

Charles Mitz[1], Chris Thome[1,5], Mary Ellen Cybulski[1], Jeroen Thompson[2], Christopher M. Somers[3], Richard G. Manzon[3], Joanna Y. Wilson[4*], and Douglas R. Boreham[1,2,5]

Abstract

We developed an incremental growth-at-temperature model to predict hatch timing in the lake whitefish under varying incubation temperatures. The model extends earlier approaches by explicitly incorporating a temperature-dependent development stage at hatching. Testing using experimentally reared embryos from Lakes Huron and Simcoe shows that the model provides significantly improved predictive accuracy for asymmetrically varying thermal regimes that mimic those encountered under real-world conditions. The simple calibration of the model to different populations and its ability to predict observed phenomena such as thermally induced mass hatching and hatching synchronization makes it a useful tool for the evaluation of thermal impacts from industrial discharges and climate change.

* corresponding author

[1]*Department of Medical Physics and Applied Radiation Sciences, McMaster University*
[2]*Bruce Power, Tiverton, ON, Canada*
[3]*Department of Biology, University of Regina*
[4]*Department of Biology, McMaster University*
[5]*Northern Ontario School of Medicine*

5.1 Introduction

The ability to reliably predict hatching under ecologically relevant conditions is essential when assessing effects such as phenological mismatch (Cushing, 1969; Genner *et al.*, 2009) that may occur if temperature changes during incubation result in a species hatching asynchronously to its food supply. Such effects are of particular concern for the lake whitefish (*Coregonus clupeaformis*), an economically and culturally important salmonid whose embryonic development takes place over winter on shallow cobble shoals (Hart, 1931; Scott and Crossman, 1973). Winter thermal stratification occurs under low energy ice-covered conditions resulting in a shallow hypothermal zone characterized by water temperatures varying from 0.5 to 1.5^{o}C (Thome *et al.*, 2016). Under such conditions, even comparatively modest temperature increases resulting from the discharge of industrial cooling water might result in advanced hatching. Also relevant is the effect of climate change which may alter the timing of winter ice cover formation and spring break-up.

While the thermal scaling of embryonic development of the lake whitefish is relatively well understood for constant temperature conditions (Hart, 1931; Price, 1940; Bidgood, 1972; Brooke, 1975, Griffiths, 1979; Berlin *et al.*, 1977; Luczynski, 1984; Luczynski and Kirklewska 1984), less research has been focused on the effect of variable thermal regimes on growth and hatch timing. Griffiths (1979), Patrick *et al.* (2013), Mueller *et al.* (2015) and Lee *et al.* (2016) investigated the effect of variable thermal regimes on whitefish development under laboratory conditions while Thome *et al.* (2016) carried out *in situ* incubation of lake whitefish near the Bruce Power Nuclear Generating Station on Lake Huron. However, there remains an apparent

disconnect between model predictions for hatching dynamics under variable thermal regimes and the observed timing of spawning and the appearance of free-swimming larvae (e.g. John and Hasler, 1956). Other impacts of variable thermal regimes remain poorly understood including the phenomenon of thermally-induced mass hatching (Griffiths, 1979; Hooper, 2006) and the observation that hatching ceases with a decline in water temperature (Luczynski, 1984; Reshetnikov and Bogdanov, 2011).

Several whitefish-specific models for variable temperature development time have been proposed (Brooke, 1975; Berlin *et al.*, 1977; Griffiths, 1979; Thome *et al.*,2016). The Brooke and Griffiths models take the form of a series of stage-wise or incremental polynomial equations while Thome *et al.* (2016) used an incremental exponential model. The modified power-law function of Price (1940) can also be used as the basis of an incremental growth-at-temperature model to predict hatching under varying temperatures. General models relating development time to variable thermal regimes include thermal sums (Neuheimer and Taggart, 2007; Chezik *et al.*, 2014).

The effect of varying temperatures on hatching dynamics remains poorly understood compared to that of constant temperatures. Assessment of phenological mismatch under varying temperature incubation is complicated by the tendency for larvae to hatch at a larger size and with smaller yolk reserves at lower temperatures (Griffths, 1979; Mueller *et al.*, 2015). This phenomenon may be accounted for by the temperature dependence of hatching scaling differently than the temperature dependence of differentiation (van der Have and de Jong, 1996; Zuo *et al.*, 2012). Alternatively, the embryos incubating at low temperatures may simply

attain a more advanced stage of development before hatching (Figure 5-1). Chapter 4 presents evidence for the later hypothesis and proposes the term heterograde for hatching that occurs at different temperature-dependent development stages.

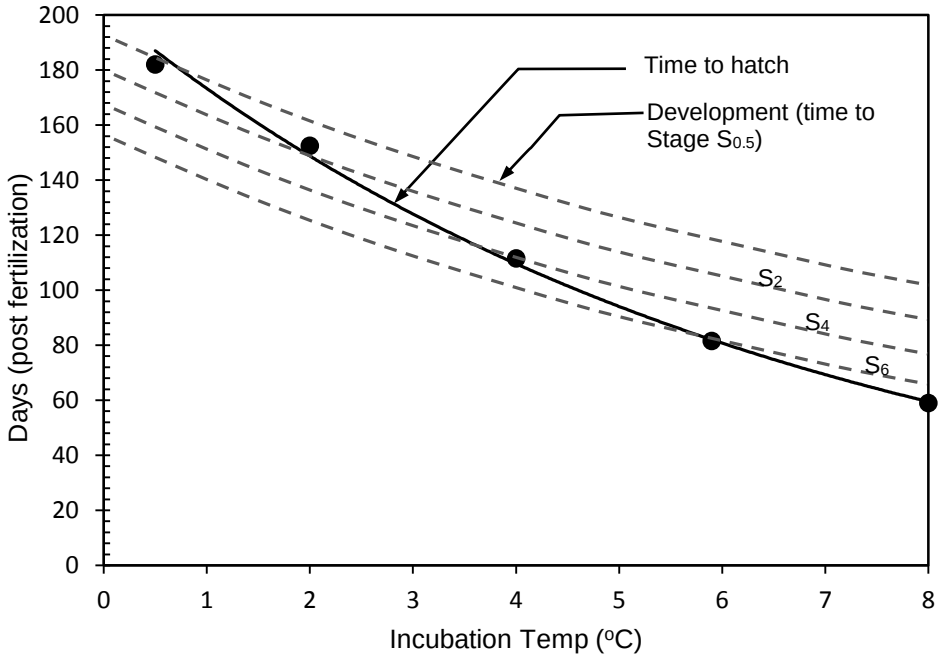


Figure 5-1: A hypothetical heterograde relationship (i.e. when embryos hatch at a different stage of development at different incubation temperatures) between constant temperature and the time to mean hatch. The solid markers (•) represent observed time to mean hatch reported in Brooke (1975) for which Q_{10}≈4.9. The broken lines scale according to Q_{10}≈3.2 and represent the time to reach a hypothetical stage (S_n) of development equivalent to that in embryos hatching normally at a specific constant incubation temperatures.

The recognition of heterograde hatching in the lake whitefish offers a basis for an improved model of hatch timing under ecologically relevant variable incubation temperatures. Existing models are predicted to substantially underestimate the time to hatch for embryos incubated under a progressively decreasing thermal regime since the embryos must grow to a more advanced stage than would be the case were hatching to occur at the same stage of

differentiation independent of temperature. Conversely, existing models would be predicted to overestimate the time to hatch under an increasing thermal regime. Natural thermal regimes tend to be asymmetric in character with water temperatures progressively decreasing post-spawning and then rising rapidly following the break-up of winter ice cover. Under cyclical or symmetrically varying thermal regimes such as those tested by Griffiths (1979), the accuracy of existing models is predicted to be little affected since the over and under-estimation tends to cancel out.

In this paper we present a heterograde model to predict embryonic development and hatch timing in lake whitefish. We compared this model to existing hatch timing models using Lake Huron and Lake Simcoe whitefish embryos incubated in laboratory conditions under a variety of constant and symmetrically and asymmetrically varying thermal regimes. We also propose a method to predict the approximate timing of fractional hatching in addition to median hatch time.

5.2 Methods and Materials

Egg Collection and Rearing Methods

Lake whitefish eggs were collected from spawning Lake Huron fish in between 3 and 6 m of ca. 7^{o}C water south of Whitefish Island (Lat. 44 42 37.74; Long. 81 18 38.94) on November 21. 2013 (18 females fertilized with milt from 30 males) and on November 29th, 2014 (3 females fertilized with milt from 5 males). Eggs were stripped from ripe females and pooled with milt from males. Eggs were dry fertilized for approximately 5 minutes, followed by wet fertilization and disinfection with Ovadine® (5 ml/L, Syndel Laboratories Ltd.). In 2014,

additional eggs were obtained from the Ontario Ministry of Natural Resources from a single female from Lake Simcoe fertilized by pooled milt from four different males on November 26, 2014. The Lake Simcoe eggs were used to avoid confounding maternal effects on the estimation of the thermal dependence of development (Chapter 4) and to test how readily our proposed model can accommodate different populations.

The fertilized eggs were transported to the McMaster University laboratory where they were reared in refrigerated coolers using apparatus and methods described in Mitz *et al.* (2014). Cooler temperatures were held at nominal temperatures of 2, 5 and 8°C with an additional 0.5°C (nominal) temperature point obtained by rearing embryos in plates on an unsaturated bed of crushed ice. The eggs were reared in 24-well or 48-well plates filled with dechlorinated municipal tap water. Embryos were moved to a clean multi-well plates during weekly water changes to prevent buildup of fungus. Viable hatches were recorded and dead embryos removed daily. Embryo mortalities were recognized by opacity on a significant portion of the egg or by the disintegration of the chorion and embryo. Photoperiod was ambient based on natural light through laboratory windows and was consistent across all treatments.

Data loggers (Schlumberger Mini-Diver and Onset Hobo) recorded water temperature every 5 minutes at multiple locations within the cooler units. Temperature was not logged for the 0°C (nominal) treatment group, although a measurement with a thermometer indicated water temperature of approximately 0.5°C. Spawning adult Lake Whitefish were collected under a scientific fishing permit from the Ontario Ministry of Natural Resources and Forestry to J.Y.Wilson.

Thermal Regimes

Embryos were shifted between the different constant temperature coolers to create different variable thermal regimes. These included cyclically variable regimes where embryos spent six days at a constant base temperature before being transferred to a different cycle temperature for one day (Lake Huron embryos in 2013-2014), or five days at a base temperature followed by two days at the cycle temperature (Lake Simcoe embryos in 2014-2015). Asymmetrically variable regimes included initial incubation at 8^{o}C for different lengths of time before transfer to 2^{o}C for the remaining development, and progressively increasing or decreasing regimes where initial development took place at either 2 or 8^{o}C for approximately 1/3rd of expected development followed by transfer to higher or lower temperatures for the remaining thirds of incubation. Asymmetrically varying regimes also included a one-time shift from a base temperature of 2 or 5^{o}C to a different temperature. The experimental regimes were selected to provide a statistically useful cross section of constant, cyclical and asymmetrically variable thermal regimes in order to allow for a robust evaluation of different hatch prediction models. The various tested thermal regimes are summarized on Table 5.1.

Model Description

We assumed a first order exponential relationship to temperature for both development and time-to-hatch. The scaling of development to temperature is modelled in the form:

$$S_{50} = \alpha e^{-\kappa T} \qquad [1]$$

where the time, S_{50} is that required for 50% of developing embryos to reach a specified stage of development at a constant temperature *T*, α is the number of days to reach a stage of development equivalent to mean hatch at T = 0 °C, and κ is the logarithmic slope constant for development. At higher temperatures, embryos hatch at an earlier development stage that may be described using a heterograde ratio (f_{het}) estimated from the relative timing of hatching following temperature shifts (Chapter 4) and related to the difference between Q_{10} for development and Q_{10} for hatching:

$$f_{het} = 1 - \left(\left(\frac{{}^{develop}Q_{10})}{{}^{hatch}Q_{10}} \right)^{\left(\frac{\Delta T}{10}\right)} \right) \qquad [2]$$

Using data from Chapter 4, we estimate that $f_{het}^{0\rightarrow 8}$ = 0.28 which means that an embryo incubating at 8°C will reach at development stage equivalent to that of hatching at 0°C at $1 - f_{het}$ or 72% of relative development at hatching for a constant temperature of 0°C.

Relative stage-wise development f_S at any time step is estimated according to:

$$f_S = \sum_{0}^{t} \frac{1}{\alpha e^{-\kappa T}} \qquad [3]$$

Relative time-to-hatch (RTH) follows a similar exponential relationship (Thome *et al.*, 2016) where β is the logarithmic slope constant for time-to-hatch under constant temperature incubation and α is the number of days to reach 50% hatch at 0°C. The predicted relative time-to-hatch f_{RTH} for any constant temperature time step is therefore:

$$f_{RTH} = \sum_{0}^{t} \frac{1}{\alpha e^{-\beta T}} \qquad [4]$$

If hatching were to occur at the same stage of development then $\kappa = \beta$. But for heterograde hatching, we must account for the difference in stage-at-hatch for higher temperatures by subtracting the difference in hatching stage at the instantaneous temperature *T* experienced at any particular time step. Thus, the relative time to hatching, f_h under varying temperatures becomes:

$$f_h = \frac{\Delta t}{f_S \alpha e^{-\kappa T} - (\alpha e^{-\beta T} - \alpha e^{-\kappa T})} \qquad [5]$$

where Δt is the cumulative number of incubation days at any time step. Equation 5 is composed of two parts, a continuous monotonic function f_S describing development, and a discontinuous non-monotonic component $(\alpha e^{-\beta T} - \alpha e^{-\kappa T})$ describing the difference in hatching stage for the instantaneous temperature *T*.

In addition to estimating the time to mean hatch, it is possible to model cumulative hatch vs. time using a modified version of the logistic function which allows the observed temporal asymmetry associated with early hatching embryos to be modelled. Fractional hatching h_x is related to f_h using an empirically fitted equation:

$$h_x = \frac{A_t}{1 + e^{-\left(\frac{f_h - f_0}{b}\right)}} \qquad [6]$$

where A_t is equal to 1.03, f_o is 1.02 and b is 0.03. A_t incorporates a degree of asymmetry by shifting the entire sigmoidal hatching curve slightly upwards to mimic the asymmetry created by a small number of early hatching embryos.

Equation 6 is independent of the model used to predict relative development and therefore may be used to predict the timing of fractional hatching with any of the incremental models considered in this paper.

Model parameters may be calibrated to a specific lake whitefish population using time to hatch under constant temperature incubation. The constant temperature data provides direct measurement of values for β (0.152 and 0.163) and α (231 and 251) for Lake Huron and Lake Simcoe populations respectively. The values for β are equivalent to $Q_{10} \approx 4.6$ and 5.0 while the value for κ is estimated (Chapter 4) to have a population-independent value of approximately 0.117 (equivalent to $Q_{10} \approx 3.2$).

Existing Hatch Prediction Models

For all models except the degree day, we used incremental growth-at-temperature to predict the time to median hatch, t_h, as a function of time steps (e.g. Alderdice & Velsen, 1978; Thome *et al.*, 2016). This method is based on a constant temperature development model $t_h = f(T)$ where the incremental growth occurring at any time step is $f(T)^{-1}$. Increments of growth over discrete time steps $\Delta t_1, \Delta t_2, \Delta t_3 ....$ at temperatures $T_1, T_2, T_3 ....$ with t_h predicted when:

$$\sum_0^t f(T)^{-1} = 1 \qquad [7]$$

IGT was used to predict hatching for all existing models except the degree day. This includes models such as (Price, 1940) which were constant temperature models in their originally published form.

The first whitefish-specific development model was proposed by Price (1940) who incubated lake whitefish eggs from Lake Erie at constant temperatures of 0.5°C to 10°C and developed a model of the form $\frac{M}{A^T}$ in which the time (t) to attain a given stage is inversely proportional to a constant (A) raised to an exponent, temperature (T), and where M is the days to hatch at zero degrees. Price determined that the best fit values for A were 1.205 between 0 and 6°C and 1.1575 between 6 and 12°C. For our model comparison, we adjusted Price's value for M for the experimental whitefish populations but we did not attempt to modify Price's value for A as we lacked an adequate basis to do so.

Brooke (1975) conducted a similar experiment using whitefish embryos from Lake Michigan and derived a polynomial equation to estimate the developmental rate for each of 20 developmental stages, including hatch, based on daily mean temperature.

$$R_j = ab^T c^{T_2} d^{T_3} \qquad [8]$$

Where the specific equation for mean hatching given by

$$LogR_j = -2.3098 + 0.0684T \qquad [9]$$

Brooke's model parameters were not adjusted for the experimental populations.

Griffiths (1979) incubated lake whitefish embryos in 16 combinations of constant and fluctuating temperature regimes ranging from 1.8 to 10.0 ºC and derived a polynomial equation for time to complete hatch (Y):

$$Y = 229.123 - 8.64305X_1^{1.3775} - 5.27425X_2^{1.2041} + 0.0645081X_1^{2.755} - 0.0772456X_2^{2.4082} + 0.25173X_1^{1.3775}X_2^{1.2041}$$

with X_1 and X_2 as the base and cycle temperatures, respectively. We applied Griffiths polynomial equation using incremental growth-at-temperature and the mean daily temperature for both the base and cycle temperatures. The timing of median hatch was based on Equation 6 with the assumption that 99% fractional hatch is essentially complete.

Thome *et al.* (2016) developed a model based on a first order exponential function to predict median hatching time for lake whitefish incubated *in situ* in Lake Huron. This equation was $t_h = 219e^{-0.159T}$ and is similar to the constant temperature application of our proposed heterograde model apart from slight differences in parameterization (β = 0.152 vs. 0.159 and $\alpha = 219$ vs. 231). The Thome model was applied using the same values for β and α used in the heterograde-exponential model for consistency and in accordance with the parameterization procedure described in Thome *et al.* (2016).

Finally, we considered thermal sums, widely known as the degree-day method. The most common form is $h_{50} = k(T)^{-1}$ where h_{50} is the incubation period (days to mean hatching) at a given mean temperature T, and k is a constant number of degree days required to reach h_{50}. The degree day is often modified by the use of a reference temperature, or biological zero (Chezik *et al.*, 2014), which may be negative. We used an average of the number of degree

days required to achieve mean hatch for 5 and 8°C constant temperature incubation to estimate k=500 which we applied to both experimental populations. We did not use a negative temperature for biological zero.

Statistical Analysis

Statistical analysis was conducted using Sigmaplot V11.0. The time to 25, 50, and 75% hatch and the time to median hatch were determined based on replicate dishes (typically 2 per group). The differences between modelled predictions and observations were compared using net and root mean squared error (RMSE) and an R-squared value determined by linear regression of observed vs. predicted hatch times. The prediction of fractional hatch was assessed by comparing the RMSE for predicted 25 and 75% hatch to the accuracy of predicted median hatch for all experimental thermal regimes. Comparisons between different predictive models used RMSE and a non-parametric Kruskal-Wallis one-way ANOVA on Ranks followed by Tukey's HSD test. Differences were considered statistically significant at $P \leq 0.05$.

5.3 Results

The time from fertilization to mean hatch for different thermal regimes and whitefish populations is summarized on Table 5.1 along with predictions derived using the proposed heterograde development model and existing alternatives. Median hatch time was similar to mean hatch but generally about 1% later owning to a slight asymmetry caused by early hatching embryos. The time from fertilization to mean hatch differed slightly between the Lake Simcoe and Lake Huron populations with the latter consistently requiring less time to reach mean hatch for a given temperature.

Table 5.1. Summary of time-to-hatch for lake whitefish embryos reared under different constant and variable thermal regimes

							Observed hatch timing (dpf)								Predicted hatch timing (dpf)							
															This study			Brooke	Griffiths	Thome	Price	GDD
No.	Pop	Experimental thermal regime	$T_e^{0.16\ 1}$	T_μ^{2}	*n*	Live hatch %	h_{25}	Mean	Median	Std Dev	h_{75}	First	Last	Duration	h_{25}	h_{50}	h_{75}	h_{50}	h_{50}	h_{50}	h_{50}	h_{50}
1	Huron	Constant (0 °C)	0.50	0.50	9	9%	201	203.0	203	3.2	205	198	208	10	211	218	226	217	223	214	210	1000
2	Huron	Constant (2 °C)	2.56	2.53	26	27%	152	156.1	158	9.7	162	134	172	38	158	159	172	173	185	156	142	198
3	Huron	Constant (5 °C)	5.07	5.07	47	49%	104	109.1	110	8.1	115	93	129	36	106	109	114	116	129	107	89	99
4	Huron	Constant (8 °C)	8.07	8.07	7	7%	65	67.7	67	3.0	71	64	72	8	67	69	71	67	74	68	70	62
5	Simcoe	Constant (2 °C)	2.00	2.00	43	45%	176	179.3	181	11.0	184	125	197	72	185	188	195	186	196	180	172	250
6	Simcoe	Constant (5 °C)	4.98	4.98	41	43%	104	115.8	113	14.8	129	89	142	53	113	114	120	100	131	110	99	100
7	Simcoe	Constant (8 °C)	7.94	7.94	11	11%	64	67.2	68	4.5	68	62	75	13	71	70	74	71	76	68	78	63
8	Huron	Cyclic (0↔2 °C)	1.06	0.96	31	65%	180	188.3	188	8.7	193	172	205	33	194	195	197	194	210	196	188	521
9	Huron	Cyclic (0↔5 °C)	1.66	1.37	28	58%	165	170.4	171	8.0	176	155	185	30	166	168	177	182	196	180	167	364
10	Huron	Cyclic (0↔8 °C)	2.48	1.79	8	17%	147	153.5	156	7.3	160	144	160	16	145	152	158	152	169	158	156	280
11	Huron	Cyclic (2↔5 °C)	3.14	3.04	28	58%	137	142.4	146	12.3	150	111	161	50	137	143	145	154	170	143	127	165
12	Huron	Cyclic (2↔8 °C)	3.86	3.50	27	56%	119	123.4	125	4.0	127	119	130	11	119	124	131	139	149	129	120	143
13	Simcoe	Cyclic (0↔2 °C)	0.95	0.92	13	14%	187	196.1	200	15.0	205	159	217	58	206	209	215	193	215	214	209	546
14	Simcoe	Cyclic (0↔5 °C)	2.18	1.80	60	63%	160	161.3	161	11.7	162	106	183	77	159	161	167	183	185	175	166	278
15	Simcoe	Cyclic (0↔8 °C)	3.64	2.60	37	77%	133	136.4	134	8.6	142	102	148	46	125	126	132	134	146	139	148	192
16	Simcoe	Cyclic (5↔0 °C)	4.17	3.91	32	67%	136	138.2	142	15.9	143	99	163	64	127	129	132	137	146	126	115	128
17	Simcoe	Cyclic (2↔5 °C)	3.05	2.88	75	78%	146	147.5	147	6.7	153	128	161	33	139	144	153	162	171	152	142	173
18	Simcoe	Cyclic (2↔8 °C)	4.14	3.56	63	66%	99	118.0	120	16.4	133	99	142	43	116	118	118	94	136	124	130	136
19	Simcoe	Cyclic (5↔8 °C)	5.96	5.80	45	46%	90	89.1	90	3.8	91	76	94	18	90	93	97	109	110	94	92	86
20	Simcoe	Cyclic (8↔0 °C)	6.85	6.19	14	29%	82	88.2	92	8.2	94	75	98	23	82	82	88	75	90	82	92	81
21	Simcoe	Cyclic (8↔2 °C)	6.70	6.19	22	46%	86	87.4	87	3.2	90	82	93	11	85	85	87	88	93	83	93	81
22	Simcoe	Cyclic (8↔5 °C)	7.21	7.06	29	30%	79	79.9	82	6.8	85	65	90	25	78	78	85	79	87	77	84	71
23	Huron	Upwardly Asymmetric (2→5→8 °C)	4.64	4.20	18		104	103.3	104	2.1	104	97	106	9	101	104	106	120	120	110	105	119
24	Simcoe	Upwardly Asymmetric (5(44)→8 °C)	6.54	6.35	25		83	84.1	83	2.4	86	79	90	11	84	84	88	91	95	85	87	79
25	Simcoe	Upwardly Asymmetric (5(60)→8 °C)	6.10	5.93	12		86	86.3	89	7.1	90	69	93	24	89	89	93	97	102	91	91	84
26	Simcoe	Upwardly Asymmetric (5(73)→8 °C)	5.68	5.55	19		89	88.4	91	5.9	91	75	94	19	92	93	97	105	107	96	93	90
27	Simcoe	Upwardly Asymmetric (2(82)→8 °C)	3.84	3.26	24		103	103.4	104	0.8	104	102	104	2	112	113	116	126	127	119	123	154
28	Simcoe	Upwardly Asymmetric (2(82)→5 °C)	3.49	3.29	20		135	139.2	142	5.3	143	128	145	17	133	135	140	147	157	141	133	152
29	Simcoe	Upwardly Asymmetric (2(93)→8 °C)	3.89	3.30	21		118	120.9	120	3.6	122	118	131	13	118	120	122	137	142	126	136	151
30	Simcoe	Upwardly Asymmetric (2(93)→5 °C)	3.24	3.05	20		139	140.8	143	3.6	143	132	144	12	138	138	143	156	161	146	138	164
31	Simcoe	Upwardly Asymmetric (2(121)→8 °C)	2.85	2.53	22		133	133.1	133	1.5	133	131	138	7	133	133	134	150	150	143	144	197
32	Simcoe	Upwardly Asymmetric (2(121)→5 °C)	2.82	2.67	23		153	153.1	153	1.3	154	148	155	7	145	146	154	168	169	156	149	188
33	Simcoe	Upwardly Asymmetric (2(137)→8 °C)	2.42	2.25	24		142	143.8	144	2.4	147	139	147	8	140	141	143	154	160	153	153	222
34	Simcoe	Upwardly Asymmetric (2(137)→5 °C)	2.42	2.33	22		153	153.1	153	1.2	154	149	155	6	150	151	156	174	175	163	156	214
35	Simcoe	Upwardly Asymmetric (2(160)→8 °C)	2.08	2.05	44		161	159.1	161	9.2	161	106	162	56	160	160	160	174	174	168	165	243
36	Simcoe	Upwardly Asymmetric (2(160)→5 °C)	2.04	2.04	22		159	159.7	160	2.1	161	155	164	9	160	161	161	181	183	172	166	246
37	Huron	Downwardly Asymmetric (8→5→2 °C)	3.54	3.23	42		160	162.9	168	15.3	171	112	181	69	142	151	158	139	153	126	112	155
38	Huron	Downwardly Asymmetric (8(10)→2 °C)	3.05	2.85	39		156	158.6	160	11.2	164	116	177	61	152	159	164	160	170	143	132	176
39	Huron	Downwardly Asymmetric (2(29)→8(10)→2 °C)	3.02	2.83	38		154	160.0	162	11.0	168	133	180	47	152	159	163	153	170	144	133	177
40	Huron	Downwardly Asymmetric (2(60)→8(10)→2 °C)	3.14	2.93	43		146	157.7	162	14.2	168	111	178	67	148	156	159	140	167	141	130	170
41	Simcoe	Downwardly Asymmetric (8→5→2 °C)	3.60	3.24	39		168	168.8	174	12.6	176	133	186	53	158	164	170	139	150	128	124	154
42	Simcoe	Downwardly Asymmetric (8(10)→2 °C)	2.55	2.34	74		171	176.3	176	9.3	182	148	204	56	175	183	187	166	180	163	160	213
43	Simcoe	Downwardly Asymmetric (8(20)→2 °C)	3.04	2.68	82		164	170.1	168	9.4	176	147	198	51	170	174	180	151	166	148	149	186
44	Simcoe	Downwardly Asymmetric (8(32)→2 °C)	3.65	3.14	44		144	160.0	161	15.5	174	131	185	54	162	165	174	111	148	130	136	159
45	Simcoe	Downwardly Asymmetric (5(44)→2 °C)	2.93	2.79	41		166	172.2	172	9.2	176	142	200	58	169	174	178	162	174	151	139	179
46	Simcoe	Downwardly Asymmetric (5(60)→2 °C)	3.26	3.09	14		163	168.6	170	9.7	172	146	184	38	164	169	175	154	165	141	127	162
47	Simcoe	Downwardly Asymmetric (5(73)→2 °C)	3.49	3.32	15		102	137.0	154	36.5	162	76	172	96	159	164	170	84	159	133	118	145
48	Simcoe	Downwardly Asymmetric (8→5→2 °C)	4.68	4.20	18		104	103.5	104	1.9	104	97	106	9	103	106	109	120	120	111	112	119

1 – exponentially weighted mean (Chapter 3) 2 – arithmetic mean h_x refers to fractional hatching with median hatching occurring at h_{50}.

For constant temperature incubation, the observed incubation times (Figure 5-2) were somewhat greater than those reported in Brooke (1975) and substantially greater than those reported by Price (1940). In all cases, the growth curves closely approximate an exponential relationship.

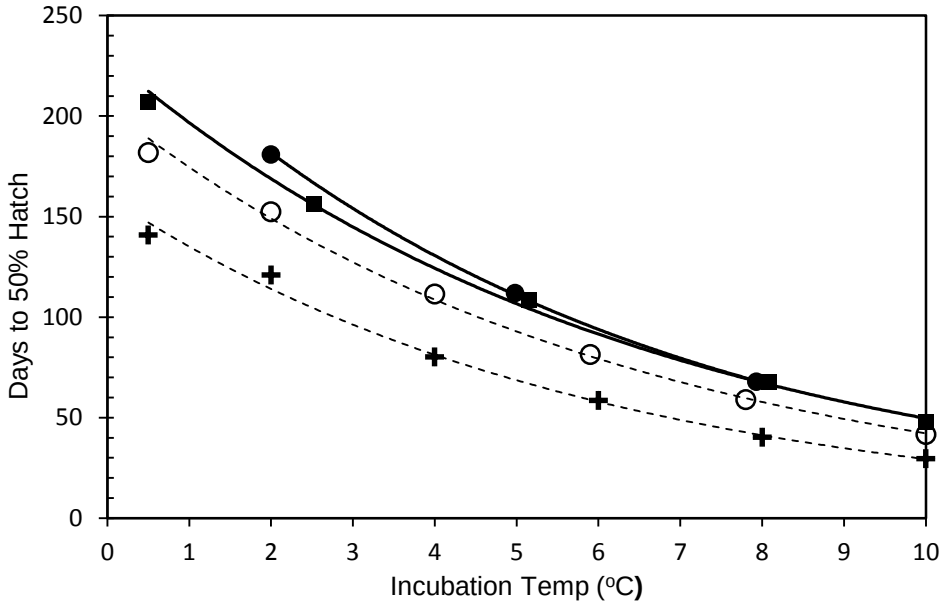


Figure 5-2: Days to median hatch at constant incubation temperature for different lake whitefish populations. The relationship between temperature and time to median hatch (t_h) closely approximates an exponential relationship; ■ – this study, Lake Huron, t_h= 231exp-0.159T, R^2=0.998); ● – this study, Lake Simcoe, t_h= 251exp-0.163T, R^2=0.998); ○ – Brooke (1975), Lake Michigan, t_h= 205exp-0.158T, R^2=0.998; + – Price (1940), Lake Erie, t_h= 160exp-0.169T, R^2=0.997).

This relationship tended to over-estimate the time to hatch under cyclical thermal regimes when using mean temperature - consistent with the effect of Jensen's inequality (Ruel and Ayres, 1999). Observed time to median hatch was less than predicted using Equation 4 with mean temperature for 12 of 15 cyclically varying regimes. For the asymmetric thermal regimes, observed timing was less than the mean temperature expectation for all (18 of 18) upwardly

shifting regimes and greater than the expectation based on Equation 4 for all but one (7 of 8) of the downwardly shifting regimes. The single exception occurred for a trial in which partial hatching had occurred prior to the downward shift.

The development times predicted using the heterograde, Thome-exponential, and Griffiths modified polynomial models were statistically indistinguishable from the observed time to hatch and from each other for constant temperature regimes. Both the Brooke and Price models generated errors in percentage terms (10 to 16%) that differed from observed timing by more than would be expected for an accurate model. The degree day model generated large percentage errors that reflect its inaccuracy at near-zero temperatures.

Model predictions deteriorated slightly with cyclical thermal regimes and no meaningful difference was observed in predictive accuracy for Lake Huron and Lake Simcoe populations. As with the constant temperature regimes, the heterograde, Thome-exponential, and Price models yielded predictions that were statistically indistinguishable from each other and from the observed timing. The degree day model again performed poorly for low cycle temperatures approaching zero.

The predictive accuracy of different models diverged for asymmetrically variable thermal regimes. While the heterograde-exponential model resulted in predicted hatch times averaging within 5% of observed for downwardly asymmetrical regimes, the existing models generated errors that exceeded 15%. It is interesting to note that the degree day model (RMSE=8.4%) actually outperformed all but the heterograde model for these regimes. For upwardly

asymmetrical regimes, the heterograde model predicted hatch timing within 2% of that observed. A graphical comparison of predictive accuracy achieved by the different models for different thermal regimes is provided on Figure 5-3 while a statistical summary of model accuracy is given in Table 5.2.

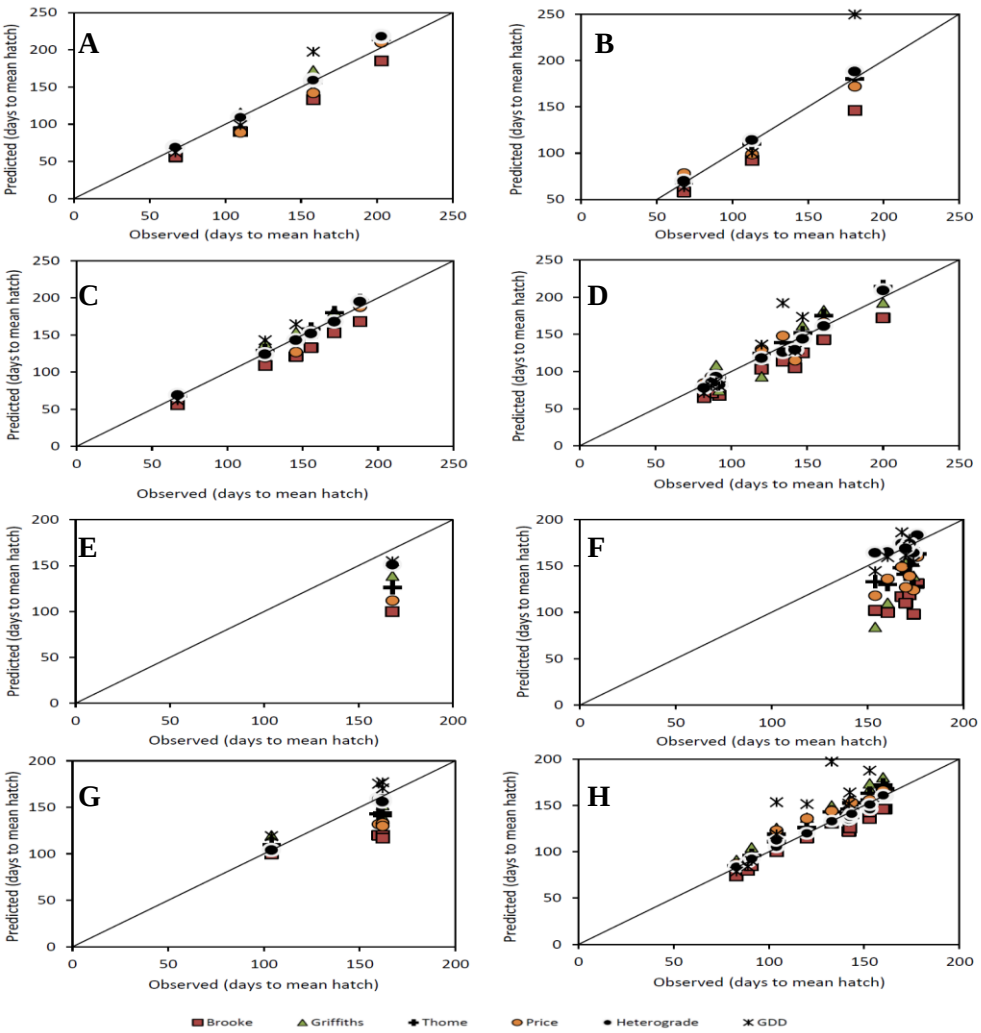


Figure 5-3: Observed vs. predicted time to median hatching for different thermal regimes. **A** and **B**: constant temperature incubation **C** and **D**: fluctuating temperature incubation; **E** and **F**: downwardly asymmetrically varying temperatures; **G** and **H**: upwardly asymmetrically varying temperatures. Panels on the left side (**A**,**C**,**E** and **G**) show data for Lake Huron (mF) embryos while the panels on the right (**B**,**D**,**F**, and **H**) show data for Lake Simcoe (sF) embryos.

Table 5.2. Predictive accuracy for different models (Huron and Simcoe Populations)

Constant Temperatures				
Model	n[2]	Mean RMSE	Std. Dev	Significance[1] (P<0.05)
Constant Temperatures				
Heterograde-exponential	7	2.80%	2.39	✓a
Brooke stage-wise	7	16.00%	3.54	
Griffiths polynomial	7	5.80%	3.73	a
Thome exponential	7	2.02%	1.80	✓a
Price	7	9.89%	5.90	
Degree day	7	70.30%	142.0	
Cyclically Varying Temperatures				
Heterograde-exponential	15	3.66%	3.04	✓a*
Brooke stage-wise	15	15.90%	4.95	
Griffiths polynomial	15	8.43%	7.30	
Thome exponential	15	5.30%	2.99	✓a*
Price	15	5.32%	5.34	✓a*
Degree day	15	51.00%	59.7	
Upwardly Asymmetrically Varying				
Heterograde-exponential	18	1.98%	2.18	✓a
Brooke stage-wise	18	10.30%	8.19	
Griffiths polynomial	18	11.90%	4.37	
Thome exponential	18	6.36%	3.90	
Price	18	7.77%	6.54	
Degree day	18	23.90%	19.60	
Downwardly Asymmetrically Varying Temperatures				
Heterograde-exponential	8	4.31%	3.10	✓a
Brooke stage-wise	8	34.70%	5.87	
Griffiths polynomial	8	18.70%	13.20	
Thome exponential	8	16.60%	6.64	
Price	8	20.70%	8.51	
Degree day	8	8.38%	6.27	a

✓- statistically indistinguishable from observed a - predictions not statistically different * - the variance in hatch time under cyclical regimes is intrinsically greater than constant temperatures or asymmetric shifts. The intrinsic uncertainty in observed hatch timing was assessed for nine groups of embryos (n between 24 and 48) reared at a temperature of 2±0.1ºC in the same shelf within the same incubator (unpublished data). Mean hatch for these was 159.56 days with a Standard Deviation of 4.2 days (2.31±2.0%). Therefore, models with a predictive accuracy < 5% are treated as statistically indistinguishable from observed.

[1] Kruskal-Wallis One Way Analysis of Variance on Ranks

Our model predicted 25% fractional hatch within 1.5% of the RMSE achieved for median hatch while the predicted time for 75% fractional hatch yielded an essentially identical RMSE to that obtained for median hatch. Other hatch fractions were not assessed due to the generally low numbers involved. Testing the different models against all regimes indicates that the heterograde model is statistically superior ($P<0.05$). A summary of model accuracy (net error) vs. precision (absolute error) is shown on Figure 5-4.

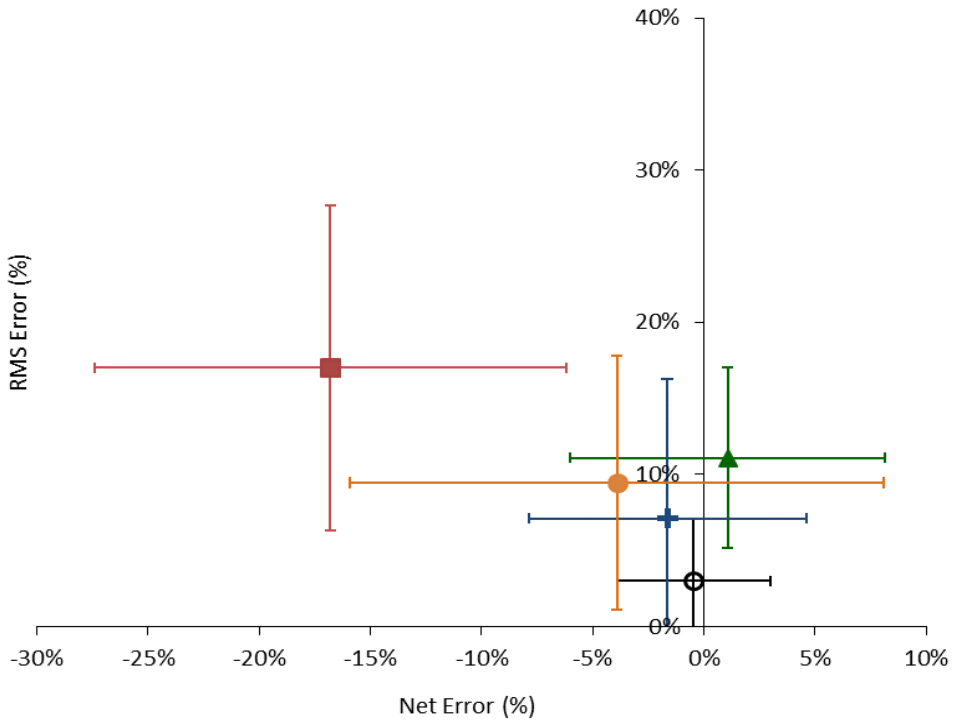


Figure 5-4: Model accuracy (net error) vs. precision (RMSE) for the model predictions for 48 different constant and variable thermal regimes. Our proposed model (o) yielded superior results compared to existing models with the lowest net error, lowest RMSE, and the smallest standard deviation. The Griffiths polynomial (▲) model tends to overestimate development time for most thermal regimes while the Brooke model (■) tended to underestimate. Both models suffer from an inability to easily calibrate their parameters to specific whitefish populations. Both the Price (●) and Thome (+) models are parameterized to the specific Lake Huron and Lake Simcoe whitefish populations and this is reflected in their improved accuracy and precision. The degree day model is not included as its errors exceed the domain and range shown.

Application of our model to the temperature data in Berlin *et al.* (1977) resulted in predicted mean hatching within 1.4% of the 135 days observed. We also tested the model using published hatch timing data from Mueller *et al.* (2015) who reared Lake Huron lake whitefish embryos under thermal regimes that included temperatures shifts at one or more of two critical developmental time points. Using this data, the model predicted the timing of 50% hatch to within an RMSE of 2.9±3.4% with a slight tendency to overestimate the time to 50% hatch (by an average of 2.2±3.0%). We further tested our model using data reported by Patrick, *et al.* (2013) who incubated lake whitefish embryos obtained from Lake Huron in a facility that replicated natural seasonal temperatures (AV) and four treatments of fluctuating increments above ambient (AV+1, AV+2, AV+3, and AV+5). Our model predicted the timing of median hatch to within 2% (0.3±0.9%). of that observed by Patrick *et al.* (2013) for four of the five regimes (AV, AV+1, AV+2, and AV+3). For the highest temperature regime (AV+5), the model overestimated the time of median hatch by nearly 20% although it is possible that this regime with daily mean temperatures between 7 and 8^{o}C included intra-day temperatures that exceeded normal tolerance limits for whitefish embryos.

5.4 Discussion

The results indicate that the improved predictive accuracy of our model is derived from its heterograde component rather than the assumed exponential dependence of development with temperature. For constant and cyclically variable regimes, where the effect of heterograde hatching is absent and moderated respectively, the Thome-exponential, Price, and Griffiths

modified polynomial models achieved accuracy generally similar to that of our model. Where temperature shifts occurred asymmetrically, the existing models consistently underperformed with RMSE exceeding 10%. Moreover the direction of the errors is consistent with that predicted by heterograde hatching. For downward temperature shifts, existing models underestimated time to hatch by 15 to 20%, while time to hatch was typically overestimated by 6 to 12% for upward temperature shifts.

The Brooke polynomial tended to underestimate development for all temperatures while the accuracy of the degree day method deteriorated at lower temperatures reflecting mathematical limitations at near zero temperatures (the degree day method predicts infinite development time at zero). The Brooke model was verified with experiments that simulated natural, variable temperature conditions for southern Lake Michigan (Berlin, *et al.*, 1977). Under these conditions, the Brooke model predicted development times to within 3.7% of that observed (140 vs. 135 days), so it is reasonable to assume that the Brooke model would have performed similarly to the Thome and Griffiths models had it been calibrated to the specific lake whitefish populations used in our experiments.

Relative development, f_h, is monotonic for all existing hatch timing models and this fundamentally limits their accuracy when temperatures vary asymmetrically. In contrast, our model allows for an instantaneous decrease in f_h following a decrease in incubation temperature and an instantaneous increase in f_h corresponding to an increase in incubation temperature. This attribute offers an explanation for the phenomenon of thermally-triggered

mass hatching in whitefish (Hooper, 2006) and for the cessation of hatching following a decrease in incubation temperature (Reshetnikov and Bogdanov, 2011).

Under heterograde hatching, the length of time spent at elevated temperatures during early development stages has a relatively minor effect on hatch timing while elevated temperatures in late development may substantially advance hatching. For example, embryos spawned in early vs late November will be exposed to elevated temperatures for a substantially longer time than those spawned in early December. Existing hatch timing models predict a substantial advance in hatching time (> 1 month) under normal winter thermal regimes. This is illustrated by Regimes 42, 43, and 44 (Figure 5-5) in which embryos were exposed to 8^{o}C for 10 to 32 days during early development. Using existing predictive models, Regime 44 (32 days at 8^{o} then 2^{o}C until hatch) would be predicted to result in a 30% decrease in incubation time under existing models but an 11.5% decrease under our heterograde model. The observed decrease in hatch timing for Regime 31 was 12.5%. The hatch advance resulting from warming of a given magnitude is, therefore, not constant but rather a function of the specific characteristics of the thermal regime being considered. This finding is consistent with that of Lee *et al.* (2016) who tested the effect of weekly 3^{o}C temperature fluctuations of 1 hour duration and found that these has no measurable effect of development stage prior embryos reaching hatching competence.

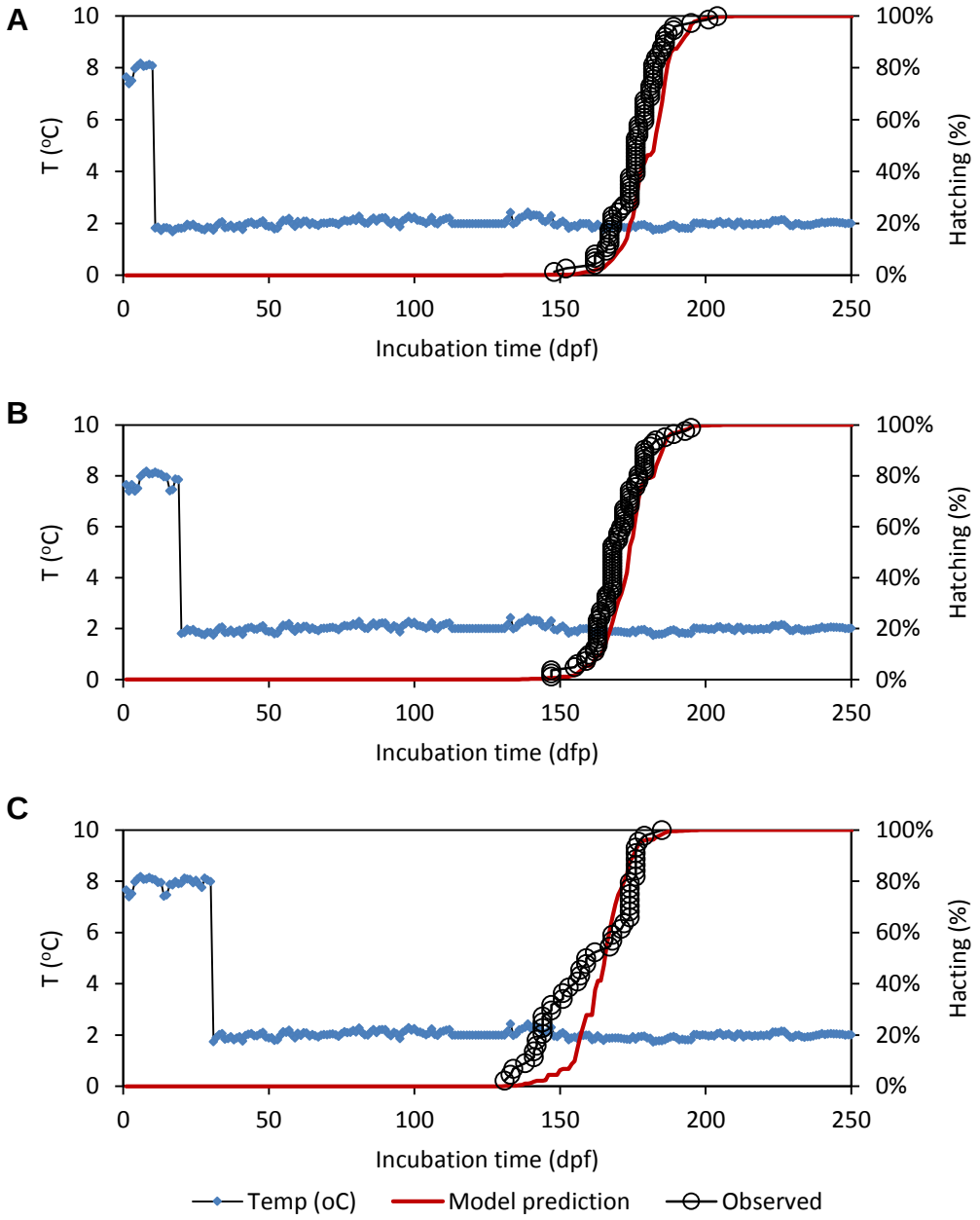


Figure 5-5: Graphical illustration of the effect of heterograde hatching on the synchronization of hatching under conditions of environmental uncertainty. Panels **A**, **B**, and **C** show experimental thermal regimes 42 to 44 where the duration of initial incubation at 8°C varies to simulate a rapid or delayed onset of free-up following spawning. The initial durations of 10, 20, and 32 days at 8 °C represent 15, 30, and 48% of total development based on an isograde development model which would predict hatching 27 and 59 days later than **A**. Instead, the observed mean hatching differed by 8 and 15 days and was in general accordance with model predictions (solid red line).

Existing models performed poorly under asymmetrically varying temperature regimes because they implicitly assume that hatching is a valid and consistent stage of development independent of incubation temperature. In contrast, our model treats development stage at hatching as a temperature-dependent variable and this allows for more accurate predictions. At present, our knowledge of the mechanism(s) by which hatching enzymes are released remains unknown and it is possible that hatching is affected by the absolute size of the embryo not just its stage of development. This factor is not incorporated in our model which limits its ability to predict size-at-hatching in addition to timing.

While hatching in the lake whitefish has been shown to be heterograde (Chapter 4) a similar temperature dependence of size at hatch has been recognized in only a handful of other species such as the Atlantic cod (Jordaan, 2002; Jordaan *et al.*, 2006). Nevertheless, we speculate that other fall-spawning Coregonid's such as the lake herring (*Coregonus artedi*) and round whitefish (*Prospium cylindraceum*) will exhibit a similar temperature dependence of development stage at hatching. A modified version of our model might perform well for these species.

In order to evaluate match/mismatch hypotheses, it is necessary to reliably predict the hatch timing under real world conditions in which temperatures may fluctuate asymmetrically over the course of development. Such regimes are ecologically relevant as they mimic the effect of a delayed or accelerated autumn when higher temperatures prevail. They also mimic the effect of transient impingement of a sinking thermal plume from once-through cooling. Our study

indicates that the use of a two-component heterograde model yields a large and statistically significant improvement in predictive accuracy for those asymmetrically varying thermal regimes most relevant to such questions.

5.5 Acknowledgements

The authors wish to thank Lisa Stoa, Emily Hulley and Shayen Sreetharan for their assistance with the experimental work. The Ontario Ministry of Natural Resources and Forestry is gratefully acknowledged for their assistance in providing whitefish embryos from Lake Simcoe. We are grateful to Bruce Power for its support of this work, both financially and technically, and for funding through a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development Grant.

5.6 References

Alderdice, D. F., & F. P. J. Velsen, 1978. Relation between temperature and incubation time for eggs of Chinook salmon (*Oncorhynchus tshawytscha*); *Journal of the Fisheries Research Board of Canada* 35: 69–75.

Berlin, W.H., L.T. Brooke and L.J. Stone, 1977. Verification of a model for predicting the effect of inconstant temperature on embryonic development of lake whitefish (*Coregonus clupeaformis*); *Technical papers of the US Fish and Wildlife Service* 92.

Bidgood, B. F. 1972. Divergent growth in lake whitefish populations from two eutrophic Alberta lakes; Ph.D. Thesis. University of Alberta, Edmonton, Alta. 106 p.

Brooke, L.T. 1975. Effect of different constant incubation temperatures on egg survival and embryonic development in lake whitefish (*Coregonus clupeaformis*). *Transactions of the American Fisheries Society* 104 (3), 555-559.

Chezik, K. A., N. P. Lester, P. A. Venturelli, and K. Tierney, 2014. Fish growth and degree-days I: Selecting a base temperature for an among-population study; *Canadian Journal of Fisheries and Aquatic Sciences* 71.9: 1303-1311.

Cushing, D. H., 1969. The regularity of the spawning season of some fishes; *Journal du Conseil pour l'Exploration de la Mer* 33:81–92.

Czerkies, P., P. Brzuzan, K. Kordalski, & M. Luczynski, 2001. Critical partial pressures of oxygen causing precocious hatching in *Coregonus lavaretus* and *C. albula* embryos; *Aquaculture* 196 (2001). 151–158.

Eme, J., C.A. Mueller, R.G. Manzon, C.M. Somers, D.R. Boreham, and J.Y. Wilson, 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings. *Comparative Biochemistry and Physiology* Part A; Molecular & Integrative Physiology. 179, 71-80.

Genner, M. J., N. C. Halliday, S. D. Simpson, A. J. Southward, S. J. Hawkins, and D. W. Sims, 2009. Temperature-driven phenological changes within a marine larval fish assemblage. *Journal of Plankton Research* 32.5 (2009): 699-708.

Griffiths, J.S. 1979. Potential effects of unstable thermal discharges on incubation of Lake Whitefish eggs; *Ontario Hydro Research Division Report No*. 79-521-K.

Hart, J.L. 1931. The spawning and early life history of the whitefish, Coregonus clupeaformis (Mitchill), in the Bay of Quinte, Ontario; *Contributions to Canadian Biology and Fisheries* 6 (1), 165-214.

Hooper, G. W., 2006: Techniques used for the intensive culture of Lake Whitefish (*Coregonus clupeaformis*) in Ontario, Canada; *Special Publication Aquaculture Association of Canada* (11): 169-172.

John, K. R., and A. D. Hasler, 1956. Observations on some factors affecting the hatching of eggs and the survival of young shallow-water Cisco, *Leucichthys Artedi* Lesueur, in Lake Mendota, Wisconsin; *Limnology and Oceanography*. 1.3 (1956): 176-194.

Jordaan, A., 2002. The effect of temperature on the development, growth and survival of Atlantic Cod (*Gadus morhua*) during early life-histories; University of Maine, Electronic Theses and Dissertations. Paper 141.

Jordaan, A., S.E. Hayhurst and L.J. Kling, 2006. The influence of temperature on the stage at hatch of laboratory reared Atlantic cod *Gadus morhua* and implications for comparisons of length and morphology; *Journal of Fish Biology* 68, 7–24.

Lee, A.H., J. Eme, Mueller, C.A., R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2016. The effects of increased constant incubation temperature and cumulative acute heat shock exposures on morphology and survival of Lake Whitefish (*Coregonus clupeaformis*) embryos; *Journal of Thermal Biology* 57 (2016) 11–20.

Luczynski, M. 1984. Improvement in the efficiency of stocking lakes with larvae of *Coregonus albula* L. by delaying hatching; *Aquaculture* 41: 99 111.

Luczynski, M., A Kirklewska, 1984. Dependence of *Coregonus albula* embryogenesis rate on the incubation temperature; *Aquaculture*, 42, 1, 43-55.

Mitz, C., C. Thome, M.E. Cybulski, L. Laframboise, C.M. Somers, R.G.Manzon, J.Y. Wilson, D.R. Boreham, 2014. A self-contained, controlled hatchery system for rearing lake whitefish embryos for experimental aquaculture; *North American Journal of Aquaculture* 76 (3), 179-184.

Mueller, C.A., J. Eme, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype and cost of development in lake whitefish (*Coregonus clupeaformis*); *Journal of Comparative Physiology*. B, Biochemical, Systemic, and Environmental Physiology. 185 (3), 315-331.

Neuheimer, A. B, and C. T. Taggart., 2007. The growing degree-day and fish size-at-age: The overlooked metric; *Canadian Journal of Fisheries and Aquatic Sciences* 64.2 (2007): 375-385.

Patrick, P.H., E. Chen, J. Parks, J. Powell, J. S. Poulton and C-L Fietsch, 2013. Effects of fixed and fluctuating temperature on hatch of Round Whitefish and Lake Whitefish eggs; *North American Journal of Fisheries Management* 33:1091-1099.

Price, J.W., 1940. Time-temperature relations in the incubation of the whitefish, *Coregonus clupeaformis* (Mitchill); *Journal of General Physiology* 23 (4), 449-468.

Ragland, G.J. and J.G. Kingsolver, 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*; *Evolutionary Ecology Research* 10: 29-44.

Reshetnikov, Y. S., and V. D. Bogdanov, 2011. Features of reproduction of whitefishes; *Journal of Ichthyology* 51.6 (2011): 432-456.

Ruel, J. J. and M. P. Ayres, 1999. Jensen's inequality predicts effects of environmental variation; *Trends in Evolution and Ecology* 14: 361-366.

Scott, W.B. and E.J. Crossman, 1973. Freshwater fishes of Canada; *Fish Research Board of Canada Bulletin* 184.

Sreetharan, S., C. Thome, C. Mitz, J. Eme, C.A. Mueller, E.N. Hulley, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2014. Embryonic development of lake whitefish (*Coregonus clupeaformis*): a staging series, analysis of growth and impacts of fixation; *Journal of Fish Biology* 87.3 (2015): 539-558.

Thome, C., C. Mitz, C.M. Somers, R.G. Manzon, J.Y. Wilson, and D.R. Boreham, 2016. Incubation of Lake Whitefish (*Coregonus clupeaformis*) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development: *Canadian Journal of Fisheries and Aquatic Sciences, http://dx.doi.org/10.1139/cjfas-2015-0286.*

Chapter 6

Is there a trade-off between hormetic growth stimulation and metabolic efficiency?

Charles Mitz[1], Christopher Thome[1,5], Mary Ellen Cybulski[1], Christopher M. Somers[3], Richard G. Manzon[3], Joanna Y. Wilson[4], and Douglas R. Boreham[1,2,5*]

Abstract

Beneficial hormetic effects have been demonstrated for low dose exposure to ionizing radiation. However, such benefits may be offset by some form of evolutionarily relevant cost because the responsible biological mechanisms are not maintained in an upregulated state but require some form of sensitizing exposure. It has been suggested that stimulation of the adaptive response mechanisms could be metabolically costly or that the hormetic response could come at a sacrifice to other physiological processes.

We exposed developing lake whitefish embryos to a fractionated regime of gamma radiation to determine whether radiation-stimulated growth in developing lake whitefish embryos was accompanied by a trade-off in metabolic efficiency. Developing lake whitefish embryos were exposed to fractionated doses of 662 keV high dose rate (0.3 Gy min^{-1}) gamma radiation. A total of four fractions, ranging from 14 mGy to 7.6 Gy per fraction, were delivered with a 14 day separation between dose fractions followed by constant temperature incubation at 2°C until measurement at 14 and 42 days following delivery of the final radiation exposure. A size-normalized yolk conversion efficiency was measured using unpreserved dry weights.

Our results show that the irradiated embryos were 8 to 10% larger than the controls but yolk conversion efficiency was statistically indistinguishable and likely slightly higher. This demonstrates that stimulated growth in developing lake whitefish embryos is not "paid for" by a trade-off in the efficiency of yolk conversion. Intrinsic energetic demands imposed by an increased metabolic rate points to phenological mismatch as a plausible cost of hormesis.

* corresponding author

[1]*Department of Medical Physics and Applied Radiation Sciences, McMaster University*
[2]*Bruce Power, Tiverton, ON, Canada*
[3]*Department of Biology, University of Regina*
[4]*Department of Biology, McMaster University*
[5]*Northern Ontario School of Medicine*

6.1 Introduction

Hormesis is a non-linear dose-response relationship in which stimulation occurs at low concentrations and inhibition at high concentrations. Exposure to low levels of ionizing radiation is known to trigger an adaptive response that includes immune stimulation and the up-regulation of long-lasting protective effects that limit damage to DNA and stimulate its repair (Ludwików *et al.*, 2002; Feinendegen *et al.*, 2013), or confer protection against subsequent high dose exposures (Boreham and Mitchel, 1991; Azzam et al., 1998; Broome *et al.*, 1999). The response may also include stimulated growth (Tang and Loke, 2015), which has been widely reported in plants (Breslavets, 1946) and was first recognized more than a century ago (Russell, 1915). Radiation enhanced growth has been less widely reported in animals although Mothersill *et al.* (2013) reported a higher condition factor in fathead minnows (*Pimephales promelas*) fed a diet containing 100 mBq g^{-1} of ^{226}Ra. An enhanced condition factor has been reported for chronic external exposures to gamma radiation in juvenile clams (*Mercenaria mercenaria*) and scallops (*Argopecten irradians*) exposed to 0.007 to 0.008 $mGyh^{-1}$(Baptist *et al.*, 1976), and in juvenile blue crabs (*Callinectes sapidus*) exposed to 32 $mGyh^{-1}$ (Engel, 1967). Enhanced growth has also been reported in southern toad, (*Anaxyrus terrestris*) larvae following exposure to low (0.13, 2.4, 21, and 222 mGy d^{-1}) doses of gamma radiation (Stark *et al.*, 2015).

The mechanisms underlying radiation-induced growth stimulation remain poorly understood. Calabrese (1999) has speculated that radiation may induce an overcompensation effect which

may be analogous to compensatory growth observed after fasting-refeeding experiments (Johnston *et al*., 2011). Alternate mechanisms include inactivation of inhibitory pathways (Miller and Miller, 1987) or even radiation as a direct input of energy (Dadachova and Casadevall, 2008). Calabrese and Baldwin (2003) argue that hormetic effects may represent the rule rather than the exception in nature as they have been observed in plants, bacteria, and vertebrates in response to more than 1,000 different chemical or environmental stressors (Costantini *et al.*, 2010).

Hormesis poses an interesting problem from an evolutionary perspective. It is clear that the biological response to a mild stress exposure increases fitness[1] in the presence of a repeated or sustained stress of similar character. However, the adaptive response must act to decrease fitness in the absence of such stress because the biological response mechanisms are not maintained in an upregulated state but rather require some form of sensitizing exposure. It has been suggested that sstimulation of the adaptive response mechanisms could be metabolically costly (Forbes, 2001; Zhang *et al.*, 2008), or that the hormetic response could come at a sacrifice to other physiological processes (Saul *et al.*, 2013; Costantini *et al.*, 2014). Little work has been done to identify and quantify such costs and no direct measurement of hormetic effects on metabolic efficiency has previously been attempted to the best of our knowledge.

[1] Fitness in this chapter refers to selective or Darwinian fitness which is a measure of the capacity of a variant genotype to outcompete and displace other genotypes within a population.

This paper presents a measurement of metabolic efficiency using dry weight yolk conversion efficiency (YCE) measurements on developing lake whitefish (*Coregonus clupeaformis*) embryos irradiated with high dose-rate gamma radiation. Radiation is an ideal experimental stressor for hormesis as it avoids potential confounding effects found in certain chemical stressors that are simultaneously toxic (at high concentrations) and important micro-nutrients. Hormetic effects from radiation exposure are presumed to function solely through stimulation of an organism's endogenous biological responses. Embryonic development is also an ideal life-stage for examining whole-organism effects of ionizing radiation because growth takes place under comparatively simple bioenergetic conditions where nutrition is supplied endogenously and factors such as water quality, and oxygen availability may be controlled with reasonable accuracy in a laboratory setting. The lake whitefish offers further advantages as a test organism due to its low intraclutch variability in egg size (Muir *et al.*, 2014) and lengthy development (Brooke, 1975; Patrick *et al.*, 2013; Eme *et al.*, 2015; Mueller *et al.*, 2015) which allows for the accurate targeting of specific developmental stages as well as enabling manual dechorionation and dissections to be completed at a consistent time point.

We exposed developing lake whitefish embryos to a fractionated regime of gamma radiation to determine whether radiation-stimulated growth in developing lake whitefish embryos was accompanied by a trade-off in metabolic efficiency. Because the differences in metabolic cost were expected to be small, we designed our experiment to maximize sensitivity to small changes in dry weight. Specific measures included the use of highly uniform eggs from a single

female and the timing of the irradiations during the eyed stage when natural mortality is particularly low. Dechorionated embryos were imaged live and unpreserved dry weights were used exclusively to avoid confounding effects resulting from chemical preservation.

6.2 Methods

Egg collection and Incubation

On November 26, 2014, Ontario Ministry of Natural Resources and Forestry (MNRF) provided us with whitefish eggs (mean dry weight 2.735±0.081 mg) from a single female from Lake Simcoe fertilized earlier that day by pooled milt from different males. The fertilized eggs were transported in an ice bath to McMaster University where they were reared in refrigerated coolers using apparatus and methods described in Mitz *et al.,* (2014). Following initial development for 40 days in McDonald Bell hatching jars at 5 °C, the embryos were transferred to petri dishes and held at a nominal 2°C (2.07 ± 0.004) for the experiments. Data loggers (Schlumberger Mini-Diver and Onset Hobo) were placed within the cooler units and set to record water temperature every 5 minutes. Photoperiod was ambient with natural lighting through the laboratory windows and was consistent across exposure groups.

The eggs were reared in 150 mm diameter petri dishes loaded with approximately 100 eggs per dish. The dishes were filled with dechlorinated municipal tap water, which was changed twice weekly and following the removal of any mortalities. The position of the petri dishes was shuffled regularly throughout incubation to minimize any effect on growth from slight variations in temperature in different areas of the cooler shelves. Viable hatches were recorded

and dead embryos removed daily. Embryo mortalities were recognized by the development of opacity on a significant portion of the egg or by the disintegration of the chorion and embryo.

Irradiations

Developing lake whitefish embryos were exposed to fractionated doses of 662 keV ^{137}Cs gamma radiation (0.3 Gy min^{-1}) ranging from 15 mGy to 8 Gy nominal dose per fraction. A total of four fractions were delivered per treatment beginning 41 days post-fertilization (dpf) in Development Stage 10 (Sreetharan *et al.,* 2015) at which time the eyes of embryos were fully pigmented and the major organs formed. For each exposure, the embryos (sham and exposure groups) were transported to and from the irradiation facility in petri dishes placed on a bed of crushed ice in insulated coolers. Irradiations were carried out in petri dishes placed on an ice slurry. Dose fractions were delivered at 14 day intervals followed by constant temperature incubation at 2°C for a further 14 (initial measurement of a small subsample) and 42 (the measurement of a larger sample of individual and pooled embryos) days before dechorionation and measurement. The larger subsample was measured (dry weight and embryo dimensions) at 128 dpf at which time the embryos had reached Development Stage 16 (Sreetharan *et al.,* 2015). Nominal doses were calculated based on previously generated calibration curves and verified using thermoluminescent dosimeters (Mirion Technologies, Irvine, California, United States). The dosimetry results showed a mean value (n=9) of 0.944±0.036 of the nominal dose. Doses presented in the balance of this chapter are the dosimeter-corrected values to two significant digits.

Measurements

Small subsamples of 10 (treatment groups) to 20 embryos (sham) were dechorionated and weighed individually at 97 dpf to determine the rate at which the embryos were growing and to determine the time required to complete the measurements on the unpreserved embryos. Larger (25 to 30 embryos per treatment group) samples were taken at 128 dpf and these provide most of the data presented in this paper.

Dechorionated larvae were anesthetized using an approximately 0.5% solution of MS-222 (ethyl 3-aminobenzoate methanesulfonate, Sigma Aldrich) prior to being imaged live using an Axio Zoom V16 microscope (Carl Zeiss) equipped with a Canon EOS Rebel T1i camera. Dimensional measurements of standard length (SL), post-ventral height, yolk length and width, and fin definition were made to the nearest 1 μm using Zeiss AxioVison digital image processing software. Following imaging, embryos were placed intact or deyolked on aluminum foil tares and dried to a constant weight overnight at a temperature of 70^{o}C in a VWR Brand Model 1500EM drying oven. Anhydrous silica gel was placed in the drying oven along with the larvae and places beside the aluminum tares during weighing (Mettler-Toledo XA105DU, ±0.01 mg) to maintain a low humidity. The order of weighing was random to reduce the potential for confounding effects resulting from humidity fluctuations during the weighing process. Measurements were taken using unpreserved embryos to avoid possible confounding effects resulting from chemical preservation (Smith & Walker, 2003; Melo *et al.*, 2010; Sreetharan *et al.*, 2015).

The relationship between embryo dry weight and somatic (i.e. yolk-free) dry weight was calculated using the weights of pooled embryos (25 yolked and 25 deyolked except for the 7.6 Gy dose fractions for which high mortality limited the number of embryos to 8 yolked and 10 deyolked). The dry weight of the yolk was estimated by subtracting the pooled yolk-free dry weight from the pooled total embryo weight. This relationship was compared to a time series of pooled yolked and deyolked dry weights to establish the rate of change experienced in the absence of irradiation.

Normalized YCE

The efficiency of yolk conversion was calculated by determining a nominal value defined by the yolk-free dry weight of the embryos divided by the amount of yolk consumed. This value was then normalized to correct for size differences between exposure groups which is necessary because maintenance metabolism scales with body weight, W_S , according to W_S^b where b is generally taken to be in the range of 0.75 (West *et al.*, 1999; Gillooly *et al.*, 2001). The fraction of yolk energy allocated to maintenance per unit time therefore increases with increasing values of W_S but at progressively decreasing rate for all values of b less than unity. In addition to normalizing YCE to take into account the greater somatic maintenance requirements associated with a larger body size, it is necessary to also account for the fact that larger embryos have spent less time for each incremental increase in dry weight than the sham.

Chapter 4 determined that the relationship between W_S somatic (yolk-free) dry weight and W_y (total yolk consumed) at time *t*, could be approximated using a hyperbolic relationship in the

form $W_S = \varepsilon W_y((1+aW_y)^{-1})$ (Figure 6-1) where a is experimentally determined and related to YCE and ε represents the limiting efficiency of yolk conversion as the maintenance allocation approaches zero. Size-related differences in metabolic maintenance allocation may be addressed by determining the difference in somatic dry weight (ΔW_S) between a given experimental group and the sham and calculating the additional yolk mass ΔW_y required to produce ΔW_S in the absence of any maintenance allocation (since all measurements were made at the same point in time):

$$\Delta W_y = \frac{\Delta W_s}{\varepsilon} \qquad [1]$$

Now we can normalize for the sham using:

$$\overset{n}{YCE} = \frac{W_s + \Delta W_s}{W_y + \left(\frac{\Delta W_s}{\varepsilon}\right)} \qquad [2]$$

This transformation corrects for the differences in somatic maintenance between groups and it reduces the apparent differences obtained using nominal YCE.

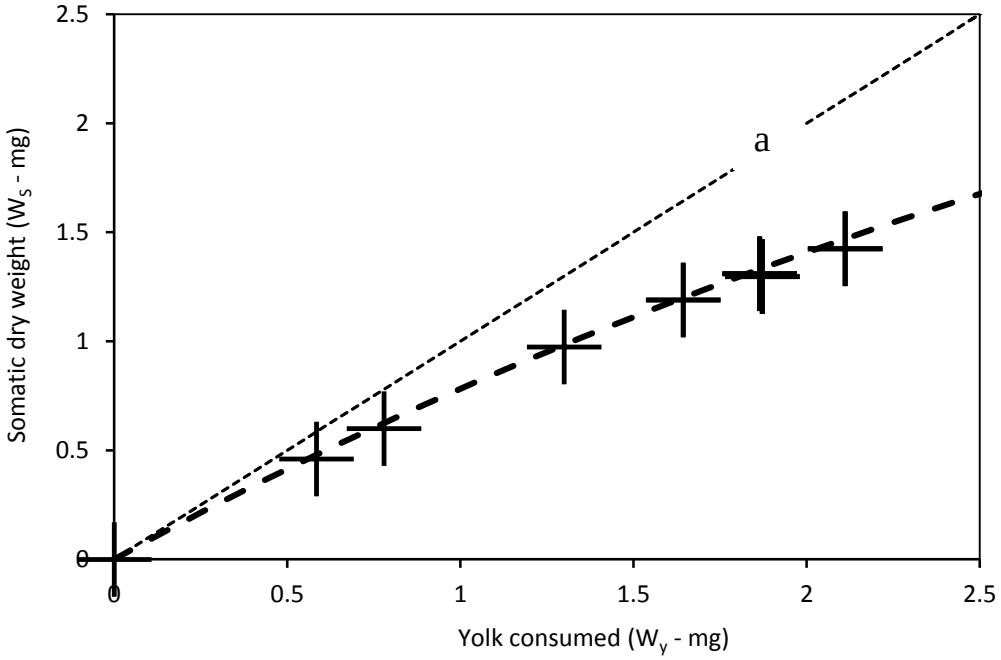


Figure 6-1: Conversion of yolk dry weight to dry weight for embryos reared at a constant 5ºC. The solid line represents 100% conversion efficiency; the dashed line is fitted to measured data and deviates from line **a** according to actual yolk conversion efficiency and the progressively greater proportion of energy devoted to somatic maintenance during later stages of embryonic development. The dashed line represents a hyperbolic regression for the relationship $W_s=\varepsilon W_y((1+aW_y)^{-1})$. Data points (+) represent pooled data for yolked and deyolked embryos at different points in development (see Chapter 4).

Statistical Analysis

Statistical analysis was conducted using Sigmaplot V11.0. Morphometric measurements and dry weights were compared using a one-way ANOVA followed by Tukey's HSD test. Differences in condition factor (dry weight normalized to SL) were compared to the sham using separate ANOVAs on SL and on condition factor. Differences were considered statistically significant at $P \leq 0.05$.

6.3 Results

The groups receiving 3.8 and 7.6 Gy dose fractions experienced high (>50%) mortality during the experiment. Mortality was uniformly low (<5%) for the sham and all other dose fractions. Embryos were not reared to the normal hatching stage but a number of the embryos receiving 3.8 and 7.6 Gy dose fractions spontaneously hatched prior to the termination of the experiment. This was in line with previous observations (unpublished) for Lake Huron whitefish showing that a single acute exposure of 6.4 Gy and higher resulted in a slightly earlier hatch compared to the sham. Neither hatch advance nor increased mortality was observed for single acute exposures of 3.7 Gy or less (data not shown).

There was a significant effect of radiation dose on the unpreserved dry weight of the embryos ($F_{9,300}$=43.1, P <0.001). Embryos receiving less than 1.9 Gy per dose fraction (i.e. less than 7.6 Gy total dose) were heavier than the sham by an average of 10.3±2.7% (Figure 6-2A). The difference in dry weight was significant for all trials except the 470 mGy dose fractions (P=0.093). Between the 14 mGy and 0.9 Gy fractions there was no significant difference in size, but a non-significant peak in the 240 mGy fractions was noted in both the early (97 dpf) and late (128 dpf) measurements (Figure 6-2). From 1.9 to 7.6 Gy per dose fraction, the embryos were progressively lighter with both groups significantly different from the sham. Growth stimulation was similar for both the 97 and 128 dpf measurements (8.5±4.0% and 10.3±2.7%).

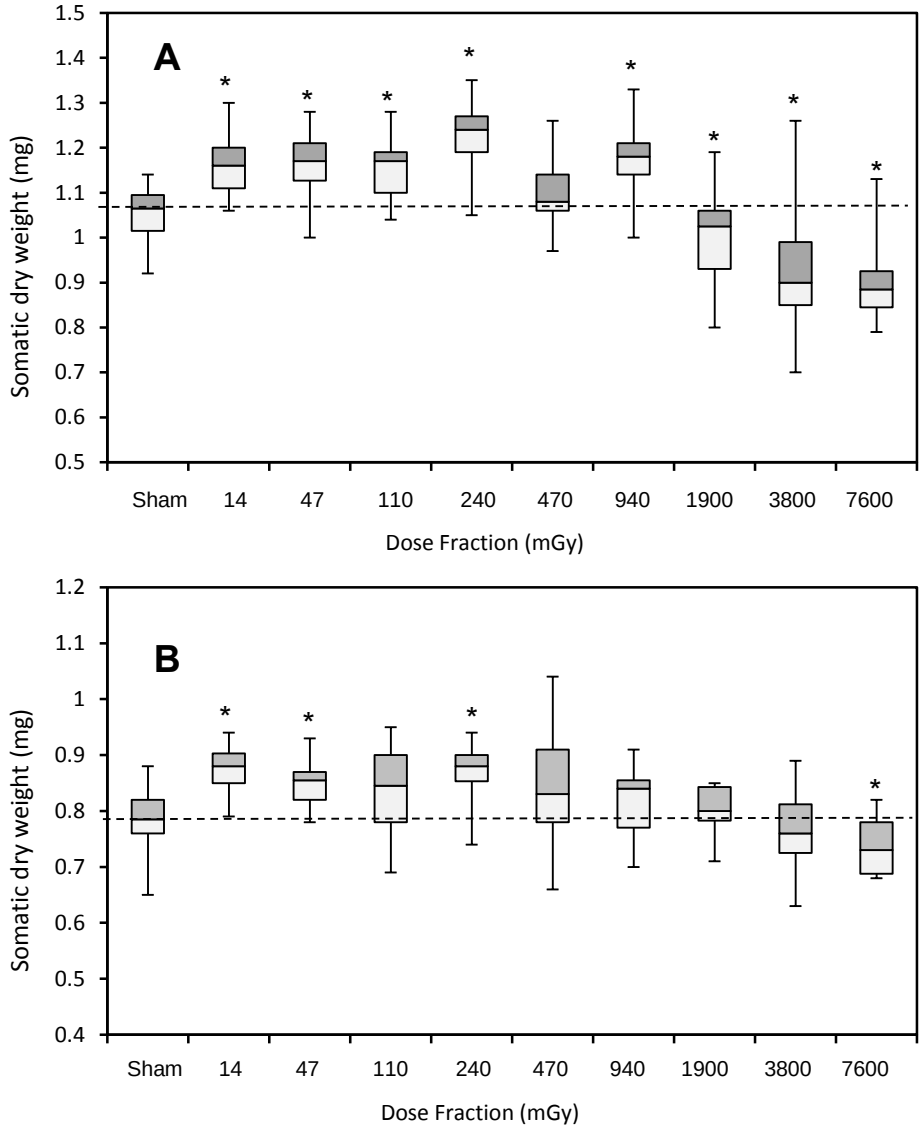


Figure 6-2: Relationship between somatic dry weight and radiation dose for whitefish embryos sampled at 128 dpf (**A**) and at 97 dpf (**B**) The absence of statistical significance at 97 dpf reflects the smaller number of embryos (n=9 to 20 per dose fraction compared to 25 to 36 for 128 dpf except for the nominal 7.6 Gy dose fractions where high mortality limited us to only 8 embryos). * = statistically significant relative to the sham.

Standard length (SL) also varied significantly with radiation exposure ($F_{9,191}$=11.1, P <0.001) although the differences were small and fewer trials were statistically different from the sham

(Figure 6-3A). The relationship between radiation exposure and total embryo weight (Figure 6-3B) showed only subtle and non-significant differences except for the 4 x 7.6 Gy group.

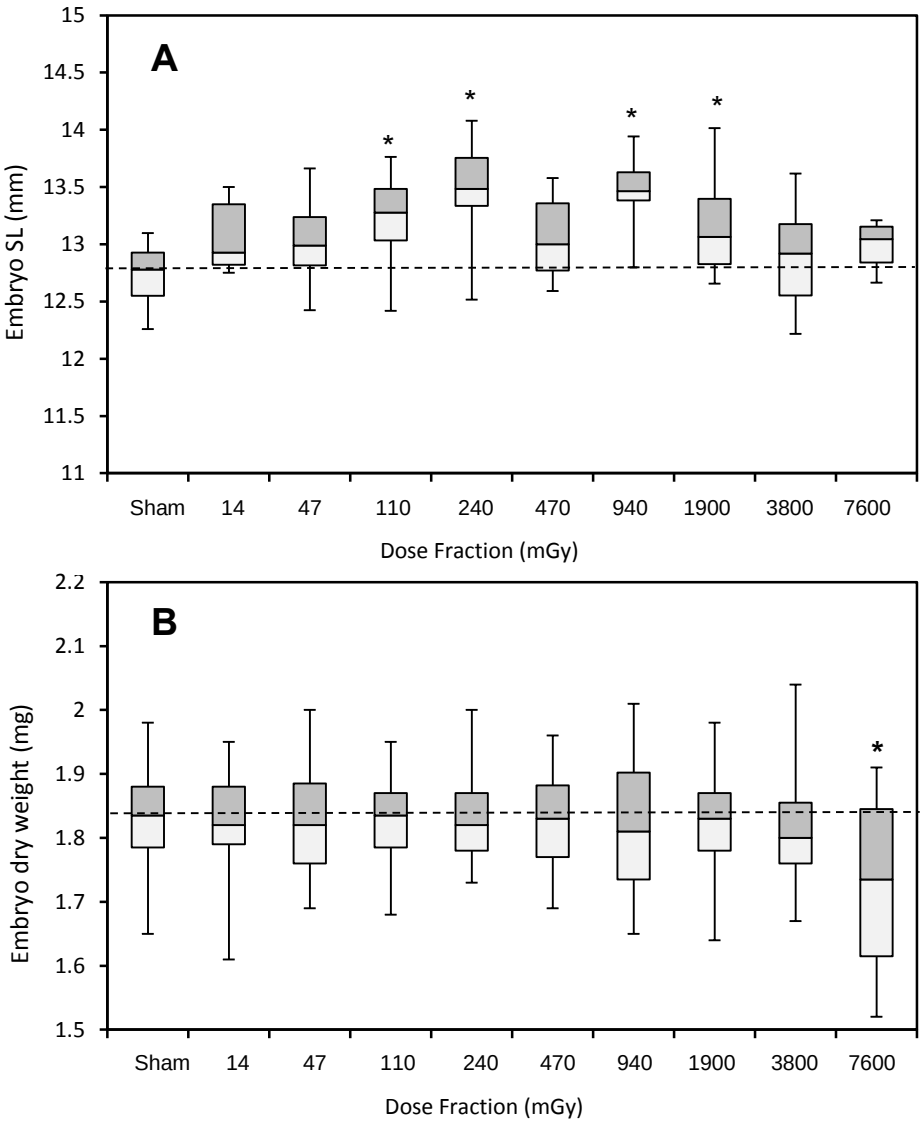


Figure 6-3: Relationship between embryo standard length (SL) and total embryo dry weight and radiation dose for whitefish embryos A: SL vs. radiation dose for whitefish embryos sampled at 128 dpf **B:** total embryo weight and radiation dose for embryos sampled at 128 dpf. * = statistically significant relative to the sham.

Figure 6-4 shows the observed differences in condition factor (dry weight normalized to SL) for the different exposure groups.

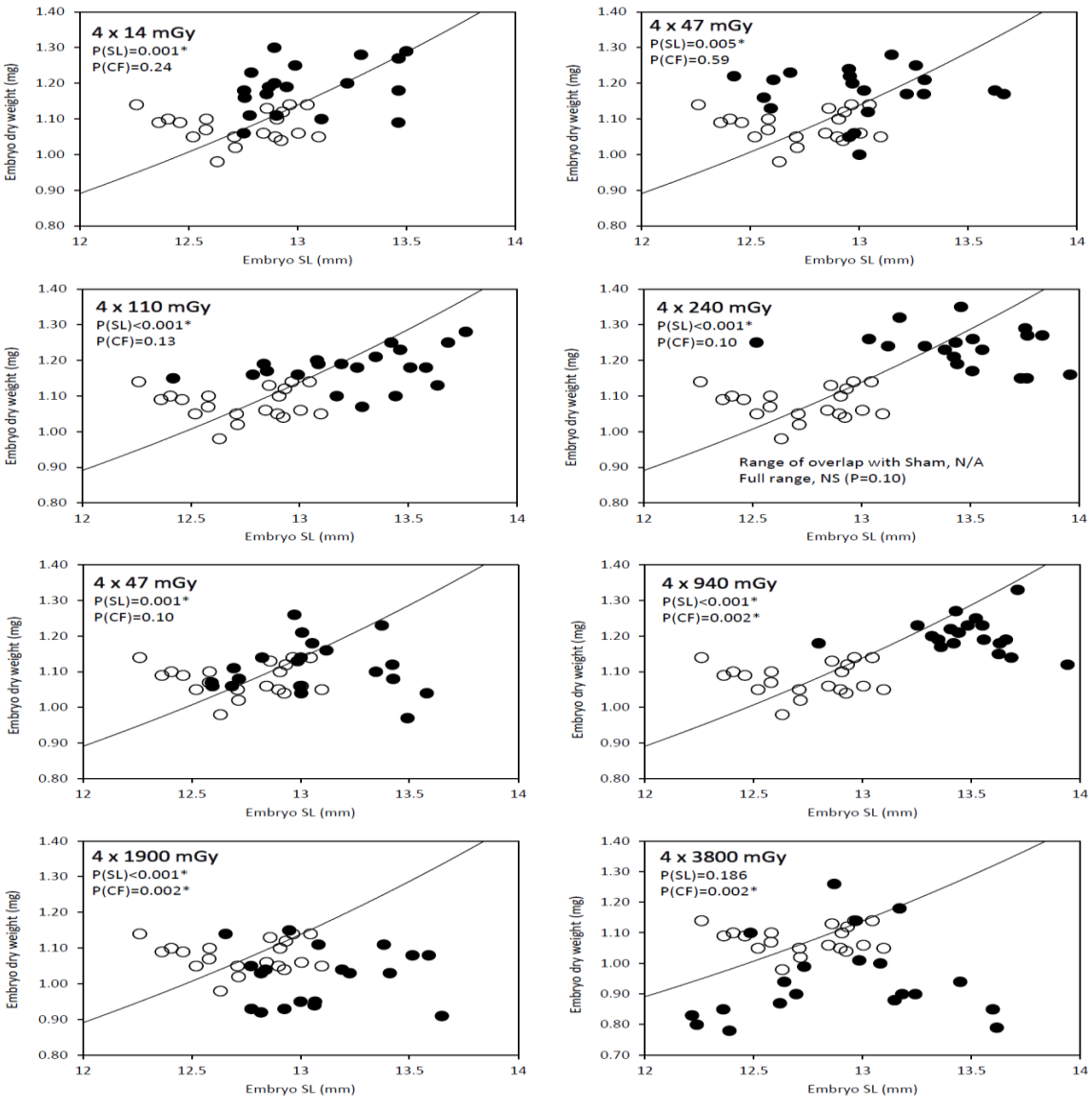


Figure 6-4: Relationship between somatic dry weight and SL for different irradiation groups (●) vs the sham (○). The solid line is a regression relationship relating SL to yolk-free dry weight (DW=0.047exp0.245SL) for 2ºC incubation based on data from Chapter 4). The first P value refers to difference in condition factor, the second to difference in SL

The 4 x 14 mGy group was significantly heavier for a given length than the sham while the difference for the 4 x 47 mGy group was near-significant. The relationship between dry weight and SL was not significantly different from the sham for the intermediate exposures (110, 240, and 470 mGy) but the 4 x 0.94 to 4 x 3.8 Gy groups were significantly lighter for a given SL. Thus, the greater dry weight achieved by the low dose fractions is partially due to the irradiated embryos being longer and partially to their higher condition factor. In the intermediate dose range (110 mGy to 0.94 Gy) the greater weight appears to result solely from increased length. Thus the results show two aspects of stimulation where the greater length occurs even at dose ranges where the condition factor is negatively affected. This association is particularly strong for the highest dose (4 x 3.8 Gy) for which we have reliable morphological measurements. For this group, SL was statistically indistinguishable from the control but the condition factor was significantly and substantially lower.

The nominal yolk conversion efficiency for the sham was calculated to be 62.3±0.52% compared to values ranging between 62.5 and 65.7% for dose fractions between 14 mGy and 0.94 Gy. With higher (i.e. > 1 Gy) dose fractions, nominal YCE declined significantly to a low of approximately 55% for the 4 x 7.6 Gy trial. Normalized YCE was more tightly constrained (Figure 6-5) with all values for dose fractions less than 1.9 Gy being higher than the sham. Within this range, the cost in metabolic efficiency (relative to the sham) ranged from -0.13% for the 4 x 0.94 Gy group to -0.90% for the 4 x 240 mGy group.

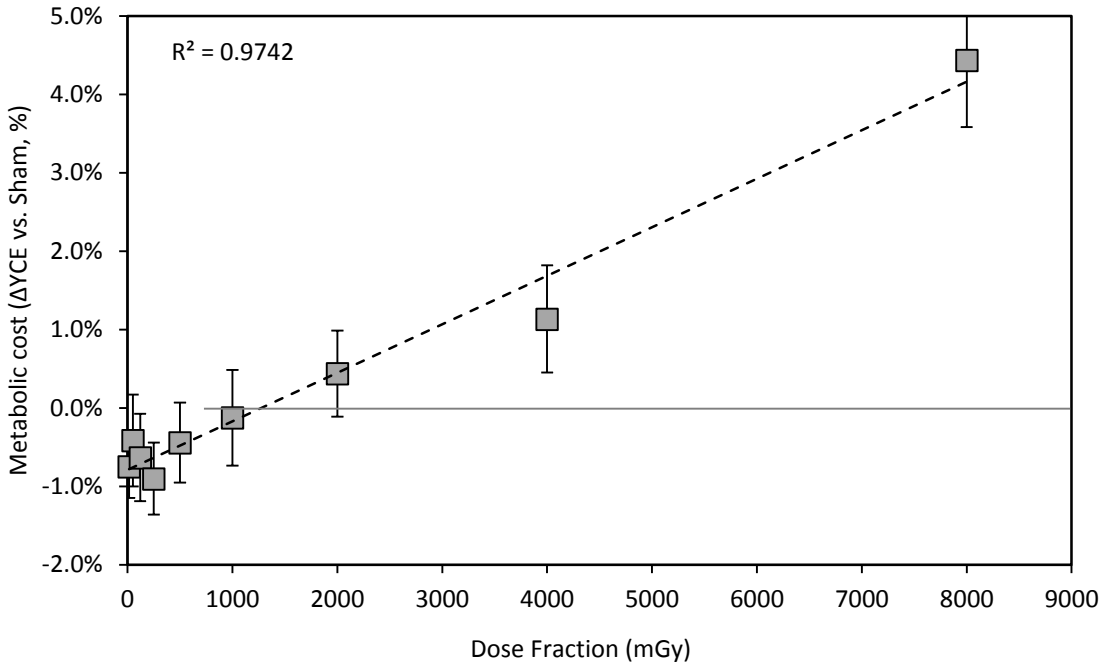


Figure 6-5: Metabolic cost for different radiation exposures normalized for differences in somatic maintenance resulting from differences in specific growth rate. The dashed line represents the best fit relationship (excluding the sham) determined by linear regression.

Figure 6-6 shows that there is an approximately linear relationship between the calculated metabolic cost and degree of growth stimulation ($F_{1,9}$=45.7, P <0.001). When the 4 x 7.6 Gy group is excluded (this dose group is clearly in the lethal range and contained numerous deformed embryos) the relationship between growth stimulation and metabolic cost is approximately linear (R^2=0.867, P <0.001) although the 4 x 0.94 Gy point is an outlier combining significant growth stimulation with a near-zero rather than negative metabolic cost. This point is affected by the anomalously low whole-embryo dry weight which is lower than the adjacent 4 x 470 mGy and 4 x 1.9 Gy groups. If the metabolic cost for the 4 x 0.94 Gy

point is recalculated using the average embryo weight for the two adjacent groups, the resulting point conforms to the linear trend. Alternatively, the 4 x 0.94 Gy group (the highest dose for which a negative metabolic cost was measured) may be affected by the reduced metabolic efficiency seen in the 4 x 1.9 Gy and higher dose groups.

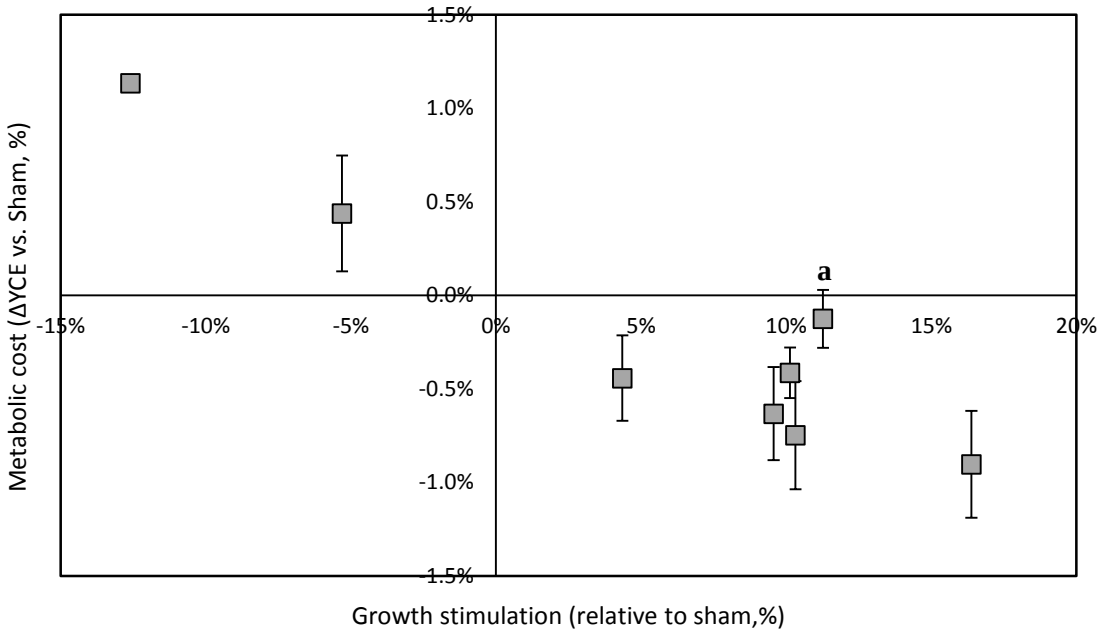


Figure 6-6: Relationship between calculated metabolic cost and growth stimulation. When the 4 x 8 Gy group is excluded the relationship between growth stimulation and metabolic cost is approximately linear suggesting that the cost may truly be slightly negative. The outlier (denoted a) is the 4 x 0.94 Gy trial which may have been affected by differential survival. Error bars denote confidence intervals (i.e. 2 x SEM).

Limitations and Uncertainty

Taken in aggregate, the observed growth stimulation was both substantial and significant. However, the 4 x 470 mGy group appears to be an outlier with SL and mean somatic weight only marginally higher than that of the sham. This might simply be the result of chance or it

might reflect the presence of some unrecognized factor affecting size in this experimental group. The early time data (i.e. the 97 dpf measurements) for this dose fraction showed growth stimulation consistent with the other exposure groups. We investigated the dry weight distribution for this group reasoning that some unrecognized pathogen would result in a size difference between replicates recognizable as from bimodality in the dry weight measurements. No such bimodality was observed in the histogram (data not shown).

The absence of a significant dose-response relationship between the different low-dose groups means that our estimation of growth stimulation is dependent on the difference between the irradiated embryos as a group and the sham. The distribution of dry weights for the sham did not meet normality criteria (Shapiro-Wilk, W=0.937, P=0.041) however the early time weights were normally distributed (P=0.408) and the substitution of median or modal dry weights for the 128 dpf sham did not affect the calculated growth stimulation.

Our calculated values for normalized YCE suggest that the low dose treatment groups (i.e. less than 0.94 Gy per fraction) converted yolk to somatic mass more efficiently than the sham (i.e. the metabolic efficiency cost is negative). Calculation of normalized YCE is sensitive to small variations in the measured total embryo weight, the confidence intervals for which are sufficiently large to shift the entire linear YCE-dose relationship (Figure 6-5) upward so that it crosses the Y axis at zero. The normalization procedure also assumes a constant value for the limiting efficiency ε. We cannot exclude the possibility of small variations in the value for ε between the sham and irradiated groups although sensitivity testing shows that small

variations in ε do not significantly affect the results. The relationship between growth stimulation/retardation and calculated metabolic cost (Figure 6-6) follows a generally linear trend. While the metabolic cost of the stimulated growth is sensitive to variations in the sham, the near-linear relationship between growth stimulation and metabolic cost appears to be robust, persisting when we substituted median and modal values for somatic and whole embryo dry weights in place of the mean. The slope to the growth-cost relationship (which must pass through the origin by definition) gives us confidence that the negative metabolic cost for stimulated growth is indeed negative although perhaps only marginally so.

6.4 Discussion

Life has coexisted with radiation and other environmental stressors for more than 3 billion years, over which time various biochemical mechanisms have evolved to protect organisms from damage. Conservation of biochemical components of the adaptive response (e.g. heat shock proteins) through deep geological time (Wang *et al.*, 2004) provides an independent argument that their function increases fitness in the presence of an environmental stress. However, the need for a sensitizing or priming exposure to trigger their expression above baseline constitutive levels means that their upregulation must decrease fitness in the absence of stress at least when measured across the reproductive lifespan of an organism. This conclusion does not depend on the assumption that hormetic effects are intrinsically beneficial in the absence of stress. In uncertain environments, a response that provided an organism with,

for example, heat shock resistance would increase fitness over time even if that resistance conferred no advantage other than protection against a possible future event.

Our experimental results demonstrate that radiation-stimulated growth in developing lake whitefish embryos is not "paid for" by a trade-off in the efficiency of yolk conversion. Growth stimulation averaging about 10% was accompanied by a slightly improved YCE. Thus, the cost in terms of metabolic efficiency appears to be slightly negative. The growth stimulation we observed is also similar in magnitude to that reported in a parallel study (Thome *et al.*, submitted) using chronic irradiation with a different whitefish population raised in a different laboratory. While we saw no evidence for a trade-off between stimulated growth and metabolic efficiency, the stimulated growth itself represents a substantial investment of energy that might decrease fitness if the energy invested in growth is diverted from other physiological processes or if it results in an embryo hatching with insufficient yolk reserves to sustain itself during the transition to exogenous feeding. This later possibility is an example of phenological mismatch (Cushing, 1969; Genner *et al.*, 2009) which has been identified as a specific concern for lake whitefish (Patrick *et al.*, 2013).

Under natural conditions lake whitefish spawn in the fall when water temperatures drop below about 10^{o}C (Hart, 1931, Scott and Crossman, 1973). Hatching takes place in spring, typically in April or May, and coincides with the spring break-up of ice cover. Growth stimulation would result in an embryo with lower yolk reserves and greater somatic maintenance demands than its unaffected siblings. Dostatni *et al.* (1999) reported that the caloric quantity of zooplankton

(the main food source for whitefish hatchlings) increased approximately 4-fold in the first three weeks following ice out with a peak in early May approximately 20-fold higher. Embryos hatching asynchronously to this natural increase in food supply may therefore experience low survival. Under typical incubation temperatures of between 0.5 and 1.5^{o}C, growth stimulation in the range of the 10% we observed would be equivalent to hatching several weeks early - not an insignificant difference given the vulnerability of lake whitefish hatchlings to starvation (Taylor and Freeberg, 1984; Reshetnikov and Bogdanov 2011; Meyer *et al.*, 2012).

While a phenological mismatch between yolk reserves and seasonal changes in prey availability adequately accounts for an evolutionarily-relevant cost specific to developing whitefish embryos, it is harder to imagine it as a generalized cost across different taxa. More plausible is energetic mismatch resulting from a sustained increase in metabolic rate. Other alternatives include trade-offs in future growth (Cedergreen, 2008), reproductive fitness (Saul *et al.*, 2013) and immune function (McClure *et al.*, 2014). The ubiquity of hormesis implies a corresponding universality underlying its cost. If not, an organism could realize an evolutionary advantage by the selective upregulation of different components of the adaptive response without the need for prior hormetic priming. It is intriguing to speculate that the seemingly different aspects of hormesis as well as apparently distinct offsetting costs might all derive from a common underlying mechanism.

Our results are limited to a single stressor experienced by a single species at a single life-stage, but the absence of any direct trade-off in metabolic efficiency points to the energetic costs of

stimulated growth as a more possible candidate for a general cost. In the absence of a trade-off in metabolic efficiency, stimulated growth must be associated with an increased metabolic rate. This increase, if sustained, would increase future energetic demands thereby increasing the vulnerability to starvation. Over the time scales on which natural selection operates, even small increases in vulnerability to starvation would be expected to significantly reduce fitness. This suggests that stimulated growth is not a hormetic benefit but an energy demand capable of altering naturally evolved growth trajectories, or requiring the diversion of resources from other physiological processes leading to a longer term decrease in fitness. It would also imply the persistence of effects beyond the duration of transient availability of excess food under natural conditions.

The past decades have produced an extensive body of research surrounding the validity of the linear no threshold model for radiation-induced stochastic effects. Numerous researchers have argued the roughly linear dose response seen at high doses cannot be extrapolated into the low dose range owing to the suite of biological response mechanisms that make up the adaptive response. While the experimental evidence unequivocally demonstrates the existence of beneficial hormetic effects invalidating the linear no threshold dose-response model, natural selection guarantees the existence of an offsetting cost and any risk model incorporating hormetic effects is necessarily incomplete without its inclusion. An understanding of the form and character of such cost is essential to the development of scientifically valid dose-response models within the low dose range.

6.5 Acknowledgements

The authors wish to thank Lisa Stoa, Emily Hulley, and Shayen Sreetharan for assistance with the experimental work and the Ontario Ministry of Natural Resources and Forestry who made our experiment possible by providing us whitefish embryos. We are grateful to Bruce Power for its support of this work, both financially and technically, and for funding through a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development Grant.

6.6 References

Azzam E, S. de Toledo, T. Gooding, and J. B. Little, 1998. Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. *Radiation Research*, 1998 150 (5): 497-504.

Baptist, J. P., D. A. Wolfe, and D. R. Colby, 1976. Effects of chronic gamma radiation on the growth and survival of juvenile clams (*Mercenaria Mercenaria*) and scallops (*Argopecten Irradians*); *Health Physics* 30.1 (1976): 79-83.

Boreham D.R. and R.E.J Mitchel, 1991. DNA lesions that signal the induction of radioresistance and DNA repair in yeast. *Radiation Research* 128:19-28

Breslavets, L.B., 1946. Plants and X-rays. Moscow; Acad. Sci. USSR Press, (Translation *American Institute of Biological Sciences* 1960)

Broome EJ, D. L. Brown and R.E.J. Mitchel, 1999. Dose responses for adaption to low doses of 60 Co γ rays and 3 H β particles in normal human fibroblasts. *Radiation Research,* 158(2), pp.181-186.

Calabrese, E. J., 1999. Evidence that hormesis represents an "overcompensation" response to a disruption in homeostasis; *Ecotoxicology and Environmental Safety* 42.2: 135-137.

Calabrese E.J. and L. A. Baldwin, 2003. Toxicology rethinks its central belief; *Nature* 421:691–692.

Cedergreen, N., 2008. Is the growth stimulation by low doses of glyphosate sustained over time?; *Environmental Pollution* 156.3 (2008): 1099-1104.

Colby, P.. J., and L. T. Brooke, 1973. Effects of temperature on embryonic development of Lake Herring (*Coregonus artedii*); *Journal of the Fisheries Research Board of Canada*; 30.6: 799-810.

Costantini, D., N. B. Metcalfe and P. Monaghan, 2010. Ecological processes in a hormetic framework; *Ecology Letters* 13, 1435-1447.

Costantini, D., P. Monaghan, and N. B. Metcalfe, 2014. Prior hormetic priming is costly under environmental mismatch; *Biology Letters* 10.2

Cushing, D. H., 1969. The regularity of the spawning season of some fishes; *Journal du Conseil pour l'Exploration de la Mer* 33:81–92.

Dadachova, E., and A. Casadevall, 2008. Ionizing radiation: How fungi cope, adapt, and exploit with the help of melanin; *Current Opinion in Microbiology* 11.6 (2008): 525-531.

Dostatni, D., A. Mamcarz, J., and P. Poczyczyński, 1999. Feeding of early- and late-hatched whitefish in illuminated cages; *Archives of Polish Fisheries*, Vol. 7, Fasc. 2, 281-295.

Eme, J., C.A. Mueller, R.G. Manzon, C.M. Somers, D.R. Boreham, and J.Y. Wilson, 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings. Comparative Biochemistry and Physiology Part A; *Molecular & Integrative Physiology*. 179, 71-80.

Engel, D.W., 1967. Effect of single and continuous exposures of gamma radiation on the survival and growth of the Blue Crab, *Callinectes sapidus*. *Radiation Research*: December 1967, Vol. 32, No. 4, 685-691.

Feinendegen, L. E., M. Pollycove, and R. D. Neumann, 2013. Hormesis by low dose radiation effects: Low-dose cancer risk modeling must recognize up-regulation of protection; *Therapeutic Nuclear Medicine* Springer 2013 ISBN 978-3-540-36718-5

Forbes, V. E., 2000. Is hormesis an evolutionary expectation?; *Functional Ecology* 14.1 (2000): 12-24.

Genner, M. J., N. C. Halliday, S. D. Simpson, A. J. Southward, S. J. Hawkins, and D. W. Sims, 2009. Temperature-driven phenological changes within a marine larval fish assemblage. *Journal of Plankton Research* 32.5 (2009): 699-708.

Hart, J.L. 1931. The spawning and early life history of the whitefish, Coregonus clupeaformis (Mitchill), in the Bay of Quinte, Ontario; *Contributions to Canadian Biology and Fisheries*. 6 (1), 165-214.

Johnston, I. A., N. I. Bower, and D. J. Macqueen, 2011. Growth and the regulation of myotomal muscle mass in Teleost fish; *Journal of Experimental Biology* 214.10 (2011): 1617-1628.

Ludwików G., X. Yun, R. A. Hoebe, N. A. P. Franken, F. Darroudi, J. Stap, C. H. van Oven, C. J. F. van Noorden and J. A. Aten, 2002. Induction of chromosome aberrations in unirradiated chromatin after partial irradiation of a cell nucleus. *International Journal of Radiation Biology*; 78, 239-247.

McClure, C.D.; W. Zhong, V. L. Hunt, F. M. Chapman, F.V. Hill and N. K. Priest, 2014. Hormesis results in trade-offs with immunity. *Evolution* 68, 2225–2233.

Meyer, S., E. Caldarone, M. Chícharo, C. Clemmesen, A. Faria, C. Faulk, A. Folkvord, G. Holt, H. Høie, P. Kanstinger, A. Malzahn, D. Moran, C. Petereit, J. Støttrup and M. Peck, 2012. On the edge of death: Rates of decline and lower thresholds of biochemical condition in food-deprived fish larvae and juveniles; *Journal of Marine Systems* 93 (2012): 11-24.

Miller M.W and W.M. Miller, 1987. Radiation hormesis in plants; *Health Physics*, 52: 607-616.

Mitz, C., C. Thome, M.E. Cybulski, L. Laframboise, C.M. Somers, R.G.Manzon, J.Y. Wilson and D.R. Boreham, 2014. A self-contained, controlled hatchery system for rearing lake whitefish embryos for experimental aquaculture; *North American Journal of Aquaculture.* 76 (3), 179-184.

Mothersill, C., R., Smith, D. Lariviere, and C. Seymour, 2013. Chronic exposure by ingestion of environmentally relevant doses of 226 Ra leads to transient growth perturbations in Fathead Minnow (*Pimephales Promelas*, Rafinesque, 1820); *International Journal of Radiation Biology* 89.11 (2013): 950-964.

Mueller, C.A., J. Eme, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype and cost of development in lake whitefish (*Coregonus clupeaformis*); *Journal of Comparative Physiology*. B, Biochemical, Systemic, and Environmental Physiology. 185 (3), 315-331.

Muir, A. M., M. T. Arts, M. A. Koops, T. B. Johnson, C. C. Krueger, T. M. Sutton and J. Rosenfeld, 2014. Reproductive life-history strategies in lake whitefish (*Coregonus clupeaformis*) from the Laurentian Great Lakes; *Canadian Journal of Fisheries and Aquatic Sciences* (2014): 1256-1269.

Patrick, P.H., E. Chen, J. Parks, J. Powell, J. S. Poulton and C-L Fietsch, 2013. Effects of fixed and fluctuating temperature on hatch of Round Whitefish and Lake Whitefish eggs; *North American Journal of Fisheries Management* 33:1091-1099.

Russell, E. J., 1915. The effect of radium on the growth of plants; *Nature* 96, 147-148

Saul N, K. Pietsch, S.R. Stürzenbaum, R. Menzel and C.E. Steinberg, 2013. Hormesis and longevity with tannins: free of charge or cost-intensive? *Chemosphere* 93:1005-1008.

Sax, K., 1963. The stimulation of plant growth by ionizing radiation; *Radiation Botany* Volume 3, Issue 3, 1963, 179–186.

Scott, W.B. and E.J. Crossman, 1973. Freshwater fishes of Canada; Fish Research Board of Canada Bulletin 184.

Sibley R.M. and P. Calow, 1989: A life cycle theory of responses to stress. Calow, P. And R. J. Berry (ed.). *Evolution, Ecology and Environmental Stress*; Symposium, London, England, Uk, June. Iii 187p. Academic Press Ltd: London, England, UK; San Diego, California, USA. Paper. 101-116.

Simon O, S. Massarin, F. Coppin T.G. Hinton and R. Gilbin, 2011. Investigating the embryo/larval toxic and genotoxic effects of γ irradiation on zebrafish eggs; *Journal of Environmental Radioactivity* 102:1039-1044.

Sreetharan, S., C. Thome, C. Mitz, J. Eme, C.A. Mueller, E.N. Hulley, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2014. Embryonic development of lake whitefish (*Coregonus clupeaformis*): a staging series, analysis of growth and impacts of fixation; *Journal of Fish Biology*.

Stark K, D.E. Scott, O. Tsyusko, D.P. Coughlin and T.G. Hinton, 2015. Multi-level effects of low dose rate ionizing radiation on Southern Toad, *Anaxyrus* [Bufo] t*errestris*. *PLoS One* 10:e0125327.

Tang, F. R., and W. K. Loke, 2015. Molecular Mechanisms of low dose ionizing radiation-induced hormesis, adaptive responses, radioresistance, bystander effects, and genomic instability; *International Journal of Radiation Biology* 91.1: 13-27

Taylor, W. W., and M. H. Freeberg. 1984. Effect of food abundance on larva1 Lake whitefish, *Coregonus clupeaformis* growth and survival; *Fish Biology*. 25: 733-741.

Thome, C., C. Mitz, C.M. Somers, R.G. Manzon, J.Y. Wilson, and D.R. Boreham, 2016. Incubation of Lake Whitefish (*Coregonus clupeaformis*) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development: *Canadian Journal of Fisheries and Aquatic Sciences, http://dx.doi.org/10.1139/cjfas-2015-0286.*

Van den Berghe, E. P., and M. Sofia Vala I., L.R. Martins, N. Imaizumi, R.J. Nunes, J. Rino, and F. Kuonen, 2010. Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis; *PLoS ONE* 5(6): e11222.

Wang, W., B. Vinocur, O. Shoseyov and A. Altman, 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response; *Trends in Plant Science* 9.5 (2004): 244-252.

Zhang, Q., J. Pi, C. G. Woods, A. M. Jarabek, H. J. Clewell, and M. E. Andersen, 2008. Hormesis and adaptive cellular control systems; *Dose-Response* 6.2 (2008): 196-208.

Chapter 7

Conclusion

This study set out to determine whether radiation-stimulated growth (growth hormesis) in the lake whitefish (*Coregonus clupeaformis*) is accompanied by an energetic trade-off. Such a trade-off could take the form of an embryo converting yolk nutrients less efficiently, or through phenological mismatch resulting from accelerated development leading to embryos hatching asynchronously with spring zooplankton bloom which constitutes their principal food source. Alternatively, the cost could include a sustained increase in metabolic rate that would impose greater energetic demands and consequently increase the vulnerability of a hatchling to starvation (also a form of phenological mismatch).

The ability to evaluate the energetic costs of hormesis required the development of methods to measure the efficiency of yolk conversion to a degree of precision unattainable using conventional methods. It also required an improved understanding of the thermal kinetics of growth and factors governing the timing of hatching under real world conditions. For this reason, much of the thesis is devoted to methods development including:

- derivation of an exponentially-weighted mean that allows a time series of variable temperatures to be converted to a constant temperature that mimics the growth capacity of the variable regime;
- a method to normalize calculated yolk conversion efficiency across development stages and between controls and groups experiencing stimulated growth; and
- the development of a model that allows hatch timing to be predicted with significantly greater accuracy than existing models.

The following subsections briefly summarize the more important findings of this thesis along with their implications and applications. Section 7.1 describes the development of rearing methods and apparatus while 7.2 is a theoretical treatment of growth under varying thermal regimes. The thermal kinetics of embryonic growth in the lake whitefish are discussed in 7.3 (heterograde hatching) and 7.4 (a predictive model describing the timing of hatching under variable thermal regimes). Section 7.5 summarizes the fundamental question of the cost of hormesis in the context of energetic constraints and using methods developed in the earlier chapters. Finally, avenues for future research are discussed in Section 7.6.

7.1 The Starting Point – Hatchery System and Methods

When we began work on this project, lake whitefish had previously only been raised in large scale apparatus using continuous water flow (Brooke, 1975; Griffiths, 1979) or aeration (Price, 1940). This type of rearing apparatus would have precluded the number of replicates that the research required and was poorly suited for experiments (by others) involving chemical effects necessitating segregated water supplies. McMaster's Nuclear Research Building also lacked aquatic laboratory facilities which posed a problem for irradiation of embryos if they had to be transported from a distant laboratory building to the radiation source. To overcome these limitations, a number of small-scale recirculating temperature-controlled rearing units were designed and constructed. The rearing units were essentially small scale hatcheries constructed within 2-door chromatography refrigerators. The units incorporated both upwelling hatching jars on a recirculated filtered water supply, and shelf space for rearing embryos in petri dishes

(Mitz *et al.*, 2014). Over the past five years, more than half a million lake whitefish embryos have been raised using this equipment by eight different researchers including more than 1,000 separate experimental groups/replicates. Use of this or similar small-scale systems could prove useful to other researchers or for restoration where specific genetic strains are required to be raised under segregated conditions with separate water supplies.

7.2 Varying temperatures – a quantitative baseline

It was recognized as early as the 1930's that non-linearity in the thermal scaling of growth resulted in variable temperature growth rates that differed from those predicted using a simple mean temperature (Kauffman, 1932). More recently, the work of Jensen (1906) was rediscovered (Ruel and Ayres, 1999) as the mathematical basis of the Kauffman effect while work by Sharpe and DeMichele (1977) and others improved on the foundations of Arrhenius and Eyring by integrating enzyme reaction kinetics. However, despite our greatly improved understanding of biological thermodynamics, growth rates under varying thermal regimes continue to defy accurate prediction. It is noteworthy that more than a century after Arrhenius, the prediction of varying temperature growth continues to be crudely approximated through the use of thermal sums. This does not derive from ignorance but from the fact that thermal sums (despite their limitations) often perform as well as or better than mechanistic models (Neuheimer and Taggart, 2007).

In order to separate potentially confounding effects from those derived from the non-linear scaling of growth to temperature (i.e. Jensen's inequality), an exponentially-weighted mean

was developed as described in Chapter 3. This measurement allows a variable thermal regime to be transformed to an effective temperature yielding a theoretically equivalent biological growth capacity. While the exponential relationship of growth to temperature is limited to a portion of a larger thermal performance curve described by the Sharpe-Schoolfield equation[1], within this portion the solution is mechanistic and provides an exact solution for variable temperature growth provided that certain bounding assumptions are met. These are:

1) growth to a consistent developmental end point (e.g. hatching) under varying temperatures may be described as the incremental sum of a continuous function of time-at-temperature T;

2) temperature dependence of growth follows an exponential relationship over the range of temperatures considered; and

3) the scaling of temperature dependent growth is the same across the development period considered regardless of absolute changes due to size-dependent effects.

The exponential mean yielded good results for constant and fluctuating thermal regimes but large errors when applied to thermal regimes that varied asymmetrically. This phenomenon has not been widely reported as most published work on the lake whitefish used constant or fluctuating thermal regimes. However, observations consistent with my results (i.e. anomalously delayed hatching following a temperature decrease) have been reported (John and

[1]For many species the exponential increase in growth rate with temperature has an upper bound beyond which growth rates begin to decline with further increases in temperature. The thermal tolerance range of lake whitefish embryos, however, appears to reside entirely within the exponential domain.

Hasler, 1956; Luczynski, 1984; Reshetnikov and Bogdanov, 2011). Interestingly, Vangansbeke *et al.*, (2015) used an analogous approach (incremental growth expectations as a quantitative baseline instead of deviations in effective temperature) to conclude that Jensen's inequality was insufficient to fully account for differences in variable temperature developmental rates for two species of predatory mites (*Phytoseiulus persimilis* and *Neoseiulus californicus*).

The recognition that Jensen's inequality was insufficient to account for hatch timing under asymmetrically variable thermal regimes forced a reassessment of the validity of using hatching as a consistent developmental end point. Suspicion that hatching stage might vary as a function of incubation temperature was reinforced by the widely reported temperature dependence of hatchling size (Brooke, 1975; Griffiths, 1979; Eme *et al.*, 2015, and Mueller *et al.*, 2015). The existence of a thermal dependence of size-at-hatch (for which I have proposed the term heterograde in Chapter 4) could account for the temperature-size rule in whitefish hatchlings and explain why hatching occurred earlier than predicted when incubation temperatures progressively rose and later than predicted when the temperatures progressively declined.

7.3 Thermal Dependence of Hatching Stage

Testing of the heterograde hypothesis was hindered by the lack of readily measurable morphological indications of development stage close to hatching. As a result, testing relied primarily on the scaling of time-to-hatch following discrete shifts in temperature with the use

of fin indentation as an additional independent measure. The scaling of time-to-hatch following discrete temperature shifts (Chapter 4) demonstrated that the development stage at hatching varied as a function of temperature. Fin definition measurements supported this finding as embryos incubated under cool temperatures hatched with larger and more deeply indented fins than those reared at warmer temperatures. While others (e.g. Jordaan *et al.*, 2006) have discovered a thermal dependence of hatching stage in other species, this thesis is the first to provide a mathematical basis to quantify it and to detect its presence in the absence of visible morphological indications of development stage.

Testing of the heterograde hypothesis also required the development of a method to normalize calculated yolk conversion efficiency across different development stages. Most published measurements of yolk conversion efficiency ignore the potential confounding effects from embryos hatching at different development stages. The normalization procedures described in Chapters 4 and 6 provide a basis for comparison of YCE across different development stages.

Heterograde hatching is suggested as a mechanism to synchronize the timing of hatching to the break-up of ice cover despite spawning over a range of dates. Heterograde hatching acts in a passive manner as the temperature increase associated with spring break-up effectively results in an instantaneous increase in relative development. While the resulting thermally-induced mass hatching mimics an active recognition and response to the temperature change, it is simply a consequence of the non-plastic scaling relationship between temperature and

hatching stage. This linkage between a non-plastic scaling relationship[2] and plasticity in the timing of physiological transitions/processes is common in nature with the most obvious example being the thermal scaling of growth or reaction rates. Confusion in the literature (see Kingsolver *et al.*, 2004; Schulte *et al.*, 2011) regarding plastic vs. non-plastic phenomena may have contributed to researchers attributing evidence of a heterograde relationship between temperature and hatching stage to plasticity.

Considerable commentary has been written surrounding the hypothesis that there is a universal thermal dependence of biological growth and metabolic rates (e.g. West *et al.*, 1999; Gillooly *et al.*, 2001; Brown *et al.*, 2004). The related concept of thermal time (Charnov and Gillooly, 2003) where temperature and time are interrelated and growth at a cool temperature can be thought of as the equivalent of slowing time. While both concepts have been criticized (Clarke, 2004; Clarke and Fraser, 2004; O'Connor *et al.*, 2007), it is interesting that the anomalously high Q_{10} for hatching in lake whitefish (Q_{10}>5) appears to be an artefact of heterograde hatching. When we account for the effect of heterograde hatching, the thermal scaling of development in the lake whitefish now falls within the typical range (Q_{10}=2 to 3) for ectotherms (Gillooly *et al.*, 2001 & 2002)

The prevalence of heterograde hatching in nature is currently unknown as it has been recognized in only a handful of species such as the Atlantic cod (Jordaan, 2002; Jordaan *et al.*,

[2]Scaling relationships of this type should properly be referred to as thermal performance curves rather than reaction norms which would describe a change in a scaling relationship across environments (or over time).

2006). However, the temperature dependence of hatchling size in other Coregonids (Colby and Brooke, 1973; Luczynski and Kirklewska, 1984;) suggests that it is more widespread than currently recognized. It also possible that heterograde hatching exists in non-coregonine salmonids as Beacham and Murray (1989) report results for sockeye salmon (*Oncorhynchus nerka*) and chinook salmon (*Oncorhynchus tshawytscha*) that are consistent with heterograde hatching and Dwyer (1987) describes an anomalously long delay in hatching after a shift from 8 to 0.9 ^{o}C in developing lake trout (*Salvelinus namaycush*) embryos. Synchronized hatching in the white sucker (*Catostomus commersoni*) reported by Hamel *et al*. (1997) is also consistent with heterograde hatching. It is possible that instances of this phenomena have previously gone unrecognized owing to the paucity of reliable morphometric stage-indicators late in development, or attributed to phenotypic plasticity for the two phenomena are not mutually exclusive.

7.4 Hatch Timing under Varying Thermal Regimes

The confirmation of heterograde hatching in the lake whitefish provided the basis for the development of a predictive hatch timing model that extends the incremental exponential approach of Thome *et al.* (2016) by explicitly incorporating a thermal dependence of development that differs from the thermal dependence of hatching. Chapter 5 presents this model which consists of a continuous monotonic component describing development stage, and a discontinuous non-monotonic component describing the difference in stage at hatching. Comparison to experimental observations and the published literature (e.g. Berlin *et al.*, 1977;

Patrick *et al.*, 2013 and Mueller *et al.*, 2015) shows that the proposed model provides improved predictive accuracy compared to conventional time-domain models. For the ecologically relevant asymmetrically varying regimes, existing models generate prediction errors three times greater than the new model which is able to predict the timing of hatching to within 5% of observed timing consistently. More broadly, a review of the literature reveals a range of models that could be used as the basis for a variable temperature incremental model including Elliott and Hurley (1997); Sharpe-Schoolfield (Sharpe and DeMichele, 1977); Logan (Logan *et al.*, 1976), Briere (Briere *et al.*, 1999) and others. While these models differ in their mathematical form, they are all continuous functions and therefore incapable of reproducing the thermal dynamics that derive from heterograde hatching. The hatch timing model presented in this thesis might form the basis of a predictive model for any species for which hatching is heterograde.

As the prediction of development time under varying temperatures is a problem that has remained unsolved for more than a century, this is a meaningful accomplishment and one that provides an important tool for researchers studying phenological mismatch. In principal, the model could be extended to predict size and yolk reserves at hatch and therefore offer a still more accurate means to evaluate the effect of climate change and cooling water discharge on whitefish recruitment. Such an extension would require a function describing stage-wise development in terms of dry weight and the incorporation of a thermal dependence of YCE.

Model improvements might also be accomplished by accounting for subtle changes in the thermal scaling of development.

7.5 Cost of Hormesis

The fact that the adaptive response is not maintained in an upregulated state is an indication that the associated hormetic benefits are not cost-free but are associated with tradeoffs that decrease fitness under normal environmental conditions. The near-universality of the adaptive response suggests a similar universality in its offsetting cost. If there is no trade-off between radiation-stimulated growth and intrinsic metabolic efficiency in lake whitefish embryos (as demonstrated in Chapter 6) then trade-offs in metabolic efficiency are unlikely in other organisms. We must therefore look elsewhere to find a candidate for an evolutionarily relevant cost.

While the observed radiation-stimulated growth was not accompanied by a trade-off in metabolic efficiency, the magnitude of growth stimulation (10%) represents a significant energetic cost in absolute terms. This absolute cost could result in phenological mismatch by accelerating the depletion of yolk reserves before the spring zooplankton bloom that accompanies spring break-up (temporal mismatch), or more subtly through a sustained increase in metabolic rate (energetic mismatch). The former possibility adequately accounts for an evolutionarily-relevant cost specific to developing whitefish embryos but it is associated with specific environmental conditions and is therefore a weak candidate for a generalized trade-off. Energetic mismatch would seem to be a more plausible candidate for a generalized

cost. The absence of a trade-off in metabolic efficiency means that the stimulated growth described in Chapter 6 must be associated with an increased metabolic rate. This increase, if sustained, would increase future energetic demands when resources might be scarce thereby increasing the vulnerability to starvation. Over the time scales on which natural selection operates, even small increases in vulnerability to starvation would be expected to significantly reduce fitness. An increased metabolic rate in times of food scarcity could also lead to a non-adaptive allocation of limited resources amongst different physiological processes as postulated by Saul *et al.* (2013) and Costantini *et al.* (2014). Therefore the energetic mismatch and allocation hypotheses should be seen as complementary rather than alternative explanations.

The cost of hormesis is a question of considerable practical relevance. Risk models based on a simple linear extrapolation of effects from high dose radiation exposures ignore adaptive mechanisms and are consequently likely to overstate the risk of low dose exposures to low LET radiation. An inaccurate estimation of risk is not merely conservative but imposes a real societal cost through the misallocation of resources and foregone benefits that could be derived from medical techniques with the potential to improve or save lives such as low dose radiation therapies (Seegenschmiedt *et al.,* 2000; Rödel *et al.,* 2007) or therapies that deliberately stimulate the adaptive response through hyperthermy (Mantso *et al.,* 2016). Improved risk models incorporating the adaptive response (e.g. Calabrese *et al.,* 2016) could be a great benefit to society however there are outstanding questions that need to be answered including the

nature of trade-offs that natural selection tells us must exist. Development of improved, scientifically-realistic, risk models incorporating hormetic effects requires that such costs be quantified along with other non-targeted effects such as the bystander effect and genomic instability.

The results of this thesis point to phenological mismatch as a plausible candidate for an evolutionarily relevant form of trade-off, and one that imposes a far lower risk in our modern food-rich human society than for natural systems where the availability of food is uncertain. On the other hand, benefits that come with trade-offs in immune function (McClure *et al.*, 2014; Costantini *et al.*, 2014) would potentially represent a more serious impediment to any beneficial use of hormetic therapies as well as representing a problematic form of non-linear risk in its own right. Other potential trade-offs should be considered including the possibility that maintaining a degree of reserve immune capacity could confer some form of selective advantage. In fully mobilizing the constituent pathways of the adaptive response an organism may achieve certain transient benefits but will have simultaneously expended this reserve capacity.

7.6 Directions for Future Research

The results of this thesis include discoveries and insights into the development of lake whitefish embryos that provide avenues for future research. The fundamental question of the cost of hormesis also remains unanswered although a plausible candidate for a general cost is presented. Future research in both areas has the potential to further our understanding of the

ecology of an important fish species and in the development of scientifically realistic risk models for sublethal exposures to environmental stressors.

The discovery of heterograde hatching (Chapter 4) and its associated predictive model (Chapter 5) allows for the prediction of both the timing and size/stage of hatching under real-world conditions but post-hatch energetics remains an area where further research is needed. Specific questions relate to the role of stage-related morphological features such as fin development and mouth gape in relation to prey species on the ability of hatchlings to transition to exogenous feeding - a time of high natural mortality (Muir *et al.*, 2010). This relationship reflects the greater metabolic energy needs of larger organisms and the timing of the spring zooplankton bloom which constitutes the principal food source for whitefish hatchlings. The interaction between morphology and energetic efficiency is also an area where current knowledge is deficient. Small hatchlings with larger yolks would be able to sustain long transitions to exogenous feeding if energy consumption remained at pre-hatching levels. However, active metabolism imposes a significantly higher energetic demand and one affected by non-linear relationships between larval length, water temperature, viscosity, and the energy expended for motility (China and Holzman, 2014). Larval size and fin development will also influence the effectiveness of predator avoidance. The methods developed in this thesis for normalization of YCE across development stages would could be extended to post-hatching energetic studies in which the energetic efficiency of locomotion and active metabolism could

be determined for different hatchling lengths at different temperatures up to the point of effective yolk exhaustion.

The possibility that an increase in metabolic rate could be a general cost of hormesis requires further investigation. If this hypothesis is correct then it would require that the elevated metabolic rate be sustained for longer than a transient availability of excess food. This could be tested using methods developed in this thesis by exposing a large cohort of embryos to a single dose of ionizing radiation and then comparing a time series of unpreserved dry weights to an unexposed control to determine the duration of the stimulatory effect and its decay kinetics. The combination of dry weight measurements with respirometry could enhance the precision of such measurements. It is also necessary that the increase in metabolic rate be a generalized component of the adaptive response which could be tested using non-radiological stressors such as heat or low concentrations of heavy metals such as cadmium which is known to trigger an adaptive response in fish (Kamunde and MacPhail, 2013). A successful outcome for experiments of this nature would then lead to the testing of other model systems to determine the generality of the increased metabolic rate.

If we look further afield, the differential scaling between growth and development examined in Chapter 4 as an explanation for the temperature-size-rule (van der Have and Dejong, 1996; Forster and Hirst, 2012; Zuo *et al.*, 2012) is likely to have an interspecies counterpart in the thermal scaling of growth. Even subtle differences in thermal scaling, if present, could be sufficient to shift the competitive balance between organisms in the presence of temperature

fluctuations. The use of temperature oscillations to shift competitive equilibria between pathogens and biological defences might lead to novel applications of medical hyperthermia to combat increasingly common antibiotic resistant pathogens (Roca *et al.*, 2015).

7.7 Summary

Living organisms convert energy into growth and reproduction. While the sources of such energy may vary up and down the food web, all life functions as a thermodynamic system. Species or individuals deriving an energetic advantage under particular environmental conditions will tend to thrive and to outcompete those that lack such advantage. It follows that environmental factors that affect the availability of energy or the efficiency of its use may give rise to biological impacts at levels well below those that cause direct mortality. The balance between energy reserves and environmental conditions is of critical importance to lake whitefish embryos used as a test organism as illustrated by the evolution of mechanisms to synchronize hatching to environmental conditions. While the relationships between energy resources, physiological processes, and environmental conditions may differ from organism to organism, they will affect fitness across all forms of life.

This thesis provides insights into the role of metabolic efficiency and phenological mismatch as factors affecting the fitness of developing lake whitefish embryos. An increased metabolic rate is advanced as a possible candidate for a universal cost of hormesis however we cannot exclude the existence of other non-energetic forms of cost. Further research in this area is

essential if simplistic linear risk models are to be supplanted by more scientifically realistic ones.

7.8 References

Beacham, T. D. and C. B. Murray, 1989. Variation in developmental biology of ockeye Salmon (*Oncorhynchus Nerka*) and Chinook Salmon (*O . Tshawytscha*) in British Columbia; *Canadian Journal of Zoology*. 67.9

Berlin, W.H., L.T. Brooke and L.J. Stone, 1977. Verification of a model for predicting the effect of inconstant temperature on embryonic development of lake whitefish (*Coregonus clupeaformis*); *Technical papers of the US Fish and Wildlife Service* 92.

Briere, J. F., P. Pracros, A. Y. Le Roux, and J. S. Pierre, 1999. A novel rate model of temperature-dependent development for arthropods; *Environmental Entomology*, Vol. 28, No. 1, 22–29.

Brooke, L.T. 1975. Effect of different constant incubation temperatures on egg survival and embryonic development in lake whitefish (*Coregonus clupeaformis*); *Transactions of the American Fisheries Society*. 104 (3), 555-559.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, & G. B. West, 2004. Toward a metabolic theory of ecology; *Ecology* 85: 1771–1789.

Calabrese, E.J., D.Y. Shamoun, J.C. Hanekamp, 2016. The integration of LNT and hormesis for cancer risk assessment optimizes public health protection; *Health Physics*: March 2016 - Volume 110 - Issue 3 - p 256–259.

Charnov, E.L. and J.F. Gillooly, 2003. Thermal time: body size, food quality and the 10ºC rule; *Evolutionary Ecology Research*, 2003, 5: 43–51.

China, V. and R. Holzman, 2014. Hydrodynamic starvation in first-feeding larval fishes; *Proceedings of the National Academy of Sciences* 111.22 (2014): 8083-8088.

Clarke, A., 2004. Is there a Universal Temperature Dependence of metabolism?; *Functional Ecology*, 18, 252-256.

Clarke, A. and K.P.P Fraser, 2004. Why does metabolism scale with temperature; *Functional Ecology*, 18, 243-251.

Colby, P. J., and L. T. Brooke, 1973. Effects of temperature on embryonic development of Lake Herring (*Coregonus Artedii*); *Journal of the Fisheries Research Board of Canada* 30.6 : 799-810.

Costantini, D., P. Monaghan, and N. B. Metcalfe, 2014. Prior hormetic priming is costly under environmental mismatch; *Biology Letters* 10.2

Dwyer, W., 1987. Effect of lowering water temperature on hatching time and survival of lake trout eggs; *The Progressive Fish-Culturist* 49:175-176.

Elliott, J. M. and M. A. Hurley, 1997. A functional model for maximum growth of Atlantic Salmon parr, *Salmo salar*, from two populations in northwest England; *Functional Ecology* 11.5 (1997): 592-603.

Eme, J., C.A. Mueller, R.G. Manzon, C.M. Somers, D.R. Boreham, and J.Y. Wilson, 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (Coregonus clupeaformis) embryos and hatchlings; *Comparative Biochemistry and Physiology* Part A: Molecular & Integrative Physiology. 179, 71-80.

Forster, J., and A. G. Hirst, 2012. The temperature-size rule emerges from ontogenetic differences between growth and development rates; *Functional Ecology* Volume 26, Issue 2, 483–492.

Gillooly, J.F., J.H. Brown, G.B. West, V.M. Savage and E.L. Charnov, 2001. Effects of size and temperature on metabolic rate. *Science* 293, 2248–2251.

Gillooly, J.F., E.L. Charnov, G.B. West, V.M. Savage and J.H. Brown, 2002. Effects of size and temperature on developmental time. *Nature* 417, 70–73.

Griffiths, J.S., 1979. Potential effects of unstable thermal discharges on incubation of Lake Whitefish eggs; Ontario Hydro Research Division Report No. 79-521-K.

Hamel, P., Magnan, P., East, P., Lapointe, M. and Laurendeau, P., 1997. Comparison of different models to predict the in situ embryonic developmental rate of fish, with special reference to White Sucker (Catostomus Commersoni); *Canadian Journal of Fisheries and Aquatic Science* 54.1 (1997): 190-197.

Jensen, J.L., 1906. Sur les fonctions convexes et les inégualités entre les valeurs moyennes; *Acta Mathmatica*. 30, 175–193.

John, K. R., and A. D. Hasler, 1956. Observations on some factors affecting the hatching of eggs and the survival of young shallow-water Cisco, *Leucichthys Artedi* Lesueur, in Lake Mendota, Wisconsin; *Limnology and Oceanography*. 1.3 (1956): 176-194.

Jordaan, A., 2002. The effect of temperature on the development, growth and survival of Atlantic Cod (*Gadus morhua*) during early life-histories; Electronic Theses and Dissertations. Paper 141.

Jordaan, A., S. E. Hayhurst, and L. J. Kling, 2006. The influence of temperature on the stage at hatch of laboratory reared *Gadus Morhua* and implications for comparisons of length and morphology; *Journal of Fish Biology* 68.1 (2006): 7-24.

Kamunde, C. and R. MacPhail, 2013. Subcellular interactions of dietary cadmium, copper and zinc in Rainbow Trout (*Oncorhynchus mykiss*); *Aquatic Toxicology* 105.3-4 (2011): 518-527.

Kaufmann, O., 1932. Einige bemerkuungen uber den ein-ßuss von temperatureschwankungen auf die entwicklungsdauer und streuung bei insekten und seine graphische darstellung durch kettelinie und hyperbel; *Zeitschrift für Morphologie und Ökologie der Tiere* 25: 353-361.

Kingsolver, J. G., 2004. Plasticity of size and growth in fluctuating thermal environments: Comparing reaction norms and performance curves; *Integrative and Comparative Biology* 44.6 (2004): 450-460.

Logan, J. A., D. J. Wollkind, S. C. Hoyt, and L. K. Tanigoshi, 1976. An analytical model for description of temperature dependent rate phenomena in arthropods; *Environmental Entomology*, Vol. 5, pp. 1133–1140, 1976.

Luczynski, M. 1984. Improvement in the efficiency of stocking lakes with larvae of *Coregonus albula* L. by delaying hatching; *Aquaculture* 41: 99-111.

Luczynski, M. and A Kirklewska, 1984. Dependence of *Coregonus albula* embryogenesis rate on the incubation temperature; *Aquaculture*, vol. 42, no. 1, 43-55.

Mantso, T., G. Goussetis, R. Franco, S. Botaitis, A. Pappa and M. Panayiotidis, 2016. Effects of hyperthermia as a mitigation strategy in DNA damage-based cancer therapies; *Seminars in Cancer Biology* 37-38 (2016): 96-105.

McClure, C.D.; W. Zhong, V. L. Hunt, F. M. Chapman, F.V. Hill and N. K. Priest, 2014. Hormesis results in trade-offs with immunity. *Evolution* 68, 2225–2233.

Mitz, C., C. Thome, M.E. Cybulski, L. Laframboise, C.M. Somers, R.G.Manzon, J.Y. Wilson and D.R. Boreham, 2014. A self-contained, controlled hatchery system for rearing lake whitefish embryos for experimental aquaculture; *North American Journal of Aquaculture.* 76 (3), 179-184.

Mueller, C.A., J. Eme, R.G. Manzon, C.M. Somers, D.R. Boreham and J.Y. Wilson, 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype and cost of development in lake whitefish (Coregonus clupeaformis); *Journal of Comparative Physiology*. B, Biochemical, Systemic, and Environmental Physiology. 185 (3), 315-331.

Muir, A. M., T. M. Sutton, M. T. Arts, R. M. Claramunt, M. P. Ebener, J. D. Fitzsimons, T. B. Johnson, R. E. Kinnunen, M. A. Koops and M. M. Sepúlveda, 2010. Does condition of Lake Whitefish spawners affect physiological condition of juveniles?; *Journal of Great Lakes Research* 36 (2010): 92-99.

Neuheimer, A. B, and C. T. Taggart., 2007. The growing degree-day and fish size-at-age: The overlooked metric; *Canadian Journal of Fisheries and Aquatic Sciences* 64.2 (2007): 375-385.

O'Connor, M. P., S. J. Kemp, S. J. Agosta, F. Hansen, A. E. Sieg, B. P. Wallace, J. N. McNair and A. E. Dunham, 2007. Reconsidering the mechanistic basis of the metabolic theory of ecology. *Oikos*, 116, 1058-1072.

Patrick, P.H., E. Chen, J. Parks, J. Powell, J. S. Poulton and C-L Fietsch, 2013. Effects of fixed and fluctuating temperature on hatch of Round Whitefish and Lake Whitefish eggs; *North American Journal of Fisheries Management* 33:1091-1099.

Price, J.W., 1940. Time-temperature relations in the incubation of the whitefish, *Coregonus clupeaformis* (Mitchill); *Journal of General Physiology*. 23 (4), 449-468.

Reshetnikov, Y. S., and V. D. Bogdanov, 2011. Features of reproduction of whitefishes; *Journal of Ichthyology* 51.6 (2011): 432-456.

Roca, I., M. Akova, F. Baquero, J. Carlet, M. Cavaleri, S. Coenen, J. Cohen, D. Findlay, I. Gyssens, O.E. Heure, G. Kahlmeter, H. Kruse, R. Laxminarayan, E. Liébana, L. López-Cerero, A. MacGowan, M. Martins, J. Rodríguez-Baño, J.-M. Rolain, C. Segovia, B. Sigauque, E. Tacconelli, E. Wellington and J. Vila, 2015. The global threat of antimicrobial resistance: science for intervention; *New Microbes and New Infections*, Volume 6, 22–29.

Rödel, F., L. Keilholz, M. Herrmann, R. Sauer and G. Hildebrandt, 2007. Radiobiological mechanisms in inflammatory diseases of low-dose radiation therapy. *International Journal of Radiation Biology*, 83(6), 357-366.

Ruel, J. J. and M. P. Ayres, 1999. Jensen's inequality predicts effects of environmental variation; *Trends in Evolution and Ecology* 14: 361-366.

Saul N, K. Pietsch, S.R. Stürzenbaum, R. Menzel and C.E. Steinberg, 2013. Hormesis and longevity with tannins: free of charge or cost-intensive?; *Chemosphere* 93:1005-1008.

Seegenschmiedt, M., A. Katalinic, H. Makoski, W. Haase, G. Gademann and E.Hassenstein, 2000. Radiation therapy for benign diseases: patterns of care study in Germany; *International journal of radiation oncology, biology, physics*, 47(1), 195-202.

Sharpe, P. J. H. and D. DeMichele. 1977. Reaction kinetics of poikilotherm development; *Journal of Theoretical Biology*, 64: 649-670.

Thome, C., C. Mitz, C.M. Somers, R.G. Manzon, J.Y. Wilson, and D.R. Boreham, 2016. Incubation of Lake Whitefish (Coregonus clupeaformis) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development; *Canadian Journal of Fisheries and Aquatic Sciences, http://dx.doi.org/10.1139/cjfas-2015-0286.*

van der Have, T. M. & G. de Jong, 1996. Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology*. 138: 329–340.

Vangansbeke, D., J. Audenaert, D. T. Nguyen, R. Verhoeven, B. Gobin, L. Tirry and P. De Clercq, 2015. Diurnal temperature variations affect development of a herbivorous arthropod pest and its predators; *PLOS ONE* 10.4 (2015): e0124898

West, G.B., J.H. Brown and B.J. Enquist, 1999. The fourth dimension of life: Fractal geometry and allometric scaling of organisms; *Science* 284: 1677–1679.

Zuo, W., M.E. Moses, G. B. West, C. Hou, and J. H. Brown, 2012. A general model for effects of temperature on ectotherm ontogenetic growth and development: *Proceedings of the Royal Society* B (2012) 279, 1840–1846.