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The effects of oxygen and temperature on the physiology of hatching stage Atlantic salmon

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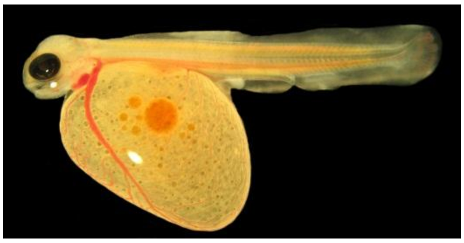

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"doing what little one can to increase the general stock of knowledge is as respectable an object of life, as one can in any likelihood pursue"

(Charles Darwin)

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Declaration of Originality

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The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Candidate = Elias Polymeropoulos (School of Zoology)

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Author 2 = Dr. Nick Elliott (Commonwealth Scientific and Industrial Research Organisation)

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Author details and their roles:

Respirometry: Correcting for diffusion and validating the use of plastic multiwell plates with integrated optodes.

Located in Chapter 2.3. Correcting for diffusion and validating the use of plastic multiwell plates with integrated optodes

Candidate was the primary author and author 1 contributed to the idea, its formalisation and development

Author 2 and author 3 assisted with refinement and presentation

Author 3 derived the mathematical model that enables general correction of oxygen diffusion occurring across polystyrene multiwell plates.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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Date:______________________

Statement of Ethical Conduct (where applicable)

"The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University."

Cover image: Atlantic salmon (*S. salar*) yolk sac alevin.

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Abbreviations

2n	=	diploid
3n	=	triploid
bpm	=	beats per minute
βO_2	=	solubility of oxygen
f_H	=	heart rate
f_V	=	ventilation rate
GH	=	growth hormone
GO_2	=	oxygen conductance
Hb	=	hemoglobin
Hsp	=	heat shock protein
KO_2	=	Krogh’s diffusion coefficient
kPa	=	kilopascal
$\dot{M}O_2$	=	metabolic rate
PO_2	=	partial pressure of oxygen
T_a	=	ambient temperature
Tx	=	transgenic
Nt	=	non transgenic

Abstract

Environmental change and its impact on the form, function and adaptive responses of fish, especially in relation to maternal effects or individual genotype, is of major interest to biologists. Oxygen levels and temperature have profound effects on the physiology of fish, and early developmental stages are particularly susceptible to environmental challenges. In this thesis the metabolic, cardiorespiratory and cellular stress responses to acute or chronic changes of these variables in Atlantic salmon (*Salmo salar*) embryos and yolk-sac alevins were investigated.

Eggs and alevins matched their metabolic demand to acute changes in oxygen levels without altering cardiorespiratory function unless oxygen reached critically low levels (5 kPa). In contrast, chronic hypoxia (10.5 kPa for 15 days), but not hyperoxia (28 kPa for 15 days) resulted in functional and structural modifications that enabled metabolic rate to return to the normoxic (21 kPa) pre-exposure level while maintained in hypoxia (10.5 kPa); this indicating a metabolic compensation. On return to acute normoxia, irrespective of the measurement temperature (4, 8, 12°C), metabolic rate was elevated above the pre-hypoxic value observed in normoxia, presumably a result of the structural and/or functional modifications that occured in hypoxia.

In addition, differences in metabolism and responses to hypoxia were influenced by maternal factors such as egg size, which in turn are determined by maternal body size. Egg size-dependent differences in metabolic rate of embryos were

present and larger embryos from repeat spawning females exhibited increased tolerance to hypoxia, as represented by lower critical oxygen levels for hatching than observed in embryos from smaller eggs from maiden spawners (13 kPa vs. 17 kPa). This result suggests an advantage in embryos from larger eggs whereby the embryo can obtain sufficient oxygen due to the larger surface area of the egg in respect to the embryo's metabolic rate.

Genetic modification for growth (growth hormone transgenesis) or triploidy (three sets of paired chromosomes) increased metabolic rate above a diploid conspecific (both by 8%). This effect was additive in triploid transgenic alevins that also displayed an altered cardiorespiratory response to acute, severe hypoxia (5 kPa). In addition, acute hypoxia did not elicit a cellular stress response, but was associated with differential (reduced) expression of cellular stress proteins (heat shock proteins) that in one case (Hsp90) was dependent on the growth hormone transgenic genotype.

Taken together, the above observations demonstrate that extrinsic as well as intrinsic factors have substantial effects on the physiology of developing fish, and that the overall response to extrinsic factors contains a temporal component. Given the associated effects are reflected in changes in metabolism, it is likely that the effects will significantly impact growth, survival and performance of the developing fish.