The effect of incubation temperature on early malformation, regionalisation and meristic characters of the vertebral column in farmed Chinook salmon (*Oncorhynchus tshawytscha*).

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Adelbert De Clercq

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ABSTRACT

Skeletal deformities are a recurrent problem in farmed Chinook salmon which limit production and have animal welfare impacts. Skeletal deformities of a variety of types are recognised especially when the external phenotype of the animal is affected. These types are well described in juvenile and adult stages of the production cycle. Which skeletal malformations affect early life stages in salmonids is less well known. Temperature is commonly manipulated in fish farming husbandry. High rearing temperatures are related to higher growth rates and in Atlantic salmon, elevated temperature has been inferred as a potential risk factor for skeletal deformities. In this thesis, malformations of the vertebral column in post-hatch to first feed life stages (500-900 degreedays) were studied in farmed Chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand. Fish were reared at a constant 4°C, 8°C and 12°C, from fertilisation to juvenile stages. The effects of rearing fish at these temperatures on malformations of the vertebral column were studied in specimens whole-mount stained for cartilage and mineralised bone, and in histological sections. The external phenotype of post-hatch stages could be linked to internal skeletal malformations such as notochord malformations, chordacentra fusions and malformations of the associated elements. In all temperature groups, externally normal specimens could have internal malformations, predominantly fused chordacentra. Conversely, externally malformed fish usually displayed internal malformations. Specimens raised at 8°C had fewest malformations, followed by specimens of the 12°C group. Specimens raised at 4°C had the highest number of malformations. This study indicates that 8°C is the best incubation temperature of those tested. In addition, the effects of rearing temperature on morphological variation of skeletal elements such as vertebrae, vestigial ribs and vestigial elements in the caudal fin were studied. Six vertebral column regions were identified. The defining characters of each of these regions remained independent of the rearing temperature. Still, the postcranial, transitional and ural regions showed temperature sensitive meristic variation of the vertebrae, vestigial ribs, arches, epurals and uroneurals. Meristic variation can foreshadow skeletal malformations that emerge late in life and thus be significant for the early diagnosis of vertebral deformities.

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I was still in the full swing of writing the Ph.D. thesis when I first drafted this section. However, I am nearing the end and one question has been tumbling through my mind many times in the last six months: “Is it truly hard to do a Ph.D. project, and if yes, why is it so hard?” I can only answer this question from my own perspective and experience(s) and thus different people may have different answers or may ask the question differently.

From a scientific viewpoint my answer to the question is fairly straightforward and simple: “No, scientifically I find a Ph.D. project not hard to do.” I feel that when one is passionate about science, passionate about working in the lab, and is curious about the results of the experiments, a Ph.D. project is not so hard to do. One question that follows is: “Was the project not scientifically challenging?” The answer to this latter question is a firm: “No, it was challenging.” Not the difficult experimental methods or statistical analyses, but the scope, range and depth of the subject were challenging. Skeletal tissues have deep roots in evolution, are observable over hundreds of millions of years and have hundreds of books describing their anatomy in currently living animals. In addition, hundreds of outside and innate factors influence the skeleton. The width and depth of this subject take gargantuan proportions. The diversity in anatomy of skeletal tissues of currently living teleost fish alone is mindboggling. Moreover, one can happily spend an entire life studying the skeleton of a single fish species, for example the salmon. With passion, drive and curiosity the daily challenges while doing science are overcome. “Than why do PhD students suffer so much during their project?”

Many a time I heard from fellow Ph.D. students, friends and people close to me say that they are frustrated, stressed, burned-out, having health issues and being outright unhappy. “Are these ‘things’ also part of doing science?” Again, for me personally the answer is straightforward and simple: “No, the suffering is not a part of doing science.” The negative emotions and also positive emotion one experiences during a Ph.D. project is part of being human. This brings me to the ‘Why it is so hard to do a Ph.D. project’. For me, science is the absolute sustenance of my rationale. I eat, drink and breath science. But, above all I am still a human being, full of emotions and believes. However, being a human is sometimes hard. That is why doing a PhD project is so hard. For me, the project was a constant search for a balance. Distancing myself from feeling and believing during scientific activities, while feeling and believing during life experiences outside science. Searching for a balance, something we all struggle with is likely part of the real valuable lessons I have learned during this Ph.D. project. As humans, we have help. The amazing people who helped and supported me as scientist and human brings me to the true purpose of this section: ‘Saying thank you’.

I have to start by thanking Freddy, Christian, Goedele, Jurgen and many other fantastic teachers of the high school I attended (Scheppers-Wetteren, Belgium). These people taught with so much care and passion it affirmed my choice of studying biology and increased my interest in science.

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Big thanks to Sam. Scientific and life mentor. Unbelievable as it may seem, I chose the subject of my Master thesis from a list of 164 subjects. I knew I wanted to do a thesis in the EvoDevo lab of Ann, but still, what are the chances. Together with Jeroen you always managed to lift the spirits. You taught me to name and classify samples, pictures and files with care. Your eye for good graphics was inspirational and I have to thank you for teaching me the workings of Photoshop, Illustrator and Amira. Yes, I know and truly understand your search for perfect symmetry when making manuscript figures. Thank you for your continued support during my PhD work.

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I just heard I had to leave the place I was living at that time and I did not have all my wits about me. A very awkward phone call (number provided by Magda) brought me to Jack McKenzie. Still, we met and I rented a room. You have given me a home in New Zealand and I would never want to trade it for anything else. Thank you for the stimulating conversations about almost any subject. Thank you for teaching me so much about sports, and to help me appreciate rugby, cricket, cycling and many other sports on a different level. In the same breath I want to say thank you to Kyoko Murakami. You have taught me so much more about the Japanese culture, about its beautiful language and about life. Thank you for introducing me to Hash House Harriers, the most quirky and weird bunch of people I know, but absolute top fun. Shumba, my furry cat friend, I will miss you tremendously.

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Adelbert

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

List of Symbols and Abbreviations

6m	six months (at sea)
°C	degrees centigrade
°d	degreedays
τ_s	Gorodilov's tau S
>	larger than
%	percent
±	plus or minus
<	smaller than
Σ	summation
antS	anterior spine
AB	Alcian blue
AC	alternating current
ALP	alkaline phosphatase
AB-PAS	Alcian blue-periodic acid Shiff
AR	Alizarin red
ARF	ADP-ribosylation factor
Atf	cyclic AMP-dependent transcription factor
Bbf	multifactor complex containing B element binding factor
BD	basidorsal
BMP	bone morphogenetic protein
BN	bent neck
BNA	base of neural arch
BTM	bent tail medium
BTS	bent tail severe
BV	basiventral
cm	centimetre
C	compression
C	compression and/or reduced intervertebral spaces
Cb	chordoblast(s)
CB	cancellous bone
CC	central canal
CHC	chordacentrum
Cltc	clathrin coated
CM	curly medium
Col	collagen

COP	coatomer protein
Creb	cyclic AMP response element-binding protein
CS	curly severe
CTM	critical thermal methodology
d	days
dd	degreedays
df	degrees of freedom
dpf	days post fertilisation
DEPC	diethyl pyrocarbonate
DIC	differential interference contrast microscopy
E	epural(s)
ECM	extracellular matrix
EEM	external elastic membrane
EMT	epithelial-mesenchymal transition
ER	endoplasmic reticulum
F	fertilisation
F	fusion
FF	first feed
Fig.	figure
FL	fork length
FW	freshwater
g	gram
glm	general linear model
h	hour(s)
H	hatching
HA	haemal arch
H_2O_2	hydrogen peroxide
Hox	homeobox
HS	haemal spine
HSP	heath shock protein
Hy	hypural(s)
ILT	incipient lethal temperature
IV	intervertebral space
K	kyphosis
KOH	potassium hydroxide
kV	kilovolt
l	left

logistf	Firth logistic regression
L	litre
L	lordosis
LKS	lordosis, kyphosis, scoliosis
LLT	lower lethal temperature
LRO	lysosome related organelles
μm	micrometre
m^3	cubic metre
mA	milliamps
min	minute
mm	millimetre
mM	millimolar
ml	millilitre
MET	mesenchymal-epithelial transition
Mfh	mesenchyme fork head
MMP	matrix metalloproteinase
nm	nanometre
nsh	notochord sheath
N	normal
NA	neural arch
NCH	notochord
NS	neural spine
NT	neural tube
NTC	notochord
NZKS	New Zealand King Salmon
opc	opistural cartilage
O.	*Oncorhynchus*
OPT	optimum temperature
pap	parapophyses
ppm	parts per million
pERK	phosphorylated extracellular signal-regulated kinase
pS6	phosphorylated S6
Pax	paired box
PBS	phosphate-buffered saline
PH	parhypural
PH	post-hatch
PHy	parhypural

PFA	paraformaldehyde
PIT	passive integrated transponder
PLP	paraformaldehyde-lysine-periodate
Pre-zyg	pre-zygapophysis
Pst-zyg	post-zygapophysis
PU	preural
r	right
R	region
R	rib
RAS	recirculating aquaculture system
sec	seconds
smolt	freshwater pre-smoltification stage
spp	species
Sox	sex-determining region Y-related high mobility group box
S	scoliosis
SC	scar tissue
SD	standard deviation
SHH	sonic hedgehog
SIG	salmon improvement group
SMM	spinal malformation medium
SMS	spinal malformation severe
ST	stegural
SW	seawater
SW-6	six months in seawater
SW-12	12 months in seawater
T	time
TEM	transmission electron microscopy
TL	total length
TM	tail malformation
TNL	TNL International Ltd
Total	total deformities
U	ural
ULT	upper lethal temperature
UN	uroneural
UPR	unfolded protein response
UV	ultraviolet
vbi	vertebrae imperfecta

V	vertebra(e)
VE	vertebral endplate
VNA/S	vestigial neural arch/spine
VS	vertical shift of the entire vertebral centrum
WT	wild type