UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics

Photoperiodic history-dependent preadaptation of the smolting gill

Novel players and SW immediate response as markers of growth and welfare

Marianne Iversen

A dissertation for the degree of Philosophiae Doctor, December 2020

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Front page image: Juvenile salmon showing the transition from parr (bottom) to smolt (top). Photo by Barbara Tomotani.

List of contents

I. Acknowledgements

Finally, it is done. It's been a long and winding road where I have experienced and learned a lot, both about salmon and about myself.

I'd like to thank those communities that I have been part of during this time. My friends and colleagues at UiT, both new and old. The professors, researchers, post-docs, technicians and fellow phd-students that I shared my time with at the Arctic Chronobiology and Physiology research group deserve a special mention, you are all brilliant! Thank you to my friends found around various UiT departments and research institutions. You have provided interesting discussion about all and nothing during those occasional long lunch breaks. Thank you to the sporting dog communities in Tromsø, for making me focus solely on my dog during training, providing that much needed thesis-free space. Thank you to my always-up-for-fun-dog Kajsa for providing relaxing hikes and bringing enthusiasm to everything we do. Thank you to my family, my parents, and my wonderful husband for always being there for me, patient, understanding and loving.

I would like to make a special dedication to my aunt Arna, whom sadly passed this December. Since I moved to Tromsø as a young student we became very close, and I will surely miss her a lot.

Finally, to my supervisors David and Even, thank you for seeing this through with me.

II. Thesis Abstract

This thesis presents a rich introduction to the evolution, life history and physiology of Atlantic salmon, with a special emphasis on the developmental transitions (termed smolting) juvenile salmon pass through has they prepare to migrate from their native freshwater habitat to the sea. The introduction provides a comprehensive background on chronobiology and osmoregulation in fishes and links this to the process of smolting as it has been described in literature.

Further, this thesis contributes three papers to the standing literature on Atlantic salmon, salmonids and smolting. Paper I focuses on the role of photoperiod history for smolting and pre-adaptation to saltwater to occur in a coordinated and organized manner, and presents results showing photoperiod history ultimately influences saltwater growth. The paper presents data on previously unstudied genes in the context of salmonids and pre-adaptation to saltwater indicating that they could be important for predicting SW-tolerance in juvenile salmon.

Paper II further illustrates the importance of photoperiod stimuli to drive smolting and preadaptation to saltwater. The data presented in paper II show clear differences in the response to saltwater between different photoperiod treatments. Not only in the number of responsive genes but also in the group of genes whose expression was influenced by saltwater exposure. Saltwater-responsive genes in the two treatments designed not to bring forth a saltwater-adapted juvenile were enriched for promoter motifs linked with a general stress response and osmoregulatory stress.

The third paper focused on the temporal and spatial expression of the so-called clock genes in salmon, also smolting. One of the main findings of this paper is that many clock genes derived from the fourth salmon specific whole genome duplication have tissue-specific expression profiles, and that their regulation in the gill correlates with smolting.

The main findings of these papers are presented and discussed together with insights from literature and unpublished data derived from the same datasets presented in the papers.

III. List of papers

Paper I

RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile Atlantic salmon

Marianne Iversen[1], Teshome Mulugeta[2], Børge Gellein Blikeng[1#], Alexander West[1], Even Jørgensen[1], Simen Rød Sandven[3] and David Hazlerigg [1]

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Paper II

Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in an anadromous salmonid

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Paper III

Diversified regulation of circadian clock gene expression following whole genome duplication

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IV. Abbreviations

AANAT	Aralkylamin N-acetyltransferase
AC	Accessory cell
ACTH	Adrenocorticotropic hormone
AORe	Ancestral ohnologue resolution
AQP	Aquaporin
ATP	Adenosine triphosphate
Bmal	Brain and Muscle ARNT-like
CA	Carbonic anhydrase
CAPN	Calpain
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
Clock	Circadian Locomoter Output Cycles Protein Kaput
CR	Corticosteroid receptors
CRH	Corticotropin-releasing hormone
Cry	Chryptochrome Circadian Regulator
CSF	Cerebrospinal fluid
DEG	Differentially expressed gene
dioX	Deiodinase X
DNA	Deoxy-ribonucleic acid
Eya	Eyes absent homolog
FAK	Focal adhesion kinease
FKBP	FK506-Binding Protein
FSH	Follicle stimulating hormone
FW	Freshwater
FXYD	FXYD Domain Containing Ion Transport Regulator
GH	Growth hormone
GHBP	Growth hormone-binding protein
GHRH	Growth hormone-releasing hormone
GIT	Gastrointestinal tract
GO	Gene ontology
GOI	Genes of interest
GR	Glucocorticoid receptor
GRE	Glucocorticoid-response element
IGF	Insulin-like growth factor
KLF	Krüppel-like factor
LBP	Light-brain-pituitary
LP	Long day (photoperiod)
LDN	Natural photoperiod
LGD	Local gene duplication
LL	Continuous light
LM	Lower mode
LORe	Linage-specific ohnologue resolution
MBH	Medio-basal hypothalamus
mOsm	milliosmole
MRC	Mitochondria-rich cell
mRNA	messenger ribonucleic acid

MSH	Melanocyte-stimulating hormone
MT	Melatonin receptor
MYA	Million years ago
NFAT	Nuclear factor of activated T-cells
NKA	Sodium-potassium ATPase
NKCC	Na-K-2Cl cotransporter
PCA	Principal component analysis
OREBP	Osmotic response element binding protein
OT	Optic tectum
PD	Pars distalis
Per	Period
POA	Preoptic area
PRL	Prolactin
PT	Pars tuberalis
PVC	Pavement cell
qPCR	quantitative polymerase chain reaction
RNA	Ribonucleic acid
SCN	Suprachiasmatic nuclei
SEM	Standard error of mean
SP	Short day (photoperiod)
Ss4R	Salmonid-specific fourth round of vertebrate whole genome duplication
SV	Saccus vasculosis
SW	Saltwater
T_3	Triiodothyronine
T_4	Thyroxine
TCTP	Translationally Controlled Tumour Protein
TH	Thyroid hormone
TPH1	Tryptophan hydroxylase 1 (gene)
TRH	Thyrotropin-releasing hormone
TRP	Transient receptor potential
TonEBP	Tonicity-responsive Enhancer Binding Protein
TSH	Thyroid-stimulating hormone (thyrotropin)
Ts3R	Teleost-specific third round of vertebrate whole genome duplication
UM	Upper mode
WGD	Whole genome duplication
WSP	Weeks of short-photoperiod

1. Introduction

Atlantic salmon (*Salmo salar*), the leaping fish, renowned for its vitality and wisdom in North-European folklore, is today under threat from global climate change, habitat destruction, and environmental pollutants. Simultaneously it is also a very numerous species, due to the massive production of domesticated salmon in aquaculture.

A key process in both wild and captive salmonid life history, known as smoltification or smolting, is the transition from a benthic parr in freshwater (FW) to a saltwater (SW)-ready smolt (parr-smolt transformation). The terms smolting and parr-smolt transformation will be used interchangeably, depending on context. This process is controlled by photoperiod, meaning that the initiation and continuation of smolting is regulated by changes in the observed photoperiod (i.e. the duration of daily exposure to light). Evidence is accumulating that salmonid smolting is dependent upon many of the same processes that are responsible for circannual rhythms of birds and mammals, for example in relation to reproduction and migration (Falcón, Migaud, Muñoz-Cueto et al., 2010; Lorgen, Casadei, Król et al., 2015; Maeda, Shimo, Nakane et al., 2015; Nakane, Ikegami, Iigo et al., 2013; Nakao, Ono, Yamamura et al., 2008; Nakao, Ono, and Yoshimura, 2008; Sáenz de Miera, Hanon, Dardente et al., 2013). This includes, but is not limited to, the actions of melatonin, thyroid hormones and deiodinases in the light-brain-pituitary (LBP) axis.

Smolting is a complex transition, involving changes in morphology, physiology and behaviour, in order to preadapt the juvenile salmon to SW. Historically much emphasis has been placed on measuring growth, plasma hormones, and hypo-osmoregulatory capacity (including expression of ion channels) during smoltification (McCormick, Regish, and Christensen, 2009; Stefansson, Björnsson, Ebbesson et al., 2008), in addition to early histology studies of the salmonid gill (Lubin, Rourke, and Bradley, 1989; Pisam, Prunet, Boeuf et al., 1988). With modern biotechnology, and the recently completed Atlantic salmon reference genome (Lien, Koop, Sandve et al., 2016), we are now capable of studying the transition by gene expression analyses, revealing new information on the pre-adaption process, and defining the SW-ready smolt within a new context of knowledge (Björnsson and Bradley, 2007).

Both the currents threats to the wild salmon strains, and its importance as a cultured species, validates the need for improved understanding of the complex life history and large phenotypic plasticity exhibited by the salmon. The physiological processes enabling the salmon's migration

between its native river and the sea, and the environmental inputs ensuring the correct phenology of these processes are of major interest to researchers, conservation managers, and industry alike.

This thesis demonstrates how photoperiodic history is of major significance to FW-SW transition and SW response, and presents new information on the gill-specific effects of photoperiodic regulation of smolting, introducing new and more accurate markers for SW-ready smolts. In the following sections I will go over fundamental aspects of salmonid evolution and life history, photoperiodic regulation, and relate this to central aspects of smoltification and gill physiology.

1.1 The Atlantic salmon –evolution and life history

The natural distribution of Atlantic salmon is limited to the temperate and subarctic regions of the North Atlantic Ocean, and its surrounding watersheds. The Atlantic salmon belongs to the subfamily Salmoninae, one of three linages under the Salmonidae family (order Salmoniformes). Out of eleven Salmonidae genera, seven are found within the Salmoninae, making it the most species rich and diverse linage, with more than 120 registered species (Fricke, Eschmeyer, and Fong, 2019). While the term salmonid usually will refer to all three subfamilies, it will here be used to refer to the Salmoninae specifically. The two other subfamilies will be referred to by their specific names, Coregoninae and Thymallinae.

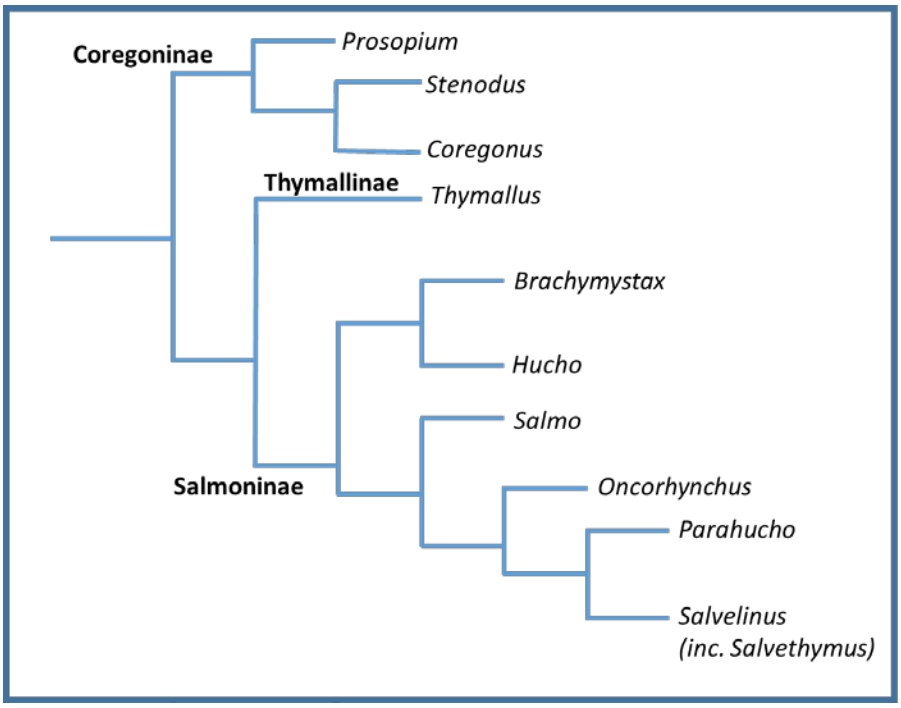

Figure 1 *Simple phylogenetic tree showing the relationship between the Salmonidae lineages.*

All three Salmonidae subfamilies show complex life history patterns, and exhibit a high level of plasticity where many species are able to coexist in a variety of morphs and follow very different life history trajectories, shaped by the surrounding ecosystem and niche availability (Dodson, Aubin-Horth, Thériault et al., 2013; Skulason and Smith, 1995). The majority of current species linages developed 30-10 million years ago (MYA), as global temperatures decreased causing large environmental changes (Macqueen and Johnston, 2014).

1.1.1 The salmonid-specific whole genome duplication

A popular hypothesis states that the species radiation occurring among the Salmonidae during the Oligo- and Miocene eras about 30-10 MYA was made possible through a much more ancient event, the salmonid-specific fourth round (Ss4R) of vertebrate whole genome duplication (Alexandrou, Swartz, Matzke et al., 2013; Berthelot, Brunet, Chalopin et al., 2014; Glasauer and Neuhauss, 2014; Robertson, Gundappa, Grammes et al., 2017; Van de Peer, Maere, and Meyer, 2009). The Ss4R whole genome duplication (WGD) occurred an estimated 100-80 MYA (Lien, Koop, Sandve et al., 2016; Macqueen and Johnston, 2014), at the base of the Salmonidae linage. This autotetraploidization event provided the Salmonidae ancestor with a large amount of redundant genetic material. WGDs are found at the base of several species rich linages because they release the genome from conservative and purifying constraints, allowing for the accumulation of stochastic mutations in redundant genes that occasionally will result in functional or regulatory changes for evolution to act upon (Ohno, 1970). Additionally, local gene duplications (LGD) are promoted during the rediploidization process following WGD (Warren, Ciborowski, Casadei et al., 2014). Duplicated genes, derived from WGD and LGD are termed ohnologues and paralogues, respectively.

Pairs of duplicated genes are rarely retained over the process of rediploidzation, but when they are they seem to differentiate in one of three ways (Conant and Wolfe, 2008; Glasauer and Neuhauss, 2014):

1. Non-functionalization; caused by accumulation of deleterious mutations.
2. Sub-functionalization; partitioning of functions held by a pleiotropic gene through mutations that optimize or disturb sub-function in one or both duplicates.
3. Neo-functionalization; one duplicate acquires a new function through mutation(s).

After WGD or LGD, reciprocal gene loss through non-functionalization and population differences in sub- and neo-functionalization of genes facilitates species divergence through building genetic isolation between groups. Notably, mutations do not have to occur in the protein-coding region of a

gene for divergence to occur. Changes in promoters, *cis*-regulatory elements, enhancers and other regulators of gene expression also have significant impacts (Arnone and Davidson, 1997; Carroll, 2008; Levine, 2010).

In the Atlantic salmon studies have shown incidences of duplicate gene pairs that appear to have diverged in regulation and/or function (Dalziel, Bittman, Mandic et al., 2014; Lappin, Shaw, and Macqueen, 2016; Lorgen, Casadei, Król et al., 2015; Lorgen, Jorgensen, Jordan et al., 2017). To complicate things further some of these duplicate gene pairs already existed as retained duplicates derived from an even older WGD-event, the teleost-specific third round of vertebrate WGD (Ts3R, 320 MYA) (Jaillon, Aury, Brunet et al., 2004; Meyer and Schartl, 1999; Meyer and Van de Peer, 2005; Taylor, Braasch, Frickey et al., 2003).

See box 1 for a brief description of an explorative analysis of the regulation of duplicated genes in the Atlantic salmon genome performed as part of this thesis work.

1.1.2 Evolution of anadromy and species radiation

There were no immediate effects of the Ss4R of WGD and subsequent LGD on the salmonid lineage (Macqueen and Johnston, 2014). Rather species radiation took place much later, as a result of climatic cooling during the Eocene-Oligocene transition(ca. 34 MYA) (Coxall and Pearson, 2007; Liu, Pagani, Zinniker et al., 2009; Macqueen and Johnston, 2014), and continuing into the current Quaternary ice age (beginning 2.6 MYA) (Ehlers and Gibbard, 2011).

Lineage-specific ohnologue resolution (LORe) has been put forward as a possible mechanisms explaining the time-lag between WGD and species radiation (Robertson, Gundappa, Grammes et al., 2017). Under LORe, rediploidization is delayed, and ohnologues diverge independently in sister lineages, providing linage specific adaptation that promotes species radiation. This in opposition to, but not excluding, ancestral ohnologue resolution (AORe) where ohnologues diverge in the ancestor to sister lineages. Robertson, Gundappa, Grammes et al. (2017) estimates that at least 4550 unique genes have evolved under LORe from the retained salmonid ohnologues, under the selective pressure of climatic cooling. Rediploidization and ohnologue resolution has continued in salmonids up to today, and is considered an ongoing process (Houston and Macqueen, 2019).

BOX 1 PHOTOPERIOD AND SALT REGULATED DUPLICATE GENES

Experimental set-up, sampling and mRNA extraction and processing as described experiment 1 in paper I.

An explorative analysis was performed using R (version) and the edgeR (version). First, genes responding to re-exposure to light at sampling points T4, T5 and T6 were found using contrasts between the different treatments for each timepoint. Secondly, genes responding to SW exposure were found using contrast between FW and SW for each timepoint and treatment. Both lists were reduced by filtering for differentially expressed genes (DEGs, FDR<0.01.). This resulted in 6 207 photoperiod- and 10 535 salt-regulated genes, a total of 14 236 unique DEGs. While 2 506 were dually regulated, 3 701 were uniquely photoperiod-regulated and 8 029 were uniquely salt-regulated. The resulting list was compared to a reference list of duplicate gene pairs detected in the salmon genome (Lien et al., 2016). The duplicated gene pairs where divided in into categories, based on the combinations of light, salt, or dually regulated genes. Categories were as follows: Light (both light-regulated), Salt (both salt-regulated), LS-LS (both genes regulated by both light and salt), LightX (one gene regulated by light, the other unknown), SaltX (one regulated by salt, the other unknown), Divided (one gene regulated by light, the other by salt), Light-LS (one gene regulate by light, the other by both light and salt), Salt-LS (one gene regulated by salt, the other by both light and salt), LS-X (one gene regulated by both light and salt, the other by unknown factor), XX (both genes regulated by unknown factors).

The ratio of nonsynonymous substitutions per nonsynonymous site (d_N) and synonymous substitutions per synonymous site (d_S) for each gene pair was calculated in order to check for stabilizing or positive selection. The analysis did not show a tendency towards positive selection for advantageous mutations. Among pairs regulated by photoperiod and/or salt 21 did show a $d_N/d_S>1$, however, further statistic testing placed these as outliers.

Differential expression could also arise from changes in promoters/transcription factors and enhancers, influencing regulation of transcription and tissue specificity. In several of the differentially expressed duplicate pairs, regulation of timing and tissue specificity might be more important for optimization of function than the protein itself. Also, positive selection in one specific domain of a gene could be masked by very strong stabilizing selection in the other coding regions. A sliding-window approach to d_N/d_S would then be more suitable.

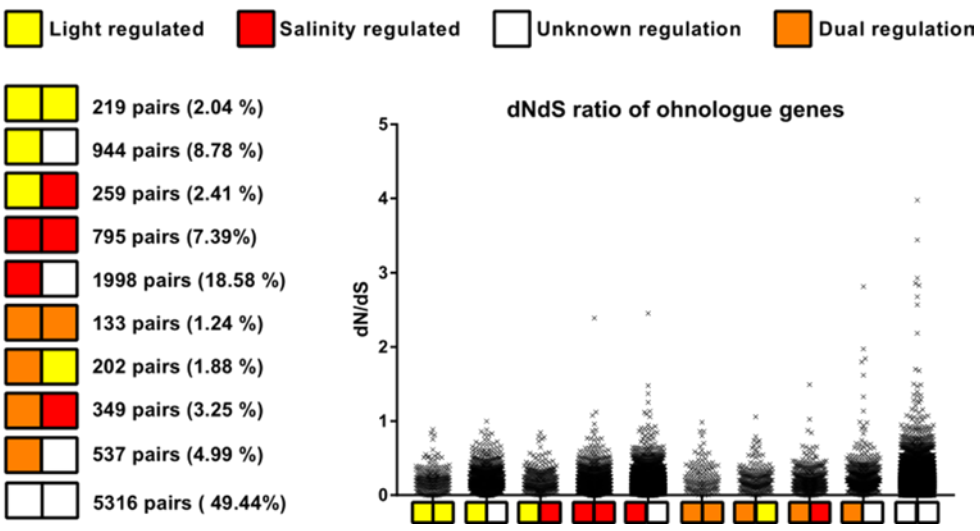

Figure i Showing the regulation of expression of ohnologue gene pairs, and the range of dN/dS ratios.

The three subfamilies of the Salmonidae split 50-40 MYA. While Thymallinae species have remained strictly limnetic, small-scale anadromy developed independently in Salmoninae and Coregoninae, 30-20 and 25-20 MYA, respectively (Alexandrou, Swartz, Matzke et al., 2013). Anadromous Coregininae typically restrict themselves to estuaries and coastal areas, displaying small-scale anadromy, while several salmonids within the Salmoninae have been shown to venture long distances at sea, exhibiting large-scale anadromy (Alexandrou, Swartz, Matzke et al., 2013; Strøm, Thorstad, Hedger et al., 2018). A popular hypothesis is that anadromy developed in FW residing Salmonina and Coregoninae in response to reduced productivity in lakes and rivers due to the climatic cooling , driving the fish to take advantage of the rich, marine production (Dodson, Laroche, and Lecomte, 2009; Ramsden, Brinkmann, Hawryshyn et al., 2003). It is believed that full SW adaptation was prevented due to the FW requirements of eggs and juveniles (Dodson, Laroche, and Lecomte, 2009; Gross, Coleman, and McDowall, 1988).

Anadromy is considered a driver of genetic isolation as one of its traits is specific homing, leading to reproductive isolation, promoting local specialization (McDowall, 2001). However, the degree of local adaptation and reproductive isolation between Atlantic salmon populations is uncertain (Fraser, Weir, Bernatchez et al., 2011; Garcia de Leaniz, Fleming, Einum et al., 2007; Taylor, 1991; Thorstad, Whoriskey, Rikardsen et al., 2010). It is possible that an isolative effect of specific homing helped accelerate salmonid species radiation together with LORe (Macqueen and Johnston, 2014; Robertson, Gundappa, Grammes et al., 2017). Macqueen and Johnston (2014) present data suggesting that more than 50% of Salmonidae species only developed over the last five million years, placing most of the species radiation after the development of anadromy. Studies of charr populations have shown them capable of rapid segregation into reproductively isolated morphs, indicating that delayed rediploidization might be reinforcing genetic isolation driven by niche segregation (Jonsson and Jonsson, 2001; Kapralova, Morrissey, Kristjánsson et al., 2011; Macqueen and Johnston, 2014; Skúlason, Snorrason, and Jónsson, 1999).

1.1.3 Life history of Atlantic salmon

Salmonids display a wide variation in types of life history strategies, including limnetic, marine and a range of anadromous lifestyles. Even within species, life histories can vary greatly due to the high plasticity exhibited among individuals. Many salmonids, such as the Arctic charr exhibit highly divergent morphs that vary in primary prey and habitat use within the same population (Garduño-Paz and Adams, 2010; Jonsson and Jonsson, 2001; Skúlason, Snorrason, and Jónsson, 1999). The Atlantic salmon is much less plastic, and mainly show variation in the timing of life history and

transitional events which can be linked to growth rate and environmental conditions such as temperature (Gurney, Bacon, Speirs et al., 2012; Johnston, McLay, Abercromby et al., 2000; Thorpe, 1989; Thorpe, Mangel, Metcalfe et al., 1998).

Mature Atlantic salmon mate and lay their eggs in the gravel of riverbeds in late autumn (Oct.-Dec.) (Fleming, 1996). Eggs hatch the following spring (May-Apr.). The alevin (i.e. the yolk-sack stage) remain hidden in the gravel and feed off the remaining yolk sack for some time before emerging from the riverbed (Fleming and Einum, 2010). At this stage, they are known as fry. The fry quickly grow and develop into parr, characterised by the prominent dark marking along the side of the body (see fig. 1). The parr is benthic and territorial (Armstrong, Huntingford, and Herbert, 1999; Keeley and Grant, 1995; Steingrímsson and Grant, 2008). Drift feeding is the primary feeding mode (Stradmeyer and Thorpe, 1987), although surface feeding and piscivory have also been observed (Cunjak, 1992; Erkinaro, Shustov, and Niemela, 1998). The period of time spent as parr varies both among and within populations (Klemetsen, Amundsen, Dempson et al., 2003). However, there is a clear latitudinal gradient, where Arctic populations can spend more than five years as parr, contrasting with lower latitude populations spending one to three years as parr (Klemetsen, Amundsen, Dempson et al., 2003). There is also large variation within cohorts, reflecting maternal effects and feed access during the fry and parr stage (Forseth, Letcher, and Johansen, 2010).

The length of the parr stage is influenced by size, growth rate and metabolic status (Rowe, Thorpe, and Shanks, 1991; Stefansson, Björnsson, Ebbesson et al., 2008; Thorpe, 1994b; Thorpe, Mangel, Metcalfe et al., 1998; Økland, Jonsson, Jensen et al., 1993). In autumn, the parr can enter one of two new developmental trajectories, precocious maturation or smolting (pre-adaption for migration to SW), or it may remain as a parr for another year (Thorpe, 1994a). It is hypothesized that a main gating window exist in autumn, and that this is followed by assessment windows and the possibility of redirecting efforts during late winter and early spring (Thorpe, Mangel, Metcalfe et al., 1998). Several authors (Heggenes and Metcalfe, 1991; Kristinsson, Saunders, and Wiggs, 1985; Thorpe, 1977; Thorpe, Talbot, and Villarreal, 1982) have described a bimodal size frequency distribution arising in the autumn. Larger parr, the upper mode (UM), accelerate growth and enter the smolting trajectory, while parr below failing to meet the size threshold, lower mode (LM), remain as parr. Precociously sexually maturing individuals would originally be among the fastest growing fish prior to the decision window; however, they join the LM after gonadal development begins (Kristinsson, Saunders, and Wiggs, 1985; Saunders, Henderson, and Glebe, 1982). The UM juveniles continue to actively feed and grow, while LM parr reduce feeding to a maintenance level, discontinue growth,

and eventually becomes quiescent in anticipation of the next spring (Metcalfe, Huntingford, and Thorpe, 1986; Metcalfe, Huntingford, and Thorpe, 1988).

The hallmarks of smoltification are well described in literature (Folmar and Dickhoff, 1980; McCormick, 1994; McCormick, Hansen, Quinn et al., 1998; McCormick and Saunders, 1987; Pisam, Prunet, Boeuf et al., 1988; Prunet, Boeuf, Bolton et al., 1989; Stefansson, Björnsson, Ebbesson et al., 2008; Wedemeyer, Saunders, and Clarke, 1980). As photoperiod increases in early spring the UM parr initiates the physiological transformation (Björnsson, Thorarensen, Hirano et al., 1989; Duston and Saunders, 1990; McCormick, Björnsson, Sheridan et al., 1995; Skilbrei, 1991; Stefansson, Bjornsson, Hansen et al., 1991), gradually becoming SW adapted. LM parr are unresponsive to the photoperiodic stimulus, and retain their parr appearance (McCormick, Shrimpton, Moriyama et al., 2007).

The smolting salmon lose their territoriality and positive rheotaxis, and start running together in shoals (Stefansson, Björnsson, Ebbesson et al., 2008). They become silvered, with a somewhat darker shade on their dorsal side, and a brighter hue ventrally (Johnston and Eales, 1967; Staley and Ewing, 1992). This form of countershading is common among marine fishes inhabiting the pelagic zone. Condition factor is typically reduced, resulting in a more elongated body shape allowing for more efficient swimming (Wedemeyer, Saunders, and Clarke, 1980). The physiological changes described in literature so far are primarily linked to changes in hormone levels and osmoregulatory function, focusing on the gill. However, alterations in the LBP axis, metabolism, the gastrointestinal tract (GIT) and olfactory epithelium have also been described (Ebbesson, Ekström, Ebbesson et al., 2003; Higgins, 1985; Lema and Nevitt, 2004; McCormick and Saunders, 1987). Smolting culminates in the migration to sea, occurring between April and July depending on latitude. The process of smolting will be presented in more detail in section 1.3.

Salmon will spend one to five years at sea (Thorstad, Whoriskey, Rikardsen et al., 2010), where they lead a pelagic life. They have recently been shown to migrate over long distances in the North Atlantic Ocean, reaching Svalbard and surrounding areas, from the coast of Norway (Hayes and Kocik, 2014; Hedger, Rikardsen, Strøm et al., 2017; Jensen, Karlsson, Fiske et al., 2014; Strøm, Thorstad, Hedger et al., 2018). Timing of maturation and homing (May-Oct.) appears to be linked to growth (Jonsson, Hansen, and Jonsson, 1991; Jonsson and Jonsson, 2007; Aas, Einum, Klemetsen et al., 2010). Maturing salmon are believed to use geomagnetism and olfaction to guide their way back to their native river (Hasler, Scholz, and Horrall, 1978; Keefer and Caudill, 2014; Moore, Freake, Thomas et al., 1990; Putman, Scanlan, Billman et al., 2014; Stabell, 1984). A large proportion of

Atlantic salmon survive the strenuous mating season and migrate back to sea, however, only a reduced number of these fish (but very variable between rivers and years) live to return to the river again due to high mortality at sea (Fleming, 1996; Jonsson and Jonsson, 2004; Niemelä, Erkinaro, Julkunen et al., 2006).

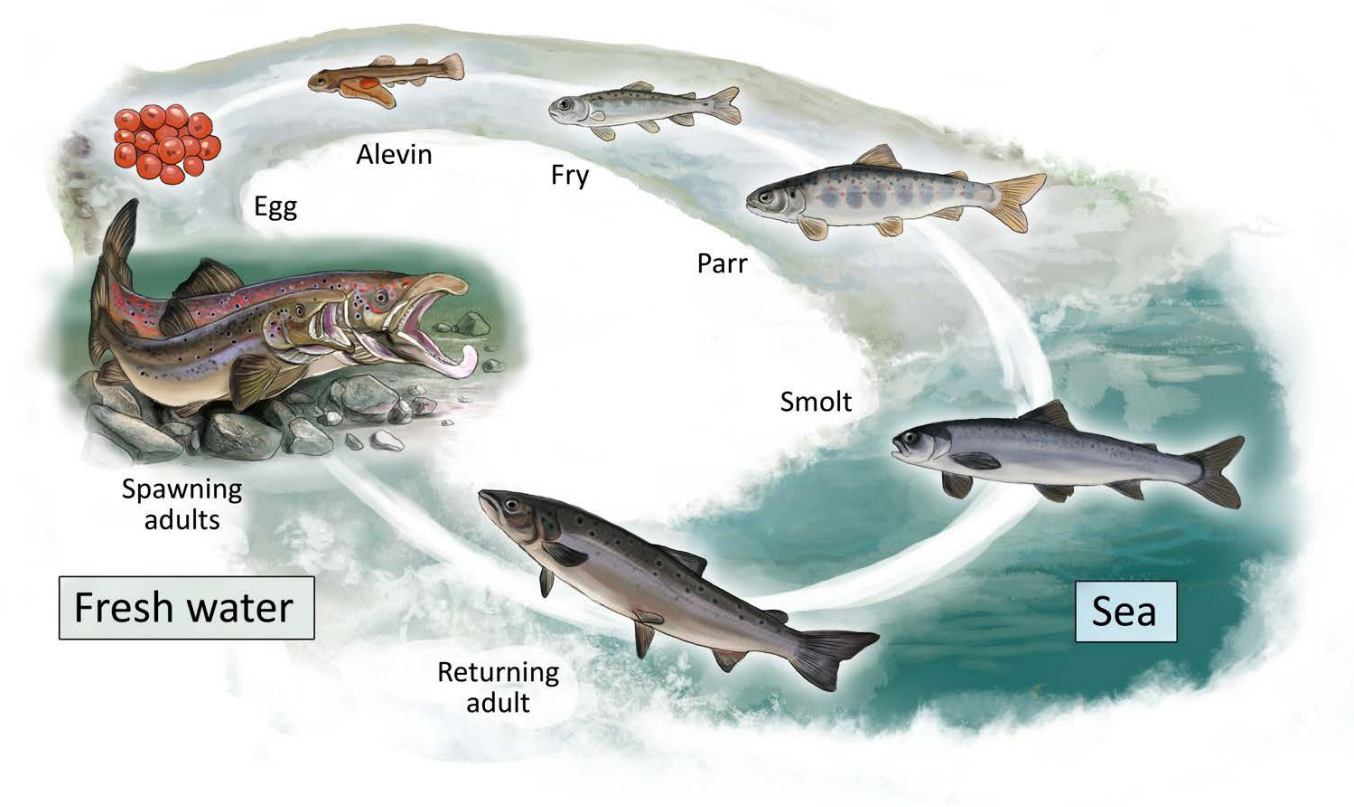

Figure 2 *The life cycle of the Atlantic salmon. Illustration by Jamie van Dalum.*

1.2 Chronobiology of fishes

Light is essential to life on Earth (Björn, 2015). It provides the energy with which the building blocks of life are created. Light is also rhythmic. It changes diurnally with the Earth's rotation around its own axis, and it changes seasonally as the tilted earth moves around the Sun. This continuous cycling between day and night, and through the seasons, creates predictable changes in access to abiotic and biotic factors. This predictability has served as the ultimate driver in the evolution of anticipatory timing mechanisms central to the survival of any organism. Through the activity of cyclical molecular mechanisms influenced by the exogenous rhythms of the environment, organisms are able to anticipate rhythmic changes in their habitat, thereby coordinating behaviour and physiology in order to optimize survival and propagation (Dunlap, Loros, and DeCoursey, 2004). In fish, this innate timing influences daily activity patterns (locomotor activity, schooling behaviour, rest), skin pigmentation, food intake, and seasonal timing of development and life-history events such as growth, migration,

and reproduction (Reebs, 2002; Zhdanova and Reebs, 2005). The mechanisms underlying this innate timing are referred to as biological clocks, and exist across all taxa from single-celled prokaryotes to giant sequoia trees and humans (Dunlap, Loros, and DeCoursey, 2004).

1.2.1 Biological clocks

In its most basic form, the biological clock consists of one or more central oscillators setting an endogenous rhythm, a pathway communicating sensory input, and a means of communicating the rhythm to the peripheral systems. While the physiological and even molecular structure of the clock might differ between organismal groups, all biological clocks have three essential qualities:

1. Continued rhythmicity in constant conditions due to the continued oscillations of a self-sustaining system (endogenous)
2. Temperature compensation[1]
3. Ability to entrain to external cues, so that the clock is in phase with the environmental rhythm

The most studied biological clock is circadian (box 2), operating with a period of approximately 24 hours, however other biological clocks such as the circatidal clock are also described (de la Iglesia and Johnson, 2013). For circadian timing, the main oscillator and endogenous timekeeper is a brain nuclei called the suprachiasmatic nucleus (SCN). The timing mechanism of circadian clocks consists of chemical interactions among proteins, DNA, and mRNA, involving complex feedback loops and neural and hormonal signalling. So far, this region has only been identified in mammals and birds, however, similar structures have been localized in the brains of teleosts (Falcón, Migaud, Muñoz-Cueto et al., 2010). The SCN receives time-giving information from photosensitive organs such as the pineal gland (hormonal and nervous input), eyes (nervous input), and deep-brain photoreceptors (reptiles and birds, nervous input) (Dunlap, Loros, and DeCoursey, 2004). While the SCN appears to be central to rhythmicity in mammals, its role appears to be less clear in birds and teleosts (Moore and Whitmore, 2014; Noche, Lu, Goldstein-Kral et al., 2011; Patton and Hastings, 2018).

1.2.2 Circannual rhythms

Circannual rhythms, exhibiting a period of approximately twelve months, allows animals to anticipate and prepare for seasonal changes in temperature, precipitation, feed access and reproductive events

[1]Temperature compensation is the ability to maintain the period length of a rhythm despite the influence of temperature on biomolecular processes.

through migration, moulting, deposition of fat, and gonadal development, amongst other behaviours and processes. Photoperiod is known to be the most significant external signal influencing circannual rhythms, linking the circadian and circannual time keeping systems together (Dardente, Wyse, Birnie et al., 2010; Ikegami and Yoshimura, 2013). It is still not entirely clear which endogenous mechanism maintain circannual rhythmicity under constant conditions, however, the

BOX 2 THE CORE CIRCADIAN CLOCK

The basic oscillator of any circadian system is based on translational-transcriptional feedback loops where mRNA and protein levels of the circadian genes oscillate over a period of approximately 24 hours. The loop originates from the transcription and translation of *Bmal1* and *Clock*. The two proteins form a heterodimer which binds to the promoter region of *Per* and *Cry*, activating their transcription. The resulting proteins also form a dimer, inhibiting the activity of Clock-Bmal, negatively affecting further transcription of *per* and *cry.* Bmal-Clock also activates the transcription of *rev-erb α*, a transcription factor inhibiting the transcription of *Bmal*. While this core clock loop is self-sustaining, the phase of the clock can be set to correspond with photoperiod, as light induces the transcription of *per* and *cry* through the activation of Tef transcription factors. The Bmal-Clock dimer also binds to the promoters of clock-controlled genes, enabling transcription. This group of genes is considered the output of the clock, and the first products in a chain enabling the core clock to communicate time to other tissues.

A significant output from the core clock is the production of *Aanat* through transcriptional activation by the Bmal-Clock dimer. This gene transcribes into the rate-limiting enzyme of the melatonin synthesis. Up to three *Aanat* genes have been reported in fish, however only one appear to be expressed in the pineal gland. AANAT can only accumulate in the dark, because only then is there enough cAMP activity to drive phosphorylation of AANAT resulting in the pAANAT forming a complex with the 14-3-3 protein. This stabilizes pAANAT and prevents its degradation by proteasomal enzymes. By controlling AANAT via the endogenous rhythmicity of Bmal-Clock and Per-Cry, most organisms are able to maintain an endogenous rhythm of melatonin secretion, even under constant darkness. The light activation of *Per* and *Cry*, and light sensitivity of AANAT, allows for the endogenous rhythm and melatonin secretion to phase align to the ambient photoperiod, enabling the organism to anticipate light-dark shifts and behave accordingly.

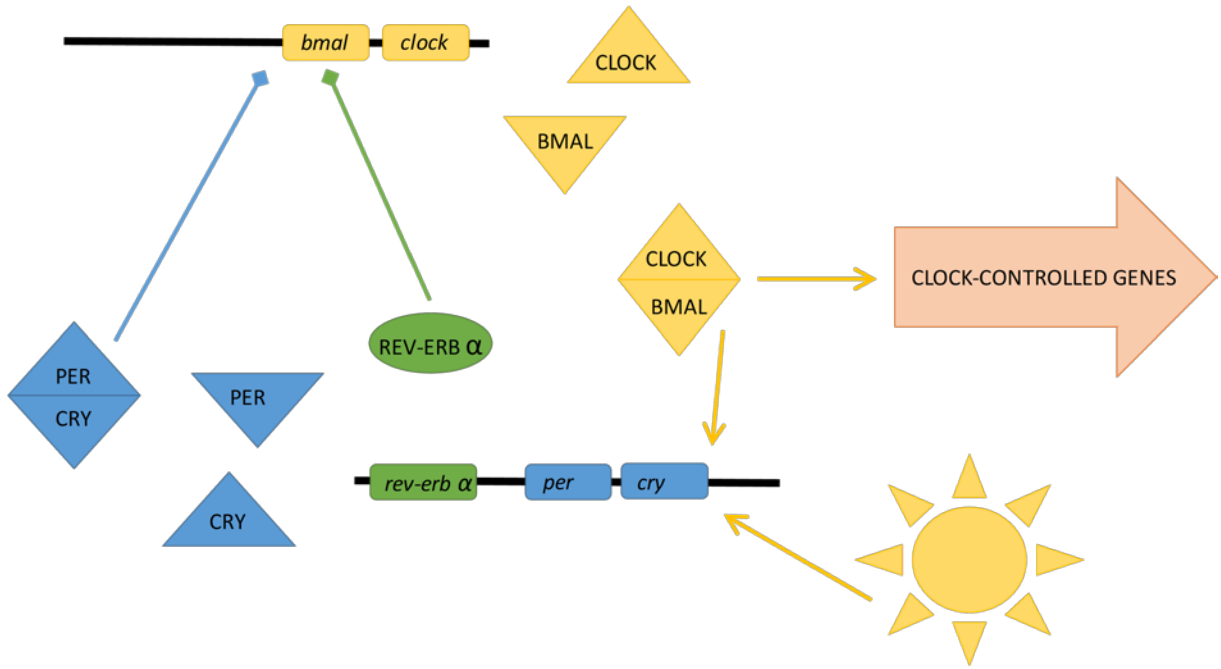

Figure ii Overview of the core circadian clock

Sources: Isorna et al. 2017, Vatine et al. 2011, Falcón et al. 2011

thyroid hormone axis and neural differentiation within the pituitary is implied (Ikegami and Yoshimura, 2013; Nakao, Ono, Yamamura et al., 2008; Nakao, Ono, and Yoshimura, 2008). While significant findings have been presented for mammals and birds (Hazlerigg and Lincoln, 2011; Lincoln and Hazlerigg, 2014; Sáenz de Miera, Monecke, Bartzen-Sprauer et al., 2014; Yoshimura, Yasuo, Watanabe et al., 2003), much less effort have been placed into understanding the physiological and molecular timekeeping within teleosts (Davie, Minghetti, and Migaud, 2009; Nakane, Ikegami, Iigo et al., 2013).

Circannual rhythms can be divided into two categories, however, they coexist on a continuous scale (Goldman, Gwinner, Karsch et al., 2004). Type I rhythms have elements of endogenous timing mechanisms but are heavily dependent on exogenous components for the rhythm to persist. Type I rhythms are most frequently observed among opportunistic and short-lived species (<2 yrs.). The true circannual rhythms, type II, have a strong endogenous component, and continue to run persistently under constant conditions. In the case of type II rhythms exogenous cues serve as synchronizers rather than permissive signals. Such strong, persistent rhythms are only observed among long-lived species (>2 yrs.). A variation on the circannual rhythms are the gated events, i.e. important developmental events that only occur once in the lifespan of an organisms. These important transitions are usually tightly linked with specific times of the year, such as the emergence of certain insects (Miyazaki, Nisimura, and Numata, 2014; Nisimura and Numata, 2003).

The smolting of Atlantic salmon is considered a gated event, requiring certain metabolic and growth-related criteria to be met (Kristinsson, Saunders, and Wiggs, 1985; Skilbrei, 1991; Thorpe, 1989; Økland, Jonsson, Jensen et al., 1993) before changes in photoperiod can induce and synchronize the process (McCormick, Shrimpton, Moriyama et al., 2007). Evidence have been presented that certain smolt-related traits behave cyclically under constant conditions (Eriksson and Lundqvist, 1982; Wagner, 1974), however, the traits appear to cycle in an uncoordinated manner.

1.2.3 The light-brain-pituitary axis of teleosts

The light-brain-pituitary (LBP) axis begins with the perception of light. One significant effect of light is the inhibition of melatonin synthesis (see box 2). Melatonin functions as a communicator of temporal information (Falcón, Migaud, Muñoz-Cueto et al., 2010; Isorna, Pedro, Valenciano et al., 2017). It is synthesised in the pineal gland, and disseminated within the brain via the cerebrospinal fluid (CFS), and to the peripheral systems through the circulatory system (Falcón, Besseau, Magnanou et al., 2011; Falcón, Migaud, Muñoz-Cueto et al., 2010).

Migaud, Davie, Martinez Chavez et al. (2007) identified three possible pathways for the regulation of pineal melatonin synthesis in teleosts. One pathway is similar to the pathway established in mammals. Light is perceived via the retinas of the eyes, and the photic information is communicated to the SCN, which signals to the pineal gland controlling melatonin synthesis. The second pathway carries more similarity to the pathway of birds and reptiles, where both the pineal gland and visual input is necessary to maintain the amplitude of the melatonin rhythm. In both these pathways, melatonin secretion is controlled by a pineal endogenous circadian clock, or oscillator, which maintains cyclic melatonin synthesis under constant darkness. In the third pathway, which is exclusive to the salmonid linage, the pineal synthesis of melatonin occurs as an acute response to darkness and is independent of any endogenous oscillator (Bolliet, Ali, Lapointe et al., 1996; Iigo, Abe, Kambayashi et al., 2007; McStay, Migaud, Vera et al., 2014).

Melatonin secreted from the pineal gland conveys information about photoperiod through binding to G-protein-coupled melatonin receptors (Falcón, Besseau, Magnanou et al., 2011; Falcón, Besseau, Sauzet et al., 2007; Falcón, Migaud, Muñoz-Cueto et al., 2010; Reppert, 1997). Three classes of melatonin receptors have been verified in teleosts, MT1, MT2 and Mel1c. In some fish species there are multiple paralogues of each of the melatonin receptors. MT1 and MT2 are widely distributed in the CNS and peripheral tissues, whereas Mel1c appears limited to the skin and retina. Melatonin levels do not only indicate the passing of night and day, variations in duration are accompanied by changes in amplitude which provide seasonal information (Masuda, Iigo, Mizusawa et al., 2003; Reiter, 1993; Vera, López-Olmeda, Bayarri et al., 2005).

In fish, as in birds and mammals, pineal melatonin acts on brain areas of the hypothalamus-pituitary axis (Isorna, Pedro, Valenciano et al., 2017). In particular the preoptic area (POA) has received attention as it is considered a putative homologue to the mammalian SCN by some authors (Falcón, Besseau, Magnanou et al., 2011; Falcón, Migaud, Muñoz-Cueto et al., 2010). The POA is situated in the hypothalamus, above the pituitary gland. The POA receives rapid nervous input on illumination from the retina and the pineal, and temporal information from the pineal melatonin signal. The integrated information is conveyed to the pituitary, affecting pituitary hormone secretion via hormone releasing factors (Falcón, Besseau, Magnanou et al., 2011). Hormones such as growth hormone (GH), prolactin (PRL), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (thyrotropin, TSH), are then transported via the circulatory system to their respective target organs, affecting among other things growth, feeding, behaviour, and reproductive status. However,

evidence have also been presented pointing to a functional role of the teleost SCN as an circadian regulator (Watanabe, Itoh, Mogi et al., 2012).

In birds and mammals evidence has accumulated that the pars tuberalis (PT), a specific region if the pituitary, functions as a seasonal sensor and regulator of seasonal activities (box 3) such as reproduction, moulting and hibernation (Dardente, Hazlerigg, and Ebling, 2014; Wood and Loudon, 2018). Teleosts do not exhibit a distinct PT, and in its place the saccus vasculosus (SV) has been put forward as a potential seasonal regulator (Maeda, Shimo, Nakane et al., 2015; Nakane, Ikegami, Iigo et al., 2013). Nakane, Ikegami, Iigo et al. (2013) fund that the coronet cells of the SV expressed opsins, TSHβ and deiodinase 2 (dio2). The presence of opsins indicate that the coronet cells of the SV are light sensitive, in addition to expressing significant components of the seasonal signalling of the PT (box 3). Assuming that the SV is analogous to the PT, TSH affects the regulation of deiodinases locally, and potentially in nearby tissues, thereby regulating levels of active TH (see box 3), thus influencing neurological structure in a seasonal manner. However, whether the SV actually represents an analogous structure to the PT remains inconclusive.

1.3 Gill function in FW and SW –opposing forces

The gill is critical to the maintenance of homeostasis in fish. Maintaining homeostasis requires control of four tasks, namely respiration, acid-base regulation, excretion of urea and osmoregulation (Evans, Piermarini, and Choe, 2005). The gill is a highly efficient organ in performing these four tasks due to its large surface area and the close contact formed between the circulating blood and external media. Fish inhabit a wide variety of aquatic habitats that differ in many aspects such a temperature, flow, substrate, nutrient levels, oxygen levels, pH and salinity. While some species are euryhaline and can cope with varying salinities most fish are obligate FW or SW species. In FW the fish produce high amounts of urine to get rid of excessive water gained through osmotic uptake. While there is a high reabsorption of ions in the renal system, the gill actively compensates for the net salt loss through active ion uptake (Evans, Piermarini, and Choe, 2005). In SW the massive salt-intake caused by drinking (to compensate for osmotic loss of water), intestinal uptake, and diffusion is countered by active extrusion of NaCl (sodium chloride) across the gill epithelium, while water retainment is achieved by production of small amounts of highly concentrated urine (Evans, Piermarini, and Choe, 2005).

BOX 3 SEASONAL ROLE OF THE PARS TUBERALIS

The classic hypothalamic-pituitary-thyroid axis consists of multiple feedback loops controlling thyroid hormone (TH) secretion from the thyroid gland and metabolism in target tissues. The hypothalamus secretes thyrotropin-releasing hormone (TRH), which causes the release of thyroid-stimulating hormone (thyrotropin, TSH), from the PD of the pituitary. TSH is circulated to the thyroid gland, which releases thyroxin (T_4), the inactive form of TH. While some triiodothyronine (T_3), the active form of TH, is also released, the majority of TH is only activated once it reaches its target tissue. There, locally controlled deiodinase enzymes balance the activity of TH. Deiodinase 2 (dio2) metabolises T_4 to T_3, and deiodinase 3 (dio3) inactivates T_3 (it can also remove T_4 directly by metabolising it to an inactive isomer of T_3)

The pars tuberalis (PT) however, lacks receptors for TRH, and the production of TSH is controlled by melatonin via its effect on the expression of *Eya3*. *Eya3* is a strongly clock-controlled gene, whose expression is in a set relationship with the onset of *Cry1*-expression (see box 2). During SP, the expression of *Eya3* is inhibited by the presence of melatonin, while under LP the high expression of *Eya3* increases the expression of *TSHβ* from PT-specific thyrotrope cells. The TSH released from the pituitary acts upon the medio-basal hypothalamus (MBH), regulating the expression of deiodinase enzymes, thus influencing the presence of active TH in the MBH. The activity of T_3 is believed to influence cellular activity and the nervous structure of the hypothalamic nuclei and the median eminence in a seasonal manner.

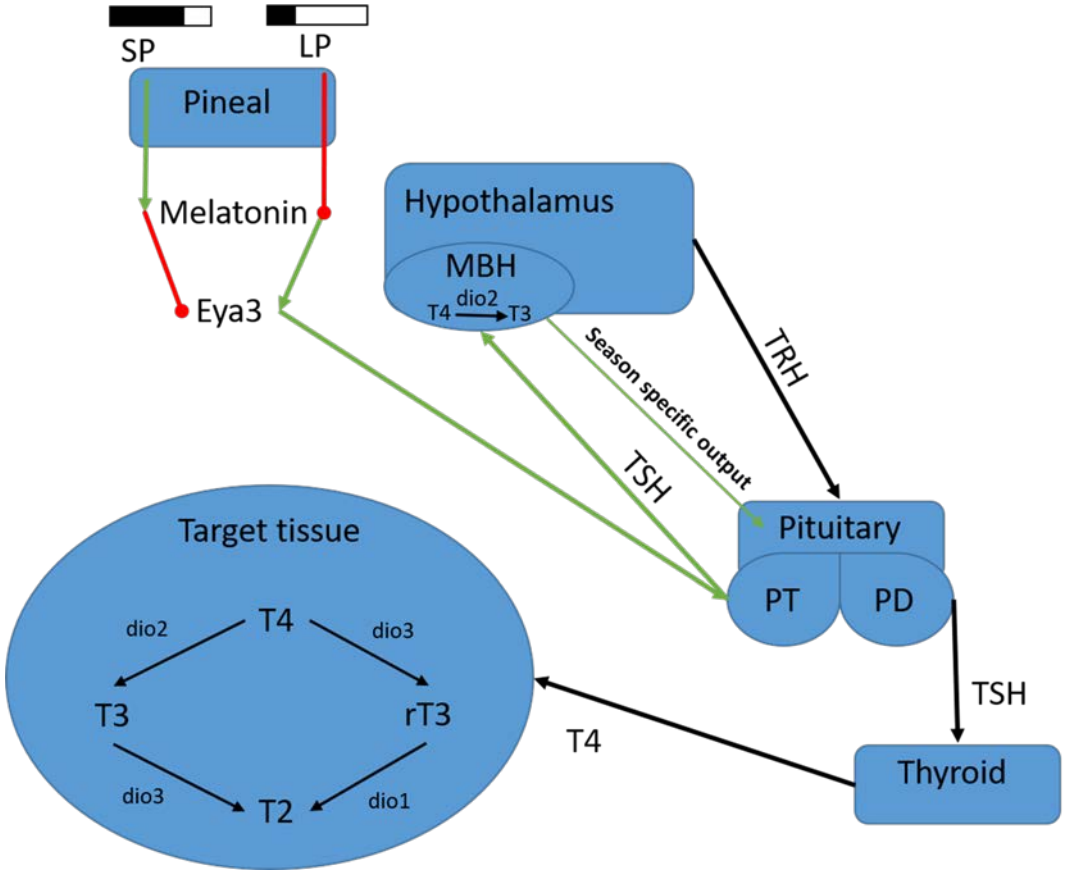

Figure iii The hypothalamic-pituitary-thyroid axis

Source: Nakao et al. 2008, Dardente et al. 2010, Dardente et al. 2014

1.3.1 Gill physiology

The main structure of the gill (fig.2) are the holobranchs (of which there are four), each consisting of a gill arch to which a pair of hemibranchs are attached (Barton and Bond, 2007). The hemibranchs consist of a column of gill filaments (primary filaments), where each filament is equipped with multiple, horizontally spaced lamella (also known as secondary filaments)(Barton and Bond, 2007). The lamella are important for maximising the epithelial surface of the gill and optimizing gas- and ion-exchange. Each hemibranchs is supplied with blood (cardiac output) via the afferent branchial artery, stretching through the gill arch (Barton and Bond, 2007; Evans, Piermarini, and Choe, 2005). Blood is circulated into the gill filaments via the afferent filamental artery and flows across the lamellar sinusoids into the efferent filamental artery, which drains into the efferent branchial artery (Barton and Bond, 2007; Evans, Piermarini, and Choe, 2005). The oxygenated blood then enters the systemic circulation via the dorsal aorta (Barton and Bond, 2007; Evans, Piermarini, and Choe, 2005).

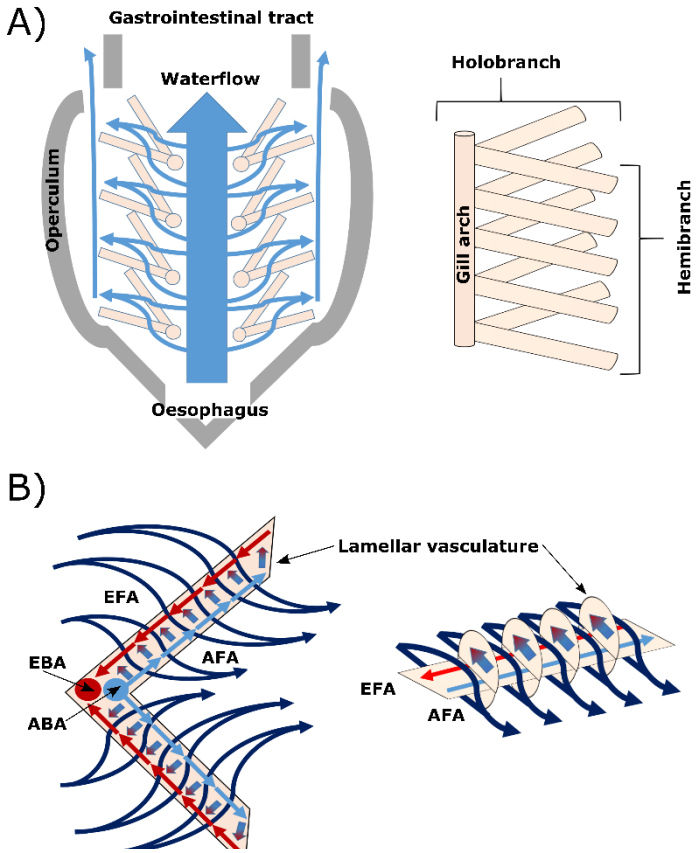

Figure 3 *Overview of (A) gill morphology showing the waterflow across the gill, and the structure of a holobranch and (B) the primary filaments and vasculature of a teleost fish, also illustrating water flow and blood flow across he lamellae, EFA –efferent filamental artery, AFA –afferent filamental artery, EBA –efferent branchial artery, ABA –afferent branchial artery.*

The gill is constructed so that water coming from the oesophagus will pass through the gill filaments and their lamella in opposite direction of the blood flow across the lamellae. This counter-current system greatly improve gas exchange as there will always be a difference in concentration between the blood and the water (Barton and Bond, 2007; Evans, Piermarini, and Choe, 2005). Gill perfusion is mainly controlled by adrenergic neurons, with serotonergic neurons also controlling perfusion of the more proximal filamental regions (Evans, Piermarini, and Choe, 2005). Angiotensin, arginine vasotocin and bradykinin are also involved, regulating vascular constriction and perfusion together with natriuretic peptides (Evans, Piermarini, and Choe, 2005). The filamental and lamellar surface consist mainly of two different epithelial

cell types; the pavement cells (PVC), and mitochondria-rich cells (MRC) (Evans, Piermarini, and Choe, 2005; Wilson and Laurent, 2002). The latter is, as the name implicates, characterized by its numerous mitochondria, and an extensive basolateral tubular system (Stefansson, Björnsson, Ebbesson et al., 2008). While respiration occurs over the PVCs of the lamellae, ion exchange (osmoregulation) is known to occur across the MRCs (Evans, Piermarini, and Choe, 2005). MRCs are found both on the lamella, and in the interlamellar space, however their precise distribution may vary (Evans, Piermarini, and Choe, 2005). MRCs are relatively large, oval cells that are rich in mitochondria. In SW they co-occur with accessory cells (AC). MRCs are highly polarized cells, with distinct apical and basolateral morphologies (Evans, Piermarini, and Choe, 2005). While various subtypes have been described in the literature, these appear to vary between species and habitat (Evans, Piermarini, and Choe, 2005; Hiroi and McCormick, 2012; Hwang and Lee, 2007); I will focus on the main functional and morphological differences between FW and SW MRCs below.

Among the other cell types inhabiting the gill we find epithelial and endothelial cells, pillar cells, mucus cells and neuroepithelial cells (Wilson and Laurent, 2002).

1.3.2 Osmoregulation in freshwater

FW-MRCs are in general smaller than the SW form and have a less extensive tubular network. They also have microvilli protruding from the apical side (Evans, Piermarini, and Choe, 2005; Hwang and Lee, 2007; Pisam, Prunet, Boeuf et al., 1988). FW-MRCs are associated with PVCs, with which they form multistranded intercellular junctions that are impermeable to ions, reducing ionic loss to FW (Evans, Piermarini, and Choe, 2005). There are two dominant models of Na+ uptake in FW-MRCs (Evans, Piermarini, and Choe, 2005; Hwang and Lee, 2007); i) the ENaC model where an apical V-type ATPase moves H+ outwards, and drives the electrophoretic uptake of Na+ via an epithelial Na+ channel, ii) the NHE model where there is electroneutral exchange of Na+ and H+ via an apical Na+/H+ exchanger, which is potentially driven by carbonic anhydrase (CA) IV. Both systems might be active in the gill; however there are still discrepancies leaving the understanding of FW-MRCs incomplete (Hwang, Lee, and Lin, 2011). Chloride uptake is executed through apical Cl^-/HCO_3^- anion exchangers, this process is driven by a V-type H+ ATPase anion exchanger and CA II (Hwang and Lee, 2007; Hwang, Lee, and Lin, 2011). However, Na-K-2Cl- cotransporter (NKCC) has also been implicated in this process. The chloride ions diffuse across the cell and is extruded into the extracellular fluid via the cystic fibrosis transmembrane conductance regulator (CFTR) channel. On the basolateral side of FW MRCs one also finds the sodium-potassium ATPase (NKA) pump, which exchanges intracellular Na+ for extracellular K+ (Evans, Piermarini, and Choe, 2005; Stefansson, Björnsson, Ebbesson et al.,

2008). It is believed that the mechanisms controlling osmolality in FW originally developed as a component of acid-base regulation. There is also uptake of divalent ions such as Ca^{2+}, Mg^{2+} and Zn^{2+} across the MRCs, however less is known about the precise mechanisms for this (Evans, Piermarini, and Choe, 2005; Hwang and Lee, 2007). FW-MRCs also contain far more aquaporins (AQP), small pore-forming water-permeable membrane proteins (Kruse, Uehlein, and Kaldenhoff, 2006), which contribute to make FW-MRCs more permeable to water than SW-MRCs (Evans, Piermarini, and Choe, 2005).

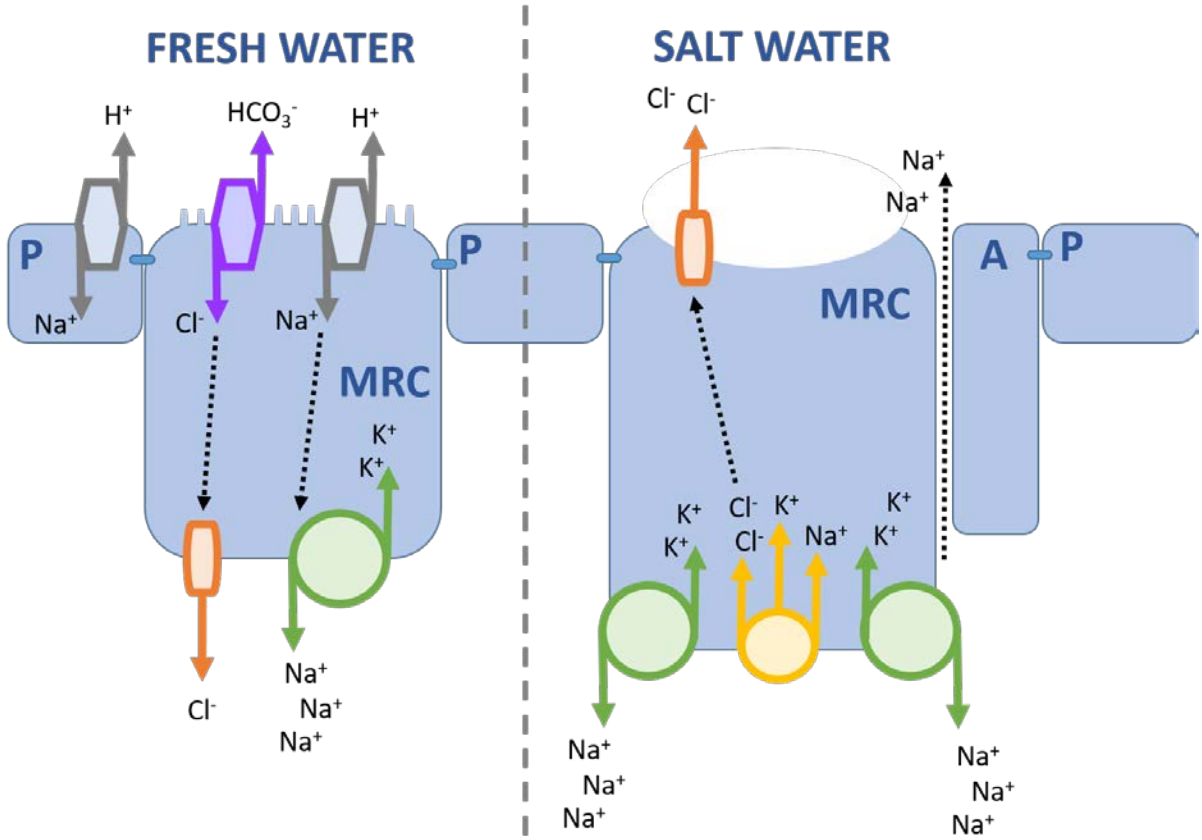

Figure 4 *Simplified diagram of FW and SW MRCs. P = PVC, A = AC*

1.3.3 Osmoregulation in saltwater

In SW, osmotic loss of water is compensated through increased drinking and intestinal NaCl-uptake in order to draw water from the intestinal lumen (Evans, Piermarini, and Choe, 2005). There is also diffusional salt-uptake across the gill. The increased salt-intake is countered by the activity of the SW-

MRC, extruding salt across the gill epithelium[2] (Evans, Piermarini, and Choe, 2005; Hwang and Lee, 2007).

SW-MRCs are larger than the FW-MRCs, and are characterized by a much more extensive and denser tubular network and a deep apical crypt (Evans, Piermarini, and Choe, 2005; Hwang and Lee, 2007; Pisam, Prunet, Boeuf et al., 1988; Stefansson, Björnsson, Ebbesson et al., 2008). They form close associations with ACs, with which they share leaky paracellular pathways (Chasiotis, Kolosov, Bui et al., 2012; Pisam, Prunet, Boeuf et al., 1988). Just below the apical membrane of SW-MRCs is a tubulo-vesicular system compromised of numerous vesicles and tubules involved in the transport of ion channels and proteins to the apical membrane (Evans, Piermarini, and Choe, 2005). SW-MRCs and the ACs are also associated with PVCs, with which they form tight junctions impermeable to ions (Evans, Piermarini, and Choe, 2005; Pisam, Prunet, Boeuf et al., 1988).

The tubular system on the basolateral side of the SW MRC is rich in NKA pumps, which are responsible for generating the electrochemical gradient driving the extrusion of ions from the cell (Evans, Piermarini, and Choe, 2005; Hiroi and McCormick, 2012; Hwang and Lee, 2007). As the NKA pump extrudes Na+ across the basolateral membrane an electrochemical gradient is created driving the basolateral NKCC to transfer chloride ions into the cell (together with Na+ and K+). The chloride ions diffuse across the cell and are actively extruded by CFTR channels in the apical membrane, generating a negative electrical load in the apical crypt (trans-epithelial electrical potential) which drive the extrusion of sodium ions via the paracellular pathways (leaky tight junctions) between the SW-MRC and AC (Evans, Piermarini, and Choe, 2005; Hwang and Lee, 2007). Both Cl- and Na+ move down their respective electrochemical gradients. K+ is recycled back across the basolateral membrane via K+-channels (Evans, Piermarini, and Choe, 2005). There is also putative excretion of Ca2+ and other divalent ions, however the details of these mechanisms are not known (Evans, Piermarini, and Choe, 2005).

1.3.4 Osmosensing and responses to osmotic stress

Osmosensing is the ability to perceive and respond to changes in intracellular composition and ionic strength (Evans, 2010; Kültz, 2012). Osmosensing is critical to fish as their blood plasma is hypotonic

[2] Teleosts are unable to produce highly concentrated urine due to the lack of a structure similar to the loop of Henle (Evans, Piermarini, and Choe, 2005).

to sea water and hypertonic to FW, making them vulnerable to osmotic stress[3]. While teleosts have developed effective mechanisms that offset the osmotic movement of water and ions in order to maintain homeostasis (Evans and Somero, 2009), these systems are often functionally restricted and cope poorly with larger salinity changes (stenohaline fish) (Fiol and Kültz, 2007; Kültz, 2015).

Euryhaline fish, such as tilapia, killifish and salmon, are able to acclimatize to different salinities through adaptive processes generating changes in the osmoregulatory system (Evans, 2010; Fiol and Kültz, 2007; Kültz, 2015). To achieve this they must first sense and bring about immediate responses to osmotic stress in order to minimize damage and stabilize cell function (Evans and Somero, 2009). Osmotic stress activates mechanisms for the stabilization of proteins, mRNA and DNA, and maintenance of cell volume and ionic strength through the movement of osmolytes and water (Kültz, 2012). These initial responses are limited by the existing constituents of the cell and depend upon protein-protein interaction and post-translational modification such as phosphorylation (Evans and Somero, 2009; Kültz, 2012). During this first phase appropriate signalling pathways communicating the direction, acuteness, magnitude and ionic nature of the osmotic disturbance are activated (Evans and Somero, 2009; Fiol and Kültz, 2007; Kültz, 2012). Following a time lag, they bring about large-scale genomic responses that includes changes to cellular processes (Evans, 2010; Evans and Somero, 2009; Hiroi and McCormick, 2012; Kültz, 2012, 2015). The latter is especially critical when experiencing a reversal of the osmotic gradient, as when moving from FW to SW (or vice versa) as this would require a shift in the phenotype of their osmoregulatory function. Large-scale and long-term responses to changes in environmental salinity are systemic and involve (neuro)endocrine pathways (Aruna, Nagarajan, and Chang, 2012; Breves, Fox, Pierce et al., 2010; Evans, 2010)

Kültz (2012) suggests that there are two mechanisms through which osmosensing is achieved; 1) Osmosensors –molecules that are directly sensitive to changes in osmolality and are able to initiate signalling pathways that contribute to reducing osmotic stress (primarily autocrine), and 2) Osmoreceptors –which are specialized cells capable of initiating para-, neuro- or endocrine signals that influence the fluid homeostasis of the organism (Kültz, 2012). A proposed third mechanism of osmosensing is through mechanical stress on the cytoskeleton and cell membrane (Evans, 2010; Pedersen, Hoffmann, and Mills, 2001). Kültz (2012), however, argues that cytoskeletal effects of

[3] Osmotic stress is caused by changes to water or electrolyte content of the cell changing the solvent capacity of the intracellular fluid and disrupting the biochemical workings of the cell (Kültz, 2012).

osmotic stress are the result of responding osmosensors such as proteins associated with cytoskeletal structure or membrane properties, rather than mechanic stress.

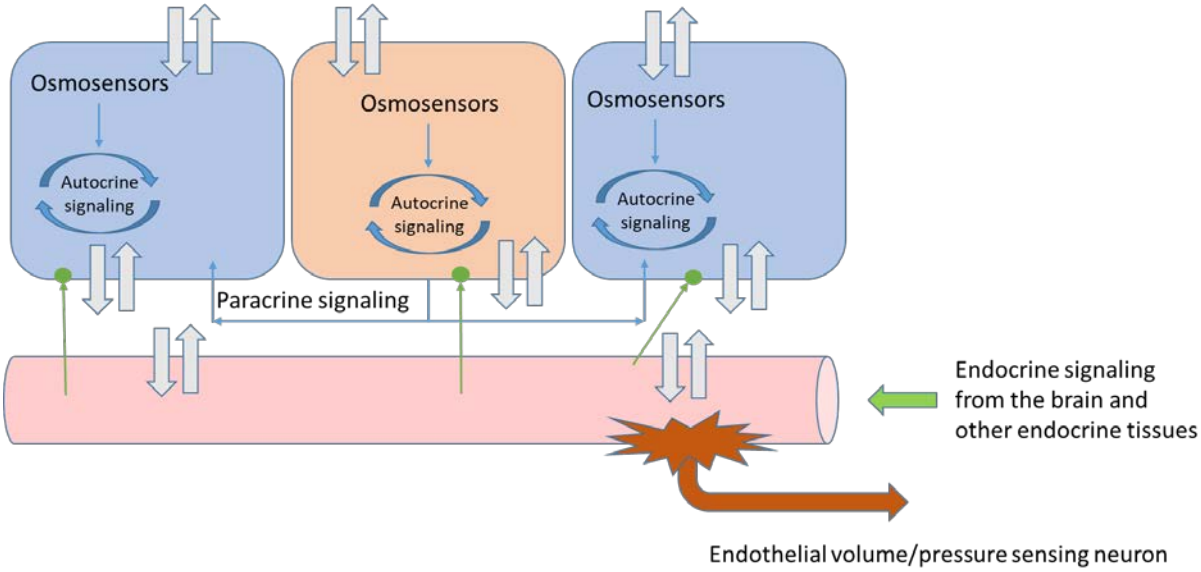

Figure 5 *Schematic showing how osmosensing occurs in the gill*

Osmosensors include a wide array of molecules affected by osmotic stress, which through various mechanisms and pathways aid in re-establishing cellular homeostasis. FK506-Binding protein 51 (FKBP-51) and translationally controlled tumour protein (TCTP) are examples of molecular chaperone proteins who are recruited away from an inhibitory role in order to deal with denatured proteins during osmotic stress (Evans and Somero, 2009). FBKP51 is part of a heteromeric complex rendering the glucocorticoid receptor (GR) inactive (Evans and Somero, 2009), the GR and cortisol have previously been strongly linked to MRC cell differentiation and the expression of important ion-regulatory proteins such as NKA α-subunits and CFTR (Kiilerich, Kristiansen, and Madsen, 2007a; McCormick, 2001). TCTP has been found to inhibit the function of NKA α-subunits (Jung, Kim, Kim et al., 2004).

Sensing concentrations of inorganic ions, such as Na^+, K^+, Ca^{2+} and Mg^{2+}, the major cation constituents of SW, is a significant component of osmosensing (Kültz, 2012). Transient receptor potential (TRP) cation channels form a large group of relatively non-selective ions channels found in most cell types, and of which some have been shown to sense Na^+, Ca^{2+} and osmolality (Bossus, Charmantier, and Lorin-Nebel, 2011; Gomis, Soriano, Belmonte et al., 2008; Liedtke, 2007; Nilius and Owsianik, 2011; Pedersen and Nilius, 2007; Zhang, Chen, Faltynek et al., 2008). Intracellular calcium concentration and calcium signalling pathways play key roles in osmosensing (Fiol, Chan, and Kültz, 2006; Fiol and Kültz, 2007; Kültz, 2012). Levels of intracellular Ca2+ is known to be involved in

pathways controlling important osmoregulatory hormones such as cortisol and PRL (Hyde, Seale, Grau et al., 2004; Seale, Richman III, Hirano et al., 2003).

Ca^{2+} is also known to be involved in the regulation of the phosphorylation state of four of the five Nuclear factor of activated T-cells (NFAT)-transcription factors (Hogan, Chen, Nardone et al., 2003; Putney, 2012), which have been implied in osmosensing. The phosphorylation state of the fifth NFAT, NFAT5 (also known as osmotic response element binding protein, OREBP), is regulated in response to osmotic stress but not Ca^{2+} (Cheung and Ko, 2013; Ferraris, Williams, Persaud et al., 2002; Woo, Lee, and Kwon, 2002). The phosphorylation state of the NFAT's is significant for nuclear transport (Irarrazabal, Gallazzini, Schnetz et al., 2010; Macian, 2005). The NFATs are of recent interest as NFAT5 response elements have been linked to the process of smolting (pre-adaptation to SW) in Atlantic salmon (Lorgen, Jorgensen, Jordan et al., 2017).

Transcriptional regulation through the up- or downregulation of transcription factor activity through post-translational modification is an important part of the secondary response to osmotic stress (Evans and Somero, 2009). For example, the phosphorylation state of focal adhesion kinase (FAK, an enzyme responding to changes in cytoskeleton dynamics), affected by osmotic stress, is known to influence the activity of the ion transporter channels NKCC and CFTR (Marshall, Katoh, Main et al., 2008; Marshall, Watters, Hovdestad et al., 2009).

Osmoreceptors exist as neurons and non-neural cells whose activity is closely controlled by the osmolality of the extracellular fluid, and as epithelial cells providing a front-line able to sense changes in environmental salinity before the physiological homeostasis is disrupted (Kültz, 2012). Osmoreceptors are present in several areas of the brain and the pituitary gland. In fish the most studied area is the rostral pars distalis (PD) of the anterior pituitary, consisting mainly of PRL-producing cells (Abraham, 1971; Emmart and Mossakowski, 1967). Upon hypo-osmotic stress these cells swell and release PRL (Grau, Nishioka, and Bern, 1981; Weber, Seale, Richman Iii et al., 2004). The role of PRL and other hormones in osmoregulation will be further discussed in section 1.4. Endothelial volume-/pressure-sensing neurons localized to the vasculature of the gill arches serve a similar role as mammalian baroreceptors (Burleson, 2009) signaling to osmoregulatory effector tissue (Kougias, Weakley, Yao et al., 2010).

Epithelial cells, such as those lining the GIT or those of the gill are particularly important for rapid responses to changes in environmental salinity (Fiol, Chan, and Kültz, 2006). Experiments on isolated gill cells show that they are capable of primary and secondary responses to osmotic stress through

auto- and paracrine signals (Kültz, 2012). Neuroepithelial cells have been identified in the gill, producing both nervous and paracrine signals in response to osmotic stress (Dunel-Erb, Bailly, and Laurent, 1982; Goniakowska-Witalińska, Zaccone, Fasulo et al., 1995; Kültz, 2012; Monteiro, Oliveira, Fontaínhas-Fernandes et al., 2010; Zaccone, Lauweryns, Fasulo et al., 1992). Gill epithelial cells are also target cells for endocrine signals emanating from the brain. Initial priming of target cells through auto- and paracrine stress responses could be a significant aspect of the cellular response (Kültz, 2012). The combination of para-, neuro- and endocrine signalling from osmoreceptors throughout the organism serves an important role in the coordination of a systemic response to osmotic disturbance (Kültz, 2012).

1.4 Photoperiodic and hormonal control of smolting

Smolting is a coordinated process changing the behaviour, morphology and physiology of UM parr (see section 1.1.3), so that it becomes a SW-ready smolt (McCormick, Hansen, Quinn et al., 1998; McCormick and Saunders, 1987; Stefansson, Bjornsson, Sundell et al., 2003; Stefansson, Björnsson, Ebbesson et al., 2008). Smolting is initiated through photoperiod signals, which are perceived and interpreted by the brain, and stimulating endocrine signalling pathways originating from the hypothalamus and pituitary (Björnsson, Thorarensen, Hirano et al., 1989; Duston and Saunders, 1990; Ebbesson, Ekström, Ebbesson et al., 2003; Saunders, Henderson, and Harmon, 1985; Stefansson, Björnsson, Ebbesson et al., 2008). It is important to not confuse this process with SW acclimation in euryhaline fish, as smolting is a pre-adaptive process occurring in salmonid fish prior to SW migration. Under constant photoperiodic conditions or with inadequate photoperiodic stimulation it has been shown that the potential smolts have poor SW survival, and lack several smolt traits (Berge, Berg, Fyhn et al., 1995; Stefansson, Bjornsson, Hansen et al., 1991; Stefansson, Nilsen, Ebbesson et al., 2007). However, some smolt processes appear to proceed spontaneously in UM juveniles, though in a less temporally coordinated manner than when a synchronizing photoperiodic stimulus is applied (Duncan and Bromage, 1998; Duston and Saunders, 1990; Eriksson and Lundqvist, 1982).

Critical to successful smoltification is the development of hypo-osmoregulatory ability in order to maintain body fluid homeostasis in SW. Teleosts maintain a narrow range of osmotic concentration in their extracellular fluid at around one-third of SW (McCormick, 2001), which is about 1000 milliosmole (mOsm) kg^{-1}. In FW the passive loss of ions and gain of water is counteracted by copious production of dilute urine, active salt intake across gills and intestines, and ion-reabsorption in the kidneys (James-Curtis and Wood, 1992; McCormick, 2001). In SW the passive loss of water and

strong ion influx is reversed by drinking SW, absorbing water and monovalent ions across the gut, and excreting NA+ and Cl- over the gills, and divalent ions via the gut and kidney (McCormick, 2001). Central to the movement of ions are the MRCs, which will be discussed in section 1.4.3.

Research into the parr-smolt transformation is undertaken on a variety of salmonids, and the findings presented below will not be limited to the Atlantic salmon and the *Salmo*-genus, but also include studies on parr-smolt transformation in the genus *Oncorhynchus*. While the timing of parr-smolt transformation may vary considerably between species, the basic physiological process is considered equal (Hoar, 1988).

1.4.1 The light-brain-pituitary axis during smoltification

Increasing photoperiod is established as a crucial cue for successful smolting to occur (Björnsson, Thorarensen, Hirano et al., 1989; McCormick, Björnsson, Sheridan et al., 1995; McCormick, Shrimpton, Moriyama et al., 2007; Saunders, Henderson, and Harmon, 1985; Stefansson, Björnsson, Ebbesson et al., 2008; Stefansson, Nilsen, Ebbesson et al., 2007). A recent study exposing juvenile salmon of high latitude origin to different photoperiod transitions suggest that the increasing photoperiod needs to surpass 16 h. of light before a complete smolt transformation can take place (Strand, Hazlerigg, and Jørgensen, 2018). Salmon from lower latitudes migrate earlier, and hence might be stimulated to smolt by shorter photoperiods.

Most teleost fish are able to perceive light both through their eyes (i.e. retina) and via a light sensitive pineal gland (Migaud, Davie, Martinez Chavez et al., 2007). In most fish exposure to a light-dark rhythm sets an circadian, endogenous clock, resulting in a rhythmic production of pineal melatonin even in the absence of light stimuli (Falcón, Besseau, Sauzet et al., 2007). Salmonids appear to lack pineal circadian regulation of melatonin production (Bolliet, Ali, Lapointe et al., 1996; Iigo, Abe, Kambayashi et al., 2007); hence, the secretion of melatonin is an acute pineal response to darkness. Porter, Randall, Bromage et al. (1998) showed that pinealectomised juvenile salmon achieved similar SW survival as the control group, but their SW survival and physical appearance of being smolts was comparatively delayed. The same study also found that melatonin implants either in complete or pinealectomised fish seemed to advance smolting (Porter, Randall, Bromage et al., 1998). Ciani, Fontaine, Maugars et al. (2019) reports the expression of five melatonin receptors (Mtrn) in the brain of Atlantic salmon, thereof three that are expressed in the pituitary. The three Mtrns expressed in the pituitary displayed daily fluctuations, suggesting a potential role in development (Ciani, Fontaine, Maugars et al., 2019). There is also evidence for beep brain opsin photoreceptors in teleost, and in particular in zebrafish (Hang, Kitahashi, and Parhar, 2016). These

could potentially also play a role in the development of smolts, however, functional evidence is lacking.

Studies of gene expression in salmon are complicated due to the large amount of duplicate genes and the difficulties of resolving between the ohnologues. West, Iversen, Jørgensen et al. (2020) found 61 clock genes in the salmon genome, of which 42 could be assigned to 21 ohnologue pairs (deriving from Ss4R). Although investigations into mRNA expression of the salmon should be interpreted with caution unless they have resolved between ohnologues, they do still provide useful information. A 2009-study comparing whole brain from salmon parr on long day (LP, 16:8) and short day (SP, 8:16) found that *Clock*, *Bmal1*, *Per2*, and *Cry2* exhibited rhythmicity under SP, but not LP (Davie, Minghetti, and Migaud, 2009). Under LP, the surveyed genes were all arrhythmic, with the exception of *Cry2* which was phase delayed (Davie, Minghetti, and Migaud, 2009). This was closely followed by a study looking at the same *Cry2*- and *Clock*-genes, in addition to *Per1-like,* in the pineal and brain, reports a similar break-down of rhythmicity during exposure to continuous day (Huang, Ruoff, and Fjelldal, 2010). The apparent break-down of circadian rhythmicity during continuous and LP is similar to observations of other Arctic animals that exhibit weak circadian clocks (Lu, Meng, Tyler et al., 2010; Steiger, Valcu, Spoelstra et al., 2013; van Oort, Tyler, Gerkema et al., 2007).

A more recent study, looking at clock genes and AANAT2 production in the pineal gland of Atlantic salmon reports lack of rhythmicity in clock-genes *Per1* and *Cry2 in vitro*, while rhythmicity is present *in vivo* (McStay, Migaud, Vera et al., 2014). This is highly indicative of an extra-pineal regulation of the pineal clock. Additionally the study found that *Aanat2*, encoding the rate limiting enzyme in melatonin synthesis (see box 2), of Atlantic salmon, had a different promoter structure compared with other teleost fish making in unresponsive to rhythmic mRNA translation regulation, i.e. there is a loss of functional circadian regulation of *Aanat2* and melatonin production in the pineal of salmonids (McStay, Migaud, Vera et al., 2014). This is in line with other studies indicating that regulation of AANAT2 occurs at the transcriptional and protein level (Bégay, Falcón, Cahill et al., 1998; Falcón, Barraud, Thibault et al., 1998).

Several structural changes, involving both dopaminergic and corticotropic systems, occur in response to photoperiodic stimulation in the juvenile salmon brain when transitioning from parr to smolt (Ebbesson, Ebbesson, Nilsen et al., 2007; Ebbesson, Ekström, Ebbesson et al., 2003; Ebbesson, Nilsen, Helvik et al., 2011; Holmqvist, Östholm, and Ekström, 1994). Studies have observed increased retinal and pineal innervation of the POA and optic nucleus of the hypothalamus. The POA is central to regulation of the pituitary through neuronal innervation (Anglade, Zandbergen, and Kah, 1993;

Cerdá-Reverter and Canosa, 2009; Holmqvist and Ekström, 1995; Peter, Yu, Marchant et al., 1990). It can be hypothesized that the increased pineal and retinal innervation of the POA is permissive of the neuroendocrine response driving the smoltification process (Ebbesson, Ebbesson, Nilsen et al., 2007; Ebbesson, Nilsen, Helvik et al., 2011; Holmqvist, Östholm, and Ekström, 1994).

In addition to the POA the SCN could be of importance to circadian and seasonal regulation (Holmqvist and Ekström, 1995; Sandbakken, Ebbesson, Stefansson et al., 2012), as has been shown for other teleost (Philp, Garcia-Fernandez, Soni et al., 2000; Watanabe, Itoh, Mogi et al., 2012). The SV, discussed in section 1.2.3, could also be involved in regulation of smolting, however, research efforts so far have been limited to seasonal regulation of reproduction (Chi, Li, Liu et al., 2017, 2019; Maeda, Shimo, Nakane et al., 2015; Nakane, Ikegami, Iigo et al., 2013).

Following the structural changes the salmon brain also experiences a surge of neurotransmitters including serotonin, dopamine, norepinephrine and glutamine during the parr-smolt transformation (Ebbesson, Smith, Co et al., 1996; Stefansson, Björnsson, Ebbesson et al., 2008). Changes in the number and distributions of opioid receptors have also been observed in the brain of smolting salmon (Ebbesson, Deviche, and Ebbesson, 1996). These changes are likely to be connected with the reorganization of neuronal brain pathways.

Following restructuring of neuronal networks is the activation of endocrine signalling pathways originating in the hypothalamus, providing the necessary stimuli of the pituitary to release the hormones soliciting the parr-smolt transformation. Hypothalamic corticotropin-releasing hormone (CRH) and growth hormone-releasing hormone (GHRH) facilitates the circulatory release of adrenocorticotropic hormone (ACTH) and GH from the pituitary gland. Dopamine and ghrelin can also function as GH secretagogues in salmonids, but their role during parr-smolt transformation is unclear (Ágústsson, Ebbesson, and Björnsson, 2000; Björnsson, Johansson, Benedet et al., 2002). Two functional GH genes, which differ in promoter elements, have been identified in salmon (von Schalburg, Yazawa, de Boer et al., 2008), however their expression has not been studied in the context of smolting.

While GH have a direct impact on the parr-smolt transformation, ACTHs primary role is to induce the release of cortisol from the intrarenal glands. Cortisol and GH are essential hormones during smolting, and appear to work in synergy to stimulate physiological development (McCormick, 2001; Pelis and McCormick, 2001; Shrimpton and McCormick, 1998a; Tipsmark, Jørgensen, Brande-Lavridsen et al., 2009; Tipsmark and Madsen, 2009).

In the classic hypothalamus-pituitary-thyroid axis thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the pituitary to release TSH resulting in increased release of thyroid hormone (TH) from the thyroid gland. Ojima and Iwata (2010) found that intracerebroventricular injections of GHRH and CRH stimulated TH release and smolt-like behaviour, while injections with TRH only induced TH release. This is in support of earlier studies showing that CRH might be important for regulation of THS in salmonid (De Groef, Van der Geyten, Darras et al., 2006; Larsen, Swanson, Dickey et al., 1998). During smolting TSH does not seem to increase, rather the thyroid gland seem to become much more sensitive to the already present TSH signal, resulting in in the characteristic TH plasma surges of smolting (Dickhoff, Folmar, and Gorbman, 1978; Larsen, Swanson, and Dickhoff, 2011; Prunet, Boeuf, Bolton et al., 1989; Robertson and McCormick, 2012; Specker and Schreck, 1984). Recently Fleming, Maugars, Lafont et al. (2019) reported that the two paralogues of the TSHβ-subunit (deriving from Ts3R) showed different expression patterns during smolting, and are expressed by distinct pituitary cell populations. What this means for the downstream processes remains to be elucidated.

In summary, the neuroendocrine development during smolting is complex, and several of its aspects such as neuronal growth and neurotransmitter surges remains poorly understood. The brief review above by no means mirror the complex feedback systems and hormonal interactions involved in the regulation of the pituitary hormones and their receptors (Björnsson, Johansson, Benedet et al., 2002; De Groef, Van der Geyten, Darras et al., 2006; Larsen, Swanson, and Dickhoff, 2011; MacKenzie, Jones, and Miller, 2009; Specker, Eales, Tagawa et al., 2000; Stefansson, Björnsson, Ebbesson et al., 2008).

1.4.2 General physiological changes during smoltification

The hormones released from the pituitary during parr-smolt transformation are released in a coordinated and specific manner, as described in Stefansson, Björnsson, Ebbesson et al. (2008). Minor peaks in plasma TH and GH can be observed prior to the neural reorganization occurring in the brain (Stefansson, Björnsson, Ebbesson et al., 2008). Following the restructuring of neuronal networks in the brain are major increases in circulating levels of the key hormones TH, cortisol and GH (Barron, 1986; McCormick, 2001; Stefansson, Björnsson, Ebbesson et al., 2008).

The increase in systemic TH occurs primarily in the form of the prohormone thyroxine (T_4), which is converted locally in target tissues to its active form triiodothyronine (T_3) by deiodination (Eales and Brown, 1993; Larsen, Swanson, and Dickhoff, 2011). It is believed that the increasing GH levels are involved through stimulation of deiodinase activity (de Luze and Leloup, 1984; Leloup and Lebel,

1993; MacLatchy, Kawauchi, and Eales, 1992). T_3 levels are also part of a negative feedback loop affecting pituitary release of TSH (Larsen, Swanson, and Dickhoff, 2011). T_3 appears to work in synergy with GH (McCormick, 2001), and has permissive role for the activities of cortisol through affecting the expression of corticosteroid receptors (CR) in peripheral tissues (Shrimpton and McCormick, 1998a; Shrimpton and McCormick, 1999).

The thyroid axis is implicated in several significant changes in appearance and behaviour during the parr-smolt transformation. Most notably, the deposition of purines in skin and scales causing the well-known silvering of smolts (Johnston and Eales, 1967; Madsen and Korsgaard, 1989; Premdas and Eales, 1976; Staley and Ewing, 1992). The silvering contributes to a counter-shading camouflage commonly seen in pelagic fish, and spares the salmon water as the purines do not need to be metabolized (Stefansson, Björnsson, Ebbesson et al., 2008). The reduction in aggressive behaviour and loss of positive rheotaxis has also been linked to TH levels (Godin, Dill, and Drury, 1974; Hutchison and Iwata, 1998; Iwata, 1995). Finally, TH is thought to play a role in olfactory imprinting (Hasler, Scholz, and Horrall, 1978; Lema and Nevitt, 2004; Nevitt and Dittman, 1998; Scholz, White, Muzi et al., 1985), believed to be a significant prerequisite for the homing behaviour of salmon (Keefer and Caudill, 2014).

The increases in T_4 and T_3 are accompanied by increased plasma GH (McCormick, Björnsson, Sheridan et al., 1995; Prunet, Boeuf, Bolton et al., 1989; Stefansson, Bjornsson, Hansen et al., 1991). Some authors describe a pattern involving two peaks in plasma GH levels (Prunet, Boeuf, Bolton et al., 1989), and it has been suggested that these distinct peaks in GH plasma content are due to changes in secretion and clearance rates (Ágústsson, Sundell, Sakamoto et al., 2001).

The major target organs of GH is the liver, intestines, kidney and gill, but GH receptors are also present in muscle and adipose tissues (Björnsson, Johansson, Benedet et al., 2002). In plasma GH exists both in free form and bound to GH binding proteins (GHBP). GHBP are likely to be important during parr-smolt transformation, as a fourfold increase in GHBP-bound GH could be detected in rainbow trout plasma after transfer to SW (Sohm, Manfroid, Pezet et al., 1998).

GH is known to have effects on growth and metabolism through mobilization of lipids and accretion of proteins, in addition to stimulating skeletal growth (Björnsson, 1997). During smolting the increased metabolism and length growth causes a characteristic reduction of condition factor, resulting in a leaner fish (Björnsson, 1997; Stefansson, Björnsson, Ebbesson et al., 2008).

Insulin-like growth factor (IGF)-1 production is induced in the liver and gill by GH (Björnsson, 1997; Björnsson, Johansson, Benedet et al., 2002). During smolting IGF-1 is involved in regulation of growth and metabolism, and osmoregulation. Evidence in salmonids suggest an endo- and paracrine role of IGF-1 in the regulation of growth, while osmoregulatory adaptions of the gill seem to be elicited mainly through para- and autocrine actions (Duan, Duguay, and Plisetskaya, 1993; Madsen and Bern, 1993; McCormick, 1996; Nordgarden, Fjelldal, Hansen et al., 2006; Wargelius, Fjelldal, Benedet et al., 2005). Both GH and IGF-1 are part of a negative feedback loop affecting GH secretion (Björnsson, Johansson, Benedet et al., 2002).

GH sensitizes the interrenal tissue to ACTH (Young, 1988), increasing cortisol production. GH also influences the action potential of cortisol through affecting tissue concentration of CR (Shrimpton, Devlin, McLean et al., 1995; Shrimpton and McCormick, 1998a). T_3 is also known to increase CR levels, and together with GH the effect is additive (Shrimpton and McCormick, 1998a).

The increases in T_4 and GH are accompanied by increased plasma cortisol levels (Barron, 1986; Shrimpton and McCormick, 1998b; Specker and Schreck, 1982; Virtanen and Soivio, 1985). As part of the stress response cortisol exerts its impact on metabolism and growth (Mommsen, Vijayan, and Moon, 1999). Research on the role of cortisol during parr-smolt transformation has, however, almost exclusively focused on the effect of cortisol on the gill and its role in osmoregulation. As a osmoregulatory hormone cortisol increases fluid adsorption in the intestine during parr-smolt transformation (Veillette, Sundell, and Specker, 1995).

Cortisol serves a dual role in osmoregulation (Madsen, 1990; McCormick, 1996, 2001), depending on whether it interacts with GH or PRL. While interaction with GH (and T_3) produces a SW phenotype, interaction with PRL produces a FW phenotype. This is particularly evident in desmolting of smolt that remain in FW, where PRL levels rise. The synergistic effect of GH and cortisol on SW adaption of the gill will be further discussed below.

1.4.3 Structural and cellular changes to gill physiology during smoltification

The gill is vital to osmoregulation, and its function changes drastically during smolting. Both the FW and SW MRCs were described in section 1.3, and this section will focus on the transitions between types during smolting.

It is unclear whether the appearance of SW-MRCs during smolting is a result of differentiation of already present FW-MRCs, or active development of new MRCs from dormant cells. Studies in

salmon indicate that both approaches might be important (McCormick, Regish, Christensen et al., 2013).

Using an electron microscope Pisam, Prunet, Boeuf et al. (1988) were among the first to described the preadaptive ultrastructural changes to the salmon gill during smolting. Pisam, Prunet, Boeuf et al. (1988) concludes that 'the Atlantic salmon develops in FW most of the ultrastructural modifications of the gill epithelium which in most euryhaline fish are triggered by exposure to sea water'. Among the primary findings of Pisam, Prunet, Boeuf et al. (1988) is the increased number and size of MRCs, and the appearance of the associated ACs during smolting. The MRCs are described to develop the extensive tubular system which is characteristic of SW-MRCs. After SW transfer, the number of MRCs connected to ACs increased, and so did the number of AC in contact with the external medium (Pisam, Prunet, Boeuf et al., 1988). SW is described to promote the connection between MRCs and ACs through stimulating the formation of multiple cytoplasmic interdigitations, forming a mosaic of cells bound via leaky, paracellular pathways (Chasiotis, Kolosov, Bui et al., 2012; Evans, Piermarini, and Choe, 2005; Pisam, Prunet, Boeuf et al., 1988).

Gill development during smolting is under strong hormonal control. In particular the interplay between GH, IGF-1 and cortisol has been implicated in the regulation of the three major salt transporters CFTR, NKCC and NKA (McCormick, 2001; Stefansson, Björnsson, Ebbesson et al., 2008).

Atlantic salmon possess two differentially regulated CFTR-isoforms. CFTR I is alone to be upregulated during smolting (Nilsen, Ebbesson, Madsen et al., 2007). Abrupt exposure to SW cause a sustained increase in the mRNA expression of CFTR I, while the response of CFTR II is smaller and transient (Singer, Clements, Semple et al., 2002). Cortisol implants have been shown to increase the mRNA expression of CFTR I in FW, whilst having no effect on CFTR II (Singer, Finstad, McCormick et al., 2003). Interestingly, cortisol injections increased both CFTR I and II in SW-acclimated gill tissue (Kiilerich, Kristiansen, and Madsen, 2007a). The study by Kiilerich, Kristiansen , and Madsen (2007a) indicates that the effect of cortisol on CFTR I was mediated through the GR signalling pathway, while the effect on CFTR-II required both glucocorticoid and mineralocorticoid signalling. Together these results point to an important role for CFTR I under the influence of cortisol during smolting. CFTR-II could be important during acute SW exposure. Evidence from other euryhaline fish indicate that CFTR redistributes from the basolateral to the apical membrane of MRCs in response to being moved from FW to SW (Hiroi and McCormick, 2012; Marshall and Singer, 2002).

NKCC have also been localized to MRCs the Atlantic salmon, and was found to increase in abundance in response to GH and cortisol (Pelis and McCormick, 2001; Pelis, Zydlewski, and McCormick, 2001).

The NKA transporter and its level of activity have received much attention in smolt research (Langdon, Thorpe, and Roberts, 1984; Madsen and Naamansen, 1989; McCormick, Björnsson, Sheridan et al., 1995; Virtanen and Soivio, 1985). Increased NKA activity is known to be a hallmark of smolting in salmonids, and has been linked to the increased levels of plasma GH , IGF-1 and cortisol (McCormick, 1996; Shrimpton and McCormick, 1999; Singer, Finstad, McCormick et al., 2003).

The NKA transporter is an oligomeric protein consisting of the subunits α, β, and FXYD (FXYD Domain containing ion transport regulator). The α-subunit consists of multiple membrane-spanning domains, including the bindings sites for cations, ATP (adenosine triphosphate) and phosphate (Geering, 1991). As the catalytic subunit it hydrolyses ATP and facilitates the exchange of cations (Na^+, K^+) across the plasma membrane (Geering, 2008). In salmonids both smolting and long-term SW exposure causes a shift from one α-isoform to another (Madsen, Kiilerich, and Tipsmark, 2009; McCormick, Regish, and Christensen, 2009; McCormick, Regish, Christensen et al., 2013; Nilsen, Ebbesson, Madsen et al., 2007; Richards, Semple, Bystriansky et al., 2003). These isoforms are known as α1a (FW) and α1b (SW) (Dalziel, Bittman, Mandic et al., 2014). The adaptive reason for this isoform shift is not known, however, molecular modelling suggest that the difference between the isoforms affects ion binding, energetics, and possibly interactions with the FXYD-subunit and membrane lipids (Bystriansky and Ballantyne, 2007; Dalziel, Bittman, Mandic et al., 2014; Jorgensen, 2008). Together cortisol and GH upregulate the expression of *α1b* in FW salmon (cortisol alone increased *α1a*), and PRL alone cause a decrease in *α1b* in SW salmon (Tipsmark and Madsen, 2009).

The β-subunit serves as a molecular chaperone, and is essential for correct packing of recently synthesized α-units and proper membrane integration of the NKA transporter (Geering, 2008). Variation in β isoform have been found to affect the potassium affinity of the α-subunit, and the structure of the β unit influences the sodium affinity of the NKA transporter (Geering, 2008). Additionally, it is suggested that the β-subunit contributes to establishing cell polarity through influencing the formation of tight junctions, cell-cell adhesion and cell motility (Geering, 2008).

FXYD proteins are small transmembrane proteins of which eight have been identified in Atlantic salmon (Tipsmark, 2008). FXYD subunits have been shown to influence the affinity of the NKA transporter for sodium, potassium and ATP, and it is suggested they serve a stabilizing function during thermal stress (Geering, 2008). Most FXYD proteins appear to show tissue specific expression

(Geering, 2008; Tipsmark, 2008), and in particular FXYD-11 have been linked to gill tissue in the salmon (Tipsmark, 2008; Tipsmark, Mahmmoud, Borski et al., 2010). Tipsmark, Mahmmoud, Borski et al. (2010) observed an increase in FXYD-11 during smolting, and in response to cortisol and GH.

In recent years other cellular components such as structural proteins have received attention as they too are implied in gill differentiation and osmoregulation through transcriptional profiling and comparisons (Houde, Akbarzadeh, Günther et al., 2019; Robertson and McCormick, 2012; Seear, Carmichael, Talbot et al., 2010). More specific efforts investigation specific proteins have also been made. Tipsmark and coauthors (2008; 2010) have investigated the regulation of claudins and AQPs during smolting in salmon. Claudins are transmembrane proteins that regulate the biophysical properties of tight junctions (Hartsock and Nelson, 2008; Van Itallie and Anderson, 2006; Van Itallie and Anderson, 2014). Permeability characteristics and trans-epithelial resistance of tight junctions are dependent on the specific claudin expressed, meaning that the claudins have strong influence on ion movement through the paracellular tight junctions (Hartsock and Nelson, 2008). The numerous and tissue specific AQPs selectively allow the passage of water and small uncharged molecules. Both claudins and AQPs show SW-induced shifts in isoform expression that are mirrored during parr-smolt transformation in salmon (Tipsmark, Kiilerich, Nilsen et al., 2008; Tipsmark, Sørensen, and Madsen, 2010).

2. Aims of the study

The overall aim of this study was to utilize the recent developments in salmon genomics to gain new insight into gill differentiation during parr-smolt transformation in Atlantic salmon, and in particular, to which degree this development is dependent on photoperiodic history. In addition, we assessed the overall performance of salmon following short and long-term SW transfer in relation to photoperiodic history.

To address this aim parr were exposed to different photoperiod regimes in two different experimental set-ups. Data was collected in order to assess growth, hypoosmoregultory capacity during short-term SW exposure, NKA activity, transcriptional shifts during the parr-smolt transformation and acute (within 24-h) transcriptional response to SW. During the long-term SW exposure, we collected data on growth and feed utilization. In the collection of transcriptional data RNAseq was used for the initial and smaller experiment, while qPCR (quantitative polymerase chain reaction) was applied to follow the transcriptional change in specific targets during the second and larger experiment.

The transcriptional profiling was coupled with other bioinformatic tools, including gene ontology (GO) analysis and transcription factor motif analysis, in order to provide novel insights into gill differentiation during parr-smolt transformation.

Some specific hypotheses were challenged during this project:

- Long-term SW performance is dependent upon photoperiodic history, and in particular the history of exposure to short photoperiod.
- Preadaption of the gill to SW is dependent upon photoperiodic history, and in particular the history of exposure to short photoperiod.
- The profile of an acute response to SW will change as the salmon smoltifies because SW no longer triggers certain osmosensing pathways.

The major findings are reported in paper I and II, and further discussed below. Some minor findings and observations will also be coupled with the discussion in order to provide a broader view of the insights following this work.

3. Summary of papers

Paper I:

RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile salmon.

Iversen M, Mulugeta T, Gellein Blikeng B, West AC, Jørgensen EH, Rød Sandve S, Hazlerigg, D.

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Anadromous salmonids go through a process termed smolting prior to migrating to a marine environment. Essential to the process of smolting is the acquisition of the ability to efficiently osmoregulate at sea. In natural systems, smolting is initiated as increasing photoperiod sets of a cascade of physiological responses mediated primarily through endocrine signals. Smolting can be achieved artificially through controlling photoperiod. Essential to smolting is the transformation from an ion-absorbing to an ion-secreting gill, this shift includes the differentiation of MRCs. The MRCs contain ion pumps such as Na^+, K^+ ATPase and CFTR, which both are involved in osmoregulation.

In this study we first profiled the transcriptome of juvenile salmon put through an artificially induced smolting process using a classical smolt inducing photoperiod regime (eight weeks of SP, 8:16,

followed by continuous light, LL). We identified a subset of novel, responsive genes (upregulated in LL post-SP) that we chose to focus on (genes of interest, GOIs), also including important ion channels. Next, we put groups of salmon through three different photoperiod regimes, varying in the length of exposure to short photoperiod (two, four and eight weeks). We used qPCR to follow the expression of the GOIs throughout the experiment. In both experiments we used 24-hours SW tests to check osmoregulatory capacity and sea water readiness. In the latter experiment juvenile salmon were also exposed to a longer sea water stay after having been put through one of the photoperiod regimes, in order to check for differences in growth as an indicator of welfare.

We observed that the response of some GOIs, and growth in sea water, was dependent upon photoperiod-history. Shorter exposure to SP reduced responsiveness of some GOIs and led to poorer growth in sea water. The response of the GOIs associated with ion pumps, and osmoregulatory capacity, did not depend upon photoperiod history, and did not relate to growth in sea water nor NKA activity. This suggest that current methods for evaluating sea water readiness are inadequate and that biomolecular approach focusing on multiple genetic markers including the photoperiod-history dependent GOIs might provide a better prediction of sea water tolerance.

Paper II:

Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in an anadromous salmonid

Iversen M, Mulugeta T, West AC, Jørgensen EH, Martin S, Rød Sandve S, Hazelrigg D.

Accepted pending minor revision: G3

The gill is the primary site of osmo-sensing and osmoregulatory control in teleost fish. Euryhaline fish are defined by their ability to tolerate varying salinities. In most euryhaline fish this is a process of acclimation. In anadromous fish, such as salmon, adaptation occurs prior to SW migration, and is termed smolting. Smolting is a photoperiod-history dependent life history transition enabling the juvenile salmon to survive in and exploit the marine environment. Smolting is controlled by a series of cascading endocrine responses to photoperiod increase in spring. The gill is a major target tissue for this endocrine response. While much is known about the pre-adaptive process taking place in the gill prior to migration, less is known about how the prepared gill responds sea water (SW) and the importance of SW exposure for completion of smolting.

Gill tissue senses salinity in at least three ways: i) increased cellular tonicity and altered intracellular ion concentrations, ii) via cell surface receptors for SW constituents, and iii) indirectly via hormonal signals. The NFAT family of transcription factors have been implicated in osmo-sensing and Ca^{2+} dependent transcriptional control. The atypical NFAT5 regulates the transcription of tonicity-responsive genes such as ion transporters and osmo-protective proteins. Two pairs of NFAT5 paralogues have been found in Atlantic salmon, where one set has been shown to be SW responsive.

We exposed juvenile Atlantic salmon to three different photoperiod regimes, where one regime was a classic smolt inducing regime. Osmoregulatory capacity was regularly checked through exposing fish to 24-hour SW challenges. The other two photoperiod regimes served as controls for LL exposure and to short photoperiod only. Transcriptional profiling revealed differentially expressed genes (DEGs) between sampling points, photoperiod regimes and salinity. The SW response of fish maintained on short photoperiod was tenfold larger that than that of the two other groups at the final sampling point. A principal component analysis (PCA) showed less divergence along both principal components between FW and SW samples from juvenile salmon put through a smolt inducing photoperiod regime, than between juveniles put through the control regimes. The family of NFAT genes also show distinctive responses to SW exposure depending on photoperiod history. Analysis of promoter motif enrichment of the transcriptome at the final sampling point show enrichment of NFAT promoter motifs in particular in downregulated genes of fish being maintained on LL or SP. Also, promoter motif enrichment analysis revealed strong enrichment of GR response elements in the fish maintained on short photoperiod.

The diminished role of both NFAT and GRE response elements in the SW response of juvenile salmons put through a smolt inducing photoperiod regime indicate reduced involvement of pathways linked to osmotic stress and osmoregulation. In this group, having completed their FW preparative phase we observe a unique set of genes stimulated by SW, representing a SW-activation phase. Further studies to understand the impact of these genes on the gill is now warranted.

Paper III:

Diversified regulation of circadian clock gene expression following whole genome duplication

West AC, Iversen M, Jørgensen EJ, Sandve SR, Hazlerigg D, Wood SH

2020 PLOS Genetics 16 (10): e1009097. https://doi.org/10.1371/journal.pgen.1009097

Existing vertebrate linages have all been through multiple rounds of WGD giving rise to the large complement of clock genes observed in current vertebrates. Paralogues arising from WGDs are known as ohnologues. The genetic redundancy these ohnologues provide has been hypothesize by Susumu Ohno to be significant for evolutionary innovation and species radiation. The genes involved in the maintenance of circadian rhythm, constituting a molecular clock, have been retained after multiple WGD, existing as many ohnologue sets. However, the evolutionary importance and the extent to which these genes are functionally divergent remain unclear. The salmonid linage has been through four rounds of WGD, and consequently the salmonid genome contains 61 clock genes (comparably 18 clock genes are found in the mouse genome) making the salmon a model species for studying the diversification of clock genes. Forty-two of these clock genes derive from the most recent and salmonid-specific WGD.

Multiple approaches, including using both publicly available data and in vivo and in vitro-experimental set-ups, for collecting information on the salmon genome and gene expression through developmental phases and in different tissues were applied.

This complex study shows tissue-specificity in the regulation of clock gene expression, and differences in robustness of rhythmicity between tissues. A striking contrast is observed between the optic tectum (OT) influenced primarily by the light-dark cycle, and the gill responding to its aqueous environment. Clear evidence for sub-functionalization is found as there exists minimal overlap between the responsive genes in the OT and the gill.

Glucocorticoids play a major role in circadian organization, however there is little evidence for its circadian role in salmonids. The glucocorticoid cortisol is also an important regulator of osmoregulation and smolting. Transcription factor binding site analysis revealed that the promoters of the SW-regulated clock genes were enriched for GREs to a much larger extent than in circadian oscillating clock genes.

Clock genes were shown to change their expression significantly in the gill during smolting in Atlantic salmon, but also in response to acute osmotic stress. Within this group of genes there were nine ohnologue pairs where both genes showed smoltification-associated regulation, while in 3 pairs only one of the ohnologues was significantly regulated. There appears to be a strong developmental component to the regulation of clock genes during smolting.

4. Transcriptomics data can provide further insight into smolting

This section is intended as an extension on paper I and includes further presentation of the clusters from figure 1c of Iversen et al. 2020. The figure shows a heatmap and a cluster analysis of 389 DEGs. The DEGs are derived from an analysis of the three latter sampling points of the three treatments in experiment 1, and subject to strict filtering criteria as described in Iversen et al. 2020. Six clusters are defined based on the expression profiles of the DEGs as expressed in the SPLL treatments, but only cluster 3, showing a distinct increase in expression in response to re-entering LL, is further investigated in the context of the paper (Iversen, Mulugeta, Gellein Blikeng et al., 2020). This section will be used to provide information on the five other clusters, so that they can be relevant for the following discussion. The clusters will be referred to as C1, C2, C3, C4, C5 and C6, and contained 75, 129, 96, 44, 32 and 12 genes, respectively. Plots of the mentioned genes can be found in appendix 1, and full gene lists for each cluster are found in appendix 2.

Paper I focus on expression in FW, however, by also looking at the expression pattern of these genes in SW, an additional dimension of information becomes available. Several clusters, as will be described in the following paragraphs, contain genes that are regulated by SW under LL or SP, but loses this SW-responsiveness as fish re-enter LL in the SPLL treatment. This is closely linked with LL inducing a change in expression level similar to that which SW would normally induce.

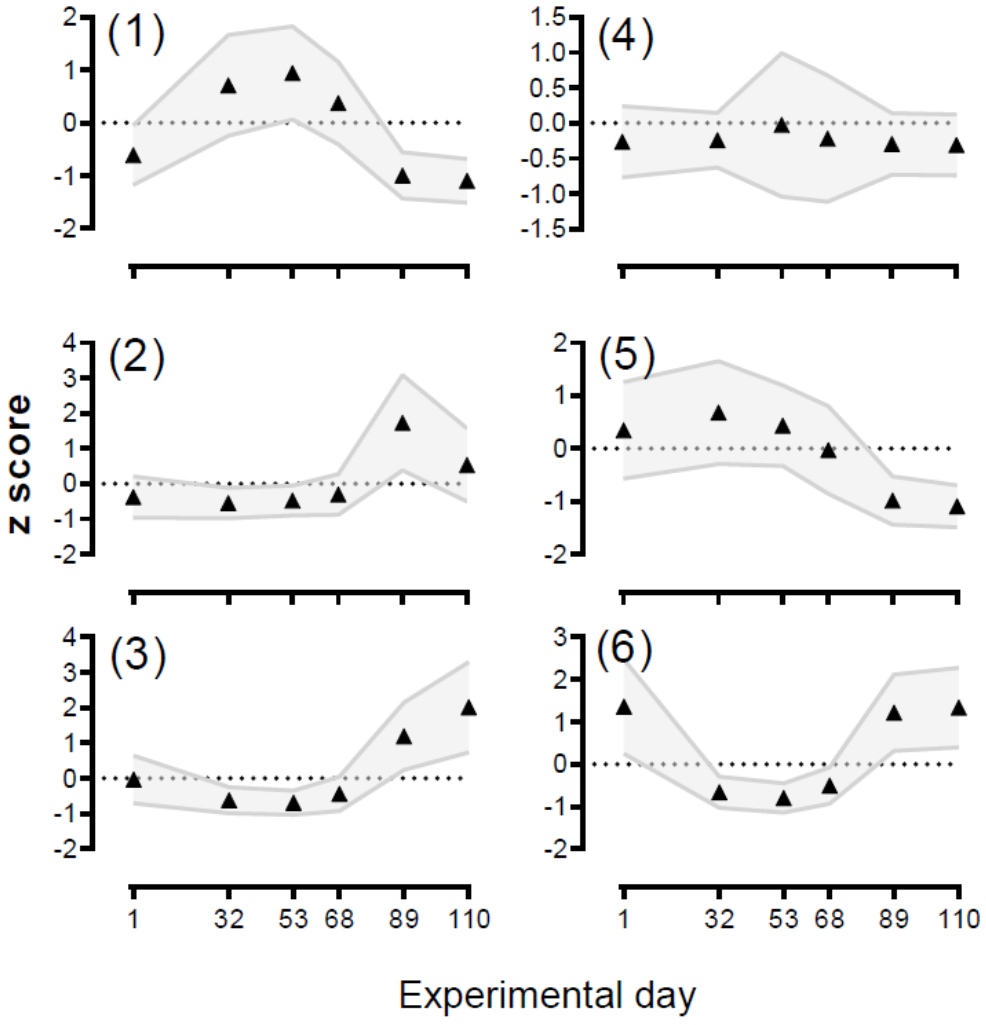


Figure 6 *Excerpt from figure 1 of Iversen et al. 2020, showing expression profiles of the six clusters. Z-score represents row-scaled and averaged counts per million for each of the six clusters of DEGs, data are mean ± SEM.*

C1 and C6 showed inverse and reversible photoperiod-dependent changes in expression over the study, with gene expression in cluster 1 increasing under SP, and gene expression in cluster 6 decreasing. In both clusters day 53 represents the largest deviation from pre-SP expression levels.

Among the genes in C1 we find both NKA *α1a* paralogues (LOC106602157, LOC106610479) and another pair of genes annotated on NCBI as NKA *α1a-like* (LOC106596139, LOC106596208). The NKA *α1a* paralogues behave similarly, increasing expression under SP. Two members of the Krüppel-like family of transcription factors are also found in clusters 1. Both are annotated as *Krüppel-like factor 15* (*KLF15*, LOC106565633 and LOC106583637). KLFs are involved in a variety of biological processes including metabolism, cell growth and differentiation (Hsieh, Fan, Sweet et al., 2018; McConnell and Yang, 2010). KLF15 is known to interact with the GR (Knoedler and Denver, 2014; Oishi and Manabe,

2018). *KLF15* has also been shown to be rhythmically expressed in mammalian tissues, under regulation by CLOCK/BMAL (Jeyaraj, Scheer, Ripperger et al., 2012). Short photoperiod induces both *KLF-15* genes, however, only one of them responds to SW when under SP, which causes a decrease in expression level. Another interesting expression pattern in this cluster is exhibited by LOC106601729 *protein Tob1-like*. SW has an inductive effect on the gene under SP. Under LL, however, SW exposure does not increase the genes expression. Tob-proteins act a transcriptional repressors across a variety of tissues and signalling pathways (Jia and Meng, 2007). Other noteworthy genes inhabiting cluster 1 includes LOC106603872, annotated as *Stanniocalcin-2*, which is downregulated upon re-entering LL. Stanniocalcin-2 is involved in calcium and phosphate homeostasis, and also implicated in the regulation of extracellular Na^+ and Cl^- (Yeung, Law, and Wong, 2012).

Expression of genes in C2 peaks distinctly, but transiently, around day 89. These genes could be central to the pre-adaptation process. Within this cluster is an *FKBP5* (LOC100196052, *fkbp5*). FKBP5 could play a role in the glucocorticoid signalling during smolting (Davies, Ning, and Sánchez, 2002; Jääskeläinen, Makkonen, and Palvimo, 2011). *Calpain 1* (*CAPN1*, LOC106611381), and an *NKCC* (LOC100136908) are also found within this cluster. Interestingly, LOC106579439, *melanocortin-2 receptor accessory protein 2A-like* (*MRAP2*) is also here. MRAP2 is an accessory protein to the G-protein coupled melanocortin receptors, the targets of ACTH and melanocyte-stimulating hormone (MSH) (Cerdá-Reverter, Agulleiro, Cortés et al., 2013; Rouault, Srinivasan, Yin et al., 2017; Webb and Clark, 2010). Two *MRAP2* paralogues have been confirmed in zebrafish (Agulleiro, Roy, Sánchez et al., 2010), and it appears that two paralogues are also present in the Atlantic salmon (Rønnestad, Gomes, Murashita et al., 2017). The zebrafish paralogues are both expressed in gill tissue, while the ACTH-receptor gene *MC2R* is not (Agulleiro, Roy, Sánchez et al., 2010). The gene annotated as *MC2R* in the salmon genome (LOC106592485, NCBI) neither appears to be expressed in the gill. While ACTH is known to be vital to cortisol release from interrenal gland during smolting, less can be asserted about the role of MSH, but it has been implicated in regulation of sodium balance and appetite (Kathpalia, Charlton, Rajagopal et al., 2011; Rønnestad, Gomes, Murashita et al., 2017; Valentin, Wiedemann, and Humphreys, 1993).

Many C2-genes are SW inducible pre-SP and under SP, for example the previously mentioned *CAPN1* and *FKBP5*. For many of these genes SW does not have an effect on expression post-SP (particularly at day 89 and beyond), as the gene expression is already increased in response to LL. Among the genes where this pattern can be distinctly observed we find LOC106608778, annotated as *insulin receptor substrate 1-B-like* (*IRS*). Hyperosmotic stress can induce cellular glucose uptake, and induce

degradation of IRS causing a disruption in insulin-regulation of glucose uptake (Gual, Le Marchand-Brustel, and Tanti, 2003). The upregulation of *IRS* once re-entering LL could thus be a pre-adaptive measure in order to prevent dysfunctional regulation of glucose metabolism. IRS have also been implicated in both the GH and the PRL signalling pathways (Yamauchi, Kaburagi, Ueki et al., 1998). There is also LOC106611586, *ELOVL fatty acid elongase 6* (*ELOVL6*), which is involved in the elongation of fatty acids into the phospholipids of the cell membrane (Weiss-Hersh, Garcia, Marosvölgyi et al., 2019). Regulation of the phospholipid composition of the plasma membrane can be important both in the context of salinity and temperature (Chen, Cui, Yan et al., 2018; Gillard, Harvey, Gjuvsland et al., 2018).

Genes in C3 increase once the fish re-enter LL and continue to stay elevated for the duration of the study. This cluster and some of its more interesting genes have been discussed in paper I (Iversen, Mulugeta, Gellein Blikeng et al., 2020). Yet, there is additional interesting genes in C3, some of which have a clear SW-response under continuous LL or under SP, similar to that of already described *FKBP5*. As SPLL fish re-enters LL FW expression increases and the SW-response becomes superfluous. Examples of C3 genes following this pattern are *forkhead box protein J1-b like* (LOC106609599, *FOXJ1*), and *caspase-3-like* (LOC106568012). While forkhead box proteins are transcription factors involved in cell proliferation and differentiation, where FOXJ1 in particular appear to be involved in the formation of cilia (Yu, Ng, Habacher et al., 2008), caspase-3 is involved in apoptosis.

Many C3 genes, however, do not respond to changes in salinity, their response appears to be induced by re-entering LL. Among these genes, following the general pattern of C3, we find *prominin-2-like* (LOC106580506, *PROM2*), and *zymogen granule membrane protein 16-like* (LOC106562679, *ZG16*). PROM2 is a transmembrane glycoprotein found in epithelial cells and is associated with lipid rafts and membrane protrusion such as cilia (Florek, Bauer, Janich et al., 2007; Jászai, Thamm, Karbanová et al., 2020; Singh, Schroeder, Scheffer et al., 2013). Prominins have been identified in goldfish where they are highly expressed in kidney and gill tissue (Walsh, Barreda, and Belosevic, 2007), and in zebrafish (Jászai, Thamm, Karbanová et al., 2020). The *ZG16-like* gene could be involved in the formation of zymogen granules and thus protein trafficking (Kleene, Dartsch, and Kern, 1999). Largely, many of the genes found in C3 are transcription factors or involved with cellular components or processes such as the plasma membrane, cytoskeleton, cell-cell communication, or transport. Overall, the nature of this cluster points to reorganization of the cellular matrix of the gill.

Genes of C4 change little, and they are both up- and downregulated. However, many of the genes in this cluster are distinctly upregulated in the SP treatment, such as LOC106575629 (*intermediate*

filament protein ON3-like), LOC106564722 and LOC106575639 (*keratin 2C type I cytoskeletal 18–like*). Annotation by similarity implies possible functions related to regulation of the cellular matrix and cell growth.

Genes of cluster 5 decrease in expression once the juvenile fish re-enters LL, and their expression remains reduced for the extent of the study. Examples are LOC106607975 (*interlukin-12 subunit-beta-like*, *IL12*), LOC106571918 (*cadherin-13-like*, *CDH13*) and LOC106602268 (*hydroperoxide isomerase ALOXE3-like*). *IL12* has also been found to be differentially expressed between FW and SW in Japanese eels (Gu, Dai, Liu et al., 2018). Cadherins are cell-cell adhesion molecules (Patel, Chen, Bahna et al., 2003). CDH13 is a special case lacking the cadherin transmembrane region; rather, it seems to serve as a signalling molecule (Hulpiau and van Roy, 2009). ALOXE3 is a lipoxygenase, implicated in epidermal differentiation and permeability (Krieg and Fürstenberger, 2014). Some genes who respond to re-entering LL are affected by SW exposure, this includes LOC106611738 (*P2Y purinoceptor 4-like*, *P2YR4*). P2YR4 is also linked to the growth and differentiation of the epidermis (Burrell, Bowler, Gallagher et al., 2003).

In C6, we find the NKA α-paralogue (LOC106575572, *NKA α1b ii*) shown in paper I (Iversen, Mulugeta, Gellein Blikeng et al., 2020), and the *claudin-10-like* (LOC106586095) gene. The latter appear to be an additional claudin to the ones detected by Tipsmark, Kiilerich, Nilsen et al. (2008). The presence of these genes in C6 could indicate why we see a change in hypo-osmoregulatory capacity once the fish enters SP and mRNA expression of these genes is reduced. In this group we also find LOC106600484, *cardiac regulatory gene junctional protein associated with coronary artery disease* (*JCAD*), which was discussed in paper II. JCAD is a involved in the regulation of endothelial cell-cell junctions (Akashi, Higashi, Masuda et al., 2011; Jones, Kaiser, Najafabadi et al., 2018). Pre-SP *JCAD* seems insensitive to SW exposure, SP exposure reduced expression levels, while post-SP *JCAD* becomes responsive to SW, causing expression levels to increase by two to four times FW levels. Interestingly, some C6 genes, including those listed here, appear unable to respond to SW-exposure under SP.

The scrutiny of these clusters reveals many candidates and possible focus areas for further investigation of gill differentiation during smolting. Some of these, through their annotation-by-similarity, reveals interesting connections and links to pathways that deserve further investigation, for example the MRAP and IRS. We also see that many of the more notable genes are involved in cell-cell adhesion or junctions and linked to both epithelial and endothelial cells. This shows that the permeability of the gill tissue is an important aspect of gill differentiation during smolting. Another interesting aspect revealed when studying the mRNA expression of these genes under the different

photoperiodic regimes, and during the 24-h SW challenge, is that many of the DEGs that are regulated in response to re-entering LL, is regulated similarly in response SW, and that the SW response is no longer needed after the light-induced regulation occurs. These genes are of particular interest as identifying their function and connections could be crucial to understanding the pre-adaption during smolting.

5. Discussion of main findings

The work presented in paper I and II confirms previous findings that successful smolting is dependent upon photoperiodic history (Björnsson, Thorarensen, Hirano et al., 1989; Duncan and Bromage, 1998; Duston and Saunders, 1990; Saunders, Henderson, and Harmon, 1985; Stefansson, Björnsson, Ebbesson et al., 2008). It also introduces novel genes whose expression in the gill is photoperiodic history-dependent (Iversen, Mulugeta, Gellein Blikeng et al., 2020). Among these genes, different photoperiodic history requirements are seen, underlining the complexity of smolting. The work of this thesis also shows that the transcriptional response to SW clearly differs between juvenile salmon kept on LL, and juvenile salmon exposed to SP before re-entering LL, despite an apparently similar capacity to hypo-osmoregulate (paper II). Notably, groups differed in the enrichment of GRE- and NFAT5-response elements in the transcriptomic response to SW exposure. Paper III clearly points to a role for differentiated clock-genes in the gill during smolt development and in the control of osmoregulation. The work of this thesis brings forward overlooked aspects of gill pre-adaption during smolting such as differentiation of cell-cell junctions and distinct control of the osmosensing pathways. These findings and their inferences are discussed below.

5.1 Novel genes linked to smolting

The novel genes introduced in paper I (Iversen, Mulugeta, Gellein Blikeng et al., 2020) show photoperiodic history-dependent expression profiles, with *tryptophan hydroxylase 1* (*TPH1*) and *CAPN2* showing the strongest pattern of history-dependence. Both *TPH1* and *CAPN2* require between four and eight weeks of SP before LL can stimulate any increase in their expression. Though the four other novel genes show photoperiod-history-dependence in the strength of their response after SP exposure, which is statistically significant, re-exposure to LL appears to brings about a rise in expression regardless of length of the SP-exposure (for example as in *S100A* and *SLCA7*). This could be understood as differences in the cellular preparedness for facilitating the expression. It is possible that this photoperiodic history-dependence can be interpreted as a need for certain processes to run their course prior to light induction. This might include both inhibitory and activational processes. The effect of SP could be similar to the effect of cold temperatures in the

vernalization process of plants (Amasino, 2004), where the juvenile salmon gains the competence to smolt, but requires exiting SP before smolting can occur.

The genes from paper I can be linked to numerous cellular pathways and functions. While it is difficult to speculate on gill-specific and smolt-related pathways and function, existing literature can provide some direction. It should, however, be pointed out that annotation of these novel genes is based on sequence similarity with that of well-mapped genomes (Lien, Koop, Sandve et al., 2016) and thus has some level of uncertainty.

As described in section 1.3.1 the gill consists of multiple cell types, serving a variety of functions. Without further histological analysis or transcriptome analysis of individual cells, we cannot be sure of the type of cell in which these novel genes are expressed. West, Mizoro, Wood et al. (2020) applied single-nuclei RNA sequencing and statistically identified 20 distinct cell types in the gill. In addition to the expected increase in MRCs during smolting, West, Mizoro, Wood et al. (2020) could also show a reduction in immune-related cell types during smolting.

CAPN2 is a calcium-dependent proteolytic enzyme. It is implicated in the terminal differentiation of cell systems, known to control the function of several cytoskeletal and focal adhesion proteins (Carragher and Frame, 2002; Franco, Perrin, and Huttenlocher, 2004; Sato and Kawashima, 2001). It is also possibly involved in the regulation of G proteins (Lombardi, Kavelaars, Penela et al., 2002; Sato and Kawashima, 2001). The potential role of CAPN2 in cell-cell junctions, cell (MRC) migration, interdigitation of the MRCs by ACs, and on G protein-gated ion channels deserves further investigation. We, for example, know that the tight junctions between MRCs become leakier during smolting (Lubin, Rourke, and Bradley, 1989; Pisam, Prunet, Boeuf et al., 1988; Sardet, Pisam, and Maetz, 1979), and CAPN2 is a candidate to be involved in this process through its interaction with the cytoskeletal proteins of the tight junctions (Bozym, Patel, White et al., 2011; Chun and Prince, 2009; Wang, Wang, Moreno-Vinasco et al., 2012). The presence of CAPN1 in C2 further signifies the importance of calpain during gill differentiation.

Some of the other genes studied in paper I, and discussed in section 4, such as *S100A* and *ST6GALNAC2*, have also been linked to the cytoskeleton, cell-cell and cell-substrate interaction (Samyn-Petit, Krzewinski-Recchi, Steelant et al., 2000; Wright, Cannon, Zimmer et al., 2009).

TPH1 shows a strong dependence upon photoperiodic history. TPH1 is the rate-limiting enzyme in the synthesis of serotonin, which is known to have a role in vasoconstriction in the gill (Nilsson and Sundin, 1998; Sundin, Nilsson, Block et al., 1995). Regulation of blood flow through the gill could be

connected to both gas exchange, acid-base balance and osmoregulation (Evans, Piermarini, and Choe, 2005). Serotonergic neuroepithelial cells have been described in the gills of zebrafish and implicated in chemoreception, where neuronal signalling potentially influences ventilation and heart rate, in addition to vasoconstriction (Jonz and Nurse, 2003).

FKBP5 also show a strong photoperiodic history-dependent response to re-entering LL. FKBP5 is part of the glucocorticoid signalling network, and in particular plays a role in the translocation of hormone-bound GRs to the nucleus (Jääskeläinen, Makkonen, and Palvimo, 2011). Thus FKBP5 could be influential in the sensitivity of the gill to cortisol (Davies, Ning, and Sánchez, 2002; Jääskeläinen, Makkonen, and Palvimo, 2011). As describe earlier the glucocorticoid network is important during smolting and in the regulation of ion pumps such as CFTR and NKA, but also regulation of paracellular permeability (Kelly and Chasiotis, 2011; Kiilerich, Pedersen, Kristiansen et al., 2011; Shrimpton and McCormick, 1999). In contrast to *CAPN2* and *TPH1*, *FKBP5* peaks already at four weeks post-SP (which is also when the C2 *FKBP5* gene peaks) designating it as an early response gene (comparatively).

5.2 Role of photoperiodic history-dependence in smolting and adaptation of the gill

Paper I illustrates that growth, and by extension welfare, in SW is dependent upon photoperiodic history. This is consistent with findings presented by Saunders, Henderson , and Harmon (1985), whom found that juvenile salmon grew well under LL in FW, but grew far less than fish kept on a natural photoperiod (LDN) when acclimated to SW. The link between photoperiodic history-dependence and growth-performance in SW is found in the pre-adaptation (or lack thereof) of the gill (Iversen, Mulugeta, Gellein Blikeng et al., 2020).

During experiment 2 of paper I all treatment groups appeared to osmoregulate and do well in the 24-h SW challenge at four and eight weeks post-SP (figure 3b, paper I), indicating their smolts status, but only the group having spent eight weeks under SP (8WSP) grew well during the prolonged SW challenge. The 8WSP group was also the only group to have significantly increased NKA activity. As described above the novel genes *TPH1*, *CAPN2*, *FKBP5*, and the ion channel *CFTR*, show the same strong photoperiodic history-dependence as NKA activity and SW growth. They are all dependent upon more than four weeks of SP exposure for LL exposure to significantly induce their expression. For *ST6GALNAC2*, *SLCA7* and *S100A* four weeks of SP appears to be sufficient. Interestingly, in view

of NKA activity, *NKA α1b* is equally induced in all three treatment groups, appearing to be independent of photoperiod history.

The surveyed genes can thus be divided in three groups;

i) More than four weeks of SP required for LL to trigger response
ii) Two to four weeks of SP sufficient for LL to trigger response
iii) Two weeks of SP sufficient for LL to trigger response

While the first group is a primary example of photoperiodic history-dependence, group three might be responsive to any period of SP exposure and cannot be categorized as photoperiodic history-dependent. For pre-adaptation of the gill, and by extension smolting, to ultimately be successful changes in gene expression, and associated pathways, must take place in a synchronized manner. It appears that this can only occur appropriately given more than four weeks of SP prior to entering LL. In a study by Duncan and Bromage (1998) they found that smoltification was incomplete in juvenile salmon exposed to less than six weeks of SP, and that the many processes of smolting showed some desynchronization in all the artificial photoperiod regimes compared to their control group on a natural light regime. This emphasizes the complexity of smolting, being a sequential program of developmental changes culminating in a SW-ready phenotype.

Entering SW with anything less than a prepared gill would place a high energy expenditure on acute osmoregulatory responses until the fish either is able to assemble the required adaptations or continue to weaken and succumbs in response to the hyperosmotic stress.

Paper II comes closer to describing what a prepared gill appears like in terms of SW response. The distinctive pattern of the PCA and the comparison of GO terms from each SW-challenged group of experiment 1 makes it clear that the LL, SP and SPLL groups exhibit different acute responses to SW, again demonstrating the importance of photoperiod-history for SW pre-adaption.

Another clear distinction between the LL, SP and SPLL gill found in paper II is the lack of NFAT- and GRE-motif enrichment among the genes expressed in the SPLL gill during SW challenge. This indicates reduced induction of hypo-osmotic pathways, and implies that the fish is less disturbed by SW, in other words, the gill is well-prepared to deal with SW (Iversen, Mulugeta, West et al., 2020). This contrast strongly with the strong enrichment of GRE-motifs among the genes expressed by the SP-

gill, and enrichment of NFAT-motifs in the LL gill, indicating the induction of acute stress-related and osmoregulatory pathways.

Together paper I and II shows that a fully pre-adapted gill requires a minimum period of between four to eight weeks of SP-exposure. This in in line with Duncan and Bromage (1998) whom conclude that five to six weeks of SP exposure is necessary for smoltification to ensue. Paper I show this by exposing the energetic cost of not having a well-prepared gill, resulting in stunted growth. Paper II illustrates that the transcriptomes of the pre-adapted gill and the unprepared gill differ drastically as exemplified by enrichment for GO terms, and NFAT- and GRE-motifs.

5.3 Diversified clock genes involved in the regulation of smolting and SW adaptation

Findings presented in paper III (West, Iversen, Jørgensen et al., 2020) indicate that clock-genes derived from WGD could be involved in the regulation of smolting. The study found that 30 clock genes were differentially expressed in the gill during smolting, and 12 of these clock genes were also found to be differentially expressed in response to SW. The majority of these genes were found to be derived from Ss4R. Promoter motif analysis showed that the SW-inducible genes were enriched for HSF1 (heat shock factor 1), FOXO1 (forkhead box O1), MAX1 (myc-associated factor X1) and glucocorticoid response elements (GREs)(West, Iversen, Jørgensen et al., 2020). While HSF1 and FOXO1 response elements are known to be responsive to IGF1 signalling, GREs are under the influence of cortisol. In mammals a strong linked has been established between cortisol and circadian coordination of the HPA-axis, but a similar link to the teleost HPI-axis has not been confirmed (Mommsen, Vijayan, and Moon, 1999). Both cortisol and IGF1 are well established as significant hormones in the developmental control of smolting and regulation of SW-adaptation in salmonids.

Many of the responsive Ss4R genes were identifiable as ohnologues, and around half of these pairs showed tissue specific within-pair divergence of expression during smolting, while divergence in diurnal expressions patters were rare (West, Iversen, Jørgensen et al., 2020). This tissue-specific divergence is likely a result of sub-functionalization (Conant and Wolfe, 2008) where selection pressure has operated on the promoter regions meeting tissue-specific requirements. This aligns with findings presented in box 1, where gene pairs differ in environmental regulation (light, salt, both, unknown) without showing changes in the coding regions of the genes. This also suggests that the primary evolutionary driver behind the divergence in regulation is tissues specific requirements in regulation of gene expression rather than functional changes in the gene itself.

Recent insights into how gene expression change during smolting and SW-adaptation indicate that the multitude of gene pairs derived from WGD-events have provided the salmonids with an excess of redundant genes facilitating the development of anadromy (Macqueen and Johnston, 2014; Robertson, Gundappa, Grammes et al., 2017).

5.4 Regulation of NKA activity

This section brings in additional data to provide a more extensive discussion on certain findings in paper I and paper II. First, I discuss the inertia of NKA activity despite *NKA α1b* being expressed and provide additional data on other constituents of the NKA ion pump. Second, I take a look at the possible role of deiodinases and NFAT in the regulation of NKA.

5.4.1 Regulation of NKA and its constituents during smolting

Reciprocal regulation of *NKA α1a* (down) and *α1b* (up) accompanied by an increase in NKA activity has long been considered a characteristic of SW-readiness in juvenile salmon (McCormick, Regish, and Christensen, 2009; Nilsen, Ebbesson, Madsen et al., 2007). A study by Christensen, Regish , and McCormick (2018), using juvenile salmon kept under LDN, concluded that protein levels of NKA α1b is the best predictor for NKA activity. In this study they did not observe any significant increase in *α1b* mRNA, alluding to the significance of post-transcriptional and translational mechanisms for protein load (Halbeisen, Galgano, Scherrer et al., 2007; Vogel and Marcotte, 2012). Christensen, Regish , and McCormick (2018) also looked at the dynamics of NKA α1a mRNA and protein. In juvenile salmon under LDN *α1a* mRNA was found to decrease early in spring, while protein levels decreased sometime later, together with peaks in cortisol, NKA activity and α1b-protein load. This implies a complex and finely timed shift from α1a to α1b NKA ion transporters in the gill.

In paper I we observed an equal increase in *α1b* mRNA in all three treatment groups at four weeks post-SP. NKA activity, however, was only significantly increased in the 8WSP group. What causes this inertia in NKA activity? Is this due to post-transcriptional or translational limitations, or is it caused by the necessity of other components such as the NKA β-subunit? Very few studies on smolting or euryhaline fish have included the other components of the NKA ion transporter such as the β-subunit, or the FXYD-proteins (Hu, Chu, Yang et al., 2017; Tipsmark, Mahmmoud, Borski et al., 2010). Both subunits modify ion affinity, contribute to stabilization, and ensures transport and membrane localisation of the NKAαβ-heterodimer (Geering, 2008; Li and Langhans, 2015). Can the lack of these components be limiting NKA activity in treatment groups exposed to four, or fewer, weeks of SP? Nilsen, Ebbesson, Madsen et al. (2007), in their experiment comparing an anadromous salmon strain

with a landlocked salmon population (under LDN), show the *NKA β1* mRNA expression to peak about a month prior to *NKA α1b* mRNA and NKA activity peaks in the anadromous strain. In Madsen, Kiilerich , and Tipsmark (2009) FW to SW transfer of post-smolts resulted in increased mRNA levels of *NKA α1b* after only three days, while NKA activity and *NKA β1* were increased after seven days. This implies that upregulation of NKA β1 is highly significant for NKA activity.

Data collected during this thesis work, but so far unpublished (appendix 2), show that during experiment 1 there is a peak in both *NKA β1* and *NKA α1b* mRNA (paper I) at four weeks post-SP. In the same time span *NKA α1a* mRNA levels decrease. There is also an induction of *NKA β233* (LOC100195568, also known as *atp1b1a*), which is found to be salt-induced in sole (Armesto, Infante, Cousin et al., 2015). Limited transcriptomics data from experiment 2 show similar patterns (appendix 3), with increased expression of *NKA β* and *NKA β233*, in both the 8WSP and the 2WSP group (induced by re-entering LL). Our experiments thus show simultaneous increases of *NKA α1b* and *NKA β*-mRNA indicating that it could not act as a limiting factor on NKA activity in our experiments. The same is true for the *FXYDs* (*11a*, *11b* and *5a*, appendix 2 and 3) (Tipsmark, Mahmmoud, Borski et al., 2010). This indicates that photoperiodic history-dependent post-transcriptional and translational processes controlling mRNA translation, protein transport and turnover could be responsible for the inertia of NKA activity in the 2WSP group. Findings also indicate that lipid microdomains in the plasma membrane might influence the functioning of NKA (Welker, Geist, Frühauf et al., 2007), and that the transitions from NKA α1a to α1b during smolting could be important for the contact with the lipid microdomain (Lingwood, Harauz, and Ballantyne, 2005). Learning more about these processes and understanding the importance of lipid microdomains for NKA activity, seems instrumental to improving our understanding of smolting, SW-preadaptation, and improving the quality of smolt in aquaculture.

5.4.2 Are deiodinases a part of the NKA regulatory system?

The regulation of NKA α1b and NKA activity (Shrimpton and McCormick, 1999) has been linked to the corticosteroid pathways, and elevated plasma levels of GH and cortisol (Kiilerich, Kristiansen, and Madsen, 2007a; Kiilerich, Pedersen, Kristiansen et al., 2011; McCormick, Björnsson, Sheridan et al., 1995; Shrimpton and McCormick, 1998a; Tipsmark and Madsen, 2009). The increase T_4 during smolting has been connected to the regulation of CR in the gill (Shrimpton and McCormick, 1998a). For TH to exert its action it needs to be converted T_3, this action is performed by dio2. Atlantic salmon have two isoforms of *dio2* (Lorgen, Casadei, Król et al., 2015). Lorgen, Casadei, Król et al.

(2015) showed that mRNA expression of *dio2a* was SW induced, while *dio2b* increased post-SP in juvenile salmon being put through a smolt-inducing photoperiod regime.

Data from experiment 1 and experiment 2 (appendix 2 and 3) show that *dio2b* is induced by increased photoperiod. Similar to *NKA α1b* and *β* it does not exhibit any particular photoperiodic history-dependence, and the response is much stronger in the 8WSP group than in the 2 WSP group. While *NKA α1* is regulated via corticosteroid signalling (Kiilerich, Kristiansen, and Madsen, 2007a; Kiilerich, Pedersen, Kristiansen et al., 2011), literature indicate, assuming it is a conserved feature, that the salmon *NKA β1* promoter can contain thyroid response elements (Feng, Orlowski, and Lingrel, 1993).

Lorgen, Casadei, Król et al. (2015) found the promoter region of *dio2a* but not *dio2b* to be enriched in NFAT5-response elements. They suggest divergent evolution of the *dio2*-paralogues supporting an organ-specific timing (brain and gill) of TH-dependent events during smoltification and migration. It can further be hypothesized that both deiodinases play important roles in the gill, as follows:

a) Acute: Induction of *dio2a*, via NFAT5, is the result of an acute SW-induced response inducing a shift in gill physiology and utilizing the readily available T_4 to produce T_3. T_3 is then involved in the transcriptional activation of SW adaptation, including upregulation of CR (Kiilerich, Kristiansen, and Madsen, 2007b; Shrimpton and McCormick, 1998a; Terrien, Fini, Demeneix et al., 2011), and possibly in the upregulation of *NKA β1*.
b) Pre-adaptive: During smolting, as plasma T_4 increases, *dio2b* is upregulated in the gill where it exerts control of the T_4-T_3-deiodination process. Increasing T_3 influences CR density (Shrimpton and McCormick, 1998a), enabling the gill to respond to the increased cortisol levels. T_3 could also here be involved in the transcriptional activation of *NKA β1*.

The thyroid hormone has long been implicated in smolting, however attempts at manipulating systemic TH levels have been inconclusive (Björnsson and Bradley, 2007). The tissue specific, and even cell-specific, control of TH deiodination through regulation of the deiodinases could be key to further understanding the role of TH in smolting (Arrojo e Drigo and Bianco, 2011; Gereben, Zavacki, Ribich et al., 2008; Larsen, Swanson, and Dickhoff, 2011; Specker, Brown, and Bern, 1992). The very basic hypothesis described above describes how local control of TH could be implied in both an acute SW-response and the pre-adaptive process of smolting through the control of the functionally diverged *dio2*-genes (Lorgen, Casadei, Król et al., 2015) and deiodinase activity.

5.5 Experimental design

When designing an experiment, one must make many decisions that affects both outcome and interpretation. One also has to consider the number of experimental animals, the potential strain on them, the capacity of the facility to keep and maintain the animals during the experiment, and capacity for sampling. When running smolting experiments one needs to consider the duration of the photoperiod treatments (and whether these should change abruptly or gradually), and total duration of the experiment.

When designing experiment 2 it was opted to have all groups enter SP simultaneously, and then stagger the re-entry to LL. It was also decided to allow only eight weeks of LL for each of the groups before transfer to SW. The results of experiment 2 show similar trends it the 4WSP and the 8WSP-group when it comes to osmoregulatory capacity, NKA activity, and expression of a subset of genes. Some of these trends are also observed in the 2WSP-group. However, within the allowed eight weeks the 2WSP and 4WSP-groups do not achieve the same levels as the 8WSP-group.

A different experimental design, allowing the 2WSP and 4WSP groups 14 and 12 weeks post-SP, respectively, to compensate for the shorter SP-exposure could have given answers as to whether the 2WSP and 4WSP groups would be able to achieve the same level of SW-readiness as the 8WW-group within 16 weeks from onset of SP. It is possible that entry to SP sets in motion certain processes that do not complete until a certain period of time has passed since SP-entry. Also, some of these processes could be inhibited from acceleration by LL until after an unknown threshold (4-8 eight weeks as indicated by paper I) when light-induction is permitted. This would also allow these processes to be on hold during longer periods of SP exposure (i.e. Arctic winter). Vernalization is a similar process taking place in plants, where prolonged cold exposure enables the plant to flower, but additional ques are usually necessary for the flowering to occur (Amasino, 2004).

In both experiments the juvenile salmon from the beginning show a surprising ability to hypo-osmoregulate during SW challenge. During the 24-h. SW test they are able to maintain plasma osmolality at a physiologically sound level. Since these are captive fish, they have been growing well, and this could have facilitated some form of pseudo-smolting providing the salmon juveniles with a more effective response to SW. This could possibly be initiated directly due to metabolic status, or through a hypothetical endogenous timer activated as fish enters the UM (Eriksson and Lundqvist, 1982), in both cases related to the bimodal size-distribution of juvenile salmon (Kristinsson, Saunders, and Wiggs, 1985). The ability to maintain a sound plasma osmolality level is quickly lost once the fish enter SP. The shorter days effectively prompt degeneration of osmoregulatory capacity,

similar to how smolts de-smoltify if they have not entered SW prior to reduction in daylength (Duston and Saunders, 1990).

Providing a gradual change in photoperiod require that the experimental facility has the required technical systems. It is, however, unclear if and how the manner of photoperiod transition affects seasonal biology as good comparative studies have not been performed. Butler, Turner, Park et al. (2010) found differences in responsiveness between Siberian hamster that had been exposed to either LDN changes or abrupt transitions. This topic needs further studies for clarification.

5.6 Future perspectives

Based on the findings presented here some questions stand out as particularly relevant for future research. While much is known about the circulatory endocrine signals and the differentiation of the MRCs, very little information exists to establish causal linkages between these two.

In order to move forward an improved understanding of the transformation of the gill and the MRCs is necessary. The gill transcriptome changes dramatically during smolting, but also when non-smoltified fish are exposed to SW. Continued efforts to study how osmotic perturbation this causes functional changes in the gill will be important. Similarly it will be significant to investigate how the historical WGDs and LGDs have influenced the evolution of smolting through subfunctionalization, or even neofunctionalization, of available redundant genes. Paralogue promoter motifs should be inspected to reveal whether there has been a divergence, separating paralogues into those that are responsive to acute SW-exposure and those that are responsive to endocrine signalling (photoperiodic history-dependent) similar to what has been observed for *dio2* (Lorgen, Casadei, Król et al., 2015).

Cell-specific approaches, such as single-cell sequencing would enable a better functional description of the different cell-types found in the gill. Though the MRC is vital to osmoregulation, other cell types such as the mucus cells, would also be expected to differentiate in preparation for SW migration. Concurrent with this histological time-series could be used to observe the functional shift in cell morphology and gill physiology during smolting.

Mapping the expressed genes onto pathways and networks linked to specific cellular activity or processes, and analysing these, would increase our understanding of the cellular processes involved in gill development during smolting and SW-tolerance.

The findings presented in this thesis have both implications and application for the aquaculture industry. The implications are that ensuring proper smoltification within each cohort of salmon benefits growth in SW, which translates to improved health and welfare (Stien, Bracke, Folkedal et al., 2013). Salmon juveniles who are not properly smoltified will at best grow poorly until harvest (Iversen, Mulugeta, Gellein Blikeng et al., 2020; Saunders, Henderson, and Harmon, 1985). The results of this thesis could contribute to targeted efforts to develop better tests for estimating smolt quality and SW readiness.

5.7 Concluding remarks

In conclusion, photoperiodic history impacts the gills capability to respond to i) increased photoperiod and ii) SW (Iversen, Mulugeta, Gellein Blikeng et al., 2020; Iversen, Mulugeta, West et al., 2020). This has ramifications for the long-term welfare and growth of the salmon in SW (Iversen, Mulugeta, Gellein Blikeng et al., 2020).

This thesis has contributed to increased understanding of the timing of smoltification and shed light onto new and important aspects of gill differentiation through the identification of novel photoperiodic history-dependent genes. It has resulted in the first paper to show a reduced osmoregulatory response, exemplified by the NFAT-family and (lack of) GRE-motif enrichment, in pre-adapted juvenile salmon. In addition, it has added weight to the importance of WGD in the development of anadromy through showing that ohnologue pairs of clock-genes have evolved tissue-specific expression profiles that correlate with smolting and responses to SW.

The work presented here also brings about many new questions for continued exploration of the salmon transcriptome during smolting. The transcriptomics dataset (ArrayExpress, ac. E-MTAB-8276) presented within all three papers contain data on about 80 000 genes expressed in the gill during the smolting process in Atlantic salmon, and will hopefully be used also in future studies to further enhance our understanding of how a SW gill is prepared during smolting.

6. References

Abraham, M. (1971). The ultrastructure of the cell types and of the neurosecretory innervation in the pituitary of *mugil cephalus* l. From fresh water, the sea, and a hypersaline lagoon: I. The rostral pars distalis. *General and Comparative Endocrinology, 17*(2), 334-350. https://doi.org/10.1016/0016-6480(71)90144-4

Agulleiro, M. J., Roy, S., Sánchez, E., Puchol, S., Gallo-Payet, N., & Cerdá-Reverter, J. M. (2010). Role of melanocortin receptor accessory proteins in the function of zebrafish melanocortin receptor type 2. *Molecular and cellular endocrinology, 320*(1-2), 145-152. https://doi.org/10.1016/j.mce.2010.01.032

Ágústsson, T., Ebbesson, L. O. E., & Björnsson, B. T. (2000). Dopaminergic innervation of the rainbow trout pituitary and stimulatory effect of dopamine on growth hormone secretion in vitro. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 127*(3), 355-364. https://doi.org/10.1016/S1095-6433(00)00265-8

Ágústsson, T., Sundell, K., Sakamoto, T., Johansson, V., Ando, M., & Bjornsson, B. T. (2001). Growth hormone endocrinology of atlantic salmon (*salmo salar*): Pituitary gene expression, hormone storage, secretion and plasma levels during parr-smolt transformation. *Journal of endocrinology, 170*(1), 227-234. Retrieved from https://joe.bioscientifica.com/downloadpdf/journals/joe/170/1/227.pdf

Akashi, M., Higashi, T., Masuda, S., Komori, T., & Furuse, M. (2011). A coronary artery disease-associated gene product, jcad/kiaa1462, is a novel component of endothelial cell–cell junctions. *Biochem Bioph Res Co, 413*(2), 224-229. https://doi.org/10.1016/j.bbrc.2011.08.073

Alexandrou, M. A., Swartz, B. A., Matzke, N. J., & Oakley, T. H. (2013). Genome duplication and multiple evolutionary origins of complex migratory behavior in salmonidae. *Molecular Phylogenetics and Evolution, 69*(3), 514-523. https://doi.org/10.1016/j.ympev.2013.07.026

Amasino, R. (2004). Vernalization, competence, and the epigenetic memory of winter. *The Plant Cell, 16*(10), 2553-2559. https://doi.org/10.1105/tpc.104.161070

Anglade, I., Zandbergen, T., & Kah, O. (1993). Origin of the pituitary innervation in the goldfish. *Cell and Tissue Research, 273*(2), 345-355. https://doi.org/10.1007/BF00312837

Armesto, P., Infante, C., Cousin, X., Ponce, M., & Manchado, M. (2015). Molecular and functional characterization of seven na+/k+-atpase β subunit paralogs in senegalese sole (*solea senegalensis* kaup, 1858). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 182*, 14-26. https://doi.org/10.1016/j.cbpb.2014.11.011

Armstrong, J. D., Huntingford, F. A., & Herbert, N. A. (1999). Individual space use strategies of wild juvenile atlantic salmon. *Journal of Fish Biology, 55*(6), 1201-1212. https://doi.org/10.1111/j.1095-8649.1999.tb02070.x

Arnone, M. I., & Davidson, E. H. (1997). The hardwiring of development: Organization and function of genomic regulatory systems. *Development, 124*(10), 1851-1864. Retrieved from https://dev.biologists.org/content/develop/124/10/1851.full.pdf

Arrojo e Drigo, R., & Bianco, A. C. (2011). Type 2 deiodinase at the crossroads of thyroid hormone action. *The International Journal of Biochemistry & Cell Biology, 43*(10), 1432-1441. https://doi.org/10.1016/j.biocel.2011.05.016

Aruna, A., Nagarajan, G., & Chang, C. F. (2012). Involvement of corticotrophin-releasing hormone and corticosteroid receptors in the brain–pituitary–gill of tilapia during the course of seawater acclimation. *Journal of Neuroendocrinology, 24*(5), 818-830. https://doi.org/10.1111/j.1365-2826.2012.02282.x

Barron, M. G. (1986). Endocrine control of smoltification in anadromous salmonids. *Journal of Endocrinology, 108*(2), 313. https://doi.org/10.1677/joe.0.1080313

Barton, M., & Bond, C. E. (2007). Circulation and gas exchange. In M. Barton & C. E. Bond (Eds.), *Bond's biology of fishes* (3rd ed., pp. 441-468). Belmont, CA.: Thomson.

Bégay, V., Falcón, J., Cahill, G. M., Klein, D. C., & Coon, S. L. (1998). Transcripts encoding two melatonin synthesis enzymes in the teleost pineal organ: Circadian regulation in pike and zebrafish, but not in trout. *Endocrinology, 139*(3), 905-912. https://doi.org/10.1210/endo.139.3.5790

Berge, A. I., Berg, A., Fyhn, H. J., Barnung, T., Hansen, T., & Stefansson, S. O. (1995). Development of salinity tolerance in underyearling smolts of atlantic salmon (*salmo salar*) reared under different photoperiods. *Can J Fish Aquat Sci, 52*(2), 243-251. https://doi.org/10.1139/f95-024

Berthelot, C., Brunet, F., Chalopin, D., Juanchich, A., Bernard, M., Noël, B., . . .Guiguen, Y. (2014). The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. *Nature Communications, 5*. https://doi.org/10.1038/ncomms4657

Björn, L. O. (2015). *Photobiology: The science of light and life* (L. O. Björn Ed. 3rd ed.). Verlag, NY: Springer.

Björnsson, B. T. (1997). The biology of salmon growth hormone: From daylight to dominance. *Fish Physiology and Biochemistry, 17*(1), 9-24. https://doi.org/10.1023/A:1007712413908

Björnsson, B. T., & Bradley, T. M. (2007). Epilogue: Past successes, present misconceptions and future milestones in salmon smoltification research. *Aquaculture, 273*(2), 384-391. https://doi.org/10.1016/j.aquaculture.2007.10.020

Björnsson, B. T., Johansson, V., Benedet, S., Einarsdottir, I. E., Hildahl, J., Agustsson, T., & Jönsson, E. (2002). Growth hormone endocrinology of salmonids: Regulatory mechanisms and mode of action. *Fish Physiology and Biochemistry, 27*(3), 227-242. https://doi.org/10.1023/B:FISH.0000032728.91152.10

Björnsson, B. T., Thorarensen, H., Hirano, T., Ogasawara, T., & Kristinsson, J. B. (1989). Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypoosmoregulatory ability of juvenile atlantic salmon (*salmo salar*) during parr-smolt transformation. *Aquaculture, 82*(1-4), 77-91. https://doi.org/10.1016/0044-8486(89)90397-9

Bolliet, V., Ali, M. A., Lapointe, F.-J., & Falcón, J. (1996). Rhythmic melatonin secretion in different teleost species: An in vitro study. *Journal of Comparative Physiology B, 165*(8), 677-683. https://doi.org/10.1007/BF00301136

Bossus, M., Charmantier, G., & Lorin-Nebel, C. (2011). Transient receptor potential vanilloid 4 in the european sea bass *dicentrarchus labrax*: A candidate protein for

osmosensing. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 160*(1), 43-51. https://doi.org/10.1016/j.cbpa.2011.04.014

Bozym, R. A., Patel, K., White, C., Cheung, K.-H., Bergelson, J. M., Morosky, S. A., & Coyne, C. B. (2011). Calcium signals and calpain-dependent necrosis are essential for release of coxsackievirus b from polarized intestinal epithelial cells. *Molecular Biology of the Cell, 22*(17), 3010-3021. https://doi.org/10.1091/mbc.e11-02-0094

Breves, J. P., Fox, B. K., Pierce, A. L., Hirano, T., & Grau, E. G. (2010). Gene expression of growth hormone family and glucocorticoid receptors, osmosensors, and ion transporters in the gill during seawater acclimation of mozambique tilapia, *oreochromis mossambicus*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 313A*(7), 432-441. https://doi.org/10.1002/jez.613

Burleson, M. L. (2009). Sensory innervation of the gills: O2-sensitive chemoreceptors and mechanoreceptors. *Acta Histochemica, 111*(3), 196-206. https://doi.org/10.1016/j.acthis.2008.11.002

Burrell, H. E., Bowler, W. B., Gallagher, J. A., & Sharpe, G. R. (2003). Human keratinocytes express multiple p2y-receptors: Evidence for functional p2y1, p2y2, and p2y4 receptors. *Journal of Investigative Dermatology, 120*(3), 440-447. https://doi.org/10.1046/j.1523-1747.2003.12050.x

Butler, M. P., Turner, K. W., Park, J. H., Schoomer, E. E., Zucker, I., & Gorman, M. R. (2010). Seasonal regulation of reproduction: Altered role of melatonin under naturalistic conditions in hamsters. *Proceedings of the Royal Society B: Biological Sciences, 277*(1695), 2867-2874. https://doi.org/10.1098/rspb.2010.0396

Bystriansky, J. S., & Ballantyne, J. S. (2007). Gill na+-k+-atpase activity correlates with basolateral membrane lipid composition in seawater- but not freshwater-acclimated arctic char (*salvelinus alpinus*). *American Journal of Physiology -Regulatory, Integrative and Comparative Physiology, 292*(2), R1043-R1051. https://doi.org/10.1152/ajpregu.00189.2005

Carragher, N. O., & Frame, M. C. (2002). Calpain: A role in cell transformation and migration. *Int J Biochem Cell Biol, 34*(12), 1539-1543. https://doi.org/10.1016/S1357-2725(02)00069-9

Carroll, S. B. (2008). Evo-devo and an expanding evolutionary synthesis: A genetic theory of morphological evolution. *Cell, 134*(1), 25-36. https://doi.org/10.1016/j.cell.2008.06.030

Cerdá-Reverter, J. M., Agulleiro, M. J., Cortés, R., Sánchez, E., Guillot, R., Leal, E., . . .Eley, M. (2013). Involvement of melanocortin receptor accessory proteins (mraps) in the function of melanocortin receptors. *General and Comparative Endocrinology, 188*, 133-136. https://doi.org/10.1016/j.ygcen.2013.01.017

Cerdá‐Reverter, J. M., & Canosa, L. F. (2009). Neuroendocrine systems of the fish brain. In N. J. Bernier, G. Van der Kraak, A. P. Farrel, & C. J. Brauner (Eds.), *Fish neuroendocrinology* (Fish physiology, Vol. 28, pp. 3-74): Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/S1546509809280010

Chasiotis, H., Kolosov, D., Bui, P., & Kelly, S. P. (2012). Tight junctions, tight junction proteins and paracellular permeability across the gill epithelium of fishes: A review. *Respiratory Physiology & Neurobiology, 184*(3), 269-281. https://doi.org/10.1016/j.resp.2012.05.020

Chen, J., Cui, Y., Yan, J., Jiang, J., Cao, X., & Gao, J. (2018). Molecular characterization of elongase of very long-chain fatty acids 6 (elovl6) genes in *misgurnus anguillicaudatus* and their potential roles in adaptation to cold temperature. *Gene, 666*, 134-144. https://doi.org/10.1016/j.gene.2018.05.019

Cheung, C. Y. K., & Ko, B. C. B. (2013). Nfat5 in cellular adaptation to hypertonic stress – regulations and functional significance. *Journal of Molecular Signaling, 8*(1), 5. https://doi.org/10.1186/1750-2187-8-5

Chi, L., Li, X., Liu, Q., & Liu, Y. (2017). Photoperiod regulate gonad development via kisspeptin/kissr in hypothalamus and saccus vasculosus of atlantic salmon (*salmo salar*). *PloS one, 12*(2), e0169569. https://doi.org/10.1371/journal.pone.0169569

Chi, L., Li, X., Liu, Q., & Liu, Y. (2019). Photoperiod may regulate growth via leptin receptor a1 in the hypothalamus and saccus vasculosus of atlantic salmon (*salmo salar*). *Animal Cells and Systems, 23*(3), 200-208. https://doi.org/10.1080/19768354.2019.1595138

Christensen, A. K., Regish, A. M., & McCormick, S. D. (2018). Shifts in the relationship between mrna and protein abundance of gill ion-transporters during smolt development and seawater acclimation in atlantic salmon (*salmo salar*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 221*, 63-73. https://doi.org/10.1016/j.cbpa.2018.03.020

Chun, J., & Prince, A. (2009). Tlr2-induced calpain cleavage of epithelial junctional proteins facilitates leukocyte transmigration. *Cell Host & Microbe, 5*(1), 47-58. https://doi.org/10.1016/j.chom.2008.11.009

Ciani, E., Fontaine, R., Maugars, G., Mizrahi, N., Mayer, I., Levavi-Sivan, B., & Weltzien, F.-A. (2019). Melatonin receptors in atlantic salmon stimulate camp levels in heterologous cell lines and show season-dependent daily variations in pituitary expression levels. *Journal of Pineal Research, 67*(3), e12590. https://doi.org/10.1111/jpi.12590

Conant, G. C., & Wolfe, K. H. (2008). Turning a hobby into a job: How duplicated genes find new functions. *Nature Reviews Genetics, 9*(12), 938-950. https://doi.org/10.1038/nrg2482

Coxall, H., & Pearson, P. (2007). The eocene-oligocene transition. In M. Williams, A. M. Haywood, F. J. Gregory, & D. N. Schmidt (Eds.), *Deep-time perspectives on climate change: Marrying the signal from computer models and biological proxies* (Vol. 2, pp. 351-387). London: The Geological Society.

Cunjak, R. (1992). Comparative feeding, growth and movements of atlantic salmon (salmo salar) parr from riverine and estuarine environments. *Ecology of Freshwater Fish, 1*(1), 26-34. https://doi.org/10.1111/j.1600-0633.1992.tb00004.x

Dalziel, A. C., Bittman, J., Mandic, M., Ou, M., & Schulte, P. M. (2014). Origins and functional diversification of salinity-responsive na+, k+ atpase α1 paralogs in salmonids. *Molecular Ecology, 23*(14), 3483-3503. https://doi.org/10.1111/mec.12828

Dardente, H., Hazlerigg, D. G., & Ebling, F. J. (2014). Thyroid hormone and seasonal rhythmicity. *Frontiers in endocrinology, 5*, 19. https://doi.org/10.3389/fendo.2014.00019

Dardente, H., Wyse, C. A., Birnie, M. J., Dupré, S. M., Loudon, A. S. I., Lincoln, G. A., & Hazlerigg, D. G. (2010). A molecular switch for photoperiod responsiveness in mammals. *Current Biology, 20*(24), 2193-2198. https://doi.org/10.1016/j.cub.2010.10.048

Davie, A., Minghetti, M., & Migaud, H. (2009). Seasonal variations in clock ‐ gene expression in atlantic salmon (*salmo salar*). *Chronobiology International, 26*(3), 379-395. https://doi.org/10.1080/07420520902820947

Davies, T. H., Ning, Y.-M., & Sánchez, E. R. (2002). A new first step in activation of steroid receptors hormone-induced switching of fkbp51 and fkbp52 immunophilins. *J Biol Chem, 277*(7), 4597-4600. Retrieved from https://www.jbc.org/content/277/7/4597.full.pdf

De Groef, B., Van der Geyten, S., Darras, V. M., & Kühn, E. R. (2006). Role of corticotropin-releasing hormone as a thyrotropin-releasing factor in non-mammalian vertebrates. *General and Comparative Endocrinology, 146*(1), 62-68. https://doi.org/10.1016/j.ygcen.2005.10.014

de la Iglesia, H. O., & Johnson, C. H. (2013). Biological clocks: Riding the tides. *Current biology, 23*(20), R921-R923. https://doi.org/10.1016/j.cub.2013.09.006

de Luze, A., & Leloup, J. (1984). Fish growth hormone enhances peripheral conversion of thyroxine to triiodothyronine in the eel (*anguilla anguilla* l.). *General and Comparative Endocrinology, 56*(2), 308-312. https://doi.org/10.1016/0016-6480(84)90045-5

Dickhoff, W. W., Folmar, L. C., & Gorbman, A. (1978). Changes in plasma thyroxine during smoltification of coho salmon, *oncorhynchus kisutch*. *General and Comparative Endocrinology, 36*(2), 229-232. https://doi.org/10.1016/0016-6480(78)90027-8

Dodson, J. J., Aubin-Horth, N., Thériault, V., & Páez, D. J. (2013). The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biological Reviews, 88*(3), 602-625. https://doi.org/10.1111/brv.12019

Dodson, J. J., Laroche, J., & Lecomte, F. (2009). Contrasting evolutionary pathways of anadromy in euteleostean fishes. *American Fisheries Society Symposium, 69*, 63-77. Retrieved from https://www.researchgate.net/profile/Julian_Dodson/publication/273458096_Contrasting_evolutionary_pathways_of_anadromy_in_euteleosltean_fishes/links/5523fec10cf2b123c516d6c7/Contrasting-evolutionary-pathways-of-anadromy-in-euteleosltean-fishes.pdf

Duan, C., Duguay, S. J., & Plisetskaya, E. M. (1993). Insulin-like growth factor i (igf-i) mrna expression in coho salmon, *oncorhynchus kisutch*: Tissue distribution and effects of growth hormone/prolactin family proteins. *Fish Physiology and Biochemistry, 11*(1-6), 371-379. https://doi.org/10.1007/BF00004587

Duncan, N. J., & Bromage, N. (1998). The effect of different periods of constant short days on smoltification in juvenile atlantic salmon (*salmo salar*). *Aquaculture, 168*(1-4), 369-386. https://doi.org/10.1016/S0044-8486(98)00363-9

Dunel-Erb, S., Bailly, Y., & Laurent, P. (1982). Neuroepithelial cells in fish gill primary lamellae. *Journal of Applied Physiology, 53*(6), 1342-1353. https://doi.org/10.1152/jappl.1982.53.6.1342

Dunlap, J. C., Loros, J. J., & DeCoursey, P. J. (2004). *Chronobiology: Biological timekeeping* (J. C. Dunlap, J. J. Loros, & P. J. DeCoursey Eds.). Sunderland, Mass: Sinauer Associates.

Duston, J., & Saunders, R. L. (1990). The entrainment role of photoperiod on hypoosmoregulatory and growth-related aspects of smolting in atlantic salmon (*salmo salar*). *Can. J. Zool., 68*(4), 707-715. https://doi.org/10.1139/z90-103

Eales, J. G., & Brown, S. B. (1993). Measurement and regulation of thyroidal status in teleost fish. *Reviews in Fish Biology and Fisheries, 3*(4), 299-347. https://doi.org/10.1007/BF00043383

Ebbesson, L. O., Deviche, P., & Ebbesson, S. O. (1996). Distribution and changes in μ - and κ - opiate receptors during the midlife neurodevelopmental period of coho salmon, *oncorhynchus kisutch*. *Journal of Comparative Neurology, 366*(3), 448-464. https://doi.org/10.1002/(SICI)1096-9861(19960311)366:3%3C448::AID-CNE6%3E3.0.CO;2-4

Ebbesson, L. O. E., Ebbesson, S. O. E., Nilsen, T. O., Stefansson, S. O., & Holmqvist, B. (2007). Exposure to continuous light disrupts retinal innervation of the preoptic nucleus during parr–smolt transformation in atlantic salmon. *Aquaculture, 273*(2–3), 345-349. https://doi.org/10.1016/j.aquaculture.2007.10.016

Ebbesson, L. O. E., Ekström, P., Ebbesson, S. O. E., Stefansson, S. O., & Holmqvist, B. (2003). Neural circuits and their structural and chemical reorganization in the light–brain–pituitary axis during parr–smolt transformation in salmon. *Aquaculture, 222*(1–4), 59-70. https://doi.org/10.1016/S0044-8486(03)00102-9

Ebbesson, L. O. E., Nilsen, T. O., Helvik, J. V., Tronci, V., & Stefansson, S. O. (2011). Corticotropin-releasing factor neurogenesis during midlife development in salmon: Genetic, environmental and thyroid hormone regulation. *Journal of Neuroendocrinology, 23*(8), 733-741. https://doi.org/10.1111/j.1365-2826.2011.02164.x

Ebbesson, S. O. E., Smith, J., Co, C., & Ebbesson, L. O. E. (1996). Transient alterations in neurotransmitter levels during a critical period of neural development in coho salmon (*oncorhyncus kisutch*). *Brain Research, 742*(1), 339-342. https://doi.org/10.1016/S0006-8993(96)00993-6

Ehlers, J., & Gibbard, P. (2011). Quaternary glaciation. In V. P. Singh, P. Singh, & U. K. Haritashya (Eds.), *Encyclopedia of snow, ice and glaciers* (pp. 873-882). Dordrecht: Springer Netherlands. Retrieved from https://doi.org/10.1007/978-90-481-2642-2_423

Emmart, E. W., & Mossakowski, M. J. (1967). The localization of prolactin in cultured cells of the rostral pars distalis of the pituitary of *fundulus heteroclitus* (linnaeus). *General and Comparative Endocrinology, 9*(3), 391-400. https://doi.org/10.1016/0016-6480(67)90036-6

Eriksson, L.-O., & Lundqvist, H. (1982). Salmonid smoltification circannual rhythms and photoperiod regulation of growth and smolting in baltic salmon (*salmo salar* l.). *Aquaculture, 28*(1), 113-121. http://doi.org/10.1016/0044-8486(82)90014-X

Erkinaro, J., Shustov, Y., & Niemela, E. (1998). Feeding strategies of atlantic salmon *salmo salar* parr occupying lacustrine and fluvial habitats in a subarctic river, northern finland. *Polskie Archiwum Hydrobiologii, 45*(2), 259-268.

Evans, D. H., Piermarini, P. M., & Choe, K. P. (2005). The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews, 85*(1), 97-177. https://doi.org/10.1152/physrev.00050.2003

Evans, T. G. (2010). Co-ordination of osmotic stress responses through osmosensing and signal transduction events in fishes. *J Fish Biol, 76*(8), 1903-1925. https://doi.org/10.1111/j.1095-8649.2010.02590.x

Evans, T. G., & Somero, G. N. (2009). Protein-protein interactions enable rapid adaptive response to osmotic stress in fish gills. *Communicative & Integrative Biology, 2*(2), 94-96. https://doi.org/10.4161/cib.7601

Falcón, J., Barraud, S., Thibault, C., & Bégay, V. (1998). Inhibitors of messenger rna and protein synthesis affect differently serotonin arylalkylamine n-acetyltransferase activity in clock-controlled and non clock-controlled fish pineal. *Brain Research, 797*(1), 109-117. https://doi.org/10.1016/S0006-8993(98)00297-2

Falcón, J., Besseau, L., Magnanou, E., Herrero, M.-J., Nagai, M., & Boeuf, G. (2011). Melatonin, the time keeper: Biosynthesis and effects in fish. *Cybium, 35*(1), 3-18.

Falcón, J., Besseau, L., Sauzet, S., & Boeuf, G. (2007). Melatonin effects on the hypothalamo–pituitary axis in fish. *Trends in Endocrinology & Metabolism, 18*(2), 81-88. http://doi.org/10.1016/j.tem.2007.01.002

Falcón, J., Migaud, H., Muñoz-Cueto, J. A., & Carrillo, M. (2010). Current knowledge on the melatonin system in teleost fish. *General and Comparative Endocrinology, 165*(3), 469-482. http://doi.org/10.1016/j.ygcen.2009.04.026

Feng, J., Orlowski, J., & Lingrel, J. B. (1993). Identification of a functional thyroid hormone response element in the upstream flanking region of the human na,k-atpase β 1 gene. *Nucleic Acids Research, 21*(11), 2619-2626. https://doi.org/10.1093/nar/21.11.2619

Ferraris, J. D., Williams, C. K., Persaud, P., Zhang, Z., Chen, Y., & Burg, M. B. (2002). Activity of the tonebp/orebp transactivation domain varies directly with extracellular nacl concentration. *Proceedings of the National Academy of Sciences, 99*(2), 739-744. https://doi.org/10.1073/pnas.241637298

Fiol, D. F., Chan, S. Y., & Kültz, D. (2006). Identification and pathway analysis of immediate hyperosmotic stress responsive molecular mechanisms in tilapia (*oreochromis mossambicus*) gill. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 1*(3), 344-356. https://doi.org/10.1016/j.cbd.2006.08.002

Fiol, D. F., & Kültz, D. (2007). Osmotic stress sensing and signaling in fishes. *The FEBS Journal, 274*(22), 5790-5798. https://doi.org/10.1111/j.1742-4658.2007.06099.x

Fleming, I. A. (1996). Reproductive strategies of atlantic salmon: Ecology and evolution. *Reviews in Fish Biology and Fisheries, 6*(4), 379-416. https://doi.org/10.1007/BF00164323

Fleming, I. A., & Einum, S. (2010). Reproductive ecology: A tale of two sexes. In Ø. Aas, S. Einum, A. Klemetsen, & J. Skurdal (Eds.), *Atlantic salmon ecology* (pp. 33-65). Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1002/9781444327755.ch2

Fleming, M. S., Maugars, G., Lafont, A. G., Rancon, J., Fontaine, R., Nourizadeh-Lillabadi, R., . . .Dufour, S. (2019). Functional divergence of thyrotropin beta-subunit paralogs gives new insights into salmon smoltification metamorphosis. *Scientific Reports, 9*(1), 4561. https://doi.org/10.1038/s41598-019-40019-5

Florek, M., Bauer, N., Janich, P., Wilsch-Braeuninger, M., Fargeas, C. A., Marzesco, A.-M., . . .Corbeil, D. (2007). Prominin-2 is a cholesterol-binding protein associated with apical and basolateral plasmalemmal protrusions in polarized epithelial cells and released into urine. *Cell and Tissue Research, 328*(1), 31-47. https://doi.org/10.1007/s00441-006-0324-z

Folmar, L. C., & Dickhoff, W. W. (1980). The parr—smolt transformation (smoltification) and seawater adaptation in salmonids: A review of selected literature. *Aquaculture, 21*(1), 1-37. https://doi.org/10.1016/0044-8486(80)90123-4

Forseth, T., Letcher, B. H., & Johansen, M. (2010). The behavioural flexibility of salmon growth. In *Atlantic salmon ecology* (pp. 145-169). Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1002/9781444327755.ch6

Franco, S., Perrin, B., & Huttenlocher, A. (2004). Isoform specific function of calpain 2 in regulating membrane protrusion. *Experimental Cell Research, 299*(1), 179-187. https://doi.org/10.1016/j.yexcr.2004.05.021

Fraser, D. J., Weir, L. K., Bernatchez, L., Hansen, M. M., & Taylor, E. B. (2011). Extent and scale of local adaptation in salmonid fishes: Review and meta-analysis. *Heredity, 106*(3), 404-420. https://doi.org/10.1038/hdy.2010.167

Fricke, R., Eschmeyer, W. N., & Fong, J. D. (2019). Eschmeyer's catalogue of fishes: Species by family/subfamily. Retrieved from http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp

Garcia de Leaniz, C., Fleming, I. A., Einum, S., Verspoor, E., Consuegra, S., Jordan, W. C., . . .Quinn, T. P. (2007). Local adaptations. In E. Verspoor, L. Stradmeyer, & J. L. Nielsen (Eds.), *The atlantic salmon: Genetics, conservation and management* (pp. 200-239): John Wiley & Sons. Retrieved from https://www.researchgate.net/publication/236177931_Local_Adaptation

Garduño-Paz, M. V., & Adams, C. E. (2010). Discrete prey availability promotes foraging segregation and early divergence in arctic charr, *salvelinus alpinus*. *Hydrobiologia, 650*(1), 15-26. https://doi.org/10.1007/s10750-009-0055-8

Geering, K. (1991). The functional role of the β -subunit in the maturation and intracellular transport of na, k-atpase. *FEBS Letters, 285*(2), 189-193. https://doi.org/10.1016/0014-5793(91)80801-9

Geering, K. (2008). Functional roles of na^+,k^+-atpase subunits. *Current Opinion in Nephrology and Hypertension, 17*(5), 526-532. https://doi.org/10.1097/MNH.0b013e3283036cbf

Gereben, B., Zavacki, A. M., Ribich, S., Kim, B. W., Huang, S. A., Simonides, W. S., . . .Bianco, A. C. (2008). Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocrine reviews, 29*(7), 898-938. https://doi.org/10.1210/er.2008-0019

Gillard, G., Harvey, T. N., Gjuvsland, A., Jin, Y., Thomassen, M., Lien, S., . . .Sandve, S. R. (2018). Life-stage-associated remodelling of lipid metabolism regulation in atlantic salmon. *Molecular Ecology, 27*(5), 1200-1213. https://doi.org/10.1111/mec.14533

Glasauer, S. M. K., & Neuhauss, S. C. F. (2014). Whole-genome duplication in teleost fishes and its evolutionary consequences. *Molecular Genetics and Genomics, 289*(6), 1045-1060. https://doi.org/10.1007/s00438-014-0889-2

Godin, J. G., Dill, P. A., & Drury, D. E. (1974). Effects of thyroid hormones on behavior of yearling atlantic salmon (*salmo salar*). *Journal of the Fisheries Research Board of Canada, 31*(11), 1787-1790. https://doi.org/10.1139/f74-227

Goldman, B., Gwinner, E., Karsch, F., Saunders, D., Zucker, I., & Gall, G. (2004). Circannual rhythms and photoperiodism. In J. Dunlap, J. Loros, & P. DeCoursey (Eds.), *Chronobiology: Biological timekeeping*. Sunderland, Mass: Sinauer Associates.

Gomis, A., Soriano, S., Belmonte, C., & Viana, F. (2008). Hypoosmotic- and pressure-induced membrane stretch activate trpc5 channels. *Journal of Physiology, 586*(23), 5633-5649. https://doi.org/10.1113/jphysiol.2008.161257

Goniakowska-Witalińska, L., Zaccone, G., Fasulo, S., Mauceri, A., Licata, A., & Youson, J. (1995). Neuroendocrine cells in the gills of the bowfin *amia calva*. An ultrastructural and immunocytochemical study. *Folia histochemica et cytobiologica, 33*(3), 171-177. Retrieved from http://europepmc.org/abstract/MED/8612869

Grau, E. G., Nishioka, R. S., & Bern, H. A. (1981). Effects of osmotic pressure and calcium ion on prolactin release in vitro from the rostral pars distalis of the tilapia *sarotherodon mossambicus*. *General and Comparative Endocrinology, 45*(3), 406-408. https://doi.org/10.1016/0016-6480(81)90082-4

Gross, M. R., Coleman, R. M., & McDowall, R. M. (1988). Aquatic productivity and the evolution of diadromous fish migration. *Science, 239*(4845), 1291-1293. https://doi.org/10.1126/science.239.4845.1291

Gu, J., Dai, S., Liu, H., Cao, Q., Yin, S., Lai, K. P., . . .Shi, H. (2018). Identification of immune-related genes in gill cells of japanese eels (*anguilla japonica*) in adaptation to water salinity changes. *Fish & Shellfish Immunology, 73*, 288-296. https://doi.org/10.1016/j.fsi.2017.12.026

Gual, P., Le Marchand-Brustel, Y., & Tanti, J. F. (2003). Positive and negative regulation of glucose uptake by hyperosmotic stress. *Diabetes & Metabolism, 29*(6), 566-575. https://doi.org/10.1016/S1262-3636(07)70071-X

Gurney, W. S. C., Bacon, P. J., Speirs, D. C., McGinnity, P., & Verspoor, E. (2012). Sea-age variation in maiden atlantic salmon spawners: Phenotypic plasticity or genetic polymorphism? *Bulletin of Mathematical Biology, 74*(3), 615-640. https://doi.org/10.1007/s11538-011-9679-8

Halbeisen, R. E., Galgano, A., Scherrer, T., & Gerber, A. P. (2007). Post-transcriptional gene regulation: From genome-wide studies to principles. *Cellular and Molecular Life Sciences, 65*(5), 798. https://doi.org/10.1007/s00018-007-7447-6

Hang, C. Y., Kitahashi, T., & Parhar, I. S. (2016). Neuronal organization of deep brain opsin photoreceptors in adult teleosts. *Frontiers in Neuroanatomy, 10*(48). https://doi.org/10.3389/fnana.2016.00048

Hartsock, A., & Nelson, W. J. (2008). Adherens and tight junctions: Structure, function and connections to the actin cytoskeleton. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1778*(3), 660-669. https://doi.org/10.1016/j.bbamem.2007.07.012

Hasler, A. D., Scholz, A. T., & Horrall, R. M. (1978). Olfactory imprinting and homing in salmon: Recent experiments in which salmon have been artificially imprinted to a synthetic chemical verify the olfactory hypothesis for salmon homing. *American Scientist, 66*(3), 347-355. Retrieved from http://www.jstor.org/stable/27848646

Hayes, S. A., & Kocik, J. F. (2014). Comparative estuarine and marine migration ecology of atlantic salmon and steelhead: Blue highways and open plains. *Reviews in Fish Biology and Fisheries, 24*(3), 757-780. https://doi.org/10.1007/s11160-014-9348-8

Hazlerigg, D. G., & Lincoln, G. A. (2011). Hypothesis: Cyclical histogenesis is the basis of circannual timing. *Journal of Biological Rhythms, 26*(6), 471-485. https://doi.org/10.1177/0748730411420812

Hedger, R. D., Rikardsen, A. H., Strøm, J. F., Righton, D. A., Thorstad, E. B., & Næsje, T. F. (2017). Diving behaviour of atlantic salmon at sea: Effects of light regimes and

temperature stratification. *Marine Ecology Progress Series, 574*, 127-140. https://doi.org/10.3354/meps12180

Heggenes, J., & Metcalfe, N. B. (1991). Bimodal size distributions in wild juvenile atlantic salmon populations and their relationship with age at smolt migration. *Journal of Fish Biology, 39*(6), 905-907. https://doi.org/10.1111/j.1095-8649.1991.tb04421.x

Higgins, P. J. (1985). Metabolic differences between atlantic salmon (*salmo salar*) parr and smolts. *Aquaculture, 45*(1), 33-53. https://doi.org/10.1016/0044-8486(85)90256-X

Hiroi, J., & McCormick, S. D. (2012). New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Respiratory Physiology & Neurobiology, 184*(3), 257-268. https://doi.org/10.1016/j.resp.2012.07.019

Hoar, W. S. (1988). The physiology of smolting salmonids. In W. S. Hoar & D. J. Randall (Eds.), *Fish physiology* (Vol. 11, pp. 275-343). Cambrigde: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/S1546509808602162

Hogan, P. G., Chen, L., Nardone, J., & Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and nfat. *Genes & Development, 17*(18), 2205-2232. https://doi.org/10.1101/gad.1102703

Holmqvist, B. I., & Ekström, P. (1995). Hypophysiotrophic systems in the brain of the atlantic salmon. Neuronal innervation of the pituitary and the origin of pituitary dopamine and nonapeptides identified by means of combined carbocyanine tract tracing and immunocytochemistry. *Journal of Chemical Neuroanatomy, 8*(2), 125-145. https://doi.org/10.1016/0891-0618(94)00041-Q

Holmqvist, B. I., Östholm, T., & Ekström, P. (1994). Neuroanatomical analysis of the visual and hypophysiotropic systems in atlantic salmon (*salmo salar*) with emphasis on possible mediators of photoperiodic cues during parr-smolt transformation. *Aquaculture, 121*(1), 1-12. https://doi.org/10.1016/0044-8486(94)90003-5

Houde, A. L. S., Akbarzadeh, A., Günther, O. P., Li, S., Patterson, D. A., Farrell, A. P., . . .Miller, K. M. (2019). Salmonid gene expression biomarkers indicative of physiological responses to changes in salinity, temperature, but not dissolved oxygen. *Journal of Experimental Biology, 222*, jeb198036. https://doi.org/10.1242/jeb.198036

Houston, R. D., & Macqueen, D. J. (2019). Atlantic salmon (salmo salar l.) genetics in the 21st century: Taking leaps forward in aquaculture and biological understanding. *Animal Genetics, 50*(1), 3-14. https://doi.org/10.1111/age.12748

Hsieh, P. N., Fan, L., Sweet, D. R., & Jain, M. K. (2018). The krüppel-like factors and control of energy homeostasis. *Endocrine Reviews, 40*(1), 137-152. https://doi.org/10.1210/er.2018-00151

Hu, Y.-C., Chu, K.-F., Yang, W.-K., & Lee, T.-H. (2017). Na+, k+-atpase β1 subunit associates with α1 subunit modulating a "higher-nka-in-hyposmotic media" response in gills of euryhaline milkfish, *chanos chanos*. *Journal of Comparative Physiology B, 187*(7), 995-1007. https://doi.org/10.1007/s00360-017-1066-9

Huang, T.-S., Ruoff, P., & Fjelldal, P. G. (2010). Diurnal expression of clock genes in pineal gland and brain and plasma levels of melatonin and cortisol in atlantic salmon parr and smolt. *Chronobiology International, 27*(9-10), 1697-1714. https://doi.org/10.3109/07420528.2010.514630

Hulpiau, P., & van Roy, F. (2009). Molecular evolution of the cadherin superfamily. *The International Journal of Biochemistry & Cell Biology, 41*(2), 349-369. https://doi.org/10.1016/j.biocel.2008.09.027

Hutchison, M. J., & Iwata, M. (1998). Effect of thyroxine on the decrease of aggressive behaviour of four salmonids during the parr-smolt transformation. *Aquaculture, 168*(1), 169-175. https://doi.org/10.1016/S0044-8486(98)00347-0

Hwang, P.-P., & Lee, T.-H. (2007). New insights into fish ion regulation and mitochondrion-rich cells. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 148*(3), 479-497. https://doi.org/10.1016/j.cbpa.2007.06.416

Hwang, P.-P., Lee, T.-H., & Lin, L.-Y. (2011). Ion regulation in fish gills: Recent progress in the cellular and molecular mechanisms. *American Journal of Physiology -Regulatory, Integrative and Comparative Physiology, 301*(1), R28-R47. https://doi.org/10.1152/ajpregu.00047.2011

Hyde, G. N., Seale, A. P., Grau, E. G., & Borski, R. J. (2004). Cortisol rapidly suppresses intracellular calcium and voltage-gated calcium channel activity in prolactin cells of the tilapia (*oreochromis mossambicus*). *American Journal of Physiology - Endocrinology and Metabolism, 286*(4), E626-E633. https://doi.org/10.1152/ajpendo.00088.2003

Iigo, M., Abe, T., Kambayashi, S., Oikawa, K., Masuda, T., Mizusawa, K., . . .Yanagisawa, T. (2007). Lack of circadian regulation of in vitro melatonin release from the pineal organ of salmonid teleosts. *General and Comparative Endocrinology, 154*(1–3), 91-97. http://dx.doi.org/10.1016/j.ygcen.2007.06.013

Ikegami, K., & Yoshimura, T. (2013). Seasonal time measurement during reproduction. *Journal of Reproduction and Development, 59*(4), 327-333. https://doi.org/10.1262/jrd.2013-035

Irarrazabal, C. E., Gallazzini, M., Schnetz, M. P., Kunin, M., Simons, B. L., Williams, C. K., . . .Ferraris, J. D. (2010). Phospholipase c-γ1 is involved in signaling the activation by high nacl of the osmoprotective transcription factor tonebp/orebp. *Proceedings of the National Academy of Sciences, 107*(2), 906-911. https://doi.org/10.1073/pnas.0913415107

Isorna, E., Pedro, N. d., Valenciano, A. I., Alonso-Gómez, Á. L., & Delgado, M. J. (2017). Interplay between the endocrine and circadian systems in fishes. *Journal of Endocrinology, 232*(3), R141-R159. https://doi.org/10.1530/joe-16-0330

Iversen, M., Mulugeta, T., Gellein Blikeng, B., West, A. C., Jørgensen, E. H., Rød Sandven, S., & Hazlerigg, D. (2020). Rna profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile atlantic salmon. *Plos One, 15*(4), e0227496. https://doi.org/10.1371/journal.pone.0227496

Iversen, M., Mulugeta, T., West, A., Jørgensen, E., Martin, S. A. M., Sandve, S. R., & Hazlerigg, D. (2020). Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in atlantic salmon (*salmo salar*). *bioRxiv*, 2020.2003.2024.006510. https://doi.org/10.1101/2020.03.24.006510

Iwata, M. (1995). Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: A review. *Aquaculture, 135*(1), 131-139. https://doi.org/10.1016/0044-8486(95)01000-9

Jaillon, O., Aury, J.-M., Brunet, F., Petit, J.-L., Stange-Thomann, N., Mauceli, E., . . .Roest Crollius, H. (2004). Genome duplication in the teleost fish tetraodon nigroviridis reveals the early vertebrate proto-karyotype. *Nature, 431*(7011), 946-957. https://doi.org/10.1038/nature03025

James-Curtis, B., & Wood, C. M. (1992). Kidney and urinary bladder responses of freshwater rainbow trout to isosmotic nacl and nahco3 infusion. *Journal of Experimental Biology, 173*(1), 181-203. Retrieved from https://jeb.biologists.org/content/jexbio/173/1/181.full.pdf

Jászai, J., Thamm, K., Karbanová, J., Janich, P., Fargeas, C. A., Huttner, W. B., & Corbeil, D. (2020). Prominins control ciliary length throughout the animal kingdom: New lessons from human prominin-1 and zebrafish prominin-3. *Journal of Biological Chemistry, 295*(18), 6007-6022. https://doi.org/10.1074/jbc.RA119.011253

Jensen, A. J., Karlsson, S., Fiske, P., Hansen, L. P., Østborg, G. M., & Hindar, K. (2014). Origin and life history of atlantic salmon (*salmo salar*) near their northernmost oceanic limit. *Canadian Journal of Fisheries and Aquatic Sciences, 71*(11), 1740-1746. https://doi.org/10.1139/cjfas-2014-0169

Jeyaraj, D., Scheer, Frank A. J. L., Ripperger, Jürgen A., Haldar, Saptarsi M., Lu, Y., Prosdocimo, Domenick A., . . .Jain, Mukesh K. (2012). Klf15 orchestrates circadian nitrogen homeostasis. *Cell Metabolism, 15*(3), 311-323. https://doi.org/10.1016/j.cmet.2012.01.020

Jia, S., & Meng, A. (2007). Tob genes in development and homeostasis. *Developmental Dynamics, 236*(4), 913-921. https://doi.org/10.1002/dvdy.21092

Johnston, C., & Eales, J. (1967). Purines in the integument of the atlantic salmon (*salmo salar*) during parr–smolt transformation. *Journal of the Fisheries Board of Canada, 24*(5), 955-964. https://doi.org/10.1139/f67-085

Johnston, I. A., McLay, H. A., Abercromby, M., & Robins, D. (2000). Phenotypic plasticity of early myogenesis and satellite cell numbers in atlantic salmon spawning in upland and lowland tributaries of a river system. *Journal of Experimental Biology, 203*(17), 2539-2552. Retrieved from https://jeb.biologists.org/content/jexbio/203/17/2539.full.pdf

Jones, P. D., Kaiser, M. A., Najafabadi, M. G., Koplev, S., Zhao, Y., Douglas, G., . . .Webb, T. R. (2018). Jcad, a gene at the 10p11 coronary artery disease locus, regulates hippo signaling in endothelial cells. *Arteriosclerosis, Thrombosis and Vascular Biology, 38*(8), 1711-1722. https://doi.org/10.1161/ATVBAHA.118.310976

Jonsson, B., & Jonsson, N. (2001). Polymorphism and speciation in arctic charr. *Journal of Fish Biology, 58*(3), 605-638. https://doi.org/10.1111/j.1095-8649.2001.tb00518.x

Jonsson, B., & Jonsson, N. (2004). Factors affecting marine production of atlantic salmon (*salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences, 61*(12), 2369-2383. https://doi.org/10.1139/f04-215

Jonsson, N., Hansen, L. P., & Jonsson, B. (1991). Variation in age, size and repeat spawning of adult atlantic salmon in relation to river discharge. *Journal of Animal Ecology, 60*(3), 937-947. https://doi.org/10.2307/5423

Jonsson, N., & Jonsson, B. (2007). Sea growth, smolt age and age at sexual maturation in atlantic salmon. *Journal of Fish Biology, 71*(1), 245-252. https://doi.org/10.1111/j.1095-8649.2007.01488.x

Jonz, M. G., & Nurse, C. A. (2003). Neuroepithelial cells and associated innervation of the zebrafish gill: A confocal immunofluorescence study. *Journal of Comparative Neurology, 461*(1), 1-17. https://doi.org/10.1002/cne.10680

Jorgensen, P. L. (2008). Importance for absorption of na^+ from freshwater of lysine, valine and serine substitutions in the α1a-isoform of na^+,k^+-atpase in the gills of rainbow

trout (*oncorhynchus mykiss*) and atlantic salmon (*salmo salar*). *Journal of Membrane Biology, 223*(1), 37. https://doi.org/10.1007/s00232-008-9111-y

Jung, J., Kim, M., Kim, M.-J., Kim, J., Moon, J., Lim, J.-S., . . .Lee, K. (2004). Translationally controlled tumor protein interacts with the third cytoplasmic domain of na,k-atpase α subunit and inhibits the pump activity in hela cells. *Journal of Biological Chemistry, 279*(48), 49868-49875. https://doi.org/10.1074/jbc.M400895200

Jääskeläinen, T., Makkonen, H., & Palvimo, J. J. (2011). Steroid up-regulation of fkbp51 and its role in hormone signaling. *Current Opinion in Pharmacology, 11*(4), 326-331. https://doi.org/10.1016/j.coph.2011.04.006

Kapralova, K. H., Morrissey, M. B., Kristjánsson, B. K., Ólafsdóttir, G. Á., Snorrason, S. S., & Ferguson, M. M. (2011). Evolution of adaptive diversity and genetic connectivity in arctic charr *(salvelinus alpinus*) in iceland. *Heredity, 106*(3), 472-487. https://doi.org/10.1038/hdy.2010.161

Kathpalia, P. P., Charlton, C., Rajagopal, M., & Pao, A. C. (2011). The natriuretic mechanism of gamma-melanocyte-stimulating hormone. *Peptides, 32*(5), 1068-1072. https://doi.org/10.1016/j.peptides.2011.02.006

Keefer, M. L., & Caudill, C. C. (2014). Homing and straying by anadromous salmonids: A review of mechanisms and rates. *Reviews in Fish Biology and Fisheries, 24*(1), 333-368. https://doi.org/10.1007/s11160-013-9334-6

Keeley, E. R., & Grant, J. W. A. (1995). Allometric and environmental correlates of territory size in juvenile atlantic salmon (*salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences, 52*(1), 186-196. https://doi.org/10.1139/f95-019

Kelly, S. P., & Chasiotis, H. (2011). Glucocorticoid and mineralocorticoid receptors regulate paracellular permeability in a primary cultured gill epithelium. *Journal of Experimental Biology, 214*(14), 2308-2318. https://doi.org/10.1242/jeb.055962

Kiilerich, P., Kristiansen, K., & Madsen, S. S. (2007a). Cortisol regulation of ion transporter mrna in atlantic salmon gill and the effect of salinity on the signaling pathway. *Journal of Endocrinology, 194*(2), 417-427. https://doi.org/10.1677/joe-07-0185

Kiilerich, P., Kristiansen, K., & Madsen, S. S. (2007b). Hormone receptors in gills of smolting atlantic salmon, *salmo salar*: Expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. *General and Comparative Endocrinology, 152*(2), 295-303. https://doi.org/10.1016/j.ygcen.2006.12.018

Kiilerich, P., Pedersen, S. H., Kristiansen, K., & Madsen, S. S. (2011). Corticosteroid regulation of na+,k+-atpase α1-isoform expression in atlantic salmon gill during smolt development. *General and Comparative Endocrinology, 170*(2), 283-289. http://dx.doi.org/10.1016/j.ygcen.2010.02.014

Kleene, R., Dartsch, H., & Kern, H.-F. (1999). The secretory lectin zg16p mediates sorting of enzyme proteins to the zymogen granule membrane in pancreatic acinar cells. *European Journal of Cell Biology, 78*(2), 79-90. https://doi.org/10.1016/S0171-9335(99)80009-0

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N., Connell, M. F., & Mortensen, E. (2003). Atlantic salmon *salmo salar* l., brown trout *salmo trutta* l. And arctic charr *salvelinus alpinus* (l.): A review of aspects of their life histories. *Ecol Freshw Fish, 12*. https://doi.org/10.1034/j.1600-0633.2003.00010.x

Knoedler, J. R., & Denver, R. J. (2014). Krüppel-like factors are effectors of nuclear receptor signaling. *General and Comparative Endocrinology, 203*, 49-59. https://doi.org/10.1016/j.ygcen.2014.03.003

Kougias, P., Weakley, S. M., Yao, Q., Lin, P. H., & Chen, C. (2010). Arterial baroreceptors in the management of systemic hypertension. *Medical Science Monitor, 16*(1), RA1-RA8. Retrieved from http://www.medscimonit.com/abstract/index/idArt/878303

Krieg, P., & Fürstenberger, G. (2014). The role of lipoxygenases in epidermis. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1841*(3), 390-400. https://doi.org/10.1016/j.bbalip.2013.08.005

Kristinsson, J. B., Saunders, R. L., & Wiggs, A. J. (1985). Growth dynamics during the development of bimodal length-frequency distribution in juvenile atlantic salmon (*salmo salar* l.). *Aquaculture, 45*(1), 1-20. https://doi.org/10.1016/0044-8486(85)90254-6

Kruse, E., Uehlein, N., & Kaldenhoff, R. (2006). The aquaporins. *Genome Biology, 7*(2), 206. https://doi.org/10.1186/gb-2006-7-2-206

Kültz, D. (2012). The combinatorial nature of osmosensing in fishes. *Physiology, 27*(4), 259-275. https://doi.org/10.1152/physiol.00014.2012

Kültz, D. (2015). Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology, 218*(12), 1907-1914. https://doi.org/10.1242/jeb.118695

Langdon, J. S., Thorpe, J. E., & Roberts, R. J. (1984). Effects of cortisol and acth on gill na+/k+-atpase, sdh and chloride cells in juvenile atlantic salmon *salmo salar* l. *Comparative Biochemistry and Physiology Part A: Physiology, 77*(1), 9-12. https://doi.org/10.1016/0300-9629(84)90004-5

Lappin, F. M., Shaw, R. L., & Macqueen, D. J. (2016). Targeted sequencing for high-resolution evolutionary analyses following genome duplication in salmonid fish: Proof of concept for key components of the insulin-like growth factor axis. *Marine Genomics, 30*, 15-26. https://doi.org/10.1016/j.margen.2016.06.003

Larsen, D. A., Swanson, P., Dickey, J. T., Rivier, J., & Dickhoff, W. W. (1998). In vitrothyrotropin-releasing activity of corticotropin-releasing hormone-family peptides in coho salmon,*oncorhynchus kisutch*. *General and Comparative Endocrinology, 109*(2), 276-285. https://doi.org/10.1006/gcen.1997.7031

Larsen, D. A., Swanson, P., & Dickhoff, W. W. (2011). The pituitary–thyroid axis during the parr–smolt transformation of coho salmon, *oncorhynchus kisutch*: Quantification of tsh β mrna, tsh, and thyroid hormones. *General and Comparative Endocrinology, 171*(3), 367-372. https://doi.org/10.1016/j.ygcen.2011.03.003

Leloup, J., & Lebel, J.-M. (1993). Triiodothyronine is necessary for the action of growth hormone in acclimation to seawater of brown (*salmo trutta*) and rainbow trout (*oncorhynchus mykiss*). *Fish Physiology and Biochemistry, 11*(1), 165-173. https://doi.org/10.1007/BF00004563

Lema, S. C., & Nevitt, G. A. (2004). Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. *Journal of Experimental Biology, 207*(19), 3317-3327. https://doi.org/10.1242/jeb.01143

Levine, M. (2010). Transcriptional enhancers in animal development and evolution. *Current Biology, 20*(17), R754-R763. https://doi.org/10.1016/j.cub.2010.06.070

Li, Z., & Langhans, S. A. (2015). Transcriptional regulators of na,k-atpase subunits. *Frontiers in Cell and Developmental Biology, 3*(66). https://doi.org/10.3389/fcell.2015.00066

Liedtke, W. (2007). Role of trpv ion channels in sensory transduction of osmotic stimuli in mammals. *Experimental Physiology, 92*(3), 507-512. https://doi.org/10.1113/expphysiol.2006.035642

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., . . .Davidson, W. S. (2016). The atlantic salmon genome provides insights into rediploidization. *Nature, 533*(7602), 200-205. https://doi.org/10.1038/nature17164

Lincoln, G., & Hazlerigg, D. (2014). Stem cell regulation of circannual rhythms. In H. Numata & B. Helm (Eds.), *Annual, lunar, and tidal clocks* (pp. 227-245): Springer Japan. Retrieved from http://dx.doi.org/10.1007/978-4-431-55261-1_12

https://link.springer.com/content/pdf/10.1007%2F978-4-431-55261-1_12.pdf

Lingwood, D., Harauz, G., & Ballantyne, J. S. (2005). Regulation of fish gill na+-k+-atpase by selective sulfatide-enriched raft partitioning during seawater adaptation. *Journal of Biological Chemistry, 280*(44), 36545-36550. https://doi.org/10.1074/jbc.M506670200

Liu, Z., Pagani, M., Zinniker, D., DeConto, R., Huber, M., Brinkhuis, H., . . .Pearson, A. (2009). Global cooling during the eocene-oligocene climate transition. *Science, 323*(5918), 1187-1190. https://doi.org/10.1126/science.1166368

Lombardi, M. S., Kavelaars, A., Penela, P., Scholtens, E. J., Roccio, M., Schmidt, R. E., . . .Heijnen, C. J. (2002). Oxidative stress decreases g protein-coupled receptor kinase 2 in lymphocytes via a calpain-dependent mechanism. *Molecular Pharmacology, 62*(2), 379-388. https://doi.org/10.1124/mol.62.2.379

Lorgen, M., Casadei, E., Król, E., Douglas, A., Birnie, M. J., Ebbesson, L. O., . . .Dardente, H. (2015). Functional divergence of type 2 deiodinase paralogs in the atlantic salmon. *Current Biology, 25*(7), 936-941. https://doi.org/10.1016/j.cub.2015.01.074

Lorgen, M., Jorgensen, E. H., Jordan, W. C., Martin, S. A. M., & Hazlerigg, D. G. (2017). Nfat5 genes are part of the osmotic regulatory system in atlantic salmon (*salmo salar*). *Marine Genomics, 31*, 25-31. https://doi.org/10.1016/j.margen.2016.06.004

Lu, W., Meng, Q.-J., Tyler, N. J. C., Stokkan, K.-A., & Loudon, A. S. I. (2010). A circadian clock is not required in an arctic mammal. *Current Biology, 20*(6), 533-537. https://doi.org/10.1016/j.cub.2010.01.042

Lubin, R. T., Rourke, A. W., & Bradley, T. M. (1989). Ultrastructural alterations in branchial chloride cells of atlantic salmon, *salmo salar*, during parr‐smolt transformation and early development in sea water. *Journal of Fish Biology, 34*(2), 259-272. https://doi.org/10.1111/j.1095-8649.1989.tb03307.x

Macian, F. (2005). Nfat proteins: Key regulators of t-cell development and function. *Nature Reviews Immunology, 5*(6), 472-484. https://doi.org/10.1038/nri1632

MacKenzie, D. S., Jones, R. A., & Miller, T. C. (2009). Thyrotropin in teleost fish. *General and Comparative Endocrinology, 161*(1), 83-89. https://doi.org/10.1016/j.ygcen.2008.12.010

MacLatchy, D. L., Kawauchi, H., & Eales, J. G. (1992). Stimulation of hepatic thyroxine 5'-deiodinase activity in rainbow trout (*oncorhynchus mykiss*) by pacific salmon growth hormone. *Comparative biochemistry and physiology. Comparative physiology, 101*(4), 689-691. https://doi.org/10.1016/0300-9629(92)90344-p

Macqueen, D. J., & Johnston, I. A. (2014). A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species

diversification. *Proceedings of the Royal Society of London B: Biological Sciences, 281*(1778). https://doi.org/10.1098/rspb.2013.2881

Madsen, S., & Bern, H. (1993). In-vitro effects of insulin-like growth factor-i on gill na+, k+-atpase in coho salmon, *oncorhynchus kisutch*. *Journal of Endocrinology, 138*(1), 23-30. https://doi.org/10.1677/joe.0.1380023

Madsen, S. S. (1990). The role of cortisol and growth hormone in seawater adaptation and development of hypoosmoregulatory mechanisms in sea trout parr (*salmo trutta trutta*). *General and Comparative Endocrinology, 79*(1), 1-11. https://doi.org/10.1016/0016-6480(90)90082-W

Madsen, S. S., Kiilerich, P., & Tipsmark, C. K. (2009). Multiplicity of expression of na^+ ,k^+ – atpaseα -subunit isoforms in the gill of atlantic salmon (*salmo salar*): Cellular localisation and absolute quantification in response to salinity change. *Journal of Experimental Biology, 212*(1), 78-88. https://doi.org/10.1242/jeb.024612

Madsen, S. S., & Korsgaard, B. (1989). Time-course effects of repetitive oestradiol-17β and thyroxine injections on the natural spring smolting of atlantic salmon, *salmo salar* l. *Journal of Fish Biology, 35*(1), 119-128. https://doi.org/10.1111/j.1095-8649.1989.tb03398.x

Madsen, S. S., & Naamansen, E. T. (1989). Plasma ionic regulation and gill na+/k+-atpase changes during rapid transfer to sea water of yearling rainbow trout, *salmo gairdneri*: Time course and seasonal variation. *Journal of Fish Biology, 34*(6), 829-840. https://doi.org/10.1111/j.1095-8649.1989.tb03367.x

Maeda, R., Shimo, T., Nakane, Y., Nakao, N., & Yoshimura, T. (2015). Ontogeny of the saccus vasculosus, a seasonal sensor in fish. *Endocrinology, 156*(11), 4238-4243. https://doi.org/10.1210/en.2015-1415

Marshall, W. S., Katoh, F., Main, H. P., Sers, N., & Cozzi, R. R. F. (2008). Focal adhesion kinase and β1 integrin regulation of na+, k+, 2cl− cotransporter in osmosensing ion transporting cells of killifish, *fundulus heteroclitus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 150*(3), 288-300. https://doi.org/10.1016/j.cbpa.2008.03.013

Marshall, W. S., & Singer, T. D. (2002). Cystic fibrosis transmembrane conductance regulator in teleost fish. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1566*(1), 16-27. https://doi.org/10.1016/S0005-2736(02)00584-9

Marshall, W. S., Watters, K. D., Hovdestad, L. R., Cozzi, R. R. F., & Katoh, F. (2009). Cftr cl– channel functional regulation by phosphorylation of focal adhesion kinase at tyrosine 407 in osmosensitive ion transporting mitochondria rich cells of euryhaline killifish. *Journal of Experimental Biology, 212*(15), 2365-2377. https://doi.org/10.1242/jeb.030015

Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K., & Tabata, M. (2003). Variations in plasma melatonin levels of the rainbow trout (*oncorhynchus mykiss*) under various light and temperature conditions. *Zoological Science, 20*(8), 1011-1016, 1016. https://doi.org/10.2108/zsj.20.1011

McConnell, B. B., & Yang, V. W. (2010). Mammalian krüppel-like factors in health and diseases. *Physiological Reviews, 90*(4), 1337-1381. https://doi.org/10.1152/physrev.00058.2009

McCormick, S. D. (1994). Ontogeny and evolution of salinity tolerance in anadromous salmonids: Hormones and heterochrony. *Estuaries, 17*(1), 26. https://doi.org/10.2307/1352332

McCormick, S. D. (1996). Effects of growth hormone and insulin-like growth factor i on salinity tolerance and gill na+, k+-atpase in atlantic salmon (*salmo salar*): Interaction with cortisol. *General and Comparative Endocrinology, 101*(1), 3-11. https://doi.org/10.1006/gcen.1996.0002

McCormick, S. D. (2001). Endocrine control of osmoregulation in teleost fish. *Integrative and Comparative Biology, 41*(4), 781-794. https://doi.org/10.1668/0003-1569(2001)041[0781:ECOOIT]2.0.CO;2

McCormick, S. D., Björnsson, B. T., Sheridan, M., Eilerlson, C., Carey, J. B., & O'Dea, M. (1995). Increased daylength stimulates plasma growth hormone and gill na+, k+-atpase in atlantic salmon (*salmo salar*). *Journal of Comparative Physiology B, 165*(4), 245-254. https://doi.org/10.1007/bf00367308

McCormick, S. D., Hansen, L. P., Quinn, T. P., & Saunders, R. L. (1998). Movement, migration, and smolting of atlantic salmon (*salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences, 55*(S1), 77-92. https://doi.org/10.1139/d98-011

McCormick, S. D., Regish, A. M., & Christensen, A. K. (2009). Distinct freshwater and seawater isoforms of na^+/k^+-atpase in gill chloride cells of atlantic salmon. *Journal of Experimental Biology, 212*(24), 3994-4001. https://doi.org/10.1242/jeb.037275

McCormick, S. D., Regish, A. M., Christensen, A. K., & Björnsson, B. T. (2013). Differential regulation of sodium–potassium pump isoforms during smolt development and seawater exposure of atlantic salmon. *Journal of Experimental Biology, 216*(7), 1142-1151. https://doi.org/10.1242/jeb.080440

McCormick, S. D., & Saunders, R. L. (1987). *Preparatory physiological adaptations for marine life of salmonids: Osmoregulation, growth, and metabolism.* Paper presented at the American Fisheries Society Symposium.

McCormick, S. D., Shrimpton, J. M., Moriyama, S., & Björnsson, B. T. (2007). Differential hormonal responses of atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. *Aquaculture, 273*(2), 337-344. https://doi.org/10.1016/j.aquaculture.2007.10.015

McDowall, R. M. (2001). Diadromy, diversity and divergence: Implications for speciation processes in fishes. *Fish and Fisheries, 2*(3), 278-285. https://doi.org/10.1046/j.1467-2960.2001.00050.x

McStay, E., Migaud, H., Vera, L. M., Sánchez-Vázquez, F. J., & Davie, A. (2014). Comparative study of pineal clock gene and aanat2 expression in relation to melatonin synthesis in atlantic salmon (*salmo salar*) and european seabass (*dicentrarchus labrax*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 169*, 77-89. https://doi.org/10.1016/j.cbpa.2013.12.011

Metcalfe, N. B., Huntingford, F. A., & Thorpe, J. E. (1986). Seasonal changes in feeding motivation of juvenile atlantic salmon (*salmo salar*). *Canadian Journal of Zoology, 64*(11), 2439-2446. https://doi.org/10.1139/z86-364

Metcalfe, N. B., Huntingford, F. A., & Thorpe, J. E. (1988). Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile atlantic salmon *salmo salar*. *J Anim Ecol, 57*(2), 463-474. https://doi.org/10.2307/4918

Meyer, A., & Schartl, M. (1999). Gene and genome duplications in vertebrates: The one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. *Current Opinion in Cell Biology, 11*(6), 699-704. https://doi.org/10.1016/S0955-0674(99)00039-3

Meyer, A., & Van de Peer, Y. (2005). From 2r to 3r: Evidence for a fish ‐ specific genome duplication (fsgd). *Bioessays, 27*(9), 937-945. https://doi.org/10.1002/bies.20293

Migaud, H., Davie, A., Martinez Chavez, C. C., & Al-Khamees, S. (2007). Evidence for differential photic regulation of pineal melatonin synthesis in teleosts. *Journal of Pineal Research, 43*(4), 327-335. https://doi.org/10.1111/j.1600-079X.2007.00480.x

Miyazaki, Y., Nisimura, T., & Numata, H. (2014). Circannual rhythms in insects. In H. Numata & B. Helm (Eds.), *Annual, lunar, and tidal clocks: Patterns and mechanisms of nature's enigmatic rhythms* (pp. 333-350). Tokyo: Springer Japan. Retrieved from https://doi.org/10.1007/978-4-431-55261-1_16

Mommsen, T. P., Vijayan, M. M., & Moon, T. W. (1999). Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries, 9*(3), 211-268. https://doi.org/10.1023/a:1008924418720

Monteiro, S. M., Oliveira, E., Fontaínhas-Fernandes, A., & Sousa, M. (2010). Fine structure of the branchial epithelium in the teleost *oreochromis niloticus*. *Journal of Morphology, 271*(5), 621-633. https://doi.org/10.1002/jmor.10821

Moore, A., Freake, S. M., Thomas, I. M., & Bone, Q. (1990). Magnetic particles in the lateral line of the atlantic salmon (*salmo salar* l.). *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 329*(1252), 11-15. https://doi.org/10.1098/rstb.1990.0145

Moore, H. A., & Whitmore, D. (2014). Circadian rhythmicity and light sensitivity of the zebrafish brain. *Plos One, 9*(1), e86176. https://doi.org/10.1371/journal.pone.0086176

Nakane, Y., Ikegami, K., Iigo, M., Ono, H., Takeda, K., Takahashi, D., . . .Yoshimura, T. (2013). The saccus vasculosus of fish is a sensor of seasonal changes in day length. *Nature Communications, 4*. https://doi.org/10.1038/ncomms3108

Nakao, N., Ono, H., Yamamura, T., Anraku, T., Takagi, T., Higashi, K., . . .Yoshimura, T. (2008). Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature, 452*(7185), 317-322. https://doi.org/10.1038/nature06738

Nakao, N., Ono, H., & Yoshimura, T. (2008). Thyroid hormones and seasonal reproductive neuroendocrine interactions. *Reproduction, 136*(1), 1-8. https://doi.org/10.1530/rep-08-0041

Nevitt, G., & Dittman, A. (1998). A new model for olfactory imprinting in salmon. *Integrative Biology: Issues, News, and Reviews, 1*(6), 215-223. https://doi.org/10.1002/(SICI)1520-6602(1998)1:6%3C215::AID-INBI3%3E3.0.CO;2-V

Niemelä, E., Erkinaro, J., Julkunen, M., Hassinen, E., Länsman, M., & Brørs, S. (2006). Temporal variation in abundance, return rate and life histories of previously spawned atlantic salmon in a large subarctic river. *Journal of Fish Biology, 68*(4), 1222-1240. https://doi.org/10.1111/j.0022-1112.2006.001012.x

Nilius, B., & Owsianik, G. (2011). The transient receptor potential family of ion channels. *Genome Biology, 12*(3), 218. https://doi.org/10.1186/gb-2011-12-3-218

Nilsen, T. O., Ebbesson, L. O. E., Madsen, S. S., McCormick, S. D., Andersson, E., Björnsson, B. T., . . .Stefansson, S. O. (2007). Differential expression of gill na^+,k^+-atpase α- and β-subunits, na^+,k^+,$2cl^-$ cotransporter and cftr anion channel in juvenile anadromous and

landlocked atlantic salmon *salmo salar*. *Journal of Experimental Biology, 210*(16), 2885-2896. https://doi.org/10.1242/jeb.002873

Nilsson, S., & Sundin, L. (1998). Gill blood flow control. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 119*(1), 137-147. https://doi.org/10.1016/S1095-6433(97)00397-8

Nisimura, T., & Numata, H. (2003). Circannual control of the life cycle in the varied carpet beetle anthrenus verbasci. *Functional Ecology*, 489-495. https://doi.org/10.1046/j.1365-2435.2003.00753.x

Noche, R. R., Lu, P.-N., Goldstein-Kral, L., Glasgow, E., & Liang, J. O. (2011). Circadian rhythms in the pineal organ persist in zebrafish larvae that lack ventral brain. *BMC Neuroscience, 12*(1), 7. https://doi.org/10.1186/1471-2202-12-7

Nordgarden, U., Fjelldal, P.-G., Hansen, T., Björnsson, B. T., & Wargelius, A. (2006). Growth hormone and insulin-like growth factor-i act together and independently when regulating growth in vertebral and muscle tissue of atlantic salmon postsmolts. *General and Comparative Endocrinology, 149*(3), 253-260. https://doi.org/10.1016/j.ygcen.2006.06.001

Ohno, S. (1970). *Evolution by gene duplication*. Berlin, Heidelberg: Springer.

Oishi, Y., & Manabe, I. (2018). Krüppel-like factors in metabolic homeostasis and cardiometabolic disease. *Frontiers in Cardiovascular Medicine, 5*(69). https://doi.org/10.3389/fcvm.2018.00069

Ojima, D., & Iwata, M. (2010). Central administration of growth hormone-releasing hormone and corticotropin-releasing hormone stimulate downstream movement and thyroxine secretion in fall-smolting coho salmon (*oncorhynchus kisutch*). *General and comparative endocrinology, 168*(1), 82-87. https://doi.org/10.1016/j.ygcen.2010.04.007

Patel, S. D., Chen, C. P., Bahna, F., Honig, B., & Shapiro, L. (2003). Cadherin-mediated cell–cell adhesion: Sticking together as a family. *Current Opinion in Structural Biology, 13*(6), 690-698. https://doi.org/10.1016/j.sbi.2003.10.007

Patton, A. P., & Hastings, M. H. (2018). The suprachiasmatic nucleus. *Current Biology, 28*(15), R816-R822. https://doi.org/10.1016/j.cub.2018.06.052

Pedersen, S. F., Hoffmann, E. K., & Mills, J. (2001). The cytoskeleton and cell volume regulation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130*(3), 385-399. https://doi.org/10.1016/s1095-6433(01)00429-9

Pedersen, S. F., & Nilius, B. (2007). Chapter ten - transient receptor potential channels in mechanosensing and cell volume regulation. In D. Häussinger & H. Sies (Eds.), *Osmosensing and osmosignaling* (Methods in enzymology, Vol. 428, pp. 183-207): Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/S0076687907280103

Pelis, R. M., & McCormick, S. D. (2001). Effects of growth hormone and cortisol on na+–k+–2cl− cotransporter localization and abundance in the gills of atlantic salmon. *General and Comparative Endocrinology, 124*(2), 134-143. http://doi.org/10.1006/gcen.2001.7703

Pelis, R. M., Zydlewski, J., & McCormick, S. D. (2001). Gill na+-k+-2cl− cotransporter abundance and location in atlantic salmon: Effects of seawater and smolting. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 280*(6), R1844-R1852. https://doi.org/10.1152/ajpregu.2001.280.6.R1844

Peter, R. E., Yu, K. L., Marchant, T. A., & Rosenblum, P. M. (1990). Direct neural regulation of the teleost adenohypophysis. *Journal of Experimental Zoology, 256*(S4), 84-89. https://doi.org/10.1002/jez.1402560415

Philp, A. R., Garcia-Fernandez, J. M., Soni, B. G., Lucas, R. J., Bellingham, J., & Foster, R. G. (2000). Vertebrate ancient (va) opsin and extraretinal photoreception in the atlantic salmon *(salmo salar*). *Journal of Experimental Biology, 203*(12), 1925-1936. Retrieved from https://jeb.biologists.org/content/jexbio/203/12/1925.full.pdf

Pisam, M., Prunet, P., Boeuf, G., & Jrambourg, A. (1988). Ultrastructural features of chloride cells in the gill epithelium of the atlantic salmon, *salmo salar*, and their modifications during smoltification. *American Journal of Anatomy, 183*(3), 235-244. https://doi.org/10.1002/aja.1001830306

Porter, M. J. R., Randall, C. F., Bromage, N. R., & Thorpe, J. E. (1998). The role of melatonin and the pineal gland on development and smoltification of atlantic salmon (*salmo salar*) parr. *Aquaculture, 168*(1–4), 139-155. http://doi.org/10.1016/S0044-8486(98)00345-7

Premdas, F. H., & Eales, J. G. (1976). The influence of tsh and acth on purine and pteridine deposition in the skin of rainbow trout (*salmo gairdneri*). *Canadian Journal of Zoology, 54*(4), 576-581. https://doi.org/10.1139/z76-067

Prunet, P., Boeuf, G., Bolton, J. P., & Young, G. (1989). Smoltification and seawater adaptation in atlantic salmon (*salmo salar*): Plasma prolactin, growth hormone, and thyroid hormones. *General and Comparative Endocrinology, 74*(3), 355-364. https://doi.org/10.1016/S0016-6480(89)80031-0

Putman, Nathan F., Scanlan, Michelle M., Billman, Eric J., O'Neil, Joseph P., Couture, Ryan B., Quinn, Thomas P., . . .Noakes, David L. G. (2014). An inherited magnetic map guides ocean navigation in juvenile pacific salmon. *Current Biology, 24*(4), 446-450. https://doi.org/10.1016/j.cub.2014.01.017

Putney, James W. (2012). Calcium signaling: Deciphering the calcium–nfat pathway. *Current Biology, 22*(3), R87-R89. https://doi.org/10.1016/j.cub.2011.12.030

Ramsden, S. D., Brinkmann, H., Hawryshyn, C. W., & Taylor, J. S. (2003). Mitogenomics and the sister of salmonidae. *Trends in Ecology & Evolution, 18*(12), 607-610. https://doi.org/10.1016/j.tree.2003.09.020

Reebs, S. G. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries, 12*(4), 349-371. https://doi.org/10.1023/a:1025371804611

Reiter, R. J. (1993). The melatonin rhythm: Both a clock and a calendar. *Experientia, 49*(8), 654-664. https://doi.org/10.1007/BF01923947

Reppert, S. M. (1997). Melatonin receptors: Molecular biology of a new family of g protein-coupled receptors. *Journal of Biological Rhythms, 12*(6), 528-531. https://doi.org/10.1177/074873049701200606

Richards, J. G., Semple, J. W., Bystriansky, J. S., & Schulte, P. M. (2003). Na+/k+-atpase α-isoform switching in gills of rainbow trout (*oncorhynchus mykiss*) during salinity transfer. *Journal of Experimental Biology, 206*(24), 4475-4486. https://doi.org/10.1242/jeb.00701

Robertson, F. M., Gundappa, M. K., Grammes, F., Hvidsten, T. R., Redmond, A. K., Lien, S., . . .Macqueen, D. J. (2017). Lineage-specific rediploidization is a mechanism to explain time-lags between genome duplication and evolutionary diversification. *Genome Biology, 18*(1), 111. https://doi.org/10.1186/s13059-017-1241-z

Robertson, L. S., & McCormick, S. D. (2012). Transcriptional profiling of the parr–smolt transformation in atlantic salmon. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 7*(4), 351-360. https://doi.org/10.1016/j.cbd.2012.07.003

Rouault, A. A. J., Srinivasan, D. K., Yin, T. C., Lee, A. A., & Sebag, J. A. (2017). Melanocortin receptor accessory proteins (mraps): Functions in the melanocortin system and beyond. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1863*(10, Part A), 2462-2467. https://doi.org/10.1016/j.bbadis.2017.05.008

Rowe, D. K., Thorpe, J. E., & Shanks, A. M. (1991). Role of fat stores in the maturation of male atlantic salmon (*salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(3), 405-413. https://doi.org/10.1139/f91-052

Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jönsson, E., & Volkoff, H. (2017). Appetite-controlling endocrine systems in teleosts. *Frontiers in Endocrinology, 8*(73). https://doi.org/10.3389/fendo.2017.00073

Sáenz de Miera, C., Hanon, E. A., Dardente, H., Birnie, M., Simonneaux, V., Lincoln, G. A., & Hazlerigg, D. G. (2013). Circannual variation in thyroid hormone deiodinases in a short-day breeder. *Journal of Neuroendocrinology, 25*(4), 412-421. https://doi.org/10.1111/jne.12013

Sáenz de Miera, C., Monecke, S., Bartzen-Sprauer, J., Laran-Chich, M.-P., Pévet, P., Hazlerigg, David G., & Simonneaux, V. (2014). A circannual clock drives expression of genes central for seasonal reproduction. *Current Biology, 24*(13), 1500-1506. http://doi.org/10.1016/j.cub.2014.05.024

Samyn-Petit, B., Krzewinski-Recchi, M.-A., Steelant, W. F. A., Delannoy, P., & Harduin-Lepers, A. (2000). Molecular cloning and functional expression of human st6galnac ii. Molecular expression in various human cultured cells. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1474*(2), 201-211. https://doi.org/10.1016/S0304-4165(00)00020-9

Sandbakken, M., Ebbesson, L., Stefansson, S., & Helvik, J. V. (2012). Isolation and characterization of melanopsin photoreceptors of atlantic salmon (*salmo salar*). *Journal of Comparative Neurology, 520*(16), 3727-3744. https://doi.org/10.1002/cne.23125

Sardet, C., Pisam, M., & Maetz, J. (1979). The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. *Journal of Cell Biology, 80*(1), 96-117. https://doi.org/10.1083/jcb.80.1.96

Sato, K., & Kawashima, S. (2001). Calpain function in the modulation of signal transduction molecules. *Biological Chemistry, 382*(5), 743. https://doi.org/10.1515/bchm.2001.382.5.743

Saunders, R. L., Henderson, E. B., & Glebe, B. D. (1982). Precocious sexual maturation and smoltification in male atlantic salmon (*salmo salar*). *Aquaculture, 28*(1), 211-229. https://doi.org/10.1016/0044-8486(82)90024-2

Saunders, R. L., Henderson, E. B., & Harmon, P. R. (1985). Effects of photoperiod on juvenile growth and smolting of atlantic salmon and subsequent survival and growth in sea cages. *Aquaculture, 45*(1), 55-66. https://doi.org/10.1016/0044-8486(85)90257-1

Scholz, A. T., White, R. J., Muzi, M., & Smith, T. (1985). Uptake of radiolabelled triiodothyronine in the brain of steelhead trout (*salmo gairdneri*) during parr-smolt transformation: Implications for the mechanism of thyroid activation of olfactory

imprinting. *Aquaculture, 45*(1), 199-214. https://doi.org/10.1016/0044-8486(85)90270-4

Seale, A. P., Richman III, N. H., Hirano, T., Cooke, I., & Grau, E. G. (2003). Evidence that signal transduction for osmoreception is mediated by stretch-activated ion channels in tilapia. *American Journal of Physiology -Cell Physiology, 284*(5), C1290-C1296. https://doi.org/10.1152/ajpcell.00532.2002

Seear, P. J., Carmichael, S. N., Talbot, R., Taggart, J. B., Bron, J. E., & Sweeney, G. E. (2010). Differential gene expression during smoltification of atlantic salmon (salmo salar l.): A first large-scale microarray study. *Marine Biotechnology, 12*(2), 126-140. https://doi.org/10.1007/s10126-009-9218-x

Shrimpton, J. M., Devlin, R. H., McLean, E., Byatt, J. C., Donaldson, E. M., & Randall, D. J. (1995). Increases in gill cytosolic corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*oncorhynchus kisutch*) treated with growth hormone and placental lactogen. *General and Comparative Endocrinology, 98*(1), 1-15. https://doi.org/10.1006/gcen.1995.1039

Shrimpton, J. M., & McCormick, S. D. (1998a). Regulation of gill cytosolic corticosteroid receptors in juvenile atlantic salmon: Interaction effects of growth hormone with prolactin and triiodothyronine. *General and Comparative Endocrinology, 112*(2), 262-274. https://doi.org/10.1006/gcen.1998.7172

Shrimpton, J. M., & McCormick, S. D. (1998b). Seasonal differences in plasma cortisol and gill corticosteroid receptors in upper and lower mode juvenile atlantic salmon. *Aquaculture, 168*(1-4), 205-219. https://doi.org/10.1016/S0044-8486(98)00350-0

Shrimpton, J. M., & McCormick, S. D. (1999). Responsiveness of gill na+/k+-atpase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. *Journal of Experimental Biology, 202*(8), 987-995. Retrieved from https://jeb.biologists.org/content/jexbio/202/8/987.full.pdf

Singer, T. D., Clements, K. M., Semple, J. W., Schulte, P. M., Bystriansky, J. S., Finstad, B., . . .McKinley, R. S. (2002). Seawater tolerance and gene expression in two strains of atlantic salmon smolts. *Canadian Journal of Fisheries and Aquatic Sciences, 59*(1), 125-135. https://doi.org/10.1139/f01-205

Singer, T. D., Finstad, B., McCormick, S. D., Wiseman, S. B., Schulte, P. M., & Scott McKinley, R. (2003). Interactive effects of cortisol treatment and ambient seawater challenge on gill na^+,k^+-atpase and cftr expression in two strains of atlantic salmon smolts. *Aquaculture, 222*(1), 15-28. https://doi.org/10.1016/S0044-8486(03)00099-1

Singh, R. D., Schroeder, A. S., Scheffer, L., Holicky, E. L., Wheatley, C. L., Marks, D. L., & Pagano, R. E. (2013). Prominin-2 expression increases protrusions, decreases caveolae and inhibits cdc42 dependent fluid phase endocytosis. *Biochemical and Biophysical Research Communications, 434*(3), 466-472. https://doi.org/10.1016/j.bbrc.2013.03.097

Skilbrei, O. T. (1991). Importance of threshold length and photoperiod for the development of bimodal length–frequency distribution in atlantic salmon (*salmo salar*). *Candian Journal of Fisheries and Aquatic Sciences, 48*(11), 2163-2172. https://doi.org/10.1139/f91-255

Skulason, S., & Smith, T. B. (1995). Resource polymorphisms in vertebrates. *Trends in Ecology & Evolution, 10*(9), 366-370. https://doi.org/10.1016/S0169-5347(00)89135-1

Skúlason, S., Snorrason, S. S., & Jónsson, B. (1999). Sympatric morphs, populations and speciation in freshwater fish with emphasis on arctic charr. In A. E. Magurran & R. M. May (Eds.), *Evolution of biological diversity* (pp. 70-92). Oxford, UK: Oxford University Press.

Sohm, F., Manfroid, I., Pezet, A., Rentier-Delrue, F., Rand-Weaver, M., Kelly, P. A., . . .Edery, M. (1998). Identification and modulation of a growth hormone-binding protein in rainbow trout (*oncorhynchus mykiss*) plasma during seawater adaptation. *General and Comparative Endocrinology, 111*(2), 216-224. https://doi.org/10.1006/gcen.1998.7106

Specker, J. L., Brown, C. L., & Bern, H. A. (1992). Asynchrony of changes in tissue and plasma thyroid hormones during the parr-smolt transformation of coho salmon. *General and Comparative Endocrinology, 88*(3), 397-405. https://doi.org/10.1016/0016-6480(92)90234-B

Specker, J. L., Eales, J. G., Tagawa, M., & Tyler, I. I. I. W. A. (2000). Parr-smolt transformation in atlantic salmon: Thyroid hormone deiodination in liver and brain and endocrine correlates of change in rheotactic behavior. *Canadian Journal of Zoology, 78*(5), 696-705. https://doi.org/10.1139/z99-258

Specker, J. L., & Schreck, C. B. (1982). Changes in plasma corticosteroids during smoltification of coho salmon, *oncorhynchus kisutch*. *General and Comparative Endocrinology, 46*(1), 53-58. https://doi.org/10.1016/0016-6480(82)90162-9

Specker, J. L., & Schreck, C. B. (1984). Thyroidal response to mammalian thyrotropin during smoltification of coho salmon (*oncorhynchus kisutch*). *Comparative Biochemistry and Physiology Part A: Physiology, 78*(3), 441-444. https://doi.org/10.1016/0300-9629(84)90574-7

Stabell, O. B. (1984). Homing and olfaction in salmonids: A critical review with special reference to the atlantic salmon. *Biological Reviews, 59*(3), 333-388. https://doi.org/10.1111/j.1469-185X.1984.tb00709.x

Staley, K., & Ewing, R. (1992). Purine levels in the skin of juvenile coho salmon (*oncorhynchus kisutch*) during parr-smolt transformation and adaptation to seawater. *Comparative biochemistry and physiology. B, Comparative biochemistry, 101*(3), 447-452. https://doi.org/10.1016/0305-0491(92)90026-n

Stefansson, S. O., Bjornsson, B. T., Hansen, T., Haux, C., Taranger, G. L., & Saunders, R. L. (1991). Growth, parr-smolt transformation, and changes in growth-hormone of atlantic salmon (*salmo salar*) reared under different photoperiods. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(11), 2100-2108. https://doi.org/10.1139/f91-249

Stefansson, S. O., Bjornsson, B. T., Sundell, K., Nyhammer, G., & McCormick, S. D. (2003). Physiological characteristics of wild atlantic salmon post-smolts during estuarine and coastal migration. *Journal of Fish Biology, 63*(4), 942-955. https://doi.org/10.1046/j.1095-8649.2003.00201.x

Stefansson, S. O., Björnsson, B. T., Ebbesson, L. O. E., & McCormick, S. D. (2008). Smoltification. In R. N. Finn & B. G. Kapoor (Eds.), *Fish larval physiology*. New Dehli: Science Publishers, Inc. Enfield (NH) & IBH Publishing Co. Pvt. Ltd.

Stefansson, S. O., Nilsen, T. O., Ebbesson, L. O. E., Wargelius, A., Madsen, S. S., Björnsson, B. T., & McCormick, S. D. (2007). Molecular mechanisms of continuous light inhibition of

atlantic salmon parr–smolt transformation. *Aquaculture, 273*(2), 235-245. https://doi.org/10.1016/j.aquaculture.2007.10.005

Steiger, S. S., Valcu, M., Spoelstra, K., Helm, B., Wikelski, M., & Kempenaers, B. (2013). When the sun never sets: Diverse activity rhythms under continuous daylight in free-living arctic-breeding birds. *Proceedings of the Royal Society B: Biological Sciences, 280*(1764), 20131016. https://doi.org/10.1098/rspb.2013.1016

Steingrímsson, S. Ó., & Grant, J. W. A. (2008). Multiple central-place territories in wild young-of-the-year atlantic salmon *salmo salar*†. *Journal of Animal Ecology, 77*(3), 448-457. https://doi.org/10.1111/j.1365-2656.2008.01360.x

Stien, L. H., Bracke, M. B. M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., . . .Kristiansen, T. S. (2013). Salmon welfare index model (swim 1.0): A semantic model for overall welfare assessment of caged atlantic salmon: Review of the selected welfare indicators and model presentation. *Reviews in Aquaculture, 5*(1), 33-57. https://doi.org/10.1111/j.1753-5131.2012.01083.x

Stradmeyer, L., & Thorpe, J. E. (1987). Feeding behaviour of wild atlantic salmon, *salmo salar* l., parr in mid- to late summer in a scottish river. *Aquaculture Research, 18*(1), 33-49. https://doi.org/10.1111/j.1365-2109.1987.tb00123.x

Strand, J. E. T., Hazlerigg, D., & Jørgensen, E. H. (2018). Photoperiod revisited: Is there a critical day length for triggering a complete parr–smolt transformation in atlantic salmon *salmo salar*? *Journal of Fish Biology, 93*(3), 440-448. https://doi.org/10.1111/jfb.13760

Strøm, J. F., Thorstad, E. B., Hedger, R. D., & Rikardsen, A. H. (2018). Revealing the full ocean migration of individual atlantic salmon. *Animal Biotelemetry, 6*(1), 2. https://doi.org/10.1186/s40317-018-0146-2

Sundin, L., Nilsson, G. E., Block, M., & Lofman, C. O. (1995). Control of gill filament blood flow by serotonin in the rainbow trout, oncorhynchus mykiss. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 268*(5), R1224-R1229. https://doi.org/10.1152/ajpregu.1995.268.5.r1224

Taylor, E. B. (1991). A review of local adaptation in salmonidac, with particular reference to pacific and atlantic salmon. *Aquaculture, 98*(1–3), 185-207. http://doi.org/10.1016/0044-8486(91)90383-I

Taylor, J. S., Braasch, I., Frickey, T., Meyer, A., & Van de Peer, Y. (2003). Genome duplication, a trait shared by 22,000 species of ray-finned fish. *Genome Research, 13*(3), 382-390. https://doi.org/10.1101/gr.640303

Terrien, X., Fini, J.-B., Demeneix, B. A., Schramm, K.-W., & Prunet, P. (2011). Generation of fluorescent zebrafish to study endocrine disruption and potential crosstalk between thyroid hormone and corticosteroids. *Aquatic Toxicology, 105*(1), 13-20. https://doi.org/10.1016/j.aquatox.2011.04.007

Thorpe, J. E. (1977). Bimodal distribution of length of juvenile atlantic salmon (*salmo salar* l.) under artificial rearing conditions. *Journal of Fish Biology, 11*(2), 175-184. https://doi.org/10.1111/j.1095-8649.1977.tb04111.x

Thorpe, J. E. (1989). Developmental variation in salmonid populations. *J Fish Biol, 35*(sA), 295-303. https://doi.org/10.1111/j.1095-8649.1989.tb03073.x

Thorpe, J. E. (1994a). An alternative view of smolting in salmonids. *Aquaculture, 121*(1), 105-113. https://doi.org/10.1016/0044-8486(94)90012-4

Thorpe, J. E. (1994b). Performance thresholds and life-history flexibility in salmonids. *Conservation Biology, 8*(3), 877-879. Retrieved from http://www.jstor.org/stable/2386536

Thorpe, J. E., Mangel, M., Metcalfe, N., & Huntingford, F. (1998). Modelling the proximate basis of salmonid life-history variation, with application to atlantic salmon, *salmo salar* l. *Evolutionary Ecology, 12*(5), 581-599. https://doi.org/10.1023/A:1022351814644

Thorpe, J. E., Talbot, C., & Villarreal, C. (1982). Bimodality of growth and smolting in atlantic salmon, *salmo salar* l. *Aquaculture, 28*(1), 123-132. https://doi.org/10.1016/0044-8486(82)90015-1

Thorstad, E. B., Whoriskey, F., Rikardsen, A. H., & Aarestrup, K. (2010). Aquatic nomads: The life and migrations of the atlantic salmon. In Ø. Aas, S. Einum, A. Klemetsen, & J. Skurdal (Eds.), *Atlantic salmon ecology* (pp. 1-32). Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1002/9781444327755.ch1

Tipsmark, C. K. (2008). Identification of fxyd protein genes in a teleost: Tissue-specific expression and response to salinity change. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 294*(4), R1367-R1378. https://doi.org/10.1152/ajpregu.00454.2007

Tipsmark, C. K., Jørgensen, C., Brande-Lavridsen, N., Engelund, M., Olesen, J. H., & Madsen, S. S. (2009). Effects of cortisol, growth hormone and prolactin on gill claudin expression in atlantic salmon. *General and Comparative Endocrinology, 163*(3), 270-277. https://doi.org/10.1016/j.ygcen.2009.04.020

Tipsmark, C. K., Kiilerich, P., Nilsen, T. O., Ebbesson, L. O. E., Stefansson, S. O., & Madsen, S. S. (2008). Branchial expression patterns of claudin isoforms in atlantic salmon during seawater acclimation and smoltification. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 294*(5), R1563-R1574. https://doi.org/10.1152/ajpregu.00915.2007

Tipsmark, C. K., & Madsen, S. S. (2009). Distinct hormonal regulation of na^+,k^+-atpase genes in the gill of atlantic salmon (*salmo salar* l.). *Journal of Endocrinology, 203*(2), 301-310. https://doi.org/10.1677/joe-09-0281

Tipsmark, C. K., Mahmmoud, Y. A., Borski, R. J., & Madsen, S. S. (2010). Fxyd-11 associates with na^+, k^+-atpase in the gill of atlantic salmon: Regulation and localization in relation to changed ion-regulatory status. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 299*(5), R1212-R1223. https://doi.org/10.1152/ajpregu.00015.2010

Tipsmark, C. K., Sørensen, K. J., & Madsen, S. S. (2010). Aquaporin expression dynamics in osmoregulatory tissues of atlantic salmon during smoltification and seawater acclimation. *Journal of Experimental Biology, 213*(3), 368-379. https://doi.org/10.1242/jeb.034785

Valentin, J. P., Wiedemann, E., & Humphreys, M. H. (1993). Natriuretic properties of melanocyte-stimulating hormones. *Journal of cardiovascular pharmacology, 22 Suppl 2*, S114-118. Retrieved from http://europepmc.org/abstract/MED/7508015

Van de Peer, Y., Maere, S., & Meyer, A. (2009). The evolutionary significance of ancient genome duplications. *Nature Reviews Genetics, 10*(10), 725-732. Retrieved from http://doi.org/10.1038/nrg2600

Van Itallie, C. M., & Anderson, J. M. (2006). Claudins and epithelial paracellular transport. *Annual Review of Physiology, 68*(1), 403-429. https:/doi.org/10.1146/annurev.physiol.68.040104.131404

Van Itallie, C. M., & Anderson, J. M. (2014). Architecture of tight junctions and principles of molecular composition. *Seminars in Cell & Developmental Biology, 36*, 157-165. https://doi.org/10.1016/j.semcdb.2014.08.011

van Oort, B. E. H., Tyler, N. J. C., Gerkema, M. P., Folkow, L., & Stokkan, K.-A. (2007). Where clocks are redundant: Weak circadian mechanisms in reindeer living under polar photic conditions. *Naturwissenschaften, 94*(3), 183-194. https://doi.org/10.1007/s00114-006-0174-2

Vatine, G., Vallone, D., Gothilf, Y., & Foulkes, N. S. (2011). It's time to swim! Zebrafish and the circadian clock. *FEBS Letters, 585*(10), 1485-1494. https://doi.org/10.1016/j.febslet.2011.04.007

Veillette, P. A., Sundell, K., & Specker, J. L. (1995). Cortisol mediates the increase in intestinal fluid absorption in atlantic salmon during parr-smolt transformation. *General and Comparative Endocrinology, 97*(2), 250-258. https://doi.org/10.1006/gcen.1995.1024

Vera, L. M., López‐Olmeda, J. F., Bayarri, M. J., Madrid, J. A., & Sánchez‐Vázquez, F. J. (2005). Influence of light intensity on plasma melatonin and locomotor activity rhythms in tench. *Chronobiology International, 22*(1), 67-78. https://doi.org/10.1081/CBI-200038157

Virtanen, E., & Soivio, A. (1985). The patterns of t3, t4, cortisol and na+□ k+-atpase during smoltification of hatchery-reared *salmo salar* and comparison with wild smolts. *Aquaculture, 45*(1), 97-109. https://doi.org/10.1016/0044-8486(85)90261-3

Vogel, C., & Marcotte, E. M. (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature Reviews Genetics, 13*(4), 227-232. https://doi.org/10.1038/nrg3185

von Schalburg, K. R., Yazawa, R., de Boer, J., Lubieniecki, K. P., Goh, B., Straub, C. A., . . .Koop, B. F. (2008). Isolation, characterization and comparison of atlantic and chinook salmon growth hormone 1 and 2. *BMC Genomics, 9*. https://doi.org/10.1186/1471-2164-9-522

Wagner, H. H. (1974). Photoperiod and temperature regulation of smolting in steelhead trout (*salmo gairdneri*). *Canadian Journal of Zoology, 52*(2), 219-234. https://doi.org/10.1139/z74-026

Walsh, J. G., Barreda, D. R., & Belosevic, M. (2007). Cloning and expression analysis of goldfish (*carassius auratus* l.) prominin. *Fish & Shellfish Immunology, 22*(4), 308-317. https://doi.org/10.1016/j.fsi.2006.04.007

Wang, T., Wang, L., Moreno-Vinasco, L., Lang, G. D., Siegler, J. H., Mathew, B., . . .Garcia, J. G. N. (2012). Particulate matter air pollution disrupts endothelial cell barrier via calpain-mediated tight junction protein degradation. *Particle and Fibre Toxicology, 9*(1), 35. https://doi.org/10.1186/1743-8977-9-35

Wargelius, A., Fjelldal, P.-G., Benedet, S., Hansen, T., Björnsson, B. T., & Nordgarden, U. (2005). A peak in gh-receptor expression is associated with growth activation in atlantic salmon vertebrae, while upregulation of igf-i receptor expression is related to increased bone density. *General and Comparative Endocrinology, 142*(1), 163-168. https://doi.org/10.1016/j.ygcen.2004.12.005

Warren, I. A., Ciborowski, K. L., Casadei, E., Hazlerigg, D. G., Martin, S., Jordan, W. C., & Sumner, S. (2014). Extensive local gene duplication and functional divergence among paralogs in atlantic salmon. *Genome Biology and Evolution, 6*(7), 1790-1805. https://doi.org/10.1093/gbe/evu131

Watanabe, N., Itoh, K., Mogi, M., Fujinami, Y., Shimizu, D., Hashimoto, H., . . .Suzuki, T. (2012). Circadian pacemaker in the suprachiasmatic nuclei of teleost fish revealed by rhythmic period2 expression. *General and Comparative Endocrinology, 178*(2), 400-407. https://doi.org/10.1016/j.ygcen.2012.06.012

Webb, T. R., & Clark, A. J. L. (2010). Minireview: The melanocortin 2 receptor accessory proteins. *Molecular Endocrinology, 24*(3), 475-484. https://doi.org/10.1210/me.2009-0283

Weber, G. M., Seale, A. P., Richman Iii, N. H., Stetson, M. H., Hirano, T., & Grau, E. G. (2004). Hormone release is tied to changes in cell size in the osmoreceptive prolactin cell of a euryhaline teleost fish, the tilapia, *oreochromis mossambicus*. *General and Comparative Endocrinology, 138*(1), 8-13. https://doi.org/10.1016/j.ygcen.2004.04.006

Wedemeyer, G. A., Saunders, R. L., & Clarke, W. C. (1980). *Environmental-factors affecting smoltification and early marine survival of anadromous salmonids* (Marine fisheries review Vol. 42): U. S. National Marine Fisheries Service.

Weiss-Hersh, K., Garcia, A. L., Marosvölgyi, T., Szklenár, M., Decsi, T., & Rühl, R. (2019). Saturated and monounsaturated fatty acids in membranes are determined by the gene expression of their metabolizing enzymes scd1 and elovl6 regulated by the intake of dietary fat. *European Journal of Nutrition*, 1-11. https://doi.org/10.1007/s00394-019-02121-2

Welker, P., Geist, B., Frühauf, J.-H., Salanova, M., Groneberg, D. A., Krause, E., & Bachmann, S. (2007). Role of lipid rafts in membrane delivery of renal epithelial na+-k+-atpase, thick ascending limb. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 292*(3), R1328-R1337. https://doi.org/10.1152/ajpregu.00166.2006

West, A. C., Iversen, M., Jørgensen, E. H., Sandve, S. R., Hazlerigg, D. G., & Wood, S. H. (2020). Diversified regulation of circadian clock gene expression following whole genome duplication. *PLOS Genetics, 16*(10), e1009097. https://doi.org/10.1371/journal.pgen.1009097

West, A. C., Mizoro, Y., Wood, S. H., Ince, L. M., Iversen, M., Jørgensen, E. H., . . .Hazlerigg, D. G. (2020). A single nuclei transcriptomic analysis of the atlantic salmon gill through smoltification and seawater transfer. *bioRxiv*, 2020.2009.2003.281337. https://doi.org/10.1101/2020.09.03.281337

Wilson, J. M., & Laurent, P. (2002). Fish gill morphology: Inside out. *Journal of Experimental Zoology, 293*(3), 192-213. https://doi.org/10.1002/jez.10124

Woo, S., Lee, S., & Kwon, M. H. (2002). Tonebp transcriptional activator in the cellular response to increased osmolality. *Pflügers Archive European Journal of Physiology, 444*(5), 579-585. https://doi.org/10.1007/s00424-002-0849-2

Wood, S., & Loudon, A. (2018). The pars tuberalis: The site of the circannual clock in mammals? *General and comparative endocrinology, 258*, 222-235. https://doi.org/10.1016/j.ygcen.2017.06.029

Wright, N. T., Cannon, B. R., Zimmer, D. B., & Weber, D. J. (2009). S100a1: Structure, function, and therapeutic potential. *Current Chemical Biology, 3*(2), 138-145. https://doi.org/10.2174/187231309788166460

Yamauchi, T., Kaburagi, Y., Ueki, K., Tsuji, Y., Stark, G. R., Kerr, I. M., . . .Kadowaki, T. (1998). Growth hormone and prolactin stimulate tyrosine phosphorylation of insulin receptor substrate-1, -2, and -3, their association with p85 phosphatidylinositol 3-kinase (pi3-kinase), and concomitantly pi3-kinase activation via jak2 kinase. *Journal of Biological Chemistry, 273*(25), 15719-15726. https://doi.org/10.1074/jbc.273.25.15719

Yeung, B. H. Y., Law, A. Y. S., & Wong, C. K. C. (2012). Evolution and roles of stanniocalcin. *Molecular and Cellular Endocrinology, 349*(2), 272-280. https://doi.org/10.1016/j.mce.2011.11.007

Yoshimura, T., Yasuo, S., Watanabe, M., Iigo, M., Yamamura, T., Hirunagi, K., & Ebihara, S. (2003). Light-induced hormone conversion of t4 to t3 regulates photoperiodic response of gonads in birds. *Nature, 426*(6963), 178-181. https://doi.org/10.1038/nature02117

Young, G. (1988). Enhanced response of the interrenal of coho salmon (*oncorhynchus kisutch*) to acth after growth hormone treatment in vivo and in vitro. *General and Comparative Endocrinology, 71*(1), 85-92. https://doi.org/10.1016/0016-6480(88)90298-5

Yu, X., Ng, C. P., Habacher, H., & Roy, S. (2008). Foxj1 transcription factors are master regulators of the motile ciliogenic program. *Nature Genetics, 40*(12), 1445-1453. https://doi.org/10.1038/ng.263

Zaccone, G., Lauweryns, J. M., Fasulo, S., Tagliafierro, G., Ainis, L., & Licata, A. (1992). Immunocytochemical localization of serotonin and neuropeptides in the neuroendocrine paraneurons of teleost and lungfish gills*. *Acta Zoologica, 73*(3), 177-183. https://doi.org/10.1111/j.1463-6395.1992.tb01185.x

Zhang, X.-F., Chen, J., Faltynek, C. R., Moreland, R. B., & Neelands, T. R. (2008). Transient receptor potential a1 mediates an osmotically activated ion channel. *European Journal of Neuroscience, 27*(3), 605-611. https://doi.org/10.1111/j.1460-9568.2008.06030.x

Zhdanova, I., & Reebs, S. (2005). Circadian rhythms in fish. In K. A. Sloman, R. W. Wilson, & S. Balshine (Eds.), *Behaviour and physiology of fish* (Vol. 24, pp. 197-238): Academic Press.

Økland, F., Jonsson, B., Jensen, A. J., & Hansen, L. P. (1993). Is there a threshold size regulating seaward migration of brown trout and atlantic salmon? *Journal of Fish Biology, 42*(4), 541-550. https://doi.org/10.1111/j.1095-8649.1993.tb00358.x

Aas, Ø., Einum, S., Klemetsen, A., & Skurdal, J. (2010). *Atlantic salmon ecology*.

7. Appendix

Appendix 1: Plots showing additional genes from the clustering of genes of interest performed as part of Paper I, and base upon their FW expression. Genes were chosen to exemplify the pattern of expression represented by each cluster, and to show that the SW response of genes within the same clusters could vary.

p.85-92.

Appendix 2: Complete list of genes and clusters from paper I.

p.93-98

Appendix 3: Plots showing the FW expression of deiodinases and components of the NKA pump, data from the 2013-experiement (presented in paper I).

p.99-102

Appendix 4: Plots showing the FW expression of deiodinases and components of the NKA pump, data from the 2017-experiement (presented in paper I).

p.103-106

Appendix 5: Paper I RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile Atlantic salmon

p.107-128

Appendix 6: Paper II Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in Atlantic salmon

p.129-160

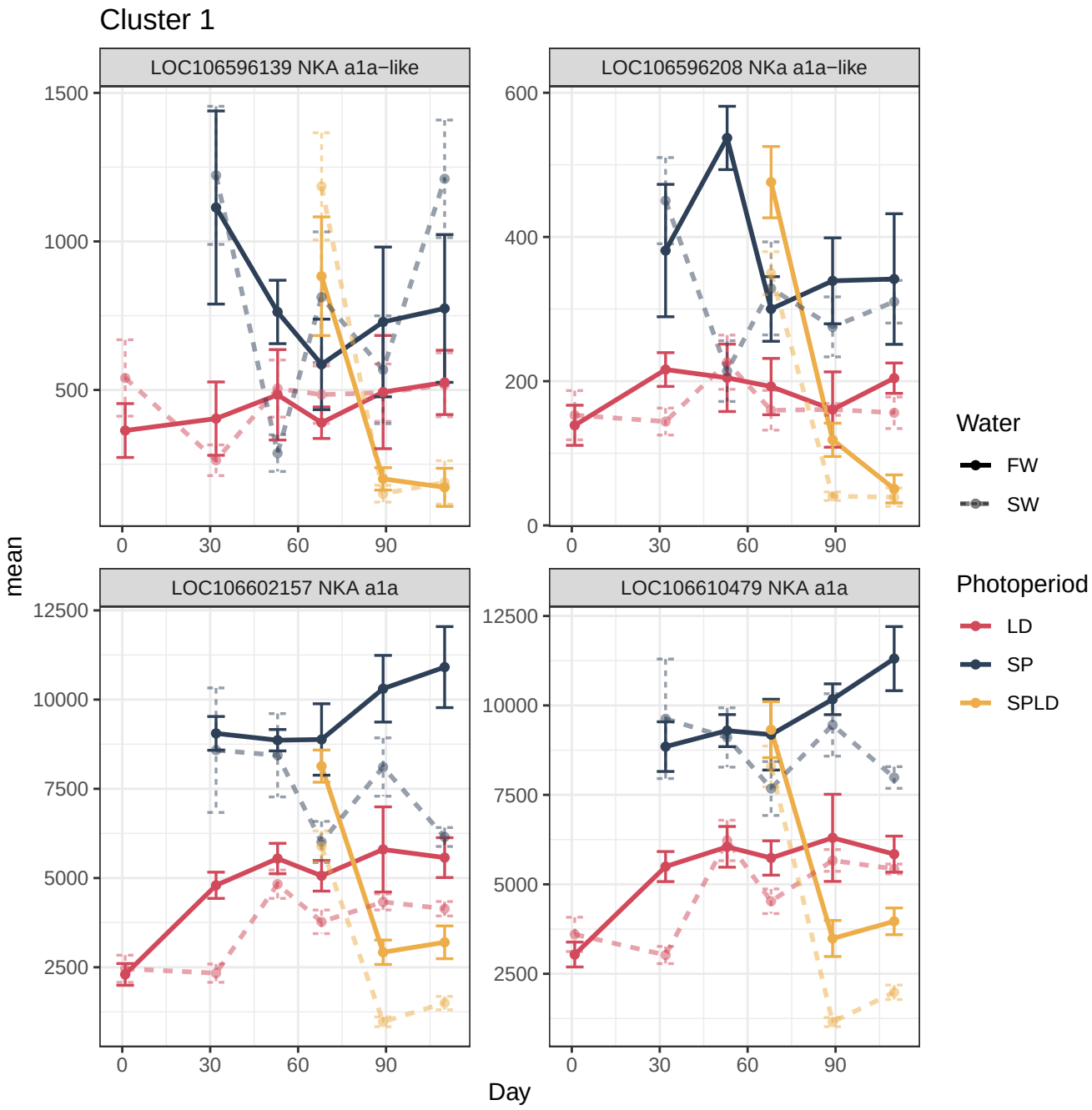
Appendix 7: Paper III Diversified regulation of circadian clock gene expression following whole genome duplication

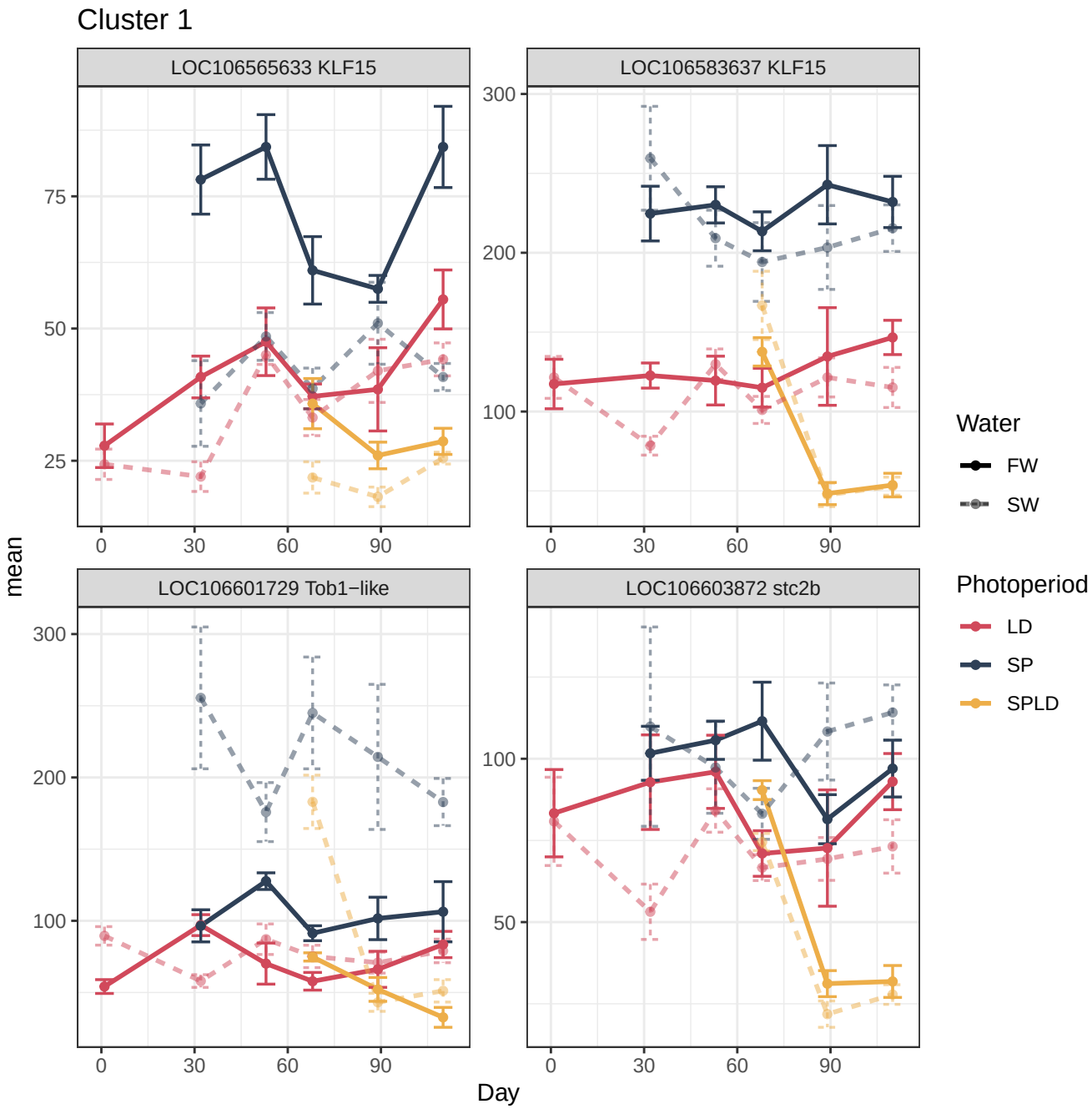
p.161-181

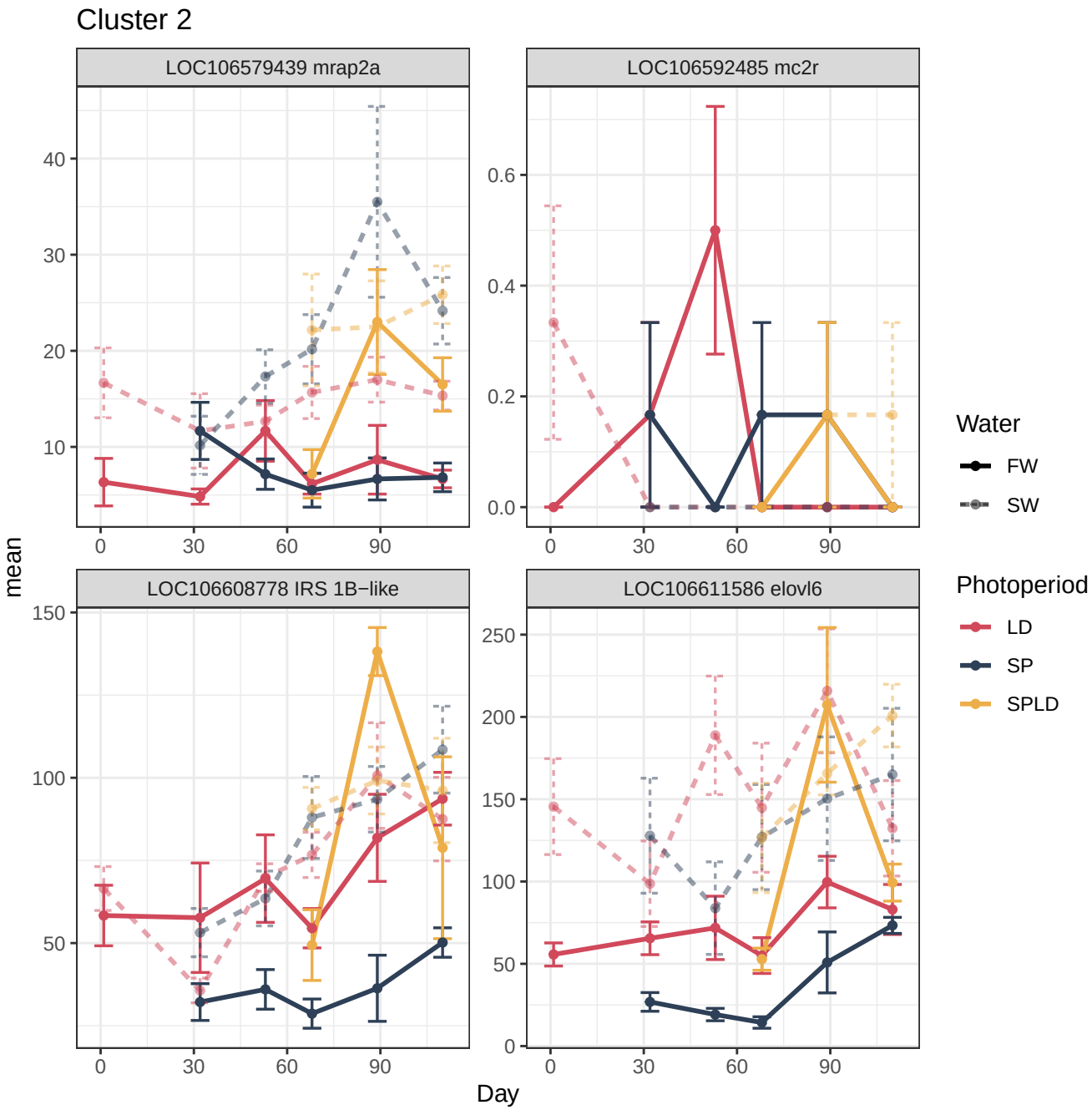
Appendix 8: Co-author declaration

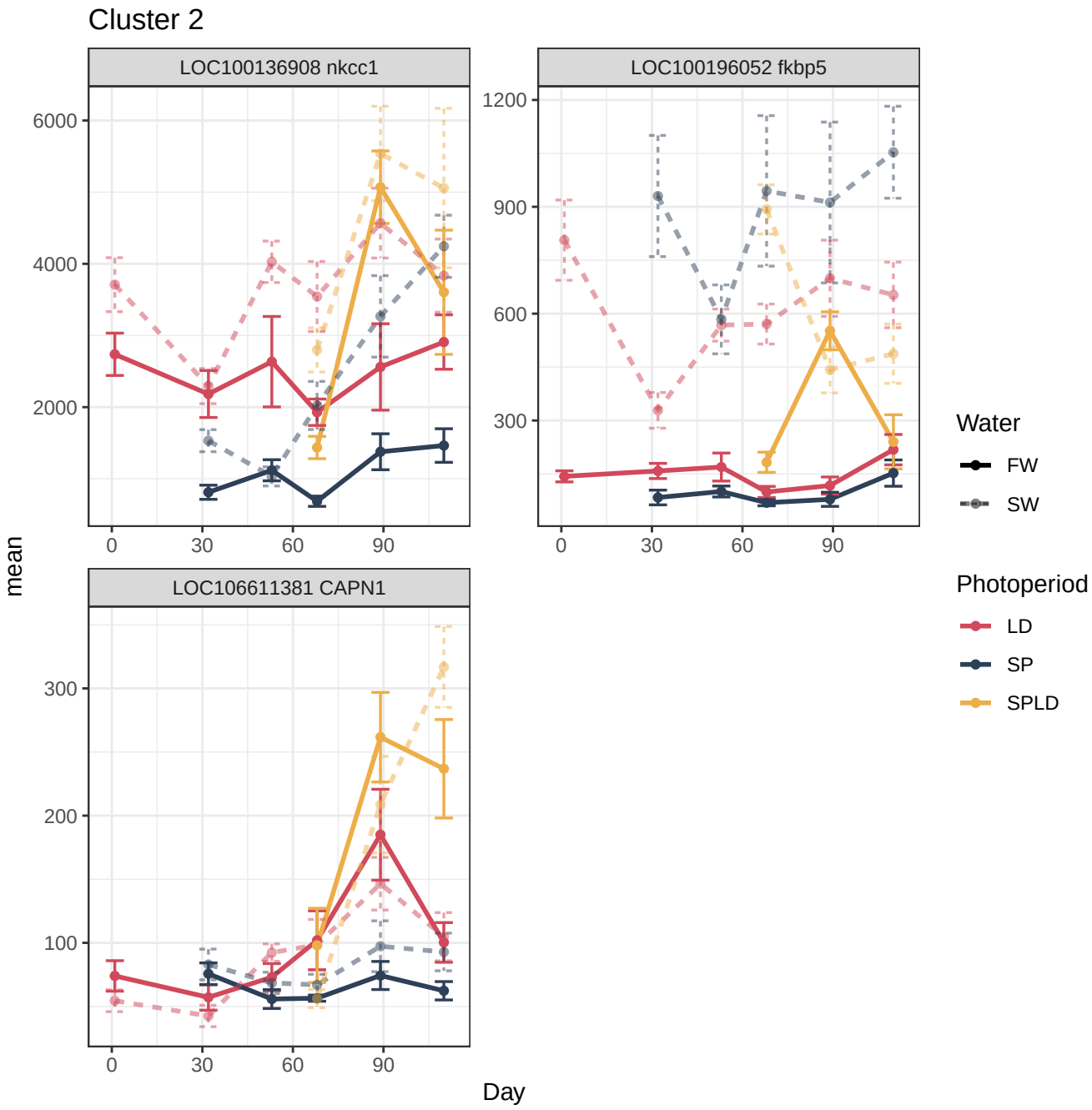
p.182

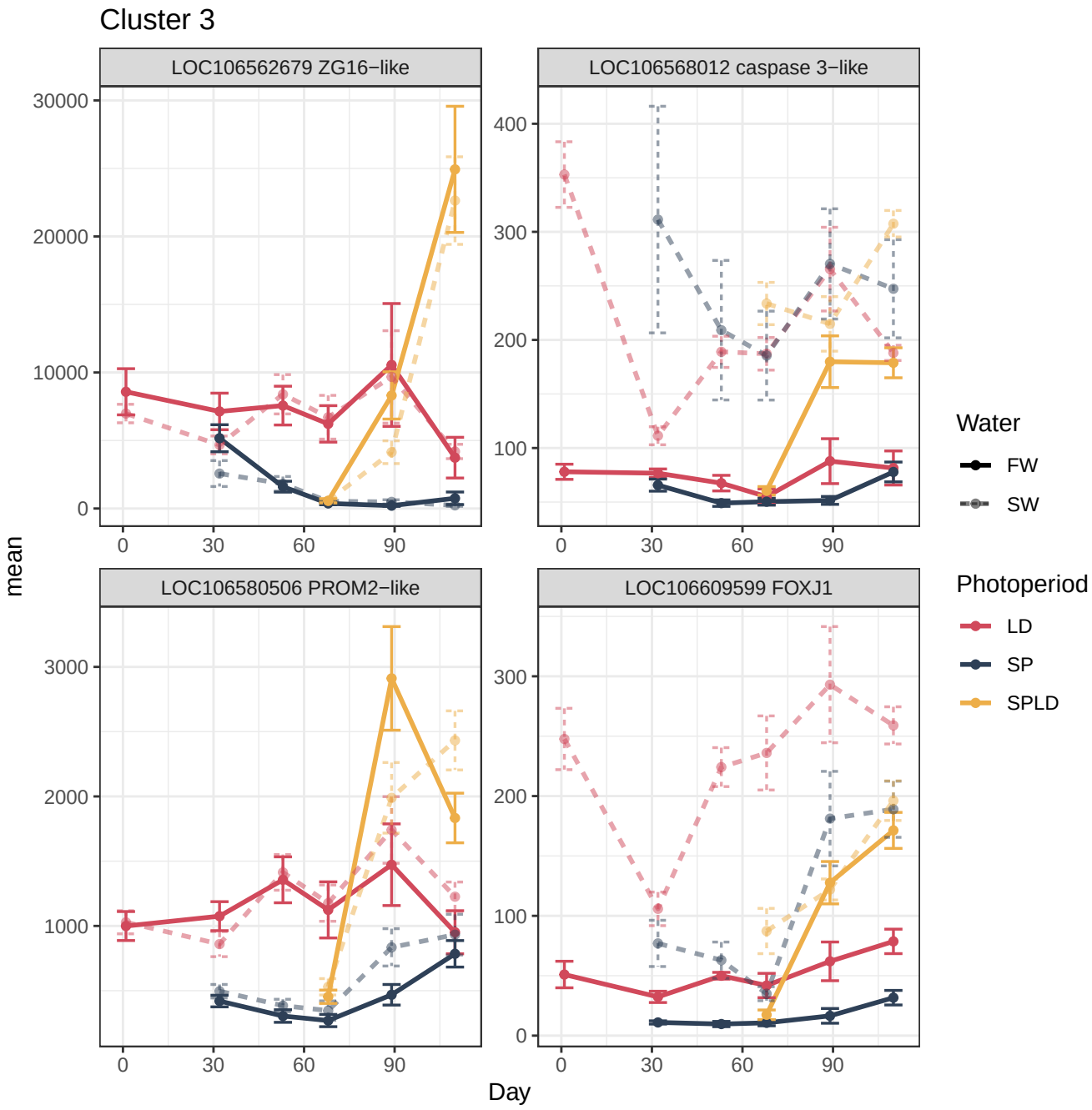
Appendix 1:
Plots showing additional genes from the clustering of genes of interest perfromed as part of Paper I, and based upon their FW expression. Genes where chosen to exemplify the pattern of expression represented by each cluster, and also to show that the SW response of genes within the same clusters could vary.

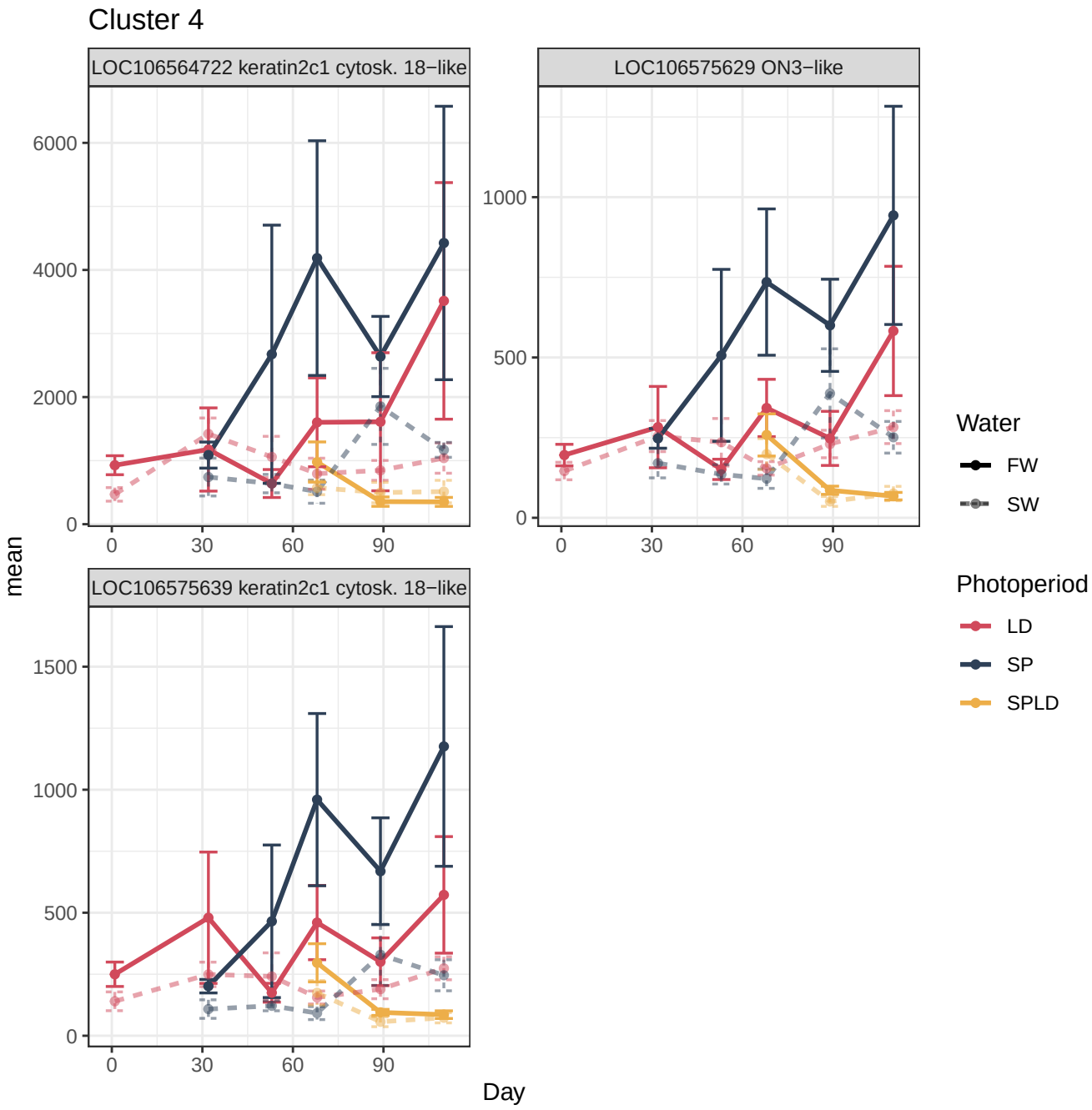


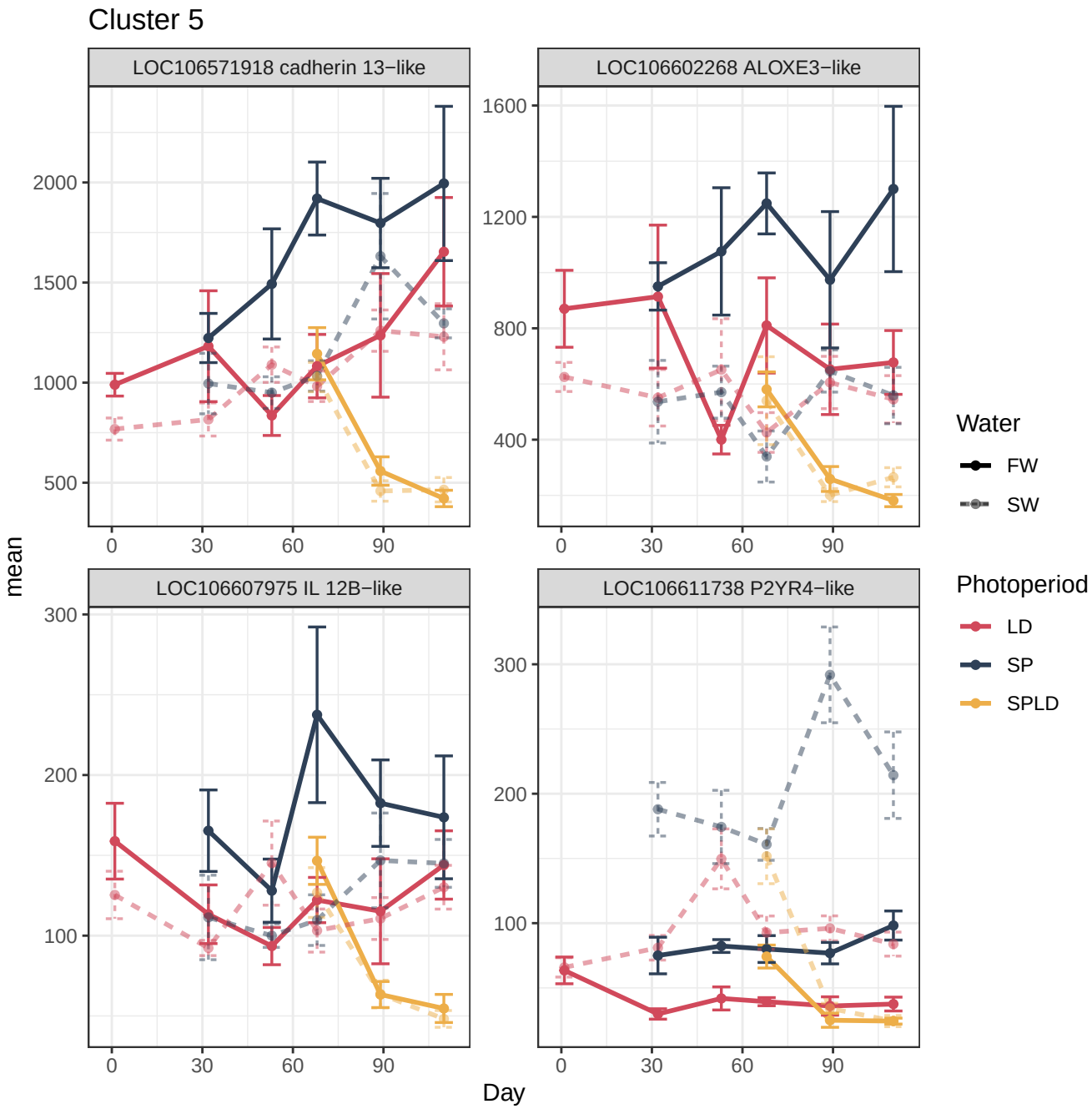

Cluster 1
LOC106565633 KLF15
LOC106583637 KLF15
LOC106601729 Tob1−like
LOC106603872 stc2b
mean
Day
Water
FW
SW
Photoperiod
LD
SP
SPLD

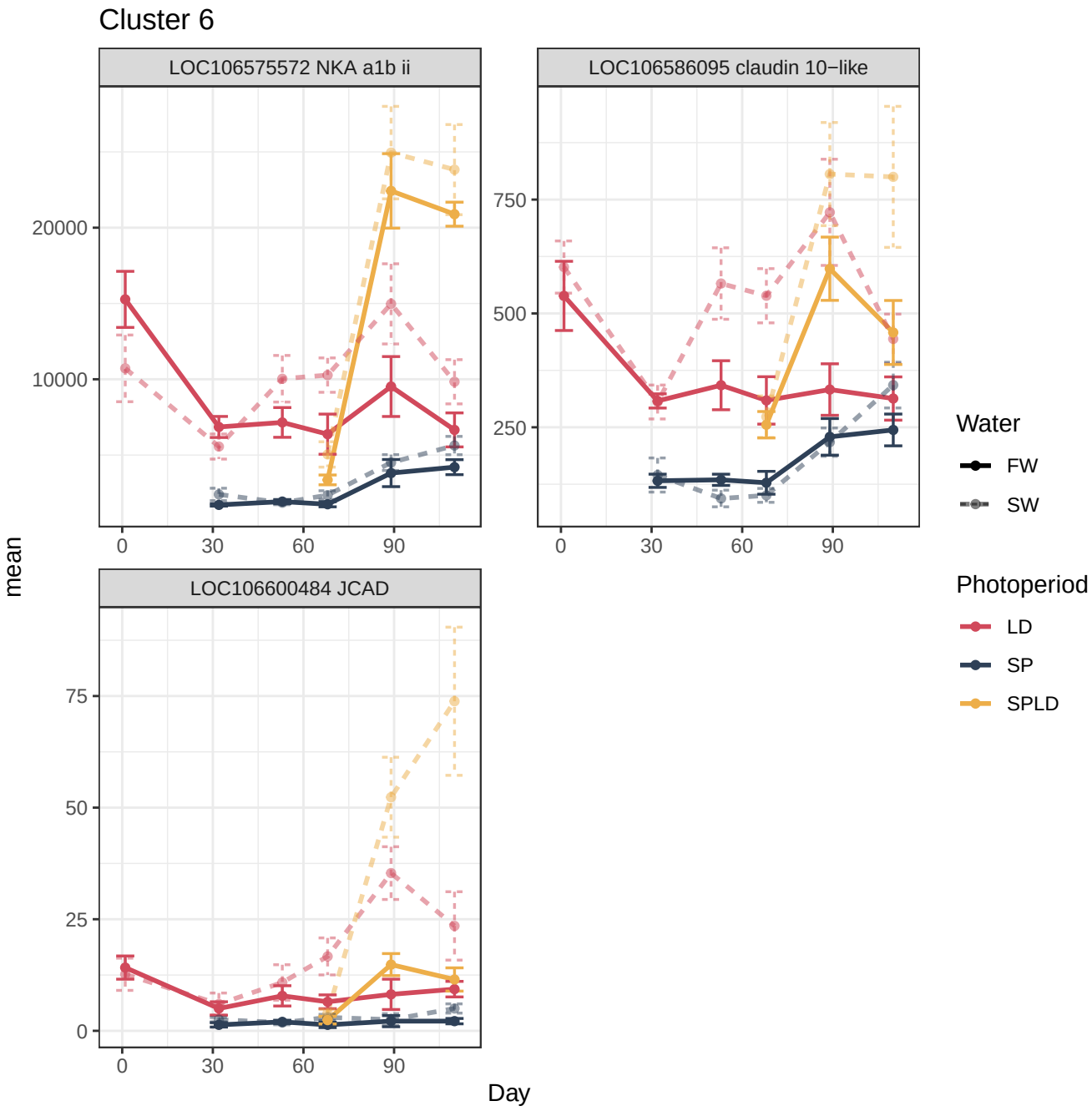

Cluster 2
LOC106579439 mrap2a
LOC106592485 mc2r
LOC106608778 IRS 1B−like
LOC106611586 elovl6
mean
Day
Water
FW
SW
Photoperiod
LD
SP
SPLD


Cluster 2
LOC100136908 nkcc1
LOC100196052 fkbp5
LOC106611381 CAPN1
mean
Day
Water
FW
SW
Photoperiod
LD
SP
SPLD


Cluster 3
LOC106562679 ZG16−like
LOC106568012 caspase 3−like
LOC106580506 PROM2−like
LOC106609599 FOXJ1
mean
Day
Water
FW
SW
Photoperiod
LD
SP
SPLD


Cluster 4
LOC106564722 keratin2c1 cytosk. 18−like
LOC106575629 ON3−like
LOC106575639 keratin2c1 cytosk. 18−like
mean
Day
Water
FW
SW
Photoperiod
LD
SP
SPLD


Cluster 5
LOC106571918 cadherin 13−like
LOC106602268 ALOXE3−like
LOC106607975 IL 12B−like
LOC106611738 P2YR4−like
mean
Day
Water
FW
SW
Photoperiod
LD
SP
SPLD


Cluster 6
LOC106575572 NKA a1b ii
LOC106586095 claudin 10−like
LOC106600484 JCAD
mean
Day
20000
10000
750
500
250
75
50
25
0
0
30
60
90
Water
FW
SW
Photoperiod
LD
SP
SPLD

Appendix 2:

Complete list of genes and clusters from Paper I.

gene_id	T1_LL	T2_SP	T3_SP	T4_SPLL	T5_SPLL	T6_SPLL	cluster	protein_id	gene	product	gene_biotype	chrom	start	end	strand	HGNC.ID
gene10327:106603774	-0,374856782	0,203056735	1,025899706	0,508929727	-0,62160311	-1,066515629	1	XP_014053320.1	klhl35	kelch-like family member 35	protein_coding	ssa04	65905419	65913819	-	
gene10426:106603872	-0,027810379	0,849534675	0,877923518	0,376225936	-1,68726025	-1,612259871	1	XP_014053551.1	LOC106603872	stanniocalcin-2-like	protein_coding	ssa04	73005881	73011182	+	STC2
gene11695:106605056	-0,243156616	0,733206778	1,056616387	0,030137347	-1,321306649	-1,556157365	1	XP_014055788.1	LOC106605056	thyrotropin-releasing hormone receptor-like	protein_coding	ssa05	47735277	47741530	-	TRHR
gene11909:106605273	-0,317498174	-0,021478335	0,88165404	0,863683534	-0,862071467	-0,946061282	1	XP_014056205.1	LOC106605273	cysteine-rich venom protein ophanin-like	protein_coding	ssa05	55463672	55470763	-	CRISP2
gene12041:106605466	-0,552441013	1,785016354	0,921189647	0,276976724	-1,272944255	-1,349786812	1	XP_014056633.1	LOC106605466	annexin A2-like	protein_coding	ssa05	58827891	58834999	-	
gene1470:106609366	-0,494026301	0,985289363	0,861225445	0,689868317	-1,351226796	-1,209137393	1	XP_014063618.1	LOC106609366	BTB/POZ domain-containing protein KCTD5-like	protein_coding	ssa01	74348053	74362429	-	KCTD17
gene14837:106608024	-0,925238238	1,244456193	1,185528074	0,221048996	-1,484911705	-1,177397785	1	XP_014061133.1	tdrp	testis development related protein	protein_coding	ssa06	67571579	67594698	-	TDRP
gene14885:106608077	-0,878621118	0,377987224	0,636889472	0,44723519	-0,818152156	-0,878711175	1	XP_014061244.1	LOC106608077	galectin-3-like	protein_coding	ssa06	70326770	70339256	+	
gene1507:106609735	-0,225779427	1,250328872	1,542798108	-0,073001797	-1,155413921	-1,330209477	1	XP_014064187.1	LOC106609735	membrane-associated progesterone receptor component 1-like	protein_coding	ssa01	75473957	75477774	-	PGRMC1
gene15104:106608311	-0,719087881	1,247215658	1,77250377	-0,411244298	-0,998877809	-0,878324106	1	XP_014061665.1	LOC106608311	SLC2A4 regulator-like	protein_coding	ssa06	82160560	82178136	+	
gene15351:106608493	-0,172248322	1,117360643	1,178125828	0,583383582	-1,577813345	-1,010024041	1	XP_014061905.1	LOC106608493	mucin-2-like	protein_coding	ssa07	3514465	3529601	+	
gene16344:106609418	-0,17578193	1,038497268	1,607738944	0,601469423	-1,13908674	-1,254869807	1	XP_014063694.1	LOC106609418	putative ferric-chelate reductase 1	protein_coding	ssa07	43369905	43379838	+	
gene17191:100380370	-0,558948004	0,50201838	1,061114797	0,360476941	-1,234836861	-1,204127652	1	XP_014064984.1	abcg2	ATP-binding cassette%2C sub-family G (WHITE)%2C member 2 (Junior blood group)	protein_coding	ssa08	16987237	17004315	-	ABCG2
gene17193:106610264	-0,952666104	0,236433227	1,053612771	0,148673407	-0,121781623	-0,793812501	1					ssa08	17022254	17031281	+	
gene17434:106610479	-1,283495194	0,888567589	0,927627466	0,894747192	-1,289290183	-1,047474156	1	XP_014065332.1	LOC106610479	sodium/potassium-transporting ATPase subunit alpha-1-like	protein_coding	ssa08	23735589	23748919	+	ATP1A1
gene19552:106612710	-0,958735973	0,846826362	1,56034196	-0,161622485	-0,974462741	-0,85671655	1	XP_014069618.1	LOC106612710	estrogen-related receptor gamma-like	protein_coding	ssa09	101702589	101709285	+	
gene21328:106560518	-1,175387412	0,973308299	0,94889631	0,489929622	-1,368612643	-1,189086363	1	XP_013978965.1	LOC106560518	leucine-rich repeat-containing protein 19-like	protein_coding	ssa10	44255706	44272393	-	
gene21663:106561069	-0,413007797	0,954918053	1,709425491	-0,380601202	-1,490130838	-1,4222112	1	XP_013980163.1	LOC106561069	protein FAM60A-like	protein_coding	ssa10	67321197	67328572	+	FAM60A
gene21905:106560848	-1,04735932	0,74714995	0,939317305	0,775375615	-0,93639137	-1,047503583	1	XP_013979696.1	LOC106560848	solute carrier family 12 member 3-like	protein_coding	ssa10	79338130	79346381	-	SLC12A3
gene22318:106561585	-1,222612919	1,112972565	1,41494309	0,507923355	-0,532319193	-1,133273663	1	XP_013981164.1	ovch2	ovochymase 2	protein_coding	ssa10	99869360	99889328	+	OVCH2
gene22775:106561930	-0,990829184	0,710640208	1,110774603	0,562660931	-0,64489565	-0,979703771	1					ssa11	8566726	8568993	+	
gene22882:106562069	-0,432554751	0,294519656	0,795696916	0,287502065	-0,861847893	-1,205370582	1	XP_013982095.1	LOC106562069	G-protein coupled receptor 64-like	protein_coding	ssa11	14495296	14500545	-	
gene23835:106562980	-0,757223334	0,563977479	0,662438033	1,379987544	-1,408675122	-1,704891734	1	XP_013983621.1	LOC106562980	G protein-coupled receptor kinase 5-like	protein_coding	ssa11	54919828	54953358	+	GRK5
gene23837:106562982	-0,624306859	0,118522731	0,898091806	0,662510424	-0,750749542	-0,66313985	1	XP_013983625.1	LOC106562982	NK1 transcription factor-related protein 2-like	protein_coding	ssa11	54975003	54976660	+	
gene26117:106565132	-0,691257165	0,618378295	1,684575002	0,844681746	-0,966009088	-1,242281442	1	XP_013987376.1	LOC106565132	solute carrier family 25 member 34-like	protein_coding	ssa12	45722406	45739005	-	
gene26543:106565633	-0,977115877	1,48259378	1,625628759	-0,539388683	-1,157242768	-0,999538837	1	XP_013988425.1	LOC106565633	Krueppel-like factor 15	protein_coding	ssa12	60671902	60681995	+	
gene281:106566099	-0,532394197	1,004388532	1,31231426	0,203248803	-0,90192031	-0,779297289	1	XP_013989429.1	LOC106566099	radical S-adenosyl methionine domain-containing protein 2-like	protein_coding	ssa01	13388973	13395473	+	RSAD2
gene28441:106567289	-1,215911061	0,254594473	0,150053248	-0,782582498	-0,90949839	-0,920404482	1	XP_013991906.1	LOC106567289	suppressor of cytokine signaling 2-like	protein_coding	ssa13	43407984	43411559	-	
gene2908:106570018	-0,770388783	0,763083306	0,648476312	1,300776922	-0,942551457	-1,637335517	1	XP_013997380.1	LOC106570018	phosphatidylinositol 4-phosphate 5-kinase-like protein 1	protein_coding	ssa01	145129221	145139255	-	PIP5KL1
gene29270:106568196	-0,509906023	1,510101758	1,823121146	0,026584124	-1,049621642	-1,158574112	1	XP_013993798.1	LOC106568196	proline dehydrogenase 1%2C mitochondrial-like	protein_coding	ssa13	87185543	87202775	+	
gene29561:106568486	-0,596111149	1,172325942	1,4030725	0,937024262	-1,266318426	-1,368871562	1	XP_013994346.1	agxt2	alanine--glyoxylate aminotransferase 2	protein_coding	ssa13	99065074	99084841	-	AGXT2
gene30114:106568851	0,142775549	0,386192758	0,745803689	0,431099266	-1,030602995	-1,213769779	1	XP_013995054.1	LOC106568851	C-type lectin domain family 10 member A-like	protein_coding	ssa14	17418878	17419828	-	CLEC4E
gene30762:106569650	-0,927764571	0,445060893	0,084904147	-0,532241122	-0,548888938	-0,666687689	1	XP_013996683.1	LOC106569650	uncharacterized LOC106569650	protein_coding	ssa14	42533497	42542445	+	
gene32891:106571565	-0,550404876	0,239389879	0,470829032	1,709880613	-0,485745543	-1,237004624	1	XP_014000232.1	LOC106571565	uncharacterized LOC106571565	protein_coding	ssa15	48287134	48298260	+	
gene3427:106574776	-0,390645637	0,609159416	0,577263731	0,286539552	-1,13179539	-0,345743395	1	XP_014006319.1	LOC106574776	protein asteroid homolog 1-like	protein_coding	ssa02	6241896	6243222	+	
gene35055:106573916	-0,062108332	1,265060876	1,039657553	0,180218788	-1,319008625	-0,656040399	1	XP_014004849.1	LOC106573916	interferon-induced protein 44-like	protein_coding	ssa16	43549790	43558483	+	
gene35273:106574173	-0,780587407	0,521428612	0,991416045	0,218168608	-0,700921363	-0,385586593	1	XP_014005317.1	LOC106574173	receptor-transporting protein 2-like	protein_coding	ssa16	53796489	53799311	-	RTP2
gene35652:106574488	-0,06371355	0,843212704	0,935986626	0,207379599	-1,087289164	-1,463383418	1	XP_014005871.1	LOC106574488	UPF0392 protein F13G3.3-like	protein_coding	ssa16	72493921	72500383	-	
gene37371:106576268	-0,266210889	0,914313735	0,699802734	0,038607471	-0,934247944	-1,01237226	1	XP_014008825.1	LOC106576268	probable polypeptide N-acetylgalactosaminyltransferase 8	protein_coding	ssa17	44458710	44470651	+	
gene3790:106578268	-0,270898042	0,34762206	1,207233308	0,130343361	-0,373348934	-0,940125557	1					ssa02	14015106	14018389	+	
gene38081:106577085	0,25879509	0,593682449	0,35592008	0,977221595	-0,908786464	-1,407998593	1	XP_014010282.1	mb21d1	Mab-21 domain containing 1	protein_coding	ssa18	15517395	15518264	-	MB21D1
gene38179:106577055	-0,863417701	0,860030089	0,488615588	0,589120171	-1,210914111	-1,257261431	1	XP_014010233.1	LOC106577055	SPARC-like	protein_coding	ssa18	21393600	21428213	+	
gene39044:106577929	-0,461541844	0,873398868	0,858719463	0,13039392	-0,937855729	-0,962455091	1	XP_014011872.1	LOC106577929	C-type mannose receptor 2-like	protein_coding	ssa18	61640062	61651933	-	
gene39536:106578443	-0,297814349	0,89252136	0,643420092	1,07047756	-1,776579017	-1,818280599	1	XP_014012715.1	LOC106578443	chondrolectin-like	protein_coding	ssa19	11000501	11016660	+	CHODL
gene40188:106579318	-0,843466933	0,18265855	0,525294723	0,679701733	-1,035094404	-1,352723972	1	XP_014014607.1	osbpl7	oxysterol binding protein-like 7	protein_coding	ssa19	50775107	50786008	-	
gene41027:106580271	-1,106159733	1,474078793	1,008963168	-0,029131042	-1,10825283	-1,500367801	1	XP_014016567.1	LOC106580271	interferon-inducible GTPase 5-like	protein_coding	ssa20	9169893	9184249	+	IRGC
gene41882:106580735	-1,303690175	0,70713882	1,29920198	-0,528861771	-0,919085705	-0,636963941	1	XP_014017558.1	LOC106580735	estrogen-related receptor gamma-like	protein_coding	ssa20	45674716	45687399	+	
gene43002:106581759	-1,28201025	1,175619036	1,423529026	0,69702016	-1,212590094	-1,148569982	1	XP_014019506.1	LOC106581759	rap guanine nucleotide exchange factor 4-like	protein_coding	ssa21	16712590	16775440	+	RAPGEF4
gene43038:106581900	-1,073602334	0,688154677	0,460984684	0,569199024	-1,207642148	-1,146053053	1	XP_014019816.1	LOC106581900	high affinity cGMP-specific 3'%2C5'-cyclic phosphodiesterase 9A-like	protein_coding	ssa21	18967938	19002549	+	PDE9A
gene44811:106583637	-0,525096947	1,234623999	1,202315851	-0,130648221	-1,471852625	-1,373508461	1	XP_014023520.1	LOC106583637	Krueppel-like factor 15	protein_coding	ssa22	47901781	47916022	-	KLF15
gene45006:106583765	-0,233801778	0,009538842	0,524861388	0,374453568	-0,667581307	-0,727586598	1	XP_014023799.1	LOC106583765	RING finger protein 208-like	protein_coding	ssa22	57671336	57676044	-	RNF186
gene45172:106583895	-0,169312828	-0,242961778	0,415757738	0,097447294	-0,603920267	-1,172940258	1					ssa23	2438165	2439810	-	
gene45418:106584143	-0,923075213	1,590090327	1,462575162	0,143170584	-1,041690885	-1,281712002	1	XP_014024535.1	LOC106584143	regulator of G-protein signaling 4-like	protein_coding	ssa23	19162773	19166021	-	RGS4
gene46127:106584858	0,065801405	0,993504945	-0,024520261	0,625572265	-1,527538157	-1,074631981	1					ssa23	49138644	49143411	-	
gene46521:106585256	-1,18674844	0,804049391	0,574742497	-0,551495842	-0,489170726	-1,169169334	1	XP_014026740.1	LOC106585256	trichohyalin-like	protein_coding	ssa24	16800734	16816849	+	
gene46585:100195784	-0,356591769	-0,177627069	0,224069144	0,411150583	-0,864420999	-1,087735396	1	NP_001134285.1	lincr	E3 ubiquitin-protein ligase LINCR	protein_coding	ssa24	19237455	19240735	+	NEURL3
gene46607:106585344	-0,30232034	0,140479634	1,239484023	-0,604780415	-0,561263401	-0,626554384	1	XP_014026910.1	LOC106585344	bridging integrator 3-like	protein_coding	ssa24	19983797	20015463	-	
gene48420:106587297	-0,68536536	0,501330699	0,16468765	-0,100531715	-1,205358248	-1,085324232	1	XP_014031027.1	LOC106587297	myosin light chain kinase 3-like	protein_coding	ssa26	13390797	13424287	-	
gene50823:106589219	0,009541948	0,146511365	0,808520366	0,473583423	-1,066436127	-1,226175061	1	XP_014034413.1	LOC106589219	toll-like receptor 13	protein_coding	ssa28	3524713	3527969	+	
gene51019:106589495	-0,419978032	-0,229720108	1,11872235	-0,012873824	-0,662275412	-1,456170861	1	XP_014035029.1	glp2r	glucagon-like peptide 2 receptor	protein_coding	ssa28	12315730	12334589	-	GLP2R
gene51351:106589791	-0,112712211	0,642704992	1,073155975	0,035221775	-0,981000661	-1,027552883	1	XP_014035607.1	hcrtr2	hypocretin (orexin) receptor 2	protein_coding	ssa28	27012367	27028212	-	HCRTR2
gene5319:106592846	-0,414699764	-0,229962077	1,378789599	0,684202752	-0,685061982	-1,17215342	1	XP_014039674.1	LOC106592846	NACHT%2C LRR and PYD domains-containing protein 1-like	protein_coding	ssa02	64429572	64434501	-	
gene5320:106592855	-1,119026251	0,590547682	0,811215133	0,313408069	-0,917726402	-1,144279223	1	XP_014039681.1	LOC106592855	NACHT%2C LRR and PYD domains-containing protein 1-like	protein_coding	ssa02	64466277	64472024	-	
gene56622:106596139	-0,407328763	1,387369913	0,55598071	0,823523576	-0,833338887	-0,868942235	1	XP_014042932.1	LOC106596139	sodium/potassium-transporting ATPase subunit alpha-1-like	protein_coding	NW_012358557.1	649	7296	+	
gene57813:106596208	-0,683535247	0,803231023	1,676439022	1,301875662	-0,874197937	-1,179968439	1	XP_014042995.1	LOC106596208	sodium/potassium-transporting ATPase subunit alpha-1-like	protein_coding	NW_012358904.1	4	7589	-	
gene6421:106599351	-1,294060491	1,474840819	1,089812462	0,580776907	-1,043493991	-1,286183471	1	XP_014046020.1	LOC106599351	E3 ubiquitin-protein ligase RNF152-like	protein_coding	ssa03	23789255	23836249	+	
gene6953:106600631	-0,82990703	0,643810508	1,574280322	0,925388222	-0,648587356	-1,003610237	1					ssa03	47255747	47257877	+	
gene7028:106600678	-0,308650039	0,22957629	0,802814952	0,565684116	-1,00594372	-0,798882818	1	XP_014047681.1	LOC106600678	noggin-2-like	protein_coding	ssa03	49438100	49440139	+	
gene71096:106592036	-0,680875861	0,860941788	0,326666585	-0,39803318	-0,976050211	-0,474538588	1	XP_014038808.1	LOC106592036	transient receptor potential cation channel subfamily M member 5-like	protein_coding	NW_012347555.1	19286	25068	-	
gene738:106601420	-0,687764401	0,91803427	1,131187618	1,101479182	-1,155519522	-1,067848172	1	XP_014049106.1	LOC106601420	prostasin-like	protein_coding	ssa01	31201130	31224360	+	PRSS27
gene75346:106591119	-0,48826984	0,874267799	0,670475795	1,169117423	-0,855545157	-1,425825697	1	XP_014037783.1	LOC106591119	myosin heavy chain%2C fast skeletal muscle-like	protein_coding	NW_012343454.1	11110	53054	+	MYH8
gene8093:106601729	-0,727391749	0,713185376	1,569830103	-0,020515749	-0,987718466	-1,401794148	1	XP_014049550.1	LOC106601729	protein Tob1-like	protein_coding	ssa03	79403525	79407403	-	TOB1
gene8520:106602157	-1,303652716	1,064537259	0,887259449	0,614125525	-1,2273379	-1,079037287	1	XP_014050103.1	LOC106602157	sodium/potassium-transporting ATPase subunit alpha-1-like	protein_coding	ssa04	1704215	1717303	+	ATP1A1
gene9168:106602753	-0,358598275	0,21384002	-0,119354786	0,962404009	-0,732008912	-0,687461911	1	XP_014051067.1	LOC106602753	mitochondrial sodium/hydrogen exchanger 9B2-like	protein_coding	ssa04	22369134	22387824	-	

gene9922:106603380	-0,580957418	-0,161441734	0,547787922	0,246420339	-0,4399105	-0,595100959	1	XP_014052454.1	LOC106603380	glutamate receptor 3	protein_coding	ssa04	45769721	45879285	-			
gene10504:106603951	-0,921879886	-0,316675707	-0,349859761	-0,435076434	1,326829474	0,440897405	2	XP_014053680.1	LOC106603951	peripheral-type benzodiazepine receptor-associated protein 1-like	protein_coding	ssa04	76469390	76645159	+	BZRAP1		
gene12771:106606117	0,53967702	-0,516573544	-0,577622491	-0,605685834	2,766526124	0,980269035	2	XP_014057715.1	LOC106606117	GMP reductase 1-like	protein_coding	ssa05	77941923	77949786	-			
gene12989:106606338	-0,627894336	-0,606676922	-0,271007647	-0,389444613	2,29448826	0,805074308	2	XP_014057967.1	fbxl16	F-box and leucine-rich repeat protein 16	protein_coding	ssa06	4967935	5013628	+	FBXL16		
gene13030:106606381	-0,574587313	-0,390496489	-0,207525701	-0,373770126	1,437561277	0,146168541	2	XP_014058011.1	LOC106606381	BAH and coiled-coil domain-containing protein 1-like	protein_coding	ssa06	6681806	6862840	+	BAHCC1		
gene13122:106606469	0,472252016	-0,813416663	-0,777764534	-0,574107207	2,432498521	1,19067601	2	XP_014058158.1	LOC106606469	arf-GAP with dual PH domain-containing protein 1-like	protein_coding	ssa06	10862335	10948849	+			
gene13405:106606801	-0,225121257	-0,03723332	-0,20336399	0,732479621	1,885735333	0,287307482	2	XP_014058630.1	LOC106606801	CMRF35-like molecule 8	protein_coding	ssa06	20201744	20207019	+			
gene15557:106608610	0,03937174	-0,48451515	-0,945527704	0,041253948	1,641058297	0,913286413	2	XP_014062115.1	LOC106608610	uncharacterized LOC106608610	protein_coding	ssa07	12955254	12961001	-	PROB1		
gene15601:106608778	-0,065944659	-0,748710104	-0,663590171	-0,231472613	1,976059779	0,350463291	2	XP_014062380.1	LOC106608778	insulin receptor substrate 1-B-like	protein_coding	ssa07	17212164	17297173	+			
gene16799:106609889	0,160630348	-0,627281137	-0,8061647	0,256895265	1,330498407	0,682493447	2	XP_014064398.1	LOC106609889	sialic acid synthase-like	protein_coding	ssa08	566749	575090	+	NANS		
gene18337:106611381	-0,399100343	-0,315687841	-0,648028213	-0,036209018	1,842508257	1,613171938	2	XP_014067018.1	LOC106611381	calpain-1 catalytic subunit-like	protein_coding	ssa09	43817341	43846449	+			
gene18572:106611586	-0,218247202	-0,753739654	-0,935734379	-0,211011367	2,273602681	0,564024771	2	XP_014067429.1	elovl6	ELOVL fatty acid elongase 6	protein_coding	ssa09	55593209	55614866	-	ELOVL6		
gene19262:106612236	-0,321213022	-0,431222296	-0,393876308	-0,214943365	2,647686139	1,706863432	2	XP_014068696.1	LOC106612236	homeobox protein EMX1-like	protein_coding	ssa09	84479997	84496246	+			
gene19346:106612328	-0,814269214	-0,430969738	-0,686404894	-0,446196922	1,382024895	0,660263266	2	XP_014068854.1	LOC106612328	golgin subfamily A member 6-like protein 22	protein_coding	ssa09	90637029	90638403	-			
gene2018:106560913	0,558627256	-0,152124284	-0,467114297	-0,443428363	1,203607844	-0,510648412	2	XP_013979825.1	LOC106560913	ABC transporter G family member 23-like	protein_coding	ssa01	107906985	107925558	+			
gene20424:106613409	-0,524102647	-0,243210125	0,012718347	-0,152486799	0,996938541	-0,091474448	2	XP_014071070.1	LOC106613409	E3 ubiquitin-protein ligase HERC2-like	protein_coding	ssa10	111651	124620	-			
gene20428:106613415	-0,564310421	-0,537530062	-0,12886642	-0,316410847	1,854312424	-0,040532144	2	XP_014071077.1	LOC106613415	E3 ubiquitin-protein ligase HERC2-like	protein_coding	ssa10	348253	369763	-	HERC2		
gene20764:106613738	-0,196868602	-0,504138556	-0,195697052	0,048225208	1,366645568	0,287129424	2	XP_014071776.1	cilp2	cartilage intermediate layer protein 2	protein_coding	ssa10	18690830	18717724	+	CILP2		
gene21031:106613976	-0,921875787	-0,624248854	-0,283956844	-0,453873121	1,367341651	0,374761055	2	XP_014072341.1	LOC106613976	dynamin-2-like	protein_coding	ssa10	28847266	28875217	+	DNM3		
gene2122:106561975	0,86298208	-0,859229084	-0,678033937	-0,261748065	1,689718175	0,088756455	2	XP_013981951.1	LOC106561975	cathepsin L1-like	protein_coding	ssa01	113367197	113372320	+	CTSL		
gene23184:106562339	-0,440765764	-0,437275019	-0,589199697	-0,445725803	1,727689191	0,406103926	2	XP_013982632.1	LOC106562339	sodium-dependent noradrenaline transporter-like	protein_coding	ssa11	25136173	25160418	+	SLC6A2		
gene23361:106562509	-0,89108241	-0,742747227	-0,264435084	-0,124865125	2,047086751	0,153792396	2	XP_013982900.1	LOC106562509	protein FAM214A-like	protein_coding	ssa11	33151939	33176670	-	FAM214A		
gene23591:106562745	0,23090676	-0,293578355	-0,44485328	-0,417743492	1,415678029	1,213727745	2	XP_013983225.1	LOC106562745	guanylyl cyclase-activating protein 2-like	protein_coding	ssa11	41601298	41606368	-	GUCA1B		
gene23644:106562800	-0,545825169	-0,500166952	-0,442916173	-0,485761448	1,525099484	0,696439528	2	XP_013983292.1	LOC106562800	eukaryotic translation initiation factor 5B-like	protein_coding	ssa11	43637470	43654843	+	PLEKHG4B		
gene24245:106563397	-0,106499274	-0,559786013	-0,713666066	-0,012949613	1,520054433	1,394583328	2	XP_013984388.1	LOC106563397	gamma-aminobutyric acid receptor subunit beta-4-like	protein_coding	ssa11	73870569	73913048	-	GABRB3		
gene24739:106563883	-0,525190109	-0,666867045	-0,293872926	-0,221797806	1,596882783	0,335981676	2	XP_013985287.1	LOC106563883	synaptojanin-1-like	protein_coding	ssa11	89225632	89233466	-	SYNJ1		
gene24774:106563914	-0,646380402	-0,449087858	-0,136326175	-0,291958243	1,838795127	0,412179264	2	XP_013985336.1	tmem135	transmembrane protein 135	protein_coding	ssa11	91296349	91330703	+	TMEM135		
gene25135:106564279	-0,692137495	-0,473115503	-0,178875127	-0,423177434	1,499203522	0,187982526	2	XP_013985798.1	LOC106564279	polycystin-1-like	protein_coding	ssa12	7798699	7815596	-	PKD1		
gene25214:106564364	-0,582906716	-0,646455545	-0,487781149	-0,392318363	1,83358861	0,595527344	2	XP_013985897.1	LOC106564364	CREB3 regulatory factor-like	protein_coding	ssa12	9309254	9320475	+			
gene25364:106564514	-0,266053899	-0,731963199	-0,614356255	-0,427906716	2,197395829	0,438606124	2	XP_013986113.1	LOC106564514	nuclear factor 1 X-type-like	protein_coding	ssa12	14333368	14335655	+	NFIX		
gene25538:106564658	-0,415397863	-0,558825392	-0,585215024	-0,602605917	2,384438743	1,246794053	2	XP_013986321.1	LOC106564658	rhomboid-related protein 3-like	protein_coding	ssa12	20878203	20940381	-			
gene2630:106567252	-0,539906111	-0,951626924	-1,095843262	-0,536673004	1,393570973	1,119032412	2	XP_013991798.1	LOC106567252	collagen alpha-1(I) chain-like	protein_coding	ssa01	132595451	132705666	-			
gene27411:106566360	-0,639625233	-0,518399207	-0,433513035	-0,50518761	1,384659254	0,344430513	2	XP_013989770.1	LOC106566360	arginine-glutamic acid dipeptide repeats protein-like	protein_coding	ssa13	4390628	4589774	-	RERE		
gene27412:106566362	0,426908068	-0,677922205	-0,728692391	-0,514447467	2,217641933	0,799828888	2	XP_013989772.1	LOC106566362	probable G-protein coupled receptor 61	protein_coding	ssa13	4696542	4705330	-	GPR61		
gene28245:106567325	1,004060866	-0,590291653	-0,677812038	-0,156375551	2,347780024	0,987590523	2	XP_013991934.1	LOC106567325	N-acetyltransferase 8-like	protein_coding	ssa13	37312808	37314625	+			
gene28380:106567236	0,051208547	-0,861916291	-0,631354314	-0,18374521	1,981535016	0,681049968	2	XP_013991753.1	LOC106567236	membrane-associated guanylate kinase%2C WW and PDZ domain-containing protein 3-like	protein_coding	ssa13	41158915	41207021	+			
gene28770:106567727	-0,378471068	-0,457744245	-0,638163697	-0,307343759	2,631002168	0,953689977	2	XP_013992835.1	LOC106567727	zinc finger and BTB domain-containing protein 16-A-like	protein_coding	ssa13	67427085	67550893	+	ZBTB16		
gene29:106561325	-0,63435702	-0,325897358	0,193584671	-0,681678227	1,378886177	1,008819093	2	XP_013981033.1	LOC106561325	M-phase inducer phosphatase 1-like	protein_coding	ssa01	1207728	1235951	+	CDC25B		
gene29598:106568520	-0,741970769	-0,557022173	-0,404514901	-0,442010522	1,744148323	0,23279626	2	XP_013994416.1	LOC106568520	protein Shroom3-like	protein_coding	ssa13	101126415	101220506	+			
gene2979:100136908	0,512832925	-1,001167422	-0,754230028	-0,48025427	1,956106867	0,985949094	2	NP_001117155.1	nkcc1a	Na/K/2Cl co-transporter	protein_coding	ssa01	147535344	147613923	-	SLC12A2		
gene31363:106570233	-0,267508123	-0,683576119	-0,445056713	-0,471211432	1,209639895	0,069518637	2	XP_013997807.1	LOC106570233	collagen alpha-1(XXII) chain-like	protein_coding	ssa14	65546821	65589387	-	COL22A1		
gene31433:106570295	0,034259064	0,009435117	-0,673981613	-0,223829164	1,765716842	1,501069897	2	XP_013997932.1	LOC106570295	protachykinin-like	protein_coding	ssa14	69043512	69048994	-	TAC1		
gene31600:100380667	0,202181301	-0,764349925	-0,967806251	0,203865901	2,146226462	0,808662765	2	XP_013998311.1	LOC100380667	cytoplasmic dynein 1 intermediate chain 1	protein_coding	ssa14	78107725	78217683	+			
gene31753:106570612	-0,625734328	-0,744522414	-0,63524548	-0,636639254	1,34942624	0,807067416	2	XP_013998549.1	LOC106570612	peptidyl-prolyl cis-trans isomerase FKBP9-like	protein_coding	ssa14	84623488	84641010	-	FKBP9		
gene31761:106570628	-0,65947628	-0,592108411	-0,069262978	-0,417807288	1,466584891	0,294622699	2	XP_013998568.1	LOC106570628	upstream-binding protein 1-like	protein_coding	ssa14	85105672	85114337	-			
gene32024:106570880	-0,600868482	-0,402520041	-0,465094788	-0,414094291	1,894980786	0,170876091	2	XP_013998922.1	LOC106570880	extracellular matrix protein FRAS1-like	protein_coding	ssa15	3638049	3876112	+	FRAS1		
gene325:106570601	-0,46058933	-0,516827068	-0,158561811	-0,317110292	2,163350495	-0,025564604	2	XP_013998571.1	ush2a	Usher syndrome 2A (autosomal recessive%2C mild)	protein_coding	ssa01	16093166	16494780	+	USH2A		
gene32763:100136382	-0,195745164	-0,363214967	-0,583382464	-0,207340928	1,003505128	0,84237207	2	XP_014000490.1	LOC100136382	thyrotropin receptor	protein_coding	ssa15	42108401	42142631	+	TSHR		
gene32887:106571568	-0,233431899	-0,585853718	-0,743232362	-0,215730143	2,49330758	0,763654006	2	XP_014000242.1	LOC106571568	ena/VASP-like protein	protein_coding	ssa15	48050577	48114633	-	EVL		
gene33460:106572020	-0,101728609	-0,950078883	-0,947842312	0,158021662	1,963154327	1,544413915	2	XP_014001195.1	LOC106572020	ATPase family AAA domain-containing protein 3-like	protein_coding	ssa15	75700186	75714615	-	ATAD3A		
gene33802:106572649	-0,719632855	-0,683114589	-0,662444487	-0,385490118	1,553011191	0,630391018	2	XP_014002491.1	LOC106572649	low-density lipoprotein receptor-related protein 1-like	protein_coding	ssa15	88639402	88652717	-			
gene33815:106572662	-0,654058018	-0,639163262	-0,382719715	-0,20953111	2,020419246	0,559532977	2	XP_014002509.1	LOC106572662	low-density lipoprotein receptor-related protein 1-like	protein_coding	ssa15	89210642	89397085	+	LRP1		
gene33828:106572681	-0,598950071	-0,508929865	-0,387646979	-0,363266122	1,359389385	0,168879921	2	XP_014002541.1	LOC106572681	paternally-expressed gene 3 protein-like	protein_coding	ssa15	90001923	90014135	-			
gene3397:106574458	-0,203608764	-0,579193355	-0,620590279	-0,162792402	2,642671277	0,518421443	2	XP_014005832.1	LOC106574458	nuclear receptor subfamily 4 group A member 3-like	protein_coding	ssa02	5574660	5616636	-			
gene34112:106572966	-0,841025612	-0,763738614	-0,291945598	0,041074924	1,361300537	0,712650174	2	XP_014003040.1	LOC106572966	RNA-binding protein 10-like	protein_coding	ssa15	102398845	102426037	-	RBM10		
gene3448:106574976	-0,619313977	-0,250124166	0,320894462	-0,327985907	1,598294989	-0,002453559	2	XP_014006588.1	LOC106574976	bone morphogenetic protein receptor type-2-like	protein_coding	ssa02	6703731	6715198	-			
gene34677:106573537	-0,712338368	-0,689004191	-0,421418342	-0,513516821	1,601191916	0,023343244	2	XP_014004134.1	LOC106573537	fibronectin-like	protein_coding	ssa16	27876433	27877895	-			
gene35261:100846967	-0,030766188	-0,444759825	-0,412301662	-0,703378255	2,128329095	1,063796496	2	NP_001239291.1	at1b1	Sodium/potassium-transporting ATPase subunit beta-1	protein_coding	ssa16	53346648	53347224	+	ATP1B1		
gene35960:106574883	-0,311599088	-0,340212997	-0,595340748	-0,440148972	0,988521789	0,108583807	2	XP_014006489.1	LOC106574883	collagen alpha-1(II) chain-like	protein_coding	ssa16	80218770	80237896	-			
gene36296:106575175	0,48905417	-0,633337891	-0,663323317	0,797070569	1,446301534	0,752935268	2	XP_014006935.1	LOC106575175	MAGUK p55 subfamily member 4-like	protein_coding	ssa17	8515332	8526227	-	MPP4		
gene38359:106577235	-0,681329801	-0,557199658	-0,650593729	-0,539908243	1,529250142	1,121428855	2	XP_014010628.1	LOC106577235	secreted frizzled-related protein 5-like	protein_coding	ssa18	30956866	30991884	+	SFRP5		
gene38360:106577261	-0,497679004	-0,441501579	-0,381631954	-0,292169867	1,14803804	0,136489734	2	XP_014010671.1	LOC106577261	probable JmjC domain-containing histone demethylation protein 2C	protein_coding	ssa18	31011130	31020159	-			
gene3897:106579313	0,653181127	-0,946131297	-0,729362905	-0,050185514	2,60347273	0,441470882	2	XP_014014605.1	LOC106579313	amine sulfotransferase-like	protein_coding	ssa02	17093976	17113429	+			
gene3919:106579417	-0,455144221	-0,246730368	-0,289782527	-0,200185163	2,169624383	0,812311127	2	XP_014014786.1	LOC106579417	t-SNARE domain-containing protein 1-like	protein_coding	ssa02	18040995	18130802	-	TSNARE1		
gene3922:106579439	-0,385950015	0,535747392	-0,223485269	-0,223608332	1,572455471	0,962605942	2	XP_014014822.1	LOC106579439	melanocortin-2 receptor accessory protein 2A-like	protein_coding	ssa02	18524466	18536546	-	MRAP2		
gene39250:106578147	-0,736328362	-0,622995182	-0,446434694	-0,360569868	1,514243458	0,42237692	2	XP_014012245.1	LOC106578147	dysferlin-like	protein_coding	ssa18	67957249	67998346	+			
gene39303:106578202	-0,612796246	-0,388506244	-0,40180692	-0,205071188	1,857361585	0,109134183	2	XP_014012318.1	gab1	GRB2-associated binding protein 1	protein_coding	ssa18	69569940	69592441	+			
gene39338:106578236	-0,718414438	-0,579473374	-0,193791186	-0,197301004	1,750209734	0,120504173	2	XP_014012361.1	LOC106578236	ephrin type-B receptor 4-like	protein_coding	ssa18	70463965	70476473	+			
gene39866:106578775	-0,716758352	-0,580268656	-0,365184849	-0,4045084	1,309239444	0,332860983	2	XP_014013402.1	LOC106578775	AT-rich interactive domain-containing protein 4B-like	protein_coding	ssa19	35405208	35425431	-			
gene40050:106578942	-0,704339207	-0,516450357	-0,486680142	-0,537486176	1,358754255	0,716886774	2	XP_014013706.1	LOC106578942	neurexin-1a-like	protein_coding	ssa19	44553824	44627142	-			
gene40825:106579714	-0,614118135	-0,711337304	-0,392683898	-0,25664169	2,024131749	0,368260616	2	XP_014015336.1	LOC106579714	extracellular sulfatase Sulf-1-like	protein_coding	ssa19	78207945	78293456	+	SULF1		
gene40891:106579778	-0,301763231	-0,481086782	-0,527800799	-0,639893505	1,863802386	0,63264386	2	XP_014015420.1	LOC106579778	cryptochrome DASH-like	protein_coding	ssa19	82936486	82950607	-			
gene42294:106581151	0,413079662	-0,953631771	-0,982522713	-0,705050735	1,905998115	0,007295781	2	XP_014018481.1	LOC106581151	cell adhesion molecule 1-like	protein_coding	ssa20	60063033	60220036	+	CADM1		
gene42296:106581149	-0,425273932	-0,774029187	-0,685168456	-0,707377186	2,389611121	1,520301563	2	XP_014018472.1	LOC106581149	protein turtle homolog B-like	protein_coding	ssa20	60271067	60350929	-	IGSF9B		
gene42649:106581490	-0,842766443	-0,497004361	-0,425575076	-0,460358696	1,952914219	0,266106225	2	XP_014019040.1	LOC106581490	pumilio homolog 1-like	protein_coding	ssa20	79313416	79323813	-			

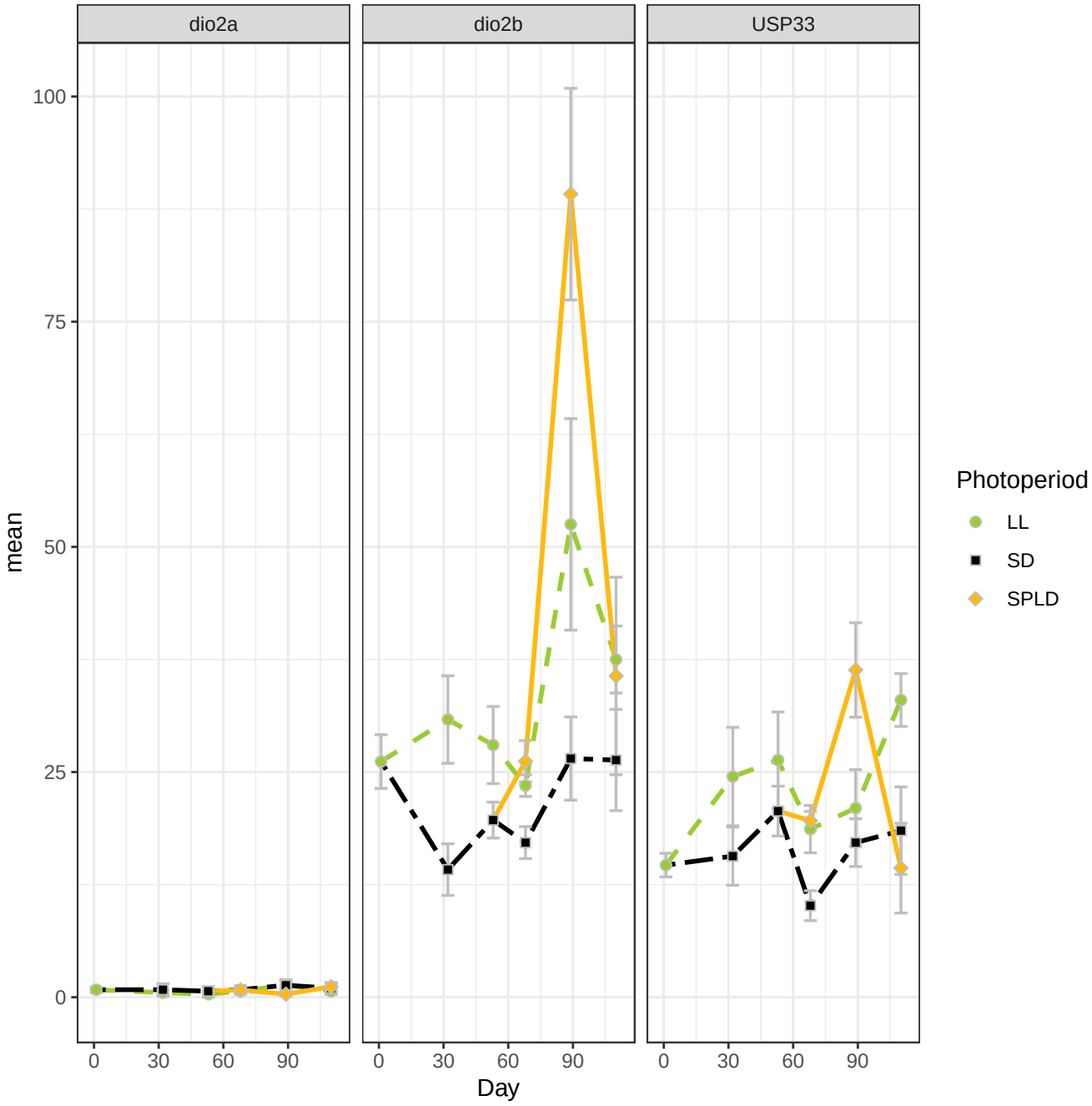
gene42754:106581599	-0,560148962	-0,373834114	-0,496228884	-0,397363419	0,821308449	0,030200221	2	XP_014019197.1	LOC106581599	uncharacterized LOC106581599	protein_coding	ssa20	85503206	85507801	+				
gene42825:106581667	-0,332927434	-0,264608707	-0,391646031	-0,42992678	1,2721456	0,070457868	2	XP_014019348.1	LOC106581667	collagen alpha-3(VI) chain-like	protein_coding	ssa21	6162387	6170570	+				
gene43175:106582012	-0,59198454	-0,557927952	-0,518458922	-0,239574436	1,602548832	0,369467346	2	XP_014020121.1	LOC106582012	importin subunit alpha-4-like	protein_coding	ssa21	24819123	24836957	-	KPNA3			
gene43754:106582584	-0,561617654	-0,561716757	-0,399910684	-0,393130163	1,536559752	0,104631541	2	XP_014021284.1	LOC106582584	disco-interacting protein 2 homolog A-like	protein_coding	ssa21	57506262	57573314	+	DIP2C			
gene43755:106582585	-0,454639182	-0,345754774	-0,358300815	-0,399654738	1,119029846	0,289714832	2					ssa21	57546852	57548845	+				
gene44058:106582889	0,059851133	-1,132996361	-1,191392904	-0,183318403	2,044237784	1,268349412	2	XP_014021951.1	LOC106582889	zinc fingers and homeoboxes protein 3-like	protein_coding	ssa22	15824874	15841763	+				
gene44582:100196052	-0,15148585	-0,609365451	-0,459493266	0,260932999	2,702156652	0,505077245	2	NP_001134553.1	fkbp5	FK506 binding protein 5	protein_coding	ssa22	37514764	37546894	+				
gene45119:106583876	-0,309165284	-0,557904738	-0,660845585	-0,350527244	1,518543922	0,813385362	2	XP_014023971.1	LOC106583876	serine/threonine-protein kinase BRSK2-like	protein_coding	ssa23	296591	322734	-	BRSK1			
gene45122:106583880	-0,483142416	-0,542922209	-0,686440802	-0,295770198	1,798264865	1,216377158	2	XP_014023974.1	LOC106583880	vesicle-fusing ATPase-like	protein_coding	ssa23	489320	540455	-	NSF			
gene45127:106583889	-0,434924723	-0,462105316	-0,668353211	0,116774043	1,968782404	1,351780049	2	XP_014023990.1	LOC106583889	heme-binding protein 2-like	protein_coding	ssa23	754401	757558	+	HEBP2			
gene4525:106586133	-0,528613538	-0,786894174	-0,668374217	-0,623407604	1,548041433	0,558482171	2	XP_014028501.1	LOC106586133	nidogen-1-like	protein_coding	ssa02	41515992	41600067	-	NID1			
gene45293:106584050	0,024137972	-0,752607806	-0,884083874	-0,339915413	2,203156789	0,600768445	2					ssa23	10957793	10964228	-				
gene46058:106584787	-0,78360908	-0,601087376	-0,467205006	-0,196280105	1,542394283	0,374427964	2	XP_014025840.1	LOC106584787	protein phosphatase 1 regulatory subunit 12A-like	protein_coding	ssa23	45957886	45981724	-				
gene46469:106585014	-0,182882143	-0,361040791	-0,737812044	0,685692388	1,340597576	0,631552576	2	XP_014026223.1	dnajb5	DnaJ (Hsp40) homolog%2C subfamily B%2C member 5	protein_coding	ssa24	15237102	15246607	+	DNAJB5			
gene47199:106585952	0,366664689	-0,900538798	-0,691628874	-0,29719446	2,284925534	0,664192752	2	XP_014028180.1	LOC106585952	WD repeat and FYVE domain-containing protein 3-like	protein_coding	ssa24	44770110	44831547	+	WDFY3			
gene47742:106586506	-0,756073299	-0,702165034	-0,36105034	-0,676790714	1,360305494	0,770201093	2	XP_014029365.1	LOC106586506	matrix-remodeling-associated protein 5-like	protein_coding	ssa25	28192435	28208840	+				
gene49223:106588001	-0,383632245	-0,403320644	-0,301614525	-0,320008328	2,493181854	-0,006818947	2					ssa26	42934102	42935068	+				
gene49224:106588002	-0,478223271	-0,439542701	-0,478083167	-0,41630415	1,231634996	0,11633305	2	XP_014032139.1	LOC106588002	uncharacterized LOC106588002	protein_coding	ssa26	42943379	42984122	+				
gene50199:106588609	-0,436582804	-0,395701832	-0,393210116	-0,509397299	1,09505858	0,215628572	2	XP_014033240.1	LOC106588609	pollen-specific leucine-rich repeat extensin-like protein 4	protein_coding	ssa27	17867055	17869619	+				
gene50231:106588657	-0,600877724	-0,853081918	-0,414123805	-0,495571343	2,081458554	0,751413081	2	XP_014033326.1	LOC106588657	forkhead box protein O6-like	protein_coding	ssa27	19583717	19639830	-				
gene50323:100380777	-0,244091205	-0,759193321	-0,815186145	-0,706203977	1,28585508	0,603860595	2	XP_014033600.1	matn1	matrilin 1%2C cartilage matrix protein	protein_coding	ssa27	23109972	23119479	-	MATN1			
gene51276:106589708	-0,640004274	-0,523864589	-0,690072115	-0,270950476	1,345148739	1,295613792	2	XP_014035456.1	LOC106589708	ankyrin repeat domain-containing protein 1-like	protein_coding	ssa28	21931890	21937283	+	ANKRD1			
gene5253:106592167	-0,732934056	-0,520754346	-0,345990053	-0,319741542	1,507095207	-0,019669285	2	XP_014038956.1	LOC106592167	polycystin-1-like	protein_coding	ssa02	62873234	62887525	+	PKD1			
gene5452:106594192	-0,232161162	-0,531294575	0,042300934	0,14051841	1,924042872	-0,374420181	2	XP_014041045.1	LOC106594192	uncharacterized LOC106594192	protein_coding	ssa02	68238275	68242476	-				
gene55695:106593606	-0,083071074	-0,344071744	-0,456535483	-0,298085778	2,033481482	0,688912537	2					NW_012350545.1	9677	13518	+				
gene56648:106590969	-0,961651235	-0,666206568	-0,381917031	-0,294266689	1,263990967	0,678848893	2	XP_014037622.1	LOC106590969	pleckstrin homology domain-containing family G member 4B-like	protein_coding	NW_012343266.1	567	46908	+	PLEKHG4B			
gene5746:106596897	-0,700523366	-0,64312592	-0,580965239	-0,640499285	1,512949115	0,460486811	2	XP_014043626.1	LOC106596897	collagen alpha-1(XI) chain-like	protein_coding	ssa03	4193218	4367779	-	COL11A1			
gene57709:106592319	-0,634295252	-0,593496357	-0,645602207	-0,319251797	1,791642118	0,650664365	2	XP_014039132.1	LOC106592319	low-density lipoprotein receptor-related protein 1-like	protein_coding	NW_012347996.1	3345	20302	+	LRP1			
gene58523:106593628	-0,682434912	-0,665855087	-0,283361982	-0,449313836	1,385509464	0,050774294	2	XP_014040469.1	LOC106593628	fibronectin-like	protein_coding	NW_012350597.1	10198	13880	+	FN1			
gene59321:106591092	-0,567215412	-0,390858112	-0,449694341	-0,440224286	1,666804823	0,045664512	2	XP_014037756.1	LOC106591092	probable JmjC domain-containing histone demethylation protein 2C	protein_coding	NW_012343424.1	5086	24628	+				
gene59595:106595386	-0,54297296	-0,165395527	-0,168094695	-0,076393274	1,857556351	-0,099971079	2	XP_014042230.1	LOC106595386	upstream-binding protein 1-like	protein_coding	NW_012355462.1	24	8128	-				
gene59783:106594629	-0,579877703	-0,530157608	-0,222759514	-0,413270861	1,413038758	-0,030780621	2	XP_014041507.1	LOC106594629	probable JmjC domain-containing histone demethylation protein 2C	protein_coding	NW_012353214.1	2110	8051	+				
gene60059:106593083	-0,69433861	-0,535829645	-0,313587516	-0,41540381	2,038654389	0,699011365	2	XP_014039896.1	LOC106593083	rho GTPase-activating protein 44-like	protein_coding	NW_012349384.1	3955	10381	-				
gene60490:106592132	-0,540603217	-0,40384974	-0,0965358	-0,243346457	1,441892856	0,288316928	2	XP_014038918.1	LOC106592132	trinucleotide repeat-containing gene 6A protein-like	protein_coding	NW_012347709.1	9251	24183	+				
gene62178:106592913	-0,863980931	-0,530418227	-0,186197696	-0,430046813	1,554379029	0,354042465	2	XP_014039737.1	LOC106592913	RNA-binding protein 47-like	protein_coding	NW_012349075.1	10004	15490	-				
gene6284:106599162	0,442010375	-1,051115213	-1,020160646	0,008077748	2,516172386	0,672500653	2	XP_014045751.1	LOC106599162	cholesterol 7-alpha-monooxygenase-like	protein_coding	ssa03	16411904	16416236	+	CYP7A1			
gene63147:106594892	-0,574142965	-0,452634239	-0,494791356	-0,176378628	1,563434336	0,005951859	2	XP_014041754.1	LOC106594892	anoctamin-5-like	protein_coding	NW_012353984.1	2216	10295	+	ANO5			
gene63322:106591806	-0,529555286	-0,667314073	-0,485782177	-0,369374396	1,71723862	0,276524131	2	XP_014038543.1	LOC106591806	Krueppel-like factor 13	protein_coding	NW_012347252.1	30038	30889	+				
gene63886:106593918	-0,615933349	-0,488198783	-0,307864778	-0,168972022	1,417029793	0,192917829	2	XP_014040769.1	LOC106593918	cytoplasmic dynein 2 heavy chain 1-like	protein_coding	NW_012351320.1	597	12533	+	DYNC2H1			
gene64341:106592807	-0,532468287	-0,729099479	-0,521229279	-0,543147905	2,348692261	1,376190424	2	XP_014039615.1	LOC106592807	high-affinity choline transporter 1-like	protein_coding	NW_012348855.1	1578	15781	+	SLC5A7			
gene64344:106592192	-0,749953983	-0,439562808	-0,055577303	-0,243238233	1,141600724	0,745414801	2	XP_014038984.1	LOC106592192	metastasis suppressor protein 1-like	protein_coding	NW_012347796.1	13053	23324	-				
gene65901:106598398	-0,695720516	-0,308017689	-0,054683496	-0,433201444	1,612463239	0,321252908	2	XP_014044911.1	LOC106598398	spectrin beta chain%2C non-erythrocytic 1-like	protein_coding	NW_012377671.1	379	1734	-				
gene66604:106595324	-0,278200626	-0,549117857	-0,635796311	-0,608521483	2,281074756	1,251547727	2	XP_014042174.1	LOC106595324	angiopoietin-1-like	protein_coding	NW_012355245.1	55	9166	+				
gene67232:106590854	-0,450548377	-0,696409503	-0,652032398	-0,423245446	1,287204398	0,602399018	2	XP_014037505.1	LOC106590854	collagen alpha-1(II) chain-like	protein_coding	NW_012342255.1	288	1828	+				
gene68772:106593570	-0,437520577	-0,568635936	-0,216235763	0,069827798	1,716865413	0,302030745	2					NW_012350450.1	2909	3670	+				
gene69505:106592371	-0,517658843	-0,391495454	-0,398473365	-0,470928885	1,178720851	0,099157387	2	XP_014039184.1	LOC106592371	uncharacterized LOC106592371	protein_coding	NW_012348096.1	469	1690	-				
gene70863:106594621	-0,463917877	-0,391502222	-0,056773978	-0,271655707	1,527999875	-0,004303599	2	XP_014041501.1	LOC106594621	trinucleotide repeat-containing gene 6A protein-like	protein_coding	NW_012353185.1	2856	11169	+				
gene71701:106593305	-0,460136059	-0,466781925	-0,50592218	0,054688864	1,46206523	0,091290633	2					NW_012349858.1	5598	6496	+				
gene7355:100195269	0,040650337	-0,672826545	-1,060030675	-0,140434099	2,05968175	1,042508543	2	NP_001133770.1	phop1	Probable phosphatase phospho1	protein_coding	ssa03	59291961	59295493	-	PHOSPHO1			
gene73910:106590752	-0,410227199	-0,682339875	-0,548242507	-0,298278851	2,507213341	0,591761693	2	XP_014037384.1	LOC106590752	nuclear factor 1 X-type-like	protein_coding	NW_012341900.1	443	31875	-	NFIX			
gene73964:106592409	-0,575755139	-0,399123472	-0,216840456	-0,445476299	1,295990077	0,674477848	2	XP_014039223.1	LOC106592409	dystrophin-like	protein_coding	NW_012348161.1	4859	20281	+	DMD			
gene75624:106595057	-0,610268361	-0,431843322	0,006012274	-0,41739139	1,879523249	0,026968187	2	XP_014041905.1	LOC106595057	protein furry homolog	protein_coding	NW_012354455.1	55	7575	-	FRY			
gene77836:106592414	-0,636418978	-0,743976923	-0,16617886	-0,173799379	1,733232488	0,436799081	2	XP_014039226.1	LOC106592414	protein furry homolog	protein_coding	NW_012348173.1	2731	19654	+				
gene9961:106603596	-0,460903084	-0,588817268	-0,631734798	-0,596074802	2,493494589	0,734002409	2	XP_014052943.1	LOC106603596	retinal guanylyl cyclase 2-like	protein_coding	ssa04	46810311	46847253	+	GUCY2D			
rna133441	-0,376860215	-0,374823478	-0,439895335	-0,399836468	1,031225102	0,276219623	2												
gene11111:106604469	-0,290119875	-0,554692317	-0,638421999	-0,208208081	0,455833289	1,788734729	3	XP_014054583.1	ky	kyphoscoliosis peptidase	protein_coding	ssa05	19725253	19732029	-	KY			
gene11514:106604849	-0,230352514	-0,905182706	-1,038857033	-0,70763487	1,703244918	1,492647127	3	XP_014055381.1	LOC106604849	arylsulfatase I-like	protein_coding	ssa05	36754069	36757784	+	ARSI			
gene1356:106607942	-0,722687822	-0,699564455	-0,723607908	-0,575484745	1,681401489	1,898110634	3	XP_014060917.1	LOC106607942	GDNF family receptor alpha-1-like	protein_coding	ssa01	68000090	68096972	+	GFRA1			
gene13645:106606883	-0,459871716	-0,579139307	-0,725258216	-0,619686313	0,662057245	2,54214536	3	XP_014058782.1	LOC106606883	glycine-rich cell wall structural protein-like	protein_coding	ssa06	24622165	24653167	+				
gene1493:106609599	0,142618183	-0,807031143	-0,854358463	-0,674865397	1,339671454	2,440732097	3	XP_014064038.1	LOC106609599	forkhead box protein J1-B-like	protein_coding	ssa01	75107702	75111902	+				
gene15067:106608259	-0,082279506	-0,469310575	-0,868350985	-0,550669151	1,043009038	1,524276353	3	XP_014061588.1	LOC106608259	actin%2C alpha cardiac	protein_coding	ssa06	79997893	80006644	+	ACTC1			
gene16076:106609244	0,33234615	-0,810625562	-0,828332024	-0,796999218	1,762709099	1,598443477	3	XP_014063281.1	LOC106609244	uncharacterized LOC106609244	protein_coding	ssa07	34079240	34086061	-				
gene16253:106609359	0,472615455	-0,905234154	-1,12650051	-0,447384375	2,070444435	1,526675008	3	XP_014063590.1	LOC106609359	gelsolin-like	protein_coding	ssa07	41280327	41288555	+	GSN			
gene16683:106609768	-0,381666538	-0,435657599	-0,567154371	-0,512163179	1,170302096	2,784365429	3	XP_014064253.1	LOC106609768	cytidine monophosphate-N-acetylneuraminic acid hydroxylase-like	protein_coding	ssa07	54913778	54931984	-				
gene16791:106609883	0,336248976	-0,176535645	-0,704851948	-0,423887544	0,925010657	2,018452399	3	XP_014064384.1	LOC106609883	beta-1%2C4-galactosyltransferase 1-like	protein_coding	ssa08	383567	387163	-				
gene17394:106610444	-0,565005996	-0,665322282	-0,511569362	0,077891108	1,152879403	2,051380983	3	XP_014065298.1	LOC106610444	C-type lectin domain family 4 member F-like	protein_coding	ssa08	22651419	22653447	+	CLEC4E			
gene18264:106611327	0,084126687	-0,666701934	-0,547183308	-0,163095233	1,997450832	1,202383395	3	XP_014066889.1	LOC106611327	vacuolar protein 8-like	protein_coding	ssa09	40362065	40369542	-				
gene18348:106611399	0,107668882	-0,823177312	-0,712076447	-0,789618219	1,635885387	1,386571688	3	XP_014067045.1	LOC106611399	dynactin subunit 1-like	protein_coding	ssa09	44194144	44254794	-	DCTN1			
gene20520:106613519	-0,145802835	-0,156071126	-0,330343891	-0,298282275	1,673565397	1,631219905	3	XP_014071339.1	ccdc181	coiled-coil domain containing 181	protein_coding	ssa10	10547754	10553869	-	CCDC181			
gene20791:106613765	0,530563345	-0,770395784	-0,609587244	0,02119666	0,903801086	1,983593289	3	XP_014071827.1	LOC106613765	cyclic AMP-responsive element-binding protein 3-like protein 3-B	protein_coding	ssa10	19861892	19869452	-	CREB3L3			
gene21183:106560392	0,46833073	-0,280405154	-0,500713547	-0,486997107	0,229156188	2,903551764	3	XP_013978745.1	LOC106560392	mucin-7-like	protein_coding	ssa10	37144041	37148396	+				
gene22493:106561651	-0,804806019	-0,764847821	-0,402025308	0,047541241	0,731529912	1,866568741	3	XP_013981286.1	LOC106561651	troponin I%2C cardiac muscle-like	protein_coding	ssa10	107741513	107748669	-	TNNI3			
gene23154:106562311	-0,295184879	-0,156933344	-0,268555193	-0,331767781	0,557797549	3,075858961	3	XP_013982581.1	LOC106562311	tryptophan 5-hydroxylase 1-like	protein_coding	ssa11	24066168	24073655	+	TPH1			
gene23459:106562604	-0,278098687	-0,34795879	-0,388234554	-0,440098587	-0,044278521	3,253141782	3	XP_013983028.1	LOC106562604	CUB and zona pellucida-like domain-containing protein 1	protein_coding	ssa11	36699035	36708871	-				
gene23530:106562679	0,381229116	-0,035053241	-0,582487803	-0,72936399	0,214081444	2,33498919	3	XP_013983134.1	LOC106562679	zymogen granule membrane protein 16-like	protein_coding	ssa11	38977534	39003641	+	ZG16			

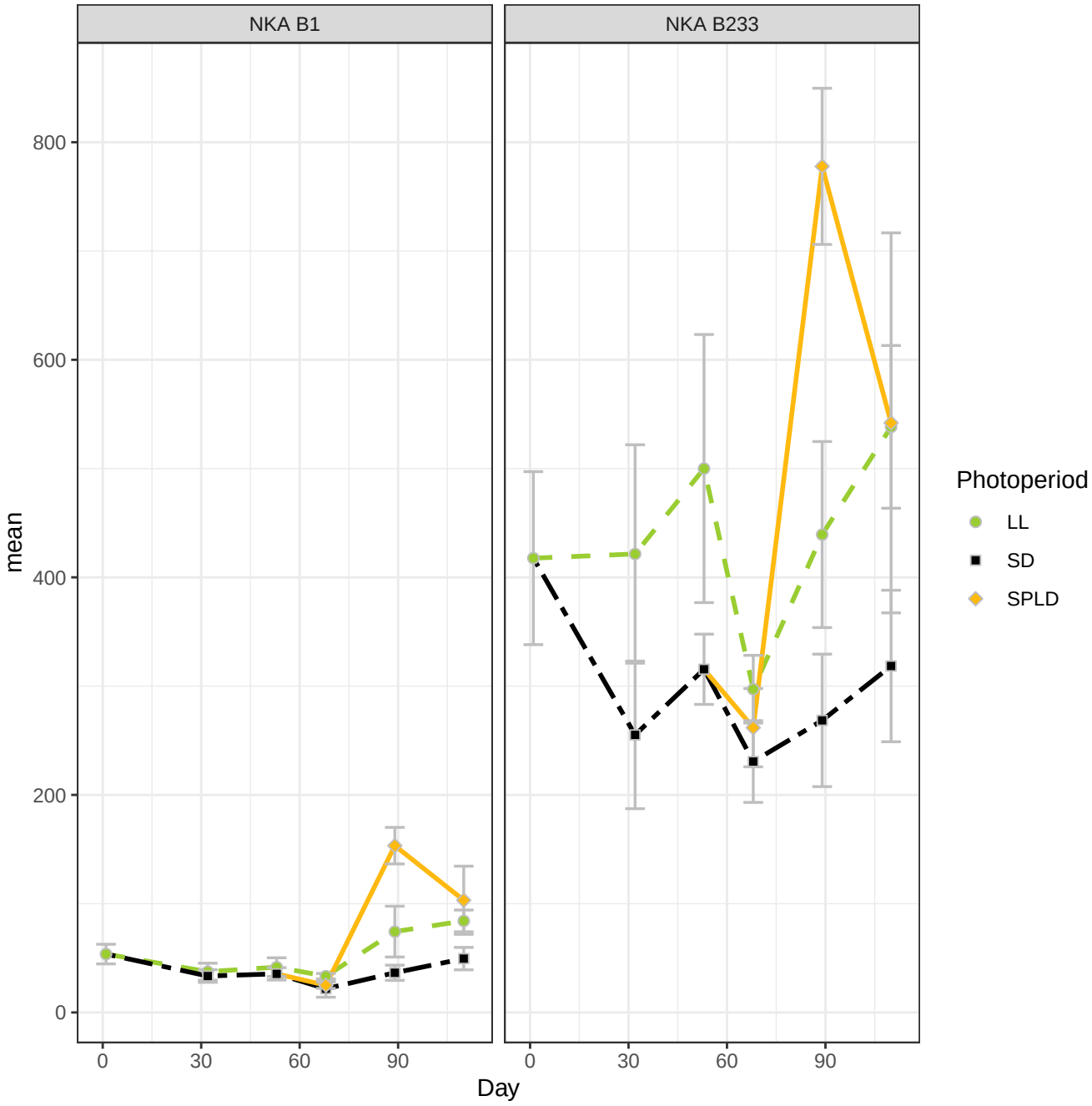
gene24159:106563217	0,027130869	-0,566440444	-0,699248689	-0,308641018	-0,150343276	2,9323709	3	XP_013984048.1	LOC106563217	protein KIAA1045 homolog	protein_coding	ssa11	71639258	71657431	+	PHF24
gene25359:106564502	-0,193377302	-0,607253422	-0,572604663	-0,191916103	1,58697626	2,354465443	3	XP_013986098.1	LOC106564502	volume-regulated anion channel subunit LRRC8C-like	protein_coding	ssa12	14161226	14170165	-	
gene2565:106566600	-0,482583658	-0,636890312	-0,800630297	-0,01852417	1,019848059	2,396238755	3	XP_013990237.1	LOC106566600	complement component C7-like	protein_coding	ssa01	129757262	129762855	+	C7
gene25926:106565075	-0,444231491	-0,586621355	-0,606287905	-0,463372253	1,692671852	2,493499652	3					ssa12	36092032	36114063	-	
gene25927:106565076	-0,283281381	-0,309414976	-0,309414976	-0,114851879	1,334985608	1,605166229	3	XP_013987184.1	LOC106565076	1%2C25-dihydroxyvitamin D(3) 24-hydroxylase%2C mitochondrial-like	protein_coding	ssa12	36126846	36140228	+	CYP24A1
gene26145:106565112	-0,659682287	-0,94124984	-0,717997005	-0,147410437	1,914611457	1,80944221	3	XP_013987325.1	LOC106565112	cryptochrome-1-like	protein_coding	ssa12	46213291	46223102	-	CRY1
gene26209:106565346	-0,503755906	-0,7369885	-0,586108327	0,061583365	2,026469743	2,061133996	3	XP_013987820.1	LOC106565346	peptidyl-prolyl cis-trans isomerase FKBP5-like	protein_coding	ssa12	49824186	49850676	-	FKBP5
gene2707:106568012	-0,037923456	-0,216942378	-0,698342832	-0,417448231	1,792315993	2,102213718	3	XP_013993472.1	LOC106568012	caspase-3-like	protein_coding	ssa01	136638230	136642742	+	
gene27282:106566233	-0,41766107	-0,192548139	-0,512896691	-0,756586069	2,099572512	1,837295542	3	XP_013989585.1	LOC106566233	plasma membrane calcium-transporting ATPase 2	protein_coding	ssa12	89314937	89511804	+	ATP2B2
gene27740:100195645	0,872400426	-0,642172477	-0,699109163	-0,680441369	0,639515786	2,697897101	3	NP_001134146.1	st2s2	Cytosolic sulfotransferase 2	protein_coding	ssa13	17615538	17618691	-	
gene27741:106566634	0,463240212	-0,8690573	-0,669167524	-0,713186046	1,035707704	2,016791495	3	XP_013990320.1	LOC106566634	cytosolic sulfotransferase 3-like	protein_coding	ssa13	17631370	17639939	+	SULT1A1
gene28132:106567030	0,046516224	-0,518788929	-0,444265996	-0,241611748	0,500731886	2,179835754	3	XP_013991309.1	ribc1	RIB43A domain with coiled-coils 1	protein_coding	ssa13	34155702	34164099	-	RIBC1
gene28231:106567109	0,405985289	-0,629322587	-0,65971889	-0,32692861	0,776658736	1,883318215	3	XP_013991486.1	LOC106567109	G0/G1 switch protein 2-like	protein_coding	ssa13	36629794	36630789	-	G0S2
gene28967:106567921	0,384191014	-0,867577044	-0,859106407	-0,889093099	1,616656082	2,042665994	3	XP_013993288.1	dscam	Down syndrome cell adhesion molecule	protein_coding	ssa13	76519046	76658168	+	DSCAM
gene29334:106568147	-0,505344248	-0,398984533	-0,399430117	-0,736845323	0,67402166	2,141815879	3	XP_013993697.1	LOC106568147	dual specificity protein phosphatase 4-like	protein_coding	ssa13	90230866	90237464	+	DUSP4
gene29410:106568325	0,315545636	-0,398659873	-0,610957872	-0,658349476	0,760204305	2,938333577	3	XP_013994046.1	LOC106568325	trypsin inhibitor CITI-1-like	protein_coding	ssa13	92154023	92154940	-	SPINK2
gene30578:106569470	0,835750043	-0,660972587	-0,782533025	-0,342000572	0,26722364	1,695059427	3	XP_013996322.1	catip	ciliogenesis associated TTC17 interacting protein	protein_coding	ssa14	35645179	35649403	+	CATIP
gene30938:106569812	0,415447738	-0,855215619	-0,773137323	-0,496010378	0,781774046	1,489614384	3	XP_013996920.1	LOC106569812	myosin heavy chain%2C fast skeletal muscle-like	protein_coding	ssa14	49560056	49586507	+	MYH8
gene31003:106570104	-0,266618816	-0,457927547	-0,592024951	-0,543165609	1,462323069	2,417148101	3	XP_013997587.1	LOC106570104	protein S100-A1-like	protein_coding	ssa14	52521492	52523724	-	
gene31218:106569928	0,183259451	-0,665676667	-0,658952064	-0,57894213	1,500703891	2,474565475	3	XP_013997156.1	LOC106569928	DEP domain-containing mTOR-interacting protein-like	protein_coding	ssa14	59968116	59987537	-	
gene31316:106570214	0,043236137	-0,869598118	-1,070466393	-0,021550758	0,934187121	1,669635075	3	XP_013997774.1	LOC106570214	1-phosphatidylinositol phosphodiesterase-like	protein_coding	ssa14	63316481	63325509	+	
gene31425:106570302	0,35218816	-0,715300643	-0,507907603	-0,320125436	0,888244115	2,291718475	3	XP_013997948.1	lrrc3b	leucine rich repeat containing 3B	protein_coding	ssa14	68838307	68845930	+	LRRC3B
gene32397:100194800	-0,550637624	-1,042610361	-1,138670378	-0,135075986	1,177274767	2,196542673	3	NP_001133301.1	tieg3	Transforming growth factor-beta-inducible early growth response protein 3	protein_coding	ssa15	25918574	25923332	+	KLF11
gene32699:106571495	-0,498164465	-0,577682737	-0,603192803	-0,357345049	0,6954084	1,433436843	3	XP_014000132.1	LOC106571495	uncharacterized protein KIAA0408-like	protein_coding	ssa15	38290441	38299256	+	KIAA0408
gene33240:106572206	-0,096209512	-0,236668421	-0,652202564	-0,435200504	1,697494181	1,793771264	3	XP_014001651.1	LOC106572206	espin-like	protein_coding	ssa15	66159728	66262843	-	ESPN
gene33704:106572553	0,737138101	-0,292390921	-0,423881719	-0,016332253	0,970351413	2,479396736	3	XP_014002308.1	fam43b	family with sequence similarity 43%2C member B	protein_coding	ssa15	85602806	85605147	-	FAM43B
gene33902:106572758	-0,245567436	-0,631102267	-0,704969599	-0,47286793	0,352265023	1,92453759	3	XP_014002694.1	LOC106572758	SRSF protein kinase 3-like	protein_coding	ssa15	93041309	93058346	-	
gene34295:100136364	0,61061284	-1,112485772	-1,156538357	-0,878471281	1,738714994	1,718665434	3	NP_001117005.1	LOC100136364	cystic fibrosis transmembrane conductance regulator I	protein_coding	ssa16	8837765	8889830	+	CFTR
gene35056:106573917	0,03235194	-0,16119577	-0,449587692	0,441463299	0,657561225	2,808560675	3	XP_014004853.1	LOC106573917	phospholipase A2 inhibitor and Ly6/PLAUR domain-containing protein-like	protein_coding	ssa16	43573507	43576556	-	PINLYP
gene35147:106574043	0,46776455	-0,770256153	-0,691195457	-0,750960342	1,644753579	1,207142195	3	XP_014005039.1	LOC106574043	multiple PDZ domain protein-like	protein_coding	ssa16	47425685	47527068	+	MPDZ
gene35521:106574429	0,192885786	-0,652796767	-0,774522568	-0,307086433	1,758957446	1,405021378	3	XP_014005787.1	LOC106574429	secretin receptor-like	protein_coding	ssa16	68135386	68149857	-	
gene37235:106576140	-0,183520597	-0,797882773	-0,822182004	-0,762350838	1,572749825	2,393947133	3	XP_014008529.1	LOC106576140	branched-chain-amino-acid aminotransferase%2C cytosolic-like	protein_coding	ssa17	39533745	39553804	+	BCAT1
gene37252:106576157	0,841029742	-0,768980383	-1,144501954	-0,925499001	1,077158535	1,64657623	3	XP_014008578.1	LOC106576157	patatin-like phospholipase domain-containing protein 2	protein_coding	ssa17	40243683	40278338	+	PNPLA2
gene37885:106576808	-0,268292308	-0,382849191	-0,443185227	-0,419439178	1,616879667	2,278456654	3	XP_014009716.1	LOC106576808	brorin-like	protein_coding	ssa18	6933636	6959882	+	VWC2
gene38562:106577459	-0,299485669	-0,645478973	-0,694929848	-0,664532522	1,478406688	2,322779253	3	XP_014010930.1	LOC106577459	ethanolamine-phosphate phospho-lyase-like	protein_coding	ssa18	40301673	40336685	-	ETNPPL
gene39069:106577945	0,334069481	-0,467663432	-0,399982651	0,087949579	0,205229655	2,292319538	3	XP_014011897.1	LOC106577945	sterile alpha motif domain-containing protein 9-like	protein_coding	ssa18	62074830	62108080	+	
gene39071:106577947	-0,299899595	-0,629096843	-0,483999327	-0,274646857	0,826854106	2,489627671	3	XP_014011901.1	LOC106577947	sterile alpha motif domain-containing protein 9-like	protein_coding	ssa18	62125144	62136314	+	
gene3920:106579424	-0,953978448	-0,461147861	-0,531577397	-0,594122093	1,689347355	1,918094789	3	XP_014014798.1	LOC106579424	brain-specific angiogenesis inhibitor 1-like	protein_coding	ssa02	18171329	18281042	+	ADGRB1
gene40102:106578986	-0,331453401	-0,372438849	-0,393596298	-0,391993462	0,618423371	3,307062408	3	XP_014013824.1	LOC106578986	glutamate receptor ionotropic%2C delta-1-like	protein_coding	ssa19	46723466	47146887	-	GRID1
gene40156:106579029	-0,268293804	-0,818375608	-0,775198639	-0,658705834	1,075052638	2,31691961	3	XP_014013933.1	LOC106579029	delta-1-pyrroline-5-carboxylate synthase-like	protein_coding	ssa19	49458178	49471950	-	ALDH18A1
gene40466:106579089	-0,078217973	-0,198169288	-0,673584123	-0,563364329	0,5850768	3,03637801	3	XP_014014111.1	LOC106579089	alpha-tectorin-like	protein_coding	ssa19	59510656	59566196	-	
gene40861:106579749	0,042331844	-1,05311629	-1,061925861	-0,493563765	1,333485827	1,259569449	3	XP_014015387.1	LOC106579749	peptidase inhibitor 15-A	protein_coding	ssa19	80581159	80585481	-	PI15
gene40931:106579802	0,741650268	-0,343779517	-0,797997559	-0,107584392	0,739761841	2,494951262	3	XP_014015468.1	LOC106579802	protein NipSnap homolog 1-like	protein_coding	ssa20	2711013	2736855	-	NIPSNAP1
gene41624:106580506	0,009558642	-0,753145154	-0,946962139	-0,728734984	2,203832885	1,01998143	3	XP_014017128.1	LOC106580506	prominin-2-like	protein_coding	ssa20	34324133	34345456	-	
gene41914:106580762	0,42444632	-0,423768208	-0,144444542	-0,536650643	1,179607979	2,422618548	3	XP_014017622.1	LOC106580762	ras-related protein Rab-1A-like	protein_coding	ssa20	46377023	46379226	+	RAB1A
gene42888:106581733	0,310020118	-0,778149519	-1,067568002	-0,695847686	1,539384515	1,92088935	3	XP_014019452.1	LOC106581733	uncharacterized LOC106581733	protein_coding	ssa21	11869034	11871002	+	
gene443:106581812	0,154615539	-0,678337511	-0,643075065	-0,494932885	1,828218544	1,403215941	3	XP_014019692.1	LOC106581812	uncharacterized protein C15orf52-like	protein_coding	ssa01	21870341	21918146	+	C15orf52
gene44614:100286414	0,340846878	-0,875469818	-0,840025345	-0,430573617	1,120958163	1,155171962	3	XP_014023277.1	LOC100286414	fibromodulin	protein_coding	ssa22	39372641	39377260	-	FMOD
gene46936:106585698	-0,673952499	-0,803855483	-0,974388845	0,489239831	1,009334398	1,749665824	3	XP_014027680.1	LOC106585698	fibrous sheath CABYR-binding protein-like	protein_coding	ssa24	30522492	30542345	-	
gene47030:106585760	-0,13923369	-0,997481276	-0,89594606	-0,371609663	1,918395919	1,085826544	3					ssa24	34872302	34881059	-	
gene47521:106586273	0,555042015	-0,914807951	-0,843166223	-0,512543744	2,152784567	1,573663685	3	XP_014028871.1	LOC106586273	pyridoxal kinase-like	protein_coding	ssa25	16370986	16381074	+	PDXK
gene47680:106586448	-0,127631627	-0,965535544	-0,877639141	-0,567809856	2,023754471	1,065834671	3	XP_014029218.1	LOC106586448	microtubule-associated protein 2-like	protein_coding	ssa25	25665903	25801143	-	
gene48700:106587461	0,041821131	-0,837828583	-0,761403169	-0,602159431	1,746256324	1,249356574	3	XP_014031344.1	LOC106587461	spondin-1-like	protein_coding	ssa26	24700731	24841401	-	SPON1
gene48721:106587482	-0,036290522	-0,634189292	-0,601819989	-0,252868988	0,093852864	1,903702947	3	XP_014031373.1	LOC106587482	myosin-binding protein C%2C cardiac-type-like	protein_coding	ssa26	25427524	25463164	+	MYBPC3
gene49001:106587770	-0,230738555	-0,373742829	-0,825143731	-0,695689419	1,276267012	1,439070449	3					ssa26	36385574	36391330	-	
gene4977:106589946	-0,475530914	-0,809489462	-0,867664813	-0,619237385	1,830884557	1,988442925	3	XP_014035882.1	LOC106589946	nuclear factor 1 X-type-like	protein_coding	ssa02	55563261	55708886	+	NFIX
gene49892:106588358	-0,577751086	-0,824202767	-0,920110094	-0,643935964	1,223983705	2,16512215	3	XP_014032726.1	mtss1	metastasis suppressor 1	protein_coding	ssa27	11081799	11129849	+	MTSS1
gene50748:106589263	-0,096024344	-0,67064108	-0,821176236	-0,618130148	1,369555242	1,127175422	3	XP_014034461.1	LOC106589263	PH and SEC7 domain-containing protein 1-like	protein_coding	ssa28	421288	537654	+	
gene50914:100135779	0,057371402	-0,83133495	-1,069551617	-0,512342557	1,445765462	1,13500293	3	NP_001119702.1	fsh-r	follicle stimulating hormone receptor	protein_coding	ssa28	7914675	7922535	+	FSHR
gene51429:106589898	-0,24305561	-0,595439083	-0,563151155	-0,509756391	2,002410074	2,193443542	3	XP_014035790.1	LOC106589898	alpha-N-acetylgalactosaminide alpha-2%2C6-sialyltransferase 2-like	protein_coding	ssa28	30838401	30854224	-	
gene515:106590180	-0,511972965	-0,728313578	-0,647397128	-0,519990221	0,762001091	2,431841837	3	XP_014036412.1	esrrb	estrogen-related receptor beta	protein_coding	ssa01	24447604	24509813	-	ESRRB
gene51540:106589985	-0,39118633	-0,418425017	-0,417364235	-0,419614013	1,855202178	2,532252763	3	XP_014035938.1	LOC106589985	calpain-2 catalytic subunit-like	protein_coding	ssa28	37948022	37974487	-	CAPN2
gene51545:106589990	-0,545281702	-0,763160039	-0,844969315	-0,312988069	0,671849202	2,162807058	3					ssa28	38379439	38385016	-	
gene51902:106590380	0,481271257	-0,89537844	-0,531589925	0,195739547	0,12411896	2,20277716	3	XP_014036827.1	LOC106590380	GTP-binding protein Rheb-like	protein_coding	ssa29	22082374	22117565	+	RHEB
gene64345:106592191	-0,748168992	-0,416794509	-0,65047679	-0,52299502	1,056638504	1,686136961	3	XP_014038983.1	LOC106592191	vegetative cell wall protein gp1-like	protein_coding	NW_012347796.1	13053	15501	-	
gene6625:106600417	-0,19584493	-0,634290081	-0,635866604	-0,628551251	1,036888134	2,697581735	3	XP_014047216.1	c8b	complement component 8%2C beta polypeptide	protein_coding	ssa03	34030881	34038200	-	C8B
gene68674:106592927	0,211304818	-0,940973715	-0,947837694	-0,935679328	0,872336226	1,956521354	3					NW_012349099.1	9665	16300	-	
gene7057:106600730	0,396773348	-0,692700189	-0,663236484	-0,702415423	2,083008966	1,979983617	3					ssa03	50697787	50705790	+	
gene7254:106600960	-0,685445806	-0,522143542	-0,575955065	-0,44629787	1,110036037	1,422427856	3	XP_014048242.1	LOC106600960	heat shock protein Hsp-16.1/Hsp-16.11-like	protein_coding	ssa03	56206872	56207842	-	
gene7302:106600778	-0,327156447	-0,371072149	-0,268080414	-0,365402735	1,663772028	1,920126113	3	XP_014047906.1	LOC106600778	cell wall protein IFF6-like	protein_coding	ssa03	57955497	57961665	+	
gene7306:106600814	-0,190722866	-0,533043342	-0,41921311	-0,141523366	1,035638136	1,522119155	3	XP_014047985.1	LOC106600814	keratin%2C type I cytoskeletal 13-like	protein_coding	ssa03	58005339	58008304	+	KRT14
gene7957:106601582	-0,493856511	-0,600115057	-0,640589363	-0,550730351	0,892913692	1,892995215	3	XP_014049367.1	LOC106601582	armadillo repeat-containing protein 5-like	protein_coding	ssa03	75583194	75621894	+	ARMC5
gene8110:106601740	0,39773671	-0,37837165	-0,796608784	-0,529628838	1,255717454	2,17324773	3	XP_014049570.1	LOC106601740	NADPH oxidase organizer 1-like	protein_coding	ssa03	79955233	79960638	+	NOXO1
gene8155:106601789	0,875693046	-0,433689541	-0,701690358	-0,565622619	1,050073007	2,429619264	3	XP_014049626.1	LOC106601789	parvalbumin beta-like	protein_coding	ssa03	81341861	81348573	-	
gene826:106602112	0,717662445	-0,647114406	-0,946523994	-0,638478108	1,166153627	1,959610149	3	XP_014050060.1	ttc6	tetratricopeptide repeat domain 6	protein_coding	ssa01	35620215	35649714	-	TTC6

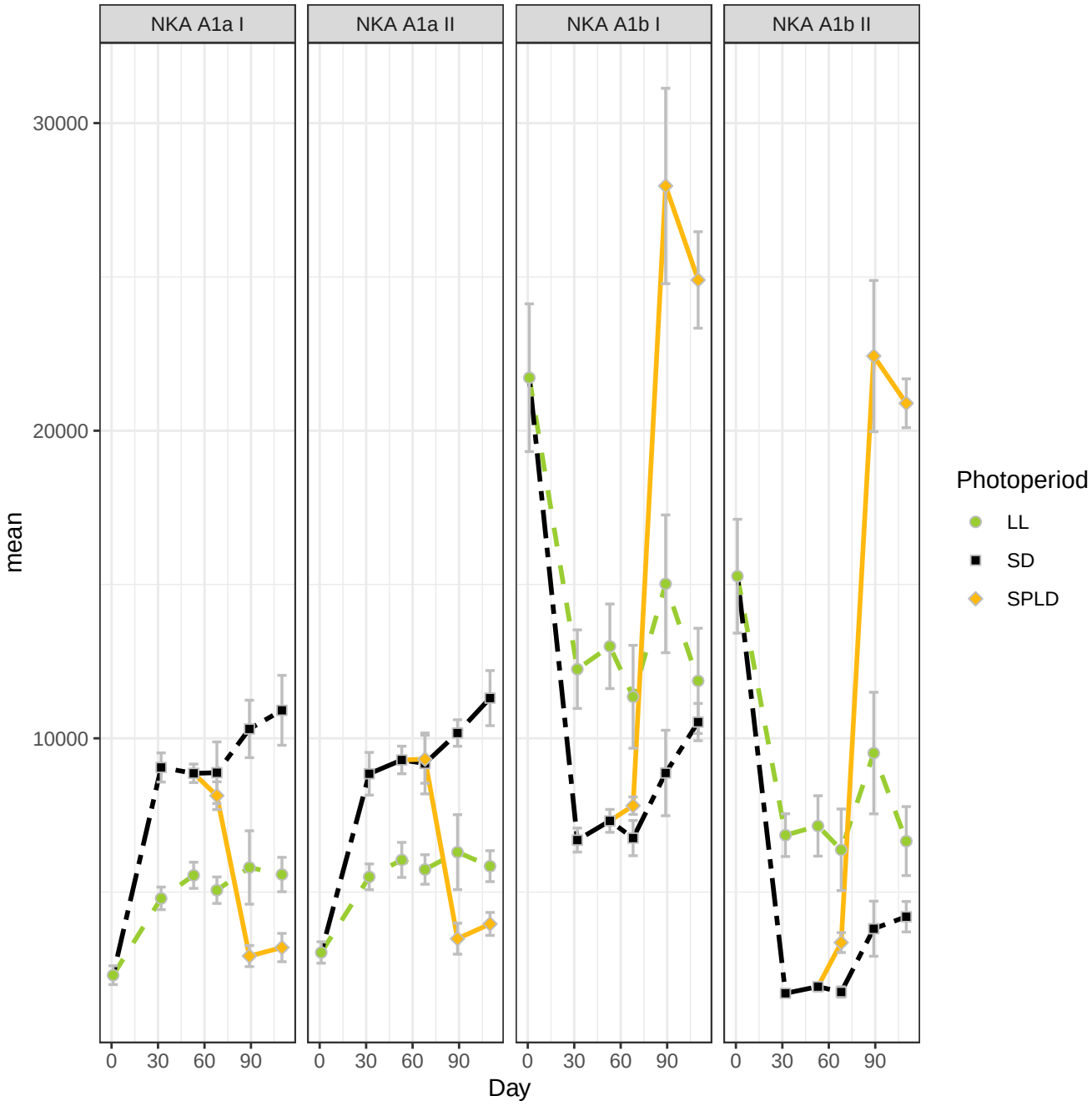
gene8495:106602131	-0,422056695	-0,778403929	-0,764436499	-0,499205177	1,864850543	1,96509206	3	XP_014050075.1	LOC106602131	high affinity choline transporter 1-like	protein_coding	ssa04	1043050	1049857	+	SLC5A7
gene11936:106605296	-0,210186651	-0,262217074	0,129598675	-0,075248662	-0,510649107	-0,651272574	4	XP_014056250.1	LOC106605296	uncharacterized LOC106605296	protein_coding	ssa05	56174074	56177299	+	
gene11985:106605344	-0,445436982	-0,445436982	-0,445436982	-0,445436982	-0,039380915	0,004710643	4	XP_014056335.1	LOC106605344	uncharacterized LOC106605344	protein_coding	ssa05	57513415	57519251	+	
gene12335:106605687	-0,406812481	-0,30592904	-0,313316294	-0,361908386	-0,034006279	-0,110656661	4	XP_014057052.1	LOC106605687	uncharacterized LOC106605687	protein_coding	ssa05	67841956	67845089	-	
gene12536:100195957	-0,063961954	-0,233538768	0,548483352	-0,219795456	-0,379082146	-0,365830203	4	XP_014057348.1	asal	Mannose-specific lectin	protein_coding	ssa05	71606954	71619995	+	
gene13024:106606371	-0,422848093	-0,441548463	-0,421064791	-0,415770497	0,030014348	-0,114714834	4	XP_014057995.1	LOC106606371	uncharacterized LOC106606371	protein_coding	ssa06	6410446	6417758	-	
gene13049:100286729	-0,070989117	-0,183050325	-0,167279944	-0,333489899	-0,338830977	-0,337970217	4	NP_001140141.1	roa2	Heterogeneous nuclear ribonucleoproteins A2/B1	protein_coding	ssa06	7554874	7557447	-	
gene15130:106608294	-0,350685953	-0,187624305	0,522266846	-0,225510062	-0,24257134	-0,189279974	4	XP_014061632.1	LOC106608294	proto-oncogene c-Fos-like	protein_coding	ssa06	83156215	83158302	+	FOS
gene152:106606833	-0,342267337	-0,376646042	-0,411603205	1,761650056	0,19627744	0,15135071	4	XP_014058717.1	LOC106606833	uncharacterized LOC106606833	protein_coding	ssa01	6596726	6625919	+	
gene18345:106611397	0,046325917	-0,448485403	-0,155258327	-0,118411339	-0,698003393	-0,755011198	4	XP_014067042.1	LOC106611397	uncharacterized LOC106611397	protein_coding	ssa09	44034491	44039519	+	
gene1886:100136575	-0,485269494	-0,482712037	-0,473771973	-0,468308914	0,020462615	0,093151643	4	NP_001117137.1	alb1	serum albumin 1	protein_coding	ssa01	101401500	101416191	+	ALB
gene25567:106564722	-0,302687239	-0,230701359	0,223158635	-0,278521368	-0,497934225	-0,496958331	4	XP_013986483.1	LOC106564722	uncharacterized LOC106564722	protein_coding	ssa12	21937901	21943131	+	
gene25607:106564757	-0,385790228	-0,470279093	-0,131529464	-0,284656324	-0,479531199	-0,444877124	4	XP_013986576.1	LOC106564757	keratin%2C type I cytoskeletal 19-like	protein_coding	ssa12	22663906	22670694	+	KRT14
gene27731:100136370	0,800390936	-0,219321214	-0,202040571	-0,292211666	-0,292211666	-0,292211666	4	NP_001117009.1	LOC100136370	rod opsin	protein_coding	ssa13	17383791	17385341	-	RHO
gene27862:106566684	0,128367607	-0,193073945	0,000788095	-0,089512394	-0,731153687	-0,689181123	4	XP_013990433.1	LOC106566684	interleukin-17 receptor B-like	protein_coding	ssa13	22206269	22224385	+	IL17RB
gene28545:100196802	-0,092599734	-0,14368027	0,873185721	-0,17793717	-0,271363533	-0,333867434	4	NP_001135296.1	egr1	early growth response 1	protein_coding	ssa13	50010466	50011397	-	
gene28581:100534607	-0,078444341	-0,227208055	-0,182815047	0,082243636	-0,59861529	-0,45044232	4	NP_001191824.1	il4/13a	interleukin 4/13A	protein_coding	ssa13	51820649	51821677	+	
gene29678:106568603	-0,174187686	-0,203404386	0,609799704	-0,228629834	-0,460202392	-0,634923848	4	XP_013994532.1	LOC106568603	endonuclease domain-containing 1 protein-like	protein_coding	ssa13	106700504	106702200	-	ENDOD1
gene32174:106571028	-0,222218225	-0,165400982	0,840568538	-0,140629379	-0,15595282	-0,260839073	4	XP_013999132.1	LOC106571028	proto-oncogene c-Fos-like	protein_coding	ssa15	14609313	14612113	-	FOS
gene3275:106573538	-0,509538239	-0,447800515	-0,511181141	-0,499414306	0,109784899	-0,019378928	4	XP_014004138.1	LOC106573538	uncharacterized LOC106573538	protein_coding	ssa02	2356770	2358108	-	
gene36715:106575629	-0,408815029	-0,247953016	0,29152925	-0,244057372	-0,680151665	-0,708280264	4	XP_014007714.1	LOC106575629	intermediate filament protein ON3-like	protein_coding	ssa17	20562725	20572107	-	
gene36716:106575633	-0,406916058	-0,435035272	0,178862766	-0,292911889	-0,599232407	-0,60091801	4					ssa17	20572524	20578931	+	
gene36721:106575639	-0,322586003	-0,374404002	0,026190566	-0,226801801	-0,595818826	-0,605056899	4	XP_014007725.1	LOC106575639	keratin%2C type I cytoskeletal 18-like	protein_coding	ssa17	20837758	20843942	-	
gene36722:106575637	-0,457513458	-0,174247303	-0,092484515	-0,155405948	-0,648938344	-0,63215701	4	XP_014007720.1	LOC106575637	intermediate filament protein ON3-like	protein_coding	ssa17	20844149	20852680	+	
gene3724:106577517	-0,405461167	-0,39092214	-0,405461167	-0,39727051	0,031423179	0,011168581	4	XP_014011091.1	LOC106577517	uncharacterized LOC106577517	protein_coding	ssa02	12357758	12360758	+	
gene38949:106577833	-0,252537336	0,094719959	0,678103998	-0,17929556	-0,389389843	-0,392709971	4	XP_014011670.1	LOC106577833	interleukin-8-like	protein_coding	ssa18	58439621	58445037	+	CXCL9
gene41976:100136573	-0,48550034	-0,45584762	-0,48550034	-0,474500684	0,033143708	0,007854859	4	XP_014017528.1	LOC100136573	apolipoprotein A-I	protein_coding	ssa20	49149151	49151678	+	
gene4257:106582922	-0,47743166	-0,437169223	-0,478098696	-0,494888181	0,079373578	0,132029832	4	XP_014022028.1	LOC106582922	fatty acid-binding protein 10-A%2C liver basic	protein_coding	ssa02	28354668	28356025	+	
gene44008:106582846	-0,850346072	-0,50664924	-0,110535225	-0,821265255	-0,672345409	-0,190026392	4	XP_014021834.1	LOC106582846	contactin-4-like	protein_coding	ssa22	13163849	13251411	-	
gene4565:106586353	-0,355291289	0,145535639	0,875326137	-0,262946834	-0,636624849	-0,594585626	4	XP_014029028.1	LOC106586353	uncharacterized LOC106586353	protein_coding	ssa02	42583121	42584942	+	
gene45814:106584519	0,016125307	0,090422255	-0,258051264	-0,041673983	-0,491654806	-0,517243866	4	XP_014025391.1	LOC106584519	troponin I%2C fast skeletal muscle-like	protein_coding	ssa23	36541741	36550314	-	TNNI2
gene47085:106585836	0,002851117	-0,719177043	-0,388371677	-0,51332022	-0,306102924	-0,400999273	4	XP_014027983.1	grid2	glutamate receptor%2C ionotropic%2C delta 2	protein_coding	ssa24	38608182	39219563	-	GRID2
gene47323:106586060	-0,157463753	-0,152151312	-0,142795576	-0,183967732	-0,250055447	-0,245402922	4	XP_014028361.1	LOC106586060	uncharacterized LOC106586060	protein_coding	ssa25	6826506	6836445	-	
gene4734:106588483	-0,210249243	-0,21229718	0,707565602	-0,18293748	-0,246575414	-0,247166896	4					ssa02	47265739	47271239	+	
gene48131:106586883	-0,316672382	-0,429893687	-0,593100025	-0,489083374	-0,082336449	-0,039678941	4	XP_014030085.1	LOC106586883	hemopexin-like	protein_coding	ssa25	47533370	47543269	-	HPX
gene48359:106587127	0,48921655	0,254597957	-0,213780656	-0,744663114	-1,061994376	-0,317293313	4					ssa26	10030234	10031155	+	
gene49891:106588285	0,250834086	0,668621761	-0,163384979	-1,022413859	-0,353781994	-0,92346223	4	XP_014032596.1	LOC106588285	secretagogin-like	protein_coding	ssa27	11058252	11066077	+	SCGN
gene5011:106590303	-0,246835421	-0,214820076	0,204329669	-0,278886199	-0,287965315	-0,331862718	4	XP_014036639.1	LOC106590303	trypsin-3-like	protein_coding	ssa02	56868745	56871573	-	
gene51215:106589658	-0,408269639	-0,25396215	-0,396325734	-0,476038082	-0,099442822	0,343236533	4	XP_014035375.1	LOC106589658	actin%2C alpha skeletal muscle 2-like	protein_coding	ssa28	19462451	19465523	-	ACTC1
gene5506:106594684	-0,232607996	-0,281587192	-0,29234921	1,736998095	0,168562528	-0,060881032	4	XP_014041557.1	LOC106594684	keratin%2C type I cytoskeletal 13-like	protein_coding	ssa02	69661251	69673197	-	
gene56561:106599980	-0,571397223	-0,178436082	-0,375080983	-0,129270589	-0,452180489	-0,560537771	4					NW_012492077.1	288	1230	+	
gene56607:106591791	-0,613364473	-0,417406658	0,202103395	0,388467723	0,346591523	-0,195653845	4					NW_012347232.1	20887	21367	-	
gene6676:106600326	-0,119284292	-0,052692248	-0,161648404	-0,406478265	-0,383873469	-0,161736271	4	XP_014047107.1	LOC106600326	calsequestrin-1-like	protein_coding	ssa03	35169354	35189711	+	CASQ1
gene9039:106602564	-0,389162233	-0,389573163	-0,405137527	-0,427659147	-0,095036759	0,059976021	4	XP_014050741.1	LOC106602564	fibrinogen gamma chain-like	protein_coding	ssa04	16886376	16892252	-	FGG
gene942:100194722	-1,064361108	0,068462323	0,390685751	-0,400188719	0,050629391	-0,597048953	4	XP_014052545.1	LOC100194722	uncharacterized LOC100194722	protein_coding	ssa01	41831690	41834961	+	
gene14787:106607975	0,241387376	0,507465628	-0,019768717	0,205666908	-0,968699471	-1,033992734	5	XP_014060991.1	LOC106607975	interleukin-12 subunit beta-like	protein_coding	ssa06	64862373	64873847	+	
gene15661:106608734	1,548460785	0,025128577	0,522249656	-0,016525742	-0,747029963	-1,124585322	5					ssa07	18601132	18604142	+	
gene18767:106611738	0,203209683	0,801414348	1,003183352	0,696158269	-1,102874949	-1,045768079	5	XP_014067736.1	LOC106611738	P2Y purinoceptor 4-like	protein_coding	ssa09	63937338	63941728	+	P2RY4
gene21210:101448057	-0,404565309	1,201895135	0,96080531	-0,523273626	-0,837100241	-1,158177075	5	NP_001266069.1	igfbp-1a1	insulin-like growth factor binding protein 1 paralog A1	protein_coding	ssa10	38367407	38369429	+	IGFBP1
gene21832:106560915	-0,032266391	0,67472951	0,290182622	0,078773207	-1,053202862	-0,861641469	5	XP_013979832.1	LOC106560915	C-C motif chemokine 8-like	protein_coding	ssa10	76254313	76255590	-	
gene23086:106562250	1,200066615	0,702020457	0,683497778	0,522393667	-1,638395361	-1,320704511	5	XP_013982489.1	LOC106562250	ammonium transporter Rh type C-like	protein_coding	ssa11	21627278	21633421	+	RHCG
gene24598:106563725	0,685803011	1,040712799	0,426453711	-0,099671028	-1,059487841	-0,966538593	5					ssa11	83239491	83249905	+	
gene25071:106564214	0,656755179	1,322761456	0,786398874	0,673971804	-1,077441504	-1,404842816	5	XP_013985737.1	LOC106564214	glycine-rich cell wall structural protein 1-like	protein_coding	ssa12	6372698	6375651	+	
gene26176:106565323	-0,472789892	0,914058347	0,805400951	-0,5609358	-1,295215519	-1,259954915	5	XP_013987747.1	LOC106565323	voltage-dependent calcium channel subunit alpha-2/delta-3-like	protein_coding	ssa12	47932845	47946980	-	CACNA2D3
gene27418:106566378	0,35585771	0,823140582	0,939053334	0,894661205	-1,479264762	-1,3714432	5	XP_013989815.1	LOC106566378	uncharacterized LOC106566378	protein_coding	ssa13	4931087	4940905	-	
gene31279:106569869	0,393386686	0,57524415	0,028841405	0,000166011	-0,777372945	-1,158876761	5					ssa14	61799242	61815450	-	
gene33086:106571918	-0,370929951	0,09591069	0,448414901	-0,071795979	-1,114360183	-1,248958334	5	XP_014000952.1	LOC106571918	cadherin-13-like	protein_coding	ssa15	60362044	60393132	+	
gene33124:106571957	0,045384827	1,269834671	0,450294085	-0,50662149	-0,882353967	-0,917914559	5	XP_014001044.1	LOC106571957	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2	protein_coding	ssa15	62385445	62390167	-	NDUFA4
gene34286:100195857	0,123608265	0,189652003	-0,046433845	-0,058626118	-0,997280256	-0,969881379	5	NP_001134358.1	cbpa1	Carboxypeptidase A1	protein_coding	ssa16	8327844	8350491	+	CPA1
gene37676:106576604	1,469298474	1,131321838	0,115487626	0,067767124	-0,780047202	-0,933923878	5	XP_014009323.1	LOC106576604	GTPase IMAP family member 7-like	protein_coding	ssa17	54852106	54852721	+	GIMAP7
gene37698:106576605	-0,104862287	0,080178345	0,209303324	-0,43798204	-0,982118444	-1,261332698	5	XP_014009324.1	LOC106576605	fish-egg lectin-like	protein_coding	ssa17	55333289	55335655	-	
gene4115:106581372	0,049268326	0,433568759	-0,134803227	-0,452990204	-0,558541859	-0,594493769	5	XP_014018867.1	LOC106581372	uncharacterized LOC106581372	protein_coding	ssa02	24458454	24465076	+	
gene4116:106581364	-0,188017274	0,590565645	-0,021794782	-0,114918118	-0,52062495	-0,639791897	5	XP_014018855.1	LOC106581364	uncharacterized LOC106581364	protein_coding	ssa02	24500570	24505363	-	
gene43872:106582708	0,106932848	0,47022203	0,838272685	-0,791994117	-1,038652101	-1,036578647	5	XP_014021516.1	LOC106582708	synaptotagmin-2-like	protein_coding	ssa22	7971682	8068956	-	
gene44890:106583693	0,573118556	0,530848752	0,225114224	-0,141684435	-1,231435798	-0,531989122	5					ssa22	52306704	52307584	+	
gene48358:106586984	0,837959494	0,454827785	0,242855745	-0,350549837	-1,064394611	-1,229510867	5	XP_014030247.1	LOC106586984	high choriolytic enzyme 1-like	protein_coding	ssa26	9996864	10017687	+	ASTL
gene48680:106587367	0,119077221	0,238335246	0,301175937	0,006544862	-0,891042609	-0,91914574	5	XP_014031185.1	LOC106587367	uncharacterized LOC106587367	protein_coding	ssa26	23359657	23408817	+	
gene4914:106589400	0,139660631	0,497342884	1,017334157	-0,275125772	-0,938611239	-1,234733865	5	XP_014034808.1	LOC106589400	tartrate-resistant acid phosphatase type 5-like	protein_coding	ssa02	53857223	53862205	+	ACP5
gene5277:106592430	0,885382288	1,562578847	0,487790989	0,314671602	-1,059847486	-1,217778265	5	XP_014039254.1	LOC106592430	GTPase IMAP family member 4-like	protein_coding	ssa02	63325948	63328591	-	GIMAP7
gene62201:106595409	0,41248323	1,118462592	0,553619043	0,843023873	-1,132493962	-1,345177506	5	XP_014042255.1	LOC106595409	GTPase IMAP family member 7-like	protein_coding	NW_012355609.1	5645	8212	+	GIMAP7
gene68156:106593189	0,757305696	0,714238156	1,179504718	0,24042822	-0,814501839	-1,324802141	5	XP_014039998.1	LOC106593189	uncharacterized LOC106593189	protein_coding	NW_012349610.1	159	8622	+	
gene68353:106591222	0,563512673	1,112580963	0,71565299	0,882659116	-1,010271605	-1,09842072	5	XP_014037909.1	LOC106591222	zymogen granule membrane protein 16-like	protein_coding	NW_012343574.1	2403	4869	+	ZG16
gene69438:100286614	0,585196704	0,448845128	-0,028915777	-0,470740334	-0,930305647	-1,099340225	5	NP_001140025.1	hce2	High choriolytic enzyme 2	protein_coding	NW_012366086.1	1	3469	-	ASTL
gene79981:106592578	0,113666383	1,141865378	0,40437224	0,120384408	-0,987985715	-1,404471584	5					NW_012348467.1	3676	5828	+	
gene8674:106602268	0,16787133	0,472461398	0,604893289	-0,349587903	-1,062300983	-1,158724228	5	XP_014050268.1	LOC106602268	hydroperoxide isomerase ALOXE3-like	protein_coding	ssa04	5828995	5854514	-	ALOXE3

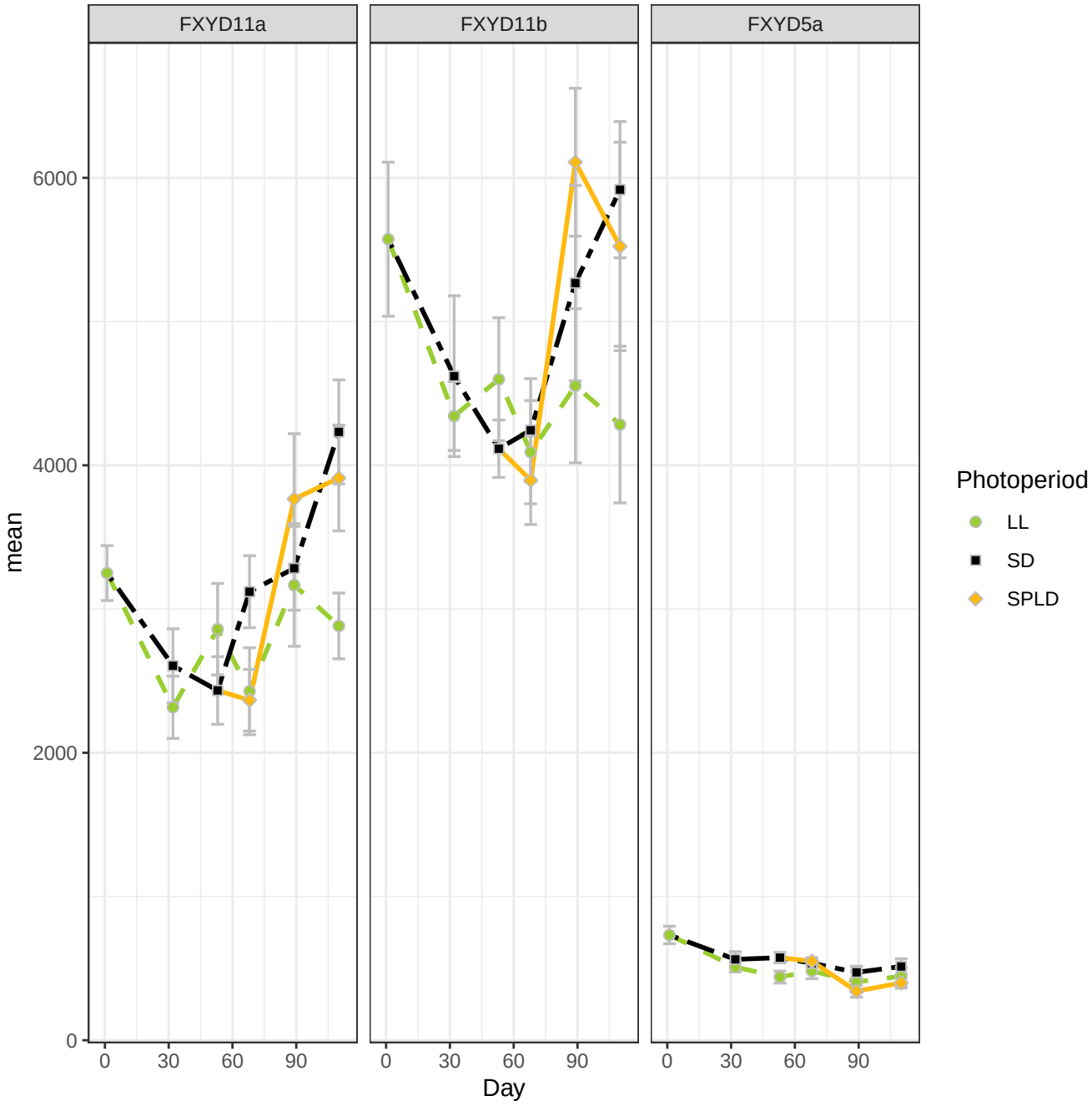
gene9637:100195688	0,151760513	0,368748131	-0,251165539	-0,635936553	-0,693028803	-0,833950506	5	XP_014051609.1	gbg8	Guanine nucleotide-binding protein GI/GS/GO subunit gamma-8	protein_coding	ssa04	35848015	35851398	+	
gene9793:106603265	0,303748353	0,265402887	0,284066887	-0,629940381	-0,694256326	-1,243195488	5	XP_014052154.1	LOC106603265	fish-egg lectin-like	protein_coding	ssa04	40443227	40445151	-	
gene17706:106610758	1,792954802	-0,629780328	-0,826222342	0,104257534	0,055169199	1,986749152	6					ssa09	14013752	14015503	+	
gene17920:106611031	1,617899773	-0,712671176	-0,810157249	-0,984225022	1,468934059	1,344047149	6	XP_014066318.1	LOC106611031	ectonucleoside triphosphate diphosphohydrolase 5-like	protein_coding	ssa09	22830680	22843733	+	ENTPD5
gene20092:100136358	2,058449826	-0,245419417	-0,33281164	-0,198983434	-0,231240296	1,270639358	6	XP_014070450.1	nos2	nitric oxide synthase 2%2C inducible	protein_coding	ssa09	121392631	121407938	-	NOS2
gene30598:106569487	1,060824474	-0,718227964	-0,738312255	-0,736290484	1,744183813	1,931602596	6	XP_013996353.1	LOC106569487	carbonic anhydrase 4-like	protein_coding	ssa14	36255144	36267130	+	
gene3471:100196478	0,719274321	-0,402519629	-0,461922311	-0,412233929	1,33505662	0,794822676	6	XP_014006826.1	necp1	Adaptin ear-binding coat-associated protein 1	protein_coding	ssa02	7353289	7361736	-	NECAP1
gene36658:106575572	1,219073939	-0,919231989	-0,89414642	-0,651766543	1,870609185	1,887817548	6	XP_014007630.1	LOC106575572	sodium/potassium-transporting ATPase subunit alpha-1-like	protein_coding	ssa17	19064834	19075775	-	ATP1A1
gene41046:106580130	1,788508483	-0,85065677	-1,02343953	-0,785642829	0,618075273	1,715013444	6	XP_014016312.1	LOC106580130	formin-binding protein 1-like	protein_coding	ssa20	11930618	11962433	-	FNBP1
gene41047:106580129	1,242822238	-0,598770565	-0,669816274	-0,635272527	1,640623971	1,599849959	6	XP_014016310.1	fibcd1	fibrinogen C domain containing 1	protein_coding	ssa20	12086759	12315059	+	FIBCD1
gene47511:106586095	1,601282202	-1,006515247	-1,021327806	-0,190834775	1,409464134	0,796658632	6	XP_014028429.1	LOC106586095	claudin-10-like	protein_coding	ssa25	16010279	16012077	-	
gene51373:106589822	0,935289011	-0,541461641	-0,87822906	-0,435657298	1,74709561	0,876439027	6	XP_014035674.1	LOC106589822	uncharacterized LOC106589822	protein_coding	ssa28	28107813	28124821	-	
gene52091:106590554	0,838724812	-0,437456745	-1,169133096	-0,484583466	1,714509101	0,958727539	6	XP_014037137.1	LOC106590554	uncharacterized LOC106590554	protein_coding	ssa29	29119823	29122471	-	
gene6804:106600484	1,454278192	-0,83799593	-0,713675099	-0,645627023	1,219092055	0,847256283	6	XP_014047334.1	LOC106600484	junctional protein associated with coronary artery disease homolog	protein_coding	ssa03	39961382	39991116	-	KIAA1462

Appendix 3:
Plots showing the FW expression of deiodinase and components of the NKA pump, data from the 2013-experiment (presented in Paper I).

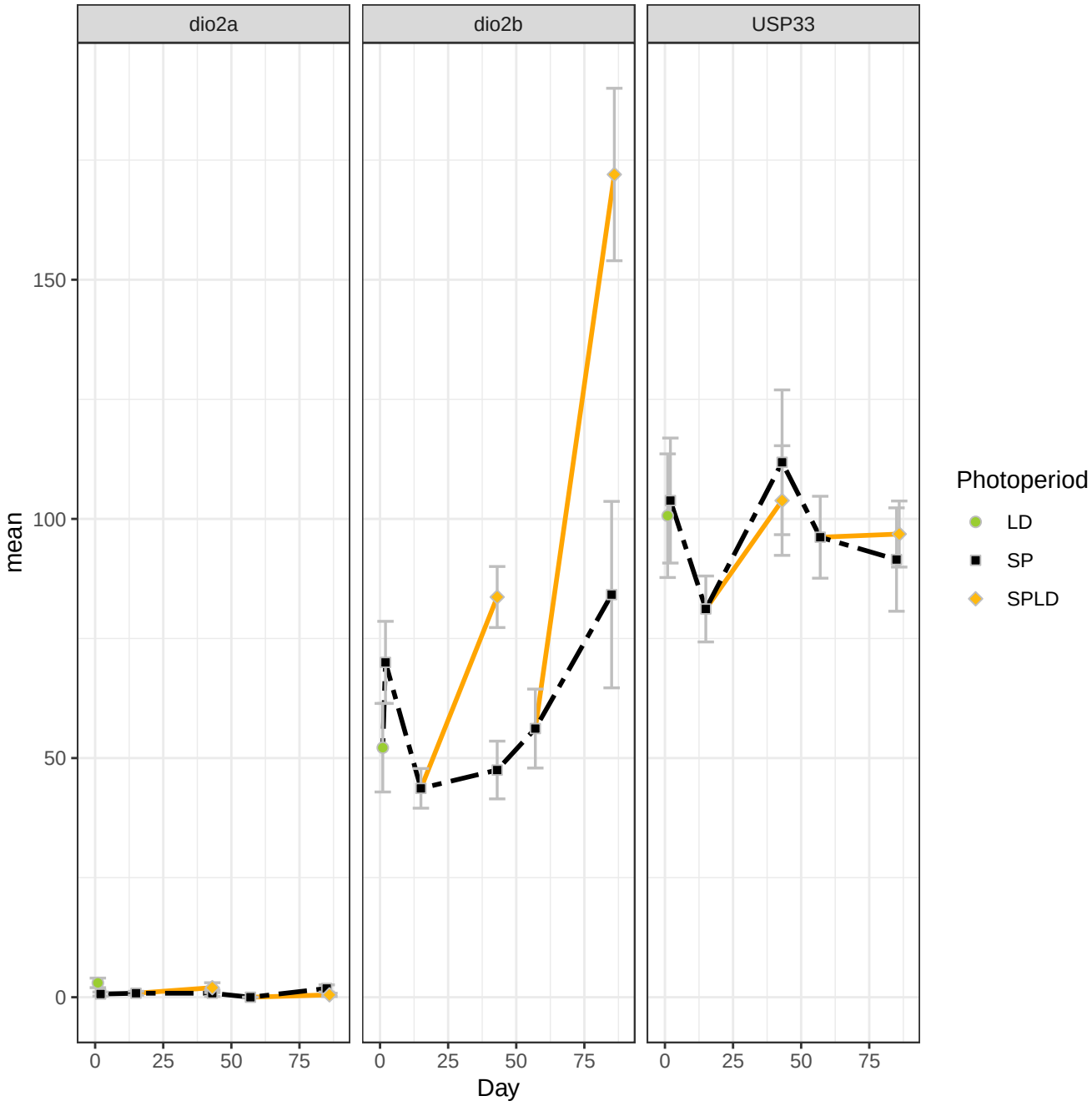


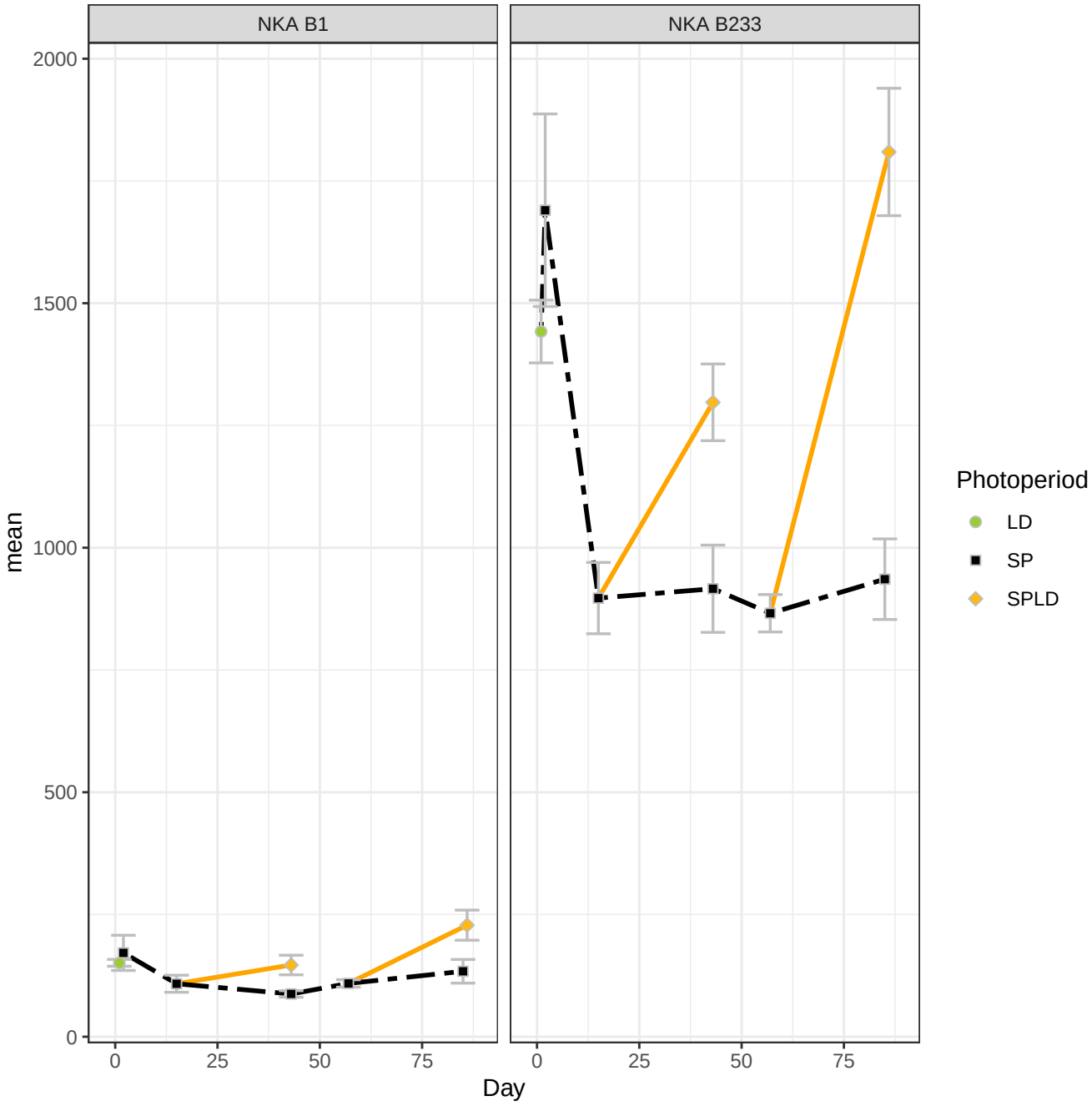

NKA B1
NKA B233
mean
800
600
400
200
0
0
30
60
90
Day
Photoperiod
LL
SD
SPLD

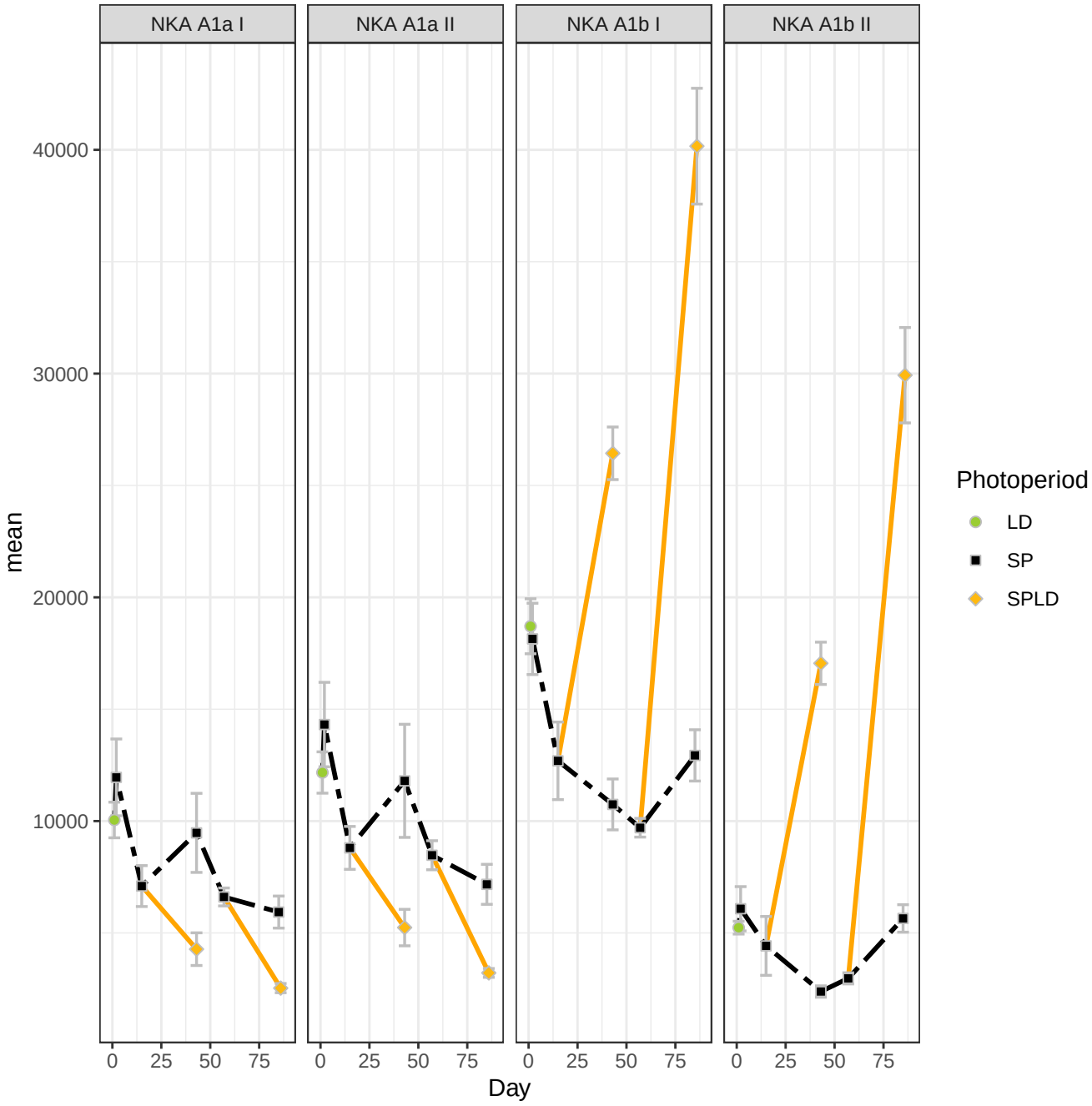

NKA A1a I
NKA A1a II
NKA A1b I
NKA A1b II
30000
20000
10000
mean
0
30
60
90
Day
Photoperiod
LL
SD
SPLD

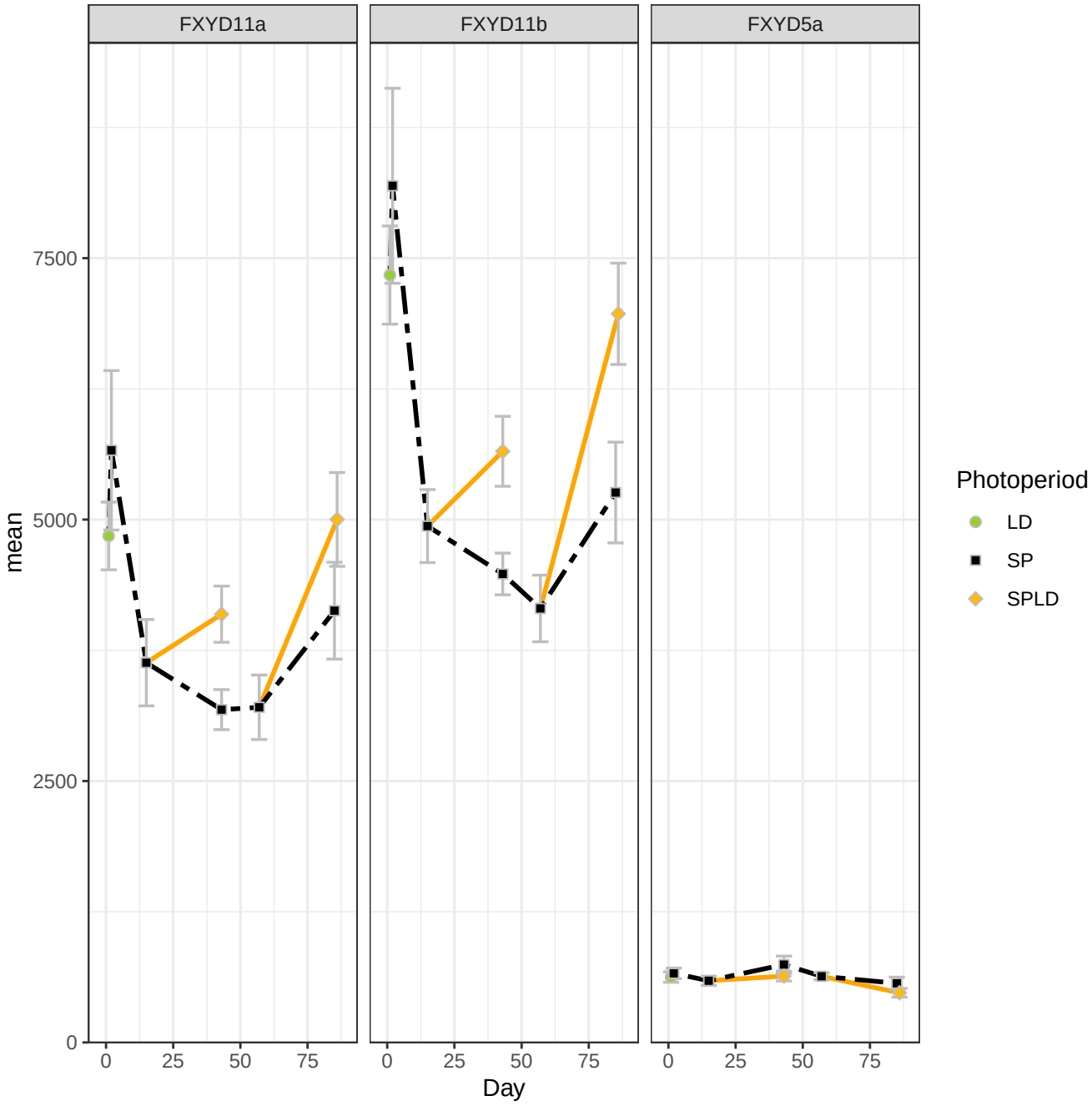

FXYD11a
FXYD11b
FXYD5a
mean
6000
4000
2000
0
0
30
60
90
Day
Photoperiod
LL
SD
SPLD

Appendix 4:
Plots showing the FW expression of deiodinase and components of the NKA pump, data from the 2017-experiment (presented in Paper I).




NKA B1
NKA B233
2000
1500
1000
500
0
mean
0
25
50
75
Day
Photoperiod
LD
SP
SPLD


NKA A1a I
NKA A1a II
NKA A1b I
NKA A1b II
mean
40000
30000
20000
10000
0
25
50
75
Day
Photoperiod
LD
SP
SPLD


FXYD11a
FXYD11b
FXYD5a
mean
7500
5000
2500
0
0
25
50
75
Day
Photoperiod
LD
SP
SPLD

Appendix 5:
Paper I

RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile Atlantic salmon

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Abstract

Atlantic salmon migrate to sea following completion of a developmental process known as smolting, which establishes a seawater (SW) tolerant phenotype. Smolting is stimulated by exposure to long photoperiod or continuous light (LL) following a period of exposure to short photoperiod (SP), and this leads to major changes in gill ion exchange and osmoregulatory function. Here, we performed an RNAseq experiment to discover novel genes involved in photoperiod-dependent remodeling of the gill. This revealed a novel cohort of genes whose expression rises dramatically in fish transferred to LL following SP exposure, but not in control fish maintained continuously on LL or on SP. A follow-up experiment revealed that the SP-history dependence of LL induction of gene expression varies considerably between genes. Some genes were inducible by LL exposure after only 2 weeks exposure to SP, while others required 8 weeks prior SP exposure for maximum responsiveness to LL. Since subsequent SW growth performance is also markedly improved following 8 weeks SP exposure, these photoperiodic history-dependent genes may be useful predictive markers for full smolt development.

Introduction

In anadromous salmonids, the transformation of freshwater resident juvenile fish (parr) into a migratory form (a smolt) which will migrate downstream migration and enter the sea is known as smoltification or smolting. Smolting entails a complex combination of physiological and behavioural changes, critical amongst which is the acquisition of the ability to efficiently maintain water and ionic balance upon entering the sea [1, 2].

In natural systems smolting is stimulated by the increasing day length (photoperiod) in spring, causing a cascade of physiological responses mediated by changes in circulating

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endocrine signals [1, 3–5]. The aquaculture industry depends on this photoperiod-dependence in the production of seawater (SW) tolerant juvenile salmon for transfer to sea cages in which rapid growth can take place. Smolting is artificially achieved by exposing juvenile salmon exceeding a minimum size threshold to short photoperiod (SP) for several weeks and then returning them to continuous light (LL). Based on observations of SW performance, it has been shown that the duration of exposure to SP should be at least six weeks long for LL to induce smolting [6]. The underlying causes of this photoperiodic history-dependence remain unknown, and untangling the role of SP exposure in smolt development is of considerable interest, as SP exposure reduces growth rates and slows aquaculture production.

The gill has a pivotal role in the energy demanding regulation of water and ionic fluxes, and it therefore undergoes extensive differentiation during the smolting process to pre-adapt to the SW environment. Within the gill, mitochondria rich cells (MRCs) are considered the primary drivers of ionic regulation, and smolting includes a pronounced shift in the location and phenotype of MRCs in the gill [7, 8]. During smolting the gill complement of MRCs shifts from an ion-absorbing FW type to an ion-secreting SW type, and the distribution of MRCs shifts from the lamellae to the gill filament itself [9]. Differences between FW and SW MRCs include a redistribution and change in composition of ionic pumps [2, 8, 10], the occurrence of an apical crypt and an extensive tubular network in the SW MRC, and the interdigitation of SW MRCs by accessory cells (ACs) [11–14].

The Na^+, K^+- ATPase (NKA) pump in the MRC cells has become an established marker for smolt state due to its marked increase in activity during smolting [1]. Further, it has become evident that its catalytic α-subunit has two protein isoforms, α1a and α1b, which are predominant in freshwater MRCs and in salt-water MRCs, respectively [2, 15–17]. Cystic fibrosis transmembrane conductance regulator I (CFTR I) is another ion channel protein considered a marker for smolt state due to upregulation of its mRNA during smolting [5, 18, 19]. Transcriptomic profiling studies have revealed additional genes associated with smolting in salmonids [20–22], and these offer the potential for increased understanding of the smolting process as well as providing novel markers.

The degree to which remodeling of gill tissue during smolting depends on photoperiodic history remains poorly characterized because extant studies have not sought to resolve history-dependent effects of photoperiod from direct effects of light or developmental age [4, 8, 23]. What is clear is that prior exposure to short photoperiod may enhance the capacity of juvenile salmon to perform well following transfer to SW [6, 24]. To assess the extent to which this priming effect of SP affects gill development, we have performed RNA profiling in smolting Atlantic salmon subjected to a range of different lighting protocols. Our data reveal a novel cohort of genes which expression is dramatically induced by exposure to LL, conditional on prior exposure to SP. Further, we show that history-dependence varies between genes which allows the identification of novel markers whose expression patterns are good predictors of subsequent SW growth performance.

Materials and methods

Animal welfare statement

The experiments were conducted as part of the continuously ongoing smolt production at Tromsø Aquaculture Research Station, approved by the Norwegian Animal Research Authority (NARA) for hold of, and experiments on salmonids, fresh- and salt-water fish and marine invertebrates. In accordance with Norwegian and European legislation related to animal research, formal approval of the experimental protocol by NARA is not required when the

experimental conditions are practices undertaken routinely during recognized animal husbandry, and no compromised welfare is expected.

Fish

Atlantic salmon (*Salmo salar*, Linnaeus, 1758, of the Aquagene commercial stain, Trondheim, Norway) were used for both experiments, and were raised from hatching in FW, on continuous light (LL, > 200 lux at water surface) at 10˚C (Experiment 1) and 4˚C (Experiment 2). Fish were fed continuously with pelleted salmon feed (Skretting, Stavanger, Norway).

Experimental set-up

During both experiments, all experimental groups were fed pellet salmon feed continuously and in excess with automatic feeders for eight hours a day, corresponding to the light phase under SP.

Experiment 1: This experiment utilized 237 juvenile salmon kept in a 500 L circular tank since start of feeding. The experiment was begun when the salmon juveniles had reached approximately 7 months of age (02.12.2013) and a mean weight of 49.5 g (s.d. ± 7.0 g, n = 6). One initial sampling (set as day 1 of the experiment, and referred to as pre-SP) was done in order to establish a pre-smolt baseline. Two days later (Day 3), 225 parr were taken from the original tank and randomly allocated into two 100 L circular tanks (FW, 8.5˚C) in separate rooms. One tank received 75 parr, and was kept on LL for the remainder of the experiment. The other tank received 150 parr, and the photoperiod was gradually decreased over a week from LL to short photoperiod of 8-h light/24-h (SP). Further samplings from both these groups were done on Days 32 and 53 (n = 6). At Day 60 half of the remaining SP group was moved to a new 100L and returned to LL (SPLL). Further sampling of the three groups were done on Days 68, 89, and 110, as shown in Fig 1.

Experiment 2: This experiment utilized 1400 fish at approximately 11 months old (beginning on 05.01.2017), weighing an average of 40.3 g (s.d. ± 9.7 g, n = 10). The juvenile salmon were distributed among eight 300 L circular tanks with FW at 7˚C and LL, and left to acclimate for one week. The total number of fish in each tank ranged from 150 to 200, depending on the number of fish to be sampled during the experiment in each tank and the need to avoid density-dependent social stress effects.

After an initial sampling at the last day of acclimation under continuous light (Day 1), fish in all tanks were transferred to SP. One group of fish remained on SP for 16 weeks (SPC group), while the three other groups were kept on SP for two, four or eight weeks (2WSP, 4WSP and 8WSP groups, respectively; collectively termed the SP-LL groups). LL exposure then continued for a further 8 weeks. All treatments were run in duplicate tanks. After the initial sampling, all SP-LL groups were sampled on the last day of SP, and at four and eight weeks post-SP. For the SP-LL groups the two post-SP sampling points corresponded to 196 and 392 degree-days (˚d) after re-entering LL. At each of these sampling points, samples were also collected from the SPC group.

24-hours Salt-Water Challenge (SWC). In both experiments, 24-h prior to each FW sampling point, randomly selected fish (n = 6 for exp.1, and n = 10 for exp.2) were transferred to 100 L tanks supplied with full strength SW (7˚C, 34‰ salinity) for 24 hours. No feed was given during this 24-h period. There were no mortalities during the SWCs. After 24 h, the fish were netted out and lethally anesthetized (10 L water container, SW, Benzocaine, 150 ppm), followed by blood sampling, decapitation and tissue dissection as described below.

Blood sampling and tissue dissection. Following lethal anesthesia (in 10 L water container, FW or SW as appropriate, Benzocaine, 150 ppm), body masses (± 0.5 g) and fork

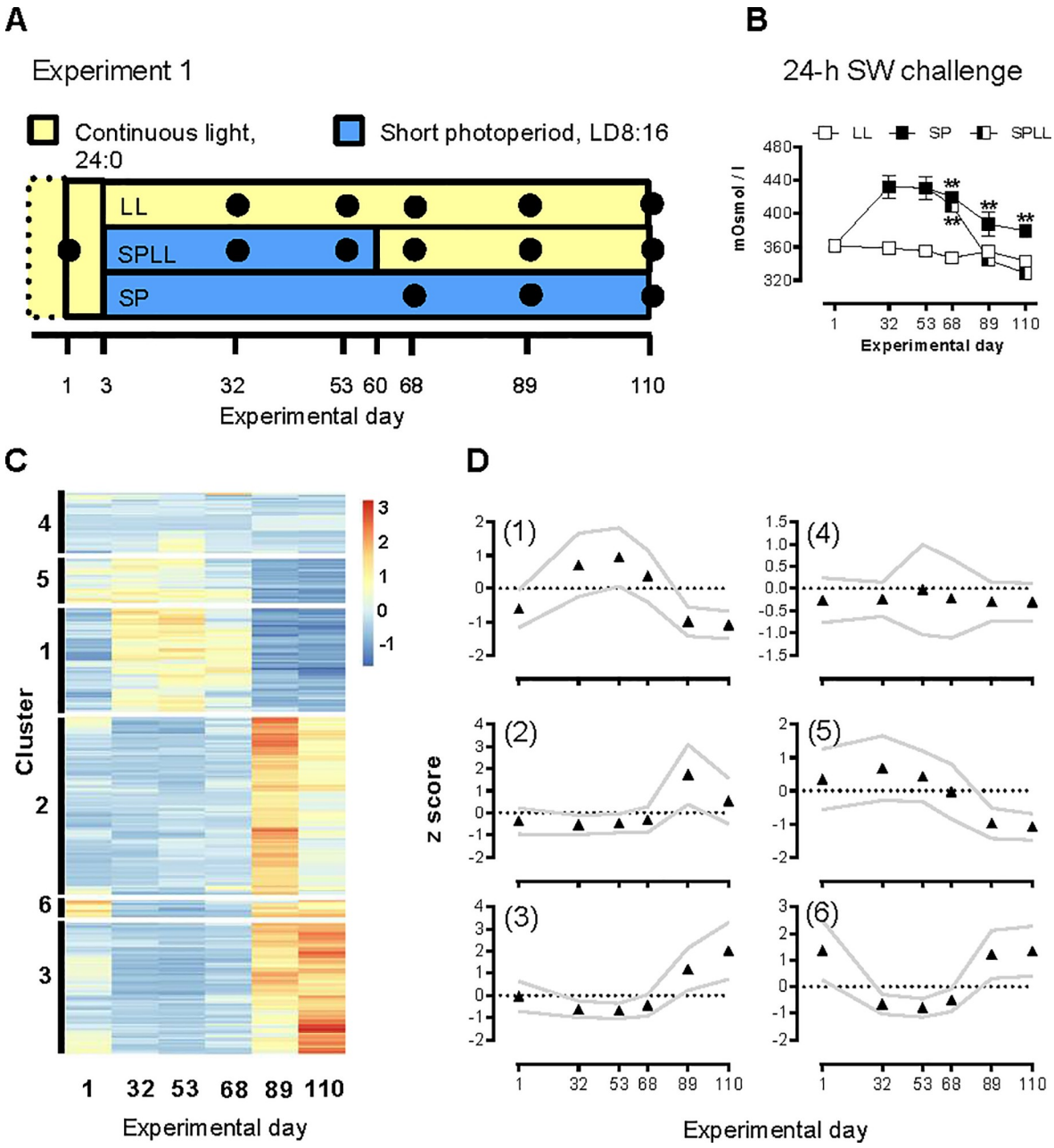

Fig 1. RNA profiling of photoperiodic history-dependent changes in gill gene expression in juvenile Atlantic salmon. A) Schematic presentation of experiment 1. Sampling time-points are indicated by black dots. B) Plasma osmolality (mOsm kg^{-1}) following a 24-h SW challenge; data are mean ± SEM of n = 6 fish per sampling point. ** significantly higher osmolality than in LL and SPLL fish at the same sampling point, $p<0.01$. Where error bars do not appear, errors lie within the symbol. C) Heatmap showing genes that are highly differentially expressed (FDR<0.01, logFC >|2|) between experimental groups over the three latter sampling points of exp.1. Hierarchical clustering has been used to generate six clusters. D) The averaged expression profile (z score) of the six clusters of DEGs, data are mean ± SEM, n = 6.

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lengths (± 0.1 cm) were recorded (For FW, n = 6 for exp.1 and n = 10 for exp.2). Blood was collected from the caudal vein into 2 ml Lithium-heparinized vacutainers (BD vacutainers®, Puls Norge, Moss, Norway), and placed on ice until it was centrifuged (6000 x *g*) for 10 min. The plasma fraction was collected and stored at -20˚C for later analysis of osmolality and

chloride concentration. Fish were then decapitated and dissected. After decapitation, the operculum on the right side of the head (caudal view) was removed and primary gill filaments were collected and placed in RNAlater® (Sigma-Aldrich, St. Louis, Missouri, USA) for transcript and qPCR analyses. Samples were stored at 4˚C for 24 h, and then kept frozen at -80˚C until further processing.

During Experiment 2, two secondary filaments (2–3 mm) were also collected and placed in 100 μL ice cold SEI buffer (0.15 M sucrose, 0.01 M Na_2EDTA, 0.05 M Imidazole, in H_2O, pH 7.3, Sigma-Aldrich, St. Louis, Missouri, USA) and immediately frozen at -80˚C until analyses of NKA activity.

Prolonged salt-water exposure following smolt induction. Following maintenance on LL for eight weeks, 30 randomly selected fish from each of the SP-LL groups (fifteen from each duplicate tank) were netted out and anaesthetized (Benzocaine, 60 ppm), and fork length and body mass was measured. After recovery fish were placed in 300 L, circular tanks supplied with full strength SW (34 ‰) at 7˚C and continuous light. Fish were fed pelleted salmon feed continuously and in excess by automatic feeders. The amount of feed eaten was monitored daily by collection of feed remnants from the tank outlet sieve. After 15 days in SW, fork length and body mass were again recorded as above, before returning the fish to the SW tanks for a further 15 days. On day 30 of SW exposure, all fish were anaesthetized with benzocaine (150 ppm, Sigma-Aldrich, St. Louis, Missouri, USA), after which fork length and body mass were recorded, and the fish decapitated. No fish died during the prolonged SW exposure. Fish from the SPC group were not subjected to extended SW exposure for animal welfare reasons associated with the anticipated lack of SW tolerance.

Analyses

Plasma osmolality and chloride levels. Thawed plasma samples were analysed using a Fiske One-Ten Osmometer (Fiske Associates, Massachusetts, USA, ± 4 mOsm kg^{-1}) and a Chloride Analyzer from CIBA Corning Diagnostics (Essex, England, ± 2.2 mmol L^{-1}).

NKA activity. NKA activity (experiment 2) was measured in gill samples by a method described by McCormick [25] and Schrock et al. [26]. The assay measures the rate of hydrolysis of ATP to ADP and P_i, which is coupled to the oxidation of NADH to NAD^+. Briefly, the gill tissue was thawed and homogenized in SEI buffer, and the supernatant assayed for ATP-ADP hydrolysis activity, with and without the NKA activity inhibitor ouabain, by spectrophotometer readings (Spectramax Plus 384, Molecular Devices Corp., California, USA) at 340 nm at 30 second intervals over 10 minutes. Protein was measured using the Pierce BCA Protein Assay kit (Thermo Fisher, Waltham, Massachusetts, USA) utilizing a bicinchoninic acid method [27]. From the measured change in ADP levels and amount of protein, NKA activity is expressed in units of activity per mg protein.

RNA extraction. Gill tissue was disrupted using TissueLyser II (QIAGEN, Hilden, Germany). Total RNA from experiment 1 was extracted using the RNeasy Plus Universal Mini Kit (QIAGEN, Hilden, Germany). For experiment 2 total RNA was extracted using a TRIzol-based method (Invitrogen, Thermo Fisher, Waltham, Massachusetts, USA) and following the manufacturer's protocol. RNA concentrations were measured using a NanoDrop ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). RNA samples were frozen at -80˚C until further processing.

Transcriptome sequencing. Sequencing libraries were prepared using the TruSeq Stranded mRNA HS kit (Illumina, San Diego, California). Library mean length was determined by a 2100 Bioanalyzer using the DNA 1000 Kit (Agilent Technologies, Santa Clara, California, USA) and library concentration was determined with the Qbit BR Kit (Thermo

Scientific, Waltham, Massachusetts, USA). Each sample was barcoded using Illumina unique indexes. Single-end 100bp sequencing of sample libraries was carried out on an Illumina HiSeq 2500 at the Norwegian Sequencing Center (University of Oslo, Oslo, Norway).

Cutadapt [28] was used to remove sequencing adapters, trim low quality bases, and remove short sequencing reads using the parameters -q 20 -O 8—minimum-length 40 (version 1.8.1). Quality control of the reads were performed with FastQC software [29]. Mapping of reads to reference genome was done using STAR software (ver. 2.4.2a) [30]. HTSEQ-count software (version 0.6.1p1) [31] was used to generate read count for annotated genes.

Transcriptome analysis. Analysis of differential gene expression in experiment 1 was performed with package edgeR (ver. 3.14.0) using R (ver. 3.4.2) and RStudio (ver. 1.0.153). Prior to higher level analyses, the raw counts were filtered, setting an expression level threshold of a minimum of one count per million reads (cpm) in five or more libraries. The counts were scaled by applying trimmed means of M-values (TMM) scaling. The data was fitted with a quasi-likelihood negative binomial generalized log-linear model. Two tests, (empirical Bayes quasi-likelihood F-tests), contrasting between the SPLD group and the LL or SP group for the three latter sampling points (days 68, 89 and 110), were applied to compare the SPLL light regime with the LL and SP regimes. Both outputs were filtered using a false discovery rate (FDR) < 0.01, and for genes to show a $\log_2$-fold change $> |1|$.

The resulting outputs from the SPLL vs. LL and SPLL vs. SP comparisons were combined to form a list of unique genes that showed significant photoperiod-dependent changes in expression. The count data (cpm) for those genes was extracted for the SPLL-group, and row-scaled by calculating z-scores. The R-package pheatmap (ver. 1.0.10) was used to clusters the genes into six clusters applying Euclidian distance measures and complete linkage clustering. The sum of squared error (SSE) and gap statistic were used to evaluate which number of clusters to use. Cluster centroids were calculated, and the correlation between centroid and genes checked for uniformity.

One cluster, appearing to represent light responding, consequently upregulated, genes was chosen for further examination. The expression profile of the genes, their expression levels, magnitude of $\log_2$-fold change and FDR value was evaluated and a small set of six genes (Table 1) for which primers could be successfully be developed and confirmed was selected for further testing of relevance in experiment 2. Gene functions were briefly investigated using GeneCards [32, 33]. Since the gene symbols of the targeted genes consist of numerical string we opted to use HGNC symbols, based on the gene description, when referring to the genes in the text.

Real-time quantitative PCR. RNA samples from experiment 2 were ethanol-precipitated and DNAse-treated according to the manufacturer's protocol (TURBO DNA-free Kit, Thermo Fisher). cDNA was constructed using the High-Capacity RNA-to-cDNA kit (Thermo Fisher, Waltham, Massachusetts, USA), following the recommended protocol.

Primers (Table 1) were designed to target all splice variants of the target genes, while not picking up ohnologue and paralogue duplicates of the targeted genes. Primer3 [34, 35] and ApE software (v2.0.51) were used for designing primers, and primers were checked against both the National Center for Biotechnology Information (NCBI, Bethesda, Maryland, USA) database using BLAST [36] and the SalmoBase database [37] for non-target hits. Primer specificity was confirmed by melt-curve analysis, and amplicon size verified by agarose gel electrophoresis. In order to establish primer amplification efficiencies a subset of samples were pooled and diluted, and analyzed by qPCR. Amplification efficiencies fell between 90% and 110%.

Real-time quantitative PCR analysis was performed using a BioRad CFX Connect Real-Time instrument (Hercules, California, USA), and SYBR Green detection. Reactions were

Table 1. Primer sequences for target genes.

Target	Gene symbol		Sequence (5' to 3')	Annealing temperature˚C	Product length (bp)
EF1A	LOC100136525	F	AGGCTGCTGAGATGGGTAAG	63	218
		R	AGCAACGATAAGCACAGCAC		
NKA a1b (ii)	LOC 106575572	F	GGGTGTGGGCATCATTTCTG	66	152
		R	CATCCAACTGTTCGGCTGAC		
CFTR I	LOC 100136364	F	CCTTCTCCAATATGGTTGAAGAGGCAAG	63	81
		R	GCACTTGGATGAGTCAGCAG		
CAPN2	LOC 106589985	F	GTTGAGGAGATCGTGGTGGA	65	118
		R	TGTTCAGAATCCTCCGCAGT		
TPH1	LOC 106562311	F	ACTTCCTCAGAGAACGCACA	63	218
		R	CTGGGAGAACTGGGCAAAAC		
S100A1	LOC 106570104	F	GGATGACCTGATGACGATGC	65	122
		R	ATCACATACTCCCCACCAGG		
ST6GALNAC2	LOC 106589898	F	CTTCGACCGCCAATATCACC	63	149
		R	ATGGCAACCTTGAGTGAGTT		
FKBP5	LOC 106565346	F	CTGGGAAAGGGTCAGGTGAT	65	264
		R	GACTGTTGATCCGTCGTTGG		
SLC5A7	LOC 106602131	F	AGGTGGGACGTGTTTCAGAT	65	203
		R	CCCGACCAACAAAACCCTTT		

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carried out on 96-well plates, with 20 ng RNA cDNA equivalent, 250 nM forward and reverse primer, and 1x Sso Advanced Universal SYBR Green Supermix (BioRad, Hercules, California, USA), in a total volume of 20 μL. After initial heating (95˚C, 30 sec.), amplification was carried out under the following conditions: 95˚C for 10 sec., and primer-specific annealing temperature for 1 min. over 40 cycles. A melting curve analysis was completed at the end of each run (0.5˚C intervals at 3 sec., from 65˚C to 95˚C).

Data analysis and statistics. Condition factor (CF) was calculated as

$$CF = W \times \left(\frac{100}{L^3}\right). \quad (1)$$

where W is wet body mass (g), and L is fork length (cm).

Specific growth rate (SGR) was calculated as

$$SGR = \left[\frac{(ln\bar{W}_T - ln\bar{W}_t)}{(T-t)}\right] \times 100. \quad (2)$$

where W_t and W_T are mass (g) at the beginning and end of the period of extended SW exposure, respectively. Similarly, the feed conversion ratio (FCR) over the same period was calculated by dividing the total amount of ingested food per tank (g, dry weight) by the increase in total biomass for each tank.

The C_t values of target genes were normalized against EF1A [38, 39] using the $\Delta\Delta C_t$ method described by Livak [40].

GraphPad Prism (ver. 7.03) was used for statistical computation of one- and two-way ANOVAs for physiological measurements and relative mRNA content for both exp.1 and exp.2. Summary statistics are given as mean ± standard deviation (S.E.M.).

Experiment 1: Effects of photoperiod regime (treatment) and time (i.e. time passed after returning to LL for the SPLL group) over the three latter sampling points were assessed by two-way ANOVA, and Tukey's test for post hoc pairwise comparisons. A one-way ANOVA

was applied to test for significant differences between the initial sampling and any other sampling, applying Dunnett's test for multiple comparisons. The statistical significance threshold was set to $p < 0.05$.

Experiment 2: Effects of photoperiod regime (treatment) and time (i.e. time passed after returning to continuous light for SP-LL groups) were assessed by two-way ANOVA, and Tukey's test for post hoc pairwise comparisons. To avoid pseudo-replication of data the initial sampling point (day 1), which is common for all groups, was excluded from the ANOVA analysis. Data from this sampling point is provided in figures for reference, and a one-way ANOVA was performed to test for any significant differences between the initial sampling point and all other samplings, applying Dunnett's test for multiple comparisons. The statistical significance threshold was set to $p<0.05$.

Results

Experiment 1

Experiment 1 is summarized in Fig 1A.

Hypo-osmoregulatory capacity. The capacity to hypo-osmoregulate in response to an acute (24-h) SW challenge was time- and photoperiod dependent (Fig 1B, $p<0.0001$ for time, photoperiod regime and the interaction term, two-way ANOVA, Fig 1B, S1 Table). On day 1, prior to SP transfer, plasma osmolality after 24-h in SW was about 360 mOsm kg^{-1}. The LL group maintained this capacity for hypo-osmoregulation throughout the experiment. Fish that were transferred to SP lost their osmoregulatory capacity by day 32, but then underwent a partial recovery when exposure to SP conti nued to the end of the study. Fish that were transferred back onto LL on day 60 had fully recovered their hypo-osmoregulatory capacity by day 89 of the experiment. Plasma chloride levels followed the same pattern (S1 Fig). Size is not believed to have influenced osmoregulatory capacity post-SP as there were no significant differences in weight between the three groups on day 110 (LL: 91.3 ± S.D, SP: 78.4 ± SD, SPLL: 81.7 ± SD, n = 6 for all groups, S4 Table).

RNA profiling of gill tissue. In order to identify novel photoperiod-dependent changes in gill gene expression we analyzed the transcriptome of the gill, focusing on expression changes across the three last sampling points of experiment 1. This revealed 389 unique transcripts showing photoperiod-dependent changes, after applying filtering criteria. Hierarchical clustering identified six major clusters with distinctive profiles (Fig 1C and 1D, S8 Table) for the SPLL group. In numerical order the clusters consisted of 75, 129, 96, 44, 32 and 12 genes.

Clusters 1 and 6 showed reversible photoperiod-dependent changes in expression over the study as a whole, with the *NKA α1b* gene being placed in cluster 6. Contrastingly, cluster 3 was distinctive in exhibiting a photoperiod-dependent increase in expression over the latter timepoints of the study, and lower expression levels at the initial sampling point. This suggested to us that this cluster comprised genes involved in photoperiodic history-dependent smolt transition. Apart from CFTR I, which is closely linked to smolting [5, 18, 19], genes in cluster 3 have not previously been linked to gill differentiation during smolting [20, 21, 41].

Fig 2 shows the TMM normalized RNA expression profiles for 6 novel smolting genes representative of cluster 3, as well as for *CFTR1* and *NKAα1b*. For all 6 novel genes the developmental change in expression over the last 3 sampling points of the study was highly dependent on photoperiodic history ($P <0.0001$ for treatment x time interaction by 2-way ANOVA, S6 Table). In all cases expression was lowest at day 1, and highest at the end of the study in fish that had been transferred to SP and then returned to LL. In fish maintained throughout on SP or LL, expression levels did not change significantly over time, and final values were markedly lower than in corresponding SPLL fish.

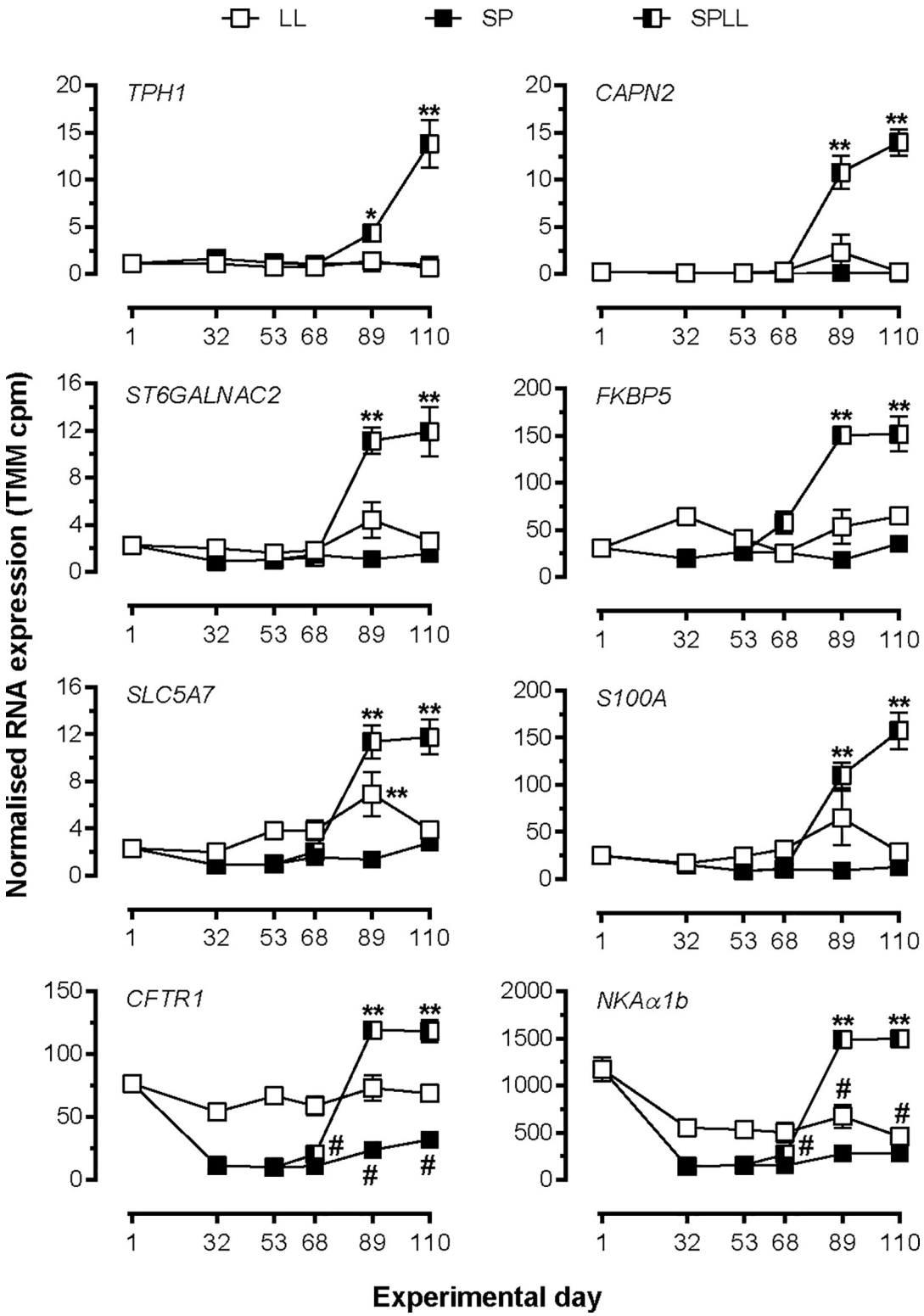

Fig 2. Temporal expression profiling of selected genes from cluster 3 in experiment 1. Data are presented as normalized (TMM) counts and are mean± SEM of n = 6 fish, except for SPLL on day 68 where n = 5. *, ** significantly higher expression than LL and SP values at the corresponding time point, $p<0.05$, 0.01, respectively; # significantly lower expression than at day 1, $p<0.05$. Where error bars do not appear, errors lie within the symbol.

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In contrast to these novel genes, the expression of both *CFTR1* and *NKAα1b* was relatively high at day 1 of the experiment and declined markedly with transfer to SP. Return of SP fish to LL led to a return to elevated values, which were 25–50% higher than day 1 values. For both genes maintenance on SP maintained low levels of expression throughout, and for *NKAα1b* continuous exposure to LL caused a progressive decline in expression so that values at the end of the study in LL fish were significantly lower than at the start of study ($p<0.0001$ by 1-way ANOVA).

Experiment 2

Experiment 2 is summarized in Fig 3A. To further characterize the apparent requirement for exposure to SP for induction of expression of the cluster 3 genes, juvenile salmon were exposed to two, four or eight weeks of SP, before being returned to LL (2WSP, 4WSP, 8WSP, respectively), and their short- and long-term SW-tolerance and gene expression were assessed. Complete information on growth and CF during the FW and SW stays can be found in S5 Table.

Hypo-osmoregulatory capacity. The ability to hypo-osmoregulate during a 24-h SW challenge was not dependent upon prior exposure to SP, but rather time spent after re-entering LL ($p<0.0001$, for main effect of time, by two-way ANOVA, Fig 3B, S2 Table). As in Experiment 1, the fish were able to hypo-osmoregulating efficiently on day 1 of the experiment, and this ability was lost within two weeks of transfer to SP, as evidenced by the increased levels of plasma osmolytes ($p<0.0001$, one-way ANOVA). The dynamics of re-establishment of hypo-osmoregulatory capacity following return to LL did not differ between the SP-LL groups, which developed smolt-like hypo-osmoregulatory capacity within four weeks of re-entering LL. The SPC group spontaneously regained its ability to osmoregulate towards day 86 after having spent more than 12 weeks under SP. Though slightly higher levels were measured on day 113, plasma osmolality levels of the SPC group were not statistically different from those measured on day 1, nor at the end-points of the SP-LL groups. Chloride plasma levels followed a similar pattern as described for plasma osmolytes.

NKA activity. Because gill NKA activity is considered a good indicator of osmoregulatory capacity and smolt status we also examined how this trait was influenced by photoperiodic history (Fig 3C). In contrast to plasma osmolality and chloride levels, gill NKA activity did not change significantly under chronic exposure to SP (SPC group). However, the development of NKA activity following return of fish to LL was highly dependent on photoperiodic history ($p<0.001$, for time x photoperiod regime, by two-way ANOVA, supplemental material S3 Table). In fish exposed to SP for two weeks no significant rise in gill NKA activity was seen during the subsequent eight weeks of LL exposure, while in the 8WSP group NKA activity rose approximately five-fold over eight weeks of LL exposure ($p<0.001$, two-way ANOVA). In 4WSP fish, an intermediate response was observed, with NKA activity rising some two-fold over the post-SP phase. Gill NKA activity does not appear do predict performance in 24-h SW challenges.

RNA profiling of gill tissue. We used qPCR to assess the expression of the six novel transcripts selected from Experiment 2, and of *CFTR I* and *NKA α1b* (Fig 4). For each of the novel cluster 3 transcripts induction of expression by exposure to LL was highly dependent on the duration of prior SP exposure ($p<0.001$, time x photoperiod regime interaction, by two-way ANOVA, S7 Table), with the strongest induction of expression consistently being observed in the 8WSP group ($p<0.001$, one-way ANOVA), with the exception of *ST6GALNAC2*. No significant increases above day 1 expression levels was seen for the 2WSP group at any point following return to LL, while in the 4WSP group one could observe intermediate increases, with

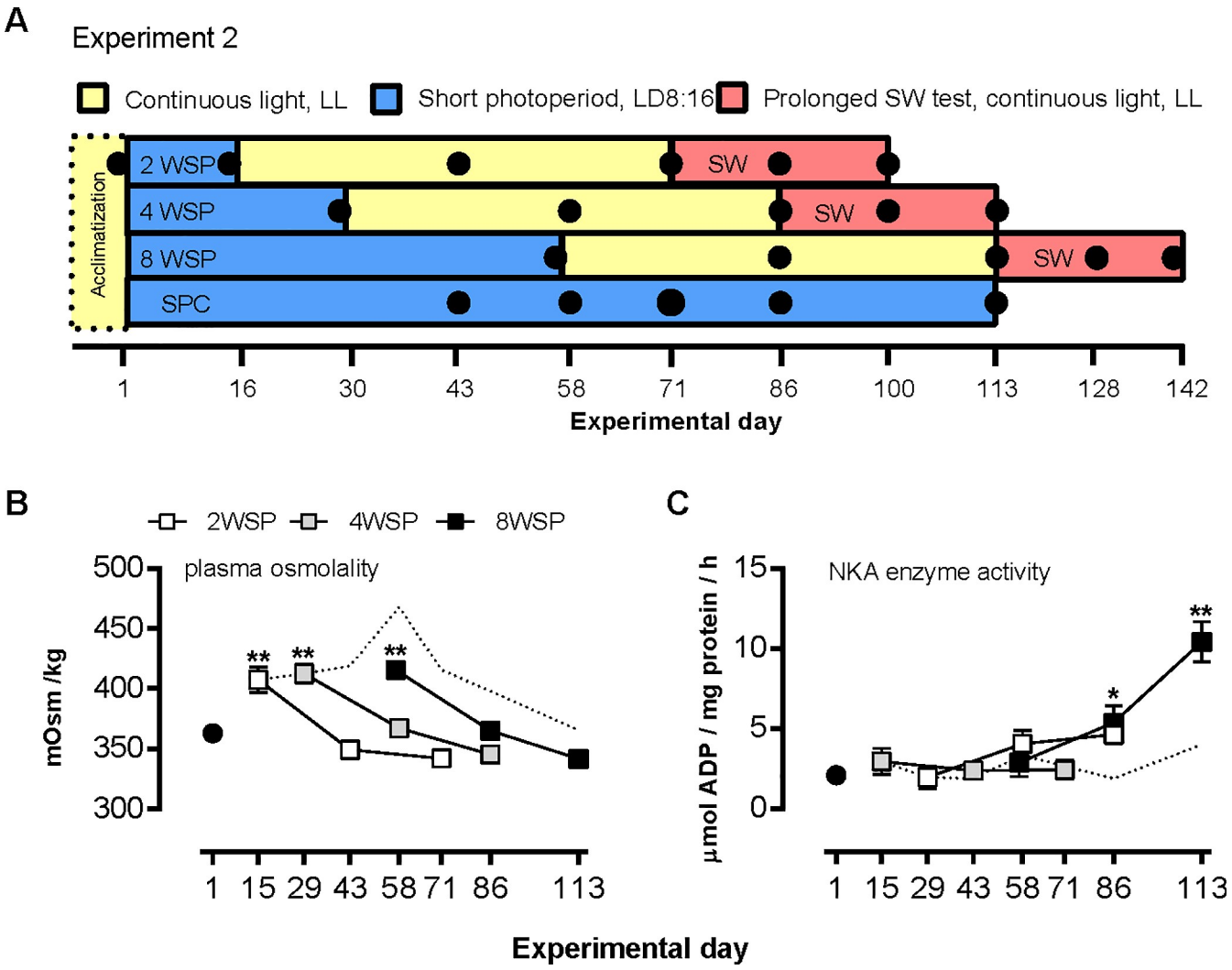

Fig 3. Effect of SP exposure duration on smolting performance parameters. (A) Experimental design for experiment 2. (B) Plasma osmolality after 24-h SW challenge tests at the indicated sampling points. Data are mean ± SEM of n = 9–10 fish per sample point. **, significantly higher values than at day 1 and four and eight weeks after return to LL, $p<0.01$. (C) Gill Na^+, K^+- ATPase activity; data are mean ± SEM of n = 6–10 fish per sampling point. *, **, significantly higher activity than at day 1 of the experiment, $p<0.05$, 0.01, respectively. Where error bars do not appear, errors lie within the symbol. The dashed line represents the SP control group.

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significant differences to pre-SP levels for *S100A1*, *ST6GALNAC2*, *SLC5A7* and *CAPN2* ($p<0.05$, one-way ANOVA).

A clear dependence on photoperiodic history was also observed for *CFTR I* ($p<0.0001$, time x photoperiod regime, two-way ANOVA, S7 Table), with insignificant changes in 2WSP and an intermediate response in 4WSP, and a very significant increase in 8WSP ($p<0.0001$, one-way ANOVA) following the SP-LL transition. Contrastingly, the induction of *NKA α1b* expression by re-entering LL was not dependent on photoperiodic history, with all three SP-LL groups showing elevated (and equal) mRNA levels, compared with pre-SP levels, after four weeks of LL exposure ($p>0.001$, one-way ANOVA).

Growth performance during extended exposure to SW. In order to assess long-term SW performance of the presumed smolts from each of the SP-LL photoperiod regimes fish from each of the SP-LL groups were transferred to SW tanks for 28 days. Initial weights at the point

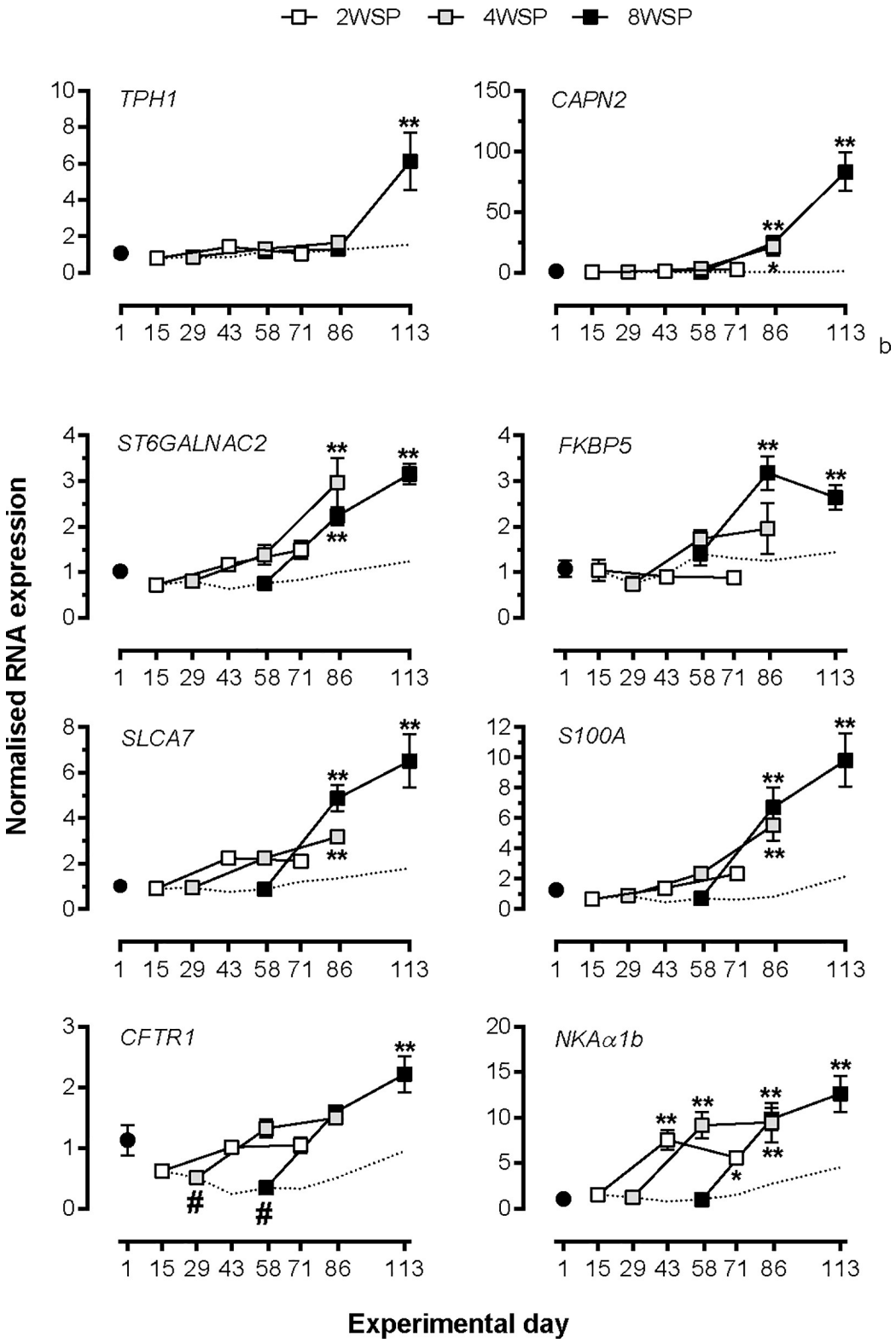

Fig 4. qPCR profiling of effect of SP exposure duration on selected cluster 3 genes. Data are normalized mRNA abundance, mean ± SEM of n = 6 fish per sampling point. *, ** significantly higher expression than LL and SP values at the corresponding time point, $p<0.05$, 0.01, respectively; # significantly lower expression than at day 1, $p<0.05$. Where error bars do not appear, errors lie within the symbol. The dashed line represents the SP control group.

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of transfer to SW did not differ significantly between the groups (Table 2), and there were no differences in CF. Contrastingly, subsequent SW growth performance was highly dependent on prior exposure to short photoperiod ($p = 0.003$ for time x photoperiod regime interaction, two-way ANOVA, S5 Table). During the four weeks in SW, fish transferred from the 2WSP group showed no significant increase in body mass, while over the same period body masse increased in the 4WSP and 8WSP groups by 19.6% and 27.3%, respectively. Moreover, fish in the 8WSP group grew significantly more, and at a higher SGR (Table 2) than fish from the 4WSP group ($P<0.01$ for final weight comparison). Total dry weight feed intake was 860 g, 944 g, and 1277 g in the 2WSP, 4WSP, and 8WSP treatment groups, respectively, leading to FRCs of 8.27, 2.31 and 2.11 (Table 2).

Discussion

Successful smolting involves the coordination of developmental and physiological processes to produce a SW-ready smolt phenotype. Confirming previous studies, we find that the successful coordination of smolting is dependent upon photoperiodic history [6, 42, 43]. Further, we have identified a cohort of genes, previously unstudied in the context of smolting, whose expression in the gills is highly history-dependent. We show that juvenile salmon exposed to four or fewer weeks of SP, followed by LL, maintain low levels of plasma osmolytes during 24-h SWCs, while experiencing poor growth during extended SW exposure. We suggest that the novel genes presented here could act as markers for SW preparedness in smolts. Further exploration of these genes would improve our understanding of the physiological and endocrine regulation of gill differentiation during smolting, and how it is controlled by photoperiod.

In both experiments, juvenile fish raised on LL were able to maintain osmotic balance during 24-h exposure to SW. Similar hypo-osmoregulatory ability has previously been observed under similar conditions [44, 45], and was attributed to a spontaneous development of salinity tolerance after exceeding a minimum body size threshold. In the present study, SP exposure suppressed salinity tolerance in all groups. Under prolonged exposure to SP, the hypo-osmoregulatory capacity spontaneously recovers, but not to the same extent as in fish that are returned to LL. The physiological reasons for this partial recovery are unknown, but could be due to endogenous processes influencing the MRCs [46], or by an improved capacity to handle osmotic stress due to increased size [47]. Regardless of photoperiodic history, LL is a strong stimulus for recovery of the capacity to hypo-osmoregulate during a 24-h SW challenge. This apparent lack of history-dependence in the response to a short-term SW challenge is consistent with previous reports [6, 42], but gives no indication of how the osmoregulatory mechanisms involved in maintaining ionic balance change during the developmental process of smolting. Based on the impaired SW growth rate of fish exposed to SP for 2 and 4 weeks prior to LL exposure, it appears that smolting entails a development of energetically efficient mechanisms for maintaining ionic balance through a process which is dependent on photoperiodic history. The reacquisition of the ability to hypo-osmoregulate during 24-h SW challenges under prolonged SP exposure could be a result of free-running, endogenous rhythms [6, 46]. A common feature of the 2WSP, 4WSP and SPC groups in Experiment 2 was the unresponsiveness of the NKA activity and in the mRNA expression of five of our six novel genes. A similar decoupling of NKA activity and hypo-osmoregulatory capacity has been observed by Berge, Berg [48] and Handeland and Stefansson [49], indicating that increased NKA activity is not a prerequisite for (short-term) salinity tolerance.

The extended SW exposure in Experiment 2 show reduced growth for the 2WSP and 4WSP, and particularly in the case of the 2WSP, a very high FCR. This indicates that growth

Table 2. Information on age and weight of fish during the prolonged SW stay. Significance as determined by two-way ANOVA. SGR -Specific growth rate, FCR -Feed conversion ratio.

	AGE (weeks)		BIOMASS (g)						
Treatment	SW entry	SW end	SW entry	SW mid-phase	SW end	Increase (%)	p-value	SGR	FCR
2WSP	56	60	67.2±10.9	68.8±11.6	70.6±11.4	5.1	n.s.	0.169	8.27
4WSP	58	62	69.4±8.7	73.8±10.2	83.0±12.5	19.6	< 0.001	0.586	2.31
8WSP	62	66	73.9±11.8	80.8±12.5	94.1±15.1	27. 3	<0.0001	0.839	2.11

In addition to differences in food intake, and weight gain, moderate changes in CF were observed (S5 Table). Upon transfer to SW the fish in the 8WSP group had a significantly lower CF than those in the 2WSP group ($p<0.05$), while an intermediate value was seen in the 4WSP fish. Only the fish in the 2WSP group showed a significant decrease in CF over the four weeks in SW ($p<0.001$).

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and FCR in SW is influenced by photoperiodic history through its control of hypo-osmoregulatory capacity. We suggest that the lack of hypo-osmoregulatory capacity, especially in the 2WSP group, causes a higher energy demand leading to reduced growth. Similar observation were made by Saunders, Henderson [43], whom found that juvenile salmon exposed to LL grew better than juveniles exposed to a natural photoperiod in FW, but in SW these grew substantially less than those exposed to natural photoperiod.

One potential caveat of our experimental design is the age difference arising between treatment groups in experiment 2. However, the juvenile salmon in experiment 1 responded with increased mRNA expression for all the novel genes at four months younger, so we do not believe that age *per se* is an underlying cause for observed differences in long-term SW tolerance. Rather, the time spent on SP prior to LL exposure leads to history-dependent effects on gene expression, NKA activity and long-term SW performance.

Smolting is a hormonally controlled process and photoperiod-dependent changes in the secretion of anterior pituitary trophic hormones (ACTH, GH and TSH), together with cortisol and IGF-1, have been reported [2, 15, 19, 50, 51]. Importantly, the same hormones are not influenced by increased day length in salmon juveniles below a threshold body size for smolting [2, 4, 52, 53]. Thus, several authors have suggested that a central hormonal mechanisms controlling the 'decision' to smolt are connected to growth and energetic status [1, 3, 54–57]. Such conditional activation is known to be a key feature of life history transitions where seasonal timing is of paramount importance [58, 59]. Such central hormonal systems may also undergo innately timed changes to control the expression of seasonal responses, even when external stimuli has been inadequate [60–64].

Photoperiodic history-dependence might also be an innate property of autonomous timers in peripheral tissues, expressed as inertia in responses to hormonal signals [65, 66]. An indication of inertia in gill tissue is observed in a paper by McCormick, Björnsson [67], where advancing the phase of the spring increase in photoperiod causes a corresponding advance in pituitary GH secretion, but not in gill NKA activity. Expression of *NKA α1b* has previously been linked with increased plasma GH and NKA activity [2, 23], however, Christensen, Regish [68] emphasize the role of NKA α1b protein rather than mRNA for NKA activity and SW tolerance. The dichotomy in photoperiodic history-dependence between *NKA* α1b mRNA expression and NKA activity in the present study, together with the aforementioned results, point to post-translational mechanisms influencing NKA activity, rather than an effect of increased NKA α1b expression. Other NKA pump components such as the NKA β subunit and FXYD proteins could be significant contributors to the stabilization and function of the NKA pump [23, 69–71]. Further examination of photoperiodic history-dependence of gill

gene expression is warranted to resolve between centrally controlled processes and peripheral mechanisms.

The genes in cluster 3 can be linked to cytoskeletal function, G-protein coupled receptor signaling, ion uptake and excretion, epidermal structure and cell adhesion. TPH1 is the rate limiting enzyme for serotonin (5HT) synthesis, serotonin is known to have vasoconstrictory effects in the gill [72], and evidence shows that serotonylation of histones can influence gene transcription [73]. Whereas the latter could be significant in terms of changes to the transcriptome, the vasoconstriction will influence blood pressure in the gill and potentially redistribute the blood flow, influencing the exchange of molecules with the environment [74]. CAPN2's role in cytoskeletal remodeling and cell motility [75] is also of interest given the extensive tubular network found in SW-ready MRCs [12, 14], and potential migration of developing MRCs from the base of the filament [76, 77]. FKBP5 can be linked to the glucocorticoid receptor regulatory network [78–80] which is known to be involved in SW acclimation [81, 82]. The glucocorticoid receptors have previously been associated with regulation of CFTR I and the NKA α-subunits in salmon [19, 83]. S100A is a calcium binding protein, implicated in the regulation of many cellular processes, including differentiation [84]. After binding with Ca^{2+}, the S100A undergoes a conformational change, allowing it to interact with a wide variety of targets, such as cytoskeletal proteins and transcription factors [84]. SLC5A7 is an sodium/substrate symporter known to mediate choline uptake in humans [85]. Cholinergic cells have been described in the gill of zebrafish (*Danio rerio*), and it is proposed that they could be significant in ventilatory control [86]. ST6GALNAC2 is a sialyltransferase, influencing cell-cell and cell-substrate interactions [87]. This breadth of potential actions of cluster 3 genes underlines the extent to which successful smolting relies upon comprehensive re-organisation of gill function, reflecting the pleiotropic role of this tissue in many essential aspects of salmonid physiology. It remains to disclose the true function of these genes in a gill specific context, with further studies of cellular localization and protein function now being required.

In conclusion, commonly used predictors of SW readiness in smolts (osmoregulation, NKA activity) appear to be inadequate when it comes to predicting actual SW performance. A biomolecular approach, simultaneously measuring mRNA levels of several confirmed marker genes potentially offers a better prediction of SW performance. Additionally, it appears that such markers exhibit a strong dependence on photoperiodic history, emphasizing the role of SP for smolt development.

Supporting information

S1 Checklist. The ARRIVE guidelines checklist.
(PDF)

S1 Table. Experiment 1 Osmolality, 2-way ANOVA. Table showing the 2-way ANOVA and multiple comparison results for the plasma osmolality measurements in experiment 1.
(PDF)

S2 Table. Experiment 2 Osmolality, 2-way ANOVA. Table showing the 2-way ANOVA and multiple comparison results for the plasma osmolality measurements in experiment 2.
(PDF)

S3 Table. Experiment 2 Na^+, K^+- ATPase activity, 2-way ANOVA. Table showing the 2-way ANOVA and multiple comparison results for the gill Na^+, K^+- ATPase activity measurements in experiment 2.
(PDF)

S4 Table. Experiment 1 weight and condition factor. Table shows the development of weight (g) and conditionu factor of the experimental groups in experiment 1.
(PDF)

S5 Table. Experiment 2 weight and condition factor FW. Table shows the development of weight (g) and condition factor of the experimental groups during the FW phase of experiment 2.
(PDF)

S6 Table. Experiment 1 Gene expression, 2-way ANOVA. Tables showing the 2-way ANOVA and multiple comparison results for the expression of the genes measured in experiment 1 and shown in Fig 2.
(PDF)

S7 Table. Experiment 2 Gene expression, 2-way ANOVA. Tables showing the 2-way ANOVA and multiple comparison results for the expression of the genes measured in experiment 2 and shown in Fig 4.
(PDF)

S8 Table. Heatmap data. Data table with z-scores, cluster number and information on each of the genes in the heatmap presented in Fig 1.
(XLSX)

S1 Fig. Plasma chloride. Graphs showing the averaged levels (±SEM) of plasma chloride (mmol l^{-1}) in the treatments groups of experiment 1 (top, n = 6) and experiment 2 (bottom, n = 10, dotted line represents the SPC group), measured after 24h SWCs.
(PDF)

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References

1. Hoar WS. The Physiology of Smolting Salmonids. In: Hoar WS, Randall DJ, editors. Fish Physiology. 11. Cambrigde: Academic Press; 1988. p. 275–343.
2. McCormick SD, Regish AM, Christensen AK, Björnsson BT. Differential regulation of sodium–potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. J Exp Biol. 2013; 216(7):1142–51. https://doi.org/10.1242/jeb.080440 PMID: 23487266
3. Stefansson SO, Björnsson BT, Ebbesson LOE, McCormick SD. Smoltification. In: Finn RN, Kapoor BG, editors. Fish Larval Physiology. New Dehli: Science Publishers, Inc. Enfield (NH) & IBH Publishing Co. Pvt. Ltd.; 2008.
4. McCormick SD, Shrimpton JM, Moriyama S, Björnsson BT. Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. Aquaculture. 2007; 273(2):337–44. https://doi.org/10.1016/j.aquaculture.2007.10.015
5. McCormick SD. Endocrine Control of Osmoregulation in Teleost Fish. Am Zool. 2001; 41(4):781–94. https://doi.org/10.1668/0003-1569(2001)041[0781:ECOOIT]2.0.CO;2
6. Duncan NJ, Bromage N. The effect of different periods of constant short days on smoltification in juvenile Atlantic salmon (*Salmo salar*). Aquaculture. 1998; 168(1–4):369–86. WOS:000077308400031.
7. Evans DH, Piermarini PM, Choe KP. The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of Nitrogenous Waste. Physiol Rev. 2005; 85 (1):97–177. https://doi.org/10.1152/physrev.00050.2003 PMID: 15618479.
8. Pelis RM, Zydlewski J, McCormick SD. Gill Na+-K+-2Cl− cotransporter abundance and location in Atlantic salmon: effects of seawater and smolting. Am J Physiol Regul Integr Comp Physiol. 2001; 280 (6):R1844–R52. https://doi.org/10.1152/ajpregu.2001.280.6.R1844 PMID: 11353691
9. Hiroi J, McCormick SD. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. Respir Physiol Neurobiol. 2012; 184(3):257–68. https://doi.org/10.1016/j.resp.2012.07.019 PMID: 22850177
10. Marshall WS, Singer TD. Cystic fibrosis transmembrane conductance regulator in teleost fish. Biochim Biophys Acta Biomembr. 2002; 1566(1):16–27. https://doi.org/10.1016/S0005-2736(02)00584-9.
11. Lubin RT, Rourke AW, Bradley TM. Ultrastructural alterations in branchial chloride cells of Atlantic salmon, *Salmo salar*, during parr-smolt transformation and early development in sea water. J Fish Biol. 1989; 34(2):259–72. https://doi.org/10.1111/j.1095-8649.1989.tb03307.x
12. Sardet C, Pisam M, Maetz J. The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. J Cell Biol. 1979; 80(1):96–117. https://doi.org/10.1083/jcb.80.1.96 PMID: 422655
13. Pisam M. Membranous systems in the "chloride cell" of teleostean fish gill; their modifications in response to the salinity of the environment. Anat Rec. 1981; 200(4):401–14. https://doi.org/10.1002/ar.1092000403 PMID: 7305008
14. Pisam M, Prunet P, Boeuf G, Jrambourg A. Ultrastructural features of chloride cells in the gill epithelium of the Atlantic salmon, *Salmo salar*, and their modifications during smoltification. Am J Anat. 1988; 183 (3):235–44. https://doi.org/10.1002/aja.1001830306 PMID: 3213829
15. Tipsmark CK, Madsen SS. Distinct hormonal regulation of Na+,K+-atpase genes in the gill of Atlantic salmon (*Salmo salar* L.). J Endocrinol. 2009; 203(2):301–10. https://doi.org/10.1677/JOE-09-0281 PMID: 19696099
16. Bystriansky JS, Richards JG, Schulte PM, Ballantyne JS. Reciprocal expression of gill Na+/K+-ATPaseα -subunit isoforms α1a and α1b during seawater acclimation of three salmonid fishes that vary in their salinity tolerance. J Exp Biol. 2006; 209(10):1848–58. https://doi.org/10.1242/jeb.02188 PMID: 16651551
17. McCormick SD, Regish AM, Christensen AK. Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. J Exp Biol. 2009; 212(24):3994–4001. https://doi.org/10.1242/jeb.037275 PMID: 19946077
18. Singer TD, Finstad B, McCormick SD, Wiseman SB, Schulte PM, Scott McKinley R. Interactive effects of cortisol treatment and ambient seawater challenge on gill Na^+,K^+-ATPase and CFTR expression in

two strains of Atlantic salmon smolts. Aquaculture. 2003; 222(1):15–28. https://doi.org/10.1016/S0044-8486(03)00099-1

19. Kiilerich P, Kristiansen K, Madsen SS. Cortisol regulation of ion transporter mRNA in Atlantic salmon gill and the effect of salinity on the signaling pathway. J Endocrinol. 2007; 194(2):417–27. https://doi.org/10.1677/JOE-07-0185 PMID: 17641289

20. Seear P, Carmichael S, Talbot R, Taggart J, Bron J, Sweeney G. Differential Gene Expression During Smoltification of Atlantic Salmon (Salmo salar L.): a First Large-Scale Microarray Study. Marine Biotechnol. 2010; 12(2):126–40. https://doi.org/10.1007/s10126-009-9218-x PMID: 19585168

21. Houde ALS, Gunther OP, Strohm J, Ming TJ, Li S, Patterson DA, et al. Discovery and validation of candidate smoltification gene expression biomarkers across multiple species and ecotypes of Pacific salmonids. bioRxiv. 2018:474692. https://doi.org/10.1101/474692

22. Hale MC, McKinney GJ, Thrower FP, Nichols KM. RNA-seq reveals differential gene expression in the brains of juvenile resident and migratory smolt rainbow trout (Oncorhynchus mykiss). Comp Biochem Physiol Part D Genomics Proteomics. 2016; 20:136–50. https://doi.org/10.1016/j.cbd.2016.07.006 PMID: 27693967.

23. Nilsen TO, Ebbesson LOE, Madsen SS, McCormick SD, Andersson E, Björnsson BT, et al. Differential expression of gill Na^+,K^+-ATPase α- and β-subunits, Na^+,K^+,$2Cl^-$ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. J Exp Biol. 2007; 210 (16):2885–96. https://doi.org/10.1242/jeb.002873 PMID: 17690237

24. Stefansson SO, Nilsen TO, Ebbesson LOE, Wargelius A, Madsen SS, Björnsson BT, et al. Molecular mechanisms of continuous light inhibition of Atlantic salmon parr–smolt transformation. Aquaculture. 2007; 273(2):235–45. https://doi.org/10.1016/j.aquaculture.2007.10.005

25. McCormick SD. Methods for Nonlethal Gill Biopsy and Measurement of Na^+, K^+-ATPase Activity. Can J Fish AquatSci. 1993; 50(3):656–8. https://doi.org/10.1139/f93-075

26. Schrock RM, Beeman JW, Rondorf DW, Haner PV. A Microassay for Gill Sodium, Potassium-Activated ATPase in Juvenile Pacific Salmonids. T Am Fish Soc. 1994; 123(2):223–9. https://doi.org/10.1577/1548-8659(1994)123<0223:AMFGSP>2.3.CO;2

27. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Anal Biochem. 1985; 150(1):76–85. Epub 1985/10/01. https://doi.org/10.1016/0003-2697(85)90442-7 PMID: 3843705.

28. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal. 2011; 17(1):3. Epub 2011-08-02. https://doi.org/10.14806/ej.17.1.200

29. Andrews S, Lindenbaum P, Howard B, Ewels P. FastQC High Throughput Sequence QC Report. 2011–2014.

30. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics (Oxford, England). 2013; 29(1):15–21. Epub 2012/10/25. https://doi.org/10.1093/bioinformatics/bts635 PMID: 23104886.

31. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics (Oxford, England). 2015; 31(2):166–9. Epub 2014/09/25. https://doi.org/10.1093/bioinformatics/btu638 PMID: 25260700.

32. Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, et al. GeneCards Version 3: the human gene integrator. Database (Oxford). 2010; 2010:baq020. Epub 2010/08/07. https://doi.org/10.1093/database/baq020 PMID: 20689021; PubMed Central PMCID: PMC2938269.

33. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics. 2016; 54:1 30 1–1 3. Epub 2016/06/21. https://doi.org/10.1002/cpbi.5 PMID: 27322403.

34. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics. 2007; 23(10):1289–91. Epub 2007/03/24. https://doi.org/10.1093/bioinformatics/btm091 PMID: 17379693.

35. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3—new capabilities and interfaces. Nucleic acids research. 2012; 40(15):e115. Epub 2012/06/26. https://doi.org/10.1093/nar/gks596 PMID: 22730293; PubMed Central PMCID: PMC3424584.

36. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3):403–10. Epub 1990/10/05. https://doi.org/10.1016/S0022-2836(05)80360-2 PMID: 2231712.

37. Samy JKA, Mulugeta TD, Nome T, Sandve SR, Grammes F, Kent MP, et al. SalmoBase: an integrated molecular data resource for Salmonid species. BMC Genomics. 2017; 18(1):482. Epub 2017/06/28. https://doi.org/10.1186/s12864-017-3877-1 PMID: 28651544; PubMed Central PMCID: PMC5485693.

38. Olsvik PA, Lie KK, Jordal A-EO, Nilsen TO, Hordvik I. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. BMC Mol Biol. 2005; 6(1):21. https://doi.org/10.1186/1471-2199-6-21 PMID: 16293192

39. Jorgensen SM, Kleveland EJ, Grimholt U, Gjoen T. Validation of Reference Genes for Real-Time Polymerase Chain Reaction Studies in Atlantic Salmon. Mar Biotechnol. 2006; 8(4):398–408. https://doi.org/10.1007/s10126-005-5164-4 PMID: 16676145

40. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262 WOS:000173949500003. PMID: 11846609

41. Sutherland BJG, Hanson KC, Jantzen JR, Koop BF, Smith CT. Divergent immunity and energetic programs in the gills of migratory and resident Oncorhynchus mykiss. Mol Ecol. 2014; 23(8):1952–64. https://doi.org/10.1111/mec.12713 PMID: 24612010

42. Duston J, Saunders RL. The entrainment role of photoperiod on hypoosmoregulatory and growth-related aspects of smolting in Atlantic salmon (*Salmo salar*). Can J Zool. 1990; 68(4):707–15. https://doi.org/10.1139/z90-103

43. Saunders RL, Henderson EB, Harmon PR. Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. Aquaculture. 1985; 45(1):55–66. https://doi.org/10.1016/0044-8486(85)90257-1.

44. Handeland SO, Imsland AK, Björnsson BT, Stefansson SO. Long-term effects of photoperiod, temperature and their interaction on growth, gill Na+, K+-ATPase activity, seawater tolerance and plasma growth-hormone levels in Atlantic salmon *Salmo salar*. J Fish Biol. 2013; 83(5):1197–209. https://doi.org/10.1111/jfb.12215 PMID: 24580662

45. Sigholt T, Åsgård T, Staurnes M. Timing of parr-smolt transformation in Atlantic salmon (*Salmo salar*): effects of changes in temperature and photoperiod. Aquaculture. 1998; 160(1):129–44. https://doi.org/10.1016/S0044-8486(97)00220-2

46. Eriksson L-O, Lundqvist H. Salmonid Smoltification Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (Salmo salar L.). Aquaculture. 1982; 28(1):113–21. http://dx.doi.org/10.1016/0044-8486(82)90014-X.

47. Parry G. Size and Osmoregulation in Salmonid Fishes. Nature. 1958; 181(4617):1218–9. https://doi.org/10.1038/1811218a0

48. Berge AI, Berg A, Fyhn HJ, Barnung T, Hansen T, Stefansson SO. Development of salinity tolerance in underyearling smolts of Atlantic salmon (*Salmo salar*) reared under different photoperiods. Can J Fish Aquat Sci. 1995; 52(2):243–51. https://doi.org/10.1139/f95-024 WOS:A1995RM82500002.

49. Handeland SO, Stefansson SO. Photoperiod control and influence of body size on off-season parr–smolt transformation and post-smolt growth. Aquaculture. 2001; 192(2):291–307. https://doi.org/10.1016/S0044-8486(00)00457-9

50. Langdon JS, Thorpe JE, Roberts RJ. Effects of cortisol and acth on gill Na+/K+-ATPase, SDH and chloride cells in juvenile atlantic salmon Salmo salar L. Comp Biochem Physiol A Physiol. 1984; 77(1):9–12. https://doi.org/10.1016/0300-9629(84)90004-51.

51. Fleming MS, Maugars G, Lafont AG, Rancon J, Fontaine R, Nourizadeh-Lillabadi R, et al. Functional divergence of thyrotropin beta-subunit paralogs gives new insights into salmon smoltification metamorphosis. Sci Rep. 2019; 9(1):4561. Epub 2019/03/16. https://doi.org/10.1038/s41598-019-40019-5 PMID: 30872608.

52. Thorpe JE, Talbot C, Villarreal C. Bimodality of growth and smolting in Atlantic salmon, Salmo salar L. Aquaculture. 1982; 28(1):123–32. https://doi.org/10.1016/0044-8486(82)90015-1.

53. Skilbrei OT. Importance of threshold length and photoperiod for the development of bimodal length frequency-distribution in Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci. 1991; 48(11):2163–72. WOS:A1991GU71800013.

54. Metcalfe NB, Huntingford FA, Thorpe JE. Feeding Intensity, Growth Rates, and the Establishment of Life-History Patterns in Juvenile Atlantic Salmon Salmo salar. J Anim Ecol. 1988; 57(2):463–74. https://doi.org/10.2307/4918

55. Thorpe JE. Developmental variation in salmonid populations. J Fish Biol. 1989; 35(sA):295–303. https://doi.org/10.1111/j.1095-8649.1989.tb03073.x

56. Thorpe J, Mangel M, Metcalfe N, Huntingford F. Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. Evol Ecol. 1998; 12(5):581–99. https://doi.org/10.1023/A:1022351814644

57. McCormick SD, Hansen LP, Quinn TP, Saunders RL. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci. 1998; 55(S1):77–92. https://doi.org/10.1139/d98-011

58. Rewitz KF, Yamanaka N, O'Connor MB. Chapter One—Developmental Checkpoints and Feedback Circuits Time Insect Maturation. In: Shi Y-B, editor. Current Topics in Developmental Biology. 103: Academic Press; 2013. p. 1–33. https://doi.org/10.1016/B978-0-12-385979-2.00001-0 PMID: 23347514

59. Rani S, Kumar V. Avian circannual systems: Persistence and sex differences. Gen Comp Endocrinol. 2013; 190:61–7. https://doi.org/10.1016/j.ygcen.2013.04.013 PMID: 23631901

60. Hazlerigg D, Loudon A. New Insights into Ancient Seasonal Life Timers. Curr Biol. 2008; 18(17):R795–R804. https://doi.org/10.1016/j.cub.2008.07.040 PMID: 18786385

61. Ikegami K, Yoshimura T. Seasonal time measurement during reproduction. J Reprod Dev. 2013; 59 (4):327–33. https://doi.org/10.1262/jrd.2013-035 PMID: 23965600; PubMed Central PMCID: PMC3944362.

62. Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, et al. Thyrotrophin in the pars tuberalis triggers photoperiodic response. Nature. 2008; 452(7185):317–22. http://www.nature.com/nature/journal/v452/n7185/suppinfo/nature06738_S1.html. https://doi.org/10.1038/nature06738 PMID: 18354476

63. Nakao N, Ono H, Yoshimura T. Thyroid hormones and seasonal reproductive neuroendocrine interactions. Reproduction. 2008; 136(1):1–8. https://doi.org/10.1530/REP-08-0041 PMID: 18515309

64. Dardente H, Wyse CA, Birnie MJ, Dupré SM, Loudon ASI, Lincoln GA, et al. A Molecular Switch for Photoperiod Responsiveness in Mammals. Curr Biol. 2010; 20(24):2193–8. https://doi.org/10.1016/j.cub.2010.10.048 PMID: 21129971

65. Brown SA, Azzi A. Peripheral Circadian Oscillators in Mammals. In: Kramer A, Merrow M, editors. Circadian Clocks. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. p. 45–66.

66. Giebultowicz JM. Peripheral clocks and their role in circadian timing: insights from insects. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2001; 356(1415):1791–9. https://doi.org/10.1098/rstb.2001.0960 PMID: 11710986

67. McCormick SD, Björnsson BT, Sheridan M, Eilerlson C, Carey JB, O'Dea M. Increased daylength stimulates plasma growth hormone and gill Na+, K+-ATPase in Atlantic salmon (Salmo salar). J Comp Physiol B. 1995; 165(4):245–54. https://doi.org/10.1007/bf00367308

68. Christensen AK, Regish AM, McCormick SD. Shifts in the relationship between mRNA and protein abundance of gill ion-transporters during smolt development and seawater acclimation in Atlantic salmon (*Salmo salar*). Comp Biochem Phys A. 2018; 221:63–73. Epub 2018/04/09. https://doi.org/10.1016/j.cbpa.2018.03.020 PMID: 29627325.

69. Geering K. Functional roles of Na+,K+-ATPase subunits. Curr Opin Nephrol Hy. 2008; 17(5):526–32. https://doi.org/10.1097/MNH.0b013e3283036cbf 00041552-200809000-00014. PMID: 18695395

70. Madsen SS, Kiilerich P, Tipsmark CK. Multiplicity of expression of Na+, K+–ATPaseα -subunit isoforms in the gill of Atlantic salmon (*Salmo salar*): cellular localisation and absolute quantification in response to salinity change. J Exp Biol. 2009; 212(1):78–88. https://doi.org/10.1242/jeb.024612 PMID: 19088213

71. Tipsmark CK, Mahmmoud YA, Borski RJ, Madsen SS. FXYD-11 associates with Na+, K+-ATPase in the gill of Atlantic salmon: regulation and localization in relation to changed ion-regulatory status. Am J Physiol-Reg I. 2010; 299(5):R1212–R23. https://doi.org/10.1152/ajpregu.00015.2010 PMID: 20702795

72. Sundin L, Nilsson GE, Block M, Lofman CO. Control of gill filament blood flow by serotonin in the rainbow trout, Oncorhynchus mykiss. Am J Physiol Regul Integr Comp Physiol. 1995; 268(5):R1224–R9. https://doi.org/10.1152/ajpregu.1995.268.5.R1224 PMID: 7771583.

73. Farrelly LA, Thompson RE, Zhao S, Lepack AE, Lyu Y, Bhanu NV, et al. Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. Nature. 2019; 567(7749):535–9. https://doi.org/10.1038/s41586-019-1024-7 PMID: 30867594

74. Nilsson S, Sundin L. Gill Blood Flow Control. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 1998; 119(1):137–47. https://doi.org/10.1016/S1095-6433(97)00397-8.

75. Carragher NO, Frame MC. Calpain: a role in cell transformation and migration. Int J Biochem Cell Biol. 2002; 34(12):1539–43. https://doi.org/10.1016/s1357-2725(02)00069-9 PMID: 12379276

76. MacKinnon M, Enesco HE. Cell renewal in the gills of the fish Barbus conchonius. Canadian Journal of Zoology. 1980; 58(4):650–3. https://doi.org/10.1139/z80-093

77. Laurent P, Dunel-Erb S, Chevalier C, Lignon J. Gill epithelial cells kinetics in a freshwater teleost, Oncorhynchus mykiss during adaptation to ion-poor water and hormonal treatments. Fish Physiol Biochem. 1994; 13(5):353–70. https://doi.org/10.1007/BF00003415 PMID: 24197072

78. Jääskeläinen T, Makkonen H, Palvimo JJ. Steroid up-regulation of FKBP51 and its role in hormone signaling. Curr Opin Pharmacol. 2011; 11(4):326–31. https://doi.org/10.1016/j.coph.2011.04.006 PMID: 21531172

79. Davies TH, Ning Y-M, Sánchez ER. A new first step in activation of steroid receptors hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem. 2002; 277(7):4597–600. https://doi.org/10.1074/jbc.C100531200 PMID: 11751894

80. Vermeer H, Hendriks-Stegeman BI, van der Burg B, van Buul-Offers SC, Jansen M. Glucocorticoid-induced increase in lymphocytic FKBP51 messenger ribonucleic acid expression: a potential marker for glucocorticoid sensitivity, potency, and bioavailability. J Clin Endocrinol Metab. 2003; 88(1):277–84. https://doi.org/10.1210/jc.2002-020354 PMID: 12519866

81. Breves JP, Fox BK, Pierce AL, Hirano T, Grau EG. Gene expression of growth hormone family and glucocorticoid receptors, osmosensors, and ion transporters in the gill during seawater acclimation of Mozambique tilapia, Oreochromis mossambicus. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology. 2010; 313A(7):432–41. https://doi.org/10.1002/jez.613 PMID: 20623800

82. Aruna A, Nagarajan G, Chang CF. Involvement of Corticotrophin-Releasing Hormone and Corticosteroid Receptors in the Brain–Pituitary–Gill of Tilapia During the Course of Seawater Acclimation. Journal of Neuroendocrinology. 2012; 24(5):818–30. https://doi.org/10.1111/j.1365-2826.2012.02282.x PMID: 22250893

83. Kiilerich P, Pedersen SH, Kristiansen K, Madsen SS. Corticosteroid regulation of Na+,K+-ATPase α1-isoform expression in Atlantic salmon gill during smolt development. Gen Comp Endocrinol. 2011; 170 (2):283–9. https://doi.org/10.1016/j.ygcen.2010.02.014 PMID: 20171217

84. Wright NT, Cannon BR, Zimmer DB, Weber DJ. S100A1: Structure, Function, and Therapeutic Potential. Curr Chem Biol. 2009; 3(2):138–45. https://doi.org/10.2174/187231309788166460 PMID: 19890475

85. Wright EM, Turk E. The sodium/glucose cotransport family SLC5. Pflügers Archiv. 2004; 447(5):510–8. https://doi.org/10.1007/s00424-003-1063-6 PMID: 12748858

86. Zachar PC, Pan W, Jonz MG. Distribution and morphology of cholinergic cells in the branchial epithelium of zebrafish (Danio rerio). Cell and Tissue Research. 2017; 367(2):169–79. https://doi.org/10.1007/s00441-016-2531-6 PMID: 27909858

87. Samyn-Petit B, Krzewinski-Recchi M-A, Steelant WFA, Delannoy P, Harduin-Lepers A. Molecular cloning and functional expression of human ST6GalNAc II. Molecular expression in various human cultured cells. Biochimica et Biophysica Acta (BBA)—General Subjects. 2000; 1474(2):201–11. https://doi.org/10.1016/S0304-4165(00)00020-9.

Appendix 6:
Paper II

Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in an anadromous salmonid

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COVER PAGE

Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in Atlantic salmon (*Salmo salar*)

Short title: seawater sensitive gill transcriptome

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Abstract

The developmental transition of juvenile salmon from a freshwater resident morph (parr) to a seawater (SW) migratory morph (smolt), known as smoltification, entails a reorganization of gill function to cope with the altered water environment. Recently, we used RNAseq to characterize the breadth of transcriptional change which takes place in the gill in the FW phase of smoltification. This highlighted the importance of extended exposure to short, winter-like photoperiods (SP) followed by a subsequent increase in photoperiod for completion of transcriptional reprogramming in FW and for efficient growth following transfer to SW. Here, we extend this analysis to examine the consequences of this photoperiodic history-dependent reprogramming for subsequent gill responses upon exposure to SW. We use RNAseq to analyse gill samples taken from fish raised on the photoperiod regimes we used previously and then challenged by SW exposure for 24-h. While fish held on constant light (LL) throughout were able to hypo-osmoregulate during a 24-h SW challenge, the associated gill transcriptional response was highly distinctive from that in fish which had experienced an 7 week period of exposure to SP followed by a return to LL (SPLL) and had consequently acquired the characteristics of fully developed smolts. Fish transferred from LL to SP, and then held on SP for the remainder of the study were unable to hypo-osmoregulate and the associated gill transcriptional response featured many transcripts apparently regulated by the glucocorticoid stress axis, and through response elements for the osmo-sensing transcription factor NFAT5. The importance of these pathways for the gill transcriptional response to SW exposure appears to diminish as a consequence of photoperiod mediated induction of the smolt phenotype.

1. Introduction

The gill is the primary site of osmo-sensing and osmoregulatory control in fish (Evans et al. 2005, Evans 2010). In both freshwater (FW) and seawater (SW), osmoregulatory systems work to counter the passive diffusion of ions and water across the gill epithelium, and balance plasma osmolality. Euryhaline fish species are defined by their ability to tolerate salinity changes through modulation of osmoregulatory function. In most cases this depends on responses to altered salinity (acclimation), while in a few species groups including salmonids and eels (genus Anguilla), sustained migrations between sea and freshwater are facilitated by preparative changes in osmoregulatory function, forming part of a key developmental life history transition (Folmar and Dickhoff 1980, Wilson et al. 2004, Kalujnaia et al. 2007, Stefansson et al. 2008).

In Atlantic salmon (*Salmo salar*) this preparatory process is commonly known as 'smoltification' or, hereafter, 'smolting'. Smolting is photoperiodically controlled so that migration to sea occurs in a spring 'smolt window', when conditions favour juvenile growth (Gross et al. 1988). Smolting does not occur before the fish exceed a certain size threshold and is presumed to relate to the capacity of juvenile fish to meet the necessary metabolic demands (Higgins 1985, Kristinsson et al. 1985, Metcalfe et al. 1988, Skilbrei 1991). During smolting the juvenile salmon develop traits that will enable them to survive in and exploit the marine environment. Following exposure to short day lengths in winter, the increase of photoperiod in spring induces a hormonal cascade influencing behavior, metabolism, growth, pigmentation and gill physiology (Duston and Saunders 1990, McCormick 1994, McCormick et al. 1998, McCormick et al. 2007). In particular, gill physiology changes in order to accommodate the expected shift in environmental salinity and osmotic drive (Pisam et al. 1988, Evans et al. 2005, Kiilerich et al. 2007, Nilsen et al. 2007, Tipsmark

et al. 2009). The mitochondria rich cell (MRC), situated on the gill lamella, is a significant component of osmoregulation (Wilson and Laurent 2002). The MRC is rich in ion transporters, and changes in both morphology and composition in response to salinity (Pisam et al. 1988, Hwang and Lee 2007, Madsen et al. 2009, Hwang et al. 2011, Hiroi and McCormick 2012). Completion of the smolting process requires entry to sea, where SW exposure triggers the final shifts in physiology and behavior (Pisam et al. 1988, Lubin et al. 1989, Nilsen et al. 2007, McCormick et al. 2013). Hence, smolting can be considered a two-step process: a FW preparative phase followed by a SW activational phase.

Recently, we performed an RNAseq experiment designed to identify the hallmarks of photoperiodically induced smolting in the gills of the Atlantic salmon (Iversen et al. 2020). By comparing RNA profiles from fish raised continuously on constant light with those that experienced a 7 week period of short photoperiod, simulating winter photoperiod, before return to constant light we were able to identify a cohort of novel genes the expression of which is winter-photoperiod dependent. In a second experiment we saw that the length of winter-photoperiod exposure was critical to these genes and to growth performance after SW transfer.

This finding provides a genome wide analysis of the well described preparative phase of smoltification but does not address the issue of further activational changes triggered in smolts during the first few days in SW (Prunet and Boeuf 1985, Handeland et al. 1996, Handeland et al. 2000, Stefansson et al. 2008), hereafter the 'SW activational phase'. SW responses are also triggered in juveniles entering SW prematurely, which have not initiated or finished the preparative phase of smolt development (Saunders et al. 1985, Stagg et al. 1989). Triggers may include osmotic stress due to the hyper-osmotic SW environment as well as direct responses to

86 changes in the concentrations of specific ions either in the gill or in internal organs such as the
87 kidney and intestine (Evans and Somero 2008, Evans 2010, Kültz 2012). However, the specific
88 response is expected to differ drastically between SW-ready smolts and unprepared juveniles
89 (Stagg et al. 1989, Houde et al. 2018). The importance of SW-exposure for completion of the
90 smolting process and establishment of a SW phenotype is clearly demonstrated by the process of
91 'de-smoltification', which occurs if migration to SW is prevented and involves a loss of tolerance
92 to SW (Stefansson et al. 1998, Arnesen et al. 2003).

93 Gill tissue may respond to SW in at least three possible ways: i) as a direct response to increased
94 cellular tonicity and altered intracellular ion concentrations ii) as a direct response via cell surface
95 receptors for SW constituents (e.g. Ca^{2+} perceived via the calcium-sensing receptor, CaSR) (Loretz
96 2008, Kültz 2012) and iii) as an indirect response via hormonal signals (e.g. cortisol, or angiotensin
97 II) which change in response to SW-exposure (McCormick 2001, Kültz 2012). In this context, the
98 'nuclear factor of activated T-cells' (NFAT) family of transcription factors have been the focus of
99 recent interest because of their implication in osmo-sensing and in Ca^{2+}-dependent
100 transcriptional control (Hogan et al. 2003, Putney 2012, Cheung and Ko 2013, Lorgen et al. 2017).
101 The NFAT family comprises four subgroups, where groups 1-4 (NFATs c1, c2, c3, c4) are Ca^{2+}-
102 stimulated, and the fifth, NFAT5, is regulated in response to extracellular tonicity (Rao et al. 1997,
103 Macian 2005, Cheung and Ko 2013). All members share a Rel-like homology domain, and bind to
104 similar binding sites in the regulatory regions of their target genes (Macian 2005).

105 NFAT5 (also known as osmotic response element binding protein, OREBP, or tonicity-responsive
106 enhancer binding protein, TonEBP), is considered the primordial NFAT, as it is the only one found
107 outside the vertebrate group (Hogan et al. 2003). NFAT5 regulates the transcription of tonicity-

responsive genes such as ion transporters and osmo-protective proteins (Woo et al. 2002, Zhou et al. 2006, Cheung and Ko 2013). Hypertonic stress increases nuclear import and retention of NFAT5 through changes in phosphorylation state, while hypotonic stress leads to nuclear export (Ferraris et al. 2002, Macian 2005, Irarrazabal et al. 2010, Cheung and Ko 2013).

Two recent studies in salmon focus attention on the role of NFAT signaling during smolting. Lorgen et al. (2015) showed that the salmonid thyroid hormone deiodinase *dio2a* was SW-inducible in gill tissue, and its promoter region was enriched for osmotic response elements (OREs / NFAT5 response elements). A subsequent survey of NFAT5 expression in Atlantic salmon (Lorgen et al. 2017) revealed four NFAT5 paralogues, NFAT5 a1 and a2, and NFAT5 b1 and b2. Of these, NFAT5b1/2 gill expression was highly induced by SW exposure. Together these studies suggest that NFAT5b1/2 could coordinate SW stimulated changes in transcription.

In the present study we have extended our transcriptomic analysis of pre-adaptive, photoperiod-induced changes in gill phenotype (Iversen et al. 2020) to consider the shifting transcriptional response to SW-exposure. Specifically, we aimed first to assess the extent of the transcriptional response to an acute SW-challenge, and to determine how this is affected by prior photoperiodic exposure. Secondarily, we sought to infer the importance of stress (glucocorticoid) and NFAT-signaling in the shifting pattern of SW-responsiveness. Our data indicate that as salmon develop into smolts these pathways are less activated upon SW-entry.

2. Materials & Methods

2.2 Fish rearing and experimental set-up

All studies were performed in accordance with Norwegian and European legislation on animal research. The experimental design has been described in detail previously (Iversen et al. 2020), and is presented schematically in fig. 1A.

Briefly, juvenile Atlantic salmon (n = 225) of the Aquagene commercial strain were raised at the Aquaculture research station in Tromsø (69.867N, 18.935E), a bespoke salmonid research facility drawing a natural freshwater supply from the Kårvikelva river. Fish were kept on continuous light from hatching until approximately 7 months old, when they weighed on average 49.5 g (s.d. = 7.0 g). Then the fish were divided into two groups of 75 and 150 fish by dip netting and placed in separate 100 l circular tanks in separate rooms (FW, 8.5°C). The group of 75 fish were kept on LL for the rest of the experiment, while for the group of 150 fish, photoperiod was incrementally reduced over a week until it reached 8-h light/24-h (SP). After 7 weeks, half of the fish transferred to SP were moved to a new 100 l circular tank and returned to LL, again by incrementally increasing photoperiod over 1 week (hereafter referred to as the SPLL group), while the remainder continued on SP. The experiment continued for a further 6 weeks. During the experiment the fish were fed continuously and in excess over the eight hours corresponding to day in the SP treatment group, using standard commercial salmon pellets (Skretting, Nutra olympic 2,0 mm).

2.3 Sampling procedure

Fish were sampled from all tanks on days 1, 32, 53, 68, 89 and 110 (n=6 for each treatment) of the study. At each sampling point another subsample of fish from each of the treatments were put through a 24-h salt-water challenge (SWC, 100 l tanks, 34 ‰, salinity, 7°C, n=6 for each treatment, as detailed in Iversen et al. (2020)), starting on the day prior to sampling. All fish were fasted for 24-h prior to each sampling point.

All fish were terminally anaesthetized (Benzocaine 150 ppm) before sampling, after which body mass (± 0.5 g) and fork length (± 0.1 cm) were measured. In the fish that had been exposed to the 24-h SWCs, blood was drained from the caudal vein into 2ml lithium heparinized vacutainers (BD vacutainers®, Puls Norge, Moss, Norway), and held on ice. Blood samples were centrifuged at 6000 x *g* for 10 minutes, and the plasma fraction collected. The plasma was kept frozen at -80°C until analyses for osmolyte content using a Fiske One-Ten Osmometer (Fiske Associates, Massachusetts, USA, ± 4 mOsm kg^{-1}).

From sampling day 68 onwards, the right operculum was removed, and a gill arch dissected out, on the sampled fish in both groups (directly from FW and after the SWC) the. The primary gill filaments were then cut from the arch and placed in RNAlater® (Sigma-Aldrich, St. Louis, Missouri, USA). Samples were stored at 4 °C for 24 h, and then kept frozen at -80°C until RNA extraction.

2.4 RNA extraction and sequencing

RNA extraction, library preparation and Illumina HiSeq analysis were performed as described previously (Iversen et al. 2020).

2.5 Transcriptome analysis of SW-sensitive gene expression

Transcriptome analysis was performed using the Edge R package (version3.14.0) and R (version 3.4.2), run in RStudio (version 1.0.153). Raw counts were filtered (expression threshold CPM>1 in five or more libraries), and scaled using trimmed means of M-values (TMM). Principal component analysis (PCA) was performed on all above threshold genes using The R Stats Package (Stats, ver. 3.4.2) (Love et al. 2014). For simplicity and interpretability of the plot, TMM normalized counts for each gene in each sample group (n=6, except for T4 SPLL FW where n=5) were averaged before generating the PCA plot.

A quasi-likelihood negative binomial generalized log-linear model was used to fit the data, and nine empirical Bayes F-tests were run contrasting between the FW and SW sampled fish for each condition on days 68, 89 and 110 of the study. Outputs were filtered requiring a false discovery rate (FDR) of 0.01, and a $\log_2$-fold change of |1|. Lists of differentially expressed genes (DEGs) from each of the sampling groups were compared across time within treatments, and between treatments at the same time point. The numbers of unique and shared DEGs are summarized in the 'Upset'-plots (UpSetR ver. 1.4.0) (Conway et al. 2017) in fig. 2.

Gene ontology analysis was performed on lists of DEGS generated by Edge R, using topGO (ver. 2.24.0) and the annotation package for the salmon genome Ssa.RefSeq.db (ver. 1.2), with a gill-specific gene universe. Fisher statistics and the 'elim'-algorithm (Alexa et al. 2006) was applied, with a significance threshold of $p<0.05$ for enrichment. Only the top 150 GO terms were included in the output. GOplot (ver. 1.0.2) (Walter et al. 2015) and ggplot2 (ver. 3.0.0) were used to visualized GO term enrichment. For each GO term (eq.1), positive z scores indicate over-

representation of upregulated genes within the GO term, and negative z scores indicate downregulated genes. Before plotting, unique GO IDs were filtered for a count>5.

Equation 1

$$z - score = \frac{(\#upregulated\ genes\ -\ \#downregulated\ genes)}{\sqrt{Total\ number\ of\ genes}}$$

2.6 NFAT family member gene expression

From the set of expressed genes (CPM > 1 in five or more libraries), 18 genes could be identified as NFAT (5 genes), NFAT-like (12 genes) or NFAT-interacting genes (1 gene) based on their SalmoBase annotation (ICSASG_v2) (Lien et al. 2016, Samy et al. 2017). Raw count data was used to calculate mean gene expression at each sampling point for all three treatments. The gene expression of the SP treatment group was then hierarchically clustered using the R-package pheatmap (ver. 1.0.10) (row scaled by z-scores, applying Euclidian distance measures and complete linkage clustering).

2.7 Motif analysis

Motif enrichment analysis was performed using SalMotifDB-shiny tool (https://cigene.no/tools/), described in detail elsewhere (Mulugeta et al. 2019). This tool accesses a database containing over 19,000 predicted transcription factor binding sites (TFBSs) found in the proximal promoter regions (-1,000/+200bp from TSS) of salmonid genes. The motif enrichment analysis utility of this

tool was used to screen for enrichment of NFAT and glucocorticoid response element (GRE) motifs in lists of DEGs.

2.8 Data sharing

All relevant data and supporting information can be found within the manuscript or its supporting information, and the full transcriptomics dataset is accessible in the ArrayExpress depository, with accession number E-MTAB-8276.

3. Results

3.1 Phenotypic attributes

To verify that the lighting protocol produced the anticipated phenotypic response, we focused on two attributes: body condition factor (CF = 100 x wet weight / length^3) and hypo-osmoregulation during a 24-h SW challenge.

Over the course of the study, CF remained stable and at a similar value in both the LL and SP groups of fish (fig. 1B). Contrastingly, a pronounced decline if CF was seen over the last 3 sampling points in the SPLL fish ($P < 0.001$ for photoperiod treatment x time interaction, 2 way ANOVA), so that, by the end of the experiment CF was approximately 20% lower in SPLL fish than in either SP or LL fish. This decline in CF is a standard hallmark of light-induced smoltification (Björnsson et al. 1989, Stefansson et al. 2008).

At the beginning of the experiment (Day 1), when all fish were acclimated to LL, exposure to a 24-h SW challenge resulted in a plasma osmolality of 361 ± 4.7 mosmol / l. In fish that remained in LL, this response was largely stable over the course of the study, with a slight but significant decline in osmolality seen in SW-challenged LL fish at the last sampling on day 110 (343 ± 5.0 mosmol / l; $P <0.05$ compared to day 1 by Tukey post hoc test).

Transfer to SP produced a sustained reduction in hypo-osmoregulatory performance in response to SW challenge (fig. 1B), with plasma osmolality at days 53 and 68 of the experiment being some 20% higher compared to day 1 ($P<0.0001$ by Tukey post-hoc test). Thereafter continued SP exposure was associated with an apparent recovery of hypo-osmoregulatory ability, so that by

day 110 plasma osmolality values following SW challenge were not significantly higher than at day 1.

Within 28 days of return to LL following exposure to SP, hypo-osmoregulatory performance recovered to levels not significantly different from those seen at day 1 (SPLL day 68 = 344 ± 2.1 mosmol / l), and the lowest values recorded in the study as a whole were in the SPLL group at day 110 (328 ± 7.1 mosmol / l).

3.2 RNA profile of the gill response to SW-challenge

To explore treatment effects on the overall RNA expression profile of the gills we performed a PCA analysis (fig. 2A). The first component separated samples by photoperiodic history and sampling time (40% variation explained, PC1) while the second component separated the FW from the SW-challenged fish (30% variation explained, PC2). On the PC1 axis the largest separation of data points was between early (T4, one week after re-entry to LL) and late (T5 and T6, 4 and 8 weeks after re-entering LL) sampling points for SPLL fish. This contrasted with low PC1 resolution for samples from fish in either the LL or SP control groups. The PC2 separation was most pronounced in SP control fish and less so in LL control fish. For the SP and LL groups divergence along PC2 appear independent of time. Contrastingly, in SPLL fish, PC2 resolution was dependent on time of sampling with major segregation between FW and SW samples at T4, one week after re-entering LL, while at both later time points resolution between FW and SW samples was greatly reduced. Overall the PCA analysis indicates that return to LL after SP exposure dampens the transcriptional response to SW exposure.

To further investigate this effect, we compared lists of genes whose expression was significantly induced or suppressed by 24-h SW challenge relative to the corresponding FW control fish (SW-DEGs; FDR < 0.01, fold-change > |1|, supplemental material S1) for the 3 photoperiod groups . At the end of the study (T6) we found some 10-fold more SW-DEGs in SP fish than in either the LL or SPLL groups (fig. 2B, C). Separate gene ontology enrichment tests were performed for genes responding to SW exposure at T6 in the three photoperiod treatments (supplemental material S3 through S6). Enriched ontologies for SP fish included up-regulated transcripts associated with chromatin silencing and suppression of transcription (e.g. histone deactylase 5, transcriptional repressor p66, NFAT5; GO:0000122 '*negative regulation of transcription by RNA polymerase 2*'), and also with formation of stress granules, indicative of translational arrest due to cellular stress (Anderson and Kedersha 2008) (e.g. ddx6, ddx3x, roquin 1; GO:0010494, '*stress granule'*).

Only 51 SW-DEGs (i.e. about 5% of the SP set) were shared across all three photoperiod treatments, and this shared group included genes involved in mitochondrial respiration (e.g. cytochrome P450 subunits, hexokinase-1), presumably reflecting the energy demand imposed by SW challenge. Correspondingly, the only significantly over-represented BP GO-term shared across the photoperiod treatments was GO:0000302, '*response to reactive oxygen species'*, encompassing six of the shared genes (fig. 2D).

While there is a similar number of SW-DEGs at T6 in the LL and SPLL treatments (150 and 125 genes, respectively), the overlap between these two groups was almost entirely limited to the universally responsive energy-related genes described above. LL-specific SW-DEGs at T6 were mainly associated with metabolism and cell signaling (f. ex. GO: 0009749 '*response to glucose*', GO:0051591 '*response to cAMP*'). In contrast to the SP and LL groups, the SPLL group had a

dramatic reduction in DEGs in response to SW between T4 and T6 (Figure 2C). Within the group of SW-induced genes unique to SPLL at the T6 time-point, the inward rectifying K+ channels KCNJ1 and KCNJ5 and 'junctional cadherin 5 associated' (JCAD, also known as KIAA1462) were the most strongly induced transcripts (supplemental material S2).

3.3 Effects of SW on the expression of NFAT family members

The highly divergent transcriptional responses to SW, including the presence of NFAT5 only in the list of SP-specific DEGs led us to explore further the regulation of expression among all members of the NFAT family of transcription factors (fig. 3, supplemental material S7 and S8). Clustering of response patterns across this gene family gave four distinctive patterns of regulation, represented by the four profile plots in fig 3. The NFAT5b cluster (fig. 3, second cluster from the top) showed strong, SP-specific SW-induction, while weaker SP-specific SW-induction of expression was also seen in the cluster typified by NFAT4c (LOC106600383) (fig. 3, first cluster from the top), but only evident at earlier sampling points (T4, T5). Contrastingly, genes typified by NFAT3c (LOC106561519) showed reduced expression in SW (fig. 3, third cluster from the top). The last cluster of genes were largely SW-unresponsive across the study as a whole (fig. 3, fourth cluster from the top).

3.4 Enrichment for NFAT- and GRE-response motifs in SW-DEGs

We used MotifDb ((Mulugeta et al. 2019) (https://salmobase.org/apps/SalMotifDB/) to determine how NFAT response elements are associated with SW-induced changes in gene expression (fig. 4A), focusing on changes occurring at the last sampling point (T6, day 110) of the experiment. This revealed enrichment of seven non-redundant motifs, of which four are associated with SW-induced gene expression changes, in the LL control fish ($p<=0.001$). Three

response elements were enriched in the SP control fish. No enrichment of NFAT elements was seen in SPLL fish at this sampling point. We also looked at presence of glucocorticoid receptor response elements (GREs, fig. 4B) due to the stress response indicated by GO-terms in the SP group, and confirmed that these were only enriched among the SW-response genes in the SP-group (fig. 4B).

4. Discussion

Recently we used RNAseq to demonstrate that photoperiodic history produces a complex suite of changes in gill function during the freshwater preparative phase of smoltification in juvenile Atlantic salmon (Iversen et al. 2020). Here we have extended that analysis to consider how photoperiodic history and associated preparative changes in gill function affects the gill response to SW exposure. SP exposure dramatically impairs the ability of juvenile salmon to hypo-osmoregulate in SW and is associated with extensive changes in gill gene expression (fig. 2), including genes predicted to be regulated by the glucocorticoid pathway (fig. 4B), indicative of cellular stress. Contrastingly, exposure of LL acclimated fish to SW results in a comparatively modest osmoregulatory disturbance over 24-h, and is associated with less extensive changes in gill gene expression (fig. 2). Nevertheless, a major effect of photoperiodic history was observed in the transcriptional response of LL acclimated fish to SW, with the response profiles of fish held on LL throughout life being highly distinctive from those fish which had experienced an 8 week period of exposure to SP prior to return to LL. The diminished role of NFAT transcriptional regulation in the SW response of SPLL fish (fig. 4A) suggests that preparative effects of SP exposure reduce the involvement of pathways linked to changes in cellular tonicity or intracellular calcium levels in the response to SW.

Previous work by Lorgen et al. (2015, 2017) showed that in the gill the SW-induced gene *dio2a* is enriched for NFAT5 response-elements, and that expression of both dio2a and NFAT5b is SW-induced in SP-acclimated Atlantic salmon juveniles. Our RNAseq analysis confirms these findings, showing that strongest SW-induction of NFAT5b is indeed seen in SP acclimated fish, as well as implicating NFAT4 and NFATc3 in the response. Given that this is the case, it is somewhat surprising that statistical enrichment for NFAT motifs is less pronounced within the SW-induced transcriptome of SP fish than in LL fish. We believe this may reflect a swamping of signal by large numbers of genes induced through stress-activated pathways, including but probably not limited to the corticoid axis revealed by GRE enrichment in SW-induced genes in SP fish. In support of this interpretation the subset of SW-induced genes shared between fish in the LL and SP T6 groups, which constitutes less than 10% of the overall SP SW-induced group (but about half of the LL SW-induced group) is highly enriched for NFAT5 elements ($p<0.01$).

Despite the superficial similarity observed between the LL and SPLL fish in ability to hypo-osmoregulate (fig. 1B) as well as the magnitude of transcriptional responses to SW exposure (fig. 2), it is clear from the GO analysis that the SW-responses of fish in these two groups are quite distinctive. We suggest that the marked enrichment of NFAT-response elements, and in particular NFAT5, in the LL group reflects a transient activation of NFAT5-responsive genes in response to SW. By contrast, in the SPLL group there is no motif enrichment for NFAT5 nor the Ca^{2+}-regulated NFATs. We interpret this lack of NFAT5 responses in SPLL as evidence for NFAT5-signaling playing a role in the activation of hypo-osmoregulation in salmon which have not developed a SW migratory phenotype. Accordingly, exposure to SP for 8 weeks prior to re-exposure to LL stimulates pre-adaptation and obviates the need for NFAT-mediated responses to SW exposure

– presumably because, even in the initial phase of SW exposure, the changes in tonicity or intracellular Ca^{2+} levels in pre-adapted gill cells are comparatively modest.

The transcriptional response of the NFAT family was not limited to NFAT5b since we also observed SW-induction of NFATc1 and c4 in the SP group, and photoperiodic history-dependent SW-suppression of NFATc3 and NFATc1 paralogous pairs in the SP and SPLL groups. In mammals, these calcium-regulated NFAT's play important roles in immune function, but also in the development, differentiation and function of various other cell types such as osteoclast and cardiac tissue (Hogan et al. 2003, Macian 2005, Ames et al. 2016). Changes in intracellular calcium leading to NFAT activation may conceivably arise as a result of Ca^{2+} production as a second messenger within the cell, or as a result of Ca^{2+} entry from the environment – and both these pathways are likely to be involved in osmosensing (Kültz 2012).

In addition, extracellular Ca^{2+} may affect gill function through the G-protein coupled calcium sensing receptor (CaSR), expressed in the MRCs and proposed to function as a salinity sensor in fish (Nearing et al. 2002, Loretz 2008, Loretz et al. 2009). While CaSR signal transduction has primarily been linked to cAMP-dependent signal transduction, the possibility of cross-talk with NFAT pathways is suggested by work on TNF secretion in the mammalian kidney tubule (Abdullah et al. 2006, Gong and Hou 2014).

Our results clearly show that NFATs are playing a minor role in SW regulated transcriptional responses in SPLL fish compared to LL and SP. This is consistent with a model where the photoperiodic treatment received (SPLL) is known to stimulate a range of smolt characteristics including improved long-term performance in SW (Saunders et al. 1985, Stefansson et al. 1991,

Berge et al. 1995, McCormick et al. 1995, McCormick et al. 2007, Stefansson et al. 2008). With the exception of day 68 (i.e. the first week after return to LL from SP, when these fish are in a transitional state), there is no SW-induction of NFAT5b-expression or any other NFATs, nor is there any enrichment of NFAT-motifs in the SW-responsive transcriptome. Nevertheless, a small number of genes were uniquely stimulated by SW in the SPLL group. These included the inward rectifying potassium channel genes KCNJ1 and KCNJ5, the former being ATP-regulated and the latter being G-protein regulated (Ho et al. 1993, Clapham 1994, Krapivinsky et al. 1995). Also, we find the cardiac regulatory gene junctional protein associated with coronary artery disease, known as JCAD. The potassium channels have been identified as key markers for SW adaptation in eels, where they have been found to be expressed in MRCs (Suzuki et al. 1999, Tse et al. 2006). JCAD is predicted to play a role in endothelial cell junctions (Akashi et al. 2011) and has been linked to the Hippo signaling pathway (Jones et al. 2018), which regulates cell proliferation and apoptosis (Halder and Johnson 2011). Both KCNJ1 and JCAD show high SW-inducibility after being exposed to the photoperiod-induced smolting (S2), and they therefore represent the final activational response to SW occurring specifically in fish that have completed a FW preparative phase in response to photoperiod. Further studies to understand the impact of these genes on gill function in SW are now warranted.

References

Abdullah, H. I., Pedraza, P. L., Hao, S., Rodland, K. D., McGiff, J. C., et al. (2006). "NFAT regulates calcium-sensing receptor-mediated TNF production." Am J Physiol-Renal **290**(5): F1110-F1117.

Akashi, M., Higashi, T., Masuda, S., Komori, T. and Furuse, M. (2011). "A coronary artery disease-associated gene product, JCAD/KIAA1462, is a novel component of endothelial cell–cell junctions." Biochem Bioph Res Co **413**(2): 224-229.

Alexa, A., Rahnenführer, J. and Lengauer, T. (2006). "Improved scoring of functional groups from gene expression data by decorrelating GO graph structure." Bioinformatics **22**(13): 1600-1607.

Ames, R. Y., Valdor, R., Abe, B. T. and Macian, F. (2016). Encyclopedia of Signaling Molecules. **N:** 1-8.
Anders, S., Pyl, P. T. and Huber, W. (2015). "HTSeq--a Python framework to work with high-throughput sequencing data." Bioinformatics **31**(2): 166-169.
Anderson, P. and Kedersha, N. (2008). "Stress granules: the Tao of RNA triage." Trends in Biochem Sci **33**(3): 141-150.
Andrews, S. (2010). "FastQC: a quality control tool for high throughput sequence data." from http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
Andrews, S., Lindenbaum, P., Howard, B. and Ewels, P. (2011-2014). FastQC High Throughput Sequence QC Report.
Arnesen, A. M., Toften, H., Agustsson, T., Stefansson, S. O., Handeland, S. O., et al. (2003). "Osmoregulation, feed intake, growth and growth hormone levels in 0+ Atlantic salmon (Salmo salar L.) transferred to seawater at different stages of smolt development." Aquaculture **222**(1): 167-187.
Berge, A. I., Berg, A., Fyhn, H. J., Barnung, T., Hansen, T., et al. (1995). "Development of salinity tolerance in underyearling smolts of Atlantic salmon (*Salmo salar*) reared under different photoperiods." Can J Fish Aquat Sci **52**(2): 243-251.
Björnsson, B. T., Thorarensen, H., Hirano, T., Ogasawara, T. and Kristinsson, J. B. (1989). "Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypoosmoregulatory ability of juvenile Atlantic salmon (Salmo salar) during parr-smolt transformation." Aquaculture **82**(1-4): 77-91.
Cheung, C. Y. K. and Ko, B. C. B. (2013). "NFAT5 in cellular adaptation to hypertonic stress – regulations and functional significance." J Mol Sign **8**(1): 5.
Clapham, D. E. (1994). "Direct G Protein Activation of Ion Channels?" Ann Rev Neurosci **17**(1): 441-464.
Conway, J. R., Lex, A. and Gehlenborg, N. (2017). "UpSetR: an R package for the visualization of intersecting sets and their properties." Bioinformatics **33**(18): 2938-2940.
Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., et al. (2013). "STAR: ultrafast universal RNA-seq aligner." Bioinformatics **29**(1): 15-21.
Duston, J. and Saunders, R. L. (1990). "The entrainment role of photoperiod on hypoosmoregulatory and growth-related aspects of smolting in Atlantic salmon (*Salmo salar*)." Can. J. Zool. **68**(4): 707-715.
Evans, D. H., Piermarini, P. M. and Choe, K. P. (2005). "The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of Nitrogenous Waste." Physiol. Rev. **85**(1): 97-177.
Evans, T. G. and Somero, G. N. (2008). "A microarray-based transcriptomic time-course of hyper- and hypo-osmotic stress signaling events in the euryhaline fish Gillichthys mirabilis: osmosensors to effectors." J Exp Biol **211**(22): 3636-3649.
Evans, T. G. (2010). "Co-ordination of osmotic stress responses through osmosensing and signal transduction events in fishes." J Fish Biol **76**(8): 1903-1925.
Ferraris, J. D., Williams, C. K., Persaud, P., Zhang, Z., Chen, Y., et al. (2002). "Activity of the TonEBP/OREBP transactivation domain varies directly with extracellular NaCl concentration." P Natl A Sci **99**(2): 739-744.

Folmar, L. C. and Dickhoff, W. W. (1980). "The parr—Smolt transformation (smoltification) and seawater adaptation in salmonids: A review of selected literature." Aquaculture 21(1): 1-37.
Gong, Y. and Hou, J. (2014). "Claudin-14 Underlies Ca++-Sensing Receptor–Mediated Ca++ Metabolism via NFAT-microRNA–Based Mechanisms." J Am Soc Nephrol 25(4): 745-760.
Gross, M. R., Coleman, R. M. and McDowall, R. M. (1988). "Aquatic Productivity and the Evolution of Diadromous Fish Migration." Science 239(4845): 1291-1293.
Halder, G. and Johnson, R. L. (2011). "Hippo signaling: growth control and beyond." Development 138(1): 9-22.
Handeland, S. O., Jarvi, T., Ferno, A. and Stefansson, S. O. (1996). "Osmotic stress, antipredator behaviour, and mortality of Atlantic salmon (Salmo salar) smolts." Can J Fish Aquat Sci 53(12): 2673-2680.
Handeland, S. O., Berge, Å., Björnsson, B. T., Lie, Ø. and Stefansson, S. O. (2000). "Seawater adaptation by out-of-season Atlantic salmon (Salmo salar L.) smolts at different temperatures." Aquaculture 181(3): 377-396.
Higgins, P. J. (1985). "Metabolic differences between Atlantic salmon (Salmo salar) parr and smolts." Aquaculture 45(1): 33-53.
Hiroi, J. and McCormick, S. D. (2012). "New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish." Respir Physiol Neurobiol 184(3): 257-268.
Ho, K., Nichols, C. G., Lederer, W. J., Lytton, J., Vassilev, P. M., et al. (1993). "Cloning and expression of an inwardly rectifying ATP-regulated potassium channel." Nature 362(6415): 31-38.
Hogan, P. G., Chen, L., Nardone, J. and Rao, A. (2003). "Transcriptional regulation by calcium, calcineurin, and NFAT." Genes Dev 17(18): 2205-2232.
Houde, A. L. S., Gunther, O. P., Strohm, J., Ming, T. J., Li, S., et al. (2018). "Discovery and validation of candidate smoltification gene expression biomarkers across multiple species and ecotypes of Pacific salmonids." bioRxiv: 474692.
Hwang, P.-P. and Lee, T.-H. (2007). "New insights into fish ion regulation and mitochondrion-rich cells." Comp. Biochem. Phys. A 148(3): 479-497.
Hwang, P.-P., Lee, T.-H. and Lin, L.-Y. (2011). "Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms." Am J Physiol Reg Integr Comp Physiol 301(1): R28-R47.
Irarrazabal, C. E., Gallazzini, M., Schnetz, M. P., Kunin, M., Simons, B. L., et al. (2010). "Phospholipase C-γ1 is involved in signaling the activation by high NaCl of the osmoprotective transcription factor TonEBP/OREBP." P Natl A Sci 107(2): 906-911.
Iversen, M., Mulugeta, T., Gellein Blikeng, B., West, A. C., Jørgensen, E. H., et al. (2020). "RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile Atlantic salmon." Plos One 15(4): e0227496.
Jones, P. D., Kaiser, M. A., Najafabadi, M. G., Koplev, S., Zhao, Y., et al. (2018). "JCAD, a Gene at the 10p11 Coronary Artery Disease Locus, Regulates Hippo Signaling in Endothelial Cells." Arteriosc Throm Vas 38(8): 1711-1722.
Kalujnaia, S., McWilliam, I. S., Zaguinaiko, V. A., Feilen, A. L., Nicholson, J., et al. (2007). "Salinity adaptation and gene profiling analysis in the European eel (Anguilla anguilla) using microarray technology." Gen Comp Endocrinol 152(2): 274-280.
Kiilerich, P., Kristiansen, K. and Madsen, S. S. (2007). "Cortisol regulation of ion transporter mRNA in Atlantic salmon gill and the effect of salinity on the signaling pathway." J. Endocrinol. 194(2): 417-427.

Krapivinsky, G., Gordon, E. A., Wickman, K., Velimirović, B., Krapivinsky, L., et al. (1995). "The G-protein-gated atrial K+ channel IKAch is a heteromultimer of two inwardly rectifying K+-channel proteins." Nature **374**(6518): 135-141.

Kristinsson, J. B., Saunders, R. L. and Wiggs, A. J. (1985). "Growth dynamics during the development of bimodal length-frequency distribution in juvenile Atlantic salmon (Salmo salar L.)." Aquaculture **45**(1): 1-20.

Kültz, D. (2012). "The Combinatorial Nature of Osmosensing in Fishes." Physiology **27**(4): 259-275.

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., et al. (2016). "The Atlantic salmon genome provides insights into rediploidization." Nature **533**(7602): 200-+.

Loretz, C. A. (2008). "Extracellular calcium-sensing receptors in fishes." Com Biochem Phys A **149**(3): 225-245.

Loretz, C. A., Pollina, C., Hyodo, S. and Takei, Y. (2009). "Extracellular calcium-sensing receptor distribution in osmoregulatory and endocrine tissues of the tilapia." Gen Comp Endocr **161**(2): 216-228.

Lorgen, M., Casadei, E., Król, E., Douglas, A., Birnie, M. J., et al. (2015). "Functional Divergence of Type 2 Deiodinase Paralogs in the Atlantic Salmon." Curr Biol **25**(7): 936-941.

Lorgen, M., Jorgensen, E. H., Jordan, W. C., Martin, S. A. M. and Hazlerigg, D. G. (2017). "NFAT5 genes are part of the osmotic regulatory system in Atlantic salmon (Salmo salar)." Mar Genom **31**: 25-31.

Love, M. I., Huber, W. and Anders, S. (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." Genome Biol **15**(12): 550-550.

Lubin, R. T., Rourke, A. W. and Bradley, T. M. (1989). "Ultrastructural alterations in branchial chloride cells of Atlantic salmon, *Salmo salar*, during parr-smolt transformation and early development in sea water." J. Fish Biol. **34**(2): 259-272.

Macian, F. (2005). "NFAT proteins: key regulators of T-cell development and function." Nat Rev Immunol **5**(6): 472-484.

Madsen, S. S., Kiilerich, P. and Tipsmark, C. K. (2009). "Multiplicity of expression of Na^+ ,K^+ – ATPaseα -subunit isoforms in the gill of Atlantic salmon (*Salmo salar*): cellular localisation and absolute quantification in response to salinity change." J. Exp. Biol. **212**(1): 78-88.

Martin, M. (2011). "Cutadapt removes adapter sequences from high-throughput sequencing reads." EMBnet.journal **17**(1): 3.

McCormick, S. D. (1994). "Ontogeny and evolution of salinity tolerance in anadromous salmonids: Hormones and heterochrony." Estuaries **17**(1): 26.

McCormick, S. D., Björnsson, B. T., Sheridan, M., Eilerlson, C., Carey, J. B., et al. (1995). "Increased daylength stimulates plasma growth hormone and gill Na+, K+-ATPase in Atlantic salmon (Salmo salar)." J Comp Physiol B **165**(4): 245-254.

McCormick, S. D., Hansen, L. P., Quinn, T. P. and Saunders, R. L. (1998). "Movement, migration, and smolting of Atlantic salmon (*Salmo salar*)." Can. J. Fish. Aquat. Sci. **55**(S1): 77-92.

McCormick, S. D. (2001). "Endocrine Control of Osmoregulation in Teleost Fish." Am. Zool. **41**(4): 781-794.

McCormick, S. D., Shrimpton, J. M., Moriyama, S. and Björnsson, B. T. (2007). "Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting." Aquaculture **273**(2): 337-344.

McCormick, S. D., Regish, A. M., Christensen, A. K. and Björnsson, B. T. (2013). "Differential regulation of sodium–potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon." J. Exp. Biol. **216**(7): 1142-1151.
Metcalfe, N. B., Huntingford, F. A. and Thorpe, J. E. (1988). "Feeding Intensity, Growth Rates, and the Establishment of Life-History Patterns in Juvenile Atlantic Salmon Salmo salar." J Anim Ecol **57**(2): 463-474.
Mulugeta, T. D., Nome, T., To, T.-H., Gundappa, M. K., Macqueen, D. J., et al. (2019). "SalMotifDB: a tool for analyzing putative transcription factor binding sites in salmonid genomes." BMC Genomics **20**(1): 694.
Nearing, J., Betka, M., Quinn, S., Hentschel, H., Elger, M., et al. (2002). "Polyvalent cation receptor proteins (CaRs) are salinity sensors in fish." P Natl A Sci **99**(14): 9231-9236.
Nilsen, T. O., Ebbesson, L. O. E., Madsen, S. S., McCormick, S. D., Andersson, E., et al. (2007). "Differential expression of gill Na^+,K^+-ATPase α- and β-subunits, Na^+,K^+,$2Cl^-$ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*." J. Exp. Biol. **210**(16): 2885-2896.
Pisam, M., Prunet, P., Boeuf, G. and Jrambourg, A. (1988). "Ultrastructural features of chloride cells in the gill epithelium of the Atlantic salmon, *Salmo salar*, and their modifications during smoltification." Am. J. Anat. **183**(3): 235-244.
Prunet, P. and Boeuf, G. (1985). "Plasma prolactin level during transfer of rainbow trout (Salmo gairdneri) and Atlantic salmon (Salmo salar) from fresh water to sea water." Aquaculture **45**(1): 167-176.
Putney, James W. (2012). "Calcium Signaling: Deciphering the Calcium–NFAT Pathway." Curr Biol **22**(3): R87-R89.
Rao, A., Luo, C. and Hogan, P. G. (1997). "Transcription factors of the NFAT family: Regulation and Function." Ann Rev Immunol **15**(1): 707-747.
Samy, J. K. A., Mulugeta, T. D., Nome, T., Sandve, S. R., Grammes, F., et al. (2017). "SalmoBase: an integrated molecular data resource for Salmonid species." BMC Genomics **18**(1): 482.
Saunders, R. L., Henderson, E. B. and Harmon, P. R. (1985). "Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages." Aquaculture **45**(1): 55-66.
Skilbrei, O. T. (1991). "Importance of Threshold Length and Photoperiod for the Development of Bimodal Length–Frequency Distribution in Atlantic Salmon (Salmo salar)." Can J Fish Aquat Sci **48**(11): 2163-2172.
Stagg, R. M., Talbot, C., Eddy, F. B. and Williams, M. (1989). "Seasonal variations in osmoregulatory and respiratory responses to seawater exposure of juvenile Atlantic salmon (Salmo salar) maintained in freshwater." Aquaculture **82**(1): 219-228.
Stefansson, S. O., Bjornsson, B. T., Hansen, T., Haux, C., Taranger, G. L., et al. (1991). "Growth, parr-smolt transformation, and changes in growth-hormone of Atlantic salmon (*salmo salar*) reared under different photoperiods." Can J Fish Aquat Sci **48**(11): 2100-2108.
Stefansson, S. O., Berge, Å. I. and Gunnarsson, G. S. (1998). "Changes in seawater tolerance and gill Na+,K+-ATPase activity during desmoltification in Atlantic salmon kept in freshwater at different temperatures." Aquaculture **168**(1): 271-277.

Stefansson, S. O., Björnsson, B. T., Ebbesson, L. O. E. and McCormick, S. D. (2008). Smoltification. Fish Larval Physiology. Finn, R. N. and Kapoor, B. G. New Dehli, Science Publishers, Inc. Enfield (NH) & IBH Publishing Co. Pvt. Ltd.

Suzuki, Y., Itakura, M., Kashiwagi, M., Nakamura, N., Matsuki, T., et al. (1999). "Identification by Differential Display of a Hypertonicity-inducible Inward Rectifier Potassium Channel Highly Expressed in Chloride Cells." J Biol Chem **274**(16): 11376-11382.

Tipsmark, C. K., Jørgensen, C., Brande-Lavridsen, N., Engelund, M., Olesen, J. H., et al. (2009). "Effects of cortisol, growth hormone and prolactin on gill claudin expression in Atlantic salmon." Gen. Comp. Endocr. **163**(3): 270-277.

Tse, W. K. F., Au, D. W. T. and Wong, C. K. C. (2006). "Characterization of ion channel and transporter mRNA expressions in isolated gill chloride and pavement cells of seawater acclimating eels." Biochem Bioph Res Co **346**(4): 1181-1190.

Walter, W., Sánchez-Cabo, F. and Ricote, M. (2015). "GOplot: an R package for visually combining expression data with functional analysis." Bioinformatics **31**(17): 2912-2914.

Wilson, J. M. and Laurent, P. (2002). "Fish gill morphology: inside out." J Exp Zool **293**(3): 192-213.

Wilson, J. M., Antunes, J. C., Bouça, P. D. and Coimbra, J. (2004). "Osmoregulatory plasticity of the glass eel of Anguilla anguilla: freshwater entry and changes in branchial ion-transport protein expression." Can J Fish Aquat Sci **61**(3): 432-442.

Woo, S., Lee, S. and Kwon, M. H. (2002). "TonEBP transcriptional activator in the cellular response to increased osmolality." Pflüg Arch Eur J Phy **444**(5): 579-585.

Zhou, X., Ferraris, J. D. and Burg, M. B. (2006). "Mitochondrial reactive oxygen species contribute to high NaCl-induced activation of the transcription factor TonEBP/OREBP." American Journal of Physiology-Renal Physiology **290**(5): F1169-F1176.

Figure legends

Figure 1 Experimental design and the effect of SW challenge on hypo-osmoregulatory capacity. A) Experimental set-up showing the three photoperiod treatments. The protocol established groups of fish with three distinctive photoperiodic histories: those raised on constant light throughout life (LL group), those transferred from LL to 8-h light / 24-h (SP) at day 3 of the study, and maintained on SP to the end of the study (SP group), and those which were returned from SP to LL after 7 full weeks of SP (SPLL group). The white / dark grey shading depicts the periods of light and dark in each 24 hour period over the course of the study. Arrows indicated the points at which tank transfers were made to establish the SP group (Day 3 of the study) and subsequently the SPLL group (Day 60 of the study). Sampling days are indicated by Xs. B) Physiological indices of smoltification: upper panel shows plasma osmolality following 24-h SW challenge, and lower panel shows body condition factor (100 x weight (g) / length(mm)^3). Note re-establishment of acute SW tolerance over last 3 sampling points in SPLL fish, in parallel with a marked decline in CF only in the SPLL group. All data are mean ± S.E.M of n = 6 observations; *** = significantly lower values in SPLL fish than in fish from the same group at T4, $p<0.001$.

Figure 2 Effect of photoperiodic history on the gill transcriptomic response to SW-challenge. A) PCA plot based on gene expression of the sampled fish. Blue indicates fish sampled from FW and red indicates fish sampled after a 24-h SW challenge. B) Venn diagram showing the number of genes whose expression was significantly induced or suppressed when compared the SW-challenged profile to the corresponding FW control (DEGs, $p<0.01$, $\log_2$-FC>|1|) found for each treatment condition at day 110 (T6), and the degree of overlap between the treatments. C) 'Upset'-plots, indicating how the number of DEGs changed across the three latter timepoints of the experiment for each of the treatments. The bar graph shows number of unique or shared genes for the treatment group(s) indicated by the table below. D) GO-term analysis of SW-sensitive gene expression at T6 for the 3 photoperiod treatments; data are shown as Bubble-plots of enriched biological process (BP) GO-terms and the number of genes linked to each term. Terms enriched across groups are indicated by color. Strongly represented GO-terms are labeled. See supplemental figure S3 for other timepoints and GO categories, and supplemental tables S4-6 for a table of GO-terms and names.

Figure 3 Photoperiodic history-dependent responses of NFAT family members to SW-challenge. The heatmap shows the expression of NFAT-genes (CPM) at days 68, 89 and 110 of the SP-treatment, and graphs on the right show representative profiles of selected NFAT-genes in the 3 photoperiod treatments.

Figure 4 Photoperiodic history-dependent promoter motif enrichment for NFAT and glucocorticoid response elements in SW-induced transcript profiles. Panels A and B show the enrichment of NFAT- and GRE-transcription motifs, respectively, in up- and down-regulated genes at day 110 of the experiment (up, do, respectively), for the three different photoperiod-treatments.

Supplemental material

S1 Differentially expressed genes between FW and SW on day 68, 89 and 110, for the different treatments (LL, SP, SPLL). Data filtered for logFC>|1| and FDR<0.01.

S2 Box plots showing the expression of genes KCNJ1, KCNJ4, and JCAD (raw counts).

S3 Additional GO plots showing GO-terms analysis of SW-sensitive genes expression on days 68, 89 and 110 for the different photoperiod treatments. Data are shown as Bubble-plots of GO-terms and the number of genes linked to each term. Terms enriched across groups are indicated by colour. Strongly represented GO-terms are labelled. For days 68 and 89 terms connected to biological processes, cellular components and molecular function are shown, for day 110 only cellular component and molecular function are shown as biological process can be viewed in figure 2.

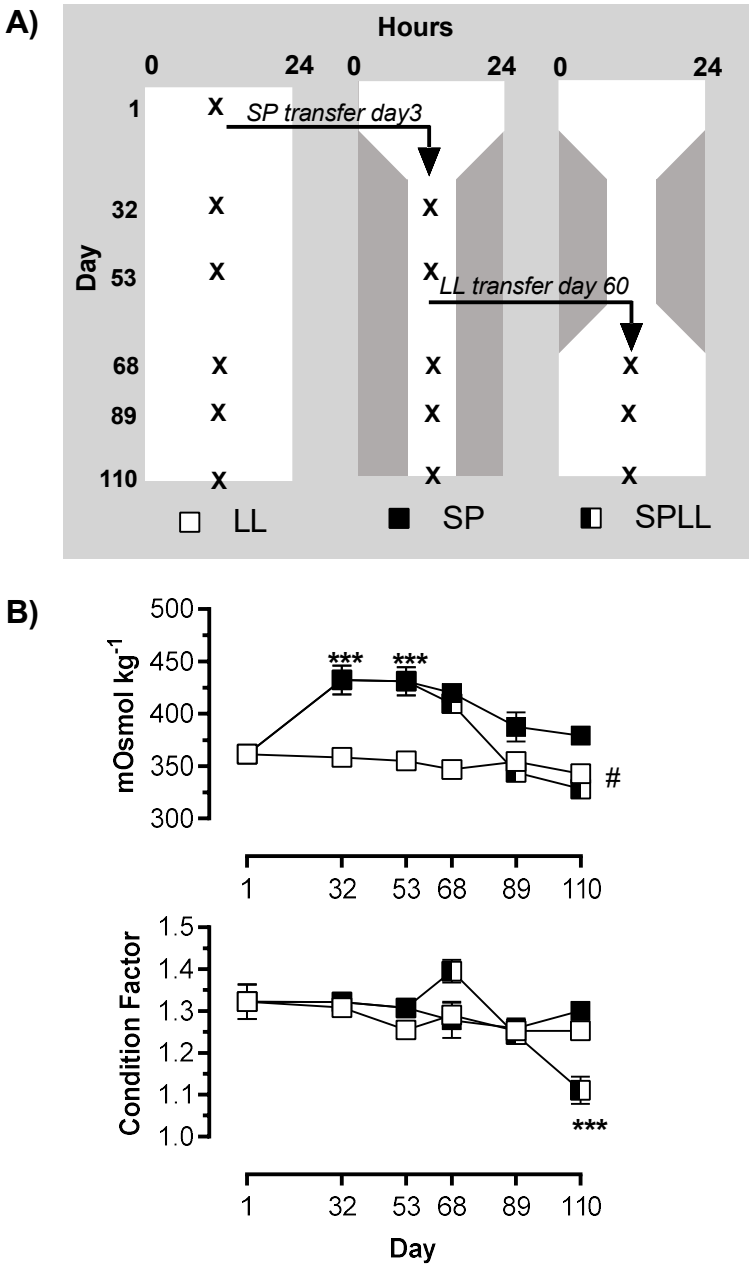
S4 Set of tables (sheet 1,2,3) showing the enriched GO-terms at day 68, for the three photoperiod treatments, adjusted p-value>0.05. Data has also been filtered for number of connected genes. Also included is an overview of the data behind GO plots for day 68 in S3, listing all GO terms, number of genes in each term, and overlap between photoperiod treatments.

S5 Set of tables (sheet 1,2,3) showing the enriched GO-terms day 89, for the three photoperiod treatments, adjusted p-value>0.05. Data has also been filtered for number of connected genes. Also included is an overview of the data behind GO plots for day 89 in S3, listing all GO terms, number of genes in each term, and overlap between photoperiod treatments.

S6 Set of tables (sheet 1,2,3) showing the enriched GO-terms on day 110, for the three photoperiod treatments, adjusted p-value>0.05. Data has also been filtered for number of connected genes. Also included is an overview of the data behind GO plots for day 110 in S3, listing all GO terms, number of genes in each term, and overlap between photoperiod treatments.

S7 Overview of NFAT-genes, including raw count data. Genes are ordered as in the heatmap.

S8 Boxplots showing the expression of the NFAT genes (raw counts).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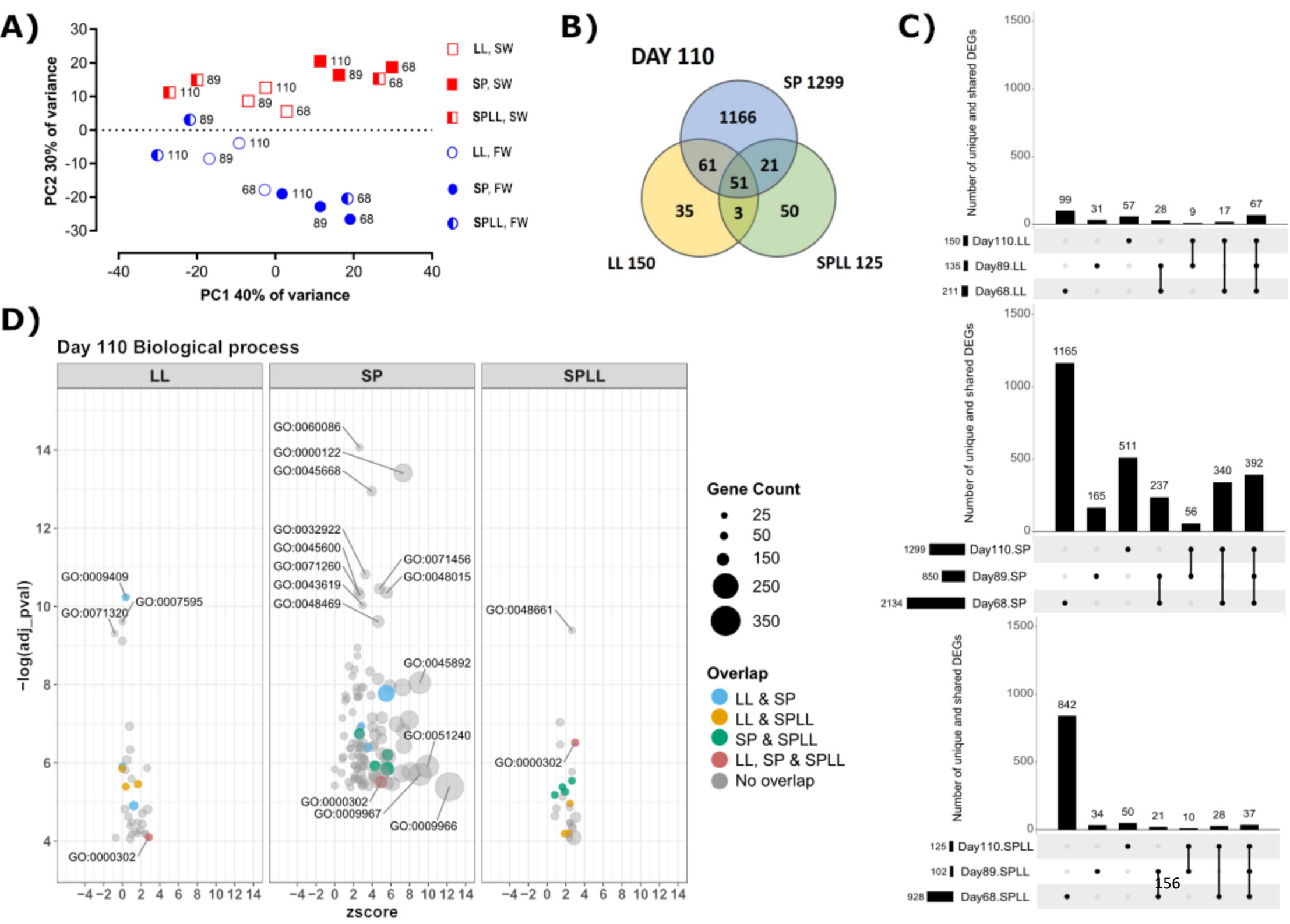
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LL, SW
SP, SW
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LL, FW
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B)
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1166
61
21
51
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3
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C)
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Day68.SP
Day110.SPLL
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Day68.SPLL
D)
Day 110 Biological process
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SPLL
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LL, SP & SPLL
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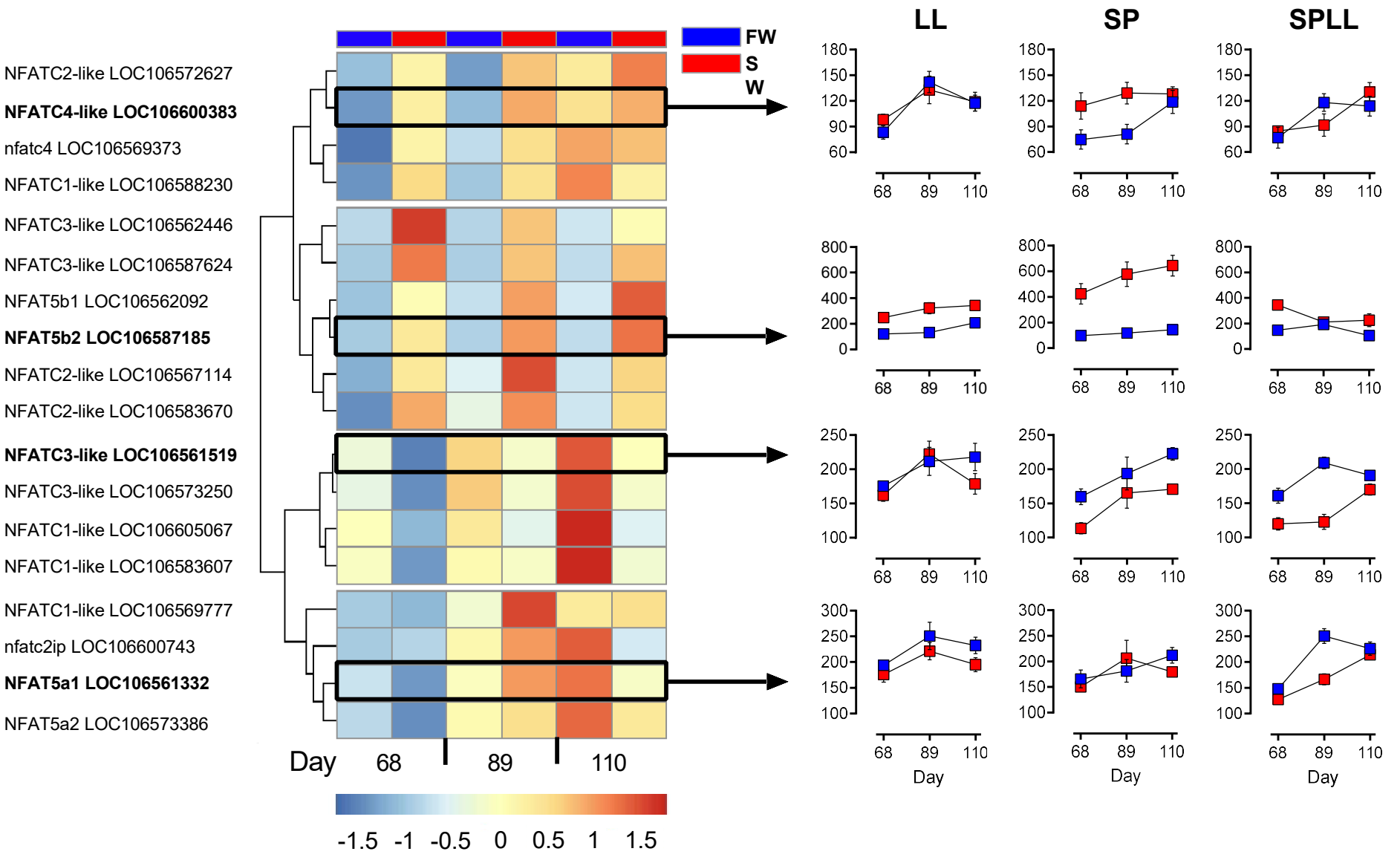
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nfatc2ip LOC106600743
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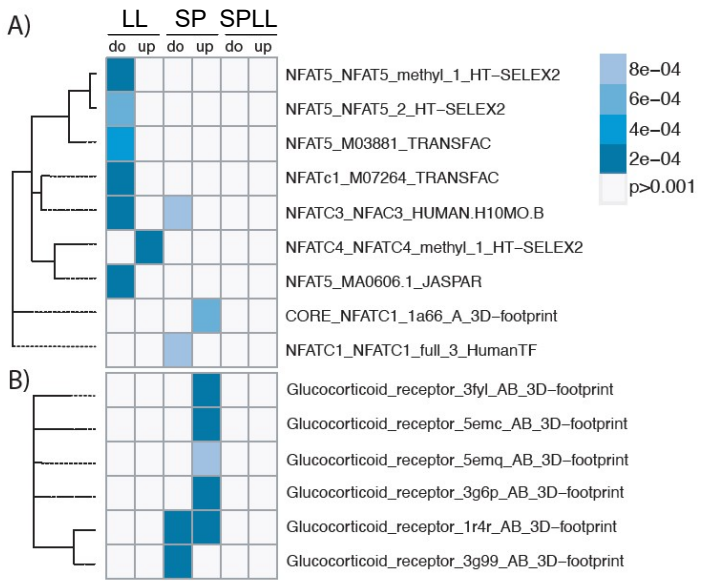
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NFAT5_NFAT5_2_HT−SELEX2
NFAT5_M03881_TRANSFAC
NFATc1_M07264_TRANSFAC
NFATC3_NFAC3_HUMAN.H10MO.B
NFATC4_NFATC4_methyl_1_HT−SELEX2
NFAT5_MA0606.1_JASPAR
CORE_NFATC1_1a66_A_3D−footprint
NFATC1_NFATC1_full_3_HumanTF
8e−04
6e−04
4e−04
2e−04
p>0.001
B)
Glucocorticoid_receptor_3fyl_AB_3D−footprint
Glucocorticoid_receptor_5emc_AB_3D−footprint
Glucocorticoid_receptor_5emq_AB_3D−footprint
Glucocorticoid_receptor_3g6p_AB_3D−footprint
Glucocorticoid_receptor_1r4r_AB_3D−footprint
Glucocorticoid_receptor_3g99_AB_3D−footprint

Appendix 7:
Paper III

Diversified regulation of circadian clock gene expression following whole genome duplication

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PLOS GENETICS

RESEARCH ARTICLE

Diversified regulation of circadian clock gene expression following whole genome duplication

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Abstract

Across taxa, circadian control of physiology and behavior arises from cell-autonomous oscillations in gene expression, governed by a networks of so-called 'clock genes', collectively forming transcription-translation feedback loops. In modern vertebrates, these networks contain multiple copies of clock gene family members, which arose through whole genome duplication (WGD) events during evolutionary history. It remains unclear to what extent multiple copies of clock gene family members are functionally redundant or have allowed for functional diversification. We addressed this problem through an analysis of clock gene expression in the Atlantic salmon, a representative of the salmonids, a group which has undergone at least 4 rounds of WGD since the base of the vertebrate lineage, giving an unusually large complement of clock genes. By comparing expression patterns across multiple tissues, and during development, we present evidence for gene- and tissue-specific divergence in expression patterns, consistent with functional diversification of clock gene duplicates. In contrast to mammals, we found no evidence for coupling between cortisol and circadian gene expression, but cortisol mediated non-circadian regulated expression of a subset of clock genes in the salmon gill was evident. This regulation is linked to changes in gill function necessary for the transition from fresh- to sea-water in anadromous fish. Overall, this analysis emphasises the potential for a richly diversified clock gene network to serve a mixture of circadian and non-circadian functions in vertebrate groups with complex genomes.

Author summary

The generation of daily (circadian) rhythms in behaviour and physiology depends on the activities of networks of so-called clock genes. In vertebrates, these have become highly complex due to a process known as whole genome duplication, which has occurred repeatedly during evolutionary history, giving rise to additional copies of key elements of

available data was used to assess the multi-tissue expression in the Atlantic salmon, these data can be found in the NCBI Sequence Read Archive (SRA): PRJNA72713 and PRJNA260929. All RNA-seq data for the smoltification experiment is available in the European nucleotide archive under project number: PRJEB34224. Nanostring data can be accessed on GEO under the project identifier GSE146530. Source data for the cortisol assay in Fig 4A is provided in S4 Table. Source data for the qPCR in Fig 5 are provided in S6 Table.

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the clock gene network. It remains unclear whether this results in functional redundancy, or whether it has permitted new roles for clock genes to emerge. Here, based on studies in the Atlantic salmon, a species with an unusually large complement of clock genes, we present evidence in favour of the latter scenario. We observe marked tissue-specific, and developmentally-dependent differences in the expression patterns of duplicated copies of key clock genes, and we identify a subset of clock genes whose expression is associated with the physiological preparation to migrate to sea, but is independent of circadian regulation. Associated with this, cortisol secretion is uncoupled from circadian organisation, contrasting with the situation in mammals. Our results indicate that whole genome duplication has permitted clock genes to diversify into non-circadian functions, and raise interesting questions about the ubiquity of mammal-like coupling between circadian and endocrine function.

Introduction

Circadian control of metabolic physiology and behaviour is a ubiquitous characteristic across taxa [1–3]. In eukaryotes, circadian control derives from a cell-autonomous molecular oscillator, assembled from a regulatory network of transcription factors, co-factors, (co-) regulators, chromatin modifiers and an array of post-translational regulators of protein function, often described collectively as 'clock genes' [1]. Clock gene oscillations coordinate the transcription of multiple genes to exert effects on global cell metabolism [1]. While the molecular clock is conserved between insects and mammals [2], the mammalian network contains many duplicated components as a consequence of both local and whole genome duplication (WGD).

Two rounds of WGD preceded the establishment of the tetrapod lineage 500 million years ago (MYA) (Fig 1A), and gave rise to the complement of clock genes seen in mammals, including multiple paralogues of *Period* and *Cryptochrome* genes. Paralogues arising from WGD are known as 'ohnologues', after Susumu Ohno, who wrote a seminal monograph hypothesising that the genetic redundancy proceeding WGD facilitates evolutionary innovation [4,5]. Nevertheless, the evolutionary importance and extent to which clock gene ohnologues are functionally divergent largely remains unclear [6–10]. Indeed the retention of multiple redundant ohnologues of core clock genes is puzzling given that the essential role of the circadian clock has not changed during the course of evolutionary history [1,2,11,12]. Conceivably, functional differences between ohnologues, achieved either by coding sequence differences or by promoter-based differences in expression level, could enable tissue-specific optimization of function, but evidence for this is sparse [11,12]. It has been suggested that preferential interactions of ancient duplicated mammalian PERIOD proteins with specific duplicated mammalian CRYPTOCHROME proteins may affect photic entrainment [13], but experimental evidence is lacking [14]. Tissue-specific functions of mammalian CKIδ/ε ohnologues in regulation of PERIOD protein stability have been suggested [15], and alterations in period (tau), amplitude and clock resetting behavior have been observed but clear distinctions of function between the ohnologues are lacking [8,16–18].

Following the two basal vertebrate WGD events (Fig 1A), subsequent rounds of WGD have occurred in several linages, resulting in highly complex genomes containing thousands of ohnologue pairs. This is exemplified by the situation found in the salmonids, which underwent two additional rounds of WGD compared to basal vertebrates; a third WGD (Ts3R) shared by all teleost fish, and a more recent, the salmonid-specific fourth round of duplication (Ss4R) taking place some 100 MYA (Fig 1A) [19]. The Ss4R event is a defining characteristic of the

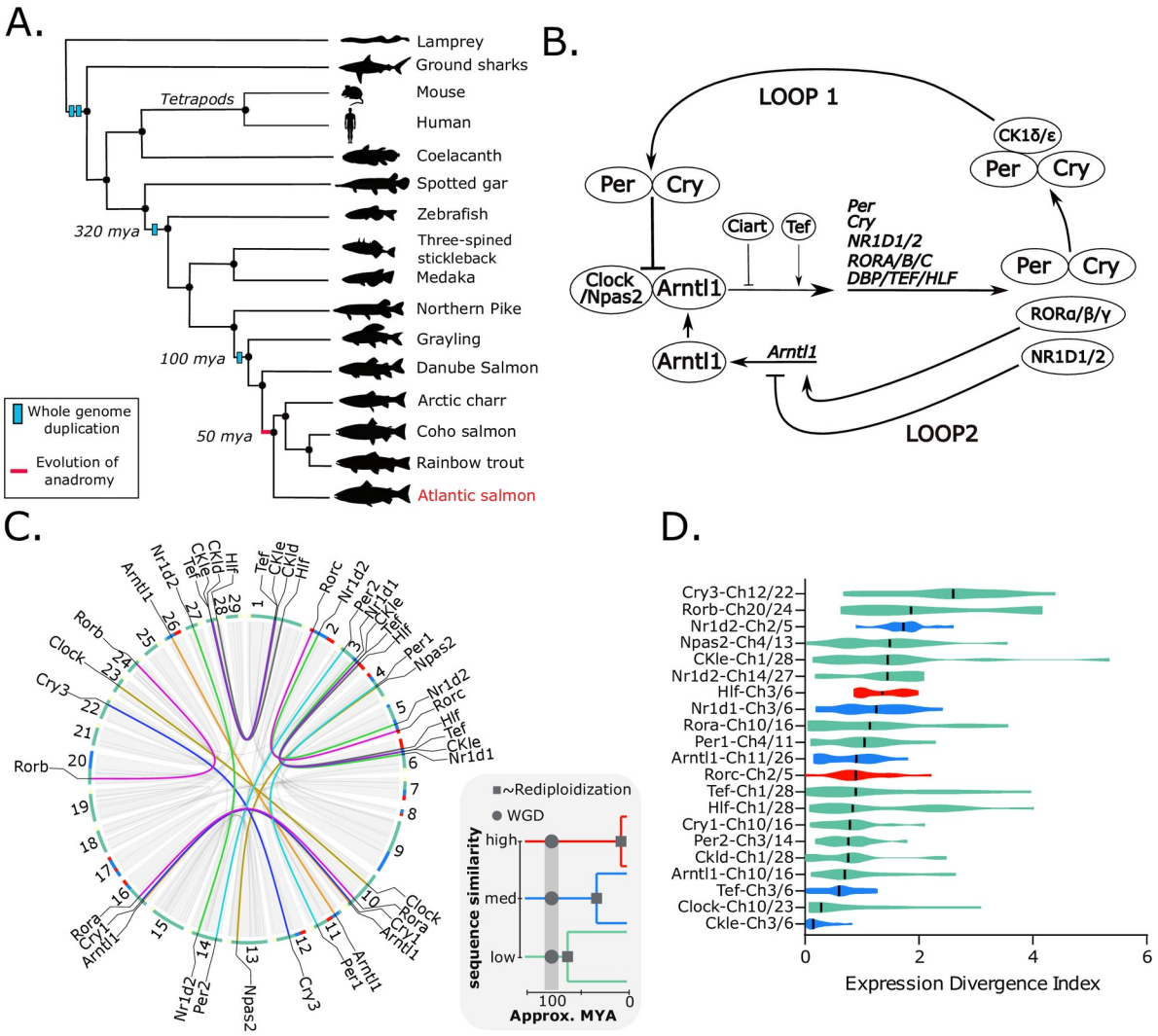

Fig 1. Clock gene Ss4R duplicate pairs are divergently expressed in different tissues. A. Phylogenetic tree highlighting WGD events and evolution of anadromy. All species represented (excluding the lamprey, ground sharks and coelacanth) were included in the phylogenetic identification of Atlantic salmon clock genes. B. The mammalian molecular clock network. C. Circos plot representing the Atlantic salmon chromosomes and the large collinear blocks of the genome that are duplicated (grey blocks/lines). Overlayed are the chromosomal positions of all Ss4R clock ohnologues, duplicated clock genes are connected by a coloured line. The colour of this line represents the different orthogroups. Around the outside of the circos plot sequence similarity of the loci in a 1mb window is shown as a coloured bar; high similarity >95% (red), medium 95–90% (blue), low ~87% (green). Sequence similarity on a genome wide level relates to approximate rediploidization time of Atlantic salmon chromosomes (see grey box). D. Differential regulation of Ss4R pairs in a panel of 11 different tissues. For each Ss4R pair, in each tissue, an expression divergence index (EDI) index was calculated (EDI = abs(log2[Gene1/Gene2)]. The graph shows a violin plot of the distribution of EDI values across all tissues. The vertical black bar represents the median value. Approximate rediploidisation time of each pair is represented by a colour: red—late, blue—mid, green—early.

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salmonid group and is theorized to have led to the evolution of anadromy; an adaptation of freshwater salmonids to spend part of their life-cycle at sea [20]. Genome-scale analysis in salmonids has begun to provide new insights into the evolutionary significance of ohnologue divergence [21,22]. Impressively, even though gene loss often occurs following duplication events (reviewed in: [23]), there remains a rich complexity of clock genes in teleosts compared to mammals. Of the 18 clock genes (as defined in Fig 1B) identified in laboratory mice the

zebrafish genome contains 30, and the Atlantic salmon genome contains 61 clock genes (S1 Table, S1 Appendix).

To understand why so many additional copies of core clock genes are retained, we have undertaken a comprehensive analysis of clock gene expression in the Atlantic salmon, exploring temporal regulation in different tissues and responsiveness to different environmental stimuli. Here, we show diversified regulation of clock ohnologues as a result of WGD, reflecting the fundamental differences in temporal organization of metabolism between tissues.

Results

Tissue-specific expression of clock gene ohnologues indicates regulatory divergence

To identify all conserved clock genes in the Atlantic salmon we extracted amino acid sequences from the well-characterized mouse clock gene network (Fig 1B) then searched for homologous sequences in Atlantic salmon [22] and 12 other vertebrates (S1 Appendix, Materials and methods, *Evolutionary analysis*). Homology relationships between protein sequences were traced back to the root of the vertebrate tree, revealing 61 canonical clock genes in the Atlantic salmon. Comparing the repertoire of clock genes in spotted gar (2 WGDs), zebrafish (3 WGDs), and Atlantic salmon (4 WGDs) (Fig 1A) we find no difference in gene retention for loop 1 versus loop 2 genes (Fig 1B). Forty-two of the 61 salmon circadian genes are duplicates arising from the salmonid specific genome duplication and can be assigned to 21 Ss4R specific ohnologue pairings (referred to as Ss4R pairs from here on), while for the remaining 19 genes no extant Ss4R duplicate can be identified suggesting gene loss after WGD (S1 Table, S1 Appendix). The chromosomal locations of the Ss4R pairs are shown on Fig 1C, and for ease of comparison the genes will be referred to by their gene name and chromosome. The S1 Table lists the specific gene identifiers and orthologues to zebrafish, medaka, spotted gar and mouse.

Following an autopolyploidization, such as the Ss4R, the tetraploid genome will accumulate mutations which block recombination and thereby accelerate duplicate divergence (referred to as rediploidization) [24]. This process has occurred at different rates in different genomic regions in salmonids. Using published data [22] on sequence similarity in 1Mbp windows across syntenic Ss4R regions we could classify the rediploidization times for our 21 Ss4R pairs from early (approx. 87% sequence similarity) to late (>95% sequence similarity) (Fig 1C) and assess whether the history of rediploidization was associated with regulatory divergence.

RNA profiling from 13 different tissues [22] demonstrated tissue-specificity of clock gene expression, and particularly highlighted the wide variety and high abundance of clock genes in the brain (S1 Fig). To assess the divergence between Ss4R pairs we calculated an expression divergence index (EDI), based on the relative expression of each member of a pair across all tissues expressed as a ratio (Fig 1D). This revealed evidence for divergent tissue-specific expression within multiple Ss4R pairs but no clear relationship to approximate time of rediploidization (Fig 1D). The Cry3-Ch12/Ch22 pair had the highest EDI, largely attributable to divergent expression in the brain and gill (S1 Fig). The three Ss4R pairs of *Nr1d1* (*Rev-erbα*) and *Nr1d2* (*Rev-erbβ*), which encode transcriptional repressors linking the circadian clock to energy metabolism [25], were also highly divergently expressed genes, again due to differences in the brain and gill (Fig 1D, S1 Fig). Hence tissue-specific expression divergence is a feature of particular aspects of the circadian clockwork.

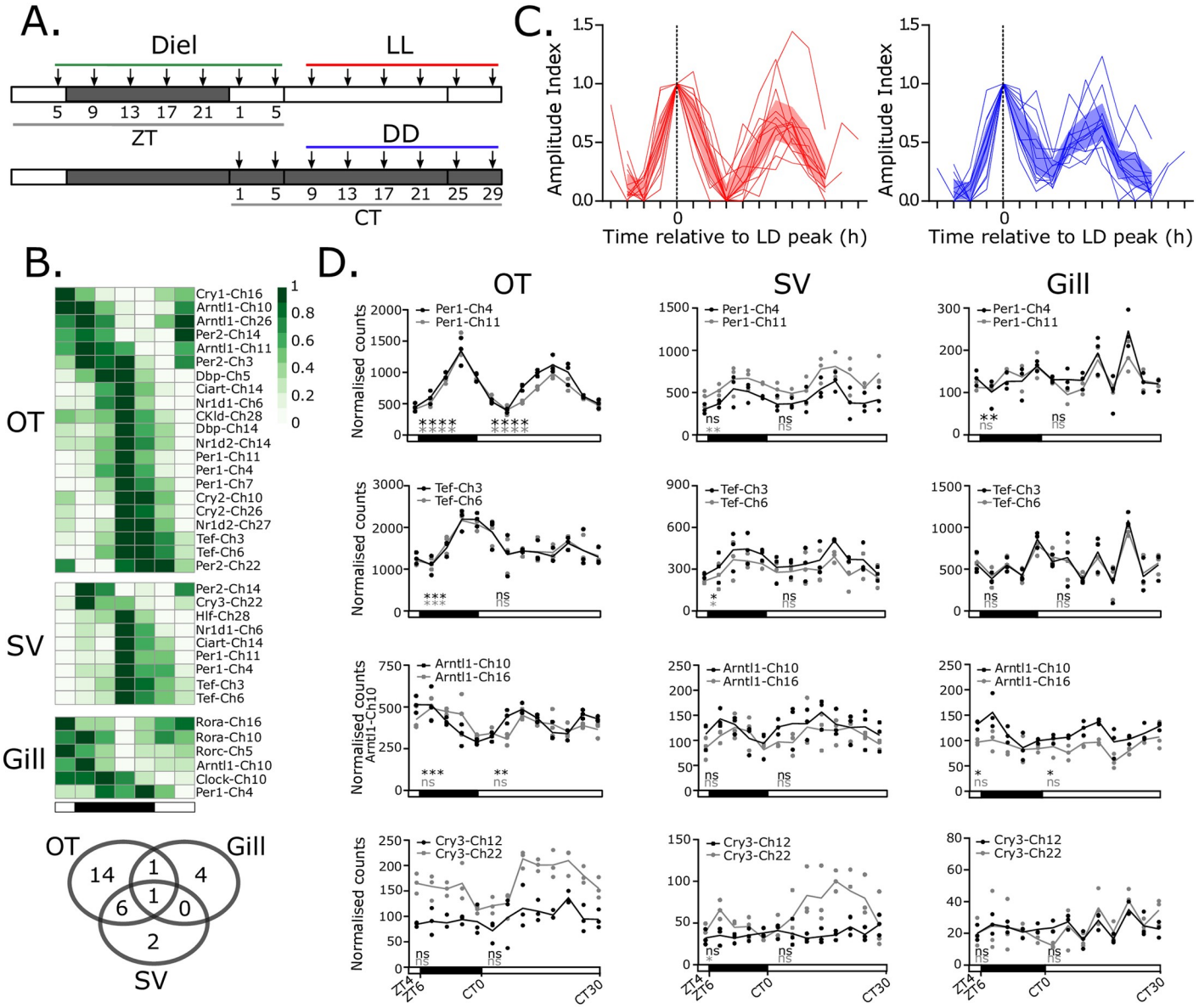

Fig 2. The molecular clock network regulation in the Atlantic salmon. A. Diel and circadian regulation experiment design, arrows indicate tissue collection points. Fish were maintained in a light dark cycle ("Diel"—short photoperiod 6 hours light: 18 dark) transitioning onto either constant light (LL) or constant dark (DD). Sampling in diel conditions was at a 4 hourly resolution starting from 5 hours after lights on (Zeitgeber time—ZT5) for 24 hours. The green line represents the time-points used in the statistical analyses of diel rhythmicity. Constant conditions are defined by circadian time (CT) which is relative to lights on in the preceding cycle. The assessment of DD or LL rhythmicity was made using samples from CT9 to CT29, indicated by the red and blue lines. N = 3 for all time-points. B. Heatmap displays all diel rhythmic genes in OT, SV and gill. Overlap between tissues is shown in the venn diagram. C. Peak-phase aligned LL and DD rhythmic genes in the OT. Shaded area shows 95% confidence limit. D. Example duplicate comparisons from OT, SV and gill. JKTcycle (adjP<0.05*, adjP<0.01**, adjP<0.001***, adjP<0.0001****).

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Circadian and light-regulated Ss4R pair expression differs between tissues but is highly similar within tissues

To test for circadian regulation of gene expression we collected samples from fish kept in a light dark cycle (diel), constant light (LL) and constant dark (DD) (Fig 2A, see Materials and methods, *Circadian experiment I*). To avoid unintended rhythmical stimuli (zeitgebers), fish were fasted from 48 hours before the first sampling point and temperature was held constant.

We focussed on three tissues with distinctive roles in salmonid physiology: the optic tectum (OT) of the brain, because it is linked to visual processing and is coupled directly and indirectly to light input [26–29]; the saccus vasculous (SV) because it has been proposed as a mediator of photoperiodic responses [30]; and the gill because it is essential for respiratory gas exchange, ion- and water balance [31]. We hypothesized that expression profiles of clock genes in these three tissues would differ reflecting tissue-specific differences in temporal metabolic demand. We analysed RNA transcript profiles using a bespoke NanoString CodeSet which could specifically identify 46 clock gene targets including 17 Ss4R pairs (S2 Table, S2A Fig and S2 Appendix).

In diel conditions, we identified 28 oscillating transcripts (JTK-cycle [32], adj.p<0.05, S1 Table) (Fig 2B). Of the three tissues studied, the OT showed by far the strongest oscillations in gene expression, both under diel and constant conditions (Fig 2B and 2C). For half of the genes identified, oscillation was only observed in the OT, and even for genes showing significant oscillation across tissues (e.g. Per1-Ch4) the amplitude of oscillation was clearly highest in the OT (Fig 2B and 2D).

In contrast, rhythmicity in both the SV and gill was much less robust. In the SV, only Nr1d1-Ch6 maintained rhythmicity and phase under DD, while in the gill only Arntl1-Ch10 maintained rhythmicity and phase under DD (S2B and S2C Fig). Hence robust circadian rhythmicity is a feature of the salmon brain, but gene expression rhythms are severely dampened in the peripheral tissues we studied.

Although differences in absolute expression levels were widely seen within Ss4R pairs—both across and within tissues, when comparing temporal dynamics of expression, within a given tissue they were typically similar (Fig 2D, S1 Table, S2 Appendix). This is exemplified by the almost superimposable expression patterns seen for the Per1-Ch4/11 pair (Fig 2D), and for the Tef-Ch3/6 pair (Fig 2D). Indeed, only two significant within-pair differences in expression profile were observed (non-linear regression p-value <0.01, S1 Table, S2D and S2E Fig): the Arntl1-Ch10/16 pair, with Arntl1-Ch10 showing more robust and higher amplitude rhythmicity than Arntl1-Ch16 in the OT (Fig 2D and S2D and S2E Fig), and the arrhythmic Cry3-Ch12/22 pair, with Cry3-Ch22 showing a light-induced increase in expression following transfer to LL in the SV (Fig 2D and S2E Fig). Interestingly, we do not observe light responses through Tef as reported in zebrafish [33].

Regulatory divergence of clock gene ohnologues within a tissue during a developmental transition

The lack of circadian regulatory divergence among Ss4R pairs led us to consider whether retention of duplicates might be related to developmental changes in tissue function. One striking example of this in salmon is the transformation of gills during smoltification from a salt retaining, water excreting organ in freshwater to a salt excreting water retaining organ in seawater [34]. This developmental transition during the anadromous lifecycle relies on hormonally-driven changes in physiology, dependent on seasonally changing day-length (photoperiod) [34]. We therefore performed a photoperiod manipulation experiment, over 110 days, to assess the impact of photoperiod-dependent developmental changes in juvenile salmon (parr) (Fig 3A, see Materials and methods, *smoltification experiment*). This protocol produces a seawater-tolerant (smolt) phenotype within 4–6 weeks of return to LL (S3 Fig) (reviewed in: [34]).

We identified 30 clock genes showing significant changes in expression over the 110 days of the experiment (FDR<0.01, S1 Table, Fig 3B); 3 clock genes were undetectable by RNAseq, while a further 28 were present but did not change significantly over time. Amongst the

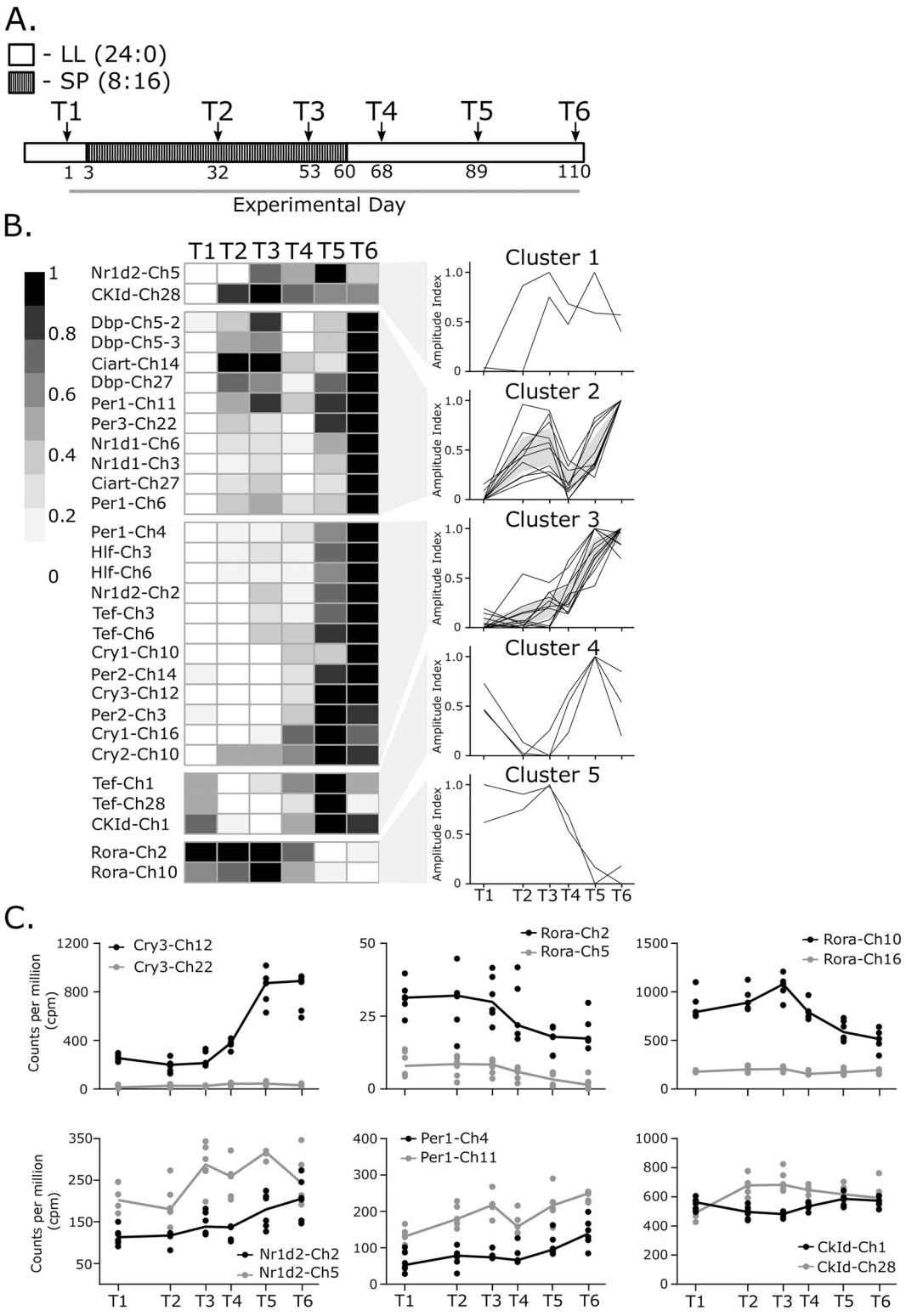

Fig 3. The molecular clock network shows ohnologue specific differences during a photoperiodically driven developmental transition. A. Photoperiodic gene expression experimental design. LL—constant light, SP—short photoperiod. Time-points for sampling (n = 6) indicated by the arrows, sampling was conducted in mid-light phase, time scale shown is the number of experimental days. B. Expression heatmap of significantly photoperiodic (FDR<0.01) clock genes in the gill. Significant genes cluster into five distinct expression patterns. Individual profiles are represented. Shaded area shows 95% confidence limit where applicable. C. RNAseq counts per million profiles for divergently regulated ohnologue pairs.

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differentially regulated genes we found 9 Ss4R pairs (18 genes) where both copies within a pair showed smoltification-associated regulation, while for 3 pairs only one copy of the pair showed significant changes in expression during smoltification.

Gene expression correlation cluster analysis of the differentially expressed genes revealed 5 distinct patterns of expression over the experiment indicating that different regulatory pathways are directing the expression of particular clock genes during smoltification (Fig 3B). Based upon this analysis we identified 6 Ss4R pairs within which evidence of developmental regulatory divergence could be observed (Fig 3C). For 3 of these Ss4R pairs only one member showed a developmental change in expression (Fig 3C, Cry3-Ch12/22, Rora-Ch2/5, Rora-Ch10/16), while for the 3 remaining Ss4R pairs the dynamics of expression, as defined by cluster analysis, differed within the pairing (Fig 3C, Nr1d2-Ch2/5, Per1a-Ch4/11, CKIδ-Ch1/28). Therefore we see strong developmental regulation of clock genes in the gill over 110 days, contrasting with the lack of circadian regulation.

Glucocorticoid signaling induces clock ohnologue expression and accounts for regulatory divergence observed in the Ss4R pair Tef-Ch3/6

While glucocorticoids play a major role in the circadian organization of mammals (reviewed in: [35]), the evidence for an analogous role in fish is unclear (References summarized in: S3 Table). Nevertheless, cortisol is a major hormonal regulator of smoltification in Atlantic salmon, steadily rising during this photoperiod-driven seasonal process [36]. We collected blood samples from fish kept in a light dark cycle (LD—6:18) and in constant conditions (LL or DD) and found no evidence of diel or circadian rhythmicity in cortisol secretion (Fig 4A, see Materials and methods, *Circadian experiment II*, S4 Table) along with weak or absent peripheral tissue clock gene circadian oscillation (S2B and S2C Fig). We hypothesized that the changes in gene expression observed in Fig 3 may be due to seasonally increasing cortisol during smoltification, therefore if we induced cortisol through a simple stress test we may induce the same clock genes seen during smoltification.

To test this we conducted a 24 hour seawater (SW) challenge test in freshwater-adapted fish (Fig 4B, see Materials and methods, *Smoltification experiment*) eliciting an osmotic stress-mediated increase in cortisol secretion (Fig 4C). Gills were collected from SW and fresh water (FW) groups. We identified 15 clock genes showing significant changes in expression in response to SW by RNAseq (FDR<0.01, S1 Table, Fig 4D). Importantly, 87% of acutely SW-responsive clock genes (13/15) also change over the chronic developmental time-scales of smoltification (Fig 3, S1 Table). Amongst the SW responsive genes we found 3 Ss4R pairs (6 genes) where both copies responded to SW, and 6 pairs where only one of the pair changed expression in SW. To assess regulatory divergence within these 9 pairs we plotted the fold change in response to SW for each copy of the pair and determined that 5 of the pairs showed significant regulatory divergence (two-way ANOVA <0.01, S1 Table, Fig 4E).

To further examine if glucocorticoid signaling, via cortisol, was responsible for the induction of clock genes in the gill we used transcription factor binding site analysis [37] on clock genes induced by SW (15 genes) compared to 43 clock genes that were SW-insensitive. SW-induced circadian genes promoters were highly enriched for HSF1 (heat shock factor 1), FOXO1 (forkhead box O1), MAX1 (myc-assocated factor X1) and glucocorticoid receptor response elements (GR) (Fig 4F, S5 Table). Smoltification and responses to SW-exposure are coordinated by multiple endocrine factors including cortisol, growth hormone (GH) and IGF-1 [38,39]. HSF-1 and FOXO-1 elements are regulated by IGF1 signaling, during stress, cellular metabolism and development [40–44]. Furthermore, the enrichment of GR implicates non-

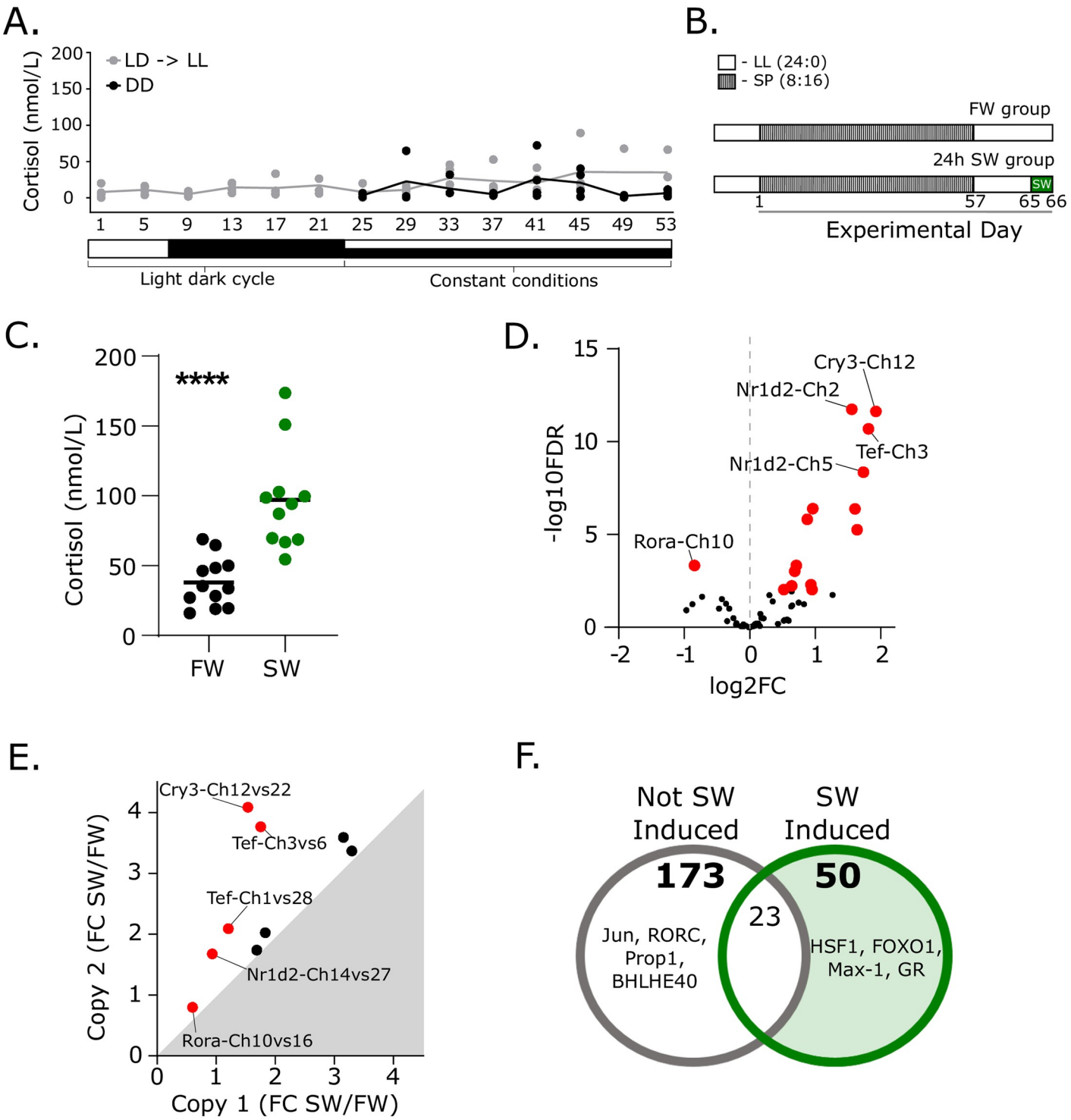

Fig 4. Stress response implicates glucocorticoid receptor signaling in clock ohnologue regulation. A. Diel and circadian profile of plasma cortisol (n = 4). Time axis is given in continuous hours since the start of the experiment, therefore 1 to 21 represent zeitgeber time (ZT) and 25 onwards is equivalent to circadian time (CT)1 to CT29. Due to the sampling protocol time-points 1 and 5 are replotted from time-points 25 and 29. B. Sea-water stress experiment design. LL—constant light, SP—short photoperiod, SW- sea-water challenge. C. Plasma cortisol concentration in blood plasma in sea-water stress experiment (n = 11–12). D. Volcano plot showing sea-water stress regulation of clock genes (n = 6). Significantly regulated transcripts (FDR<0.01) are shown in red. FC—fold change. E. Differential sea-water stress regulation of ohnologue pairs. Significantly different pairs (Analysis of genes where one or both genes are significantly regulated by seawater (FDR<0.01), then submitted to a two-way ANOVA, with sea-water regulation and interaction, p<0.05) are shown in red. F. Predicted transcription factor promoter binding analysis. Both sea-water induced and not-induced gene cohorts were analysed. 50 motifs were specific to the sea-water induced cohort. The top four motifs in each group are displayed.

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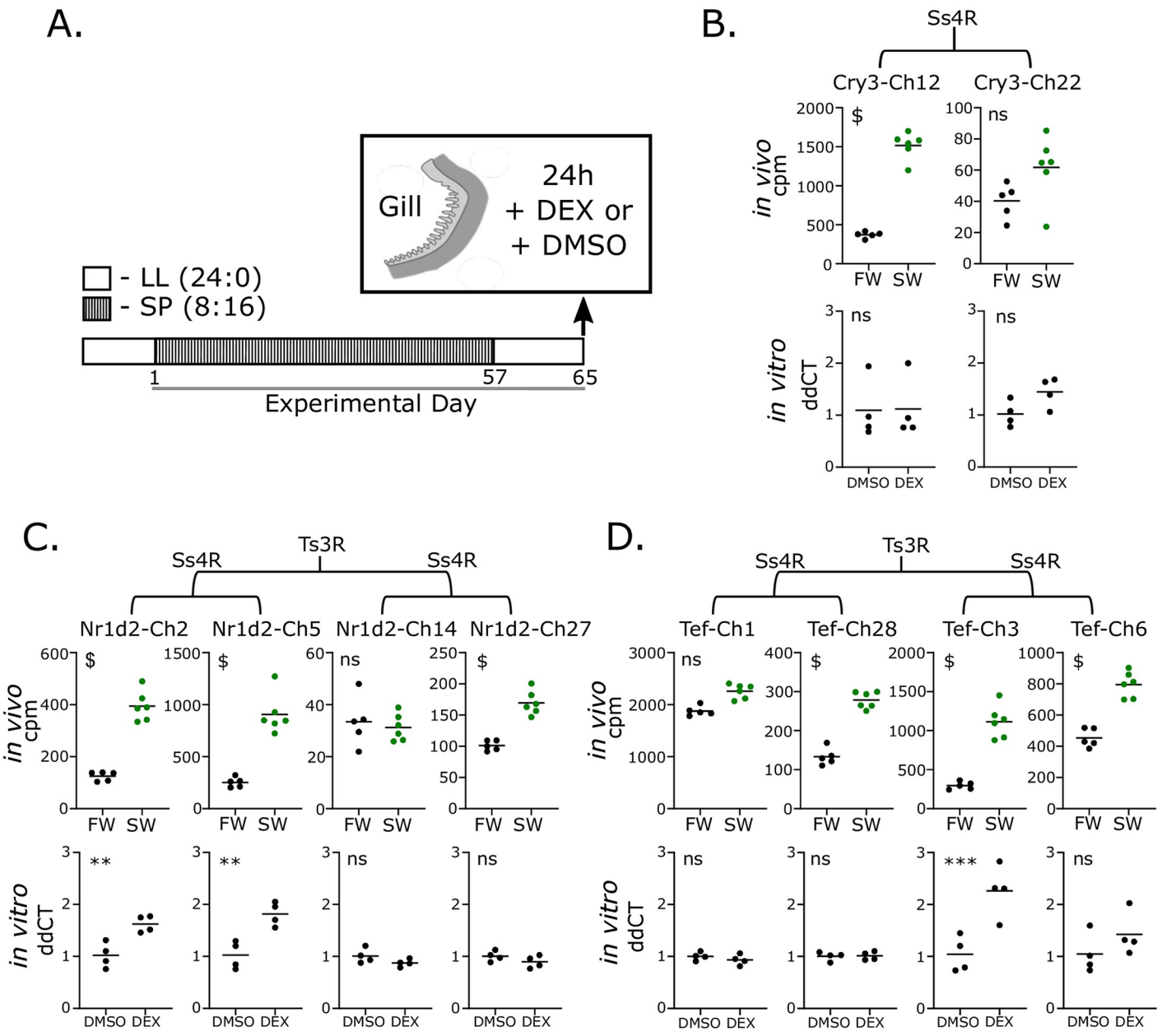

Fig 5. In-vitro validation of glucocorticoid stimulated clock ohnologue expression. A. Dexamethasone-dependent transcript regulation experimental design. After photoperiod manipulation *in vivo*, whole gill arches (n = 4) were removed and treated for 24 hours with the glucocorticoid receptor agonist dexamethasone (DEX), or dimethlysulphoxide (DMSO; vehicle control). B. Comparative regulation of Cry3-Ch12/22 taken from *in vivo* sea-water stress experiment and *in vitro* dexamethasone treatment. C. As in B for Nr1d2-Ch2/5 & Nr1d2-Ch14/27. D. As in B for Tef-Ch1/28 & Tef-Ch3/6. Braches indicate phylogenetic relationship between gene sets.

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circadian glucocorticoid signaling in the induction of clock genes in the gill during smoltification and stress.

Next we wanted to experimentally test the *in-silico* association with glucocorticoid signaling and ask whether differential sensitivity to glucocorticoid signaling might account for the differential regulation observed within Ss4R pairs. We treated isolated gill arches with dexamethasone (DEX; a GR agonist) for 24 hours and then measured the gene expression of the 5 seawater stress regulated Ss4R pairs using qPCR (Fig 5A, S6 Table). We validated the

experiment by assessment of a positive control gene: *Tsc22d3* (*Gilz*) [45](S4 Fig). We found that whilst some of the genes are glucocorticoid sensitive (3/10), supporting the *in-silico* association, the majority are not induced by glucocorticoids (Fig 5B–5D). However, we do demonstrate divergence in the regulation of the Tef-Ch3/6 pair in response to glucocorticoids (Fig 5D).

Discussion

Divergent regulation of gene expression is a major contributor to the evolution of novel adaptations and species diversity [46,47]. Our analysis of the circadian clock network in the Atlantic salmon reveals clear evidence of diversified expression regulation among the many retained copies of canonical clock genes that have arisen through successive WGD events. Diversified regulation is seen in tissue-specific expression patterns (Fig 1), and in response to developmental and environmental stimuli (Figs 3, 4 and 5). Ultimately, these differences likely reflect the uneven influence of diel factors on metabolism and cellular processes in central and peripheral tissues in a cold-blooded fish. Altered sensitivity to GR-signalling, likely mediated through the cortisol axis, emerges as one proximate cause of differences in tissue-specific expression dynamics (Figs 4 and 5). Overall, this analysis emphasises the potential for a richly diversified clock gene network to serve non-circadian functions in vertebrate groups with complex genomes.

A striking contrast emerges between clock gene expression regulation in the OT and the gill. The OT is a brain site which is coupled to light input both through retinal afferents, photoreceptor expression, and indirect melatonin-mediated input via OT melatonin receptors [26–29]. In contrast, the gill is directly exposed to the water environment and continuously handles the osmotic and energetic challenges that this presents [48–50]. Thus while both tissues are highly metabolically active and heavily reliant on ATPase activity to maintain function, the principal environmental influence for the OT is rhythmic light dark input, while for the gill it is continuous osmotic challenge.

Given these differences in tissue function, it is not surprising to observe different complements of clock genes in the two tissues and dramatic differences in temporal dynamics (Fig 2). In the diel-sensitive OT a dominant diel / circadian influence on expression is seen, while in the gill this is a much weaker influence. Nonetheless, clock gene expression in the gill is dynamically regulated, both over the chronic developmental time-scales of smoltification, and acutely in response to osmotic stress. The finding that the complement of genes showing highly sensitive changes in expression in the gill is largely non-overlapping with that under light / circadian control in the OT is clear evidence for diverged expression regulation within the circadian network.

Previous work has identified several Atlantic salmon clock genes that cycle in diel conditions [51–53]. While confirming these earlier findings, our analysis goes far beyond them by showing how the entire clock network is regulated under diel and constant conditions in several tissues. We show that only the OT shows persistent rhythmicity under constant conditions, not the SV or gill (Fig 2). These results sit in contrast to the zebrafish circadian system in which tissue autonomous clock gene oscillation are seen in all tissues investigated [54]. Specific elements of the zebrafish clock appear to be fundamentally different to the Atlantic salmon clock. For example zebrafish Cry1a is acutely light-induced through a D-box in it's promoter, via reactive oxygen species (ROS) and the JNK/MAPK pathway [55]. Importantly this light-mediated ROS inducibility of zebrafish Cry1a appears to be absent in cave fish and mammals [55]. Zebrafish Cry1a has also been reported to pause the circadian mechanism under LL [9], whereas the closest salmon orthologues to zebrafish Cry1a, Cry1-Ch10 and Cry1-Ch16, are

not light-induced and show persistent rhythmicity in the OT under LL (S1 Table). Instead the salmon orthologues to zebrafish Cry3b, Cry3-Ch22 and Cry3-Ch12, are acutely induced by light and salt, respectively. Light induction of Zebrafish Tefa [33] is not seen in the closest salmon orthologues Tef-Ch1 and Tef-Ch28, however, the zebrafish Tefb orthologues, Tef-Ch3 and Tef-Ch6 are seawater induced. In zebrafish, Tefb transcription is induced in response to light and UV exposure and then mediates the expression of other clock genes, initiating a DNA damage repair cascade [56]. This suggests a zebrafish-specific adaptation to UV light exposure, potentially related to spawning environment [56]. In our study we found no enrichment for genes associated with oxidative stress in either the seawater challenge or photoperiodic treatment groups but the photoperiod-driven smoltification induction of all four Tef genes is noteworthy, as is the seawater specific induction of the Ss4r pair Tef-Ch3 and Ch6, and the apparent regulatory divergence of these duplicates in glucocorticoid responsiveness. These species differences indicate plasticity of the circadian clock in response to different environmental stressors depending on life history.

Within the Atlantic salmon the distinct tissue-specific expression dynamics we observe and the smoltification and stress-related induction of clock genes focuses attention on cortisol [34,35,57]. In mammals cortisol is a major player in circadian organization, acting as an internal zeitgeber through which the hypothalamic–pituitary–adrenal (HPA) axis can coordinate daily changes in tissue activity throughout the organism (reviewed in: [35]). Because the teleost hypothalamic–pituitary–interrenal (HPI) axis is functionally analogous to the HPA axis [57], there has been a widespread assumption that cortisol plays a similar circadian coordination function in teleosts [58], although definitive evidence for this is lacking (S3 Table) [59]. Indeed our study implies that it is unlikely that cortisol plays a circadian role in salmonids–we found no evidence for circadian or even diel changes in cortisol secretion in Atlantic salmon (Fig 4A), and GREs are less enriched in circadian oscillating clock genes than in non-oscillating, development- and SW-sensitive clock genes (Fig 4F). Hence while our data strongly implicate cortisol in the dynamic expression of a subset of Atlantic salmon clock genes, this seems to have nothing to do with circadian function *per se*, and more to do with a role for these genes in non-circadian influences of the HPI axis. It is interesting to speculate that this shifting relationship between glucocorticoids and clock genes could be a contributory factor for the evolution of anadromy and the regulation of its seasonal timing. Furthermore, this finding raises interesting questions about the ubiquity and evolutionary origins of mammal-like coupling between the HPA axis and circadian function.

While evidence of functional divergence in ancient duplicated cryptochromes, and, sequence divergence of Ts3R cryptochromes in zebrafish have been observed [60] we expected that Ss4R generated significant genetic complexity in the salmonid circadian clock and therefore asked how expression patterns diverged within Ss4R pairs. Intriguingly, the answer to this question was highly dependent upon context. We saw many examples of pronounced within-Ss4R pair differences in terms of tissue-specific expression, and some 50% of identifiable Ss4R pairs showed within-pair divergence in expression during smoltification. But within-pair divergence in daily expression patterns was hardly observed. Why might this be so? We suggest this may reflect a difference in the way that selection pressures have operated on promoter regions to, on the one hand modulate tissue-specific expression and, on the other daily temporal regulation. According to this view duplication would confer freedom to diverge, thereby meeting differing tissue-specific requirements. Conversely, the daily temporal patterning may be so fundamental to cell function that any mutations leading to deviation from the ancestral dynamics were strongly selected against. This conjecture will require detailed analysis of regions of promoter conservation / divergence among Ss4R pairs.

Materials and methods

Ethics statement

Fish handling and euthanasia was performed by competent persons and in accordance with the European Union Regulations concerning the protection and welfare of experimental animals (European directive 91/492/CCE). The experiment was approved by the Norwegian Committee on Ethics in Animal Experimentation (ID 3630).

Evolutionary analysis

To identify gene orthologs and ohnologs we generated protein sequence homology based orthogroups using the Orthofinder pipeline [61]. For each orthogroup we used the resulting protein tree topology to manually annotate pairs of salmon ohnologs based on the following criteria: (i) salmon ohnologs should form a monophyletic clade only containing genes from other salmonid species, (ii) this monophyletic salmonid clade must have Northern pike as the sister group, and (iii) putative ohnolog pairs had to be conserved in minimum one other salmonid species. Finally, we only retained putative ohnolog if their genomic positions were defined as syntenic regions originating from the Ss4R as defined in Lien *et al.* [22]. Maximum likelihood gene trees with bootstrap values for all genes included in the study are presented in S1 Appendix. These trees include the following species: human, mouse, spotted gar, zebrafish, stickleback, medaka, northern pike, grayling, danube salmon, arctic charr, coho salmon, rainbow trout, Atlantic salmon. The relevant scripts, input files and resulting trees are also available here. For quick reference S1 Table presents the salmon gene loci and their corresponding orthologues in Zebrafish, spotted gar, medaka and mouse.

Multi-tissue analysis

Publically available data was used to assess the multi-tissue expression in the Atlantic salmon (one adult male, kept in freshwater), these data can be found in the NCBI Sequence Read Archive (SRA): PRJNA72713 and PRJNA260929.

Animal husbandry

Juvenile Atlantic salmon (*Salmo salar*, Linnaeus, 1758) of the Aquagene commercial strand (Trondheim, Norway) were used in all experiments. Fish were held under constant light (LL; >200 lux), at 10˚C from hatching onwards, and kept in 500 L tanks from first feeding. The fish were approximately 7 months old when the experiments were initiated. Up until that time the fish had been feed continuously with pelleted salmon feed (Skretting, Stavanger, Norway), from automatic feeders.

Circadian experiment I

Fish were maintained in 500L freshwater were transferred from LL to a short photoperiod (SP; 6L:18D) light schedule for 8 weeks before the start of the experiment. Short photoperiods provide a strong zeitgeber (timing signal) entraining the fish to a light dark cycle. In order to test for a circadian rhythm it is necessary to remove any zeitgebers, light, temperature and food are all potential zeitgebers which we controlled for. Two weeks before sampling, fish were distributed to two separate 150L tanks and water temperature was maintained at 14˚C (±0.5). Fish were fasted for 48 hours prior to the experiment and throughout the sampling. To identify genes which were driven by an endogenous circadian rhythm we sampled under diel (SP; 6L:18D), constant light (LL) and constant dark conditions (DD). Sampling was at 4 hour resolution starting at ZT5 (zeitgeber time; time since lights on) in diel conditions (n = 3 per time

point, 7 time-points), transitioning onto either LL or DD. In LL sampling was from CT9 (circadian time) at a 4 hour resolution (n = 3 per time-point, 6 time-points) because ZT 1 and 5 in this design can also be defined as CT1 and 5. In DD, samples were collected from CT1 at a 4 hour resolution (n = 3 per timepoint, 8 time-points) (Fig 2A). For the statistical analysis ZT5 to ZT5 was used for diel conditions, and CT9 to CT29 for constant conditions. Collections during the dark phase were conducted under dim red light. During sampling fish were netted out and euthanized by an overdose of benzocaine (150ppm). Weight and length were recorded and no significant variation noted. Optic tectum, gill and saccus vasculous were dissected and snap frozen on dry ice. RNA was extracted for subsequent nanostring profiling.

Circadian experiment II

Fish were maintained in 500L freshwater were transferred from LL to a short photoperiod (SP; 6L:18D) light schedule for 20 weeks before the start of the experiment. Two weeks before sampling, fish were distributed to two separate 150L tanks. Fish were fasted for 48 hours prior to the experiment and throughout the sampling. Temperature was maintained at an average of 8.5˚C ±1. Sampling was at a 4 hour resolution starting one hour after lights on (ZT1), maintaining the fish under diel conditions (SP; 6L:18D) for 24 hours and then switching to either LL or DD, and sampling at 4h resolution for a further 29 hours (n = 4 per time-point) (Fig 4A). Collections during the dark phase were conducted under dim red light. During sampling fish were netted out and euthanized by an overdose of benzocaine (150ppm). Weight and length were recorded and no significant variation noted. Blood was collected at each time-point from the caudal vein in heparinized vacutainers. Blood samples were centrifuged at 500 x g for 15 min to collect plasma for subsequent hormone analysis.

Smoltification experiment and seawater tests

Using a standard aquaculture and research method we used photoperiod to induce the developmental transition of juvenile salmon (parr) to seawater prepared fish (smolts). This method requires switching parr on constant light to short photoperiod (SP; 6L:18D) for at least 8 weeks before returning the fish to constant light where over 4 weeks they gain osmo-regulatory capacity in seawater (Fig 3A). Fish were maintained in 150L freshwater tanks at an average 8.5˚C ±1 and were transferred from LL to SP (8L:16:D) for 8 weeks before return to LL for 7 weeks. Fish were sampled at six time-points in fresh-water shown in Fig 3A (T1-T6, n = 6 per time-point), these time-points are designed to capture the photoperiodically induced transition from parr to smolt. Fish were fasted for 48 hours prior to sampling. Gills were collected into RNAlater (Sigma-Aldrich, St. Louis, Missouri, USA), storing at 4˚C for 24 hours, before being transferred to -80˚C. RNA was extracted and used for RNA-seq (Data shown in Fig 3).

To validate the photoperiod protocol, seawater challenge tests were conducted on a randomly selected subgroup that were transferred to a 100L tank supplied with seawater (34‰ salinity) for 24 hours. Fish were netted out after 24 hours and euthanized by an overdose of benzocaine (150ppm). Blood samples were collected from the caudal vein in heparinized vacutainers and plasma was collected and stored at -20˚C. To test the osmolality of blood thawed plasma samples were analysed for osmolyte content using a Fiske One-Ten Osmometer (Fiske Associates, Massachusetts, USA, ± 4 mOsm kg-1). These tests were conducted from T2 to T6 (n = 12 per time-point), and confirm that from T5 (29 days after the return to LL) the fish had osmo-regulatory capacity in seawater (S3 Fig). Gills were collected at each time-point after seawater challenge (n = 6 per time-point), RNA was extracted and RNA-seq performed as above. All data are available from the European nucleotide archive under project number: PRJEB34224. This study presents data from the freshwater groups at all time-points (Fig 3)

and seawater challenged fish at T4 only (Fig 4B). T4 is 8 days after return to LL and therefore these fish do not have osmo-regulatory capacity in seawater and are used as a stress test comparison to fish from the same time-point kept in freshwater (Fig 4B–4F).

In-vitro Gill Culture

Juvenile Atlantic Salmon were prepared as in the smoltification experiment, sampling at the equivalent of T4 (8 days after return to LL) (Fig 5A). Following euthanasia whole gill arches were rapidly dissected (biological replicates, n = 4 per treatment), excess mucus was removed by careful blotting onto tissue paper before the arches were transferred individually into 50 ml of pre-prepared control or treatment media. The prepared media consisted of Leibovitz L-15 (Lonza) supplemented with non-essential amino acids (1%, 100x Lonza), sodium-pyruvate (1%, 100x Lonza), 0.05 mg/ml gentamycin (Sigma) and 20% fetal bovine serum (FBS, sigma). The experimental group was supplemented with 0.1M dexamethasone diluted in DMSO (dimethyl-sulphoxide, Sigma) to a final concentration of 0.1μM. The control group contained an equivalent concentration of DMSO (0.1%). The excised gill arches were incubated for 24 hours at 4˚C, gill filaments were removed with a scalpel and snap frozen on dry ice before being stored at -80˚C. RNA was extracted for qPCR analysis.

RNA extraction

RNA extraction for RNAseq was performed using a TRIzol-based method (Invitrogen, Thermo Fisher, Waltham, Massachusetts, USA), and in accordance with the manufacturers recommendation. Resulting RNA concentrations and quality were checked using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). RNA was stored at -80˚C.

For nanostring and qPCR, SVs were extracted using QIAgen RNeasy micro kit, OT and gill tissues were extracted using QIAgen RNeasy mini kit according to the manufacturers instructions. RNA concentration was quantified and quality confirmed using the Experion Automated Electrophoresis System (BioRad).

Nanostring

Custom nanostring codesets were designed by Nanostring Technologies Inc. using the Atlantic Salmon reference genome (Cigene), accession numbers and target sequences are shown in S2 Table. Codesets were processed by the Univerisity of Manchester Genomic Technologies Core Facility. This technology is based on the use of fluorescent barcoded probes which bind specifically to the target molecule. Importantly these barcodes should only bind one at a time to each target molecule, therefore the number of fluorescent barcodes reflects the number of RNA molecules of your target gene. A spike control with a known number of RNA molecules was also used to normalise across samples and runs. Therefore the units are counts normalised to spike-in positive controls. Data was processed using nSolver 4.0 software (Nanostring). Data can be accessed on GEO under the project identifier GSE146530.

Transcriptome sequencing and assembly

Libraries were prepared using TruSeq Stranded mRNA HS kit (Illumina, San Diego, California, USA). Mean library length was determined using the 2100 Bioanalyzer with the DNA 1000 kit (Agilent Technologies, Santa Clara, California, USA). Library concentrations was determined using the Qubit BR kit (Thermo Scientific, Waltham, Massachusetts, USA). Samples were barcoded with Illumina unique indexes. The Illumina HiSeq 2500 was used to

perform single-end 100-bp sequencing of samples at the Norwegian Sequencing Centre (University of Oslo, Oslo, Norway).

Cutadapt (ver. 1.8.1) was used for removal of sequencing adapters and trimming of low quality bases (parameters–q 20, -O 8 -minimum-length 40). Quality control was performed with FastQC software. Reads were mapped onto the references genome using STAR software (ver. 2.4.2a). Read counts for annotated genes were generated using the HTSEQ-count software (ver. 0.6.1p1).

All RNAseq data for the smoltification experiment is available in the European nucleotide archive under project number: PRJEB34224.

Analysis of differentially expressed genes

Analysis of differential gene expression was performed with package edgeR (ver. 3.14.0) using R (ver. 3.4.2) and RStudio (ver. 1.0.153). Prior to analysis of differential expression, the raw counts were filtered, setting an expression level threshold of a minimum of one count per million reads (cpm) in five or more libraries, resulting in a list of 33 951 expressed genes. The counts were scaled by applying trimmed means of M-values (TMM) scaling. Exact tests were then performed to find genes that were differentially expressed between FW-kept and 24-hour SW challenged fish. An ANOVA-like test was performed to find genes that were differentially expressed over T1-T6 FW time-points. The test results were filtered for a false discovery rate (FDR) to be less than 0.01 to identify significantly differentially expressed genes. Clustering analysis was performed using Pearson correlation.

Heatmaps were generated in R using custom scripts for pheatmap. Transcription factor binding site analysis was conducted using SalmotifDB [37].

qPCR

cDNA was synthesised from sample total RNA using high capacity RNA to cDNA kit (Applied Biosystems). qPCR was performed using GoTaq Master Mix (Promega) and a 96 well thermal cycler (Applied Biosystems). Relative gene expression was quantified by the ΔΔCT method using *Ef1a* as reference gene. Primer sequences are listed in S2 Table and source data is in S6 Table.

Hormone assays

Cortisol ELISA assays were performed by Stockgrand (UK). Source data in S4 Table.

Statistical analyses

RNAseq analysis is detailed above. Mean difference comparisons were carried out using Student's t-test (two-sided, unpaired), two-way ANOVA with post hoc tests as appropriate (Graphpad Prism 8.1.2). The expression divergence index (EDI) index was calculated as follows: EDI = abs(log2[Gene1/Gene2)].

The R package JTK cycle was used to assess rhythmicity of transcripts under LD and constant light or dark conditions [32]. For statistical comparison of gene expression between ohnologue pairs in the circadian experiment, expression was normalized to group mean then best fit sixth-order centered polynomial curves were generated by non-linear regression analysis and shared characteristics tested with extra sum of squares F test (Graphpad Prism 8.0).

Supporting information

S1 Fig. Tissue specific expression of clock ohnologues. A. PCA plot showing the relative tissue differences when considering clock ohnologue expression. B. Heatmap showing the tissue specific expression of clock ohnologues.
(TIF)

S2 Fig. Nanostring clock gene expression and circadian phase aligned plots. A. Heatmap showing the mesor expression for each clock ohnologue in three tissues. Grey indicates the gene is not expressed. B. Phase aligned plots for the gill. C. Phase aligned plot for the SV. D. Arntl1-Ch10/16 comparison: plot of non-linear regression using a sixth-order centered polynomial to fit the data and compare individual curves. P-value is the result of extra sum-of-squares F test. E. As above for Cry3-Ch12/22.
(TIF)

S3 Fig. Osmoregulatory capacity during the smoltification experiment. Osmolality (mOsm kg-1) is displayed for fish in freshwater (FW—blue) and seawater (SW—green) (n = 6). This plot show osmoregulatory capacity develops by the two latest timepoints (T5 and T6).
(TIF)

S4 Fig. Gene expression of Tsc22d3-Ch3, a positive control gene for DEX treatment. A. Gene expression of Tsc22dd3-Ch3 *in vivo* sea-water stress experiment (RNAseq counts per million (cpm)) and B. *in vitro* dexamethasone treatment (qPCR).
(TIF)

S1 Table. Clock genes identified in Atlantic Salmon, orthogroups, duplicates, significances for the circadian, smoltification and seawater challenge experiments.
(XLSX)

S2 Table. Nanostring codeset design and qPCR primers.
(XLSX)

S3 Table. Summary of previous studies measuring cortisol in fish.
(XLSX)

S4 Table. Cortisol source data for Fig 4A.
(XLSX)

S5 Table. SalmotifDB results—Transcription factor binding site analysis.
(XLSX)

S6 Table. qPCR source data for Fig 5.
(XLSX)

S1 Appendix. Evolutionary gene trees for circadian clock genes.
(PDF)

S2 Appendix. Nanostring circadian profiles for all genes.
(PDF)

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Author Contributions

Conceptualization: Alexander C. West, David G. Hazlerigg, Shona H. Wood.

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References

1. Shi M, Zheng X. Interactions between the circadian clock and metabolism: there are good times and bad times. Acta Biochim Biophys Sin (Shanghai). 2013; 45: 61–69. https://doi.org/10.1093/abbs/gms110 PMID: 23257295
2. Rosbash M. The Implications of Multiple Circadian Clock Origins. Young Michael, editor. PLoS Biol. 2009; 7: e1000062. https://doi.org/10.1371/journal.pbio.1000062 PMID: 19296723
3. Young MW, Kay SA. Time zones: a comparative genetics of circadian clocks. Nat Rev Genet. 2001; 2: 702–715. https://doi.org/10.1038/35088576 PMID: 11533719
4. Taylor JS, Raes J. Duplication and divergence: the evolution of new genes and old ideas. Annu Rev Genet. 2004; 38: 615–43. https://doi.org/10.1146/annurev.genet.38.072902.092831 PMID: 15568988
5. Ohno S. Evolution by Gene Duplication. Berlin, Heidelberg: Springer Berlin Heidelberg; 1970. https://doi.org/10.1007/978-3-642-86659-3
6. van der Horst GTJ, Muijtjens M, Kobayashi K, Takano R, Kanno S, Takao M, et al. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature. 1999; 398: 627–630. https://doi.org/10.1038/19323 PMID: 10217146
7. Erzberger A, Hampp G, Granada AE, Albrecht U, Herzel H. Genetic redundancy strengthens the circadian clock leading to a narrow entrainment range. J R Soc Interface. 2013; 10: 20130221. https://doi.org/10.1098/rsif.2013.0221 PMID: 23676895
8. Pilorz V, Cunningham PS, Jackson A, West AC, Wager TT, Loudon ASI, et al. A Novel Mechanism Controlling Resetting Speed of the Circadian Clock to Environmental Stimuli. Curr Biol. 2014; 24: 766–773. https://doi.org/10.1016/j.cub.2014.02.027 PMID: 24656826
9. Tamai TK, Young LC, Whitmore D. Light signaling to the zebrafish circadian clock by Cryptochrome 1a. Proc Natl Acad Sci. 2007; 104: 14712–14717. https://doi.org/10.1073/pnas.0704588104 PMID: 17785416
10. Shi S, Hida A, McGuinness OP, Wasserman DH, Yamazaki S, Johnson CH. Circadian Clock Gene Bmal1 Is Not Essential; Functional Replacement with its Paralog, Bmal2. Curr Biol. 2010; 20: 316–321. https://doi.org/10.1016/j.cub.2009.12.034 PMID: 20153195
11. Tauber E, Last KS, Olive PJW, Kyriacou CP. Clock Gene Evolution and Functional Divergence. J Biol Rhythms. 2004; 19: 445–458. https://doi.org/10.1177/0748730404268775 PMID: 15534324

12. Looby P, Loudon ASI. Gene duplication and complex circadian clocks in mammals. Trends Genet. 2005; 21: 46–53. https://doi.org/10.1016/j.tig.2004.11.012 PMID: 15680514

13. Daan S, Albrecht U, Van der Horst GTJ, Illnerová H, Roenneberg T, Wehr TA, et al. Assembling a Clock for All Seasons: Are There M and E Oscillators in the Genes? J Biol Rhythms. 2001; 16: 105–116. https://doi.org/10.1177/074873001129001809 PMID: 11302553

14. Hastings M. Modeling the Molecular Calendar. J Biol Rhythms. 2001; 16: 117–123. https://doi.org/10.1177/074873001129001818 PMID: 11302554

15. Meng Q-J, Logunova L, Maywood ES, Gallego M, Lebiecki J, Brown TM, et al. Setting Clock Speed in Mammals: The CK1ε tau Mutation in Mice Accelerates Circadian Pacemakers by Selectively Destabilizing PERIOD Proteins. Neuron. 2008; 58: 78–88. https://doi.org/10.1016/j.neuron.2008.01.019 PMID: 18400165

16. Meng Q-J, Maywood ES, Bechtold DA, Lu W-Q, Li J, Gibbs JE, et al. Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. Proc Natl Acad Sci. 2010; 107: 15240–15245. https://doi.org/10.1073/pnas.1005101107 PMID: 20696890

17. Lee H, Chen R, Lee Y, Yoo S, Lee C. Essential roles of CKIδ and CKIε in the mammalian circadian clock. Proc Natl Acad Sci. 2009; 106: 21359–21364. https://doi.org/10.1073/pnas.0906651106 PMID: 19948962

18. Etchegaray J-P, Machida KK, Noton E, Constance CM, Dallmann R, Di Napoli MN, et al. Casein Kinase 1 Delta Regulates the Pace of the Mammalian Circadian Clock. Mol Cell Biol. 2009; 29: 3853–3866. https://doi.org/10.1128/MCB.00338-09 PMID: 19414593

19. Macqueen DJ, Johnston IA. A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proc R Soc B Biol Sci. 2014; 281: 20132881. https://doi.org/10.1098/rspb.2013.2881 PMID: 24452024

20. Alexandrou MA, Swartz BA, Matzke NJ, Oakley TH. Molecular Phylogenetics and Evolution Genome duplication and multiple evolutionary origins of complex migratory behavior in Salmonidae. Mol Phylogenet Evol. 2013; 69: 514–523.

21. Robertson FM, Gundappa MK, Grammes F, Hvidsten TR, Redmond AK, Lien S, et al. Lineage-specific rediploidization is a mechanism to explain time-lags between genome duplication and evolutionary diversification. Genome Biol. 2017; 18: 111. https://doi.org/10.1186/s13059-017-1241-z PMID: 28615063

22. Lien S, Koop BF, Sandve SR, Miller JR, Matthew P, Leong JS, et al. The Atlantic salmon genome provides insights into rediploidization. Nature. 2016; 533: 200–205. https://doi.org/10.1038/nature17164 PMID: 27088604

23. Moriyama Y, Koshiba-takeuchi K. Significance of whole-genome duplications on the emergence of evolutionary novelties. 2018; 17: 329–338. https://doi.org/10.1093/bfgp/ely007 PMID: 29579140

24. Li WH. Rate of gene silencing at duplicate loci: a theoretical study and interpretation of data from tetraploid fishes. Genetics. 1980; 95: 237–58. PMID: 7429144

25. Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, et al. Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. Nature. 2012; 485: 123–127. https://doi.org/10.1038/nature11048 PMID: 22460952

26. Holmqvist BI, Östholm T, Ekström P. Neuroanatomical analysis of the visual and hypophysiotropic systems in Atlantic salmon (Salmo salar) with emphasis on possible mediators of photoperiodic cues during parr-smolt transformation. Aquaculture. 1994; 121: 1–12. https://doi.org/10.1016/0044-8486(94)90003-5

27. Meyer RL. Eye-in-water electrophysiological mapping of goldfish with and without tectal lesions. Exp Neurol. 1977; 56: 23–41. https://doi.org/10.1016/0014-4886(77)90136-4 PMID: 558902

28. Sandbakken M, Ebbesson L, Stefansson S, Helvik JV. Isolation and characterization of melanopsin photoreceptors of atlantic salmon (Salmo salar). J Comp Neurol. 2012; 520: 3727–3744. https://doi.org/10.1002/cne.23125 PMID: 22522777

29. Mazurais D, Brierley I, Anglade I, Drew J, Randall C, Bromage N, et al. Central melatonin receptors in the rainbow trout: Comparative distribution of ligand binding and gene expression. J Comp Neurol. 1999; 409: 313–324. https://doi.org/10.1002/(sici)1096-9861(19990628)409:2<313::aid-cne11>3.0.co;2-1 PMID: 10379923

30. Nakane Y, Ikegami K, Iigo M, Ono H, Takeda K, Takahashi D, et al. The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nat Commun. 2013; 4: 2108. https://doi.org/10.1038/ncomms3108 PMID: 23820554

31. Evans DH, Piermarini PM, Choe KP. The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of Nitrogenous Waste. Physiol Rev. 2005; 85: 97–177. https://doi.org/10.1152/physrev.00050.2003 PMID: 15618479

32. Hughes ME, Hogenesch JB, Kornacker K. JTK_CYCLE: An Efficient Nonparametric Algorithm for Detecting Rhythmic Components in Genome-Scale Data Sets. J Biol Rhythms. 2010; 25: 372–380. https://doi.org/10.1177/0748730410379711 PMID: 20876817

33. Vatine G, Vallone D, Appelbaum L, Mracek P, Ben-Moshe Z, Lahiri K, et al. Light Directs Zebrafish period2 Expression via Conserved D and E Boxes. Kramer A, editor. PLoS Biol. 2009; 7: e1000223. https://doi.org/10.1371/journal.pbio.1000223 PMID: 19859524

34. Björnsson BT, Stefansson SO, McCormick SD. Environmental endocrinology of salmon smoltification. Gen Comp Endocrinol. 2011; 170: 290–298. https://doi.org/10.1016/j.ygcen.2010.07.003 PMID: 20627104

35. Dumbell R, Matveeva O, Oster H. Circadian Clocks, Stress, and Immunity. Front Endocrinol (Lausanne). 2016; 7. https://doi.org/10.3389/fendo.2016.00037 PMID: 27199894

36. Langhorne P, Simpson TH. The interrelationship of cortisol, Gill (Na + K) ATPase, and homeostasis during the Parr-Smolt transformation of atlantic salmon (Salmo salar L.). Gen Comp Endocrinol. 1986; 61: 203–213. https://doi.org/10.1016/0016-6480(86)90198-x PMID: 3007267

37. Mulugeta TD, Nome T, To T-H, Gundappa MK, Macqueen DJ, Våge DI, et al. SalMotifDB: a tool for analyzing putative transcription factor binding sites in salmonid genomes. BMC Genomics. 2019; 20: 694. https://doi.org/10.1186/s12864-019-6051-0 PMID: 31477007

38. McCormick SD. Effects of Growth Hormone and Insulin-like Growth Factor I on Salinity Tolerance and Gill Na+, K+-ATPase in Atlantic Salmon (Salmo salar): Interaction with Cortisol. Gen Comp Endocrinol. 1996; 101: 3–11. https://doi.org/10.1006/gcen.1996.0002 PMID: 8713639

39. McCormick SD. Endocrine Control of Osmoregulation in Teleost Fish. Am Zool. 2001; 41: 781–794. https://doi.org/10.1093/icb/41.4.781

40. Accili D, Arden KC. FoxOs at the Crossroads of Cellular Metabolism, Differentiation, and Transformation. Cell. 2004; 117: 421–426. https://doi.org/10.1016/s0092-8674(04)00452-0 PMID: 15137936

41. Barthel A, Schmoll D, Unterman TG. FoxO proteins in insulin action and metabolism. Trends Endocrinol Metab. 2005; 16: 183–189. https://doi.org/10.1016/j.tem.2005.03.010 PMID: 15860415

42. Dong XC, Copps KD, Guo S, Li Y, Kollipara R, DePinho RA, et al. Inactivation of Hepatic Foxo1 by Insulin Signaling Is Required for Adaptive Nutrient Homeostasis and Endocrine Growth Regulation. Cell Metab. 2008; 8: 65–76. https://doi.org/10.1016/j.cmet.2008.06.006 PMID: 18590693

43. Li J, Labbadia J, Morimoto RI. Rethinking HSF1 in Stress, Development, and Organismal Health. Trends Cell Biol. 2017; 27: 895–905. https://doi.org/10.1016/j.tcb.2017.08.002 PMID: 28890254

44. Barna J, Princz A, Kosztelnik M, Hargitai B, Takács-Vellai K, Vellai T. Heat shock factor-1 intertwines insulin/IGF-1, TGF-β and cGMP signaling to control development and aging. BMC Dev Biol. 2012; 12: 32. https://doi.org/10.1186/1471-213X-12-32 PMID: 23116063

45. Ronchetti S, Migliorati G, Riccardi C. GILZ as a Mediator of the Anti-Inflammatory Effects of Glucocorticoids. Front Endocrinol (Lausanne). 2015; 6. https://doi.org/10.3389/fendo.2015.00170 PMID: 26617572

46. Carroll SB. Evo-Devo and an Expanding Evolutionary Synthesis: A Genetic Theory of Morphological Evolution. Cell. 2008; 134: 25–36. https://doi.org/10.1016/j.cell.2008.06.030 PMID: 18614008

47. Carroll SB. Endless Forms. Cell. 2000; 101: 577–580. https://doi.org/10.1016/s0092-8674(00)80868-5 PMID: 10892643

48. McCormick SD, Saunders R. Preparatory Physiological Adaptations for Marine Life of Salmonids: Osmoregulation, Growth, and Metabolism. Am Fish Soc Symp. 1987; 1: 211–229.

49. McCarthy ID. Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. J Fish Biol. 2000; 57: 224–238. https://doi.org/10.1111/j.1095-8649.2000.tb00788.x

50. McCormick SD, Moyes CD, Ballantyne JS. Influence of salinity on the energetics of gill and kidney of Atlantic salmon (Salmo salar). Fish Physiol Biochem. 1989; 6: 243–254. https://doi.org/10.1007/BF01875027 PMID: 24221555

51. Davie A, Minghetti M, Migaud H. Seasonal Variations in Clock-Gene Expression in Atlantic Salmon (Salmo salar). Chronobiol Int. 2009; 26: 379–395. https://doi.org/10.1080/07420520902820947 PMID: 19360485

52. Huang T, Ruoff P, Fjelldal PG. Diurnal expression of clock genes in pineal gland and brain and plasma levels of melatonin and cortisol in Atalantic salmon parr and smolts. Chronobiol Int. 2010; 27: 1697–1714. https://doi.org/10.3109/07420528.2010.514630

53. Huang T, Ruoff P, Fjelldal PG. Effect of continuous light on daily levels of plasma melatonin and cortisol and expression of clock genes in the pineal gland, brain, and liver in Atalantic salmon postsmolts. Chronobiol Int. 2010; 27: 1715–1734. https://doi.org/10.3109/07420528.2010.521272

54. Davies WIL, Tamai TK, Zheng L, Fu JK, Rihel J, Foster RG, et al. An extended family of novel vertebrate photopigments is widely expressed and displays a diversity of function. Genome Res. 2015; 25: 1666–1679. https://doi.org/10.1101/gr.189886.115 PMID: 26450929

55. Pagano C, Siauciunaite R, Idda ML, Ruggiero G, Ceinos RM, Pagano M, et al. Evolution shapes the responsiveness of the D-box enhancer element to light and reactive oxygen species in vertebrates. Sci Rep. 2018; 8: 13180. https://doi.org/10.1038/s41598-018-31570-8 PMID: 30181539

56. Gavriouchkina D, Fischer S, Ivacevic T, Stolte J, Benes V, Dekens MPS. Thyrotroph Embryonic Factor Regulates Light-Induced Transcription of Repair Genes in Zebrafish Embryonic Cells. Riley BB, editor. PLoS One. 2010; 5: e12542. https://doi.org/10.1371/journal.pone.0012542 PMID: 20830285

57. Wendelaar Bonga SE. The stress response in fish. Physiol Rev. 1997; 77: 591–625. https://doi.org/10.1152/physrev.1997.77.3.591 PMID: 9234959

58. Isorna E, De Pedro N, Valenciano AI, Alonso-gómez ÁL. Interplay between the endocrine and circadian systems in fishes. 2017. https://doi.org/10.1530/JOE-16-0330 PMID: 27999088

59. Mommsen TP, Vijayan MM, Moon TW. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish. 1999; 9: 211–268.

60. Liu C, Hu J, Qu C, Wang L, Huang G, Niu P, et al. Molecular evolution and functional divergence of zebrafish (Danio rerio) cryptochrome genes. Sci Rep. 2015; 5: 8113. https://doi.org/10.1038/srep08113 PMID: 25630924

61. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol. 2015; 1–14.

Appendix 8:
Co-author contributions

List of papers and contributions

Name of candidate: Marianne Iversen

Papers
The following papers are included in my PhD thesis:

I: RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile Atlantic salmon (https://doi.org/10.1371/journal.pone.0227496)

II: Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in Atlantic salmon (*Salmo salar*) (under revision, G3)

III: Diversified regulation of circadian clock gene expression following whole genome duplication (https://doi.org/10.1371/journal.pgen.1009097)

Contributions

	Paper I	**Paper II**	**Paper III**
Concept and idea	MI, DH, EJ	MI, DH, SM	AW, DH, SW
Study design and methods	MI, DH, EJ, SRS	MI, DH, TM, SRS	AW, MI, SRS, EJ, SW
Data gathering and interpretation	MI, TM, BGB, AW, EJ, DH, SRS	MI, DH, EJ, AW, TM, SRS	AW, MI, EJ, SRS, DH, SW
Manuscript preparation	MI, EJ, DH	MI, DH	AW, MI, EJ, SRS, DH, SW

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