Influence of temperature and feeding on early sexual maturation commitment in male Atlantic salmon (*Salmo salar*, L.) during the freshwater stage

Thesis for the degree

Master of science in Aquaculture Biology

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January 2021

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Acknowledgements

First and foremost, I wish to express my sincerest gratitude to my main supervisors, Professor Sigurd O. Handeland and Researcher II Pablo Balseiro Vigo, during this project: for their excellent guidance and support, critical feedback, and late evening guidance over the phone during the COVID-19 lockdown. Additionally, profoundly appreciate the help from Pablo Balseiro Vigo on conducting parts of the laboratory work during the lockdown. The persistent support from supervisors has been invaluable for being able to realize this thesis. Furthermore, I want to thank Enrique Pino Martinez (Ph.D.) for letting me take part in the sampling for his Ph.D. project and for providing guidance and RStudio expertise. I would also like to thank Chief engineer Cindy Pedrosa for her assistance and guidance within the laboratory. Furthermore, I would like to thank Sigurd Stefansson and Albert K. D. Imsland for their read through and critical feedback in the final phase of writing this thesis. Last but not least, I would like to thank my family and friends for their continuous support throughout this process.

This study and master thesis were conducted in context of collaboration between the University of Bergen (UiB, Bergen), Department of biological science (BIO) and the Integrative Fish Biology Group in the Norwegian Research Center AS (NORCE, Bergen), and it was supported by the research projects "*Tidlig Modning hos Postsmolt fra RAS Anlegg*" (SAFT I; 286597) and "*Kapasitetsløft for Bærekraftig og Innovativ Sjømatproduksjon*" (KABIS; 280782).

Markus Førde Braanaas
January 20th, 2021

Glossary of species (alphabetic order):

Acanthopagrus schlegelii	-	Blackhead seabream
Anguilla japonica	-	Japanese eel
Cyprinus carpio	-	Common carp
Danio rerio	-	Zebrafish
Oncorhynchus mykiss	-	Rainbow trout
Oreochromis niloticus	-	Nile tilapia
Oryzias latipes	-	Medaka
Salmo salar	-	Atlantic salmon
Salvelinus alpinus	-	Arctic charr

Abbreviation list (alphabetic order):

11-KT	-	11-Ketotestosterone
A_{diff}	-	Differentiated type A spermatogonia
Amh	-	Anti-Müllerian hormone
Amhr2	-	Anti-Müllerian hormone type II transmembrane receptor
Ar	-	Androgen receptor
Aund	-	Undifferentiated type A spermatogonia
B	-	Type B spermatogonia
BPG-axis	-	Brain-Pituitary-Gonad axis
CF	-	Condition factor
d°C	-	Day degrees
DHP	-	17α, 20β-dihydroxy-4-pregnen-3-one
DNA	-	Deoxyribonucleic acid
Ef1a	-	Elongation factor 1-alfa
FCR	-	Feed Conversion Ratio
Fsh	-	Follicle-stimulating hormone
Fshr	-	Follicle-stimulating hormone receptor
FTS	-	Flow-Through System
Gnrh	-	Gonadotropin-releasing hormone
Gsdf1	-	Gonadal soma-derived factor 1
Gsdf2	-	Gonadal soma-derived factor 2
GSI (%)	-	Gonadosomatic Index
H0	-	Null-hypothesis

HA	-	Alternative hypothesis
HSI (%)	-	Hepatosomatic Index
Igf3	-	Insulin-like growth factor 3
Inha	-	Inhibin subunit alpha
Kiss1/Kiss-r	-	Kisspeptin/Kisspeptin-receptor
LD	-	Light, Darkness
Lh	-	Luteinizing hormone
Lhr	-	Luteinizing hormone receptor
MTA2	-	Metastasis-associated protein 2
NORCE	-	Norwegian Research Center
qPCR	-	Quantitative polymerase chain reaction
RAS	-	Recirculating Aquaculture System
RIN	-	Ribonucleic acid integrity number
RNA	-	Ribonucleic acid
SC (p.)	-	Primary spermatocyte
SC (s.)	-	Secondary spermatocyte
SCB	-	Sertoli cell barrier
SDG	-	Sustainable Development Goals
SGR	-	Specific growth rate
ST	-	Spermatid
STF	-	Seminiferous tubular fluid
SZ	-	Spermatozoa
TAN	-	Total ammonia nitrogen
UN	-	United Nations

Nomenclature:

This thesis applies the nomenclature recommendations form ZFIN (https://wiki.zfin.org/display/general/ZFIN+Zebrafish+Nomenclature+Conventions):

- *Gene/protein fish* – Gene: *fsh* (italicized, small letters). Protein: Fsh (first letter in uppercase).
- *Gene/protein human* – Gene: *FSH* (italicized, uppercase). Protein: FSH (uppercase).
- *Gene/protein rat* – Gene: *Fsh* (italicized, first letter in uppercase). Protein: FSH (uppercase).

Reference:

Literature having more than two authors are written et al., in the text and in the bibliography.

Abstract

The intensification of Atlantic salmon (*Salmo salar*) post-smolt production to enhance growth performance has promoted a rise in precocious male maturation rates. Unlike traditional parr maturation, which seems to be highly dependent on genetic background, post-smolt maturation seem to be more linked to intensive rearing conditions. This study focused on the relationship between different temperatures and feeding rations on precocious male maturation and early gonad development in Atlantic salmon pre-smolts. Early gonad development was used as an indicator for post-smolt maturation. Juvenile salmon (n = 1800) were reared at three different temperatures (8, 12.5, and 18°C) and two different feeding rations (67%, 100%), producing six experimental groups (8-67%, 8-100%, 12.5-67%. 12.5-100%, 18-67%, 18-100%). An LD24:0 photoperiod was maintained throughout the experiment with a five-week winter signal (LD12:12) induction in February-March to promote developmental events. Growth (body weight, CF), hepatosomatic index (HSI), gonadosomatic index (GSI), gonadotropin receptors transcription (*fshr*, *lhr*), gonadotropin-responsive transcription (*amh, gdsf1, gsdf2, igf3*), plasma 11-Ketotestosterone (11-KT) concentrations, and spermatogenic activity were used as indicators for maturational advancements. According to the present results, the intensive rearing groups (18°C-100%, 18°C-67%, 12.5°C-100%) experienced a high developmental rate, stimulating spermatogenetic advancement. In comparison, less intensive rearing groups (12.5°C-67%, 8°C-100%, 8°C-67%) displayed lesser physiological development with corresponding low or no spermatogenetic advances. Results suggest high temperatures (18°C) to be one of the main contributors to trigger precocious male maturation in Atlantic salmon, controlling the rate and magnitude of gonadal development independently of the feeding ration. Intermediate temperature (12.5°C) seems to be more dependent on intensive feed rations to fully mature, as the full-fed group displayed a moderate percentage of maturational advancements with corresponding physiological development than the restricted feeding group. This proposes that the relevance of feed rations on precocious male maturation may be dependent on temperature. Low rearing temperatures (8°C) seemed to impair the maturational process independently of feeding rations, further supporting the importance of temperature as a precocious maturation trigger. For the salmon industry, this means that intensive rearing may enhance growth, but at the cost of a high proportion of early maturation. By rearing fish at lower intensities (12.5°C-67%, 8°C-100%, 8°C-67%), it is possible to achieve growth and avoid maturation simultaneously.

1. Introduction

1.1. Background

Norwegian production of Atlantic salmon (*Salmo salar* Linnaeus, 1758) has become a highly advanced industrial industry since its inception in the early 1970s. Production has developed from extensive to intensive, of which farmed salmon has become the fourth greatest export commodity in Norway (Liu et al., 2011; Statistisk sentralbyrå, 2020). According to SINTEF, an increase of production from one (2010) to five million ton round weight is estimated achievable by 2050 (Olafsen et al., 2012). This is in line with The World Bank, which concludes in the 2013 Aquaculture Prospect that global aquaculture production must increase from 47.1 (2006) to 93.6 million metric tons by 2030 to feed the growing population (World Bank, 2013).

Biological and environmental challenges related to production have on the other hand become a limiting factor for further growth within the Norwegian aquaculture sector. Persistent problems with escapees from open-net pens with associated genetic introgression of farmed salmon into wild salmon populations (Glover et al., 2013; Karlsson et al., 2016) and emissions of organic waste into the environment (Olsen et al., 2008) are considered great environmental threats. Biological issues, such as infectious diseases, salmon lice (*Lepeophtheirus salmonis*) (Krøyer, 1837) infestations (Kabata, 1974; Brandal et al., 1976; Costello, 2006) and associated resistance development to chemotherapy and antibiotic treatments (Wright, 2005; Torrissen et al., 2013; Watts et al., 2017), also characterize production by negatively impacting fish growth, welfare, and mortality rates.

In line with the United Nations (UN) Sustainable Development Goals (SDGs), the current aim is to reduce the adverse effects on the marine ecosystem through reducing waste generation (SDG 12.5) and marine pollution (SDG 14.1) while ensuring sustainable food production systems (SDG 2.4) and economic productivity (SDG 8.2) (Hambrey, 2017; UN, 2020). Innovations within production technology and strategy have made it possible to extend the onshore production time while reducing the grow-out phase in the sea, consequently reducing the impact on the marine ecosystem while reducing biological issues associated with the grow-out stage of production (Fig. 1A). These innovations include intensive post-smolt production in Recirculating Aquaculture Systems (RAS).

Land-based RAS is a closed-containment aquaculture production system with integrated artificial control over production parameters, allowing partial recirculation of water through mechanical and biological treatments (Fig. 1B). The degree of recirculation is directly correlated with the complexities of the systems,

of which the range typically varies between 95-99% (Bregnballe, 2015; Fjellheim et al., 2016). Recirculation of production water reduces water and energy consumption as well as the release of waste and nutrients into the environment (Piedrahita, 2003; Martins et al., 2011; Badiola et al., 2018). Initially, RAS has been utilized throughout the freshwater phase of the salmon production cycle, rearing salmon smolt up to 100 g before sea transfer. However, modern technology has enabled extended onshore production by including the early seawater phase, making it possible to rear post-smolt up to 1 000 g. As the prolonged production onshore shortens the grow-out phase in open-net pens, it will in consequence reduce salmon lice susceptibility, risk of infectious diseases, and escapees, ultimately reducing production costs (Dalsgaard et al., 2013; Holan and Kolarevic, 2015). As post-smolts are assumed to be better adapted to seawater, physically larger, and more robust than smolts, the survival rates increase during the grow-out phase of production (Holan and Kolarevic, 2015). This form of production allows growth within the Norwegian salmon industry through increased opportunity for more efficient utilization of available MPB (Maximum Permitted Biomass) (Holm, 2015), thus being a possible solution to current regulations and demands for sustainability.

On the other side, RAS is expensive to establish and entails high operation costs, challenging current requirements for employee competence while increasing the dependence on alarm systems and back-up solutions (Lazur et al., 2003; Fjellheim et al., 2016). Complex systems are sensitive to suboptimal conditions which may entail negative consequences for production if not detected and fixed. Examples are acute bad water quality due to TAN (total ammonia nitrogen) accumulation (Schreier et al., 2010), or the production of toxic hydrogen sulfide in pipes (Letelier-Gordo et al., 2020). Furthermore, biological issues have also emerged, such as the increase of early sexual maturation events in male post-smolts reared under intensive RAS conditions (Imsland et al., 2014; Melo et al., 2014; Good and Davidson, 2016). Maturation ceases feed intake and causes growth rates to stagnate (Kadri et al., 1996; McClure et al., 2007) as acquired energy is reallocated to gonadal growth, gametogenesis, and the development of secondary sex characteristics, ultimately degrading fillet quality (Fleming, 1998; Hendry and Beall, 2004; Taranger et al., 2010). Moreover, maturation also compromises the development of the hypo-osmoregulatory ability, thus leading to fish welfare issues and increased mortality rates when transferred to open-net pens in sea (Hou et al., 1999; Harris and Bird, 2000; Law et al., 2001; McQuillan et al., 2003; Makino et al., 2007; Taranger et al., 2010). The problem of precocious male puberty is therefore assumed to have the potential to cause significant biological and economic losses for the aquaculture industry, compromising the economic viability of RAS while increasing disease susceptibility and lowering fish welfare (Aksnes et al., 1986; Johnston et al., 2006; McClure et al., 2007).

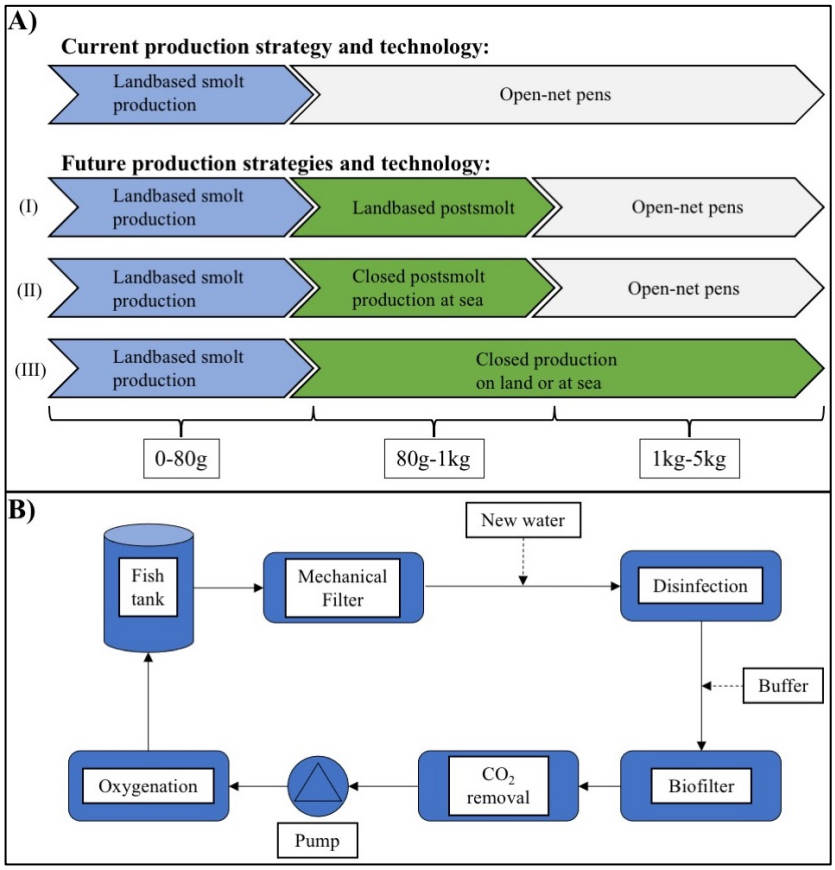

Figure 1: (A) Schematic of current and possible future production strategies. (B) Recirculating Aquaculture System (RAS) step simplification. *Progression of the RAS schematic is as follows: (1) Fish tank – water flows through the water outlet into the tubes leading it to the (2) mechanical filter. The mechanical filter removes organic matter of various sizes depending on the filter, before being (3) disinfected for potential pathogens. Water then goes to the (4) biofilter, where nitrifying bacteria converts TAN via nitrite to nitrate. CO_2 is then removed (5) from the production water and oxygenated (6) before leading it back to the fish tank. During the cycle, amounts of new water are added and amounts of production water removed or lost. Water is buffered after disinfection due to the acidifying nature of disinfection.*

1.2. The Atlantic salmon life cycle

The Atlantic salmon is a cold water adapted teleost of the salmonid family Salmonidae, which includes species such as Rainbow trout (*Oncorhynchus mykiss*) (Walbaum, 1792) and Arctic charr (*Salvelinus alpinus*) (Linnaeus, 1758). Like many other species of this family, the Atlantic salmon is a euryhaline species that exhibits an anadromous life cycle in which it hatches in freshwater and migrates to the sea to grow before moving upstream to spawn in autumn (Fig. 2). Fertilized eggs (roe) are buried in gravel on the riverbed after spawning and completes embryogenesis before hatching as alevins (yolk sac fry) the following spring. Alevins stay underneath the gravel until most of their nutritional yolk sac is consumed, further developing physiologically and morphologically, before emerging to initiate first feeding during the summer months as fry. Over autumn, the fry develops into juvenile salmon parr, characterized by parr marks (vertical stripes) and spotted camouflage. The parr stays in the river to grow for a period ranging from two to seven years (depending on genetic factors and environmental conditions such as growth rate,

size, temperature, photoperiod, etc.) before migrating into the North Atlantic Ocean during spring and early summer (Stefansson et al., 2002). Prior to seaward migration, parr undergoes a series of morphological (e.g. reduced condition factor and silvering), physiological (e.g. shift in osmoregulatory capacity and hormonal change), and behavioral (e.g. negative rheotaxis, reduced territoriality, and developed schooling traits) changes, enabling it to survive, grow and wander in the ocean (Hansen, 1998; Stefansson et al., 2002). This process is known as smoltification and is a preadaptation to the new osmotic environment. After smoltification and seaward migration, the Atlantic salmon spend one to three years growing until sufficient energy is stored to migrate from the oceanic feeding grounds upstream the native river in which they hatched to spawn as a mature adult (Fig. 2) (Stefansson et al., 2002).

There are two main alternative life histories to smoltification: (1) desmoltification and (2) male parr maturation ("dwarf males") (Stefansson et al., 2008). Smolts that abandon the preparatory changes towards a marine life due to being prevented from exposure to seawater undergo desmoltification (e.g. when missing the "smolt-window") (Stefansson et al., 2008). Desmoltification ultimately causes the fish to remain in the river until the next chance to undergo smoltification. Some male parr commits to staying in the rivers to breed. These adopt an elusive breeding strategy, in which they achieve sexual maturation early, avoiding physical competition (Thorpe, 1994). To understand the underlying factors that affect early sexual maturation of male post-smolt in RAS, it is important to recognize the different biological aspects of sexual maturation in Atlantic salmon.

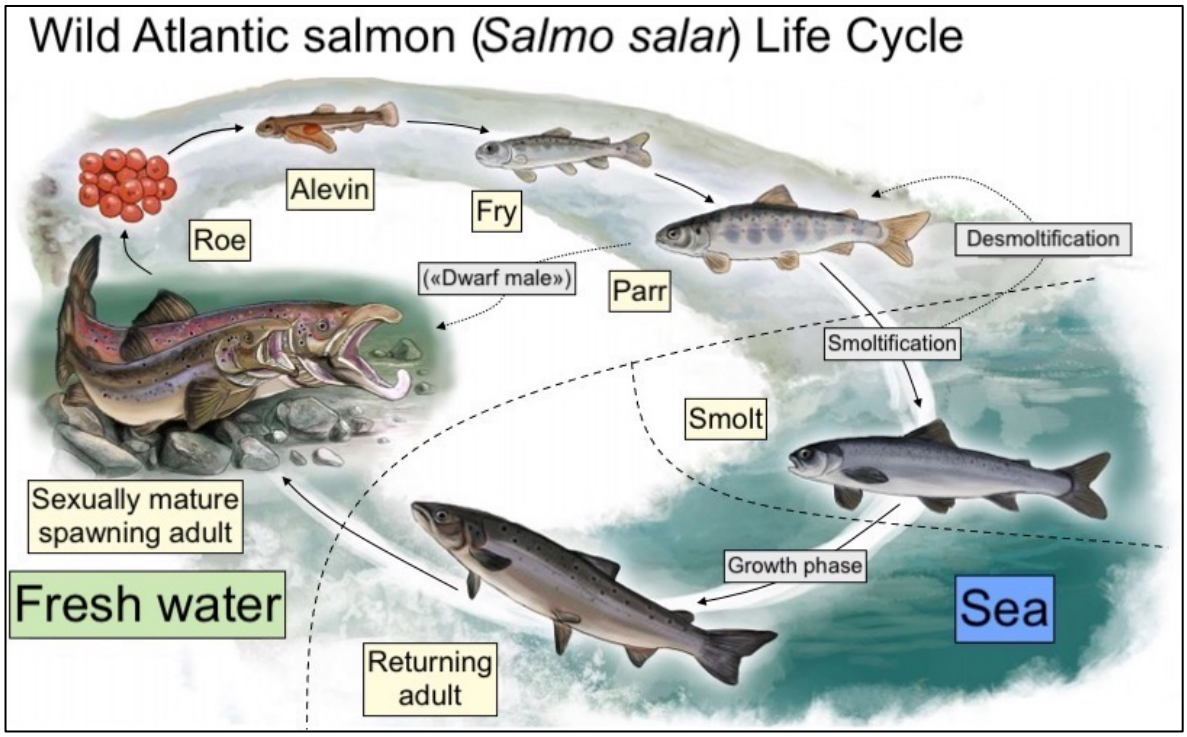

Figure 2: Schematic overview of the Atlantic salmon life cycle, from roe to sexually mature adults capable of reproduction. *Dotted lines illustrated the different osmotic life phases. Dotted arrows illustrate alternative life histories. Modified after Jayme van Dalum.*

1.3.Sexual maturation in Atlantic salmon

Life history variation in Atlantic salmon shows considerable plasticity in terms of timing and routing of the life cycle (Hutchings and Jones, 1998). The life history variation applies to puberty at various life stages, as there is significant phenotypic and genotypic variation in age and size at puberty in cultivated species (Wild et al., 1994; Hutchings and Jones, 1998). Puberty is defined as the process by which morphological, physiological, and behavioral changes transform an immature juvenile into a mature adult capable of sexual reproduction (Taranger et al., 2010). The proportion of male Atlantic salmon undergoing early sexual maturation is much greater than in females. The reason is assumed to be that maturation is an energetically expensive process, of which it requires a greater amount of energy to develop ovaries and eggs than to develop testies and sperm (Thorpe, 1994; Taranger et al., 2010; Imsland et al., 2014). The commitment to mature is made several months in advance of spawning, based on biological thresholds such as size, growth rate, energy status, genetics, and external cues entrained with inner circadian rhythms (Thorpe, 1994; Taranger et al., 2010). Somatic growth rates and appetite are higher during the earliest stages of sexual maturation, increasing the condition factor as a consequence of high lipid reserves (Taranger et al., 2010). Somatic growth occurs in parallel with increased plasma sex steroid levels (e.g. 11-Ketotestosterone and testosterone), affecting gametogenesis (Taranger et al., 2010). However, in later stages, appetite and feed intake cease (Kadri et al., 1996; McClure et al., 2007). Hormonal changes such as the increased leptin production which inhibits hunger, negatively affect the FCR (feed conversion ratio), leading to weight loss and growth reduction (Kadri et al., 1996; McClure et al., 2007). Weight loss and reduced growth rate are direct consequences of energy being reallocated to gonadal development, gametogenesis, and the development of secondary sex characteristics (Hendry and Beall, 2004; Taranger et al., 2010). A measurable example is the diminishing lipid storages in adipose tissue in the liver due to energy relocation. As energy is redirected, the hepatosomatic index (HSI) decreases in parallel with an increasing gonadosomatic index (GSI). Approximately 59% of the total energy reserves are reallocated into the maturation process and breeding, regardless of gender (Fleming, 1998).

Endocrine changes during gonadal maturation decrease the immune competence, as the immunomodulatory role of hormones associated with maturation change (Hou et al., 1999; Harris and Bird, 2000; Law et al., 2001; McQuillan et al., 2003; Taranger et al., 2010). This subsequently increases the susceptibility of diseases and thus may potentially negatively affect health. In conjunction with the agnostic behavior associated with puberty-related competition and territoriality, the risk of secondary infections and parasite infections due to skin damage increases as the immune system is down modulated (Fleming, 1996; Skarstein et al., 2001). Furthermore, early maturation prevents the development of, or compromises the hypo-osmoregulatory ability by hindering alternations in the branchial ion transport (e.g.

Na^+/K^+-ATPase α-subunit 1a → Na^+/K^+-ATPase α-subunit 1b) associated with the development of seawater tolerance (Makino et al., 2007; McCormick et al., 2009; Taranger et al., 2010; Morro et al., 2019). This ultimately increases the uptake of sodium and chloride ions from the environment while water uptake is reduced, subsequently compromising homeostasis if in the sea (Elgen, 2011).

1.4. Endocrine control of sexual maturation

Sexual maturation is characterized by the endocrine activation of the two main functions in gonads: (1) the ability to produce fertile gametes and (2) to synthesize reproductive hormones (e.g. steroid hormones: progestogens, androgens, and estrogens) (Schulz et al., 2010; Taranger et al., 2010). Developmental changes within gonads enable the production of haploid (n) gametes from diploid (2n) germ cells, a process collectively known as gametogenesis (Schulz et al., 2010). Spermatogenesis is the male-specific form of gametogenesis, of which spermatogonial stem cells forms the basis for the male gamete spermatozoa through a variety of species-dependent mitotic and meiotic proliferations (Maugars and Schmitz, 2008a; Schulz et al., 2010). Gametes are the cellular basis of fertility, of which the first batch produced may be considered the endpoint of sexual maturation (Okuzawa, 2002).

The series of complex changes associated with sexual maturation and gonadal function is driven by two main regulatory inputs. Of primary relevance is the endocrine BPG-axis (brain-pituitary-gonad) with its associated feedback systems, which regulate gonad development, function, and maintenance through endocrine signaling (Fig. 3) (Taranger et al., 2010). Secondly, any other system that provides the premise for reproductive development, such as those which regulate growth and energy metabolism (Taranger et al., 2010). The commitment to undergo sexual maturation depends on regulatory inputs and biological thresholds, influenced by internal (e.g. genetic, size, energy status, growth factor, biological clock) and external (e.g. photoperiod, temperature, diet) cues, activating the BPG-axis (Hutchings and Jones, 1998; Bromage et al., 2001; Taranger et al., 2010). The kisspeptin/kisspeptin-receptor (Kiss1/Kiss-r) system is assumed to regulate the release of gonadotropin-releasing hormone (Gnrh) from the neurohypophysis through mediating external and internal cues into regulatory inputs for activating pituitary gonadotropin release (Gopurappilly et al., 2013). Through Gnrh neurons, Gnrh stimulates the anterior pituitary (adenohypophysis) to produce and release gonadotropins (follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh)) from *proximal pars distalis* into the blood plasma (Schulz et al., 2010). Gonadotropins regulate downstream targets such as in the gonads, ultimately regulating steroidogenesis and gametogenesis (Dufour and Rousseau, 2007; Rocha et al., 2009; Taranger et al., 2010; Schulz and Nóbrega, 2011). The biological functions of Fsh and Lh peptide hormones depend on their binding to and

activation of specific G protein-coupled receptors (Fshr, Lhr) located on the surface of gonad somatic cells (e.g. Sertoli cells and Leydig cells) (Rocha et al., 2009; Levavi-Sivan et al., 2010). During the initiation and early stages of spermatogenesis, the pituitary expression and release of Fsh increases before being reduced before spawning, while plasma levels of Lh remain low until final maturation and spermiation (spermiogenesis), suggesting their relevance at different stages of sexual maturation (Maugars and Schmitz, 2008a; Rocha et al., 2009; Schulz et al., 2010; Sambroni et al., 2013b).

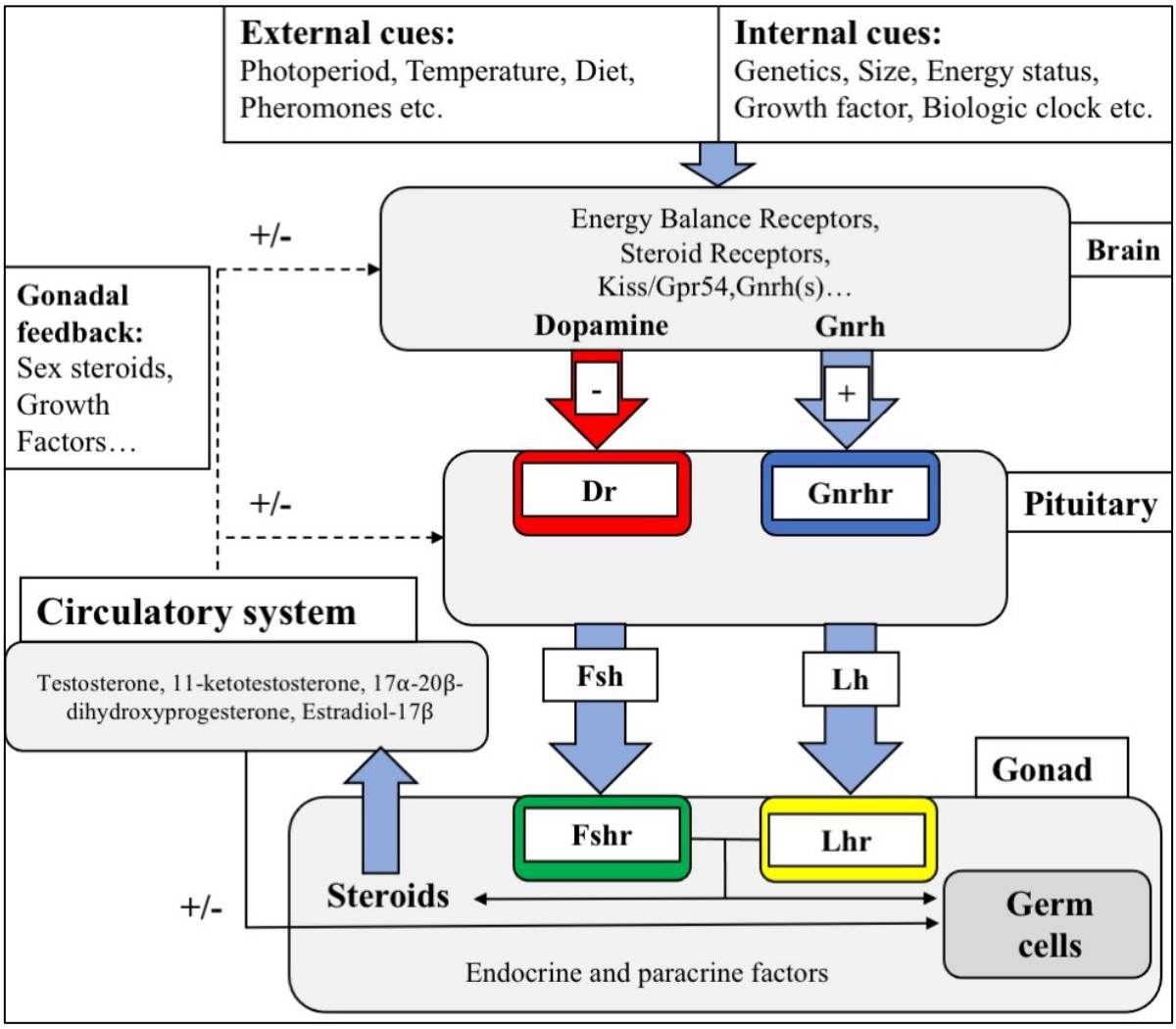

Figure 3: Schematic representation of selected regulatory pathways in the BPG-axis during sexual maturation in Atlantic salmon. *External cues and internal factors (including the endocrine feedback systems associated with gonads) activates the Gnrh neuron through assumed Kiss1/Kiss-r stimuli, stimulating the production/release of gonadotropin (Fsh and Lh) from the anterior pituitary (adenohypophysis) through activation of Gnrhr. Released Fsh and Lh stimulate germ cell development through activation of and interactions with Fshr and Lhr localized on Sertoli and Leydig cells, which accounts for gonad steroidogenesis (e.g. production of testosterone and 11-KT) and spermatogenesis, thus controlling germ cell growth, development and survival. Sex steroids and growth factors produced have feedback properties working at the brain and/or pituitary level, in which they may stimulate or inhibit the production and/or secretion of Fsh and Lh. Modified after (Taranger et al., 2010).*

1.5. Morphology of salmonid testes

Salmonids have an anastomosing tubular testies, characterized by germinal compartments forming loops in which they branch and rejoin (Parenti and Grier, 2004; Uribe et al., 2014). The testies are elongated paired organs attached cranially to the dorsal abdominal wall by the mesorchium (Schulz et al., 2010; Schulz and Nóbrega, 2011; Kryvi and Poppe, 2016; Yoshida, 2016). During maturation, there is a significant growth of the gonads, of which it fills much of the abdominal cavity when sexually mature.

The testies consists of two main compartments: (1) the intertubular and (2) the tubular compartment (Fig. 4). The intertubular compartment (1) contains Leydig cells, blood/lymphatic vessels, macrophages, mast cells, and neural and connective tissue elements, including the peritubular myoid cells (Schulz et al., 2010; Schulz and Nóbrega, 2011). Leydig cells play an essential role in steroidogenesis, of which its primary function is to convert cholesterol into androgens for the maintenance of spermatogenesis and extratesticular androgenic functions (Diemer et al., 2003). The Leydig cells are stimulated by Lh (and to some degree Fsh) to produce two major androgens: 11-Ketotestosterone (11-KT) and testosterone (Diemer et al., 2003). 11-KT plays a major role in all stages of spermatogenesis and affects the development of secondary sex characteristics, reproductive behavior, and stimulates Sertoli cells through interactions with androgen receptor (Ar) to produce growth factors (Borg, 1994). Testosterone influences reproduction through feedback mechanisms on the hypothalamus and pituitary with respect to Gnrh, though it also affects specific steps of spermatogenesis and is a precursor to 11-KT during steroidogenesis (Tilbrook and Clarke, 2001). The tubular compartment (2) consists of the germinal epithelium, which contains Sertoli cells and germ cells (Schulz et al., 2010; Schulz and Nóbrega, 2011). Sertoli cells are the first cells to differentiate in testies, and their most prominent trait is to facilitate germ cell development, physiological function, and survival, thus functioning as "nursing" cells (Griswold, 1995; DiNapoli and Capel, 2008; Schulz et al., 2010; Schulz and Nóbrega, 2011). Sertoli cells also phagocytize apoptotic germ cells, residual bodies discarded by spermatids, and sperm (Almeida et al., 2008; Schulz et al., 2010). During meiosis, Sertoli cells form the Sertoli cell barrier (SCB), of which germinal cells transits the tight junctions between the Sertoli cells, which creates a microenvironment providing immune privilege to passing germ cells while protecting and nursing the cells until fully developed (Smith and Braun, 2012). Throughout all stages of spermatogenesis, Sertoli cells and germ cells communicate through direct contact and paracrine factors, suppressing or triggering germ cell proliferation and differentiation (Kerr, 1995; DiNapoli and Capel, 2008). The survival of germ cells throughout spermatogenesis strictly depends on their continuous direct contact with Sertoli cells, as they provide nutritional, structural, and regulatory support (Walker and Cheng, 2005; Schulz and Nóbrega, 2011). Therefore, there is a spermatogenic

ceiling, of which the number of Sertoli cells determines the amount of fertile gametes produced (Nóbrega et al., 2008; Schulz et al., 2010).

1.6.Spermatogenesis

Spermatogenesis is a highly coordinated process in which diploid (2n) spermatogonia transform through mitotic and meiotic proliferation and differentiation into highly specialized motile haploid (n) spermatozoa (Schulz et al., 2010). The process may be divided into three different phases: (1) the mitotic phase (spermatogonial phase), (2) the meiotic phase, and (3) the spermiogenic phase (spermiogenesis) (Fig. 4). The mitotic phase includes the proliferation of different species-specific generations of spermatogonia (undifferentiated male germ cells), of which undifferentiated type A spermatogonia (A_{und}) with high potential of self-renewal transforms into differentiated type A spermatogonia (A_{diff}) with reduced potential for self-renewal and thereafter type B spermatogonia (B) (Ando et al., 2000). After the final mitotic division, type B spermatogonia differentiate into primary spermatocytes (SC(p.), entering meiosis (Schulz et al., 2010). In parallel, immature Sertoli cells proliferate through hormone-mediated cues (e.g. Fsh, Igf1, activin), establishing the Sertoli cell barrier (SCB) (Schulz et al., 2005; Meroni et al., 2019). The meiotic phase initiates the meiotic division of the primary spermatocytes to form secondary spermatocytes (SC(s.)) and thereafter spermatids (ST), and is induced by 11-KT (Miura et al., 1991; Taranger et al., 2010). The final stage of spermatogenesis is the spermatogenic phase and is characterized by the differentiation of spermatids into motile spermatozoa (SZ). Once the anastomosing tubular cysts are full of spermatozoa, the tight junction contact between germ cells and Sertoli cells are broken, releasing the spermatozoa into the tubular lumen (Schulz et al., 2010). This process is called spermiation. Once released into the tubular lumen, spermatozoa survives and acquires mobility through contact with the seminiferous tubular fluid (STF) produced and released by the Sertoli cells before spawning (Rato et al., 2010).

The process of spermatogenesis in male Atlantic salmon is to a high degree controlled by androgens and gene expression modulation through gonadotropin stimuli of Leydig cells and Sertoli cells (Fig. 5). Fsh regulates Sertoli cell activity, such as the structural, nutritional, and regulatory support of germ cell development in terms of gene expression (Sambroni et al., 2013a). Expression and release of Fsh increase during spermatogonial proliferation for then to be reduced before spawning, suggesting specific relevance at early stages of spermatogenesis (Maugars and Schmitz, 2008a; Rocha et al., 2009; Schulz et al., 2010; Sambroni et al., 2013b). Lh is the primary regulatory stimulant of Leydig cell steroidogenesis (Schulz, 2003). The level of plasma Lh levels remain low during gonad development and early stages of spermatogenesis, but increase during spermiogenesis, suggesting relevance at late stages of sexual

maturation (Maugars and Schmitz, 2008a; Rocha et al., 2009; Schulz et al., 2010; Sambroni et al., 2013b). Sex steroids (e.g. testosterone and 11-KT) produced may exert negative feedback controls on all levels of the BPG-axis (Nagahama, 1994). In salmonids, both Lh and Fsh stimulate the conversion of cholesterol to testosterone and 11-KT in Leydig cells. Lh is on the other hand a more potent stimulator of the intermediate 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) production, which is an indispensable hormone for initiating meiosis and late stages of spermatogenesis as it is shown to induce DNA replication in spermatogonia (Swanson et al., 2003; Schulz et al., 2010). This stimulatory effect further suggests the specific relevance of Lh at the late stages of spermatogenesis (Swanson et al., 2003).

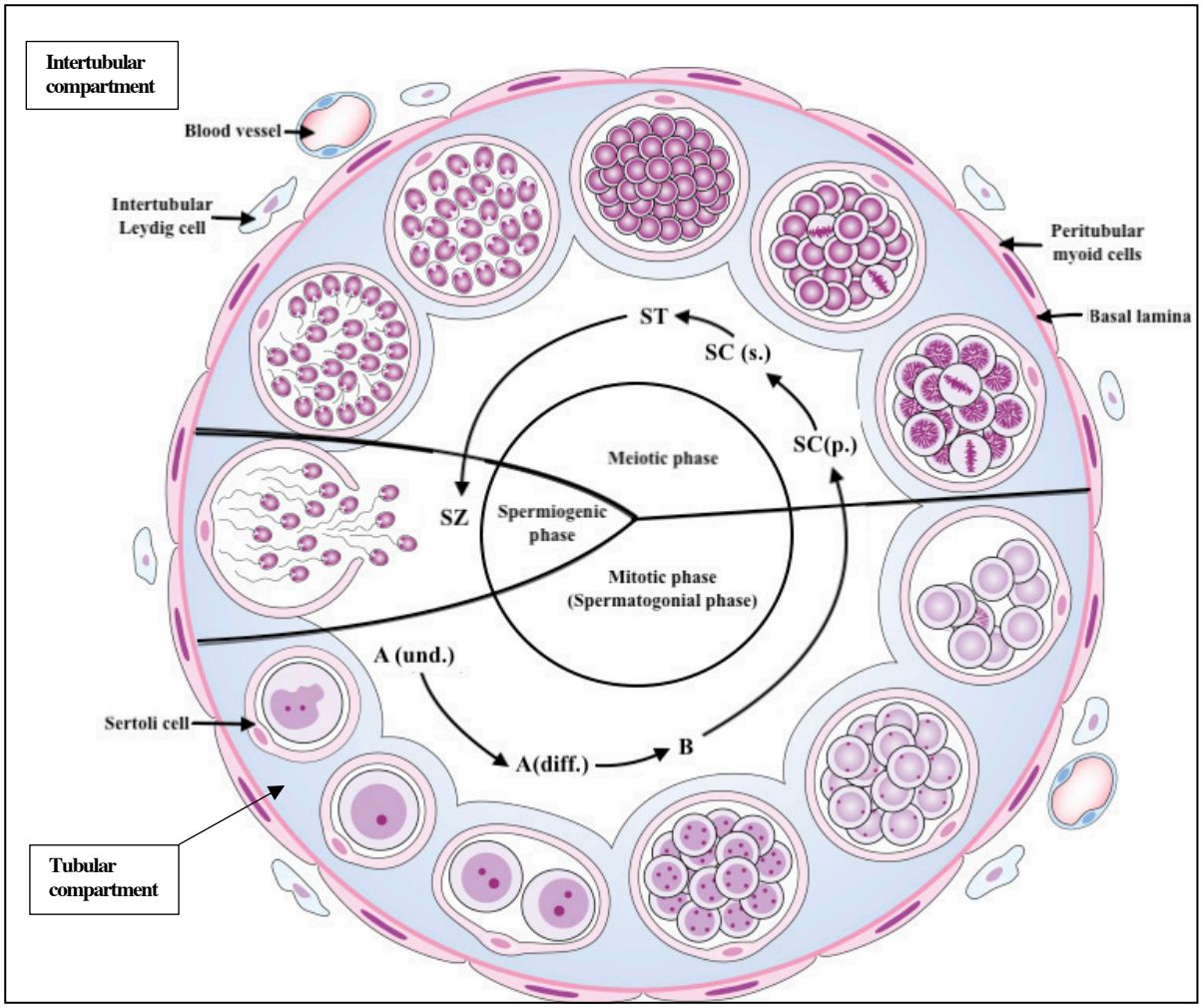

Figure 4: Schematic representation of spermatogenesis in the germinal epithelium in Atlantic salmon testis. *Progression of schematic spermatogenesis follows: (1) mitotic phase, (2) meiotic phase, and (3) spermiogenic phase. The tubular compartment contains Sertoli cells and germ cells. Basal lamina and peritubular myoid cells limit the tubular compartment from the intertubular compartment containing Leydig cells, blood vessels, etc. A(und.) – undifferentiated type A spermatogonia, A(diff.) – differentiated type A spermatogonia, B – type B spermatogonia, SC (p.) - primary spermatocytes, SC (s.) – secondary spermatocytes, ST – spermatids, SZ – spermatozoa. Modified after: (Schulz et al., 2010; Norris and Carr, 2013; Melo et al., 2014).*

1.7. Gonadotropin-responsive gene expression

Fsh and Lh regulate spermatogenesis directly or indirectly through the BPG-axis, stimulating multiple regulatory pathways which controls transcription of specific genes in for instance the TGF-β pathway (*amh, inha, gsdf1, gsdf2*) and the IGF pathway (*igf3*) (Sambroni et al., 2013b; Zheng et al., 2018). Sertoli cell derived Anti-Müllerian hormone (Amh) regulates self-renewal of undifferentiated type A spermatogonia and inhibits further germ cell proliferation and differentiation into type B spermatogonia (Skaar et al., 2011; Pfennig et al., 2015). Furthermore, Amh exerts negative regulation of androgen secretion from Leydig cell, inhibiting 11-KT induced proliferation of type B spermatogonia, thus the entry into the meiotic phase of spermatogenesis (Skaar et al., 2011; Pfennig et al., 2015). Transcription of *amh* is downregulated by increased levels of Fsh and androgens, allowing commitment of germ cells to differentiate and proliferate from early spermatogenesis and onwards (Maugars and Schmitz, 2008a; Schulz et al., 2010; Sambroni et al., 2013b). In parallel, Inhibin subunit alpha (*inha*) transcription is upregulated by Fsh and Lh. Inha has an inhibitory effect on Amh and exerts negative feedback control on Fsh release (Sambroni et al., 2013b; Kleppe et al., 2020). Two forms of gonadal soma-derived factors (Gsdf1, Gsdf2) found in salmonids are expressed in the Sertoli cells surrounding type A spermatogonia, and stimulates primordial germ cell proliferation and mitotic activity (Sawatari et al., 2007; Sambroni et al., 2013b; Kleppe et al., 2020). The transcription of *gsdf1* is downregulated by Lh and androgens during later stages of spermatogenesis, permitting onwards differentiation and proliferation of germ cells (Lareyre et al., 2008; Chen et al., 2013; Kleppe et al., 2020). The transcription of *gsdf2* is recently discovered and is only described in salmonids (Lareyre et al., 2008). Gsdf2 encodes for proteins that are essential for the function of the TGF-β pathway (Lareyre et al., 2008), however limited research has been done on Gsdf2 and its functions, although it is assumed to be associated with spermatogenesis (Lareyre et al., 2008; Sambroni et al., 2013b). Insulin-like growth factor 3 (*igf3*, also called *igf1b*) transcription in Sertoli cells are upregulated by Fsh and androgens during spermatogenesis, promoting spermatogonial proliferation and differentiation, thus the entry into meiosis (Wang et al., 2008; Sambroni et al., 2013b; Melo et al., 2015; Nóbrega et al., 2015) (Fig. 5).

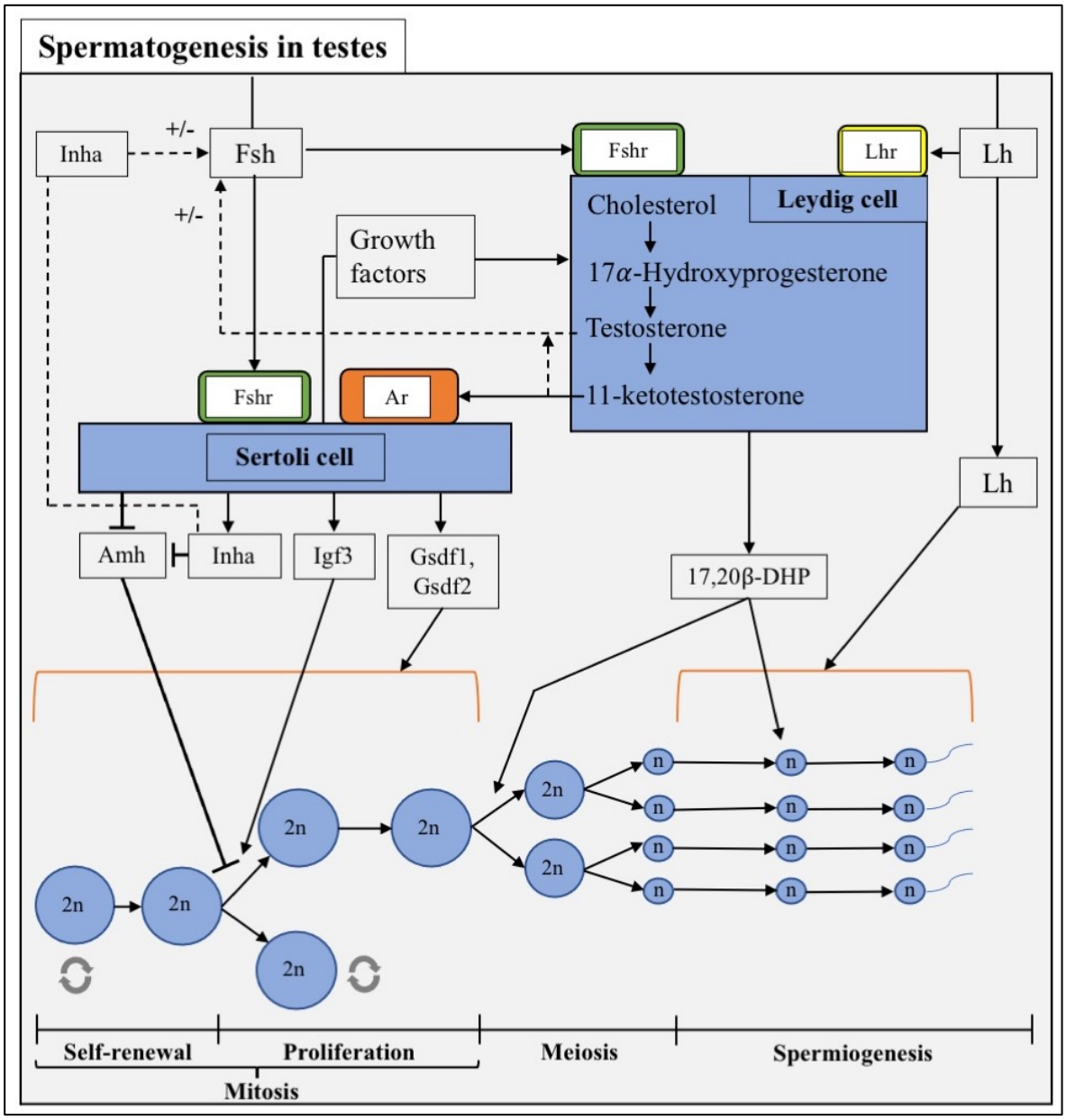

Figure 5: Gonadotropin regulation of paracrine production in testes during spermatogenesis. *Stimuli of the BPG-axis mediated gonadotropin (Fsh, Lh) production and release which regulate spermatogenesis. Fsh mainly modulate mitotic phase processes through regulating gene transcription in Sertoli cells and to some degree steroidogenesis in Leydig cells. Lh control processes related to the spermatogenetic phase and steroidogenesis. Conversion of cholesterol to 11-KT in Leydig cells during steroidogenesis is regulated by both gonadotropins, with Lh being a more potent 17, 20β-DHP stimulator which affects meiosis and spermiogenesis. Synthesized 11-KT provides feedback control on Fsh release. Both Fsh and 11-KT stimulates Sertoli cells through receptor interactions (Fshr, AR) to produce growth factors (e.g. Amh, Gsdf1, Gsdf2, Inha, and Igf3), regulating gene transcription in Sertoli cells which affects early phases of spermatogenesis. Transcription of amh is downregulated by increased levels of Fsh and 11-KT, reducing the antagonistic effect on spermatogenesis advancement. High levels of Fsh and/or Lh causes upregulation of inha transcription, which exerts an inhibitory effect on Amh function while providing feedback control on Fsh release. The transcription of gsdf1 is downregulated by Lh and 11-KT, which permits onwards differentiation and proliferation of germ cells from the mitotic phase. The transcriptional regulation of gsdf2 during sexual maturation is not fully known to the authors knowledge, although it is expected to be downregulated in parallel to gsdf1 transcription as it encodes essential proteins for the TGF-b pathway. Transcription of igf3 is upregulated by Fsh and 11-KT in parallel to the transcriptional downregulation of amh, promoting spermatogonial proliferation and differentiation, thus the entry into the meiotic phase of spermatogenesis. The growth factors produced regulate Leydig cell development and steroidogenesis. Modified after: (Schulz et al., 2010) and (Norris and Carr, 2013).*

1.8. Post-smolt production

Based on the changes implemented in the Norwegian aquaculture production regulations concerning smolt size restrictions in 2011 (Anonymous, 2011), the interest in producing larger smolt and post-smolt in RAS has increased (Bjørndal et al., 2018). The post-smolt stage (200 – 1 000 g) is defined as the first period after smoltification and the establishment of seawater tolerance, thus differs from smolt production (~100g), which primarily is freshwater oriented (CtrlAqua, 2020). The intensification of rearing conditions in RAS to enhance growth rates is assumed to have promoted the problem of early sexual maturation in male post-smolts, and may be linked to the species plasticity in timing and routing of puberty at various life stages (Wild et al., 1994; Hutchings and Jones, 1998; Imsland et al., 2014; Melo et al., 2014; Good and Davidson, 2016). Early puberty at different life stages has been a persistent problem in salmon farming; however, unlike traditional cases of early puberty as parr, maturation of post-smolts does not occur in nature. The aquaculture company Bremnes Seashore AS registered 10 % mature male post-smolt in their facility Bremnes Trovåg (Bremnes Seashore AS, Trovåg, Rogaland, Norway) in 2017-2018, resulting in a major loss in production. Preliminary studies indicate that the issue observed in Bremnes Trovåg may be linked to the use of high temperatures and high-energy feed during production to achieve a high specific growth rate (SGR). Early maturation during the production of post-smolt in RAS can become a production barrier, as it may compromise the economic and biological practicability of the systems, thus making it difficult to fully justify the use of the system biologically and economically (Davidson et al., 2016).

1.9. Factors affecting maturation

1.9.1.1. Energy budget, feeding, and growth rate

The energy budget is an important factor, ultimately deciding the size and age of sexual maturation. This is reflected in Atlantic salmon males who undergo puberty during the freshwater phase. They initially tend to be among the largest individuals in a population, until repressing growth during maturation, ultimately ending up smaller than individuals committing to smoltify (Skilbrei, 1989). Energy levels and storage (lipids and glycogen) is obtained through the diet, of which the energy is initially used for physiological maintenance before somatic growth. Intensive feeding, optimal temperatures, and continuous light cause elevated energy levels, subsequently increasing the risk of early sexual maturation. Previous studies on early sexual maturation have shown that the degree of maturation is higher in groups which experience high growth rates, linking maturation to a physiological threshold which must be exceeded depending on energy storage and fed availability (Rowel et al., 1991; Kadri et al., 1996; Norrgård et al., 2014). As rearing conditions are intensified in RAS with high feeding regimes and energy-rich feed, it may affect the rate of sexual maturation in post-smolts.

1.9.1.2. Photoperiod

Annual variations in environmental factors have been shown to have a synchronizing effect on the reproductive cycles, acting as cues for developmental events (Taylor, 1991; Hutchings and Jones, 1998; Bromage et al., 2001; Taranger et al., 2010). Variations in photoperiod are regarded as the most important cue for developmental events in Atlantic salmon. Photoperiod is a proximate factor providing seasonal cues for reproduction, synchronizing maturation with seasonal change, ensuring spawning during favorable conditions for the offspring (Bromage et al., 2001). Changes in daylength serve as a zeitgeber, affecting the circannual endogenous rhythms through levels of circulating melatonin. These changes will initiate or postpone developmental events, depending on the physiological state in relation to genetically determined developmental thresholds (Duston and Bromage, 1988; Taranger et al., 1999,2010; Bromage et al., 2001). Seasonal timing of maturation suggests that directional change in daylength is important (Randall et al., 1998). Different light regimes (photoperiods, light intensity, and quality) are utilized in aquaculture production to induce smoltification, enhance growth or delay puberty. However, early sexual maturation of post-smolts remains a problem even when using traditional light control in RAS. This may suggest that other factors such as temperature may have a critical role in early sexual maturation than earlier expected.

1.9.1.3. Water temperature

Nearly all fish are ectotherm poikilotherms, meaning that the rate of all biological processes is regulated by the ambient temperature. For instance, during winter when water temperatures are low, appetite and growth ceases due to low metabolic rate, while during summer when water temperatures are high, appetite and growth rate increase due to the increased metabolic rate (Schmidt-Nielsen, 1997). Ultimately, it may modulate rates of spermatogenesis, allowing or inhibiting puberty. Previous studies on the effect of temperature and photoperiod on early sexual maturation in Atlantic salmon have consistently shown that the percentage of sexual maturation is higher when the salmon is farmed at high temperatures (Fjelldal et al., 2011; Imsland et al., 2014; Good and Davidson, 2016). As temperature is a factor utilized especially in RAS to maximize the growth rate of farmed fish, it may affect the rates of sexual maturation in male Atlantic salmon post-smolts.

1.10. Objective

It is essential to solve the problem of early sexual maturation in RAS if we are to ensure a sustainable conversion of the traditional smolt strategy while reaching the national growth target by 2050. To our knowledge, little research has been conducted on triggering factors of the early sexual maturation of male post-smolts reared in intensive RAS conditions. Due to the high degree of control in RAS, it should be possible to control, refine and/or eliminate environmental triggers by defining parametric thresholds of early maturation once properly identified (Good and Davidson, 2016). This study focused on the relationship between different temperatures and feeding ration (simulating RAS intensive conditions) on precocious male maturation and early gonad development in Atlantic salmon pre-smolt. Early gonad development is used as an indicator for post-smolt maturation.

This experiment was based on the following hypotheses:

- **$H0_1$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on body weight development.
- **HA_1:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on body weight development.
- **$H0_2$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on body weight development.
- **HA_2:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on body weight development.

- **$H0_3$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on condition factor (CF) development.
- **HA_3:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on condition factor (CF) development.
- **$H0_4$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on condition factor (CF) development.
- **HA_4:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on condition factor (CF) development.

- **$H0_5$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Hepatosomatic index (HSI) development.

- **HA_5:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on Hepatosomatic index (HSI) development.
- **$H0_6$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Hepatosomatic index (HSI) development.
- **HA_6:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on Hepatosomatic index (HSI) development.

- **$H0_7$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Gonadosomatic index (GSI) development.
- **HA_7:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on Gonadosomatic index (GSI) development.
- **$H0_8$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Gonadosomatic index (GSI) development.
- **HA_8:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on Gonadosomatic index (GSI) development.

- **$H0_9$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Follicle-stimulating hormone receptor (*fshr*) gene transcription.
- **HA_9:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on Follicle-stimulating hormone receptor (*fshr*) gene transcription.
- **$H0_{10}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Follicle-stimulating hormone receptor (*fshr*) gene transcription.
- **HA_{10}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on Follicle-stimulating hormone receptor (*fshr*) gene transcription.

- **$H0_{11}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Luteinizing hormone receptor (*lhr*) gene transcription.
- **HA_{11}:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on Luteinizing hormone receptor (*lhr*) gene transcription.
- **$H0_{12}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Luteinizing hormone receptor (*lhr*) gene transcription.
- **HA_{12}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on Luteinizing hormone receptor (*lhr*) gene transcription.

- **$H0_{13}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Anti-Müllerian hormone (*amh*) gene transcription.
- **HA_{13}:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on Anti-Müllerian hormone (*amh*) gene transcription.
- **$H0_{14}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Anti-Müllerian hormone (*amh*) gene transcription.
- **HA_{14}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on Anti-Müllerian hormone (*amh*) gene transcription.

- **$H0_{15}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on gonadal soma-derived factor 1 (*gsdf1*) gene transcription.
- **HA_{15}:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on gonadal soma-derived factor 1 (*gsdf1*) gene transcription.
- **$H0_{16}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on gonadal soma-derived factor 1 (*gsdf1*) gene transcription.
- **HA_{16}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on gonadal soma-derived factor 1 (*gsdf1*) gene transcription.

- **$H0_{17}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on gonadal soma-derived factor 2 (*gsdf2*) gene transcription.
- **HA_{17}:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on gonadal soma-derived factor 2 (*gsdf2*) gene transcription.
- **$H0_{18}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on gonadal soma-derived factor 2 (*gsdf2*) gene transcription.
- **HA_{18}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on gonadal soma-derived factor 2 (*gsdf2*) gene transcription.

- **$H0_{19}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on insulin-like growth factor 3 (*igf3*) gene transcription.
- **HA_{19}:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on insulin-like growth factor 3 (*igf3*) gene transcription.

- **$H0_{20}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on insulin-like growth factor 3 (*igf3*) gene transcription.
- **HA_{20}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on insulin-like growth factor 3 (*igf3*) gene transcription.

- **$H0_{21}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on plasma 11-Ketotestosterone (11-KT) concentrations.
- **HA_{21}:** Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have a significant effect on plasma 11-KT concentrations.
- **$H0_{22}$:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on plasma 11-Ketotestosterone (11-KT) concentrations.
- **HA_{22}:** Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have a significant effect on plasma 11-KT concentrations.

2. Materials and methods

2.1. Fish Stock

Juvenile Atlantic salmon pre-smolt (*Salmo salar* L.) were obtained from the commercial aquaculture company Bremnes Seashore AS. Pre-smolts are defined as juvenile salmons that have not yet completed the parr-smolt transformation (Houde et al., 2018) due to being maintained in fresh water (Stefansson et al., 2008). Fertilized roe with origin in the Erfjord stain was acquired from SalmoBreed AS (Bergen, Norway) and reared at Bremnes Trovåg RAS facility (Bremnes Seashore AS, Trovåg, Norway) following standard commercial production protocols. Roe hatched in mid-April 2018 at ~500 d°C (day degrees), and alevins were kept in hatching cabinets until the yolk sac had been absorbed (~ 400 d°C post hatching), developing into fry. When developed into parr (3-4 g), the fish was transferred into 8 m diameter tanks (100 m^3). On 27.09.18, the pre-smolt (n = 1800, 5.5-month-old, average weight: 23.1 ± 7.2 g,) were transported from the Bremnes Trovåg RAS to the flow-through facility (FTS) at Høyteknologisenteret (UiB, BIO, Bergen).

2.2. Experimental design

The experiment consisted of a 3x2 factorial design, of which three different temperatures (8, 12.5, and 18 °C) and two different feeding ratios (67% and full feeding) produced six experimental groups reared in duplicates (8-67%, 8-100%, 12.5-67%. 12.5-100%, 18-67%, 18-100%) (Fig. 6). Pre-smolt was randomly distributed between 12-flow-through tanks of 0.5 m^3 (n = 150/tank). Fish were acclimated for one week at 12.5°C, full feed ratio, specific flow rate 0.5 L/kg/min, and LD24:0 (Light 24h, darkness 0h) photoperiod for physiological stabilization and to avoid additional stress after transportation. Feed ration was calculated based upon estimations of specific growth rate (SGR) = 1.7% and food conversion ratio (FCR) = 1.2 to ensure sufficient feeding. Tanks were checked and flushed for excess feed daily throughout the experimental period.

In the period 4.10.18 - 6.10.18, temperatures were gradually adjusted to 8°C and 18°C in two pairs of tanks per feeding group, while the third pair were kept at 12.5°C. Water flow was adjusted to 0.18 L/s (11L/min) in all tanks to keep oxygen saturation above 80 %. After the temperatures were stabilized at their respective levels, the feed ratio in the restrictive feeding group was reduced to 67 %. To avoid aggression and establishment of hierarchies among fish (Mccarthy et al., 1992), the restrictive feeding ratio was achieved by including fasting every three days. Feed rations were re-calculated for each tank after every sampling point, based on temperature, mean weight, and stocking density. SGR and FCR needed for these calculations were estimated by using a table published by Skretting for Atlantic salmon

(www.skretting.no). Throughout the experiment, salmon were fed appropriate 2 and 3 mm formulated commercial feed from Biomar® provided by Bremnes Seashore AS over a two-hour feeding cycle. The LD24:0 photoperiod was utilized for four months to ensure high growth rates, until it on 4.02.19 were reduced to LD12:12 to provide the winter signal for smoltification/maturation. The winter signal was maintained for five weeks (04.02.19 – 11.03.19), and thereafter, set back to LD24:0 and maintained until the end of the experiment.

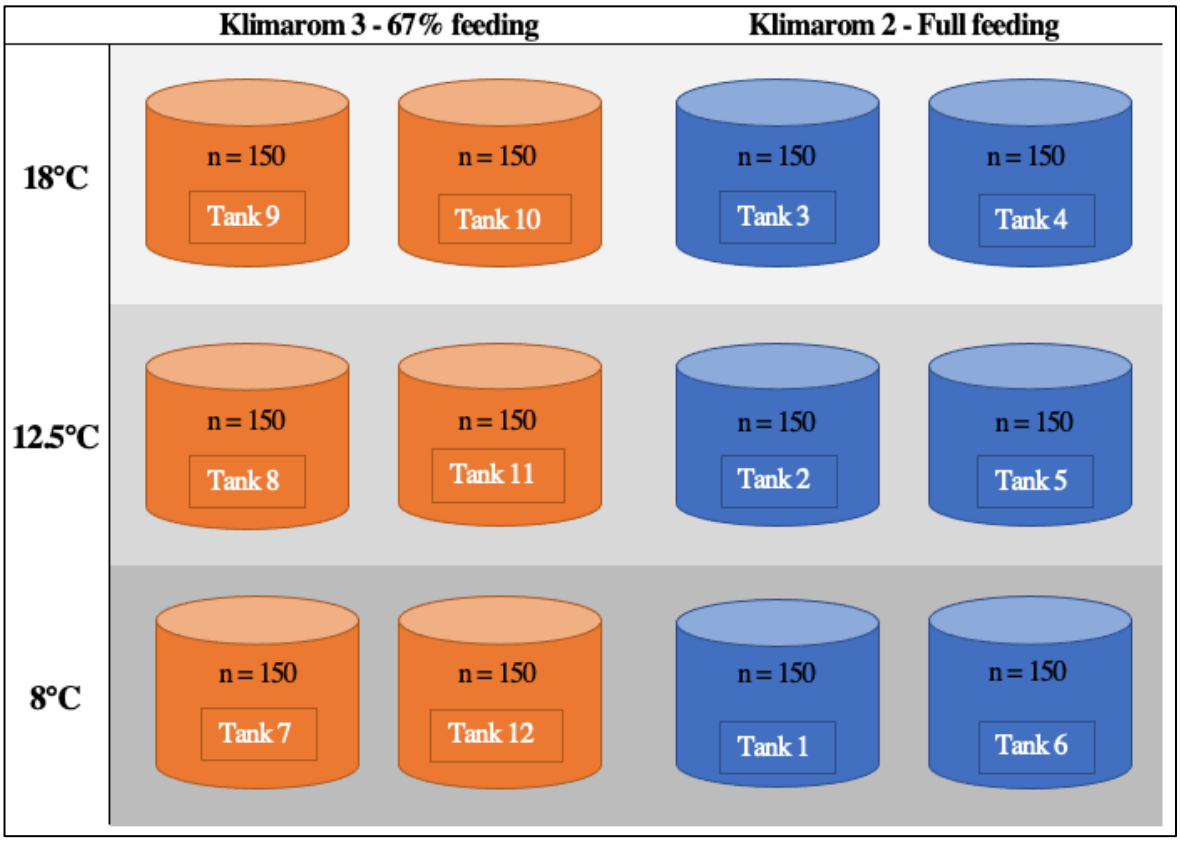

Figure 6: Experimental design. *The experimental setup followed a 3x2 factorial design with temperature and feeding regime as factors run in duplicate tanks. Three different temperatures (8, 12.5, 18°C) and two feeding regimes (100%, 67%) were used to investigate the extent to which the factors contributed to early sexual maturation. A mixed gender population of n = 1800 Atlantic salmon was divided among 12 separate tanks (n=150 per tank).*

2.3. Sampling protocol

Throughout the experiment, eight individual samplings were conducted, each of which lasted two days (Fig. 7). For each sampling point, a total of 72 male fish (six fish per tank) were randomly selected for biometric measurements and tissue samplings. All fish were quickly netted out and humanly euthanized using an overdose of benzocaine (Benzoak vet.® 20%, ACD Pharma AS, Norway). As the experimental groups contained a mixed gender population, female salmon were discarded from the experiment.

Blood was manually extracted from the caudal vein using sterile heparinized syringes to avoid blood coagulation. Blood samples were centrifuged (5000 rpm, 4 min), separating plasma from the blood cells. Plasma was collected in a separate tube, immediately frozen in dry ice, and kept at -80°C until sex steroid analysis (11-Ketotestosterone). Before dissecting the fish, morphological measurements of body weight (grams, g) and fork length (centimeters, cm) were measured to the nearest 0.1 g/cm respectively (with Kern PRS6200-2 and length scale respectively) and used for calculating condition factor (CF). Fish were then dissected, and gonads examined to record gender and degree of maturation.

Testes were excised and weighted to the nearest 0.01 g (VWR LPC-213i) for calculations of gonadosomatic index (GSI). Depending on size, whole or anterior sections of testis were preserved appropriated to downstream application. One testis was preserved in RNAlater following manufacturer instructions (Sigma-Aldrich, St. Louis, MO, US) for gene transcription analysis, while the other was fixed in formalin (4% formalin, 0.08 M sodium phosphate, pH 7.0) for histological image analysis. The liver was extracted and weighted to the nearest 0.01 g (VWR LPC-213i) for calculations of hepatosomatic index (HSI). All samples were stored at -80°C until analysis.

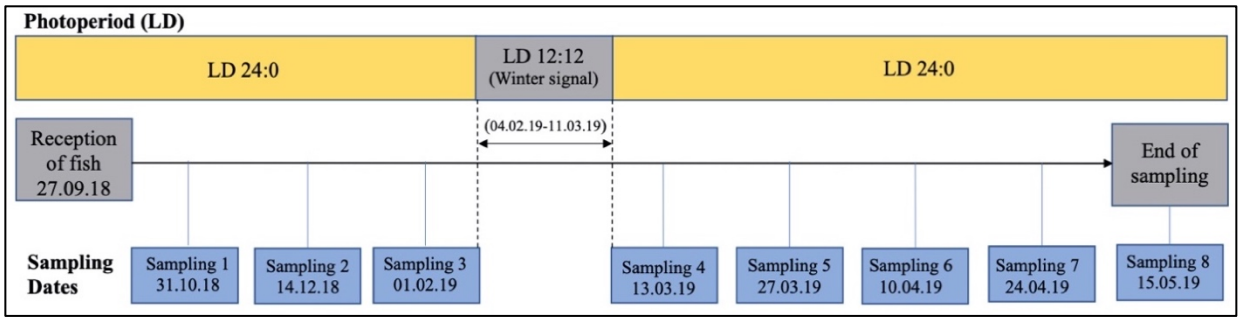

Figure 7: Schematic representation of the experimental timeline and sampling time points. *Using a 3x2 factorial design dependent on two feeding regimes (100%, 67%) and three temperatures (8, 12.5, 18°C). The 12 different tanks were sampled at eight representative timepoints: sampling 1 (31.10.18), sampling 2 (14.12.18), sampling 3 (01.02.19), sampling 4 (13.03.19), sampling 5 (27.03.19), sampling 6 (10.04.19), sampling 7 (24.04.19) and sampling 8 (15.05.19). For each sampling, male Atlantic salmon (n = 72) were randomly selected, euthanized, measured, and dissected according to sampling protocol.*

2.4. Condition factor, gonadosomatic index and hepatosomatic index

Fulton's condition factor (CF) (Froese, 2006) was calculated in order to assess the condition of the fish in terms of energetic reserves as a predictor of developmental changes, using the following equation:

$$\mathrm{CF} = \left(\frac{\text{Total body weight (g)}}{(\text{Fork length (cm)})^3}\right) * 100$$

Gonadosomatic index (GSI) was calculated in order to assess the degree of sexual maturation, expressing gonad weight as a portion of total body weight, using the following equation:

$$\text{GSI (\%)} = \left(\frac{\text{Gonad weight (g)}}{\text{Total body weight (g)}}\right) * 100$$

An arbitrary scale following data dispersion and published literature was used to divide samples into four categories based on GSI (%): "*immature*" (GSI ≤ 0.06), "*early stage*" (0.06 < GSI ≤ 0.1), "*maturing*" (0.1 < GSI ≤ 1) and "*mature*" (1 < GSI) (Thorpe, 1994; Peterson and Harmon, 2005).

Hepatosomatic index (HSI) were calculated in order to assess any decrease in fat deposition associated with sexual maturation, expressing liver weight as a portion of total body weight, using the following equation:

$$\text{HSI (\%)} = \left(\frac{\text{Liver weight (g)}}{\text{Total body weight (g)}}\right) * 100$$

2.5. Histological image analysis

Gonad tissue samples (n = 144) were processed and evaluated histologically in accordance with Fjelldal et al. (2018) by Marianne Kraugerud at Fish Vet Group Norge (Oslo, Norway) (Fig. 8). Formalin fixed tissues were processed in a Thermo Scientific Excelsior tissue processor and embedded in paraffin Histowax using a Tissue – Tek, TEC 5 (Sakura) embedding center. Embedded tissue was sectioned at 1.5-2 µm using a Leica RM 2255 Microtome, sections were mounted on glass slides and stained with Hematoxylin-Eosin (HE). Stained slides were scanned in an Aperio ScanScope® AT Turbo slide scanner and read using Aperio ImageScope® (Leica) (magnification: 10X). Tissue slides were graded from 1 – 6 for sexual maturity (Fjelldal et al., 2018).

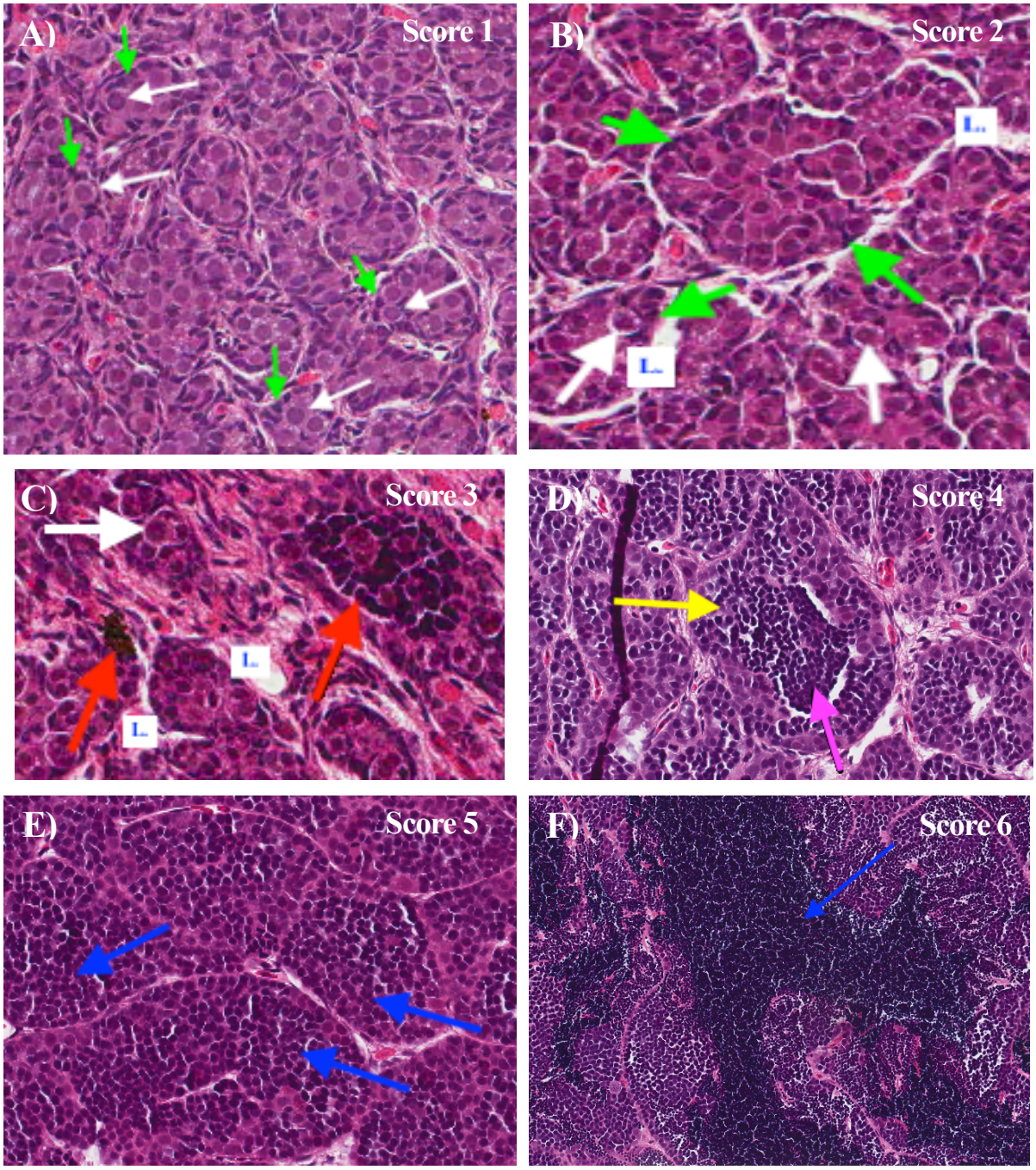

Figure 8: Testis development of Atlantic salmon presenting the different gonad scores. ***(A)*** *Score 1; Type A spermatogonia (white arrows) are the most developed type of germ cell. Sertoli cell nuclei (green arrow) belonging to the Sertoli cells are located in the spermatogenetic tubules, not in direct contact with the germ cells, thus the Sertoli cell barrier is not yet formed.* ***(B)*** *Score 2; Type A spermatogonia (white arrows) are the most developed type of germ cell. Sertoli cell nuclei (green arrow) still not in direct contact with germ cells. Spermatogenic tubules show lumina (L.) to some degree.* ***(C)*** *Score 3; testis contain type A spermatogonia (white arrows) and type B spermatogonia (red arrows), of which type B spermatogonia is the most developed type of germ cell. Lumina is visible throughout tubuli.* ***(D)*** *Score 4; Spermatocytes (yellow arrows) and spermatids (purple arrows) are the most developed type of germ cell.* ***(E)*** *Score 5; Spermatozoa (blue arrows) is the most developed type of germ cell, with earlier germ cell generations present.* ***(F)*** *Score 6; Spermatozoa (blue arrows) is the most developed type of germ cell and is present in vast numbers in tubuli. Histological images are stained with Hematoxylin-Eosin (HE) (Fjelldal et al., 2018).*

2.6. Transcription of mRNA

2.6.1. Total RNA isolation

Isolation of total RNA from gonad tissue was performed using the QIAsymphony SP automated nucleic acid extraction robot (Qiagen, Hilde, Germany) and TRI reagent (Invitrogen, Carlsbad, USA) method. The QIAsymphony provides accurate purification with a high RNA yield, however extraction yield may be insufficient when using small tissue inputs. The TRI reagent method was utilized on small gonad tissue samples to ensure high RNA yields.

2.6.1.1. Automated RNA isolation with QIAsymphony SP robot

Total RNA was isolated from testis tissue samples using the QIAsymphony SP automated nucleic acid extraction robot (Qiagen, Hilde, Germany) in conjunction with the QIAsymphony RNA kit (Qiagen), following the manufacturers protocol. Prior to the QIAsymphony procedure, manual disruption and homogenization of tissue were performed. Gonad samples were thawed, dried, weighed, and cut to fit the set procedure threshold of 20 – 25 mg. Thereafter tissue was homogenized in 450 µl RLT plus lysis buffer (Qiagen) and 0.5% (v/v) reagent DX (Qiagen), using stainless-steel beads (5 mm) (Qiagen) in a Precellys 24 Tissue Homogenizer (Bertin technologies, Versailles, France) (5000 rpm, 15 s), followed by incubation at room temperature for 5 minutes. Samples were homogenized in batches and stored at -20°C until reaching a sufficient number for automated RNA purification. A total of 276 tissue samples was purified, of which 92 samples were purified per cycle of isolation.

2.6.1.2. Manual RNA isolation using TRI-reagent

Total RNA was manually isolated from 156 testicular tissue samples using the TRI-reagent (TRIzol) method (Invitrogen, Carlsbad, USA) following a standard protocol based upon improvements of a single-step report by Chomczynski (Chomczynski, 1993). Gonads were homogenized (5000 rpm, 15 s) in 1 mL TRI-reagent using stainless-steel beads (5 mm) in a Precellys 24 Tissue Homogenizer (Bertin Technologies) to disrupt cells and cell components, followed by incubation for 5 minutes at room temperature. Homogenized tissue was added 200 µl chloroform (Sigma-Aldrich) and vortexed. The aqueous phase (~ 400 – 450 µl) was collected after centrifugation at 16000 g for 15 min at 4ºC in Eppendorf 5415R centrifuge (Eppendorf AG, Hamburg, Germany) and mixed with 500 ul isopropanol. Precipitated RNA was collected by centrifugation at 16000 g for 15 min at 4ºC in Eppendorf 5415R centrifuge and washed with 1 mL ethanol (80 %) before being centrifuged at 9000 g for 5 min at 4°C, followed by being resuspended in nuclease free water.

2.6.1.3. *Quantification and purity measurement of total RNA*

Total RNA concentration (ng/µl) was measured using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, USA) with the Qubit RNA BR assay kit (Thermo Fisher Scientific) using 2 µl total RNA as input. Measurements were conducted following manufacturer instructions (Thermo Fisher Scientific). The total RNA purity was measured using the NanoDrop One microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific) using 1 µl total RNA. The ratio of absorbance for samples was ~ 2 and < 2 for 260/280 nm ($A_{260/280}$) and 260/230 nm ($A_{260/230}$) respectively, which indicated sufficient purity of contaminants (Desjardins and Conklin, 2010). Purified RNA was thereafter stored in a -80°C freezer until downstream analysis was performed.

2.6.2. Normalization and first-strand complementary DNA (cDNA) synthesis

First-strand complementary DNA (cDNA) synthesis was performed in a total volume of 20 µl using the SuperScript™ III Reverse Transcriptase kit (Invitrogen, Carlsbad, USA) in accordance with the manufacturer protocol. All pipetting was performed using the Hamilton Microlab STARlet pipetting robot (Hamilton, Nevada, USA). Isolated total RNA (300 ng) was diluted in 11 µl ultra-pure water, before adding 1 µl Oligo$(dT)_{20}$ primer (50 µM) and 1 µl dNTP mix (0.5 mM) to each sample (total volume 13 µl). After incubation at 65°C for 5 min in the C1000 Touch Thermal Cycler (Bio-Rad Laboratories, CA, US), samples were placed on ice for one min to limit the formation of secondary structures. A total of 4 µl of 5X First-Strand Buffer (Tris-HCL (250 mM at pH 8.3), KCL (375 mM), $MgCl_2$ (15 mM)), 1 µl dithiothreitol (DTT; 5 mM), 1 µl RNaseOUT™ Recombinant RNase Inhibitor (40 units) and 1 µl SuperScript™ III RT (200 units/µl) was then added to the samples, resulting in the final volume of 20 µl. The cDNA synthesis was then performed in accordance with the manufacturers: incubation at 50°C for 60 min, followed by inactivation at 70°C for 15 minutes and then down to 4°C before storage at -20°C until analysis.

2.6.3. Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) was performed to quantify the relative mRNA abundance of *fshr, lhr, amh*, *gsdf1*, *gsdf2*, and *igf3* using the C1000 Touch Thermal Cycler, CFX96 Real-Time Detection System (Bio-Rad Laboratories, CA, US) and CFX Manager software (version 3.1) (Bio-Rad Laboratories). All pipetting was performed using the Hamilton Microlab STARlet pipetting robot. The RT-qPCR reactions were carried out in duplicates in a total volume of 12.5 µl using 6.25 µl iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories), 0.5 µl of each forward and reverse primer (0.2 µM) (Table 1), 2.5 µl cDNA, and 3.25 µl ultra-pure water. The RT-qPCR reactions were

performed in 96-well plates (Bio-Rad Laboratories), with the thermal cycling program described in Table 2. All plates included a "non-template control" (NTC) for checking for contamination and a common pool sample (cDNA pool used for intercalibration among plates.

Table 1: ***Primers used in gonad RT-qPCR.*** The specific primers and their respective nucleic acid sequences used for each RT-qPCR assay to measure mRNA abundance in the present study. F = forward primer, R = reverse primer.

Gene	Primer sequence (5' → 3')		Gene Accession number
amh	F	CAAAAACACCAGAGACAGGACAA	AY722411.1
	R	TATCCGTTGAGAAAAGCACCA	
fshr	F	CACTGCCATTGTGCTAAC	NM 001123610.1
	R	AGCCTGATGATGGATGAC	
gsdf1	F	GCGACTGACAGACTTACTTC	XM_014138924.1
	R	TACAGCCACTGCTTTGTC	
gsdf2	F	TGATGGTTGTGCTCTCTAG	XM_014172058.1
	R	CTTGGCAACTGTTCAGAGTG	
igf3	F	ACTGCGCAAAGCCAAAGC	Middleton et al, 2019 (https://doi.org/10.1016/j.ygcen.2019.05.010)
	R	GAAATTGCTCCTCCATAACTTGCT	
lhr	F	CCTGAGAAGAGTCCAGCATATAGA	Maugars and Schmitz (2008)
	R	GAAGATTTCATTGAGGTCGAGAAG	
ef1a	F	CCCCTCCAGGACGTTTACAAA	Olsvik et al., 2005
	R	CACACGGCCCACAGGTACA	

Table 2: ***Thermal cycle program used in the present study.***

Step	Process	(°C)	Time pr. cycle	Cycles	Description
1	Initiation denaturation	95	3 min	1	High-temperature incubation, separating *initial* cDNA nucleic acid.
2	Denaturation	95	15 s	40	High-temperature incubation, separating *quantified* cDNA nucleic acid double chains.
3	Annealing, extension, and fluorescence read	60	1 min		Complementary primer sequences to single nucleic acid chains hybridize. DNA polymerase catalyzes the synthesis of double-stranded DNA, extending the primer. SYBR green supermix binds to double strand DNA and produces a detectable fluorescent signal measured by a fluorescent light detection camera at the end of the period.
4	Melting curve: Denaturation	95	10 s	1	Melting curve generation: denaturation of all PCR products at 95°C for 10 s, followed by re-hybridization through gradual temperature increase (0.5°C every 5 s) from 65°C. For each increment, fluorescent signals are measured. Detection of fluorescent signals at the end of the period.
5	Melting curve: Re-hybridization	65-95	5 s	60	

Prior to the mRNA expression analysis, all primers were validated for efficiency and optimal dilution by running two-fold dilution series using a pooled cDNA sample taken from a selection of samples covering the experimental period and the different treatment groups. Based on the dilution series, all RT-qPCR assays were run with cDNA dilution 1 to 60. Primer amplification efficiency (E) was determined by the slope of the regression line generated from dilution curves.

Quantification of mRNA abundance was completed using the mean of the duplicate target Ct values and the corresponding mean of the duplicate reference Ct values used for normalization (*ef1a*). The following formulas were used for calculations (Pfaffl, 2012):

1) Primer Amplification Efficiency (E):

$$E = 10^{\left(\frac{-1}{slope}\right)}$$

2) Relative mRNA quantification of *fshr*, *lhr*, *amh*, *gsdf1*, *gsdf2*, *igf3:*

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta Ct_{\text{mean target}}}}{(E_{\text{ref}})^{\Delta Ct_{\text{mean ref}}}}$$

E = Primer amplification efficiency.

$\mathbf{E_{target}}$ = RT-qPCR efficiency of the target genes (*amh*, *fshr*, *gsdf1*, *gsdf2*, *igf3*, and *lhr*).

$\mathbf{E_{ref}}$ = RT-qPCR efficiency of the reference gene (*ef1a*).

Ct = Cycle threshold, number of cycles required for the fluorescent signal to cross the threshold.

$\Delta\mathbf{Ct_{mean\ target}}$ = Mean Ct values of the target gene in replicates (*amh*, *fshr*, *gsdf1*, *gsdf2*, *igf3,* and *lhr*).

$\Delta\mathbf{Ct_{mean\ ref}}$ = The mean Ct values of the reference gene in replicates (*ef1a*).

The coefficient of variance (CV) was calculated for each set of duplicates to account for the precision of technical replicated measurements. The following formula was utilized for calculating the CV (Brown, 1998):

$$\mathrm{CV} = \left(\frac{\text{Int. SD Ct}}{\text{Int. mean Ct}}\right) \text{x } 100\%$$

Int. SD Ct = Intercalibrated Standard Deviation (SD) cycle threshold for replicated targets.

Int. mean Ct = Intercalibrated mean cycle threshold for replicated targets.

Duplicate values with CV below 1.5% were considered consistent enough for downstream analysis. The repeated samples were analyzed in triplicates in case of one amplification failed. Samples with CV>1.5% were repeated in triplicates. For genes with generally low relative expression (*fshr*, *lhr,* and *igf3*), the CV threshold was increased to 2.5%. For data analysis purposes, Ct-values of samples with too little transcription to be reliably quantified were substituted by an arbitrary value lower than the less transcribed samples with precise measures for that particular gene (*fshr* = 37, *igf3* = 38.5, *lhr* = 39). Samples with CV over the threshold were disregarded.

2.7. Plasma 11-Ketotestosterone analysis

A total of 407 gonad tissue samples were processed for 11-Ketotestosterone analysis conducted by Birgitta Norberg at the Institute of Marine Research (IMR, Bergen, Norway). The method was conducted in accordance with descriptions from Fjelldal et al. (2018). Sex steroids were extracted from blood plasma following a methodology modified from Pankhurst and Carragher (Pankhurst and Carragher, 1992). Blood plasma (100 µl) was mixed with ethyl acetate ($C_4H_8O_2$), vortexed, and centrifuged. The organic phase was collected. Extracts were dissolved in 1 mL buffer (0.1 mol L^{-1} phosphate, 0.4 mol L^{-1} NaCl, 1 mmol L^{-1} EDTA) by heating (60°C for 10 min). The extracted and dissolved sex steroids were then stored at -20°C until analysis using the enzyme-linked immunoadsorption assay (ELISA) (Cuisset et al., 1994). ED80 and ED20 strand values were 0.04 ng mL^{-1} and 1.00 ng mL^{-1} respectively, and detection limit for the assay was 0.005 ng mL^{-1} (Fjelldal et al., 2018). Samples with binding concentrations higher than the standard curve were diluted and re-run until they fitted the linear part of the standard curve. Internal standards for 11-KT were prepared mature male Atlantic salmon plasma. Accepted intraassay CV was 10%. Acetylcholine esterase-labeled tracers and microplates precoated with monoclonal mouse antirabbit lgG were obtained from Cayman Chemicals (USA). Standard steroids were purchased from Sigma Aldrich® (Sigma reference standards).

2.8. Statistical analysis

All statistical analysis on collected data was performed using the TIBCO Statistica™ v. 13 (TIBCO Software Inc, Palo Alto, CA, US) software. All figures were generated using RStudio v. 1.2.1335 (RStudio, Inc, Boston, MA, US) and R v.3.6.1 (R core team, Vienna, Austria), utilizing the following packages: ggplot2 (Wickham, 2016), dplyr (Wickham et al., 2020), and scales (Wickham, 2018). Statistical outliers with values greater than 1.5 times the interquartile range were excluded from the datasets using the Tukey fence method in Microsoft® Excel v. 16.41 (Microsoft, Redmond, Washington, US). The distributions of all response variables (fork length, body weight, CF, GSI, HSI, relative mRNA abundance, and plasma 11-Ketotestosterone) were checked for normality and homogeneity of variance using the Shapiro-Wilk test and the Levene test respectively.

General Linear Models (three-way random effects nested ANOVA) analysis were fitted between each of the response variables (see above) and the predictor variables "time", "temperature", and "feeding regime", with replicate tanks (random effect) as a nested factor within the predictor variables. In addition, a three-way factorial ANOVA analysis was applied between the same variables to determine any significant interactions between variables. Further downstream statistical analysis was performed within each of the feeding groups separately, using two-way and one-way models. Two-way random effects nested ANOVA with replicate tanks (random effect) nested within temperature and time (fixed effects) were used to test mean values among and within groups across the different time points. In addition, a two-way ANOVA with time and temperature as predictors was conducted to determine if there any significant interactions on the response variables within the different feeding groups. Upon confirmation of any significant differences, two one-way models were applied and fitted to the data. The first model was a one-way random effects nested ANOVA with *feeding regime* and *time* as conditions, of which replicate tanks (random effect) were nested within temperatures (fixed effect) to determine any significant differences between groups at given time points. The second model was a one-way random effects nested ANOVA with *feeding regime* and *temperatures* as conditions, of which replicate tanks (random effect) were nested within time (fixed effect) to determine any significant differences between time points within each of the temperature groups. Tukey HSD post-hoc tests based on each of the one-way models were applied to identify where significant differences between groups occurred. A two-way ANOVA with temperature as condition and feeding regime and time as predictors were performed to determine differences between feeding regimes at given temperatures. If significant differences occurred, a Tukey HSD post-hoc test was applied to identify where the differences between groups occurred.

A significance level of $\alpha=0.05$ was used for all statistical models and Tukey HSD post-hoc tests. Graphically, different small letters were used to indicate significant differences between temperature groups at given time points, while asterisks were used to indicate significant differences within temperature groups between consecutive time points ($p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)). All statistical results generated are given in Appendix II. Data in all graphical illustrations are represented by the means of each group ± the Standard Error of Means (SEM) (Appendix I).

3. Results

3.1. Biometry

3.1.1. Body weight development

The three-way random effects nested ANOVA model fitted for body weight showed significance dependence for all predictors ($p < 0.001$). Interactions between time*temperature ($p < 0.001$), time*feeding regime ($p < 0.01$), temperature*feeding regime ($p < 0.001$), and time*temperature*feeding regime ($p < 0.01$) did also influence body weight. All treatment groups exhibited a gradual increase in body weight (mean ± SEM) throughout the experiment, with individuals reared at 12.5°C and 18°C displaying higher growth rate and mean body weight than those reared at 8°C (Tukey HSD post-hoc tests, see significance in Fig. 9) (see Appendix II).

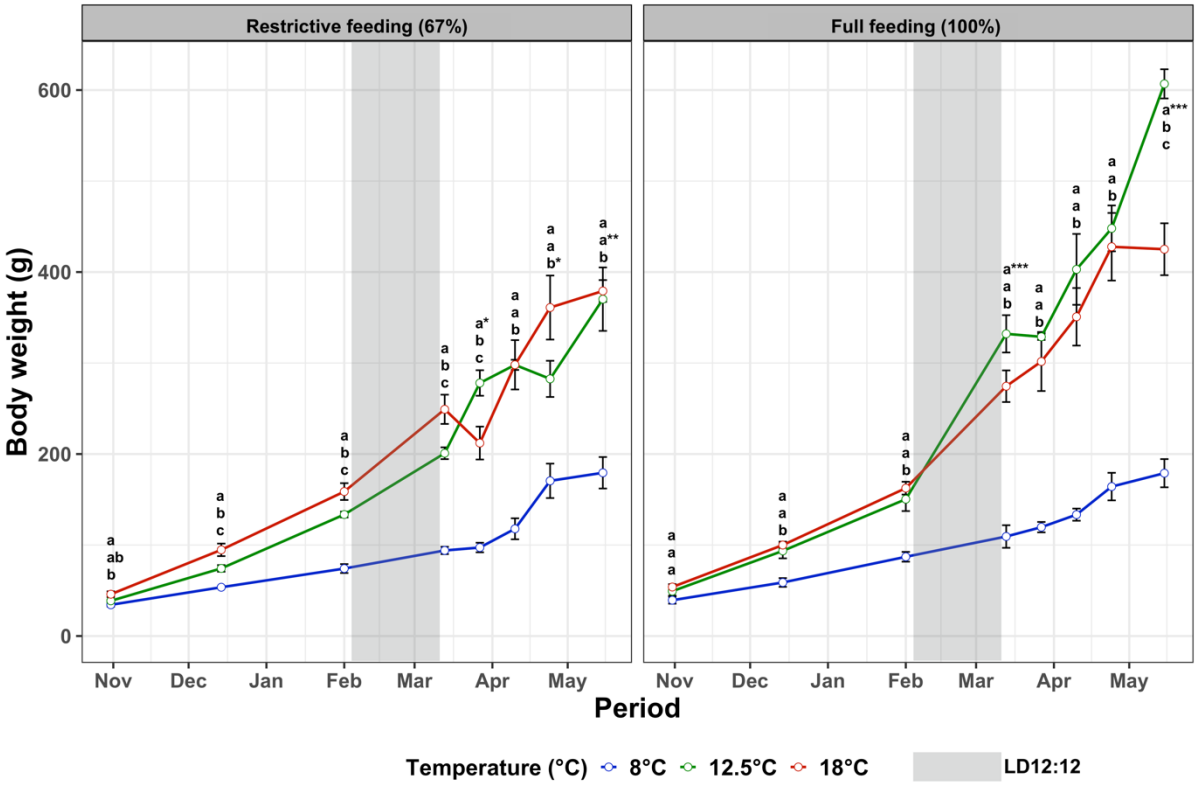

Figure 9: Body weight. *Average body weight development in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (*$p<0.05$; **$p<0.01$; ***$p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 5 – 13.*

3.1.1.1. Restrictive feeding group

The two-way nested effect ANOVA showed significant dependence of all predictors ($p < 0.001$) and their two-way interactions ($p < 0.001$). All groups exhibited an increase in body weight throughout the experiment ($p < 0.001$) (Fig 9). The average body weight in the 8°C group was considerably lower compared to the other temperature groups at every sampling point from December 14th to May 15th ($p < 0.05$). The 12.5°C group showed significant growth from 38.6±1.4 g on October 31st to 133.5±3.2 g on February 1st ($p < 0.05$), followed by a further increase to 370.2±34.8 g on May 15th ($p < 0.001$). In comparison, the 18°C group displayed a higher growth trend than the 12.5°C group from December 14th to March 13th ($p < 0.05$), experiencing considerable growth from 158.7±0.2 g on February 1st to 379.1±11.9 g on May 15th. At the final sampling, the 12.5°C and 18°C groups displayed an approximately equal mean body weight compared to the significantly lower 8°C group ($p < 0.001$).

3.1.1.2. Full fed group

As in the restrictive feeding groups, all predictors and their two-way interactions significantly affected body weight development ($p < 0.001$). All groups experienced growth during the experimental period ($p < 0.001$) (Fig. 9). Body weight development in the 8°C group was noticeably lower than the other temperature groups at all sampling points from December 14th to May 15th ($p < 0.05$). The 12.5°C group experienced a substantial increase during the winter signal from 46.2±4.7 g on October 31st to 332.1±20.5 g ($p < 0.001$) on March 13th, and further augmented to 606.8±16 g on May 15th ($p < 0.001$). In parallel, the 18°C group exhibited growth from 53.8±3.1 g on October 31st to 274.5±17.3 g on March 13th ($p < 0.001$), followed by a further increase to 425.0±28.5 g on May 15th ($p < 0.01$). At the end of the experiment, the 12.5°C group had a significantly higher average weight than the 8°C and 18°C group ($p < 0.001$), while the 18°C in turn exhibited a substantially higher body weight than the 8°C group ($p < 0.001$).

3.1.1.3. Comparison of temperature groups between feeding regimes

There were significant differences in body weight between the full and restrictive fed salmon reared at 12.5°C ($p < 0.001$) and 18°C ($p < 0.01$), of which the full-fed individuals displayed the highest overall mean body weight (Fig. 9). Over time, the 12.5°C full-fed group showed a higher mean weight than its corresponding restrictive group on March 13th ($p < 0.001$) and during the period of April 24th to May 15th ($p < 0.001$). The most prominent difference was recorded on May 15th, where the full-fed group (606.8±16 g) was substantially larger than its corresponding restrictive group (370± 34.8 g) ($p < 0.001$). The 18°C groups did not display significant differences over time, although the full-fed group had an overall higher

mean weight. No overall or over time differences were found between the 8°C full-fed group and its corresponding restrictive feeding group.

3.1.2. Condition factor (CF) development

Outputs from the three-way random effects nested ANOVA model with univariate tests of significance fitted for CF showed significance dependence for all predictors ($p < 0.01$) except for time. Results from the three-way factorial ANOVA displayed significant interactions between temperature*time ($p < 0.001$), temperature*feeding regime ($p < 0.01$), and feeding regime*time ($p < 0.01$). The 18°C groups displayed a significantly higher CF (mean ± SEM) than the lower temperature groups for most of the experiment (Tukey HSD post-hoc tests, see significance in Fig. 10) (see Appendix II).

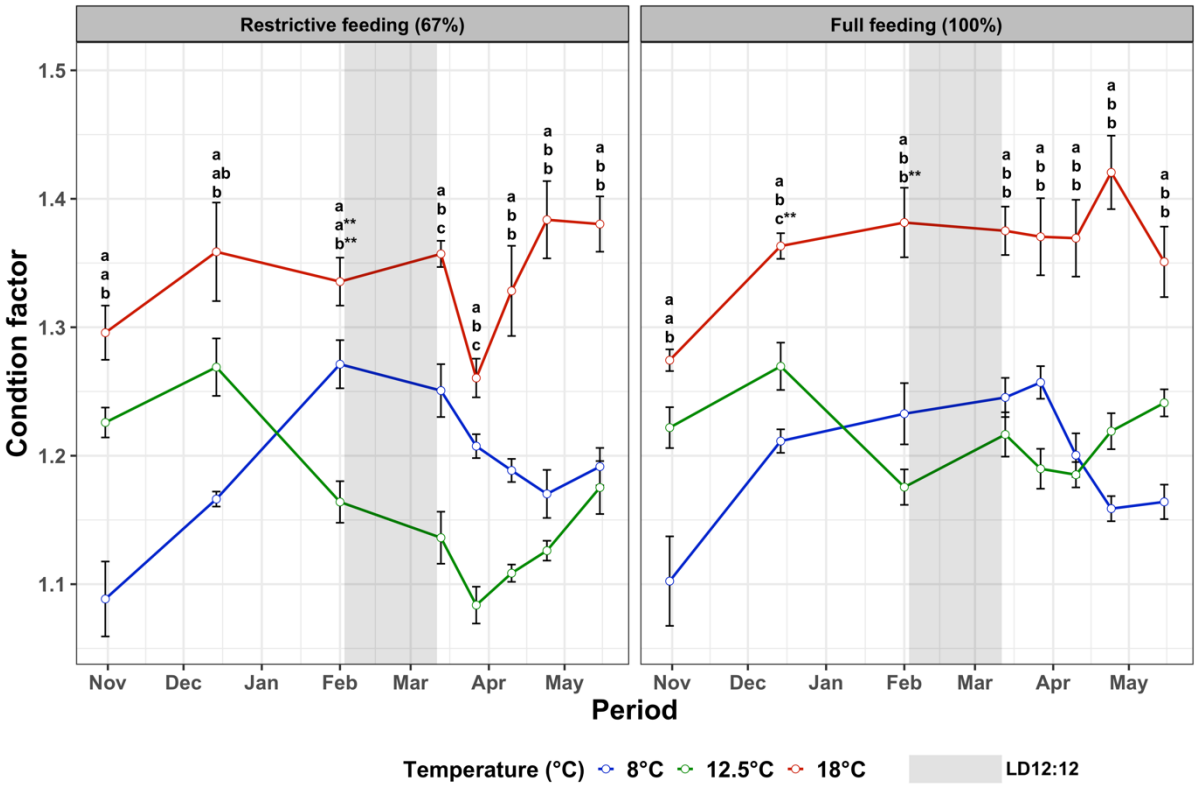

Figure 10: Condition factor (CF). *Changes in average condition factor in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 4 – 13.*

3.1.2.1. Restrictive feeding group

The two-way random effects nested ANOVA showed that the CF was significantly affected by temperature ($p < 0.001$) and differed between replicate tanks ($p < 0.001$). The two-way interactions temperature*time did affect the response value ($p < 0.001$). From October 31st to February 1st, the 8°C group experienced an increase in CF from 1.08±0.03 to 1.27±0.02 ($p < 0.001$), followed by a drop to 1.19±0.01 on May 15th ($p < 0.001$) (Fig. 10). In contrast, the 12.5°C group exhibited a decline in CF from 1.22±0.01 on October 31st to 1.08±0.01 on March 27th ($p < 0.001$), and thereupon a substantial rise to 1.17±0.02 until May 15th ($p < 0.001$). The 18°C group displayed the highest CF throughout the experiment, exhibiting a significantly higher CF than the lower temperature groups during the period March 13th to May 15th ($p < 0.05$).

3.1.2.2. Full fed group

As in the restrictive feeding group, CF was significantly affected by temperature ($p < 0.001$) and differed between replicate tanks ($p < 0.001$). From October 31st to March 27th, the 8°C group displayed a considerable rise in CF from 1.10±0.03 to 1.25±0.01 ($p < 0.001$), followed by a decline to 1.16±0.01 on May 15th ($p < 0.001$) (Fig. 10). In comparison, the 12.5°C group exhibited a significant decline from 1.26±0.01 on December 31st to 1.17±0.01 on February 1st ($p < 0.01$), although not experiencing any overall significant development throughout the experiment. As in the restrictive feeding group, the 18°C group displayed a generally higher CF trend than the lower temperature groups, exhibiting a significantly higher CF from December 14th until May 15th ($p < 0.005$). At the end of the experiment, no significant differences were observed between the 8°C and 12.5°C group, of which both groups were significantly lower in CF compared to the 18°C group ($p < 0.05$).

3.1.2.3. Comparison of temperature groups between feeding regimes

Significant differences in CF were only found between the different 12.5°C feeding groups, of which fully fed salmon displayed an overall higher mean CF than those restrictively fed ($p < 0.001$) (Fig. 10). Over time, the full-fed group exhibited a significantly higher CF from March 13th to April 24th ($p < 0.05$).

3.1.3. Hepatosomatic index (HSI (%)) development

The three-way random effects nested ANOVA showed significant dependence of time ($p < 0.001$), temperature ($p < 0.001$), and feeding regime ($p < 0.05$) as predictors on HSI. The three-way factorial ANOVA displayed significant interactions between temperature*time ($p < 0.001$) and feeding regime*time on the HSI development. All groups displayed a gradual decrease in HSI (mean ± SEM) throughout the experiment. The 12.5°C and 18°C groups had significantly lower HSI than the 8°C groups at most time points, with only a few exceptions after the winter signal induction (Tukey HSD post-hoc tests, see significance in Fig. 11) (see Appendix II).

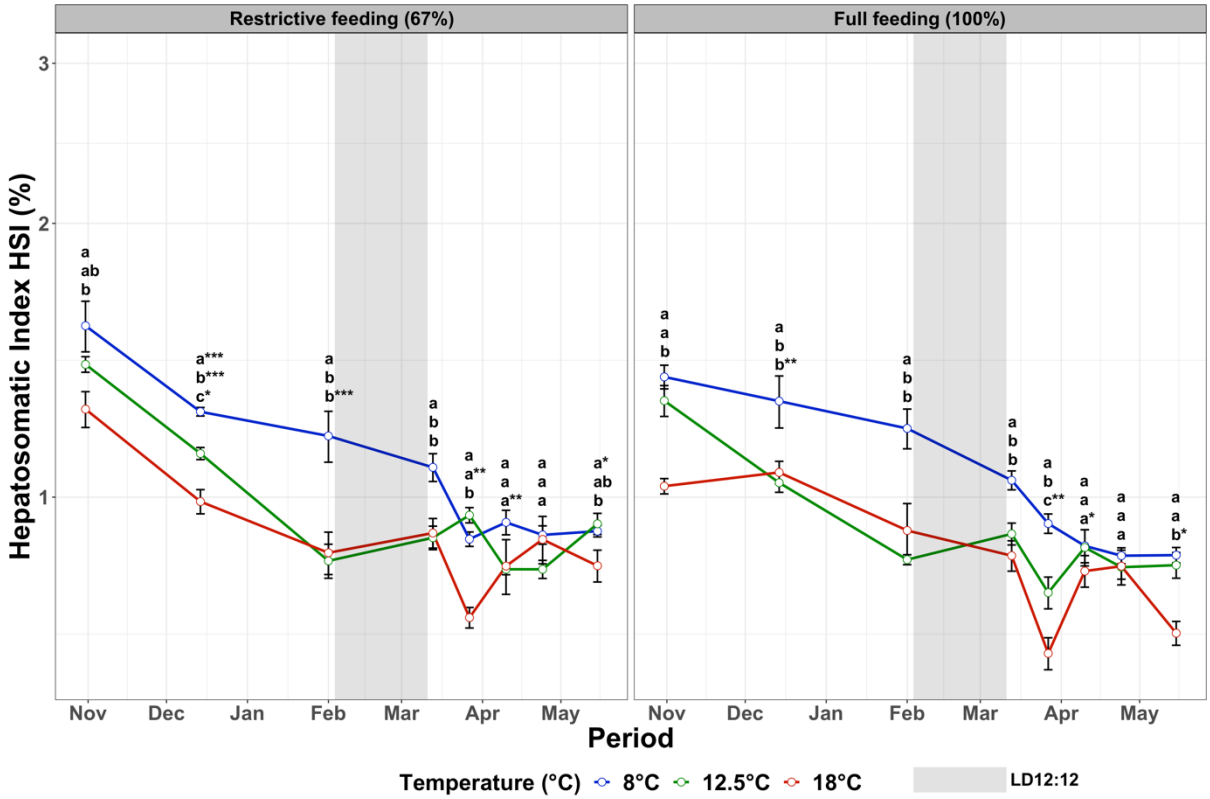

Figure 11: Hepatosomatic index (HSI (%)). *Changes in average HSI in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 4 – 12.*

3.1.3.1. Restrictive feeding group

All predictor variables and their two-way interactions significantly affected the response value ($p < 0.001$). All groups exhibited a decrease in HSI throughout the experiment ($p < 0.001$) (Fig. 11). From October 31st to February 1st, the 8°C group showed a significant decline from 1.54±0.09 to 1.16±0.07 ($p < 0.001$), followed by a further decline to 0.91±0.01 on May 15th ($p < 0.01$). The 12.5°C group exhibited a strong decline from 1.39±0.02 on October 31st to 0.85±0.03 on February 1st ($p < 0.001$), followed by stabilizing on a flat level. The 18°C group exhibited a similar trend to that of the 12.5°C group during the prewinter signal period, of which HSI dropped from 1.24±0.05 to 0.86±0.04 ($p < 0.001$), followed by no further change until May 15th. At the end of the experiment, the 18°C displayed a significantly lower HSI (0.84±0.03) than the 12.5°C group (0.93±0.02) ($p < 0.05$), whereas no differences were observed between the 12.5°C and 8°C group.

3.1.3.2. Full fed group

As in the restrictive feeding group, all predictors and their two-way interactions were significant ($p < 0.001$). All temperature groups experienced a decrease in HSI ($p < 0.001$) (Fig. 11). From October 31st to March 13th, the 8°C group exhibited a decline in HSI from 1.356±0.04 to 1.04±0.02 ($p < 0.001$), followed by a further decline to 0.86±0.01 on May 15th ($p < 0.01$). The 12.5°C displayed a decrease from 1.27±0.04 on October 31st to 0.91±0.02 on March 13th, followed by no further development until May 15th. The 18°C group showed a decline in the period October 31st to March 13th from 1.02±0.02 to 0.86±0.03 ($p < 0.05$), followed by a further decline to 0.70±0.02 on May 15th ($p < 0.01$). At the end of the experiment, the 18°C displayed a significantly lower HSI than the lower temperature groups ($p < 0.01$), whereas the 8°C and 12.5°C did not significantly differ from each other.

3.1.3.3. Comparison of temperature groups between feeding regimes

There were significant differences in HSI between the full and restrictive fed salmon reared at 12.5°C ($p < 0.01$) and 18°C ($p < 0.05$), of which the full-fed individuals displayed the lowest overall mean HSI (Fig. 11). Over time, the 12.5°C full-fed group showed a lower mean HSI than its corresponding restrictive group on March 27th ($p < 0.001$). The 18°C groups did not display significant differences over time, although the full-fed group had an overall lower mean HSI at post-winter signal. No differences were found between the 8°C full-fed group and its corresponding restrictive feeding group.

3.1.4. Gonadosomatic index (GSI (%)) development

Results from the three-way random factor nested ANOVA model with univariate tests of significance showed significant dependence of time ($p < 0.01$) and temperature ($p < 0.01$) as predictors affecting GSI. Replicate tanks as a random factor nested within the different predictors had a significant effect ($p < 0.001$). The three-way factorial ANOVA displayed interactions between temperature*time ($p < 0.001$) affecting the response variable. All groups exhibited low GSI (mean ± SEM) throughout the experiment, with the exception of the 18°C groups which exhibited a significantly higher index value than the other temperature groups, displaying a breaking point for development from March 27th (Tukey HSD post-hoc tests, see significance in Fig. 12) (see Appendix II).

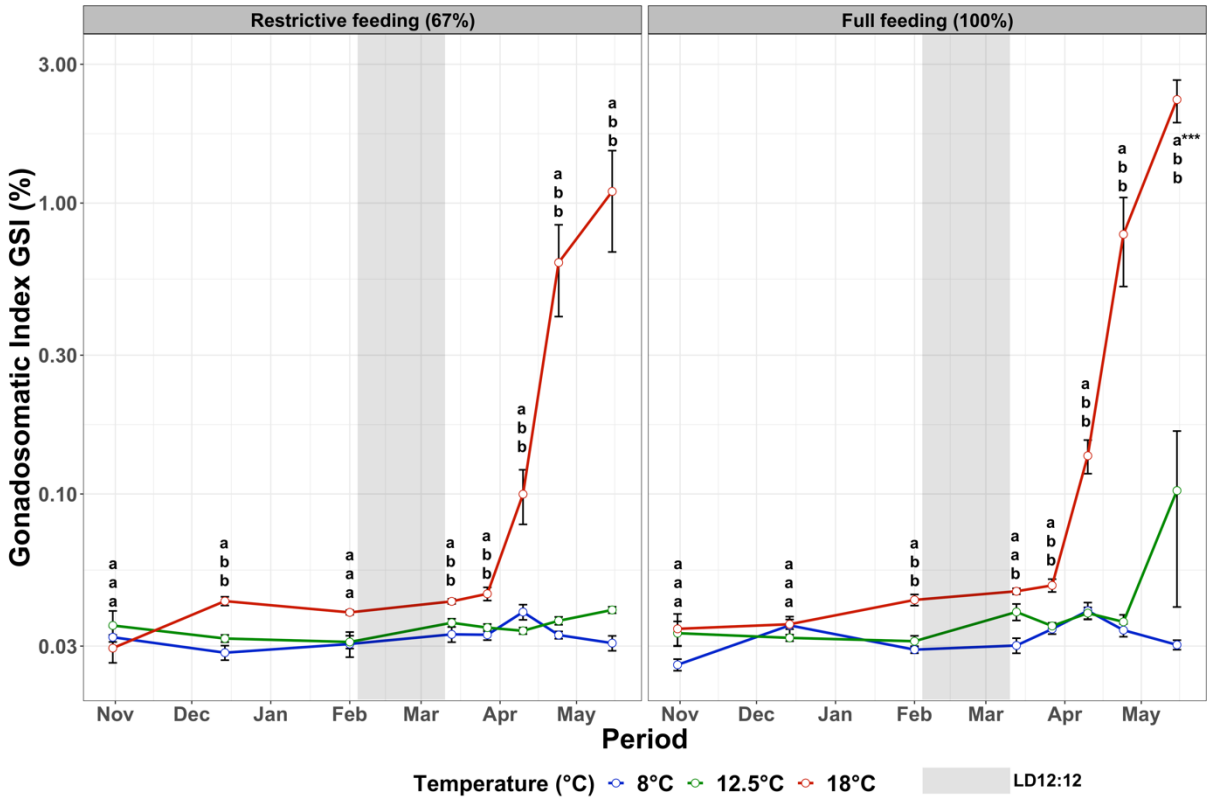

Figure 12: Gonadosomatic index (GSI (%)). *Changes in average GSI in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 5 – 13.*

3.1.4.1. Restrictive feeding group

The two-way random effects nested ANOVA showed that temperature and replicate tanks significantly affected GSI ($p < 0.05$). Interactions between time*temperature ($p < 0.001$) had a significant effect on response development. There was no noticeable change in GSI development observed in the 8°C and 12.5°C groups throughout the experiment. GSI remained within the range recorded on October 31st, with no differences between the 8/12.5°C groups (Fig. 12). The 18°C was significantly higher than the lower temperature groups on December 14th ($p < 0.01$) and from March 13th until May 15th ($p < 0.05$). At the end of the experiment, the 18°C groups displayed a GSI of 1.09±0.41, being significantly higher ($p < 0.05$) than the 8°C (0.030±0.001) and the 12.5°C (0.039±0.001) groups.

3.1.4.2. Full fed group

As in the restrictive group, both temperature and replicate tanks significantly affected GSI ($p < 0.05$), as well as the interaction between time*temperature ($p < 0.001$). There were no significant differences among the 8°C and 12.5°C group throughout the experiment, although a peak in GSI was observed in the 12.5°C group on May 15th (Fig. 12). The 18°C displayed a significantly higher GSI on February 1st ($p < 0.001$) and from March 27th until May 15th ($p < 0.05$), exhibiting an increase from 0.78±0.26 on April 24th to 2.26±0.37 on May 15th ($p < 0.001$). At the end of the experiment, the 18°C group displayed a considerably higher mean GSI compared to the 8°C (0.030±0.001) and 12.5°C (0.10±0.06) groups which did not significantly differ from each other.

3.1.4.3. Comparison of temperature groups between feeding regimes

There were no differences in overall mean GSI between feeding rations. Significant differences over time occurred only in the 18°C group, of which the full-fed group was higher than its corresponding restrictive feeding group on May 15th ($p < 0.001$) (Fig. 12).

3.1.4.4. Percentage of male maturation

None of the 8°C feeding regime groups displayed any degree of maturation development throughout the experiment and remained 100% immature (GSI ≤ 0.06). For this reason, the 8°C groups are not included in Fig. 13. There was no observed degree of maturation in the 12.5°C restrictive feeding group throughout the experiment. In the 12.5°C full-fed group, maturation occurred only at the end of the experiment on May 15th, represented by 16.6% maturing males ($0.1 < GSI \leq 1$) (Fig. 13). In contrast, a rapid increase in maturation occurred in the 18°C groups from April 10th, of which the percentage of early-stage ($0.6 < GSI \leq 0.1$) and maturing males was highest in the fully fed group. Cases of mature males (GSI > 1) first

occurred on April 27th, of which the 18°C groups had 30% mature individuals in the sampled population. The degree of maturing males was on the other hand highest in the fully fed group (50%). On the last sampling, the fully fed 18°C group showed 84.6% mature males, compared to the 33.4% observed in the restrictive group which showed an increase of early-stage and maturing males (Fig. 13). The degree of maturation observed in the fully fed groups displayed a higher degree of maturation development than the restrictive feeding groups in general.

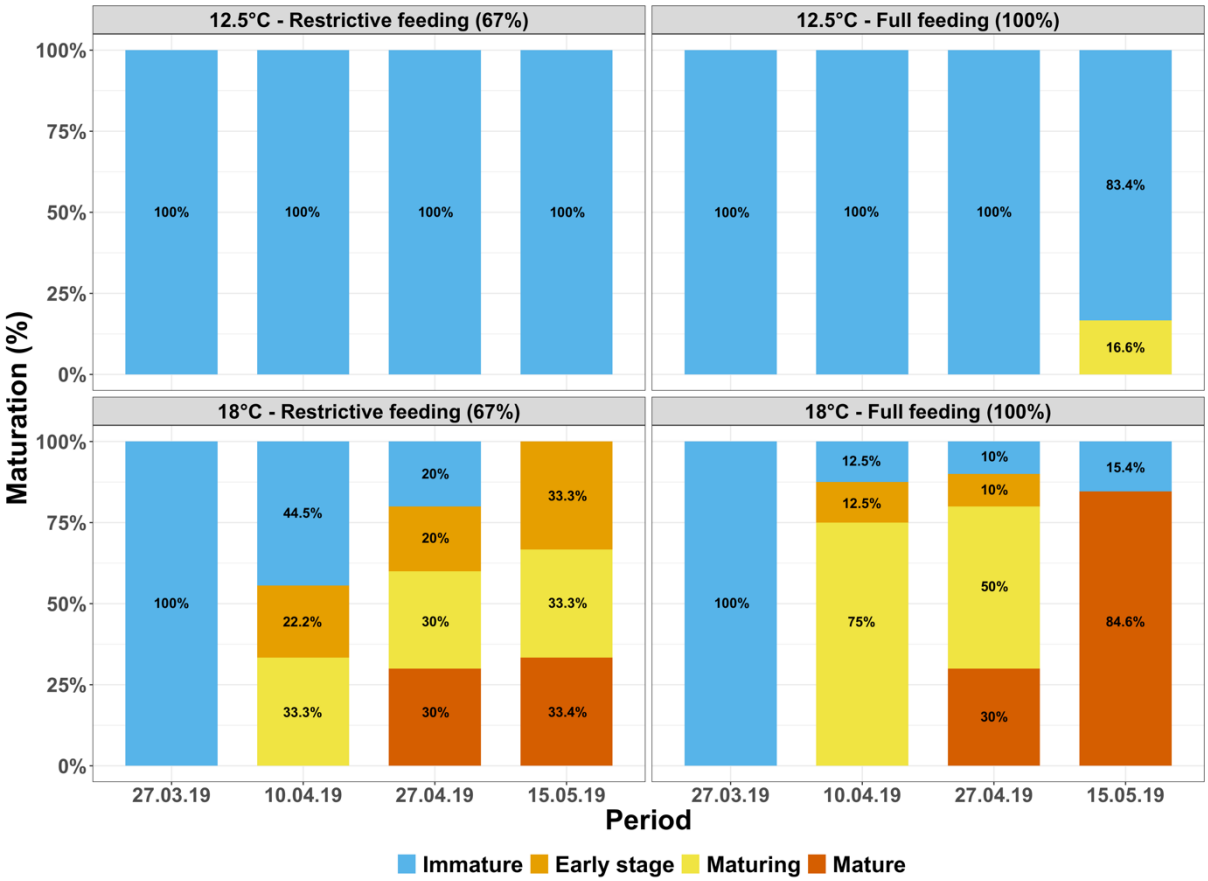

Figure 13: Percentage of sexual maturation in the 12.5°C and 18°C groups during the period March 27th to May 15th. *Four categories of maturation were established based upon GSI (%), following previous literature (Thorpe, 1994; Peterson and Harmon, 2005): "immature" (GSI ≤0.06), "Early stage" (0.06 < GSI ≤0.1), "Maturing" (0.1 < GSI ≤1), and "Mature" (GSI > 1).*

3.2. Gonad histological image analysis

The gonad histological image analysis revealed an overall absence of germ cell stage development in the 8°C groups independently of feed ration (Fig. 14). In comparison, the 12.5°C groups exhibited progress in germ cell development, especially after introducing the winter signal. Salmon reared at 12.5°C restrictive feeding displayed type B spermatogonia as the furthest developed germ cell stage at post-winter signal, showing an overall increasing trend during the winter signal before stabilizing on a higher level than before the photoperiod shift. The 12.5°C full-fed group displayed spermatozoa as the most advanced germ cell stage at the end of the experiment, exhibiting a generally higher developmental trend than its corresponding restrictive feeding group. The developmental trend of germ cells was highest in the 18°C groups, where all individuals showed a large number of fully developed spermatozoa in *tubuli* (score 6) on May 15th. Developed type B spermatogonia were revealed within both feeding rations prior to the winter signal induction on December 14th and February 1st, followed by a steady developmental increase after introducing the winter signal.

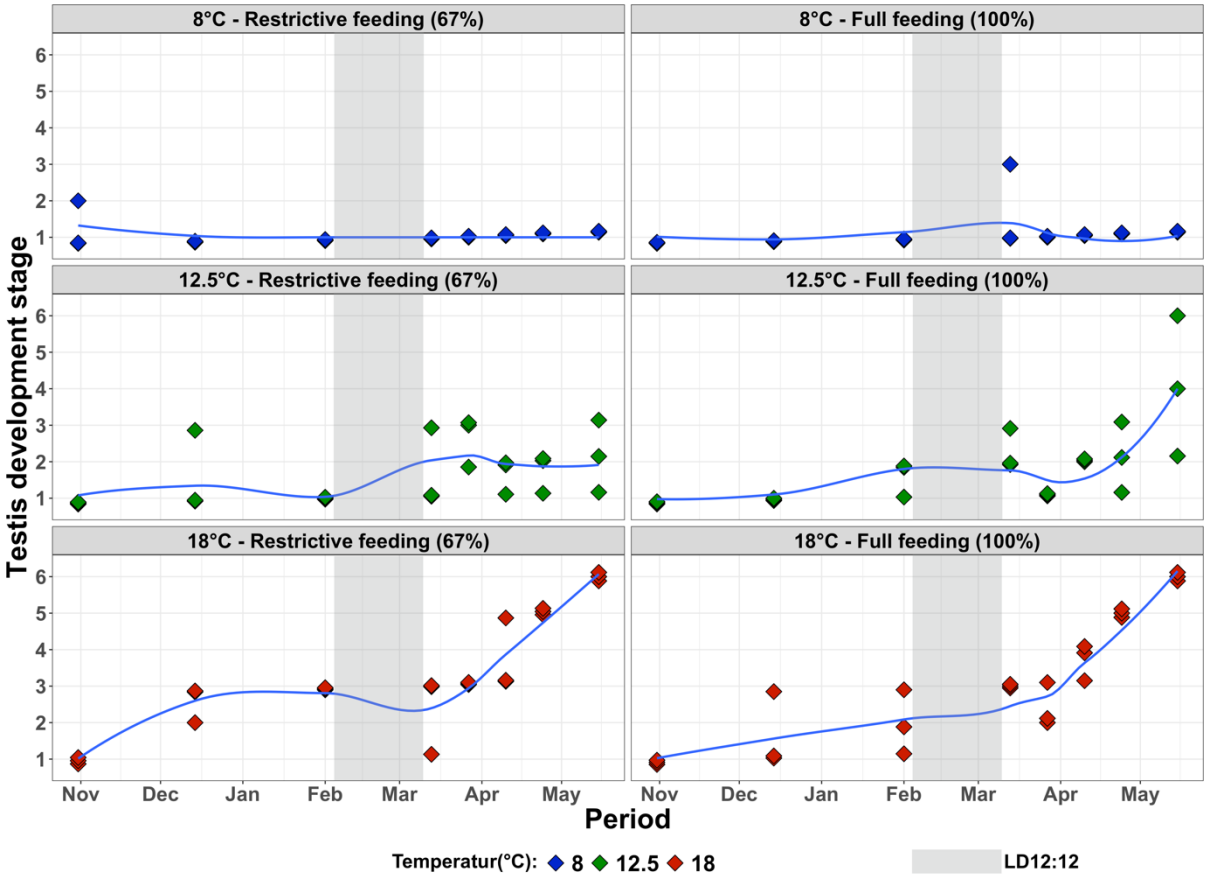

Figure 14: Germ cell stage development based upon gonad histological image analysis. *Scores based upon Fjelldal et al. (2018) describing furthest developed germ cell types observed: (1) - Type A spermatogonia without visible lumen; (2) – Type A spermatogonia with initiated lumen formation; (3) – Type B spermatogonia; (4) – Spermatocytes and/or spermatids; (5) – Spermatozoa with early germ cell generations present; (6) – Spermatozoa in large numbers in tubuli. A LOESS line is included to represents the developmental trend of observations. Each sampling point is represented by n = 3.*

3.3. Relative mRNA abundance in testis

3.3.1. Relative mRNA abundance of *fshr* in the testies

Results from the three-way random effect nested ANOVA model showed significant dependence of time ($p < 0.001$) and temperature ($p < 0.05$) as predictors on relative *fshr* mRNA abundance. Replicate tanks as a random effect nested within the predictors affected mRNA abundance significantly ($p < 0.001$). Interactions between time*temperature ($p < 0.001$) and time*feeding regime ($p < 0.05$) had an impact on relative mRNA abundance. All groups displayed a variety of relative *fshr* mRNA abundance (mean ± SEM) adjustments. However, all groups showed a transcriptional downregulation after introducing the winter signal, especially the full-fed 18°C group (Tukey HSD post-hoc tests, see significance in Fig. 15) (see Appendix II).

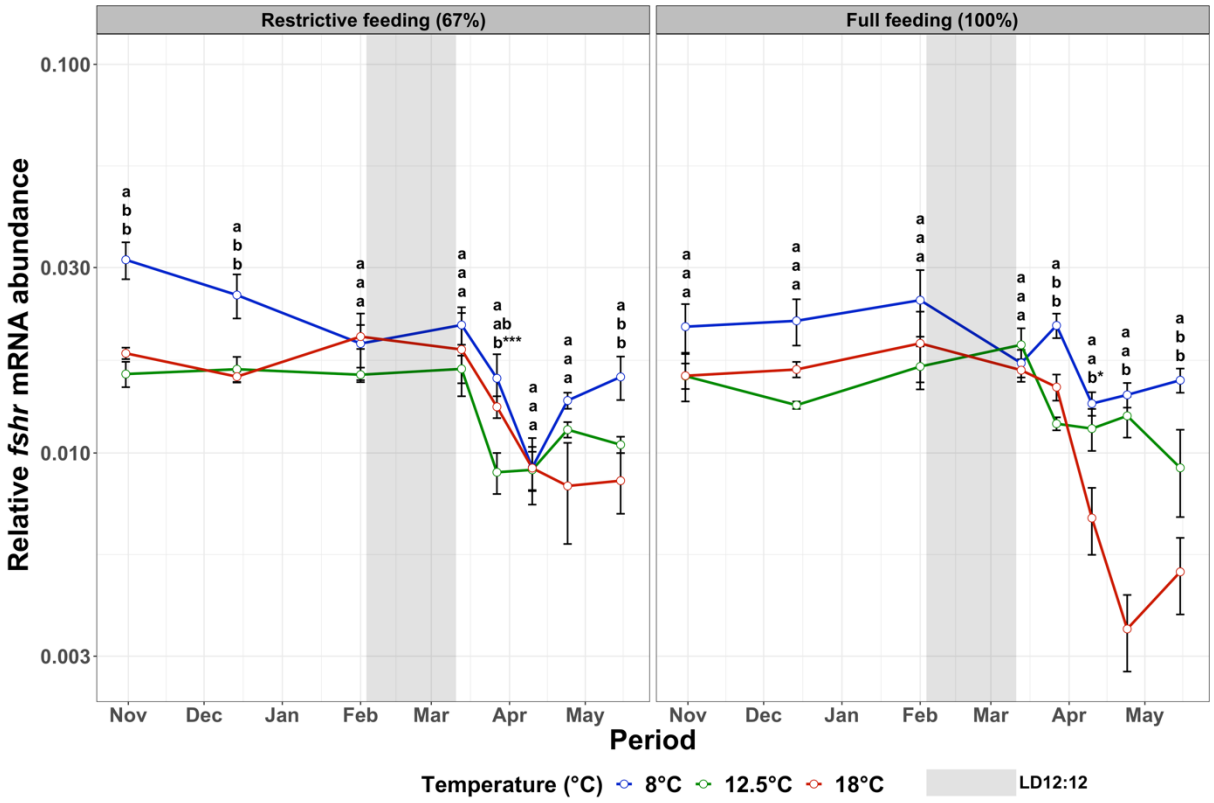

Figure 15: Relative *fshr* mRNA abundance. *Changes in average relative fshr mRNA abundance in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 3 – 12.*

3.3.1.1. Restrictive feeding group

Results from the two-way random effects nested ANOVA showed a dependence of time ($p < 0.001$) and temperature ($p < 0.001$) on relative *fshr* mRNA abundance. The two-way interactions between time*temperature also impacted relative abundance ($p < 0.05$). The 8°C group displayed an overall decrease in transcription from October until May ($p < 0.01$) (Fig. 15). The 12.5°C group exhibited the same transcriptional pattern as the 8°C group but lower abundance, experiencing a significant downregulation from 0.016±0.001 on October 31st to 0.008±0.001 on March 27th ($p < 0.001$). The group exhibited no further development until May 15th, displaying an overall decrease throughout the experiment ($p < 0.05$). The 18°C group showed an overall downregulation in transcription throughout the experiment, however not significant. At the end of the experiment, the 8°C group had a higher relative *fshr* mRNA expression (0.015±0.002) ($p < 0.05$) compared with the 12.5°C (0.0105±0.0005) and 18°C (0.008±0.001) groups.

3.3.1.2. Full fed group

As in the restrictive feeding group, predictors and their two-way interaction significantly affected relative *fshr* abundance ($p < 0.001$). The 8°C and 12.5°C groups did not display any transcriptional development throughout the experiment (Fig. 15). The 18°C group experienced a significant downregulation in transcription from 0.015±0.001 on October 31st to 0.006±0.001 on April 10th ($p < 0.01$), followed by no further development until May 15th. At the end of the experiment, the 8°C group was significantly higher in relative *fshr* mRNA abundance (0.015±0.001) ($p < 0.05$) than the 12.5°C (0.009±0.002) and 18°C (0.004±0.001) groups.

3.3.1.3. Comparison of temperature groups between feeding regimes

No significant differences were observed between temperature parallels receiving different feed rations.

3.3.2. Relative mRNA abundance of *lhr* in the testies

The three-way random effects nested ANOVA with univariate tests of significance revealed significant dependence of time ($p < 0.05$), temperature ($p < 0.01$), and feeding regime ($p < 0.05$) as predictors on relative *lhr* mRNA abundance. Interactions between time*temperature ($p < 0.001$), time*feeding regime ($p < 0.05$), and time*temperature*feeding regime ($p < 0.05$) did significantly affect the relative mRNA abundance. All groups displayed a variety of relative *lhr* abundance (mean ± SEM) adjustments throughout the experiment. In general, there was a flat relative receptor transcription with a downregulation in groups experiencing a high percentage of maturation (Tukey HSD post-hoc tests, see significance in Fig. 16) (see Appendix II).

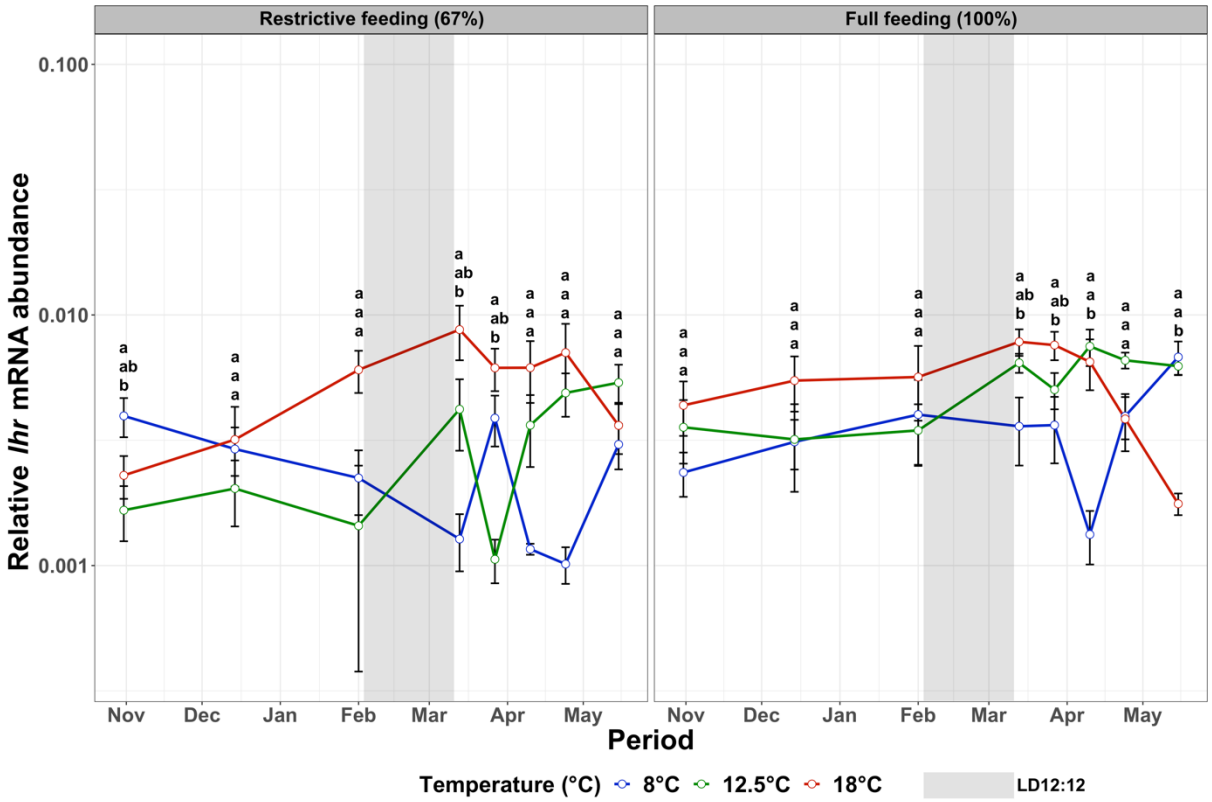

Figure 16: Relative *lhr* mRNA abundance. *Changes in average relative lhr mRNA abundance in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 2 – 11.*

3.3.2.1. Restrictive feeding group

The two-way random effects nested ANOVA displayed significant dependence of temperature ($p < 0.01$) and the two-way interactions between time*temperature ($p < 0.05$). There was no significant trend within temperature groups throughout the experiment, of which no differences were found between initial and final relative *lhr* mRNA abundance measured (Fig. 16). On October 31st, there was a significant difference between temperature groups, of which the 8°C group displayed a considerably higher transcription than the 12.5°C group ($p < 0.05$), while the 18°C group did not differ from any of the groups. On March 13th, there was a noticeable difference between the 18°C and 8°C groups, of which the 18°C group exhibited a significantly higher *lhr* transcription ($p < 0.01$). On March 27th, there was a difference between the 18°C and 12.5°C group, of which the 18°C group was significantly higher in relative mRNA abundance ($p < 0.01$). No significant difference was shown during the period from April 10th to May 15th. The 18°C group showed a higher mRNA abundance than the other temperature groups from December 14th to April 24th, while experiencing a non-significant drop after the winter signal induction.

3.3.2.2. Full fed group

Relative *lhr* mRNA abundance development was significantly dependent on temperature, replicate tanks ($p < 0.05$), and the two-way interaction between time*temperature ($p < 0.001$). No significant differences among groups were observed before the winter signal induction (Fig. 16). During the period March 13th to April 10th, there was a considerable difference between the 18°C and 8°C group, of which the 18°C group had significantly higher relative *lhr* abundance than the 8°C group ($p < 0.05$). On April 10th, no differences were shown between the 12.5°C and 18°C group, although both exhibited a significantly higher transcription of *lhr* than the 8°C group ($p < 0.05$). No differences between groups were observed on April 24th, while on May 15th, the 18°C group was significantly lower in relative transcription than the 12.5°C and 8°C group ($p < 0.001$).

3.3.2.3. Comparison of temperature groups between feeding regimes

Overall significant differences were found in the 8°C ($p < 0.05$) and 12.5°C ($p < 0.01$) groups, of which relative *lhr* mRNA abundance was highest in the full-fed groups.

3.3.3. Relative mRNA abundance of *amh* in the testis

Outputs from the three-way random effects nested ANOVA model fitted for relative *amh* mRNA abundance showed significant dependence on time ($p < 0.001$) and temperature ($p < 0.001$). Random effects of replicate tanks nested within the different predictors significantly impacted mRNA abundance ($p < 0.001$). Significant interactions between temperature*feeding regime ($p < 0.01$), temperature*time ($p < 0.001$), and temperature*feeding regime*time ($p < 0.001$) had an effect on relative mRNA abundance. Relative transcription (mean ± SEM) stayed at a stable level until introducing the winter signal. The 8°C groups had the highest relative expression throughout the experiment, independent of the feeding group. In contrast, the 18°C groups exhibited the lowest relative abundance at all sampling points, showing a downregulatory breaking point from March 27th, causing a significant drop in relative transcription (Tukey HSD post-hoc tests, see significance in Fig. 17) (see Appendix II).

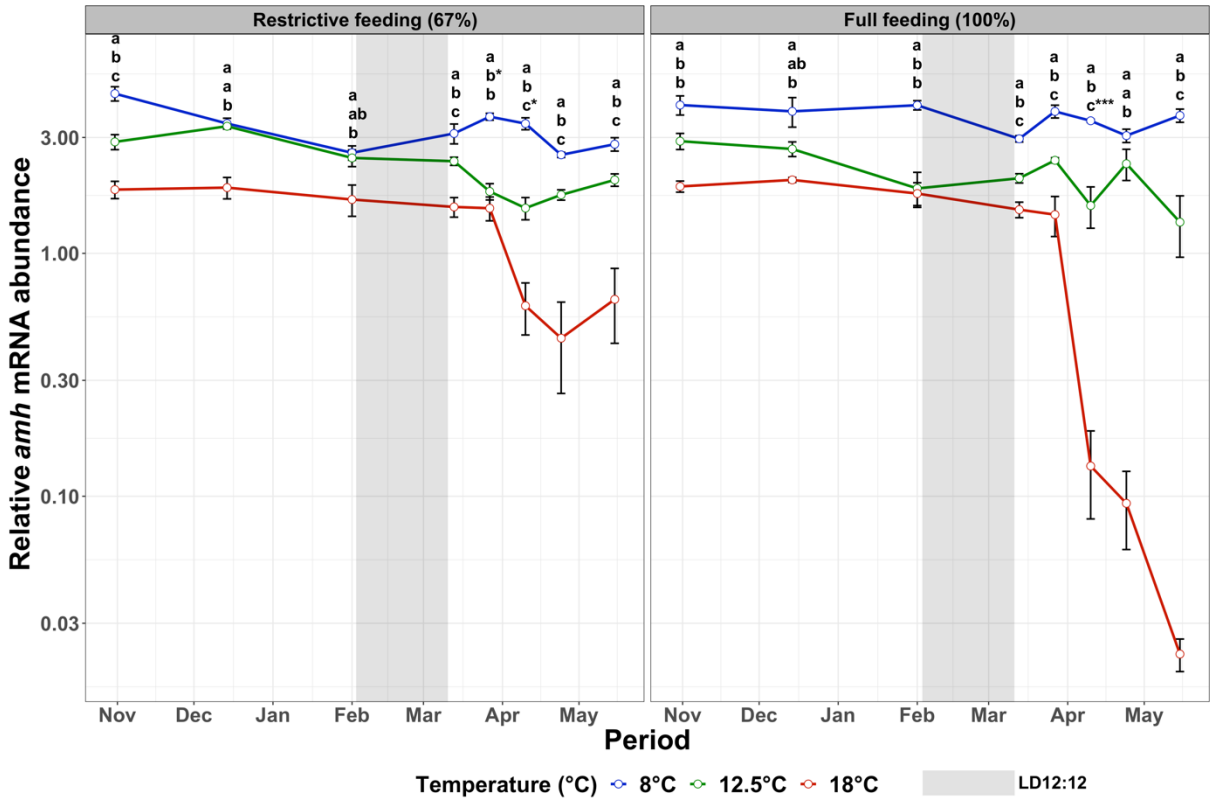

Figure 17: Relative *amh* mRNA abundance. *Changes in average relative amh mRNA abundance in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 3 – 12.*

3.3.3.1. Restrictive feeding group

The two-way random effects nested ANOVA showed that temperature, time, and replicate tanks significantly affected *amh* abundance ($p < 0.01$). Interactions between time*temperature ($p < 0.001$) had a significant effect on response development. All temperature groups displayed downregulation of relative *amh* mRNA abundance ($p < 0.01$) (Fig. 17). The relative *amh* mRNA abundance was generally highest in the 8°C group, exhibiting a significantly higher transcription than the 18°C group throughout the experiment ($p < 0.05$). The 12.5°C group experienced an intermediate decline in transcription from 2.8±0.2 on October 31st to 1.7±0.1 on March 27th ($p < 0.05$). The 18°C group displayed a stable low expression until experiencing a downregulation of relative *amh* abundance on April 10th ($p < 0.05$), remaining low in relative abundance until May 15th. At the end of the experiment, all groups differed from each other ($p < 0.05$).

3.3.3.2. Full fed group

As in the restrictive feeding group, all predictors ($p < 0.001$) and their two-way interactions ($p < 0.001$) affected mRNA abundance. The relative *amh* mRNA abundance was highest in the 8°C group which experienced no transcriptional changes while exhibiting a significantly higher abundance than the 18°C group ($p < 0.05$) throughout the experiment (Fig. 17). The 12.5°C group displayed a generally lower transcription than the 8°C group ($p < 0.05$), except for on December 14th and April 24th. The group experienced a transcriptional decline from October 31st to May 15th ($p < 0.05$), with no noteworthy expressional changes at time points in between. The 18°C group exhibited a stable low transcription until experiencing a major drop from 1.44±0.27 on March 27th to 0.022±0.003 on May 15th ($p < 0.001$). At the end of the experiment, all temperature groups significantly differed from each other, with the 18°C group displaying the absolute lowest abundance at 0.020±0.003 ($p < 0.001$).

3.3.3.3. Comparison of temperature groups between feeding regimes

There were significant differences in relative *amh* abundance between the full and restrictive fed salmon reared at 8°C ($p < 0.01$), with the full-fed individuals displaying the highest relative *amh* mRNA abundance. No significant differences were observed between 12.5°C and 18°C temperature parallels receiving different feed rations.

3.3.4. Relative mRNA abundance of *gsdf1* in the testies

Results for the three-way random effects ANOVA model showed significant dependence of time ($p < 0.001$) and temperature ($p < 0.001$) as predictors on relative *gsdf1* mRNA abundance. Random effects of replicate tanks nested within the different predictors significantly affect relative abundance ($p < 0.001$). Interactions between time*temperature ($p < 0.001$), time*feeding regime ($p < 0.001$), and time*temperature*feeding regime (three-way factorial ANOVA; $p < 0.01$) affected the relative mRNA abundance. All groups experienced a decrease in relative *gsdf1* mRNA abundance (mean ± SEM) after introducing the winter signal, especially the 18°C groups which displayed a breaking point on March 27th (Tukey HSD post-hoc tests, see significance in Fig. 18) (see Appendix II).

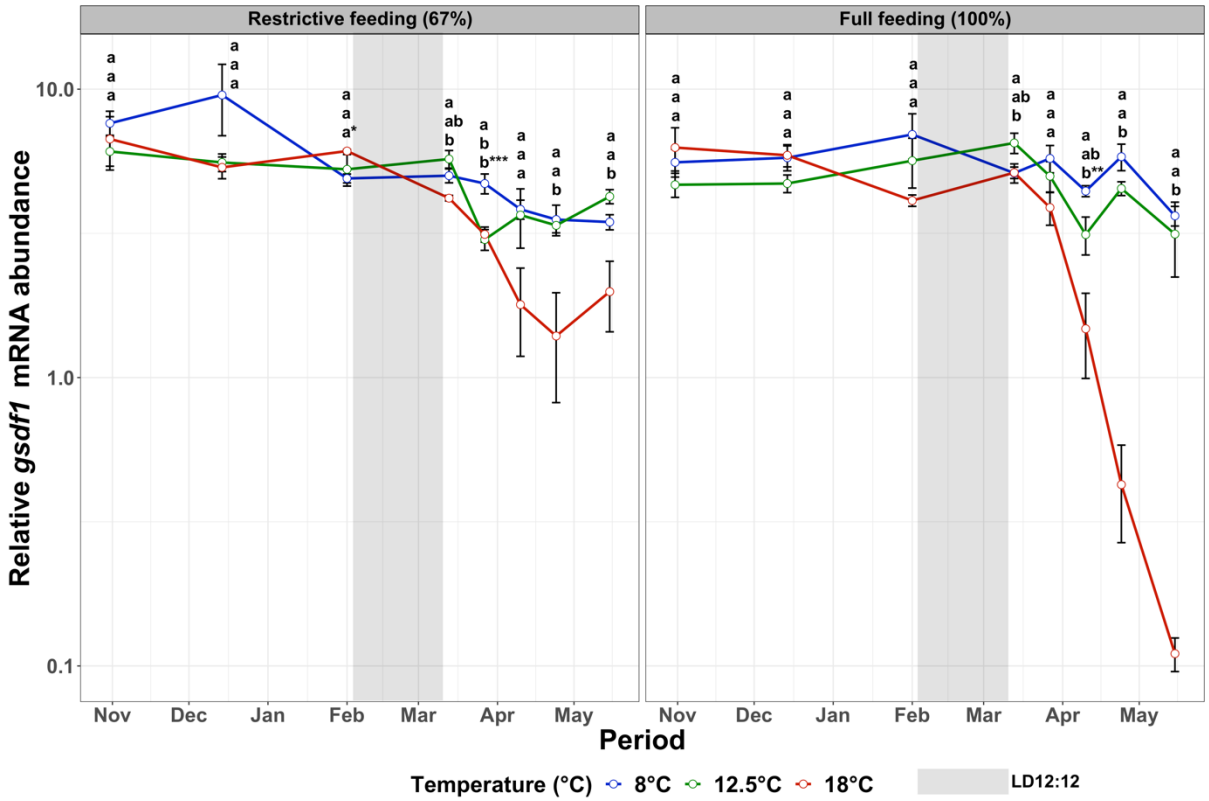

Figure 18: Relative *gsdf1* mRNA abundance. *Changes in average relative gsdf1 mRNA abundance in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 3 – 12.*

3.3.4.1. Restrictive feeding group

The two-way random effects nested ANOVA showed a dependence of time ($p < 0.001$) and temperature ($p < 0.05$) on relative *gsdf1* mRNA abundance, of which replicate tanks as a random factor nested within the predictors had an effect ($p < 0.001$). The two-way interaction between time*temperature also impacted transcription ($p < 0.05$). The 8°C group experienced a decline in relative abundance from 9.5±2.6 on December 14th to 4.9±0.1 on February 1st ($p < 0.05$), followed by further downregulation until May 15th, displaying a considerably lower transcription compared to that on October 31st ($p < 0.05$) (Fig. 18). The 12.5°C group did not exhibit any downregulation until March 27th. Relative abundance decreased from 5.7±0.4 to 3.0±0.2 ($p < 0.001$), followed by no further changes until May 15th, exhibiting no significant difference in expression from October 31st. The 18°C groups experienced a downregulation in expression from October 31st to March 27th ($p < 0.001$), followed by a further non-significant decline towards May 15th, exhibiting a significantly lower relative abundance than what was observed at prewinter signal ($p < 0.01$). At the end of the experiment, the 18°C group displayed a lower expression (1.9±0.5) than the 8°C (3.4±0.2) and 12.5°C (4.2±0.2) group ($p < 0.05$).

3.3.4.2. Full fed group

As in the restrictive feeding group, all predictor variables and their two-way interactions significantly affected the response value ($p < 0.01$). There was no significant difference between groups before the initiation of the winter signal. The 8°C and 12.5°C groups displayed a stable transcription of *gsdf1* throughout the experiment ($p > 0.05$) (Fig. 18). The 18°C group experienced a downregulation in relative mRNA abundance from 3.88±0.51 on March 27th to 0.11±0.01 on May 15th, exhibiting a considerably lower abundance than the lower temperature groups from April 24th ($p < 0.001$).

3.3.4.3. Comparison of temperature groups between feeding regimes

No significant differences were observed between temperature parallels receiving different feed rations.

3.3.5. Relative mRNA abundance of *gsdf2* in the testies

Results from the three-way random effects nested ANOVA model with univariate tests for significance showed a dependence of time ($p < 0.001$) and temperature ($p < 0.001$) as predictors on relative mRNA abundance. Replicate tanks as random effects nested within the different predictors significantly affected relative mRNA abundance ($p < 0.05$). Interactions between time*temperature ($p < 0.001$) and time*temperature*feeding regime ($p < 0.01$) affected relative abundance. All groups displayed a variety of relative *gsdf2* abundance (mean ± SEM) adjustments (Tukey HSD post-hoc tests, see significance in Fig. 19) (see Appendix II).

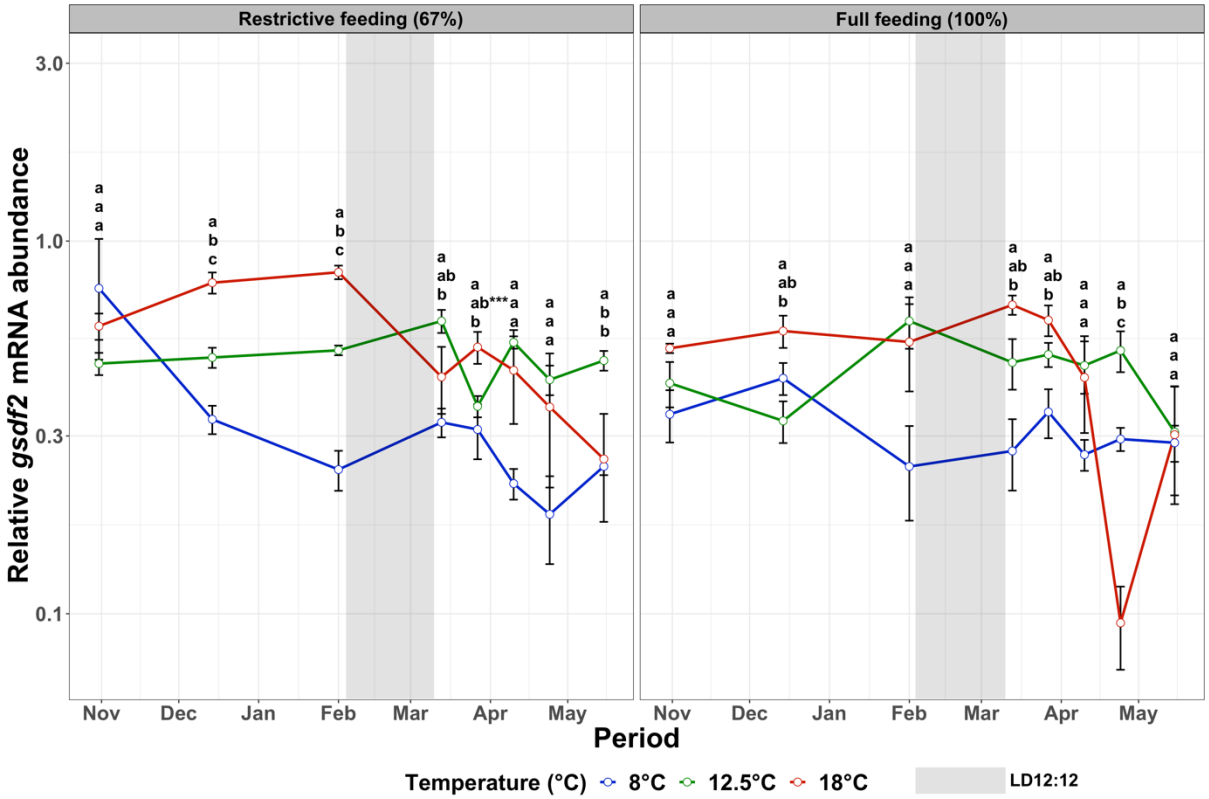

Figure 19: Relative *gsdf2* mRNA abundance. *Changes in average relative gsdf2 mRNA abundance in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 3 – 13.*

3.3.5.1. Restrictive feeding group

The two-way random effects nested ANOVA showed a dependence of time ($p < 0.05$) and temperature ($p < 0.01$) on relative *gsdf2* mRNA abundance, of which replicate tanks as a random factor nested within the predictors had an effect ($p < 0.05$). The two-way interaction between time*temperature also impacted relative expression ($p < 0.001$). The 8°C group exhibited a generally lower transcription than the higher temperature groups, experiencing a downregulation from 0.7±0.2 on October 31st to 0.20±0.03 on February 1st, followed by no further development until May 15th (Fig. 19). The 12.5°C group experienced no noteworthy development in relative *gsdf2* mRNA abundance throughout the experiment, although exhibiting a generally higher transcription than the 8°C group from December 14th. The 18°C group experienced no significant development throughout the experiment, although exhibiting the highest transcription on December 14th ($p < 0.05$) and February 1st ($p < 0.001$), followed by an insignificant downregulation until May 15th. At the end of the experiment, the 12.5°C group displayed a considerably higher relative expression (0.47±0.03) than the 8°C (0.24±0.01) and 18°C (0.26±0.08) group ($p < 0.05$), which did not differ from each other.

3.3.5.2. Full fed group

As in the restrictive feeding group, all predictors and their two-way interactions significantly affected relative *gsdf2* mRNA abundance ($p < 0.05$). There were no significant upregulatory or downregulatory developmental trends in relative *gsdf2* transcription within any of the temperature groups from October 31st to May 15th (Fig. 19). The 18°C group displayed a significantly higher transcription than the 12.5°C group on December 14th ($p < 0.05$) and the 8°C group on March 13th ($p < 0.001$) and March 27th ($p < 0.01$). All temperature groups differed from each other on April 24th, of which the 12.5°C group displayed a statistically higher transcription than the 8°C ($p < 0.01$) and 18°C ($p < 0.001$) group. On May 15th, no differences in relative *gsdf2* mRNA abundance were observed.

3.3.5.3. Comparison of temperature groups between feeding regimes

Differences between temperature groups and their corresponding feeding regime parallels only occurred in the 8°C groups. The 8°C restrictive feeding group was significantly higher than its full-fed counterpart on November 31st ($p < 0.05$).

3.3.6. Relative mRNA abundance of *igf3* in the testies

Outputs from the three-way random effects nested ANOVA model with univariate tests of significance for relative *igf3* mRNA abundance showed significant dependence of time ($p < 0.05$) and temperature ($p < 0.001$). Interactions between time*temperature ($p < 0.001$) affected the relative abundance. All groups displayed a variety of relative *igf3* mRNA abundance (mean ± SEM) adjustments, of which after the winter signal initiation, an upregulation in transcription was observed in the 12.5°C and 18°C groups in both feeding ratios, displaying a breaking point on March 13th (Tukey HSD post-hoc tests, see significance in Fig. 20) (see Appendix II).

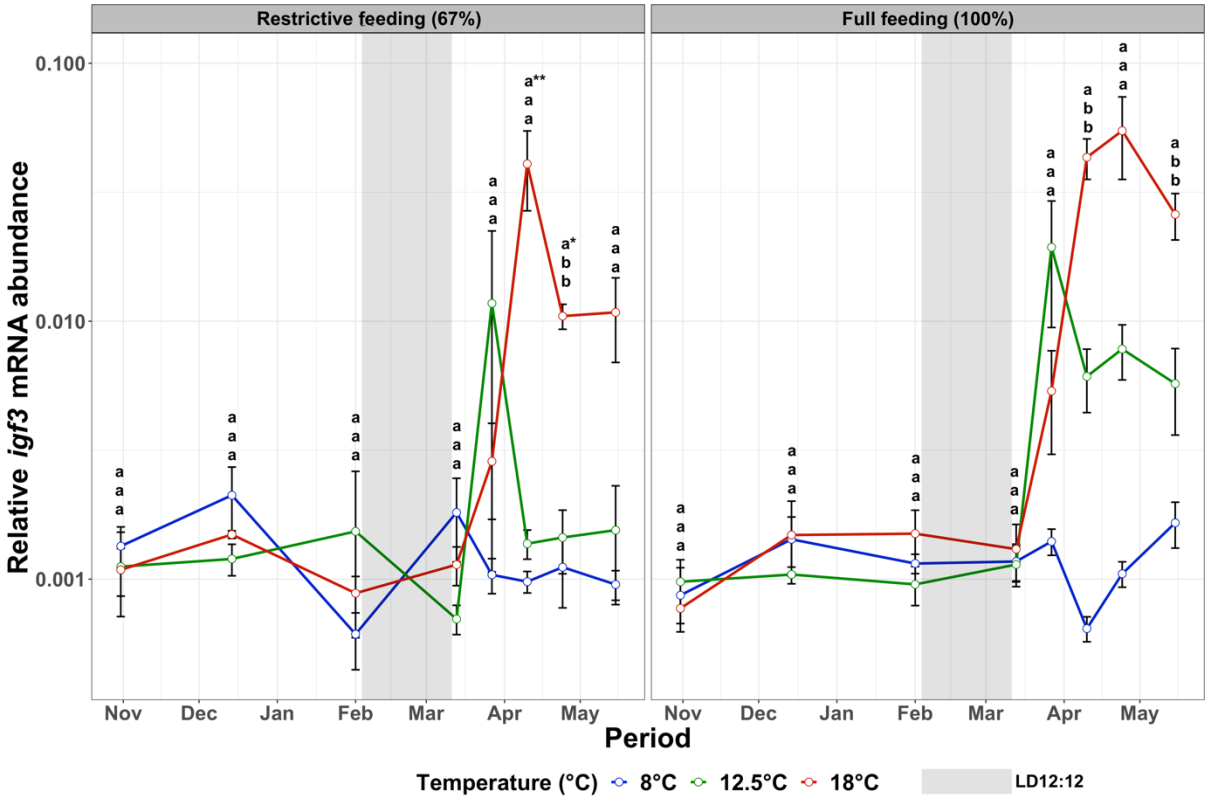

Figure 20: Relative *igf3* mRNA abundance. *Changes in average relative igf3 mRNA abundance in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 5 – 13.*

3.3.6.1. Restrictive feeding group

The two-way random effects nested ANOVA showed no significant dependence on predictor variables on mRNA abundance, though interactions between time*temperature had an effect on the response ($p < 0.01$). No significant differences between temperature groups were observed prior to the winter signal induction (Fig. 20). The 8°C group experienced no development in transcription at post-winter signal, while the 12.5°C group displayed no difference to the 8°C group, although exhibiting a higher relative *igf3* mRNA abundance trend. During the period March 27th to April 10th, the 18°C group displayed a significant increase from 0.0028±0.0011 to 0.0497±0.0139 ($p < 0.01$), followed by a significant decrease to 0.0104±0.0011 ($p < 0.05$) on April 24th. The 18°C group showed significantly higher transcription than the other temperature groups on April 24th ($p < 0.001$). No significant differences among groups were observed on May 15th, although the 18°C group showed a higher transcriptional trend than the other groups.

3.3.6.2. Full fed group

The two-way random effects nested ANOVA displayed a significant effect of temperature ($p < 0.05$) as a predictor on relative mRNA abundance. The two-way interaction between time*temperature significantly affected the response value ($p < 0.05$). No significant differences between groups were observed throughout the experiment, except for on April 10th and May 15th, of which the 18°C group showed a significantly higher relative *igf3* mRNA abundance than the other temperature groups ($p < 0.05$) (Fig. 20). Both the 12.5°C and 18°C group displayed a non-significant increase in relative expression during the post-winter signal period. The expression trend was temperature dependent, of which high temperature groups exhibited the highest transcription after initiating the winter signal.

3.3.6.3. Comparison of temperature groups between feeding regimes

There was no overall significant difference between *igf3* expression between temperature groups and their corresponding feeding regime counterparts. Over time, there was a significant difference in relative abundance between the 18°C parallels, of which the full-fed group was significantly higher than its corresponding restrictively fed group on April 24th ($p < 0.05$) (Fig. 20).

3.4. Plasma 11-Ketotestosterone (11-KT)

Results from the three-way random effect nested ANOVA model with univariate tests of significance showed a dependence of time ($p < 0.001$), temperature ($p < 0.001$), and feeding regime ($p < 0.05$) as predictors plasma 11-KT. Interactions between time*temperature ($p < 0.001$) affected the level of 11-KT. All groups exhibited low 11-KT levels (mean ± SEM) until the post-winter signal period, followed by an upregulation especially in the 18°C groups displaying significantly higher concentrations than the lower temperature groups with some exceptions, of which showing a breaking point from March 13th (Tukey HSD post-hoc tests, see significance in Fig. 21) (see Appendix II).

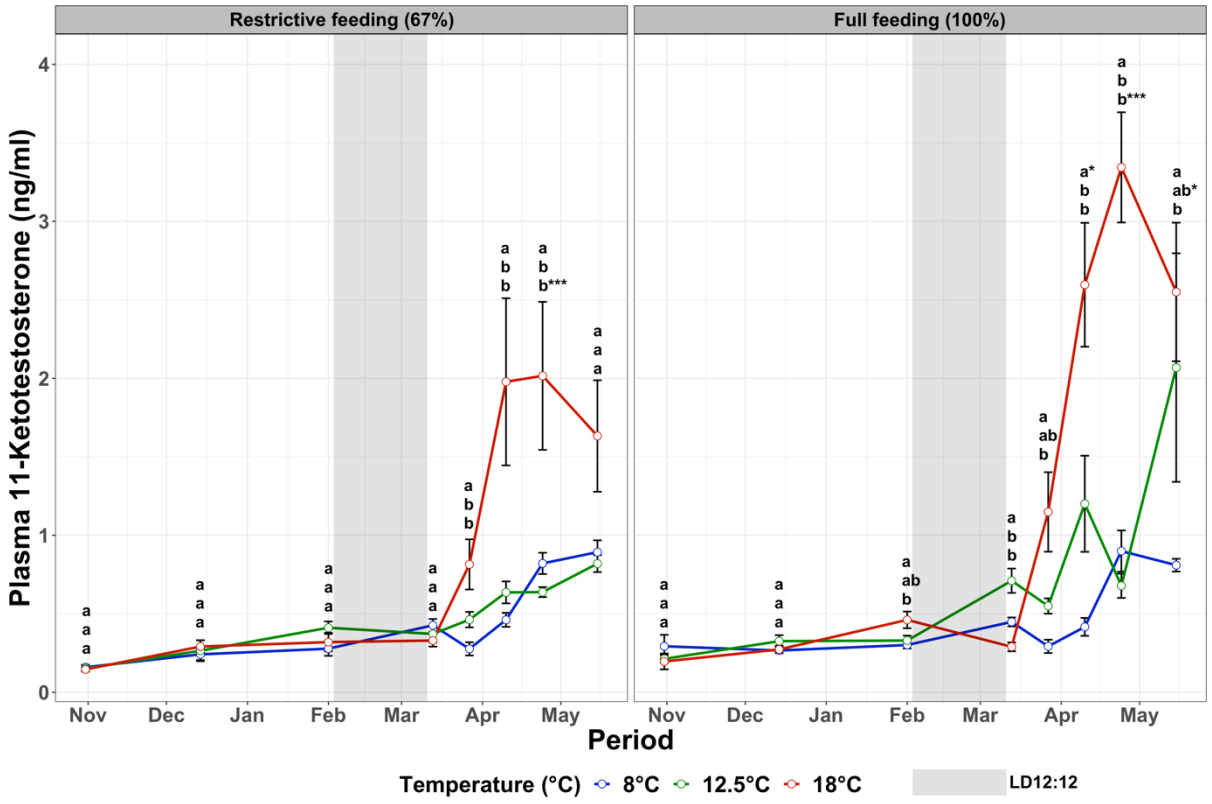

Figure 21: Plasma 11-Ketotestosterone (ng/ml) concentrations. *Changes in average plasma 11-Ketotestosterone in juvenile Atlantic salmon reared at different intensities in terms of feeding regimes (67% and 100%) and temperatures (8, 12.5, and 18°C) during the period between October 31st and May 15th. Different small letters indicate significant ($p < 0.05$) differences among treatment groups at each sampling point. Asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) indicate significant changes between consecutive sampling points. Outliers with values greater than 1.5 times the interquartile range were excluded. Each data point is represented as mean ± SEM and n = 5 – 13.*

3.4.1.1. Restrictive feeding group

The two-way random effects nested ANOVA displayed significant dependence of time and temperature ($p < 0.001$) as predictors on plasma 11-KT development. Interactions between time*temperature affected the response value ($p < 0.001$). No significant difference between temperature groups was observed at any sampling point during the prewinter signal period and until March 13th (Fig. 21). On March 27th, a significant difference between the 18°C vs. 8°C and 12.5°C group was shown, of which the 18°C group had a significantly higher 11-KT concentration than the other groups ($p < 0.05$). The 8°C group displayed a substantial increase from 0.462±0.044 ng/mg to 0.821±0.067 ng/mg ($p < 0.001$) during the period from April 10th to April 24th. During the same period, the 18°C group was significantly higher than the other temperature groups ($p < 0.05$). Plasma 11-KT levels in the 18°C group stagnated from April 10th, and on May 15th, the group showed no significant difference towards the other temperature groups.

3.4.1.2. Full fed group

The two-way random effects nested ANOVA showed dependence of time ($p < 0.001$) and temperature ($p < 0.001$) and their two-way interaction ($p < 0.001$) on plasma 11-KT levels, of which replicate tanks as a random factor nested within the predictors had an effect ($p < 0.001$). No significant differences in 11-KT levels were observed between temperatures until February 1st, of which the 18°C group was significantly higher than the 8°C group ($p < 0.05$), while the 12.5°C group had no significant differences to the other groups (Fig. 21). On March 13th, the 12.5°C group was significantly higher than the other temperature groups ($p < 0.01$). At all sampling points during the period from March 27th to April 24th, the 18°C group was significantly higher than the 8°C and 12.5°C group ($p < 0.05$), while showing a significant increase from 1.149±0.253 ng/ml on March 27th to 2.596±0.394 ng/ml ($p < 0.05$) on April 10th. The 8°C group displayed a significant increase in plasma 11-KT levels from 0.417±0.057 ng/ml on April 10th to 0.9±0.131 ng/ml ($p < 0.001$) on April 24th. Between April 24th and May 15th, a significant increase within the 12.5°C group from 0.678±0.077 ng/ml to 2.068±0.727 ng/ml ($p < 0.05$) was observed, though it resulted in no significant differences between from the other temperature groups, while the 18°C group was significantly higher than the 8°C group ($p < 0.05$) (Fig. 21).

3.4.1.3. Comparison of temperature groups between feeding regimes

There were significant differences in plasma 11-KT levels between the full and restrictive fed salmon reared at 12.5°C ($p < 0.01$) and 18°C ($p < 0.05$), of which the full-fed individuals displayed the highest overall 11-KT levels. Over time, the 12.5°C full-fed group was significantly higher than its corresponding restrictive feeding group on May 15th ($p < 0.001$) (Fig. 21).

4. Discussion

4.1. Methodological considerations

4.1.1. Experimental design and rearing conditions

Reception and random distribution of fish on September 27th were followed by one week of acclimatization. Transportation and handling function as stressors that initiate various degrees of acute stress responses in anadromous salmonids (Nikinmaa et al., 1983; Schreck et al., 1989; Barton and Iwama, 1991; Wendelaar Bonga, 1997; Barton, 2000). Acclimating before conducting the study was therefore necessary to allow the fish to physiologically stabilize, promoting fish welfare and reproducible experimental results. Following acclimatization, temperatures and then feeding ratios were gradually adjusted to the experimental levels. The restrictive feeding ratio was achieved by including fasting every three days, hence avoiding aggression and the establishment of hierarchies among fish associated with feed competition, while retaining consistent growth among individuals (Symons, 1968; Mccarthy et al., 1992; Adams et al., 2000). Earlier studies show that growth rate, food intake, and feed efficiency ratio are significantly affected by temperature, of which derivations from species optimal temperature reduce feed intake (Handeland et al., 2008). In order to adapt feeding ratios depending on the ectothermic metabolic rate of Atlantic salmon reared at different temperatures (Schmidt-Nielsen, 1997; Enders and Boisclair, 2016), feed rations were recalculated after each sampling point. As Atlantic salmon are visible feeders, an LD24:0 photoperiod regime was maintained in all treatment groups to promote growth throughout the experiment, with a five-week winter signal induction in February-March to induce developmental events (Bromage et al., 2001; Nordgarden et al., 2003; Taranger et al., 2010; Strand et al., 2018).

Atlantic salmon was reared in two different rooms containing six tanks each. Preferably, all individuals should have been reared in the same room to reduce random variability. This was on the other hand not possible, due to limited tank and room capacity. To cope for the potential variability, rooms had an identical setup in which all production parameters were kept the same, except for the feeding rations. The separation of feeding rations between rooms was decided upon to enable the possible inclusion of other studies. The current experiment took place in an FTS, as RAS facilities were unavailable during the experimental period. By not including water recirculation as a factor, system associated elements such as steroid and pheromone buildup could not be measured as a possible maturation trigger. However, intensification of rearing conditions seems to be the main factor to promote unwanted early sexual maturation in male Atlantic salmon produced in RAS (Imsland et al., 2014; Melo et al., 2014; Good and Davidson, 2016). An FTS provides an equal opportunity to intensify production parameters as a RAS and is therefore regarded as adequate given the scope of this experiment. Moreover, the present study was conducted using pre-

smolts to investigate the effect of intensive rearing conditions on maturation occurring at the post-smolt stage. The reason for using pre-smolts in the present experiment was to detect early signs of maturational development, as the commitment to mature is made several months in advance of the event, and thus before the post-smolt stage, justifying its use in the current experiment. Other environmental factors important at later aquaculture stages, as exposition to higher salinity, were not considered in the present study.

4.1.2. Total RNA isolation and quality

Testis tissue samples were fixed in RNAlater (Ambion Inc., Austin, TX, US) consecutively during dissection and stored at -80°C until downstream analysis. RNAlater stabilizes RNA *in situ* while inactivating RNases (ribonuclease) enzymes which catalyzes fragmentation of RNA, thus ensuring adequate quality for isolation and RT-qPCR (Bustin and Nolan, 2004; Pfaffl, 2004; Fleige and Pfaffl, 2006; Tröβe et al., 2010; Bennike et al., 2016). Automated and manual total RNA extraction was performed using the QIAsymphony SP system and Trizol method, respectively. Although QIAsymphony SP performs with high accuracy and prediction, extracting total RNA from lysate tissue samples with high yield and quality (Kruhøffer et al., 2010), it has limitations with small tissue inputs (Pers.com. Chief engineer Cindy Pinto Pedrosa). Therefore, the TRI-reagent method was utilized, as it ensures higher RNA yield from small tissue samples. Hence, it is considered justified to combine both RNA purification methods, given the various size range of testies tissue and low yield values from the QIAsymphony SP.

Successful quantification of mRNA in RT-qPCR relies on high-quality total RNA in terms of quantity, purity, and integrity (Nolan et al., 2006). RNA quantity and purity were evaluated using the Qubit 3.0 Fluorometer and NanoDrop One microvolume UV-Vis spectrophotometer. The Qubit provides an accurate quantification as all levels of RNA can be precisely detected without the interference of contaminants. The quantification accuracy is due to the target-specific dye following the Qubit BR RNA assay kit which emits fluorescence when bound to RNA (Garcia-Elias et al., 2017). The NanoDrop provides a measurement of potential contamination in the sample by differentiating between wavelengths of UV absorptions. According to Desjardins and Conklin (2010), a ratio of absorbance between ~ 2 and < 2 for 260/280 nm ($A_{260/280}$) and 260/230 nm ($A_{260/230}$), indicated sufficient purity of contaminants. The current study did unfortunately not evaluate the integrity of extracted total RNA. As mentioned above, in the presence of RNase, the RNA will be fragmented and degraded, thus potentially compromising results from downstream analysis (Schroeder et al., 2006). For this reason, the integrity of RNA may vary and should be monitored by accessing the RNA integrity number (RIN) before further applications. RIN is based upon the ratio of 28S:18S ribosomal RNA (rRNA), of which the number generated range from 1

(low integrity – degraded) to 10 (high integrity - intact) (Schroeder et al., 2006). To cope for the unknown integrity of RNA, the present study designed amplicons of small size as close to the 3' end as possible, thereby ensuring that RNA still could be used in case of degradational events.

4.1.3. Real-Time quantitative PCR (RT-qPCR)

In the present study, RT-qPCR on RNA-based cDNA was conducted using the intercalating SYBR green agent to determine the relative transcription of gonadotropin and gonadotropin-responsive genes. Target DNA sequences are copied during the RT-qPCR, of which the fluorescent intercalating agent emits fluorescence signals when bound to the minor groove of amplified dsDNA (Pfaffl, 2004). The quantity of fluorescence signals emitted is therefore proportional to the amount of dsDNA formed. Intercalating agents used in optical quantification is a flexible and specific method (given specific primers), requiring low contamination to ensure reliable RT-qPCR results (Bustin, 2000). It could be argued that using a more specific optical method, such as the TaqMan assay, would have been more suitable for this study (Tajadini et al., 2014). TaqMan has the advantage of multiplexing, allowing to test several genes simultaneously by using different fluorochromes, although requiring an accurate set up to be used. However, the TaqMan assay would have been an expensive and advanced method to implement compared to the cost beneficial easy-to-use SYBR green. A comparative study by Tajadini et al., (2014) concluded that the performance and quantity of the SYBR green method could be comparable to the TaqMan method if optimized. Hence, the use of the intercalating agent is considered justified given its cost beneficial easy-to-use properties combined with its potential performance and quality.

Quantification of mRNA to access the transcriptional activation status of genes is a common practice when investigating responses over time during developmental events in biological research (Pfaffl, 2004,2012; Nolan et al., 2006). In most biological studies, there is no need for measuring the absolute amount of mRNA, as relative quantity often provides a sufficient method for assessing gene regulation (Pfaffl, 2001; Čikoš et al., 2007). Relative quantification measures the change in mRNA expression by analyzing transcriptional changes of a gene across several samples, expressing the target gene relative to a reference gene (Pfaffl, 2001; Čikoš et al., 2007). The relevance for mRNA quantification is its downstream application in cells, providing the premises for a physiological response. According to the central dogma of molecular biology, genes are transcribed into mRNA in the cell nucleus and translated in cytoplasmic ribosomes into proteins, which provides the physiological response of set genes (Morange, 2009). RT-qPCR provides adequate fluorescence data for calculating relative mRNA quantity. However, RT-qPCR does not account for any post-transcriptional regulations, which has been suggested as the reason for the only partial correlation between mRNA levels and expressed proteins (Greenbaum et al., 2003; Laloo et

al., 2009; Becker et al., 2018). Thus, quantified mRNA may not fully represent the amount of protein expression. Despite this, RT-qPCR with calculations of relative mRNA transcription still provides an adequate method for investigating the general physiological change by accessing the transcriptional regulation of gene expression (Pfaffl, 2001). Therefore, it is considered sufficient in the present study.

The coefficient of variance (CV) for relative mRNA transcription was calculated for the theoretical replicates to assess the degree of data inconsistency and precision (Taylor et al., 2019). The CV threshold used for the first round of RT-qPCR was set at 1.5% for all genes. The average of technical replicates with low variation (CV < 1.5%) was used for calculating relative quantity. When measurements were above the CV threshold, or if one of the theoretical replicates did not generate a value or provided imprecise measures, the sample had to be repeated. When samples exceeded the threshold in the reference gene, all analysis of the specific sample was repeated in the second round of RT-qPCR. In the second round of RT-qPCR measurements, technical triplicates were used instead of duplicates. The use of triplicates increases the confidence in the observed Ct values. Furthermore, as variation increases when measured values are low, the CV threshold was increased to 2.5% for specific genes (*fshr, lhr, igf3*) to avoid filtering out samples unnecessarily. Samples exceeding the CV threshold in the second round of RT-qPCR were filtered out from further analysis. Moreover, samples with gene expression lower than what could be measured were substituted with an arbitrary value lower than the less expressed samples with precise measures. Previous literature has set a threshold for discarding Ct values above 35, however this is not specific as the threshold varies from gene to gene. Thus, the substitutions were set at Ct 37, 39, and 38.5 for *fshr*, *lhr,* and *igf3* respectively. The reason for substitution with arbitrary values was to be able to analyze the data as these genes were low expressed in gonads at some sampling points and groups. Ideally, repeated cDNA synthesis and RT-qPCR analysis should have been done on these samples, including more theoretical replicates. However, due to time limitations, this was not possible, as well as it would not solve the issue if these genes were in fact low expressed.

The majority of primers used were designed for the present study, while others were chosen from previous literature based upon the conjunction of probes such as in the TaqMan assays, which is not used in the current experiment. Melting profiles of the PCR product were assessed at the end of amplification to discard signal measured from putative primer dimers and hairpins, which can be an issue especially in samples with a high Ct. Moreover, PCR product melting analysis was used to confirm the non-amplification of artifacts such as short amplicon primer dimers, which could occur due to homology between primer sequences (Poritz and Ririe, 2014). This can be a product of leaving the reaction mixture for too long or due to primers not being specific enough.

4.1.4. Statistical analysis

Data generated in the present study had properties of which General Linear Model (GLM) Analysis of Variance (ANOVA) tests were considered suitable fitted for the statistical analysis. Furthermore, the dataset had properties of a random effects nested ANOVA, containing a random categorical factor (replicate tanks) nested within treatments. The ANOVA models require fulfillment of three main assumptions: (1) independence of variables, (2) the dependent variable should be continuous and approximately normal distributed, and (3) homoscedasticity (Ståhle and Wold, 1989). The current design consisted of a dependent continuous variable which could be measured on a scale that can be subdivided using increments. Moreover, it consisted of independent categorical predictor variables (time, temperature, and feeding regime) and several dependent response variables (body weight, condition factor, HSI, GSI, gonadotropin receptor- and gonadotropin-responsive gene transcription). The Shapiro-Wilk test was utilized to assess the normality of data. The test is more powerful in handling different sampling sizes than the Anderson-Darling test, Lilliefors test, and Kolmogorov-Smirnov test, justifying its use in this current experiment (Mohd Razali and Bee Wah, 2011). The Levene F-test was utilized to examine whether there was homogeneity of variance. The Levene F-test is less sensitive to departures from normality than other methods such as the Bartless test. The basis of the Levene test, F-statistics, is quite robust against violations of the homogeneity of variance assumption (Box and Andersen, 1955; Lindman, 1974). In biology studies where variation is expected to be high, it has been suggested that the assumption of homogeneity of variance should be accepted when $F \leq 20$ (Høisæter, 1989), this suggestion was followed in the present study.

Transformation of data is a common practice in biostatistics, used to decrease variability and confirm the normality assumption (Gurka et al., 2006; Wang et al., 2014). Data transformation comes at the cost of reduced interpretability (Wang et al., 2014; Schielzeth et al., 2020). Schielzeth et al., (2020) suggest that data transformation in mixed effect models might not be necessary, as violations of the normality assumption often pose little impact on the results. Therefore, it was decided upon not transforming the data as slight non-normality pose little impact on general linear models (per.com. Professor Albert K. D. Imsland).

Atlantic salmon (n = 6 - 13) were sampled from each treatment group at eight individual time points to assess sexual maturation development. The F-statistics in ANOVA is a robust test for mean differences as long as the number of samples for each group is greater than 10. Low statistical power is associated with using small sampling sizes and may result in an increased probability of committing Type I (false-positive H_0) and/or Type II (false-negative H_0) statistical errors (Cohen, 1992; Nayak, 2010; Faber and Fonseca,

2014). The combination of outlier treatments and cases of invalid RT-qPCR values resulted in a varying number of observations per sampling point. This is suspected of having possibly caused a Type I error in the *lhr* transcription results, as the significance of feeding ration may have been a result of the highly variable number of samples in the 8°C and 12.5°C restrictive groups (n = 2 – 11). The present study should preferably have had a larger sampling size per treatment group to cope for low statistical power, as conclusions based on a small selection do not necessarily reflect the whole population. However, this was not possible due to time and tank capacity limitations.

Potential random tank effects were accounted for by including replicate tanks for each of the treatment groups. Applying replicate tanks in biological studies that address aspects of growth, development, and behavior has shown to be necessary when controlling for random effects associated with variety within and between tanks (Ruohonen, 1998; Ling and Cotter, 2003; Thorarensen et al., 2015; Johnsson and Näslund, 2018). By not taking into consideration the potential random tank effects, it may cause committing Type I and/or Type II statistical error (Banerjee et al., 2009). Hence, the use of replicate tanks to reduce variance and improve the significance of the results is considered justified in this study. In nested designs, the potential effects of replicates are of less importance if the effects of treatments are still significant. The number of samples varied to some degree between replicate tanks and time points, resulting in an unbalanced design (Appendix I). Furthermore, due to outlier treatment and cases of invalid RT-qPCR values combined with the low number of sampled individuals from replicate tanks, the 12.5°C restrictive feeding group had n = 0 individuals from its replicate tank on February 1st. Therefore, random effects could not be accounted for in this group on February 1st. Although a balanced design makes statistical analysis easier to work with in terms of having large statistical power and being less susceptible to heterogeneity of variance, it is rarely the case in biological studies.

4.2. Discussion of results

4.2.1. Percentage of maturation

4.2.1.1. Effect of intensive rearing conditions on gonadosomatic index development

The GSI was on a stable low level in all treatment groups before the winter signal induction, followed by a temperature dependent development from mid-March. The 18°C groups experienced a substantial increase in GSI from mid-March to mid-May, exhibiting the highest proportion of early sexual maturation of all treatment groups (restrictive feeding ratio - 33.4%; full feeding ratio - 84.6%). The large-scale maturation suggests that individuals had reached the physiological state passing the genetically determined thresholds for committing to maturation earlier than the lower temperature groups (Rowe and Thorpe, 1990). Thus, it would be expected that some of the acquired energy were reallocated to gonad development, something which is supported by the reduction of HSI in parallel with the subsequential increase of GSI after introducing the winter signal. In contrast, the 12.5°C full-fed group experienced a slight increase in GSI from late-April to mid-May, reflecting the moderate percentage of maturing males at the end of the experiment (16.6%). The expression of somatic growth in terms of weight gain was significantly higher in the 12.5°C full-fed group compared to the 18°C groups at the end of the experiment. This proposes that individuals used acquired energy for enhancing somatic growth and maintenance rather than for gonad development, which could explain the substantial growth observed from early-February, further supported by the low GSI. Type B spermatogonia were identified in the 12.5°C groups from mid-March. This proposes that some individuals had committed to early maturation, something which is reflected by the percentage of maturing individuals observed at the end of the experiment. This could further indicate that the biological threshold for initiating the maturational process was reached in some individuals, which could be seen in context with the small peaks in GSI after the winter signal induction. As type B spermatogonia were the furthest developed germ cell stage observed until mid-May, it could indicate that underlying factors prevented or delayed the pace of germ cell development, assuming that maturation takes priority over other developmental events (Thorpe, 1994). The proposed delay in germ cell development is supported by the GSI profile, which only displayed a slight and variable peak from late-April to mid-May. This is in line with the moderate percentage of maturing individuals observed in the 12.5°C full-fed group at the end of the experiment. In comparison, GSI development seemed to be absent in less intensive rearing groups (12.5°C-67%, 8°C-100%, 8°C-67%). Therefore, the present study proposes a link between intensive rearing conditions and GSI development, of which temperature seems to control the magnitude and pace of puberty.

4.2.1.2. Photoperiodic regulation of maturation and GSI development

The proposed temperature dependent rise in GSI occurred shortly after the winter signal induction. This can be seen in line with Imsland et al. (2014), which found that temperature controls the magnitude of the photoperiod effect on maturation (Imsland et al., 2014). Photoperiodic cues serve as a *zeitgeber* in Atlantic salmon, influencing the circannual endogenous rhythms which provoke, continues, or postpone developmental events depending on the physiological thresholds such as energy storage (Duston and Bromage, 1988; Hutchings and Jones, 1998; Bromage et al., 2001; Berrill et al., 2003; Taranger et al., 2010). As photoperiodic cues regulate the seasonality of the Atlantic salmon life cycle, it was expected that a winter signal induction would either initiate developmental events or inhibit them (Berrill et al., 2003; King et al., 2003; Skilbrei and Heino, 2011). The present study supports the synchronizing effect of photoperiodic cues on timing and routing of the life cycle, of which early maturational development either was enhanced or inhibited, seemingly depending on temperature. This proposes that temperature in combination with photoperiodic cues may be the main drivers of early sexual maturation in male Atlantic salmon, given that temperature seems to modulate the degree of maturation, while photoperiod seems to provide an environmental cue for development (Fjelldal et al., 2011; Imsland et al., 2014; Good and Davidson, 2016).

Surprisingly, the 18°C groups displayed a significantly higher GSI with corresponding type B spermatogonia development than the other treatment groups at specific time points prior to the photoperiod shift (restrictive feeding group - mid-December; full feeding group - early-February). This could indicate a possible photoperiod independent commitment to early maturation. Therefore, it could be assumed that the 18°C groups had reached the genetically determined biological thresholds prior to the winter signal, which may be seen in the context of the early significant peaks in GSI and the observations of type B spermatogonia. Fjelldal et al. (2018) suggested that shifts in photoperiod are not strictly necessary for committing to maturation as long as physiological thresholds are reached. Present findings of type B spermatogonia as the furthest developed germ cell stage prior to the winter signal supports Fjelldal et al. (2018), while also confirming the activation of the BPG-axis due to the spermatogenetic advancement. Interestingly, as type B spermatogonia were the most advanced germ cell stage observed prior to the winter signal induction, it might propose a photoperiod induced mechanism for continuing or inhibiting the further differentiation into primary spermatocytes. However, the current study did not provide a control group for photoperiod and could therefore not confirm or refute such a photoperiodic mechanism on entering the meiotic phase of spermatogenesis. Hence, we propose the worth of further research on the photoperiodic effect on spermatogenetic advances in Atlantic salmon during early sexual maturation.

Overall, intensive rearing conditions (18°C-100%, 18°C-67%, 12.5°C-100%) cause a high proportion of early sexual maturation, compared to the less intensive rearing groups (12.5°C-67%, 8°C-100%, 8°C-67%) which displayed an absence of development. The photoperiod shift seemed to provide an environmental cue for initiating, continuing, or inhibiting the maturational process, of which intensive rearing groups experienced a rise in GSI after the winter signal induction. The degree and rate of maturation seemed to be regulated by temperature, which would be seen in context with the ectothermic poikilothermic nature of salmonids. This finding is supported by previous studies that found temperatures to a high degree affect gonad growth and the percentage of early maturation in Atlantic salmon (Fjelldal et al., 2011; Imsland et al., 2014; Good and Davidson, 2016). Due to the evident effect of temperature on early maturation and GSI development, the $H0_7$ is rejected for the HA_7. Feeding ration did seem to have a modulating effect on the degree and pace of development in individuals reared at moderate temperatures, of which the maturational progression in the restrictive feeding groups was lower or absent compared to the fully fed groups. However, the feed ration modulation did not significantly affect the overall GSI development, and therefore $H0_8$ is accepted.

4.2.2. Morphometric development: Weight, condition factor and hepatosomatic index

4.2.2.1. Growth development

There was an overall increase in growth (judged as weight gain) followed by the increase of growth rate after initiating the winter signal in all treatment groups. The acceleration would be seen in context of the photoperiod shift acting as a trigger mechanism for commitment to developmental events as earlier mentioned. As anticipated, growth rates in the 12.5°C and 18°C groups were significantly higher than in the 8°C groups throughout the experiment in both feeding regimes. This indicates the importance of temperature as one of the main factors influencing growth (Schmidt-Nielsen, 1997; Viadero, 2005). Temperature regulates the metabolic rate of salmonids, ultimately regulating the rate of feed transformation and utilization, thus influencing growth depending on the species-specific optimum, which in Atlantic salmon is approximately 14°C (Handeland et al., 2008). Not surprisingly, feed ration did also significantly affect overall growth, of which the growth rate was generally higher in the full-fed groups. Differences among temperature groups were identified within each feeding regime, of which high temperatures combined with intensive feeding experienced the most growth. Previous studies investigating the link between early sexual maturation and feeding regimes show high maturational development in groups with rapid growth (Rowe and Thorpe, 1990; Norrgård et al., 2014). This is coherent with the current study, as a higher percentage of maturational development was observed in the full-fed

groups than the restricted fed groups, linking feed availability and utilization with growth and thus sexual maturation.

An important distinction between the full-fed 12.5°C and 18°C groups became clear from late-April to mid-May, as growth stagnated in the 18°C group while the 12.5°C experienced a substantial rise in growth rate. This can be seen in context with the degree of maturation observed within each of the groups. Atlantic salmon males which undergo early puberty initially tend to be among the largest individuals in a population until growth is suppressed during maturation, ultimately ending up at a smaller size compared to those which has committed to smoltification (Skilbrei, 1989; Hendry and Beall, 2004; Taranger et al., 2010). The 18°C full-fed group exhibited a high percentage of maturation during the period of late-April to mid-May, something which could reflect the stagnated growth observed compared to the 12.5°C group. High growth rate with corresponding low GSI in the 12.5°C full-fed groups compared to the 18°C full-fed groups with lower growth but with high GSI might suggest that maturation is related to energy acquisition or lipid storage rather than growth rate and general size. This notion is coherent with previous research (Rowel et al., 1991; Thorpe, 1994; Kadri et al., 1996). In comparison, the restrictively fed 12.5°C and 18°C groups displayed no significant growth differences at the end of the experiment. This may reflect the lower degree of maturational advancement experienced within the groups, as well as it could be connected to the lower nutritional input. It should be noted that growth in the 18°C seemed to flatten out at the end of the experiment, while the 12.5°C seemed to experience a rise in growth rate. Therefore, it is possible that a similar trend would have occurred if the experiment went on for a longer period. However, this is only speculation. Salmon reared at 8°C displayed a low but significant growth throughout the experiment, independently of feeding intensity. Growth was significantly lower than the higher temperature groups. Low growth rates often delay or inhibit the maturational process due to energy being used to maintain fitness, combined with the strong seasonal optimum of spawning (Taranger et al., 2010). This suggests that the 8°C groups possibly were impaired to the maturational process, which is supported by the lack of spermatogenetic advancement and GSI development.

4.2.2.2. Condition factor development

The CF was significantly higher in the 18°C groups than the lower temperature groups from mid-December (full feeding) and mid-March (restrictive feeding) and onwards, indicating high energy reserves/lipid content (Herbinger and Friars, 1991). Different CF profiles between feeding ratios were identified, of which the intensive feeding group displayed a generally higher CF compared to the restrictive feeding group. As somatic growth rates and appetite are higher during the earliest stages of sexual

maturation, it increases the CF as a consequence of high lipid reserves (Taranger et al., 2010). This could be seen in connection with the evident difference in CF depending on feeding rations, as the full-fed 18°C group experienced a higher proportion of maturation compared to the corresponding restrictive group. Furthermore, during maturation the high CF reflects testis development and reallocation of energy rather than large energy reserves (Herbinger and Friars, 1991). This could be seen in connection with the lesser growth observed in the 18°C full-fed group compared to that in the 12.5°C full-fed group from mid-March. It could also be seen in context of the early significant peaks in GSI, reflecting energy being reallocated for gonad development. Peterson and Harmon (2005) found a correlation between CF and GSI, of which CF had to exceed 1.3 for initiating early maturation (Peterson and Harmon, 2005). This finding is consistent with the percentage of maturation in the 18°C groups in present study, as salmon exhibited CF > 1.3 throughout the experiment with only a few exceptions.

4.2.2.3. Hepatosomatic index development

All treatment groups experienced a feed and temperature dependent decrease in HSI throughout the experimental period, of which high temperature groups displayed the lowest index values. It would be expected that some energy was reallocated to gonadal development during maturation in intensively reared groups (18°C-100%, 18°C-67%, 12.5°C-100%), which is supported by the reduction in HSI in parallel with the subsequential rise in GSI after the winter signal induction. However, less intensive groups (12.5°C-67%, 8°C-100%, 8°C-67%) did also experience decreasing HSI without exhibiting any signs of early sexual maturation. Considering the low CF and repression of HSI in immature individuals exhibiting low GSI, it might indicate that fish prepared for an alternative developmental event such as smoltification (Pino et al., in preparation).

Overall, morphometric findings suggest that the age of commitment to maturation may be affected by temperature and feeding intensity, of which temperature regulates the metabolic pace of feed transformation and utilization, ultimately controlling the effect of feeding rations. Results suggest that adipose levels or rate of acquiring energy to a greater extent affect maturation than growth rate and general size, given that the high growth rate in the 12.5°C groups led to a lower percentage of maturational development compared to the 18°C groups which experienced lower growth. The present study found an overall temperature and feeding ration dependent development in growth, CF and HSI, thus assumed having a decisive role on sexual maturation. Due to the significant effect of temperatures and feeding rations on growth, CF and HSI, the $H0_1$-$H0_6$ is rejected for their corresponding alternative hypotheses (HA_1-HA_6).

4.2.3. Gonadotropin receptors and gonadotropin-responsive gene expression

4.2.3.1. Downregulation of fsh transcription during intensive rearing conditions

The Fsh-Fshr interactions stimulate early phases of spermatogenesis by modulating Sertoli and Leydig cell activity, regulating downstream transcription of genes and the synthesis of androgen, thus promoting the proliferation of spermatogonia (Rocha et al., 2009; Levavi-Sivan et al., 2010). An increase in the pituitary release of Fsh with coherent Fshr interactions is therefore associated with early phases of precocious male puberty. The stimulatory effect of Fsh on spermatogenesis is not only found in fish (Diemer et al., 2003; Schulz et al., 2010; Taranger et al., 2010; Schulz and Nóbrega, 2011; Sambroni et al., 2013b), but is also a common in primates (Simorangkir et al., 2009), rodents (O'Shaughnessy et al., 2010), and amphibians (Maekawa et al., 1995). Relative quantification of pituitary *fsh* transcripts will be used in the present discussion to evaluate Fsh-Fshr interactions (Pino et al., in prep.). Furthermore, Fsh-Fshr mediated upregulatory *igf3* transcription and 11-KT synthesis will be used as an indicator for reflecting receptor-ligand interactions when discussing the *fshr* transcript. It should be noted that transcription of *igf3* and 11-KT synthesis are not solely affected by Fsh-Fshr interactions (e.g. 11-KT also stimulated by Lh-Lhr). However it is considered sufficient for reflecting Fsh-Fshr interactions at early spermatogenesis as secretion of Lh remains low during the early phases of maturation (Maugars and Schmitz, 2008a; Rocha et al., 2009; Schulz et al., 2010; Sambroni et al., 2013b).

The transcription of *fshr* was high in immature testies before introducing the winter signal, followed by a transcriptional downregulation from mid-March in parallel with spermatogonial proliferation and further germ cell development in maturing individuals. Schulz et al. (2019) concluded that changes in transcription levels of gonadotropin and androgen receptors are not relevant for entering into puberty for Atlantic salmon, as functional receptors are already expressed in immature testies and ready to respond to associated ligand (Schulz et al., 2019). This supports the high relative *fshr* mRNA expression found in immature testis in the present study. The decline in relative receptor mRNA abundance contradicts previous studies which found an upregulation of *fshr* transcription during maturation (Maugars and Schmitz, 2008b; Sambroni et al., 2013b). Interestingly, there was an upregulation of *fsh* transcription levels in parallel with the downregulation of *fshr* transcripts (Pino et al., in prep.). This was simultaneous with the upregulation of relative *igf3* mRNA expression and plasma 11-KT levels, indicating Fsh-Fshr interactions. It therefore seems to be a correlation between transcriptional upregulation of gonadotropin and downregulation of receptors, thus proposing negative feedback between ligand and receptor. The proposed negative feedback mechanism is in line with previous studies on the negative correlation between *fshr* and *fsh* transcription in rats and humans (Themmen et al., 1991; Monaco et al., 1995;

Maguire et al., 1997; Griswold et al., 2001; Zhang et al., 2012). Zhang et al. (2012) found that the cascade of FSH induced activation of androgen receptors causes an upregulation of Metastasis-associated protein 2 (MTA2) expression, which represses FSHR transcription in response to FSH in humans. Griswold et al. (2001) demonstrated that continuous Sertoli cell stimulation of FSH causes desensitization, which through multiple steps causes downregulation of *Fshr* transcription in parallel with FSH-FSHR interactions in rats. This proposes a similar form for negative feedback mechanism in Atlantic salmon. However, to the authors knowledge, little research has been done on such a mechanism in salmonids. It is worth to of further investigate this mechanism, with particular regards to the molecular pathways causing the feedback, as it may contribute to a better understanding of the maturational process in Atlantic salmon.

In the currents study, temperature had a significant effect on relative *fshr* mRNA expression, of which high temperatures caused steep declines in transcription after inducing the winter signal, followed by steady low transcription until mid-May. Feeding ration did not significantly affect the transcription of *fshr*, although a steeper reduction in relative mRNA expression was observed in the 18°C full-fed group compared to its corresponding restrictive feeding group. In parallel with the downregulation of *fshr* transcription, there was a substantial increase in relative *fsh* transcript levels (Pino et al., in prep.). Furthermore, individuals reared at high temperatures displayed the highest increase in relative *igf3* mRNA abundance and plasma 11-KT levels, exhibiting spermatogonial proliferation and further germ cell development from mid-March and onwards. This suggests increased Fsh-Fshr interactions, causing alterations in Sertoli and Leydig cells gene transcription. The upregulation of gonadotropin and gonadotropin-responsive gene transcription supports the notion of a negative feedback mechanism between Fsh *stimuli* and *fshr* transcription. The 12.5°C groups displayed a lower decline in *fshr* transcripts with corresponding upregulation of relative *fsh, igf3,* and plasma 11-KT levels after the winter signal induction. This would reflect the difference in maturational development between the 12.5°C and 18°C groups. The 12.5°C restrictive feeding group exhibited low transcription of *fsh*, while the full feeding group showed a steep increase after the winter signal. As an increase in Fsh-Fshr interactions are necessary to initiate and progress spermatogenesis, the difference between feeding groups in 12.5°C could explain the absence of maturational development in the 12.5°C restrictive feeding group. In comparison, the 8°C groups displayed little variation in *fsh* and *fshr* transcription levels. A non-significant increase in *igf3* transcripts and plasma 11-KT levels were identified in the 8°C groups, suggesting few Fsh-Fshr interactions. This could reflect the lack of spermatogonial proliferation, thus may support the notion of the 8°C groups being impaired to the maturational process.

Despite not including a control group for photoperiod, the current study displayed a general downregulation of relative *fshr* mRNA abundance in all treatment groups after the winter signal induction, of which magnitude of regulation seemed to be correlated to temperature. There was an immediate upregulation of *fsh* transcription with coherent downregulation of *fshr* transcript levels after the winter signal. The regulation of transcripts followed the same temperature dependent pattern. This supports the notion of negative feedback between ligand and receptor, as well as the proposed relevance of photoperiodic variation on timing and routing of sexual maturation. It also reinforces the notion of photoperiod and temperature acting as primary drivers of early sexual maturation (Fjelldal et al., 2011; Imsland et al., 2014; Good and Davidson, 2016). Current results found a temperature regulatory effect on *fshr* transcription, of which feed ration had no significant impact. Due to the significant effect of temperature on relative *fshr* transcription, the $H0_9$ is rejected for the HA_9, while the $H0_{10}$ concerning the role of feeding ratio is accepted as it did not provide any significant effect on transcription.

4.2.3.2. Downregulation of lhr transcription during intensive rearing conditions

The Lh-Lhr interactions are the main stimulator of steroidogenesis in Leydig cells during sexual maturation, controlling meiosis and final stages of spermatogenesis mainly through mediating androgen synthesis (Swanson et al., 2003). A rise in the pituitary release of Lh with coherent Lhr interactions is therefore associated with later phases of spermatogenesis. Relative quantification of pituitary *lh* mRNA abundance will be used in the present discussion to evaluate Lh-Lhr interactions (Pino et al., in prep.). The *lhr* was transcribed throughout the experiment supporting the conclusion of Schulz et al. (2019) on the relevance of receptors at early maturation. High temperatures have been found to affect gonadal development and function by inhibiting or reducing the pituitary transcription of *lh* or impairing the DHP production, consequently affecting the degree of maturation (Taranger et al., 2003; Vikingstad et al., 2016). The relative transcription of *lh* remained lowest in high temperature groups until after introducing the winter signal, followed by a significant upregulation until mid-May (Pino et al., in prep.). The upregulation in transcription reflects the percentage of maturation and spermatogenetic advancements observed, as entry into the meiotic phase of spermatogenesis only first occurred after the winter signal induction. This supports the inhibitory effect of high temperatures on *lh* transcription, while it also suggests the effect of photoperiodic cues as triggering mechanisms which promote maturational advancement.

The 18°C groups displayed a generally higher *lhr* transcription than the other treatment groups prior to the winter signal, followed by a decline from mid-March with corresponding upregulation of *lh* transcription levels. This could propose a negative feedback mechanism as suggested for the *fshr* transcription. In

contrast, the 12.5°C groups exhibited an increase in receptor mRNA transcription from mid-Mach. The full-fed group experienced an upregulation of relative *lh* transcripts, while the restrictively fed group displayed no transcriptional change. This suggests that individuals in the full-fed group entered the meiotic phase of spermatogenesis, supported by the gonad histological image analysis and percentage of maturing individuals observed at the end of the experiment. The lack of transcriptional upregulation of *lh* in the restrictively fed group seems to have inhibited further advancement and completion of germ cell development, which supports the role of Lh on regulating meiosis and final stages of spermatogenesis (Swanson et al., 2003; Maugars and Schmitz, 2008a; Rocha et al., 2009; Schulz et al., 2010; Sambroni et al., 2013b). The relative transcription of *lhr* in the 12.5°C groups was generally lower than the 18°C groups except for at the last sampling point. The 8°C groups displayed the lowest relative *lhr* mRNA abundance of all groups with corresponding low stable relative *lh* expression. This reflects the lack of spermatogenetic advances, supporting the notion of the 8°C groups possibly being impaired to the maturational process.

In general, there was a flat relative receptor expression with a downregulation in groups experiencing high percentage of maturation (18°C-100%, 18°C-67%) in parallel with an increase of pituitary *lh* transcription. This supports a possible negative feedback mechanism between ligand and receptor. As temperature and feeding intensity exerted a significant effect on relative *lhr* expression, the $H0_{11}$-$H0_{12}$ must be rejected for their corresponding alternative hypotheses (HA_{11}-HA_{12}).

4.2.3.3. Transcriptional regulation of amh and 11-KT synthesis

The Sertoli cell secreted Amh modulates self-renewal of undifferentiated type A spermatogonia while inhibiting further proliferation and differentiation into type B spermatogonia (Skaar et al., 2011; Pfennig et al., 2015). The signaling protein has also been proposed to inhibit the development and function of Leydig cells, thus inhibiting 11-KT induced proliferation of type B spermatogonia and the entry into the meiotic phase of spermatogenesis (Skaar et al., 2011; Pfennig et al., 2015). Transcription is downregulated during sexual maturation, resulting in a reduction of the associated antagonistic effect on spermatogenesis, hence providing the premise for further advancements of germ cell development (Miura et al., 2002; Skaar et al., 2011; Pfennig et al., 2015). As anticipated, *amh* transcriptions in the current study were high in immature testis prior to the winter signal, followed by being downregulated during maturation, allowing further germ cell advancements. This expression profile is in line with previous literature on sexual maturation in male Atlantic salmon (Maugars and Schmitz, 2008a), but is also described in other species such as Japanese eel (*Anguilla japonica*) (Miura et al., 2002), Zebrafish (*Danio rerio*) (Skaar et al., 2011; Pfennig et al., 2015), and Rainbow trout (*Oncorhynchus mykiss*) (Sambroni et al., 2013b). The

transcriptional downregulation was only observed after inducing the winter signal, supporting the proposed function of photoperiod on regulating the BPG-axis and thus spermatogenesis (Duston and Bromage, 1988; Hutchings and Jones, 1998; Bromage et al., 2001; Berrill et al., 2003; Taranger et al., 2010). Temperature had a significant effect on relative *amh* mRNA expression, of which high temperature groups displayed the most downregulation of *amh* transcripts from mid-March, suggesting the regulatory effect of temperature. Feeding regimes did not significantly affect *amh* transcription, although the restrictive feeding groups showed a weaker declining trend than the full-fed groups.

Transcription of *amh* in the 18°C groups was significantly lower than the other temperature groups at all time points from mid-March, of which the full-fed group displayed the highest repression of transcription during spermiogenesis. In parallel, there was an increase in GSI and the percentage of maturing individuals. This confirms energy reallocation to gonad development and the activation of the BPG-axis, which in turn is in line with the reduced antagonistic effect of Amh detected to progress spermatogenesis (Maugars and Schmitz, 2008a; Skaar et al., 2011; Pfennig et al., 2015). The 12.5°C groups experienced an intermediate decline in *amh* transcription after the winter signal induction, expressing higher transcription than the 18°C groups while being significantly lower than the 8°C groups. The 8°C groups showed no specific changes in transcription. The process of sexual maturation in the 8°C groups was therefore most likely impaired as earlier suggested, given that the necessary downregulation of *amh* transcription for spermatogonial proliferation was not present, something which is further supported by the other gonadotropin-responsive gene expression profiles. The different *amh* transcription profiles are consistent with the gonad histological image analysis, which indicates that type B spermatogonial was the furthest developed germ cell stage in the high temperature groups from early-March, followed by further advancement in parallel with the downregulated relative *amh* mRNA abundance.

Interestingly, differences in *amh* transcription between temperature groups were evident even prior to the photoperiod shift, suggesting that the degree of transcription in general is temperature dependent. Cases of type B spermatogonia were observed in the 12.5°C and 18°C groups prior to the winter signal before the transcriptional repression of *amh*. This might indicate that transcription of *amh* was low enough in individuals reared at high temperatures to differentiate type A spermatogonia into type B spermatogonia without being exposed to a photoperiod shift. This could be seen in context with the 18°C groups displayed the lowest *amh* mRNA abundance while exhibiting the most cases of type B spermatogonia before the winter signal. However, this conflicts with earlier findings stating that photoperiod has a decisive role in maturational timing (Schulz et al., 2019). Based on the percentage of maturation, none of the treatment groups showed signs of maturation prior to the winter signal, supporting Schulz et al. (2019). However,

the arbitrary maturation scale may not be precise enough to detect early spermatogenetic phase developments as it only accounts for GSI development. Noticeably, type B spermatogonia were the furthest developed germ cell stage observed before and directly after initiating the winter signal. Plasma 11-KT levels significantly increased in high temperature groups immediately after inducing the winter signal, suggesting proliferation of type B spermatogonia and entry into the meiotic phase of spermatogenesis. Therefore, a photoperiod shift seemed to be a necessary cue for regulating the temperature dependent 11-KT synthesis, thus needed for germ cell advances into the meiotic phase. Our findings propose that differentiation to type B spermatogonia is possible without change in photoperiod given the temperature dependent transcription of *amh*, but that a shift is necessary for further advancement of spermatogenesis. This further supports the notion of temperature and photoperiod as main factors triggering and regulating sexual maturation. This notion deserves further research, as the present study is not sufficient to confirm or refute such a mechanism given the lack of a photoperiod control group.

The downregulation of *amh* transcription is associated with the upregulation of Fsh and androgens synthesis (Maugars and Schmitz, 2008a; Schulz et al., 2010; Sambroni et al., 2013b). The Fsh-Fshr mediated transcriptional change in Sertoli cells, and upregulates the transcription and release of *inha* in Rainbow trout and possibly in Atlantic salmon, which has an inhibitory effect on *amh* (Sambroni et al., 2013b). The current study did not quantify *inha*, but the repression of *amh* transcription may indicate a possible increase of *inha* expression or other *amh* inhibitory substances. The downregulation of *amh* transcription levels occurred shortly after the repression of *fshr* and corresponding upregulation of *fsh*, reflecting Fsh-Fshr interactions which downregulate the *amh* expression (Maugars and Schmitz, 2008a; Schulz et al., 2010; Sambroni et al., 2013b). It has been proposed that Amh inhibits Leydig cell development and steroidogenesis thus inhibiting the proliferation of type B spermatogonia, of which an increase of plasma 11-KT is to be expected alongside a transcriptional downregulation of *amh* (Maugars and Schmitz, 2008a; Morais et al., 2017). Findings of Amh type II transmembrane receptors (Amhr2) on Leydig cells in Medaka (*Oryzias latipes*) (Klüver et al., 2007) and Blackhead seabream (*Acanthopagrus schlegelii*) (Wu et al., 2010) support the proposed inhibitory role of Amh on Leydig cells. Moreover, Skaar et al. (2011) found Amh to have a suppressive role on steroidogenesis in Leydig cells of Zebrafish (Skaar et al., 2011). Overall, this might suggest an equal role of Amh on steroidogenesis in salmonids. Schulz et al. (2010) state that *amh* transcription is suppressed by testosterone and 11-KT (Schulz et al., 2010). The plasma 11-KT profile was upregulated in parallel with the transcriptional decline of *amh* in all groups, supporting the proposed correlation between *amh* transcription and 11-KT synthesis, of which Amh may have an antagonistic role on steroidogenesis, while androgens when produced might downregulates *amh* transcription.

As for *amh* transcription, plasma 11-KT levels were significantly affected by temperature, but also feeding ration. The 18°C groups displayed the highest plasma 11-KT level from mid-March, showing a significant increase until peaking in late-April, promoting spermatogenesis. The restrictive fed 18°C group did on the other hand display stagnant levels from early-April to mid-May, reflecting the lesser proportion of fully matured individuals compared to the corresponding full-fed group. The 12.5°C full-fed group displayed a similar response, but seemingly delayed and with high variation, possibly explaining the low percentage of maturational development. The 8°C groups and the restrictively fed 12.5°C group only displayed a small increase in 11-KT during the same period, reflecting the low or no advancement in spermatogenesis. In general, it seems that restrictive feeding caused a lower response in plasma 11-KT levels than the full feeding regimes, most likely due to the lower nutritional conditions. The degree to which 11-KT was upregulated correlate with the downregulation of *amh* transcription, being mainly regulated by temperature. Overall, present results show a significant effect of temperature on relative transcription of *amh*, thus the $H0_{13}$ is rejected for HA_{13}. As feeding rations did not provide any significant effect, the $H0_{14}$ is kept. For 11-KT, both temperature and feeding intensities affected plasma levels, thus the $H0_{21}$-$H0_{22}$ are rejected for their corresponding alternative hypotheses (HA_{21}-HA_{22}).

4.2.3.4. Temperature dependent regulation of gsdf1 and gsdf2 transcription

Transcription of *gsdf1* was high in all treatment groups prior to the winter signal induction, followed by being downregulated in maturing individuals from mid-March. The transcriptional levels of *gsdf1* were significantly affected by temperature, of which the 18°C groups exhibited a substantial decline after the winter signal induction, exhibiting a significantly lower relative mRNA expression compared to the lower temperature groups from April. In comparison, the 8°C and 12.5°C groups only showed a slight reduction in *gsdf1* transcription without exerting statistical difference among each other. Feeding rations did not significantly affect *gsdf1* transcription, although restrictive feeding groups showed weaker trends than its counterpart from mid-March, especially in the 18°C groups. The repression of *gsdf1* at post-winter signal is in agreement with previous literature on early sexual maturation of Atlantic salmon (Schulz et al., 2010). The *gsdf1* is transcribed explicitly in female granulosa and male Sertoli cells, of which high levels exert a stimulatory effect on primordial germ cell proliferation and mitotic activity during salmonid maturation, followed by being downregulated as spermatogenesis progresses (Sawatari et al., 2007; Lareyre et al., 2008; Schulz et al., 2010; Sambroni et al., 2013b; Kleppe et al., 2020). This regulatory effect has been described not only in Atlantic salmon (Schulz et al., 2010; Kleppe et al., 2020), but also in species such as Rainbow trout (Sawatari et al., 2007; Sambroni et al., 2013b), Zebrafish (Gautier et al., 2011), Medaka

(Shibata et al., 2010) and Nile tilapia (*Oreochromis niloticus*) (Kaneko et al., 2015). The *gsdf1* transcription profiles are consistent with the gonad histological image analysis, which indicate type B spermatogonia as the furthest developed germ cell stage prior to the downregulation. Further advances in germ cell stage development were observed (spermatocytes and spermatozoa) during the *gsdf1* transcription repression, indicating the initiation of the meiotic and later on spermatogenetic phase of spermatogenesis. This is supported by previous studies that found repression necessary to advance germ cells into the meiotic phase of spermatogenesis (Lareyre et al., 2008; Chen et al., 2013; Kleppe et al., 2020). The downregulation of *gsdf1* transcription was significant in the 18°C groups, which can be seen in context germ cell stage advances from early-April in both feeding groups. In contrast, the 12.5°C full-fed group experienced a minor decline in *gsdf1* transcripts, which may reflect the meiotic and spermatogenetic advances only being observed in mid-May. The photoperiod shift seemed to act as a cue accelerating the temperature dependent downregulations of *gsdf1*, as expression declined immediately after the winter signal induction. This supports the combined role of photoperiod manipulation and temperatures as possible main triggers of sexual maturation.

In comparison, the *gsdf2* transcription profile was highly variable throughout the experiment, showing few significant differences between the experimental groups while exhibiting no overall significant change (except for the 8°C restrictively fed group experiencing an overall downregulation). Transcription of *gsdf2* is, unlike *gsdf1*, restricted to the male testis and Sertoli cells, and is to the authors knowledge only described in salmonids (Schulz et al., 2010). Gsdf2 encodes for proteins that are essential for the function of the TGF-β pathway (Lareyre et al., 2008), suggesting a possible regulatory effect on central spermatogenesis TGF-β members such as Gsdf1, Amh and Inha. However, there is limited research on Gsdf2 and its functions, given its recent discovery in salmonids (Lareyre et al., 2008; Sambroni et al., 2013b). The present study shows no distinct differences in *gsdf2* transcription, although the overall expression profile exhibited a slight but insignificant decrease in abundance during sexual maturation. Interestingly, there were significant differences between all full-fed temperature groups in late-April, of which the 18°C group experienced a considerable drop in transcription. However, as the group experienced an immediate increase, it is uncertain whether this affected the spermatogenesis advancement, especially given that the 12.5°C full-fed group did not experience a drop in *gsdf2* transcription but still showed signs of maturational progress at the end of the experiment. Temperature did have a significant effect on expression; however, a clear pattern is inconspicuous. The feeding ration did not provide any significant impact on relative *gsdf2* mRNA expression.

Relative *gsdf1* and *gsdf2* transcription are downregulated by Lh and androgens, providing the premise for onwards differentiation and proliferation of germ cells (Lareyre et al., 2008; Chen et al., 2013; Kleppe et al., 2020). There seems to be a correlation between the repression of *gsdf1* transcription and the parallel upregulation of 11-KT synthesis, which supports the notion of androgens downregulating *gsdf1* (Lareyre et al., 2008; Chen et al., 2013; Kleppe et al., 2020). High temperature groups displayed the most downregulation of relative *gsdf1* mRNA abundance while exhibiting the highest upregulation of 11-KT, suggesting that the degree of regulation is temperature dependent. Even though the present study did not find any clear pattern on how temperatures affect *gsdf2* transcription, or identified a significant transcriptional development during maturation, a possible effect of *gsdf2* should not be neglected. Hence, we propose further research on relative *gsdf2* gene expression and its function in Atlantic salmon during early sexual maturation, with particular regards to whether *gsdf2* have a regulatory role on spermatogenesis relevant genes in the TGF-β pathway during early puberty. Overall, as temperature had a significant effect on *gsdf1* and *gsdf2* transcription, the $H0_{15}$ and $H0_{17}$ must be rejected for HA_{15} and HA_{17}. The $H0_{16}$ and $H0_{18}$ concerning the role of feeding ratio must be kept as no effect were recorded.

4.2.3.5. Temperature dependent upregulation of igf3 transcription

The *igf3* transcription profile showed low mRNA levels prior to the winter signal induction, followed by a temperature dependent upregulation from mid-March. The transcriptional rise in *igf3* observed after a photoperiod shift is in line with previous literature on sexual maturation in Atlantic salmon (Melo et al., 2015), but is also described in species such as Rainbow trout (Sambroni et al., 2013b), Zebrafish (Nóbrega et al., 2015), Common carp (*Cyprinus carpio*) (Song et al., 2016) and Nile tilapia (Wang et al., 2008). The teleost restricted Igf3 is paralogous to mammalian IGF1, and is expressed predominantly in gonad tissue (Wang et al., 2008). The upregulation of Igf3 promotes spermatogonial germ cell differentiation, thus the entry into the meiotic phase of spermatogenesis (Wang et al., 2008; Sambroni et al., 2013b; Melo et al., 2015; Nóbrega et al., 2015). Temperature had a significant effect on relative *igf3* mRNA expression, of which high temperatures seemed to be correlated with high transcription. All groups (except for the 8°C groups) displayed an increase in abundance from mid-March. Expression was highest in the 18°C groups, exhibiting significantly higher *igf3* transcription than the lower temperature groups at given time points after the winter signal induction. The 12.5°C groups showed a lesser increase in transcription and did not significantly differ from the 8°C groups at any time point, independently of the feed ration. The feeding ration did not have any overall effect on relative *igf3* mRNA expression.

The *igf3* transcription profile is in agreement with the results from the gonad histological image analysis, as an increase of type B spermatogonia and further advances of germ cell stage development was confirmed during the upregulation. Fsh and androgens upregulate *igf3* transcription in Sertoli cells during sexual maturation (Wang et al., 2008; Sambroni et al., 2013b; Melo et al., 2015; Nóbrega et al., 2015). Sambroni et al. (2013) found that relative *igf3* expression strongly relies on Fsh regulation in Rainbow trout, while Nóbrega et al. (2015) further suggest the role of Fsh-mediated 11-KT release from Leydig cells on *igf3* transcriptions. The current study is in line with these findings, of which an increase in plasma 11-KT levels appeared in parallel to the upregulation of relative *igf3* expression. The transcription profile of *igf3* was quite similar to that of 11-KT. A transcriptional breaking point in the 12.5°C and 18°C groups was observed in mid-March, of which both groups displayed an immediate upregulation of *igf3* transcripts with corresponding plasma 11-KT levels. Low transcription of *igf3* and 11-KT seemed to detain or postpone the maturational process and were only found in immature individuals (12.5°C-67%, 8°C-100%, 8°C-67%), while upregulated transcription was found in males exhibiting spermatogonial proliferation and further germ cell development (18°C-100%, 18°C-67%, 12.5°C-100%). Schulz et al. (2019) found that early signs of testies maturation are associated with increased *igf3* transcription, and are correlated with elevated plasma 11-KT levels (Schulz et al., 2019). Present results support the proposed correlation between *igf3* abundance and plasma 11-KT levels on pubertal advancement. Our results suggest that rate and magnitude of expression are connected with rearing temperature, as high temperature groups displayed the highest upregulatory transcription of *igf3* with coherent 11-KT levels. In contrast, lower temperature groups exhibited lower overall expression.

Interestingly, the transcription profiles of *igf3* and *amh* seemed to be inverted, of which upregulation of *igf3* appeared in parallel with the downregulation of *amh*. This implies the different regulatory roles of Fsh and androgens on the transcription of these genes, ultimately regulating spermatogenesis. As mentioned previously, Amh modulates the self-renewal of type A spermatogonia while inhibiting type B spermatogonia differentiation while exerting negative regulation of androgen secretion from Leydig cells (Skaar et al., 2011; Pfennig et al., 2015). In contrast, Igf3 promotes spermatogonial proliferation and differentiation, and facilitates the entry into meiosis (Wang et al., 2008; Sambroni et al., 2013b; Melo et al., 2015; Nóbrega et al., 2015). This illustrates the opposite roles of these genes, of which downregulation of *amh* transcription with a coherent upregulation of relative *igf3* mRNA expression is necessary for maturational progression. Furthermore, *igf3* upregulation occurs simultaneously with *gsdf1* downregulation, especially in high temperature groups. As previously mentioned, Gsdf1 exert stimulatory effect on primordial germ cell proliferation and mitotic activity during salmonid maturation, of which downregulation is necessary to progress spermatogenesis (Sawatari et al., 2007; Lareyre et al., 2008;

Schulz et al., 2010; Sambroni et al., 2013b; Kleppe et al., 2020). The downregulation of *amh* and *gsdf1* transcripts in parallel with the *igf3* upregulation supports the observations of increasing type B spermatogonia and further germ cell advancements from mid-March in the 12.5°C full-fed group and 18°C groups. The temperature had a significant effect on *igf3*, *amh* and *gsdf1* transcription levels, of which high temperatures seemed to regulate the general magnitude of transcription independently of the feed ration. These results indicate the general connection between gonadotropin-sensitive genes expressed during sexual maturation and show how they differ in the regulation of the spermatogenetic process. Furthermore, results support the proposition of temperature in combination with photoperiod cues being main drivers of early sexual maturation, as transcriptional breaking points in gene expression occurred after the winter signal induction, of which temperatures regulated the degree of gene transcription (Fjelldal et al., 2011; Imsland et al., 2014; Good and Davidson, 2016). Overall, due to the findings of a temperature dependent *igf3* transcription, the $H0_{19}$ is rejected for the HA_{19}. As feeding intensity did not significantly affect relative mRNA abundance, the $H0_{20}$ is accepted.

5. Concluding remarks

The intensification of rearing conditions during Atlantic salmon post-smolt production in RAS has promoted a rise in precocious male maturation rates. As the commitment to mature is made several months in advance of spawning, it is assumed that the developmental process is initiated during the pre-smolt phase in freshwater. The freshwater phase is characterized by the use of intensive feeding and temperature conditions to enhance growth rates. The present study focused on the relationship between different feed and temperature intensities on precocious male maturation and early gonad development in Atlantic salmon pre-smolt. High temperature (18°C) appeared to promote precocious male puberty and spermatogenesis through an early activation of the BPG-axis with corresponding gonadotropin-responsive gene transcription and sex steroid synthesis in Sertoli cells and Leydig cells, regardless of feeding ration. The effect of high temperatures did further appear to promote high developmental rates and energy acquisition independently of nutritional input. This consequently entails reaching the genetically determined biological thresholds for committing to mature early, thus causing spermatogenetic advances. High growth rate and CF before the winter signal were followed by a decrease in HSI in parallel with a subsequent rise in GSI, reflecting the proportion of fully matured individuals at the end of the experiment (restrictive feeding ratio - 33.4%; full feeding ratio - 84.6%). On a transcriptional level, the maturation was represented by downregulation of *fshr* and *lhr* mRNA abundance caused by negative feedback mechanisms associated with high gonadotropin levels. Frequent ligand-receptor interactions caused a high gonadotropin-responsive gene transcription response (*amh, gsdf1, gsdf2, igf3*) and upregulated 11-KT

synthesis, ultimately raising the pace of spermatogenic advancement. In contrast, low temperature (8°C) caused a significantly lower growth rate and physiological development, which is likely correlated with the temperature dependent low metabolic rate, hence impairing the maturational process regardless of feeding ration. Intermediate temperature (12.5°C) did on the other hand appear to be dependent on intensive feed rations to commit to maturation. This is reflected by the full-fed group which displayed a moderate percentage of maturational advancements (16.6%) with corresponding physiological and transcriptional development at the end of the experiment, compared to the general absence of development in the restricted feeding group. This proposes that the relevance of feeding rations on the commitment to mature is dependent on temperature. Present results support the role of temperature being one of the primary contributors to triggering early sexual maturation in male Atlantic salmon, influencing the magnitude and rate of development to a greater degree than feeding rations.

The induction of a winter signal was in the present study found to trigger an increased temperature dependent physiological response in terms of morphological development (body weight, CF, HSI, GSI), plasma 11-KT levels, and transcription of gonadotropin (*fshr*, *lhr*) and gonadotropin-responsive genes (*amh*, *gsdf1*, *gsdf2*, *igf3*). Intensive rearing groups (18°C-100%, 18°C-67%, 12.5°C-100%) experienced an increased developmental rate after the winter signal, stimulating spermatogenetic advancement. In contrast, less intensive conditions (12.5°C-67%, 8°C-100%, 8°C-67%) experienced minor physiological development with corresponding low or no spermatogenetic advances. These results support the role of photoperiod as a triggering mechanism for developmental advancement intertwined with biological (energy status, growth factor) and other environmental (temperature, diet) factors. Furthermore, as the present study found spermatogonial proliferation and differentiation in high temperature groups prior to the winter signal, it could suggest that the initiation of maturation is partly independent of a photoperiod cue. High temperature groups displayed the lowest transcription of *amh* throughout the experiment, suggesting a reduced antagonistic effect of Amh with subsequential proliferation and differentiation of spermatogonia. Overall, present results indicate an elevated risk of precocious male post-smolt maturation during intensive rearing conditions, of which temperature are considered the main factor regulating the rate and magnitude of maturation and spermatogenesis.

The following hypotheses were accepted/rejected in present study:

- **$H0_1$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on body weight development, *is rejected*. Present study found a significant effect of temperature on body weight development, thus **HA_1** *is accepted.*
- **$H0_2$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on body weight development, is rejected. Present study found a significant effect of feeding ration on body weight development, thus **HA_2** *is accepted*.

- **$H0_3$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on condition factor (CF) development, *is rejected*. Present study found a significant effect of temperature on CF development, thus **HA_3** *is accepted.*
- **$H0_4$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on condition factor (CF) development, *is rejected*. Present study found a significant effect of feeding ration on CF development, thus **HA_4** *is accepted*.

- **$H0_5$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Hepatosomatic index (HSI) development, *is rejected*. Present study found a significant effect of temperature on HSI development, thus **HA_5** *is accepted.*
- **$H0_6$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Hepatosomatic index (HSI) development, *is rejected*. Present study found a significant effect of feeding ration on HSI development, thus **HA_6** *is accepted*.

- **$H0_7$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Gonadosomatic index (GSI) development. Present study found a significant effect of temperature on GSI development, thus **HA_7** *is accepted.*
- **$H0_8$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Gonadosomatic index (GSI) development, *is accepted*.

- **$H0_9$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Follicle-stimulating hormone receptor (*fshr*) gene transcription, *is rejected*. Present study found a significant effect of temperature on relative *fshr* transcription, thus **HA_9** *is accepted.*

- **$H0_{10}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Follicle-stimulating hormone receptor (*fshr*) gene transcription, *is accepted*.

- **$H0_{11}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Luteinizing hormone receptor (*lhr*) gene transcription, *is rejected*. Present study found a significant effect of temperature on relative *lhr* transcription, thus **HA_{11}** *is accepted.*
- **$H0_{12}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Luteinizing hormone receptor (*lhr*) gene transcription, *is rejected*. Present study found a significant effect of feeding ration on relative *lhr* transcription, thus **HA_{12}** *is accepted.*

- **$H0_{13}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on Anti-Müllerian hormone (*amh*) gene transcription, *is rejected*. Present study found a significant effect of temperature on relative *amh* transcription, thus **HA_{13}** *is accepted.*
- **$H0_{14}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on Anti-Müllerian hormone (*amh*) gene transcription, *is accepted*.

- **$H0_{15}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on gonadal soma-derived factor 1 (*gsdf1*) gene transcription, *is rejected*. Present study found a significant effect of temperature on relative *gsdf1* transcription, thus **HA_{15}** *is accepted.*
- **$H0_{16}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on gonadal soma-derived factor 1 (*gsdf1*) gene transcription, *is accepted*.

- **$H0_{17}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on gonadal soma-derived factor 2 (*gsdf2*) gene transcription, *is rejected*. Present study found a significant effect of temperature on relative *gsdf2* transcription, thus **HA_{17}** *is accepted.*
- **$H0_{18}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on gonadal soma-derived factor 2 (*gsdf2*) gene transcription, *is accepted*.

- **$H0_{19}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on insulin-like growth factor 3 (*igf3*) gene transcription, *is rejected*. Present study found a significant effect of temperature on relative *igf3* transcription, thus **HA_{19}** *is accepted.*
- **$H0_{20}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on insulin-like growth factor 3 (*igf3*) gene transcription, *is accepted*.

- **$H0_{21}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different temperatures have no significant effect on plasma 11-Ketotestosterone (11-KT) concentrations, *is rejected*. Present study found a significant effect of temperature on plasma 11-KT concentrations, thus **HA_{21}** *is accepted.*
- **$H0_{22}$**: Rearing of Atlantic salmon pre-smolt and exposing them to different feeding rations have no significant effect on plasma 11-Ketotestosterone (11-KT) concentrations, *is rejected*. Present study found a significant effect of feeding rations on plasma 11-KT concentrations, thus **HA_{22}** *is accepted.*

6. Application for aquaculture

The salmon industry uses intensive rearing conditions to promote growth while reducing the overall production time, however at the cost of an increased proportion of unwanted precocious male maturation. Maturation causes reduced growth, hinders the development of the hypo-osmoregulatory ability, and increases agnostic behavior. This ultimately reduces fish welfare while increasing disease susceptibility and risk of secondary infections due to associated decreased immune competence, thus increasing mortality rates. It is suspected that some maturing individuals can survive the grow-out phase in open-net pens. This is a problem in itself, as maturing individuals are highly susceptible to diseases, which can affect the rest of the population in the open-net pens. Furthermore, in case of escapees, early maturing salmon may cause genetic introgression of farmed salmon into wild stock populations, being a great threat for the wild stock stain. The scenario of maturing males surviving the grow-out phase is also unwanted as fish farmers feed fish with highly reduced growth potential. Furthermore, as energy is reallocated to gonadal growth rather than somatic growth, the fillet quality is degraded, downgrading fillet value or making it unsellable. Therefore, there is associated a substantial economic loss in producing maturing salmon in addition to the biological issues. The current demands for sustainable development and issues related to the grow-out phase in open-net pens in the sea have led to new production strategies, such as the prolonged land-based production of post-smolts in RAS. From an economic point of view, the early post-smolt

maturation is of great concern when produced in RAS. This is mainly due to the large financial investment in establishing these complex systems combined with high operational costs.

Findings from the present study suggest implications for modern intensive aquaculture production and are not restricted to RAS production alone. Overall, the results indicate an elevated risk of precocious male maturation during intensive rearing conditions as modern production combined the use of high temperatures, intensive feeding regimes, and photoperiod manipulation to enhance growth rates and initiate smoltification. Results indicate temperature as a critical factor in triggering early sexual maturation, of which high temperatures stimulate early activation of the BPG-axis with corresponding gonadotropin-responsive gene expression, ultimately increasing the proportion of early sexual maturation, independently of nutritional input. Low and intermediate temperatures appear to inhibit or cause maturational advances to a lesser degree. Furthermore, results propose that Atlantic salmon reared at intermediate temperatures with high nutritional input may initiate the maturational process during the freshwater stage. This suggests that these individuals would have become fully mature at later stages, such as during the post-smolt stage. All considered, intensive rearing conditions in terms of temperature and feeding ration combined with photoperiod manipulation used during the freshwater phase of the Atlantic salmon production cycle should be of concern, as it increases the risk of early maturation. This should especially be of concern when producing large smolt, as they are significantly larger than traditional smolt. Size and rate of energy acquisition are biological thresholds for committing to maturation. Hence, the intensive production strategy increases the risk of maturation. A need for lowering rearing temperatures in modern intensive production of Atlantic salmon is evident, as the present study found high temperatures to trigger large-scale precocious male maturation. Based upon the current result, an upper temperature limit of 12°C during freshwater production may be recommended to avoid the initiation and progression of the maturational process. This limit is based on the findings of higher rearing temperatures entailing increased risk for pubertal commitment in male salmons. Furthermore, energy input seems to have a more decisive role during rearing at intermediate temperatures, of which high energy inputs may cause maturational advancements. Therefore, less energy-rich feed, or restrictive feeding rations (given avoiding aggression and the establishment of hierarchies among fish associated with feed competition), may be a practical method for reducing maturation in combination with rearing at the upper temperature limit to prevent early male puberty. A potential alternative is to switch over to an all-female production, given that commitment to maturation is significantly delayed due to higher energy requirements associated with female gametogenesis.

The maintenance of stable rearing temperatures is a however challenging task due to seasonal variations affecting the temperature of intake-water. This poses an issue especially during the summer months when temperatures are high, as regulatory systems in RAS and FTS may struggle to maintain appropriate temperature levels, resulting in high temperatures promoting early sexual maturation. During the summer of 2020, an aquaculture company in western Norway explains this problem in their RAS facility, of which the heating of water was faster than for the system to regulate. The consequence of this came in hindsight, of which a high proportion of post-smolt males underwent early sexual maturation (10-15%). This reflects the need to develop proper systems that can handle high seasonal variations in temperature while also supporting the present findings of high temperatures affecting early maturation.

7. Further perspectives

- The present study compared the relationship between three different temperatures and two different feeding regimes. If this experiment were to be repeated, it would have been interesting to include another temperature group at 14°C. This could further clarify and strengthen the role of temperature on maturational commitment found in the present study. We hypothesize that the proportion of early maturation is directly correlated with increasing temperatures, given that temperatures regulate the metabolic rate and the pace of development.

- The present study found distinct differences in the growth development of individuals committing to maturation and smoltification. The trend in the Norwegian aquaculture production strategy is to increase the smolt size (< 1 000 g) to reduce the grow-out phase in the sea ("storsmolt strategien"). During the production of large smolt, factors are intensified to promote growth and size. Previous studies have been conducted on how different internal and external factors affect maturation, however, less research has been conducted on the relationship between size and maturation. The current study found no significant body weight differences between the 12.5°C and 18°C full-fed salmons prior to the winter signal. However, after the winter signal induction, the 18°C had committed to mature while the 12.5°C seemingly had committed to smoltify. We suggest the worth of further studying how size development in large smolt affects the initiation of maturation during intensive production, given that commitment to maturation and smoltification seems to occur at same sizes. In-depth knowledge of this connection can be particularly useful, given the intensive production of larger smolt.

- During sexual maturation, pheromones and steroids are released into the water to synchronize the final maturation of germ cells and spawning behavior. It is hypothesized that the release may occur during precocious male maturation as well, of which it could have a triggering effect on the timing of maturational commitment in the rest males in a population. If this is the case, pheromones and steroids will accumulate in RAS, ultimately causing a mass initiation of maturation. However, this is only speculations as the current study did not measure pheromone steroid build-up as a possible maturational triggering factor. Therefore, the present study suggests the worth of further research on pheromones and steroids and their potential effect during precocious male maturation. A possible confirmation could provide a measure for combating precocious puberty in RAS during intensive post-smolt production.

- There is a need for an early indicator for maturational commitment in modern intensive aquaculture production, as early identification may provide a possibility to counteract or delay the developmental process. For this reason, the current findings might therefore be of particular interest due to the distinct gonadotropin-responsive *amh* gene transcription profile identified at the different intensive conditions. The present study found significant differences in *amh* transcription profiles between all temperature groups prior to the winter signal, of which high temperatures seemed to be correlated with low expression and early spermatogenic advancement. As transcriptional alterations in *amh* associated with early spermatogenetic advancement were identified several months prior to maturation, it could provide an early indicator for maturational commitment. It would be interesting to further examine the role and transcriptional properties of *amh* during early male maturation, especially during intensive rearing conditions. If current results are strengthened by further research, an *amh* transcription threshold for early commitment to maturation could be identified. This would provide an important tool for determining early commitment to maturation for the salmon industry, as commitment could be identified at earlier phases, making it possible to countermeasure the process.

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Appendix I. Overview of measurements

I - I Body weight

Table I - 1: *Body weight (grams, g); mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean (g)	SEM (g)	Min. (g)	Max. (g)
1 (31.10.18)	67%	8	5	34.31	1.917	30	40.8
		12.5	5	38.66	1.42	35.3	43.9
		18	6	45.93	3.149	35.1	54.46
	100%	8	6	39.36	3.801	28.5	50.7
		12.5	6	49.25	4.754	35.8	67.4
		18	6	53.84	3.165	41.7	62
2 (14.12.18)	67%	8	5	53.59	1.014	50.1	55.87
		12.5	6	74.30	3.630	61.05	84.27
		18	6	94.71	6.885	76.7	120
	100%	8	6	58.85	4.819	45.7	74.5
		12.5	6	93.55	8.21	68	125.8
		18	6	99.99	3.748	88.9	113.4
3 (01.02.19)	67%	8	6	74.15	4.908	60.89	93.6
		12.5	6	133.51	3.215	121.1	140
		18	6	158.75	9.245	120.7	178.5
	100%	8	6	87.073	5.362	67.4	99.2
		12.5	6	150.36	13.034	102	193.1
		18	6	162.51	7.019	140	180.2
4 (13.03.19)	67%	8	9	94.03	4.0139	73.4	115.49
		12.5	9	200.87	6.472	160	221
		18	10	249.15	16.074	175	323
	100%	8	10	109.36	12.420	55.76	175.08
		12.5	10	332.1	20.501	244	468
		18	9	274.51	17.382	192.66	366.89
5 (27.03.19)	67%	8	10	97.25	5.265	75.06	119.21
		12.5	10	278.07	14.023	221	339
		18	9	212.06	18.062	122.1	315
	100%	8	9	119.63	5.725	94.44	154.82
		12.5	6	328.83	3.439	318	342
		18	10	301.68	32.433	143.1	470.4
6 (10.04.19)	67%	8	10	117.83	11.546	63.62	185.11
		12.5	7	297.99	5.591	283.96	324.96
		18	10	298.07	27.116	187.54	458.51
	100%	8	10	133.38	6.696	113.85	169.15
		12.5	10	402.94	38.943	251.04	621.55
		18	10	350.82	31.624	212.57	506.79
7 (24.04.19)	67%	8	8	170.55	18.918	113.4	272.7
		12.5	10	282.6	19.879	191	405
		18	10	361	35.198	215	538
	100%	8	10	164.21	15.15	81.76	239
		12.5	10	447.99	25.213	330	551
		18	10	427.79	37.145	260.85	613.72
8 (15.05.19)	67%	8	10	179.4	17.296	110	284
		12.5	10	370.2	34.859	221	585
		18	11	379.18	11.998	300	453
	100%	8	10	178.96	15.5	113	276.5
		12.5	6	606.81	16.049	564.89	666
		18	13	425.07	28.522	269	608

I - II Fork length

Table I - 2: *Fork length (centimeter, cm); mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean (cm)	SEM (cm)	Min. (cm)	Max. (cm)
1 (31.10.18)	67%	8	5	14.58	0.190	14	15.2
		12.5	6	14.86	0.326	14.2	16.3
		18	5	15.46	0.267	14.8	16.2
	100%	8	6	15.2	0.367	14.2	16.5
		12.5	6	15.75	0.54	14.2	17.6
		18	6	16.15	0.3	15.2	17
2 (14.12.18)	67%	8	5	16.6	0.114	16.2	16.8
		12.5	6	18	0.243	17.2	18.7
		18	6	19.05	0.362	18	20.2
	100%	8	6	16.86	0.468	15.6	18.3
		12.5	6	19.55	0.699	17.7	22.5
		18	6	19.41	0.262	18.7	20.4
3 (01.02.19)	67%	8	6	17.96	0.429	17	19.5
		12.5	6	22.4	0.208	21.9	23
		18	6	22.78	0.493	20.7	23.9
	100%	8	5	19.48	0.287	18.5	20.3
		12.5	6	23.28	0.685	20.8	25.6
		18	6	22.73	0.424	21.5	23.9
4 (13.03.19)	67%	8	9	19.56	0.275	18.2	21
		12.5	8	26.25	0.211	25.5	27.5
		18	10	26.28	0.619	23.2	28.9
	100%	8	10	20.35	0.756	16.5	24
		12.5	10	30.02	0.657	26.5	33.5
		18	9	27.01	0.543	24.4	29.5
5 (27.03.19)	67%	8	10	20.03	0.385	18.2	21.5
		12.5	10	29.44	0.579	27.2	31.5
		18	10	25.98	0.831	21.5	30.5
	100%	8	10	21.37	0.322	19.9	23.2
		12.5	6	30.1	0.146	29.5	30.5
		18	9	28.28	0.773	25.8	32.7
6 (10.04.19)	67%	8	10	21.27	0.718	17.4	24.9
		12.5	6	29.78	0.107	29.5	30.2
		18	10	27.97	0.728	23.8	31.9
	100%	8	10	22.27	0.374	20.7	23.9
		12.5	10	31.94	0.955	27.6	35.9
		18	10	29.23	0.835	25.4	33.5
7 (24.04.19)	67%	8	8	24.225	0.915	21.5	28.5
		12.5	10	29.22	0.680	25.5	32.8
		18	10	29.37	0.817	25.3	33
	100%	8	9	24.455	0.653	21.8	27.5
		12.5	10	33.2	0.482	31.2	35.5
		18	10	30.89	0.873	27.4	35.4
8 (15.05.19)	67%	8	10	24.48	0.801	20.8	29.2
		12.5	11	31.9	0.953	27	37.5
		18	12	30.39	0.330	28.8	32.8
	100%	8	10	24.68	0.695	21.5	28.5
		12.5	6	36.31	0.404	35	37.5
		18	13	31.38	0.602	28.5	35.5

I - III Condition factor (CF)

Table I - 3: *Condition factor (CF); mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	6	1.088	0.029	0.992	1.161
		12.5	5	1.225	0.011	1.187	1.25
		18	5	1.295	0.021	1.25	1.374
	100%	8	6	1.102	0.034	0.995	1.206
		12.5	5	1.221	0.015	1.180	1.255
		18	4	1.274	0.008	1.261	1.299
2 (14.12.18)	67%	8	6	1.166	0.005	1.141	1.178
		12.5	6	1.268	0.022	1.199	1.358
		18	6	1.358	0.038	1.254	1.5
	100%	8	4	1.211	0.009	1.191	1.234
		12.5	5	1.269	0.018	1.223	1.305
		18	6	1.363	0.009	1.335	1.402
3 (01.02.19)	67%	8	6	1.271	0.018	1.202	1.327
		12.5	5	1.163	0.016	1.117	1.211
		18	6	1.335	0.018	1.265	1.387
	100%	8	6	1.232	0.023	1.176	1.304
		12.5	6	1.175	0.013	1.133	1.217
		18	6	1.381	0.027	1.273	1.454
4 (13.03.19)	67%	8	10	1.250	0.02	1.170	1.387
		12.5	10	1.136	0.02	1.053	1.257
		18	10	1.357	0.01	1.321	1.401
	100%	8	10	1.245	0.015	1.173	1.305
		12.5	10	1.216	0.017	1.141	1.311
		18	10	1.375	0.018	1.258	1.452
5 (27.03.19)	67%	8	9	1.207	0.009	1.159	1.245
		12.5	10	1.083	0.014	1.023	1.157
		18	10	1.26	0.015	1.177	1.338
	100%	8	10	1.257	0.012	1.198	1.323
		12.5	9	1.189	0.015	1.099	1.258
		18	10	1.37	0.029	1.277	1.579
6 (10.04.19)	67%	8	10	1.188	0.009	1.145	1.225
		12.5	9	1.108	0.006	1.083	1.142
		18	10	1.328	0.035	1.156	1.525
	100%	8	10	1.2	0.016	1.121	1.283
		12.5	9	1.185	0.009	1.141	1.231
		18	10	1.369	0.029	1.194	1.499
7 (24.04.19)	67%	8	8	1.17	0.018	1.103	1.254
		12.5	9	1.126	0.007	1.09	1.152
		18	10	1.383	0.03	1.209	1.567
	100%	8	9	1.158	0.009	1.128	1.206
		12.5	8	1.219	0.014	1.175	1.285
		18	10	1.42	0.028	1.268	1.54
8 (15.05.19)	67%	8	10	1.191	0.014	1.14	1.291
		12.5	11	1.175	0.02	1.077	1.307
		18	12	1.38	0.021	1.255	1.49
	100%	8	10	1.164	0.013	1.111	1.251
		12.5	6	1.241	0.01	1.219	1.281
		18	13	1.35	0.027	1.162	1.48

I - IV Hepatosomatic index (HSI)

Table I - 4: *Hepatosomatic index (%); mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	6	1.543	0.098	1.274	1.871
		12.5	4	1.399	0.027	1.34	1.471
		18	6	1.249	0.056	1.115	1.467
	100%	8	5	1.356	0.04	1.27	1.485
		12.5	5	1.276	0.049	1.101	1.372
		18	5	1.028	0.019	0.988	1.095
2 (14.12.18)	67%	8	5	1.241	0.013	1.195	1.276
		12.5	5	1.116	0.017	1.052	1.15
		18	6	0.988	0.03	0.912	1.102
	100%	8	5	1.275	0.083	1.068	1.481
		12.5	6	1.037	0.024	0.971	1.138
		18	6	1.064	0.03	0.962	1.158
3 (01.02.19)	67%	8	6	1.167	0.075	0.951	1.378
		12.5	6	0.851	0.036	0.718	0.965
		18	6	0.868	0.046	0.743	1.063
	100%	8	6	1.19	0.059	1.037	1.396
		12.5	4	0.853	0.01	0.823	0.873
		18	6	0.919	0.064	0.642	1.053
4 (13.03.19)	67%	8	10	1.078	0.037	0.931	1.302
		12.5	9	0.902	0.026	0.775	1.013
		18	10	0.913	0.034	0.774	1.119
	100%	8	10	1.043	0.024	0.9	1.183
		12.5	10	0.911	0.025	0.783	1.058
		18	10	0.862	0.0331	0.681	1.016
5 (27.03.19)	67%	8	9	0.899	0.016	0.833	0.993
		12.5	10	0.955	0.018	0.87	1.049
		18	10	0.737	0.019	0.648	0.846
	100%	8	10	0.935	0.022	0.811	1.054
		12.5	9	0.785	0.031	0.657	0.92
		18	10	0.673	0.027	0.583	0.817
6 (10.04.19)	67%	8	10	0.938	0.029	0.828	1.105
		12.5	10	0.833	0.011	0.765	0.89
		18	10	0.839	0.058	0.58	1.17
	100%	8	10	0.883	0.037	0.681	1.033
		12.5	10	0.88	0.04	0.692	1.12
		18	10	0.829	0.033	0.676	0.984
7 (24.04.19)	67%	8	8	0.908	0.021	0.817	0.986
		12.5	9	0.833	0.019	0.728	0.908
		18	10	0.898	0.053	0.643	1.224
	100%	8	9	0.862	0.017	0.801	0.964
		12.5	10	0.837	0.036	0.658	1.009
		18	10	0.839	0.027	0.729	0.971
8 (15.05.19)	67%	8	10	0.917	0.013	0.839	0.981
		12.5	11	0.934	0.025	0.819	1.056
		18	12	0.84	0.033	0.69	1.029
	100%	8	10	0.863	0.016	0.795	0.971
		12.5	6	0.841	0.027	0.777	0.942
		18	12	0.708	0.021	0.608	0.813

I - V Gonadosomatic index (GSI)

Table I - 5: *Gonadosomatic index (%); mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	6	0.032	0.002	0.021	0.04
		12.5	6	0.035	0.004	0.023	0.053
		18	6	0.029	0.003	0.021	0.043
	100%	8	5	0.025	0.001	0.022	0.028
		12.5	6	0.033	0.003	0.02	0.043
		18	6	0.034	0.004	0.023	0.052
2 (14.12.18)	67%	8	5	0.028	0.001	0.023	0.032
		12.5	5	0.031	0.0009	0.028	0.034
		18	5	0.042	0.001	0.039	0.046
	100%	8	5	0.035	0.002	0.03	0.0437
		12.5	5	0.032	0.0008	0.029	0.034
		18	6	0.035	0.001	0.031	0.039
3 (01.02.19)	67%	8	6	0.03	0.003	0.021	0.041
		12.5	6	0.03	0.001	0.025	0.035
		18	5	0.039	0.0007	0.037	0.04
	100%	8	6	0.029	0.0008	0.025	0.031
		12.5	5	0.031	0.001	0.026	0.035
		18	6	0.043	0.001	0.037	0.05
4 (13.03.19)	67%	8	10	0.032	0.001	0.022	0.041
		12.5	10	0.036	0.001	0.03	0.042
		18	10	0.042	0.0008	0.038	0.047
	100%	8	10	0.03	0.001	0.02	0.038
		12.5	10	0.039	0.002	0.031	0.054
		18	7	0.046	0.001	0.04	0.048
5 (27.03.19)	67%	8	10	0.032	0.001	0.025	0.038
		12.5	9	0.034	0.001	0.03	0.038
		18	10	0.045	0.002	0.035	0.059
	100%	8	9	0.034	0.001	0.029	0.043
		12.5	9	0.035	0.0009	0.03	0.039
		18	10	0.048	0.002	0.038	0.059
6 (10.04.19)	67%	8	10	0.039	0.002	0.029	0.052
		12.5	9	0.033	0.0008	0.029	0.037
		18	9	0.099	0.021	0.048	0.223
	100%	8	10	0.039	0.002	0.029	0.055
		12.5	9	0.038	0.001	0.031	0.05
		18	8	0.135	0.017	0.049	0.204
7 (24.04.19)	67%	8	7	0.032	0.0009	0.028	0.035
		12.5	9	0.036	0.001	0.032	0.041
		18	10	0.624	0.217	0.045	2.072
	100%	8	9	0.034	0.001	0.027	0.045
		12.5	9	0.036	0.002	0.025	0.045
		18	10	0.78	0.264	0.057	2.589
8 (15.05.19)	67%	8	10	0.03	0.001	0.022	0.039
		12.5	8	0.039	0.001	0.033	0.043
		18	12	1.096	0.417	0.06	3.757
	100%	8	10	0.03	0.001	0.024	0.035
		12.5	6	0.102	0.061	0.033	0.411
		18	13	2.268	0.378	0.043	4.09

I - VI Relative *follicle stimulating hormone receptor* (*fshr*) mRNA transcription

Table I - 6: *Relative fshr mRNA abundance; mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint used for RT-qPCR is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	4	3.14E-02	3.42E-03	2.44E-02	3.84E-02
		12.5	5	1.60E-02	1.20E-03	1.28E-02	1.91E-02
		18	3	1.81E-02	6.12E-04	1.72E-02	1.92E-02
	100%	8	5	2.11E-02	3.03E-03	1.18E-02	3.07E-02
		12.5	5	1.58E-02	2.20E-03	9.26E-03	2.30E-02
		18	6	1.58E-02	1.19E-03	1.19E-02	1.89E-02
2 (14.12.18)	67%	8	6	2.55E-02	3.31E-03	1.98E-02	3.91E-02
		12.5	5	1.64E-02	1.24E-03	1.30E-02	1.89E-02
		18	5	1.57E-02	4.80E-04	1.40E-02	1.69E-02
	100%	8	7	2.19E-02	2.98E-03	1.17E-02	3.41E-02
		12.5	4	1.33E-02	2.89E-04	1.27E-02	1.39E-02
		18	5	1.64E-02	7.55E-04	1.49E-02	1.90E-02
3 (01.02.19)	67%	8	6	1.91E-02	3.72E-03	5.15E-03	3.11E-02
		12.5	3	1.59E-02	6.83E-04	1.48E-02	1.71E-02
		18	5	1.99E-02	1.41E-03	1.59E-02	2.40E-02
	100%	8	5	2.47E-02	4.82E-03	1.41E-02	3.91E-02
		12.5	5	1.67E-02	2.11E-03	1.30E-02	2.48E-02
		18	5	1.91E-02	3.95E-03	1.22E-02	3.46E-02
4 (13.03.19)	67%	8	6	2.14E-02	2.36E-03	1.62E-02	3.07E-02
		12.5	10	1.65E-02	1.34E-03	9.26E-03	2.22E-02
		18	6	1.85E-02	4.46E-03	1.07E-02	3.97E-02
	100%	8	6	1.70E-02	1.39E-03	1.15E-02	2.06E-02
		12.5	6	1.90E-02	1.95E-03	1.30E-02	2.60E-02
		18	8	1.63E-02	1.09E-03	1.24E-02	2.15E-02
5 (27.03.19)	67%	8	8	1.56E-02	2.38E-03	5.59E-03	2.59E-02
		12.5	9	8.92E-03	1.08E-03	4.31E-03	1.30E-02
		18	10	1.31E-02	8.51E-04	8.97E-03	1.75E-02
	100%	8	7	2.13E-02	1.54E-03	1.47E-02	2.79E-02
		12.5	6	1.19E-02	4.59E-04	9.97E-03	1.32E-02
		18	8	1.48E-02	1.14E-03	8.87E-03	1.94E-02
6 (10.04.19)	67%	8	7	9.17E-03	1.19E-03	5.81E-03	1.33E-02
		12.5	4	9.05E-03	1.02E-03	7.80E-03	1.21E-02
		18	8	9.14E-03	1.78E-03	2.98E-03	1.47E-02
	100%	8	5	1.34E-02	9.34E-04	1.05E-02	1.63E-02
		12.5	10	1.16E-02	1.43E-03	3.65E-03	1.94E-02
		18	10	6.80E-03	1.33E-03	1.69E-03	1.24E-02
7 (24.04.19)	67%	8	3	1.36E-02	6.50E-04	1.26E-02	1.48E-02
		12.5	10	1.15E-02	5.27E-04	9.63E-03	1.45E-02
		18	9	8.23E-03	2.39E-03	1.52E-03	1.73E-02
	100%	8	9	1.41E-02	8.53E-04	8.90E-03	1.96E-02
		12.5	9	1.25E-02	1.51E-03	6.15E-03	1.92E-02
		18	8	3.53E-03	7.87E-04	1.43E-03	7.19E-03
8 (15.05.19)	67%	8	8	1.57E-02	2.01E-03	1.01E-02	2.88E-02
		12.5	10	1.05E-02	5.09E-04	8.55E-03	1.40E-02
		18	10	8.49E-03	1.51E-03	1.52E-03	1.42E-02
	100%	8	7	1.54E-02	1.10E-03	1.13E-02	1.90E-02
		12.5	6	9.16E-03	2.32E-03	5.42E-04	1.43E-02
		18	12	4.94E-03	1.10E-03	1.55E-03	1.16E-02

I - VII Relative *luteinizing hormone receptor* (*lhr*) mRNA transcription

Table I - 7: *Relative lhr mRNA abundance; mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint used for RT-qPCR is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	4	3.95E-03	7.04E-04	2.25E-03	5.25E-03
		12.5	5	1.66E-03	4.12E-04	2.12E-04	2.67E-03
		18	5	2.29E-03	4.45E-04	7.52E-04	3.24E-03
	100%	8	6	2.35E-03	4.72E-04	6.06E-04	3.79E-03
		12.5	6	3.56E-03	1.01E-03	1.33E-03	7.72E-03
		18	6	4.36E-03	1.07E-03	1.85E-03	8.59E-03
2 (14.12.18)	67%	8	6	2.92E-03	6.39E-04	7.92E-04	5.30E-03
		12.5	5	2.03E-03	5.97E-04	9.67E-04	4.26E-03
		18	6	3.18E-03	1.12E-03	4.88E-04	7.90E-03
	100%	8	7	3.12E-03	6.99E-04	8.20E-04	6.14E-03
		12.5	5	3.19E-03	1.22E-03	6.09E-04	6.57E-03
		18	6	5.47E-03	1.36E-03	1.04E-03	1.01E-02
3 (01.02.19)	67%	8	6	2.24E-03	6.46E-04	1.12E-04	4.08E-03
		12.5	2	1.44E-03	1.06E-03	3.77E-04	2.50E-03
		18	6	6.04E-03	1.16E-03	2.08E-03	9.00E-03
	100%	8	6	4.00E-03	1.50E-03	1.05E-03	1.05E-02
		12.5	5	3.46E-03	9.38E-04	7.85E-04	6.26E-03
		18	6	5.65E-03	1.87E-03	1.34E-03	1.34E-02
4 (13.03.19)	67%	8	7	1.28E-03	3.28E-04	3.86E-07	2.34E-03
		12.5	8	4.20E-03	1.33E-03	2.93E-04	9.54E-03
		18	7	8.74E-03	2.15E-03	1.07E-03	1.63E-02
	100%	8	7	3.59E-03	1.09E-03	5.57E-04	8.09E-03
		12.5	7	6.44E-03	5.64E-04	4.07E-03	8.10E-03
		18	10	7.81E-03	9.48E-04	2.30E-03	1.25E-02
5 (27.03.19)	67%	8	9	3.87E-03	8.90E-04	3.62E-04	9.01E-03
		12.5	7	1.06E-03	2.10E-04	2.89E-04	1.73E-03
		18	9	6.15E-03	1.19E-03	1.62E-03	1.05E-02
	100%	8	10	3.63E-03	1.08E-03	3.66E-04	9.16E-03
		12.5	7	5.03E-03	8.32E-04	1.80E-03	8.82E-03
		18	9	7.58E-03	9.85E-04	3.50E-03	1.14E-02
6 (10.04.19)	67%	8	3	1.16E-03	5.87E-05	1.05E-03	1.23E-03
		12.5	6	3.63E-03	1.16E-03	1.03E-03	7.28E-03
		18	8	6.16E-03	1.70E-03	7.19E-04	1.46E-02
	100%	8	6	1.33E-03	3.21E-04	6.64E-04	2.47E-03
		12.5	8	7.50E-03	1.25E-03	1.24E-03	1.11E-02
		18	10	6.50E-03	1.49E-03	5.54E-04	1.52E-02
7 (24.04.19)	67%	8	3	1.01E-03	1.69E-04	7.62E-04	1.34E-03
		12.5	11	4.88E-03	9.61E-04	7.07E-04	8.67E-03
		18	10	7.07E-03	2.14E-03	1.00E-03	1.86E-02
	100%	8	9	3.94E-03	7.58E-04	7.21E-04	6.93E-03
		12.5	7	6.59E-03	4.83E-04	4.68E-03	8.79E-03
		18	10	3.85E-03	9.88E-04	6.28E-04	9.51E-03
8 (15.05.19)	67%	8	10	3.04E-03	6.25E-04	7.41E-04	5.77E-03
		12.5	10	5.37E-03	9.62E-04	1.25E-03	9.67E-03
		18	10	3.62E-03	8.39E-04	8.32E-04	9.01E-03
	100%	8	8	6.79E-03	1.04E-03	1.93E-03	1.13E-02
		12.5	5	6.25E-03	4.77E-04	5.23E-03	7.81E-03
		18	11	1.76E-03	1.75E-04	9.59E-04	2.68E-03

I - VIII Relative *Anti-Müllerian hormone* (*amh*) mRNA transcription

Table I - 8: *Relative Amh mRNA abundance; mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint used for RT-qPCR is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	5	4.545	0.306	3.543	5.372
		12.5	6	2.876	0.204	2.127	3.484
		18	6	1.83	0.149	1.295	2.322
	100%	8	6	4.079	0.369	2.688	4.993
		12.5	5	2.896	0.22	2.294	3.423
		18	5	1.885	0.099	1.616	2.099
2 (14.12.18)	67%	8	5	3.423	0.175	2.929	3.929
		12.5	4	3.336	0.058	3.255	3.508
		18	6	1.865	0.189	1.134	2.369
	100%	8	7	3.842	0.534	1.516	5.635
		12.5	6	2.689	0.186	1.997	3.19
		18	5	2.006	0.047	1.837	2.104
3 (01.02.19)	67%	8	5	2.593	0.175	2.252	3.266
		12.5	3	2.471	0.196	2.086	2.732
		18	6	1.666	0.245	1.056	2.484
	100%	8	5	4.072	0.178	3.596	4.582
		12.5	6	1.851	0.305	0.945	3.053
		18	5	1.763	0.190	1.375	2.335
4 (13.03.19)	67%	8	7	3.115	0.292	1.828	4.043
		12.5	10	2.394	0.092	1.873	2.82
		18	7	1.553	0.144	1.046	2.054
	100%	8	6	2.962	0.088	2.693	3.334
		12.5	8	2.038	0.089	1.713	2.355
		18	10	1.513	0.111	0.955	2.056
5 (27.03.19)	67%	8	6	3.652	0.118	3.38	4.153
		12.5	10	1.797	0.138	0.946	2.311
		18	10	1.534	0.172	0.853	2.673
	100%	8	10	3.840	0.233	2.608	4.686
		12.5	7	2.416	0.060	2.271	2.729
		18	9	1.443	0.271	0.52	2.423
6 (10.04.19)	67%	8	6	3.421	0.193	2.687	3.94
		12.5	4	1.535	0.161	1.086	1.836
		18	10	0.608	0.147	0.012	1.245
	100%	8	4	3.514	0.049	3.388	3.599
		12.5	10	1.573	0.304	0.547	3.297
		18	8	0.133	0.053	0.007	0.462
7 (24.04.19)	67%	8	4	2.544	0.063	2.367	2.65
		12.5	9	1.741	0.085	1.3	2.082
		18	10	0.447	0.182	0.009	1.408
	100%	8	10	3.052	0.193	2.148	4.037
		12.5	10	2.338	0.343	0.197	4.16
		18	9	0.093	0.033	0.019	0.289
8 (15.05.19)	67%	8	10	2.814	0.178	1.984	3.519
		12.5	11	2.006	0.117	1.535	2.833
		18	12	0.647	0.221	0.01	2.32
	100%	8	8	3.694	0.231	2.560	4.525
		12.5	7	1.344	0.381	0.008	2.54
		18	10	0.022	0.003	0.007	0.038

I - IX Relative *gonadal soma-derived factor 1* (*gsdf1*) mRNA transcription

Table I - 9: *Relative gsdf1 mRNA abundance; mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint used for RT-qPCR is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	5	7.613	0.772	5.973	10.326
		12.5	6	6.081	0.839	4.170	8.796
		18	6	6.717	1.311	3.615	10.878
	100%	8	6	5.575	0.618	3.611	7.545
		12.5	5	4.662	0.447	3.316	5.950
		18	6	6.275	1.072	2.929	9.315
2 (14.12.18)	67%	8	6	9.545	2.649	4.623	20.324
		12.5	6	5.570	0.391	4.633	7.259
		18	6	5.355	0.463	4.264	7.067
	100%	8	7	5.784	0.574	3.210	7.933
		12.5	5	4.708	0.331	3.917	5.557
		18	6	5.898	0.521	4.177	7.634
3 (01.02.19)	67%	8	5	4.904	0.174	4.566	5.574
		12.5	3	5.273	0.661	4.300	6.534
		18	4	6.103	0.077	5.927	6.234
	100%	8	6	6.966	1.249	4.294	11.940
		12.5	6	5.650	1.110	1.562	9.542
		18	5	4.110	0.186	3.756	4.741
4 (13.03.19)	67%	8	7	5.012	0.278	3.734	6.007
		12.5	9	5.725	0.402	4.431	8.351
		18	6	4.189	0.093	3.927	4.542
	100%	8	7	5.117	0.391	3.355	6.253
		12.5	8	6.508	0.526	4.109	8.578
		18	10	5.135	0.234	4.370	6.532
5 (27.03.19)	67%	8	8	4.707	0.373	3.311	6.099
		12.5	10	3.011	0.249	1.679	4.399
		18	8	3.137	0.189	2.324	3.833
	100%	8	10	5.753	0.625	3.604	8.739
		12.5	9	4.993	0.612	2.777	8.063
		18	10	3.886	0.512	1.716	6.323
6 (10.04.19)	67%	8	6	3.832	0.291	2.932	4.608
		12.5	6	3.660	0.851	0.119	5.937
		18	10	1.791	0.606	0.032	5.538
	100%	8	5	4.432	0.194	3.704	4.820
		12.5	10	3.132	0.470	1.405	6.092
		18	10	1.476	0.483	0.118	4.345
7 (24.04.19)	67%	8	4	3.531	0.429	2.532	4.552
		12.5	10	3.370	0.196	2.243	4.321
		18	10	1.393	0.575	0.032	4.814
	100%	8	10	5.831	0.615	2.781	9.335
		12.5	8	4.524	0.249	3.488	5.497
		18	9	0.425	0.158	0.031	1.414
8 (15.05.19)	67%	8	9	3.461	0.208	2.405	4.377
		12.5	10	4.244	0.239	2.977	5.536
		18	12	1.986	0.545	0.046	4.861
	100%	8	7	3.638	0.284	2.292	4.446
		12.5	7	3.144	0.913	0.039	5.993
		18	11	0.110	0.015	0.045	0.215

I - X Relative *gonadal soma-derived factor 2* (*gsdf2*) mRNA transcription

Table I - 10: *Relative gsdf2 mRNA abundance; mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime. temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint used for RT-qPCR is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	5	0.747	0.266	0.004	1.612
		12.5	6	0.469	0.032	0.341	0.551
		18	6	0.591	0.048	0.440	0.748
	100%	8	5	0.343	0.055	0.172	0.463
		12.5	5	0.416	0.058	0.323	0.642
		18	4	0.516	0.015	0.471	0.537
2 (14.12.18)	67%	8	5	0.332	0.029	0.254	0.396
		12.5	6	0.487	0.030	0.432	0.594
		18	6	0.773	0.050	0.587	0.905
	100%	8	6	0.428	0.042	0.286	0.584
		12.5	6	0.329	0.042	0.203	0.480
		18	6	0.574	0.057	0.369	0.747
3 (01.02.19)	67%	8	4	0.244	0.030	0.155	0.282
		12.5	3	0.509	0.015	0.479	0.530
		18	5	0.825	0.035	0.753	0.958
	100%	8	6	0.248	0.071	0.028	0.413
		12.5	6	0.610	0.096	0.373	0.969
		18	7	0.536	0.141	0.002	1.073
4 (13.03.19)	67%	8	6	0.326	0.029	0.199	0.389
		12.5	10	0.610	0.043	0.422	0.832
		18	7	0.432	0.088	0.042	0.625
	100%	8	7	0.273	0.059	0.031	0.492
		12.5	7	0.472	0.073	0.105	0.701
		18	8	0.674	0.040	0.535	0.853
5 (27.03.19)	67%	8	9	0.312	0.052	0.019	0.541
		12.5	9	0.360	0.024	0.250	0.468
		18	10	0.519	0.051	0.250	0.810
	100%	8	10	0.348	0.052	0.060	0.620
		12.5	9	0.496	0.037	0.331	0.661
		18	11	0.614	0.058	0.403	0.968
6 (10.04.19)	67%	8	6	0.223	0.021	0.172	0.312
		12.5	5	0.535	0.020	0.467	0.592
		18	8	0.450	0.127	0.017	0.798
	100%	8	6	0.267	0.025	0.166	0.347
		12.5	9	0.464	0.074	0.146	0.864
		18	9	0.431	0.125	0.030	1.084
7 (24.04.19)	67%	8	4	0.185	0.049	0.054	0.281
		12.5	11	0.425	0.039	0.175	0.586
		18	10	0.359	0.141	0.008	1.223
	100%	8	10	0.294	0.021	0.189	0.372
		12.5	10	0.509	0.064	0.163	0.790
		18	8	0.095	0.024	0.012	0.216
8 (15.05.19)	67%	8	8	0.249	0.013	0.193	0.284
		12.5	11	0.478	0.029	0.356	0.647
		18	11	0.260	0.084	0.006	0.866
	100%	8	8	0.288	0.032	0.161	0.423
		12.5	7	0.307	0.099	0.006	0.632
		18	13	0.302	0.106	0.019	1.145

I - XI Relative *insulin-like growth factor 3* (*igf3*) mRNA transcription

Table I - 11: *Relative igf3 mRNA abundance; mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime. temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint used for RT-qPCR is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean	SEM	Min.	Max.
1 (31.10.18)	67%	8	3	1.34E-03	2.52E-04	8.46E-04	1.66E-03
		12.5	3	1.12E-03	4.02E-04	6.53E-04	1.92E-03
		18	5	1.09E-03	2.28E-04	5.22E-04	1.74E-03
	100%	8	4	8.66E-04	2.41E-04	4.50E-04	1.54E-03
		12.5	5	9.77E-04	2.12E-04	4.94E-04	1.63E-03
		18	2	7.71E-04	9.77E-05	6.73E-04	8.68E-04
2 (14.12.18)	67%	8	4	2.12E-03	6.05E-04	1.13E-03	3.86E-03
		12.5	6	1.20E-03	1.67E-04	7.66E-04	1.76E-03
		18	3	1.49E-03	5.15E-05	1.44E-03	1.59E-03
	100%	8	5	1.43E-03	3.14E-04	5.58E-04	2.20E-03
		12.5	3	1.04E-03	1.54E-05	1.01E-03	1.07E-03
		18	3	1.48E-03	5.24E-04	8.65E-04	2.52E-03
3 (01.02.19)	67%	8	3	6.12E-04	1.89E-05	5.92E-04	6.50E-04
		12.5	2	1.53E-03	1.09E-03	4.46E-04	2.62E-03
		18	6	8.82E-04	1.42E-04	4.49E-04	1.24E-03
	100%	8	6	1.15E-03	9.91E-05	8.29E-04	1.44E-03
		12.5	5	9.55E-04	1.64E-04	5.40E-04	1.39E-03
		18	4	1.50E-03	3.49E-04	8.42E-04	2.14E-03
4 (13.03.19)	67%	8	6	1.81E-03	6.47E-04	5.49E-04	4.54E-03
		12.5	3	7.01E-04	9.12E-05	5.23E-04	8.25E-04
		18	6	1.14E-03	1.95E-04	5.60E-04	1.86E-03
	100%	8	5	1.17E-03	1.96E-04	5.36E-04	1.60E-03
		12.5	6	1.14E-03	2.04E-04	6.58E-04	2.08E-03
		18	7	1.31E-03	3.23E-04	2.96E-04	2.73E-03
5 (27.03.19)	67%	8	7	1.04E-03	1.61E-04	6.16E-04	1.76E-03
		12.5	6	1.17E-02	1.07E-02	4.66E-04	6.50E-02
		18	6	2.86E-03	1.16E-03	7.67E-04	7.78E-03
	100%	8	7	1.40E-03	1.63E-04	7.51E-04	2.03E-03
		12.5	7	1.94E-02	9.89E-03	4.23E-04	6.46E-02
		18	4	5.36E-03	2.32E-03	9.26E-04	1.13E-02
6 (10.04.19)	67%	8	5	9.78E-04	9.36E-05	7.64E-04	1.27E-03
		12.5	5	1.37E-03	1.78E-04	8.95E-04	1.77E-03
		18	8	4.07E-02	1.39E-02	8.63E-04	9.35E-02
	100%	8	4	6.43E-04	7.14E-05	4.85E-04	7.82E-04
		12.5	7	6.11E-03	1.68E-03	2.07E-03	1.51E-02
		18	9	4.32E-02	7.72E-03	7.82E-03	8.25E-02
7 (24.04.19)	67%	8	4	1.11E-03	3.39E-04	6.08E-04	2.06E-03
		12.5	6	1.45E-03	4.01E-04	1.08E-04	2.64E-03
		18	7	1.05E-02	1.16E-03	7.68E-03	1.58E-02
	100%	8	5	1.05E-03	1.18E-04	8.04E-04	1.50E-03
		12.5	3	7.80E-03	1.88E-03	4.05E-03	9.92E-03
		18	10	5.48E-02	1.93E-02	5.39E-03	1.71E-01
8 (15.05.19)	67%	8	6	9.54E-04	1.25E-04	4.37E-04	1.25E-03
		12.5	4	1.55E-03	7.52E-04	4.49E-04	3.77E-03
		18	10	1.08E-02	3.90E-03	4.64E-04	3.37E-02
	100%	8	6	1.65E-03	3.34E-04	6.14E-04	2.48E-03
		12.5	5	5.73E-03	2.11E-03	1.94E-03	1.29E-02
		18	13	2.60E-02	5.30E-03	2.80E-03	6.75E-02

I - XII Plasma 11-Ketotestosterone (11-KT) concentration

Table I - 12: *Plasma 11-KT (ng/ml); mean, standard error of mean (SEM), minimum (min.) and maximum (max.) values measured for each sampling point dependent on group (feeding regime, temperature) in Atlantic salmon. Number of individuals sampled (n) in each group within each timepoint is included.*

Sampling (date)	Feeding regime	Temperature (°C)	n	Mean (ng/ml)	SEM (ng/ml)	Min. (ng/ml)	Max. (ng/ml)
1 (31.10.18)	67%	8	6	0.16	0.015	0.12	0.22
		12.5	5	0.152	0.016	0.11	0.21
		18	5	0.146	0.013	0.11	0.18
	100%	8	6	0.293	0.072	0.12	0.54
		12.5	6	0.213	0.024	0.13	0.27
		18	6	0.196	0.05	0.03	0.39
2 (14.12.18)	67%	8	5	0.242	0.036	0.13	0.35
		12.5	6	0.265	0.066	0.1	0.51
		18	6	0.291	0.039	0.16	0.41
	100%	8	6	0.266	0.015	0.21	0.32
		12.5	5	0.326	0.038	0.22	0.44
		18	6	0.273	0.023	0.22	0.37
3 (01.02.19)	67%	8	6	0.278	0.045	0.16	0.45
		12.5	6	0.411	0.039	0.29	0.53
		18	6	0.32	0.059	0.17	0.54
	100%	8	6	0.301	0.022	0.25	0.38
		12.5	6	0.33	0.031	0.22	0.44
		18	6	0.461	0.053	0.28	0.61
4 (13.03.19)	67%	8	10	0.427	0.04	0.26	0.65
		12.5	10	0.372	0.053	0.1	0.64
		18	9	0.33	0.038	0.17	0.54
	100%	8	8	0.448	0.028	0.32	0.58
		12.5	10	0.711	0.077	0.36	1.06
		18	10	0.29	0.028	0.14	0.42
5 (27.03.19)	67%	8	10	0.277	0.042	0.03	0.46
		12.5	9	0.463	0.049	0.21	0.7
		18	10	0.815	0.159	0.2	1.66
	100%	8	10	0.293	0.042	0.03	0.43
		12.5	8	0.55	0.048	0.33	0.78
		18	10	1.149	0.253	0.25	2.39
6 (10.04.19)	67%	8	10	0.462	0.044	0.28	0.74
		12.5	9	0.636	0.069	0.39	1.04
		18	10	1.978	0.532	0.21	5.01
	100%	8	10	0.417	0.057	0.19	0.66
		12.5	9	1.201	0.306	0.36	3.28
		18	10	2.596	0.394	0.26	4.23
7 (24.04.19)	67%	8	8	0.821	0.067	0.58	1.19
		12.5	7	0.638	0.031	0.51	0.73
		18	10	2.016	0.471	0.28	4.25
	100%	8	10	0.9	0.131	0.48	1.61
		12.5	9	0.678	0.077	0.31	0.98
		18	8	3.343	0.351	1.6	4.94
8 (15.05.19)	67%	8	10	0.893	0.075	0.64	1.31
		12.5	10	0.82	0.054	0.59	1.19
		18	11	1.632	0.355	0.3	3.4
	100%	8	9	0.81	0.04	0.63	1.06
		12.5	7	2.068	0.727	0.63	5.12
		18	13	2.55	0.441	0.03	4.51

Appendix II. Statistical analysis

Overall note: *Replicate tanks are represented as "parallel" in statistical tables.*

II – I Body weight

a. Overall tests:

Table II - 1: *Test results of a **three-way random effects nested ANOVA** for body weight, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	14301837,6	1	14301837,6	89,7459442	12606,062	1134,52064	0
Parallel (Temp.*feed*Time)	Random	1155782,92	85	13597,4462	295	3466,30039	3,9227547	0
Time	Fixed	3453753,81	7	493393,401	87,9109208	12963,3237	38,0607176	0
Temperature	Fixed	1599150,74	2	799575,369	89,5878052	12635,6931	63,2791067	0
Feeding regime	Fixed	176157,65	1	176157,65	89,7459442	12606,062	13,9740428	0,00032578
Error		1022558,62	295	3466,30039				

Table II - 2: *Test results of a **three-way factorial ANOVA** for body weight, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	14862132,6	1	14862132,6	4086,68094	0
Time	3690283,95	7	527183,422	144,961057	0
Temperature	1643966,59	2	821983,297	226,022979	0
Feed regime	192269,682	1	192269,682	52,8689165	2,4334E-12
Time*Temp.	633611,069	14	45257,9335	12,4446968	0
Time*Feed.	80643,5157	7	11520,5022	3,16782377	0,0029265
Temp.*Feed.	105371	2	52685,4919	14,487073	0,000001
Time*Temp.*Feed.	124829,384	14	8916,3846	2,45176248	0,00262536
Error	1247396,49	343	3636,72447		

b. Restrictive feeding (67%)

Table II - 3: *Test results of a **two-way random effects nested ANOVA** for body weight, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	5668178,18	1	5668178,18	40,4691785	7267,42197	779,943453	0
Parallel (Temp.*Time)	Random	292313,642	38	7692,46427	146	2671,41376	2,87954805	3,0174E-06
Time	Fixed	1342480,01	7	191782,858	3,89E+01	7526,44112	25,4812142	1,148E-12
Temperature	Fixed	523933,866	2	261966,933	40,4217796	7274,82115	36,0100857	1,0472E-09
Error		390026,408	146	2671,41376				

Table II - 4: *Test results of a **two-way factorial ANOVA** for body weight, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	5782267,25	1	5782267,25	2066,41472	0
Time	1344715,71	7	192102,244	68,6517738	0
Temperature	527074,684	2	263537,342	94,1806075	0
Temp.*Time	206643,945	14	14760,2818	5,27489689	2,93E-08
Error	475696,105	170	2798,21238		

Table II - 5: Test results of **one-*way random effects nested ANOVAs*** *for body weight, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	24074,12	1	24074,12	3,75488	4,06901	5916,453	0,000000
	Parallel (temp)	Random	11,01	3	3,67	10,00000	40,03405	0,092	0,962962
	Temperature	Fixed	391,91	2	195,95	3,39800	3,88458	50,444	0,002976
	Error		400,34	10	40,03				
2 (14.12.18)	Intercept	Fixed	91733,98	1	91733,98	3,05694	148,4279	618,0374	0,000125
	Parallel (temp)	Random	446,01	3	148,67	11,00000	126,5570	1,1747	0,363558
	Temperature	Fixed	4461,60	2	2230,80	3,02623	148,5581	15,0163	0,026841
	Error		1392,13	11	126,56				
3 (01.02.19)	Intercept	Fixed	268527,2	1	268527,2	3,00000	225,4182	1191,240	0,000053
	Parallel (temp)	Random	676,3	3	225,4	12,00000	243,4014	0,926	0,457851
	Temperature	Fixed	22634,6	2	11317,3	3,00000	225,4182	50,206	0,004941
	Error		2920,8	12	243,4				
4 (13.04.19)	Intercept	Fixed	908957,8	1	908957,8	3,08247	276,108	3292,032	0,000009
	Parallel (temp)	Random	819,7	3	273,2	22,00000	1209,686	0,226	0,877378
	Temperature	Fixed	117909,8	2	58954,9	3,04270	274,725	214,596	0,000531
	Error		26613,1	22	1209,7				
5 (27.03.19)	Intercept	Fixed	1121195	1	1121195	3,00235	7360,644	152,3229	0,001142
	Parallel (temp)	Random	22141	3	7380	23,00000	936,708	7,8792	0,000860
	Temperature	Fixed	168621	2	84310	3,00113	7370,939	11,4382	0,039447
	Error		21544	23	937				
6 (10.04.19)	Intercept	Fixed	1324089	1	1324089	3,67019	2242,533	590,4436	0,000034
	Parallel (temp)	Random	6481	3	2160	21,00000	3476,477	0,6215	0,608942
	Temperature	Fixed	196946	2	98473	3,26490	2195,026	44,8619	0,004222
	Error		73006	21	3476				
7 (24.04.19)	Intercept	Fixed	1968170	1	1968170	4,05938	958,452	2053,488	0,000001
	Parallel (temp)	Random	2513	3	838	22,00000	7481,862	0,112	0,952189
	Temperature	Fixed	162501	2	81250	3,46061	892,559	91,031	0,001018
	Error		164601	22	7482				
8 (15.05.19)	Intercept	Fixed	2987522	1	2987522	3,00496	17477,89	170,9316	0,000958
	Parallel (temp)	Random	52581	3	17527	25,00000	3981,95	4,4016	0,012839
	Temperature	Fixed	253583	2	126791	3,00258	17501,36	7,2447	0,070958
	Error		99549	25	3982				

Table II - 6: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for body weight between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,543528	0,031077
	12	0,543528		0,189222
	18	0,031077	0,189222	
2 (14.12.18)	8		0,028108	0,000405
	12	0,028108		0,023585
	18	0,000405	0,023585	
3 (01.02.19)	8		0,000249	0,000191
	12	0,000249		0,039615
	18	0,000191	0,039615	
4 (13.03.19)	8		0,000138	0,000136
	12	0,000138		0,016710
	18	0,000136	0,016710	
5 (27.03.19)	8		0,000133	0,000133
	12	0,000133		0,000399
	18	0,000133	0,000399	
6 (10.04.19)	8		0,000147	0,000142
	12	0,000147		0,999997
	18	0,000142	0,999997	
7 (24.04.19)	8		0,031588	0,000467
	12	0,031588		0,129426
	18	0,000467	0,129426	
8 (15.05.19)	8		0,000132	0,000132
	12	0,000132		0,943407
	18	0,000132	0,943407	

Table II - 7: *Test results of **Tukey HSD post-hoc tests** of difference for body weight between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,970475	0,400461	0,996808	0,997790	0,365326
	1	12	0,970475		0,799412	0,860536	0,999831	0,760989
	1	18	0,400461	0,799412		0,295444	0,740483	1,000000
	2	8	0,996808	0,860536	0,295444		0,961027	0,270095
	2	12	0,997790	0,999831	0,740483	0,961027		0,703726
	2	18	0,365326	0,760989	1,000000	0,270095	0,703726	
2 (14.12.18)	1	8		0,999638	0,625052	0,352663	0,007406	0,090069
	2	8	0,999638		0,355317	0,158111	0,002079	0,030212
	1	12	0,625052	0,355317		0,990463	0,051560	0,597218
	2	12	0,352663	0,158111	0,990463		0,128279	0,890603
	1	18	0,007406	0,002079	0,051560	0,128279		0,523966
	2	18	0,090069	0,030212	0,597218	0,890603	0,523966	
3 (01.02.19)	1	8		0,974803	0,021872	0,004996	0,000318	0,001230
	2	8	0,974803		0,006539	0,001644	0,000213	0,000496
	1	12	0,021872	0,006539		0,941272	0,086270	0,487186
	2	12	0,004996	0,001644	0,941272		0,331421	0,930587
	1	18	0,000318	0,000213	0,086270	0,331421		0,825957
	2	18	0,001230	0,000496	0,487186	0,930587	0,825957	
4 (13.03.19)	1	8		0,996932	0,002841	0,001749	0,000158	0,000146
	2	8	0,996932		0,001757	0,001134	0,000155	0,000146
	1	12	0,002841	0,001757		1,000000	0,531384	0,212392
	2	12	0,001749	0,001134	1,000000		0,440948	0,151351
	1	18	0,000158	0,000155	0,531384	0,440948		0,982739
	2	18	0,000146	0,000146	0,212392	0,151351	0,982739	
5 (27.03.19)	1	8		0,982952	0,000141	0,000141	0,000141	0,002639
	2	8	0,982952		0,000141	0,000141	0,000142	0,012704
	1	12	0,000141	0,000141		0,109313	0,238063	0,000148
	2	12	0,000141	0,000141	0,109313		0,999772	0,007960
	1	18	0,000141	0,000142	0,238063	0,999772		0,007085
	2	18	0,002639	0,012704	0,000148	0,007960	0,007085	
6 (10.04.19)	1	8		0,982827	0,000797	0,016387	0,000367	0,003057
	2	8	0,982827		0,001138	0,032328	0,000444	0,005299
	1	12	0,000797	0,001138		0,999815	0,996374	0,982847
	2	12	0,016387	0,032328	0,999815		0,987772	0,999858
	1	18	0,000367	0,000444	0,996374	0,987772		0,851686
	2	18	0,003057	0,005299	0,982847	0,999858	0,851686	
7 (24.04.19)	1	8		0,994410	0,328823	0,313603	0,024529	0,038094
	2	8	0,994410		0,485417	0,463997	0,029069	0,048016
	1	12	0,328823	0,485417		1,000000	0,626420	0,763434
	2	12	0,313603	0,463997	1,000000		0,648651	0,783092
	1	18	0,024529	0,029069	0,626420	0,648651		0,999895
	2	18	0,038094	0,048016	0,763434	0,783092	0,999895	
8 (15.05.19)	1	8		0,841869	0,000259	0,131302	0,009164	0,002363
	2	8	0,841869		0,000136	0,003332	0,000261	0,000151
	1	12	0,000259	0,000136		0,024144	0,384996	0,583446
	2	12	0,131302	0,003332	0,024144		0,698543	0,380528
	1	18	0,009164	0,000261	0,384996	0,698543		0,997690
	2	18	0,002363	0,000151	0,583446	0,380528	0,997690	

Table II - 8: *Test results of **one-way random effects nested ANOVAs** for body weight within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	589144,6	1	589144,6	9,30384	1248,579	471,8522	0,000000
	Parallel (Time)	Random	10067,8	8	1258,5	47,00000	1135,571	1,1082	0,374987
	Time	Fixed	131473,5	7	18781,9	8,15042	1257,209	14,9394	0,000468
	Error		53371,8	47	1135,6				
12.5	Intercept	Fixed	2511899	1	2511899	8,46983	6391,994	392,9759	0,000000
	Parallel (Time)	Random	53683	8	6710	47,00000	2426,101	2,7659	0,013660
	Time	Fixed	687349	7	98193	8,06058	6665,986	14,7304	0,000523
	Error		114027	47	2426				
18	Intercept	Fixed	3236002	1	3236002	9,65865	2832,310	1142,531	0,000000
	Parallel (Time)	Random	21919	8	2740	52,00000	4281,305	0,640	0,740519
	Time	Fixed	780640	7	111520	8,19806	2751,979	40,524	0,000010
	Error		222628	52	4281				

Table II - 9: *Test results of **Tukey HSD post-hoc tests** of difference for body weight within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,984202	0,523620	0,049336	0,027078	0,001108	0,000129	0,000129
	2	0,984202		0,971102	0,398948	0,282029	0,022466	0,000138	0,000129
	3	0,523620	0,971102		0,949309	0,883661	0,216690	0,000201	0,000138
	4	0,049336	0,398948	0,949309		0,999999	0,783674	0,000734	0,000165
	5	0,027078	0,282029	0,883661	0,999999		0,868089	0,000937	0,000172
	6	0,001108	0,022466	0,216690	0,783674	0,868089		0,036298	0,004002
	7	0,000129	0,000138	0,000201	0,000734	0,000937	0,036298		0,999285
	8	0,000129	0,000129	0,000138	0,000165	0,000172	0,004002	0,999285	
12.5	1		0,929412	0,048952	0,000141	0,000129	0,000129	0,000129	0,000129
	2	0,929412		0,440922	0,000434	0,000129	0,000129	0,000129	0,000129
	3	0,048952	0,440922		0,183671	0,000150	0,000139	0,000143	0,000129
	4	0,000141	0,000434	0,183671		0,027027	0,006661	0,015691	0,000129
	5	0,000129	0,000129	0,000150	0,027027		0,991078	0,999999	0,003007
	6	0,000129	0,000129	0,000139	0,006661	0,991078		0,998224	0,080547
	7	0,000129	0,000129	0,000143	0,015691	0,999999	0,998224		0,005516
	8	0,000129	0,000129	0,000129	0,000129	0,003007	0,080547	0,005516	
18	1		0,898125	0,076307	0,000138	0,000437	0,000135	0,000135	0,000135
	2	0,898125		0,690213	0,000855	0,026318	0,000138	0,000135	0,000135
	3	0,076307	0,690213		0,153553	0,778992	0,003250	0,000139	0,000135
	4	0,000138	0,000855	0,153553		0,918026	0,704772	0,008019	0,000917
	5	0,000437	0,026318	0,778992	0,918026		0,102337	0,000321	0,000147
	6	0,000135	0,000138	0,003250	0,704772	0,102337		0,397869	0,107976
	7	0,000135	0,000135	0,000139	0,008019	0,000321	0,397869		0,998220
	8	0,000135	0,000135	0,000135	0,000917	0,000147	0,107976	0,998220	

c. Full feeding group (100%):

Table II - 10: *Test results of a **two-way random effects nested ANOVA** for body weight, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	8800733,89	1	8800733,89	40,0702113	16634,0948	529,078017	0
Parallel (Temp.*Time)	Random	686104,733	38	18055,3877	149	4245,1826	4,25314748	1,1102E-10
Temperature	Fixed	1171794,44	2	585897,221	39,9181119	16727,6466	35,0256815	1,6443E-09
Time	Fixed	2213871,25	7	316267,322	39,2357876	17167,0312	18,4229479	1,3453E-10
Error		632532,208	149	4245,1826				

Table II - 11: *Test results of a **two-way factorial ANOVA** for body weight, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	9305346,62	1	9305346,62	2086,0751	0
Time	2440352,39	7	348621,77	78,1541219	0
Temperature	1235388,41	2	617694,205	138,474852	0
Time*Temp.	546936,552	14	39066,8966	8,75802735	3,10E-14
Error	771700,389	173	4460,69589		

Table II - 12: *Test results of **one-way random effects nested ANOVAs** for body weight, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	40590,65	1	40590,65	3,00000	23,3602	1737,598	0,000030
	Parallel (Temp)	Random	70,08	3	23,36	12,00000	111,8494	0,209	0,888318
	Temperature	Fixed	657,12	2	328,56	3,00000	23,3602	14,065	0,029917
	Error		1342,19	12	111,85				
2 (14.12.18)	Intercept	Fixed	127411,5	1	127411,5	3	418,9519	304,1197	0,000411
	Parallel (Temp)	Random	1256,9	3	419,0	12	156,9894	2,6687	0,094943
	Temperature	Fixed	5877,4	2	2938,7	3	418,9519	7,0144	0,073945
	Error		1883,9	12	157,0				
3 (01.02.19)	Intercept	Fixed	319928,0	1	319928,0	3,00000	548,0084	583,8012	0,000155
	Parallel (Temp)	Random	1644,0	3	548,0	12,00000	482,8073	1,1350	0,374131
	Temperature	Fixed	19690,4	2	9845,2	3,00000	548,0084	17,9654	0,021392
	Error		5793,7	12	482,8				
4 (13.04.19)	Intercept	Fixed	1644237	1	1644237	3,27327	227,704	7220,925	0,000001
	Parallel (Temp)	Random	656	3	219	23,00000	3165,712	0,069	0,975849
	Temperature	Fixed	266402	2	133201	3,12990	222,996	597,325	0,000091
	Error		72811	23	3166				
5 (27.03.19)	Intercept	Fixed	1407594	1	1407594	3,33174	5805,759	242,4479	0,000315
	Parallel (Temp)	Random	17788	3	5929	19,00000	4189,638	1,4153	0,269231
	Temperature	Fixed	205132	2	102566	3,13408	5876,598	17,4533	0,020004
	Error		79603	19	4190				
6 (10.04.19)	Intercept	Fixed	2623456	1	2623456	3	23839,57	110,0463	0,001850
	Parallel (Temp)	Random	71519	3	23840	24	6625,86	3,5980	0,028117
	Temperature	Fixed	408855	2	204428	3	23839,57	8,5751	0,057446
	Error		159021	24	6626				
7 (24.04.19)	Intercept	Fixed	3546171	1	3546171	3,00183	8856,541	400,4013	0,000272
	Parallel (Temp)	Random	26571	3	8857	24,00000	7311,737	1,2114	0,326993
	Temperature	Fixed	492954	2	246477	3,00090	8856,831	27,8290	0,011556
	Error		175482	24	7312				
8 (15.05.19)	Intercept	Fixed	2999033	1	2999033	5,42525	6380,778	470,0105	0,000002
	Parallel (Temp)	Random	19663	3	6554	23,00000	5938,940	1,1036	0,367828
	Temperature	Fixed	561966	2	280983	3,66728	6489,597	43,2975	0,002813
	Error		136596	23	5939				

Table II - 13: *Test results of **Tukey HSD post-hoc tests** of difference for body weight between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,275703	0,083668
	12	0,275703		0,737806
	18	0,083668	0,737806	
2 (14.12.18)	8		0,001314	0,000435
	12	0,001314		0,656990
	18	0,000435	0,656990	
3 (01.02.19)	8		0,000994	0,000350
	12	0,000994		0,615964
	18	0,000350	0,615964	
4 (13.03.19)	8		0,000133	0,000135
	12	0,000133		0,087722
	18	0,000135	0,087722	
5 (27.03.19)	8		0,000157	0,000157
	12	0,000157		0,700367
	18	0,000157	0,700367	
6 (10.04.19)	8		0,000129	0,000136
	12	0,000129		0,341088
	18	0,000136	0,341088	
7 (24.04.19)	8		0,000129	0,000130
	12	0,000129		0,858448
	18	0,000130	0,858448	
8 (15.05.19)	8		0,000133	0,000133
	12	0,000133		0,000348
	18	0,000133	0,000348	

Table II - 14: *Test results of **Tukey HSD post-hoc tests** of difference for body weight between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,770920	0,527697	0,989166	0,630352	0,304025
	1	12	0,770920		0,997593	0,975838	0,999823	0,943203
	1	18	0,527697	0,997593		0,850559	0,999967	0,997069
	2	8	0,989166	0,975838	0,850559		0,918416	0,611488
	2	12	0,630352	0,999823	0,999967	0,918416		0,986248
	2	18	0,304025	0,943203	0,997069	0,611488	0,986248	
2 (14.12.18)	1	8		0,029182	0,100851	0,579947	0,700908	0,042953
	1	12	0,029182		0,971684	0,001992	0,291290	0,999890
	1	18	0,100851	0,971684		0,006376	0,677964	0,994474
	2	8	0,579947	0,001992	0,006376		0,076305	0,002814
	2	12	0,700908	0,291290	0,677964	0,076305		0,393690
	2	18	0,042953	0,999890	0,994474	0,002814	0,393690	
3 (01.02.19)	1	8		0,188847	0,009374	0,944967	0,030996	0,068058
	1	12	0,188847		0,486836	0,045898	0,867909	0,985315
	1	18	0,009374	0,486836		0,002320	0,976565	0,836468
	2	8	0,944967	0,045898	0,002320		0,007151	0,015600
	2	12	0,030996	0,867909	0,976565	0,007151		0,996454
	2	18	0,068058	0,985315	0,836468	0,015600	0,996454	
4 (13.03.19)	1	8		0,000156	0,001332	0,999913	0,000156	0,001790
	1	12	0,000156		0,782201	0,000169	1,000000	0,486560
	1	18	0,001332	0,782201		0,002139	0,775412	0,998624
	2	8	0,999913	0,000169	0,002139		0,000168	0,002995
	2	12	0,000156	1,000000	0,775412	0,000168		0,478579
	2	18	0,001790	0,486560	0,998624	0,002995	0,478579	
5 (27.03.19)	1	8		0,009145	0,000340	0,994106	0,000881	0,016200
	1	12	0,009145		0,999422	0,029115	0,999997	0,844397
	1	18	0,000340	0,999422		0,001491	0,999813	0,384836
	2	8	0,994106	0,029115	0,001491		0,004187	0,074302
	2	12	0,000881	0,999997	0,999813	0,004187		0,601231
	2	18	0,016200	0,844397	0,384836	0,074302	0,601231	
6 (10.04.19)	1	8		0,000145	0,001504	0,998837	0,019376	0,022445
	1	12	0,000145		0,392590	0,000140	0,054609	0,047552
	1	18	0,001504	0,392590		0,000659	0,880180	0,852926
	2	8	0,998837	0,000140	0,000659		0,007927	0,009242
	2	12	0,019376	0,054609	0,880180	0,007927		1,000000
	2	18	0,022445	0,047552	0,852926	0,009242	1,000000	
7 (24.04.19)	1	8		0,000190	0,003313	0,999830	0,002781	0,000423
	1	12	0,000190		0,559597	0,000158	0,608116	0,983896
	1	18	0,003313	0,559597		0,002151	1,000000	0,910816
	2	8	0,999830	0,000158	0,002151		0,001779	0,000275
	2	12	0,002781	0,608116	1,000000	0,001779		0,935007
	2	18	0,000423	0,983896	0,910816	0,000275	0,935007	
8 (15.05.19)	1	8		0,003144	0,000281	0,950380	0,000141	0,003046
	1	12	0,003144		0,798739	0,001025	0,990335	0,344878
	1	18	0,000281	0,798739		0,000153	0,029966	0,677414
	2	8	0,950380	0,001025	0,000153		0,000141	0,000423
	2	12	0,000141	0,990335	0,029966	0,000141		0,000940
	2	18	0,003046	0,344878	0,677414	0,000423	0,000940	

Table II - 15: *Test results of **one-way random effects nested ANOVAs** for body weight within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	780359,6	1	780359,6	9,00342	1021,252	764,1205	0,000000
	Parallel (time)	Random	8131,8	8	1016,5	51,00000	1106,346	0,9188	0,508906
	Time	Fixed	123218,0	7	17602,6	8,12085	1017,087	17,3069	0,000279
	Error		56423,6	51	1106,3				
12.5	Intercept	Fixed	4451449	1	4451449	8,80927	9656,69	460,9706	0,000000
	Parallel (time)	Random	83906	8	10488	44,00000	3716,18	2,8223	0,012851
	Time	Fixed	1385491	7	197927	8,09493	10376,93	19,0738	0,000199
	Error		163512	44	3716				
18	Intercept	Fixed	4453079	1	4453079	9,72559	6020,591	739,6415	0,000000
	Parallel (time)	Random	47131	8	5891	54,00000	7640,676	0,7711	0,629579
	Time	Fixed	1120662	7	160095	8,22162	5909,730	27,0900	0,000047
	Error		412597	54	7641				

Table II - 16: *Test results of **Tukey HSD post-hoc tests** of difference for body weight within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,970124	0,225546	0,003821	0,000856	0,000164	0,000131	0,000131
	2	0,970124		0,819563	0,085369	0,022297	0,001731	0,000133	0,000131
	3	0,225546	0,819563		0,895477	0,585258	0,147380	0,001104	0,000181
	4	0,003821	0,085369	0,895477		0,997438	0,739461	0,012034	0,000652
	5	0,000856	0,022297	0,585258	0,997438		0,984831	0,090314	0,006833
	6	0,000164	0,001731	0,147380	0,739461	0,984831		0,445873	0,063604
	7	0,000131	0,000133	0,001104	0,012034	0,090314	0,445873		0,973740
	8	0,000131	0,000131	0,000181	0,000652	0,006833	0,063604	0,973740	
12.5	1		0,908838	0,103332	0,000133	0,000133	0,000133	0,000133	0,000133
	2	0,908838		0,739520	0,000133	0,000133	0,000133	0,000133	0,000133
	3	0,103332	0,739520		0,000147	0,000311	0,000133	0,000133	0,000133
	4	0,000133	0,000133	0,000147		1,000000	0,183944	0,002649	0,000133
	5	0,000133	0,000133	0,000311	1,000000		0,288961	0,010093	0,000133
	6	0,000133	0,000133	0,000133	0,183944	0,288961		0,716611	0,000134
	7	0,000133	0,000133	0,000133	0,002649	0,010093	0,716611		0,000327
	8	0,000133	0,000133	0,000133	0,000133	0,000133	0,000134	0,000327	
18	1		0,983435	0,395658	0,000447	0,000156	0,000134	0,000134	0,000134
	2	0,983435		0,916409	0,008635	0,001106	0,000151	0,000134	0,000134
	3	0,395658	0,916409		0,247846	0,059641	0,002710	0,000138	0,000135
	4	0,000447	0,008635	0,247846		0,997345	0,557099	0,007950	0,004972
	5	0,000156	0,001106	0,059641	0,997345		0,910446	0,041483	0,029402
	6	0,000134	0,000151	0,002710	0,557099	0,910446		0,511618	0,478826
	7	0,000134	0,000134	0,000138	0,007950	0,041483	0,511618		1,000000
	8	0,000134	0,000134	0,000135	0,004972	0,029402	0,478826	1,000000	

d. Comparison of temperature groups between feeding regimes

Table II - 17: *Test results of **factorial ANOVAs** for body weight comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	1386502	1	1386502	1234,902	0,000000
	Time	255939	7	36563	32,565	0,000000
	Feeding regime	2299	1	2299	2,048	0,155139
	Feed*Time	2870	7	410	0,365	0,920690
	Error	127995	114	1123		
12.5	Intercept	7525955	1	7525955	1939,833	0,000000
	Time	2186972	7	312425	80,528	0,000000
	Feeding regime	243692	1	243692	62,812	0,000000
	Feed*Time	167028	7	23861	6,150	0,000005
	Error	415127	107	3880		
18	Intercept	7623892	1	7623892	1320,671	0,000000
	Time	1871346	7	267335	46,310	0,000000
	Feeding regime	44440	1	44440	7,698	0,006398
	Feed*Time	27698	7	3957	0,685	0,684144
	Error	704275	122	5773		

Table II - 18: *Test results of **Tukey HSD post-hoc tests** for body weight comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		1,00	0,84	0,12	0,06	0,00	0,00	0,00	1,00	1,00	0,41	0,01	0,00	0,00	0,00	0,00
		2	1,00		1,00	0,72	0,57	0,05	0,00	0,00	1,00	1,00	0,96	0,17	0,05	0,00	0,00	0,00
		3	0,84	1,00		1,00	0,99	0,46	0,00	0,00	0,91	1,00	1,00	0,80	0,43	0,06	0,00	0,00
		4	0,12	0,72	1,00		1,00	0,97	0,00	0,00	0,15	0,82	1,00	1,00	0,96	0,44	0,00	0,00
		5	0,06	0,57	0,99	1,00		0,99	0,00	0,00	0,08	0,68	1,00	1,00	0,99	0,54	0,00	0,00
		6	0,00	0,05	0,46	0,97	0,99		0,08	0,01	0,00	0,07	0,92	1,00	1,00	1,00	0,15	0,01
		7	0,00	0,00	0,00	0,00	0,00	0,08		1,00	0,00	0,00	0,00	0,02	0,14	0,60	1,00	1,00
		8	0,00	0,00	0,00	0,00	0,00	0,01	1,00		0,00	0,00	0,00	0,00	0,02	0,16	1,00	1,00
	100	1	1,00	1,00	0,91	0,15	0,08	0,00	0,00	0,00		1,00	0,50	0,01	0,00	0,00	0,00	0,00
		2	1,00	1,00	1,00	0,82	0,68	0,07	0,00	0,00	1,00		0,98	0,22	0,06	0,00	0,00	0,00
		3	0,41	0,96	1,00	1,00	1,00	0,92	0,00	0,00	0,50	0,98		1,00	0,89	0,36	0,00	0,00
		4	0,01	0,17	0,80	1,00	1,00	1,00	0,02	0,00	0,01	0,22	1,00		1,00	0,96	0,03	0,00
		5	0,00	0,05	0,43	0,96	0,99	1,00	0,14	0,02	0,00	0,06	0,89	1,00		1,00	0,23	0,02
		6	0,00	0,00	0,06	0,44	0,54	1,00	0,60	0,16	0,00	0,00	0,36	0,96	1,00		0,79	0,17
		7	0,00	0,00	0,00	0,00	0,00	0,15	1,00	1,00	0,00	0,00	0,00	0,03	0,23	0,79		1,00
		8	0,00	0,00	0,00	0,00	0,00	0,01	1,00	1,00	0,00	0,00	0,00	0,00	0,02	0,17	1,00	
12.5	67	1		1,00	0,47	0,00	0,00	0,00	0,00	0,00	1,00	0,99	0,20	0,00	0,00	0,00	0,00	0,00
		2	1,00		0,96	0,02	0,00	0,00	0,00	0,00	1,00	1,00	0,75	0,00	0,00	0,00	0,00	0,00
		3	0,47	0,96		0,79	0,00	0,00	0,00	0,00	0,59	1,00	1,00	0,00	0,00	0,00	0,00	0,00
		4	0,00	0,02	0,79		0,35	0,15	0,26	0,00	0,00	0,10	0,98	0,00	0,02	0,00	0,00	0,00
		5	0,00	0,00	0,00	0,35		1,00	1,00	0,09	0,00	0,00	0,01	0,85	0,97	0,00	0,00	0,00
		6	0,00	0,00	0,00	0,15	1,00		1,00	0,59	0,00	0,00	0,00	1,00	1,00	0,06	0,00	0,00
		7	0,00	0,00	0,00	0,26	1,00	1,00		0,13	0,00	0,00	0,01	0,92	0,99	0,00	0,00	0,00
		8	0,00	0,00	0,00	0,00	0,09	0,59	0,13		0,00	0,00	0,00	0,99	1,00	1,00	0,29	0,00
	100	1	1,00	1,00	0,59	0,00	0,00	0,00	0,00	0,00		1,00	0,28	0,00	0,00	0,00	0,00	0,00
		2	0,99	1,00	1,00	0,10	0,00	0,00	0,00	0,00	1,00		0,97	0,00	0,00	0,00	0,00	0,00
		3	0,20	0,75	1,00	0,98	0,01	0,00	0,01	0,00	0,28	0,97		0,00	0,00	0,00	0,00	0,00
		4	0,00	0,00	0,00	0,00	0,85	1,00	0,92	0,99	0,00	0,00	0,00		1,00	0,45	0,01	0,00
		5	0,00	0,00	0,00	0,02	0,97	1,00	0,99	1,00	0,00	0,00	0,00	1,00		0,62	0,03	0,00
		6	0,00	0,00	0,00	0,00	0,00	0,06	0,00	1,00	0,00	0,00	0,00	0,45	0,62		0,96	0,00
		7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,29	0,00	0,00	0,00	0,01	0,03	0,96		0,00
		8	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
18	67	1		1,00	0,42	0,00	0,00	0,00	0,00	0,00	1,00	1,00	0,36	0,00	0,00	0,00	0,00	0,00
		2	1,00		0,99	0,01	0,20	0,00	0,00	0,00	1,00	1,00	0,98	0,00	0,00	0,00	0,00	0,00
		3	0,42	0,99		0,62	0,99	0,03	0,00	0,00	0,55	0,99	1,00	0,22	0,02	0,00	0,00	0,00
		4	0,00	0,01	0,62		1,00	0,99	0,08	0,01	0,00	0,01	0,69	1,00	0,98	0,17	0,00	0,00
		5	0,00	0,20	0,99	1,00		0,50	0,00	0,00	0,01	0,27	1,00	0,94	0,42	0,01	0,00	0,00
		6	0,00	0,00	0,03	0,99	0,50		0,90	0,51	0,00	0,00	0,05	1,00	1,00	0,98	0,01	0,01
		7	0,00	0,00	0,00	0,08	0,00	0,90		1,00	0,00	0,00	0,00	0,49	0,93	1,00	0,84	0,82
		8	0,00	0,00	0,00	0,01	0,00	0,51	1,00		0,00	0,00	0,00	0,14	0,60	1,00	0,99	0,99
	100	1	1,00	1,00	0,55	0,00	0,01	0,00	0,00	0,00		1,00	0,49	0,00	0,00	0,00	0,00	0,00
		2	1,00	1,00	0,99	0,01	0,27	0,00	0,00	0,00	1,00		0,99	0,00	0,00	0,00	0,00	0,00
		3	0,36	0,98	1,00	0,69	1,00	0,05	0,00	0,00	0,49	0,99		0,27	0,03	0,00	0,00	0,00
		4	0,00	0,00	0,22	1,00	0,94	1,00	0,49	0,14	0,00	0,00	0,27		1,00	0,71	0,00	0,00
		5	0,00	0,00	0,02	0,98	0,42	1,00	0,93	0,60	0,00	0,00	0,03	1,00		0,99	0,02	0,01
		6	0,00	0,00	0,00	0,17	0,01	0,98	1,00	1,00	0,00	0,00	0,00	0,71	0,99		0,65	0,61
		7	0,00	0,00	0,00	0,00	0,00	0,01	0,84	0,99	0,00	0,00	0,00	0,00	0,02	0,65		1,00
		8	0,00	0,00	0,00	0,00	0,00	0,01	0,82	0,99	0,00	0,00	0,00	0,00	0,01	0,61	1,00	

II - II Condition factor (CF)

a. Overall tests:

Table II - 19: *Test results of a **three-way random effects nested ANOVA** for condition factor, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	529,5689	1	529,5689	94,0276	0,008656	61178,98	0,000000
Parallel (Temp.*feed*Time)	Random	0,7952	85	0,0094	295,0000	0,003558	2,63	0,000000
Time	Fixed	0,1313	7	0,0188	88,2659	0,009078	2,07	0,055503
Temperature	Fixed	1,9554	2	0,9777	93,9934	0,008658	112,92	0,000000
Feeding regime	Fixed	0,0612	1	0,0612	94,0276	0,008656	7,07	0,009198
Error		1,0497	295	0,0036				

Table II - 20: *Test results of a **three-way factorial ANOVA** for condition factor, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	551,4091	1	551,4091	152678,1	0,000000
Time	0,1425	7	0,0204	5,6	0,000004
Temperature	2,0463	2	1,0232	283,3	0,000000
Feed regime	0,0701	1	0,0701	19,4	0,000014
Time*Temp.	0,4093	14	0,0292	8,1	0,000000
Time*Feed.	0,0785	7	0,0112	3,1	0,003464
Temp.*Feed.	0,0353	2	0,0176	4,9	0,008128
Time*Temp.*Feed.	0,0515	14	0,0037	1,0	0,433683
Error	1,2388	343	0,0036		

b. Restrictive feeding (67%)

Table II - 21: *Test results of a **two-way random effects nested ANOVA** for condition factor, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	274,3105	1	274,3105	40,3262	0,009701	28276,11	0,000000
Parallel (Temp.*Time)	Random	0,3905	38	0,0103	151,0000	0,003406	3,02	0,000001
Time	Fixed	0,1426	7	0,0204	38,5432	0,010131	2,01	0,078692
Temperature	Fixed	1,0795	2	0,5397	40,3145	0,009704	55,62	0,000000
Error		0,5144	151	0,0034				

Table II - 22: *Test results of a **two-way factorial ANOVA** for condition factor, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	278,2395	1	278,2395	80282,80	0,000000
Time	0,1417	7	0,0202	5,84	0,000004
Temperature	1,0986	2	0,5493	158,50	0,000000
Temp.*Time	0,2984	14	0,0213	6,15	0,000000
Error	0,6065	175	0,0035		

Table II - 23: Test results of **one-*way random effects nested ANOVAs*** *for condition factor, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	22,43082	1	22,43082	3,01763	0,006531	3434,674	0,000010
	Parallel (temp)	Random	0,01975	3	0,00658	10,00000	0,001739	3,786	0,047493
	Temperature	Fixed	0,12809	2	0,06405	3,00944	0,006555	9,770	0,048292
	Error		0,01739	10	0,00174				
2 (14.12.18)	Intercept	Fixed	28,78804	1	28,78804	3,00000	0,000483	59592,80	0,000000
	Parallel (temp)	Random	0,00145	3	0,00048	12,00000	0,004892	0,10	0,959192
	Temperature	Fixed	0,11131	2	0,05566	3,00000	0,000483	115,21	0,001457
	Error		0,05870	12	0,00489				
3 (01.02.19)	Intercept	Fixed	26,28943	1	26,28943	3,06419	0,001932	13604,79	0,000001
	Parallel (temp)	Random	0,00580	3	0,00193	11,00000	0,001854	1,04	0,411990
	Temperature	Fixed	0,07632	2	0,03816	3,02956	0,001933	19,74	0,018294
	Error		0,02040	11	0,00185				
4 (13.04.19)	Intercept	Fixed	46,04441	1	46,04441	3,00415	0,001762	26130,78	0,000001
	Parallel (temp)	Random	0,00528	3	0,00176	24,00000	0,003291	0,54	0,662601
	Temperature	Fixed	0,24411	2	0,12205	3,00205	0,001762	69,28	0,003076
	Error		0,07899	24	0,00329				
5 (27.03.19)	Intercept	Fixed	40,34445	1	40,34445	3,05532	0,000635	63572,84	0,000000
	Parallel (temp)	Random	0,00189	3	0,00063	23,00000	0,001877	0,34	0,799382
	Temperature	Fixed	0,16354	2	0,08177	3,02650	0,000633	129,26	0,001173
	Error		0,04317	23	0,00188				
6 (10.04.19)	Intercept	Fixed	41,53537	1	41,53537	3,02806	0,002537	16369,70	0,000001
	Parallel (temp)	Random	0,00759	3	0,00253	23,00000	0,004950	0,51	0,678395
	Temperature	Fixed	0,23308	2	0,11654	3,01376	0,002534	45,98	0,005520
	Error		0,11385	23	0,00495				
7 (24.04.19)	Intercept	Fixed	39,38198	1	39,38198	3,07078	0,005102	7719,491	0,000003
	Parallel (temp)	Random	0,01534	3	0,00511	21,00000	0,004281	1,194	0,336095
	Temperature	Fixed	0,34845	2	0,17422	3,03427	0,005108	34,111	0,008324
	Error		0,08991	21	0,00428				
8 (15.05.19)	Intercept	Fixed	50,51478	1	50,51478	3,01470	0,011604	4353,220	0,000007
	Parallel (temp)	Random	0,03502	3	0,01167	27,00000	0,003406	3,427	0,031132
	Temperature	Fixed	0,28404	2	0,14202	3,00721	0,011639	12,202	0,036048
	Error		0,09197	27	0,00341				

Table II - 24: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for condition factor between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,000911	0,000213
	12	0,000911		0,058049
	18	0,000213	0,058049	
2 (14.12.18)	8		0,062329	0,001376
	12	0,062329		0,107354
	18	0,001376	0,107354	
3 (01.02.19)	8		0,004633	0,060859
	12	0,004633		0,000288
	18	0,060859	0,000288	
4 (13.03.19)	8		0,000573	0,001133
	12	0,000573		0,000129
	18	0,001133	0,000129	
5 (27.03.19)	8		0,000137	0,035686
	12	0,000137		0,000133
	18	0,035686	0,000133	
6 (10.04.19)	8		0,053392	0,000642
	12	0,053392		0,000133
	18	0,000642	0,000133	
7 (24.04.19)	8		0,364263	0,000141
	12	0,364263		0,000140
	18	0,000141	0,000140	
8 (15.05.19)	8		0,800530	0,000127
	12	0,800530		0,000127
	18	0,000127	0,000127	

Table II - 25: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for condition factor between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,205063	0,027410	0,120665	0,239508	0,004071
	1	12	0,205063		0,753912	0,003037	0,999984	0,112629
	1	18	0,027410	0,753912		0,000641	0,889003	0,544571
	2	8	0,120665	0,003037	0,000641		0,005339	0,000297
	2	12	0,239508	0,999984	0,889003	0,005339		0,199929
	2	18	0,004071	0,112629	0,544571	0,000297	0,199929	
2 (14.12.18)	1	8		0,569892	0,039968	0,999856	0,320456	0,040152
	1	12	0,569892		0,490761	0,708429	0,995789	0,492260
	1	18	0,039968	0,490761		0,060006	0,764268	1,000000
	2	8	0,999856	0,708429	0,060006		0,435782	0,060280
	2	12	0,320456	0,995789	0,764268	0,435782		0,765707
	2	18	0,040152	0,492260	1,000000	0,060280	0,765707	
3 (01.02.19)	1	8		0,026996	0,355041	0,952696	0,159173	0,975385
	1	12	0,026996		0,001210	0,103312	0,974635	0,008654
	1	18	0,355041	0,001210		0,105718	0,008273	0,739961
	2	8	0,952696	0,103312	0,105718		0,441297	0,636218
	2	12	0,159173	0,974635	0,008273	0,441297		0,058458
	2	18	0,975385	0,008654	0,739961	0,636218	0,058458	
4 (13.03.19)	1	8		0,005242	0,053460	0,997695	0,076134	0,114670
	1	12	0,005242		0,000141	0,035020	0,862706	0,000147
	1	18	0,053460	0,000141		0,040744	0,000216	0,999178
	2	8	0,997695	0,035020	0,040744		0,279749	0,082991
	2	12	0,076134	0,862706	0,000216	0,279749		0,000345
	2	18	0,114670	0,000147	0,999178	0,082991	0,000345	
5 (27.03.19)	1	8		0,000966	0,804247	0,991517	0,001256	0,337546
	1	12	0,000966		0,000172	0,006734	0,999996	0,000144
	1	18	0,804247	0,000172		0,506195	0,000184	0,962637
	2	8	0,991517	0,006734	0,506195		0,008836	0,156859
	2	12	0,001256	0,999996	0,000184	0,008836		0,000146
	2	18	0,337546	0,000144	0,962637	0,156859	0,000146	
6 (10.04.19)	1	8		0,790545	0,174622	1,000000	0,416522	0,020621
	1	12	0,790545		0,010025	0,695873	0,992399	0,000945
	1	18	0,174622	0,010025		0,116699	0,001474	0,876013
	2	8	1,000000	0,695873	0,116699		0,286649	0,009716
	2	12	0,416522	0,992399	0,001474	0,286649		0,000220
	2	18	0,020621	0,000945	0,876013	0,009716	0,000220	
7 (24.04.19)	1	8		0,373390	0,055474	0,497471	0,379802	0,017231
	1	12	0,373390		0,000233	0,999837	0,999999	0,000165
	1	18	0,055474	0,000233		0,000307	0,000305	0,987211
	2	8	0,497471	0,999837	0,000307		0,999458	0,000181
	2	12	0,379802	0,999999	0,000305	0,999458		0,000183
	2	18	0,017231	0,000165	0,987211	0,000181	0,000183	
8 (15.05.19)	1	8		0,990228	0,001180	0,951985	0,244417	0,002186
	1	12	0,990228		0,002808	0,615153	0,048935	0,005554
	1	18	0,001180	0,002808		0,000145	0,000130	0,999722
	2	8	0,951985	0,615153	0,000145		0,628828	0,000169
	2	12	0,244417	0,048935	0,000130	0,628828		0,000131
	2	18	0,002186	0,005554	0,999722	0,000169	0,000131	

Table II - 26: *Test results of **one-way random effects nested ANOVAs** for condition factor within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	86,09534	1	86,09534	8,33083	0,004094	21031,41	0,000000
	Parallel (Time)	Random	0,03353	8	0,00419	49,00000	0,001913	2,19	0,044374
	Time	Fixed	0,13893	7	0,01985	8,04344	0,004178	4,75	0,021592
	Error		0,09374	49	0,00191				
12.5	Intercept	Fixed	78,59679	1	78,59679	8,63892	0,004732	16611,06	0,000000
	Parallel (Time)	Random	0,04024	8	0,00503	49,00000	0,001884	2,67	0,016168
	Time	Fixed	0,18922	7	0,02703	8,07141	0,004993	5,41	0,014604
	Error		0,09231	49	0,00188				
18	Intercept	Fixed	111,8349	1	111,8349	12,85969	0,002655	42124,65	0,000000
	Parallel (Time)	Random	0,0184	8	0,0023	53,00000	0,006195	0,37	0,931632
	Time	Fixed	0,1150	7	0,0164	8,50627	0,002339	7,03	0,005550
	Error		0,3283	53	0,0062				

Table II - 27: *Test results of **Tukey HSD post-hoc tests** of difference for condition factor within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,061858	0,000133	0,000133	0,000236	0,001390	0,023080	0,000949
	2	0,061858		0,003110	0,010656	0,631615	0,974429	1,000000	0,949759
	3	0,000133	0,003110		0,983857	0,127593	0,013276	0,002192	0,019187
	4	0,000133	0,010656	0,983857		0,397760	0,048450	0,007139	0,070486
	5	0,000236	0,631615	0,127593	0,397760		0,980247	0,655534	0,992781
	6	0,001390	0,974429	0,013276	0,048450	0,980247		0,986560	1,000000
	7	0,023080	1,000000	0,002192	0,007139	0,655534	0,986560		0,968457
	8	0,000949	0,949759	0,019187	0,070486	0,992781	1,000000	0,968457	
12.5	1		0,724384	0,339664	0,009674	0,000137	0,000441	0,003460	0,391816
	2	0,724384		0,005061	0,000138	0,000133	0,000133	0,000134	0,002328
	3	0,339664	0,005061		0,936490	0,028987	0,319809	0,767515	0,999722
	4	0,009674	0,000138	0,936490		0,146420	0,859902	0,999606	0,454326
	5	0,000137	0,000133	0,028987	0,146420		0,913734	0,414435	0,000462
	6	0,000441	0,000133	0,319809	0,859902	0,913734		0,988576	0,026010
	7	0,003460	0,000134	0,767515	0,999606	0,414435	0,988576		0,211774
	8	0,391816	0,002328	0,999722	0,454326	0,000462	0,026010	0,211774	
18	1		0,886744	0,990299	0,842699	0,991349	0,994701	0,466276	0,479851
	2	0,886744		0,999586	1,000000	0,254658	0,994991	0,998580	0,999339
	3	0,990299	0,999586		0,999467	0,592573	1,000000	0,932724	0,945111
	4	0,842699	1,000000	0,999467		0,132008	0,991395	0,994688	0,997012
	5	0,991349	0,254658	0,592573	0,132008		0,539205	0,019992	0,017093
	6	0,994701	0,994991	1,000000	0,991395	0,539205		0,763795	0,780676
	7	0,466276	0,998580	0,932724	0,994688	0,019992	0,763795		1,000000
	8	0,479851	0,999339	0,945111	0,997012	0,017093	0,780676	1,000000	

c. Full feeding (100%)

Table II - 28: *Test results of a **two-way random effects nested ANOVA** for condition factor, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	256,3161	1	256,3161	45,1211	0,006501	39427,24	0,000000
Parallel (Temp.*Time)	Random	0,2651	38	0,0070	144,0000	0,003718	1,88	0,004351
Temperature	Fixed	0,9195	2	0,4597	45,0970	0,006502	70,70	0,000000
Time	Fixed	0,0751	7	0,0107	40,4216	0,006794	1,58	0,169451
Error		0,5353	144	0,0037				

Table II - 29: *Test results of a **two-way factorial ANOVA** for condition factor, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	273,3917	1	273,3917	72643,03	0,000000
Time	0,0802	7	0,0115	3,04	0,004868
Temperature	0,9888	2	0,4944	131,37	0,000000
Time*Temp.	0,1681	14	0,0120	3,19	0,000178
Error	0,6323	168	0,0038		

Table II - 30: *Test results of **one-way random effects nested ANOVAs** for condition factor, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	18,24353	1	18,24353	3,148130	0,007178	2541,438	0,000011
	Parallel (Temp)	Random	0,02283	3	0,00761	9,000000	0,002165	3,516	0,062187
	Temperature	Fixed	0,06951	2	0,03475	3,063803	0,007415	4,687	0,116910
	Error		0,01948	9	0,00216				
2 (14.12.18)	Intercept	Fixed	20,82530	1	20,82530	3,705773	0,000743	28046,43	0,000000
	Parallel (Temp)	Random	0,00217	3	0,00072	9,000000	0,000957	0,76	0,545976
	Temperature	Fixed	0,05350	2	0,02675	3,300060	0,000732	36,52	0,005610
	Error		0,00861	9	0,00096				
3 (01.02.19)	Intercept	Fixed	28,72459	1	28,72459	3,00000	0,000859	33457,62	0,000000
	Parallel (Temp)	Random	0,00258	3	0,00086	12,00000	0,003517	0,24	0,863926
	Temperature	Fixed	0,13570	2	0,06785	3,00000	0,000859	79,03	0,002542
	Error		0,04221	12	0,00352				
4 (13.04.19)	Intercept	Fixed	49,07549	1	49,07549	3	0,005775	8497,580	0,000003
	Parallel (Temp)	Random	0,01733	3	0,00578	24	0,002597	2,224	0,111363
	Temperature	Fixed	0,14267	2	0,07133	3	0,005775	12,352	0,035635
	Error		0,06232	24	0,00260				
5 (27.03.19)	Intercept	Fixed	44,96117	1	44,96117	3,02773	0,008936	5031,732	0,000006
	Parallel (Temp)	Random	0,02698	3	0,00899	23,00000	0,003733	2,409	0,093051
	Temperature	Fixed	0,14477	2	0,07239	3,01278	0,008966	8,073	0,061626
	Error		0,08586	23	0,00373				
6 (10.04.19)	Intercept	Fixed	45,16556	1	45,16556	3,02377	0,003494	12925,73	0,000001
	Parallel (Temp)	Random	0,01047	3	0,00349	23,00000	0,004474	0,78	0,517051
	Temperature	Fixed	0,20148	2	0,10074	3,01140	0,003493	28,84	0,010863
	Error		0,10291	23	0,00447				
7 (24.04.19)	Intercept	Fixed	42,77526	1	42,77526	3,05126	0,003743	11429,04	0,000001
	Parallel (Temp)	Random	0,01123	3	0,00374	21,00000	0,003822	0,98	0,421454
	Temperature	Fixed	0,35455	2	0,17728	3,02569	0,003742	47,37	0,005207
	Error		0,08027	21	0,00382				
8 (15.05.19)	Intercept	Fixed	38,56785	1	38,56785	8,62158	0,001713	22509,18	0,000000
	Parallel (Temp)	Random	0,00335	3	0,00112	23,00000	0,005812	0,19	0,900644
	Temperature	Fixed	0,20152	2	0,10076	5,03477	0,001375	73,26	0,000190
	Error		0,13367	23	0,00581				

Table II - 31: *Test results of **Tukey HSD post-hoc tests** of difference for condition factor between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,005645	0,000891
	12	0,005645		0,263977
	18	0,000891	0,263977	
2 (14.12.18)	8		0,049136	0,000253
	12	0,049136		0,002051
	18	0,000253	0,002051	
3 (01.02.19)	8		0,256717	0,002673
	12	0,256717		0,000333
	18	0,002673	0,000333	
4 (13.03.19)	8		0,428473	0,000145
	12	0,428473		0,000129
	18	0,000145	0,000129	
5 (27.03.19)	8		0,063015	0,001197
	12	0,063015		0,000135
	18	0,001197	0,000135	
6 (10.04.19)	8		0,873752	0,000153
	12	0,873752		0,000141
	18	0,000153	0,000141	
7 (24.04.19)	8		0,135376	0,000140
	12	0,135376		0,000141
	18	0,000140	0,000141	
8 (15.05.19)	8		0,145727	0,000145
	12	0,145727		0,020456
	18	0,000145	0,020456	

Table II - 32: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for condition factor between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,007062	0,018886	0,082398	0,029137	0,001699
	1	12	0,007062		0,973891	0,522136	0,993893	0,831152
	1	18	0,018886	0,973891		0,419386	0,881599	0,999996
	2	8	0,082398	0,522136	0,419386		0,882229	0,115279
	2	12	0,029137	0,993893	0,881599	0,882229		0,626054
	2	18	0,001699	0,831152	0,999996	0,115279	0,626054	
2 (14.12.18)	1	8		0,528764	0,001056	0,999656	0,264262	0,005391
	1	12	0,528764		0,021055	0,627101	0,999130	0,130335
	1	18	0,001056	0,021055		0,007124	0,018196	0,706465
	2	8	0,999656	0,627101	0,007124		0,432427	0,028241
	2	12	0,264262	0,999130	0,018196	0,432427		0,139030
	2	18	0,005391	0,130335	0,706465	0,028241	0,139030	
3 (01.02.19)	1	8		0,878378	0,031537	0,998890	0,937916	0,109393
	1	12	0,878378		0,005196	0,696508	0,999967	0,017956
	1	18	0,031537	0,005196		0,058637	0,006954	0,970979
	2	8	0,998890	0,696508	0,058637		0,792739	0,194491
	2	12	0,937916	0,999967	0,006954	0,792739		0,024297
	2	18	0,109393	0,017956	0,970979	0,194491	0,024297	
4 (13.03.19)	1	8		0,953368	0,000335	0,520685	0,963385	0,002291
	1	12	0,953368		0,000157	0,137130	0,567385	0,000381
	1	18	0,000335	0,000157		0,015195	0,001698	0,932687
	2	8	0,520685	0,137130	0,015195		0,934147	0,115086
	2	12	0,963385	0,567385	0,001698	0,934147		0,015378
	2	18	0,002291	0,000381	0,932687	0,115086	0,015378	
5 (27.03.19)	1	8		0,983168	0,001755	0,958346	0,566613	0,309162
	1	12	0,983168		0,001532	0,729624	0,976150	0,160698
	1	18	0,001755	0,001532		0,012021	0,000153	0,180944
	2	8	0,958346	0,729624	0,012021		0,155313	0,785044
	2	12	0,566613	0,976150	0,000153	0,155313		0,008655
	2	18	0,309162	0,160698	0,180944	0,785044	0,008655	
6 (10.04.19)	1	8		0,993669	0,030319	0,767075	0,736494	0,031228
	1	12	0,993669		0,013692	0,979094	0,971624	0,014093
	1	18	0,030319	0,013692		0,001450	0,001282	1,000000
	2	8	0,767075	0,979094	0,001450		1,000000	0,001493
	2	12	0,736494	0,971624	0,001282	1,000000		0,001324
	2	18	0,031228	0,014093	1,000000	0,001493	0,001324	
7 (24.04.19)	1	8		0,860969	0,000156	0,995998	0,874452	0,000524
	1	12	0,860969		0,000306	0,553281	1,000000	0,006461
	1	18	0,000156	0,000306		0,000149	0,000294	0,582217
	2	8	0,995998	0,553281	0,000149		0,573893	0,000195
	2	12	0,874452	1,000000	0,000294	0,573893		0,005990
	2	18	0,000524	0,006461	0,582217	0,000195	0,005990	
8 (15.05.19)	1	8		0,983301	0,012713	0,988206	0,726407	0,009385
	1	12	0,983301		0,336851	0,858050	0,997829	0,316835
	1	18	0,012713	0,336851		0,002803	0,354486	1,000000
	2	8	0,988206	0,858050	0,002803		0,379129	0,001975
	2	12	0,726407	0,997829	0,354486	0,379129		0,322278
	2	18	0,009385	0,316835	1,000000	0,001975	0,322278	

Table II - 33: *Test results of **one-way random effects nested ANOVAs** for condition factor within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	77,68187	1	77,68187	9,01356	0,004993	15558,01	0,000000
	Parallel (time)	Random	0,04428	8	0,00554	49,00000	0,001931	2,87	0,010692
	Time	Fixed	0,14407	7	0,02058	8,07907	0,005485	3,75	0,041118
	Error		0,09462	49	0,00193				
12.5	Intercept	Fixed	76,57300	1	76,57300	9,20513	0,001705	44909,95	0,000000
	Parallel (time)	Random	0,01367	8	0,00171	42,00000	0,001652	1,03	0,426248
	Time	Fixed	0,03520	7	0,00503	8,16128	0,001708	2,94	0,075042
	Error		0,06940	42	0,00165				
18	Intercept	Fixed	103,9251	1	103,9251	13,49294	0,005249	19800,12	0,000000
	Parallel (time)	Random	0,0390	8	0,0049	53,00000	0,007006	0,70	0,693811
	Time	Fixed	0,0599	7	0,0086	8,41822	0,004910	1,74	0,221126
	Error		0,3713	53	0,0070				

Table II - 34: *Test results of **Tukey HSD post-hoc tests** of difference for condition factor within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,007876	0,000246	0,000134	0,000133	0,001904	0,247738	0,141382
	2	0,007876		0,994868	0,892156	0,652004	0,999884	0,497571	0,609890
	3	0,000246	0,994868		0,999245	0,959423	0,843705	0,047071	0,071012
	4	0,000134	0,892156	0,999245		0,998849	0,322458	0,002110	0,003310
	5	0,000133	0,652004	0,959423	0,998849		0,099439	0,000420	0,000599
	6	0,001904	0,999884	0,843705	0,322458	0,099439		0,452174	0,589683
	7	0,247738	0,497571	0,047071	0,002110	0,000420	0,452174		0,999995
	8	0,141382	0,609890	0,071012	0,003310	0,000599	0,589683	0,999995	
12.5	1		0,586478	0,570513	0,999997	0,846716	0,737538	1,000000	0,993202
	2	0,586478		0,009463	0,275943	0,021620	0,012429	0,383626	0,939634
	3	0,570513	0,009463		0,524112	0,997471	0,999812	0,505010	0,123799
	4	0,999997	0,275943	0,524112		0,838523	0,700487	1,000000	0,935574
	5	0,846716	0,021620	0,997471	0,838523		0,999997	0,813653	0,270803
	6	0,737538	0,012429	0,999812	0,700487	0,999997		0,677599	0,180630
	7	1,000000	0,383626	0,505010	1,000000	0,813653	0,677599		0,971180
	8	0,993202	0,939634	0,123799	0,935574	0,270803	0,180630	0,971180	
18	1		0,720998	0,502078	0,469367	0,529959	0,544920	0,082299	0,748086
	2	0,720998		0,999944	0,999994	1,000000	1,000000	0,884932	0,999989
	3	0,502078	0,999944		1,000000	0,999996	0,999992	0,984591	0,995286
	4	0,469367	0,999994	1,000000		1,000000	1,000000	0,924160	0,997078
	5	0,529959	1,000000	0,999996	1,000000		1,000000	0,880067	0,999287
	6	0,544920	1,000000	0,999992	1,000000	1,000000		0,867442	0,999516
	7	0,082299	0,884932	0,984591	0,924160	0,880067	0,867442		0,506184
	8	0,748086	0,999989	0,995286	0,997078	0,999287	0,999516	0,506184	

d. Comparison of temperature groups between feeding regimes

Table II - 35: *Test results of **factorial ANOVAs** for condition factor comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	171,3465	1	171,3465	73384,36	0,000000
	Feeding regime	0,0007	1	0,0007	0,29	0,593413
	Time	0,2616	7	0,0374	16,01	0,000000
	Feed*Time	0,0263	7	0,0038	1,61	0,139260
	Error	0,2662	114	0,0023		
12.5	Intercept	159,8339	1	159,8339	79315,21	0,000000
	Feeding regime	0,0819	1	0,0819	40,65	0,000000
	Time	0,1759	7	0,0251	12,47	0,000000
	Feed*Time	0,0440	7	0,0063	3,12	0,004883
	Error	0,2156	107	0,0020		
18	Intercept	224,7545	1	224,7545	36223,53	0,000000
	Feeding regime	0,0203	1	0,0203	3,28	0,072701
	Time	0,1258	7	0,0180	2,90	0,007798
	Feed*Time	0,0640	7	0,0091	1,47	0,183228
	Error	0,7570	122	0,0062		

Table II - 36: *Test results of **Tukey HSD post-hoc tests** for condition factor comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,29	0,00	0,00	0,00	0,01	0,14	0,01	1,00	0,01	0,00	0,00	0,00	0,00	0,31	0,17
		2	0,29		0,02	0,07	0,96	1,00	1,00	1,00	0,63	0,99	0,57	0,12	0,03	0,99	1,00	1,00
		3	0,00	0,02		1,00	0,48	0,09	0,02	0,12	0,00	0,86	0,99	1,00	1,00	0,26	0,00	0,00
		4	0,00	0,07	1,00		0,85	0,24	0,05	0,32	0,00	0,99	1,00	1,00	1,00	0,61	0,01	0,01
		5	0,00	0,96	0,48	0,85		1,00	0,97	1,00	0,01	1,00	1,00	0,94	0,67	1,00	0,74	0,84
		6	0,01	1,00	0,09	0,24	1,00		1,00	1,00	0,06	1,00	0,92	0,39	0,12	1,00	0,99	1,00
		7	0,14	1,00	0,02	0,05	0,97	1,00		1,00	0,41	0,99	0,56	0,09	0,02	0,99	1,00	1,00
		8	0,01	1,00	0,12	0,32	1,00	1,00	1,00		0,04	1,00	0,96	0,48	0,17	1,00	0,98	1,00
	100	1	1,00	0,63	0,00	0,00	0,01	0,06	0,41	0,04		0,05	0,00	0,00	0,00	0,01	0,68	0,50
		2	0,01	0,99	0,86	0,99	1,00	1,00	0,99	1,00	0,05		1,00	1,00	0,97	1,00	0,91	0,95
		3	0,00	0,57	0,99	1,00	1,00	0,92	0,56	0,96	0,00	1,00		1,00	1,00	1,00	0,23	0,31
		4	0,00	0,12	1,00	1,00	0,94	0,39	0,09	0,48	0,00	1,00	1,00		1,00	0,77	0,01	0,02
		5	0,00	0,03	1,00	1,00	0,67	0,12	0,02	0,17	0,00	0,97	1,00	1,00		0,40	0,00	0,00
		6	0,00	0,99	0,26	0,61	1,00	1,00	0,99	1,00	0,01	1,00	1,00	0,77	0,40		0,88	0,95
		7	0,31	1,00	0,00	0,01	0,74	0,99	1,00	0,98	0,68	0,91	0,23	0,01	0,00	0,88		1,00
		8	0,17	1,00	0,00	0,01	0,84	1,00	1,00	1,00	0,50	0,95	0,31	0,02	0,00	0,95	1,00	
12.5	67	1		0,97	0,71	0,03	0,00	0,00	0,01	0,77	1,00	0,97	0,89	1,00	0,99	0,96	1,00	1,00
		2	0,97		0,02	0,00	0,00	0,00	0,00	0,01	0,93	1,00	0,04	0,65	0,08	0,05	0,79	1,00
		3	0,71	0,02		1,00	0,10	0,69	0,98	1,00	0,80	0,03	1,00	0,74	1,00	1,00	0,73	0,26
		4	0,03	0,00	1,00		0,40	0,99	1,00	0,82	0,05	0,00	0,94	0,01	0,41	0,57	0,02	0,00
		5	0,00	0,00	0,10	0,40		1,00	0,79	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00
		6	0,00	0,00	0,69	0,99	1,00		1,00	0,09	0,00	0,00	0,27	0,00	0,02	0,04	0,00	0,00
		7	0,01	0,00	0,98	1,00	0,79	1,00		0,53	0,02	0,00	0,77	0,00	0,18	0,29	0,00	0,00
		8	0,77	0,01	1,00	0,82	0,00	0,09	0,53		0,86	0,02	1,00	0,76	1,00	1,00	0,76	0,24
	100	1	1,00	0,93	0,80	0,05	0,00	0,00	0,02	0,86		0,95	0,94	1,00	1,00	0,98	1,00	1,00
		2	0,97	1,00	0,03	0,00	0,00	0,00	0,00	0,02	0,95		0,06	0,72	0,12	0,07	0,83	1,00
		3	0,89	0,04	1,00	0,94	0,01	0,27	0,77	1,00	0,94	0,06		0,92	1,00	1,00	0,91	0,46
		4	1,00	0,65	0,74	0,01	0,00	0,00	0,00	0,76	1,00	0,72	0,92		1,00	0,98	1,00	1,00
		5	0,99	0,08	1,00	0,41	0,00	0,02	0,18	1,00	1,00	0,12	1,00	1,00		1,00	0,99	0,72
		6	0,96	0,05	1,00	0,57	0,00	0,04	0,29	1,00	0,98	0,07	1,00	0,98	1,00		0,97	0,58
		7	1,00	0,79	0,73	0,02	0,00	0,00	0,00	0,76	1,00	0,83	0,91	1,00	0,99	0,97		1,00
		8	1,00	1,00	0,26	0,00	0,00	0,00	0,00	0,24	1,00	1,00	0,46	1,00	0,72	0,58	1,00	
18	67	1		1,00	1,00	0,99	1,00	1,00	0,80	0,82	1,00	0,99	0,92	0,90	0,94	0,95	0,22	0,99
		2	1,00		1,00	1,00	0,54	1,00	1,00	1,00	0,96	1,00	1,00	1,00	1,00	1,00	0,98	1,00
		3	1,00	1,00		1,00	0,90	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,77	1,00
		4	0,99	1,00	1,00		0,30	1,00	1,00	1,00	0,93	1,00	1,00	1,00	1,00	1,00	0,92	1,00
		5	1,00	0,54	0,90	0,30		0,86	0,04	0,03	1,00	0,45	0,18	0,08	0,12	0,13	0,00	0,31
		6	1,00	1,00	1,00	1,00	0,86		0,97	0,98	1,00	1,00	1,00	0,99	1,00	1,00	0,39	1,00
		7	0,80	1,00	1,00	1,00	0,04	0,97		1,00	0,59	1,00	1,00	1,00	1,00	1,00	1,00	1,00
		8	0,82	1,00	1,00	1,00	0,03	0,98	1,00		0,60	1,00	1,00	1,00	1,00	1,00	1,00	1,00
	100	1	1,00	0,96	1,00	0,93	1,00	1,00	0,59	0,60		0,93	0,76	0,72	0,79	0,80	0,12	0,95
		2	0,99	1,00	1,00	1,00	0,45	1,00	1,00	1,00	0,93		1,00	1,00	1,00	1,00	0,99	1,00
		3	0,92	1,00	1,00	1,00	0,18	1,00	1,00	1,00	0,76	1,00		1,00	1,00	1,00	1,00	1,00
		4	0,90	1,00	1,00	1,00	0,08	0,99	1,00	1,00	0,72	1,00	1,00		1,00	1,00	1,00	1,00
		5	0,94	1,00	1,00	1,00	0,12	1,00	1,00	1,00	0,79	1,00	1,00	1,00		1,00	0,99	1,00
		6	0,95	1,00	1,00	1,00	0,13	1,00	1,00	1,00	0,80	1,00	1,00	1,00	1,00		0,99	1,00
		7	0,22	0,98	0,77	0,92	0,00	0,39	1,00	1,00	0,12	0,99	1,00	1,00	0,99	0,99		0,76
		8	0,99	1,00	1,00	1,00	0,31	1,00	1,00	1,00	0,95	1,00	1,00	1,00	1,00	1,00	0,76	

II - III Hepatosomatic index (HSI)

a. Overall tests:

Table II - 37: *Test results of a **three-way random effects nested ANOVA** for HSI (%), including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	330,0387	1	330,0387	91,3179	0,026369	12516,08	0,000000
Parallel (Temp.*feed*Time)	Random	2,4089	85	0,0283	296,0000	0,009108	3,11	0,000000
Time	Fixed	6,9191	7	0,9884	87,0544	0,027646	35,75	0,000000
Temperature	Fixed	1,9647	2	0,9824	91,1695	0,026410	37,20	0,000000
Feeding regime	Fixed	0,1741	1	0,1741	91,3179	0,026369	6,60	0,011796
Error		2,6959	296	0,0091				

Table II - 38: *Test results of a **three-way factorial ANOVA** for HSI (%), including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	335,7993	1	335,7993	31958,03	0,000000
Time	7,0411	7	1,0059	95,73	0,000000
Temperature	1,9929	2	0,9965	94,83	0,000000
Feed regime	0,1865	1	0,1865	17,75	0,000032
Time*Temp.	0,9597	14	0,0685	6,52	0,000000
Time*Feed.	0,2683	7	0,0383	3,65	0,000821
Temp.*Feed.	0,0047	2	0,0023	0,22	0,800506
Time*Temp.*Feed.	0,2073	14	0,0148	1,41	0,146231
Error	3,6146	344	0,0105		

b. Restrictive feeding (67%)

Table II - 39: *Test results of a **two-way random effects nested ANOVA** for HSI (%), including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	176,2572	1	176,2572	40,2910	0,030053	5864,830	0,000000
Parallel (Temp.*Time)	Random	1,2194	38	0,0321	150,0000	0,009611	3,339	0,000000
Time	Fixed	4,1152	7	0,5879	38,5219	0,031586	18,612	0,000000
Temperature	Fixed	0,8787	2	0,4394	40,2414	0,030093	14,600	0,000017
Error		1,4417	150	0,0096				

Table II - 40: *Test results of a **two-way factorial ANOVA** for HSI (%), including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	178,8442	1	178,8442	15415,50	0,000000
Time	4,1452	7	0,5922	51,04	0,000000
Temperature	0,9145	2	0,4573	39,41	0,000000
Temp.*Time	0,6424	14	0,0459	3,95	0,000007
Error	2,0187	174	0,0116		

Table II - 41: Test results of **one-*way random effects nested ANOVAs*** *for HSI (%), including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	30,13258	1	30,13258	3,02828	0,090156	334,2254	0,000337
	Parallel (temp)	Random	0,27917	3	0,09306	10,00000	0,011822	7,8717	0,005468
	Temperature	Fixed	0,25905	2	0,12953	3,01211	0,091788	1,4111	0,369467
	Error		0,11822	10	0,01182				
2 (14.12.18)	Intercept	Fixed	19,15147	1	19,15147	3,22068	0,001061	18051,54	0,000000
	Parallel (temp)	Random	0,00311	3	0,00104	10,00000	0,003385	0,31	0,820699
	Temperature	Fixed	0,17046	2	0,08523	3,11757	0,001049	81,25	0,002045
	Error		0,03385	10	0,00338				
3 (01.02.19)	Intercept	Fixed	16,67358	1	16,67358	3	0,031307	532,5890	0,000178
	Parallel (temp)	Random	0,09392	3	0,03131	12	0,015049	2,0803	0,156357
	Temperature	Fixed	0,38004	2	0,19002	3	0,031307	6,0696	0,088212
	Error		0,18059	12	0,01505				
4 (13.04.19)	Intercept	Fixed	26,27929	1	26,27929	3,00455	0,027768	946,3720	0,000075
	Parallel (temp)	Random	0,08344	3	0,02781	23,00000	0,008837	3,1473	0,044485
	Temperature	Fixed	0,15558	2	0,07779	3,00223	0,027791	2,7991	0,206004
	Error		0,20326	23	0,00884				
5 (27.03.19)	Intercept	Fixed	21,51499	1	21,51499	3,12887	0,000524	41022,04	0,000000
	Parallel (temp)	Random	0,00155	3	0,00052	23,00000	0,003552	0,15	0,931820
	Temperature	Fixed	0,25623	2	0,12811	3,06156	0,000520	246,55	0,000414
	Error		0,08170	23	0,00355				
6 (10.04.19)	Intercept	Fixed	22,45979	1	22,45979	3,00294	0,011279	1991,359	0,000025
	Parallel (temp)	Random	0,03383	3	0,01128	24,00000	0,014898	0,757	0,529216
	Temperature	Fixed	0,07048	2	0,03524	3,00145	0,011278	3,125	0,184668
	Error		0,35755	24	0,01490				
7 (24.04.19)	Intercept	Fixed	20,06290	1	20,06290	3,12785	0,009082	2209,006	0,000015
	Parallel (temp)	Random	0,02706	3	0,00902	21,00000	0,013588	0,664	0,583586
	Temperature	Fixed	0,03013	2	0,01507	3,06178	0,009050	1,665	0,324124
	Error		0,28535	21	0,01359				
8 (15.05.19)	Intercept	Fixed	25,99661	1	25,99661	3,01846	0,018214	1427,288	0,000039
	Parallel (temp)	Random	0,05493	3	0,01831	27,00000	0,006710	2,729	0,063542
	Temperature	Fixed	0,06001	2	0,03001	3,00906	0,018263	1,643	0,329368
	Error		0,18116	27	0,00671				

Table II - 42: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for HSI (%) between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,150689	0,002402
	12	0,150689		0,131964
	18	0,002402	0,131964	
2 (14.12.18)	8		0,017463	0,000265
	12	0,017463		0,011804
	18	0,000265	0,011804	
3 (01.02.19)	8		0,002188	0,003280
	12	0,002188		0,966633
	18	0,003280	0,966633	
4 (13.03.19)	8		0,001416	0,001971
	12	0,001416		0,966009
	18	0,001971	0,966009	
5 (27.03.19)	8		0,122483	0,000142
	12	0,122483		0,000133
	18	0,000142	0,000133	
6 (10.04.19)	8		0,153728	0,191103
	12	0,153728		0,991593
	18	0,191103	0,991593	
7 (24.04.19)	8		0,390821	0,981181
	12	0,390821		0,453464
	18	0,981181	0,453464	
8 (15.05.19)	8		0,881016	0,090098
	12	0,881016		0,027217
	18	0,090098	0,027217	

Table II - 43: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for HSI (%) between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,999009	0,546726	0,009099	0,945237	0,999699
	1	12	0,999009		0,453588	0,031872	0,996319	0,988059
	1	18	0,546726	0,453588		0,000982	0,237734	0,702122
	2	8	0,009099	0,031872	0,000982		0,069554	0,006121
	2	12	0,945237	0,996319	0,237734	0,069554		0,862105
	2	18	0,999699	0,988059	0,702122	0,006121	0,862105	
2 (14.12.18)	1	8		0,118183	0,002923	1,000000	0,311815	0,004049
	1	12	0,118183		0,422807	0,171117	0,930722	0,536586
	1	18	0,002923	0,422807		0,006394	0,072568	0,999844
	2	8	1,000000	0,171117	0,006394		0,415857	0,008717
	2	12	0,311815	0,930722	0,072568	0,415857		0,104684
	2	18	0,004049	0,536586	0,999844	0,008717	0,104684	
3 (01.02.19)	1	8		0,361262	0,238026	0,278389	0,402883	0,765138
	1	12	0,361262		0,999563	0,009691	0,999999	0,972143
	1	18	0,238026	0,999563		0,005879	0,998519	0,892396
	2	8	0,278389	0,009691	0,005879		0,011186	0,033713
	2	12	0,402883	0,999999	0,998519	0,011186		0,983666
	2	18	0,765138	0,972143	0,892396	0,033713	0,983666	
4 (13.03.19)	1	8		0,041984	0,000938	0,338157	0,002411	0,113309
	1	12	0,041984		0,768099	0,906047	0,932007	0,987882
	1	18	0,000938	0,768099		0,196675	0,998349	0,336205
	2	8	0,338157	0,906047	0,196675		0,364287	0,997414
	2	12	0,002411	0,932007	0,998349	0,364287		0,569623
	2	18	0,113309	0,987882	0,336205	0,997414	0,569623	
5 (27.03.19)	1	8		0,908733	0,004260	0,994391	0,828035	0,002060
	1	12	0,908733		0,000293	0,583994	0,999942	0,000202
	1	18	0,004260	0,000293		0,008722	0,000226	0,999413
	2	8	0,994391	0,583994	0,008722		0,458936	0,004016
	2	12	0,828035	0,999942	0,000226	0,458936		0,000176
	2	18	0,002060	0,000202	0,999413	0,004016	0,000176	
6 (10.04.19)	1	8		0,691935	0,333952	0,999609	0,690908	0,982372
	1	12	0,691935		0,985693	0,787848	1,000000	0,960007
	1	18	0,333952	0,985693		0,388024	0,985872	0,682403
	2	8	0,999609	0,787848	0,388024		0,786840	0,997996
	2	12	0,690908	1,000000	0,985872	0,786840		0,959636
	2	18	0,982372	0,960007	0,682403	0,997996	0,959636	
7 (24.04.19)	1	8		0,998204	0,997731	0,999450	0,920452	0,989501
	1	12	0,998204		1,000000	0,952073	0,984721	0,841260
	1	18	0,997731	1,000000		0,946333	0,986947	0,829930
	2	8	0,999450	0,952073	0,946333		0,686717	0,999507
	2	12	0,920452	0,984721	0,986947	0,686717		0,504051
	2	18	0,989501	0,841260	0,829930	0,999507	0,504051	
8 (15.05.19)	1	8		0,724034	0,276658	0,996052	1,000000	0,999993
	1	12	0,724034		0,007168	0,903677	0,581868	0,543553
	1	18	0,276658	0,007168		0,058125	0,206659	0,231384
	2	8	0,996052	0,903677	0,058125		0,987055	0,980070
	2	12	1,000000	0,581868	0,206659	0,987055		1,000000
	2	18	0,999993	0,543553	0,231384	0,980070	1,000000	

Table II - 44: *Test results of **one-way random effects nested ANOVAs** for HSI (%) within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	68,48984	1	68,48984	8,16881	0,044149	1551,351	0,000000
	Parallel (Time)	Random	0,37239	8	0,04655	48,00000	0,007473	6,229	0,000016
	Time	Fixed	2,36818	7	0,33831	8,02038	0,046241	7,316	0,005833
	Error		0,35870	48	0,00747				
12.5	Intercept	Fixed	53,63797	1	53,63797	10,05062	0,004641	11558,60	0,000000
	Parallel (Time)	Random	0,03746	8	0,00468	48,00000	0,004320	1,08	0,390395
	Time	Fixed	1,26817	7	0,18117	8,20584	0,004678	38,73	0,000012
	Error		0,20738	48	0,00432				
18	Intercept	Fixed	54,73767	1	54,73767	8,96062	0,020566	2661,588	0,000000
	Parallel (Time)	Random	0,16716	8	0,02089	54,00000	0,016215	1,289	0,269019
	Time	Fixed	1,13002	7	0,16143	8,11581	0,020851	7,742	0,004675
	Error		0,87559	54	0,01621				

Table II - 45: *Test results of **Tukey HSD post-hoc tests** of difference for HSI (%) within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,000144	0,000134	0,000134	0,000134	0,000134	0,000134	0,000134
	2	0,000144		0,848118	0,024445	0,000134	0,000135	0,000134	0,000134
	3	0,000134	0,848118		0,492705	0,000140	0,000250	0,000159	0,000154
	4	0,000134	0,024445	0,492705		0,001121	0,014823	0,003382	0,003139
	5	0,000134	0,000134	0,000140	0,001121		0,975033	0,999998	0,999784
	6	0,000134	0,000135	0,000250	0,014823	0,975033		0,996141	0,999439
	7	0,000134	0,000134	0,000159	0,003382	0,999998	0,996141		0,999999
	8	0,000134	0,000134	0,000154	0,003139	0,999784	0,999439	0,999999	
12.5	1		0,000134	0,000134	0,000134	0,000134	0,000134	0,000134	0,000134
	2	0,000134		0,000134	0,000141	0,001211	0,000134	0,000134	0,000251
	3	0,000134	0,000134		0,812830	0,062912	0,999488	0,999536	0,215969
	4	0,000134	0,000141	0,812830		0,651393	0,319135	0,349035	0,954527
	5	0,000134	0,001211	0,062912	0,651393		0,003152	0,004338	0,996041
	6	0,000134	0,000134	0,999488	0,319135	0,003152		1,000000	0,018955
	7	0,000134	0,000134	0,999536	0,349035	0,004338	1,000000		0,024570
	8	0,000134	0,000251	0,215969	0,954527	0,996041	0,018955	0,024570	
18	1		0,017455	0,000208	0,000229	0,000134	0,000135	0,000174	0,000134
	2	0,017455		0,726413	0,941779	0,007647	0,330847	0,864466	0,296396
	3	0,000208	0,726413		0,997312	0,491683	0,999855	0,999809	0,999843
	4	0,000229	0,941779	0,997312		0,058380	0,899920	0,999996	0,882142
	5	0,000134	0,007647	0,491683	0,058380		0,619444	0,107897	0,559392
	6	0,000135	0,330847	0,999855	0,899920	0,619444		0,967888	1,000000
	7	0,000174	0,864466	0,999809	0,999996	0,107897	0,967888		0,961561
	8	0,000134	0,296396	0,999843	0,882142	0,559392	1,000000	0,961561	

c. Full feeding (100%)

Table II - 46: *Test results of a **two-way random effects nested ANOVA** for HSI (%), including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	154,3983	1	154,3983	41,4466	0,021535	7169,643	0,000000
Parallel (Temp.*Time)	Random	0,8773	38	0,0231	146,0000	0,008591	2,688	0,000013
Temperature	Fixed	1,0922	2	0,5461	41,3449	0,021575	25,312	0,000000
Time	Fixed	3,1055	7	0,4436	39,0547	0,022569	19,657	0,000000
Error		1,2543	146	0,0086				

Table II - 47: *Test results of a **two-way factorial ANOVA** for HSI (%), including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	157,4924	1	157,4924	16776,47	0,000000
Time	3,1667	7	0,4524	48,19	0,000000
Temperature	1,0816	2	0,5408	57,61	0,000000
Time*Temp.	0,5357	14	0,0383	4,08	0,000004
Error	1,5959	170	0,0094		

Table II - 48: *Test results of **one-way random effects nested ANOVAs** for HSI (%), including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	21,48110	1	21,48110	3,000000	0,007286	2948,340	0,000014
	Parallel (Temp)	Random	0,02186	3	0,00729	9,000000	0,007636	0,954	0,454992
	Temperature	Fixed	0,28018	2	0,14009	3,000000	0,007286	19,228	0,019467
	Error		0,06872	9	0,00764				
2 (14.12.18)	Intercept	Fixed	21,17224	1	21,17224	3,26762	0,004147	5105,550	0,000002
	Parallel (Temp)	Random	0,01205	3	0,00402	11,00000	0,015878	0,253	0,857627
	Temperature	Fixed	0,18956	2	0,09478	3,12252	0,004077	23,246	0,013328
	Error		0,17466	11	0,01588				
3 (01.02.19)	Intercept	Fixed	15,04830	1	15,04830	3,06781	0,037930	396,7377	0,000240
	Parallel (Temp)	Random	0,11669	3	0,03890	10,00000	0,011823	3,2899	0,066481
	Temperature	Fixed	0,34141	2	0,17070	3,02900	0,038474	4,4369	0,125852
	Error		0,11823	10	0,01182				
4 (13.04.19)	Intercept	Fixed	26,45544	1	26,45544	3	0,009628	2747,830	0,000015
	Parallel (Temp)	Random	0,02888	3	0,00963	24	0,007626	1,262	0,309546
	Temperature	Fixed	0,17622	2	0,08811	3	0,009628	9,151	0,052847
	Error		0,18303	24	0,00763				
5 (27.03.19)	Intercept	Fixed	17,35998	1	17,35998	3,02241	0,017215	1008,439	0,000065
	Parallel (Temp)	Random	0,05202	3	0,01734	23,00000	0,005819	2,980	0,052424
	Temperature	Fixed	0,35163	2	0,17581	3,01033	0,017283	10,173	0,045785
	Error		0,13385	23	0,00582				
6 (10.04.19)	Intercept	Fixed	22,42540	1	22,42540	3,00000	0,006820	3288,260	0,000012
	Parallel (Temp)	Random	0,02046	3	0,00682	24,00000	0,014473	0,471	0,705160
	Temperature	Fixed	0,01871	2	0,00936	3,00000	0,006820	1,372	0,377451
	Error		0,34734	24	0,01447				
7 (24.04.19)	Intercept	Fixed	20,63614	1	20,63614	3,00460	0,024046	858,1950	0,000086
	Parallel (Temp)	Random	0,07231	3	0,02410	23,00000	0,005979	4,0308	0,019281
	Temperature	Fixed	0,00350	2	0,00175	3,00221	0,024075	0,0728	0,931421
	Error		0,13752	23	0,00598				
8 (15.05.19)	Intercept	Fixed	15,94903	1	15,94903	3,56029	0,005609	2843,271	0,000003
	Parallel (Temp)	Random	0,01739	3	0,00580	22,00000	0,004132	1,403	0,268521
	Temperature	Fixed	0,15871	2	0,07935	3,21409	0,005718	13,878	0,026232
	Error		0,09090	22	0,00413				

Table II - 49: *Test results of **Tukey HSD post-hoc tests** of difference for HSI (%) between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,363897	0,000730
	12	0,363897		0,003977
	18	0,000730	0,003977	
2 (14.12.18)	8		0,024496	0,045382
	12	0,024496		0,924897
	18	0,045382	0,924897	
3 (01.02.19)	8		0,002057	0,004080
	12	0,002057		0,633378
	18	0,004080	0,633378	
4 (13.03.19)	8		0,006645	0,000403
	12	0,006645		0,434298
	18	0,000403	0,434298	
5 (27.03.19)	8		0,000891	0,000133
	12	0,000891		0,010929
	18	0,000133	0,010929	
6 (10.04.19)	8		0,997763	0,574526
	12	0,997763		0,614282
	18	0,574526	0,614282	
7 (24.04.19)	8		0,767980	0,800842
	12	0,767980		0,998117
	18	0,800842	0,998117	
8 (15.05.19)	8		0,793464	0,000162
	12	0,793464		0,001303
	18	0,000162	0,001303	

Table II - 50: *Test results of **Tukey HSD post-hoc tests** of difference for HSI (%) between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,999999	0,016623	0,875635	0,790226	0,065129
	1	12	0,999999		0,018924	0,840443	0,830137	0,073477
	1	18	0,016623	0,018924		0,007197	0,191109	0,994357
	2	8	0,875635	0,840443	0,007197		0,340479	0,024221
	2	12	0,790226	0,830137	0,191109	0,340479		0,453800
	2	18	0,065129	0,073477	0,994357	0,024221	0,453800	
2 (14.12.18)	1	8		0,429709	0,487897	0,957770	0,421545	0,653208
	1	12	0,429709		0,999997	0,189063	1,000000	0,998170
	1	18	0,487897	0,999997		0,218345	0,999994	0,999614
	2	8	0,957770	0,189063	0,218345		0,185085	0,314218
	2	12	0,421545	1,000000	0,999994	0,185085		0,997798
	2	18	0,653208	0,998170	0,999614	0,314218	0,997798	
3 (01.02.19)	1	8		0,322593	0,946618	0,190535	0,279781	0,167264
	1	12	0,322593		0,722046	0,010988	0,999998	0,999941
	1	18	0,946618	0,722046		0,052709	0,661770	0,506530
	2	8	0,190535	0,010988	0,052709		0,009377	0,003818
	2	12	0,279781	0,999998	0,661770	0,009377		0,999999
	2	18	0,167264	0,999941	0,506530	0,003818	0,999999	
4 (13.03.19)	1	8		0,410499	0,408097	0,857671	0,530263	0,020568
	1	12	0,410499		1,000000	0,052165	0,999947	0,628110
	1	18	0,408097	1,000000		0,051685	0,999942	0,630759
	2	8	0,857671	0,052165	0,051685		0,079992	0,001413
	2	12	0,530263	0,999947	0,999942	0,079992		0,503032
	2	18	0,020568	0,628110	0,630759	0,001413	0,503032	
5 (27.03.19)	1	8		0,002638	0,000948	0,957053	0,079493	0,000145
	1	12	0,002638		0,999965	0,014232	0,334389	0,649877
	1	18	0,000948	0,999965		0,006317	0,277721	0,381308
	2	8	0,957053	0,014232	0,006317		0,369824	0,000182
	2	12	0,079493	0,334389	0,277721	0,369824		0,003562
	2	18	0,000145	0,649877	0,381308	0,000182	0,003562	
6 (10.04.19)	1	8		1,000000	0,814545	0,964853	0,950340	0,913857
	1	12	1,000000		0,825390	0,968789	0,955334	0,920982
	1	18	0,814545	0,825390		0,997766	0,999047	0,999887
	2	8	0,964853	0,968789	0,997766		1,000000	0,999954
	2	12	0,950340	0,955334	0,999047	1,000000		0,999994
	2	18	0,913857	0,920982	0,999887	0,999954	0,999994	
7 (24.04.19)	1	8		0,861466	0,999847	1,000000	0,312924	0,985692
	1	12	0,861466		0,683887	0,816895	0,023382	0,430569
	1	18	0,999847	0,683887		0,999880	0,383773	0,998134
	2	8	1,000000	0,816895	0,999880		0,267208	0,984481
	2	12	0,312924	0,023382	0,383773	0,267208		0,632666
	2	18	0,985692	0,430569	0,998134	0,984481	0,632666	
8 (15.05.19)	1	8		0,983098	0,002884	0,982543	0,574599	0,003776
	1	12	0,983098		0,007943	0,838073	0,410561	0,009719
	1	18	0,002884	0,007943		0,014526	0,198691	0,999995
	2	8	0,982543	0,838073	0,014526		0,908726	0,018942
	2	12	0,574599	0,410561	0,198691	0,908726		0,239832
	2	18	0,003776	0,009719	0,999995	0,018942	0,239832	

Table II - 51: *Test results of **one-way random effects nested ANOVAs** for HSI (%) within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	64,82377	1	64,82377	8,97757	0,014780	4385,805	0,000000
	Parallel (time)	Random	0,12322	8	0,01540	49,00000	0,008816	1,747	0,111122
	Time	Fixed	1,79293	7	0,25613	8,10527	0,015327	16,711	0,000321
	Error		0,43198	49	0,00882				
12.5	Intercept	Fixed	44,41255	1	44,41255	8,98219	0,015722	2824,778	0,000000
	Parallel (time)	Random	0,13339	8	0,01667	44,00000	0,008058	2,069	0,059800
	Time	Fixed	0,96055	7	0,13722	8,11999	0,016542	8,295	0,003716
	Error		0,35455	44	0,00806				
18	Intercept	Fixed	46,84319	1	46,84319	9,40004	0,010465	4476,213	0,000000
	Parallel (time)	Random	0,08506	8	0,01063	53,00000	0,008825	1,205	0,314267
	Time	Fixed	1,00672	7	0,14382	8,15424	0,010611	13,553	0,000665
	Error		0,46773	53	0,00883				

Table II - 52: *Test results of **Tukey HSD post-hoc tests** of difference for HSI (%) within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,870165	0,090719	0,000136	0,000133	0,000133	0,000133	0,000133
	2	0,870165		0,804942	0,001124	0,000133	0,000133	0,000133	0,000133
	3	0,090719	0,804942		0,071748	0,000208	0,000134	0,000133	0,000133
	4	0,000136	0,001124	0,071748		0,188363	0,008826	0,002689	0,002082
	5	0,000133	0,000133	0,000208	0,188363		0,920535	0,690262	0,679452
	6	0,000133	0,000133	0,000134	0,008826	0,920535		0,999621	0,999692
	7	0,000133	0,000133	0,000133	0,002689	0,690262	0,999621		1,000000
	8	0,000133	0,000133	0,000133	0,002082	0,679452	0,999692	1,000000	
12.5	1		0,001703	0,000133	0,000133	0,000133	0,000133	0,000133	0,000133
	2	0,001703		0,051797	0,144675	0,000210	0,030238	0,002252	0,010574
	3	0,000133	0,051797		0,956821	0,905713	0,999602	0,999987	0,999999
	4	0,000133	0,144675	0,956821		0,068022	0,994087	0,599858	0,805131
	5	0,000133	0,000210	0,905713	0,068022		0,312064	0,905947	0,928696
	6	0,000133	0,030238	0,999602	0,994087	0,312064		0,959732	0,990187
	7	0,000133	0,002252	0,999987	0,599858	0,905947	0,959732		1,000000
	8	0,000133	0,010574	0,999999	0,805131	0,928696	0,990187	1,000000	
18	1		0,998144	0,544662	0,041782	0,000134	0,006957	0,012480	0,000135
	2	0,998144		0,150330	0,002752	0,000134	0,000394	0,000690	0,000134
	3	0,544662	0,150330		0,936575	0,000253	0,589822	0,725080	0,001093
	4	0,041782	0,002752	0,936575		0,001035	0,993324	0,999406	0,008008
	5	0,000134	0,000134	0,000253	0,001035		0,010873	0,005270	0,986715
	6	0,006957	0,000394	0,589822	0,993324	0,010873		0,999997	0,073606
	7	0,012480	0,000690	0,725080	0,999406	0,005270	0,999997		0,038763
	8	0,000135	0,000134	0,001093	0,008008	0,986715	0,073606	0,038763	

d. Comparison of temperature groups between feeding regimes

Table II - 53: *Test results of **factorial ANOVAs** for HSI (%) comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	136,2598	1	136,2598	11970,48	0,000000
	Feeding regime	0,0378	1	0,0378	3,32	0,071235
	Time	3,9929	7	0,5704	50,11	0,000000
	Feed*Time	0,1132	7	0,0162	1,42	0,203704
	Error	1,2863	113	0,0114		
12.5	Intercept	100,0590	1	100,0590	14747,11	0,000000
	Feeding regime	0,0697	1	0,0697	10,27	0,001780
	Time	2,1475	7	0,3068	45,22	0,000000
	Feed*Time	0,1733	7	0,0248	3,65	0,001428
	Error	0,7328	108	0,0068		
18	Intercept	101,6985	1	101,6985	7839,990	0,000000
	Feeding regime	0,0846	1	0,0846	6,525	0,011859
	Time	1,8783	7	0,2683	20,686	0,000000
	Feed*Time	0,2059	7	0,0294	2,268	0,033194
	Error	1,5955	123	0,0130		

Table II - 54: *Test results of **Tukey HSD post-hoc tests** for HSI (%) comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,23	0,01	0,00	0,00	0,00	0,00	0,00	0,00
		2	0,00		1,00	0,29	0,00	0,00	0,00	0,00	0,94	1,00	1,00	0,07	0,00	0,00	0,00	0,00
		3	0,00	1,00		0,96	0,00	0,01	0,00	0,00	0,22	0,95	1,00	0,66	0,00	0,00	0,00	0,00
		4	0,00	0,29	0,96		0,03	0,21	0,08	0,07	0,00	0,07	0,81	1,00	0,19	0,01	0,00	0,00
		5	0,00	0,00	0,00	0,03		1,00	1,00	1,00	0,00	0,00	0,00	0,21	1,00	1,00	1,00	1,00
		6	0,00	0,00	0,01	0,21	1,00		1,00	1,00	0,00	0,00	0,00	0,69	1,00	1,00	0,97	0,97
		7	0,00	0,00	0,00	0,08	1,00	1,00		1,00	0,00	0,00	0,00	0,37	1,00	1,00	1,00	1,00
		8	0,00	0,00	0,00	0,07	1,00	1,00	1,00		0,00	0,00	0,00	0,38	1,00	1,00	1,00	1,00
	100	1	0,23	0,94	0,22	0,00	0,00	0,00	0,00	0,00		1,00	0,43	0,00	0,00	0,00	0,00	0,00
		2	0,01	1,00	0,95	0,07	0,00	0,00	0,00	0,00	1,00		0,99	0,01	0,00	0,00	0,00	0,00
		3	0,00	1,00	1,00	0,81	0,00	0,00	0,00	0,00	0,43	0,99		0,37	0,00	0,00	0,00	0,00
		4	0,00	0,07	0,66	1,00	0,21	0,69	0,37	0,38	0,00	0,01	0,37		0,65	0,08	0,03	0,02
		5	0,00	0,00	0,00	0,19	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,65		1,00	0,98	0,98
		6	0,00	0,00	0,00	0,01	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,08	1,00		1,00	1,00
		7	0,00	0,00	0,00	0,00	1,00	0,97	1,00	1,00	0,00	0,00	0,00	0,03	0,98	1,00		1,00
		8	0,00	0,00	0,00	0,00	1,00	0,97	1,00	1,00	0,00	0,00	0,00	0,02	0,98	1,00	1,00	
12.5	67	1		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,68	0,00	0,00	0,00	0,00	0,00	0,00	0,00
		2	0,00		0,00	0,00	0,04	0,00	0,00	0,01	0,16	0,97	0,00	0,00	0,00	0,00	0,00	0,00
		3	0,00	0,00		1,00	0,51	1,00	1,00	0,82	0,00	0,01	1,00	0,99	0,98	1,00	1,00	1,00
		4	0,00	0,00	1,00		0,99	0,90	0,92	1,00	0,00	0,15	1,00	1,00	0,18	1,00	0,94	0,99
		5	0,00	0,04	0,51	0,99		0,08	0,11	1,00	0,00	0,86	0,77	1,00	0,00	0,80	0,12	0,36
		6	0,00	0,00	1,00	0,90	0,08		1,00	0,27	0,00	0,00	1,00	0,75	1,00	1,00	1,00	1,00
		7	0,00	0,00	1,00	0,92	0,11	1,00		0,32	0,00	0,00	1,00	0,78	1,00	1,00	1,00	1,00
		8	0,00	0,01	0,82	1,00	1,00	0,27	0,32		0,00	0,52	0,95	1,00	0,01	0,98	0,34	0,68
	100	1	0,68	0,16	0,00	0,00	0,00	0,00	0,00	0,00		0,00	0,00	0,00	0,00	0,00	0,00	0,00
		2	0,00	0,97	0,01	0,15	0,86	0,00	0,00	0,52	0,00		0,06	0,20	0,00	0,03	0,00	0,01
		3	0,00	0,00	1,00	1,00	0,77	1,00	1,00	0,95	0,00	0,06		1,00	0,99	1,00	1,00	1,00
		4	0,00	0,00	0,99	1,00	1,00	0,75	0,78	1,00	0,00	0,20	1,00		0,08	1,00	0,82	0,96
		5	0,00	0,00	0,98	0,18	0,00	1,00	1,00	0,01	0,00	0,00	0,99	0,08		0,47	0,99	1,00
		6	0,00	0,00	1,00	1,00	0,80	1,00	1,00	0,98	0,00	0,03	1,00	1,00	0,47		1,00	1,00
		7	0,00	0,00	1,00	0,94	0,12	1,00	1,00	0,34	0,00	0,00	1,00	0,82	0,99	1,00		1,00
		8	0,00	0,00	1,00	0,99	0,36	1,00	1,00	0,68	0,00	0,01	1,00	0,96	1,00	1,00	1,00	
18	67	1		0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,26	0,00	0,00	0,00	0,00	0,00	0,00
		2	0,01		0,91	1,00	0,00	0,45	0,98	0,39	1,00	1,00	1,00	0,73	0,00	0,32	0,44	0,00
		3	0,00	0,91		1,00	0,67	1,00	1,00	1,00	0,61	0,18	1,00	1,00	0,07	1,00	1,00	0,26
		4	0,00	1,00	1,00		0,05	0,99	1,00	0,98	0,90	0,42	1,00	1,00	0,00	0,96	0,99	0,00
		5	0,00	0,00	0,67	0,05		0,82	0,11	0,75	0,00	0,00	0,13	0,50	1,00	0,91	0,82	1,00
		6	0,00	0,45	1,00	0,99	0,82		1,00	1,00	0,16	0,01	0,99	1,00	0,08	1,00	1,00	0,34
		7	0,00	0,98	1,00	1,00	0,11	1,00		1,00	0,78	0,25	1,00	1,00	0,00	0,99	1,00	0,01
		8	0,00	0,39	1,00	0,98	0,75	1,00	1,00		0,13	0,01	0,99	1,00	0,05	1,00	1,00	0,25
	100	1	0,10	1,00	0,61	0,90	0,00	0,16	0,78	0,13		1,00	0,97	0,36	0,00	0,10	0,16	0,00
		2	0,26	1,00	0,18	0,42	0,00	0,01	0,25	0,01	1,00		0,69	0,05	0,00	0,01	0,01	0,00
		3	0,00	1,00	1,00	1,00	0,13	0,99	1,00	0,99	0,97	0,69		1,00	0,00	0,98	0,99	0,02
		4	0,00	0,73	1,00	1,00	0,50	1,00	1,00	1,00	0,36	0,05	1,00		0,02	1,00	1,00	0,11
		5	0,00	0,00	0,07	0,00	1,00	0,08	0,00	0,05	0,00	0,00	0,00	0,02		0,14	0,08	1,00
		6	0,00	0,32	1,00	0,96	0,91	1,00	0,99	1,00	0,10	0,01	0,98	1,00	0,14		1,00	0,49
		7	0,00	0,44	1,00	0,99	0,82	1,00	1,00	1,00	0,16	0,01	0,99	1,00	0,08	1,00		0,34
		8	0,00	0,00	0,26	0,00	1,00	0,34	0,01	0,25	0,00	0,00	0,02	0,11	1,00	0,49	0,34	

II - IV Gonadosomatic index (GSI)

a. Overall tests:

Table II - 55: *Test results of a **three-way random effects nested ANOVA** for GSI (%), including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	6,25696	1	6,256956	90,0557	0,545286	11,47463	0,001048
Parallel (Temp.*feed*Time)	Random	49,83292	85	0,586270	286,0000	0,161601	3,62789	0,000000
Time	Fixed	13,52458	7	1,932083	87,3895	0,565760	3,41502	0,002840
Temperature	Fixed	6,98340	2	3,491698	90,0211	0,545537	6,40047	0,002518
Feeding regime	Fixed	0,27752	1	0,277521	90,0557	0,545286	0,50895	0,477440
Error		46,21781	286	0,161601				

Table II - 56: *Test results of a **three-way factorial ANOVA** for GSI (%), including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	6,59110	1	6,591100	39,27403	0,000000
Time	14,69881	7	2,099830	12,51214	0,000000
Temperature	7,30064	2	3,650319	21,75096	0,000000
Feed regime	0,31789	1	0,317894	1,89422	0,169649
Time*Temp.	31,12753	14	2,223395	13,24842	0,000000
Time*Feed.	1,91133	7	0,273047	1,62699	0,126764
Temp.*Feed.	0,55893	2	0,279464	1,66522	0,190715
Time*Temp.*Feed.	3,74814	14	0,267724	1,59527	0,078541
Error	56,05301	334	0,167823		

b. Restrictive feeding (67%)

Table II - 57: *Test results of a **two-way random effects nested ANOVA** for GSI (%), including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	1,97221	1	1,972213	43,0812	0,252589	7,807997	0,007734
Parallel (Temp.*Time)	Random	9,92129	38	0,261086	145,0000	0,168618	1,548392	0,035068
Time	Fixed	3,19059	7	0,455799	39,7661	0,257901	1,767343	0,121268
Temperature	Fixed	1,86606	2	0,933029	43,0712	0,252604	3,693651	0,033074
Error		24,44959	145	0,168618				

Table II - 58: *Test results of a **two-way factorial ANOVA** for GSI (%), including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	2,02917	1	2,029171	12,55652	0,000511
Time	3,31268	7	0,473241	2,92841	0,006427
Temperature	1,91318	2	0,956588	5,91937	0,003276
Temp.*Time	7,05997	14	0,504284	3,12051	0,000237
Error	27,31091	169	0,161603		

Table II - 59: Test results of **one-*way random effects nested ANOVAs*** *for GSI (%), including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,018827	1	0,018827	3,00000	0,000037	504,5619	0,000193
	Parallel (temp)	Random	0,000112	3	0,000037	12,00000	0,000083	0,4504	0,721684
	Temperature	Fixed	0,000101	2	0,000050	3,00000	0,000037	1,3512	0,381597
	Error		0,000994	12	0,000083				
2 (14.12.18)	Intercept	Fixed	0,017036	1	0,017036	3,000000	0,000004	4500,616	0,000007
	Parallel (temp)	Random	0,000011	3	0,000004	9,000000	0,000011	0,332	0,802440
	Temperature	Fixed	0,000555	2	0,000277	3,000000	0,000004	73,288	0,002840
	Error		0,000103	9	0,000011				
3 (01.02.19)	Intercept	Fixed	0,018746	1	0,018746	3,24080	0,000009	2143,903	0,000011
	Parallel (temp)	Random	0,000026	3	0,000009	11,00000	0,000030	0,281	0,838243
	Temperature	Fixed	0,000250	2	0,000125	3,11033	0,000009	14,501	0,026503
	Error		0,000333	11	0,000030				
4 (13.04.19)	Intercept	Fixed	0,041558	1	0,041558	3,00081	0,000046	908,9929	0,000080
	Parallel (temp)	Random	0,000137	3	0,000046	24,00000	0,000017	2,7497	0,064856
	Temperature	Fixed	0,000440	2	0,000220	3,00040	0,000046	4,8098	0,115892
	Error		0,000399	24	0,000017				
5 (27.03.19)	Intercept	Fixed	0,040675	1	0,040675	3,01651	0,000032	1282,191	0,000046
	Parallel (temp)	Random	0,000095	3	0,000032	23,00000	0,000028	1,123	0,360370
	Temperature	Fixed	0,000918	2	0,000459	3,00792	0,000032	14,471	0,028617
	Error		0,000650	23	0,000028				
6 (10.04.19)	Intercept	Fixed	0,089265	1	0,089265	3,01507	0,000736	121,2346	0,001568
	Parallel (temp)	Random	0,002206	3	0,000735	22,00000	0,001402	0,5247	0,669852
	Temperature	Fixed	0,022733	2	0,011367	3,00772	0,000736	15,4464	0,026181
	Error		0,030835	22	0,001402				
7 (24.04.19)	Intercept	Fixed	1,244138	1	1,244138	20,08403	0,011929	104,2911	0,000000
	Parallel (temp)	Random	0,000080	3	0,000027	20,00000	0,212640	0,0001	0,999998
	Temperature	Fixed	2,061160	2	1,030580	20,19662	0,005239	196,7089	0,000000
	Error		4,252805	20	0,212640				
8 (15.05.19)	Intercept	Fixed	4,24687	1	4,246866	3,20047	0,948823	4,475933	0,119012
	Parallel (temp)	Random	2,85865	3	0,952883	24,00000	0,840145	1,134189	0,355170
	Temperature	Fixed	7,95092	2	3,975458	3,09744	0,950858	4,180917	0,131853
	Error		20,16347	24	0,840145				

Table II - 60: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for GSI (%) between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,825124	0,869748
	12	0,825124		0,531007
	18	0,869748	0,531007	
2 (14.12.18)	8		0,299779	0,000392
	12	0,299779		0,001711
	18	0,000392	0,001711	
3 (01.02.19)	8		0,987348	0,059471
	12	0,987348		0,075907
	18	0,059471	0,075907	
4 (13.03.19)	8		0,213451	0,000171
	12	0,213451		0,003622
	18	0,000171	0,003622	
5 (27.03.19)	8		0,718441	0,000187
	12	0,718441		0,000747
	18	0,000187	0,000747	
6 (10.04.19)	8		0,946929	0,005355
	12	0,946929		0,003232
	18	0,005355	0,003232	
7 (24.04.19)	8		0,999863	0,043069
	12	0,999863		0,030200
	18	0,043069	0,030200	
8 (15.05.19)	8		0,999784	0,031341
	12	0,999784		0,047195
	18	0,031341	0,047195	

Table II - 61: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for GSI (%) between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,998778	0,975792	0,987802	0,988712	0,988064
	1	12	0,998778		0,999230	0,999869	0,915648	0,999877
	1	18	0,975792	0,999230		0,999998	0,767921	0,999997
	2	8	0,987802	0,999869	0,999998		0,820046	1,000000
	2	12	0,988712	0,915648	0,767921	0,820046		0,821460
	2	18	0,988064	0,999877	0,999997	1,000000	0,821460	
2 (14.12.18)	1	8		0,998503	0,018393	0,999298	0,817585	0,016308
	1	12	0,998503		0,033220	0,969066	0,961127	0,027876
	1	18	0,018393	0,033220		0,005780	0,061935	0,994787
	2	8	0,999298	0,969066	0,005780		0,545131	0,006076
	2	12	0,817585	0,961127	0,061935	0,545131		0,050459
	2	18	0,016308	0,027876	0,994787	0,006076	0,050459	
3 (01.02.19)	1	8		0,996806	0,719859	0,951235	0,999970	0,690098
	1	12	0,996806		0,465048	0,998492	0,999689	0,462049
	1	18	0,719859	0,465048		0,285305	0,621545	0,999939
	2	8	0,951235	0,998492	0,285305		0,981428	0,299451
	2	12	0,999970	0,999689	0,621545	0,981428		0,601465
	2	18	0,690098	0,462049	0,999939	0,299451	0,601465	
4 (13.03.19)	1	8		0,492763	0,000796	0,134857	0,061334	0,000390
	1	12	0,492763		0,062984	0,944325	0,851352	0,029427
	1	18	0,000796	0,062984		0,415231	0,469636	0,999260
	2	8	0,134857	0,944325	0,415231		0,999954	0,253024
	2	12	0,061334	0,851352	0,469636	0,999954		0,285342
	2	18	0,000390	0,029427	0,999260	0,253024	0,285342	
5 (27.03.19)	1	8		0,986767	0,009370	0,808762	0,605230	0,000916
	1	12	0,986767		0,063110	0,993970	0,949490	0,007308
	1	18	0,009370	0,063110		0,136615	0,254563	0,904916
	2	8	0,808762	0,993970	0,136615		0,999214	0,015690
	2	12	0,605230	0,949490	0,254563	0,999214		0,034637
	2	18	0,000916	0,007308	0,904916	0,015690	0,034637	
6 (10.04.19)	1	8		0,999827	0,616375	1,000000	0,999849	0,074485
	1	12	0,999827		0,460277	0,999945	1,000000	0,042272
	1	18	0,616375	0,460277		0,477217	0,415631	0,806795
	2	8	1,000000	0,999945	0,477217		0,999957	0,032245
	2	12	0,999849	1,000000	0,415631	0,999957		0,029205
	2	18	0,074485	0,042272	0,806795	0,032245	0,029205	
7 (24.04.19)	1	8		1,000000	0,654163	1,000000	1,000000	0,645624
	1	12	1,000000		0,373379	1,000000	1,000000	0,363712
	1	18	0,654163	0,373379		0,365345	0,434981	1,000000
	2	8	1,000000	1,000000	0,365345		1,000000	0,355797
	2	12	1,000000	1,000000	0,434981	1,000000		0,425124
	2	18	0,645624	0,363712	1,000000	0,355797	0,425124	
8 (15.05.19)	1	8		1,000000	0,128629	1,000000	1,000000	0,920120
	1	12	1,000000		0,202613	1,000000	1,000000	0,949009
	1	18	0,128629	0,202613		0,069892	0,094864	0,458109
	2	8	1,000000	1,000000	0,069892		1,000000	0,880728
	2	12	1,000000	1,000000	0,094864	1,000000		0,904536
	2	18	0,920120	0,949009	0,458109	0,880728	0,904536	

Table II - 62: *Test results of **one-way random effects nested ANOVAs** for GSI (%) within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,060206	1	0,060206	9,55396	0,000031	1960,728	0,000000
	Parallel (Time)	Random	0,000243	8	0,000030	48,00000	0,000035	0,874	0,545157
	Time	Fixed	0,000605	7	0,000086	8,19362	0,000030	2,841	0,081299
	Error		0,001669	48	0,000035				
12.5	Intercept	Fixed	0,070116	1	0,070116	9,05189	0,000018	3816,838	0,000000
	Parallel (Time)	Random	0,000146	8	0,000018	46,00000	0,000020	0,933	0,499220
	Time	Fixed	0,000407	7	0,000058	8,12640	0,000018	3,176	0,062595
	Error		0,000902	46	0,000020				
18	Intercept	Fixed	3,77789	1	3,777894	10,76432	0,370769	10,18934	0,008799
	Parallel (Time)	Random	2,86093	8	0,357616	51,00000	0,479353	0,74604	0,650821
	Time	Fixed	11,37470	7	1,624958	8,29734	0,359268	4,52297	0,023375
	Error		24,44702	51	0,479353				

Table II - 63: *Test results of **Tukey HSD post-hoc tests** of difference for GSI (%) within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,965764	0,999665	0,999997	0,999999	0,298100	1,000000	0,999710
	2	0,965764		0,999123	0,862884	0,877429	0,031573	0,916547	0,996834
	3	0,999665	0,999123		0,992740	0,994545	0,098797	0,997153	1,000000
	4	0,999997	0,862884	0,992740		1,000000	0,258474	1,000000	0,990010
	5	0,999999	0,877429	0,994545	1,000000		0,239445	1,000000	0,992668
	6	0,298100	0,031573	0,098797	0,258474	0,239445		0,341037	0,040677
	7	1,000000	0,916547	0,997153	1,000000	1,000000	0,341037		0,996708
	8	0,999710	0,996834	1,000000	0,990010	0,992668	0,040677	0,996708	
12.5	1		0,898801	0,692802	0,999974	0,999996	0,998437	0,999045	0,536294
	2	0,898801		0,999980	0,661224	0,939996	0,991692	0,530277	0,047076
	3	0,692802	0,999980		0,355264	0,749656	0,918294	0,252527	0,011066
	4	0,999974	0,661224	0,355264		0,997413	0,955540	0,999992	0,600795
	5	0,999996	0,939996	0,749656	0,997413		0,999903	0,981494	0,253818
	6	0,998437	0,991692	0,918294	0,955540	0,999903		0,879011	0,114345
	7	0,999045	0,530277	0,252527	0,999992	0,981494	0,879011		0,790853
	8	0,536294	0,047076	0,011066	0,600795	0,253818	0,114345	0,790853	
18	1		1,000000	1,000000	1,000000	1,000000	0,999999	0,709439	0,060765
	2	1,000000		1,000000	1,000000	1,000000	1,000000	0,785602	0,103100
	3	1,000000	1,000000		1,000000	1,000000	1,000000	0,780262	0,100792
	4	1,000000	1,000000	1,000000		1,000000	1,000000	0,571082	0,017503
	5	1,000000	1,000000	1,000000	1,000000		1,000000	0,576710	0,017944
	6	0,999999	1,000000	1,000000	1,000000	1,000000		0,718542	0,038291
	7	0,709439	0,785602	0,780262	0,571082	0,576710	0,718542		0,752623
	8	0,060765	0,103100	0,100792	0,017503	0,017944	0,038291	0,752623	

c. Full feeding (100%)

Table II - 64: *Test results of a **two-way random effects nested ANOVA** for GSI (%), including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	4,53278	1	4,532776	39,2973	0,845073	5,363765	0,025864
Parallel (Temp.*Time)	Random	34,75035	38	0,914483	141,0000	0,154385	5,923409	0,000000
Temperature	Fixed	5,62623	2	2,813117	39,2718	0,846305	3,323997	0,046382
Time	Fixed	11,48100	7	1,640143	38,6525	0,877808	1,868452	0,101773
Error		21,76822	141	0,154385				

Table II - 65: *Test results of a **two-way factorial ANOVA** for GSI (%), including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	4,84900	1	4,849002	27,83670	0,000000
Time	12,83811	7	1,834016	10,52855	0,000000
Temperature	5,94264	2	2,971320	17,05748	0,000000
Time*Temp.	27,77647	14	1,984033	11,38975	0,000000
Error	28,74211	165	0,174195		

Table II - 66: *Test results of **one-way random effects nested ANOVAs** for GSI (%), including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,016232	1	0,016232	3,07809	0,000057	285,0037	0,000390
	Parallel (Temp)	Random	0,000171	3	0,000057	11,00000	0,000066	0,8577	0,491496
	Temperature	Fixed	0,000205	2	0,000102	3,03595	0,000057	1,8003	0,305092
	Error		0,000729	11	0,000066				
2 (14.12.18)	Intercept	Fixed	0,018075	1	0,018075	3,07698	0,000014	1316,398	0,000037
	Parallel (Temp)	Random	0,000041	3	0,000014	10,00000	0,000016	0,870	0,488487
	Temperature	Fixed	0,000033	2	0,000017	3,04116	0,000014	1,215	0,409460
	Error		0,000158	10	0,000016				
3 (01.02.19)	Intercept	Fixed	0,019794	1	0,019794	3,02995	0,000021	954,9667	0,000069
	Parallel (Temp)	Random	0,000063	3	0,000021	11,00000	0,000009	2,2299	0,141930
	Temperature	Fixed	0,000681	2	0,000341	3,01381	0,000021	16,3817	0,024035
	Error		0,000103	11	0,000009				
4 (13.04.19)	Intercept	Fixed	0,038885	1	0,038885	3,22367	0,000034	1127,197	0,000033
	Parallel (Temp)	Random	0,000103	3	0,000034	21,00000	0,000039	0,872	0,471231
	Temperature	Fixed	0,001130	2	0,000565	3,09618	0,000034	16,422	0,022473
	Error		0,000827	21	0,000039				
5 (27.03.19)	Intercept	Fixed	0,041356	1	0,041356	3,03104	0,000044	941,6266	0,000070
	Parallel (Temp)	Random	0,000132	3	0,000044	22,00000	0,000027	1,6143	0,214702
	Temperature	Fixed	0,001179	2	0,000589	3,01557	0,000044	13,3970	0,031606
	Error		0,000600	22	0,000027				
6 (10.04.19)	Intercept	Fixed	0,126442	1	0,126442	3,05448	0,001130	111,8813	0,001667
	Parallel (Temp)	Random	0,003407	3	0,001136	21,00000	0,000733	1,5496	0,231238
	Temperature	Fixed	0,044329	2	0,022165	3,02640	0,001133	19,5630	0,018580
	Error		0,015391	21	0,000733				
7 (24.04.19)	Intercept	Fixed	2,147421	1	2,147421	3,17587	0,080716	26,60472	0,012263
	Parallel (Temp)	Random	0,237295	3	0,079098	22,00000	0,274822	0,28782	0,833681
	Temperature	Fixed	3,489413	2	1,744707	3,08776	0,079914	21,83239	0,015065
	Error		6,046089	22	0,274822				
8 (15.05.19)	Intercept	Fixed	14,88071	1	14,88071	3,27080	2,045797	7,273796	0,067366
	Parallel (Temp)	Random	6,73267	3	2,24422	23,00000	0,682797	3,286811	0,038852
	Temperature	Fixed	32,82237	2	16,41118	3,10719	2,158248	7,603938	0,063540
	Error		15,70433	23	0,68280				

Table II - 67: *Test results of **Tukey HSD post-hoc tests** of difference for GSI (%) between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,332015	0,238762
	12	0,332015		0,966929
	18	0,238762	0,966929	
2 (14.12.18)	8		0,414930	0,990241
	12	0,414930		0,324663
	18	0,990241	0,324663	
3 (01.02.19)	8		0,542989	0,000207
	12	0,542989		0,000297
	18	0,000207	0,000297	
4 (13.03.19)	8		0,010001	0,000226
	12	0,010001		0,084283
	18	0,000226	0,084283	
5 (27.03.19)	8		0,937775	0,000148
	12	0,937775		0,000167
	18	0,000148	0,000167	
6 (10.04.19)	8		0,998153	0,000141
	12	0,998153		0,000141
	18	0,000141	0,000141	
7 (24.04.19)	8		0,999958	0,013992
	12	0,999958		0,014296
	18	0,013992	0,014296	
8 (15.05.19)	8		0,984336	0,000135
	12	0,984336		0,000184
	18	0,000135	0,000184	

Table II - 68: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for GSI (%) between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,539103	0,360994	0,998684	0,957892	0,957392
	1	12	0,539103		0,999151	0,841734	0,934484	0,935146
	1	18	0,360994	0,999151		0,681723	0,798007	0,799184
	2	8	0,998684	0,841734	0,681723		0,999106	0,999080
	2	12	0,957892	0,934484	0,798007	0,999106		1,000000
	2	18	0,957392	0,935146	0,799184	0,999080	1,000000	
2 (14.12.18)	1	8		0,999751	0,951659	0,674823	0,999885	0,859531
	1	12	0,999751		0,992317	0,827222	0,993880	0,954188
	1	18	0,951659	0,992317		0,973803	0,827413	0,999448
	2	8	0,674823	0,827222	0,973803		0,439528	0,998032
	2	12	0,999885	0,993880	0,827413	0,439528		0,662749
	2	18	0,859531	0,954188	0,999448	0,998032	0,662749	
3 (01.02.19)	1	8		0,844216	0,000322	0,987753	0,933205	0,006666
	1	12	0,844216		0,001020	0,992381	0,999995	0,041997
	1	18	0,000322	0,001020		0,000551	0,001996	0,201546
	2	8	0,987753	0,992381	0,000551		0,998834	0,017368
	2	12	0,933205	0,999995	0,001996	0,998834		0,062639
	2	18	0,006666	0,041997	0,201546	0,017368	0,062639	
4 (13.03.19)	1	8		0,196615	0,022552	0,931464	0,799534	0,055421
	1	12	0,196615		0,718678	0,031228	0,855999	0,963615
	1	18	0,022552	0,718678		0,003684	0,198561	0,985980
	2	8	0,931464	0,031228	0,003684		0,276552	0,008111
	2	12	0,799534	0,855999	0,198561	0,276552		0,437281
	2	18	0,055421	0,963615	0,985980	0,008111	0,437281	
5 (27.03.19)	1	8		0,989827	0,174739	0,737168	0,996931	0,005928
	1	12	0,989827		0,077459	0,986640	0,999913	0,003002
	1	18	0,174739	0,077459		0,005733	0,038592	0,547173
	2	8	0,737168	0,986640	0,005733		0,901223	0,000230
	2	12	0,996931	0,999913	0,038592	0,901223		0,000757
	2	18	0,005928	0,003002	0,547173	0,000230	0,000757	
6 (10.04.19)	1	8		0,998719	0,000156	0,982169	0,999944	0,010266
	1	12	0,998719		0,000204	0,999842	0,999958	0,031443
	1	18	0,000156	0,000204		0,000192	0,000161	0,356341
	2	8	0,982169	0,999842	0,000192		0,996577	0,037247
	2	12	0,999944	0,999958	0,000161	0,996577		0,015267
	2	18	0,010266	0,031443	0,356341	0,037247	0,015267	
7 (24.04.19)	1	8		1,000000	0,215193	1,000000	1,000000	0,637316
	1	12	1,000000		0,154978	1,000000	1,000000	0,566666
	1	18	0,215193	0,154978		0,088573	0,110997	0,934697
	2	8	1,000000	1,000000	0,088573		1,000000	0,447709
	2	12	1,000000	1,000000	0,110997	1,000000		0,490969
	2	18	0,637316	0,566666	0,934697	0,447709	0,490969	
8 (15.05.19)	1	8		1,000000	0,073188	1,000000	0,999965	0,000182
	1	12	1,000000		0,296807	1,000000	0,999994	0,002810
	1	18	0,073188	0,296807		0,073231	0,152372	0,046564
	2	8	1,000000	1,000000	0,073231		0,999965	0,000182
	2	12	0,999965	0,999994	0,152372	0,999965		0,000343
	2	18	0,000182	0,002810	0,046564	0,000182	0,000343	

Table II - 69: *Test results of **one-way random effects nested ANOVAs** for GSI (%) within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,059689	1	0,059689	8,57648	0,000057	1052,486	0,000000
	Parallel (time)	Random	0,000479	8	0,000060	48,00000	0,000023	2,657	0,016861
	Time	Fixed	0,000954	7	0,000136	8,06600	0,000060	2,290	0,133494
	Error		0,001083	48	0,000023				
12.5	Intercept	Fixed	0,091212	1	0,091212	10,14436	0,001612	56,56952	0,000019
	Parallel (time)	Random	0,012373	8	0,001547	43,00000	0,002416	0,64009	0,739660
	Time	Fixed	0,012911	7	0,001844	8,27606	0,001556	1,18533	0,402250
	Error		0,103900	43	0,002416				
18	Intercept	Fixed	10,51027	1	10,51027	8,62879	0,838639	12,53254	0,006749
	Parallel (time)	Random	6,96103	8	0,87013	50,00000	0,433265	2,00831	0,064502
	Time	Fixed	46,43623	7	6,63375	8,09886	0,864788	7,67095	0,004852
	Error		21,66324	50	0,43326				

Table II - 70: *Test results of **Tukey HSD post-hoc tests** of difference for GSI (%) within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,051873	0,942633	0,723196	0,046853	0,000194	0,061829	0,686942
	2	0,051873		0,400928	0,492348	0,999940	0,708007	0,999664	0,530440
	3	0,942633	0,400928		0,999922	0,452481	0,002168	0,528401	0,999794
	4	0,723196	0,492348	0,999922		0,543702	0,001188	0,632579	1,000000
	5	0,046853	0,999940	0,452481	0,543702		0,241902	1,000000	0,589650
	6	0,000194	0,708007	0,002168	0,001188	0,241902		0,186456	0,001468
	7	0,061829	0,999664	0,528401	0,632579	1,000000	0,186456		0,677509
	8	0,686942	0,530440	0,999794	1,000000	0,589650	0,001468	0,677509	
12.5	1		1,000000	1,000000	0,999997	1,000000	0,999999	1,000000	0,245124
	2	1,000000		1,000000	0,999994	1,000000	0,999996	1,000000	0,279290
	3	1,000000	1,000000		0,999987	1,000000	0,999992	1,000000	0,265746
	4	0,999997	0,999994	0,999987		1,000000	1,000000	1,000000	0,223838
	5	1,000000	1,000000	1,000000	1,000000		1,000000	1,000000	0,181514
	6	0,999999	0,999996	0,999992	1,000000	1,000000		1,000000	0,239387
	7	1,000000	1,000000	1,000000	1,000000	1,000000	1,000000		0,198151
	8	0,245124	0,279290	0,265746	0,223838	0,181514	0,239387	0,198151	
18	1		1,000000	1,000000	1,000000	1,000000	0,999992	0,371820	0,000132
	2	1,000000		1,000000	1,000000	1,000000	0,999993	0,373998	0,000132
	3	1,000000	1,000000		1,000000	1,000000	0,999996	0,387078	0,000132
	4	1,000000	1,000000	1,000000		1,000000	0,999996	0,333340	0,000132
	5	1,000000	1,000000	1,000000	1,000000		0,999993	0,224666	0,000132
	6	0,999992	0,999993	0,999996	0,999996	0,999993		0,449552	0,000132
	7	0,371820	0,373998	0,387078	0,333340	0,224666	0,449552		0,000180
	8	0,000132	0,000132	0,000132	0,000132	0,000132	0,000132	0,000180	

d. Comparison of temperature groups between feeding regimes

Table II - 71: *Test results of **factorial ANOVAs** for GSI (%) comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	0,124177	1	0,124177	4003,491	0,000000
	Feeding regime	0,000000	1	0,000000	0,009	0,925389
	Time	0,001316	7	0,000188	6,062	0,000005
	Feed*Time	0,000289	7	0,000041	1,331	0,242361
	Error	0,003474	112	0,000031		
12.5	Intercept	0,174822	1	0,174822	156,4610	0,000000
	Feeding regime	0,002137	1	0,002137	1,9129	0,169579
	Time	0,016335	7	0,002334	2,0885	0,051054
	Feed*Time	0,011632	7	0,001662	1,4871	0,179764
	Error	0,117322	105	0,001117		
18	Intercept	13,91507	1	13,91507	29,10779	0,000000
	Feeding regime	0,89386	1	0,89386	1,86980	0,174119
	Time	52,76752	7	7,53822	15,76857	0,000000
	Feed*Time	6,62298	7	0,94614	1,97915	0,063618
	Error	55,93222	117	0,47805		

Table II - 72: *Test results of **Tukey HSD post-hoc tests** for GSI (%) comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		1,00	1,00	1,00	1,00	0,50	1,00	1,00	0,88	1,00	1,00	1,00	1,00	0,41	1,00	1,00
		2	1,00		1,00	0,98	0,99	0,04	0,99	1,00	1,00	0,85	1,00	1,00	0,88	0,03	0,91	1,00
		3	1,00	1,00		1,00	1,00	0,16	1,00	1,00	0,99	0,99	1,00	1,00	1,00	0,12	1,00	1,00
		4	1,00	0,98	1,00		1,00	0,44	1,00	1,00	0,62	1,00	1,00	1,00	1,00	0,34	1,00	1,00
		5	1,00	0,99	1,00	1,00		0,41	1,00	1,00	0,64	1,00	1,00	1,00	1,00	0,31	1,00	1,00
		6	0,50	0,04	0,16	0,44	0,41		0,56	0,06	0,00	1,00	0,05	0,03	0,85	1,00	0,79	0,04
		7	1,00	0,99	1,00	1,00	1,00	0,56		1,00	0,76	1,00	1,00	1,00	1,00	0,46	1,00	1,00
		8	1,00	1,00	1,00	1,00	1,00	0,06	1,00		0,97	0,98	1,00	1,00	0,99	0,04	1,00	1,00
	100	1	0,88	1,00	0,99	0,62	0,64	0,00	0,76	0,97		0,35	1,00	0,99	0,33	0,00	0,39	0,99
		2	1,00	0,85	0,99	1,00	1,00	1,00	1,00	0,98	0,35		0,90	0,94	1,00	0,99	1,00	0,95
		3	1,00	1,00	1,00	1,00	1,00	0,05	1,00	1,00	1,00	0,90		1,00	0,93	0,03	0,95	1,00
		4	1,00	1,00	1,00	1,00	1,00	0,03	1,00	1,00	0,99	0,94	1,00		0,96	0,02	0,98	1,00
		5	1,00	0,88	1,00	1,00	1,00	0,85	1,00	0,99	0,33	1,00	0,93	0,96		0,77	1,00	0,97
		6	0,41	0,03	0,12	0,34	0,31	1,00	0,46	0,04	0,00	0,99	0,03	0,02	0,77		0,69	0,02
		7	1,00	0,91	1,00	1,00	1,00	0,79	1,00	1,00	0,39	1,00	0,95	0,98	1,00	0,69		0,98
		8	1,00	1,00	1,00	1,00	1,00	0,04	1,00	1,00	0,99	0,95	1,00	1,00	0,97	0,02	0,98	
12.5	67	1		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,05
		2	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,05
		3	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,03
		4	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,02
		5	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,02
		6	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,01
		7	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,02
		8	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,06
	100	1	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	0,04
		2	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	0,05
		3	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	0,05
		4	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	0,03
		5	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	0,02
		6	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	0,04
		7	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		0,02
		8	0,05	0,05	0,03	0,02	0,02	0,01	0,02	0,06	0,04	0,05	0,05	0,03	0,02	0,04	0,02	
18	67	1		1,00	1,00	1,00	1,00	1,00	0,95	0,15	1,00	1,00	1,00	1,00	1,00	1,00	0,76	0,00
		2	1,00		1,00	1,00	1,00	1,00	0,98	0,25	1,00	1,00	1,00	1,00	1,00	1,00	0,85	0,00
		3	1,00	1,00		1,00	1,00	1,00	0,97	0,24	1,00	1,00	1,00	1,00	1,00	1,00	0,84	0,00
		4	1,00	1,00	1,00		1,00	1,00	0,88	0,04	1,00	1,00	1,00	1,00	1,00	1,00	0,56	0,00
		5	1,00	1,00	1,00	1,00		1,00	0,88	0,04	1,00	1,00	1,00	1,00	1,00	1,00	0,57	0,00
		6	1,00	1,00	1,00	1,00	1,00		0,95	0,10	1,00	1,00	1,00	1,00	1,00	1,00	0,73	0,00
		7	0,95	0,98	0,97	0,88	0,88	0,95		0,97	0,95	0,96	0,96	0,94	0,89	0,98	1,00	0,00
		8	0,15	0,25	0,24	0,04	0,04	0,10	0,97		0,16	0,16	0,17	0,12	0,04	0,17	1,00	0,00
	100	1	1,00	1,00	1,00	1,00	1,00	1,00	0,95	0,16		1,00	1,00	1,00	1,00	1,00	0,77	0,00
		2	1,00	1,00	1,00	1,00	1,00	1,00	0,96	0,16	1,00		1,00	1,00	1,00	1,00	0,77	0,00
		3	1,00	1,00	1,00	1,00	1,00	1,00	0,96	0,17	1,00	1,00		1,00	1,00	1,00	0,78	0,00
		4	1,00	1,00	1,00	1,00	1,00	1,00	0,94	0,12	1,00	1,00	1,00		1,00	1,00	0,73	0,00
		5	1,00	1,00	1,00	1,00	1,00	1,00	0,89	0,04	1,00	1,00	1,00	1,00		1,00	0,57	0,00
		6	1,00	1,00	1,00	1,00	1,00	1,00	0,98	0,17	1,00	1,00	1,00	1,00	1,00		0,84	0,00
		7	0,76	0,85	0,84	0,56	0,57	0,73	1,00	1,00	0,77	0,77	0,78	0,73	0,57	0,84		0,00
		8	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	

II - V Relative *follicle stimulating hormone receptor* (*fshr*) mRNA transcription

a. Overall tests:

Table II - 73: *Test results of a **three-way random effects nested ANOVA** for fshr gene transcription, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	0,059672	1	0,059672	110,2292	0,000034	1778,043	0,000000
Parallel (Temp.*feed*Time)	Random	0,002996	84	0,000036	229,0000	0,000024	1,480	0,011977
Time	Fixed	0,003954	7	0,000565	106,2947	0,000034	16,704	0,000000
Temperature	Fixed	0,001771	2	0,000886	108,9686	0,000034	26,325	0,000000
Feeding regime	Fixed	0,000010	1	0,000010	108,9869	0,000034	0,299	0,585922
Error		0,005519	229	0,000024				

Table II - 74: *Test results of a **three-way factorial ANOVA** for fshr gene transcription, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	0,065488	1	0,065488	2766,755	0,000000
Time	0,004489	7	0,000641	27,094	0,000000
Temperature	0,001941	2	0,000971	41,007	0,000000
Feed regime	0,000015	1	0,000015	0,621	0,431206
Time*Temp.	0,001025	14	0,000073	3,093	0,000173
Time*Feed.	0,000396	7	0,000057	2,387	0,021948
Temp.*Feed.	0,000067	2	0,000033	1,414	0,244950
Time*Temp.*Feed.	0,000404	14	0,000029	1,219	0,260544
Error	0,006533	276	0,000024		

b. Restrictive feeding (67%):

Table II - 75: *Test results of a **two-way random effects nested ANOVA** for fshr gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	0,029288	1	0,029288	50,6246	0,000033	875,3867	0,000000
Parallel (Temp.*Time)	Random	0,001304	37	0,000035	113,0000	0,000026	1,3564	0,113698
Time	Fixed	0,002241	7	0,000320	45,3897	0,000034	9,4035	0,000000
Temperature	Fixed	0,000842	2	0,000421	50,3094	0,000033	12,5644	0,000038
Error		0,002936	113	0,000026				

Table II - 76: *Test results of a **two-way factorial ANOVA** for fshr gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	0,032072	1	0,032072	1234,488	0,000000
Time	0,002563	7	0,000366	14,091	0,000000
Temperature	0,000890	2	0,000445	17,134	0,000000
Temp.*Time	0,000706	14	0,000050	1,942	0,027054
Error	0,003533	136	0,000026		

Table II - 77: *Test results of* ***one-way random effects nested ANOVAs*** *for fshr gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,004843	1	0,004843	3,696206	0,000013	359,9938	0,000081
	Parallel (temp)	Random	0,000039	3	0,000013	6,000000	0,000022	0,5816	0,648456
	Temperature	Fixed	0,000590	2	0,000295	3,391372	0,000013	22,3135	0,011174
	Error		0,000133	6	0,000022				
2 (14.12.18)	Intercept	Fixed	0,005672	1	0,005672	3,02543	0,000053	106,7970	0,001863
	Parallel (temp)	Random	0,000160	3	0,000053	10,00000	0,000020	2,6259	0,108027
	Temperature	Fixed	0,000333	2	0,000166	3,01361	0,000053	3,1247	0,184158
	Error		0,000204	10	0,000020				
3 (01.02.19)	Intercept	Fixed	0,004653	1	0,004653	1,731554	0,000018	256,1937	0,006950
	Parallel (temp)	Random	0,000038	2	0,000019	9,000000	0,000047	0,4056	0,678184
	Temperature	Fixed	0,000034	2	0,000017	1,646784	0,000018	0,9561	0,530164
	Error		0,000421	9	0,000047				
4 (13.04.19)	Intercept	Fixed	0,004860	1	0,004860	4,94022	0,000040	120,8829	0,000117
	Parallel (temp)	Random	0,000114	3	0,000038	16,00000	0,000051	0,7443	0,541217
	Temperature	Fixed	0,000028	2	0,000014	3,95448	0,000039	0,3580	0,719608
	Error		0,000814	16	0,000051				
5 (27.03.19)	Intercept	Fixed	0,004336	1	0,004336	3,02036	0,000057	75,78741	0,003108
	Parallel (temp)	Random	0,000173	3	0,000058	21,00000	0,000014	4,13719	0,018817
	Temperature	Fixed	0,000250	2	0,000125	3,00988	0,000058	2,17650	0,260216
	Error		0,000293	21	0,000014				
6 (10.04.19)	Intercept	Fixed	0,001208	1	0,001208	12,60170	0,000005	237,8657	0,000000
	Parallel (temp)	Random	0,000007	3	0,000002	13,00000	0,000019	0,1272	0,942303
	Temperature	Fixed	0,000000	2	0,000000	6,68655	0,000003	0,0370	0,963852
	Error		0,000243	13	0,000019				
7 (24.04.19)	Intercept	Fixed	0,001903	1	0,001903	15,57554	0,000011	166,6717	0,000000
	Parallel (temp)	Random	0,000016	3	0,000005	16,00000	0,000026	0,1995	0,895197
	Temperature	Fixed	0,000077	2	0,000039	6,91258	0,000007	5,1944	0,041963
	Error		0,000424	16	0,000026				
8 (15.05.19)	Intercept	Fixed	0,003701	1	0,003701	3,07308	0,000017	219,3380	0,000586
	Parallel (temp)	Random	0,000051	3	0,000017	22,00000	0,000018	0,9170	0,448902
	Temperature	Fixed	0,000240	2	0,000120	3,03364	0,000017	7,1151	0,071538
	Error		0,000404	22	0,000018				

Table II - 78: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for fshr gene transcription between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,006673	0,023160
	12	0,006673		0,821596
	18	0,023160	0,821596	
2 (14.12.18)	8		0,019510	0,012846
	12	0,019510		0,966908
	18	0,012846	0,966908	
3 (01.02.19)	8		0,788073	0,979238
	12	0,788073		0,707920
	18	0,979238	0,707920	
4 (13.03.19)	8		0,400561	0,765818
	12	0,400561		0,851229
	18	0,765818	0,851229	
5 (27.03.19)	8		0,004117	0,375854
	12	0,004117		0,056888
	18	0,375854	0,056888	
6 (10.04.19)	8		0,998942	0,999923
	12	0,998942		0,999374
	18	0,999923	0,999374	
7 (24.04.19)	8		0,801332	0,282815
	12	0,801332		0,376064
	18	0,282815	0,376064	
8 (15.05.19)	8		0,046035	0,005075
	12	0,046035		0,553320
	18	0,005075	0,553320	

Table II - 79: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for fshr gene transcription between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,101860	0,340755	0,845648	0,073654	0,203668
	1	12	0,101860		0,999995	0,083989	0,982312	0,999702
	1	18	0,340755	0,999995		0,191087	0,987972	0,999999
	2	8	0,845648	0,083989	0,191087		0,061865	0,131001
	2	12	0,073654	0,982312	0,987972	0,061865		0,949802
	2	18	0,203668	0,999702	0,999999	0,131001	0,949802	
2 (14.12.18)	1	8		0,913575	0,823674	0,137661	0,887846	0,835794
	1	12	0,913575		0,999893	0,031345	0,999968	0,999550
	1	18	0,823674	0,999893		0,022295	1,000000	0,999998
	2	8	0,137661	0,031345	0,022295		0,043902	0,036263
	2	12	0,887846	0,999968	1,000000	0,043902		0,999996
	2	18	0,835794	0,999550	0,999998	0,036263	0,999996	
3 (01.02.19)	1	8		0,999859	0,991213	0,908125	0,953946	
	1	12	0,999859		0,973492	0,848298	0,916094	
	1	18	0,991213	0,973492		0,992836	0,997954	
	2	8	0,908125	0,848298	0,992836		0,999993	
	2	12	N=0	N=0	N=0	N=0	N=0	
	2	18	0,953946	0,916094	0,997954	0,999993		
4 (13.03.19)	1	8		0,919515	0,682705	0,990022	0,646943	0,999902
	1	12	0,919515		0,973686	1,000000	0,992196	0,980448
	1	18	0,682705	0,973686		0,996366	0,999526	0,806364
	2	8	0,990022	1,000000	0,996366		0,999659	0,997418
	2	12	0,646943	0,992196	0,999526	0,999659		0,820210
	2	18	0,999902	0,980448	0,806364	0,997418	0,820210	
5 (27.03.19)	1	8		0,003043	0,199496	0,035811	0,003090	0,023680
	1	12	0,003043		0,202501	0,707118	0,999909	0,820504
	1	18	0,199496	0,202501		0,905861	0,237650	0,811044
	2	8	0,035811	0,707118	0,905861		0,792368	0,999917
	2	12	0,003090	0,999909	0,237650	0,792368		0,892627
	2	18	0,023680	0,820504	0,811044	0,999917	0,892627	
6 (10.04.19)	1	8		1,000000	0,997911	0,996817	0,999710	0,999999
	1	12	1,000000		0,999684	0,999513	0,999950	1,000000
	1	18	0,997911	0,999684		1,000000	0,999995	0,999033
	2	8	0,996817	0,999513	1,000000		0,999982	0,998321
	2	12	0,999710	0,999950	0,999995	0,999982		0,999926
	2	18	0,999999	1,000000	0,999033	0,998321	0,999926	
7 (24.04.19)	1	8		0,999920	0,974995	1,000000	0,994875	0,873844
	1	12	0,999920		0,950310	0,999479	0,994973	0,652024
	1	18	0,974995	0,950310		0,918611	0,998800	0,991084
	2	8	1,000000	0,999479	0,918611		0,978323	0,682869
	2	12	0,994875	0,994973	0,998800	0,978323		0,905411
	2	18	0,873844	0,652024	0,991084	0,682869	0,905411	
8 (15.05.19)	1	8		0,822547	0,172010	0,864154	0,832121	0,761201
	1	12	0,822547		0,760141	0,195776	1,000000	0,999995
	1	18	0,172010	0,760141		0,014798	0,748405	0,825084
	2	8	0,864154	0,195776	0,014798		0,202645	0,158776
	2	12	0,832121	1,000000	0,748405	0,202645		0,999990
	2	18	0,761201	0,999995	0,825084	0,158776	0,999990	

Table II - 80: *Test results of **one-way random effects nested ANOVAs** for fshr gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,013314	1	0,013314	10,26225	0,000050	264,8228	0,000000
	Parallel (Time)	Random	0,000419	8	0,000052	32,00000	0,000039	1,3517	0,254858
	Time	Fixed	0,001682	7	0,000240	8,23245	0,000052	4,6110	0,022450
	Error		0,001240	32	0,000039				
12.5	Intercept	Fixed	0,007421	1	0,007421	12,83131	0,000006	1347,708	0,000000
	Parallel (Time)	Random	0,000034	7	0,000005	41,00000	0,000008	0,601	0,751360
	Time	Fixed	0,000516	7	0,000074	8,12146	0,000005	14,583	0,000521
	Error		0,000335	41	0,000008				
18	Intercept	Fixed	0,008547	1	0,008547	14,98298	0,000021	411,8956	0,000000
	Parallel (Time)	Random	0,000144	8	0,000018	40,00000	0,000034	0,5298	0,826952
	Time	Fixed	0,000932	7	0,000133	8,64513	0,000018	7,2550	0,004736
	Error		0,001360	40	0,000034				

Table II - 81: *Test results of **Tukey HSD post-hoc tests** of difference for fshr gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,815609	0,075305	0,229895	0,004996	0,000190	0,014870	0,005451
	2	0,815609		0,641413	0,939264	0,094921	0,001179	0,161898	0,102826
	3	0,075305	0,641413		0,998351	0,961145	0,112589	0,912063	0,967969
	4	0,229895	0,939264	0,998351		0,674709	0,025843	0,655675	0,697789
	5	0,004996	0,094921	0,961145	0,674709		0,507119	0,999782	1,000000
	6	0,000190	0,001179	0,112589	0,025843	0,507119		0,964208	0,482686
	7	0,014870	0,161898	0,912063	0,655675	0,999782	0,964208		0,999668
	8	0,005451	0,102826	0,967969	0,697789	1,000000	0,482686	0,999668	
12.5	1		0,999996	1,000000	0,999983	0,001796	0,017373	0,107240	0,023664
	2	0,999996		0,999996	1,000000	0,000817	0,009035	0,054499	0,010748
	3	1,000000	0,999996		0,999989	0,015014	0,057409	0,294256	0,106530
	4	0,999983	1,000000	0,999989		0,000156	0,001997	0,007934	0,000925
	5	0,001796	0,000817	0,015014	0,000156		1,000000	0,527816	0,926484
	6	0,017373	0,009035	0,057409	0,001997	1,000000		0,834800	0,988189
	7	0,107240	0,054499	0,294256	0,007934	0,527816	0,834800		0,994070
	8	0,023664	0,010748	0,106530	0,000925	0,926484	0,988189	0,994070	
18	1		0,999309	0,999835	1,000000	0,901394	0,342148	0,213647	0,228304
	2	0,999309		0,943081	0,993511	0,991862	0,509277	0,317100	0,337857
	3	0,999835	0,943081		0,999887	0,417333	0,044554	0,017999	0,018747
	4	1,000000	0,993511	0,999887		0,646154	0,087652	0,036077	0,037712
	5	0,901394	0,991862	0,417333	0,646154		0,830432	0,600816	0,632735
	6	0,342148	0,509277	0,044554	0,087652	0,830432		0,999979	0,999998
	7	0,213647	0,317100	0,017999	0,036077	0,600816	0,999979		1,000000
	8	0,228304	0,337857	0,018747	0,037712	0,632735	0,999998	1,000000	

c. Full feeding (100%)

Table II - 82: *Test results of a **two-way random effects nested ANOVA** for fshr gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	0,030591	1	0,030591	47,7220	0,000029	1073,303	0,000000
Parallel (Temp.*Time)	Random	0,001122	38	0,000030	116,0000	0,000022	1,326	0,128976
Temperature	Fixed	0,001046	2	0,000523	46,9157	0,000029	18,299	0,000001
Time	Fixed	0,001962	7	0,000280	45,7498	0,000029	9,775	0,000000
Error		0,002583	116	0,000022				

Table II - 83: *Test results of a **two-way factorial ANOVA** for fshr gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	0,033505	1	0,033505	1563,805	0,000000
Time	0,002308	7	0,000330	15,391	0,000000
Temperature	0,001148	2	0,000574	26,800	0,000000
Time*Temp.	0,000706	14	0,000050	2,353	0,005881
Error	0,003000	140	0,000021		

Table II - 84: *Test results of **one-way random effects nested ANOVAs** for fshr gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,004770	1	0,004770	3,03703	0,000038	127,0369	0,001415
	Parallel (Temp)	Random	0,000113	3	0,000038	10,00000	0,000021	1,8043	0,209971
	Temperature	Fixed	0,000135	2	0,000068	3,01982	0,000038	1,7968	0,306172
	Error		0,000209	10	0,000021				
2 (14.12.18)	Intercept	Fixed	0,004407	1	0,004407	12,96874	0,000002	1864,088	0,000000
	Parallel (Temp)	Random	0,000002	3	0,000001	10,00000	0,000038	0,014	0,997691
	Temperature	Fixed	0,000201	2	0,000101	12,28947	0,000002	65,725	0,000000
	Error		0,000384	10	0,000038				
3 (01.02.19)	Intercept	Fixed	0,005732	1	0,005732	3,000000	0,000066	86,51633	0,002631
	Parallel (Temp)	Random	0,000199	3	0,000066	9,000000	0,000074	0,89412	0,480812
	Temperature	Fixed	0,000136	2	0,000068	3,000000	0,000066	1,02515	0,457831
	Error		0,000667	9	0,000074				
4 (13.04.19)	Intercept	Fixed	0,005505	1	0,005505	4,05560	0,000004	1352,608	0,000003
	Parallel (Temp)	Random	0,000011	3	0,000004	14,00000	0,000016	0,223	0,878856
	Temperature	Fixed	0,000031	2	0,000015	3,58019	0,000004	3,961	0,123773
	Error		0,000227	14	0,000016				
5 (27.03.19)	Intercept	Fixed	0,003961	1	0,003961	7,76853	0,000003	1150,677	0,000000
	Parallel (Temp)	Random	0,000007	3	0,000002	15,00000	0,000011	0,203	0,892829
	Temperature	Fixed	0,000247	2	0,000123	4,62889	0,000003	44,515	0,000949
	Error		0,000172	15	0,000011				
6 (10.04.19)	Intercept	Fixed	0,002502	1	0,002502	4,21122	0,000012	206,8601	0,000097
	Parallel (Temp)	Random	0,000034	3	0,000011	19,00000	0,000017	0,6655	0,583487
	Temperature	Fixed	0,000189	2	0,000094	3,42633	0,000012	8,0663	0,050579
	Error		0,000327	19	0,000017				
7 (24.04.19)	Intercept	Fixed	0,002508	1	0,002508	3,03587	0,000005	467,5079	0,000200
	Parallel (Temp)	Random	0,000016	3	0,000005	20,00000	0,000013	0,4141	0,744730
	Temperature	Fixed	0,000531	2	0,000265	3,01853	0,000005	49,5607	0,004917
	Error		0,000258	20	0,000013				
8 (15.05.19)	Intercept	Fixed	0,001868	1	0,001868	6,13653	0,000013	145,2059	0,000017
	Parallel (Temp)	Random	0,000034	3	0,000011	19,00000	0,000018	0,6419	0,597477
	Temperature	Fixed	0,000498	2	0,000249	4,24024	0,000012	20,5382	0,006587
	Error		0,000338	19	0,000018				

Table II - 85: *Test results of **Tukey HSD post-hoc tests** of difference for fshr gene transcription between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,202492	0,181993
	12	0,202492		0,999946
	18	0,181993	0,999946	
2 (14.12.18)	8		0,116988	0,327775
	12	0,116988		0,739916
	18	0,327775	0,739916	
3 (01.02.19)	8		0,345914	0,580346
	12	0,345914		0,895095
	18	0,580346	0,895095	
4 (13.03.19)	8		0,684930	0,945659
	12	0,684930		0,462566
	18	0,945659	0,462566	
5 (27.03.19)	8		0,000589	0,005666
	12	0,000589		0,286946
	18	0,005666	0,286946	
6 (10.04.19)	8		0,700256	0,023731
	12	0,700256		0,048111
	18	0,023731	0,048111	
7 (24.04.19)	8		0,600987	0,000157
	12	0,600987		0,000275
	18	0,000157	0,000275	
8 (15.05.19)	8		0,039820	0,000267
	12	0,039820		0,140196
	18	0,000267	0,140196	

Table II - 86: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for fshr gene transcription between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,106498	0,273944	0,559875	0,577118	0,282212
	1	12	0,106498		0,924366	0,640871	0,623446	0,917439
	1	18	0,273944	0,924366		0,980383	0,975705	1,000000
	2	8	0,559875	0,640871	0,980383		1,000000	0,983340
	2	12	0,577118	0,623446	0,975705	1,000000		0,979156
	2	18	0,282212	0,917439	1,000000	0,983340	0,979156	
2 (14.12.18)	1	8		0,590406	0,786163	0,999994	0,586618	0,918337
	1	12	0,590406		0,995687	0,695979	1,000000	0,989679
	1	18	0,786163	0,995687		0,876331	0,995416	0,999982
	2	8	0,999994	0,695979	0,876331		0,692433	0,958456
	2	12	0,586618	1,000000	0,995416	0,692433		0,989210
	2	18	0,918337	0,989679	0,999982	0,958456	0,989210	
3 (01.02.19)	1	8		0,476400	0,534770	0,809354	0,705701	0,982066
	1	12	0,476400		0,999584	0,990635	0,990322	0,871157
	1	18	0,534770	0,999584		0,999300	0,999461	0,935756
	2	8	0,809354	0,990635	0,999300		1,000000	0,993646
	2	12	0,705701	0,990322	0,999461	1,000000		0,986066
	2	18	0,982066	0,871157	0,935756	0,993646	0,986066	
4 (13.03.19)	1	8		0,893695	0,999907	0,998751	0,987982	0,999997
	1	12	0,893695		0,915059	0,956979	0,989865	0,767741
	1	18	0,999907	0,915059		0,999957	0,996163	0,998110
	2	8	0,998751	0,956979	0,999957		0,999677	0,988813
	2	12	0,987982	0,989865	0,996163	0,999677		0,941480
	2	18	0,999997	0,767741	0,998110	0,988813	0,941480	
5 (27.03.19)	1	8		0,210455	0,494990	0,999496	0,027893	0,132746
	1	12	0,210455		0,853867	0,129532	0,999972	0,971994
	1	18	0,494990	0,853867		0,276598	0,639368	0,980337
	2	8	0,999496	0,129532	0,276598		0,007715	0,045386
	2	12	0,027893	0,999972	0,639368	0,007715		0,912839
	2	18	0,132746	0,971994	0,980337	0,045386	0,912839	
6 (10.04.19)	1	8		0,749549	0,326505	0,992928	0,997878	0,197706
	1	12	0,749549		0,917182	0,950559	0,817433	0,722605
	1	18	0,326505	0,917182		0,537551	0,276686	0,998055
	2	8	0,992928	0,950559	0,537551		0,999950	0,336491
	2	12	0,997878	0,817433	0,276686	0,999950		0,136323
	2	18	0,197706	0,722605	0,998055	0,336491	0,136323	
7 (24.04.19)	1	8		0,758772	0,004265	0,997696	0,994858	0,008620
	1	12	0,758772		0,046354	0,872192	0,923859	0,092349
	1	18	0,004265	0,046354		0,002205	0,003994	0,999278
	2	8	0,997696	0,872192	0,002205		0,999997	0,005022
	2	12	0,994858	0,923859	0,003994	0,999997		0,008900
	2	18	0,008620	0,092349	0,999278	0,005022	0,008900	
8 (15.05.19)	1	8		0,999649	0,017624	0,999966	0,184174	0,021101
	1	12	0,999649		0,314541	0,999969	0,765891	0,383974
	1	18	0,017624	0,314541		0,013817	0,734776	0,999202
	2	8	0,999966	0,999969	0,013817		0,181632	0,015837
	2	12	0,184174	0,765891	0,734776	0,181632		0,856918
	2	18	0,021101	0,383974	0,999202	0,015837	0,856918	

Table II - 87: *Test results of **one-way random effects nested ANOVAs** for fshr gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,016105	1	0,016105	8,85898	0,000024	681,1614	0,000000
	Parallel (time)	Random	0,000186	8	0,000023	35,00000	0,000032	0,7179	0,674332
	Time	Fixed	0,000650	7	0,000093	8,12060	0,000023	3,9723	0,034989
	Error		0,001136	35	0,000032				
12.5	Intercept	Fixed	0,007355	1	0,007355	10,65424	0,000019	385,4295	0,000000
	Parallel (time)	Random	0,000153	8	0,000019	35,00000	0,000019	1,0081	0,447722
	Time	Fixed	0,000307	7	0,000044	8,42125	0,000019	2,2974	0,128308
	Error		0,000663	35	0,000019				
18	Intercept	Fixed	0,008448	1	0,008448	10,84686	0,000010	825,3896	0,000000
	Parallel (time)	Random	0,000077	8	0,000010	46,00000	0,000017	0,5630	0,802348
	Time	Fixed	0,002067	7	0,000295	8,36744	0,000010	30,4846	0,000026
	Error		0,000784	46	0,000017				

Table II - 88: *Test results of **Tukey HSD post-hoc test** of difference for fshr gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,999998	0,971670	0,929601	1,000000	0,407428	0,371078	0,673922
	2	0,999998		0,988252	0,786045	0,999999	0,212016	0,154860	0,416202
	3	0,971670	0,988252		0,358048	0,966182	0,059891	0,037519	0,127750
	4	0,929601	0,786045	0,358048		0,875361	0,962535	0,975702	0,999515
	5	1,000000	0,999999	0,966182	0,875361		0,289695	0,229086	0,536310
	6	0,407428	0,212016	0,059891	0,962535	0,289695		0,999998	0,998747
	7	0,371078	0,154860	0,037519	0,975702	0,229086	0,999998		0,999820
	8	0,673922	0,416202	0,127750	0,999515	0,536310	0,998747	0,999820	
12.5	1		0,988266	0,999974	0,920947	0,817235	0,643866	0,867166	0,225182
	2	0,988266		0,935442	0,476944	0,999651	0,997370	0,999984	0,819916
	3	0,999974	0,935442		0,986968	0,611929	0,402488	0,661041	0,113323
	4	0,920947	0,476944	0,986968		0,122424	0,040958	0,116769	0,008755
	5	0,817235	0,999651	0,611929	0,122424		1,000000	0,999997	0,955052
	6	0,643866	0,997370	0,402488	0,040958	1,000000		0,999795	0,959865
	7	0,867166	0,999984	0,661041	0,116769	0,999997	0,999795		0,832815
	8	0,225182	0,819916	0,113323	0,008755	0,955052	0,959865	0,832815	
18	1		0,999997	0,878984	0,999998	0,999787	0,002744	0,000168	0,000216
	2	0,999997		0,963325	1,000000	0,996911	0,002559	0,000173	0,000230
	3	0,878984	0,963325		0,929019	0,585361	0,000174	0,000130	0,000130
	4	0,999998	1,000000	0,929019		0,994627	0,000456	0,000132	0,000134
	5	0,999787	0,996911	0,585361	0,994627		0,004256	0,000176	0,000229
	6	0,002744	0,002559	0,000174	0,000456	0,004256		0,703852	0,963611
	7	0,000168	0,000173	0,000130	0,000132	0,000176	0,703852		0,994697
	8	0,000216	0,000230	0,000130	0,000134	0,000229	0,963611	0,994697	

d. Comparison of temperature groups between feeding regimes

Table II - 89: *Test results of **factorial ANOVAs** for fshr gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	0,032433	1	0,032433	902,7404	0,000000
	Feeding regime	0,000002	1	0,000002	0,0626	0,803122
	Time	0,001948	7	0,000278	7,7442	0,000000
	Feed*Time	0,000594	7	0,000085	2,3623	0,029853
	Error	0,002982	83	0,000036		
12.5	Intercept	0,016694	1	0,016694	1281,338	0,000000
	Feeding regime	0,000009	1	0,000009	0,721	0,398096
	Time	0,000879	7	0,000126	9,635	0,000000
	Feed*Time	0,000091	7	0,000013	0,997	0,438534
	Error	0,001186	91	0,000013		
18	Intercept	0,017729	1	0,017729	764,5596	0,000000
	Feeding regime	0,000073	1	0,000073	3,1620	0,078349
	Time	0,002922	7	0,000417	18,0011	0,000000
	Feed*Time	0,000124	7	0,000018	0,7661	0,616945
	Error	0,002365	102	0,000023		

Table II - 90: *Test results of* ***Tukey HSD post-hoc tests*** *for fshr gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,98	0,13	0,41	0,00	0,00	0,02	0,00	0,44	0,46	0,95	0,03	0,35	0,00	0,00	0,01
		2	0,98		0,89	1,00	0,17	0,00	0,29	0,18	1,00	1,00	1,00	0,52	1,00	0,09	0,04	0,18
		3	0,13	0,89		1,00	1,00	0,20	1,00	1,00	1,00	1,00	0,97	1,00	1,00	0,97	0,97	1,00
		4	0,41	1,00	1,00		0,91	0,04	0,90	0,93	1,00	1,00	1,00	1,00	1,00	0,70	0,63	0,91
		5	0,00	0,17	1,00	0,91		0,78	1,00	1,00	0,96	0,80	0,36	1,00	0,89	1,00	1,00	1,00
		6	0,00	0,00	0,20	0,04	0,78		1,00	0,76	0,07	0,01	0,00	0,59	0,02	1,00	0,96	0,85
		7	0,02	0,29	1,00	0,90	1,00	1,00		1,00	0,94	0,82	0,46	1,00	0,89	1,00	1,00	1,00
		8	0,00	0,18	1,00	0,93	1,00	0,76	1,00		0,97	0,82	0,38	1,00	0,91	1,00	1,00	1,00
	100	1	0,44	1,00	1,00	1,00	0,96	0,07	0,94	0,97		1,00	1,00	1,00	1,00	0,80	0,76	0,96
		2	0,46	1,00	1,00	1,00	0,80	0,01	0,82	0,82	1,00		1,00	0,98	1,00	0,54	0,43	0,80
		3	0,95	1,00	0,97	1,00	0,36	0,00	0,46	0,38	1,00	1,00		0,75	1,00	0,20	0,13	0,37
		4	0,03	0,52	1,00	1,00	1,00	0,59	1,00	1,00	1,00	0,98	0,75		1,00	1,00	1,00	1,00
		5	0,35	1,00	1,00	1,00	0,89	0,02	0,89	0,91	1,00	1,00	1,00	1,00		0,66	0,57	0,89
		6	0,00	0,09	0,97	0,70	1,00	1,00	1,00	1,00	0,80	0,54	0,20	1,00	0,66		1,00	1,00
		7	0,00	0,04	0,97	0,63	1,00	0,96	1,00	1,00	0,76	0,43	0,13	1,00	0,57	1,00		1,00
		8	0,01	0,18	1,00	0,91	1,00	0,85	1,00	1,00	0,96	0,80	0,37	1,00	0,89	1,00	1,00	
12.5	67	1		1,00	1,00	1,00	0,05	0,26	0,65	0,31	1,00	1,00	1,00	0,99	0,89	0,67	0,93	0,15
		2	1,00		1,00	1,00	0,03	0,17	0,48	0,19	1,00	1,00	1,00	1,00	0,78	0,51	0,84	0,09
		3	1,00	1,00		1,00	0,24	0,49	0,89	0,65	1,00	1,00	1,00	1,00	0,97	0,90	0,99	0,38
		4	1,00	1,00	1,00		0,00	0,06	0,16	0,03	1,00	0,98	1,00	0,99	0,52	0,17	0,55	0,02
		5	0,05	0,03	0,24	0,00		1,00	0,97	1,00	0,07	0,81	0,02	0,00	0,97	0,97	0,77	1,00
		6	0,26	0,17	0,49	0,06	1,00		1,00	1,00	0,30	0,95	0,13	0,00	1,00	1,00	0,97	1,00
		7	0,65	0,48	0,89	0,16	0,97	1,00		1,00	0,71	1,00	0,39	0,01	1,00	1,00	1,00	1,00
		8	0,31	0,19	0,65	0,03	1,00	1,00	1,00		0,37	1,00	0,14	0,00	1,00	1,00	1,00	1,00
	100	1	1,00	1,00	1,00	1,00	0,07	0,30	0,71	0,37		1,00	1,00	0,98	0,92	0,74	0,96	0,18
		2	1,00	1,00	1,00	0,98	0,81	0,95	1,00	1,00	1,00		0,99	0,52	1,00	1,00	1,00	0,92
		3	1,00	1,00	1,00	1,00	0,02	0,13	0,39	0,14	1,00	0,99		1,00	0,70	0,41	0,76	0,06
		4	0,99	1,00	1,00	0,99	0,00	0,00	0,01	0,00	0,98	0,52	1,00		0,07	0,01	0,07	0,00
		5	0,89	0,78	0,97	0,52	0,97	1,00	1,00	1,00	0,92	1,00	0,70	0,07		1,00	1,00	0,99
		6	0,67	0,51	0,90	0,17	0,97	1,00	1,00	1,00	0,74	1,00	0,41	0,01	1,00		1,00	1,00
		7	0,93	0,84	0,99	0,55	0,77	0,97	1,00	1,00	0,96	1,00	0,76	0,07	1,00	1,00		0,93
		8	0,15	0,09	0,38	0,02	1,00	1,00	1,00	1,00	0,18	0,92	0,06	0,00	0,99	1,00	0,93	
18	67	1		1,00	1,00	1,00	0,97	0,33	0,16	0,18	1,00	1,00	1,00	1,00	1,00	0,05	0,00	0,01
		2	1,00		0,99	1,00	1,00	0,56	0,29	0,32	1,00	1,00	1,00	1,00	1,00	0,07	0,00	0,01
		3	1,00	0,99		1,00	0,43	0,01	0,00	0,00	0,99	1,00	1,00	0,99	0,88	0,00	0,00	0,00
		4	1,00	1,00	1,00		0,74	0,04	0,01	0,01	1,00	1,00	1,00	1,00	0,99	0,00	0,00	0,00
		5	0,97	1,00	0,43	0,74		0,93	0,68	0,72	1,00	1,00	0,64	0,99	1,00	0,21	0,01	0,01
		6	0,33	0,56	0,01	0,04	0,93		1,00	1,00	0,44	0,38	0,03	0,19	0,60	1,00	0,60	0,86
		7	0,16	0,29	0,00	0,01	0,68	1,00		1,00	0,19	0,17	0,01	0,06	0,29	1,00	0,81	0,97
		8	0,18	0,32	0,00	0,01	0,72	1,00	1,00		0,21	0,19	0,01	0,06	0,31	1,00	0,71	0,94
	100	1	1,00	1,00	0,99	1,00	1,00	0,44	0,19	0,21		1,00	1,00	1,00	1,00	0,04	0,00	0,00
		2	1,00	1,00	1,00	1,00	1,00	0,38	0,17	0,19	1,00		1,00	1,00	1,00	0,03	0,00	0,00
		3	1,00	1,00	1,00	1,00	0,64	0,03	0,01	0,01	1,00	1,00		1,00	0,97	0,00	0,00	0,00
		4	1,00	1,00	0,99	1,00	0,99	0,19	0,06	0,06	1,00	1,00	1,00		1,00	0,01	0,00	0,00
		5	1,00	1,00	0,88	0,99	1,00	0,60	0,29	0,31	1,00	1,00	0,97	1,00		0,05	0,00	0,00
		6	0,05	0,07	0,00	0,00	0,21	1,00	1,00	1,00	0,04	0,03	0,00	0,01	0,05		0,99	1,00
		7	0,00	0,00	0,00	0,00	0,01	0,60	0,81	0,71	0,00	0,00	0,00	0,00	0,00	0,99		1,00
		8	0,01	0,01	0,00	0,00	0,01	0,86	0,97	0,94	0,00	0,00	0,00	0,00	0,00	1,00	1,00	

II - VI Relative *luteinizing hormone receptor* (*lhr*) mRNA transcription

a. Overall tests:

Table II - 91: *Test results of a **three-way random effects nested ANOVA** for lhr gene transcription, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	0,005247	1	0,005247	97,2911	0,000014	377,6195	0,000000
Parallel (Temp.*feed*Time)	Random	0,001200	83	0,000014	246,0000	0,000010	1,5180	0,007629
Time	Fixed	0,000146	7	0,000021	92,2018	0,000014	1,4840	0,182664
Temperature	Fixed	0,000292	2	0,000146	96,6239	0,000014	10,4974	0,000075
Feeding regime	Fixed	0,000078	1	0,000078	96,7254	0,000014	5,6090	0,019857
Error		0,002342	246	0,000010				

Table II - 92: *Test results of a **three-way factorial ANOVA** for lhr gene transcription, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	0,005245	1	0,005245	568,3747	0,000000
Time	0,000147	7	0,000021	2,2790	0,028366
Temperature	0,000301	2	0,000151	16,3111	0,000000
Feed regime	0,000093	1	0,000093	10,0887	0,001652
Time*Temp.	0,000543	14	0,000039	4,2048	0,000001
Time*Feed.	0,000011	7	0,000002	0,1761	0,989955
Temp.*Feed.	0,000066	2	0,000033	3,5915	0,028782
Time*Temp.*Feed.	0,000189	14	0,000014	1,4665	0,122621
Error	0,002694	292	0,000009		

b. Restrictive feeding (67%):

Table II - 93: *Test results of a **two-way random effects nested ANOVA** for lhr gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	0,001885	1	0,001885	43,5109	0,000015	126,3377	0,000000
Parallel (Temp.*Time)	Random	0,000560	36	0,000016	117,0000	0,000011	1,4699	0,064537
Time	Fixed	0,000084	7	0,000012	38,8897	0,000015	0,7823	0,606068
Temperature	Fixed	0,000230	2	0,000115	43,6931	0,000015	7,7291	0,001337
Error		0,001239	117	0,000011				

Table II - 94: *Test results of a **two-way factorial ANOVA** for lhr gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	0,001785	1	0,001785	167,4724	0,000000
Time	0,000068	7	0,000010	0,9071	0,502989
Temperature	0,000247	2	0,000124	11,5991	0,000022
Temp.*Time	0,000318	14	0,000023	2,1290	0,013630
Error	0,001481	139	0,000011		

Table II - 95: *Test results of **one-way random effects nested ANOVAs** for lhr gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,000081	1	0,000081	3,493372	0,000001	93,49609	0,001212
	Parallel (temp)	Random	0,000003	3	0,000001	8,000000	0,000001	0,62183	0,620462
	Temperature	Fixed	0,000007	2	0,000004	3,203038	0,000001	4,38212	0,121126
	Error		0,000011	8	0,000001				
2 (14.12.18)	Intercept	Fixed	0,000121	1	0,000121	3,07514	0,000004	32,75163	0,009894
	Parallel (temp)	Random	0,000011	3	0,000004	11,00000	0,000004	0,89127	0,475950
	Temperature	Fixed	0,000004	2	0,000002	3,03459	0,000004	0,52881	0,635284
	Error		0,000046	11	0,000004				
3 (01.02.19)	Intercept	Fixed	0,000142	1	0,000142	10,87482	0,000001	132,4306	0,000000
	Parallel (temp)	Random	0,000000	2	0,000000	9,00000	0,000006	0,0294	0,971112
	Temperature	Fixed	0,000056	2	0,000028	10,92559	0,000001	27,4223	0,000055
	Error		0,000055	9	0,000006				
4 (13.04.19)	Intercept	Fixed	0,000507	1	0,000507	3,02494	0,000019	26,52727	0,013902
	Parallel (temp)	Random	0,000057	3	0,000019	16,00000	0,000015	1,27507	0,316582
	Temperature	Fixed	0,000222	2	0,000111	3,01308	0,000019	5,79556	0,092751
	Error		0,000240	16	0,000015				
5 (27.03.19)	Intercept	Fixed	0,000336	1	0,000336	3,42155	0,000002	178,2057	0,000452
	Parallel (temp)	Random	0,000005	3	0,000002	19,00000	0,000008	0,2202	0,881150
	Temperature	Fixed	0,000104	2	0,000052	3,18901	0,000002	28,3134	0,009331
	Error		0,000155	19	0,000008				
6 (10.04.19)	Intercept	Fixed	0,000166	1	0,000166	8,15915	0,000007	24,23948	0,001094
	Parallel (temp)	Random	0,000014	3	0,000005	11,00000	0,000017	0,27206	0,844295
	Temperature	Fixed	0,000047	2	0,000023	4,79690	0,000006	4,22596	0,087451
	Error		0,000189	11	0,000017				
7 (24.04.19)	Intercept	Fixed	0,000423	1	0,000423	2,65103	0,000037	11,59756	0,050920
	Parallel (temp)	Random	0,000080	2	0,000040	19,00000	0,000023	1,75799	0,199295
	Temperature	Fixed	0,000089	2	0,000044	2,55352	0,000037	1,20419	0,428196
	Error		0,000434	19	0,000023				
8 (15.05.19)	Intercept	Fixed	0,000437	1	0,000437	3,00043	0,000024	18,33167	0,023400
	Parallel (temp)	Random	0,000072	3	0,000024	24,00000	0,000005	5,18995	0,006618
	Temperature	Fixed	0,000015	2	0,000007	3,00022	0,000024	0,30719	0,756191
	Error		0,000110	24	0,000005				

Table II - 96: *Test results of **Tukey HSD post-hoc tests** of difference for lhr gene transcription between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,044238	0,143591
	12	0,044238		0,681656
	18	0,143591	0,681656	
2 (14.12.18)	8		0,757522	0,972637
	12	0,757522		0,631321
	18	0,972637	0,631321	
3 (01.02.19)	8		0,918278	0,060040
	12	0,918278		0,109215
	18	0,060040	0,109215	
4 (13.03.19)	8		0,335146	0,006451
	12	0,335146		0,090857
	18	0,006451	0,090857	
5 (27.03.19)	8		0,150139	0,234332
	12	0,150139		0,006007
	18	0,234332	0,006007	
6 (10.04.19)	8		0,685738	0,220749
	12	0,685738		0,516552
	18	0,220749	0,516552	
7 (24.04.19)	8		0,443474	0,158846
	12	0,443474		0,556518
	18	0,158846	0,556518	
8 (15.05.19)	8		0,058271	0,819106
	12	0,058271		0,184524
	18	0,819106	0,184524	

Table II - 97: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for lhr gene transcription between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,099437	0,399586	0,988196	0,582671	0,534633
	1	12	0,099437		0,965503	0,609103	0,853408	0,738920
	1	18	0,399586	0,965503		0,920523	0,999213	0,996358
	2	8	0,988196	0,609103	0,920523		0,979447	0,983498
	2	12	0,582671	0,853408	0,999213	0,979447		0,999998
	2	18	0,534633	0,738920	0,996358	0,983498	0,999998	
2 (14.12.18)	1	8		0,999630	0,852931	0,998578	0,998886	0,997340
	1	12	0,999630		0,706140	0,980789	0,999998	0,999987
	1	18	0,852931	0,706140		0,969244	0,731107	0,623065
	2	8	0,998578	0,980789	0,969244		0,976233	0,956194
	2	12	0,998886	0,999998	0,731107	0,976233		1,000000
	2	18	0,997340	0,999987	0,623065	0,956194	1,000000	
3 (01.02.19)	1	8		0,996922	0,429930	0,999999	0,323385	
	1	12	0,996922		0,363651	0,995043	0,278332	
	1	18	0,429930	0,363651		0,453780	0,999186	
	2	8	0,999999	0,995043	0,453780		0,343258	
	2	1	N=0	N=0	N=0	N=0	N=0	
	2	18	0,323385	0,278332	0,999186	0,343258		
4 (13.03.19)	1	8		0,920893	0,033710	0,998051	0,967132	0,579606
	1	12	0,920893		0,172632	0,771582	0,999960	0,981660
	1	18	0,033710	0,172632		0,024191	0,128009	0,436358
	2	8	0,998051	0,771582	0,024191		0,856964	0,410781
	2	12	0,967132	0,999960	0,128009	0,856964		0,948151
	2	18	0,579606	0,981660	0,436358	0,410781	0,948151	
5 (27.03.19)	1	8		0,779599	0,633712	1,000000	0,736585	0,958169
	1	12	0,779599		0,111612	0,745003	1,000000	0,316257
	1	18	0,633712	0,111612		0,585436	0,077193	0,961688
	2	8	1,000000	0,745003	0,585436		0,691099	0,947920
	2	12	0,736585	1,000000	0,077193	0,691099		0,246974
	2	18	0,958169	0,316257	0,961688	0,947920	0,246974	
6 (10.04.19)	1	8		0,987110	0,756207	1,000000	0,996727	0,974831
	1	12	0,987110		0,895646	0,972583	0,999896	0,999988
	1	18	0,756207	0,895646		0,552201	0,784823	0,945296
	2	8	1,000000	0,972583	0,552201		0,993321	0,946631
	2	12	0,996727	0,999896	0,784823	0,993321		0,998833
	2	18	0,974831	0,999988	0,945296	0,946631	0,998833	
7 (24.04.19)	1	2	N=0	N=0	N=0	N=0	N=0	
	1	12		0,382721	0,877336	0,995678	1,000000	
	1	18	0,382721		0,125039	0,546060	0,382158	
	2	8	0,877336	0,125039		0,700664	0,877717	
	2	12	0,995678	0,546060	0,700664		0,995627	
	2	18	1,000000	0,382158	0,877717	0,995627		
8 (15.05.19)	1	8		0,923776	0,999814	0,974966	0,098121	0,999586
	1	12	0,923776		0,790753	0,999513	0,009441	0,979032
	1	18	0,999814	0,790753		0,884677	0,121630	0,989334
	2	8	0,974966	0,999513	0,884677		0,007580	0,997612
	2	12	0,098121	0,009441	0,121630	0,007580		0,032132
	2	18	0,999586	0,979032	0,989334	0,997612	0,032132	

Table II - 98: *Test results of **one-way random effects nested ANOVAs** for lhr gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,000227	1	0,000227	20,51994	0,000001	165,9129	0,000000
	Parallel (Time)	Random	0,000006	7	0,000001	33,00000	0,000004	0,2442	0,970549
	Time	Fixed	0,000050	7	0,000007	8,48610	0,000001	7,3414	0,004827
	Error		0,000121	33	0,000004				
12.5	Intercept	Fixed	0,000413	1	0,000413	8,83141	0,000010	41,57500	0,000129
	Parallel (Time)	Random	0,000074	7	0,000011	39,00000	0,000007	1,54614	0,180754
	Time	Fixed	0,000115	7	0,000016	7,74120	0,000010	1,60124	0,264599
	Error		0,000266	39	0,000007				
18	Intercept	Fixed	0,001659	1	0,001659	8,96560	0,000020	81,93799	0,000008
	Parallel (Time)	Random	0,000163	8	0,000020	45,00000	0,000019	1,07389	0,398197
	Time	Fixed	0,000241	7	0,000034	8,12726	0,000020	1,69524	0,235979
	Error		0,000852	45	0,000019				

Table II - 99: *Test results of **Tukey HSD post-hoc tests** of difference for lhr gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,989421	0,855730	0,361292	1,000000	0,556434	0,492000	0,991814
	2	0,989421		0,998449	0,781303	0,978634	0,894760	0,849135	1,000000
	3	0,855730	0,998449		0,984042	0,734117	0,992520	0,983926	0,990964
	4	0,361292	0,781303	0,984042		0,161608	1,000000	0,999999	0,578830
	5	1,000000	0,978634	0,734117	0,161608		0,423001	0,356539	0,979208
	6	0,556434	0,894760	0,992520	1,000000	0,423001		1,000000	0,807026
	7	0,492000	0,849135	0,983926	0,999999	0,356539	1,000000		0,742125
	8	0,991814	1,000000	0,990964	0,578830	0,979208	0,807026	0,742125	
12.5	1		0,999998	1,000000	0,681714	0,999921	0,912767	0,325004	0,189232
	2	0,999998		0,999994	0,822160	0,998156	0,969654	0,476920	0,300744
	3	1,000000	0,999994		0,877847	1,000000	0,967243	0,676754	0,531193
	4	0,681714	0,822160	0,877847		0,304505	0,999903	0,999195	0,979901
	5	0,999921	0,998156	1,000000	0,304505		0,642276	0,074543	0,034588
	6	0,912767	0,969654	0,967243	0,999903	0,642276		0,979144	0,897120
	7	0,325004	0,476920	0,676754	0,999195	0,074543	0,979144		0,999871
	8	0,189232	0,300744	0,531193	0,979901	0,034588	0,897120	0,999871	
18	1		0,999973	0,842119	0,209026	0,753634	0,771157	0,488688	0,999230
	2	0,999973		0,944879	0,318323	0,896392	0,906256	0,666828	0,999999
	3	0,842119	0,944879		0,949938	1,000000	1,000000	0,999784	0,958890
	4	0,209026	0,318323	0,949938		0,933152	0,942549	0,993588	0,272972
	5	0,753634	0,896392	1,000000	0,933152		1,000000	0,999779	0,907409
	6	0,771157	0,906256	1,000000	0,942549	1,000000		0,999832	0,919137
	7	0,488688	0,666828	0,999784	0,993588	0,999779	0,999832		0,640607
	8	0,999230	0,999999	0,958890	0,272972	0,907409	0,919137	0,640607	

c. Full feeding (100%)

Table II - 100: *Test results of a **two-way random effects nested ANOVA** for lhr gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	0,003573	1	0,003573	41,7679	0,000014	263,7255	0,000000
Parallel (Temp.*Time)	Random	0,000530	38	0,000014	129,0000	0,000009	1,6304	0,023156
Temperature	Fixed	0,000117	2	0,000059	41,2169	0,000014	4,3033	0,020078
Time	Fixed	0,000090	7	0,000013	40,9626	0,000014	0,9453	0,482996
Error		0,001104	129	0,000009				

Table II - 101: *Test results of a **two-way factorial ANOVA** for lhr gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	0,003758	1	0,003758	473,9655	0,000000
Time	0,000093	7	0,000013	1,6847	0,116575
Temperature	0,000113	2	0,000056	7,1260	0,001099
Time*Temp.	0,000421	14	0,000030	3,7887	0,000017
Error	0,001213	153	0,000008		

Table II - 102: *Test results of* ***one-way random effects nested ANOVAs*** *for lhr gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,000211	1	0,000211	3,00000	0,000003	80,22921	0,002936
	Parallel (Temp)	Random	0,000008	3	0,000003	12,00000	0,000005	0,49555	0,692133
	Temperature	Fixed	0,000012	2	0,000006	3,00000	0,000003	2,32784	0,245305
	Error		0,000064	12	0,000005				
2 (14.12.18)	Intercept	Fixed	0,000262	1	0,000262	3,79928	0,000002	164,3037	0,000289
	Parallel (Temp)	Random	0,000004	3	0,000001	12,00000	0,000008	0,1710	0,913933
	Temperature	Fixed	0,000023	2	0,000011	3,37223	0,000002	7,4599	0,057785
	Error		0,000101	12	0,000008				
3 (01.02.19)	Intercept	Fixed	0,000319	1	0,000319	3,05110	0,000017	19,17984	0,021279
	Parallel (Temp)	Random	0,000050	3	0,000017	11,00000	0,000013	1,30874	0,320674
	Temperature	Fixed	0,000014	2	0,000007	3,02354	0,000017	0,42784	0,686091
	Error		0,000140	11	0,000013				
4 (13.04.19)	Intercept	Fixed	0,000752	1	0,000752	5,24047	0,000002	460,4401	0,000003
	Parallel (Temp)	Random	0,000004	3	0,000001	18,00000	0,000008	0,1660	0,917899
	Temperature	Fixed	0,000074	2	0,000037	4,15435	0,000001	24,8252	0,004892
	Error		0,000140	18	0,000008				
5 (27.03.19)	Intercept	Fixed	0,000694	1	0,000694	9,58118	0,000001	579,9571	0,000000
	Parallel (Temp)	Random	0,000002	3	0,000001	20,00000	0,000010	0,0666	0,976992
	Temperature	Fixed	0,000072	2	0,000036	5,57213	0,000001	40,1253	0,000491
	Error		0,000201	20	0,000010				
6 (10.04.19)	Intercept	Fixed	0,000560	1	0,000560	3,54293	0,000008	71,11472	0,001815
	Parallel (Temp)	Random	0,000023	3	0,000008	18,00000	0,000015	0,50840	0,681463
	Temperature	Fixed	0,000134	2	0,000067	3,27261	0,000008	8,67625	0,049178
	Error		0,000268	18	0,000015				
7 (24.04.19)	Intercept	Fixed	0,000526	1	0,000526	3,23249	0,000004	128,7792	0,001032
	Parallel (Temp)	Random	0,000012	3	0,000004	20,00000	0,000006	0,6347	0,601315
	Temperature	Fixed	0,000041	2	0,000021	3,11857	0,000004	5,0956	0,104067
	Error		0,000127	20	0,000006				
8 (15.05.19)	Intercept	Fixed	0,000507	1	0,000507	4,22324	0,000002	219,4511	0,000084
	Parallel (Temp)	Random	0,000007	3	0,000002	18,00000	0,000003	0,6293	0,605492
	Temperature	Fixed	0,000134	2	0,000067	3,51682	0,000002	30,0277	0,006158
	Error		0,000062	18	0,000003				

Table II - 103: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for lhr gene transcription between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,644452	0,321797
	12	0,644452		0,823936
	18	0,321797	0,823936	
2 (14.12.18)	8		0,999120	0,344402
	12	0,999120		0,422310
	18	0,344402	0,422310	
3 (01.02.19)	8		0,966290	0,710463
	12	0,966290		0,584120
	18	0,710463	0,584120	
4 (13.03.19)	8		0,166065	0,017448
	12	0,166065		0,585769
	18	0,017448	0,585769	
5 (27.03.19)	8		0,649627	0,034782
	12	0,649627		0,271598
	18	0,034782	0,271598	
6 (10.04.19)	8		0,021823	0,046103
	12	0,021823		0,849779
	18	0,046103	0,849779	
7 (24.04.19)	8		0,118499	0,996095
	12	0,118499		0,093592
	18	0,996095	0,093592	
8 (15.05.19)	8		0,865495	0,000184
	12	0,865495		0,000931
	18	0,000184	0,000931	

Table II - 104: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for lhr gene transcription between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,943002	0,637351	0,999999	0,999502	0,995855
	1	12	0,943002		0,981812	0,917701	0,991155	0,998360
	1	18	0,637351	0,981812		0,586384	0,807258	0,883031
	2	8	0,999999	0,917701	0,586384		0,998323	0,991023
	2	12	0,999502	0,991155	0,807258	0,998323		0,999973
	2	18	0,995855	0,998360	0,883031	0,991023	0,999973	
2 (14.12.18)	1	8		0,999924	0,898096	0,990305	0,999940	0,989806
	1	12	0,999924		0,885753	0,999675	1,000000	0,978913
	1	18	0,898096	0,885753		0,663877	0,848911	0,998078
	2	8	0,990305	0,999675	0,663877		0,999067	0,872657
	2	12	0,999940	1,000000	0,848911	0,999067		0,972237
	2	18	0,989806	0,978913	0,998078	0,872657	0,972237	
3 (01.02.19)	1	8		0,981489	0,955964	0,793907	0,947026	0,979647
	1	12	0,981489		1,000000	0,996596	0,999998	0,785171
	1	18	0,955964	1,000000		0,997244	1,000000	0,662296
	2	8	0,793907	0,996596	0,997244		0,998218	0,419261
	2	12	0,947026	0,999998	1,000000	0,998218		0,640129
	2	18	0,979647	0,785171	0,662296	0,419261	0,640129	
4 (13.03.19)	1	8		0,857813	0,191331	0,999992	0,671168	0,459752
	1	12	0,857813		0,943265	0,881988	1,000000	0,998946
	1	18	0,191331	0,943265		0,172327	0,874299	0,980874
	2	8	0,999992	0,881988	0,172327		0,684507	0,454679
	2	12	0,671168	1,000000	0,874299	0,684507		0,998245
	2	18	0,459752	0,998946	0,980874	0,454679	0,998245	
5 (27.03.19)	1	8		0,976107	0,576848	1,000000	0,993911	0,340950
	1	12	0,976107		0,991608	0,966793	0,999494	0,956826
	1	18	0,576848	0,991608		0,532970	0,856969	0,999635
	2	8	1,000000	0,966793	0,532970		0,988710	0,304338
	2	12	0,993911	0,999494	0,856969	0,988710		0,648495
	2	18	0,340950	0,956826	0,999635	0,304338	0,648495	
6 (10.04.19)	1	8		0,206865	0,412974	0,999620	0,813517	0,729999
	1	12	0,206865		0,993794	0,115170	0,905146	0,847368
	1	18	0,412974	0,993794		0,254155	0,992861	0,987455
	2	8	0,999620	0,115170	0,254155		0,645593	0,530014
	2	12	0,813517	0,905146	0,992861	0,645593		1,000000
	2	18	0,729999	0,847368	0,987455	0,530014	1,000000	
7 (24.04.19)	1	8		0,360494	0,944020	0,743754	0,256238	0,969791
	1	12	0,360494		0,738775	0,920444	0,999999	0,666942
	1	18	0,944020	0,738775		0,994610	0,607843	0,999991
	2	8	0,743754	0,920444	0,994610		0,844790	0,983125
	2	12	0,256238	0,999999	0,607843	0,844790		0,526527
	2	18	0,969791	0,666942	0,999991	0,983125	0,526527	
8 (15.05.19)	1	8		0,795068	0,003985	0,891442	0,995843	0,001409
	1	12	0,795068		0,278478	0,998298	0,964739	0,163363
	1	18	0,003985	0,278478		0,039940	0,024532	0,998997
	2	8	0,891442	0,998298	0,039940		0,996114	0,015142
	2	12	0,995843	0,964739	0,024532	0,996114		0,010051
	2	18	0,001409	0,163363	0,998997	0,015142	0,010051	

Table II - 105: *Test results of **one-way random effects nested ANOVAs** for lhr gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,000699	1	0,000699	8,68589	0,000005	135,0804	0,000001
	Parallel (time)	Random	0,000041	8	0,000005	43,00000	0,000007	0,7006	0,689047
	Time	Fixed	0,000125	7	0,000018	8,10793	0,000005	3,4852	0,049620
	Error		0,000314	43	0,000007				
12.5	Intercept	Fixed	0,001223	1	0,001223	8,94897	0,000003	458,8134	0,000000
	Parallel (time)	Random	0,000021	8	0,000003	34,00000	0,000006	0,4365	0,890618
	Time	Fixed	0,000102	7	0,000015	8,13957	0,000003	5,6081	0,012866
	Error		0,000201	34	0,000006				
18	Intercept	Fixed	0,001838	1	0,001838	10,02138	0,000006	291,3215	0,000000
	Parallel (time)	Random	0,000048	8	0,000006	52,00000	0,000011	0,5292	0,829109
	Time	Fixed	0,000285	7	0,000041	8,23977	0,000006	6,7384	0,007005
	Error		0,000589	52	0,000011				

Table II - 106: *Test results of **Tukey HSD post-hoc tests** of difference for lhr gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,999586	0,961901	0,990603	0,982545	0,997771	0,949068	0,070282
	2	0,999586		0,998866	0,999976	0,999929	0,931142	0,998614	0,173392
	3	0,961901	0,998866		0,999994	0,999995	0,678559	1,000000	0,550656
	4	0,990603	0,999976	0,999994		1,000000	0,799765	0,999996	0,323803
	5	0,982545	0,999929	0,999995	1,000000		0,716956	0,999997	0,238070
	6	0,997771	0,931142	0,678559	0,799765	0,716956		0,599730	0,011525
	7	0,949068	0,998614	1,000000	0,999996	0,999997	0,599730		0,389870
	8	0,070282	0,173392	0,550656	0,323803	0,238070	0,011525	0,389870	
12.5	1		0,999996	1,000000	0,421588	0,955432	0,085077	0,355799	0,610509
	2	0,999996		1,000000	0,332475	0,893813	0,065902	0,277926	0,503596
	3	1,000000	1,000000		0,442449	0,951740	0,102284	0,378324	0,618082
	4	0,421588	0,332475	0,442449		0,957030	0,989193	1,000000	1,000000
	5	0,955432	0,893813	0,951740	0,957030		0,524318	0,926796	0,988454
	6	0,085077	0,065902	0,102284	0,989193	0,524318		0,995864	0,983964
	7	0,355799	0,277926	0,378324	1,000000	0,926796	0,995864		0,999997
	8	0,610509	0,503596	0,618082	1,000000	0,988454	0,983964	0,999997	
18	1		0,999098	0,997567	0,499613	0,611849	0,918830	0,999990	0,793633
	2	0,999098		1,000000	0,875981	0,931421	0,998901	0,981093	0,385722
	3	0,997567	1,000000		0,915035	0,956866	0,999702	0,965844	0,325224
	4	0,499613	0,875981	0,915035		1,000000	0,987186	0,166684	0,003339
	5	0,611849	0,931421	0,956866	1,000000		0,996671	0,255841	0,007478
	6	0,918830	0,998901	0,999702	0,987186	0,996671		0,647720	0,042722
	7	0,999990	0,981093	0,965844	0,166684	0,255841	0,647720		0,845625
	8	0,793633	0,385722	0,325224	0,003339	0,007478	0,042722	0,845625	

d. Comparison of temperature groups between feeding regimes

Table II - 107: *Test results of **factorial ANOVAs** for lhr gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	0,000854	1	0,000854	161,1609	0,000000
	Feeding regime	0,000032	1	0,000032	5,9714	0,016469
	Time	0,000102	7	0,000015	2,7506	0,012271
	Feed*Time	0,000075	7	0,000011	2,0179	0,061155
	Error	0,000482	91	0,000005		
12.5	Intercept	0,001544	1	0,001544	242,2238	0,000000
	Feeding regime	0,000111	1	0,000111	17,3363	0,000073
	Time	0,000202	7	0,000029	4,5357	0,000234
	Feed*Time	0,000030	7	0,000004	0,6783	0,689905
	Error	0,000561	88	0,000006		
18	Intercept	0,003517	1	0,003517	240,7016	0,000000
	Feeding regime	0,000000	1	0,000000	0,0024	0,960952
	Time	0,000407	7	0,000058	3,9765	0,000639
	Feed*Time	0,000108	7	0,000015	1,0552	0,397184
	Error	0,001651	113	0,000015		

Table II - 108: *Test results of* ***Tukey HSD post-hoc tests*** *for lhr gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		1,00	1,00	0,89	1,00	0,97	0,95	1,00	1,00	1,00	1,00	1,00	1,00	0,92	1,00	0,81
		2	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,15
		3	1,00	1,00		1,00	0,99	1,00	1,00	1,00	1,00	1,00	0,99	1,00	1,00	1,00	0,99	0,03
		4	0,89	1,00	1,00		0,67	1,00	1,00	0,97	1,00	0,98	0,74	0,88	0,77	1,00	0,62	0,00
		5	1,00	1,00	0,99	0,67		0,92	0,88	1,00	1,00	1,00	1,00	1,00	1,00	0,76	1,00	0,41
		6	0,97	1,00	1,00	1,00	0,92		1,00	1,00	1,00	1,00	0,93	0,98	0,96	1,00	0,91	0,04
		7	0,95	1,00	1,00	1,00	0,88	1,00		0,99	1,00	0,99	0,90	0,96	0,93	1,00	0,86	0,03
		8	1,00	1,00	1,00	0,97	1,00	1,00	0,99		1,00	1,00	1,00	1,00	1,00	0,99	1,00	0,06
	100	1	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	0,99	0,04
		2	1,00	1,00	1,00	0,98	1,00	1,00	0,99	1,00	1,00		1,00	1,00	1,00	0,99	1,00	0,16
		3	1,00	1,00	0,99	0,74	1,00	0,93	0,90	1,00	1,00	1,00		1,00	1,00	0,81	1,00	0,66
		4	1,00	1,00	1,00	0,88	1,00	0,98	0,96	1,00	1,00	1,00	1,00		1,00	0,92	1,00	0,36
		5	1,00	1,00	1,00	0,77	1,00	0,96	0,93	1,00	1,00	1,00	1,00	1,00		0,85	1,00	0,24
		6	0,92	1,00	1,00	1,00	0,76	1,00	1,00	0,99	1,00	0,99	0,81	0,92	0,85		0,72	0,00
		7	1,00	1,00	0,99	0,62	1,00	0,91	0,86	1,00	0,99	1,00	1,00	1,00	1,00	0,72		0,45
		8	0,81	0,15	0,03	0,00	0,41	0,04	0,03	0,06	0,04	0,16	0,66	0,36	0,24	0,00	0,45	
12.5	67	1		1,00	1,00	0,92	1,00	1,00	0,58	0,36	1,00	1,00	1,00	0,11	0,64	0,01	0,08	0,25
		2	1,00		1,00	0,98	1,00	1,00	0,76	0,54	1,00	1,00	1,00	0,20	0,80	0,02	0,16	0,39
		3	1,00	1,00		0,99	1,00	1,00	0,92	0,81	1,00	1,00	1,00	0,50	0,92	0,18	0,45	0,64
		4	0,92	0,98	0,99		0,55	1,00	1,00	1,00	1,00	1,00	1,00	0,94	1,00	0,41	0,90	0,99
		5	1,00	1,00	1,00	0,55		0,90	0,14	0,06	0,92	0,99	0,96	0,01	0,22	0,00	0,01	0,05
		6	1,00	1,00	1,00	1,00	0,90		1,00	0,99	1,00	1,00	1,00	0,82	1,00	0,27	0,75	0,94
		7	0,58	0,76	0,92	1,00	0,14	1,00		1,00	1,00	1,00	1,00	1,00	1,00	0,68	0,99	1,00
		8	0,36	0,54	0,81	1,00	0,06	0,99	1,00		0,99	0,97	0,99	1,00	1,00	0,92	1,00	1,00
	100	1	1,00	1,00	1,00	1,00	0,92	1,00	1,00	0,99		1,00	1,00	0,79	1,00	0,24	0,72	0,93
		2	1,00	1,00	1,00	1,00	0,99	1,00	1,00	0,97	1,00		1,00	0,70	1,00	0,19	0,62	0,86
		3	1,00	1,00	1,00	1,00	0,96	1,00	1,00	0,99	1,00	1,00		0,81	1,00	0,29	0,75	0,93
		4	0,11	0,20	0,50	0,94	0,01	0,82	1,00	1,00	0,79	0,70	0,81		1,00	1,00	1,00	1,00
		5	0,64	0,80	0,92	1,00	0,22	1,00	1,00	1,00	1,00	1,00	1,00	1,00		0,88	1,00	1,00
		6	0,01	0,02	0,18	0,41	0,00	0,27	0,68	0,92	0,24	0,19	0,29	1,00	0,88		1,00	1,00
		7	0,08	0,16	0,45	0,90	0,01	0,75	0,99	1,00	0,72	0,62	0,75	1,00	1,00	1,00		1,00
		8	0,25	0,39	0,64	0,99	0,05	0,94	1,00	1,00	0,93	0,86	0,93	1,00	1,00	1,00	1,00	
18	67	1		1,00	0,96	0,24	0,91	0,92	0,64	1,00	1,00	0,99	0,99	0,38	0,49	0,82	1,00	1,00
		2	1,00		1,00	0,40	0,98	0,99	0,84	1,00	1,00	1,00	1,00	0,59	0,71	0,95	1,00	1,00
		3	0,96	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,69
		4	0,24	0,40	1,00		0,99	1,00	1,00	0,33	0,79	0,98	0,99	1,00	1,00	1,00	0,41	0,02
		5	0,91	0,98	1,00	0,99		1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,44
		6	0,92	0,99	1,00	1,00	1,00		1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,50
		7	0,64	0,84	1,00	1,00	1,00	1,00		0,81	0,99	1,00	1,00	1,00	1,00	1,00	0,88	0,12
		8	1,00	1,00	1,00	0,33	0,99	0,99	0,81		1,00	1,00	1,00	0,52	0,66	0,95	1,00	1,00
	100	1	1,00	1,00	1,00	0,79	1,00	1,00	0,99	1,00		1,00	1,00	0,93	0,97	1,00	1,00	0,99
		2	0,99	1,00	1,00	0,98	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	0,87
		3	0,99	1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	0,82
		4	0,38	0,59	1,00	1,00	1,00	1,00	1,00	0,52	0,93	1,00	1,00		1,00	1,00	0,61	0,04
		5	0,49	0,71	1,00	1,00	1,00	1,00	1,00	0,66	0,97	1,00	1,00	1,00		1,00	0,74	0,07
		6	0,82	0,95	1,00	1,00	1,00	1,00	1,00	0,95	1,00	1,00	1,00	1,00	1,00		0,97	0,27
		7	1,00	1,00	1,00	0,41	0,99	1,00	0,88	1,00	1,00	1,00	1,00	0,61	0,74	0,97		1,00
		8	1,00	1,00	0,69	0,02	0,44	0,50	0,12	1,00	0,99	0,87	0,82	0,04	0,07	0,27	1,00	

II – VII Relative *Anti-Müllerian hormone* (*amh*) mRNA transcription

a. Overall tests:

Table II - 109: *Test results of a **three-way random effects nested ANOVA** for amh gene transcription, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	1554,490	1	1554,490	95,3071	0,710093	2189,135	0,000000
Parallel (Temp.*feed*Time)	Random	64,373	84	0,766	253,0000	0,336051	2,280	0,000000
Time	Fixed	63,880	7	9,126	92,3870	0,722974	12,623	0,000000
Temperature	Fixed	251,777	2	125,888	94,2406	0,714684	176,146	0,000000
Feeding regime	Fixed	0,098	1	0,098	94,8857	0,711892	0,137	0,711623
Error		85,021	253	0,336				

Table II - 110: *Test results of a **three-way factorial ANOVA** for amh gene transcription, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	1620,403	1	1620,403	4756,798	0,000000
Time	68,696	7	9,814	28,809	0,000000
Temperature	269,514	2	134,757	395,588	0,000000
Feed regime	0,056	1	0,056	0,163	0,686428
Time*Temp.	25,277	14	1,805	5,300	0,000000
Time*Feed.	3,032	7	0,433	1,271	0,264153
Temp.*Feed.	4,398	2	2,199	6,456	0,001799
Time*Temp.*Feed.	13,613	14	0,972	2,854	0,000475
Error	102,195	300	0,341		

b. Restrictive feeding (67%)

Table II - 111: *Test results of a **two-way random effects nested ANOVA** for amh gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	744,9394	1	744,9394	42,5484	0,495430	1503,622	0,000000
Parallel (Temp.*Time)	Random	19,7015	37	0,5325	125,0000	0,254036	2,096	0,001327
Time	Fixed	33,3062	7	4,7580	40,4064	0,508309	9,360	0,000001
Temperature	Fixed	98,7691	2	49,3845	41,6304	0,500761	98,619	0,000000
Error		31,7545	125	0,2540				

Table II - 112: *Test results of a **two-way factorial ANOVA** for amh gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	768,3328	1	768,3328	3158,174	0,000000
Time	35,1113	7	5,0159	20,617	0,000000
Temperature	104,7772	2	52,3886	215,339	0,000000
Temp.*Time	15,4499	14	1,1036	4,536	0,000001
Error	36,0060	148	0,2433		

Table II - 113: Test results of **one-*way random effects nested ANOVAs*** *for amh gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	157,7438	1	157,7438	3,06967	0,263555	598,5233	0,000128
	Parallel (temp)	Random	0,7903	3	0,2634	11,00000	0,274161	0,9609	0,445331
	Temperature	Fixed	19,6534	2	9,8267	3,03208	0,263492	37,2940	0,007329
	Error		3,0158	11	0,2742				
2 (14.12.18)	Intercept	Fixed	118,1237	1	118,1237	3,546435	0,057702	2047,136	0,000005
	Parallel (temp)	Random	0,1634	3	0,0545	9,000000	0,173924	0,313	0,815573
	Temperature	Fixed	8,0495	2	4,0248	3,273470	0,056131	71,703	0,001982
	Error		1,5653	9	0,1739				
3 (01.02.19)	Intercept	Fixed	65,74329	1	65,74329	1,557123	0,061252	1073,330	0,003383
	Parallel (temp)	Random	0,13475	2	0,06737	9,000000	0,279870	0,241	0,790957
	Temperature	Fixed	2,53307	2	1,26653	1,421958	0,059161	21,408	0,086810
	Error		2,51883	9	0,27987				
4 (13.04.19)	Intercept	Fixed	119,2633	1	119,2633	6,05986	0,048561	2455,955	0,000000
	Parallel (temp)	Random	0,1064	3	0,0355	18,00000	0,284542	0,125	0,944282
	Temperature	Fixed	8,1317	2	4,0659	4,56558	0,042705	95,207	0,000190
	Error		5,1218	18	0,2845				
5 (27.03.19)	Intercept	Fixed	133,0313	1	133,0313	3,94064	0,098686	1348,028	0,000004
	Parallel (temp)	Random	0,2732	3	0,0911	20,00000	0,227391	0,400	0,754184
	Temperature	Fixed	18,5760	2	9,2880	3,37237	0,094286	98,509	0,001020
	Error		4,5478	20	0,2274				
6 (10.04.19)	Intercept	Fixed	59,94806	1	59,94806	3,99813	0,190783	314,2212	0,000060
	Parallel (temp)	Random	0,56773	3	0,18924	14,00000	0,201181	0,9407	0,447313
	Temperature	Fixed	29,72174	2	14,86087	3,49452	0,190078	78,1829	0,001256
	Error		2,81654	14	0,20118				
7 (24.04.19)	Intercept	Fixed	40,00765	1	40,00765	4,64912	0,209354	191,1003	0,000059
	Parallel (temp)	Random	0,66604	3	0,22201	17,00000	0,170699	1,3006	0,306561
	Temperature	Fixed	13,15542	2	6,57771	3,46189	0,217467	30,2469	0,006422
	Error		2,90188	17	0,17070				
8 (15.05.19)	Intercept	Fixed	108,8020	1	108,8020	3,03350	0,515120	211,2167	0,000666
	Parallel (temp)	Random	1,5497	3	0,5166	27,00000	0,343205	1,5051	0,235668
	Temperature	Fixed	27,0723	2	13,5362	3,01643	0,515851	26,2405	0,012372
	Error		9,2665	27	0,3432				

Table II - 114: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for amh gene transcription between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,000873	0,000202
	12	0,000873		0,013644
	18	0,000202	0,013644	
2 (14.12.18)	8		0,948143	0,000590
	12	0,948143		0,001172
	18	0,000590	0,001172	
3 (01.02.19)	8		0,946797	0,042518
	12	0,946797		0,133429
	18	0,042518	0,133429	
4 (13.03.19)	8		0,034298	0,000227
	12	0,034298		0,013146
	18	0,000227	0,013146	
5 (27.03.19)	8		0,000145	0,000145
	12	0,000145		0,446127
	18	0,000145	0,446127	
6 (10.04.19)	8		0,000206	0,000181
	12	0,000206		0,009481
	18	0,000181	0,009481	
7 (24.04.19)	8		0,012944	0,000161
	12	0,012944		0,000165
	18	0,000161	0,000165	
8 (15.05.19)	8		0,010628	0,000127
	12	0,010628		0,000142
	18	0,000127	0,000142	

Table II - 115: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for amh gene transcription between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,076483	0,000557	0,999980	0,005520	0,000756
	1	12	0,076483		0,048814	0,163937	0,577953	0,077554
	1	18	0,000557	0,048814		0,001521	0,523971	0,999643
	2	8	0,999980	0,163937	0,001521		0,015602	0,002172
	2	12	0,005520	0,577953	0,523971	0,015602		0,687852
	2	18	0,000756	0,077554	0,999643	0,002172	0,687852	
2 (14.12.18)	1	8		0,998630	0,006022	0,948019	0,967104	0,008706
	1	12	0,998630		0,020482	0,997327	0,999047	0,029256
	1	18	0,006022	0,020482		0,040083	0,035164	0,999653
	2	8	0,948019	0,997327	0,040083		0,999999	0,057674
	2	12	0,967104	0,999047	0,035164	0,999999		0,050579
	2	18	0,008706	0,029256	0,999653	0,057674	0,050579	
3 (01.02.19)	1	8		0,975138	0,162112	0,960227	0,217407	
	1	12	0,975138		0,349455	0,999890	0,448391	
	1	18	0,162112	0,349455		0,520769	0,999477	
	2	8	0,960227	0,999890	0,520769		0,625340	
	2	12	N=0	N=0	N=0	N=0	N=0	
	2	18	0,217407	0,448391	0,999477	0,625340		
4 (13.03.19)	1	8		0,462846	0,007072	0,999255	0,301754	0,008384
	1	12	0,462846		0,166542	0,523122	0,999535	0,240909
	1	18	0,007072	0,166542		0,020362	0,260298	0,998598
	2	8	0,999255	0,523122	0,020362		0,389620	0,027749
	2	12	0,301754	0,999535	0,260298	0,389620		0,373749
	2	18	0,008384	0,240909	0,998598	0,027749	0,373749	
5 (27.03.19)	1	8		0,000293	0,000230	0,996018	0,000405	0,000157
	1	12	0,000293		0,999783	0,000689	0,999805	0,824987
	1	18	0,000230	0,999783		0,000461	0,994028	0,930039
	2	8	0,996018	0,000689	0,000461		0,001078	0,000190
	2	12	0,000405	0,999805	0,994028	0,001078		0,680770
	2	18	0,000157	0,824987	0,930039	0,000190	0,680770	
6 (10.04.19)	1	8		0,007663	0,000175	0,670875	0,030097	0,000168
	1	12	0,007663		0,421290	0,000802	0,981159	0,301071
	1	18	0,000175	0,421290		0,000159	0,125416	0,999343
	2	8	0,670875	0,000802	0,000159		0,002666	0,000159
	2	12	0,030097	0,981159	0,125416	0,002666		0,081449
	2	18	0,000168	0,301071	0,999343	0,000159	0,081449	
7 (24.04.19)	1	8		0,743015	0,018950	0,995766	0,743019	0,002072
	1	12	0,743015		0,009776	0,098845	1,000000	0,000334
	1	18	0,018950	0,009776		0,000237	0,017101	0,428481
	2	8	0,995766	0,098845	0,000237		0,115024	0,000159
	2	12	0,743019	1,000000	0,017101	0,115024		0,000557
	2	18	0,002072	0,000334	0,428481	0,000159	0,000557	
8 (15.05.19)	1	8		0,133250	0,000131	0,881459	0,076315	0,000215
	1	12	0,133250		0,000790	0,535325	0,999969	0,049695
	1	18	0,000131	0,000790		0,000132	0,000723	0,473355
	2	8	0,881459	0,535325	0,000132		0,373736	0,000494
	2	12	0,076315	0,999969	0,000723	0,373736		0,054675
	2	18	0,000215	0,049695	0,473355	0,000494	0,054675	

Table II - 116: *Test results of **one-way random effects nested ANOVAs** for amh gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	432,1310	1	432,1310	11,30012	0,169520	2549,151	0,000000
	Parallel (Time)	Random	1,2464	8	0,1558	32,00000	0,310199	0,502	0,845380
	Time	Fixed	14,7836	7	2,1119	8,46695	0,158014	13,366	0,000579
	Error		9,9264	32	0,3102				
12.5	Intercept	Fixed	244,6008	1	244,6008	9,78208	0,132501	1846,025	0,000000
	Parallel (Time)	Random	0,9312	7	0,1330	42,00000	0,129719	1,026	0,427853
	Time	Fixed	14,1316	7	2,0188	7,68454	0,132875	15,193	0,000602
	Error		5,4482	42	0,1297				
18	Intercept	Fixed	99,85709	1	99,85709	9,54523	0,263564	378,8727	0,000000
	Parallel (Time)	Random	2,07389	8	0,25924	51,00000	0,321174	0,8072	0,599345
	Time	Fixed	20,85926	7	2,97989	8,20742	0,259874	11,4667	0,001171
	Error		16,37988	51	0,32117				

Table II - 117: *Test results of **Tukey HSD post-hoc tests** of difference for amh gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,056810	0,000224	0,002733	0,176778	0,040159	0,000294	0,000194
	2	0,056810		0,296503	0,978746	0,997052	1,000000	0,297318	0,498996
	3	0,000224	0,296503		0,747604	0,062817	0,251560	1,000000	0,995652
	4	0,002733	0,978746	0,747604		0,665279	0,973040	0,725886	0,953020
	5	0,176778	0,997052	0,062817	0,665279		0,995733	0,071250	0,102735
	6	0,040159	1,000000	0,251560	0,973040	0,995733		0,257549	0,430275
	7	0,000294	0,297318	1,000000	0,725886	0,071250	0,257549		0,990681
	8	0,000194	0,498996	0,995652	0,953020	0,102735	0,430275	0,990681	
12.5	1		0,507504	0,753990	0,188376	0,000150	0,000152	0,000143	0,000686
	2	0,507504		0,055828	0,001726	0,000135	0,000135	0,000135	0,000137
	3	0,753990	0,055828		0,999979	0,111490	0,029245	0,071054	0,504113
	4	0,188376	0,001726	0,999979		0,012979	0,005232	0,006615	0,236957
	5	0,000150	0,000135	0,111490	0,012979		0,918684	0,999971	0,883790
	6	0,000152	0,000135	0,029245	0,005232	0,918684		0,979026	0,351640
	7	0,000143	0,000135	0,071054	0,006615	0,999971	0,979026		0,725806
	8	0,000686	0,000137	0,504113	0,236957	0,883790	0,351640	0,725806	
18	1		1,000000	0,999632	0,986893	0,970733	0,002853	0,000580	0,002837
	2	1,000000		0,998647	0,974064	0,946766	0,001988	0,000421	0,001954
	3	0,999632	0,998647		0,999962	0,999819	0,014900	0,002945	0,015619
	4	0,986893	0,974064	0,999962		1,000000	0,028097	0,005438	0,029598
	5	0,970733	0,946766	0,999819	1,000000		0,013349	0,002030	0,013170
	6	0,002853	0,001988	0,014900	0,028097	0,013349		0,998232	1,000000
	7	0,000580	0,000421	0,002945	0,005438	0,002030	0,998232		0,991159
	8	0,002837	0,001954	0,015619	0,029598	0,013170	1,000000	0,991159	

c. Full feeding (100%)

Table II - 118: *Test results of a **two-way random effects nested ANOVA** for amh gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	810,6009	1	810,6009	42,2034	0,910840	889,9490	0,000000
Parallel (Temp.*Time)	Random	37,0011	38	0,9737	128,0000	0,416145	2,3398	0,000221
Temperature	Fixed	158,6824	2	79,3412	42,1377	0,911699	87,0257	0,000000
Time	Fixed	33,0486	7	4,7212	40,7449	0,930748	5,0725	0,000334
Error		53,2666	128	0,4161				

Table II - 119: *Test results of a **two-way factorial ANOVA** for amh gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	855,8146	1	855,8146	1965,341	0,000000
Time	36,1658	7	5,1665	11,865	0,000000
Temperature	171,1381	2	85,5691	196,506	0,000000
Time*Temp.	24,0788	14	1,7199	3,950	0,000009
Error	66,1889	152	0,4355		

Table II - 120: *Test results of **one-way random effects nested ANOVAs** for amh gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	136,5578	1	136,5578	3,19023	0,171628	795,6607	0,000062
	Parallel (Temp)	Random	0,5048	3	0,1683	10,00000	0,475042	0,3542	0,787245
	Temperature	Fixed	12,6835	2	6,3418	3,10142	0,170072	37,2887	0,006776
	Error		4,7504	10	0,4750				
2 (14.12.18)	Intercept	Fixed	137,3175	1	137,3175	3,10846	1,011746	135,7233	0,001148
	Parallel (Temp)	Random	3,0468	3	1,0156	12,00000	0,835627	1,2154	0,346379
	Temperature	Fixed	9,1660	2	4,5830	3,05141	1,013741	4,5209	0,122340
	Error		10,0275	12	0,8356				
3 (01.02.19)	Intercept	Fixed	101,6730	1	101,6730	3,18109	0,141141	720,3675	0,000074
	Parallel (Temp)	Random	0,4157	3	0,1386	10,00000	0,372613	0,3719	0,775140
	Temperature	Fixed	16,9546	2	8,4773	3,09657	0,139953	60,5722	0,003292
	Error		3,7261	10	0,3726				
4 (13.04.19)	Intercept	Fixed	104,6459	1	104,6459	3,21109	0,104398	1002,376	0,000041
	Parallel (Temp)	Random	0,3161	3	0,1054	18,00000	0,082247	1,281	0,310923
	Temperature	Fixed	7,8903	2	3,9451	3,10701	0,104869	37,620	0,006645
	Error		1,4804	18	0,0822				
5 (27.03.19)	Intercept	Fixed	152,3983	1	152,3983	3,08912	1,237119	123,1880	0,001370
	Parallel (Temp)	Random	3,8743	3	1,2914	20,00000	0,321448	4,0175	0,021741
	Temperature	Fixed	27,0916	2	13,5458	3,03763	1,267642	10,6858	0,042212
	Error		6,4290	20	0,3214				
6 (10.04.19)	Intercept	Fixed	48,64030	1	48,64030	16,10606	0,175202	277,6245	0,000000
	Parallel (Temp)	Random	0,21396	3	0,07132	16,00000	0,518300	0,1376	0,936089
	Temperature	Fixed	26,22338	2	13,11169	7,97792	0,110848	118,2852	0,000001
	Error		8,29281	16	0,51830				
7 (24.04.19)	Intercept	Fixed	92,54269	1	92,54269	3,00650	1,044390	88,60936	0,002518
	Parallel (Temp)	Random	3,13725	3	1,04575	23,00000	0,474308	2,20479	0,114811
	Temperature	Fixed	42,28991	2	21,14495	3,00319	1,045081	20,23283	0,018082
	Error		10,90909	23	0,47431				
8 (15.05.19)	Intercept	Fixed	69,46947	1	69,46947	3,28203	0,467660	148,5469	0,000761
	Parallel (Temp)	Random	1,41350	3	0,47117	19,00000	0,402695	1,1700	0,347262
	Temperature	Fixed	59,96120	2	29,98060	3,13012	0,469486	63,8583	0,002902
	Error		7,65120	19	0,40269				

Table II - 121: *Test results of **Tukey HSD post-hoc tests** of difference for amh gene transcription between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,043006	0,001133
	12	0,043006		0,099012
	18	0,001133	0,099012	
2 (14.12.18)	8		0,099886	0,012931
	12	0,099886		0,456903
	18	0,012931	0,456903	
3 (01.02.19)	8		0,000514	0,000527
	12	0,000514		0,969737
	18	0,000527	0,969737	
4 (13.03.19)	8		0,000174	0,000149
	12	0,000174		0,003268
	18	0,000149	0,003268	
5 (27.03.19)	8		0,000281	0,000145
	12	0,000281		0,007704
	18	0,000145	0,007704	
6 (10.04.19)	8		0,001011	0,000169
	12	0,001011		0,001915
	18	0,000169	0,001915	
7 (24.04.19)	8		0,073507	0,000133
	12	0,073507		0,000133
	18	0,000133	0,000133	
8 (15.05.19)	8		0,000145	0,000144
	12	0,000145		0,001379
	18	0,000144	0,001379	

Table II - 122: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for amh gene transcription between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,510608	0,012331	0,977853	0,147541	0,042056
	1	12	0,510608		0,336493	0,842973	0,981593	0,593644
	1	18	0,012331	0,336493		0,035085	0,585351	0,999059
	2	8	0,977853	0,842973	0,035085		0,379637	0,108161
	2	12	0,147541	0,981593	0,585351	0,379637		0,859745
	2	18	0,042056	0,593644	0,999059	0,108161	0,859745	
2 (14.12.18)	1	8		0,110473	0,100843	0,591314	0,484107	0,052114
	1	12	0,110473		0,998785	0,847515	0,915778	0,997719
	1	18	0,100843	0,998785		0,719864	0,803632	1,000000
	2	8	0,591314	0,847515	0,719864		0,999968	0,620967
	2	12	0,484107	0,915778	0,803632	0,999968		0,722136
	2	18	0,052114	0,997719	1,000000	0,620967	0,722136	
3 (01.02.19)	1	8		0,044175	0,036147	1,000000	0,011108	0,015236
	1	12	0,044175		0,995739	0,025462	0,896991	0,962876
	1	18	0,036147	0,995739		0,022907	0,997343	0,999899
	2	8	1,000000	0,025462	0,022907		0,005661	0,007942
	2	12	0,011108	0,896991	0,997343	0,005661		0,999856
	2	18	0,015236	0,962876	0,999899	0,007942	0,999856	
4 (13.03.19)	1	8		0,001076	0,000179	0,998184	0,008713	0,000160
	1	12	0,001076		0,894630	0,002408	0,586889	0,426427
	1	18	0,000179	0,894630		0,000222	0,058191	0,913124
	2	8	0,998184	0,002408	0,000222		0,022833	0,000166
	2	12	0,008713	0,586889	0,058191	0,022833		0,007585
	2	18	0,000160	0,426427	0,913124	0,000166	0,007585	
5 (27.03.19)	1	8		0,385423	0,000423	0,034225	0,288486	0,005017
	1	12	0,385423		0,305966	0,002701	0,999400	0,755314
	1	18	0,000423	0,305966		0,000146	0,038056	0,933326
	2	8	0,034225	0,002701	0,000146		0,000392	0,000149
	2	12	0,288486	0,999400	0,038056	0,000392		0,299957
	2	18	0,005017	0,755314	0,933326	0,000149	0,299957	
6 (10.04.19)	1	8		0,152363	0,008840	1,000000	0,269957	0,007162
	1	12	0,152363		0,153755	0,011578	0,988058	0,112841
	1	18	0,008840	0,153755		0,000329	0,053905	0,999977
	2	8	1,000000	0,011578	0,000329		0,032980	0,000285
	2	12	0,269957	0,988058	0,053905	0,032980		0,038372
	2	18	0,007162	0,112841	0,999977	0,000285	0,038372	
7 (24.04.19)	1	8		0,567152	0,000310	0,669843	0,999629	0,000387
	1	12	0,567152		0,005979	0,024049	0,328106	0,007447
	1	18	0,000310	0,005979		0,000141	0,000170	0,999993
	2	8	0,669843	0,024049	0,000141		0,803318	0,000142
	2	12	0,999629	0,328106	0,000170	0,803318		0,000192
	2	18	0,000387	0,007447	0,999993	0,000142	0,000192	
8 (15.05.19)	1	8		0,086544	0,000152	0,999703	0,000237	0,000152
	1	12	0,086544		0,012910	0,054679	0,459269	0,012864
	1	18	0,000152	0,012910		0,000151	0,147420	1,000000
	2	8	0,999703	0,054679	0,000151		0,000193	0,000151
	2	12	0,000237	0,459269	0,147420	0,000193		0,146832
	2	18	0,000152	0,012864	1,000000	0,000151	0,146832	

Table II - 123: *Test results of **one-way random effects nested ANOVAs** for amh gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	618,7316	1	618,7316	9,40545	0,846969	730,5247	0,000000
	Parallel (time)	Random	7,1468	8	0,8933	40,00000	0,525996	1,6984	0,128840
	Time	Fixed	9,3770	7	1,3396	8,14626	0,887749	1,5090	0,285774
	Error		21,0398	40	0,5260				
12.5	Intercept	Fixed	244,9103	1	244,9103	8,95975	0,659074	371,5975	0,000000
	Parallel (time)	Random	5,3127	8	0,6641	43,00000	0,583863	1,1374	0,358571
	Time	Fixed	11,3967	7	1,6281	8,14906	0,663251	2,4547	0,114061
	Error		25,1061	43	0,5839				
18	Intercept	Fixed	67,58969	1	67,58969	13,05354	0,067385	1003,038	0,000000
	Parallel (time)	Random	0,46289	8	0,05786	45,00000	0,158237	0,366	0,933171
	Time	Fixed	39,05793	7	5,57970	8,54514	0,059080	94,443	0,000000
	Error		7,12065	45	0,15824				

Table II - 124: *Test results of **Tukey HSD post-hoc tests** of difference for amh gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,998888	1,000000	0,162359	0,998081	0,924865	0,139260	0,974271
	2	0,998888		0,999362	0,383772	1,000000	0,995826	0,366706	0,999922
	3	1,000000	0,999362		0,213370	0,998922	0,941763	0,197550	0,982915
	4	0,162359	0,383772	0,213370		0,296184	0,933261	0,999997	0,578792
	5	0,998081	1,000000	0,998922	0,296184		0,994308	0,255396	0,999874
	6	0,924865	0,995826	0,941763	0,933261	0,994308		0,957987	0,999906
	7	0,139260	0,366706	0,197550	0,999997	0,255396	0,957987		0,580209
	8	0,974271	0,999922	0,982915	0,578792	0,999874	0,999906	0,580209	
12.5	1		0,999823	0,338664	0,512336	0,958825	0,052983	0,880947	0,024296
	2	0,999823		0,557543	0,759448	0,998013	0,114329	0,985486	0,052634
	3	0,338664	0,557543		0,999806	0,882720	0,996497	0,916506	0,930156
	4	0,512336	0,759448	0,999806		0,978244	0,900658	0,990412	0,653588
	5	0,958825	0,998013	0,882720	0,978244		0,350874	0,999999	0,175876
	6	0,052983	0,114329	0,996497	0,900658	0,350874		0,349912	0,998643
	7	0,880947	0,985486	0,916506	0,990412	0,999999	0,349912		0,170135
	8	0,024296	0,052634	0,930156	0,653588	0,175876	0,998643	0,170135	
18	1		0,999711	0,999697	0,682634	0,499057	0,000131	0,000131	0,000131
	2	0,999711		0,976907	0,335713	0,206688	0,000131	0,000131	0,000131
	3	0,999697	0,976907		0,942330	0,833208	0,000131	0,000131	0,000131
	4	0,682634	0,335713	0,942330		0,999939	0,000131	0,000131	0,000131
	5	0,499057	0,206688	0,833208	0,999939		0,000131	0,000131	0,000131
	6	0,000131	0,000131	0,000131	0,000131	0,000131		0,999999	0,998916
	7	0,000131	0,000131	0,000131	0,000131	0,000131	0,999999		0,999931
	8	0,000131	0,000131	0,000131	0,000131	0,000131	0,998916	0,999931	

d. Comparison of temperature groups between feeding regimes

Table II - 125: *Test results of **factorial ANOVAs** for amh gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	1136,684	1	1136,684	2541,404	0,000000
	Feeding regime	3,239	1	3,239	7,242	0,008522
	Time	17,676	7	2,525	5,646	0,000021
	Feed*Time	7,386	7	1,055	2,359	0,029504
	Error	39,359	88	0,447		
12.5	Intercept	489,5412	1	489,5412	1330,339	0,000000
	Feeding regime	0,4044	1	0,4044	1,099	0,297025
	Time	21,3147	7	3,0450	8,275	0,000000
	Feed*Time	7,3808	7	1,0544	2,865	0,009088
	Error	36,7982	100	0,3680		
18	Intercept	166,2591	1	166,2591	715,1670	0,000000
	Feeding regime	0,7642	1	0,7642	3,2872	0,072501
	Time	58,8482	7	8,4069	36,1624	0,000000
	Feed*Time	2,3179	7	0,3311	1,4244	0,202482
	Error	26,0373	112	0,2325		

Table II - 126: *Test results of* ***Tukey HSD post-hoc tests*** *for amh gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,38	0,00	0,03	0,69	0,30	0,00	0,00	1,00	0,91	1,00	0,02	0,86	0,63	0,01	0,67
		2	0,38		0,84	1,00	1,00	1,00	0,84	0,95	0,96	1,00	0,98	1,00	1,00	1,00	1,00	1,00
		3	0,00	0,84		0,99	0,40	0,79	1,00	1,00	0,03	0,12	0,05	1,00	0,07	0,79	1,00	0,24
		4	0,03	1,00	0,99		0,99	1,00	0,99	1,00	0,42	0,80	0,52	1,00	0,70	1,00	1,00	0,95
		5	0,69	1,00	0,40	0,99		1,00	0,43	0,53	1,00	1,00	1,00	0,91	1,00	1,00	0,93	1,00
		6	0,30	1,00	0,79	1,00	1,00		0,80	0,92	0,94	1,00	0,96	1,00	1,00	1,00	1,00	1,00
		7	0,00	0,84	1,00	0,99	0,43	0,80		1,00	0,05	0,15	0,07	1,00	0,10	0,79	1,00	0,28
		8	0,00	0,95	1,00	1,00	0,53	0,92	1,00		0,03	0,15	0,06	1,00	0,07	0,92	1,00	0,30
	100	1	1,00	0,96	0,03	0,42	1,00	0,94	0,05	0,03		1,00	1,00	0,24	1,00	0,99	0,20	1,00
		2	0,91	1,00	0,12	0,80	1,00	1,00	0,15	0,15	1,00		1,00	0,58	1,00	1,00	0,55	1,00
		3	1,00	0,98	0,05	0,52	1,00	0,96	0,07	0,06	1,00	1,00		0,32	1,00	1,00	0,30	1,00
		4	0,02	1,00	1,00	1,00	0,91	1,00	1,00	1,00	0,24	0,58	0,32		0,45	1,00	1,00	0,80
		5	0,86	1,00	0,07	0,70	1,00	1,00	0,10	0,07	1,00	1,00	1,00	0,45		1,00	0,39	1,00
		6	0,63	1,00	0,79	1,00	1,00	1,00	0,79	0,92	0,99	1,00	1,00	1,00	1,00		1,00	1,00
		7	0,01	1,00	1,00	1,00	0,93	1,00	1,00	1,00	0,20	0,55	0,30	1,00	0,39	1,00		0,80
		8	0,67	1,00	0,24	0,95	1,00	1,00	0,28	0,30	1,00	1,00	1,00	0,80	1,00	1,00	0,80	
12.5	67	1		1,00	1,00	0,98	0,06	0,06	0,05	0,27	1,00	1,00	0,22	0,44	0,99	0,01	0,94	0,00
		2	1,00		0,88	0,39	0,00	0,01	0,00	0,02	1,00	0,95	0,02	0,05	0,54	0,00	0,30	0,00
		3	1,00	0,88		1,00	0,95	0,81	0,91	1,00	1,00	1,00	0,99	1,00	1,00	0,66	1,00	0,35
		4	0,98	0,39	1,00		0,69	0,56	0,59	0,98	0,98	1,00	0,93	1,00	1,00	0,18	1,00	0,05
		5	0,06	0,00	0,95	0,69		1,00	1,00	1,00	0,09	0,26	1,00	1,00	0,78	1,00	0,82	0,98
		6	0,06	0,01	0,81	0,56	1,00		1,00	0,99	0,08	0,21	1,00	0,99	0,61	1,00	0,67	1,00
		7	0,05	0,00	0,91	0,59	1,00	1,00		1,00	0,07	0,20	1,00	1,00	0,69	1,00	0,73	1,00
		8	0,27	0,02	1,00	0,98	1,00	0,99	1,00		0,33	0,68	1,00	1,00	0,99	0,96	1,00	0,66
	100	1	1,00	1,00	1,00	0,98	0,09	0,08	0,07	0,33		1,00	0,26	0,49	0,99	0,01	0,95	0,00
		2	1,00	0,95	1,00	1,00	0,26	0,21	0,20	0,68	1,00		0,56	0,82	1,00	0,04	1,00	0,01
		3	0,22	0,02	0,99	0,93	1,00	1,00	1,00	1,00	0,26	0,56		1,00	0,95	1,00	0,97	0,98
		4	0,44	0,05	1,00	1,00	1,00	0,99	1,00	1,00	0,49	0,82	1,00		1,00	0,96	1,00	0,69
		5	0,99	0,54	1,00	1,00	0,78	0,61	0,69	0,99	0,99	1,00	0,95	1,00		0,28	1,00	0,09
		6	0,01	0,00	0,66	0,18	1,00	1,00	1,00	0,96	0,01	0,04	1,00	0,96	0,28		0,28	1,00
		7	0,94	0,30	1,00	1,00	0,82	0,67	0,73	1,00	0,95	1,00	0,97	1,00	1,00	0,28		0,08
		8	0,00	0,00	0,35	0,05	0,98	1,00	1,00	0,66	0,00	0,01	0,98	0,69	0,09	1,00	0,08	
18	67	1		1,00	1,00	1,00	1,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	0,98	0,00	0,00	0,00
		2	1,00		1,00	1,00	0,99	0,00	0,00	0,00	1,00	1,00	1,00	0,99	0,95	0,00	0,00	0,00
		3	1,00	1,00		1,00	1,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00
		4	1,00	1,00	1,00		1,00	0,01	0,00	0,01	1,00	0,96	1,00	1,00	1,00	0,00	0,00	0,00
		5	1,00	0,99	1,00	1,00		0,00	0,00	0,00	0,99	0,92	1,00	1,00	1,00	0,00	0,00	0,00
		6	0,00	0,00	0,00	0,01	0,00		1,00	1,00	0,00	0,00	0,00	0,01	0,02	0,77	0,61	0,33
		7	0,00	0,00	0,00	0,00	0,00	1,00		1,00	0,00	0,00	0,00	0,00	0,00	0,99	0,97	0,84
		8	0,00	0,00	0,00	0,01	0,00	1,00	1,00		0,00	0,00	0,00	0,01	0,02	0,60	0,41	0,18
	100	1	1,00	1,00	1,00	1,00	0,99	0,00	0,00	0,00		1,00	1,00	0,99	0,96	0,00	0,00	0,00
		2	1,00	1,00	1,00	0,96	0,92	0,00	0,00	0,00	1,00		1,00	0,88	0,77	0,00	0,00	0,00
		3	1,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00	1,00	1,00		1,00	1,00	0,00	0,00	0,00
		4	1,00	0,99	1,00	1,00	1,00	0,01	0,00	0,01	0,99	0,88	1,00		1,00	0,00	0,00	0,00
		5	0,98	0,95	1,00	1,00	1,00	0,02	0,00	0,02	0,96	0,77	1,00	1,00		0,00	0,00	0,00
		6	0,00	0,00	0,00	0,00	0,00	0,77	0,99	0,60	0,00	0,00	0,00	0,00	0,00		1,00	1,00
		7	0,00	0,00	0,00	0,00	0,00	0,61	0,97	0,41	0,00	0,00	0,00	0,00	0,00	1,00		1,00
		8	0,00	0,00	0,00	0,00	0,00	0,33	0,84	0,18	0,00	0,00	0,00	0,00	0,00	1,00	1,00	

II – VIII Relative *gonadal soma-derived factor 1* (*gsdf1*) mRNA transcription

a. Overall tests:

Table II - 127: *Test results of a **three-way random effects nested ANOVA** for gsdf1 gene transcription, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	6485,853	1	6485,853	93,3421	5,713940	1135,093	0,000000
Parallel (Temp.*feed*Time)	Random	511,283	84	6,087	260,0000	2,694466	2,259	0,000000
Time	Fixed	541,391	7	77,342	90,7221	5,808785	13,315	0,000000
Temperature	Fixed	160,088	2	80,044	92,1731	5,755434	13,908	0,000005
Feeding regime	Fixed	0,328	1	0,328	92,7753	5,733897	0,057	0,811584
Error		700,561	260	2,694				

Table II - 128: *Test results of a **three-way factorial ANOVA** for gsdf1 gene transcription, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	6659,048	1	6659,048	2347,694	0,000000
Time	571,171	7	81,596	28,767	0,000000
Temperature	165,983	2	82,991	29,259	0,000000
Feed regime	0,861	1	0,861	0,304	0,582037
Time*Temp.	158,035	14	11,288	3,980	0,000003
Time*Feed.	76,293	7	10,899	3,843	0,000505
Temp.*Feed.	4,194	2	2,097	0,739	0,478282
Time*Temp.*Feed.	86,944	14	6,210	2,189	0,008179
Error	870,781	307	2,836		

b. Restrictive feeding (67%):

Table II - 129: *Test results of a **two-way random effects nested ANOVA** for gsdf1 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	3105,165	1	3105,165	41,5087	5,913350	525,1109	0,000000
Parallel (Temp.*Time)	Random	235,118	37	6,355	125,0000	2,734819	2,3236	0,000290
Time	Fixed	367,761	7	52,537	39,8904	6,056930	8,6739	0,000002
Temperature	Fixed	51,278	2	25,639	40,4564	6,005052	4,2696	0,020779
Error		341,852	125	2,735				

Table II - 130: *Test results of a **two-way factorial ANOVA** for gsdf1 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	3226,043	1	3226,043	994,3780	0,000000
Time	368,459	7	52,637	16,2245	0,000000
Temperature	56,832	2	28,416	8,7587	0,000254
Temp.*Time	96,816	14	6,915	2,1316	0,013135
Error	480,154	148	3,244		

Table II - 131: *Test results of **one-way random effects nested ANOVAs** for gsdf1 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	775,8682	1	775,8682	3,02287	12,41405	62,49919	0,004105
	Parallel (temp)	Random	37,5131	3	12,5044	11,00000	4,28437	2,91861	0,081771
	Temperature	Fixed	7,0363	2	3,5181	3,01055	12,46244	0,28230	0,772029
	Error		47,1281	11	4,2844				
2 (14.12.18)	Intercept	Fixed	838,0171	1	838,0171	3	27,62931	30,33073	0,011786
	Parallel (temp)	Random	82,8879	3	27,6293	12	11,55366	2,39139	0,119591
	Temperature	Fixed	66,7878	2	33,3939	3	27,62931	1,20864	0,412107
	Error		138,6440	12	11,5537				
3 (01.02.19)	Intercept	Fixed	345,1071	1	345,1071	0,231649	0,025355	13611,15	0,243277
	Parallel (temp)	Random	0,1282	2	0,0641	7,000000	0,452645	0,14	0,870391
	Temperature	Fixed	3,3653	2	1,6826	0,050491	0,012608	133,45	0,805388
	Error		3,1685	7	0,4526				
4 (13.04.19)	Intercept	Fixed	467,5293	1	467,5293	4,12609	0,297177	1573,236	0,000002
	Parallel (temp)	Random	0,7976	3	0,2659	16,00000	0,897137	0,296	0,827496
	Temperature	Fixed	7,4089	2	3,7044	3,62281	0,284014	13,043	0,022113
	Error		14,3542	16	0,8971				
5 (27.03.19)	Intercept	Fixed	327,6314	1	327,6314	3,30773	0,231291	1416,534	0,000018
	Parallel (temp)	Random	0,6708	3	0,2236	20,00000	0,735201	0,304	0,822062
	Temperature	Fixed	13,9872	2	6,9936	3,16239	0,227723	30,711	0,008481
	Error		14,7040	20	0,7352				
6 (10.04.19)	Intercept	Fixed	198,8554	1	198,8554	8,70288	0,443642	448,2335	0,000000
	Parallel (temp)	Random	0,7818	3	0,2606	16,00000	3,532610	0,0738	0,973206
	Temperature	Fixed	20,9455	2	10,4727	6,17636	0,368758	28,4000	0,000769
	Error		56,5218	16	3,5326				
7 (24.04.19)	Intercept	Fixed	118,8679	1	118,8679	4,01948	2,755470	43,13889	0,002733
	Parallel (temp)	Random	9,7130	3	3,2377	18,00000	1,426991	2,26887	0,115278
	Temperature	Fixed	21,2786	2	10,6393	3,27493	3,069941	3,46564	0,155470
	Error		25,6858	18	1,4270				
8 (15.05.19)	Intercept	Fixed	308,4854	1	308,4854	3,08464	1,932011	159,6706	0,000931
	Parallel (temp)	Random	5,8090	3	1,9363	25,00000	1,665843	1,1624	0,343772
	Temperature	Fixed	26,6234	2	13,3117	3,04480	1,934029	6,8829	0,074190
	Error		41,6461	25	1,6658				

Table II - 132: *Test results of **Tukey HSD post-hoc tests** of difference for gsdf1 gene transcription between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,465438	0,760182
	12	0,465438		0,857486
	18	0,760182	0,857486	
2 (14.12.18)	8		0,148550	0,124350
	12	0,148550		0,993488
	18	0,124350	0,993488	
3 (01.02.19)	8		0,742142	0,074293
	12	0,742142		0,301519
	18	0,074293	0,301519	
4 (13.03.19)	8		0,319832	0,289940
	12	0,319832		0,018767
	18	0,289940	0,018767	
5 (27.03.19)	8		0,001424	0,004320
	12	0,001424		0,948508
	18	0,004320	0,948508	
6 (10.04.19)	8		0,986423	0,120825
	12	0,986423		0,163648
	18	0,120825	0,163648	
7 (24.04.19)	8		0,971851	0,019058
	12	0,971851		0,004545
	18	0,019058	0,004545	
8 (15.05.19)	8		0,397384	0,040325
	12	0,397384		0,001224
	18	0,040325	0,001224	

Table II - 133: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for gsdf1 gene transcription between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,971529	0,854480	0,996255	0,984724	0,514936
	1	12	0,971529		0,456975	0,855210	0,999998	0,897089
	1	18	0,854480	0,456975		0,992655	0,510675	0,108838
	2	8	0,996255	0,855210	0,992655		0,891013	0,369449
	2	12	0,984724	0,999998	0,510675	0,891013		0,857798
	2	18	0,514936	0,897089	0,108838	0,369449	0,857798	
2 (14.12.18)	1	8		0,999852	0,999956	0,155000	1,000000	0,999979
	1	12	0,999852		1,000000	0,105358	0,999674	0,999999
	1	18	0,999956	1,000000		0,114705	0,999880	1,000000
	2	8	0,155000	0,105358	0,114705		0,165575	0,119550
	2	12	1,000000	0,999674	0,999880	0,165575		0,999933
	2	18	0,999979	0,999999	1,000000	0,119550	0,999933	
3 (01.02.19)	1	8		0,990785	0,489041	0,981596	0,449306	
	1	12	0,990785		0,694689	0,881557	0,650826	
	1	18	0,489041	0,694689		0,340719	0,999991	
	2	8	0,981596	0,881557	0,340719		0,312417	
	2	1	N=0	N=0	N=0	N=0	N=0	
	2	18	0,449306	0,650826	0,999991	0,312417		
4 (13.03.19)	1	8		0,990919	0,925551	1,000000	0,609061	0,734695
	1	12	0,990919		0,741479	0,994660	0,933375	0,455110
	1	18	0,925551	0,741479		0,973643	0,305096	0,999987
	2	8	1,000000	0,994660	0,973643		0,790865	0,909450
	2	12	0,609061	0,933375	0,305096	0,790865		0,091657
	2	18	0,734695	0,455110	0,999987	0,909450	0,091657	
5 (27.03.19)	1	8		0,080478	0,353034	0,999861	0,278828	0,184905
	1	12	0,080478		0,957419	0,017569	0,962371	0,999016
	1	18	0,353034	0,957419		0,143728	1,000000	0,997586
	2	8	0,999861	0,017569	0,143728		0,093095	0,058790
	2	12	0,278828	0,962371	1,000000	0,093095		0,998588
	2	18	0,184905	0,999016	0,997586	0,058790	0,998588	
6 (10.04.19)	1	8		0,999811	0,536592	0,999998	1,000000	0,746571
	1	12	0,999811		0,708045	0,999984	0,999954	0,885062
	1	18	0,536592	0,708045		0,604082	0,579536	0,998137
	2	8	0,999998	0,999984	0,604082		1,000000	0,806410
	2	12	1,000000	0,999954	0,579536	1,000000		0,785443
	2	18	0,746571	0,885062	0,998137	0,806410	0,785443	
7 (24.04.19)	1	8		0,994762	0,999969	0,922931	0,970550	0,633351
	1	12	0,994762		0,825497	0,972607	0,998032	0,021422
	1	18	0,999969	0,825497		0,490444	0,588135	0,212525
	2	8	0,922931	0,972607	0,490444		0,998764	0,012313
	2	12	0,970550	0,998032	0,588135	0,998764		0,008912
	2	18	0,633351	0,021422	0,212525	0,012313	0,008912	
8 (15.05.19)	1	8		0,994762257	0,999969041	0,922931091	0,970550333	0,633351245
	1	12	0,994762257		0,825496927	0,972607448	0,998032014	0,021422388
	1	18	0,999969041	0,825496927		0,490443978	0,58813534	0,212524812
	2	8	0,922931091	0,972607448	0,490443978		0,998764019	0,012312624
	2	12	0,970550333	0,998032014	0,58813534	0,998764019		0,008912157
	2	18	0,633351245	0,021422388	0,212524812	0,012312624	0,008912157	

Table II - 134: *Test results of **one-way random effects nested ANOVAs** for gsdf1 gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	1191,471	1	1191,471	8,68450	10,13366	117,5756	0,000002
	Parallel (Time)	Random	85,389	8	10,674	34,00000	4,60417	2,3182	0,041947
	Time	Fixed	194,930	7	27,847	8,08193	10,60253	2,6265	0,098854
	Error		156,542	34	4,604				
12.5	Intercept	Fixed	1139,860	1	1139,860	9,95455	0,842255	1353,342	0,000000
	Parallel (Time)	Random	5,406	7	0,772	45,00000	1,566315	0,493	0,834586
	Time	Fixed	76,168	7	10,881	8,28116	0,805397	13,510	0,000622
	Error		70,484	45	1,566				
18	Intercept	Fixed	798,8136	1	798,8136	8,89386	5,542266	144,1312	0,000001
	Parallel (Time)	Random	47,5069	8	5,9384	46,00000	2,496225	2,3789	0,030978
	Time	Fixed	211,2133	7	30,1733	8,11298	5,881654	5,1301	0,016939
	Error		114,8263	46	2,4962				

Table II - 135: *Test results of **Tukey HSD post-hoc tests** of difference for gsdf1 gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,808981	0,499072	0,453413	0,285425	0,102096	0,119565	0,027891
	2	0,808981		0,021575	0,012106	0,004428	0,001380	0,002827	0,000259
	3	0,499072	0,021575		1,000000	1,000000	0,990428	0,977994	0,924791
	4	0,453413	0,012106	1,000000		0,999993	0,973080	0,952247	0,834967
	5	0,285425	0,004428	1,000000	0,999993		0,994365	0,984612	0,927825
	6	0,102096	0,001380	0,990428	0,973080	0,994365		0,999999	0,999977
	7	0,119565	0,002827	0,977994	0,952247	0,984612	0,999999		1,000000
	8	0,027891	0,000259	0,924791	0,834967	0,927825	0,999977	1,000000	
12.5	1		0,996400	0,983311	0,999393	0,000636	0,032347	0,003039	0,110052
	2	0,996400		0,999975	0,999998	0,006013	0,167767	0,028130	0,460607
	3	0,983311	0,999975		0,999373	0,135368	0,608680	0,311067	0,912291
	4	0,999393	0,999998	0,999373		0,000686	0,056204	0,004054	0,191802
	5	0,000636	0,006013	0,135368	0,000686		0,971288	0,998055	0,368745
	6	0,032347	0,167767	0,608680	0,056204	0,971288		0,999819	0,984272
	7	0,003039	0,028130	0,311067	0,004054	0,998055	0,999819		0,769945
	8	0,110052	0,460607	0,912291	0,191802	0,368745	0,984272	0,769945	
18	1		0,807144	0,998732	0,127769	0,002955	0,000134	0,000130	0,000135
	2	0,807144		0,995505	0,902141	0,182396	0,001784	0,000468	0,002413
	3	0,998732	0,995505		0,573085	0,065294	0,000886	0,000315	0,001175
	4	0,127769	0,902141	0,573085		0,917682	0,087902	0,026231	0,123108
	5	0,002955	0,182396	0,065294	0,917682		0,625761	0,302173	0,750131
	6	0,000134	0,001784	0,000886	0,087902	0,625761		0,999197	0,999991
	7	0,000130	0,000468	0,000315	0,026231	0,302173	0,999197		0,986911
	8	0,000135	0,002413	0,001175	0,123108	0,750131	0,999991	0,986911	

c. Full feeding (100%)

Table II - 136: *Test results of a **two-way random effects nested ANOVA** for gsdf1 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	3390,794	1	3390,794	41,6778	4,710978	719,7643	0,000000
Parallel (Temp.*Time)	Random	185,857	38	4,891	135,0000	2,657102	1,8407	0,005935
Temperature	Fixed	118,896	2	59,448	41,5612	4,716162	12,6052	0,000053
Time	Fixed	238,481	7	34,069	40,2503	4,776626	7,1324	0,000015
Error		358,709	135	2,657				

Table II - 137: *Test results of a **two-way factorial ANOVA** for gsdf1 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	3446,120	1	3446,120	1402,701	0,000000
Time	271,170	7	38,739	15,768	0,000000
Temperature	117,156	2	58,578	23,843	0,000000
Time*Temp.	153,938	14	10,996	4,476	0,000001
Error	390,627	159	2,457		

Table II - 138: *Test results of* ***one-way random effects nested ANOVAs*** *for gsdf1 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	506,3210	1	506,3210	4,92359	0,216114	2342,845	0,000000
	Parallel (Temp)	Random	0,5057	3	0,1686	11,00000	4,495715	0,037	0,989747
	Temperature	Fixed	6,5250	2	3,2625	3,85709	0,190640	17,113	0,012081
	Error		49,4529	11	4,4957				
2 (14.12.18)	Intercept	Fixed	510,2294	1	510,2294	3,28760	0,857607	594,9453	0,000079
	Parallel (Temp)	Random	2,5107	3	0,8369	12,00000	1,807092	0,4631	0,713271
	Temperature	Fixed	4,4224	2	2,2112	3,13561	0,846853	2,6111	0,215019
	Error		21,6851	12	1,8071				
3 (01.02.19)	Intercept	Fixed	518,7631	1	518,7631	5,24905	0,326729	1587,748	0,000000
	Parallel (Temp)	Random	0,7375	3	0,2458	11,00000	7,608785	0,032	0,991751
	Temperature	Fixed	21,2048	2	10,6024	4,00050	0,283383	37,414	0,002574
	Error		83,6966	11	7,6088				
4 (13.04.19)	Intercept	Fixed	730,2129	1	730,2129	3,12337	1,499030	487,1237	0,000156
	Parallel (Temp)	Random	4,5225	3	1,5075	19,00000	1,174961	1,2830	0,308797
	Temperature	Fixed	7,7173	2	3,8586	3,06642	1,502871	2,5675	0,221270
	Error		22,3243	19	1,1750				
5 (27.03.19)	Intercept	Fixed	671,3784	1	671,3784	3,23601	1,060163	633,2783	0,000080
	Parallel (Temp)	Random	3,0960	3	1,0320	23,00000	3,593670	0,2872	0,834157
	Temperature	Fixed	17,9880	2	8,9940	3,10797	1,045083	8,6060	0,054051
	Error		82,6544	23	3,5937				
6 (10.04.19)	Intercept	Fixed	198,3506	1	198,3506	19,71178	0,324694	610,8849	0,000000
	Parallel (Temp)	Random	0,2776	3	0,0925	19,00000	2,179210	0,0425	0,987978
	Temperature	Fixed	30,5656	2	15,2828	10,98254	0,184276	82,9344	0,000000
	Error		41,4050	19	2,1792				
7 (24.04.19)	Intercept	Fixed	352,2313	1	352,2313	3,01328	3,468822	101,5421	0,002042
	Parallel (Temp)	Random	10,4416	3	3,4805	21,00000	1,375477	2,5304	0,084768
	Temperature	Fixed	153,9300	2	76,9650	3,00654	3,474754	22,1498	0,015879
	Error		28,8850	21	1,3755				
8 (15.05.19)	Intercept	Fixed	135,5752	1	135,5752	3,21317	3,150306	43,03556	0,005808
	Parallel (Temp)	Random	9,8269	3	3,2756	19,00000	1,505552	2,17569	0,124342
	Temperature	Fixed	74,4736	2	37,2368	3,11749	3,203849	11,62252	0,035872
	Error		28,6055	19	1,5056				

Table II - 139: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for gsdf1 gene transcription between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,762025	0,837672
	12	0,762025		0,446860
	18	0,837672	0,446860	
2 (14.12.18)	8		0,387819	0,987344
	12	0,387819		0,342399
	18	0,987344	0,342399	
3 (01.02.19)	8		0,695120	0,245038
	12	0,695120		0,638528
	18	0,245038	0,638528	
4 (13.03.19)	8		0,056533	0,999452
	12	0,056533		0,038487
	18	0,999452	0,038487	
5 (27.03.19)	8		0,662797	0,092153
	12	0,662797		0,425350
	18	0,092153	0,425350	
6 (10.04.19)	8		0,266651	0,004676
	12	0,266651		0,053581
	18	0,004676	0,053581	
7 (24.04.19)	8		0,070786	0,000140
	12	0,070786		0,000141
	18	0,000140	0,000141	
8 (15.05.19)	8		0,735478	0,000164
	12	0,735478		0,000297
	18	0,000164	0,000297	

Table II - 140: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for gsdf1 gene transcription between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,997963	0,999697	0,999916	0,970733	0,999507
	1	12	0,997963		0,982164	0,999904	0,999815	0,978877
	1	18	0,999697	0,982164		0,995256	0,897929	1,000000
	2	8	0,999916	0,999904	0,995256		0,993490	0,993795
	2	12	0,970733	0,999815	0,897929	0,993490		0,887000
	2	18	0,999507	0,978877	1,000000	0,993795	0,887000	
2 (14.12.18)	1	8		0,735161	1,000000	0,922728	0,735270	0,983635
	1	12	0,735161		0,754016	0,998377	0,999984	0,980886
	1	18	1,000000	0,754016		0,925002	0,747369	0,982289
	2	8	0,922728	0,998377	0,925002		0,994699	0,999702
	2	12	0,735270	0,999984	0,747369	0,994699		0,968768
	2	18	0,983635	0,980886	0,982289	0,999702	0,968768	
3 (01.02.19)	1	8		0,997682	0,773066	1,000000	0,978303	0,899395
	1	12	0,997682		0,942558	0,996375	0,999748	0,984765
	1	18	0,773066	0,942558		0,750854	0,987656	0,999987
	2	8	1,000000	0,996375	0,750854		0,972105	0,885435
	2	12	0,978303	0,999748	0,987656	0,972105		0,998285
	2	18	0,899395	0,984765	0,999987	0,885435	0,998285	
4 (13.03.19)	1	8		0,996977	0,999913	0,995724	0,364580	0,968984
	1	12	0,996977		0,999674	0,915052	0,662903	0,786902
	1	18	0,999913	0,999674		0,962202	0,337292	0,854015
	2	8	0,995724	0,915052	0,962202		0,106291	0,999762
	2	12	0,364580	0,662903	0,337292	0,106291		0,043290
	2	18	0,968984	0,786902	0,854015	0,999762	0,043290	
5 (27.03.19)	1	8		0,999994	0,831859	1,000000	0,944813	0,435399
	1	12	0,999994		0,940643	0,999986	0,987878	0,667496
	1	18	0,831859	0,940643		0,818566	0,999100	0,982000
	2	8	1,000000	0,999986	0,818566		0,937314	0,419854
	2	12	0,944813	0,987878	0,999100	0,937314		0,885138
	2	18	0,435399	0,667496	0,982000	0,419854	0,885138	
6 (10.04.19)	1	8		0,953416	0,317188	0,999642	0,951054	0,253499
	1	12	0,953416		0,558593	0,754794	1,000000	0,444842
	1	18	0,317188	0,558593		0,099628	0,566881	0,999957
	2	8	0,999642	0,754794	0,099628		0,748215	0,072175
	2	12	0,951054	1,000000	0,566881	0,748215		0,452604
	2	18	0,253499	0,444842	0,999957	0,072175	0,452604	
7 (24.04.19)	1	8		0,096456	0,000148	0,119446	0,035218	0,000148
	1	12	0,096456		0,000475	0,998862	0,995893	0,000444
	1	18	0,000148	0,000475		0,000176	0,001290	0,999422
	2	8	0,119446	0,998862	0,000176		0,936729	0,000180
	2	12	0,035218	0,995893	0,001290	0,936729		0,001118
	2	18	0,000148	0,000444	0,999422	0,000180	0,001118	
8 (15.05.19)	1	8		0,691638	0,008513	0,994068	0,817065	0,005626
	1	12	0,691638		0,001869	0,931078	0,173929	0,001389
	1	18	0,008513	0,001869		0,005190	0,076622	1,000000
	2	8	0,994068	0,931078	0,005190		0,555329	0,003588
	2	12	0,817065	0,173929	0,076622	0,555329		0,054866
	2	18	0,005626	0,001389	1,000000	0,003588	0,054866	

Table II - 141: *Test results of **one-way random effects nested ANOVAs** for gsdf1 gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	1581,034	1	1581,034	9,85054	1,681440	940,2857	0,000000
	Parallel (time)	Random	12,754	8	1,594	42,00000	3,313810	0,4811	0,862598
	Time	Fixed	46,201	7	6,600	8,26787	1,607956	4,1046	0,031006
	Error		139,180	42	3,314				
12.5	Intercept	Fixed	1156,253	1	1156,253	9,76866	1,912871	604,4595	0,000000
	Parallel (time)	Random	14,711	8	1,839	42,00000	3,078091	0,5974	0,774437
	Time	Fixed	58,155	7	8,308	8,24204	1,849966	4,4908	0,024172
	Error		129,280	42	3,078				
18	Intercept	Fixed	714,7338	1	714,7338	12,96149	0,654971	1091,245	0,000000
	Parallel (time)	Random	4,4525	8	0,5566	51,00000	1,769586	0,315	0,957019
	Time	Fixed	348,1670	7	49,7381	8,51854	0,568645	87,468	0,000000
	Error		90,2489	51	1,7696				

Table II - 142: *Test results of **Tukey HSD post-hoc tests** of difference for gsdf1 gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,999999	0,884706	0,999806	1,000000	0,965828	0,999994	0,550238
	2	0,999999		0,936881	0,997002	1,000000	0,905165	1,000000	0,368987
	3	0,884706	0,936881		0,606714	0,897275	0,318227	0,925300	0,039285
	4	0,999806	0,997002	0,606714		0,996305	0,998040	0,992471	0,792496
	5	1,000000	1,000000	0,897275	0,996305		0,884227	1,000000	0,288384
	6	0,965828	0,905165	0,318227	0,998040	0,884227		0,850609	0,994978
	7	0,999994	1,000000	0,925300	0,992471	1,000000	0,850609		0,247314
	8	0,550238	0,368987	0,039285	0,792496	0,288384	0,994978	0,247314	
12.5	1		1,000000	0,981288	0,593658	0,999972	0,752199	1,000000	0,814764
	2	1,000000		0,985815	0,623917	0,999990	0,723543	1,000000	0,790918
	3	0,981288	0,985815		0,983911	0,996265	0,127972	0,931091	0,196788
	4	0,593658	0,623917	0,983911		0,638285	0,004856	0,338386	0,013057
	5	0,999972	0,999990	0,996265	0,638285		0,312975	0,999304	0,436779
	6	0,752199	0,723543	0,127972	0,004856	0,312975		0,703484	1,000000
	7	1,000000	1,000000	0,931091	0,338386	0,999304	0,703484		0,792164
	8	0,814764	0,790918	0,196788	0,013057	0,436779	1,000000	0,792164	
18	1		0,999682	0,150107	0,712134	0,021673	0,000131	0,000131	0,000131
	2	0,999682		0,357445	0,951315	0,087665	0,000132	0,000131	0,000131
	3	0,150107	0,357445		0,850205	0,999986	0,014801	0,000320	0,000155
	4	0,712134	0,951315	0,850205		0,429330	0,000133	0,000131	0,000131
	5	0,021673	0,087665	0,999986	0,429330		0,004125	0,000149	0,000131
	6	0,000131	0,000132	0,014801	0,000133	0,004125		0,674786	0,287707
	7	0,000131	0,000131	0,000320	0,000131	0,000149	0,674786		0,999489
	8	0,000131	0,000131	0,000155	0,000131	0,000131	0,287707	0,999489	

d. Comparison of temperature groups between feeding regimes

Table II - 143: *Test results of **factorial ANOVAs** for gsdf1 gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	2909,649	1	2909,649	679,6432	0,000000
	Feeding regime	0,095	1	0,095	0,0223	0,881652
	Time	163,298	7	23,328	5,4491	0,000030
	Feed*Time	89,590	7	12,799	2,9895	0,007145
	Error	393,865	92	4,281		
12.5	Intercept	2283,083	1	2283,083	1059,092	0,000000
	Feeding regime	0,061	1	0,061	0,028	0,866385
	Time	103,479	7	14,783	6,857	0,000001
	Feed*Time	39,780	7	5,683	2,636	0,015168
	Error	219,881	102	2,156		
18	Intercept	1532,787	1	1532,787	673,8585	0,000000
	Feeding regime	5,128	1	5,128	2,2545	0,136019
	Time	520,956	7	74,422	32,7182	0,000000
	Feed*Time	34,972	7	4,996	2,1964	0,039643
	Error	257,035	113	2,275		

Table II - 144: *Test results of* ***Tukey HSD post-hoc tests*** *for gsdf1 gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,97	0,78	0,73	0,51	0,18	0,22	0,04	0,96	0,98	1,00	0,78	0,96	0,53	0,97	0,10
		2	0,97		0,03	0,01	0,00	0,00	0,00	0,00	0,09	0,10	0,72	0,02	0,05	0,01	0,06	0,00
		3	0,78	0,03		1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95	1,00	1,00	1,00	1,00	1,00
		4	0,73	0,01	1,00		1,00	1,00	1,00	0,98	1,00	1,00	0,94	1,00	1,00	1,00	1,00	1,00
		5	0,51	0,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	0,81	1,00	1,00	1,00	1,00	1,00
		6	0,18	0,00	1,00	1,00	1,00		1,00	1,00	0,98	0,94	0,40	1,00	0,91	1,00	0,88	1,00
		7	0,22	0,00	1,00	1,00	1,00	1,00		1,00	0,98	0,93	0,43	1,00	0,90	1,00	0,88	1,00
		8	0,04	0,00	1,00	0,98	1,00	1,00	1,00		0,85	0,68	0,11	0,97	0,54	1,00	0,49	1,00
	100	1	0,96	0,09	1,00	1,00	1,00	0,98	0,98	0,85		1,00	1,00	1,00	1,00	1,00	1,00	0,95
		2	0,98	0,10	1,00	1,00	1,00	0,94	0,93	0,68	1,00		1,00	1,00	1,00	1,00	1,00	0,85
		3	1,00	0,72	0,95	0,94	0,81	0,40	0,43	0,11	1,00	1,00		0,96	1,00	0,81	1,00	0,24
		4	0,78	0,02	1,00	1,00	1,00	1,00	1,00	0,97	1,00	1,00	0,96		1,00	1,00	1,00	0,99
		5	0,96	0,05	1,00	1,00	1,00	0,91	0,90	0,54	1,00	1,00	1,00	1,00		1,00	1,00	0,78
		6	0,53	0,01	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,81	1,00	1,00		1,00	1,00
		7	0,97	0,06	1,00	1,00	1,00	0,88	0,88	0,49	1,00	1,00	1,00	1,00	1,00	1,00		0,73
		8	0,10	0,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95	0,85	0,24	0,99	0,78	1,00	0,73	
12.5	67	1		1,00	1,00	1,00	0,01	0,26	0,04	0,54	0,97	0,97	1,00	1,00	0,99	0,02	0,84	0,04
		2	1,00		1,00	1,00	0,07	0,66	0,23	0,93	1,00	1,00	1,00	1,00	1,00	0,11	0,99	0,20
		3	1,00	1,00		1,00	0,59	0,97	0,84	1,00	1,00	1,00	1,00	1,00	1,00	0,68	1,00	0,76
		4	1,00	1,00	1,00		0,01	0,37	0,05	0,70	1,00	1,00	1,00	1,00	1,00	0,02	0,95	0,05
		5	0,01	0,07	0,59	0,01		1,00	1,00	0,88	0,79	0,75	0,05	0,00	0,22	1,00	0,71	1,00
		6	0,26	0,66	0,97	0,37	1,00		1,00	1,00	1,00	1,00	0,59	0,04	0,94	1,00	1,00	1,00
		7	0,04	0,23	0,84	0,05	1,00	1,00		0,99	0,96	0,95	0,19	0,00	0,55	1,00	0,95	1,00
		8	0,54	0,93	1,00	0,70	0,88	1,00	0,99		1,00	1,00	0,89	0,10	1,00	0,94	1,00	0,98
	100	1	0,97	1,00	1,00	1,00	0,79	1,00	0,96	1,00		1,00	1,00	0,69	1,00	0,87	1,00	0,92
		2	0,97	1,00	1,00	1,00	0,75	1,00	0,95	1,00	1,00		1,00	0,73	1,00	0,84	1,00	0,90
		3	1,00	1,00	1,00	1,00	0,05	0,59	0,19	0,89	1,00	1,00		1,00	1,00	0,09	0,99	0,16
		4	1,00	1,00	1,00	1,00	0,00	0,04	0,00	0,10	0,69	0,73	1,00		0,75	0,00	0,34	0,00
		5	0,99	1,00	1,00	1,00	0,22	0,94	0,55	1,00	1,00	1,00	1,00	0,75		0,31	1,00	0,48
		6	0,02	0,11	0,68	0,02	1,00	1,00	1,00	0,94	0,87	0,84	0,09	0,00	0,31		0,82	1,00
		7	0,84	0,99	1,00	0,95	0,71	1,00	0,95	1,00	1,00	1,00	0,99	0,34	1,00	0,82		0,90
		8	0,04	0,20	0,76	0,05	1,00	1,00	1,00	0,98	0,92	0,90	0,16	0,00	0,48	1,00	0,90	
18	67	1		0,97	1,00	0,23	0,00	0,00	0,00	0,00	1,00	1,00	0,26	0,80	0,03	0,00	0,00	0,00
		2	0,97		1,00	0,99	0,33	0,00	0,00	0,00	1,00	1,00	0,99	1,00	0,88	0,00	0,00	0,00
		3	1,00	1,00		0,84	0,11	0,00	0,00	0,00	1,00	1,00	0,84	1,00	0,49	0,00	0,00	0,00
		4	0,23	0,99	0,84		1,00	0,15	0,04	0,22	0,55	0,84	1,00	1,00	1,00	0,05	0,00	0,00
		5	0,00	0,33	0,11	1,00		0,88	0,52	0,95	0,02	0,07	1,00	0,29	1,00	0,61	0,03	0,00
		6	0,00	0,00	0,00	0,15	0,88		1,00	1,00	0,00	0,00	0,28	0,00	0,15	1,00	0,84	0,44
		7	0,00	0,00	0,00	0,04	0,52	1,00		1,00	0,00	0,00	0,09	0,00	0,03	1,00	0,99	0,85
		8	0,00	0,00	0,00	0,22	0,95	1,00	1,00		0,00	0,00	0,38	0,00	0,21	1,00	0,59	0,20
	100	1	1,00	1,00	1,00	0,55	0,02	0,00	0,00	0,00		1,00	0,57	0,98	0,16	0,00	0,00	0,00
		2	1,00	1,00	1,00	0,84	0,07	0,00	0,00	0,00	1,00		0,84	1,00	0,42	0,00	0,00	0,00
		3	0,26	0,99	0,84	1,00	1,00	0,28	0,09	0,38	0,57	0,84		1,00	1,00	0,12	0,00	0,00
		4	0,80	1,00	1,00	1,00	0,29	0,00	0,00	0,00	0,98	1,00	1,00		0,89	0,00	0,00	0,00
		5	0,03	0,88	0,49	1,00	1,00	0,15	0,03	0,21	0,16	0,42	1,00	0,89		0,04	0,00	0,00
		6	0,00	0,00	0,00	0,05	0,61	1,00	1,00	1,00	0,00	0,00	0,12	0,00	0,04		0,98	0,78
		7	0,00	0,00	0,00	0,00	0,03	0,84	0,99	0,59	0,00	0,00	0,00	0,00	0,00	0,98		1,00
		8	0,00	0,00	0,00	0,00	0,00	0,44	0,85	0,20	0,00	0,00	0,00	0,00	0,00	0,78	1,00	

II - IX Relative *gonadal soma-derived factor 2* (*gsdf2*) mRNA transcription

a. Overall tests:

Table II - 145: *Test results of a **three-way random effects nested ANOVA** for gsdf2 gene transcription, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	56,54699	1	56,54699	100,3773	0,062953	898,2429	0,000000
Parallel (Temp.*feed*Time)	Random	5,47182	84	0,06514	259,0000	0,046476	1,4016	0,023814
Time	Fixed	1,85662	7	0,26523	94,9764	0,063603	4,1701	0,000483
Temperature	Fixed	1,87146	2	0,93573	98,6526	0,063154	14,8166	0,000002
Feeding regime	Fixed	0,08072	1	0,08072	100,0120	0,062995	1,2813	0,260363
Error		12,03730	259	0,04648				

Table II - 146: *Test results of a **three-way factorial ANOVA** for gsdf2 gene transcription, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	58,40885	1	58,40885	1332,036	0,000000
Time	1,90027	7	0,27147	6,191	0,000001
Temperature	1,85864	2	0,92932	21,194	0,000000
Feed regime	0,10396	1	0,10396	2,371	0,124658
Time*Temp.	2,30050	14	0,16432	3,747	0,000008
Time*Feed.	0,42620	7	0,06089	1,389	0,209516
Temp.*Feed.	0,02461	2	0,01230	0,281	0,755549
Time*Temp.*Feed.	1,37986	14	0,09856	2,248	0,006440
Error	13,41788	306	0,04385		

b. Restrictive feeding (67%):

Table II - 147: *Test results of a **two-way random effects nested ANOVA** for gsdf2 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	28,85661	1	28,85661	43,5931	0,071506	403,5532	0,000000
Parallel (Temp.*Time)	Random	2,78878	37	0,07537	124,0000	0,044919	1,6779	0,018749
Time	Fixed	1,26311	7	0,18044	41,0138	0,072873	2,4762	0,032370
Temperature	Fixed	1,06915	2	0,53457	41,8913	0,072390	7,3847	0,001790
Error		5,57000	124	0,04492				

Table II - 148: *Test results of a **two-way factorial ANOVA** for gsdf2 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	29,96255	1	29,96255	683,5001	0,000000
Time	1,37082	7	0,19583	4,4673	0,000156
Temperature	1,04972	2	0,52486	11,9730	0,000015
Temp.*Time	1,91475	14	0,13677	3,1199	0,000278
Error	6,44403	147	0,04384		

Table II - 149: *Test results of* ***one-way random effects nested ANOVAs*** *for gsdf2 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	5,728999	1	5,728999	3,07821	0,096042	59,65075	0,004118
	Parallel (temp)	Random	0,287597	3	0,095866	11,00000	0,111941	0,85639	0,492094
	Temperature	Fixed	0,145486	2	0,072743	3,03601	0,095948	0,75815	0,540678
	Error		1,231356	11	0,111941				
2 (14.12.18)	Intercept	Fixed	4,674169	1	4,674169	3,04009	0,012512	373,5652	0,000279
	Parallel (temp)	Random	0,037703	3	0,012568	11,00000	0,007540	1,6668	0,231154
	Temperature	Fixed	0,551753	2	0,275877	3,01848	0,012542	21,9964	0,015862
	Error		0,082938	11	0,007540				
3 (01.02.19)	Intercept	Fixed	3,223408	1	3,223408	1,785292	0,006050	532,8147	0,003218
	Parallel (temp)	Random	0,011640	2	0,005820	7,000000	0,003517	1,6546	0,257955
	Temperature	Fixed	0,722637	2	0,361319	1,724679	0,006125	58,9878	0,025829
	Error		0,024622	7	0,003517				
4 (13.04.19)	Intercept	Fixed	4,228023	1	4,228023	5,25906	0,007522	562,1156	0,000002
	Parallel (temp)	Random	0,018029	3	0,006010	17,00000	0,029617	0,2029	0,892940
	Temperature	Fixed	0,330172	2	0,165086	4,12705	0,006824	24,1916	0,005256
	Error		0,503484	17	0,029617				
5 (27.03.19)	Intercept	Fixed	4,385149	1	4,385149	3,38235	0,001125	3897,927	0,000003
	Parallel (temp)	Random	0,003187	3	0,001062	22,00000	0,021376	0,050	0,984949
	Temperature	Fixed	0,223365	2	0,111682	3,19611	0,001095	101,994	0,001273
	Error		0,470261	22	0,021376				
6 (10.04.19)	Intercept	Fixed	2,856700	1	2,856700	15,19816	0,002563	1114,495	0,000000
	Parallel (temp)	Random	0,000778	3	0,000259	13,00000	0,071112	0,004	0,999680
	Temperature	Fixed	0,289819	2	0,144910	15,99323	0,001421	101,995	0,000000
	Error		0,924452	13	0,071112				
7 (24.04.19)	Intercept	Fixed	1,770210	1	1,770210	4,18520	0,141209	12,53608	0,022265
	Parallel (temp)	Random	0,498891	3	0,166297	19,00000	0,077647	2,14170	0,128613
	Temperature	Fixed	0,142143	2	0,071071	3,31181	0,157583	0,45101	0,671078
	Error		1,475299	19	0,077647				
8 (15.05.19)	Intercept	Fixed	3,133999	1	3,133999	3,90650	0,006041	518,8282	0,000027
	Parallel (temp)	Random	0,016202	3	0,005401	24,00000	0,035733	0,1511	0,927936
	Temperature	Fixed	0,334475	2	0,167238	3,39190	0,005689	29,3946	0,007205
	Error		0,857590	24	0,035733				

Table II - 150: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for gsdf2 gene* transcription *between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,388283	0,729044
	12	0,388283		0,806121
	18	0,729044	0,806121	
2 (14.12.18)	8		0,033290	0,000203
	12	0,033290		0,000535
	18	0,000203	0,000535	
3 (01.02.19)	8		0,001712	0,000228
	12	0,001712		0,000586
	18	0,000228	0,000586	
4 (13.03.19)	8		0,014056	0,525018
	12	0,014056		0,119380
	18	0,525018	0,119380	
5 (27.03.19)	8		0,767875	0,014522
	12	0,767875		0,067142
	18	0,014522	0,067142	
6 (10.04.19)	8		0,170372	0,291617
	12	0,170372		0,844654
	18	0,291617	0,844654	
7 (24.04.19)	8		0,325736	0,552893
	12	0,325736		0,852599
	18	0,552893	0,852599	
8 (15.05.19)	8		0,039171	0,990585
	12	0,039171		0,032157
	18	0,990585	0,032157	

Table II - 151: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for gsdf2 gene transcription between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,447833	0,898568	0,680348	0,695098	0,705652
	1	12	0,447833		0,945158	0,999912	0,997181	0,996562
	1	18	0,898568	0,945158		0,991607	0,997660	0,998112
	2	8	0,680348	0,999912	0,991607		0,999966	0,999947
	2	12	0,695098	0,997181	0,997660	0,999966		1,000000
	2	18	0,705652	0,996562	0,998112	0,999947	1,000000	
2 (14.12.18)	1	8		0,119337	0,002621	0,999985	0,745847	0,000386
	1	12	0,119337		0,216690	0,149548	0,674302	0,016205
	1	18	0,002621	0,216690		0,004739	0,020939	0,582982
	2	8	0,999985	0,149548	0,004739		0,740683	0,000668
	2	12	0,745847	0,674302	0,020939	0,740683		0,001751
	2	18	0,000386	0,016205	0,582982	0,000668	0,001751	
3 (01.02.19)	1	8		0,005379	0,000345	0,754935	0,000246	
	1	12	0,005379		0,009577	0,022304	0,001342	
	1	18	0,000345	0,009577		0,000575	0,661708	
	2	8	0,754935	0,022304	0,000575		0,000278	
	2	12	N=0	N=0	N=0	N=0	N=0	
	2	18	0,000246	0,001342	0,661708	0,000278		
4 (13.03.19)	1	8		0,301480	0,999570	0,999131	0,196598	0,907006
	1	12	0,301480		0,568594	0,328751	0,999679	0,875029
	1	18	0,999570	0,568594		0,989845	0,423959	0,987135
	2	8	0,999131	0,328751	0,989845		0,236252	0,837912
	2	12	0,196598	0,999679	0,423959	0,236252		0,741420
	2	18	0,907006	0,875029	0,987135	0,837912	0,741420	
5 (27.03.19)	1	8		0,983597	0,324762	1,000000	0,999504	0,307987
	1	12	0,983597		0,731768	0,979887	0,998796	0,711461
	1	18	0,324762	0,731768		0,269014	0,437769	1,000000
	2	8	1,000000	0,979887	0,269014		0,999385	0,253352
	2	12	0,999504	0,998796	0,437769	0,999385		0,416790
	2	18	0,307987	0,711461	1,000000	0,253352	0,416790	
6 (10.04.19)	1	8		0,808931	0,918610	1,000000	0,709536	0,838089
	1	12	0,808931		0,998863	0,795551	1,000000	0,999414
	1	18	0,918610	0,998863		0,908769	0,996924	0,999999
	2	8	1,000000	0,795551	0,908769		0,692518	0,822426
	2	12	0,709536	1,000000	0,996924	0,692518		0,997987
	2	18	0,838089	0,999414	0,999999	0,822426	0,997987	
7 (24.04.19)	1	8		0,953076	0,756610	1,000000	0,959508	0,999997
	1	12	0,953076		0,952957	0,836778	1,000000	0,560576
	1	18	0,756610	0,952957		0,416366	0,922847	0,163745
	2	8	1,000000	0,836778	0,416366		0,848366	0,999800
	2	12	0,959508	1,000000	0,922847	0,848366		0,560883
	2	18	0,999997	0,560576	0,163745	0,999800	0,560883	
8 (15.05.19)	1	8		0,725824	1,000000	0,999979	0,338368	1,000000
	1	12	0,725824		0,656521	0,618264	0,985992	0,653918
	1	18	1,000000	0,656521		0,999926	0,244123	1,000000
	2	8	0,999979	0,618264	0,999926		0,252830	0,999990
	2	12	0,338368	0,985992	0,244123	0,252830		0,258048
	2	18	1,000000	0,653918	1,000000	0,999990	0,258048	

Table II - 152: *Test results of **one-way random effects nested ANOVAs** for gsdf2 gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	4,111664	1	4,111664	10,84946	0,033670	122,1166	0,000000
	Parallel (Time)	Random	0,256891	8	0,032111	31,00000	0,047257	0,6795	0,705808
	Time	Fixed	0,878269	7	0,125467	8,37610	0,032347	3,8787	0,035497
	Error		1,464962	31	0,047257				
12.5	Intercept	Fixed	12,46003	1	12,46003	9,46632	0,008824	1412,045	0,000000
	Parallel (Time)	Random	0,06029	7	0,00861	46,00000	0,010356	0,832	0,566527
	Time	Fixed	0,34307	7	0,04901	7,87001	0,008697	5,635	0,013762
	Error		0,47636	46	0,01036				
18	Intercept	Fixed	15,80246	1	15,80246	9,44893	0,070161	225,2314	0,000000
	Parallel (Time)	Random	0,55684	8	0,06961	47,00000	0,077206	0,9016	0,523114
	Time	Fixed	1,85401	7	0,26486	8,19141	0,069686	3,8007	0,038881
	Error		3,62868	47	0,07721				

Table II - 153: *Test results of **Tukey HSD post-hoc tests** of difference for gsdf2 gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,084059	0,031019	0,056340	0,022364	0,008324	0,011397	0,007417
	2	0,084059		0,998549	1,000000	1,000000	0,990141	0,969200	0,997125
	3	0,031019	0,998549		0,998822	0,999453	1,000000	0,999932	1,000000
	4	0,056340	1,000000	0,998822		1,000000	0,990708	0,969800	0,997489
	5	0,022364	1,000000	0,999453	1,000000		0,993362	0,974846	0,998631
	6	0,008324	0,990141	1,000000	0,990708	0,993362		0,999993	0,999999
	7	0,011397	0,969200	0,999932	0,969800	0,974846	0,999993		0,999700
	8	0,007417	0,997125	1,000000	0,997489	0,998631	0,999999	0,999700	
12.5	1		0,999987	0,999239	0,153226	0,474925	0,960598	0,988070	1,000000
	2	0,999987		0,999986	0,293272	0,283429	0,993699	0,924994	1,000000
	3	0,999239	0,999986		0,799972	0,372955	0,999970	0,902368	0,999756
	4	0,153226	0,293272	0,799972		0,000194	0,873192	0,003143	0,081832
	5	0,474925	0,283429	0,372955	0,000194		0,063940	0,850074	0,190916
	6	0,960598	0,993699	0,999970	0,873192	0,063940		0,487875	0,966930
	7	0,988070	0,924994	0,902368	0,003143	0,850074	0,487875		0,917205
	8	1,000000	1,000000	0,999756	0,081832	0,190916	0,966930	0,917205	
18	1		0,945714	0,858048	0,967460	0,999633	0,980347	0,736482	0,291199
	2	0,945714		0,999986	0,366579	0,643753	0,398059	0,098440	0,014589
	3	0,858048	0,999986		0,258868	0,488898	0,282147	0,065534	0,010123
	4	0,967460	0,366579	0,258868		0,998153	1,000000	0,999430	0,902115
	5	0,999633	0,643753	0,488898	0,998153		0,999488	0,897156	0,408665
	6	0,980347	0,398059	0,282147	1,000000	0,999488		0,996870	0,818718
	7	0,736482	0,098440	0,065534	0,999430	0,897156	0,996870		0,991635
	8	0,291199	0,014589	0,010123	0,902115	0,408665	0,818718	0,991635	

c. Full feeding (100%)

Table II - 154: *Test results of a **two-way random effects nested ANOVA** for gsdf2 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	27,63776	1	27,63776	44,5940	0,057989	476,6038	0,000000
Parallel (Temp.*Time)	Random	2,24342	38	0,05904	135,0000	0,047906	1,2324	0,193309
Temperature	Fixed	0,80960	2	0,40480	44,5046	0,058002	6,9792	0,002310
Time	Fixed	1,03651	7	0,14807	41,3403	0,058474	2,5323	0,029017
Error		6,46730	135	0,04791				

Table II - 155: *Test results of a **two-way factorial ANOVA** for gsdf2 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	28,46223	1	28,46223	648,9231	0,000000
Time	1,00789	7	0,14398	3,2828	0,002755
Temperature	0,82001	2	0,41000	9,3479	0,000145
Time*Temp.	1,73687	14	0,12406	2,8285	0,000831
Error	6,97385	159	0,04386		

Table II - 156: *Test results of **one-way random effects nested ANOVAs** for gsdf2 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	2,461920	1	2,461920	3,107989	0,005884	418,4046	0,000204
	Parallel (Temp)	Random	0,017470	3	0,005823	8,000000	0,014072	0,4138	0,747781
	Temperature	Fixed	0,068387	2	0,034193	3,050475	0,005852	5,8431	0,090509
	Error		0,112579	8	0,014072				
2 (14.12.18)	Intercept	Fixed	3,450470	1	3,450470	3,05293	0,005529	624,0426	0,000124
	Parallel (Temp)	Random	0,016494	3	0,005498	12,00000	0,015662	0,3510	0,789205
	Temperature	Fixed	0,179993	2	0,089996	3,02536	0,005513	16,3245	0,023936
	Error		0,187946	12	0,015662				
3 (01.02.19)	Intercept	Fixed	4,163432	1	4,163432	3,02708	0,071229	58,45102	0,004503
	Parallel (Temp)	Random	0,213574	3	0,071191	13,00000	0,080843	0,88061	0,476520
	Temperature	Fixed	0,460635	2	0,230317	3,01412	0,071211	3,23429	0,177758
	Error		1,050964	13	0,080843				
4 (13.04.19)	Intercept	Fixed	4,645040	1	4,645040	3,52118	0,006703	692,9411	0,000036
	Parallel (Temp)	Random	0,018903	3	0,006301	16,00000	0,027822	0,2265	0,876587
	Temperature	Fixed	0,601581	2	0,300790	3,25167	0,006500	46,2728	0,004086
	Error		0,445158	16	0,027822				
5 (27.03.19)	Intercept	Fixed	6,778044	1	6,778044	3,20357	0,014644	462,8477	0,000142
	Parallel (Temp)	Random	0,043241	3	0,014414	24,00000	0,028072	0,5134	0,676894
	Temperature	Fixed	0,380171	2	0,190085	3,09429	0,014522	13,0893	0,030913
	Error		0,673736	24	0,028072				
6 (10.04.19)	Intercept	Fixed	3,410836	1	3,410836	3,37352	0,045990	74,16520	0,002069
	Parallel (Temp)	Random	0,134590	3	0,044863	18,00000	0,078381	0,57237	0,640438
	Temperature	Fixed	0,151945	2	0,075973	3,15875	0,045358	1,67495	0,319237
	Error		1,410866	18	0,078381				
7 (24.04.19)	Intercept	Fixed	2,465978	1	2,465978	3,11971	0,009028	273,1336	0,000384
	Parallel (Temp)	Random	0,026811	3	0,008937	22,00000	0,018721	0,4774	0,701269
	Temperature	Fixed	0,766982	2	0,383491	3,05591	0,008980	42,7046	0,005844
	Error		0,411862	22	0,018721				
8 (15.05.19)	Intercept	Fixed	2,045645	1	2,045645	10,37798	0,020933	97,72385	0,000001
	Parallel (Temp)	Random	0,035473	3	0,011824	22,00000	0,098827	0,11965	0,947580
	Temperature	Fixed	0,001209	2	0,000605	6,59635	0,016816	0,03595	0,964876
	Error		2,174186	22	0,098827				

Table II - 157: *Test results of **Tukey HSD post-hoc tests** of difference for gsdf2 gene transcription between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,614809	0,137171
	12	0,614809		0,457369
	18	0,137171	0,457369	
2 (14.12.18)	8		0,385052	0,152073
	12	0,385052		0,014059
	18	0,152073	0,014059	
3 (01.02.19)	8		0,107835	0,202102
	12	0,107835		0,887248
	18	0,202102	0,887248	
4 (13.03.19)	8		0,095796	0,000872
	12	0,095796		0,079559
	18	0,000872	0,079559	
5 (27.03.19)	8		0,154160	0,003797
	12	0,154160		0,281428
	18	0,003797	0,281428	
6 (10.04.19)	8		0,397098	0,522323
	12	0,397098		0,966166
	18	0,522323	0,966166	
7 (24.04.19)	8		0,005555	0,014718
	12	0,005555		0,000139
	18	0,014718	0,000139	
8 (15.05.19)	8		0,992147	0,994167
	12	0,992147		0,999468
	18	0,994167	0,999468	

Table II - 158: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for gsdf2 gene transcription between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,732769	0,533215	0,998361	0,994953	0,633804
	1	12	0,732769		0,998686	0,855878	0,896568	0,999957
	1	18	0,533215	0,998686		0,652267	0,708651	0,999953
	2	8	0,998361	0,855878	0,652267		0,999995	0,761318
	2	12	0,994953	0,896568	0,708651	0,999995		0,812586
	2	18	0,633804	0,999957	0,999953	0,761318	0,812586	
2 (14.12.18)	1	8		0,812363	0,402331	0,994840	0,996942	0,705702
	1	12	0,812363		0,088697	0,661729	0,974361	0,198449
	1	18	0,402331	0,088697		0,838449	0,271379	0,994651
	2	8	0,994840	0,661729	0,838449		0,948289	0,975590
	2	12	0,996942	0,974361	0,271379	0,948289		0,515996
	2	18	0,705702	0,198449	0,994651	0,975590	0,515996	
3 (01.02.19)	1	8		0,770095	0,756152	0,904477	0,965939	0,999666
	1	12	0,770095		1,000000	0,247669	0,993453	0,864333
	1	18	0,756152	1,000000		0,238272	0,991856	0,852324
	2	8	0,904477	0,247669	0,238272		0,499199	0,740041
	2	12	0,965939	0,993453	0,991856	0,499199		0,992966
	2	18	0,999666	0,864333	0,852324	0,740041	0,992966	
4 (13.03.19)	1	8		0,414171	0,048499	0,999505	0,620181	0,034750
	1	12	0,414171		0,961542	0,511516	0,974140	0,922895
	1	18	0,048499	0,961542		0,056722	0,402140	0,999961
	2	8	0,999505	0,511516	0,056722		0,747696	0,039676
	2	12	0,620181	0,974140	0,402140	0,747696		0,306095
	2	18	0,034750	0,922895	0,999961	0,039676	0,306095	
5 (27.03.19)	1	8		0,928274	0,502054	0,954710	0,929542	0,150641
	1	12	0,928274		0,994304	0,551884	0,999978	0,807232
	1	18	0,502054	0,994304		0,121692	0,951901	0,945787
	2	8	0,954710	0,551884	0,121692		0,463195	0,025662
	2	12	0,929542	0,999978	0,951901	0,463195		0,538608
	2	18	0,150641	0,807232	0,945787	0,025662	0,538608	
6 (10.04.19)	1	8		0,720472	0,682322	0,998439	0,926766	0,997679
	1	12	0,720472		1,000000	0,918238	0,991963	0,893842
	1	18	0,682322	1,000000		0,902743	0,989368	0,871070
	2	8	0,998439	0,918238	0,902743		0,995432	1,000000
	2	12	0,926766	0,991963	0,989368	0,995432		0,993683
	2	18	0,997679	0,893842	0,871070	1,000000	0,993683	
7 (24.04.19)	1	8		0,617696	0,284520	0,998548	0,131798	0,205424
	1	12	0,617696		0,009181	0,286749	0,871842	0,005692
	1	18	0,284520	0,009181		0,377361	0,000895	0,999953
	2	8	0,998548	0,286749	0,377361		0,031078	0,272659
	2	12	0,131798	0,871842	0,000895	0,031078		0,000587
	2	18	0,205424	0,005692	0,999953	0,272659	0,000587	
8 (15.05.19)	1	8		1,000000	0,999601	0,995691	0,996788	0,998685
	1	12	1,000000		0,999968	0,999225	0,999522	0,999868
	1	18	0,999601	0,999968		0,999880	0,999959	0,999999
	2	8	0,995691	0,999225	0,999880		1,000000	0,999976
	2	12	0,996788	0,999522	0,999959	1,000000		0,999996
	2	18	0,998685	0,999868	0,999999	0,999976	0,999996	

Table II - 159: *Test results of **one-way random effects nested ANOVAs** for gsdf2 gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	5,172933	1	5,172933	8,81682	0,018874	274,0719	0,000000
	Parallel (time)	Random	0,153108	8	0,019138	42,00000	0,014796	1,2935	0,273062
	Time	Fixed	0,155178	7	0,022168	8,11779	0,019098	1,1608	0,414378
	Error		0,621415	42	0,014796				
12.5	Intercept	Fixed	10,70645	1	10,70645	10,72316	0,016576	645,9002	0,000000
	Parallel (time)	Random	0,12096	8	0,01512	43,00000	0,041439	0,3649	0,933316
	Time	Fixed	0,45349	7	0,06478	8,39697	0,015354	4,2195	0,027908
	Error		1,78187	43	0,04144				
18	Intercept	Fixed	12,85475	1	12,85475	14,07450	0,034699	370,4671	0,000000
	Parallel (time)	Random	0,23249	8	0,02906	50,00000	0,081280	0,3575	0,937761
	Time	Fixed	2,19364	7	0,31338	8,66863	0,029817	10,5099	0,001260
	Error		4,06401	50	0,08128				

Table II - 160: *Test results of **Tukey HSD post-hoc tests** of difference for gsdf2 gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,938662	0,899381	0,975306	1,000000	0,967406	0,995515	0,992391
	2	0,938662		0,197878	0,322247	0,900901	0,320321	0,409655	0,405751
	3	0,899381	0,197878		0,999950	0,755467	0,999994	0,995542	0,998760
	4	0,975306	0,322247	0,999950		0,913404	1,000000	0,999967	0,999998
	5	1,000000	0,900901	0,755467	0,913404		0,899308	0,973770	0,964286
	6	0,967406	0,320321	0,999994	1,000000	0,899308		0,999863	0,999985
	7	0,995515	0,409655	0,995542	0,999967	0,973770	0,999863		1,000000
	8	0,992391	0,405751	0,998760	0,999998	0,964286	0,999985	1,000000	
12.5	1		0,996565	0,760706	0,999730	0,996383	0,999879	0,990113	0,983481
	2	0,996565		0,271980	0,906685	0,773787	0,910889	0,682766	0,999999
	3	0,760706	0,271980		0,922803	0,960833	0,867379	0,976984	0,158336
	4	0,999730	0,906685	0,922803		0,999998	1,000000	0,999958	0,793160
	5	0,996383	0,773787	0,960833	0,999998		0,999973	1,000000	0,597347
	6	0,999879	0,910889	0,867379	1,000000	0,999973		0,999708	0,790030
	7	0,990113	0,682766	0,976984	0,999958	1,000000	0,999708		0,488438
	8	0,983481	0,999999	0,158336	0,793160	0,597347	0,790030	0,488438	
18	1		0,999983	1,000000	0,983977	0,998913	0,999656	0,258482	0,891566
	2	0,999983		0,999998	0,997887	0,999994	0,978950	0,056784	0,538544
	3	1,000000	0,999998		0,981059	0,999208	0,995507	0,075884	0,655886
	4	0,983977	0,997887	0,981059		0,999801	0,650535	0,004006	0,093957
	5	0,998913	0,999994	0,999208	0,999801		0,839879	0,006225	0,157806
	6	0,999656	0,978950	0,995507	0,650535	0,839879		0,251844	0,966211
	7	0,258482	0,056784	0,075884	0,004006	0,006225	0,251844		0,735001
	8	0,891566	0,538544	0,655886	0,093957	0,157806	0,966211	0,735001	

d. Comparison of temperature groups between feeding regimes

Table II - 161: *Test results of **factorial ANOVAs** for gsdf2 gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	9,895290	1	9,895290	352,7838	0,000000
	Feeding regime	0,006220	1	0,006220	0,2218	0,638860
	Time	0,786577	7	0,112368	4,0061	0,000746
	Feed*Time	0,493368	7	0,070481	2,5128	0,020987
	Error	2,496375	89	0,028049		
12.5	Intercept	23,27981	1	23,27981	992,4637	0,000000
	Feeding regime	0,03030	1	0,03030	1,2919	0,258317
	Time	0,34022	7	0,04860	2,0720	0,053016
	Feed*Time	0,41472	7	0,05925	2,5258	0,019341
	Error	2,43949	104	0,02346		
18	Intercept	28,97891	1	28,97891	386,0655	0,000000
	Feeding regime	0,10065	1	0,10065	1,3408	0,249326
	Time	3,29354	7	0,47051	6,2682	0,000003
	Feed*Time	0,91635	7	0,13091	1,7440	0,105773
	Error	8,48202	113	0,07506		

Table II - 162: *Test results of* ***Tukey HSD post-hoc tests*** *for gsdf2 gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,02	0,00	0,01	0,00	0,00	0,00	0,00	0,02	0,14	0,00	0,00	0,00	0,00	0,00	0,00
		2	0,02		1,00	1,00	1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
		3	0,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	0,94	1,00	1,00	1,00	1,00	1,00	1,00
		4	0,01	1,00	1,00		1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
		5	0,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00
		6	0,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	0,75	1,00	1,00	0,99	1,00	1,00	1,00
		7	0,00	0,99	1,00	0,99	1,00	1,00		1,00	0,99	0,66	1,00	1,00	0,95	1,00	1,00	1,00
		8	0,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	0,82	1,00	1,00	1,00	1,00	1,00	1,00
	100	1	0,02	1,00	1,00	1,00	1,00	1,00	0,99	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00
		2	0,14	1,00	0,94	1,00	0,99	0,75	0,66	0,82	1,00		0,89	0,95	1,00	0,95	0,97	0,97
		3	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,89		1,00	1,00	1,00	1,00	1,00
		4	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95	1,00		1,00	1,00	1,00	1,00
		5	0,00	1,00	1,00	1,00	1,00	0,99	0,95	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00
		6	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95	1,00	1,00	1,00		1,00	1,00
		7	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,97	1,00	1,00	1,00	1,00		1,00
		8	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,97	1,00	1,00	1,00	1,00	1,00	
12.5	67	1		1,00	1,00	0,92	0,99	1,00	1,00	1,00	1,00	0,97	0,97	1,00	1,00	1,00	1,00	0,87
		2	1,00		1,00	0,97	0,97	1,00	1,00	1,00	1,00	0,92	0,99	1,00	1,00	1,00	1,00	0,75
		3	1,00	1,00		1,00	0,98	1,00	1,00	1,00	1,00	0,95	1,00	1,00	1,00	1,00	1,00	0,86
		4	0,92	0,97	1,00		0,04	1,00	0,30	0,83	0,61	0,04	1,00	0,90	0,96	0,77	0,98	0,01
		5	0,99	0,97	0,98	0,04		0,80	1,00	0,94	1,00	1,00	0,15	0,99	0,88	0,99	0,75	1,00
		6	1,00	1,00	1,00	1,00	0,80		0,99	1,00	1,00	0,68	1,00	1,00	1,00	1,00	1,00	0,45
		7	1,00	1,00	1,00	0,30	1,00	0,99		1,00	1,00	1,00	0,56	1,00	1,00	1,00	1,00	0,97
		8	1,00	1,00	1,00	0,83	0,94	1,00	1,00		1,00	0,86	0,94	1,00	1,00	1,00	1,00	0,62
	100	1	1,00	1,00	1,00	0,61	1,00	1,00	1,00	1,00		1,00	0,76	1,00	1,00	1,00	1,00	1,00
		2	0,97	0,92	0,95	0,04	1,00	0,68	1,00	0,86	1,00		0,12	0,95	0,78	0,95	0,65	1,00
		3	0,97	0,99	1,00	1,00	0,15	1,00	0,56	0,94	0,76	0,12		0,96	0,99	0,91	1,00	0,04
		4	1,00	1,00	1,00	0,90	0,99	1,00	1,00	1,00	1,00	0,95	0,96		1,00	1,00	1,00	0,81
		5	1,00	1,00	1,00	0,96	0,88	1,00	1,00	1,00	1,00	0,78	0,99	1,00		1,00	1,00	0,52
		6	1,00	1,00	1,00	0,77	0,99	1,00	1,00	1,00	1,00	0,95	0,91	1,00	1,00		1,00	0,80
		7	1,00	1,00	1,00	0,98	0,75	1,00	1,00	1,00	1,00	0,65	1,00	1,00	1,00	1,00		0,36
		8	0,87	0,75	0,86	0,01	1,00	0,45	0,97	0,62	1,00	1,00	0,04	0,81	0,52	0,80	0,36	
18	67	1		1,00	0,99	1,00	1,00	1,00	0,96	0,57	1,00	1,00	1,00	1,00	1,00	1,00	0,08	0,74
		2	1,00		1,00	0,67	0,91	0,71	0,22	0,03	0,99	1,00	0,97	1,00	1,00	0,57	0,00	0,05
		3	0,99	1,00		0,52	0,80	0,55	0,15	0,02	0,95	0,98	0,91	1,00	0,99	0,42	0,00	0,04
		4	1,00	0,67	0,52		1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,94	0,99	1,00	0,57	1,00
		5	1,00	0,91	0,80	1,00		1,00	0,99	0,72	1,00	1,00	1,00	1,00	1,00	1,00	0,10	0,88
		6	1,00	0,71	0,55	1,00	1,00		1,00	0,98	1,00	1,00	1,00	0,96	1,00	1,00	0,41	1,00
		7	0,96	0,22	0,15	1,00	0,99	1,00		1,00	1,00	0,98	0,99	0,53	0,74	1,00	0,80	1,00
		8	0,57	0,03	0,02	1,00	0,72	0,98	1,00		0,97	0,66	0,77	0,10	0,18	0,99	1,00	1,00
	100	1	1,00	0,99	0,95	1,00	1,00	1,00	1,00	0,97		1,00	1,00	1,00	1,00	1,00	0,47	0,99
		2	1,00	1,00	0,98	1,00	1,00	1,00	0,98	0,66	1,00		1,00	1,00	1,00	1,00	0,10	0,82
		3	1,00	0,97	0,91	1,00	1,00	1,00	0,99	0,77	1,00	1,00		1,00	1,00	1,00	0,14	0,90
		4	1,00	1,00	1,00	0,94	1,00	0,96	0,53	0,10	1,00	1,00	1,00		1,00	0,90	0,00	0,18
		5	1,00	1,00	0,99	0,99	1,00	1,00	0,74	0,18	1,00	1,00	1,00	1,00		0,98	0,01	0,30
		6	1,00	0,57	0,42	1,00	1,00	1,00	1,00	0,99	1,00	1,00	1,00	0,90	0,98		0,46	1,00
		7	0,08	0,00	0,00	0,57	0,10	0,41	0,80	1,00	0,47	0,10	0,14	0,00	0,01	0,46		0,95
		8	0,74	0,05	0,04	1,00	0,88	1,00	1,00	1,00	0,99	0,82	0,90	0,18	0,30	1,00	0,95	

II – X Relative *insulin-like growth factor 3* (*igf3*) mRNA transcription

a. Overall tests:

Table II - 163: *Test results of a **three-way random effects nested ANOVA** for igf3 gene transcription, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	0,007390	1	0,007390	121,5857	0,000371	19,92063	0,000018
Parallel (Temp.*feed*Time)	Random	0,031838	84	0,000379	164,0000	0,000338	1,12178	0,264879
Time	Fixed	0,006671	7	0,000953	114,4928	0,000372	2,56018	0,017354
Temperature	Fixed	0,005525	2	0,002762	120,4424	0,000371	7,44302	0,000896
Feeding regime	Fixed	0,000622	1	0,000622	121,3076	0,000371	1,67599	0,197917
Error		0,055412	164	0,000338				

Table II - 164: *Test results of a **three-way factorial ANOVA** for igf3 gene transcription, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	0,007899	1	0,007899	26,69318	0,000001
Time	0,006736	7	0,000962	3,25219	0,002673
Temperature	0,005650	2	0,002825	9,54687	0,000107
Feed regime	0,000721	1	0,000721	2,43713	0,119991
Time*Temp.	0,015907	14	0,001136	3,83992	0,000008
Time*Feed.	0,001740	7	0,000249	0,84013	0,555149
Temp.*Feed.	0,000672	2	0,000336	1,13601	0,323055
Time*Temp.*Feed.	0,003073	14	0,000219	0,74174	0,730703
Error	0,062435	211	0,000296		

b. Restrictive feeding (67%):

Table II - 165: *Test results of a **two-way random effects nested ANOVA** for igf3 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	0,001862	1	0,001862	47,27191	0,000233	7,988833	0,006877
Parallel (Temp.*Time)	Random	0,009138	37	0,000247	77,00000	0,000165	1,498273	0,068700
Time	Fixed	0,002493	7	0,000356	44,68017	0,000236	1,508428	0,189078
Temperature	Fixed	0,001377	2	0,000688	46,09231	0,000234	2,935714	0,063087
Error		0,012692	77	0,000165				

Table II - 166: *Test results of a **two-way factorial ANOVA** for igf3 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	0,001869	1	0,001869	11,83558	0,000849
Time	0,002137	7	0,000305	1,93285	0,072078
Temperature	0,001275	2	0,000638	4,03802	0,020584
Temp.*Time	0,006037	14	0,000431	2,73052	0,001903
Error	0,015793	100	0,000158		

Table II - 167: *Test results of* ***one-way random effects nested ANOVAs*** *for igf3 gene transcription, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,000015	1	0,000015	3,333457	0,000000	42,32575	0,005298
	Parallel (temp)	Random	0,000001	3	0,000000	5,000000	0,000000	1,43474	0,337133
	Temperature	Fixed	0,000000	2	0,000000	3,195076	0,000000	0,20597	0,823881
	Error		0,000001	5	0,000000				
2 (14.12.18)	Intercept	Fixed	0,000029	1	0,000029	3,198250	0,000001	29,85040	0,010176
	Parallel (temp)	Random	0,000003	3	0,000001	7,000000	0,000000	3,48691	0,078695
	Temperature	Fixed	0,000002	2	0,000001	3,096020	0,000001	1,01002	0,459547
	Error		0,000002	7	0,000000				
3 (01.02.19)	Intercept	Fixed	0,000008	1	0,000008	4,066849	0,000000	49,23349	0,002042
	Parallel (temp)	Random	0,000000	2	0,000000	6,000000	0,000000	0,29420	0,755293
	Temperature	Fixed	0,000001	2	0,000001	3,465478	0,000000	3,14255	0,166547
	Error		0,000003	6	0,000000				
4 (13.04.19)	Intercept	Fixed	0,000019	1	0,000019	9,415445	0,000000	57,12748	0,000027
	Parallel (temp)	Random	0,000001	3	0,000000	9,000000	0,000001	0,12239	0,944563
	Temperature	Fixed	0,000003	2	0,000001	5,638297	0,000000	5,74136	0,043661
	Error		0,000013	9	0,000001				
5 (27.03.19)	Intercept	Fixed	0,000485	1	0,000485	3,06305	0,000218	2,228130	0,230532
	Parallel (temp)	Random	0,000653	3	0,000218	13,00000	0,000215	1,011027	0,419245
	Temperature	Fixed	0,000406	2	0,000203	3,03163	0,000218	0,932429	0,483498
	Error		0,002797	13	0,000215				
6 (10.04.19)	Intercept	Fixed	0,004016	1	0,004016	3,32920	0,000632	6,353315	0,077853
	Parallel (temp)	Random	0,001881	3	0,000627	12,00000	0,000750	0,836127	0,499602
	Temperature	Fixed	0,007980	2	0,003990	3,18554	0,000630	6,333781	0,077616
	Error		0,008997	12	0,000750				
7 (24.04.19)	Intercept	Fixed	0,000244	1	0,000244	4,30670	0,000003	76,13600	0,000677
	Parallel (temp)	Random	0,000009	3	0,000003	11,00000	0,000005	0,61084	0,621873
	Temperature	Fixed	0,000338	2	0,000169	3,52137	0,000003	54,81100	0,002222
	Error		0,000054	11	0,000005				
8 (15.05.19)	Intercept	Fixed	0,000330	1	0,000330	3,52753	0,000158	2,087286	0,231111
	Parallel (temp)	Random	0,000553	3	0,000184	14,00000	0,000059	3,129657	0,059564
	Temperature	Fixed	0,000424	2	0,000212	3,22201	0,000172	1,236559	0,399462
	Error		0,000825	14	0,000059				

Table II - 168: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for igf3 gene transcription between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,854829	0,778822
	12	0,854829		0,996106
	18	0,778822	0,996106	
2 (14.12.18)	8		0,081122	0,349786
	12	0,081122		0,741438
	18	0,349786	0,741438	
3 (01.02.19)	8		0,354858	0,840636
	12	0,354858		0,503043
	18	0,840636	0,503043	
4 (13.03.19)	8		0,430309	0,615090
	12	0,430309		0,868104
	18	0,615090	0,868104	
5 (27.03.19)	8		0,415015	0,972995
	12	0,415015		0,562138
	18	0,972995	0,562138	
6 (10.04.19)	8		0,999746	0,061943
	12	0,999746		0,064719
	18	0,061943	0,064719	
7 (24.04.19)	8		0,969896	0,000270
	12	0,969896		0,000224
	18	0,000270	0,000224	
8 (15.05.19)	8		0,992153	0,063314
	12	0,992153		0,138745
	18	0,063314	0,138745	

Table II - 169: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for igf3 gene transcription between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,873398	1,000000	0,996869	0,879747	0,986278
	1	12	0,873398		0,873933	0,990196	0,473738	0,623758
	1	18	1,000000	0,873933		0,996916	0,879106	0,986092
	2	8	0,996869	0,990196	0,996916		0,778814	0,921413
	2	12	0,879747	0,473738	0,879106	0,778814		0,990075
	2	18	0,986278	0,623758	0,986092	0,921413	0,990075	
2 (14.12.18)	1	8		0,997967	0,999122	0,155064	0,925123	0,999979
	1	12	0,997967		1,000000	0,182041	0,677151	0,999983
	1	18	0,999122	1,000000		0,234109	0,774756	0,999995
	2	8	0,155064	0,182041	0,234109		0,035801	0,348652
	2	12	0,925123	0,677151	0,774756	0,035801		0,933091
	2	18	0,999979	0,999983	0,999995	0,348652	0,933091	
3 (01.02.19)	1	8		0,671427	0,930692	1,000000	0,999985	
	1	12	0,671427		0,944842	0,782086	0,647606	
	1	18	0,930692	0,944842		0,961950	0,931621	
	2	8	1,000000	0,782086	0,961950		0,999970	
	2	12	N=0	N=0	N=0	N=0	N=0	
	2	18	0,999985	0,647606	0,931621	0,999970		
4 (13.03.19)	1	8		0,976709	0,938157	0,999552	0,911308	0,999228
	1	12	0,976709		0,999999	0,948295	1,000000	0,996599
	1	18	0,938157	0,999999		0,894546	0,999957	0,993744
	2	8	0,999552	0,948295	0,894546		0,863750	0,990962
	2	12	0,911308	1,000000	0,999957	0,863750		0,983041
	2	18	0,999228	0,996599	0,993744	0,990962	0,983041	
5 (27.03.19)	1	8		1,000000	0,999996	1,000000	0,460615	0,999980
	1	12	1,000000		0,999998	1,000000	0,531113	0,999989
	1	18	0,999996	0,999998		0,999989	0,698752	1,000000
	2	8	1,000000	1,000000	0,999989		0,507307	0,999958
	2	12	0,460615	0,531113	0,698752	0,507307		0,545350
	2	18	0,999980	0,999989	1,000000	0,999958	0,545350	
6 (10.04.19)	1	8		1,000000	0,817004	1,000000	1,000000	0,233459
	1	12	1,000000		0,828250	1,000000	1,000000	0,240950
	1	18	0,817004	0,828250		0,733559	0,741530	0,622863
	2	8	1,000000	1,000000	0,733559		1,000000	0,156274
	2	12	1,000000	1,000000	0,741530	1,000000		0,159476
	2	18	0,233459	0,240950	0,622863	0,156274	0,159476	
7 (24.04.19)	1	8		1,000000	0,033482	0,999779	0,993499	0,012655
	1	12	1,000000		0,005737	0,998555	0,971481	0,002162
	1	18	0,033482	0,005737		0,004396	0,004492	0,872014
	2	8	0,999779	0,998555	0,004396		0,998669	0,001616
	2	12	0,993499	0,971481	0,004492	0,998669		0,001595
	2	18	0,012655	0,002162	0,872014	0,001616	0,001595	
8 (15.05.19)	1	8		1,000000	0,070432	1,000000	0,999439	0,997203
	1	12	1,000000		0,068322	1,000000	0,999341	0,996642
	1	18	0,070432	0,068322		0,072615	0,542830	0,075859
	2	8	1,000000	1,000000	0,072615		0,999526	0,997676
	2	12	0,999439	0,999341	0,542830	0,999526		1,000000
	2	18	0,997203	0,996642	0,075859	0,997676	1,000000	

Table II - 170: *Test results of **one-way random effects nested ANOVAs** for igf3 gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,000047	1	0,000047	11,84100	0,000000	100,9552	0,000000
	Parallel (Time)	Random	0,000003	8	0,000000	22,00000	0,000001	0,5563	0,801507
	Time	Fixed	0,000008	7	0,000001	8,47982	0,000000	2,5643	0,099878
	Error		0,000017	22	0,000001				
12.5	Intercept	Fixed	0,000210	1	0,000210	9,59505	0,000099	2,113151	0,177951
	Parallel (Time)	Random	0,000663	7	0,000095	20,00000	0,000138	0,686115	0,682540
	Time	Fixed	0,000531	7	0,000076	7,53176	0,000096	0,791990	0,615445
	Error		0,002762	20	0,000138				
18	Intercept	Fixed	0,003648	1	0,003648	10,17070	0,000302	12,09097	0,005802
	Parallel (Time)	Random	0,002434	8	0,000304	35,00000	0,000283	1,07415	0,403180
	Time	Fixed	0,010775	7	0,001539	8,22878	0,000304	5,06450	0,017056
	Error		0,009913	35	0,000283				

Table III - 171: *Test results of **Tukey HSD post-hoc tests** of difference for igf3 gene transcription within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,937551	0,966635	0,993727	0,999563	0,998988	0,999965	0,998107
	2	0,937551		0,368172	0,999349	0,533141	0,547195	0,738728	0,475890
	3	0,966635	0,368172		0,544243	0,995975	0,998973	0,994302	0,999184
	4	0,993727	0,999349	0,544243		0,755023	0,761708	0,912375	0,690271
	5	0,999563	0,533141	0,995975	0,755023		1,000000	1,000000	1,000000
	6	0,998988	0,547195	0,998973	0,761708	1,000000		0,999998	1,000000
	7	0,999965	0,738728	0,994302	0,912375	1,000000	0,999998		0,999991
	8	0,998107	0,475890	0,999184	0,690271	1,000000	1,000000	0,999991	
12.5	1		1,000000	1,000000	1,000000	0,897145	1,000000	1,000000	1,000000
	2	1,000000		1,000000	1,000000	0,771583	1,000000	1,000000	1,000000
	3	1,000000	1,000000		1,000000	0,957620	1,000000	1,000000	1,000000
	4	1,000000	1,000000	1,000000		0,878015	1,000000	1,000000	1,000000
	5	0,897145	0,771583	0,957620	0,878015		0,821218	0,791284	0,872024
	6	1,000000	1,000000	1,000000	1,000000	0,821218		1,000000	1,000000
	7	1,000000	1,000000	1,000000	1,000000	0,791284	1,000000		1,000000
	8	1,000000	1,000000	1,000000	1,000000	0,872024	1,000000	1,000000	
18	1		1,000000	1,000000	1,000000	1,000000	0,004797	0,978256	0,961642
	2	1,000000		1,000000	1,000000	1,000000	0,029167	0,993554	0,989210
	3	1,000000	1,000000		1,000000	0,999999	0,002423	0,967574	0,942085
	4	1,000000	1,000000	1,000000		1,000000	0,002614	0,972016	0,949286
	5	1,000000	1,000000	0,999999	1,000000		0,004370	0,991316	0,982478
	6	0,004797	0,029167	0,002423	0,002614	0,004370		0,027020	0,013483
	7	0,978256	0,993554	0,967574	0,972016	0,991316	0,027020		1,000000
	8	0,961642	0,989210	0,942085	0,949286	0,982478	0,013483	1,000000	

c. Full feeding (100%)

Table II - 172: *Test results of a **two-way random effects nested ANOVA** for igf3 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	0,006252	1	0,006252	59,63713	0,000458	13,65207	0,000480
Parallel (Temp.*Time)	Random	0,017089	38	0,000450	87,00000	0,000491	0,91582	0,610460
Temperature	Fixed	0,004554	2	0,002277	59,47911	0,000458	4,97238	0,010073
Time	Fixed	0,005874	7	0,000839	54,90674	0,000456	1,83832	0,098238
Error		0,042720	87	0,000491				

Table II - 173: *Test results of a **two-way factorial ANOVA** for igf3 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	0,006896	1	0,006896	16,41082	0,000095
Time	0,006421	7	0,000917	2,18295	0,040998
Temperature	0,004924	2	0,002462	5,85898	0,003811
Time*Temp.	0,013166	14	0,000940	2,23813	0,010310
Error	0,046642	111	0,000420		

Table II - 174: *Test results of* ***one-way random effects nested ANOVAs*** *for igf3 gene transcription, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,000007	1	0,000007	4,700693	0,000000	47,40126	0,001264
	Parallel (Temp)	Random	0,000000	3	0,000000	5,000000	0,000000	0,53365	0,679016
	Temperature	Fixed	0,000000	2	0,000000	3,644617	0,000000	0,14358	0,870919
	Error		0,000001	5	0,000000				
2 (14.12.18)	Intercept	Fixed	0,000019	1	0,000019	3,181620	0,000001	27,18068	0,011833
	Parallel (Temp)	Random	0,000002	3	0,000001	5,000000	0,000000	2,62454	0,162538
	Temperature	Fixed	0,000001	2	0,000000	3,106377	0,000001	0,47385	0,661292
	Error		0,000001	5	0,000000				
3 (01.02.19)	Intercept	Fixed	0,000020	1	0,000020	3,491868	0,000000	101,3062	0,001060
	Parallel (Temp)	Random	0,000001	3	0,000000	9,000000	0,000000	1,0761	0,407062
	Temperature	Fixed	0,000001	2	0,000001	3,210028	0,000000	2,8630	0,193357
	Error		0,000002	9	0,000000				
4 (13.04.19)	Intercept	Fixed	0,000025	1	0,000025	5,99862	0,000000	682,8308	0,000000
	Parallel (Temp)	Random	0,000000	3	0,000000	12,00000	0,000001	0,0486	0,985128
	Temperature	Fixed	0,000000	2	0,000000	4,36932	0,000000	1,7066	0,283337
	Error		0,000006	12	0,000001				
5 (27.03.19)	Intercept	Fixed	0,001272	1	0,001272	14,69515	0,000025	50,63553	0,000004
	Parallel (Temp)	Random	0,000011	3	0,000004	12,00000	0,000347	0,01098	0,998333
	Temperature	Fixed	0,001209	2	0,000604	14,25658	0,000013	47,41132	0,000001
	Error		0,004166	12	0,000347				
6 (10.04.19)	Intercept	Fixed	0,004284	1	0,004284	16,55458	0,000077	55,78087	0,000001
	Parallel (Temp)	Random	0,000068	3	0,000023	14,00000	0,000310	0,07298	0,973494
	Temperature	Fixed	0,007142	2	0,003571	10,70553	0,000045	80,12970	0,000000
	Error		0,004339	14	0,000310				
7 (24.04.19)	Intercept	Fixed	0,006005	1	0,006005	10,93822	0,001161	5,170354	0,044137
	Parallel (Temp)	Random	0,002050	3	0,000683	12,00000	0,002627	0,260205	0,852676
	Temperature	Fixed	0,011263	2	0,005632	7,21816	0,000955	5,894836	0,030364
	Error		0,031520	12	0,002627				
8 (15.05.19)	Intercept	Fixed	0,001688	1	0,001688	3,58622	0,000474	3,559846	0,140437
	Parallel (Temp)	Random	0,001789	3	0,000596	18,00000	0,000149	3,998805	0,024071
	Temperature	Fixed	0,002563	2	0,001281	3,31415	0,000520	2,463128	0,221055
	Error		0,002685	18	0,000149				

Table II - 175: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for igf3 gene transcription between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,941143	0,973101
	12	0,941143		0,875015
	18	0,973101	0,875015	
2 (14.12.18)	8		0,611570	0,988862
	12	0,611570		0,598922
	18	0,988862	0,598922	
3 (01.02.19)	8		0,744340	0,451729
	12	0,744340		0,199084
	18	0,451729	0,199084	
4 (13.03.19)	8		0,997249	0,945338
	12	0,997249		0,910220
	18	0,945338	0,910220	
5 (27.03.19)	8		0,210480	0,938860
	12	0,210480		0,476941
	18	0,938860	0,476941	
6 (10.04.19)	8		0,874801	0,003504
	12	0,874801		0,002626
	18	0,003504	0,002626	
7 (24.04.19)	8		0,982322	0,177340
	12	0,982322		0,375611
	18	0,177340	0,375611	
8 (15.05.19)	8		0,847515	0,002256
	12	0,847515		0,014754
	18	0,002256	0,014754	

Table II - 176: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for igf3 gene transcription between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,999960	0,999157	0,953586	0,975285	0,980870
	1	12	0,999960		0,994671	0,878220	0,921911	0,949575
	1	18	0,999157	0,994671		0,999076	0,999823	0,999607
	2	8	0,953586	0,878220	0,999076		0,999994	1,000000
	2	12	0,975285	0,921911	0,999823	0,999994		0,999999
	2	18	0,980870	0,949575	0,999607	1,000000	0,999999	
2 (14.12.18)	1	8		0,669013	0,885734	0,706058	0,806970	0,596991
	1	12	0,669013		0,341323	0,999875	1,000000	0,999983
	1	18	0,885734	0,341323		0,353443	0,459907	0,303283
	2	8	0,706058	0,999875	0,353443		0,999970	0,998252
	2	12	0,806970	1,000000	0,459907	0,999970		0,999991
	2	18	0,596991	0,999983	0,303283	0,998252	0,999991	
3 (01.02.19)	1	8		0,999830	0,997025	0,999997	0,963606	0,401219
	1	12	0,999830		0,978279	0,999020	0,991253	0,325758
	1	18	0,997025	0,978279		0,999206	0,834577	0,561573
	2	8	0,999997	0,999020	0,999206		0,942357	0,437270
	2	12	0,963606	0,991253	0,834577	0,942357		0,223157
	2	18	0,401219	0,325758	0,561573	0,437270	0,223157	
4 (13.03.19)	1	8		1,000000	0,999338	0,999560	0,999904	0,996986
	1	12	1,000000		0,999465	0,999669	0,999949	0,996871
	1	18	0,999338	0,999465		1,000000	0,999995	0,999996
	2	8	0,999560	0,999669	1,000000		0,999999	0,999986
	2	12	0,999904	0,999949	0,999995	0,999999		0,999840
	2	18	0,996986	0,996871	0,999996	0,999986	0,999840	
5 (27.03.19)	1	8		0,789145	0,999939	1,000000	0,823491	0,999638
	1	12	0,789145		0,927274	0,759964	0,999982	0,950786
	1	18	0,999939	0,927274		0,999961	0,950676	0,999999
	2	8	1,000000	0,759964	0,999961		0,793110	0,999707
	2	12	0,823491	0,999982	0,950676	0,793110		0,969496
	2	18	0,999638	0,950786	0,999999	0,999707	0,969496	
6 (10.04.19)	1	8		0,999187	0,252359	1,000000	0,999895	0,341831
	1	12	0,999187		0,105423	0,996215	0,999956	0,162643
	1	18	0,252359	0,105423		0,042772	0,049028	0,997838
	2	8	1,000000	0,996215	0,042772		0,999467	0,065374
	2	12	0,999895	0,999956	0,049028	0,999467		0,076161
	2	18	0,341831	0,162643	0,997838	0,065374	0,076161	
7 (24.04.19)	1	8		0,999993	0,624340	1,000000	0,999997	0,935514
	1	12	0,999993		0,887115	0,999989	1,000000	0,992466
	1	18	0,624340	0,887115		0,488686	0,700308	0,943738
	2	8	1,000000	0,999989	0,488686		0,999993	0,889158
	2	12	0,999997	1,000000	0,700308	0,999993		0,965805
	2	18	0,935514	0,992466	0,943738	0,889158	0,965805	
8 (15.05.19)	1	8		1,000000	0,877416	0,999997	0,998482	0,025976
	1	12	1,000000		0,950484	1,000000	0,999274	0,131885
	1	18	0,877416	0,950484		0,648721	0,954137	0,029368
	2	8	0,999997	1,000000	0,648721		0,987545	0,002496
	2	12	0,998482	0,999274	0,954137	0,987545		0,010769
	2	18	0,025976	0,131885	0,029368	0,002496	0,010769	

Table II - 177: *Test results of **one-way random effects nested ANOVAs** for igf3 gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	0,000053	1	0,000053	8,47183	0,000000	137,4246	0,000002
	Parallel (time)	Random	0,000003	8	0,000000	26,00000	0,000000	1,9443	0,095678
	Time	Fixed	0,000004	7	0,000001	8,05844	0,000000	1,5929	0,262915
	Error		0,000005	26	0,000000				
12.5	Intercept	Fixed	0,000927	1	0,000927	32,17877	0,000027	34,97548	0,000001
	Parallel (time)	Random	0,000042	8	0,000005	25,00000	0,000172	0,03070	0,999987
	Time	Fixed	0,001728	7	0,000247	17,84923	0,000008	30,29780	0,000000
	Error		0,004302	25	0,000172				
18	Intercept	Fixed	0,009504	1	0,009504	27,45806	0,000676	14,05890	0,000838
	Parallel (time)	Random	0,003877	8	0,000485	36,00000	0,001067	0,45414	0,879682
	Time	Fixed	0,021489	7	0,003070	9,96236	0,000514	5,97104	0,006164
	Error		0,038413	36	0,001067				

Table II - 178: *Test results of **Tukey HSD post-hoc tests** of difference for igf3 gene transcription within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,596672	0,975041	0,969978	0,575898	0,996436	0,998590	0,170194
	2	0,596672		0,968360	0,983982	1,000000	0,208308	0,882883	0,990125
	3	0,975041	0,968360		1,000000	0,971492	0,668292	0,999947	0,547140
	4	0,969978	0,983982	1,000000		0,986990	0,665378	0,999855	0,651741
	5	0,575898	1,000000	0,971492	0,986990		0,178818	0,881950	0,969584
	6	0,996436	0,208308	0,668292	0,665378	0,178818		0,877163	0,034729
	7	0,998590	0,882883	0,999947	0,999855	0,881950	0,877163		0,382447
	8	0,170194	0,990125	0,547140	0,651741	0,969584	0,034729	0,382447	
12.5	1		1,000000	1,000000	1,000000	0,287723	0,997218	0,995835	0,998979
	2	1,000000		1,000000	1,000000	0,487776	0,999118	0,998057	0,999633
	3	1,000000	1,000000		1,000000	0,286362	0,997141	0,995749	0,998938
	4	1,000000	1,000000	1,000000		0,242690	0,996864	0,995622	0,998911
	5	0,287723	0,487776	0,286362	0,242690		0,570369	0,899374	0,642036
	6	0,997218	0,999118	0,997141	0,996864	0,570369		0,999999	1,000000
	7	0,995835	0,998057	0,995749	0,995622	0,899374	0,999999		0,999999
	8	0,998979	0,999633	0,998938	0,998911	0,642036	1,000000	0,999999	
18	1		1,000000	1,000000	1,000000	1,000000	0,711188	0,413979	0,969136
	2	1,000000		1,000000	1,000000	1,000000	0,550314	0,237392	0,935457
	3	1,000000	1,000000		1,000000	1,000000	0,420222	0,138942	0,889490
	4	1,000000	1,000000	1,000000		0,999999	0,210385	0,038888	0,741798
	5	1,000000	1,000000	1,000000	0,999999		0,542781	0,205565	0,952197
	6	0,711188	0,550314	0,420222	0,210385	0,542781		0,993651	0,921750
	7	0,413979	0,237392	0,138942	0,038888	0,205565	0,993651		0,436113
	8	0,969136	0,935457	0,889490	0,741798	0,952197	0,921750	0,436113	

d. Comparison of temperature groups between feeding regimes

Table II - 179: *Test results of **factorial ANOVAs** for igf3 gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	0,000109	1	0,000109	241,2325	0,000000
	Feeding regime	0,000000	1	0,000000	0,2377	0,627548
	Time	0,000006	7	0,000001	1,9772	0,071967
	Feed*Time	0,000005	7	0,000001	1,6759	0,130887
	Error	0,000029	64	0,000000		
12.5	Intercept	0,001060	1	0,001060	8,183015	0,005811
	Feeding regime	0,000131	1	0,000131	1,015074	0,317736
	Time	0,001922	7	0,000275	2,120262	0,054898
	Feed*Time	0,000171	7	0,000024	0,188170	0,986867
	Error	0,007769	60	0,000129		
18	Intercept	0,013122	1	0,013122	20,89430	0,000016
	Feeding regime	0,001328	1	0,001328	2,11488	0,149472
	Time	0,024717	7	0,003531	5,62247	0,000023
	Feed*Time	0,006141	7	0,000877	1,39686	0,216921
	Error	0,054637	87	0,000628		

Table II - 180: *Test results of* ***Tukey HSD post-hoc tests*** *for igf3 gene transcription comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		0,98	0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	1,00	1,00
		2	0,98		0,23	1,00	0,45	0,47	0,75	0,37	0,40	0,98	0,68	0,76	0,94	0,16	0,58	1,00
		3	0,99	0,23		0,47	1,00	1,00	1,00	1,00	1,00	0,95	1,00	1,00	0,94	1,00	1,00	0,70
		4	1,00	1,00	0,47		0,78	0,79	0,96	0,68	0,71	1,00	0,94	0,97	1,00	0,36	0,88	1,00
		5	1,00	0,45	1,00	0,78		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95
		6	1,00	0,47	1,00	0,79	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95
		7	1,00	0,75	1,00	0,96	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
		8	1,00	0,37	1,00	0,68	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,91
	100	1	1,00	0,40	1,00	0,71	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	0,90
		2	1,00	0,98	0,95	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	0,93	1,00	1,00
		3	1,00	0,68	1,00	0,94	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00	0,99
		4	1,00	0,76	1,00	0,97	1,00	1,00	1,00	1,00	1,00	1,00	1,00		1,00	1,00	1,00	1,00
		5	1,00	0,94	0,94	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		0,91	1,00	1,00
		6	0,99	0,16	1,00	0,36	1,00	1,00	1,00	1,00	1,00	0,93	1,00	1,00	0,91		1,00	0,61
		7	1,00	0,58	1,00	0,88	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		0,98
		8	1,00	1,00	0,70	1,00	0,95	0,95	1,00	0,91	0,90	1,00	0,99	1,00	1,00	0,61	0,98	
12.5	67	1		1,00	1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,61	1,00	1,00	1,00
		2	1,00		1,00	1,00	0,96	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,26	1,00	1,00	1,00
		3	1,00	1,00		1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,84	1,00	1,00	1,00
		4	1,00	1,00	1,00		0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,57	1,00	1,00	1,00
		5	0,99	0,96	1,00	0,99		0,98	0,97	0,99	0,97	0,99	0,97	0,96	1,00	1,00	1,00	1,00
		6	1,00	1,00	1,00	1,00	0,98		1,00	1,00	1,00	1,00	1,00	1,00	0,36	1,00	1,00	1,00
		7	1,00	1,00	1,00	1,00	0,97	1,00		1,00	1,00	1,00	1,00	1,00	0,28	1,00	1,00	1,00
		8	1,00	1,00	1,00	1,00	0,99	1,00	1,00		1,00	1,00	1,00	1,00	0,49	1,00	1,00	1,00
	100	1	1,00	1,00	1,00	1,00	0,97	1,00	1,00	1,00		1,00	1,00	1,00	0,32	1,00	1,00	1,00
		2	1,00	1,00	1,00	1,00	0,99	1,00	1,00	1,00	1,00		1,00	1,00	0,60	1,00	1,00	1,00
		3	1,00	1,00	1,00	1,00	0,97	1,00	1,00	1,00	1,00	1,00		1,00	0,32	1,00	1,00	1,00
		4	1,00	1,00	1,00	1,00	0,96	1,00	1,00	1,00	1,00	1,00	1,00		0,26	1,00	1,00	1,00
		5	0,61	0,26	0,84	0,57	1,00	0,36	0,28	0,49	0,32	0,60	0,32	0,26		0,71	0,98	0,79
		6	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,71		1,00	1,00
		7	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,98	1,00		1,00
		8	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,79	1,00	1,00	
18	67	1		1,00	1,00	1,00	1,00	0,30	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,19	0,02	0,88
		2	1,00		1,00	1,00	1,00	0,62	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,48	0,11	0,98
		3	1,00	1,00		1,00	1,00	0,22	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,12	0,01	0,80
		4	1,00	1,00	1,00		1,00	0,22	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,12	0,01	0,82
		5	1,00	1,00	1,00	1,00		0,29	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,17	0,01	0,88
		6	0,30	0,62	0,22	0,22	0,29		0,60	0,47	0,81	0,62	0,44	0,17	0,62	1,00	1,00	0,99
		7	1,00	1,00	1,00	1,00	1,00	0,60		1,00	1,00	1,00	1,00	1,00	1,00	0,42	0,04	0,99
		8	1,00	1,00	1,00	1,00	1,00	0,47	1,00		1,00	1,00	1,00	1,00	1,00	0,28	0,02	0,99
	100	1	1,00	1,00	1,00	1,00	1,00	0,81	1,00	1,00		1,00	1,00	1,00	1,00	0,72	0,30	0,99
		2	1,00	1,00	1,00	1,00	1,00	0,62	1,00	1,00	1,00		1,00	1,00	1,00	0,48	0,11	0,98
		3	1,00	1,00	1,00	1,00	1,00	0,44	1,00	1,00	1,00	1,00		1,00	1,00	0,31	0,04	0,94
		4	1,00	1,00	1,00	1,00	1,00	0,17	1,00	1,00	1,00	1,00	1,00		1,00	0,09	0,00	0,76
		5	1,00	1,00	1,00	1,00	1,00	0,62	1,00	1,00	1,00	1,00	1,00	1,00		0,47	0,09	0,99
		6	0,19	0,48	0,12	0,12	0,17	1,00	0,42	0,28	0,72	0,48	0,31	0,09	0,47		1,00	0,97
		7	0,02	0,11	0,01	0,01	0,01	1,00	0,04	0,02	0,30	0,11	0,04	0,00	0,09	1,00		0,33
		8	0,88	0,98	0,80	0,82	0,88	0,99	0,99	0,99	0,99	0,98	0,94	0,76	0,99	0,97	0,33	

II – XI Plasma 11-Ketotestosterone (11-KT) concentrations

a. Overall tests:

Table II - 181: *Test results of a **three-way random effects nested ANOVA** for plasma 11-KT levels, including all treatment groups throughout the experiment (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	P
Intercept	Fixed	189,1379	1	189,1379	92,5971	0,962538	196,4991	0,000000
Parallel (Temp.*feed*Time)	Random	85,7010	85	1,0082	292,0000	0,474221	2,1261	0,000002
Time	Fixed	85,2607	7	12,1801	88,7630	0,984419	12,3729	0,000000
Temperature	Fixed	32,6283	2	16,3142	92,4380	0,963403	16,9339	0,000001
Feeding regime	Fixed	4,5457	1	4,5457	92,5971	0,962538	4,7226	0,032320
Error		138,4727	292	0,4742				

Table II - 182: *Test results of a **three-way factorial ANOVA** for plasma 11-KT levels, including all treatment throughout the experiment (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	P
Intercept	198,5656	1	198,5656	437,2322	0,000000
Time	92,7971	7	13,2567	29,1907	0,000000
Temperature	32,7271	2	16,3636	36,0318	0,000000
Feed regime	5,3322	1	5,3322	11,7413	0,000686
Time*Temp.	53,8460	14	3,8461	8,4690	0,000000
Time*Feed.	5,2907	7	0,7558	1,6643	0,116793
Temp.*Feed.	2,5107	2	1,2553	2,7642	0,064443
Time*Temp.*Feed.	7,5320	14	0,5380	1,1847	0,285126
Error	154,4084	340	0,4541		

b. Restrictive feeding (67%):

Table II - 183: *Test results of a **two-way random effects nested ANOVA** for plasma 11-KT levels, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	67,24427	1	67,24427	43,5034	0,524913	128,1055	0,000000
Parallel (Temp.*Time)	Random	20,37106	38	0,53608	146,0000	0,405397	1,3224	0,122848
Time	Fixed	26,79330	7	3,82761	39,8522	0,532035	7,1943	0,000014
Temperature	Fixed	9,34050	2	4,67025	43,4571	0,524997	8,8958	0,000578
Error		59,18798	146	0,40540				

Table II - 184: *Test results of a **two-way factorial ANOVA** for plasma 11-KT levels, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	69,07654	1	69,07654	181,9232	0,000000
Time	27,76936	7	3,96705	10,4478	0,000000
Temperature	9,53788	2	4,76894	12,5597	0,000008
Temp.*Time	15,00977	14	1,07213	2,8236	0,000801
Error	64,54927	170	0,37970		

Table II - 185: *Test results of **one-way random effects nested ANOVAs** for plasma 11-KT levels, including all temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,349719	1	0,349719	3,01763	0,002954	118,4003	0,001618
	Parallel (temp)	Random	0,008933	3	0,002978	10,00000	0,000787	3,7853	0,047521
	Temperature	Fixed	0,000748	2	0,000374	3,00944	0,002965	0,1261	0,885955
	Error		0,007867	10	0,000787				
2 (14.12.18)	Intercept	Fixed	1,188935	1	1,188935	3,07730	0,013333	89,17533	0,002266
	Parallel (temp)	Random	0,039930	3	0,013310	11,00000	0,015362	0,86642	0,487398
	Temperature	Fixed	0,005755	2	0,002877	3,03559	0,013320	0,21601	0,817128
	Error		0,168983	11	0,015362				
3 (01.02.19)	Intercept	Fixed	2,040200	1	2,040200	3	0,025744	79,24817	0,002990
	Parallel (temp)	Random	0,077233	3	0,025744	12	0,011561	2,22681	0,137646
	Temperature	Fixed	0,055833	2	0,027917	3	0,025744	1,08438	0,442184
	Error		0,138733	12	0,011561				
4 (13.04.19)	Intercept	Fixed	3,986651	1	3,986651	3,02008	0,014638	272,3528	0,000466
	Parallel (temp)	Random	0,043872	3	0,014624	23,00000	0,020474	0,7143	0,553490
	Temperature	Fixed	0,041932	2	0,020966	3,00985	0,014631	1,4330	0,365408
	Error		0,470898	23	0,020474				
5 (27.03.19)	Intercept	Fixed	7,785024	1	7,785024	3,01011	0,168878	46,09841	0,006462
	Parallel (temp)	Random	0,507345	3	0,169115	23,00000	0,092214	1,83395	0,169156
	Temperature	Fixed	1,484900	2	0,742450	3,00485	0,169001	4,39316	0,128221
	Error		2,120915	23	0,092214				
6 (10.04.19)	Intercept	Fixed	29,84207	1	29,84207	3,16274	0,102000	292,5681	0,000318
	Parallel (temp)	Random	0,29872	3	0,09957	23,00000	1,118626	0,0890	0,965356
	Temperature	Fixed	13,48809	2	6,74405	3,07945	0,100767	66,9272	0,002901
	Error		25,72840	23	1,11863				
7 (24.04.19)	Intercept	Fixed	29,88297	1	29,88297	3,19169	1,394372	21,43113	0,016544
	Parallel (temp)	Random	4,27114	3	1,42371	19,00000	0,843288	1,68829	0,203262
	Temperature	Fixed	9,21488	2	4,60744	3,09473	1,408738	3,27062	0,172476
	Error		16,02248	19	0,84329				
8 (15.05.19)	Intercept	Fixed	37,06667	1	37,06667	3,34135	0,040010	926,4459	0,000033
	Parallel (temp)	Random	0,11412	3	0,03804	25,00000	0,581188	0,0655	0,977679
	Temperature	Fixed	4,21138	2	2,10569	3,17580	0,039068	53,8975	0,003542
	Error		14,52970	25	0,58119				

Table II - 186: *Test results of **Tukey HSD post-hoc tests** of difference for plasma 11-KT levels between temperature groups within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (Date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,886296	0,697265
	12	0,886296		0,939351
	18	0,697265	0,939351	
2 (14.12.18)	8		0,949882	0,789836
	12	0,949882		0,926892
	18	0,789836	0,926892	
3 (01.02.19)	8		0,121720	0,784278
	12	0,121720		0,335861
	18	0,784278	0,335861	
4 (13.03.19)	8		0,670654	0,320917
	12	0,670654		0,800500
	18	0,320917	0,800500	
5 (27.03.19)	8		0,390659	0,001831
	12	0,390659		0,048450
	18	0,001831	0,048450	
6 (10.04.19)	8		0,931564	0,010660
	12	0,931564		0,029000
	18	0,010660	0,029000	
7 (24.04.19)	8		0,922196	0,033182
	12	0,922196		0,017612
	18	0,033182	0,017612	
8 (15.05.19)	8		0,975141	0,087186
	12	0,975141		0,055776
	18	0,087186	0,055776	

Table II - 187: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for plasma 11-KT levels between temperature parallel groups, within the restrictive feeding group (67%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,973747	0,534804	0,178407	0,151400	0,427132
	1	12	0,973747		0,901313	0,457583	0,365795	0,778292
	1	18	0,534804	0,901313		0,944804	0,840428	0,998463
	2	8	0,178407	0,457583	0,944804		0,998463	0,998463
	2	12	0,151400	0,365795	0,840428	0,998463		0,975929
	2	18	0,427132	0,778292	0,998463	0,998463	0,975929	
2 (14.12.18)	1	8		0,985843	0,992796	0,999253	0,985843	1,000000
	1	12	0,985843		0,770621	0,999348	0,717933	0,986581
	1	18	0,992796	0,770621		0,912543	0,999998	0,979215
	2	8	0,999253	0,999348	0,912543		0,876826	0,999608
	2	12	0,985843	0,717933	0,999998	0,876826		0,963505
	2	18	1,000000	0,986581	0,979215	0,999608	0,963505	
3 (01.02.19)	1	8		0,962655	0,999895	0,389548	0,999895	0,784578
	1	12	0,962655		0,991359	0,123088	0,991359	0,353666
	1	18	0,999895	0,991359		0,288660	1,000000	0,660133
	2	8	0,389548	0,123088	0,288660		0,288660	0,975458
	2	12	0,999895	0,991359	1,000000	0,288660		0,660133
	2	18	0,784578	0,353666	0,660133	0,975458	0,660133	
4 (13.03.19)	1	8		0,655836	0,865107	0,998591	0,999999	0,727551
	1	12	0,655836		0,998928	0,916610	0,743623	1,000000
	1	18	0,865107	0,998928		0,987064	0,915054	0,999456
	2	8	0,998591	0,916610	0,987064		0,999636	0,938897
	2	12	0,999999	0,743623	0,915054	0,999636		0,799917
	2	18	0,727551	1,000000	0,999456	0,938897	0,799917	
5 (27.03.19)	1	8		0,775043	0,004860	0,998790	0,920081	0,453706
	1	12	0,775043		0,141586	0,928897	0,998702	0,997834
	1	18	0,004860	0,141586		0,011893	0,044804	0,241980
	2	8	0,998790	0,928897	0,011893		0,990508	0,686682
	2	12	0,920081	0,998702	0,044804	0,990508		0,948859
	2	18	0,453706	0,997834	0,241980	0,686682	0,948859	
6 (10.04.19)	1	8		0,999624	0,191435	1,000000	0,999821	0,405628
	1	12	0,999624		0,323184	0,999821	1,000000	0,595654
	1	18	0,191435	0,323184		0,137096	0,239317	0,995381
	2	8	1,000000	0,999821	0,137096		0,999935	0,336108
	2	12	0,999821	1,000000	0,239317	0,999935		0,497649
	2	18	0,405628	0,595654	0,995381	0,336108	0,497649	
7 (24.04.19)	1	8		0,999756	0,953620	1,000000	0,999999	0,101768
	1	12	0,999756		0,780639	0,998565	0,999992	0,022581
	1	18	0,953620	0,780639		0,940348	0,954993	0,263784
	2	8	1,000000	0,998565	0,940348		0,999984	0,050983
	2	12	0,999999	0,999992	0,954993	0,999984		0,161211
	2	18	0,101768	0,022581	0,263784	0,050983	0,161211	
8 (15.05.19)	1	8		0,999975	0,492779	0,999080	1,000000	0,614883
	1	12	0,999975		0,611178	0,999977	0,999949	0,726656
	1	18	0,492779	0,611178		0,614413	0,366619	0,999990
	2	8	0,999080	0,999977	0,614413		0,998170	0,745588
	2	12	1,000000	0,999949	0,366619	0,998170		0,503149
	2	18	0,614883	0,726656	0,999990	0,745588	0,503149	

Table II - 188: *Test results of **one-way random effects nested ANOVAs** for plasma 11-KT levels within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	11,47291	1	11,47291	9,47272	0,020082	571,3023	0,000000
	Parallel (Time)	Random	0,15820	8	0,01977	49,00000	0,024336	0,8126	0,594950
	Time	Fixed	3,90382	7	0,55769	8,17218	0,019814	28,1458	0,000043
	Error		1,19244	49	0,02434				
12.5	Intercept	Fixed	12,55966	1	12,55966	9,78056	0,017385	722,4580	0,000000
	Parallel (Time)	Random	0,13474	8	0,01684	46,00000	0,024883	0,6769	0,709200
	Time	Fixed	2,51647	7	0,35950	8,22553	0,016918	21,2491	0,000120
	Error		1,14462	46	0,02488				
18	Intercept	Fixed	54,12364	1	54,12364	10,63825	0,672580	80,47165	0,000003
	Parallel (Time)	Random	5,06836	8	0,63354	51,00000	1,114724	0,56834	0,798760
	Time	Fixed	37,21473	7	5,31639	8,28528	0,638337	8,32851	0,003417
	Error		56,85092	51	1,11472				

Table II - 189: *Test results of **Tukey HSD post-hoc tests** of difference for plasma 11-KT levels within temperature groups through time in the restrictive feeding group (67%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,987625	0,889258	0,034190	0,828050	0,010349	0,000133	0,000133
	2	0,987625		0,999937	0,390060	0,999903	0,190090	0,000133	0,000133
	3	0,889258	0,999937		0,593353	1,000000	0,325140	0,000133	0,000133
	4	0,034190	0,390060	0,593353		0,399007	0,999627	0,000191	0,000133
	5	0,828050	0,999903	1,000000	0,399007		0,162714	0,000133	0,000133
	6	0,010349	0,190090	0,325140	0,999627	0,162714		0,000431	0,000135
	7	0,000133	0,000133	0,000133	0,000191	0,000133	0,000431		0,976693
	8	0,000133	0,000133	0,000133	0,000133	0,000133	0,000135	0,976693	
12.5	1		0,932783	0,143051	0,202560	0,019396	0,000168	0,000213	0,000129
	2	0,932783		0,741745	0,889080	0,272673	0,001334	0,002473	0,000130
	3	0,143051	0,741745		0,999690	0,998436	0,146745	0,187628	0,000332
	4	0,202560	0,889080	0,999690		0,908623	0,014212	0,026024	0,000131
	5	0,019396	0,272673	0,998436	0,908623		0,299707	0,368252	0,000403
	6	0,000168	0,001334	0,146745	0,014212	0,299707		1,000000	0,209219
	7	0,000213	0,002473	0,187628	0,026024	0,368252	1,000000		0,298227
	8	0,000129	0,000130	0,000332	0,000131	0,000403	0,209219	0,298227	
18	1		0,999998	0,999994	0,999985	0,940296	0,049080	0,041500	0,175965
	2	0,999998		1,000000	1,000000	0,978053	0,059202	0,049742	0,217793
	3	0,999994	1,000000		1,000000	0,984015	0,067236	0,056656	0,240605
	4	0,999985	1,000000	1,000000		0,972460	0,026976	0,021814	0,132745
	5	0,940296	0,978053	0,984015	0,972460		0,234742	0,201183	0,640802
	6	0,049080	0,059202	0,067236	0,026976	0,234742		1,000000	0,994961
	7	0,041500	0,049742	0,056656	0,021814	0,201183	1,000000		0,990497
	8	0,175965	0,217793	0,240605	0,132745	0,640802	0,994961	0,990497	

c. Full feeding (100%)

Table II - 190: *Test results of a **two-way random effects nested ANOVA** for plasma 11-KT levels, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Intercept	Fixed	126,6831	1	126,6831	40,3355	1,421665	89,10903	0,000000
Parallel (Temp.*Time)	Random	56,8174	38	1,4952	146,0000	0,543046	2,75335	0,000008
Temperature	Fixed	25,9304	2	12,9652	40,2319	1,424654	9,10058	0,000549
Time	Fixed	62,4522	7	8,9217	39,2503	1,454171	6,13528	0,000070
Error		79,2847	146	0,5430				

Table II - 191: *Test results of a **two-way factorial ANOVA** for plasma 11-KT levels, including all temperature groups within the full feeding group (100%) (Sampling 1-8), together with significant interactions between treatments. Significant effects (p < 0.05) are highlighted in red.*

Effect	SS	DF	MS	F	p
Intercept	135,1400	1	135,1400	255,6647	0,000000
Time	69,9223	7	9,9889	18,8975	0,000000
Temperature	25,9418	2	12,9709	24,5390	0,000000
Time*Temp.	46,2430	14	3,3031	6,2489	0,000000
Error	89,8591	170	0,5286		

Table II - 192: *Test results of* ***one-way random effects nested ANOVAs*** *for plasma 11-KT levels, including all temperature groups within the full feeding group (100%) (Sampling 1-8). Significant effects (p < 0.05) are highlighted in red.*

Sampling (date)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
1 (31.10.18)	Intercept	Fixed	0,989356	1	0,989356	3	0,029956	33,02745	0,010465
	Parallel (Temp)	Random	0,089867	3	0,029956	12	0,013661	2,19276	0,141758
	Temperature	Fixed	0,032044	2	0,016022	3	0,029956	0,53487	0,632897
	Error		0,163933	12	0,013661				
2 (14.12.18)	Intercept	Fixed	1,429662	1	1,429662	3,01642	0,009208	155,2710	0,001085
	Parallel (Temp)	Random	0,027853	3	0,009284	11,00000	0,002285	4,0635	0,036062
	Temperature	Fixed	0,016933	2	0,008467	3,00758	0,009249	0,9154	0,489195
	Error		0,025133	11	0,002285				
3 (01.02.19)	Intercept	Fixed	2,390756	1	2,390756	3,00000	0,001078	2218,227	0,000021
	Parallel (Temp)	Random	0,003233	3	0,001078	12,00000	0,010478	0,103	0,956813
	Temperature	Fixed	0,087478	2	0,043739	3,00000	0,001078	40,582	0,006730
	Error		0,125733	12	0,010478				
4 (13.04.19)	Intercept	Fixed	6,467000	1	6,467000	3,12356	0,015260	423,7885	0,000193
	Parallel (Temp)	Random	0,045362	3	0,015121	22,00000	0,027783	0,5443	0,657137
	Temperature	Fixed	0,901508	2	0,450754	3,05676	0,015185	29,6833	0,009947
	Error		0,611215	22	0,027783				
5 (27.03.19)	Intercept	Fixed	10,62371	1	10,62371	5,31410	0,059170	179,5459	0,000027
	Parallel (Temp)	Random	0,14017	3	0,04672	22,00000	0,269224	0,1735	0,913138
	Temperature	Fixed	3,82913	2	1,91456	3,88634	0,051989	36,8267	0,002978
	Error		5,92293	22	0,26922				
6 (10.04.19)	Intercept	Fixed	57,10172	1	57,10172	3,11003	0,154176	370,3676	0,000245
	Parallel (Temp)	Random	0,45566	3	0,15189	23,00000	0,895438	0,1696	0,915793
	Temperature	Fixed	24,27665	2	12,13833	3,05260	0,152988	79,3417	0,002336
	Error		20,59508	23	0,89544				
7 (24.04.19)	Intercept	Fixed	71,68566	1	71,68566	3,10190	0,135584	528,7170	0,000144
	Parallel (Temp)	Random	0,40225	3	0,13408	21,00000	0,403963	0,3319	0,802344
	Temperature	Fixed	36,14564	2	18,07282	3,04998	0,134824	134,0478	0,001066
	Error		8,48323	21	0,40396				
8 (15.05.19)	Intercept	Fixed	69,20204	1	69,20204	3,43061	3,006611	23,01662	0,012598
	Parallel (Temp)	Random	9,41004	3	3,13668	23,00000	1,885106	1,66393	0,202477
	Temperature	Fixed	15,01792	2	7,50896	3,19929	3,071997	2,44433	0,226823
	Error		43,35744	23	1,88511				

Table II - 193: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for plasma 11-KT levels between temperature groups within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling (date)	(°C)	{1}	{2}	{3}
1 (31.10.18)	8		0,483615	0,356205
	12	0,483615		0,967114
	18	0,356205	0,967114	
2 (14.12.18)	8		0,146431	0,968520
	12	0,146431		0,208570
	18	0,968520	0,208570	
3 (01.02.19)	8		0,882272	0,046762
	12	0,882272		0,106599
	18	0,046762	0,106599	
4 (13.03.19)	8		0,008552	0,133974
	12	0,008552		0,000160
	18	0,133974	0,000160	
5 (27.03.19)	8		0,557763	0,003632
	12	0,557763		0,058838
	18	0,003632	0,058838	
6 (10.04.19)	8		0,190863	0,000212
	12	0,190863		0,010584
	18	0,000212	0,010584	
7 (24.04.19)	8		0,732788	0,000140
	12	0,732788		0,000140
	18	0,000140	0,000140	
8 (15.05.19)	8		0,185884	0,020240
	12	0,185884		0,737961
	18	0,020240	0,737961	

Table II - 194: *Test results of* ***Tukey HSD post-hoc tests*** *of difference for plasma 11-KT levels between temperature parallel groups, within the full feeding group (100%) (Sampling 1-8). Significant differences (p < 0.05) are highlighted in red.*

Sampling	Parallel	(°C)	{1}	{2}	{3}	{4}	{5}	{6}
1 (31.10.18)	1	8		0,290540	0,417516	0,417516	0,669715	0,133282
	2	8	0,290540		0,999713	0,999713	0,973662	0,994094
	1	12	0,417516	0,999713		1,000000	0,996995	0,961483
	2	12	0,417516	0,999713	1,000000		0,996995	0,961483
	1	18	0,669715	0,973662	0,996995	0,996995		0,801384
	2	18	0,133282	0,994094	0,961483	0,961483	0,801384	
2 (14.12.18)	1	8		0,999974	0,054189	0,999974	0,997701	0,927646
	2	8	0,999974		0,069107	1,000000	0,989068	0,967509
	1	12	0,054189	0,069107		0,069107	0,029369	0,198329
	2	12	0,999974	1,000000	0,069107		0,989068	0,967509
	1	18	0,997701	0,989068	0,029369	0,989068		0,744879
	2	18	0,927646	0,967509	0,198329	0,967509	0,744879	
3 (01.02.19)	1	8		0,999715	0,999036	1,000000	0,418390	0,616197
	2	8	0,999715		0,985602	0,999454	0,291329	0,459939
	1	12	0,999036	0,985602		0,999454	0,616197	0,811677
	2	12	1,000000	0,999454	0,999454		0,438864	0,639120
	1	18	0,418390	0,291329	0,616197	0,438864		0,999036
	2	18	0,616197	0,459939	0,811677	0,639120	0,999036	
4 (13.03.19)	1	8		1,000000	0,066524	0,497946	0,703540	0,764429
	2	8	1,000000		0,076222	0,538282	0,663657	0,726872
	1	12	0,066524	0,076222		0,797040	0,001503	0,001926
	2	12	0,497946	0,538282	0,797040		0,026305	0,033702
	1	18	0,703540	0,663657	0,001503	0,026305		0,999997
	2	18	0,764429	0,726872	0,001926	0,033702	0,999997	
5 (27.03.19)	1	8		0,999579	0,991227	0,899676	0,182634	0,054163
	2	8	0,999579		0,999273	0,978221	0,304710	0,101464
	1	12	0,991227	0,999273		0,999976	0,798008	0,519493
	2	12	0,899676	0,978221	0,999976		0,655620	0,287928
	1	18	0,182634	0,304710	0,798008	0,655620		0,987701
	2	18	0,054163	0,101464	0,519493	0,287928	0,987701	
6 (10.04.19)	1	8		1,000000	0,708590	0,826147	0,006590	0,028978
	2	8	1,000000		0,750064	0,861253	0,007877	0,034323
	1	12	0,708590	0,750064		0,999715	0,217769	0,525889
	2	12	0,826147	0,861253	0,999715		0,095261	0,301878
	1	18	0,006590	0,007877	0,217769	0,095261		0,985857
	2	18	0,028978	0,034323	0,525889	0,301878	0,985857	
7 (24.04.19)	1	8		0,999969	0,999977	0,919922	0,000303	0,000778
	2	8	0,999969		1,000000	0,945134	0,000174	0,000271
	1	12	0,999977	1,000000		0,965094	0,000249	0,000563
	2	12	0,919922	0,945134	0,965094		0,000154	0,000176
	1	18	0,000303	0,000174	0,000249	0,000154		0,991846
	2	18	0,000778	0,000271	0,000563	0,000176	0,991846	
8 (15.05.19)	1	8		0,999999	1,000000	0,462375	0,864236	0,121320
	2	8	0,999999		0,999999	0,352566	0,784286	0,069077
	1	12	1,000000	0,999999		0,694793	0,949344	0,339583
	2	12	0,462375	0,352566	0,694793		0,958142	0,973517
	1	18	0,864236	0,784286	0,949344	0,958142		0,549214
	2	18	0,121320	0,069077	0,339583	0,973517	0,549214	

Table II - 195: *Test results of **one-way random effects nested ANOVAs** for plasma 11-KT levels within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	Effect (F/R)	SS	DF	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
8	Intercept	Fixed	13,44366	1	13,44366	10,39715	0,017280	778,0041	0,000000
	Parallel (time)	Random	0,12707	8	0,01588	49,00000	0,045416	0,3497	0,941423
	Time	Fixed	3,80636	7	0,54377	8,29734	0,016073	33,8316	0,000019
	Error		2,22539	49	0,04542				
12.5	Intercept	Fixed	27,31926	1	27,31926	9,09980	0,551724	49,51619	0,000057
	Parallel (time)	Random	4,39661	8	0,54958	44,00000	0,585923	0,93797	0,495589
	Time	Fixed	11,08031	7	1,58290	8,15972	0,549912	2,87846	0,079158
	Error		25,78060	44	0,58592				
18	Intercept	Fixed	116,5927	1	116,5927	9,69447	0,771895	151,0473	0,000000
	Parallel (time)	Random	6,0508	8	0,7563	53,00000	0,967523	0,7817	0,620580
	Time	Fixed	93,6610	7	13,3801	8,22639	0,758642	17,6370	0,000241
	Error		51,2787	53	0,9675				

Table II - 196: *Test results of **Tukey HSD post-hoc tests** of difference for plasma 11-KT levels within temperature groups through time in the full feeding group (100%). Significant effects (p < 0.05) are highlighted in red.*

(°C)	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
8	1		0,999999	1,000000	0,874583	1,000000	0,948412	0,000160	0,000848
	2	0,999999		0,999992	0,758431	0,999998	0,867948	0,000143	0,000449
	3	1,000000	0,999992		0,902641	1,000000	0,964279	0,000168	0,001050
	4	0,874583	0,758431	0,902641		0,781746	0,999984	0,001259	0,021484
	5	1,000000	0,999998	1,000000	0,781746		0,894142	0,000134	0,000202
	6	0,948412	0,867948	0,964279	0,999984	0,894142		0,000276	0,004764
	7	0,000160	0,000143	0,000168	0,001259	0,000134	0,000276		0,982784
	8	0,000848	0,000449	0,001050	0,021484	0,000202	0,004764	0,982784	
12.5	1		0,999997	0,999995	0,908802	0,991429	0,244657	0,940496	0,001955
	2	0,999997		1,000000	0,982688	0,999558	0,462086	0,990635	0,007557
	3	0,999995	1,000000		0,977235	0,999440	0,395312	0,987757	0,004311
	4	0,908802	0,982688	0,977235		0,999832	0,855341	1,000000	0,016912
	5	0,991429	0,999558	0,999440	0,999832		0,655240	0,999968	0,008820
	6	0,244657	0,462086	0,395312	0,855341	0,655240		0,830098	0,344219
	7	0,940496	0,990635	0,987757	1,000000	0,999968	0,830098		0,016746
	8	0,001955	0,007557	0,004311	0,016912	0,008820	0,344219	0,016746	
18	1		1,000000	0,999772	1,000000	0,573641	0,000552	0,000138	0,000397
	2	1,000000		0,999977	1,000000	0,672036	0,000832	0,000142	0,000603
	3	0,999772	0,999977		0,999974	0,873752	0,002516	0,000165	0,001860
	4	1,000000	1,000000	0,999974		0,522419	0,000196	0,000135	0,000161
	5	0,573641	0,672036	0,873752	0,522419		0,035368	0,000581	0,027313
	6	0,000552	0,000832	0,002516	0,000196	0,035368		0,746638	1,000000
	7	0,000138	0,000142	0,000165	0,000135	0,000581	0,746638		0,625616
	8	0,000397	0,000603	0,001860	0,000161	0,027313	1,000000	0,625616	

d. Comparison of temperature groups between feeding regimes

Table II - 197: *Test results of **factorial ANOVAs** for plasma 11-KT levels comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Effect	SS	DF	MS	F	P
8	Intercept	25,38080	1	25,38080	781,3488	0,000000
	Feeding regime	0,01377	1	0,01377	0,4240	0,516282
	Time	7,77180	7	1,11026	34,1793	0,000000
	Feed*Time	0,12419	7	0,01774	0,5462	0,797778
	Error	3,70310	114	0,03248		
12.5	Intercept	43,56285	1	43,56285	146,7949	0,000000
	Feeding regime	2,42182	1	2,42182	8,1609	0,005152
	Time	16,95509	7	2,42216	8,1620	0,000000
	Feed*Time	5,20591	7	0,74370	2,5061	0,020100
	Error	31,45657	106	0,29676		
18	Intercept	165,9986	1	165,9986	167,0444	0,000000
	Feeding regime	5,4463	1	5,4463	5,4807	0,020878
	Time	125,0423	7	17,8632	17,9757	0,000000
	Feed*Time	7,4061	7	1,0580	1,0647	0,390467
	Error	119,2487	120	0,9937		

Table II - 198: *Test results of **Tukey HSD post-hoc tests** for plasma 11-KT levels comparing corrsponding temperature groups through time between feeding regimes. Significant effects (p < 0.05) are highlighted in red.*

(°C)	Feed. (%)	Samp.	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
8	67	1		1,00	1,00	0,25	1,00	0,10	0,00	0,00	1,00	1,00	0,99	0,20	0,99	0,31	0,00	0,00
		2	1,00		1,00	0,88	1,00	0,68	0,00	0,00	1,00	1,00	1,00	0,81	1,00	0,92	0,00	0,00
		3	1,00	1,00		0,97	1,00	0,83	0,00	0,00	1,00	1,00	1,00	0,93	1,00	0,98	0,00	0,00
		4	0,25	0,88	0,97		0,89	1,00	0,00	0,00	0,99	0,94	0,99	1,00	0,95	1,00	0,00	0,00
		5	1,00	1,00	1,00	0,89		0,63	0,00	0,00	1,00	1,00	1,00	0,82	1,00	0,93	0,00	0,00
		6	0,10	0,68	0,83	1,00	0,63		0,01	0,00	0,91	0,76	0,94	1,00	0,76	1,00	0,00	0,01
		7	0,00	0,00	0,00	0,00	0,00	0,01		1,00	0,00	0,00	0,00	0,01	0,00	0,00	1,00	1,00
		8	0,00	0,00	0,00	0,00	0,00	0,00	1,00		0,00	0,00	0,00	0,00	0,00	0,00	1,00	1,00
	100	1	1,00	1,00	1,00	0,99	1,00	0,91	0,00	0,00		1,00	1,00	0,97	1,00	0,99	0,00	0,00
		2	1,00	1,00	1,00	0,94	1,00	0,76	0,00	0,00	1,00		1,00	0,88	1,00	0,96	0,00	0,00
		3	0,99	1,00	1,00	0,99	1,00	0,94	0,00	0,00	1,00	1,00		0,98	1,00	1,00	0,00	0,00
		4	0,20	0,81	0,93	1,00	0,82	1,00	0,01	0,00	0,97	0,88	0,98		0,90	1,00	0,00	0,01
		5	0,99	1,00	1,00	0,95	1,00	0,76	0,00	0,00	1,00	1,00	1,00	0,90		0,98	0,00	0,00
		6	0,31	0,92	0,98	1,00	0,93	1,00	0,00	0,00	0,99	0,96	1,00	1,00	0,98		0,00	0,00
		7	0,00	0,00	0,00	0,00	0,00	0,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00		1,00
		8	0,00	0,00	0,00	0,00	0,00	0,01	1,00	1,00	0,00	0,00	0,00	0,01	0,00	0,00	1,00	
12.5	67	1		1,00	1,00	1,00	1,00	0,97	0,98	0,67	1,00	1,00	1,00	0,88	1,00	0,06	0,93	0,00
		2	1,00		1,00	1,00	1,00	1,00	1,00	0,83	1,00	1,00	1,00	0,97	1,00	0,10	0,99	0,00
		3	1,00	1,00		1,00	1,00	1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	0,31	1,00	0,00
		4	1,00	1,00	1,00		1,00	1,00	1,00	0,90	1,00	1,00	1,00	0,99	1,00	0,09	1,00	0,00
		5	1,00	1,00	1,00	1,00		1,00	1,00	0,99	1,00	1,00	1,00	1,00	1,00	0,25	1,00	0,00
		6	0,97	1,00	1,00	1,00	1,00		1,00	1,00	0,98	1,00	1,00	1,00	1,00	0,70	1,00	0,00
		7	0,98	1,00	1,00	1,00	1,00	1,00		1,00	0,99	1,00	1,00	1,00	1,00	0,79	1,00	0,00
		8	0,67	0,83	0,99	0,90	0,99	1,00	1,00		0,72	0,95	0,93	1,00	1,00	0,98	1,00	0,00
	100	1	1,00	1,00	1,00	1,00	1,00	0,98	0,99	0,72		1,00	1,00	0,92	1,00	0,06	0,96	0,00
		2	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,95	1,00		1,00	1,00	1,00	0,24	1,00	0,00
		3	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,93	1,00	1,00		0,99	1,00	0,17	1,00	0,00
		4	0,88	0,97	1,00	0,99	1,00	1,00	1,00	1,00	0,92	1,00	0,99		1,00	0,84	1,00	0,00
		5	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00		0,51	1,00	0,00
		6	0,06	0,10	0,31	0,09	0,25	0,70	0,79	0,98	0,06	0,24	0,17	0,84	0,51		0,80	0,13
		7	0,93	0,99	1,00	1,00	1,00	1,00	1,00	1,00	0,96	1,00	1,00	1,00	1,00	0,80		0,00
		8	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13	0,00	
18	67	1		1,00	1,00	1,00	1,00	0,08	0,06	0,30	1,00	1,00	1,00	1,00	0,90	0,00	0,00	0,00
		2	1,00		1,00	1,00	1,00	0,09	0,08	0,37	1,00	1,00	1,00	1,00	0,95	0,00	0,00	0,00
		3	1,00	1,00		1,00	1,00	0,11	0,09	0,41	1,00	1,00	1,00	1,00	0,96	0,00	0,00	0,00
		4	1,00	1,00	1,00		1,00	0,04	0,03	0,23	1,00	1,00	1,00	1,00	0,92	0,00	0,00	0,00
		5	1,00	1,00	1,00	1,00		0,40	0,35	0,88	1,00	1,00	1,00	1,00	1,00	0,01	0,00	0,01
		6	0,08	0,09	0,11	0,04	0,40		1,00	1,00	0,06	0,09	0,21	0,02	0,89	0,99	0,24	0,99
		7	0,06	0,08	0,09	0,03	0,35	1,00		1,00	0,05	0,07	0,18	0,02	0,85	1,00	0,28	1,00
		8	0,30	0,37	0,41	0,23	0,88	1,00	1,00		0,26	0,35	0,61	0,15	1,00	0,69	0,03	0,66
	100	1	1,00	1,00	1,00	1,00	1,00	0,06	0,05	0,26		1,00	1,00	1,00	0,89	0,00	0,00	0,00
		2	1,00	1,00	1,00	1,00	1,00	0,09	0,07	0,35	1,00		1,00	1,00	0,94	0,00	0,00	0,00
		3	1,00	1,00	1,00	1,00	1,00	0,21	0,18	0,61	1,00	1,00		1,00	0,99	0,01	0,00	0,00
		4	1,00	1,00	1,00	1,00	1,00	0,02	0,02	0,15	1,00	1,00	1,00		0,86	0,00	0,00	0,00
		5	0,90	0,95	0,96	0,92	1,00	0,89	0,85	1,00	0,89	0,94	0,99	0,86		0,10	0,00	0,08
		6	0,00	0,00	0,00	0,00	0,01	0,99	1,00	0,69	0,00	0,00	0,01	0,00	0,10		0,97	1,00
		7	0,00	0,00	0,00	0,00	0,00	0,24	0,28	0,03	0,00	0,00	0,00	0,00	0,00	0,97		0,92
		8	0,00	0,00	0,00	0,00	0,01	0,99	1,00	0,66	0,00	0,00	0,00	0,00	0,08	1,00	0,92	