Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

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Effects of light and temperature regimes on the sexual maturation of male lumpfish (*Cyclopterus lumpus*).

Master's thesis in Marine Costal Development
Supervisor: Elin Kjørsvik, Frank Thomas Mlingi
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NTNU
Kunnskap for ei betre verd

Abstract

Nowadays salmon lice (*Lepeophtheirus salmonis*) constitutes one of the biggest threats to the welfare of farmed Atlantic salmon (*Salmo salar*). Lumpfish (*Cyclopterus lumpus*) are currently deployed in sea cages with salmon as an effective biological treatment against salmon lice. However most of the lumpfish used nowadays come from a wild caught broodstock, therefore there is a need to establish a captive broodstock to supply the demand for lumpfish in farms all year around and to alleviate the pressure on the wild lumpfish population.

This thesis was part of a pilot experiment running from September 2017 to March 2018 which aimed at investigating the biological mechanisms of sexual maturation in lumpfish. Lumpfish were reared under continuous daylight (CDL) for 18 months prior to the experiment and then split in four groups. Two groups were kept at CDL for the whole duration of the pilot experiment while the other two were exposed to short daylight (SDL) for 4 months before switching back to CDL. In addition, the water temperature in one group for each treatment (SDL0T and CDL0T) was kept ambient while the others had ambient +3°C (SDL3T and CDL3T). Temperature manipulation was done in late January 2018 when the photoperiod in all groups was changed to CDL.

Sexual maturation in males was investigated by estimating both the gonadosomatic index (GSI) and the spermatogenic maturity index (SMI). Sexual maturation in lumpfish did not appear to be significantly affected by photoperiod or temperature manipulation within this experiment. Water temperature was found to have an effect on the last stages of spermatogenesis in lumpfish.

Acknowledgements

This Master's thesis was written at the Department of Biology, NTNU Trondheim. This was part of the pilot experiment for the CycloBreed project (#901418) which is funded by FHF (The Norwegian Seafood Research Fund) and it is currently being conducted by NTNU in partnership with AquaGen AS and Nofima AS. The samplings were performed at the Nofima AS facility in Tromsø and the histological analyses were performed at the NTNU Centre of Fishery and Aquaculture (SeaLab).

First and foremost, I would like to express my gratitude to my supervisors Elin Kjørsvik and Frank Thomas Mlingi for their guidance and feedback which not only made this thesis possible but an exciting journey. I also want to give a special thanks to Tora Bardal for her precious help in the lab and with the digital slide scanner.

Finally, I'd like to thank my sister Claudia, for her invaluable help with SPSS without which I'd still be stuck on YouTube tutorials.

Ai miei genitori, per avermi sostenuto in tutti questi anni, per aver creduto in me e a questo mio sogno Norvegese, per aver appoggiato la mia scelta di un secondo Master e soprattutto, per non avere mai dubitato di quel bambino che leggeva i libri di storia naturale sognando di diventare uno scenziato. Vi voglio bene. Grazie.

Trondheim, May 2019

Emanuele Guercini

Table of content

Abbreviations

BPG axis: Brain – pituitary – gonad axis**.**

CDL: Continuous daylight

EA: Excluded area

FSH: Follicle – stimulating hormone

GTHs**:** Gonadotropins

GnRH: Gonadotropin – releasing hormone

GSI: Gonadosomatic index

LH: Luteinizing – Hormone

MIS: Maturation – inducing steroids

SG: Spermatogonia

SC: Spermatocytes

ST: Spermatids

SZ: Spermatozoa

SDL: Short daylight

SDL/CDL-3T: ambient water temperature +3°C

SDL/CDL)-0T: ambient water temperature

SMI: Spermatogenic maturity index

Ts: Testicular somatic cells

VTG: Vitellogenin

Introduction

Role of lumpfish in aquaculture

Salmonid aquaculture is globally threatened by sea lice, particularly the *Caligus ssp.* and the salmon lice (L*epeophtheirus salmonis*). The cost of treatments against salmon lice is a considerable limiting factor in Norwegian aquaculture [1] reaching US$ 436 million in 2011, which amounted approximately to 9% of the production value [2]. The use of cleaner fish is nowadays the only environmentally friendly de–lousing treatment [3, 4]. Specifically, the ballan wrasse (*Labrus bergylta)* and the lumpfish (*Cyclopterus lumpus)* have been successfully implemented in aquaculture due to their ability to prey upon salmon lice and therefore reduce lice infection within the salmon pens [5-8]. Lumpfish in particular fares better in the colder Norwegian waters temperature due its wide range of temperature tolerance [9] as opposed to ballan wrasse [10]. The production and exploitation of lumpfish as cleaner fish has increased considerably in the latest years. In 2017, 29723 lumpfish individuals were deployed in farms throughout Norway as reported from the Norwegian directorate of fisheries [11]. However, lumpfish production nowadays still relies on wild caught broodstock [12, 13]. Hence, there is a need to make lumpfish production more sustainable, to close the production cycle by creating a captive lumpfish broodstock and eventually produce fertilized lumpfish eggs all year round. Therefore, furthering our knowledge on the lumpfish reproductive biology is paramount to fully understand the mechanisms which control it.

This thesis in part of a pilot experiment within the CycloBreed project. CycloBreed was funded by FHF (The Norwegian Seafood Research Fund, Project #901418) and it is currently being conducted by NTNU in partnership with AquaGen AS and Nofima AS. One of the main objectives of CycloBreed is to acquire the knowledge on lumpfish reproductive biology by studying gonad development under different photoperiod and temperature treatments. Successful manipulation of the lumpfish sexual maturation could help in creating a captive broodstook.

Lumpfish reproductive biology

Lumpfish have a wide geographical distribution ranging from the Barents Sea to the Adriatic Sea in the Mediterranean basin [14]. They are a semipelagic species which perform seasonal breeding migrations shoreward [15, 16]. In nature adult lumpfish prefer temperature ranges between 4 – 7°C [17], although spawning happens in shallow waters when temperature is around 4°C [18, 19]. Lumpfish are determined batch spawners, producing no more than 2 batches of eggs per spawning season. This season is thought to last from late March until July [15, 16, 20, 21] although there are evidence of August spawning, thus the actual length is currently unknown [20].

In nature lumpfish are thought to reach sexual maturation when 5 – 6 years old, although males might spawn at the age of 4 years [15, 16]. Sexual dimorphism is highlighted during the spawning season when male exhibit a red or pinkish pigmentation while females display a blue – green colouration [15, 16, 22-24]. After spawning males guard the eggs for 6 – 10 weeks [16] providing parental care behaviours such as pectoral fanning, and the expelling of water from the mouth towards the egg mass [22].

Sexual maturation in temperate fish

Fish reproductive cycle is regulated by a hormonal cascade along the brain – pituitary – gonad axis (BPG – axis) (Figure 1) and a range of internal and external factors, for example fish growth and adiposity or temperature and photoperiod [25]. The brain integrates internal and external factors responding with neuroendocrine signals. The gonadotropin – releasing hormone (GnRH) is a neurohormone synthesized in the hypothalamus which acts directly on the pituitary gland in turn triggering the release of pituitary gonadotropins (GTHs): the follicle – stimulating hormone (FSH) and luteinizing – hormone (LH). These are released in the blood and play a major role in controlling gametogenesis upon reaching the fish gonads. Specifically, fish gonads are the endocrine organ responsible for the synthesis of sex steroid hormones. Steroidogenesis takes place in the somatic cells of the gonads: the granulosa and theca cells in the ovary and the interstitial Leydig cells and Sertoli cells in the testes. The major hormones responsible in the regulation of fish gametogenesis are estrogen E_2 in females and the androgen 11-KT in males, stimulated by FSH. The E_2 plays an additional role in females by stimulating the synthesis of VTG (Vitellogenin) from the liver. Sex steroids also regulate GHTs release exerting positive or negative feedback on BPG – axis. Final oocyte maturation (FOM) in females and sperm maturation in males are regulated by LH, secreted from the pituitary gland, which in turns stimulates the secretion of the maturation – inducing steroids (MIS). Both LH and MIS are important for the final gonadal maturation. Eventually ovulation in the females and spermiation in males are triggered by the interplay of MIS and LH [26-29].

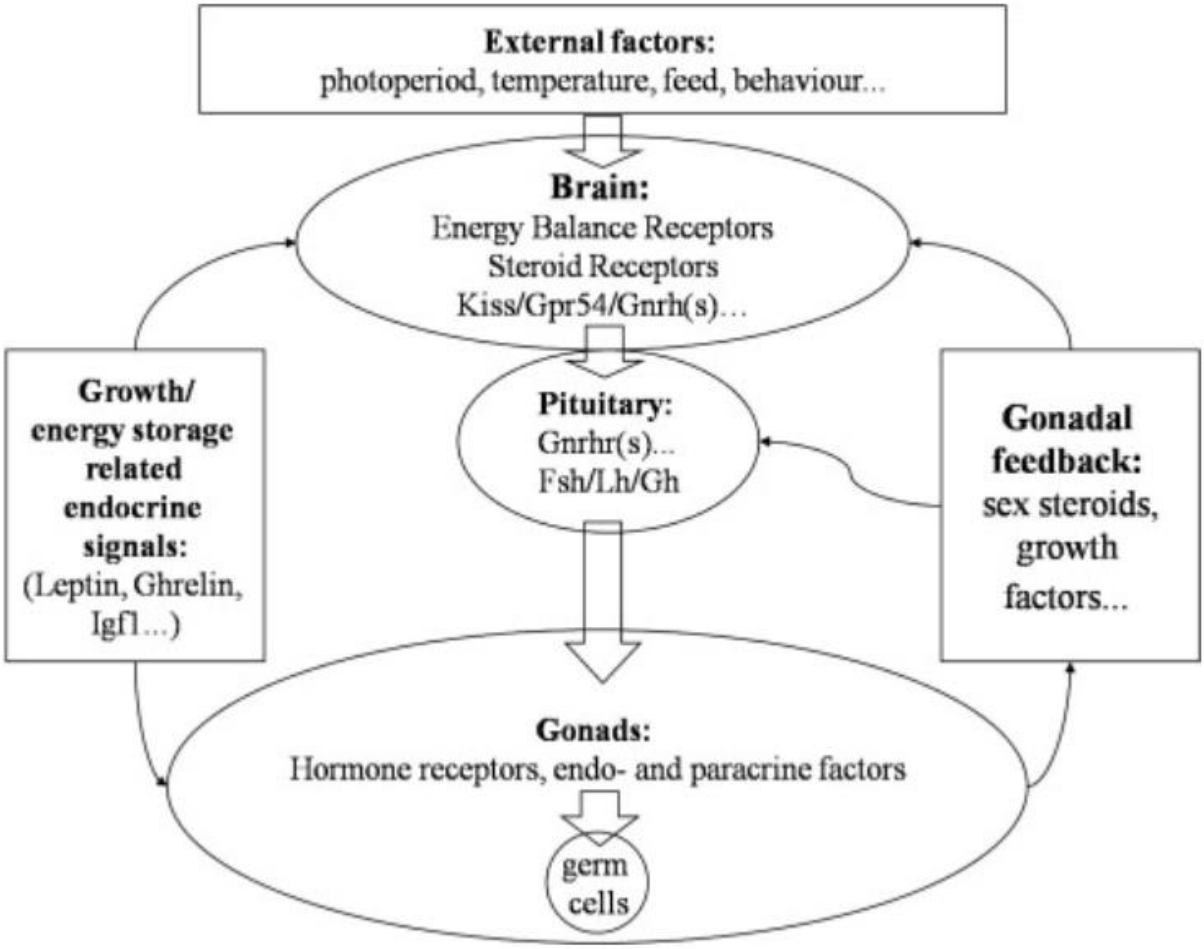


Figure 1. External factors are integrated in the fish brain, causing it to produce GnRH. This in turn stimulates the production of gonadotropins from the pituitary gland which enter the blood stream and after binding to the gonad receptors regulate gametogenesis [25].

The reproduction cycle of fish is regulated and synchronised by the seasonal environmental variation in relation to food availability and local climatic conditions. Factors responsible for sexual maturation in fish can be classified into ultimate and proximate factors. Proximate factors provide seasonal cues, such as the change in daylight (photoperiod) for reproduction whereas the ultimate factors are those determining the optimal reproductive timing (prey availability for the offspring in combination with temperatures conditions) [25].

In temperate regions, the variation of photoperiod and/or temperature are the main environmental cues triggering sexual maturation in fish [25, 30]. Especially changes in photoperiod have been regarded as the key proximate factor controlling reproduction and spawning [30], for it acts on the fish annual endogenous rhythms controlling a "gating" mechanisms or a "critical time window" [25] during which sexual maturation can start or continue depending on the physiological state of the fish (for example stage of gonadal development) [25, 31].

Moreover, it is the change of photoperiod, rather than the actual day length which is responsible for triggering sexual maturation in rainbow trout [29, 30].

Water temperature plays a direct role throughout the sexual maturation of fish because it regulates the physiological endocrine processes [25].

Changes from optimal temperature during sexual maturation can impair gametogenesis [29]. Particularly, too high temperature ranges have been linked to inhibition of sperm release in Atlantic salmon (*Salmo salar*) [32], impaired spermatogenesis [33] and reduced egg viability in rainbow trout (*Oncorhynchus mykiss*) [34]. However, water temperature has also been shown to play an important role as proximate factor for sexual maturity in many fish species [29]. Cold water allows and/or advances spawning in Atlantic salmon and in sea bass (*Dicentrarchus labrax*) [35, 36].

Manipulation of sexual maturation

Environmental manipulation can be used to achieve spawning outside the fish natural spawning season by mimicking the natural condition, through a complete cycle of environmental changes [29].

Photoperiod manipulation alters the timing of sexual maturation, whereas the water temperature dictates the final maturation and ovulation as shown in female rainbow trout. Specifically rainbow trout has the ability to delay the timing of the final maturation and ovulation when temperatures are significantly different from optimal range, although exposure to significantly different temperature regimes has an impact on egg quality [34]. Therefore, to obtain an out-of-season spawning both photoperiod and temperature should be manipulated [29].

Photoperiod manipulation comprises of changes in light regimes (Figure 2) to artificially shorten daylight (SDL) or artificially lengthen daylight. Sometimes the light regime used in photoperiod manipulation is continuous light (CDL). There are several ways of manipulating the photoperiod. A phase shifted photoperiod, which consists in the displacing of one year by certain number of months, will lead to a shift in maturation and spawning compared to natural conditions. A compressed photoperiod, which is compressing one year in less than 12 months, will advance maturation whereas an expanded photoperiod, which is when year is expanded to more than 12 months, will delay maturation.

Exposure of fish to CDL for a long period of time has been shown to delay sexual maturation in favour to somatic growth in Atlantic cod (*Gadus morhua*) [37, 38], European sea bass (*Dicentrarchus labrax*) [36, 39] in Atlantic salmon (*Salmo salar*) [40, 41] and Atlantic halibut (*Hippoglossus hippoglossus*) [42].

On the other hand, the change in photoperiod from long to short day light (SDL - autumn signal) or from short to long (spring signal) provides the cue for fish to commit to sexual maturation [25, 43-47].

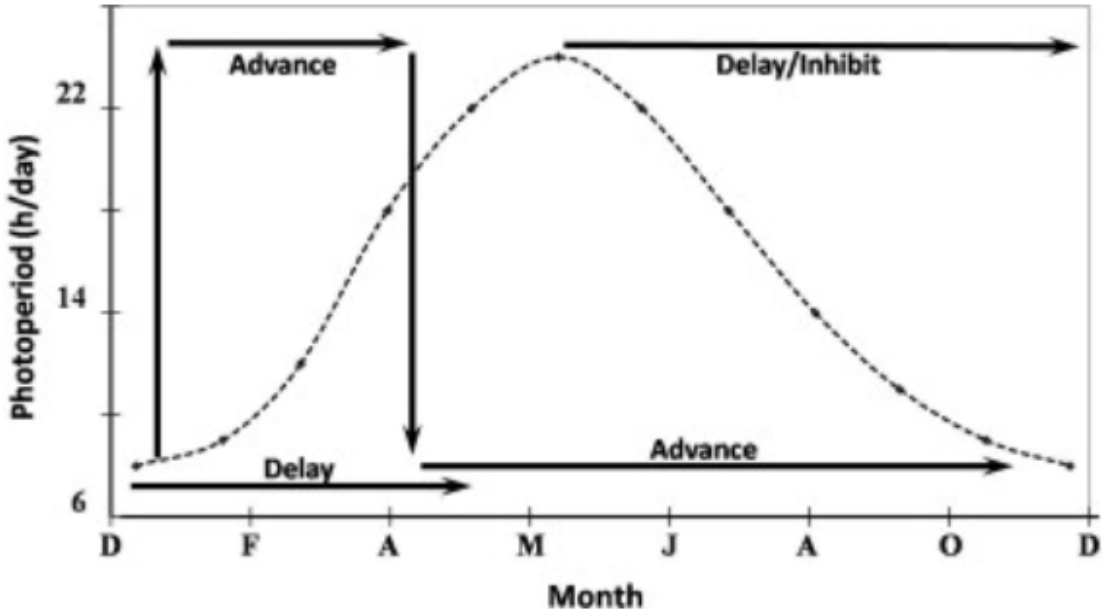


Figure 2. Effects of photoperiod manipulation on sexual maturation in salmonids. The dotted line represents the annual natural photoperiod cycle at high latitudes, and arrows represent artificial changes in photoperiod which affect timing of maturation [25].

Spermatogenesis in fish

Spermatogenesis is the process in which diploid cells (spermatogonia) proliferate and differentiate eventually giving rise to the mature spermatozoa [48]. Prior to spermatogenesis the immature testes contain spermatogonial stem cells which proliferate by mitotic division. At the start of spermatogenesis (phase I), some spermatogonial stem cells commit to produce spermatogonia. Every spermatogonium goes through several cycles of mitotic divisions, during which, the cytokinesis is incomplete and daughter cells maintain direct cytoplasmic bridges between them. This in turn creates a cluster which takes the name spermatocyst. All the cells within the cluster come from a single clone of the original spermatogonium. These cysts are enveloped by Sertoli cells which separate the different spermatocysts. Spermatogonia within the cysts slowly divide giving rise to spermatogonia – A [29]. Specifically, undifferentiated spermatogonia – A generates differentiated spermatogonia – A which share the same morphological characteristics but have reduced self – renewal potential than the former. Subsequently, the differentiated spermatogonia – A give rise to the rapidly dividing spermatogonia – B, thus irreversibly committing to sexual maturation (Figure 3) [49]. The product of the last mitotic division of spermatogonia – B, are called primary spermatocytes, which are the cells that will enter meiosis (phase II) [29]. The primary spermatocytes are divided in leptotene/zygotene, pachytene and diplotene primary spermatocytes. They are characterized by their nucleus morphology and size, observable from light microscope analyses. Pachytene spermatocytes are the most common stage due their relatively longer duration [49]. Primary spermatocytes undergo the first meiosis comprising the DNA duplication and recombination of the genetic information. This will lead to the formation of the secondary spermatocytes [29]. Secondary spermatocytes are rare for they quickly enter in meiosis II [49] without DNA replication, thus forming haploid cells called spermatids [29]. Three types of spermatids can be classified based on their nuclear condensation: early spermatids "E1", intermediate spermatids "E2" and final spermatids "E3" [49]. Spermiogenesis is the last phase (phase III) in which the spermatids differentiate into flagellated spermatozoa. During spermiogenesis there is a drastic reduction size of approximately 80% due to nucleus condensation and extrusion of cytoplasmic content to the Sertoli cells [29]. At the end of spermiogenesis, the intercellular bridges are broken and the spermatozoa are released in the lumen due to a dynamic change in the junctional complex between Sertoli cells [49]. Several germ stages can be present in the testes at any given time, ranging from immature spermatogonia to spermatozoa. Whereas at full spermiation (spawning period) the testes are usually mostly occupied by spermatozoa, in the early season higher percentage of less mature germ stages is present [29].

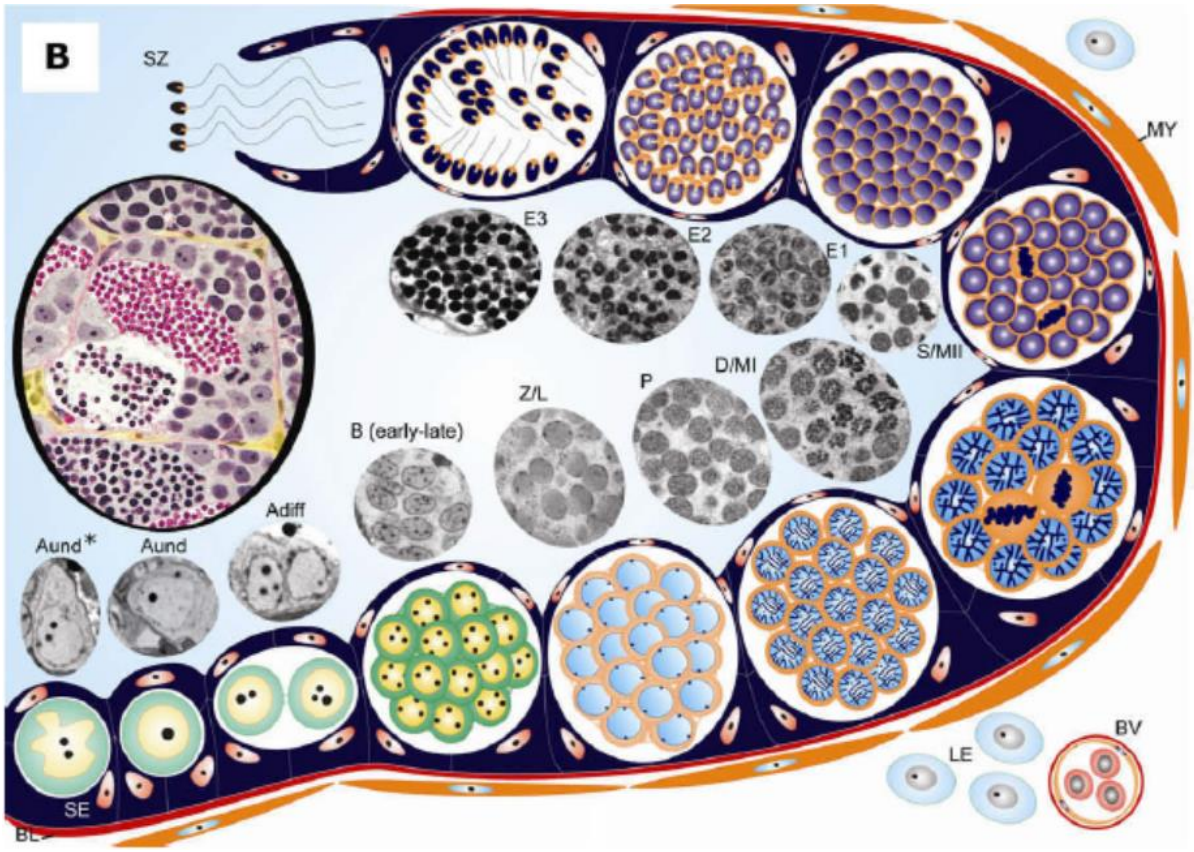


Figure 3. Spermatogenesis in zebrafish. In this illustration, the three stages of "Type A" spermatogonia (SG) are defined as "Aund*" for undifferentiated stem cells, "Aund" for undifferentiated spermatogonia and "Adiff" for differentiated spermatogonia. "Type B" spermatogonia is represented by "B (early–late)". Spermatocytes (SC) are divided in: Leptotenic/zygotenic primary spermatocytes "L/Z", pachytenic primary spermatocytes "P" and diplotenic spermatocytes/metaphase I "D/MI". The secondary spermatocytes/metaphase II are labelled "S/MII". Spermatids (ST) are divided into early, intermediate and final spermatids "E1, E2, and E3". Spermatozoa "SZ". Sertoli cells are labelled as "SE", Leydidi cells as "LE" blood vessel as "BV" basal lamina as "BL" and the peritubular myoid cells as "MY".

Aims and Hypothesis.

The overall aim of the CycloBreed project is to acquire the necessary knowledge on the lumpfish reproductive biology to establish a captive broodstock and a captive broodstock management protocol. This in turn will lead to the production of fertilized lumpfish eggs all year around and eventually reduce the pressure on wild lumpfish population.

This thesis was part of a pilot experiment within CycloBreed which aimed at investigating whether lumpfish would respond to photoperiod and temperature manipulation during the time of sexual maturation and how.

Within this pilot experiment, the aim of this thesis was to investigate and stage spermatogenesis in male lumpfish, and to investigate the effects of photoperiod and temperature manipulation on testis development. The following hypotheses were postulated:

"*Short daylight (SDL) exposure will induce earlier sexual maturation in male lumpfish compared to continuous daylight (CDL)*".

"*Increased water temperature will delay final sexual maturation in male lumpfish compared to ambient temperature*"

In this thesis, lumpfish sexual maturation was assessed with the gonadosomatic index (GSI) combined with histological analysis of the testes.

Materials and Methods

Experimental fish

Lumpfish were reared from larvae and grown for 18 months (April 2016 – September 2017) at the Nofima facility in Tromsø at ambient water temperature (4 – 9°C) and continuous day light photoperiod (CDL, L:D = 24:0 hours).

Experimental Settings

In September 2017, 300 18 months old lumpfish were transferred into four 1500 l tanks (A1 – A4). The fish density was 75 fish per tank comprising a ratio of 1:1 between male and female individuals. The temperature within the four tanks was kept ambient between 4 – 9°C and fish were exposed to continuous day light (CDL). Photoperiod was manipulated from the end of September 2017, dividing the four tanks in two groups: Photoperiod in tanks A1 and A2 was changed to short day light (SDL, L:D = 8:16 hours), while the photoperiod in tanks A3 and A4 was kept at CDL. Temperature in all tanks was kept ambient (4 – 9°C) until the end of January 2018. The photoperiod in all tanks was then set to CDL, and the water temperature of A1 and A3 was increased to ambient +3°C, while in A2 and A4 it was kept ambient. These final settings were kept until the end of the experiment in March 2018.

Sampling

A total of 4 sampling days were planned for this experiment. The fist sampling occurred on the 21st of September 2018, when the lumpfish were transferred in the four tanks. Two fish (from each tank) were sampled, belonging to the "Initial" condition. The second sampling was set on the 17th of January 2018, before the photoperiod was changed back to continuous day light in all tanks and before the temperature was increased. Lumpfish sampled in January belonged to SDL and CDL treatments for tanks A1, A2 and A3, A4 respectively. Four fish from each tank (8 per treatment) were sampled. The last samplings took place on the 6th of February and on the 7th of March 2018 (Data from lumpfish in A1 and A2 was labelled SDL3T and SDL0T respectively while A3 and A4 was labelled CDL3T and CDL0T respectively). Eight fish from each tank (8 per treatment) were sampled for a total of 32 fish per sampling day. A total of 88 individuals used in this experiment. Experimental settings and sampling are summarized in Figure 4 and Table 1.

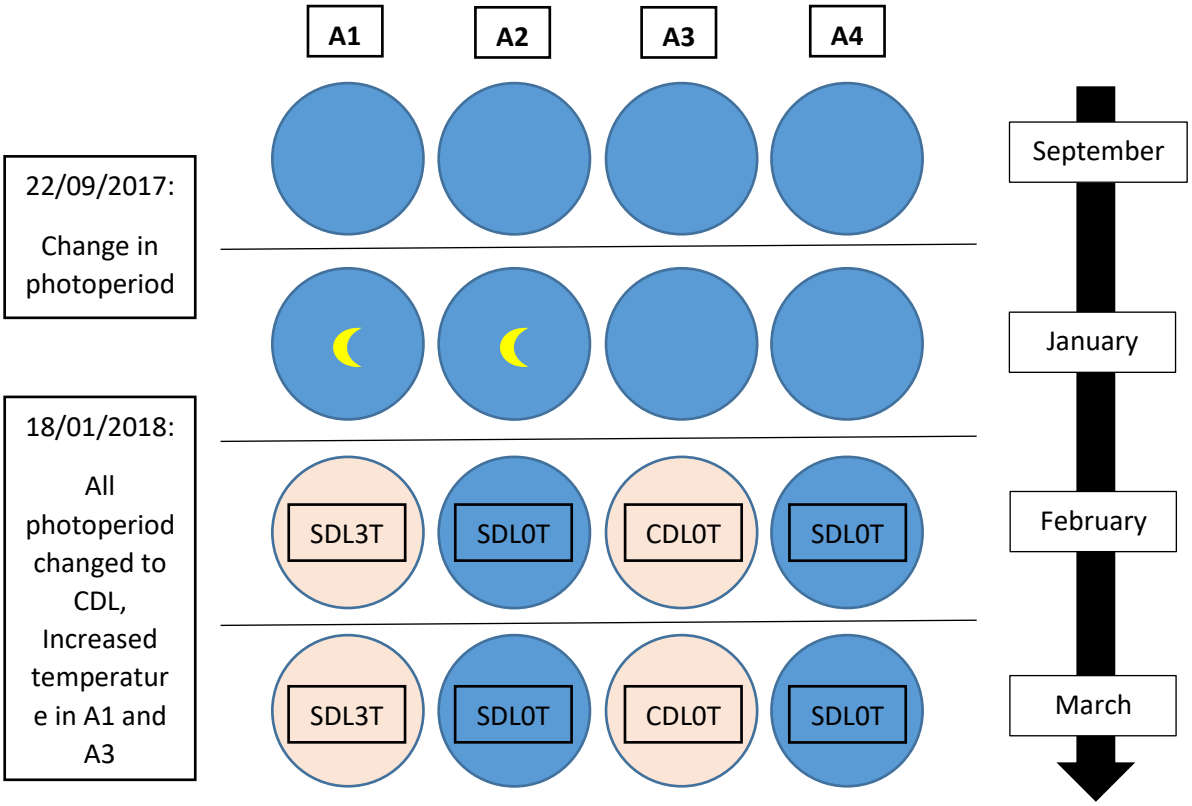


Figure 4. Experimental settings over time. The change in experimental conditions and the date of the change is reported on the left. The time line is represented by the black arrow on the right. Tank settings changed over time and are shown in the middle. Tanks are arranged from A1 to A4. A black line separates the different sampling dates. Tanks are shown in blue where the water temperature is ambient and orange where the water temperature is ambient +3°C. The moon symbol on A1 and A2 in the second row, represent the SDL photoperiod those tanks were exposed to.

Table 1. Sampled fish per treatment over time. Experimental conditions are listed on the left, sampling date is shown in the middle along with the number of fish sampled from each treatment. Total fish number sampled per day is reported on the bottom and total fish used throughout the experiment is shown in the bottom right corner. Data is missing from one fish in CDL3T from February.

	#Fish sampled by date				
	21/09/2017	17/01/2018	6/02/2018	7/03/2018	Total fish used
Condition	CDL	SDL + CDL	CDL + Temperature increase		
Initial	8				
SDL		8			
CDL		8			
SDL3T			8	8	
SDL0T			8	8	
CDL3T			7	8	
CDL0T			8	8	
Total	8	16	31	32	87

Histological sample preparation and gonadosomatic index

Sampled lumpfish were euthanized by an anaesthetic overdose (Aqui-S, AQUI-S New Zealand Ltd). Total length and body weight were measured for each individual. After harvesting and weighting the fish gonads, a small piece was carved, placed into labelled embedding cassettes (Simport Histonette II Biopsy processing/embedding cassettes®) and fixed in a 10% formalin in PBS buffer solution at 4°C. The gonadosomatic index (GSI) was estimated for each fish. GSI is a widely used tool to investigate sexual maturation in animals [50] and it is calculated with the following formula:

$$GSI = \left(\frac{Gonad\ weight}{Total\ tissue\ weight}\right) \times 100$$

Eventually, all the collected tissue was stored in a cooling room at 4°C until ready to ship to Trondheim for analyses. Upon arrival, samples were dehydrated with the Leica TP 1020 Tissue Processor® embedded in paraffin and stored in the fridge. The microtome Leica 2055 Autocut® was used to cut 4µm thick sections. The section thickness of 4µm is standard in histology and it was chosen based on the work of Virtanen in 2016 [51]. Prior to the staining process, samples were placed into an incubator to dry over night at 37°C.

Lumpfish growth

Lumpfish growth in this experiment was investigated by using the specific growth rate and the Fulton's condition factor. Lumpfish specific growth rate was calculated with the following formula:

$$SGR\% = \left[\frac{(\ln W_f - lnW_i)}{T}\right] \times 100$$

Where W_f and W_i are the average final and initial weight of lumpfish, T is the time in days. Fulton's condition factor (K) was calculated as following:

$$K = \frac{W}{L^3}$$

Where W is the body weight of the fish in grams and L is the total length in cm.

Histological staining

Sections were stained with hematoxylin and eosin, which is one of the most common staining methods for histological analyses [52], and also previously used by Virtanen in 2016 [51]. Full description of the staining protocol can be found in Appendix 1.

Imaging and staging

The histological sections were scanned using the NanoZoomer® (Hamamatsu®) digital slide scanner. For each sample, a 20x magnification scan was made and it was analysed with the Hamamatsu NDP.view2 software. Ten 20x magnification microphotographs and ten 40x magnification microphotographs were taken for every section. All the microphotographs were selected randomly along the tissue and without any overlapping. The 20x magnification images were used as a visual overview of the cell composition within the gonad tissue, whereas the 40x magnification microphotographs were used for image analysis (Figure 5).

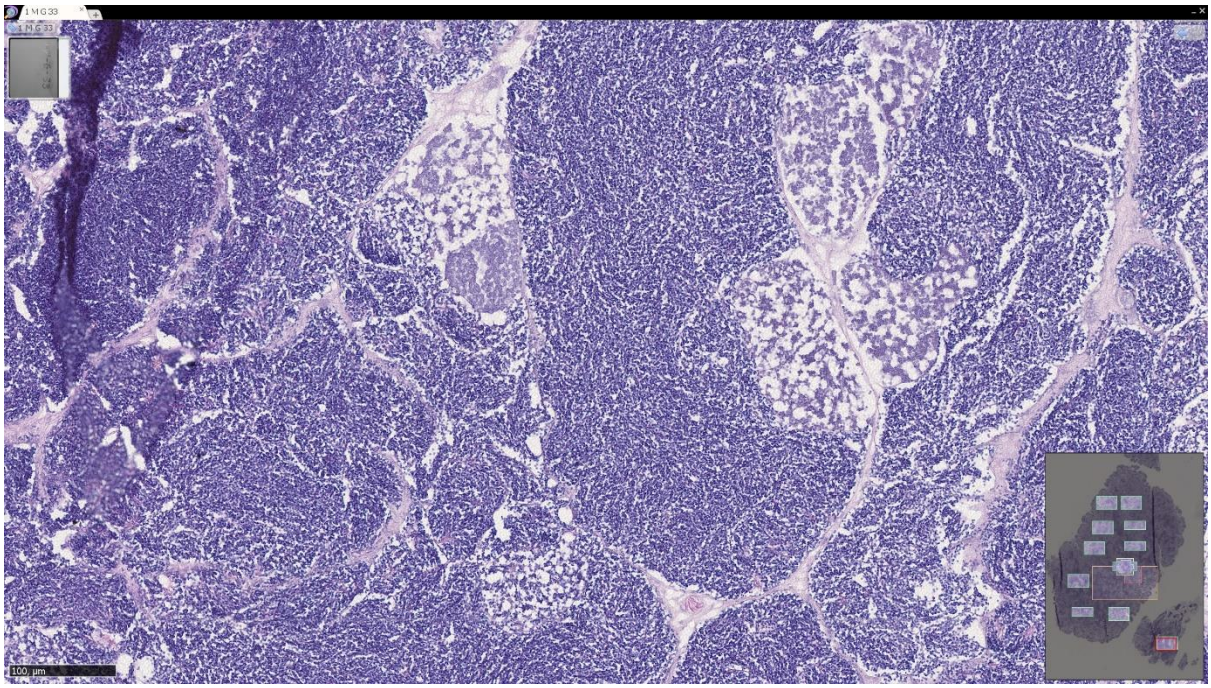
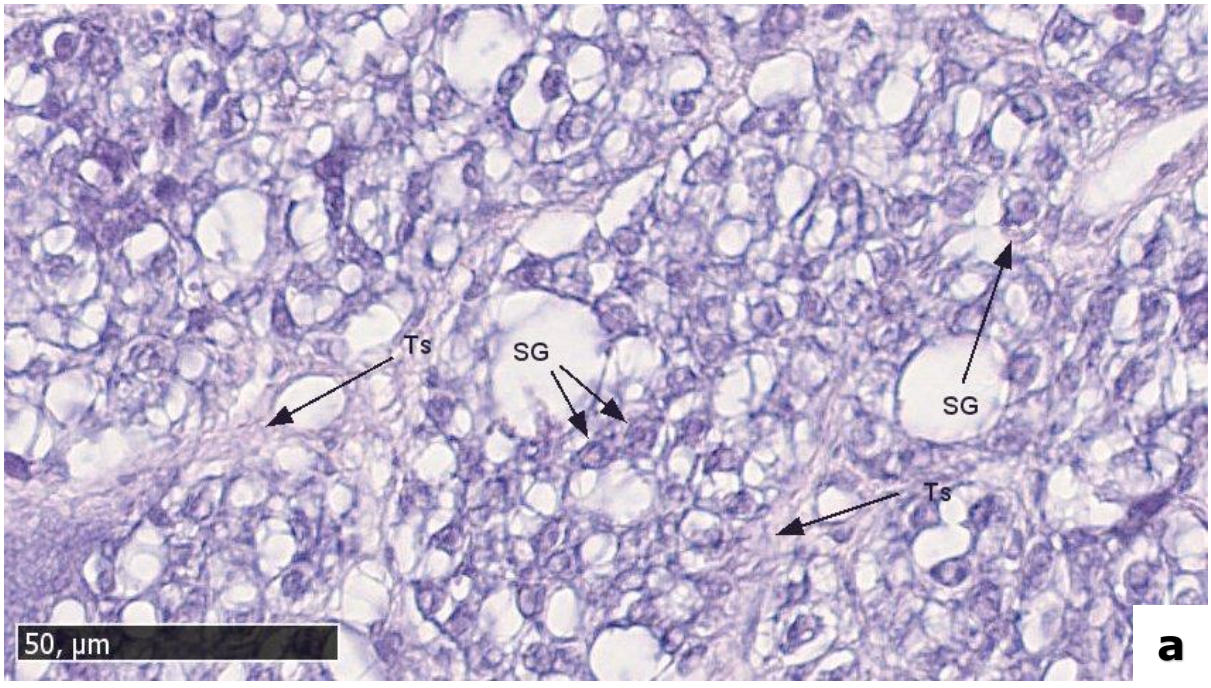
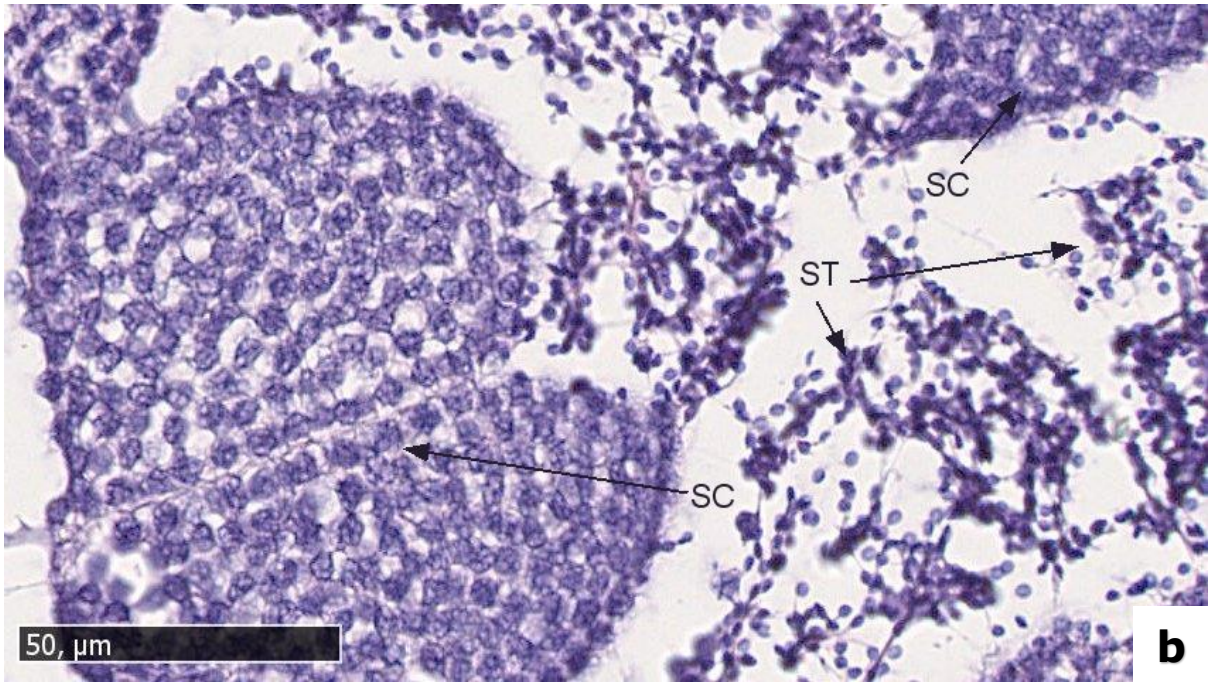


Figure 5. Screenshot of a 20x magnification microphotograph scale bar = 100µm. The miniature of the overall scanned tissue is shown on the lower right corner. The red rectangle represented the current area displayed on screen and the yellow rectangle represented the starting point, which was discarded. The same process was repeated to acquire 40x magnification microphotographs.

The criteria for the identification of the different germ cell stages in this experiment, were based from an adaptation of the work of Shulz in 2010 [49]. The criteria mainly revolved on observing nuclear characteristics, such as size, shape, and chromosome condensation. Because no distinction was made between spermatogonia A and B in this experiment, markers for identification included the large size of the cell, the shape of the nucleus and the overall weak staining pattern originating from a uniformly distributed chromatin. Spermatocytes had a larger, rounder and denser nucleus than spermatogonia, which showed the denser heterochromatin as "small dots" and/or “bands” on the nuclear membrane in turn leading to a darker stain. Spermatids, being characterized by the breaking of the intercellular and cytoplasmic bridge culminating into cyst opening, appeared to be more spread within in the lumen with denser nuclear compaction. Eventually, spermatozoa were identified by their very small, spherical and condensed nucleus, the presence of a flagellum, the lack of organelles and the high density within the lumen of the sperm ampullae (Figure 6). Five cell categories were chosen to differentiate the gonad tissue [49]. Testicular somatic cells (Ts) included connective tissue, red blood cells Sertoli and Leydig cells. Germ cells were divided in four categories: spermatogonia (SG), spermatocytes (SC), spermatids (ST) and spermatozoa (SZ). Excluded area (EA) was taken in account for blank portions of the sections.





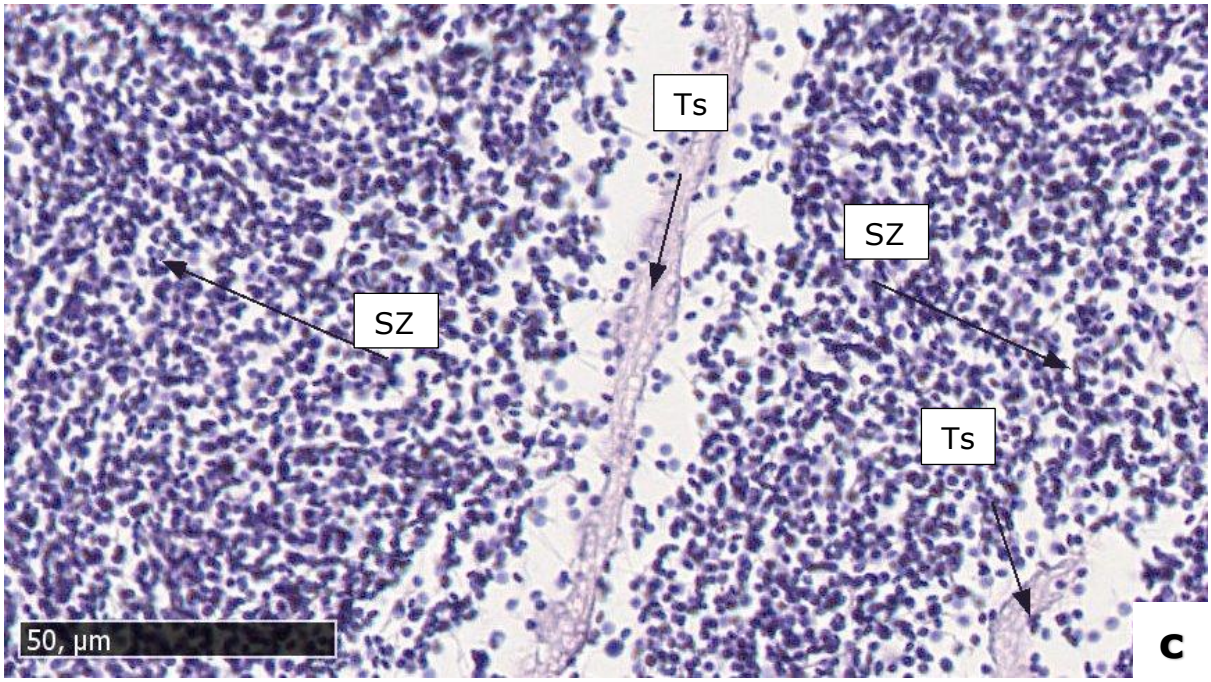


Figure 6. Morphology of the germ cell stages taken in account within this experiment. The first two microphotographs (a and b) are taken from fish sampled on January 2018, the last (c) from September 2017. In the first microphotograph (a), most of the tissue was composed by spermatogonia (SG). The second microphotograph (b) shows a combination of spermatocytes and spermatids (SC and ST) while the most predominant germ stage in the last microphotograph (c) was spermatozoa (SZ).

Area fraction of the different cell categories was calculated for every 40x magnification microphotograph. The grid plug – in of ImageJ® (National Institutes of Health, Bethesda, Maryland, USA) was used to establish a point grid of 112 crosses over the microphotographs, based on the previous work of Virtanen in 2016 on Atlantic salmon (*Salmo salar*) [51]. The category of the cells was determined at the intersection of the upper left corner of each cross over the image. The Multi-point tool was then used to mark each cell category (Figure 7). After the total number of the different categories per microphotograph was acquired, data was stored in Microsoft Office® Excel 2013 and the area fraction was calculated using the following formula:

$$F = (\frac{n_i}{112 - EA})$$

Where n_i is the relative number of the crosses counted for the different cell categories counted in the picture, 112 are the total crosses in the grid and EA is the excluded area. F was calculated for each cell type along the tissue.

After obtaining F, it was possible to calculate the Spermatogenic Maturity Index (SMI). The SMI equation was introduced by Tomkiewicz work on the European eel in 2011 [53] as a mean to quantify sexual maturation based on histological analysis. It takes into consideration the area fraction of the different cell categories and it multiplies them by a weight factor of 0, 0.25, 0.5, 0.75 and 1. The value obtained from this equation ranges from 0 to 1. A SMI value close to 1 suggest a sexually mature fish. The equation is as follows:

$$SMI = 0.0F_{Ts} + 0.25F_{SG} + 0.5F_{SC} + 0.75F_{ST} + 1F_{SZ}$$

Where F is the area fraction of the respective cells in the tissue (testicular somatic cells, spermatogonia, spermatocytes, spermatids and spermatozoa) multiplied by the weight factor of 0, 0.25, 0.5, 0.75 and 1 respectively. Eventually, the predominant germ stage in each sample was estimated. The predominant stage was any cell category which concentration within the microphotograph was > 50% (table 2).

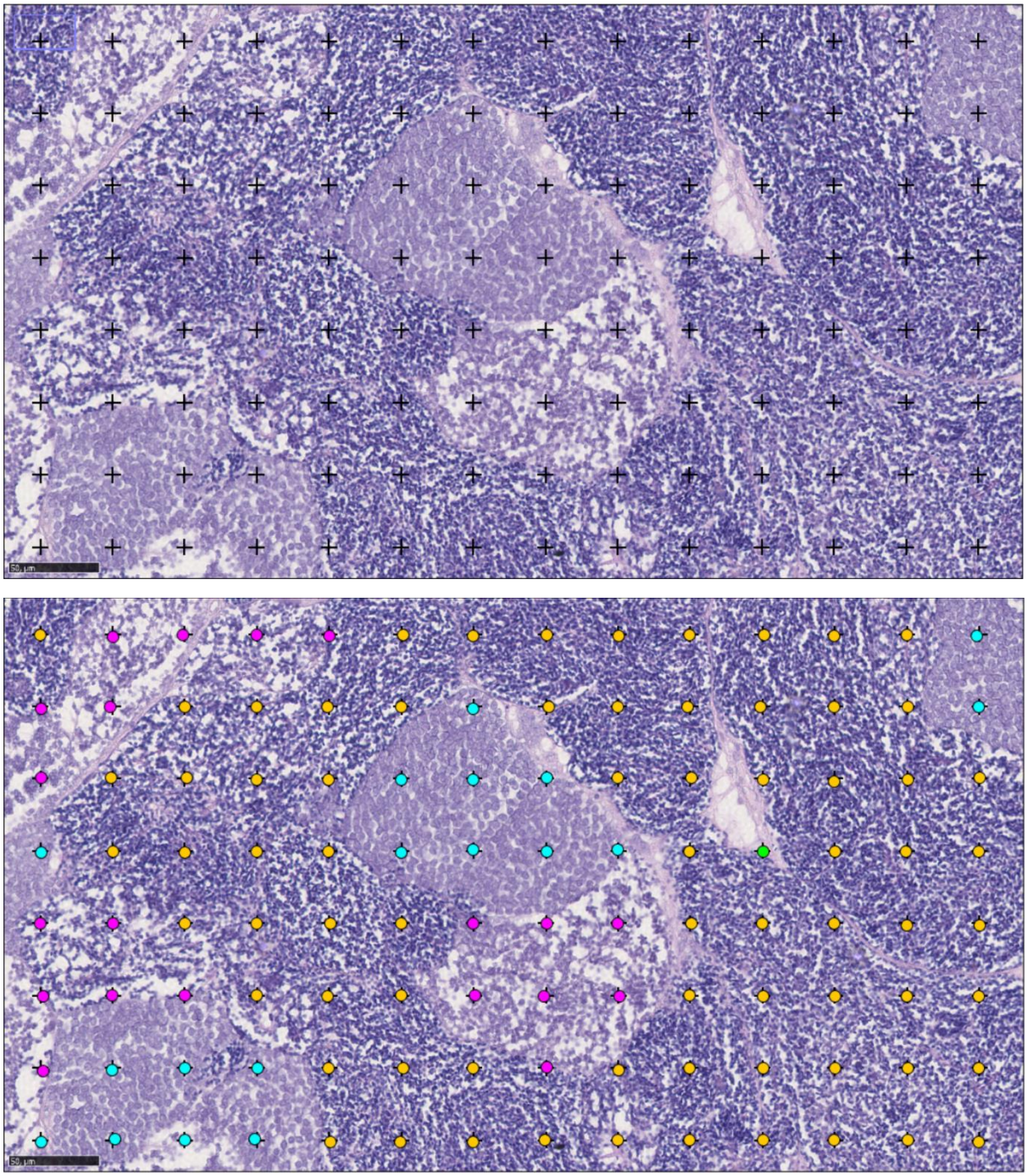


Figure 7. Gonad section taken from the sampling on the 17th of January. The 112-points grid placement is shown in the upper microphotograph. The multi-point tool is shown in the lower microphotograph. Testicular somatic cell is shown as green dot, spermatocytes are shown as light blue dots, spermatids are shown as purple dots and spermatozoa are shown as yellow dots.

Table 2. Summary of image analysis from Figure 7. This table is divided in three sections: Area fraction, SMI and germ cell predominance. Within the area fraction section, cell type and assigned category are shown on the left. The relative number refers to the different coloured dots from the multi-point tool counted in the microphotographs. Area fraction calculation and area fraction score (F) is shown. The weight factor (W) of the SMI is reported next. SMI score (in bold) was obtained by summing the multiplied the area fractions (F) by the weight factors (W). The prevalent germ stage in this example was spermatozoa, as shown in the last column of the table.

		Area Fraction				**SMI**	**Predominance**
Cell type	Category (i)	Relative number (n_i)	(n_i/(112-EA))	F	W	FxW	x>50%
EA	1	0	0/112	0	/	/	/
Ts	2	1	1/122	0.01	0	0	/
SG	3	0	0/112	0	0.25	0	/
SC	4	18	18/112	0.16	0.5	0.08	8%
ST	5	20	20/112	0.18	0.75	0.135	14%
SZ	6	73	73/112	0.65	1	0.65	**65%**
Total	/	112	/	1	/	**0.865**	/

Statistics

The program used to run statistical analyses in this experiment was PAST® 3.22 (1999-2018). IBM® SPSS® Statics, Version 25 (IBM Corp. Armonk, NY) was used to plot graphs. Tables were made with Microsoft Office® Excel 2013.

The Pearson correlation coefficient was used to investigate the relationship between total length and body weight and subsequently. The Shapiro – Wilk normality test was used to investigate the data distribution. Due to some data being non-parametric, the Kruskal – Wallis test (Kruskal – Wallis, $p \leq 0.05$) was chosen to investigate significant differences in data distribution. Specifically, it was used to find significant differences in the SGR%, Fulton's k, GSI, SMI and area fraction of lumpfish exposed to the different treatments. Two – way ANOVA was used in the last two sampling days to investigate the effects of the combination of light and temperature on lumpfish.

Results

Temperature profile

Initially the ambient temperature in each tank was around 9°C. Ambient temperature gradually decreased throughout the experiment. From January, the tanks A1 and A3 received ambient +3°C (Figure 8). The detailed temperature profile of each tank in this pilot experiment is reported in the appendix (Appendix 2)

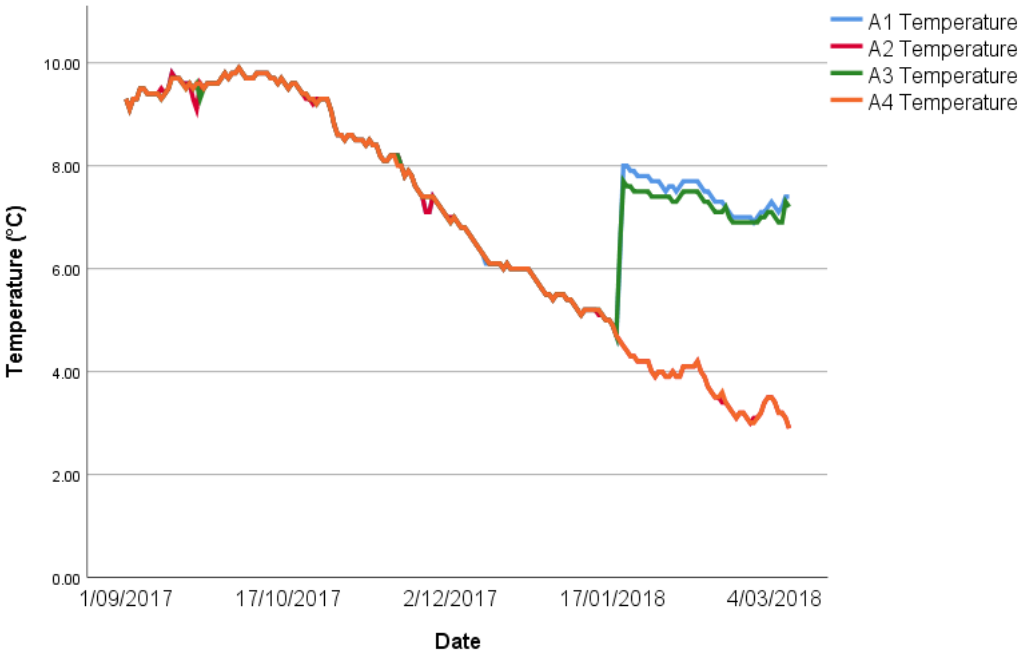


Figure 8. Temperature changes in the tanks over the course of the pilot experiment. Temperature (°C) shown on the Y – axis and time is shown on the X – axis. The peaks shown after the 17th of January in tanks A1 and A3 represent the change from ambient water temperature to ambient +3°C.

Male growth

Initially, male lumpfish had a mean body weight of 442 ± 185 g and a mean total length of 22.1 ± 2.7 cm. The biggest lumpfish were found in the tank exposed to CDL3T treatment in the last sampling (07/03/2018), having a mean weight of 922.2 ± 329.0 g and a mean length of 28 ± 2.1 cm. Evidence for a strong positive linear correlation between total length and body weight was found (Figure 9) (Pearson's r = 0.89). Morphometric data including fish weight, length and gonad weight is reported in the appendix (Appendix 3)

The specific growth rate of male lumpfish exposed to different treatments was not significantly different within each sampling day (Figure 10). Moreover, no significant changes in specific growth rates were found over time (Kruskal – Wallis, $p \leq 0.05$) although a large variation in size between fish was found. Additionally, no differences in the Fulton's condition factor were found between the fish exposed to the different treatments, nor over time (Kruskal – Wallis, $p \leq 0.05$) (Figure 11).

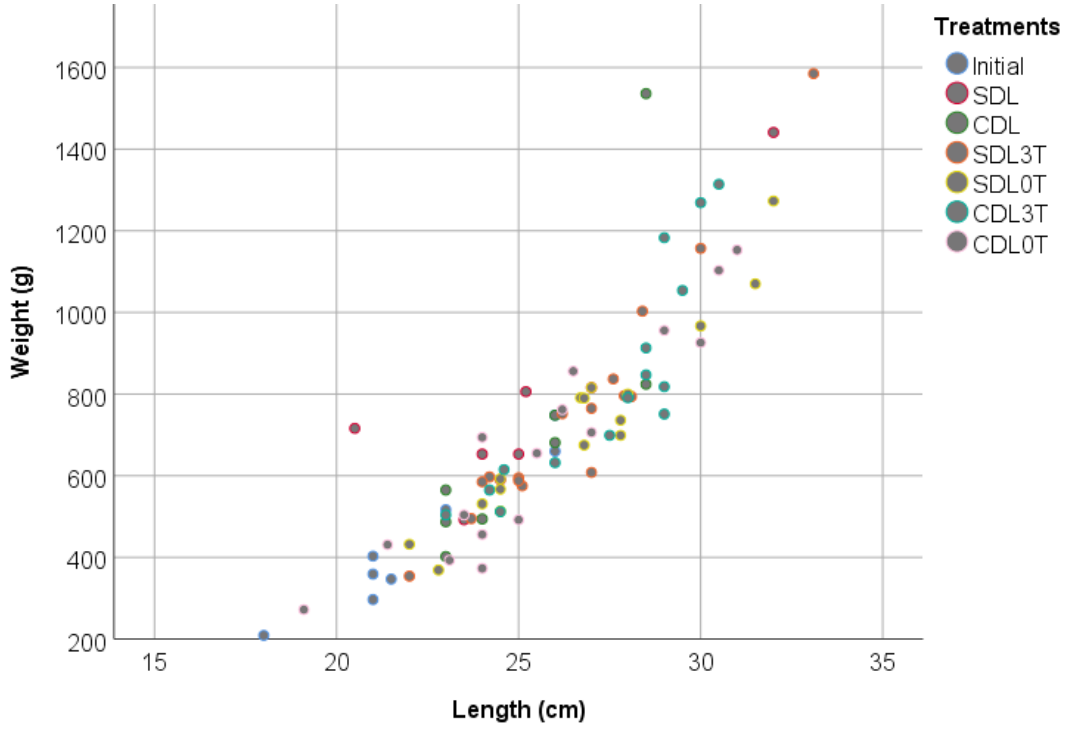


Figure 9. Lumpfish weight in grams (Y – axis) plotted against lumpfish length in centimetres (X – axis) to. Values were sorted by treatment.

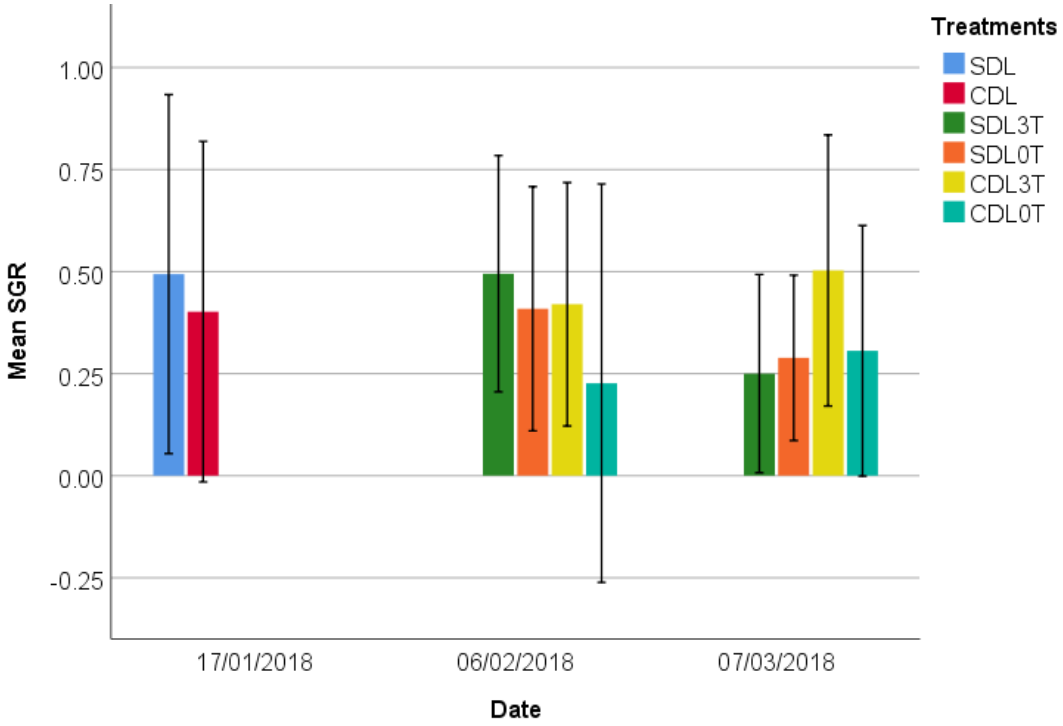


Figure 10. Mean SGR of male lumpfish (Y – axis) exposed to the different treatment over time (X – axis). Bars represent standard deviation. No significant differences were found between fish exposed to different photoperiod and temperature treatments. No significant changes in SGR were observed.

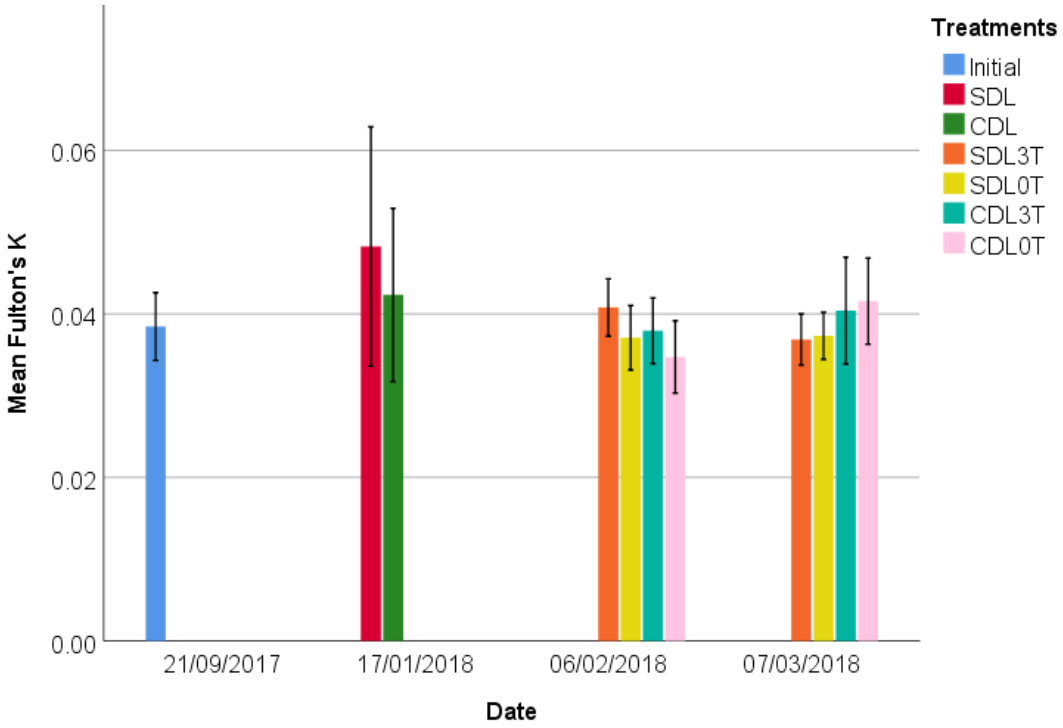


Figure 11. Mean condition factor (K) of male lumpfish (Y – axis) exposed to the different conditions over time (X – axis). Bars represent standard deviation. No significant differences were found between fish exposed to different photoperiod and temperature conditions. No significant changes in K were observed.

Gonadosomatic Index

The highest mean gonadosomatic index (8.9 ± 8.2) was observed in male lumpfish from the first sampling (21/09/17), although a high variance in data was recorded (Figure 12). The mean GSI in January (17/01/2018) was significantly higher ($p = 0.004$) in fish exposed to short daylight than in those exposed to continuous daylight (Kruskal – Wallis, $p \leq 0.05$).

In the sampling of February (06/02/2018), the mean GSI of lumpfish exposed to SDL0T was significantly higher than in those exposed to CDL0T ($p = 0.046$). However, no significant differences were found in the mean GSI of lumpfish from the other treatments. Eventually, no significant differences in GSI were found in the last two sampling days.

No significant changes in mean GSI was observed throughout the duration of the experiment (Kruskal – Wallis, $p \leq 0.05$). Moreover, no significant difference in mean GSI was found in fish exposed to the combination of continuous light and increased temperature (Two – way ANOVA, $p \leq 0.05$).

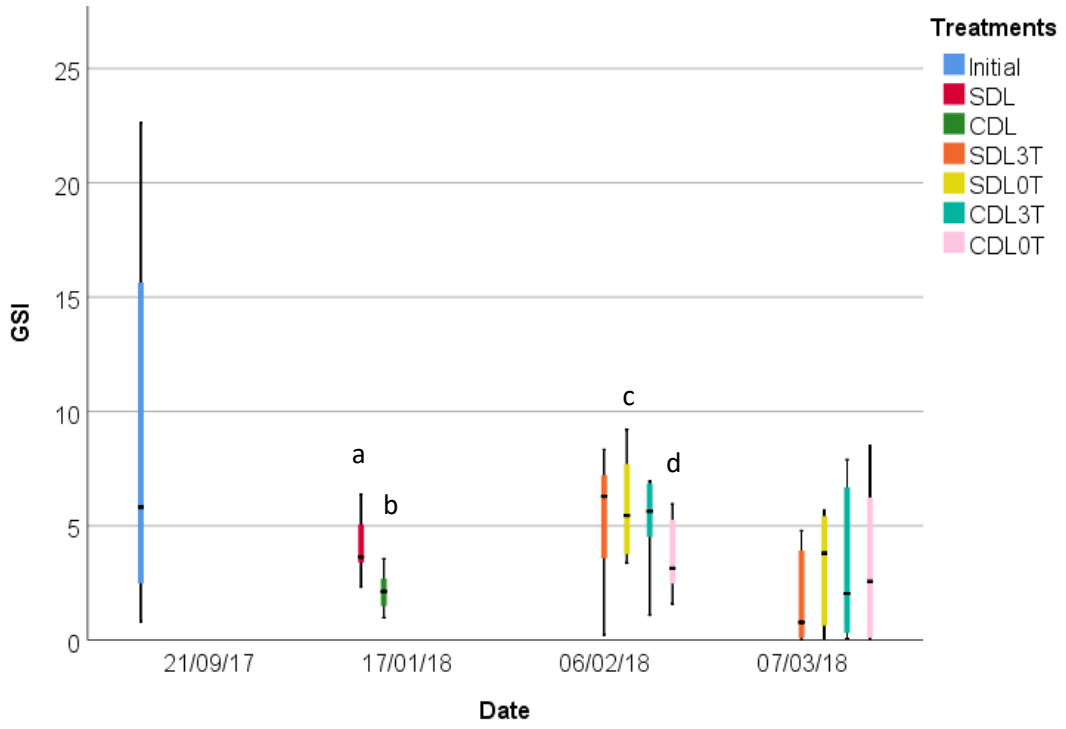


Figure 12. GSI values in lumpfish exposed to different treatments over time. GSI score is presented on the Y – axis while time (in sampling date) is shown on the X – axis. Statistical difference in GSI between SDL and CDL can be observed (a and b) in the second sampling (17/01/2018) and in the third (c and d) (06/02/2018).

Table 3. Individual GSI values of lumpfish sampled in each treatment over time. Average GSI value and standard deviation are reported at the bottom of the table. Data from CDL3T was only available for 7 lumpfish in March.

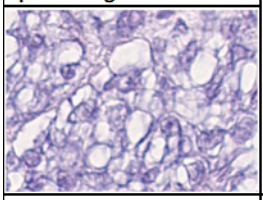
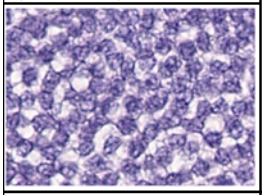
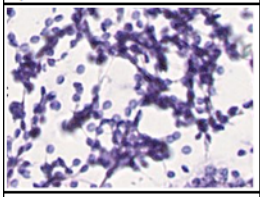
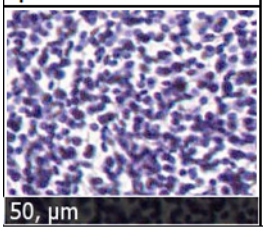
Date	21/09/2017	17/01/2018		6/02/2018				7/03/2018			
#Fish	Initial	SDL	CDL	SDL3T	SDL0T	CDL3T	CDL0T	SDL3T	SDL0T	CDL3T	CDL0T
1	2.47	6.37	1.94	0.23	3.37	1.09	3.54	0.14	1.15	0.11	3.39
2	0.81	8.74	1.39	7.22	5.22	4.51	1.58	0.13	3.97	7.65	0.086
3	13.5	3.41	2.32	7.20	9.21	11.0	2.65	0.063	5.24	2.03	1.75
4	2.48	3.37	3.56	6.34	5.66	6.76	2.29	3.71	5.69	0.53	0.094
5	17.8	3.72	3.04	1.62	3.82	4.51	5.95	4.78	3.63	7.88	8.52
6	22.6	2.31	0.98	6.22	8.19	5.32	5.61	1.41	5.59	5.71	5.73
7	8.50	3.69	1.58	8.33	3.70	5.95	4.91	0.024	0.11	0.065	0.05
8	3.14	3.56	2.30	5.54	7.20	6.94	2.74	4.11	0.056		6.74
Average	8.91	4.40	2.14	5.34	5.80	5.75	3.66	1.80	3.18	3.42	3.29
ST. Dev.	8.19	2.10	0.86	2.87	2.20	2.79	1.63	2.06	2.41	3.55	3.35

Spermatogenesis in lumpfish

Spermatogonia and spermatocytes were easily distinguishable in male lumpfish based on their peculiar morphology and staining pattern (Table 4). Specifically, spermatogonia were larger and found in cysts. The chromatin in spermatogonia was not condensed resulting to an even and weaker stain compared to spermatocytes. The latter were rounder and smaller than spermatogonia. The most characteristic trait of spermatocytes was the highly condensed heterochromatin, which appeared as darker dots or bars in the nucleus of spermatocytes. In turn, this led to a noticeably darker staining.

Lumpfish spermatids were very easily distinguishable from the previous germ stages because they appeared significantly smaller, spread in the lumen, resulting from the breaking of the cytoplasmic bridges and the cyst opening. They had a very a condensed nucleus and sometimes they appeared flagellated. However, spermatids morphology was very similar to the morphology of the spermatozoa. The key feature to distinguish between spermatids and spermatozoa was the distribution within the lumen of the gonad. Specifically, spermatids, although free from the cysts, were often found grouped in "grape – like" formation (table 4). Upon closer inspection spermatozoa appeared slightly smaller than spermatids due to the very small, spherical and condensed nucleus. Spermatozoa in this experiment were almost always found at high density with showing a homogeneous distribution within the lumen. Lumpfish Sertoli cells were only occasionally observed and appeared very small.

Table 4. Microphotographs of different germ stages. Difference in morphology and staining pattern is easily observable between spermatogonia and spermatids. Spermatids and spermatozoa are significantly smaller than the earlier stages. Example of the spermatids "grape – like" formation is shown. Spermatozoa, when found, were observed to occupy most of the lumen in a homogeneous manner.

Spermatogonia 	**Spermatogonia were the largest in lumpfish. They were found in cysts. The nucleus was round and presented uniformly distributed chromatin. This resulted in a weaker stain compared to the other stages.**
Spermatocytes 	**Spermatocytes had a larger, rounder and denser nucleus than spermatogonia. They were found in cysts. The heterochromatin was tightly packed and appeared as small dots or bands in the nucleus. This lead to a darker stain pattern compared to spermatogonia.**
Spermatids 	**Spermatids were the first haploid germ stage. The size of the cells decreased drastically. Spermatids were found spread in the lumen after the breaking of the cytoplasmic bridges culminating in the cyst opening. The nucleus was very compact and round. Some spermatids had already developed a flagellum.**
Spermatozoa 	**Spermatozoa were characterized by the presence of a fully formed flagellum, a very small, spherical and condensed nucleus and the lack of organelles. Spermatozoa were tightly packed within the lumen of the sperm ampullae.**

Area fraction

Male lumpfish were found to be already sexually mature from the start of the experiment (21/09/2017) having the highest mean spermatozoa in the area fraction (Figure 13 and 14) recorded throughout the pilot experiment. After the change to SDL photoperiod, a significant drop in mean spermatozoa was found in the area fraction from January (17/01/2018) compared to September ($p = 0.035$). Particularly, a higher abundance in mean spermatids was observed in the area fraction of lumpfish exposed to SDL compared to CDL, although this difference was not significant (Kruskal – Wallis, $p \leq 0.05$).

Significant difference in the area fraction of lumpfish exposed to different temperature treatments was found in February (06/02/2018). Specifically, lumpfish sampled from the SDL3T treatment had the highest mean spermatids and the lowest mean spermatozoa in the area fraction observed throughout this pilot experiment (Figure 13 and 14). Particularly, the mean spermatids in the area fraction of SDL3T was significantly higher (Kruskal – Wallis, $p \leq 0.05$) compared to those found in lumpfish exposed to SDL0T and CDL0T ($p = 0.011$ and $p = 0.027$ respectively) (Figure 13 and 14). However, no difference in the area fraction was found between CDL3T and the ambient temperature treatments or between SDL3T and CDL3T.

Lumpfish, regardless of the different treatments, had a significantly higher mean spermatogonia area fraction in the last sampling (07/03/2018) compared to the earlier sampling days, suggesting that they were reaching the end of their spawning period. Lumpfish in the CDL0T treatment had significantly higher spermatozoa in the area fraction compared to SDL3T ($p = 0.045$), although no other differences were found between the lumpfish exposed to the different treatments in March.

No significant change in the mean spermatozoa in the area fraction of lumpfish was found since January regardless of the different treatments. Area fraction values for each fish can be found in the appendix (Appendix 4).

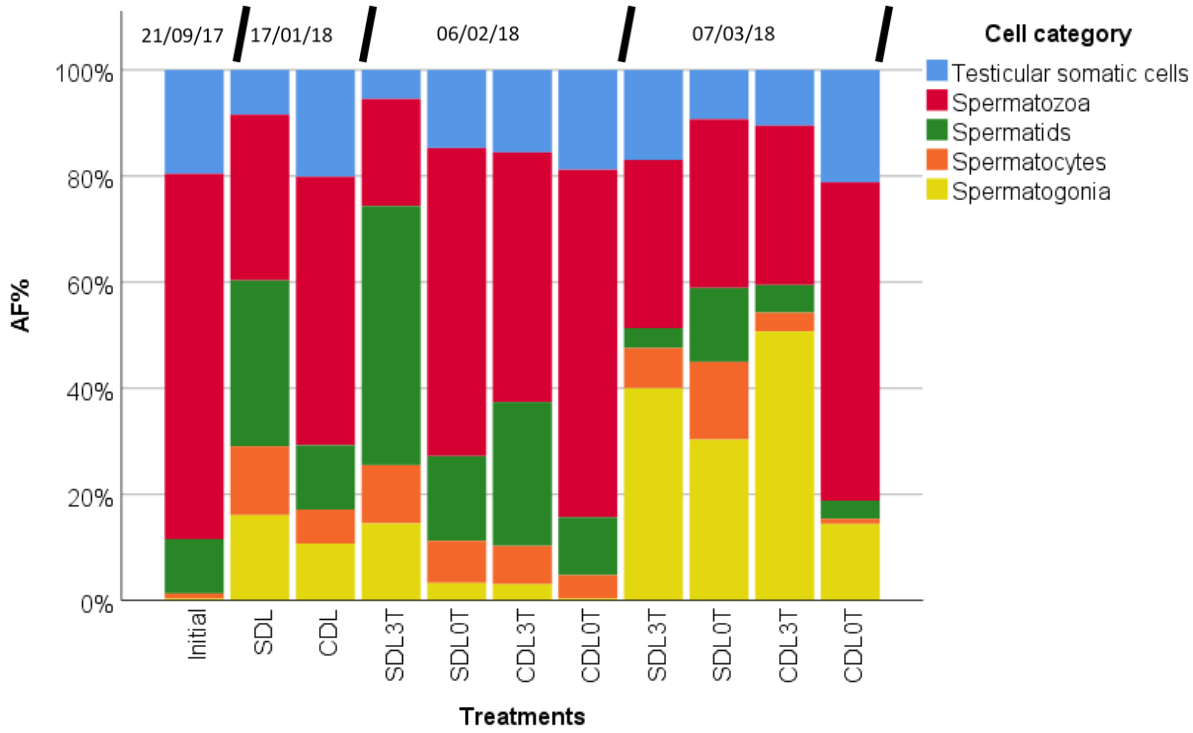


Figure 13. Mean percentage of the different area fraction in each fish over time. Spermatogonia (SG%) is shown in yellow, spermatocytes (SC%) is shown in orange, spermatids (ST%) is shown in green, spermatozoa (SZ%) is shown in red and finally testicular somatic cells (Ts%) is shown in blue.

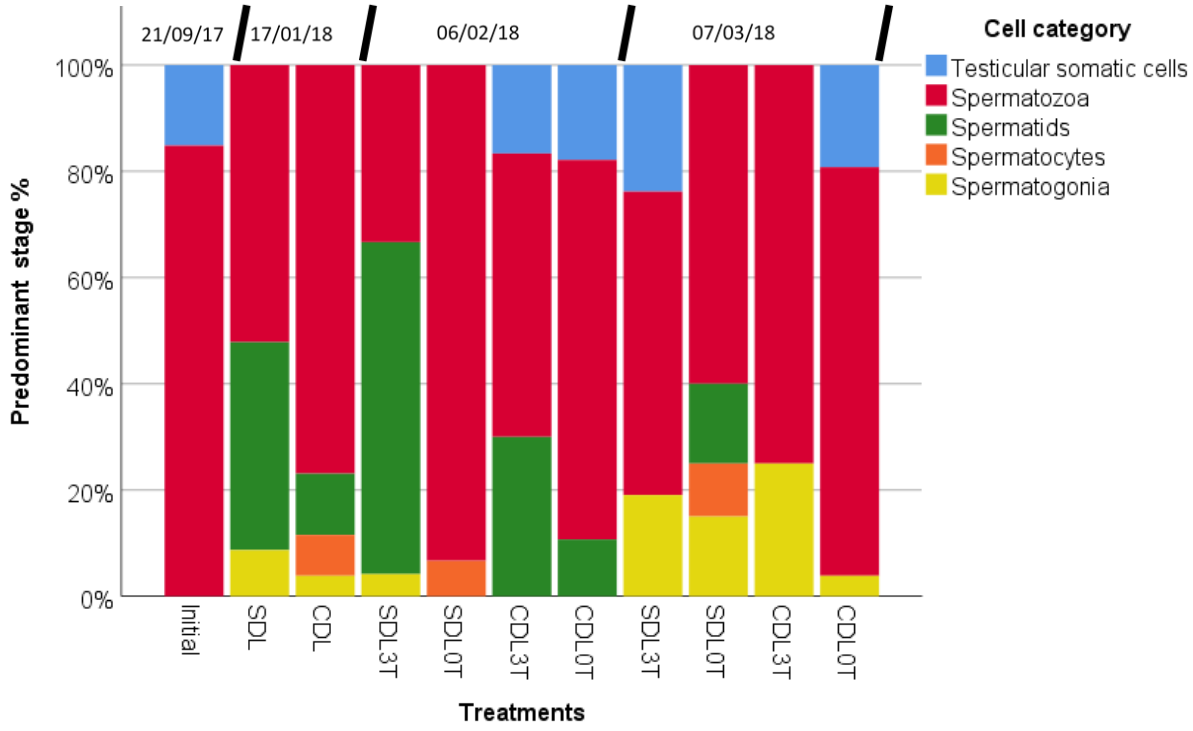


Figure 14. Mean predominant stage per fish (cell category>50% in AF) for each treatment over time. Spermatogonia (SG) is shown in yellow, spermatocytes (SC) is shown in orange, spermatids (ST) is shown in green, spermatozoa (SZ) is shown in red and finally testicular somatic cells (Ts) is shown in blue. Ts was predominant when fish were spent (majority of the area in the section was empty lumen with some leftover spermatozoa)

Spermatogenic Maturity Index

The highest mean spermatogenic maturity index (0.77 ± 0.17) was observed in the male lumpfish from the start of the experiment (21/09/17). High variance in SMI score was found throughout the experiment (Figure 15), especially in the last sampling (07/03/2018) where the SMI ranged from a minimum of 0.15 to a maximum of 0.94 in CDL0T (Table 5). This was due to a larger fraction of male found to be spent to a different degree.

No significant differences in SMI from lumpfish exposed to the different treatments were found on any sampling day (Kruskal – Wallis, $p \leq 0.05$). Moreover, no significant changes in SMI were observed throughout the duration of the experiment (Kruskal – Wallis, $p \leq 0.05$). No significant differences in sexual maturity were found when lumpfish were exposed to the combination of continuous light and increased temperature (Two – way ANOVA, $p \leq 0.05$).

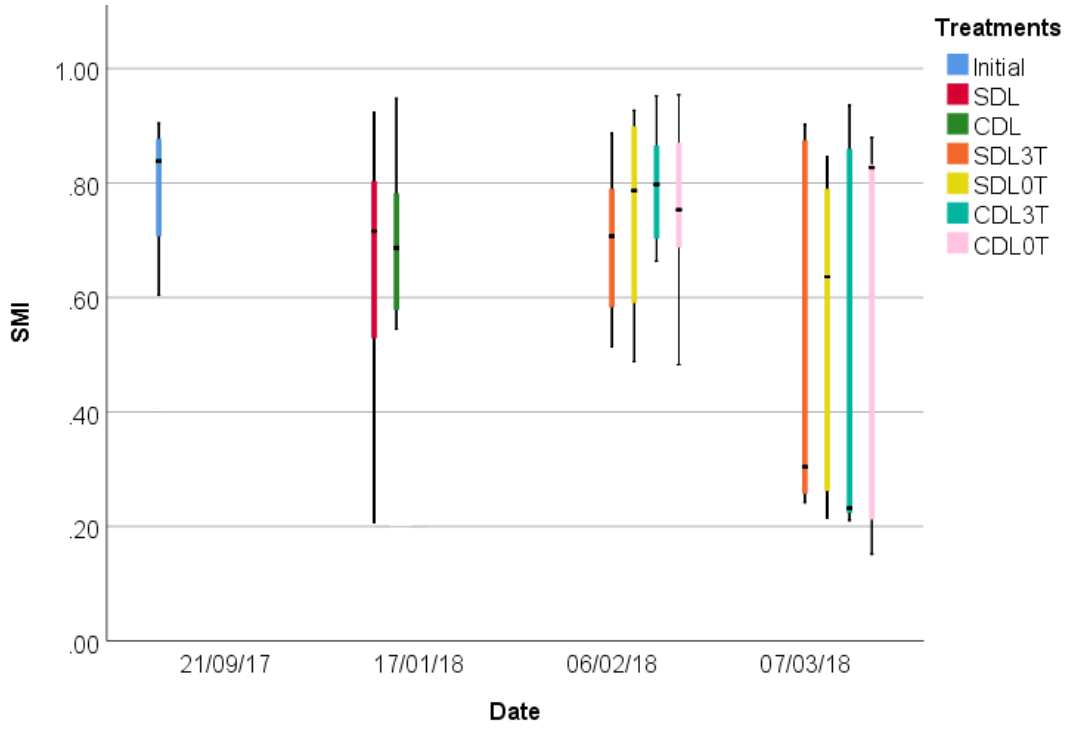


Figure 15. SMI values in lumpfish exposed to different treatments over time. SMI score is presented on the Y-axis while time (in sampling date) is shown on the X-axis.

Table 5. Individual SMI values calculated for lumpfish sampled in each treatment over time. Average SMI value and standard deviation are reported at the bottom of the table. Data from CDL0T in February and for CDL3T and CDL0T in March were only available for 7 lumpfish.

Date	21/09/2017	17/01/2018		6/02/2018				7/03/2018			
#Fish	Initial	SDL	CDL	SDL3T	SDL0T	CDL3T	CDL0T	SDL3T	SDL0T	CDL3T	CDL0T
1	0.60	0.81	0.95	0.23	0.49	0.06	0.86	0.74	0.90	0.78	0.94
2	0.88	0.66	0.61	0.68	0.91	0.95	0.77	0.89	0.59	0.21	0.21
3	0.88	0.21	0.67	0.89	0.89	0.82	0.88	0.24	0.30	0.23	0.82
4	0.90	0.40	0.71	0.82	0.93	0.74	0.70	0.24	0.80	0.23	0.15
5	0.42	0.77	0.20	0.51	0.55	0.82	0.95	0.28	0.22	0.22	0.83
6	0.84	0.92	0.54	0.74	0.84	0.91	0.68	0.86	0.85	0.89	0.88
7	0.83	0.79	0.70	0.65	0.63	0.66	0.48	0.27	0.21	0.83	0.83
8	0.81	0.66	0.85	0.76	0.73	0.78		0.33	0.68		
Average	0.77	0.65	0.66	0.66	0.75	0.72	0.76	0.48	0.57	0.48	0.67
St. Dev.	0.17	0.24	0.22	0.21	0.17	0.28	0.16	0.29	0.29	0.33	0.33

Discussion

Growth

In this pilot experiment no significant differences (Kruskal – Wallis, $p \leq 0.05$) were found in male lumpfish mean SGR% and Futon's condition factor (K) when exposed to the different photoperiod treatments (SDL and CDL). Although the temperature profile in this pilot experiment was well within lumpfish optimal temperature range for growth (8.9°C to 15.6°C) [9], no significant growth (SGR% and K) was observed from the start of the experiment (21/09/2017) (Figure 10 and 11). Moreover, the increase of 3°C after the second sampling (17/01/2018) did not cause any significant change in lumpfish growth compared to those kept at ambient water temperature.

Lumpfish were already sexually mature from the start of this pilot experiment (21/09/2017). Sexual maturation has been linked to impaired somatic growth in other marine fish species [38, 42, 54]. Specifically, the lack of significant differences in the growth of sexually mature male lumpfish was also observed by Imsland *et al*., [23, 24] when they were investigating the effects of different photoperiods treatments on the lumpfish sexual maturation. Therefore, it is possible to speculate that the lack of significant somatic growth regardless of the treatments was due to lumpfish being already sexually mature prior to this pilot experiment.

Sexual maturation

Lumpfish were grown for 18 months on a continuous light photoperiod (CDL) prior to the experiment and they were found to be already sexually mature when sampled for the first time (21/09/2017). Furthermore, there was evidence of lumpfish spawning from December 2017 (Appendix 2) and throughout this pilot experiment regardless of the treatment. The characteristic male lumpfish spawning colouration and running milt, regardless the treatment, was also previously described in Imsland *et al*., [23, 24], however spawning in males was happened earlier (September [23] and from June [24]) compared to this pilot experiment.

This suggests that lumpfish reach sexual maturation, when reared in captivity, in less than 18 months and even when reared under continuous light photoperiod. This is in contrast with multiple studies where marine fish species such as Atlantic cod (*Gadus morhua*) [37, 38], European sea bass (*Dicentrarchus labrax*) [36, 39], Atlantic salmon (*Salmo salar*) [40, 41] and Atlantic halibut (*Hippoglossus hippoglossus*) [42] have been shown to delay their sexual maturation in favour to somatic growth when exposed to a continuous light photoperiod.

In addition, based on their spawning from December 2017 until the March 2018 (Appendix 2), it can be speculated that the CDL exposure for 18 months led to a phase shifted sexual maturation, since lumpfish is known to spawn from late March until July in nature [15, 16, 20, 21].

The highest mean GSI and SMI of lumpfish (Figure 12 and 15) was recorded at the start of the experiment (21/09/2017) due to the large portion of spermatozoa in the area fraction (Figures 13 and 14). This was also observed in the rainbow trout (*Oncorhynchus mykiss*), where the highest GSI value was found in fish with the highest spermatozoa predominance [55]. The slight drop in mean GSI values from September 2017 to January 2018, could be caused by the spawning observed in December 2017 (Appendix 2).

A significantly higher mean GSI (Kruskal – Wallis, $p \leq 0.05$) was recorded in lumpfish exposed to SDL treatment compared to CDL in January (17/01/2018). Particularly, there was a higher spermatids abundance in the area fraction of lumpfish from the SDL treatment compared to CDL, where lumpfish had a higher spermatozoa predominance (Figures 13 and 14). However, the difference in area fraction from the two treatments was not significant and resulted to a very similar SMI score in both treatments. In this regard, studies on Atlantic salmon [51], sea trout (*Salmo trutta*) [56] and kichiji rockfish (*Sebastolobus macrochir*) [57], concluded that the GSI did not always correspond to the correct maturation stage in males, rather, the estimation of sexual maturation should be based on histological analysis or on a combination GSI and histological analyses [51, 56, 57].

Lumpfish spermatids are very similar in size and morphology to the spermatozoa (Table 4), contrary to what is found in salmonids where the difference in size is more apparent [51]. The three-stages metamorphosis from spermatids to mature spermatozoa comprising the loss of the residual body in the last stages of spermiogenesis described by Shulz *et al*., [49] was thought to be responsible for the decrease in final GSI recorded in Atlantic Salmon (*Salmo salar*) [51] and previously in brown trout (*Salmo trutta fario*) [58] when reaching final sexual maturation. However, in this experiment, the shedding of the spermatids residual body during spermiation does not seem to significantly lower the mean GSI in lumpfish. Specifically, this was also observed in February (06/02/2018) when the significantly higher spermatids predominance found in the mean area fraction of lumpfish exposed to SDL3T (Figure 13 and 14), did not result in any significant differences in mean GSI compared to the mean GSI recorded in the other treatments.

No changes in mean GSI were observed over time from February to March (07/03/2018). No significant difference (Kruskal – Wallis, $p \leq 0.05$) in spermatozoa was found in the area fraction of lumpfish from February and March.

However, a significantly higher mean spermatogonia area fraction was observed in all lumpfish, regardless of the treatment in the last sampling (07/03/2018) (Figures 13 and 14). This suggests the start of testes recrudescence; lumpfish were approaching the end of their spawning season.

The mean SMI of lumpfish from any treatment was high throughout the pilot experiment and did not change significantly over time (Kruskal – Wallis, $p \leq 0.05$). The high variations in SMI recorded in the last sampling (07/03/2018), might be due to the combination of very mature males with area fractions dominated by spermatozoa, spent males and males which were going through testes recrudescence. The area fraction of spent male lumpfish was dominated by the empty lumen, previously occupied by spermatozoa prior to spawning, testicular somatic cells and spermatogonia (Appendix 5). Although spent males were found in every sampling day regardless of the treatments, immature/recrudescent males were observed predominantly in the last sampling (07/03/2018).

Tomkiewic's SMI equation assigns the a weight factor of 0 to testicular somatic cells and 0.25 to spermatogonia [53]. Therefore, it is very difficult to distinguish whether a low SMI results from an immature male (area fraction dominated by early germ stages) or a spent male (area fraction dominated by empty lumen, testicular somatic cells and sometimes spermatogonia). Therefore, histological analysis of the gonads should be the primary tool to investigate spermatogenesis in male lumpfish.

Overall in this pilot experiment, lumpfish were found spawning regardless of the photoperiod treatments, as it was found when male lumpfish reached sexual maturation in the experiments of Imsland *et al*., [23, 24]. No significant differences in mean GSI and SMI values, resulting from four months of short photoperiod after continuous light exposure, were found between lumpfish sampled in September 2017 and January 2018 (Kruskal – Wallis, $p \leq 0.05$). However, in January (17/01/2018) there was a significant reduction in spermatozoa in the area fraction of lumpfish exposed to SDL compared to September 2017, probably due to the spawning of December 2017 (Appendix 2).

Increasing the ambient water temperature by 3°C in SDL3T and CDL3T after the sampling of January, did not cause any differences in sexual maturation compared to ambient water temperature treatments (SDL0T and CDL0T) in this pilot experiment based on mean GSI and SMI (Kruskal – Wallis, $p \leq 0.05$). However, upon histological analysis it seemed that the increase in temperature induced a higher predominance of spermatids in lumpfish exposed to SDL3T and CDL3T treatments compared to ambient temperature treatment. Temperature has been shown to play an important role as proximate factor influencing gametogenesis in many fish species [29]. In nature, adult lumpfish prefer temperature ranges between 4 – 7°C [17] and spawning happens in shallow waters when temperature

is around 4°C [18, 19], therefore the sudden peak to 8°C after January in SDL3T and CDL3T (Figure 8) might have slowed down spermatogenesis compared to the lumpfish exposed to ambient temperature treatments (Figure 13 and 14) as previously observed when increasing the temperature during sexual maturation in Atlantic salmon (*Salmo salar*) [32], and in rainbow trout (*Oncorhynchus mykiss*) [34]. This was particularly noticeable on fish exposed to the SDL3T treatment in February (06/02/2018) having significantly higher spermatids in the area fraction compared to those exposed to the ambient temperature treatments (SDL0T and CDL0T) (Kruskal – Wallis, $p \leq 0.05$).

On the other hand, the ambient water temperature was around 4°C from January (Figure 8). Cold water could have advanced the spermiogenesis in lumpfish as previously seen in Atlantic salmon (*Salmo salar*) and in sea bass (*Dicentrarchus labrax*) [35, 36]. This could explain the higher spermatozoa predominance in the area fraction of lumpfish exposed to the ambient treatments compared to those exposed to ambient +3°C in the last samplings.

However, the effects of increased temperature were less noticeable in the following sample in March (07/03/2018) compared to ambient (Kruskal – Wallis, $p \leq 0.05$), which could also be due to lumpfish approaching the end of their spawning season.

Results from testosterone and 11-KT analysis from the same male lumpfish in this pilot experiment showed no significant differences in sexual maturation of lumpfish exposed to the different treatments (Mlingi 2019 – unpublished data). However, female lumpfish tend to respond with a change in photoperiod, suggesting that light changes might be more important in the synchronisation of female lumpfish sexual maturation. Particularly, it was found that female lumpfish spawned earlier when cued with a shorter photoperiod after continuous light exposure [23, 24] (Mlingi 2019 – unpublished data).

Conclusion

In this pilot experiment, 18 months old male lumpfish reared under continuous photoperiod (CDL) were found to be already sexually mature from the start of the experiment (21/09/2017) and observed spawning from December 2017. CDL exposure for 18 months prior to the experiment is thought to be responsible for the phase shift in lumpfish spawning form early summer (in natural condition) to December 2017 in this experiment. The lack of significant differences in growth in all treatments was probably due to lumpfish being already sexually mature.

Change in photoperiod to SDL resulted to a significantly higher mean GSI in male lumpfish from compared to those exposed to CDL. However, the SMI values between SDL and CDL where very similar suggesting that the fish exposed to the different photoperiods were equally sexually mature. No significant differences in SMI of lumpfish were found in this experiment, regardless of the treatments. A clearer picture on the differences in lumpfish sexual maturation stages between treatments, was obtained upon investigating the histology of the testes (area fraction). The warmer water temperature in February (06/02/2018) might have slowed down spermatogenesis in fish from SDL3T treatments compared to those at ambient. Although the difference was less pronounced in March 2018.

The higher abundance of spent and recrudescent males found in the last sampling (07/03/2018), suggested that lumpfish were reaching the end of their phase shifted spawning season.

To truly investigate the effects of photoperiod and temperature manipulation on the sexual maturation of lumpfish it will be paramount in the future to start with non-sexually mature fish.

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Appendix 1

Tissue staining

The hematoxylin and eosin staining is used to better understand and differentiate the cells within the sampled tissue. The staining principle revolves around applying two chemical compounds to the sections. First hemalum, which is a complex created with the interaction of aluminium ions and the oxidised state of hematoxylin, is applied. This dyes in blue all the cell membranes, which have a basic positive charge, most notably cell nuclei. Then a counter stain, eosin-y, is applied to the sections. This in turn, will dye the acidic positive tissues in a red/pink colour most notably, the cytoplasm, collagen and red blood cells.

Prior to staining, paraffin had to be removed from the tissue sample. This was achieved by immersion in Tissue-Clear® (Sakura Finetek®) for 5 minutes. The process was repeated three times. Then, the tissue samples were dipped 4 times in ethanol at a concentration of 100%, 100%, 96% and 70% respectively for 2 minutes and set in distilled water for 5 minutes.

The first stain (Mayer's Hematoxylin) was applied via immersion for 3 minutes. Tissue samples were then placed under running water for 3 minutes to allow the development of the stain. Excess hematoxylin was removed by quickly dipping the tissue samples in a solution containing 1% of HCl in 70% ethanol and washing them under running water for 3 minutes.

The second stain was applied to the tissue sample via immersion for 2 minutes in a solution containing Eosin at 0.5% concentration. Excess of Eosin was washed away by dipping the tissue samples in tap and distilled water.

Dehydration of the sections was the last step. It required the tissue samples to be dipped in a 70% ethanol solution, then soaked for 30 second in 100% ethanol before immerging them into two 100% ethanol baths for 2 minutes each.

Eventually the sections were immersed in three different Tissue-Clear® baths for 5 minutes each. After the staining procedure, a cover slip was mounted over the section using Neo-Mount® (Merk®). Samples were left to dry one day prior to imaging.

Appendix 2

Temperature profile

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gjelder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	NOFIMA		

Kar nr.: A1

Avdeling:	A	Antall:	Min. O_2 (%)	80	Fôrtype/batch nr.: SK 5,0mm
Måned og År:	sep.17	Vekt:	Temp. (°C)	nat	Fôrregime-kanal: 48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet: SV	Flow (l/min):		Lysregime-kanal: 24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,3	—	0	✓		IN
2	9,1	—	0	✓		IN
3	9,3	—	0	✓		IN
4	9,3	—	0	✓		IN
5	9,5	—	0	✓		IN
6	9,5	87	0	✓		IN
7	9,4	—	0	✓		IN
8	9,4	—	0	✓		IN
9	9,4	-	0	✓		BE
10	9,4	-	0	✓		BE
11	9,3	—	0	✓		IN
12	9,4	—	0	✓		IN
13	9,5	87	0	✓		IN
14	9,8	-	0	✓		BE
15	9,7	-	0	✓		JJJ
16	9,7	-	0	✓		BE
17	9,6	-	0	✓		BE
18	9,6	-	0	✓		BE
19	9,6	—	0	✓		IN
20	9,5	87	1	✓		IN
21	9,6	-	0	✓	PRØVETAKMING – 5 STK	IN
22	9,6	-	0	✓		HH
23	9,5	—	0	✓		JHJ
24	9,6	—	0	✓		JHJ
25	9,6	-	0	✓		IN
26	9,6	—	0	✓		IN
27	9,6	97	0	✓		IN
28	9,7	-	0	✓		IN
29	9,8	—	0	✓		IN
30	9,7	-	0	✓		JJJ
31						

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	NAFIMA		

Kar nr.: A2

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Förtype/batch nr.:	**SK 5,0mm**
Måned og År:	**sep.17**	Vekt:		Temp. (°C)	**nat**	Förregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**24t**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,3	–	0	✓		IN
2	9,1	–	0	✓		IN
3	9,3	–	0	✓		IN
4	9,3	–	0	✓		IN
5	9,5	–	0	✓		IN
6	9,5	90	0	✓		IN
7	9,4	–	0	✓		IN
8	9,4	–	0	✓		IN
9	9,4	–	0	✓		BE
10	9,4	–	0	✓		BE
11	9,3	–	0	✓		IN
12	9,4	–	0	✓		IN
13	9,5	88	0	✓		IN
14	9,3	–	0	✓		BE
15	9,7	–	0	✓		JNJ
16	9,7	–	0	✓		BE
17	9,6	–	0	✓		BE
18	9,6	–	0	✓		BE
19	9,6	–	0	✓		IN
20	9,5	88	0	✓		IN
21	9,6	–	0	✓	PRØVETAKNING 4 STK	IN
22	9,6	–	0	✓		HT
23	9,5	–	0	✓		Jej
24	9,6	–	0	✓		Jej
25	9,6	–	1	✓		IN
26	9,6	–	0	✓		IN
27	9,6	93	0	✓		IN
28	9,7	–	0	✓		IN
29	9,8	–	1	✓		IN
30	9,7	–	0	✓		JNJ
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	NOFIMA		

Kar nr.: A3

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	sep.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,3	–	0	✓		IN
2	9,1	–	0	✓		IN
3	9,3	–	1	✓		IN
4	9,3	–	0	✓		IN
5	9,5	–	0	✓		IN
6	9,5	95	0	✓		IN
7	9,4	–	0	✓		IN
8	9,4	–	0	✓		IN
9	9,4	–	0	✓		BE
10	9,4	–	0	✓		BE
11	9,3	–	0	✓		IN
12	9,4	–	0	✓		IN
13	9,5	93	0	✓		IN
14	9,7	–	0	✓		BE
15	9,7	–	0	✓		JJ
16	9,7	–	0	✓		BE
17	9,6	–	0	✓		BE
18	9,5	–	0	✓		BE
19	9,6	–	0	✓		IN
20	9,5	89	0	✓		IN
21	9,6	–	0	✓	PÅFYLE TAKNING 4 STK	IN
22	9,8	–	0	✓		HH
23	9,5	–	0	✓		JEJ
24	9,6	–	0	✓		JEJ
25	9,6	–	0	✓		IN
26	9,6	–	0	✓		IN
27	9,6	92	0	✓		IN
28	9,7	–	0	✓		IN
29	9,8	–	0	✓		IN
30	9,7	–	0	✓		JJ
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17 27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A4

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	sep.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,3	—	0	✓		IN
2	9,1	—	0	✓		IN
	9,3	-	0	✓		IN
4	9,3	—	0	✓		IN
5	9,5	—	0	✓		IN
6	9,5	90	0	✓		IN
7	9,4	—	1	✓		IN
8	9,4	—	0	✓		IN
9	9,4	—	0	✓		BE
10	9,4	-	0	✓		BE
11	9,3	—	0	✓		IN
12	9,4	—	0	✓		IN
13	9,5	87	0	✓		IN
14	9,7	—	0	✓		BE
15	9,7	-	0	✓		JJ
16	9,7	—	0	✓		BE
	9,6	-	0	✓		BE
18	9,5	—	0	✓		BE
19	9,6	—	0	✓		IN
20	9,5	86	0	✓		IN
21	9,6	—	0	✓	PRØVETAKING 4 STK.	IN
22	9,6	—	0	✓		HA
23	9,5	—	0	✓		Jej
24	9,6	-	0	✓		Jej
25	9,6	-	0	✓		IN
26	9,6	—	0	✓		IN
27	9,6	92	0	✓		IN
28	9,7	—	0	✓		IN
29	9,8	—	0	✓		JJJ
30	9,7	-	0	✓		
31						

Koder: — = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/17	Ansv. forskningstekniker:	IVAR NEVERMO
Forsøkshaver:	NOFIMA		

Kar nr.: A1

Avdeling:	A	Antall:	Min. O_2 (%) 80	Fôrtype/batch nr.:	sk amber 5,0mm
Måned og År:	okt.17	Vekt:	Temp. (°C) nat.	Fôrregime-kanal:	4 & 24t
Art og Generasjon:	Rognkjeks	Vannkvalitet: SV	Flow (l/min):	Lysregime-kanal:	TROMSØLYS

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,8	–	0	✓		JJJ
2	9,8	–	0	✓		W
	9,9	–	0	✓		W
4	9,8	91	0	✓		W
5	9,7	–	0	✓		W
6	9,7	–	0	✓		IN
7	9,7	–	0	✓		BE
8	9,8	–	0	✓		
9	9,8	–	0	✓		JØ
10	9,8	–	0	✓		IS
11	9,8	92	0	✓		ISAK
12	9,7	–	0	✓		ISAC
13	9,7	–	0	✓		ISAC
14	9,6	–	0	✓		HT
15	9,7	–	0	✓		HT
16	9,6	–	0	✓		W
	9,5	–	0	✓		W
18	9,6	90	0	✓		IN
19	9,6	–	0	✓		W
20	9,5	–	0	✓		IN
21	9,4	–	0	✓		AHS
22	9,4	–	0	✓		W
23	9,3	–	0	✓		W
24	9,3	–	0	✓		W
25	9,2	88	0	✓		W
26	9,3	–	0	✓		W
27	9,3	–	0	✓		W
28	9,3	–	0	✓		JØ
29	9,1	–	0	✓		JØ
30	8,8	–	0	✓		W
31	8,6	–	0	✓		W

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	IVAR NEVERMO
Forsøkshaver:	NOFIMA		

Kar nr.: A2

Avdeling:	A	Antall:		Min. O_2 (%) 80	Fôrtype/batch nr.: sk amber 5,0mm
Måned og År:	okt.17	Vekt:		Temp. (°C) nat.	Fôrregime-kanal: 48 24t
Art og Generasjon:	Rognkjeks	Vannkvalitet:	SV	Flow (l/min):	Lysregime-kanal: TROMSØLYS

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,8	–	0	✓		JJJ
2	9,8	–	0	✓		IN
3	9,9	–	0	✓		IN
4	9,8	87	0	✓		IN
5	9,7	–	0	✓		IN
6	9,7	–	0	✓		IN
7	9,7	–	0	✓		BE
8	9,8	–	0	✓		BE
9	9,8	–	0	✓		I
10	9,8	–	0	✓		IS
11	9,8	93	0	✓		ISAC
12	9,7	–	0	✓		ISAC
13	9,7	–	0	✓		ISAC
14	9,6	–	0	✓		HT
15	9,7	–	0	✓		HT
16	9,6	–	0	✓		IN
17	9,5	–	0	✓		IN
18	9,6	99	0	✓		IN
19	9,6	–	0	✓		IN
20	9,5	–	0	✓		IN
21	9,4	–	0	✓		AHS
22	9,4	–	0	✓		AHS
23	9,3	–	0	✓		IN
24	9,3	–	0	✓		IN
25	9,2	88	0	✓		IN
26	9,3	–	0	✓		IN
27	9,3	–	0	✓		IN
28	9,3	–	0	✓		
29	9,1	–	0	✓		JEJ
30	8,8	–	0	✓		JEJ
31	8,1	–	0	✓		IN

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	IVAR NEVERMO
Forsøkshaver:	NOFIMA		

Kar nr.: A3

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	sk amber 5,0mm
Måned og År:	okt.17	Vekt:		Temp. (°C)	nat.	Fôrregime-kanal:	48 B4:
Art og Generasjon:	Rognkjeks	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24E

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,8	–	0	✓		JJ
2	9,8	–	0	✓		IN
3	9,9	–	0	✓		IN
4	9,8	89	0	✓		IN
5	9,7	–	0	✓		IN
6	9,7	–	0	✓		IN
7	9,7	–	0	✓		BE
8	9,8	–	0	✓		BE
9	9,8	–	0	✓		I
10	9,8	–	0	✓		IJ
11	9,8	91	0	✓		ISAC
12	9,7	–	0	✓		ISAC
13	9,7	–	0	✓		ISAC
14	9,6	–	0	✓		HA
15	9,7	–	0	✓		HA
16	9,6	–	0	✓		IN
17	9,5	–	0	✓		IN
18	9,6	91	0	✓		IN
19	9,6	–	0	✓		IN
20	9,5	–	0	✓		IN
21	9,4	–	0	✓		AHS
22	9,4	–	0	✓		NHS
23	9,3	–	0	✓		IN
24	9,3	–	0	✓		IN
25	9,2	89	0	✓		IN
26	9,3	–	0	✓		IN
27	9,3	–	0	✓		IN
28	9,3	–	0	✓		JE
29	9,1	–	0	✓		JE
30	8,8	–	0	✓		IN
31	8,6	–	0	✓		IN

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	IVAR NEVERMO
Forsøkshaver:	NOFIMA		

Kar nr.: A4

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	sk amber 5,0mm
Måned og År:	okt.17	Vekt:		Temp. (°C)	nat.	Fôrregime-kanal:	4t 24t
Art og Generasjon:	Rognkjeks	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	9,8	–	0	✓		JJJ
2	9,8	–	0	✓		IN
3	9,9	–	0	✓		IN
4	9,8	89	0	✓		IN
5	9,7	–	0	✓		IN
6	9,7	–	0	✓		IN
7	9,7	–	0	✓		IBÉ
8	9,8	–	0	✓		IBÉ
9	9,8	–	0	✓		JS
10	9,8	–	0	✓		JS
11	9,8	93	0	✓		LSAC
12	9,7	–	0	✓		LSAC
13	9,7	–	0	✓		LSAC
14	9,6	–	0	✓		AH
15	9,7	–	0	✓		AH
16	9,6	–	0	✓		IN
17	9,5	–	0	✓		IN
18	9,6	89	0	✓		IN
19	9,6	–	0	✓		IN
20	9,5	–	0	✓		IN
21	9,4	–	0	✓		AHS
22	9,4	–	0	✓		AHS
23	9,3	–	0	✓		IN
24	9,3	–	0	✓		IN
25	9,2	88	0	✓		IN
26	9,3	–	0	✓		IN
27	9,3	–	0	✓		IN
28	9,3	–	0	✓		JEJ
29	9,1	–	0	✓		JEJ
30	8,8	–	0	✓		IN
31	8,6	–	1	✓		IN

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A1

Avdeling:	A	Antall:	78	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	nov.17	Vekt:	216g	Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	Tromsølys

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	8,6	90	0	✓		IW
2	8,5	–	0	✓		IW
	8,6	–	0	✓		IW
4	8,6	–	0	✓		HHS
5	8,5	–	0	✓		HHS
6	8,5	–	0	✓		IW
7	8,5	–	0	✓		IW
8	8,4	–	0	✓		IW
9	8,5	91	0	✓		IW
10	8,4	–	0	✓		IW
11	8,4	–	0	✓		JEJ
12	8,2	–	0	✓		JEJ
13	8,1	–	0	✓		IW
14	8,1	–	0	✓		IW
15	8,2	90	0	✓		IW
16	8,2	–	0	✓		IW
	8,2	–	0	✓		IW
18	8,0	–	0	✓		BE
19	7,8	–	0	✓		BE
20	7,9	–	0	✓		IW
21	7,8	–	0	✓		IW
22	7,6	91	0	✓		IW
23	7,5	–	0	✓		IW
24	7,4	–	0	✓		IW
25	7,4	–	0	✓		HH
26	7,4	–	0	✓		HH
27	7,4	–	0	✓		IW
28	7,3	–	0	✓		IW
29	7,2	93	0	✓		IW
30	7,1	–	0	✓		IW
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A2

Avdeling:	A	Antall:	78	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	nov.17	Vekt:	216g	Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	Tromsølys

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	8,6	88	0	✓		
2	8,5	–	0	✓		
3	8,6	–	0	✓		
4	8,6	–	0	✓		
5	8,5	–	0	✓		
6	8,5	–	0	✓		
7	8,5	–	0	✓		
8	8,4	–	0	✓		
9	8,5	90	0	✓		
10	8,4	–	0	✓		
11	8,4	–	0	✓		
12	8,2	–	0	✓		
13	8,1	–	0	✓		
14	8,1	–	0	✓		
15	8,2	90	0	✓		
16	8,2	–	0	✓		
17	8,2	–	0	✓		
18	8,0	–	0	✓		
19	7,8	–	0	✓		
20	7,9	–	0	✓		
21	7,8	–	0	✓		
22	7,6	90	0	✓		
23	7,5	–	0	✓		
24	7,4	–	0	✓		
25	7,4	–	0	✓		
26	7,4	–	0	✓		
27	7,4	–	0	✓		
28	7,3	–	0	✓		
29	7,2	92	0	✓		
30	7,1	–	0	✓		
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A3

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	nov.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	8,6	91	0	✓		
2	8,5	–	0	✓		
	8,6	–	0	✓		
4	8,6	–	0	✓		
5	8,5	–	0	✓		
6	8,5	–	0	✓		
7	8,5	–	0	✓		
8	8,4	–	0	✓		
9	8,5	89	0	✓		
10	8,4	–	0	✓		
11	8,4	–	0	✓		
12	8,2	–	0	✓		
13	8,1	–	0	✓		
14	8,1	–	0	✓		
15	8,2	90	0	✓		
16	8,2	–	0	✓		
17	8,2	–	0	✓		
18	8,0	–	0	✓		BE
19	7,8	–	0	✓		BE
20	7,9	–	0	✓		
21	7,8	–	0	✓		
22	7,6	89	0	✓		
23	7,5	–	0	✓		
24	7,4	–	0	✓		
25	7,4	–	0	✓		
26	7,4	–	0	✓		
27	7,4	–	0	✓		
28	7,3	–	0	✓		
29	7,2	93	0	✓		
30	7,1	–	0	✓		
31						

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkjent av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gjelder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A4

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	nov.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	8,6	91	0	V		W
2	8,5	–	0	V		W
	8,6	–	0	V		W
4	8,6	–	0	V		W
5	8,5	–	0	V		W
6	8,5	–	0	V		W
7	8,5	–	0	V		W
8	8,4	–	0	V		W
9	8,5	91	0	V		W
10	8,4	–	0	V		W
11	8,4	–	0	V		JF
12	8,2	–	0	V		JF
13	8,1	–	0	V		W
14	8,1	–	0	V		W
15	8,2	91	0	V		W
16	8,2	–	0	V		W
	8,0	–	0	V		W
18	8,0	–	0	V		BC
19	7,8	–	0	V		BC
20	7,9	–	0	V		W
21	7,8	–	0	V		W
22	7,6	91	0	V		W
23	7,5	–	0	V		W
24	7,4	–	0	V		W
25	7,4	–	0	V		HT
26	7,4	–	0	V		HT
27	7,4	–	0	V		W
28	7,3	–	0	V		W
29	7,2	96	0	V		W
30	7,1	–	0	V		W
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A1

Avdeling:	A	Antall:	66	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	des.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	Tromsølys

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,0	—	0	V		W
2	6,9	-	0	V		BE
	7,0	-	0	V		BE
4	6,9	—	0	V		W
5	6,8	—	0	V		W
6	6,8	93	0	V		W
7	6,7	-	0	V		W
8	6,6	—	0	V		W
9	6,5	—	0	V		W
10	6,4	—	0	V		W
11	6,3	—	0	V		W
12	6,2	—	0	V		W
13	6,1	91	0	V		IW
14	6,1	—	0	V		W
15	6,1	—	0	V		IW
16	6,1	—	0	V		JEJ
	6,0	—	0	V		JEJ
18	6,1	—	0	V		W
19	6,0	—	0	V		W
20	6,0	93	0	V		W
21	6,0	—	0	V		W
22	6,0	—	0	V		W
23	6,0	—	0	V		JEJ
24	6,0	—	0	V		JEJ
25	5,9	—	0	V		JEJ
26	5,8	—	0	V		
27	5,7	92	0	✓	O_2 = 94	JJJ
28	5,6	—	0	✓		W
29	5,5	-	0	✓		JJJ
30	5,5	—	0	V		JEJ
31	5,4	—	0	V		JEJ

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkjent av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gjelder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forsikningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A2

Avdeling:	A	Antall:	68	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	des.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	Tromsølys

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,0	–	0	✓		IW
2	7,0	–	0	✓		BE
3	7,0	–	0	✓		BE
4	6,9	–	0	✓		IW
5	6,8	–	0	✓		IW
6	6,8	93	0	✓		IW
7	6,7	–	0	✓		IW
8	6,6	–	0	✓		IW
9	6,5	–	0	✓		IW
10	6,4	–	0	✓		IW
11	6,3	–	0	✓		IW
12	6,2	–	0	✓		IW
13	6,1	91	0	✓		IW
14	6,1	–	0	✓		IW
15	6,1	–	0	✓		IW
16	6,1	–	0	✓		JEJ
17	6,0	–	0	✓		JEJ
18	6,1	–	0	✓		JEJ
19	6,0	–	0	✓		IW
20	6,0	92	0	✓		IW
21	6,0	–	0	✓		IW
22	6,0	–	0	✓		IW
23	6,0	–	0	✓		JEJ
24	6,0	–	0	✓		JEJ
25	5,9	–	0	✓	Gyting	JEJ
26	5,8	–	0	✓		JEJ
27	5,7	92	0	✓		JEJ
28	5,6	–	0	✓		IW
29	5,5	–	0	✓		JEJ
30	5,5	–	0	✓		JEJ
31	5,4	–	0	✓		JEJ

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A3

Avdeling:	A	Antall:	54	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	des.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,0	–	0	✓		IW
2	6,9	–	0	✓		IBE
	7,0	–	0	✓		IBE
4	6,9	–	0	✓		IW
5	6,8	–	0	✓		IW
6	6,8	89	0	✓		IW
7	6,7	–	0	✓	1 TATT UT – SVIMER	IW
8	6,6	–	0	✓		IW
9	6,5	–	0	✓		IW
10	6,4	–	0	✓		IW
11	6,3	–	1	✓		IW
12	6,2	–	0	✓		IW
13	6,1	91	0	✓		IW
14	6,1	–	0	✓		IW
15	6,1	–	0	✓		IW
16	6,1	–	0	✓		JEJ
	6,0	–	0	✓		JEJ
18	6,1	–	0	✓		IW
19	6,0	–	0	✓		IW
20	6,0	92	0	✓		IW
21	6,0	–	0	✓		IW
22	6,0	–	1	✓		IW
23	6,0	–	0	✓		JEJ
24	6,0	–	0	✓		JEJ
25	5,9	–	0	✓		JEJ
26	5,8	–	0	✓		JEJ
27	5,7	94	0	✓		JEJ
28	5,6	–	0	✓		IW
29	5,5	–	0	✓		JEJ
30	5,5	–	0	✓		JEJ
31	5,4	–	0	✓		JEJ

Koder: – = ingen registrering. ✓ = sjekkel. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk				Skjema
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A4

Avdeling:	A	Antall:	65	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	des.17	Vekt:		Temp. (°C)	nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,0	–	0	✓		IW
2	6,9	–	0	✓		BE
	7,0	–	0	✓		BE
4	6,9	–	0	✓		IW
5	6,8	–	0	✓		IW
6	6,8	91	0	✓	GYTING / EGG I KARET	IW
7	6,7	–	0	✓		IW
8	6,6	–	0	✓		IW
9	6,5	–	0	✓		IW
10	6,4	–	0	✓		IW
11	6,3	–	0	✓		IW
12	6,2	–	0	✓		IW
13	6,1	92	0	✓		IW
14	6,1	–	0	✓	GYTING / EGG I KARET	IW
15	6,1	–	0	✓		IW
16	6,1	–	0	✓	Gyting / Egg i karet	JEJ
	6,0	–	0	✓		JEJ
18	6,1	–	0	✓		IW
19	6,0	–	0	✓		IW
20	6,0	93	0	✓		IW
21	6,0	–	0	✓		IW
22	6,0	–	0	✓		IW
23	6,0	–	0	✓		JEJ
24	6,0	–	0	✓		JEJ
25	5,9	–	0	✓		JEJ
26	5,8	–	0	✓		JEJ
27	5,7	91	0	✓		JJJ
28	5,6	–	0	✓		IW
29	5,5	–	0	✓	Gyting / Egg i karet	JJJ
30	5,5	–	0	✓		JEJ
31	5,4	–	0	✓		JEJ

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A1

Avdeling:	A	Antall:	Min. O_2 (%)	80	Fôrtype/batch nr.: SK 5,0mm
Måned og År:	jan.18	Vekt:	Temp. (°C)	nat 7-8 °C	Fôrregime-kanal: 48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet: SV	Flow (l/min):		Lysregime-kanal: Tromsølys

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	5,5	–	0	✓		Jej
2	5,5	–	0	✓		IN
	5,5	92	0	✓		IN
4	5,4	–	0	✓		IN
5	5,4	–	0	✓		IN
6	5,3	–	0	✓		HT
7	5,2	–	0	✓		HT
8	5,1	–	0	✓		IN
9	5,2	–	0	✓		IN
10	5,2	90	0	✓		IN
11	5,2	–	0	✓		IN
12	5,2	–	0	✓		IN
13	5,2	–	0	✓		AHS
14	5,1	–	0	✓		AHS
15	5,0	–	0	✓		IN
16	5,0	–	0	✓		IN
	4,9	94	0	✓	PRØVETAKNING = 5 STK, 24t LYS	IN
18	4,7	–	0	✓	TEMP ↑ –	IN
19	6,2	–	0	✓	TEMP ↑ – 3°C OVER NAT.	IN
20	8,0	–	0	✓		Jej
21	8,0	–	0	✓		Jej
22	7,9	–	0	✓		IN
23	7,9	–	0	✓		IN
24	7,8	91	0	✓		IN
25	7,8	–	0	✓		IN
26	7,8	–	0	✓		IN
27	7,8	–	0	✓		IN
28	7,7	–	0	✓		IN
29	7,7	–	0	✓		IN
30	7,7	–	0	✓		HT
31	7,6	92	0			HT

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A2

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**jan.18**	Vekt:		Temp. (°C)	**nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**Tromsølys**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	5,5	—	0	✓		IEJ
2	5,5	—	0	✓		IEJ
3	5,5	92	0	✓		IN
4	5,4	—	0	✓		IN
5	5,4	—	0	✓		IN
6	5,3	—	0	✓		HT
7	5,2	—	0	✓		HT
8	5,1	—	0	✓		IN
9	5,2	—	0	✓		IN
10	5,2	91	0	✓		IN
11	5,2	—	0	✓		IN
12	5,2	—	0	✓		IN
13	5,1	—	0	✓		AHS
14	5,1	—	0	✓		AHS
15	5,0	—	0	✓		IN
16	5,0	—	0	✓		IN
17	4,9	94	0	✓	PRØVETAKING - 8 STK. , 24t LYS	IN
18	4,7	—	0	✓		IN
19	4,6	—	0	✓		IN
20	4,5	—	0	✓		IEJ
21	4,4	—	0	✓		IEJ
22	4,3	—	0	✓		IN
23	4,3	—	0	✓		IN
24	4,2	91	0	✓		IN
25	4,2	—	0	✓		IN
26	4,2	—	0	✓		IN
27	4,2	—	0	✓		IN
28	4,0	—	0	✓		IN
29	3,9	—	0	✓		IN
30	4,0	—	0	✓		HT
31	4,0	90	0	✓		HT

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A3

Avdeling:	A	Antall:		Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	jan.18	Vekt:		Temp. (°C)	nat 7-8	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	5,5	–	0	✓		JEJ
2	5,5	–	0	✓		IN
3	5,5	92	0	✓		IN
4	5,4	–	0	✓		IN
5	5,4	–	0	✓		IN
6	5,3	–	0	✓		HTS
7	5,2	–	0	✓		HTS
8	5,1	–	0	✓		IN
9	5,2	–	0	✓		IN
10	5,2	90	0	✓		IN
11	5,2	–	0	✓		IN
12	5,2	–	0	✓		IN
13	5,2	–	0	✓		AHS
14	5,1	–	0	✓		AHS
15	5,0	–	0	✓		IN
16	5,0	–	0	✓		IN
17	4,9	95	0	✓	PRØVETAKNING – 8 STK	IN
18	4,7	–	0	✓	TEMP 4 – 6,2°C	IN
19	6,4	–	0	✓	TEMP ↑ – 3,1°C OVER NAT.	IN
20	7,7	–	0	✓		JEJ
21	7,6	–	0	✓		JEJ
22	7,6	–	0	✓		IN
23	7,5	–	0	✓	GYTING	IN
24	7,5	92	0	✓		IN
25	7,5	–	0	✓		IN
26	7,5	–	0	✓		IN
27	7,5	–	0	✓		IN
28	7,4	–	0	✓		IN
29	7,4	–	0	✓	GYTING	IN
30	7,4	–	0	✓		AHS
31	7,4	92	0	✓	1 DØD HANN	AHS

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A4

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**jan.18**	Vekt:		Temp. (°C)	**nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**24t**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	5,6	—	0	✓	Gyting	
2	5,5	—	0	✓		
	5,5	94	0	✓		
4	5,4	—	0	✓		
5	5,4	—	0	✓		
6	5,3	-	0	✓		
7	5,2	-	0	✓		
8	5,1	—	0	✓		
9	5,2	—	0	✓		
10	5,2	93	0	✓		
11	5,2	-	0	✓		
12	5,2	—	0	✓		
13	5,2	-	0	✓		
14	5,1	-	0	✓		
15	5,0	—	0	✓		
16	5,0	—	0	✓		
	4,9	95	0	✓	PRØVETAKNING – 8 STK	
18	4,7	-	1	✓		
19	4,6	—	0	✓		
20	4,5	-	0	✓		
21	4,4	-	0	✓		
22	4,3	—	0	✓		
23	4,3	-	0	✓		
24	4,2	94	0	✓		
25	4,2	—	0	✓		
26	4,2	—	0	✓		
27	4,2	—	0	✓		
28	4,0	—	0	✓		
29	3,9	—	0	✓		
30	4.0	-	0	✓		
31	4.0	93	0	✓		

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk				Skjema
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	H17/27	Ansv. forskningstekniker:	Ivar Nevermo
Forsøkshaver:	Nofima		

Kar nr.: A1

Avdeling:	A	Antall:	66	Min. O_2 (%)	80	Fôrtype/batch nr.:	SK 5,0mm
Måned og År:	feb.18	Vekt:		Temp. (°C)	3 gr over nat	Fôrregime-kanal:	48t
Art og Generasjon:	Rognkjeks-16	Vannkvalitet:	SV	Flow (l/min):		Lysregime-kanal:	24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,5	–	0	✓		IN
2	7,6	–	0	✓		IN
3	7,6	–	0	✓		BE
4	7,5	–	0	✓		BE
5	7,6	–	0	✓		IN
6	7,7	–	0	✓	PRØVETAKNING [illegible] 16 STK	IN
7	7,7	94	0	✓	~~PRØVETAKNING 8 STK~~	IN
8	7,7	–	0	✓		IN
9	7,7	–	0	✓		IN
10	7,7	–	0	✓		HT
11	7,6	–	0	✓		HT
12	7,5	–	0	✓		IN
13	7,5	–	0	✓		IN
14	7,4	90	0	✓		IN
15	7,3	–	0	✓		IN
16	7,3	–	0	✓		IN
17	7,3	–	0	✓		AHR
18	7,2	–	0	✓		AHR
19	7,1	–	0	✓		IN
20	7,0	–	0	✓	EGG/GYTING	IN
21	7,0	96	0	✓		IN
22	7,0	–	0	✓		IN
23	7,0	–	0	✓		IN
24	7,0	–	0	✓		JHJ
25	7,0	–	0	✓		JHJ
26	6,9	–	0	✓	EGG/GYTING	IN
27	7,0	–	0	✓	EGG/GYTING	IN
28	7,1	93	0	✓		IN
29						
30						
31						

Koder: – = ingen registrering. ✓ = sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A2

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**feb.18**	Vekt:		Temp. (°C)	**nat.**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**24t**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	3,9	–	0	✓		
2	3,9	–	0	✓		
3	4,0	–	0	✓		BE
4	3,9	–	0	✓		BE
5	3,9	–	0	✓		
6	4,1	–	0	✓	PRØVETAKNING, 8 STK	
7	4,1	94	0	✓	PRØVETAKN., 8 STK	
8	4,1	–	0	✓		
9	4,1	–	0	✓		
10	4,2	–	0	✓		
11	4,0	–	0	✓		
12	3,9	–	0	✓		
13	3,7	–	0	✓		
14	3,6	94	0	✓		
15	3,5	–	0	✓		
16	3,5	–	0	✓		
17	3,6	–	0	✓		MS
18	3,4	–	0	✓		MS
19	3,3	–	0	✓		
20	3,2	–	0	✓		
21	3,1	97	0	✓		
22	3,2	–	0	✓		
23	3,2	–	0	✓		
24	3,1	–	0	✓		JEJ
25	3,0	–	0	✓		JEJ
26	3,1	–	0	✓		
27	3,1	–	0	✓		
28	3,2	93	0	✓		
29						
30						
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A3

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**feb.18**	Vekt:		Temp. (°C)	**3 gr over nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**24t**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,4	–	0	✓		IW
2	7,4	–	0	✓		IW
3	7,3	–	0	✓		BE
4	7,3	–	0	✓		BE
5	7,4	–	0	✓		IW
6	7,5	–	0	✓	PRØVETAKNING, 8 STK	IW
7	7,5	96	0	✓	PRØVETAKN., 8 STK	IW
8	7,5	–	0	✓		IW
9	7,5	–	0	✓		IW
10	7,5	–	0	✓		HH
11	7,4	–	0	✓		HH
12	7,3	–	0	✓		IW
13	7,3	–	0	✓		IW
14	7,2	97	0	✓		IW
15	7,1	–	0	✓		IW
16	7,1	–	0	✓		IW
17	7,1	–	0	✓		JØS
18	7,2	–	0	✓		JØS
19	7,0	–	0	✓		IW
20	6,9	–	0	✓		IW
21	6,9	99	0	✓		IW
22	6,9	–	0	✓		IW
23	6,9	–	0	✓		IW
24	6,9	–	0	✓		JEJ
25	6,9	–	0	✓	Gyting	JEJ
26	6,8	–	0	✓		IW
27	6,9	–	0	✓		IW
28	7,0	95	0	✓		IW
29						
30						
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A4

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**feb.18**	Vekt:		Temp. (°C)	**nat.**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**24t**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	3,9	–	0	✓		
2	3,9	–	0	✓		
	4,0	–	0	✓	Egg i karet.	BE
4	3,9	–	0	✓		BE
5	3,9	–	0	✓		
6	4,1	–	0	✓	PRØVETAKING. 8 STK	
7	4,1	97	0	✓	PRØVE TAKN. 8 STK	
8	4,1	–	0	✓		
9	4,1	–	0	✓		
10	4,2	–	0	✓		
11	4,0	–	0	✓		
12	3,9	–	0	✓		
13	3,7	–	0	✓		
14	3,6	96	0	✓		
15	3,5	–	0	✓		
16	3,5	–	0	✓		
	3,6	–	0	✓		
18	3,4	–	0	✓		
19	3,3	–	0	✓		
20	3,2	–	0	✓		
21	3,1	99	0	✓	EGG/GYTING	
22	3,2	–	0	✓		
23	3,2	–	0	✓		
24	3,1	–	0	✓		
25	3,0	–	0	✓		
26	3,0	–	0	✓		
27	3,1	–	0	✓		
28	3,2	97	0	✓		
29						
30						
31						

Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A1

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**mar.18**	Vekt:		Temp. (°C)	**3 gr over nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	~~Nat.~~ 24 t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,1	–	0	✓		W
2	7,2	–	0	✓		W
3	7,3	–	0	✓		JTJ
4	7,2	–	0	✓		JTJ
5	7,1	–	0	✓	GYTING / EGG	W
6	7,2	–	0	✓		W
7	7,4	88	0	✓	UTTAK – 16 STK	W
8	7,4	–	0	✓	AVSLUTTET	W
9						
10						
11						
12						
13						
14						
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Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id:	Skjema.6.1
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A2

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**mar.18**	Vekt:		Temp. (°C)	**nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	~~Nat.~~ 24t

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	3,4	–	0	✓		✓
2	3,5	–	0	✓		✓
3	3,5	–	0	✓		✓
4	3,4	–	0	✓		✓
5	3,7	–	0	✓		✓
6	3,2	–	0	✓		✓
7	3,1	91	0	✓	PRØVETAKNING – 16 STK	✓
8	2,9	–	0	✓	AVSLUTTET	✓
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Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign.

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gjelder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A3

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**mar.18**	Vekt:		Temp. (°C)	**3 gr over nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**Nat.** ~~24t~~

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	7,0	–	1	✓		
2	7,1	–	0	✓		
3	7,1	–	0	✓		
4	7,0	–	0	✓		
5	6,9	–	1	✓		
6	6,9	–	0	✓		
7	7,3	93	0	✓	PRØVE-TAKN. NY – 16 STK	
8	7,2	–	0	✓	AVSLUTTET	
9						
10						
11						
12						
13						
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Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Havbruksstasjonen i Tromsø AS			Dok id: Skjema.6.1	
Karjournal fisk			Skjema	
Skrevet av: RIH, AH, IN, HT, AHD	Godkient av: Are Homann-Danielsen	Sist revidert: 01.10.2014	Version: 1.02	Gielder fra: 04.09.2014

Forsøksnr.:	**H17/27**	Ansv. forskningstekniker:	**Ivar Nevermo**
Forsøkshaver:	**Nofima**		

Kar nr.: A4

Avdeling:	**A**	Antall:		Min. O_2 (%)	**80**	Fôrtype/batch nr.:	**SK 5,0mm**
Måned og År:	**mar.18**	Vekt:		Temp. (°C)	**nat**	Fôrregime-kanal:	**48t**
Art og Generasjon:	**Rognkjeks-16**	Vannkvalitet:	**SV**	Flow (l/min):		Lysregime-kanal:	**24t**

Dato	°C	O_2	Dødfisk	Flow	Merknader:	Sign.
1	3,4	–	0	✓		
2	3,5	–	0	✓		
3	3,5	–	0	✓		
4	3,4	–	0	✓		
5	3,2	–	0	✓		
6	3,2	–	0	✓		
7	3,1	95	0	✓	PRØVETAKING – 16 STK	
8	2,9	–	0	✓	AVSLUTTET	
9						
10						
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Koder: – = ingen registrering. ✓= sjekket. ↑/↓ = justert opp/ned.

Dato/sign. _____

Appendix 3

Body weight, total length gonad weight of each fish

	21/09/2017		
	Initial		
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	659.9	26.0	16.3
2	296.7	21.0	2.4
3	346.8	21.5	46.7
4	402.9	21.0	10.0
5	208.8	18.0	37.2
6	516.8	23.0	116.9
7	359.0	21.0	30.5
8	748.3	26.0	23.5
Average	409.6515456	22.04222457	22.10192546
St. Dev.	185.1128846	2.72472148	35.93199678

	17/01/2018		
	SDL		
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	653	25	41.6
2	1441	32	126
3	816	27	27.8
4	716	20.5	24.1
5	492	23.5	18.3
6	653	24	15.1
7	590	24.5	21.8
8	806	25.2	28.7
Average	733.7205959	25.0326408	29.71060652
St. Dev.	291.210497	3.304299148	36.48012179

17/01/2018			
CDL			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	681	26	13.2
2	402	23	5.6
3	565	23	13.1
4	824	28.5	29.3
5	494	24	15
6	1536	28.5	15
7	748	26	11.8
8	487	23	11.2
Average	658.2065405	25.15526494	13.05488131
St. Dev.	360.4411731	2.360387377	6.76308256

6/02/2018			
SDL3T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	1003	28.4	2.3
2	1157	30	83.53
3	765	27	55.1
4	752	26.2	47.7
5	354	22	5.75
6	1585	33.1	98.6
7	597	24.2	49.75
8	837	27.6	46.4
Average	811.0184528	27.12595092	30.02700492
St. Dev.	373.6104732	3.406480169	33.27188064

6/02/2018			
SDL0T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	816	27	27.53
2	791	26.7	41.28
3	1273	32	117.28
4	513	24.5	29.05
5	369	22.8	14.1
6	592	24.5	48.47
7	736	27.8	27.2
8	1070	31.5	77.05
Average	720.3652582	26.928113	39.17362027
St. Dev.	294.0495585	3.29588488	33.90839213

6/02/2018			
CDL0T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	565	24.2	6.17
2	751	29	33.9
3	818	29	89.67
4	791	28	53.44
5	504	23	22.71
6	615	24.6	32.7
7	913	28.5	54.3
8	1054	29.5	73.2
Average	731.3990663	26.86194166	36.21464938
St. Dev.	184.4845619	2.593811316	27.31522204

6/02/2018			
CDL0T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	706	27	25
2	393	23.1	6.2
3	373	24	9.9
4	926	30	21.17
5	492	25	29.27
6	655	25.5	36.74
7	1153	31	56.6
8	272	19.1	7.44
Average	560.0995329	25.33074345	18.66185772
St. Dev.	301.5103647	3.815546207	17.12024783

7/03/2018			
SDL3T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	595	25	0.83
2	608	27	0.8
3	575	25.1	0.36
4	588	25	21.84
5	495	23.7	23.67
6	796	27.9	11.26
7	794	28.1	0.19
8	585	24	24.07
Average	621.9350659	25.67576853	2.988554597
St. Dev.	107.7019697	1.711932909	11.23363286

7/03/2018			
SDL0T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	967	30	11.15
2	531	24	21.06
3	675	26.8	35.38
4	699	27.8	39.75
5	432	22	15.68
6	790	26.8	44.18
7	800	28	0.84
8	567	24.5	0.32
Average	663.3774865	26.12457353	9.410304005
St. Dev.	171.4117328	2.572346066	17.13417471

7/03/2018			
CDL3T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	699	27.5	0.74
2	632	26	48.33
3	512	24.5	10.41
4	847	28.5	4.5
5	1183	29	93.27
6	1269	30	72.4
7	1314	30.5	0.85
Average	870.2159739	27.92668843	9.944272861
St. Dev.	329.0763408	2.160246899	38.33339337

7/03/2018			
CDL0T			
#Fish	Body weight (g)	Total length (cm)	Gonad weight (g)
1	431	21.4	14.6
2	456	24	0.39
3	504	23.5	8.8
4	762	26.2	0.72
5	694	24	59.1
6	856	26.5	49.08
7	1103	30.5	0.53
8	956	29	64.47
Average	682.8680329	25.48406125	6.59431632
St. Dev.	245.8784543	3.021323031	27.9397865

Appendix 4

Area Fraction

21/09/2017 - Initial								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0.01363	0	0	0	0.01133	0	0	0.00315
SC	0.00187	0	0	0.00504	0.01102	0.04932	0	0.01072
ST	0.01513	0.25309	0	0.03232	0	0.33392	0	0.1866
SZ	0.58818	0.68859	0.87616	0.87742	0.41004	0.56752	0.83433	0.66365
TsC	0.38119	0.05833	0.12384	0.08522	0.56761	0.04923	0.16567	0.13588

17/01/2018 - SDL								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0.00089	0.00632	0.82641	0.43804	0.00832	0	0	0.00959
SC	0.16162	0.29624	0	0.31698	0.00412	0.00741	0.00919	0.24497
ST	0.31978	0.65027	0	0.17551	0.0519	0.08832	0.52073	0.69945
SZ	0.49255	0.02079	0	0	0.73067	0.85333	0.39795	0.00849
TsC	0.02515	0.02637	0.17359	0.06948	0.22045	0.05094	0.07213	0.0375

17/01/2018 - CDL								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0	0.01129	0.02358	0.01277	0.81057	0	0	0.00093
SC	0	0	0.31286	0.19859	0	0	0	0
ST	0.03485	0.01319	0.28095	0.56855	0	0.01043	0	0.06104
SZ	0.92182	0.59861	0.29789	0.18381	0	0.53682	0.70238	0.80528
TsC	0.04333	0.37804	0.08472	0.03628	0.18943	0.44789	0.29762	0.13275

6/02/2018 - SDL3T								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0.91133	0.02165	0.00536	0.0018	0.10069	0.00562	0.09174	0.02516
SC	0	0.09241	0.01696	0.01884	0.39932	0.04119	0.16742	0.142
ST	0	0.79885	0.32167	0.3684	0.38501	0.70085	0.72744	0.60276
SZ	0	0.025	0.63548	0.53466	0	0.1908	0	0.23009
TsC	0.08867	0.06209	0.02054	0.0763	0.11498	0.06154	0.0134	0

6/02/2018 - SDL0T								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0.24601	0.00187	0	0	0.00224	0.0018	0.00108	0.00982
SC	0.42155	0.01696	0.02573	0.02411	0	0.03992	0	0.10544
ST	0.28725	0.09076	0.25142	0.16397	0	0.13187	0	0.34767
SZ	0	0.83486	0.68529	0.79189	0.55099	0.7209	0.62897	0.41776
TsC	0.07312	0.05554	0.03756	0.02003	0.44677	0.1064	0.36995	0.06484

6/02/2018 - CDL3T								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0.09714	0	0.00463	0.03169	0	0.00283	0.10442	0.00272
SC	0	0	0.0397	0.13089	0	0.04013	0.14787	0.21986
ST	0	0.01964	0.54788	0.53658	0	0.09449	0.61341	0.35884
SZ	0.03279	0.93733	0.38328	0.26679	0.81852	0.82135	0.1035	0.39989
TsC	0.87007	0.04303	0.02451	0.03404	0.18148	0.0412	0.0308	0.01869

6/02/2018 - CDL0T							
#Fish	1	2	3	4	5	6	7
Categories							
SG	0.00185	0	0	0	0	0.0225	0.00303
SC	0	0	0	0	0	0.3071	0
ST	0.22372	0	0.00818	0.00536	0.02276	0.50315	0
SZ	0.68921	0.76676	0.87736	0.69516	0.93681	0.13884	0.48185
TsC	0.08521	0.23324	0.11446	0.29948	0.04042	0.0284	0.51512

7/03/2018 - SDL3T								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0	0	0.97474	0.96027	0.73352	0	0	0.52459
SC	0	0.00192	0	0.00357	0.18996	0.01171	0	0.39852
ST	0	0.10557	0	0	0	0.19281	0	0
SZ	0.74004	0.81073	0	0	0	0.70766	0.27079	0
TsC	0.25996	0.08178	0.02526	0.03616	0.07652	0.09139	0.72921	0.05532

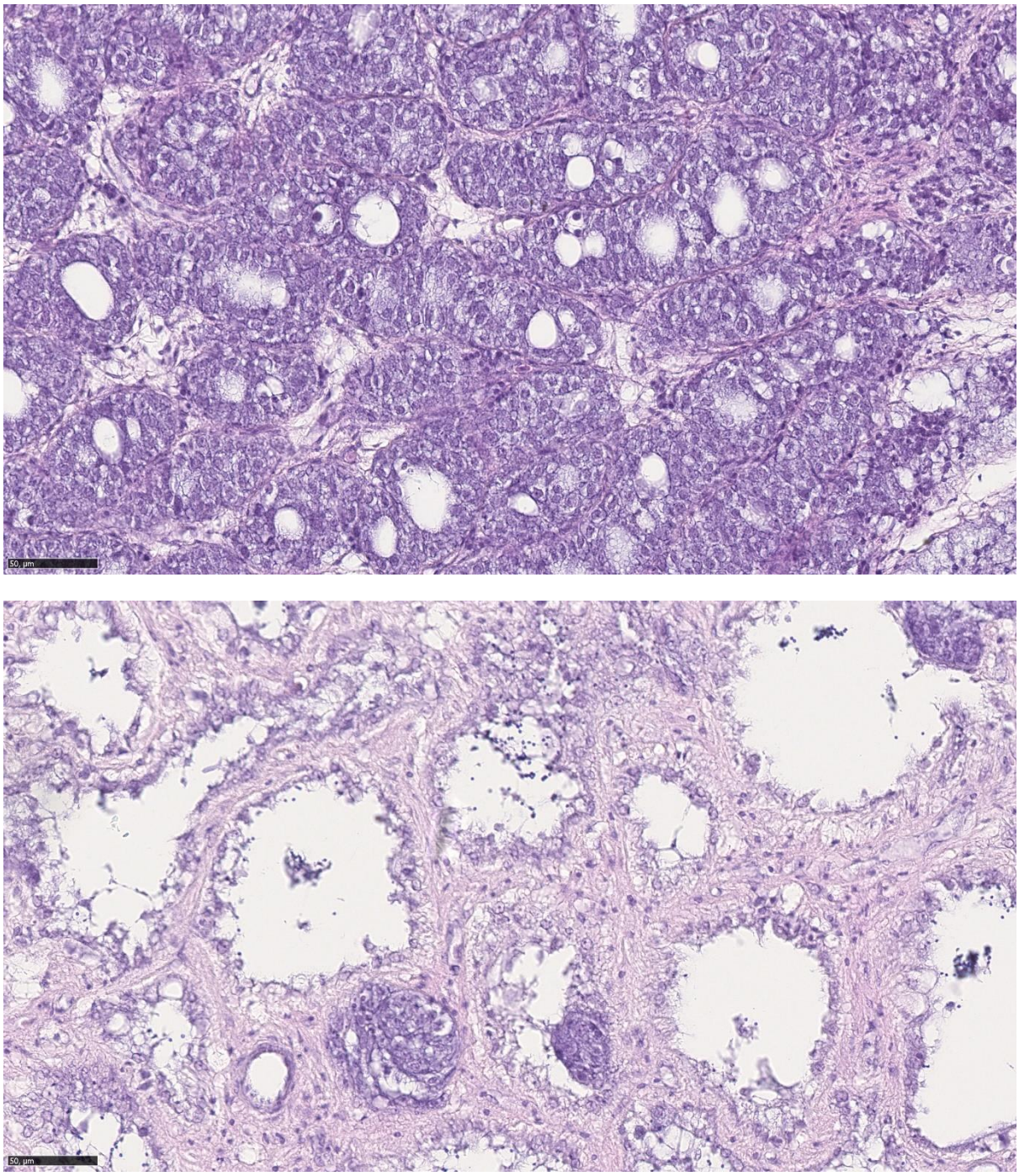
7/03/2018 - SDL0T								
#Fish	1	2	3	4	5	6	7	8
Categories								
SG	0.00182	0	0.67074	0	0.88825	0	0.85759	0
SC	0.03143	0.58087	0.26855	0	0	0	0	0.28868
ST	0.18894	0.40503	0	0	0	0	0	0.52061
SZ	0.74409	0	0	0.80296	0	0.84553	0	0.14344
TsC	0.03372	0.0141	0.0377	0.19704	0.11175	0.15447	0.14241	0.05091

7/03/2018 - CDL3T							
#Fish	1	2	3	4	5	6	7
Categories							
SG	0.03619	0.84221	0.89885	0.92599	0.84974	0	0
SC	0.22232	0	0.01081	0.00089	0.00893	0.00275	0
ST	0.32288	0	0	0	0	0.04035	0
SZ	0.41499	0	0	0	0	0.86187	0.82634
TsC	0.00362	0.15779	0.09034	0.07312	0.14133	0.09503	0.17366

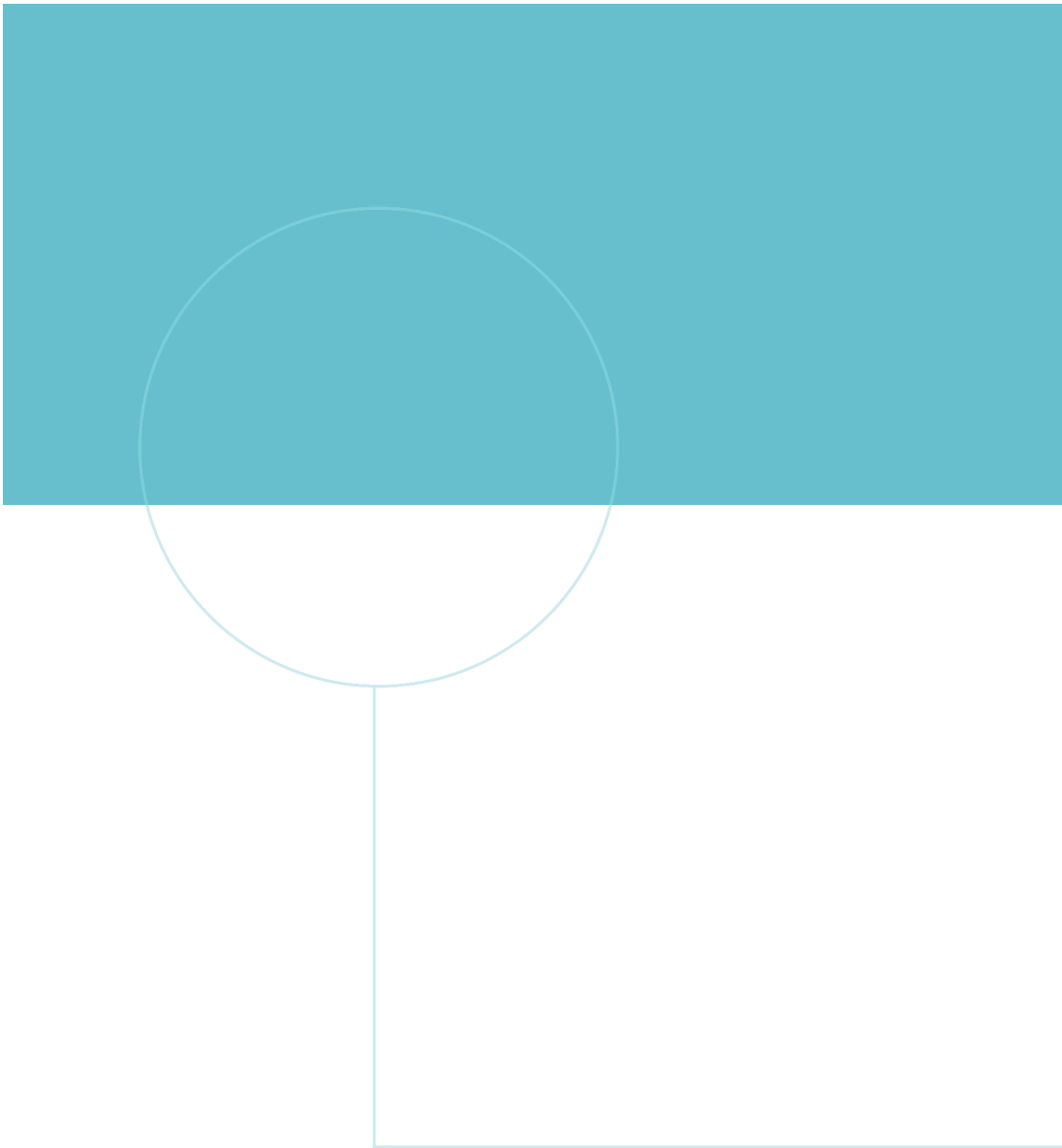
7/03/2018 - CDL0T							
#Fish	1	2	3	4	5	6	7
Categories							
SG	0	0.82307	0	0.18763	0	0	0
SC	0.02255	0.0125	0	0.03119	0	0	0
ST	0.13424	0	0	0	0	0.00446	0.10089
SZ	0.82351	0	0.82273	0.08909	0.83288	0.87641	0.75536
TsC	0.0197	0.16443	0.17727	0.69208	0.16712	0.11912	0.14375

Appendix 5

Example of recrudescent and spent males



40x microphotographs of a recrudescent male (top) and a spent male (bottom) from March (07/03/2018)



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