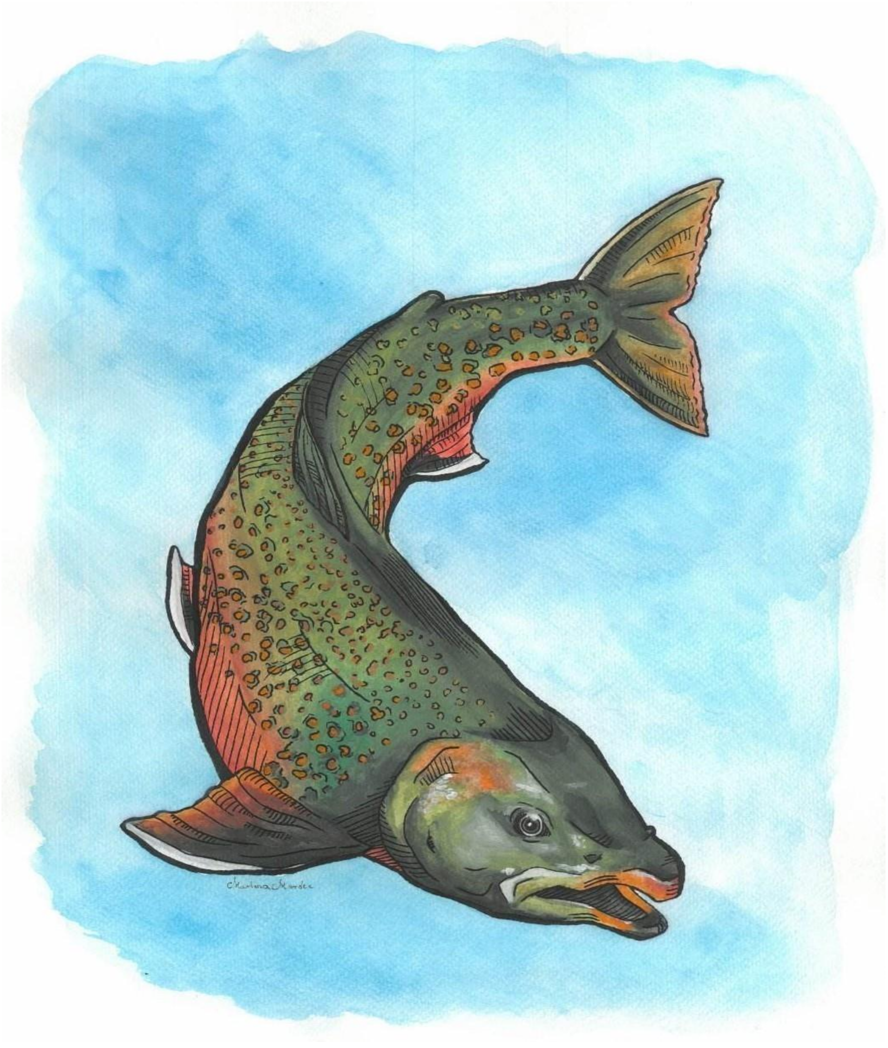
Faculty of Biosciences, Fisheries and Economics

Department of Arctic and Marine Biology

„Circannual rhythms in Arctic charr (*Salvelinus alpinus*)”

Magdalena Maria Pełko

BIO-3950 Master’s thesis in Biology, May 2023



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Supervisors

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David Hazlerigg, UiT – The Arctic University of Tromsø

Cover drawing by Marlena Mordec

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Tromsø – May 2022

Abstract

The Arctic charr (*Salvelinus alpinus*) is a true arctic species and is considered to be the northernmost freshwater fish. Since this species is being known for its great variability in size, phenotype, colour, ecology and history, some of them are landlocked (which stay in freshwater all their life) and others are anadromous (they undertake summer migrations to sea and then they come back to freshwater). Some fish from salmonid family (to which Arctic charr also belongs) as rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar) display circannual rhythms in maturation and reproduction.

Circannual rhythms are endogenous biological oscillations which underlie a wide range of seasonal processes. Reproductive activities are then well-timed to period of year, where spring and summer conditions favor rearing offspring. During winter metabolic rates may be reduced which results in weight loss. Photoperiod is one of most well-known cues, which shows the least year to year variability and is a major source of predictive environmental information in controlling various seasonal activities. Better understanding of fish physiology makes it easier to breed fish in aquaculture and lower the mortality of fish.

For this study more than 200 fish were put in 8 tanks (2 tanks for each of four treatments) in SNP (simulated natural photoperiod), SP (short photoperiod with 6 hours of light and 18 hours of dark), IP (intermediate photoperiod) and LL (constant photoperiod with 24 hours of light). The first hypothesis was about growth and reproductive status of research animals being under circannual control. The second one hypothesized that these circannual characteristics are dependent upon photoperiod. The last one suggested that circannual rhythm characteristics are dependent on the life history of an animal.

It was shown that although Arctic charr is a very variable species, there is a rhythmicity in weight, length and reproductive status, which are not damped in any of all these 4 treatments.. Photoperiod has an effect on reproductive status, where fish in constant light had different timing of reproductive status comparing to SNP, SP and SP treatments. However it didn’t have an effect on maturation of fish. The SP treatment might be one where fish maintain circannual rhythms in reproductive status for 2 or even more cycles, however, there was only 16% of fish confirming this hypothesis. Arctic charr’s circannual rhythms is then depending strongly on the individual life history stage of the animal.

Keywords: *Salvelinus alpinus*, circannual rhythm, biological rhythms,

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1 Introduction

1.1 Chronobiology

Since the Earth rotates on its axis relative to the sun every 24 hours with an iclination of ~23.45 degrees from the plane of its orbit around the sun, as a result, there are day and night and leads to seasons and different climates.

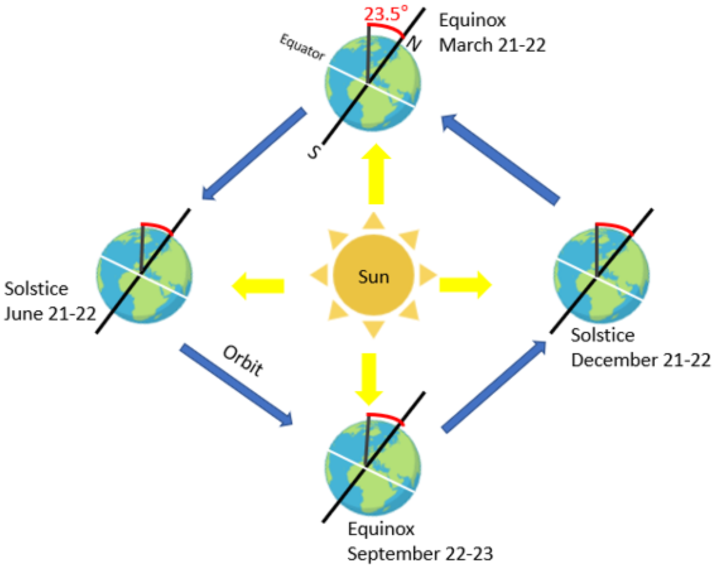


Figure 1: The Earth’s axis through the seasons, The earth's *axis of rotation tilts* by 23.5° relative to the plane of the earth's orbit around the sun (right)

Chronobiology is described as studying the biological rhythms and the mechanisms of biological timekeeping. It is relevant to the other fields, especially medicine, pharmacology, and drug delivery, and it helps to better understand how different organisms adapted to their environments (Smolensky and Peppas, 2007). The term “biological rhythm” means a self-sustaining oscillation of endogenous origin (defined by the characteristics of period, amplitude, and phase). There are many types of biological rhythms, but these four are probably the most well-known. The first ones are circadian rhythms, driven by an internal clock (situated in the hypothalamus) and last about 24 hours (Arendt, 1998). The main function is that they provide an external local time and then allow organism to program its activities (then they can occur at an appropriate time). Circadian rhythms are controlled by self-sustained oscillators (they are temperature compensated, which means that they run with the same period at different constant temperatures) and continue to oscillate in constant conditions. Entrainment is the most important property by which the phase relationship of the clock can be determined. Second ones are diurnal rhythms, which are circadian clocks synchronized with day/night cycles. Third ones, the ultradian rhythms are "short-term rhythms" with a frequency of 10^{-3} to $5x20^{-5}$ Hz – periods in the range of 20 minutes to 6 hours (Daan and Aschoff, 1981). Lastly, infradian rhythms which period lasts longer than one day, with a frequency of less than one cycle in 24 hours (Brown *et al.*, 1982). In some fish, like for example in rainbow trout, there is a biological activity that oscillates under constant environmental conditions with period length close to but not exactly equal to 24h, which is called circadian rhythm (Dunlap, *et al.*, 2004). In zebrafish (which is the most well-known studied fish) the environmental light signal sets the clock to the same phase in all cells and tissues in vivo. It might occur that light *in vivo* could set all the body clocks to the same phase. Then various hormonal and neuronal cues apply subtle or less to adjustments to this timing (Frøland Steindal and Whitmore, 2019).

1.1.1 Circannual rhythms

Some animals (especially long-lived ones) are well adapted to live in seasonally changing environments. They have evolved control systems which let them adapt to annual fluctuations of biologically significant factors. These systems define circannual rhythms which are endogenous biological oscillations that underlie a wide range of seasonal processes (Helm and Stevenson, 2014). During spring and summer (in temperate zones) when the conditions favor rearing the offspring, the reproductive activities of organisms are timed accordingly to that period of year. When winter comes many animals reduce their metabolic activities by even going into a state of hibernation or by simply leaving their home area in late summer or early autumn to a place with a more favorable climate. This and other activities constitute annual "phase maps," which can specify when different processes of an individual, population or species can occur (Lack 1950; Murton and Westwood 1977; Farner and Follett 1979). The most well-known examples for showing circannual rhythms are African stonechat and golden-mantled ground squirrel (Gwinner, 1991; cf Lee and Zucker, 1991). In East African Stonechats, not only the moult but also the duration of the annual breeding season depends primarily on an endogenous circannual program. This is indicated by the observation that in a constant equatorial photoperiod the duration of the reproductively active phase, termed 'reproductive window', is like the reproductive window of freeliving conspecifics (Gwinner 1991). The reproductive window is defined as the interval between successive phases of gonadal growth and regression. Since in their captive birds' nutritional conditions were constant and much better than those of their conspecifics in nature, these observations suggest that favourable feeding conditions have no major effect on the duration of the width of the reproductive window (Gwinner, 1991). In golden-mantled ground squirrels under natural conditions, long days and short nightly melatonin signals are producing phase delays which facilitate entrainment to a tau of 12 months (Hiebert *et al.*, 1995).

Ultimate factors are environmental variables which, in evolution, exert selection pressure to restrict an activity to a particular time of the year (Baker, 1938a). The best example is a large food supply. It is sufficient for raising the offspring and to cover the energetic costs of the parents. The reproductive season’s critical phases always coincide with that factor. It is the best seen among food specialists in extreme environments. The young red-backed sandpiper (*Calidris alpina*) in Alaska normally hatches when adult dipterans (the major food items of the chicks) emerge. If it hatches before or after this time, it has high chance that it will not survive (Holmes 1966).

There are also proximate factors which assure the coincidence of abundant trophic resources and maximum food requirement, the preceding processes of gonadal development and many others to be initiated long in advance (Baker, 1938a). Ambient temperature is very often used as this factor in many mid and high-latitude species – especially poikilotherms (Danilevskii, 1965). Other environmental variables also contain predictive information, and they could be also used as proximate cue (in temperate and arctic regions). Photoperiod (the light fraction of the 24-hour day) is an even more reliable cue. Among all other environmental factors, it shows the least year to year variability. It makes it the major source of predictive environmental information in controlling various seasonal activities (Immelmann, 1973).

Circannual rhythms have been mostly studied in birds. It was discovered that high-latitude birds can be subdivided into a photosensitive and photorefractory half-cycle. Photosensitive half-cycle is physiologically characterized by at least some secretion of GnRH (gonadotrophin releasing hormone and can respond to long days. In photorefractory half-cycle there is an absence of GnRH and no respond to long photoperiods with that gonadal development. To transit to photosensitive half-cycle, there must be shorter amount of light and longer amount of dark (Gwinner, 2003). And to transit back to photorefractory half-cycle there must be a long photoperiod. From research on European starlings (Sturnus vulgaris) the easiest way to see both transitions are to make an experiment where there is 12 hours of light and 12 hours of dark. It happens because this photoperiod is short enough to transit to photosensitive half-cycle and long enough to come back to photorefractory one. These two half-cycles with their photoperiod requirements are parts of basic circannual calendar and shows relationship between endogenous components and photoperiod - where photoperiod sets limits to expression of circannual rhythms – (Gwinner, 1996).

However, for some migratory birds in which circannual rhythmicity is synchronized by the annual photoperiodic cycle it determines when the animals come into general migratory condition. In Figure 2 below there could be seen the variation in annual amount of light in northern hemisphere and how is it changing with latitudes. The stimuli that release or inhibit migration are provided by the food condition or the weather (Gwinner 1986). In higher latitudes (Figure 2) photoperiodic stimulation in the same animals leads only to a partial development of the ovary. For final follicular development and vitellogenesis is required supplementary information from the environment (as adequate food) (Immelmann 1973; Murton and Westwood 1977; Farner and Follet 1979).

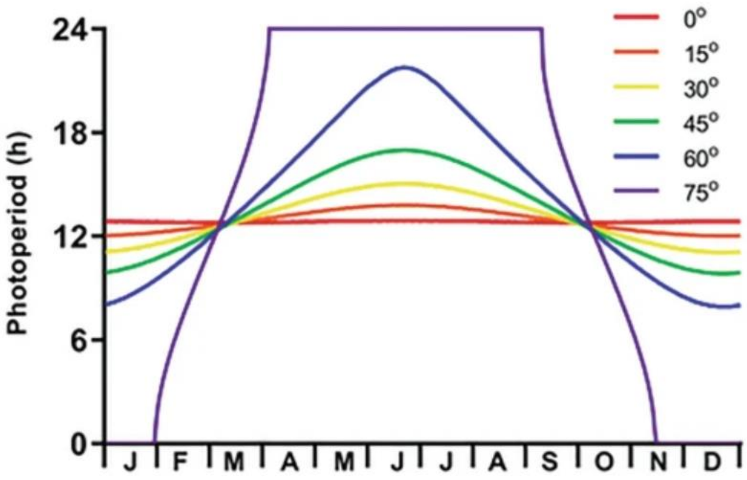


Figure 2: Annual variations in day length in northern hemisphere

1.1.2 Circannual rhythms in salmonids

In the salmonid family there are only a few examples with evidence of circannual rhythm. In 1991 Duston and Bromage showed that female rainbow trout (Figure 3a) maintained under constant 6 hours of light and 18 hours of darkness, constant temperature and constant food ration exhibit a rhythm of gonadal maturation and spawning. It is self-sustaining for at least three cycles, and it free-runs with a periodicity that approximates. However, it can vary significantly from 12 months if there is no zeitgeber (time cue). It is known that the rhythm can be entrained by changes in photoperiod (Duston and Bromage, 1987). In one studies salmon (Figure 3b) which had spent two or more winters at sea returned earlier than the grilse to coastal Norwegian waters. The postspawners descended downstream earlier than the smolts and the older and larger smolts descended earlier in the spring than younger and smaller smolts (Jonsson *et al*., 1990).

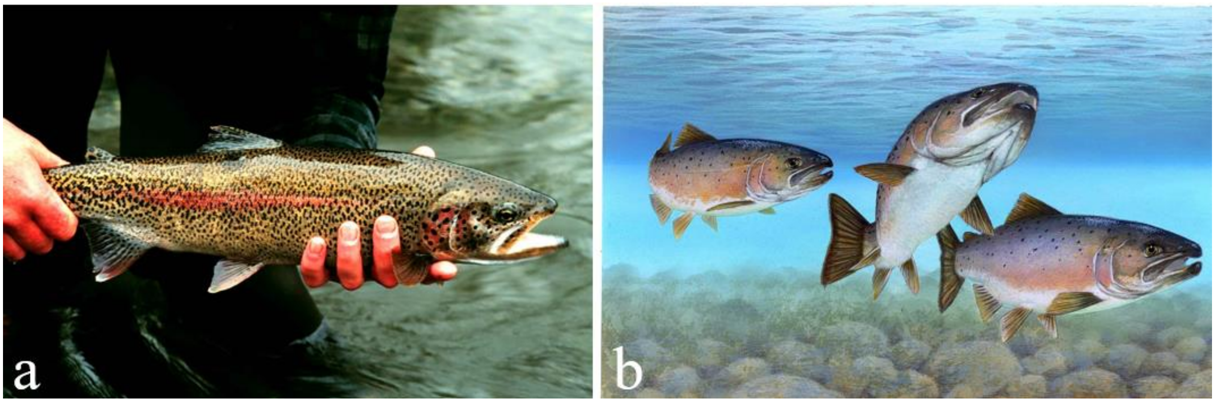


Figure 3: Salmonids which show circannual rhythms, a=Rainbow trout (*Oncorhynchus mykiss*), b=Atlantic salmon (*Salmo salar*)

1.2 Arctic environment

Arctic means the region under Arctos (polar star), even though there is no general agreement on the limits of this region. In other words, this is a polar circle which is parallel to latitude $23^{o}28'$N from the pole and defined by the angle between the axis of rotation of the earth and the ecliptic (Blix, 2005). These areas are colder than the rest of the world because of the less amount of solar radiation, which is received per year (which is mostly reflected rather than absorbed). The period of continuous light in summer and the period without sun, they both increase from one day (per year) at the polar circle to 6 months (per year) at the pole (Blix, 2005). The most well-known definition for defining the Arctic region is that this is an area enclosed by 10^{o}C isotherm for the warmest summer month (most often it is July), in the northern hemisphere. It encircles the Arctic Ocean and includes some islands like Greenland and Svalbard as also countries like the northern 2/3 of Iceland and most of the northern coast and all off lying islands of Russia, Canada and Alaska and the most northern parts of Scandinavia (Blix, 2005).

1.3 Arctic charr

Arctic charr (*Salvelinus alpinus*) is the world's northernmost freshwater fish species (Johnson, 1980). This species has a bewildering phenotypic and ecological diversity throughout its circumpolar range. It shows extreme life history diversity at the species, population and even individual (ontogenetic) levels. Arctic charr (Figure 4) is the most variable of all vertebrates in range, size at maturity, phenotype (color and form), in behavior, ecology and in life history (Klemetsen, 2010).

Figure 4: Arctic charr, Fot: Mathias Leines Dahle

Arctic charr is a true Artic species, because of its distribution (Figure 5) which is circumpolar and confined to the north of the 10^{o}C July isotherm (Klemetsen et al., 2003). It can live in water with temperatures that range between 0.1^{o}C as a minimum in wintertime and 3.6^{o}C as a maximum in late summer (Svenning *et al*., 2007).

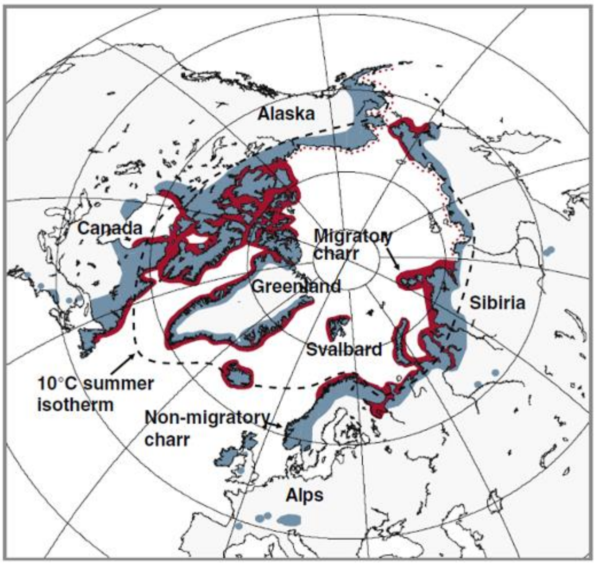


Figure 5: Distribution of Arctic charr by Jørgensen and Johnsen, 2014, blue dashed line shows 10^{o}C isotherm

Some individuals of Arctic charr are landlocked (they stay all the time in freshwater, which is visualized on Figure 6 in smaller lake) and others have an anadromous life strategy (Figure 6) and undertake annual, short-lasting migrations during summer (Gross *et al.*, 1988). They are visualized in the biggest lake on the drawning (Figure 6). During this time, they increase in body weight during their summer residence in seawater (Mathisen and Berg, 1968; Berg and Berg, 1989; Finstad and Heggberget, 1993; Jørgensen *et al.*, 1997; Rikardsen et al., 2000). However, when they come back to lake for autumn, later during winter, they lose weight (Dutil, 1986; Boivin and Power, 1990; Finstad and Heggberget, 1993; Jørgensen *et al.*, 1997).

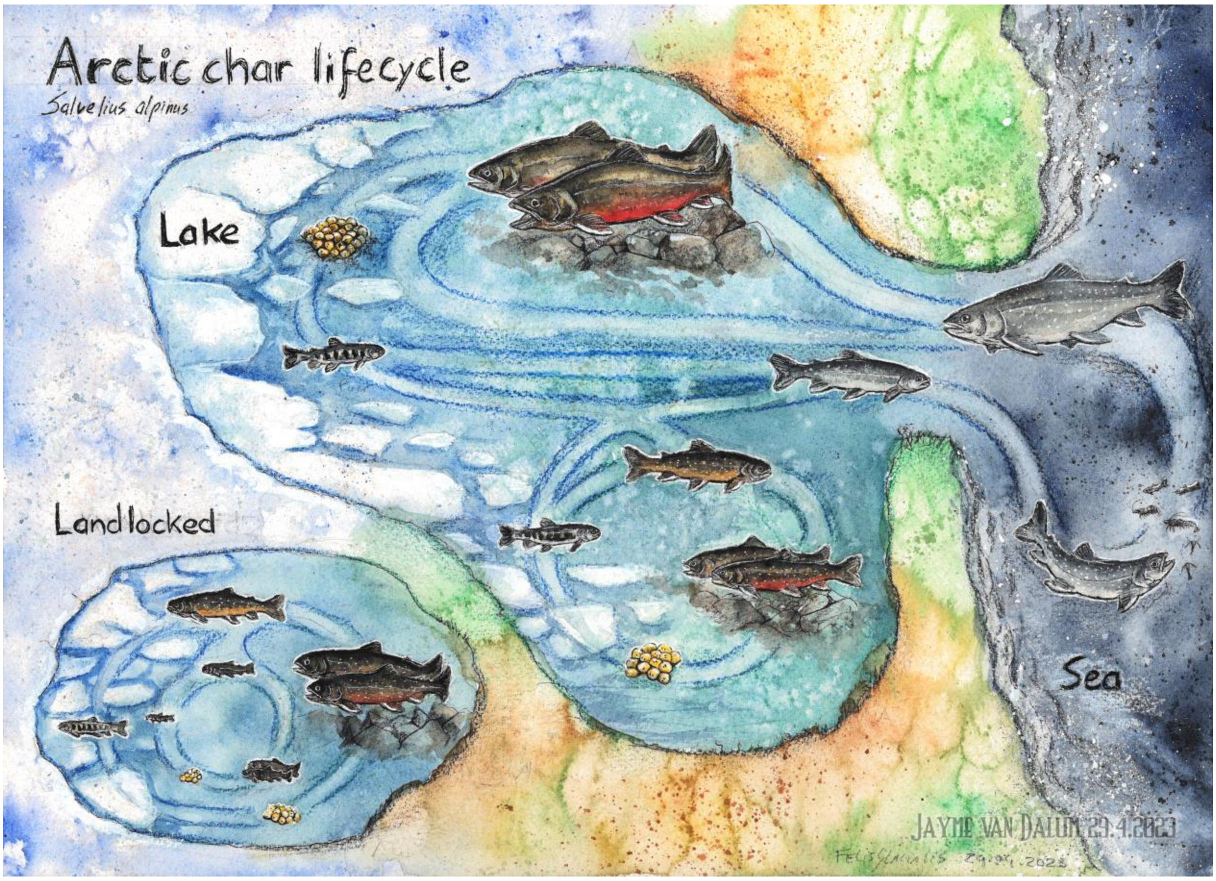


Figure 6: Arctic char lifecycle by Jayme Van Dalum 29.04.2023

Findings from a study by Hawley et al. from 2017 of full-year analysis of diel and seasonal activity of Arctic charr have shown variability in the strength of activity rhythms in two morphs. They also confirmed different activity rhythms in feeding, where there is a summer satiation and winter deprivation. It was also shown that similar to Svalbard reindeer the output rhythms of an internal circadian clock may be temporarily uncoupled or unsynchronized in constant treatments of photoperiods - constant light or dark – (van Oort *et al.*, 2005).

1.4 Aim of the study

In this experiment the main task was to analyze the growth and reproductive activity of Arctic charr in different photoperiods from the data which was collected to investigate if these animals show circannual rhythms. These rhythms are mostly known in birds or mammals, and they are least studied in fish (few examples in salmonids). Since timing of animal being able to reproduce is depending on annual seasons in arctic region and length with weight are related to amount of available food (which also depends on time of a year), the results should give reliable information to confirm one of the three hypothesis and predictions. There will be a control group with animals raised under simulated natural photoperiod (SNP). A short photoperiod (SP) will be like that during extended winter. A long constant photoperiod (LL) with 24 hours of light will show light conditions during extended summer. Intermediate photoperiod (12:12) will be important for confirming circannual rhythms because in those conditions it is possible to notice two half-cycles which are basic parts for those rhythms. These four photoperiods were chosen, so there would be a higher chance that in one of the constant photoperiods Arctic charr will show circannual rhythms.

The first hypothesis is about growth and reproductive status of Arctic charr being under circannual control. In that case animals were placed in constant photoperiod will show circannual cycles of growth and reproductive status. The second hypothesis says that circannual characteristics are dependent upon photoperiod. It will be proved if animals held on constant light will show different period length circannual rhythms than animals which were held on constant short or intermediate photoperiod. The last hypothesis suggests that circannual rhythm characteristics depend on the life history of an animal. There would be differences in circannual rhythm expression in different lightning conditions in mature males, mature females, and immature Arctic charr.

2 Methods

2.1 Animals

There were 210 individuals of 3 years old Hammerfest strain Arctic charr which were born and raised in the experimental facility in Kårvika. Each one of them was individually pit tagged to allow for individual monitoring over the whole experiment. PIT tagging is a method for tracking movements of fish for better understand their ecology (Dare, 2003; Baras *et al.,* 1999) and consist of small glass capsules, which enclose a uniquely-coded microchip surrounded by a copper wire coil. When being exposed to electromagnetic field, the copper coil powers the microchip which transmits a distinctive signal which can be detected by a low frequency antenna (Castro-Santos *et. al*, 1996; Grieve *et al.* 2018). The procedures were under FOTS ID 16431.

2.2 Experimental set up

A number of 210 individuals were kept under different lightning conditions for 21 months (from 11.07.2018 till 11.09.2020). Each fish has a unique identifier (PIT tag ID) and was assigned to a 300L tank (A, B, C, D, E, F, G, H) - two tanks were assigned to each photoperiod treatment (SNP – simulated natural photoperiod, SP – short day, where there are 6 hours of light and 18 hours of darkness, 12:12 and LL-long day meaning continuous light). Tank A, B (SNP), tank C, D (SP HOLD), tank E, F (12:12) and tank G, H (LL). The first two tanks will be used as controlling groups since the lightning will be like the natural in environmental condition (with changing photoperiod to longer light in summer and shortening amount of night in months where should be winter). Tank C and D will have similar light conditions as in extended winter to see if shorter photoperiod will cause the fish to either have a peak in weight/length or be in reproductive status earlier next year. Tank E and F will have a role in the period between summer and winter, and as stated in introduction important for confirming circannual rhythms (because in those conditions it is possible to notice two half-cycles - basic parts for those rhythms). And the last two tanks will have constant conditions and act as an extended summer (Figure 7).

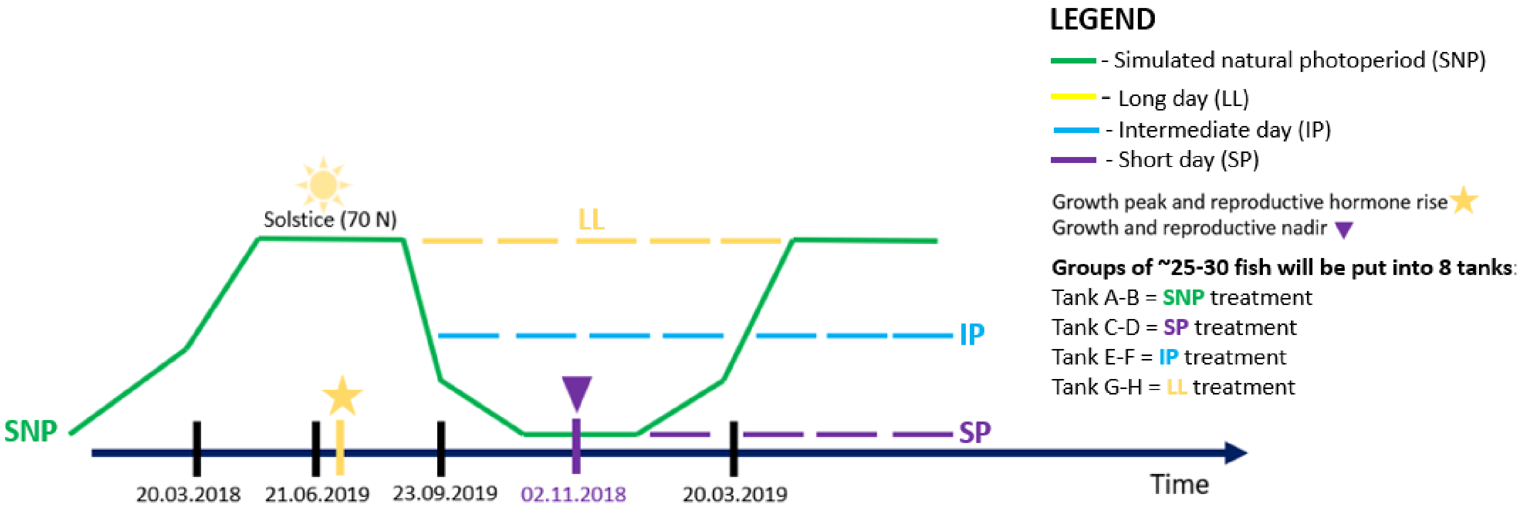


Figure 7: Presentation of duration of each treatment in specified tanks

Each identifier contains information under which lightning photoperiod fish were kept and was it an immature (0), mature female (MF) or mature male (MM) at the end of experiment. Every month all fish were weighted and measured individually, and each tank had its feed intake monitored over a 3-day period. All the data was then given to me, since I was not participating in any part of the experiment. The differences especially in weight and length among individuals in all tanks could have been easily seen (Figure 8).

Figure 8:Variability in size and coloration in different tanks, Fot: Shona Wood

In each tank there were a minimum of 24 fish. To avoid hierarchy and stabilize growth there was a minimum 40kg/m^3 density for fish and high current water. Fish at the start weighed about 500g, meaning there was 12 kg of fish per tank.

2.3 Calculations

Monthly change in weight was calculated by subtracting the weight of the weight at the end of a given month from the weight at the end of the preceeding month. The next monthly change would be calculated from the difference of 3rd and 2nd month of an experiment. And the same for following months. For data from length, there was used raw data with values written each month after measuring the fish during an experiment.

Not all fish were weighed and measured through 21 months. Some were measured for a shorter time period because they died before the experiment was finished. In this case in the months where the fish was not measured there is a blank cell, with no variable in this, even no 0.

Next, the duration of periods (span in days between first noticed peak in length/weight or reproductive status and second one) were calculated by using excel. Peaks were defined by following its definition from publication from 2022 by Baccini et al., where to state that a point on a graph can be considered a peak, it has to have one point with lower value before that point and the value had to be higher than 50g (Figure 9). Since there were many values above 50g (and there was great variation between individuals in all tanks), which showed that fish gained weight but not as much as 200g or more, they were still included as peaks to better quantify the number of them (Baccini et al., 2022). However only fish with 2 peaks in length and 2 and 3 peaks in weight were later analyzed for the period. In Table 2 below it is shown with red circle which points on the graph meet the criteria to be considered as peaks. Peaks were calculated by using the formula in excel which found the maximum value in chosen range. The same was done for length data (but to state the peak the value had to be higher than 1cm). For reproductive status data the periods and phases were calculated only.

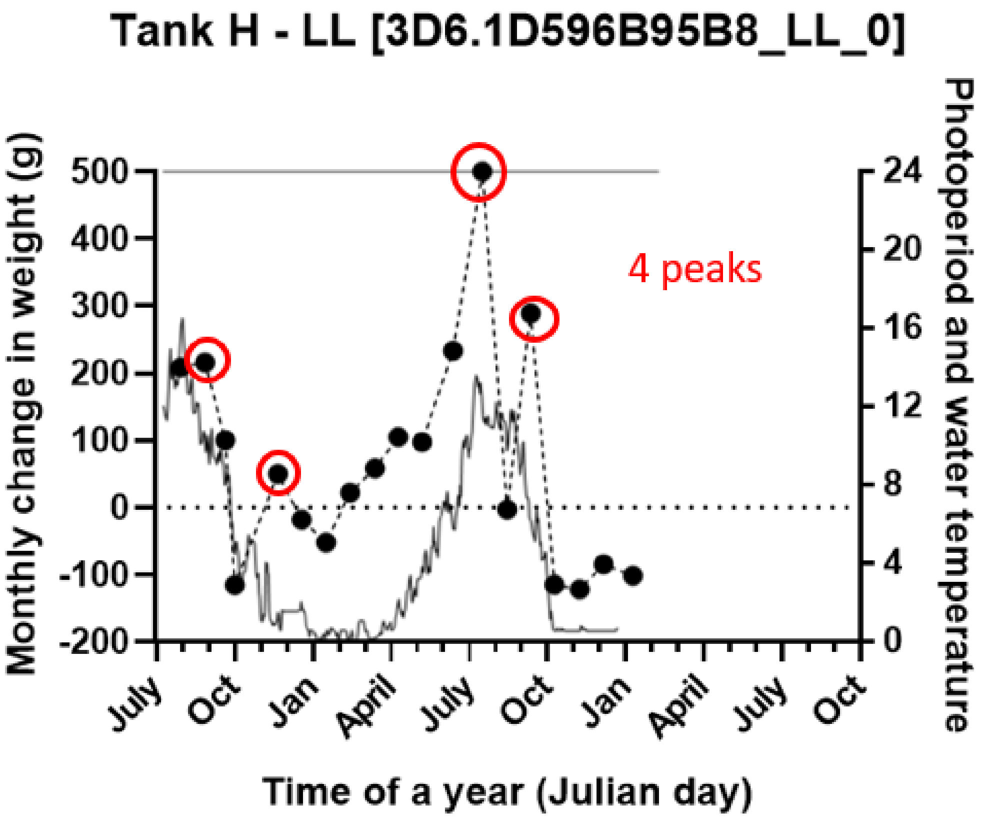


Figure 9: Example of phase shift and period

To be ale to present dates on X axis, it was converted to Julian day (Table 1), where 191 is the first day of the experiment, and 984 is the last day. In Julian day 1st of January is 0, and the 31st od December is 365 in each year.

MONTH/DAY/YEAR	JULIAN DAY
7/11/2018	191
7/30/2018	210
8/27/2018	238
9/20/2018	262
10/1/2018	273
11/20/2018	323
12/18/2018	351
1/15/2019	379
2/12/2019	407
3/13/2019	436
4/9/2019	463
5/7/2019	491
6/12/2019	527
7/16/2019	561
8/14/2019	590
9/10/2019	617
10/8/2019	645
11/6/2019	674
12/4/2019	702
1/7/2020	736
2/17/2020	777
5/7/2020	857
6/24/2020	905
8/25/2020	967
9/1/2020	984

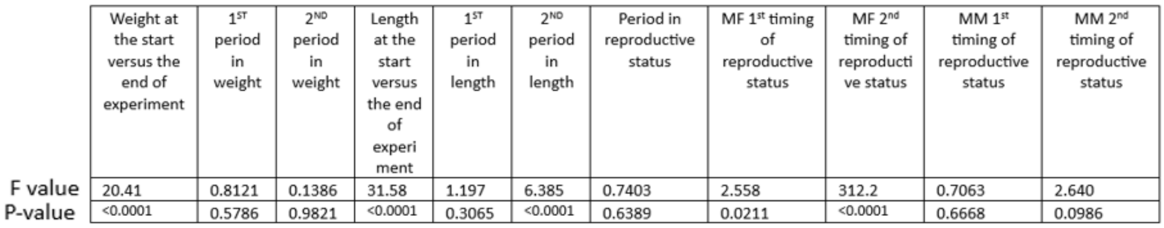
Table 1: Conversion from month/day/year to Julian day

2.4 Graphs design

The graphs of monthly changes in weight through the whole experiment for each fish, for weight at the beginning and end for each tank and duration of period for each tank were made in GraphPad Prism 7. The same was done for length data. Graphs with timing of reproductive status and duration of period in all tanks were also completed in GraphPad Prism 7. The graphs of length and reproductive status were also made in the same program. However, the number of peaks in weight and length and percentage of fish showing at least one peak in length and weight were done in Microsoft Office Excel. Graph showing number of mature and immature fish was made in R program.

2.5 Statistical analyses

Analysis for number of mature and immature was done in R statistic. All the other analysis (for weight, length and reproductive status data) were done by using One-way Anova and Multiple comparisons in GraphPad Prism 7.



	Weight at the start versus the end of experiment	1ST period in weight	2ND period in weight	Length at the start versus the end of experiment	1ST period in length	2ND period in length	Period in reproductive status	MF 1st timing of reproductive status	MF 2nd timing of reproductive status	MM 1st timing of reproductive status	MM 2nd timing of reproductive status
F value	20.41	0.8121	0.1386	31.58	1.197	6.385	0.7403	2.558	312.2	0.7063	2.640
P-value	<0.0001	0.5786	0.9821	<0.0001	0.3065	<0.0001	0.6389	0.0211	<0.0001	0.6668	0.0986

Table 2: F and P values from all analyses for results section

3 Results

3.1 Arctic charr's growth rate

The data from almost two years of experiment shows that Arctic charr is variable among individuals and shows rhythmicity in weight and length. However all fish in all of eight tanks showed rhythmicity in simulated natural photoperiod (SNP), short day photoperiod (SP), intermediate day photoperiod (IP) and long day photoperiod (LL).

3.1.1 Arctic charr's weight is variable between individuals and shows no significant difference between different photoperiods

There was a group analysis (Table 2) done for all tanks, where the weight of group of fish in each tank was compared with the one at the beginning and at the end of the experiment. It showed that there was no significant difference (by both p-value and F-value) in the average weight in each tanks, which means that the experiment is balanced. On average there was an increase in weight per tank at the end of the experiment, which seems not to be different between photoperiodic treatments. On each box with whiskers there is a weight in the beginning and next to it weight at the end in each tank (for example A START and A END). Each black dot is an individual fish, and the whiskers shows the variance in weight between individuals in each tank (how they differ from each other). Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

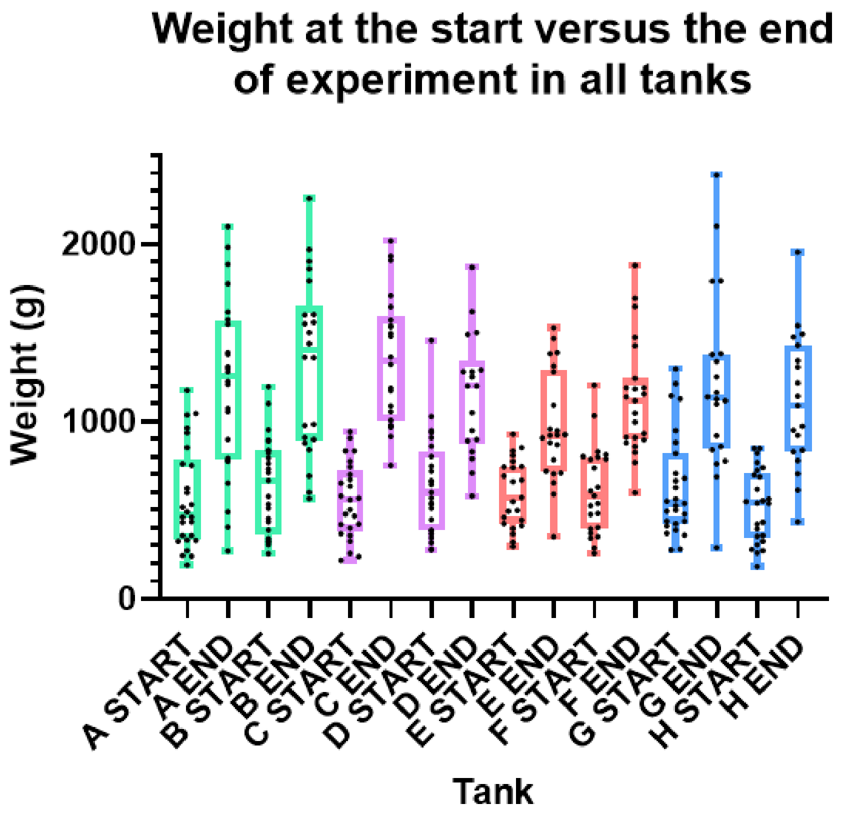


Figure 10: Weight of Arctic charr in every tank at the beginning and end of experiment

However since there was a high variability among individuals noted from individual analyses, the statement above about no significant difference might be misleading (Figure 11,12,13 and 14). Moreover the highest recorded weights were in LL treatment (the record was 2624g). Then the highest value equal to 2098g were in SNP, and then in IP between 1500-1951g and in SP between 1300-1846g (Appendix 1). In SNP there were a few fish which at the end of experiment weighed less than 500g. In other treatments there were almost no fish weighing less than 500g (Appendix 1). Overall it seems that fish usually didn't gain more than 800g and didn't lose more than 200g. On each box with whiskers there is monthly change in weight shown for each tank. Each black dot is an individual fish, and the whiskers shows the variance in weight between individuals in each tank (how they differ from each other). Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

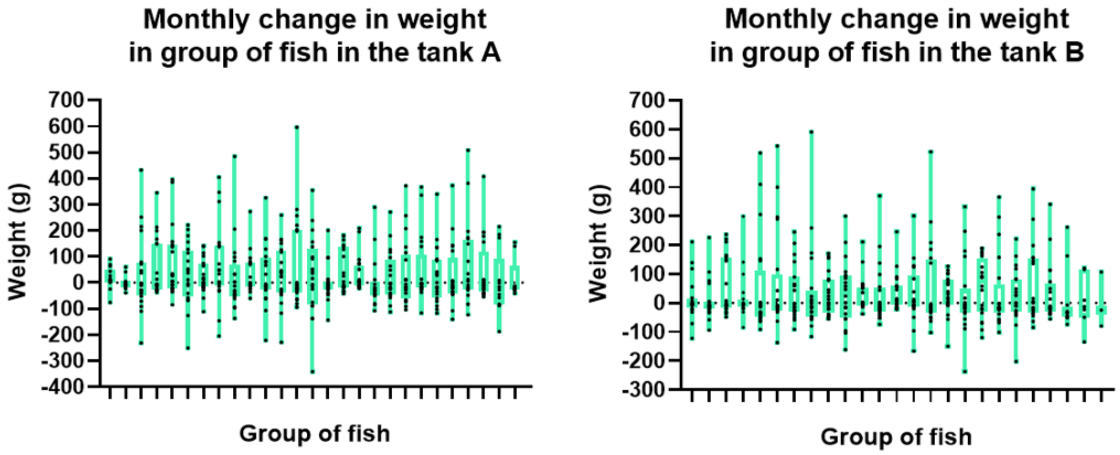


Figure 11: Monthly change in weight for the two groups in fish in SNP treatment

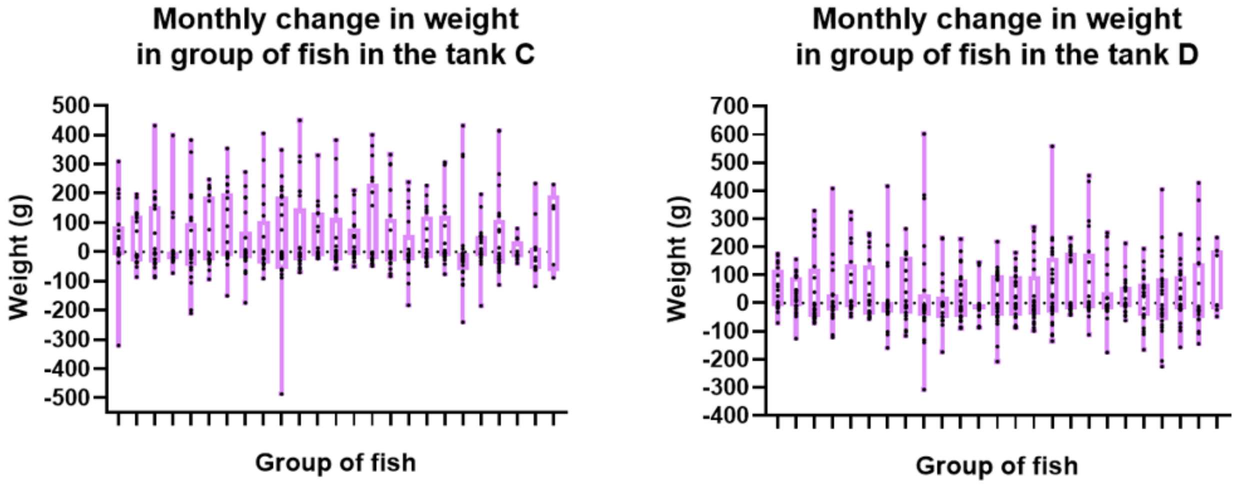


Figure 12: Monthly change in weight for the two groups in fish in SP treatment

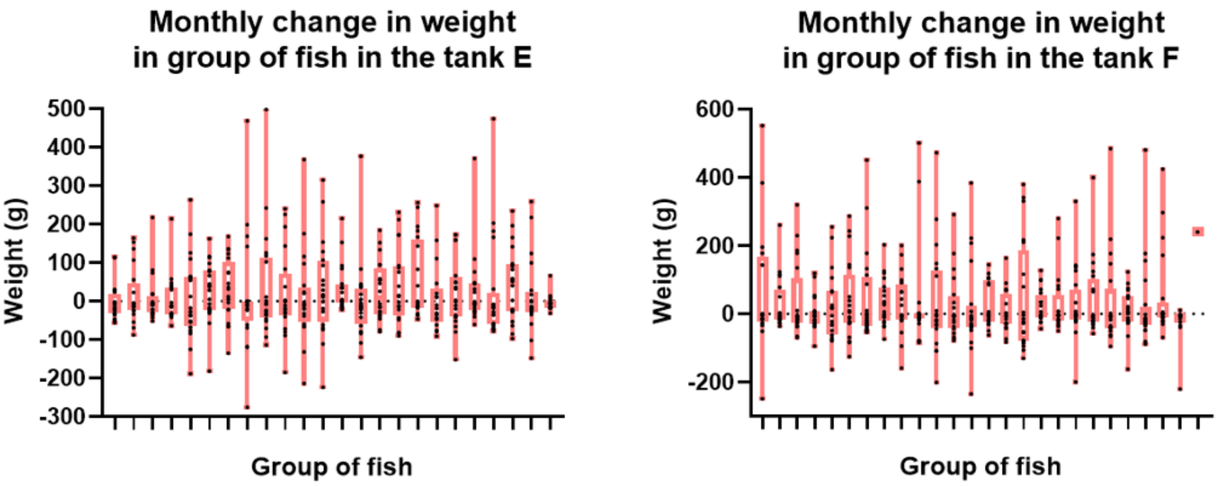


Figure 13: Monthly change in weight for the two groups in fish in IP treatment

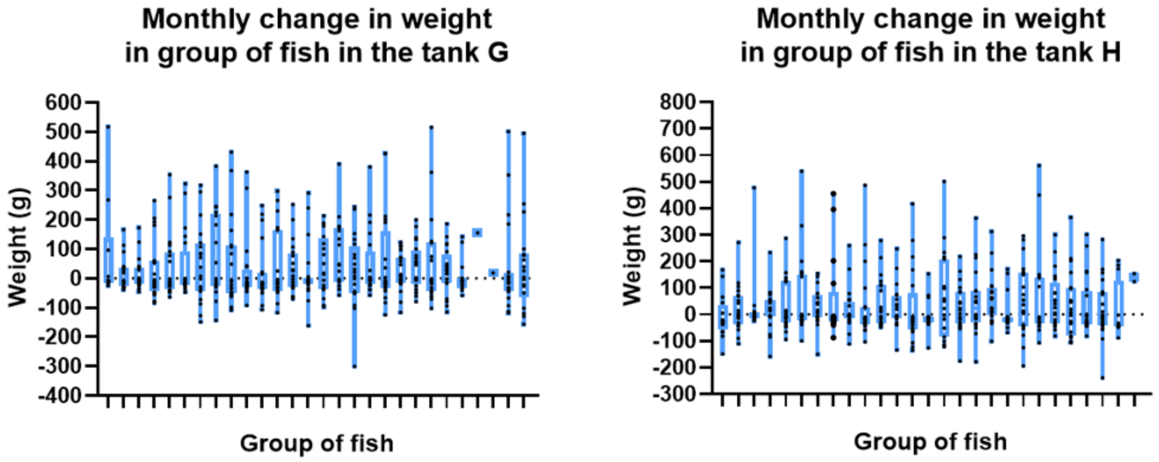


Figure 14:Monthly change in weight for the two groups in fish in LL treatment

3.1.2 Individual level analysis of Arctic charr shows rhythmicity in weight change

From analyzing all graphs of monthly change in weight in fish, all of the individuals showed nonlinear weight change through almost two years of experiment. Moreover in simulated natural photoperiod (SNP) it could be seen that the highest peak in weight was before the highest value of temperature (Figure 15). In short (SP) and long (LL) photoperiod the highest peak in weight was usually after the highest value of the temperature (Figure 15). In intermediate photoperiod (IP) the highest peak in weight was well-timed with occurrence of the highest temperature of temperature and the both happened at the same time (Figure 15).

LEGEND

Green line = simulated natural photoperiod (SNP), Purple line = short photoperiod (SP),

Light pink line = intermediate photoperiod (IP), Blue line = long photoperiod (LL)

Line with dots = monthly weight change in fish, Black line = monthly change in water temperature in tank

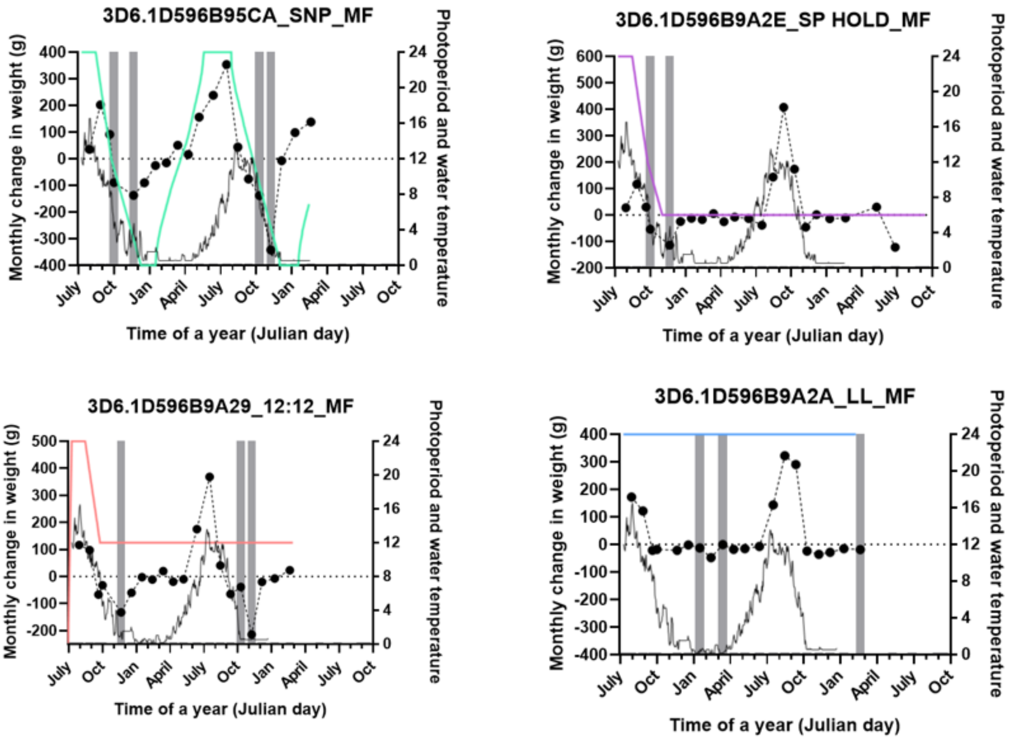


Figure 15:Possible influence of water temperature on fish’s monthly change in weight

The highest number of peaks (sometimes even 4,5 or 6) was shown by fish in SP treatment, especially the mature ones. In SNP treatment in all sexes and for all immatures number of peak was mostly equal to 2 (Figure 16). In IP treatment the number of peaks was between 1-2 for all fish. The highest variability in peaks in weight can be seen in LL treatment, where it is very difficult to see any trend for any of sexes or immature ones (Figure 16).

LEGEND

The X axis represents the sum of peaks, the Y axis shows how many fish had the exact number of peaks in weight, Tank A-B present simulated natural photoperiod (SNP) – green color, Tank C-D short photoperiod (SP) – purple color, Tank E-F intermediate photoperiod (IP) – light pink color, Tank G-H long photoperiod (LL) – blue color

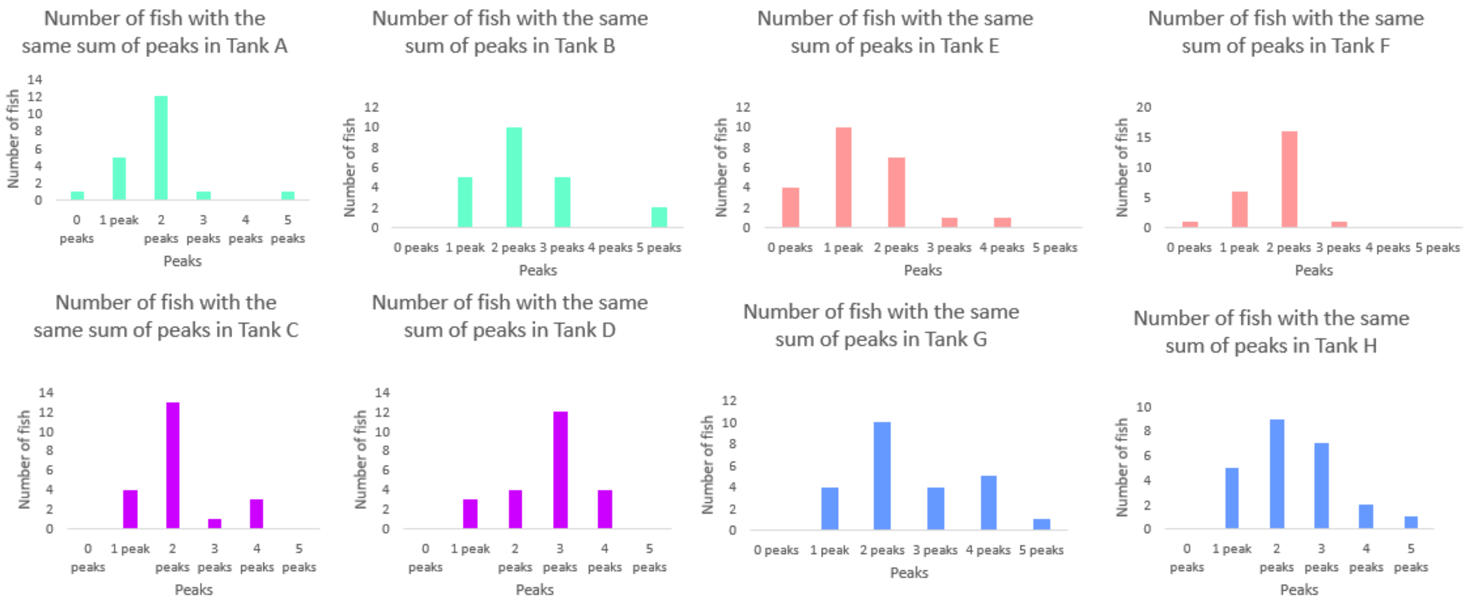
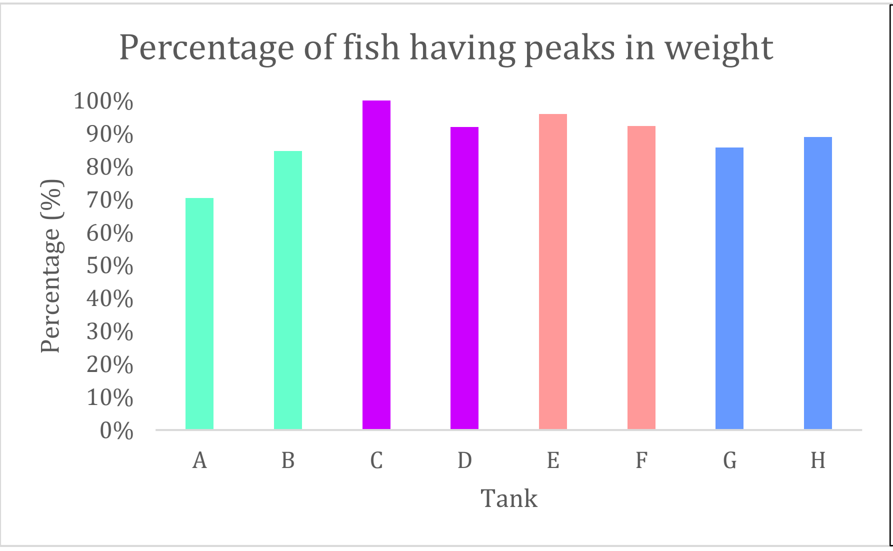


Figure 16: Variability in number of peaks in weight in all eight tanks

However in SNP treatment 70-80% fish showed at least one peak, whereas in rest treatments more than 80% fish showed at least one peak. (Figure 17).



LEGEND

The X axis represent the tanks

The Y axis represents the percentage of fish having peaks in weight

Tank A-B = SNP treatment (

Tank C-D = SP treatment

Tank E-F = IP treatment

Tank G-H = LL treatment

Figure 17: Percentage of fish showing at least one peak in all tanks

3.1.3 Duration of period in weight varies in SNP, SP, IP and LL treatments

After group analysis (Table 2) in duration of period (between first and second peak in weight) in each tank it was shown that fish from SNP, SP and IP treatment showed rhythmicity in weight for two cycles and that there is a significant difference between photoperiods (by looking at p-value and F-value). The first period for all these tanks was between 300-400 days (similar to duration of a year). In SNP treatment first period was the closest to duration of a year (Figure 18). In SP and IP the first period was sometimes reaching 400 days (Figure 18). In LL treatment fish showed only one period lasting between 250-350 days, which was sometimes a bit shorter than duration of a year (Figure 18). There was no significant difference between all treatments. On each box with whiskers there is variation in duration of first period in weight shown for each tank. Each black dot is an individual fish, and the whiskers shows the variance in duration of its first period in weight - whiskers shows difference between individuals in each tank - and how they differ from each other). Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

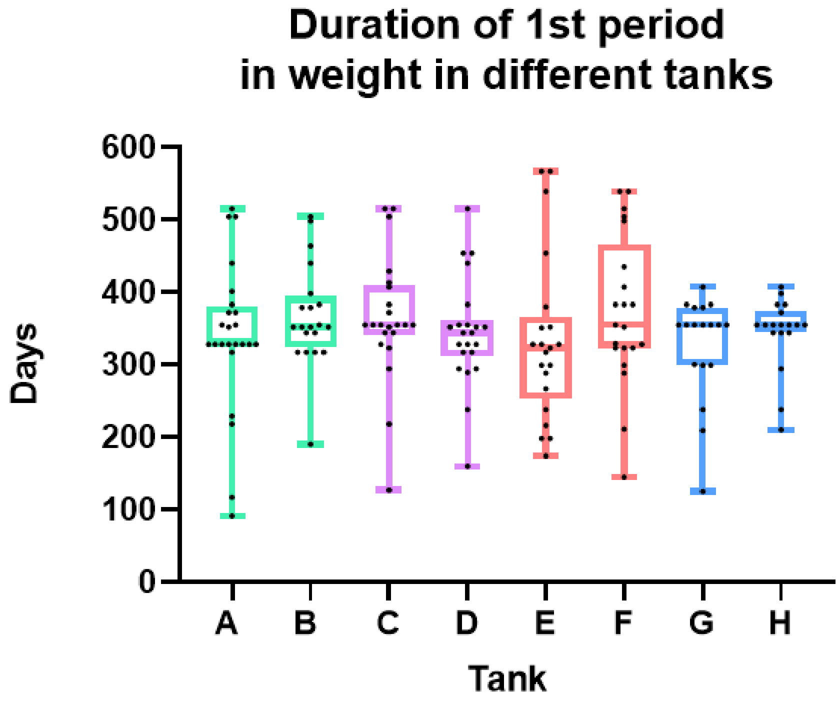


Figure 18: Duration of first period in weight in all tanks

Group analysis (Table 2) for span of second photoperiod (between second and third peak) in weight also showed significant difference (in p-value and F-value) between the tanks. Second period for simulated natural photoperiod treatment (SNP) was between 150-250 days, in short photoperiod (SP) between 150-400 days and for intermediate photoperiod (IP) between 50-250 days (Figure 19). In all cases second period was shorter than the first one. In long photoperiod (LL) second period in weight was not noted. On each box with whiskers there is variation in duration of second period in weight shown for each tank. Each black dot is an individual fish, and the whiskers shows the variance in duration of its second period in weight - whiskers shows difference between individuals in each tank - and how they differ from each other). Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color.

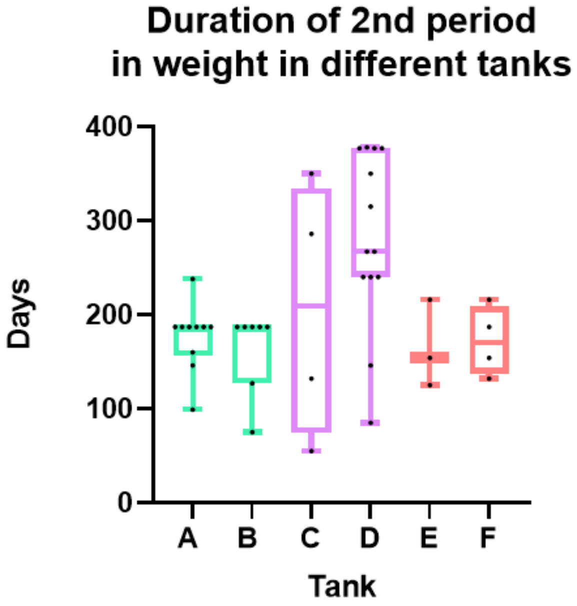


Figure 19: Duration of first period in weight in all tanks

However four fish from short photoperiod (SP) treatment had 3 periods in weight and because of small number of fish it was impossible to make group analysis. Although in only one fish its third period in weight was close to duration of a year, whereas in rest three ones it was shorter and lasted less than 200 days (Figure 20). The whiskers are showing the variation in duration of third period between these four individuals.

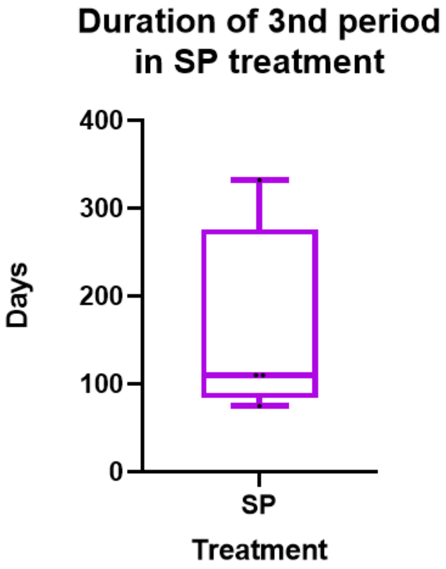


Figure 20: Duration of third period in weight in SP treatment

3.1.4 Arctic charr's length does not show significant difference between different treatments

Changes in length are not as variable as weight data, probably because changes in length through the months, cannot go as dramatically lower or higher (comparing one month to previous one), as it is with weight (Appendix 2). Group analysis (Table 2) in the length at the beginning and at the end of experiment among all tanks didn't show significant difference (in p-value and F-value) between tanks. The biggest changes in length could be seen in SP treatment (where fish gained 18 cm difference through whole experiment), then in SNP, LL and IP where the highest difference was usually 10 cm (Appendix 2, Figure 21). Significant difference between length at the beginning and end of experiment was noted (with higher values of fish-length at the end of the experiment). On each box with whiskers there is length at the beginning and next to it at the end of the experiment shown for each tank (for example A START and A END). Each black dot is an individual fish, and the whiskers shows the variance in weight between individuals in each tank (how they differ from each other). Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

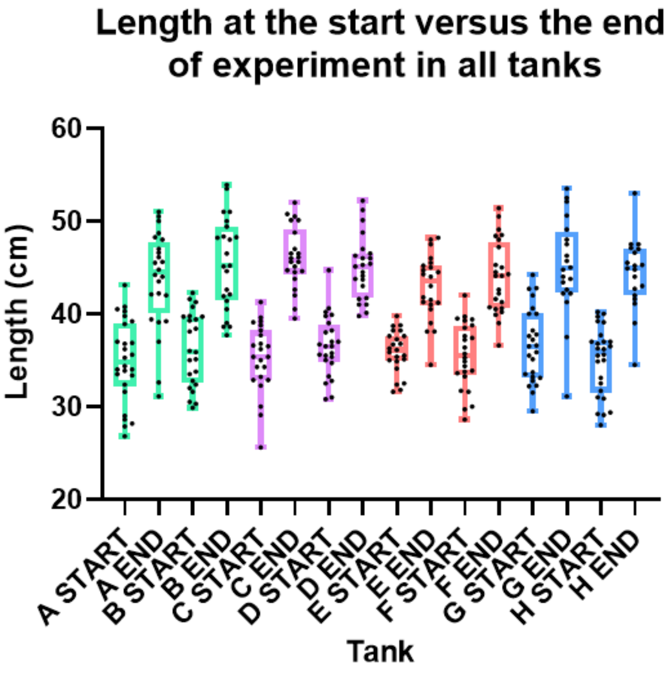


Figure 21: Length of Arctic charr in every tank at the beginning and end of experiment

However the rate of growing in length also varies among individuals - similarly to growth in weight - (Table 22, 23, 24 and 25). Similarly to graphs of monthly change in weight on each box with whiskers there is monthly change in weight shown for each tank. Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

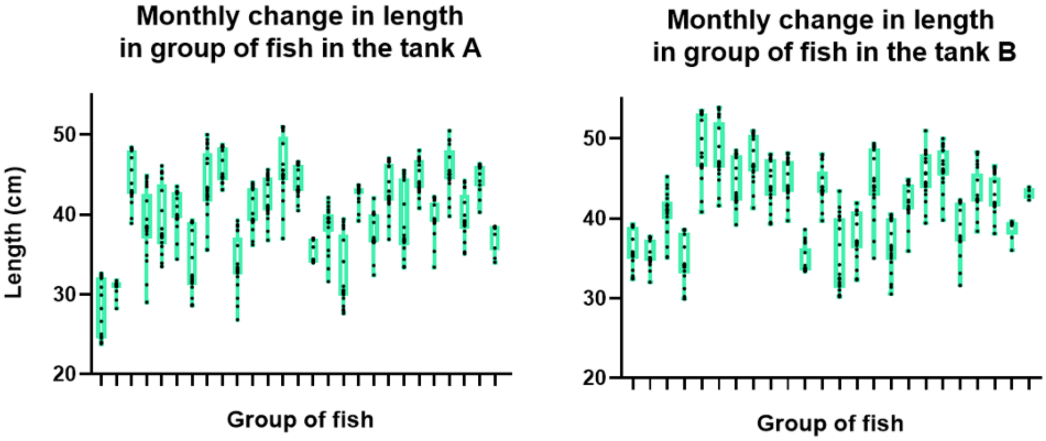


Figure 22: Group analysis of monthly change in weight in SNP treatment

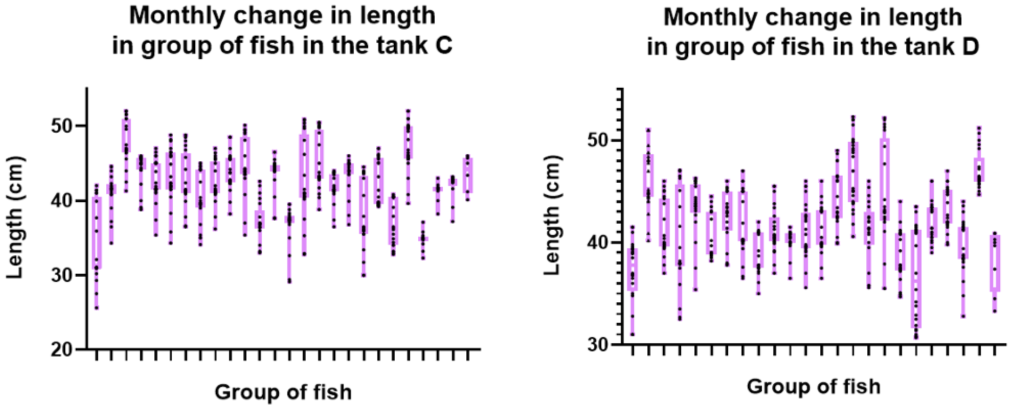


Figure 23: Group analysis of monthly change in weight in SP treatment

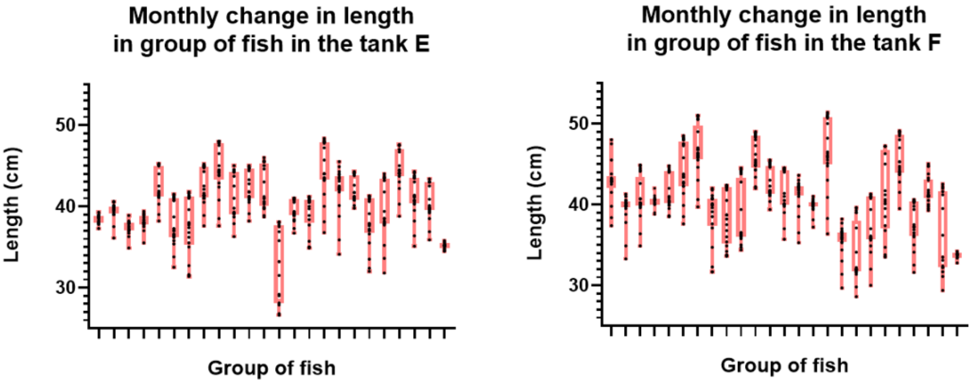


Figure 24: Group analysis of monthly change in weight in IP treatment

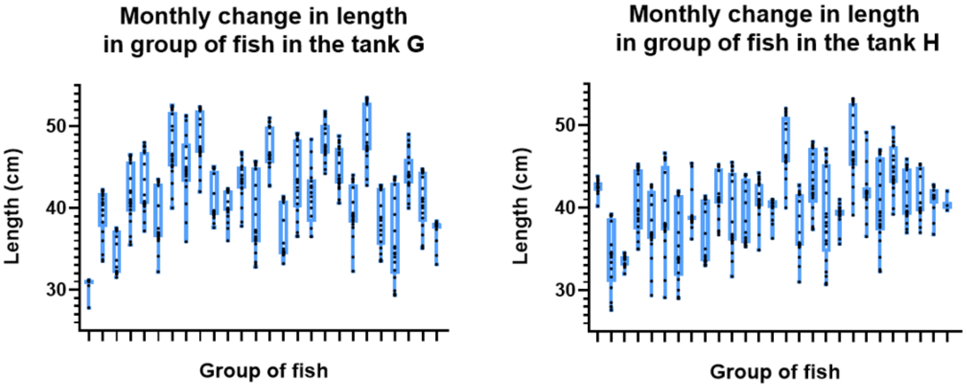


Figure 25: Group analysis of monthly change in weight in LL treatment

3.1.5 Individual level analysis of Arctic charr shows rhythmicity in length change

From analyzing all graphs of monthly change in length in fish, all of the individuals showed nonlinear weight change through almost two years of experiment. In all treatments it can be seen that the highest peak in length was after the highest water temperature (Figure 26).

LEGEND

The X axis represents the sum of peaks, the Y axis shows how many fish had the exact number of peaks in weight, Tank A-B present simulated natural photoperiod (SNP) – green color, Tank C-D short photoperiod (SP) – purple color, Tank E-F intermediate photoperiod (IP) – light pink color, Tank G-H long photoperiod (LL) – blue color

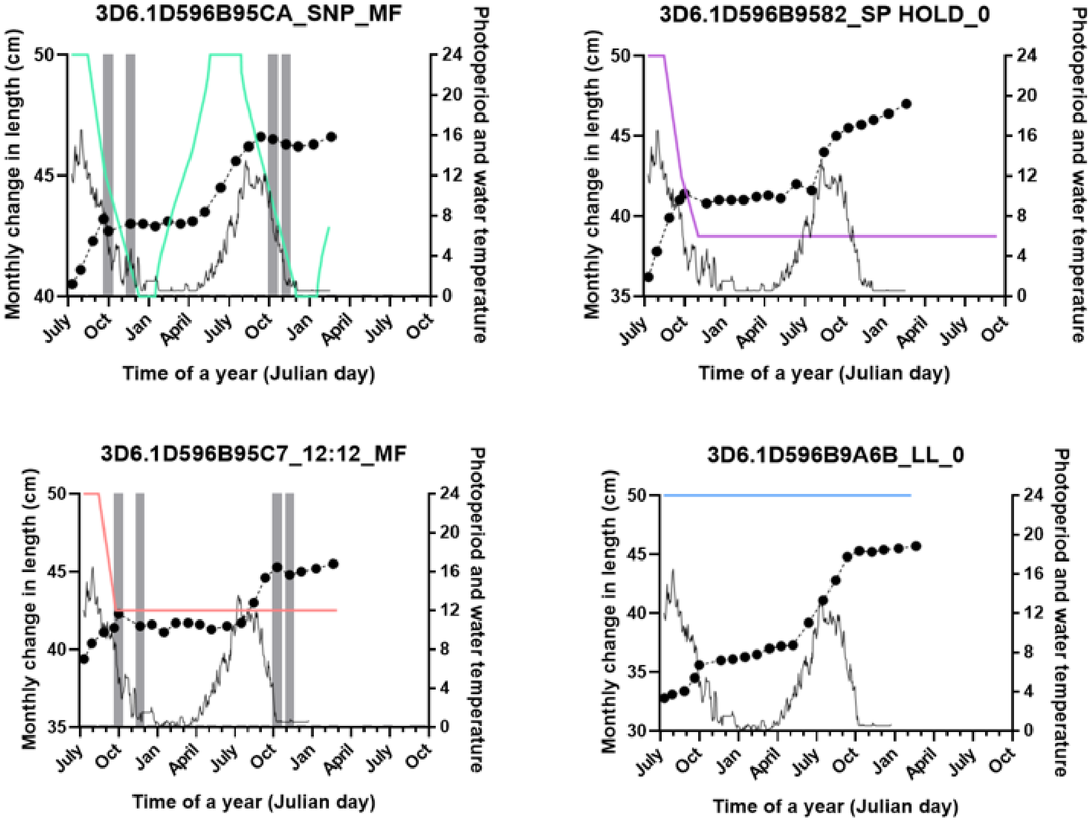


Figure 26:Possible influence of water temperature on fish's monthly change in length

For fish-length data number of peaks for all treatments (Figure 27) were mostly equal to 2, which makes it less variable than number of peaks in weight. In almost all tanks the percentage of fish showing peaks in length was 100% (Figure 28).

LEGEND

The X axis represents the sum of peaks, the Y axis shows how many fish had the exact number of peaks in weight, Tank A-B present simulated natural photoperiod (SNP) – green color, Tank C-D short photoperiod (SP) – purple color, Tank E-F intermediate photoperiod (IP) – light pink color, Tank G-H long photoperiod (LL) – blue color

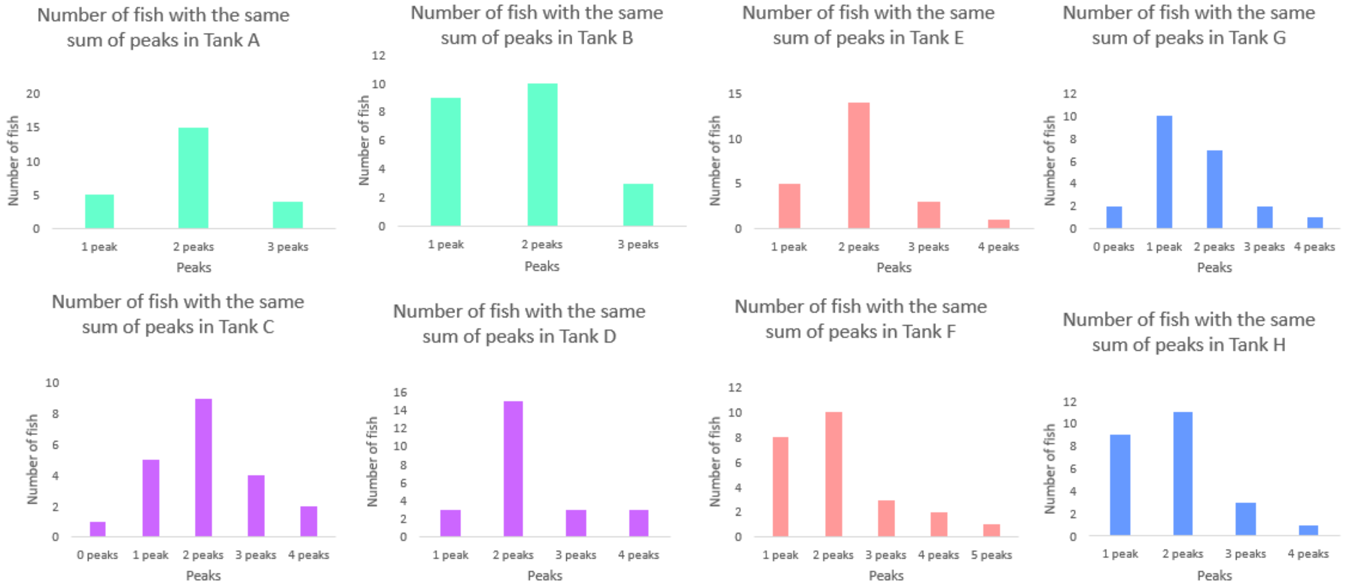
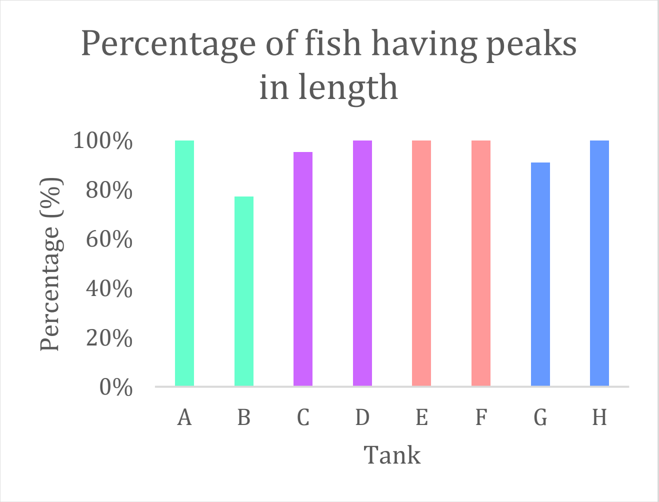


Figure 27: Variability in number of peaks in weight in all eight tanks



LEGEND

The X axis represent the tanks

The Y axis represents the percentage of fish having peaks in weight

Tank A-B = SNP treatment

Tank C-D = SP treatment

Tank E-F = IP treatment

Tank G-H = LL treatment

Figure 28: Percentage of fish showing at least one peak in all tanks

3.1.6 Duration of period in length varies in four different treatments

Group analysis (Table 2) showed significant difference in duration of first period (between first and second peak) in p-value and F value. In SNP treatment similarly to weight data, the duration of first period in fish-length was the closest to duration of a year, ranging between 300-350 days (Figure 29). In SP treatment duration of a first period (similarly to weight) was a bit too long, between 300-400 days (Figure 29). In IP and LL treatment the first period was between 250-350 days, sometimes being too short (Figure 30). Each box with whiskers shows variation in span of first period in length among individuals in each tank. The longer the whiskers the biggest variation in tank. Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

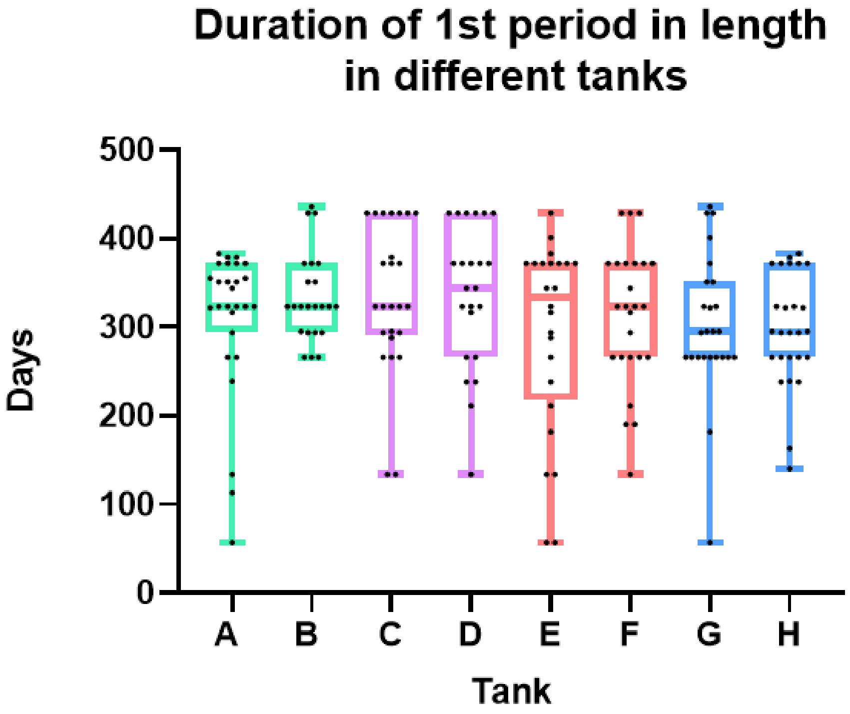


Figure 29: Duration of first period in length in all tanks

Group analysis (Table 2) for second period in length (between second and third peak) also showed significant difference in p-value and F value between tanks. In the second period, the biggest variation of duration was seen in SP treatment where it was between 100-300 days (Figure 30). In SNP and LL it was ranging between 50-150 days, which is definitely shorter than duration of first period (Figure 30). For fish from IP treatment duration of period was the least variable, because it was between 100-200 days (Figure 30). Each box with whiskers shows variation in span of second period in length among individuals in each tank. Similarly to first period in length the longer the whiskers the biggest variation in tank. Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

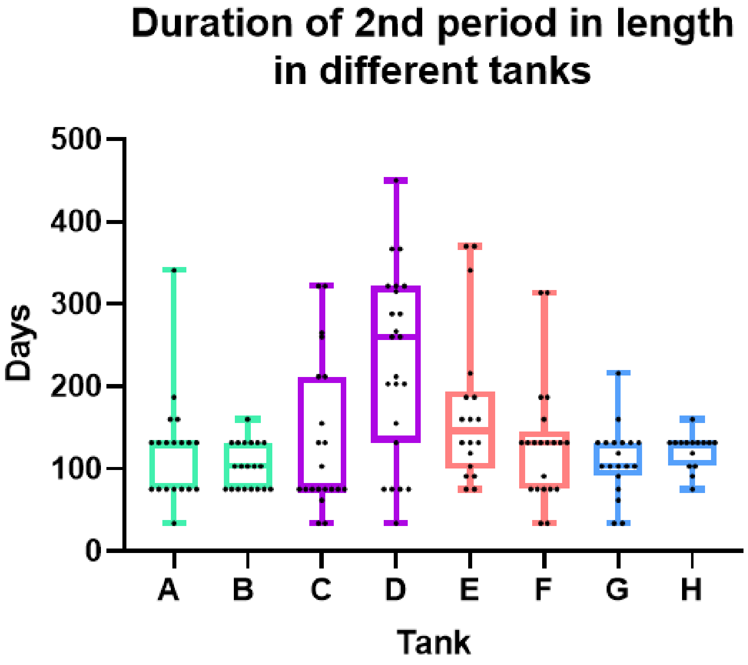


Figure 30: Duration of second period in length in all tanks

3.2 Arctic charr’s similarities in both sex in maturation and differences in timing of reproductive status

Group analysis(Table 2) showed no significant difference between mature and immature fish in four different treatments. Maturation of Arctic charr is not depending on photoperiod, since there was no difference in SNP, SP, IP and LL treatments, which showed in both tanks higher or lower number of mature fish. Both sexes of Arctic charr’s display similar rhythmicity of timing of timing of first reproductive status and differences in second one. In whole experiment mostly all fish were two times in reproductive status, whereas a few mature females were three times in reproductive status. However, comparing to the growth rate, in reproductive status there was mostly only one period, not two.

3.2.1 Arctic charr’s maturation is similar in all treatments

Group analysis (Table 2) in maturation of fish in all tanks didn’t show highly significant difference (in p-value and F value) between different tanks. The highest number of mature fish can be seen in tank B,D, E and H . In these four tanks more than half of the fish in the tanks are mature. In tank A,C,F and G the number of mature fish is slightly bigger than the number of immature fish. From analyzing if there is any significant difference between percent of maturation in different tanks, it was concluded that there is no linear correlation between maturation of fish and amount of light (Figure 31).

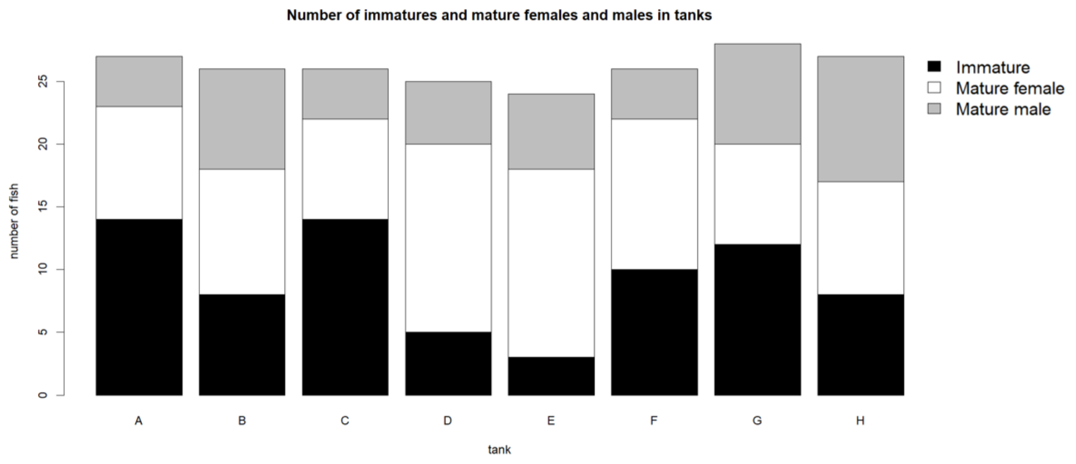


Figure 31: Number of mature and immature fish in each tank

3.2.2 Arctic charr's both mature females and males shows rhythmicity in reproductive status

Group analysis (Table 2) from period in reproductive status showed significant difference among tanks (in p-value and F value). In SNP, IP and LL fish had only one period - between first and second reproductive status - (Figure 32). The shortest duration of period was found in IP treatment, ranging between 322-372 days and being the closest to duration of a year. In SNP and LL it was slightly longer with duration between 322-401 and 322-426. In SP treatment in first period mostly the duration was ranging between 322-400 with one fish having period lasting for 694 days. Each box with whiskers represents fish from different tanks and each black dot is different individual fish. The longer the whiskers the biggest variation in tank. Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

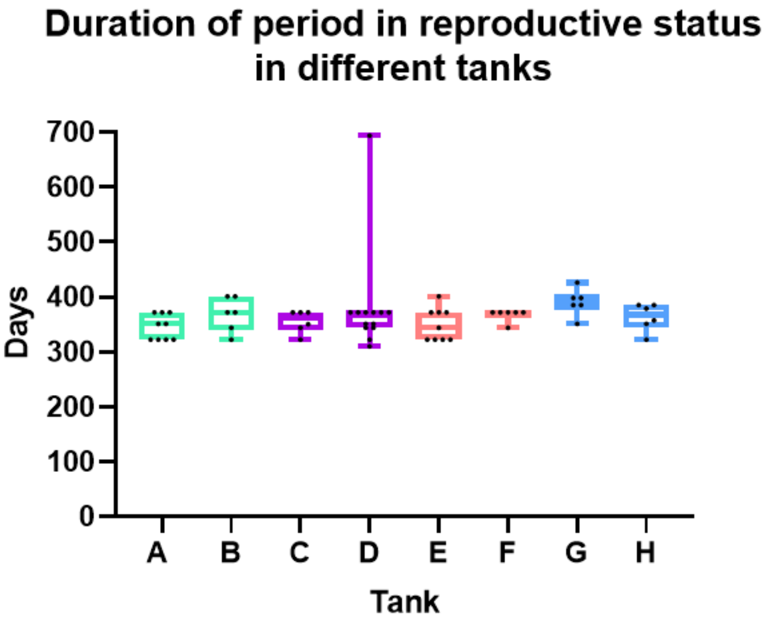


Figure 32: Duration of period in reproductive status in different tanks

Group analysis (Table 2) from first timing of reproductive status in both mature females and males showed a statistical significant difference in p-value and F value. Most fish in SP, IP and LL treatment both mature males and females were during first reproductive status before autumn 2019, so before a year after start of an experiment (Figure 33). Although most of fish in SNP treatment were in reproductive status in beginning of 2019, with some individuals having it later in autumn 2019 (Table 33). Similarly to figure with period in reproductive status for both figures with first and second timing in reproductive status for mature males and females, each box with whisker represents different tank (with black dots as individual fish). Tank A-B represents simulated natural photoperiod (SNP) with green color. Tank C-D represents short photoperiod (SP) with purple color. Tank E-F represents intermediate photoperiod (IP) with light pink color. Tank G-H represents long photoperiod (LL) with blue color.

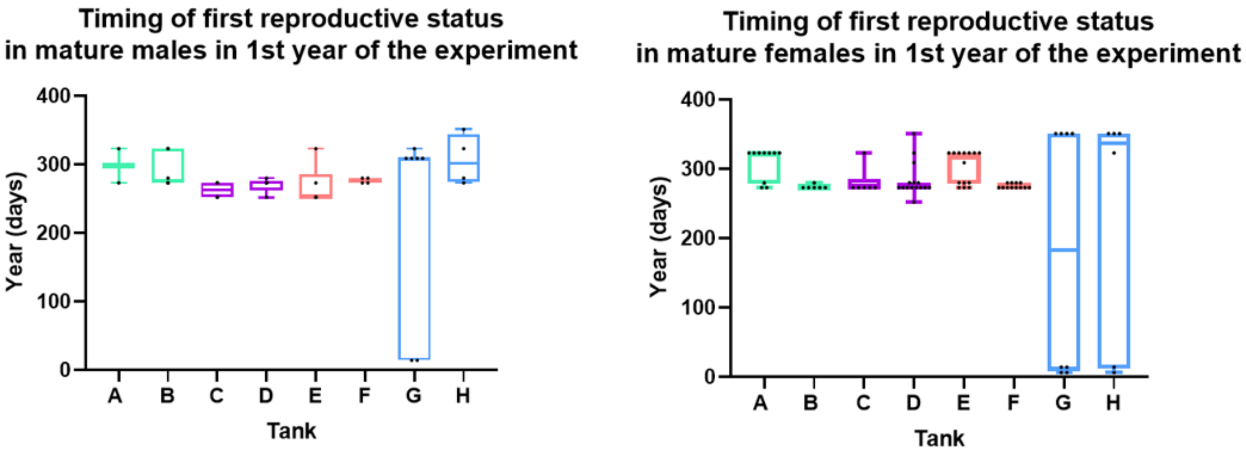


Figure 33: Timing of first reproductive status in mature males and females

Group of analysis (Table 2) from second timing of reproductive status showed the highest difference (in p-value and F value) between mature males and females (Figure 34). For mature males in SNP, SP and IP treatment the timing of second reproductive status was almost the same (close to 300 day, which is autumn). However in LL treatment mature males were in second reproductive status in first two months of winter. Mature females were more variable. Fish from SP treatment were mature the fastest in spring/summer. Then fish from SNP and IP treatment being in second reproductive status in summer/autumn and lastly fish from LL treatment which were in second reproductive status in autumn (Figure 34).

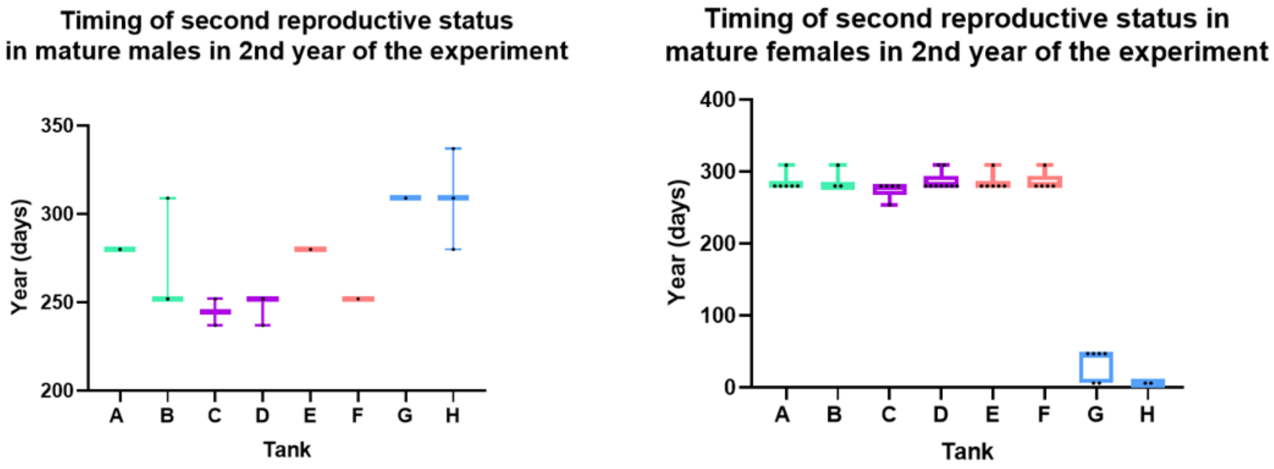


Figure 34: Timing of second reproductive status in mature males and females

There was no group analysis in third timing in reproductive status because there were only four fish from D tank (SP treatment). However their third reproductive status occurred more than 200 days after second reproductive status (Figure 35).

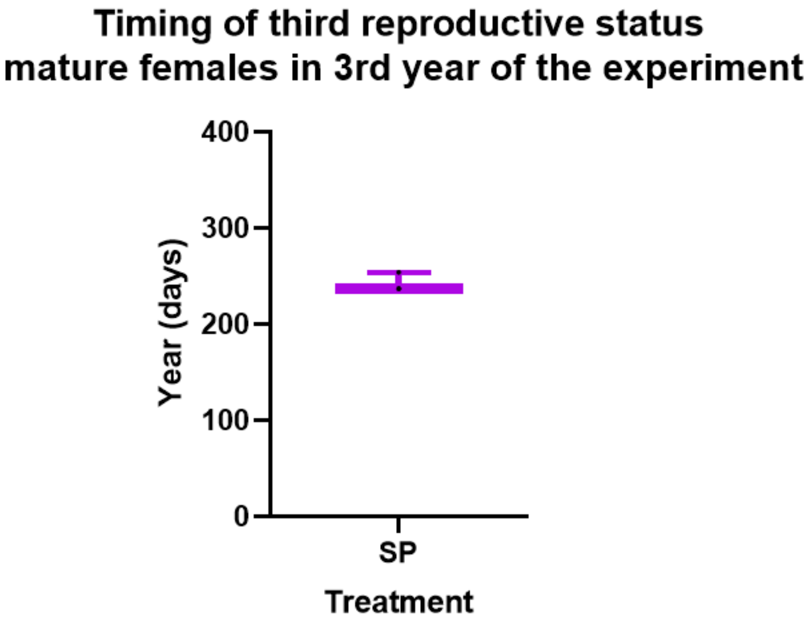


Figure 35: Timing of third reproductive status in mature males and females

4. Discussion

4.1 Maximum values of weight and length occur between spring and summer

All along the experiment Arctic charr in simulated natural photoperiod, short day, intermediate day and long day presented highest growth during spring-summer, but mostly from June to August and the lowest growth during winter months. These findings are similar to the ones recorded by Jobling in 1987 and on immature salmon studied by Eriksson and Lundqvist in 1982. From living in Arctic region, Arctic charr could probably show high dependence on water temperature. On graphs of monthly change in weight and length it could be seen that the highest values for weight and length followed the summer and early autumn months, with the water temperature usually above 10^{o}C. However it is just observation and there should be done some analysis to prove if it is really reliable observation. In the research done by Swift in 1964 and by Jobling in 1983, they indicated that rates of growth of yearling and under yearling charr increase with increasing temperature and reach a maximum between 12-15^{o}C. Future studies can be done to see if the same rates of temperatures are favouring growth in older Arctic charr. Comparable findings where fish were dependent on temperature, were also noticed in cod. Earlier in the period 1965-1992 one group of cod's body length increased in elevated water temperatures close to 5^{o}C (Nakken,1994). In Barents Sea cold temperatures appear to be limiting growth performance and recruitment. Before 2000, during period of cooling, there was a collapse of cod population on Georges Bank and close to Newfoundland. It is similar in warmer regions, when the water temperature are too high (O'Brien, 2000).

In 2009 Deane and Woo reviewed study of importance and regulation of fish growth hormone (GH), during the exposure to stress. Salinity and variations in water temperature have been shown to modulate fish GH. Moreover pollutants like xenoestrogens and heavy metals are also affecting rate of GH, most probably via interference with the GH receptor and/or GH transcription. Handling, confinement and nutritional stress in aquaculture are also impacting GH levels (Deane and Woo, 2009). Since in this almost 2 year experiment of Hammerfest strain of Arctic, there was freshwater, salinity didn't have a great impact on growth rate of fish. In aquaculture it is highly recommended that the Arctic charr should be kept in either freshwater or brackish water, whereas it can be held in seawater only during summer months. The reason for that is because different strains of Arctic charr show contrasting salinity tolerances and hypoosmoregulatory abilities and it can then lead to depression of feeding (Arnesen *et al.*, 1993). Furthermore Finstad et al. found in 1989 recorded high mortalities in Arctic charr which were exposed to sea water at 1°C. On the other hand fish held at 8°C performed much lower rates of mortality. It was then suggested that the water temperature in addition to seasonal changes which occur in hypo-osmoregulatory ability, could be an important factor for the Arctic charr's ability for survival the winter in seawater (Findstad *et al.*, 1989a; Jobling *et al.*, 1993).

4.2 Timing of reproductive status is depending on photoperiod and it is different for mature females and males

In the data it was shown that timing of reproductive status of both mature females and males in Arctic charr is different during second timing of reproductive status. Although in both case they are dependent on photoperiod. First reproductive status for both sexes in SNP treatment occurs in autumn, in SP treatment in early autumn, in IP treatment in late autumn and in LL treatment mostly in late autumn (a few fish had their first reproductive status in winter). Second reproductive status especially for mature females occurred earlier than the first one in all treatments. In SNP and IP treatment fish had their second reproductive status in summer/autumn, in SP treatment in summer and in LL treatment in middle of autumn. However in mature males in SNP, SP and IP treatments the timing of second reproductive status was the same and happened in autumn and the earliest second reproductive status could be noticed in winter in LL treatment. It shows that Arctic charr in all treatments maintained maturation and continued being in reproductive status, which may suggest endogenous rhythms which are mainly controlling that and photoperiod can only display supplementary role. Arctic charr shows similarity to rainbow trout which could spawn successfully in both constant short and long days (Duston and Bromage 1987). These two photoperiods are thought to be the most stimulatory to gonadal development however possibly all salmonids and other fish require different lengths of photoperiod at different stages of the annual reproductive cycle (Bromage *et al.* 1982b, 1984). In both fish it was seen that changes in daylength in all treatments altered the timing of reproductive status (Arctic charr) and maturation (Rainbow trout) (Duston and Bromage 1987).). Similar endogenous rhythms were also observed in two other fish - catfish (*Siluriformes*) and three-spined stickleback (*Gasterosteus aculeatus)* - (Sundararaj *et al.*, 1982; Baggerman, 1980).

4.3 Circannual rhythm depending on the life history of an animal

These differences between treatments when it comes to weight and length and especially to reproductive status indicate that circannual rhythms characteristic is depending on the life history of an animal. Especially it can be seen in reproductive status in females where the second reproductive status occurred earlier than the first one in all treatments. This rhythmicity might be similar to one of other species in salmonids, Atlantic salmon, whose circannual changes occur at earlier times of the year with increasing age. However, since the Arctic charr is the most variable vertebrates when it comes to its ecology, life history and especially phenotype, that probably there will not be seen one trend of rhythmicity but a few (Jonsson et al. 1990). Only 4 from 31 fish (which makes it approximately 13%), where 3 were mature females and 1 mature males had 3 times of reproductive status. Both periods were similar to each other, with duration between 11-12 months, which is close to duration of year. Unfortunately, since they make 13% of fish in only SP treatment it is possible that some phenotypes can maintain circannual rhythms in constant conditions (in this case 6 hours of light and 18 hours of dark) by exhibition of reproductive status. It is similar to rainbow trout which was in the same treatment of light and were able to exhibit a rhythms of gonadal maturation and spawning and sustained for 3 cycles and then free-runed approximately (Duston and Bromage, 1991). To better understand if Arctic charr shows circannual rhythmicity (especially in reproduction) the research on that should be done for at least four years in the same photoperiods (the number was chosen because rainbow trout can maintain maturation and spawning for 3 cycles and then it free runs). If during first three years of experiment Arctic charr will show circannual rhythms it would be more reliable than comparing studies of almost two years of research on this fish. It would be also interesting if after these three years this fish will free-run like rainbow trout or not. Moreover since a few fish of Arctic charr had third reproductive status in short photoperiod, probably in this treatment animals will maintain circannual rhythms.

5. Conclusions

In this experiment it was shown that Arctic charr is a very variable species, whose differences in life history, phenotypes and ecology make it very difficult to state if this species show circannual rhythms. However, they show rhythmicity in weight, length and reproductive status (they are not damped) in all four photoperiods (SNP, SP, IP and LL). The SP treatment with 6 hours of light and 8 hours of darkness where 16% of fish showed a possibility of maintaining circannual rhythm for at least two cycles, and it can be the one which in the future can give more and better results (the number of fish in experiment has to be higher) to stating whether Arctic charr shows circannual rhythms in reproductive status for more than two years.

Moreover, this study confirms that by manipulating photoperiod, with shortening hours of light, fish will be more often in reproductive status, whereas by providing light for 24 hours the reproductive status will last longer and occur faster in relation to previous year. It may be possible that Arctic charr's growth rate and reproductive status are highly dependent on water temperature, where temperatures above 10^{o}C increase growth rate, and temperatures below that allow fish to be in reproductive status. However further research is needed to confirm this. To study whether more individuals of Arctic charr indicate having circannual rhythms the research should probably last at least 3-4 years and the number of fish should be higher in case of some percentage of mortality during experiment. Moreover it might be more reliable to do research on blood samples to look at cortisol or any other hormones of fish.

6. References

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Figure 2: Annual variations in day length in northern hemisphere, source: https://bibsys-almaprimo.hosted.exlibrisgroup.com/primo-explore/openurl?sid=google&auinit=AC&aulast=West&atitle=Calendar%20Timing%20in%20Teleost%20Fish&id=doi:10.1007%2F978-3-030-55643-3_7&vid=UBTO&institution=UBTO&url_ctx_val=&url_ctx_fmt=null&isSerivcesPage=true, 09.05.2023, permission to use from Alex West

Figure 3a: https://pixnio.com/fauna-animals/fishes/trout-fishes-pictures/rainbow-trout-fish-onchorhynchus-mykiss-detailed-photography, Author: Lisac Mark, USFWS , License: Free to use CC0, 07.05.2023

Figure 3b: https://pixnio.com/fauna-animals/fishes/salmon-fish-pictures/atlantic-salmon-atlantic-fish , Author: Timothy Knepp, USFWS, License: Free to use CC0, 07.05.2023

Figure 4: Arctic charr, Fot: Mathias Leines Dahle

Figure 5: Distribution of Arctic charr by Jørgensen and Johnsen, 2014, blue dashed line shows 10ºC isotherm, 09.05.2023, permission to use from Even Jørgensen and Helge Johnsen

Figure 8:Variability in size and coloration in different tanks, Fot: Shona Wood

APPENDIX 1
(Monthly change in weight and reproductive status in Arctic charr)

LEGEND FOR APPENDIX 1 & 2

▮ 3D6.24D596B959D = reproductive status
-●- 3D6.1D596B9578_12:12_MF = monthly change in length or weight
— = SNP (simulated natural photoperiod)
— = LL (long photoperiod)
— = IP (intermediate photoperiod)
— = SP (short photoperiod)

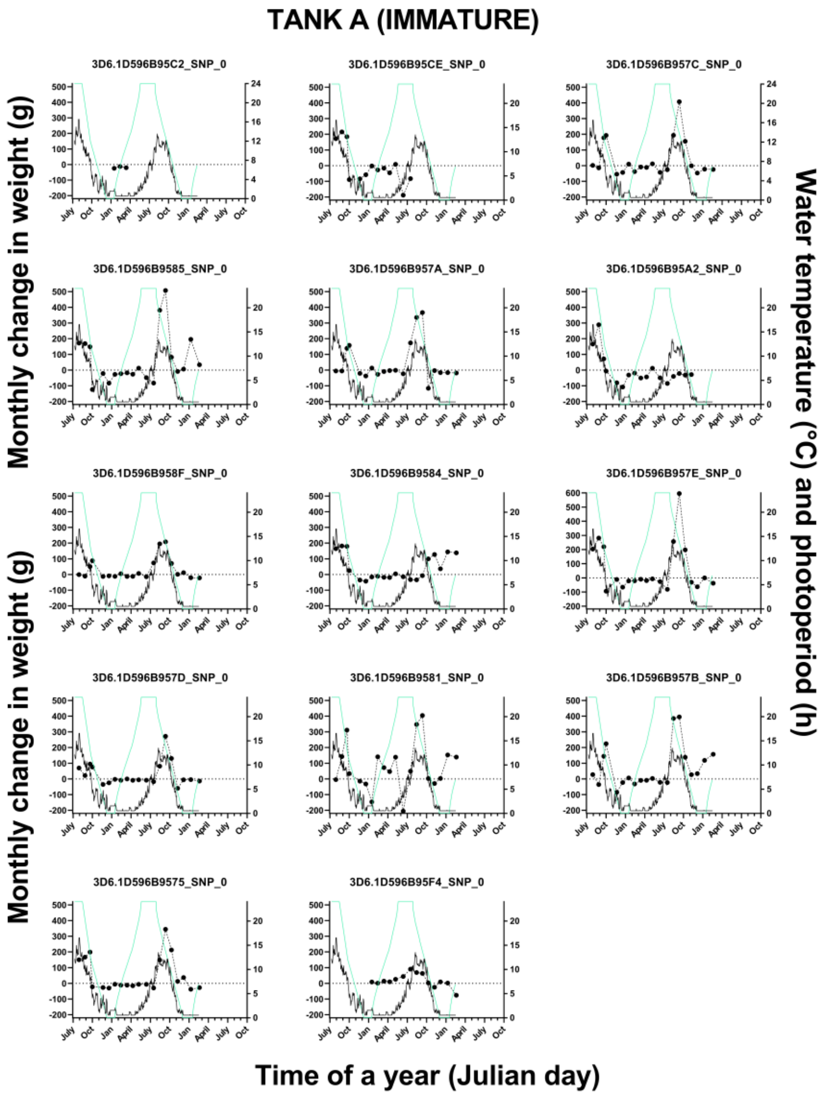

Figure 36: Monthly change in weight in immature Arctic charr in Tank A

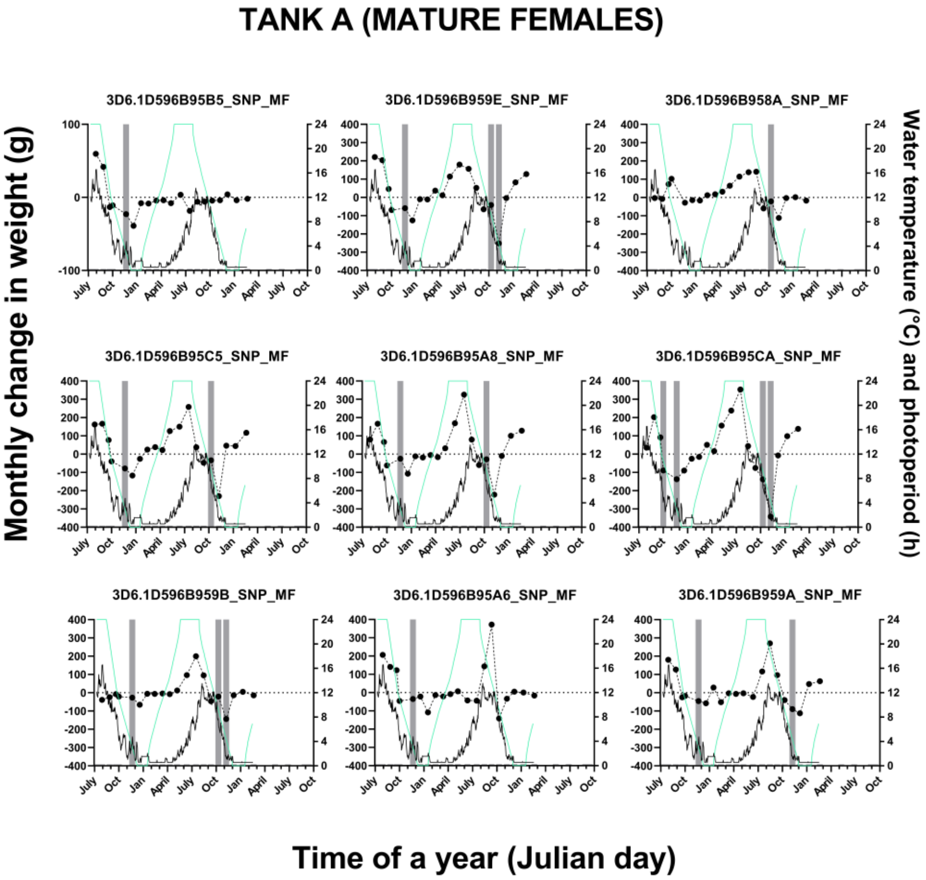

Figure 37: Monthly change in weight in mature females of Arctic charr in Tank A

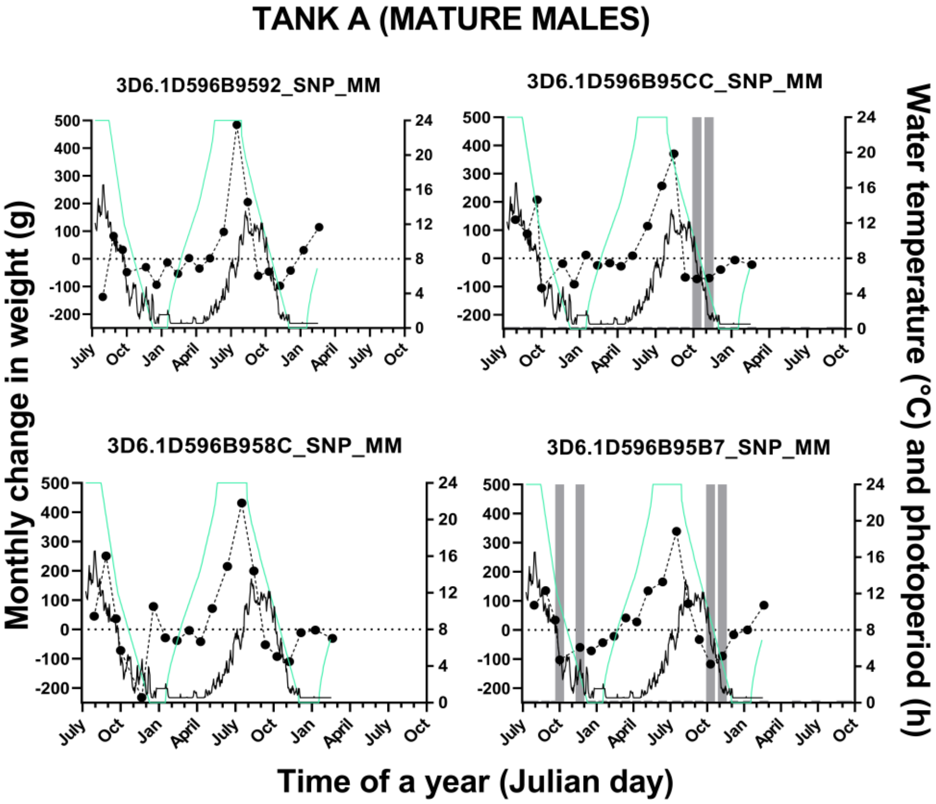

Figure 38: Monthly change in weight in mature males of Arctic charr in Tank A

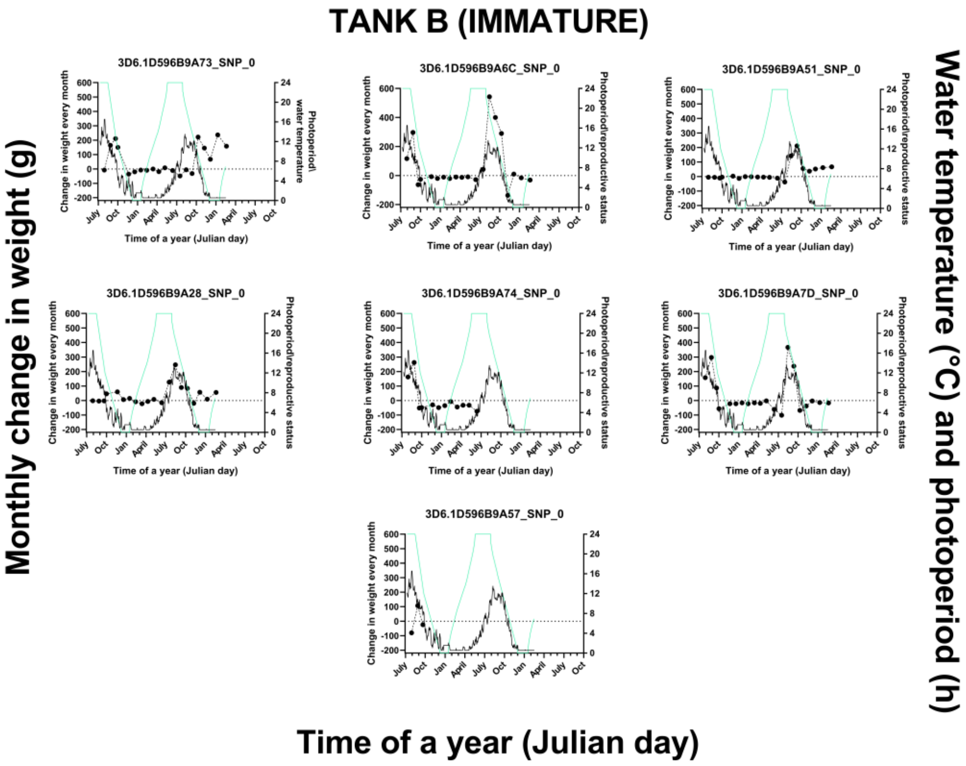

Figure 39: Monthly change in weight in immature Arctic charr in Tank B

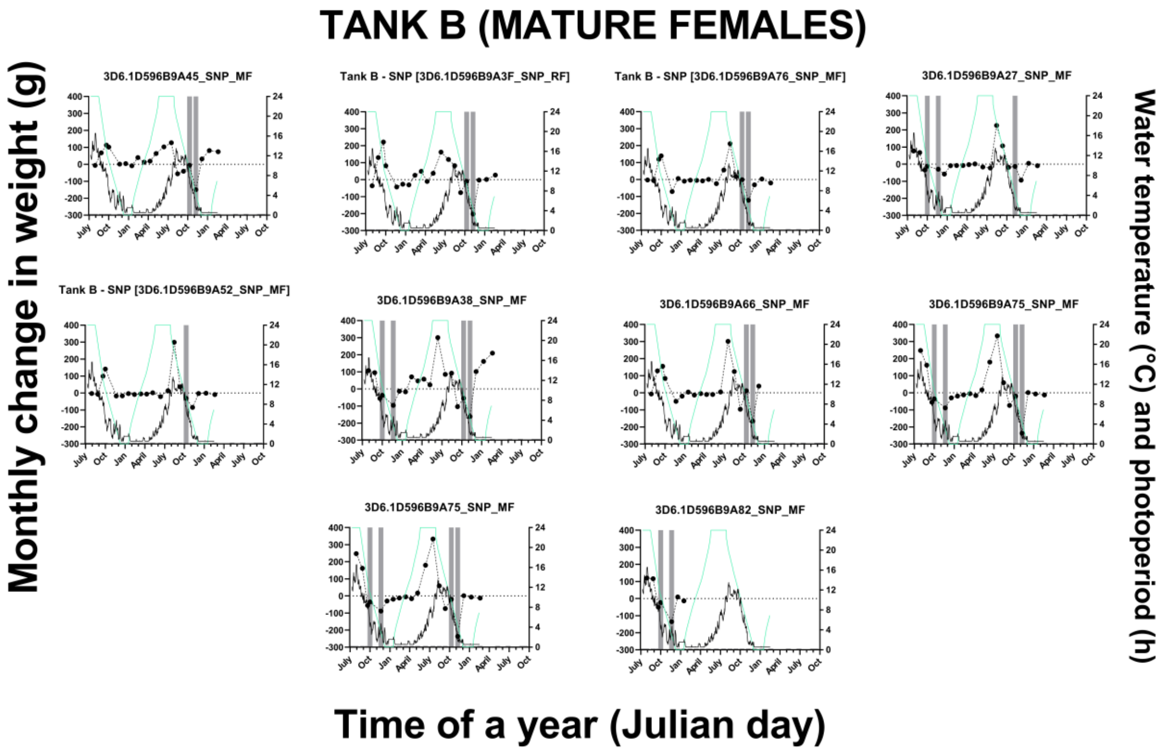

Figure 40: Monthly change in weight in mature females of Arctic charr in Tank B

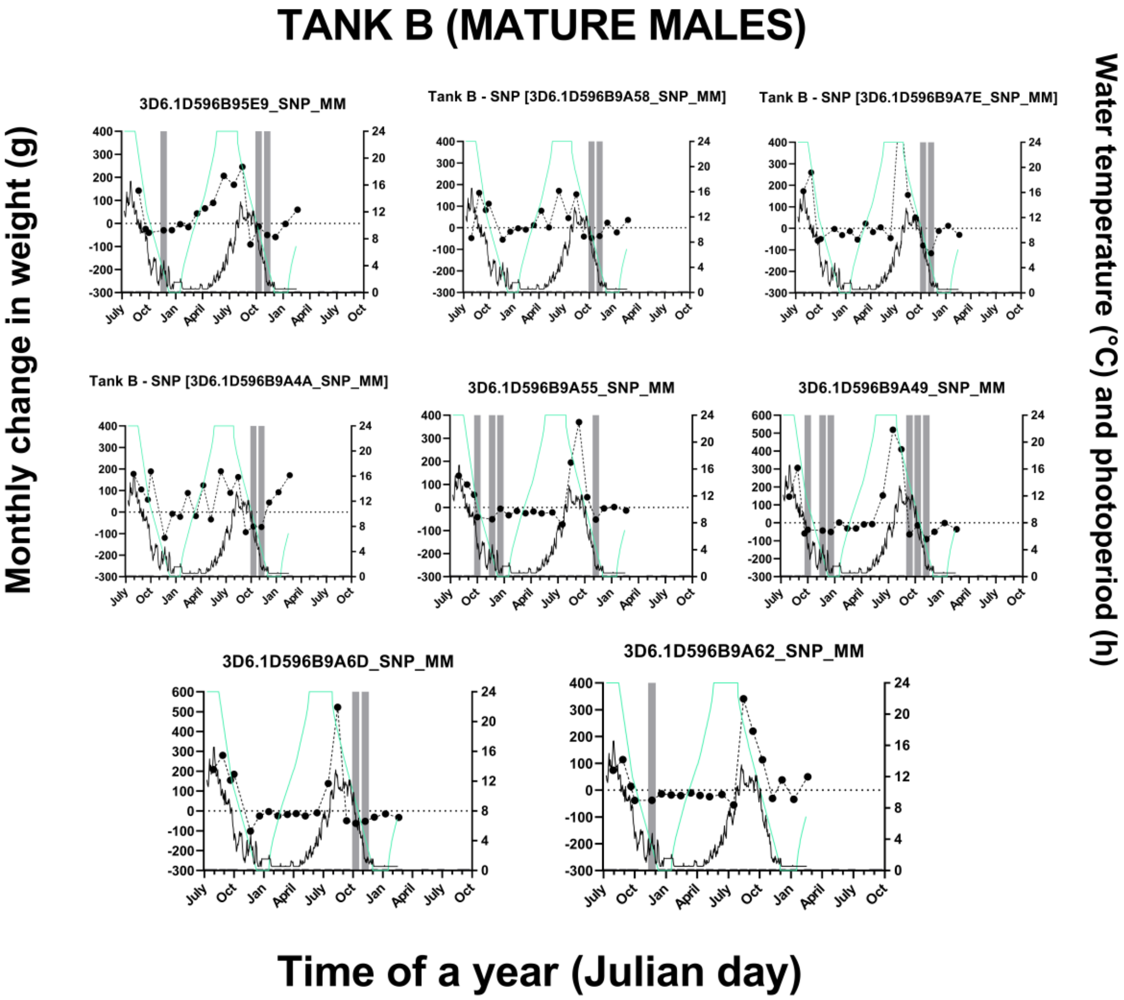

Figure 41: Monthly change in weight in mature males of Arctic charr in Tank A

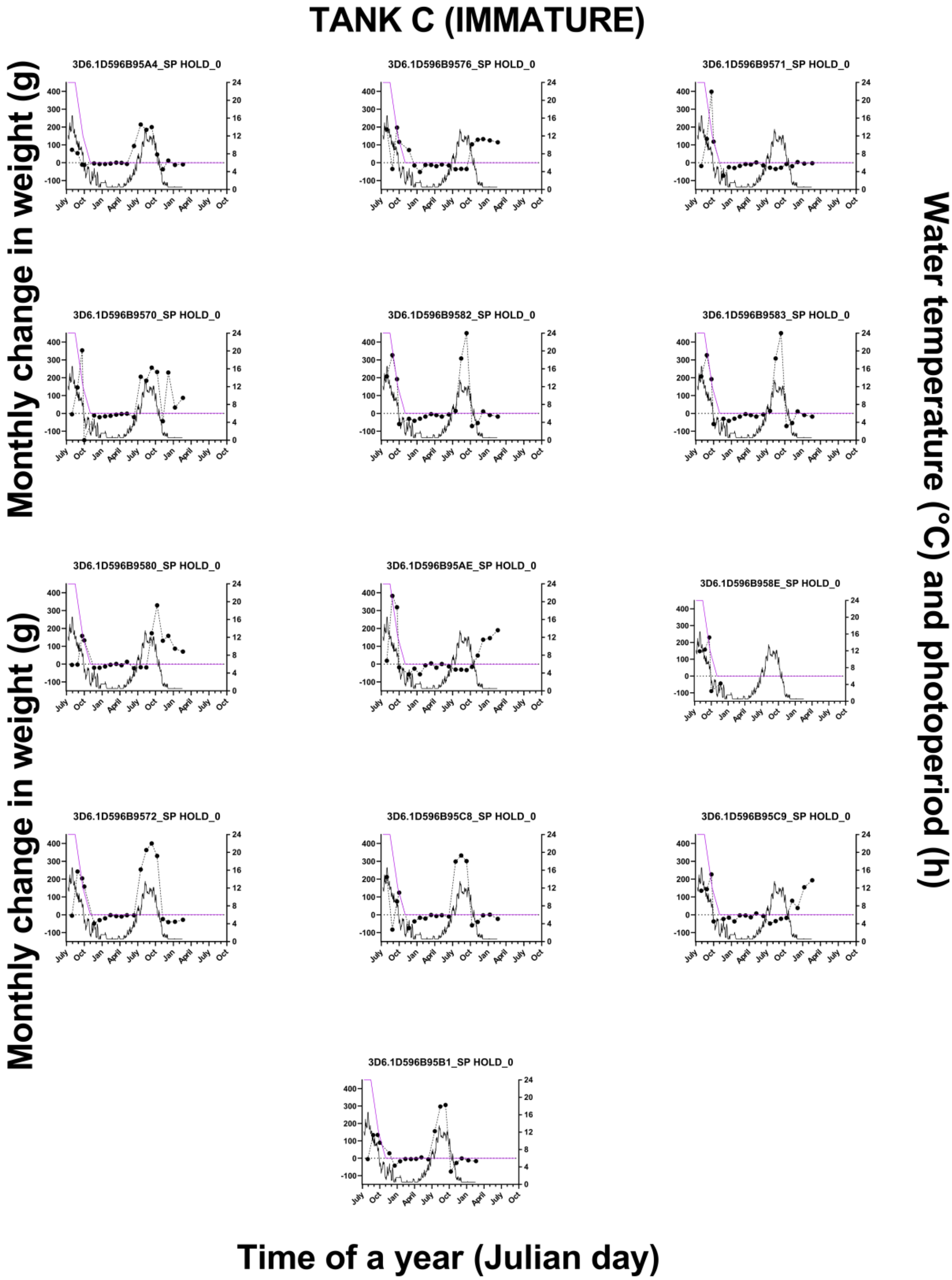

Figure 42: Monthly change in weight in immature Arctic charr in Tank C

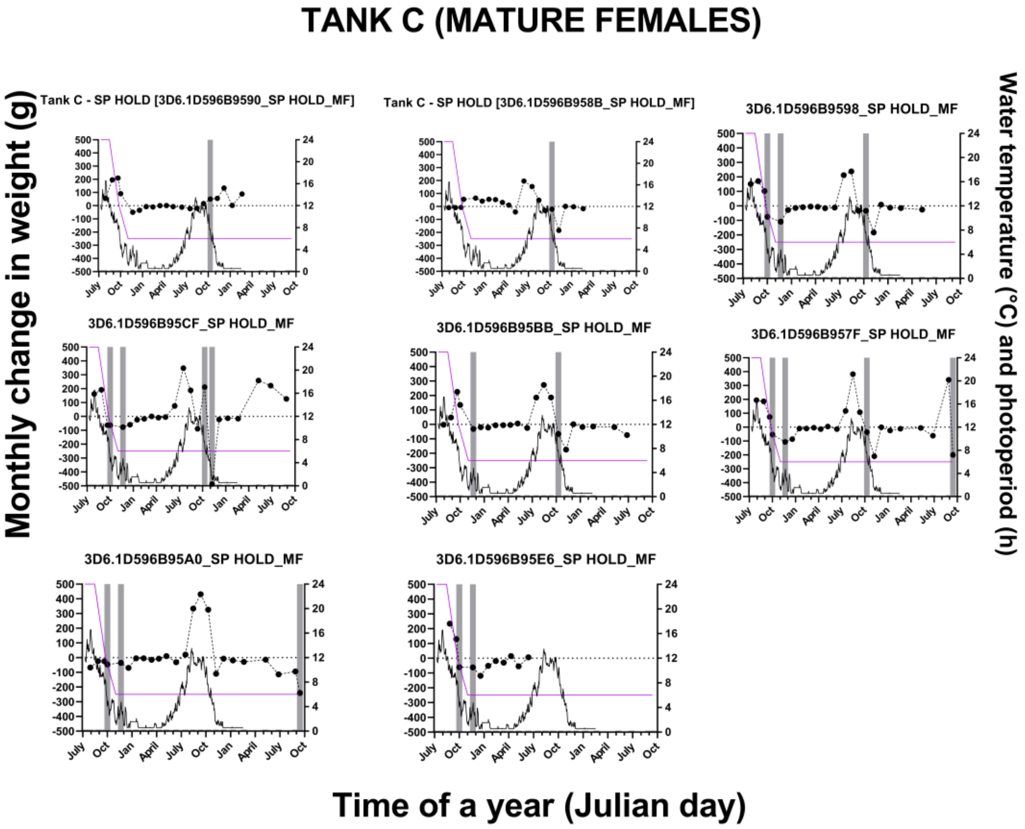

Figure 43: Monthly change in weight in mature females of Arctic charr in Tank C

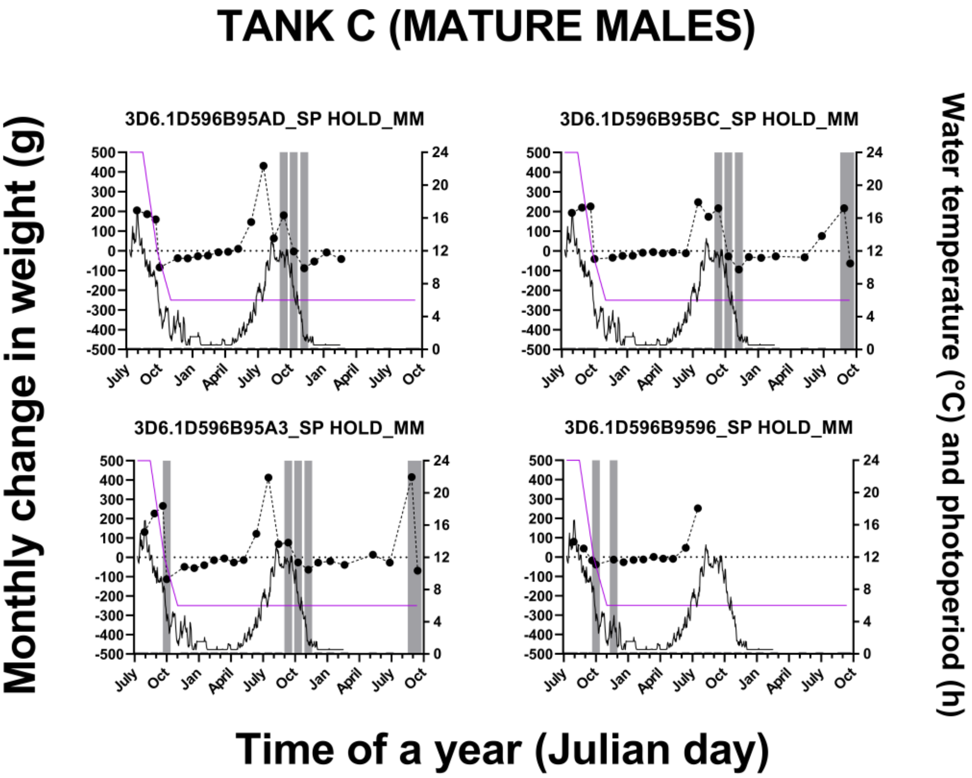

Figure 44: Monthly change in weight in mature males of Arctic charr in Tank A

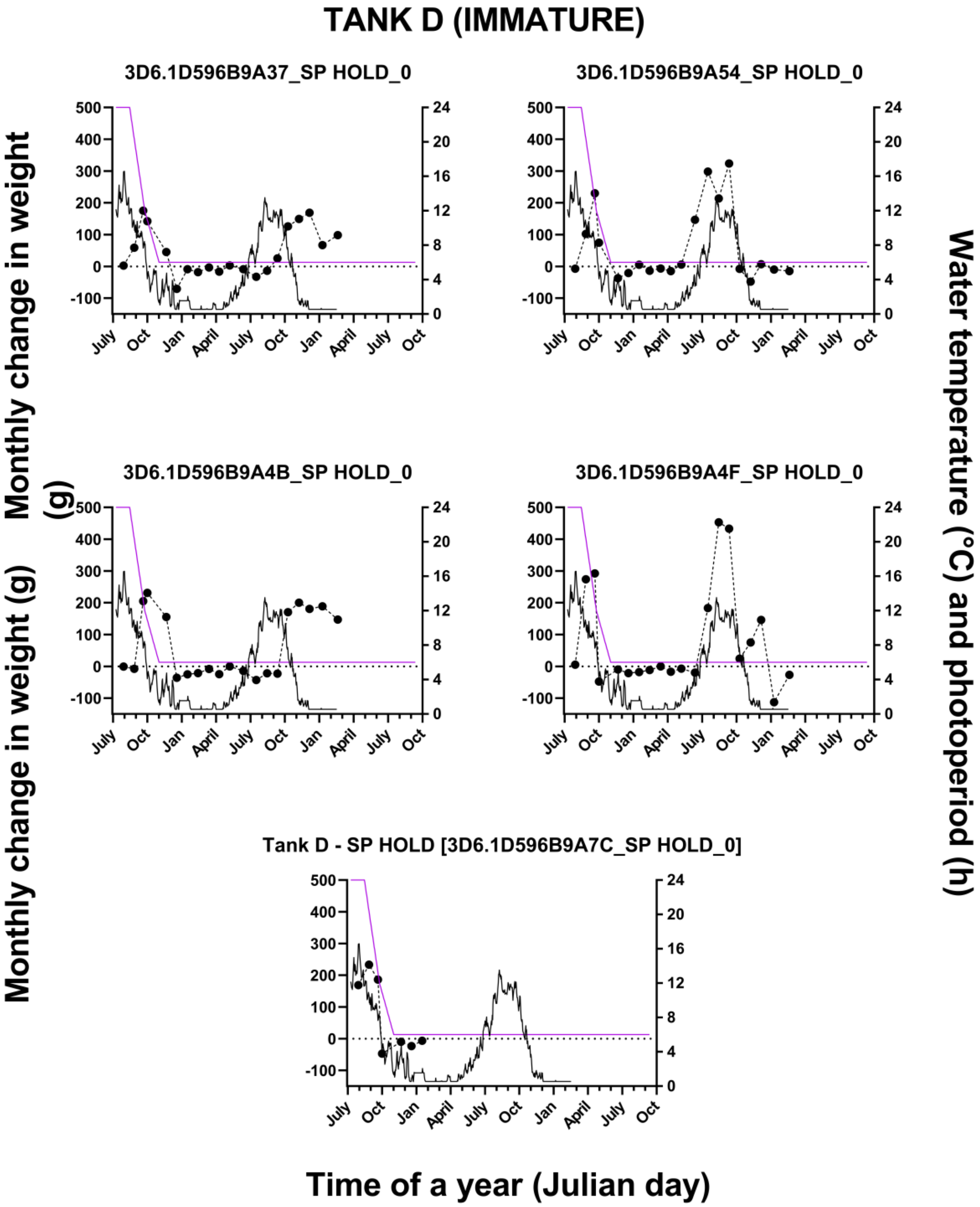

Figure 45: Monthly change in weight in immature Arctic charr in Tank D

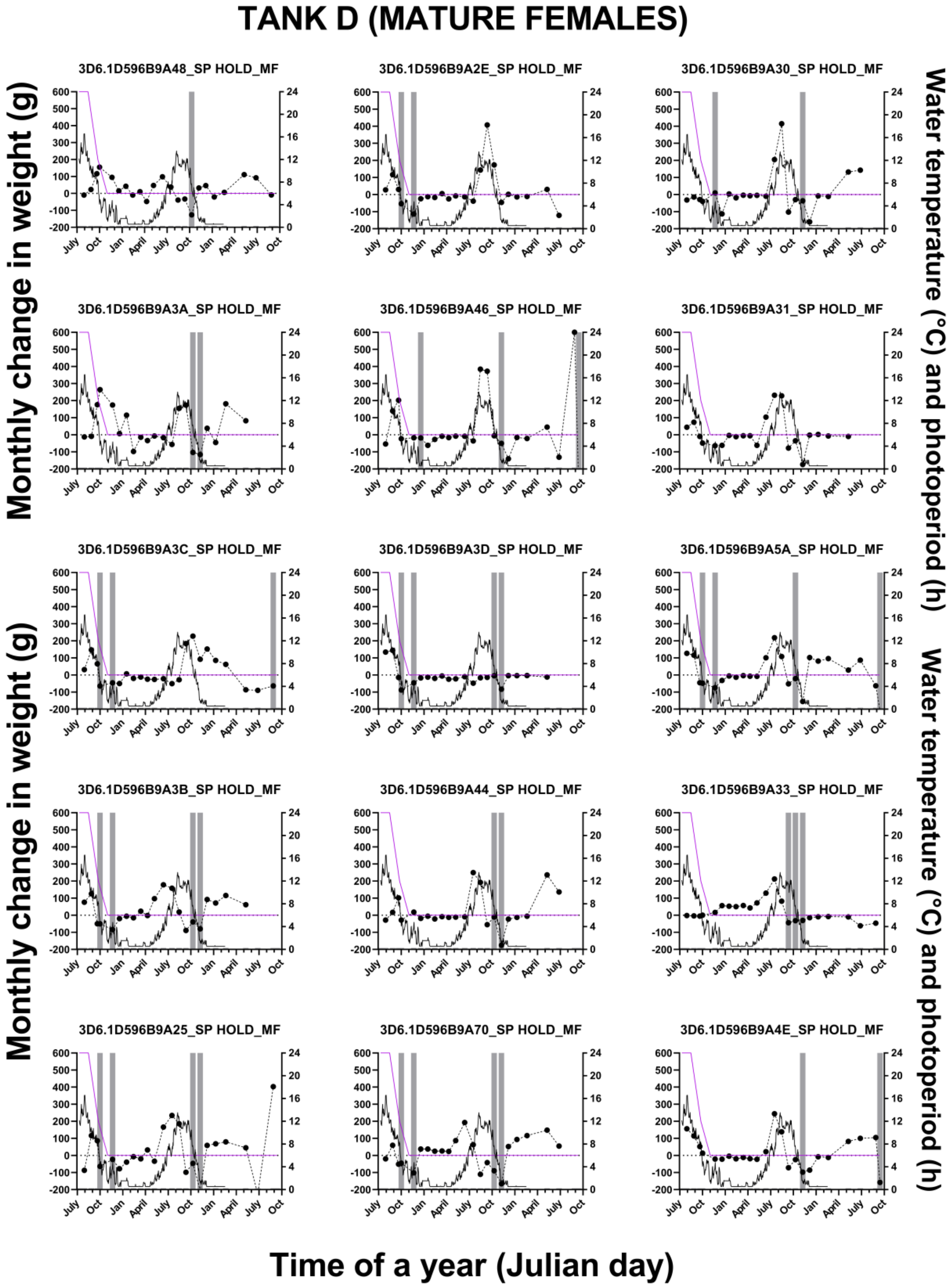

Figure 46: Monthly change in weight in mature females of Arctic charr in Tank D

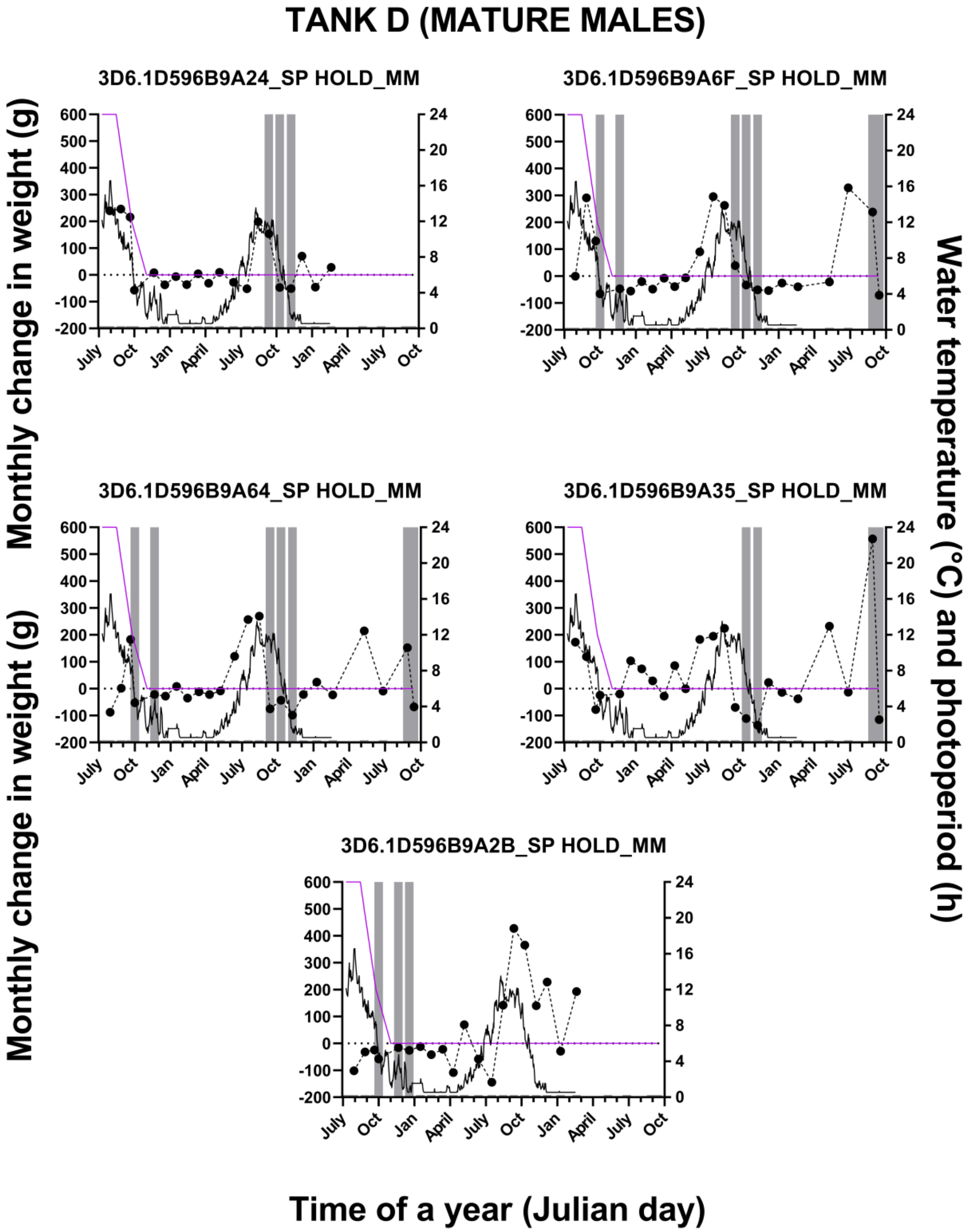

Figure 47: Monthly change in weight in mature males of Arctic charr in Tank D

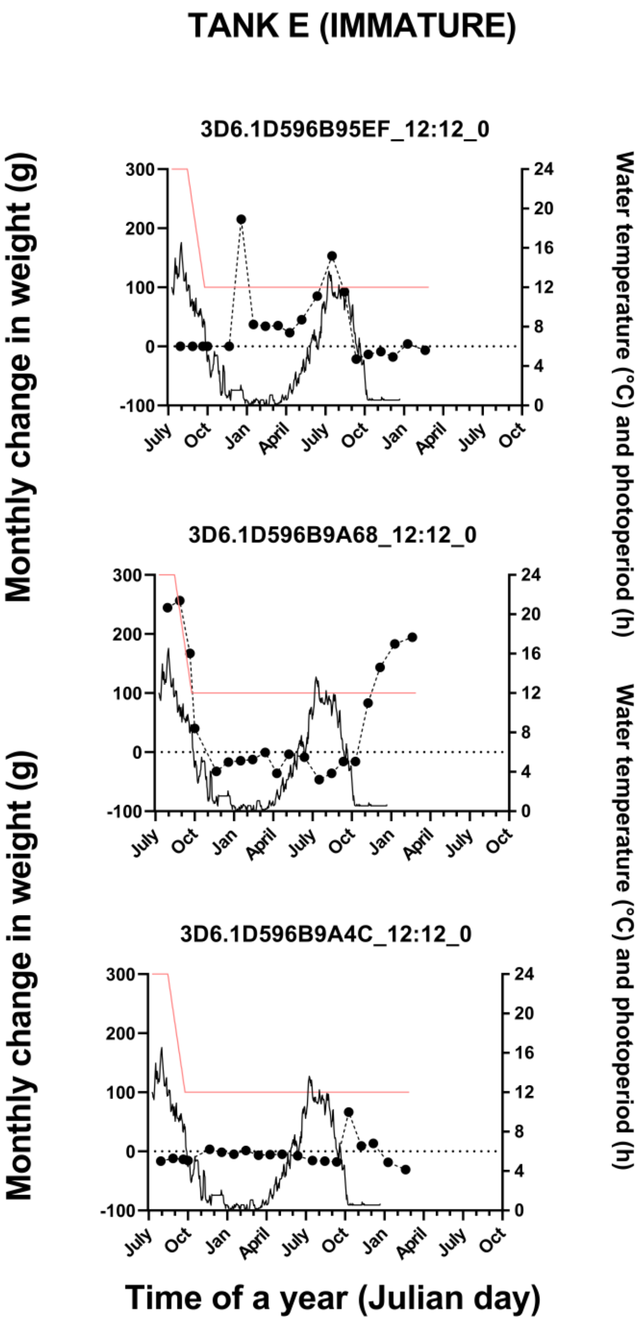

Figure 48: Monthly change in weight in immature Arctic charr in Tank E

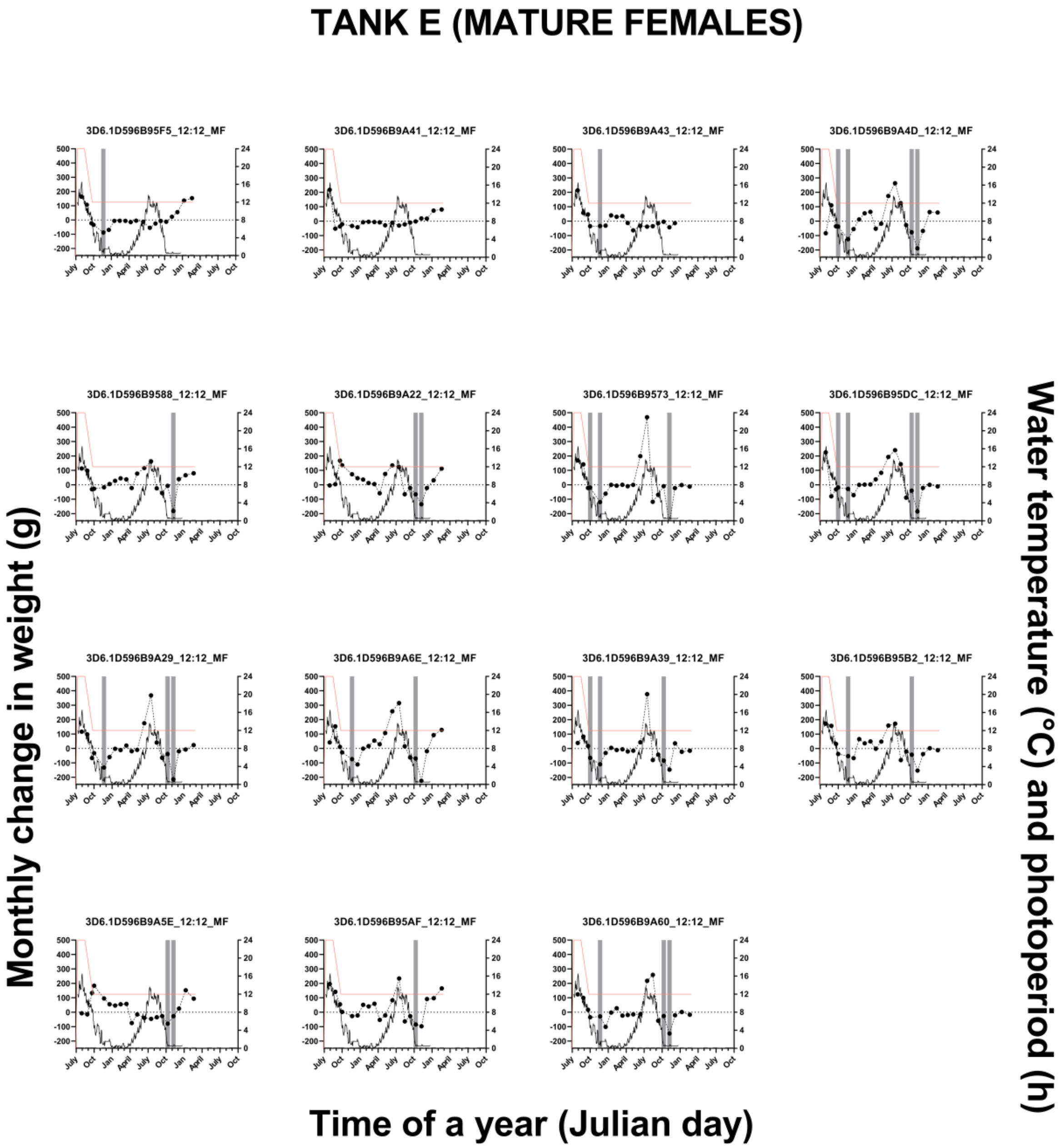

Figure 49: Monthly change in weight in mature females of Arctic charr in Tank E

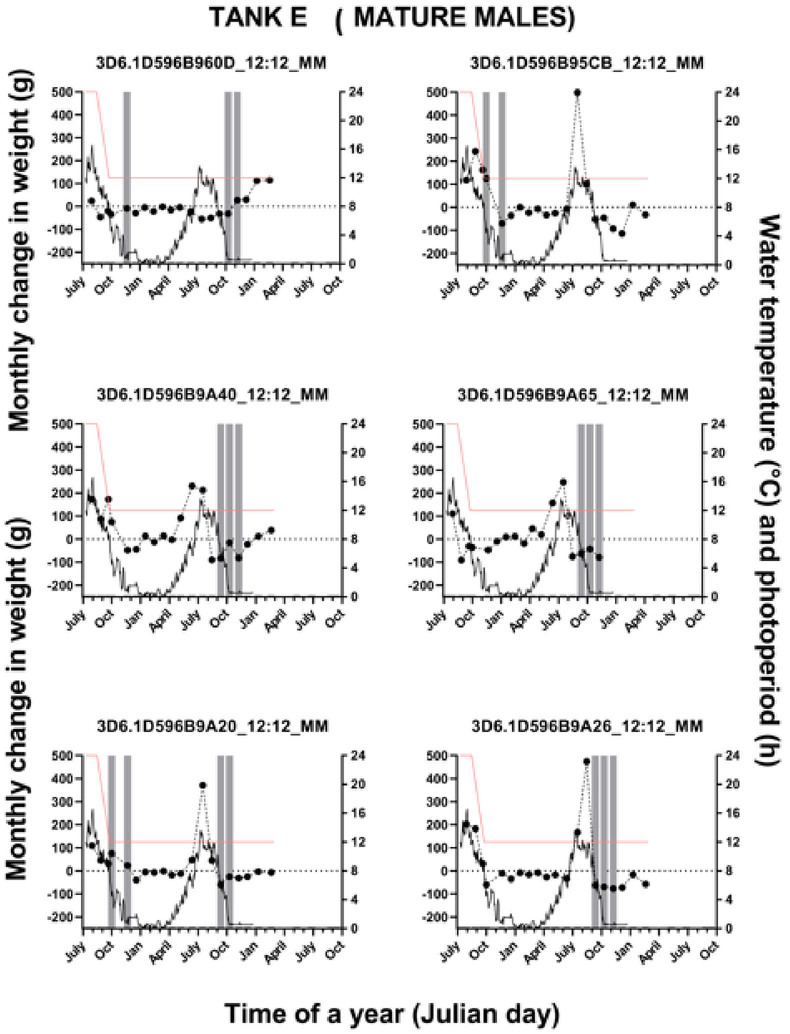

Figure 50: Monthly change in weight in mature males of Arctic charr in Tank E

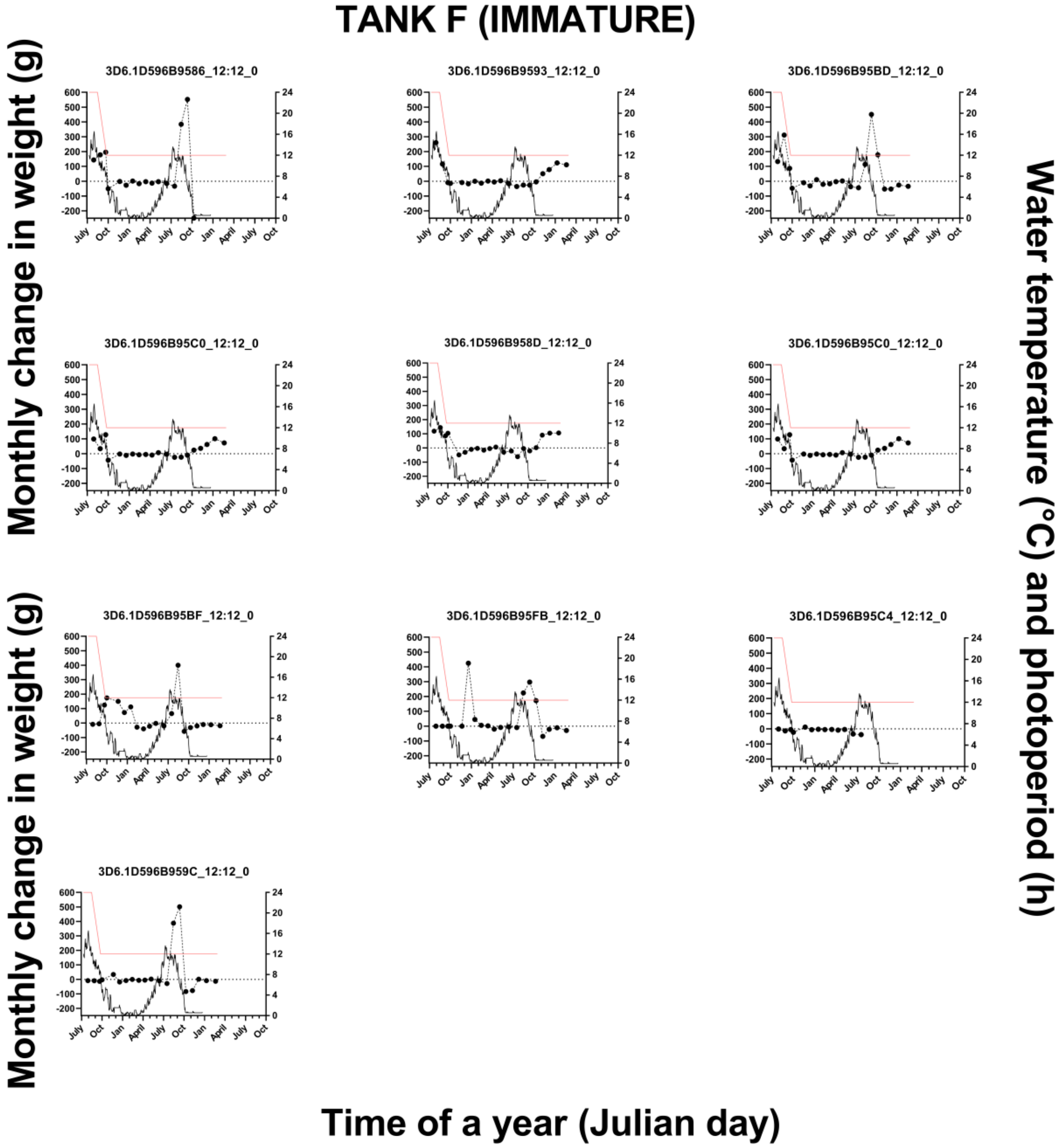

Figure 51: Monthly change in weight in immature Arctic charr in Tank F

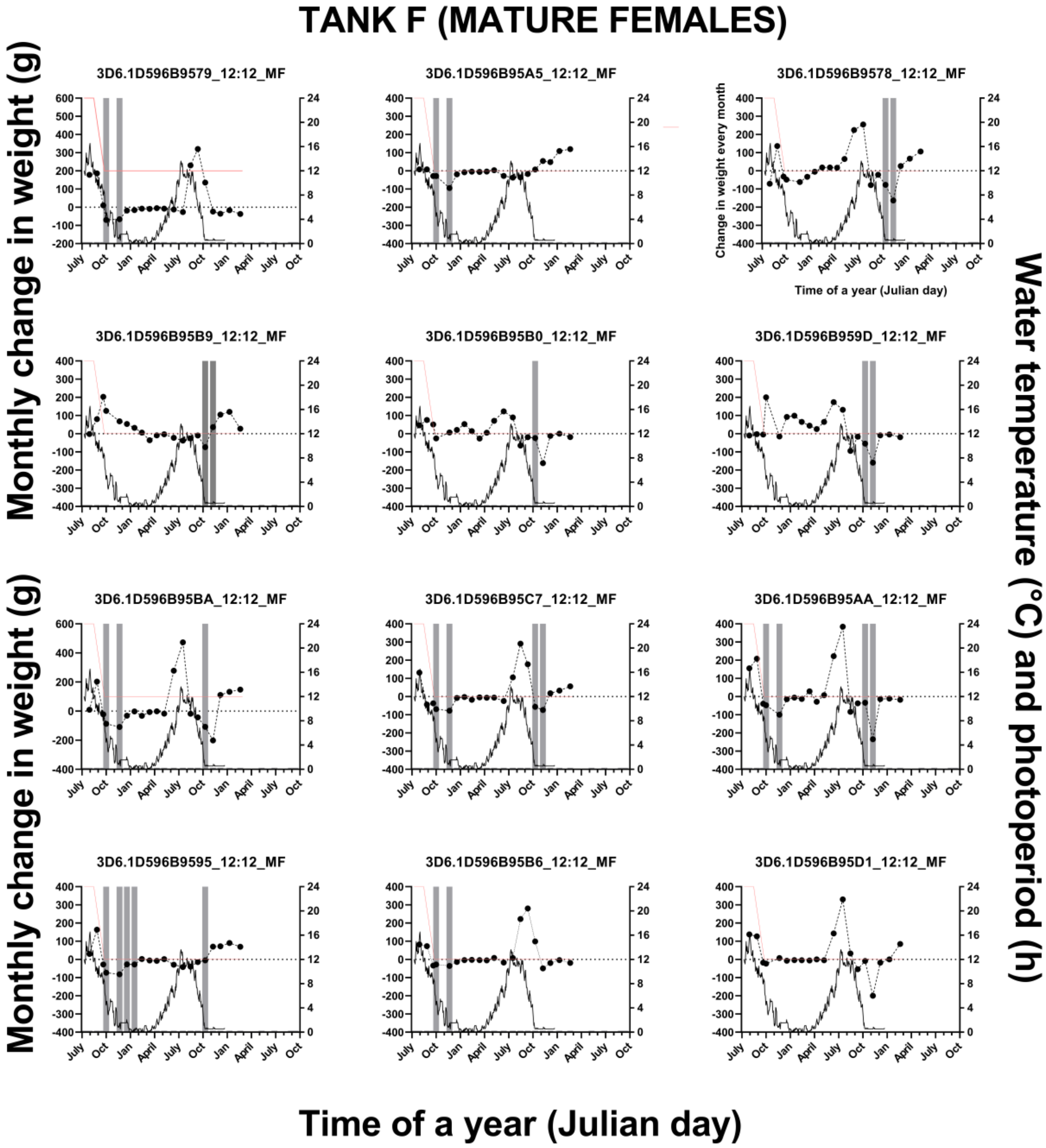

Figure 52: Monthly change in weight in mature females of Arctic charr in Tank F

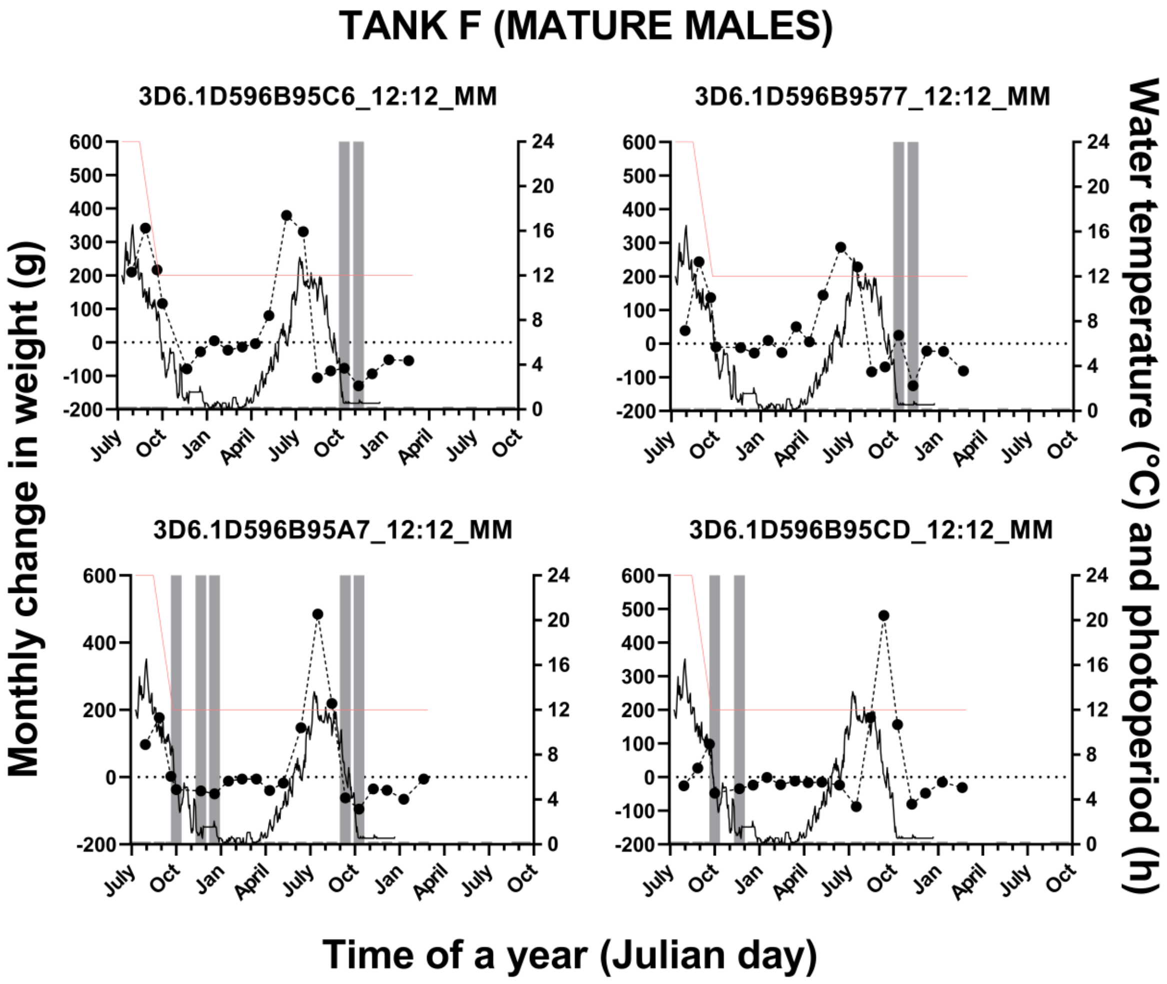

Figure 53: Monthly change in weight in mature males of Arctic charr in Tank F

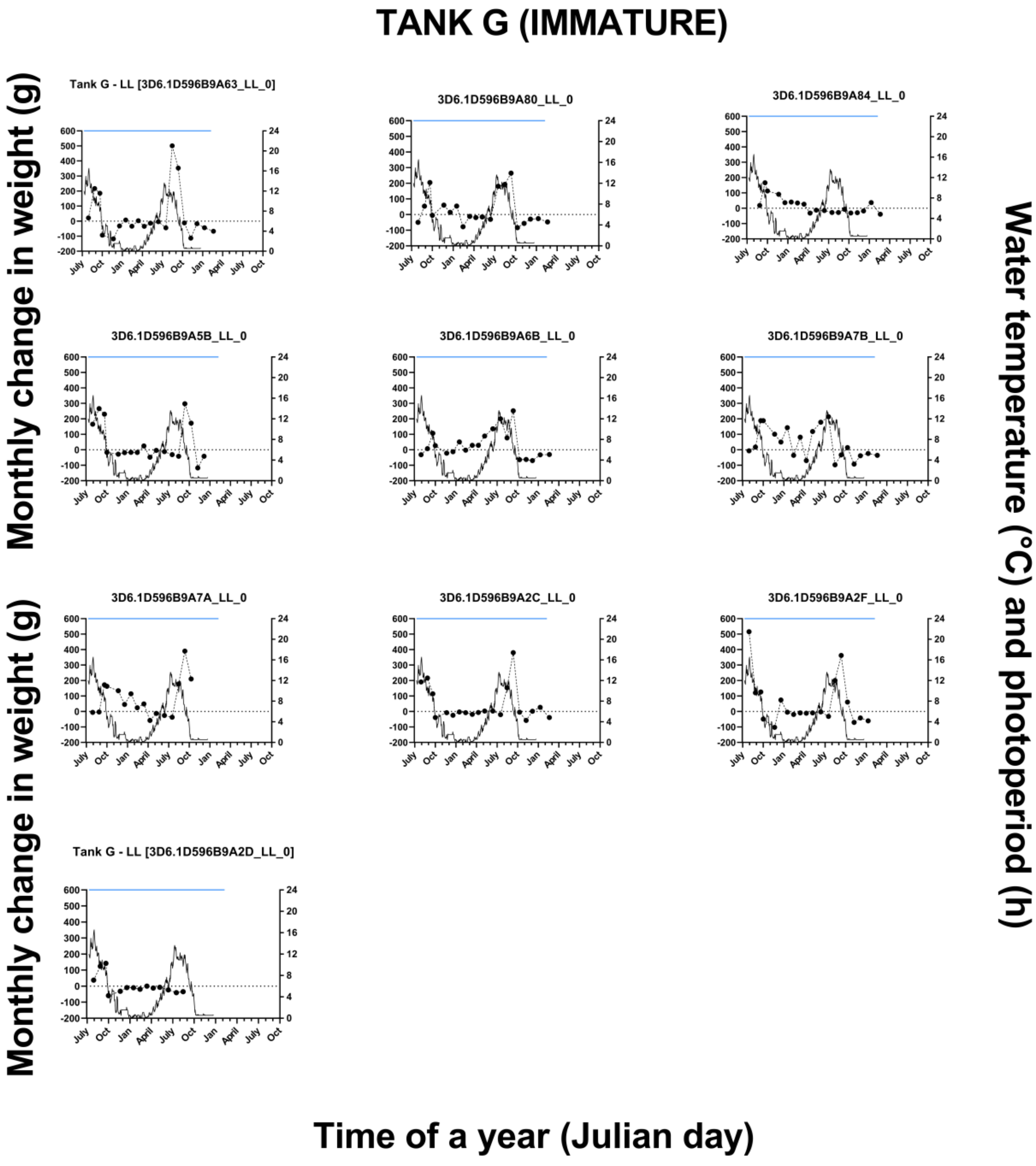

Figure 54: Monthly change in weight in immature Arctic charr in Tank G

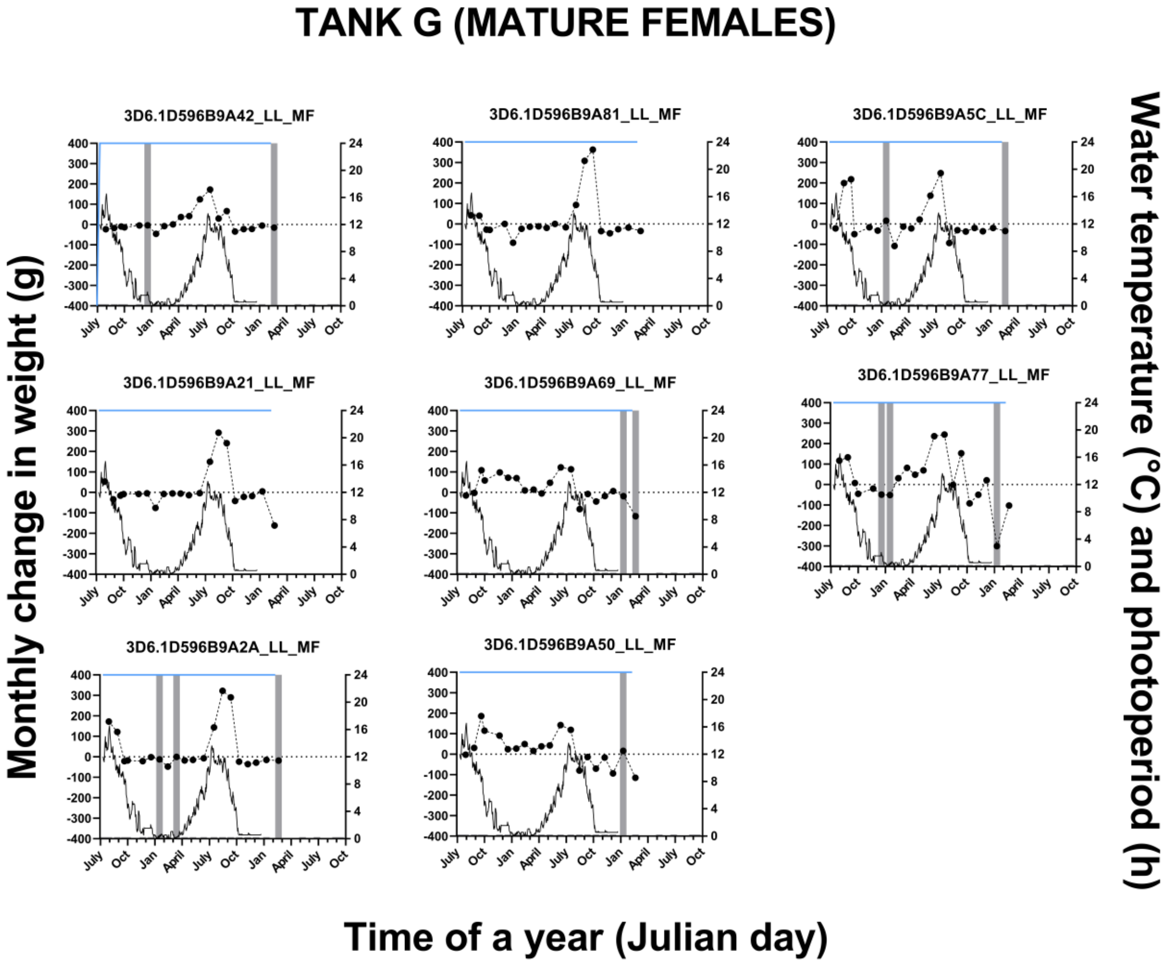

Figure 55: Monthly change in weight in mature females of Arctic charr in Tank G

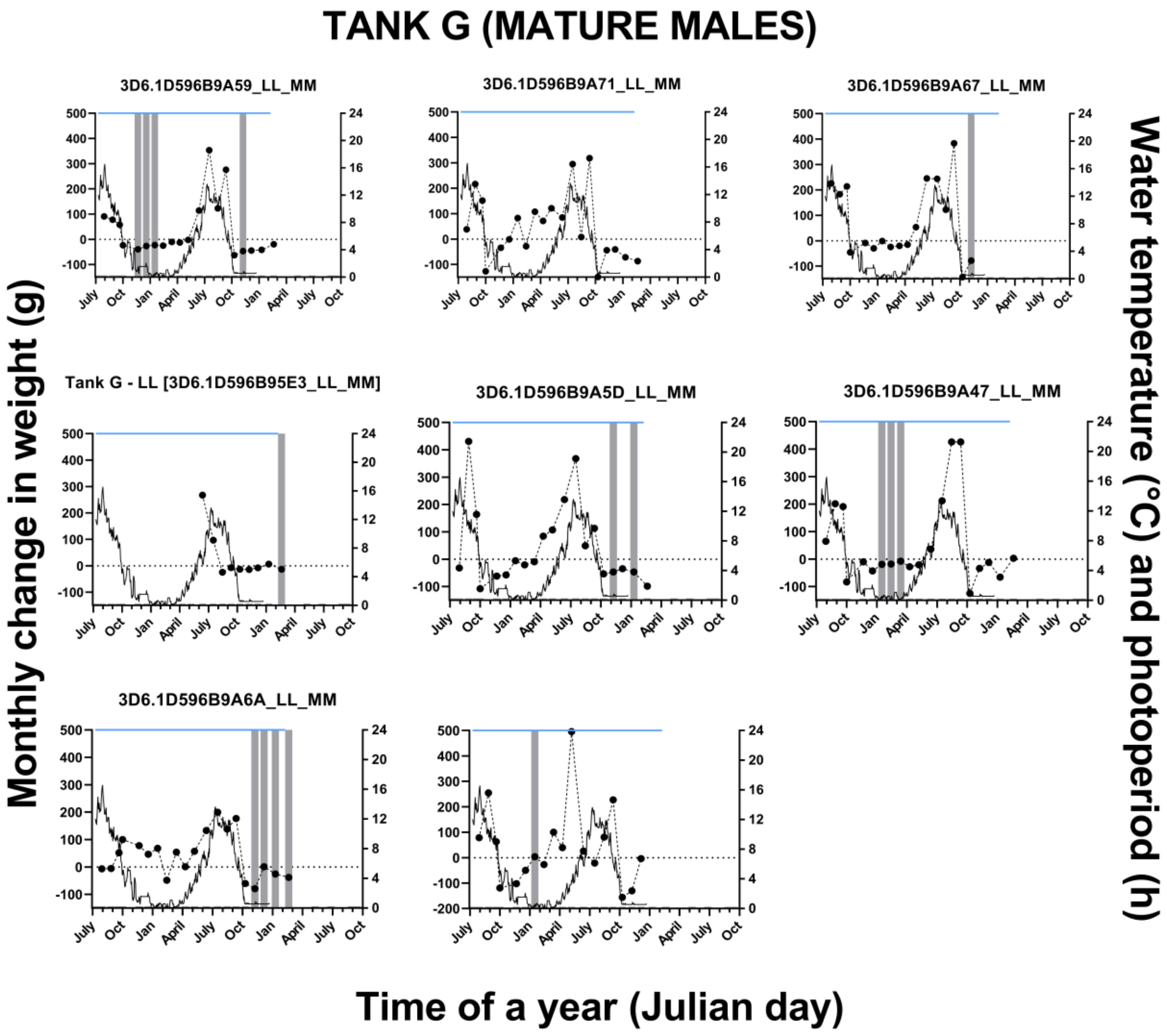

Figure 56: Monthly change in weight in mature males of Arctic charr in Tank G

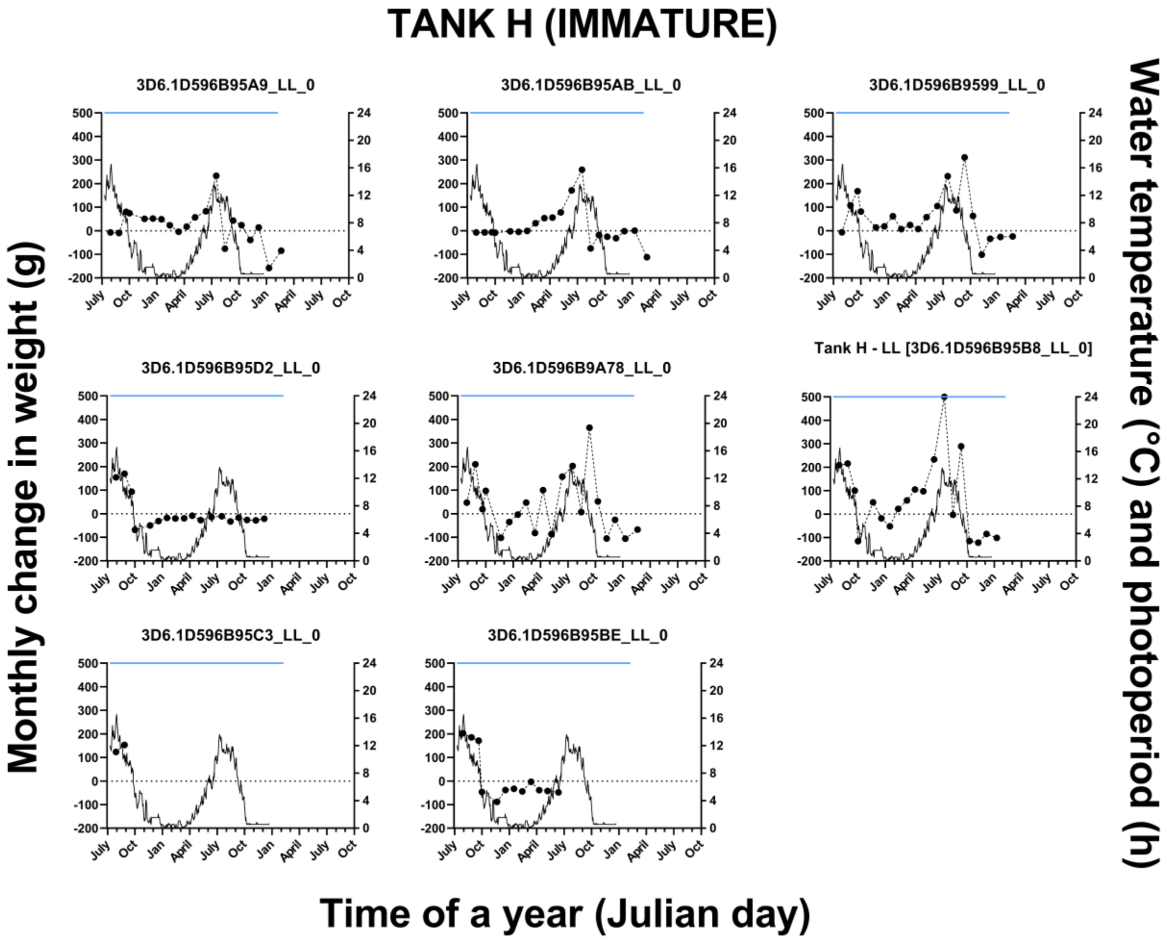

Figure 57: Monthly change in weight in immature Arctic charr in Tank H

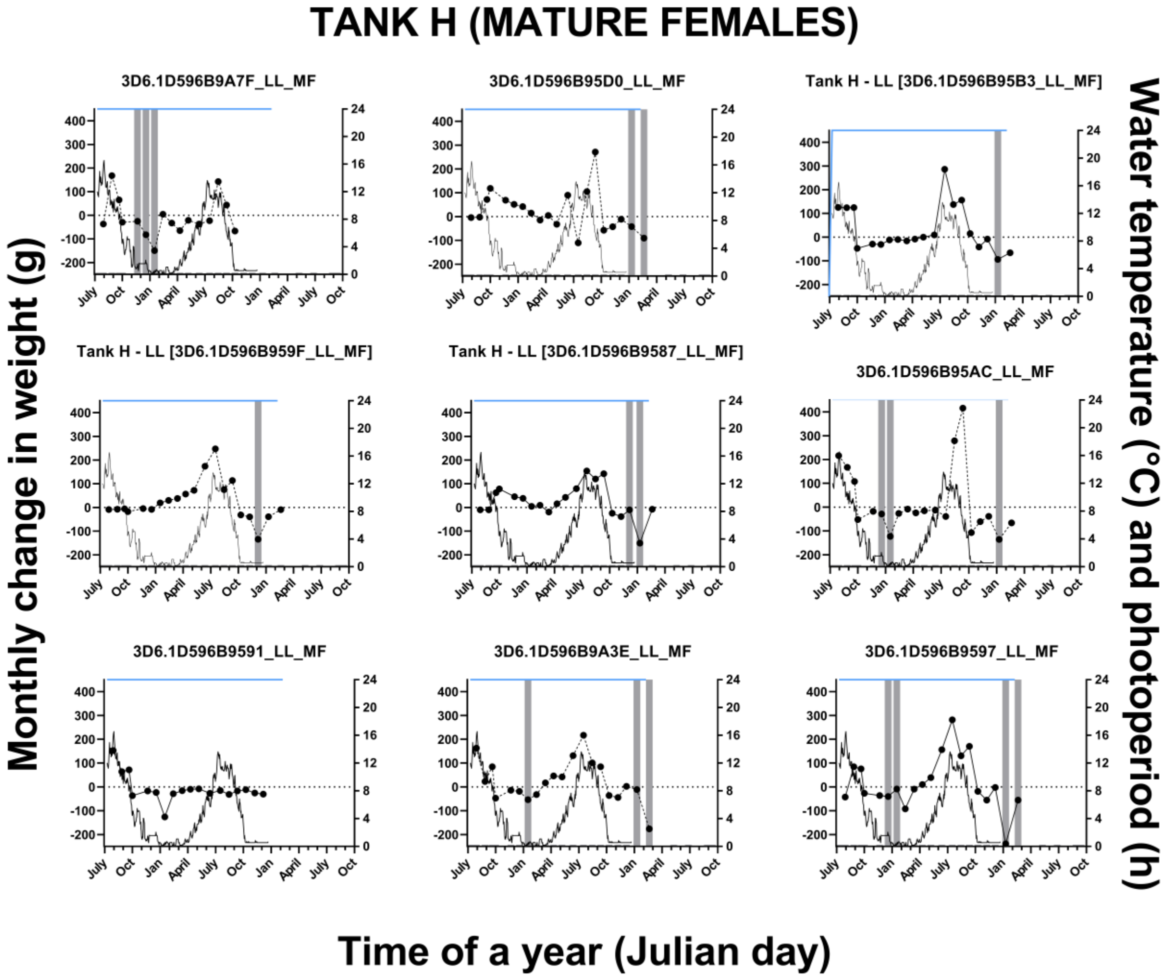

Figure 58: Monthly change in weight in mature females of Arctic charr in Tank H

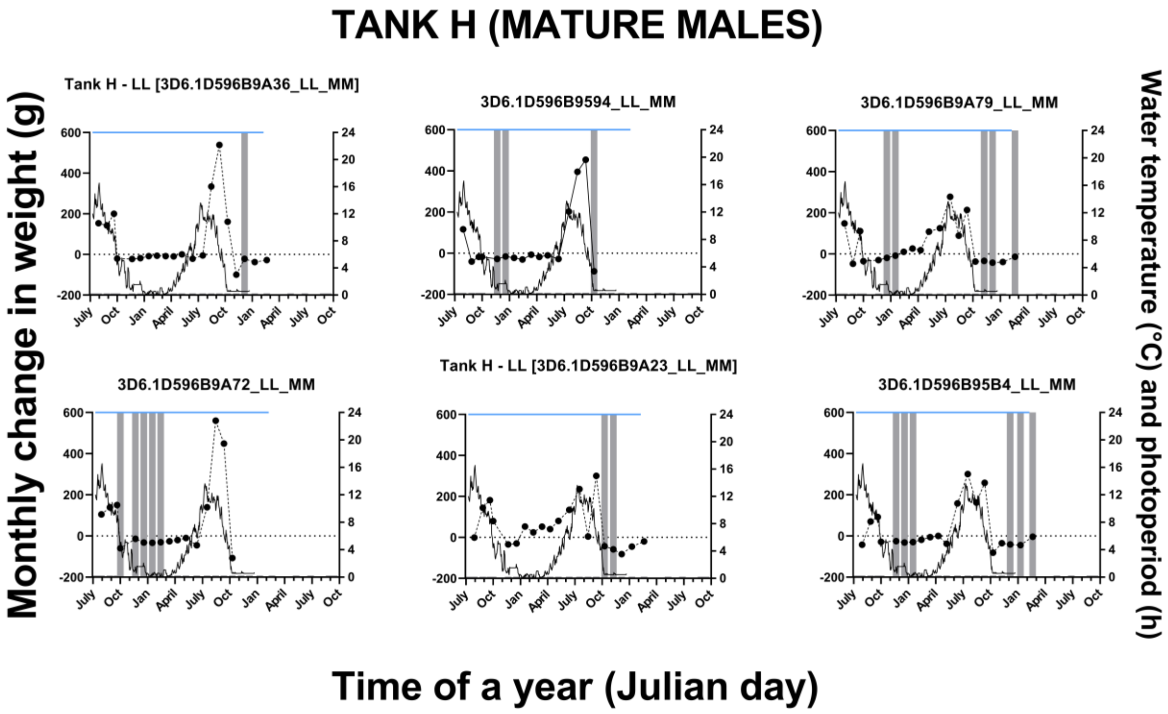

Figure 59: Monthly change in weight in mature males of Arctic charr in Tank H

APPENDIX 2

(Monthly change in length and reproductive status in Arctic charr)

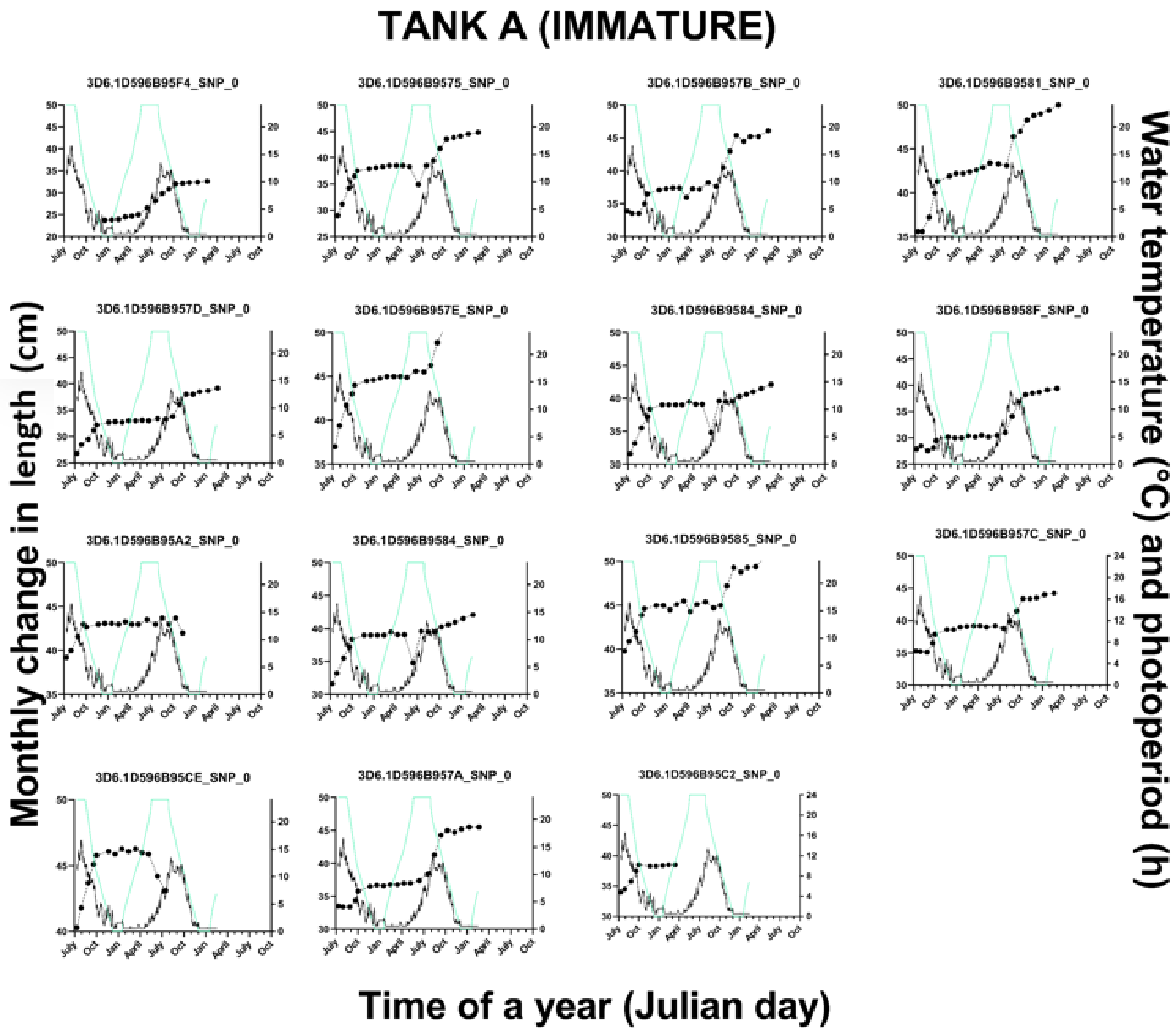

Figure 60: Monthly change in length in immature Arctic charr in Tank A

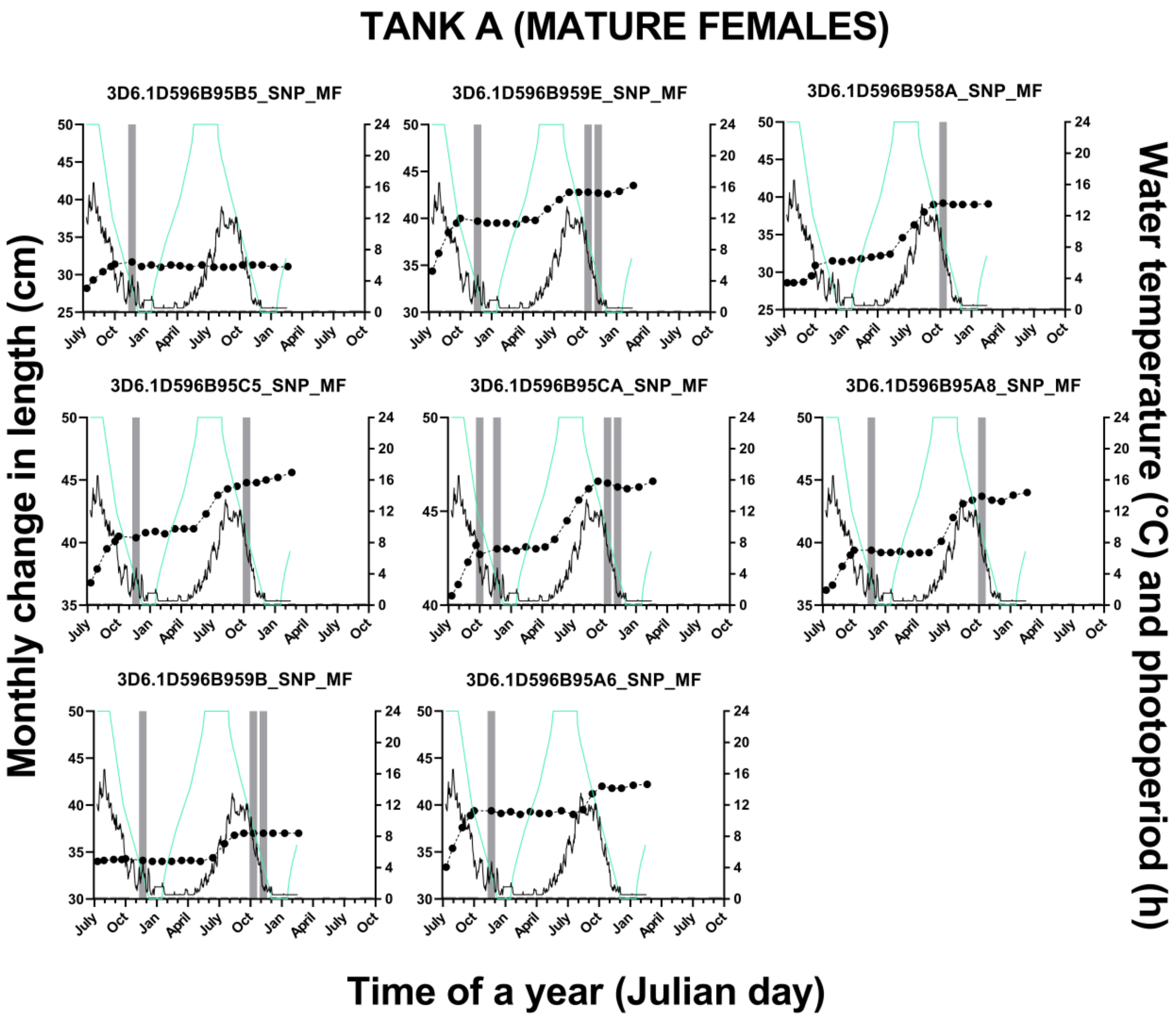

Figure 61: Monthly change in length in mature females of Arctic charr in Tank A

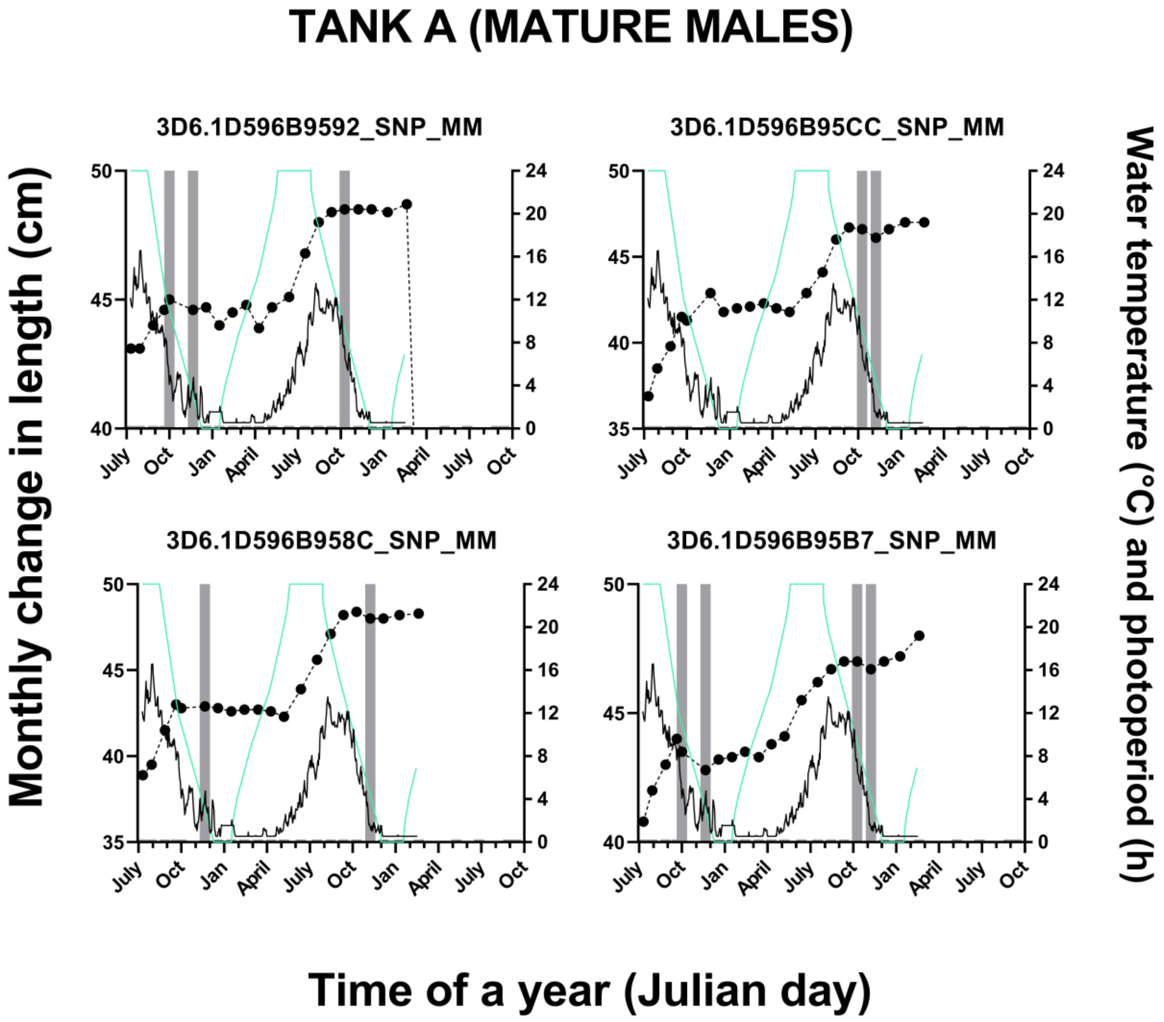

Figure 62: Monthly change in length in mature males of Arctic charr in Tank A

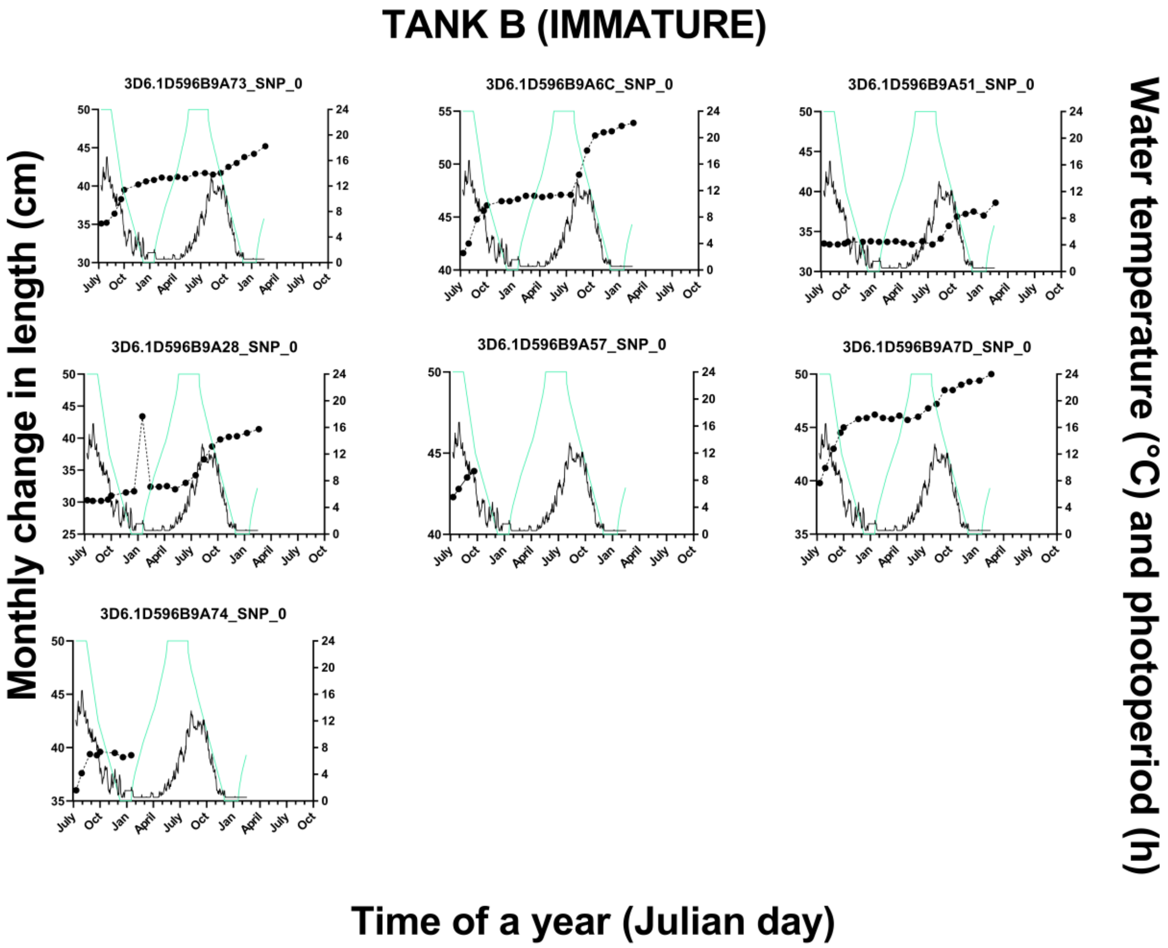

Figure 63: Monthly change in length in immature Arctic charr in Tank B

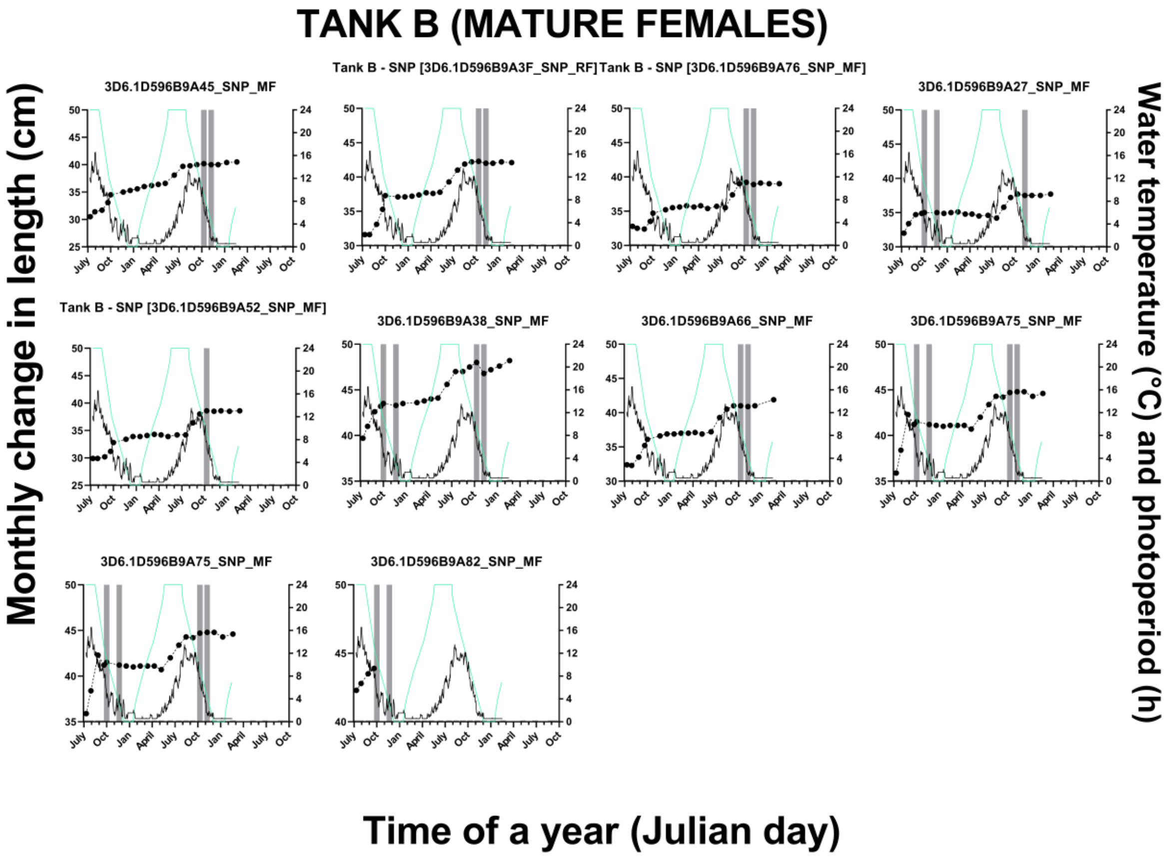

Figure 64: Monthly change in length in mature females of Arctic charr in Tank B

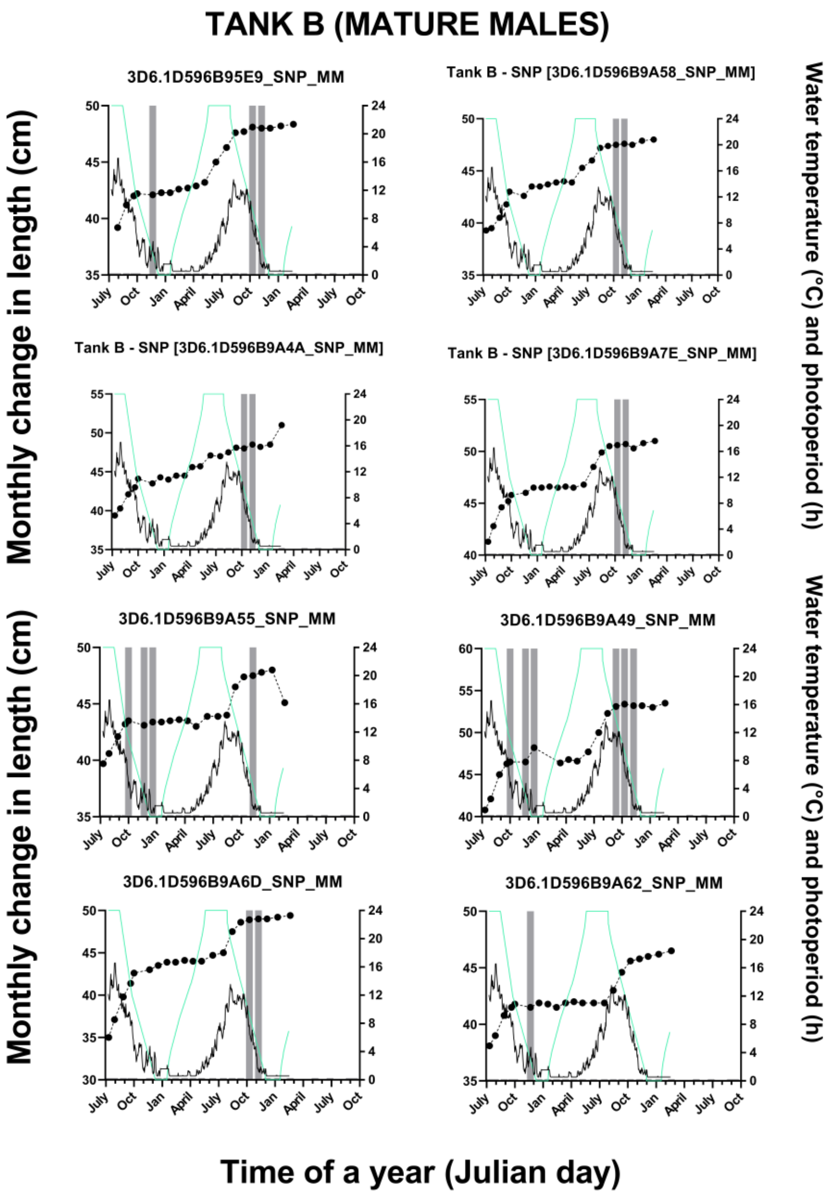

Figure 65: Monthly change in length in mature males of Arctic charr in Tank B

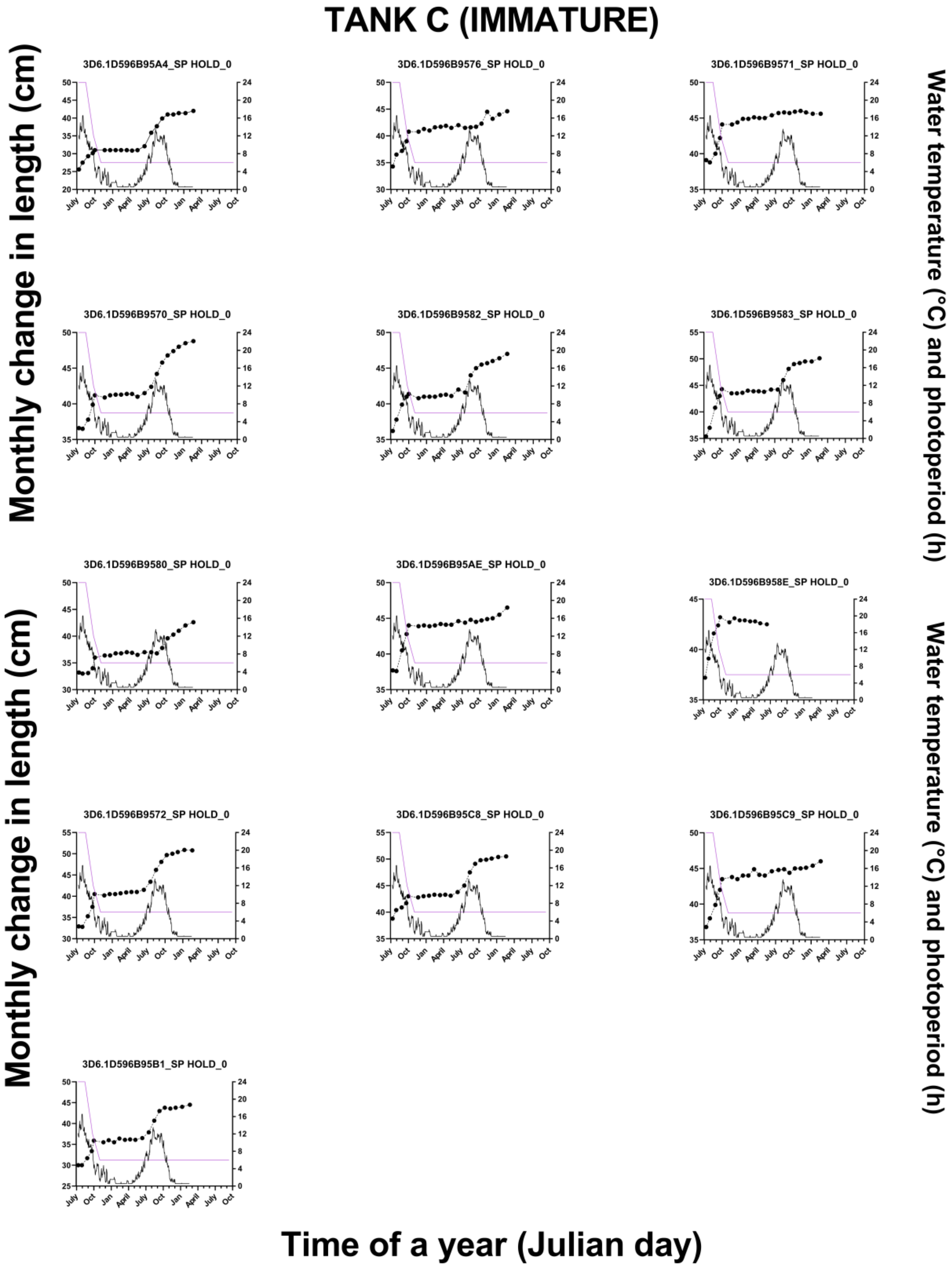

Figure 66: Monthly change in length in immature Arctic charr in Tank C

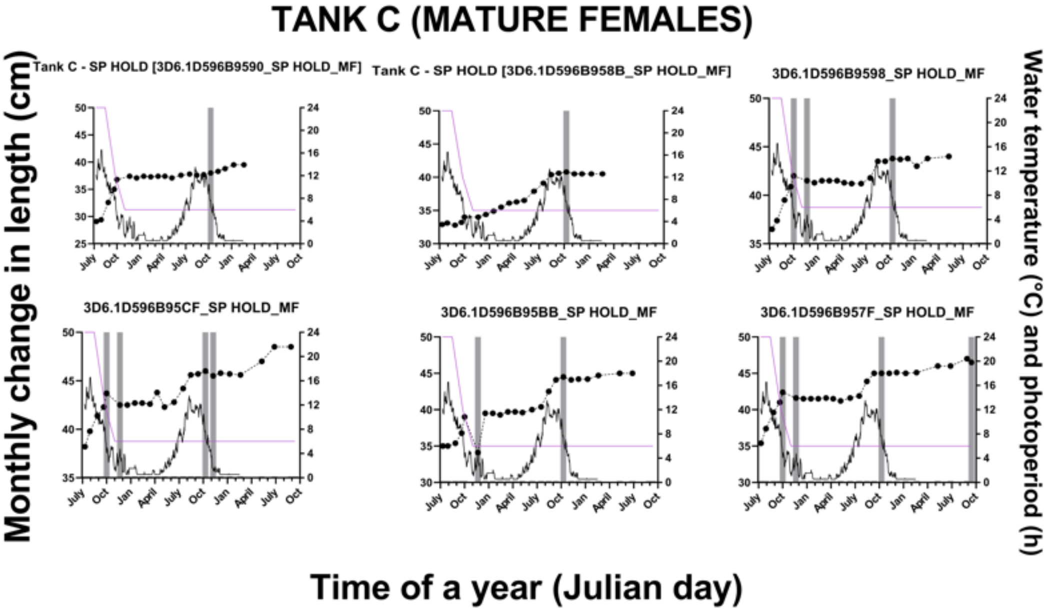

Figure 67: Monthly change in length in mature females of Arctic charr in Tank C

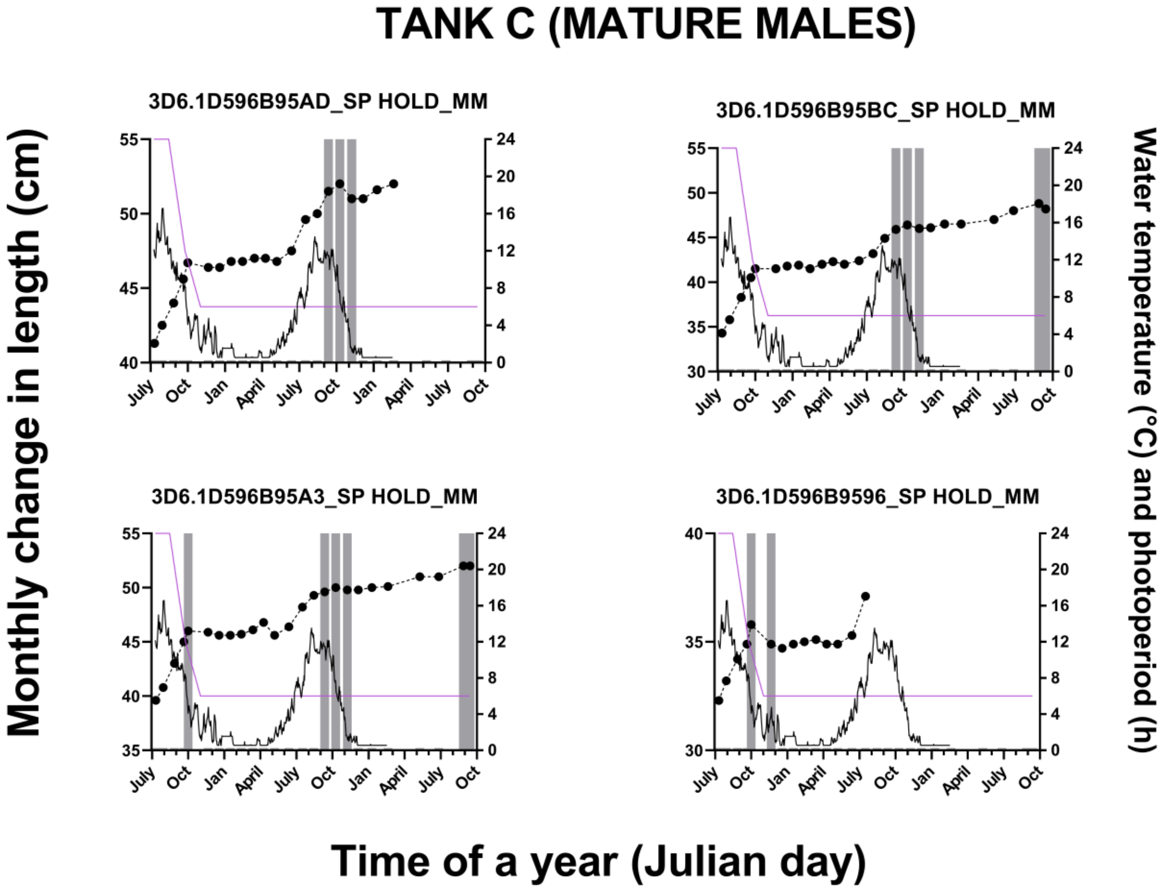

Figure 68: Monthly change in length in mature males of Arctic charr in Tank C

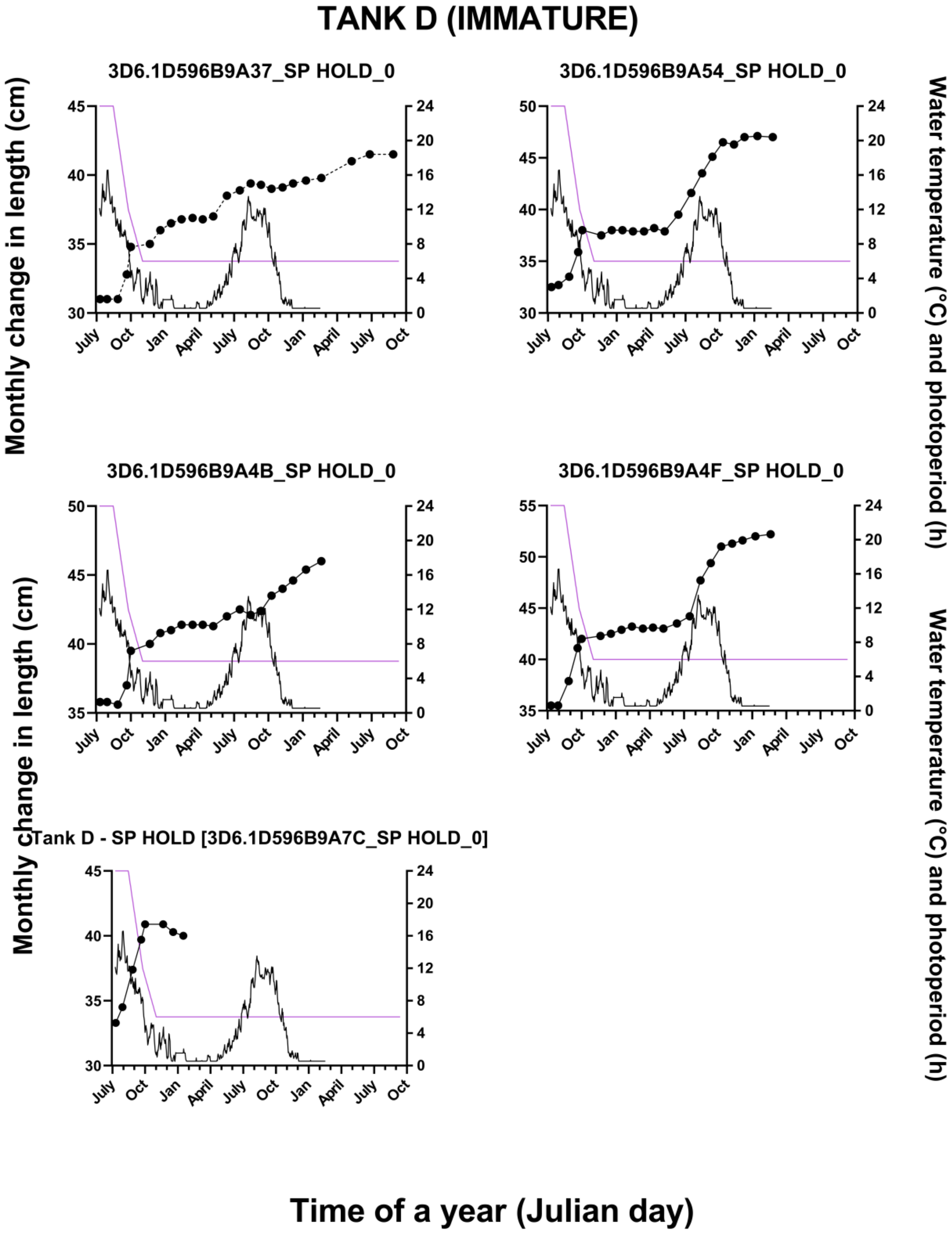

Figure 69: Monthly change in length in immature Arctic charr in Tank D

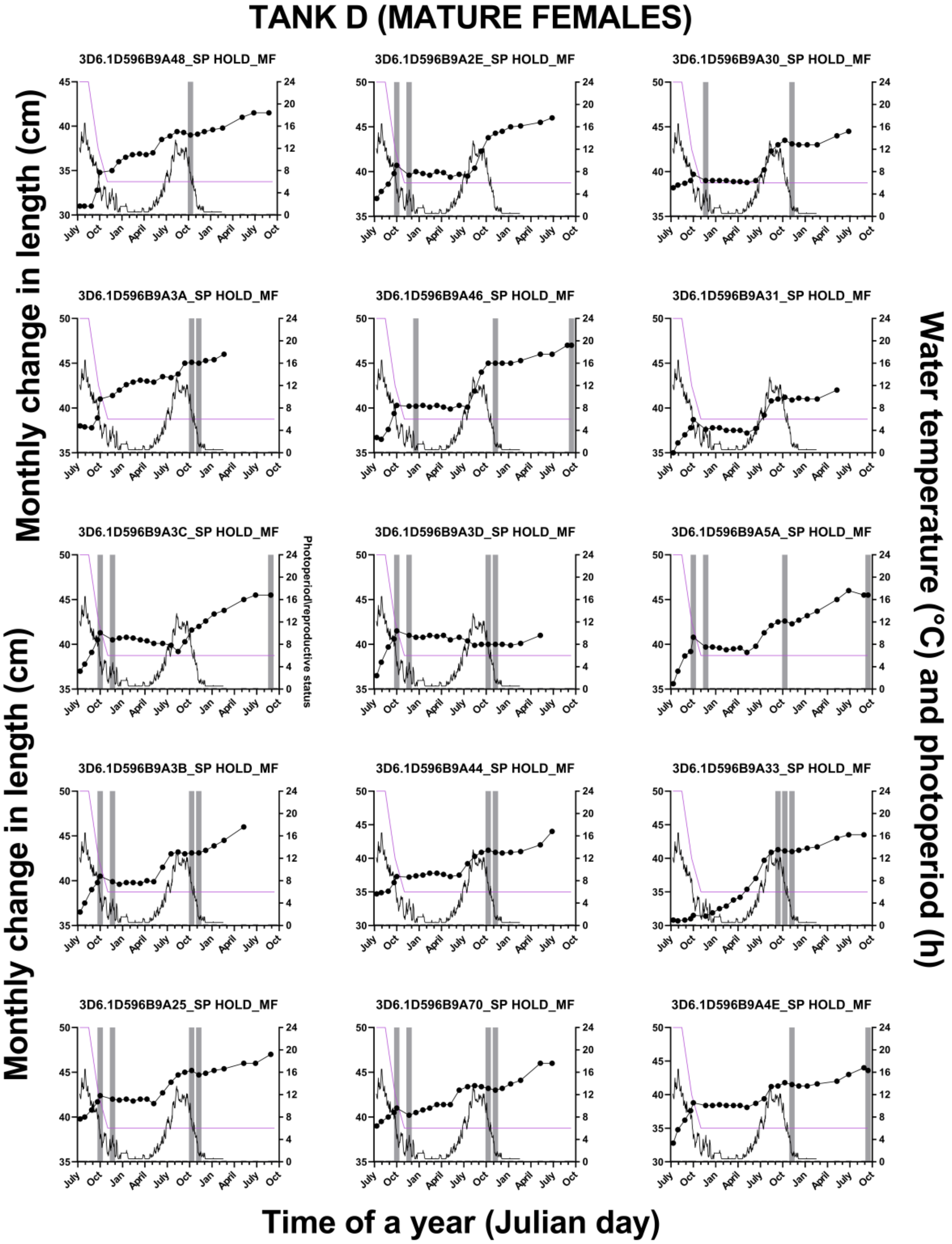

Figure 70: Monthly change in length in mature feamles of Arctic charr in Tank D

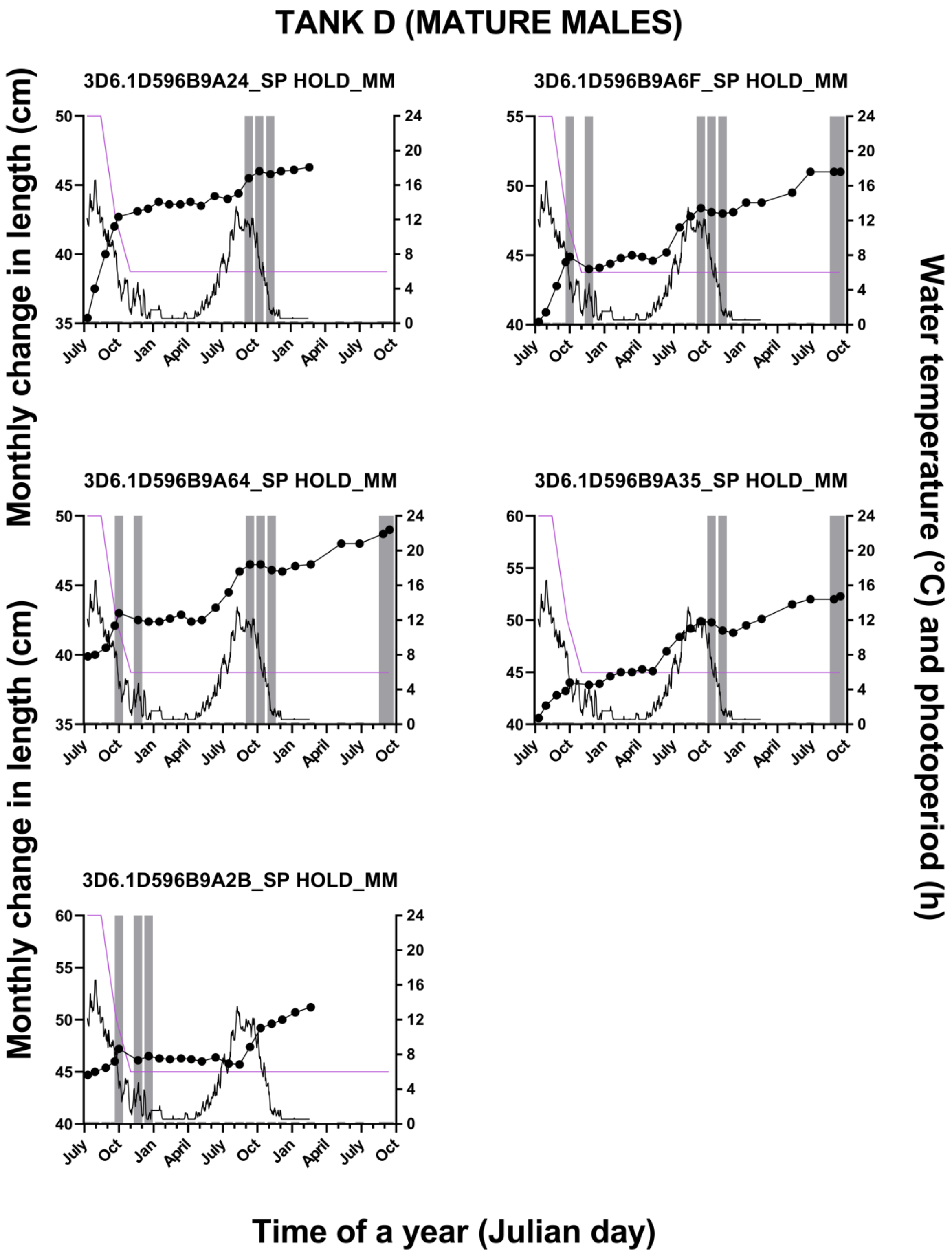

Figure 71: Monthly change in length in mature males of Arctic charr in Tank D

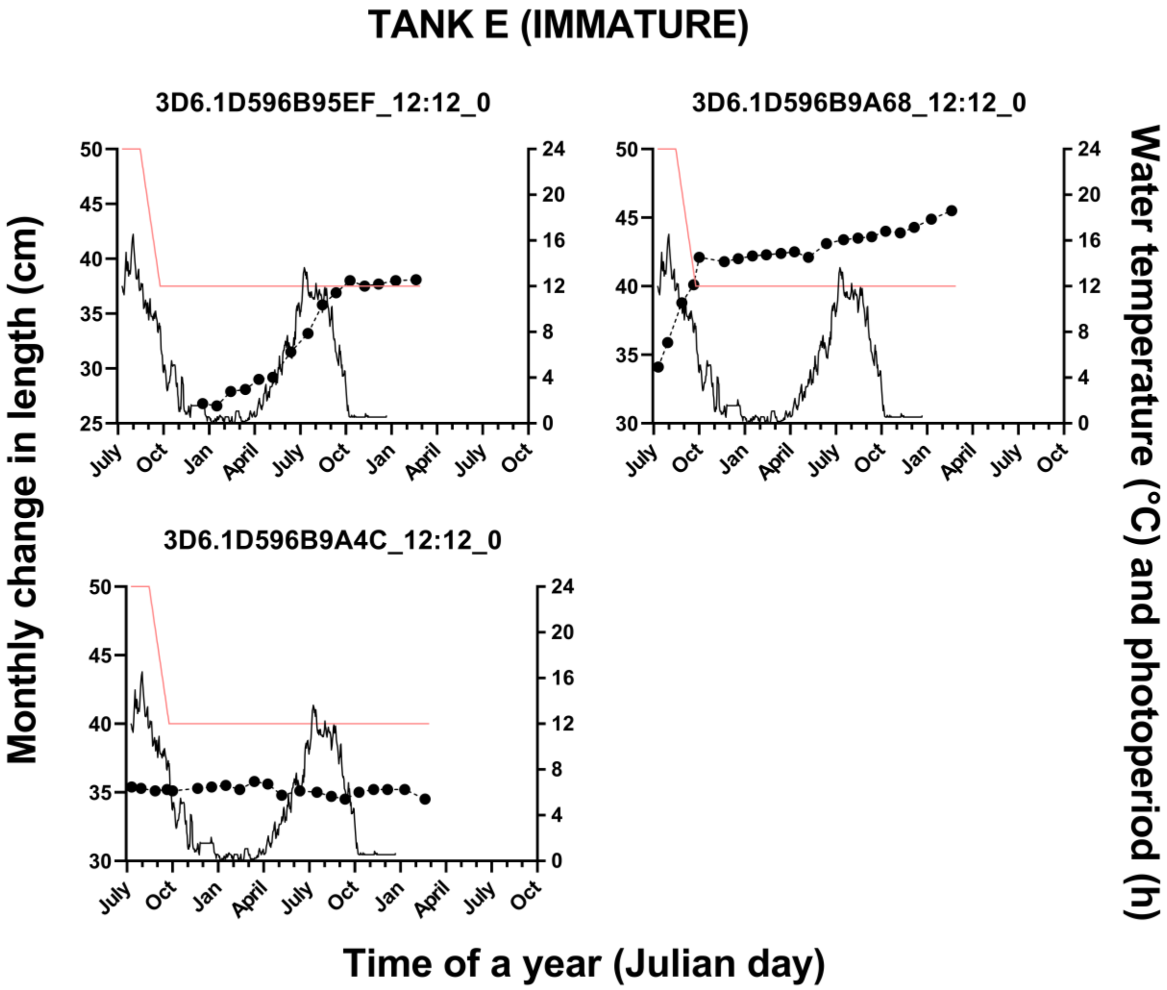

Figure 72: Monthly change in length in immature Arctic charr in Tank E

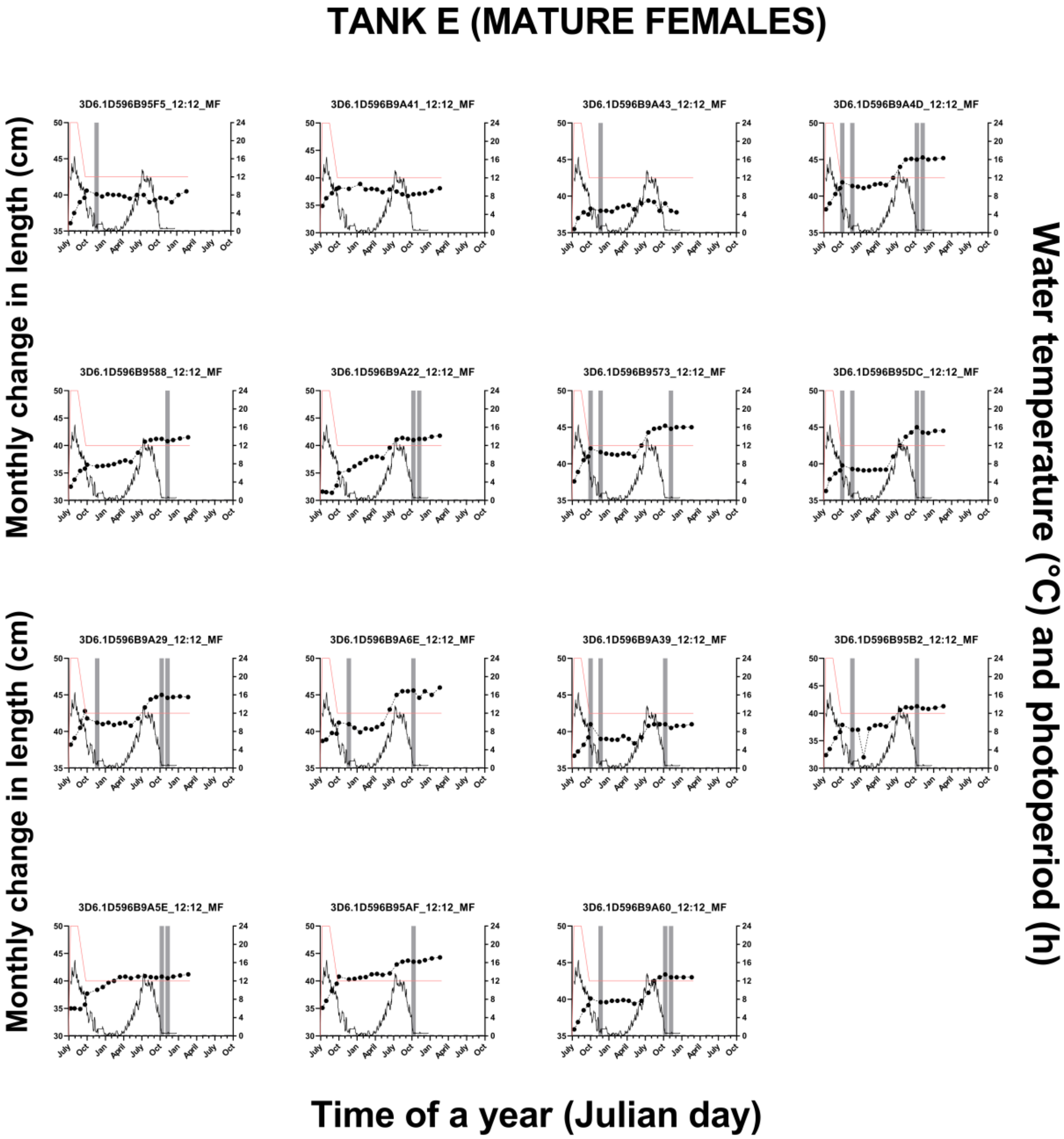

Figure 73: Monthly change in length in mature females of Arctic charr in Tank E

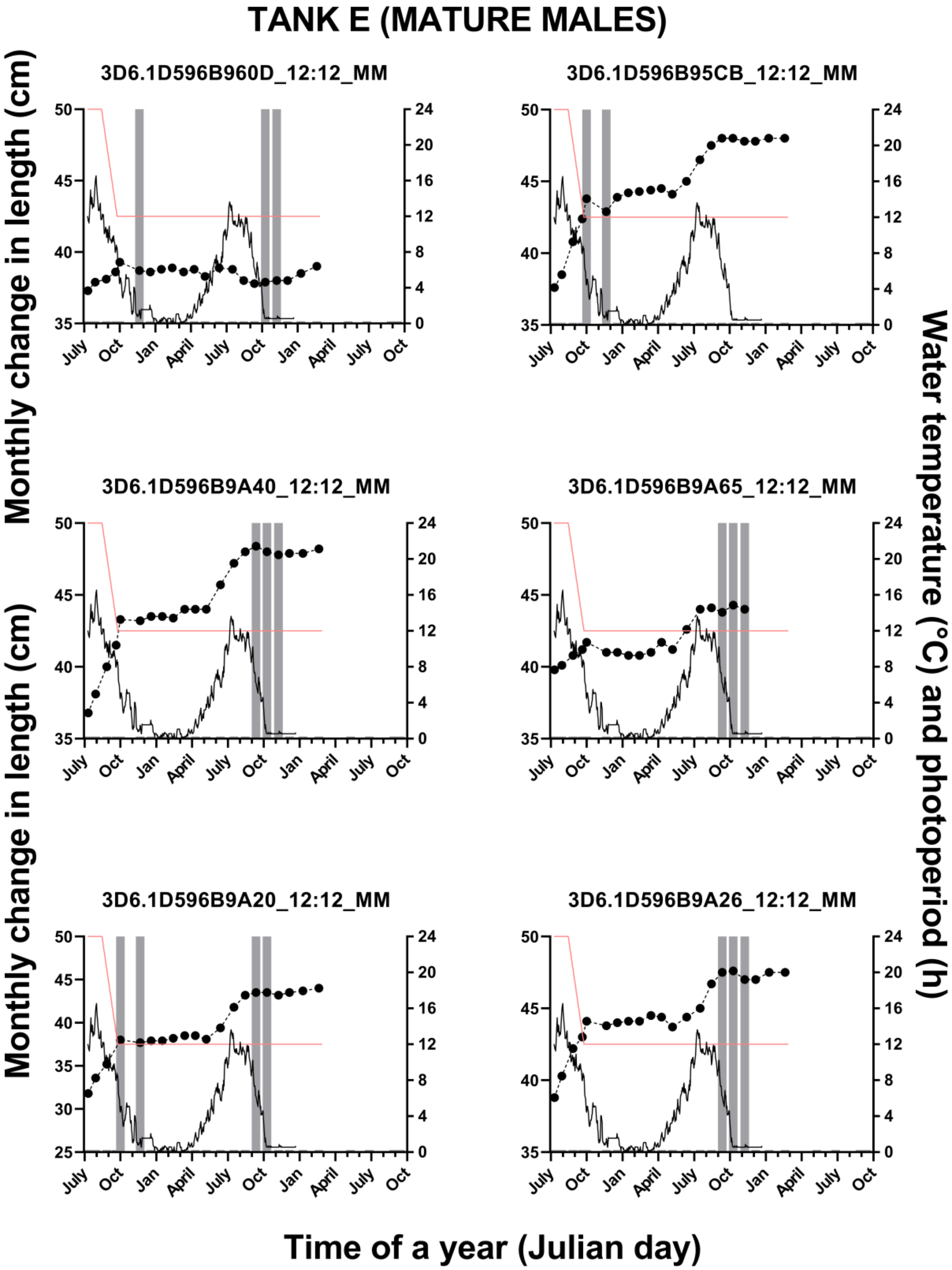

Figure 74: Monthly change in length in mature males of Arctic charr in Tank E

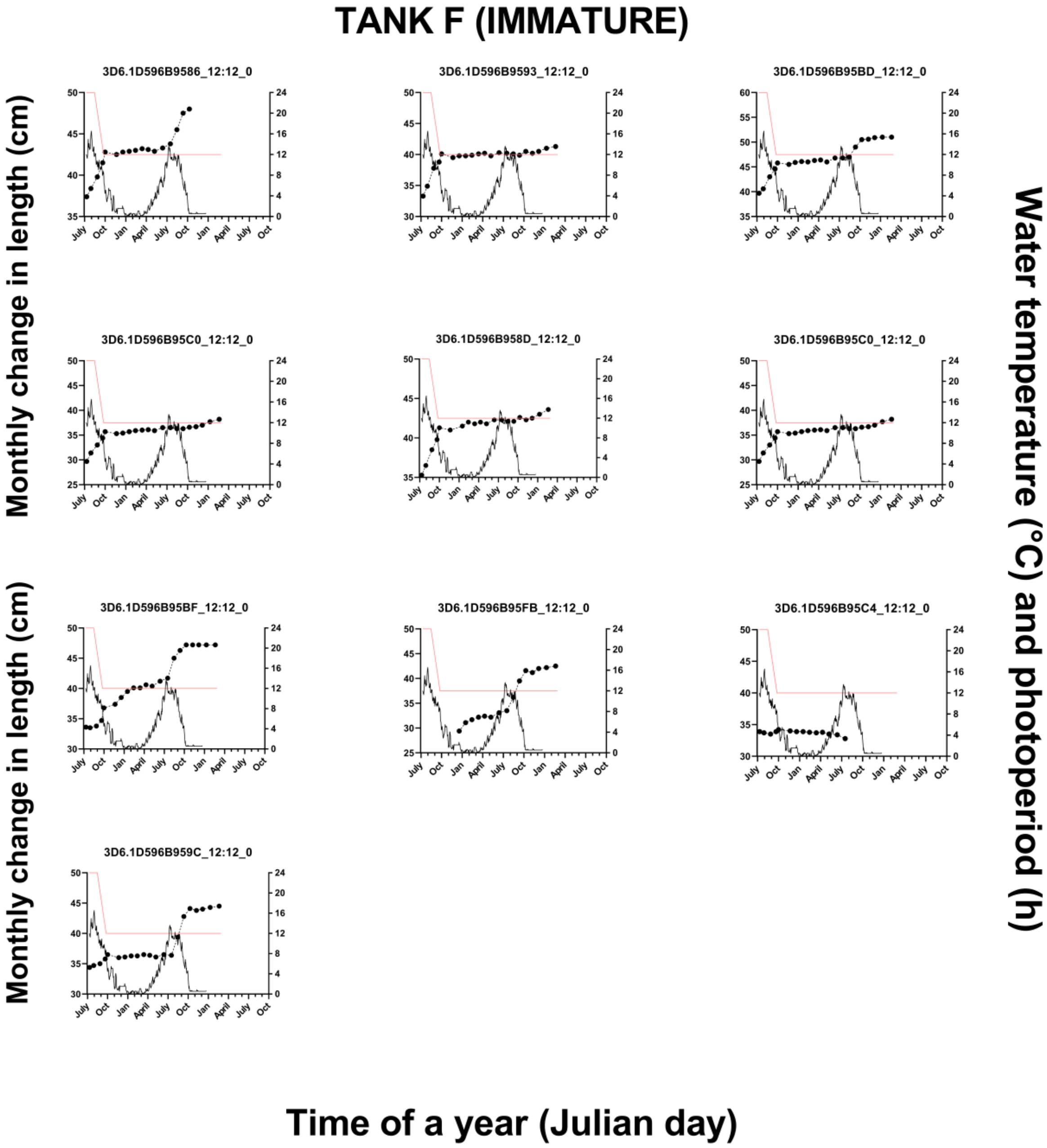

Figure 75: Monthly change in length in immature Arctic charr in Tank F

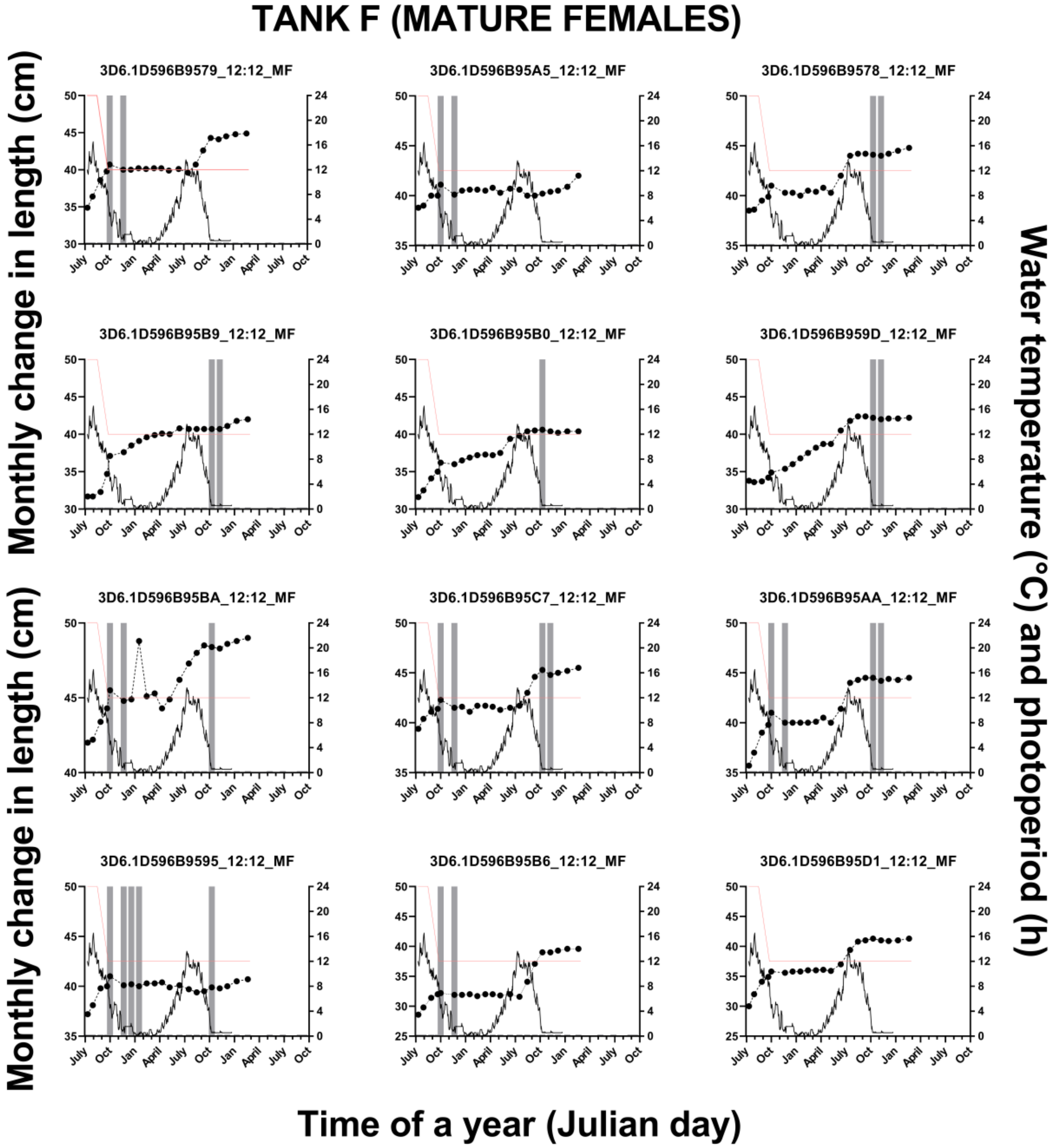

Figure 76: Monthly change in length in mature females Arctic charr in Tank F

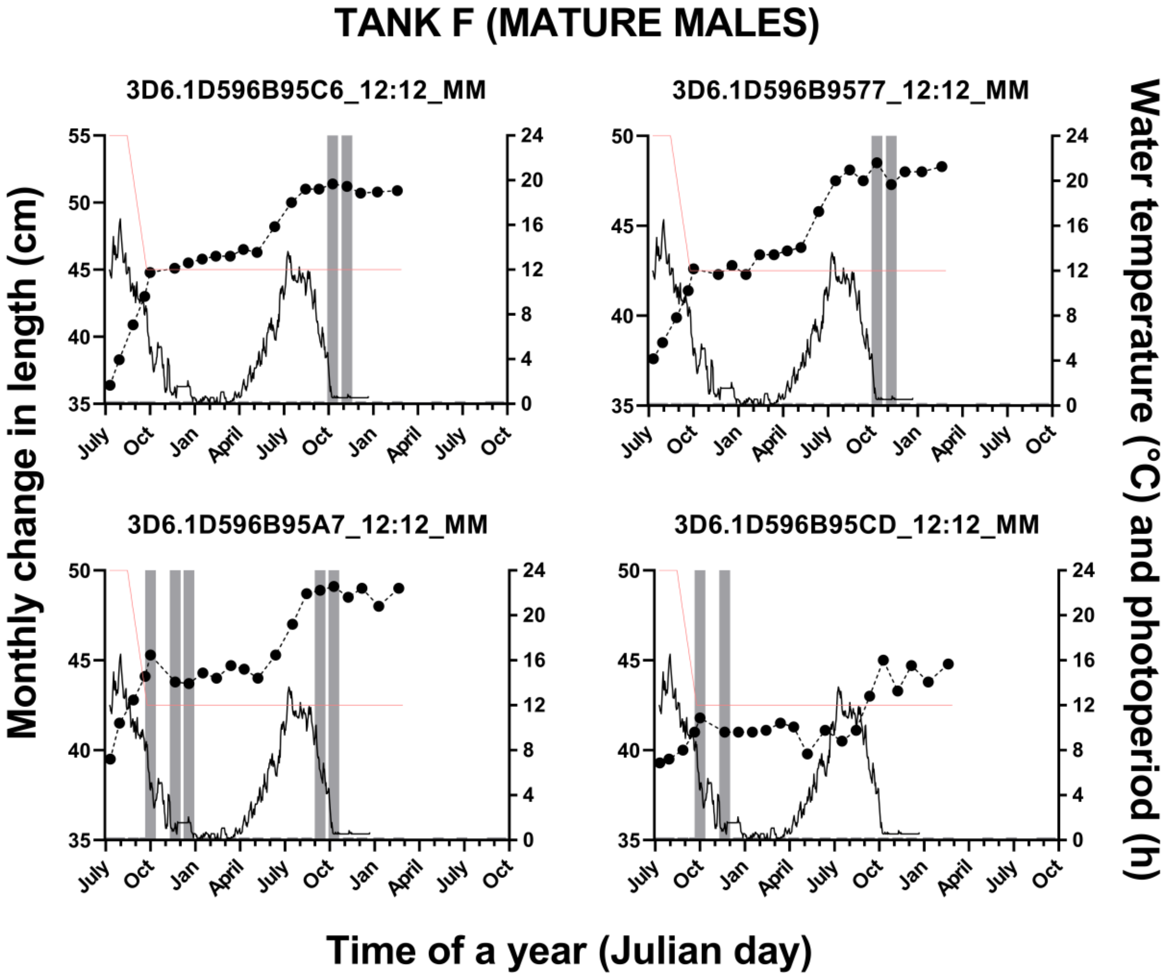

Figure 77: Monthly change in length in mature males of Arctic charr in Tank F

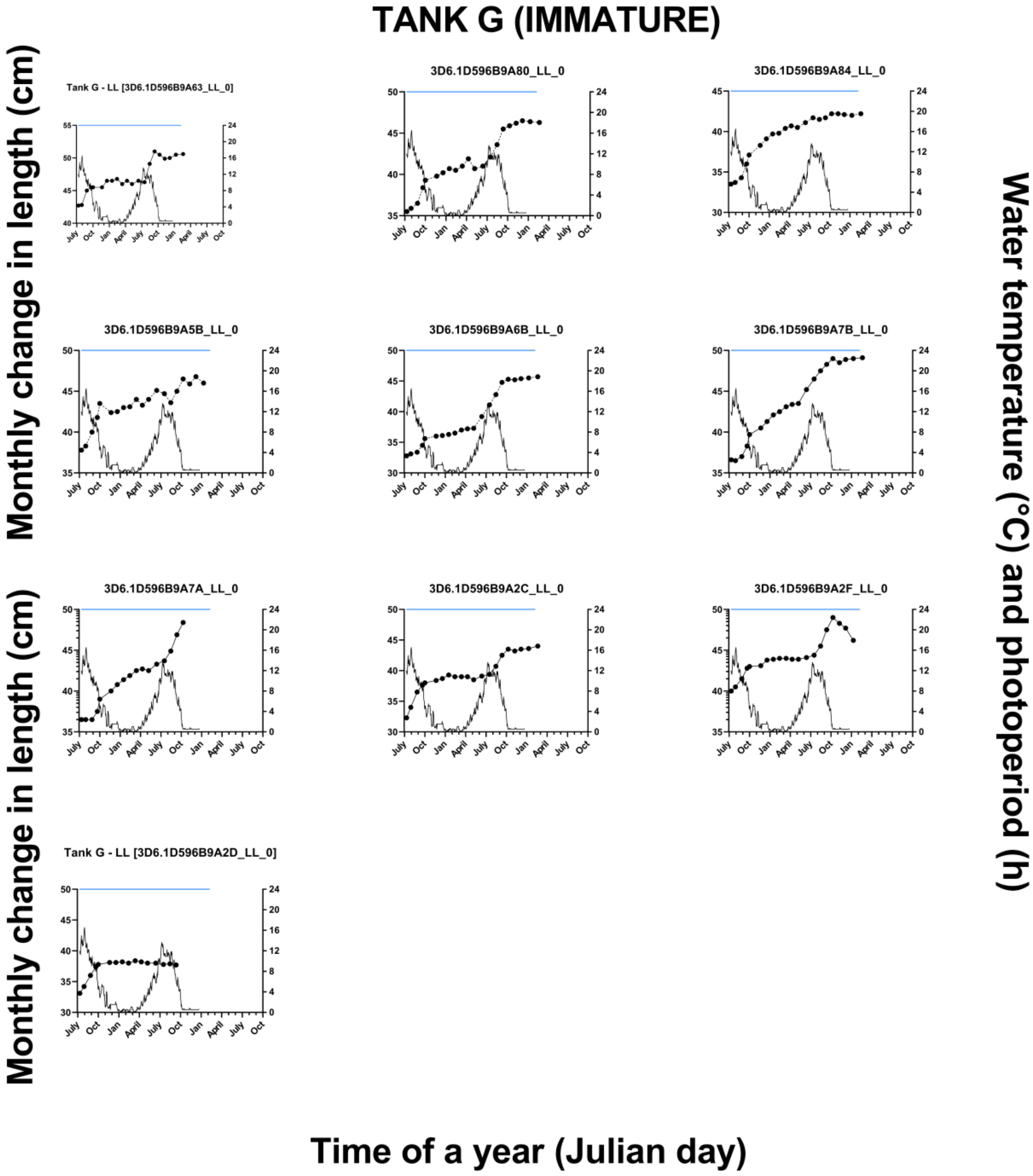

Figure 78: Monthly change in length in immature Arctic charr in Tank G

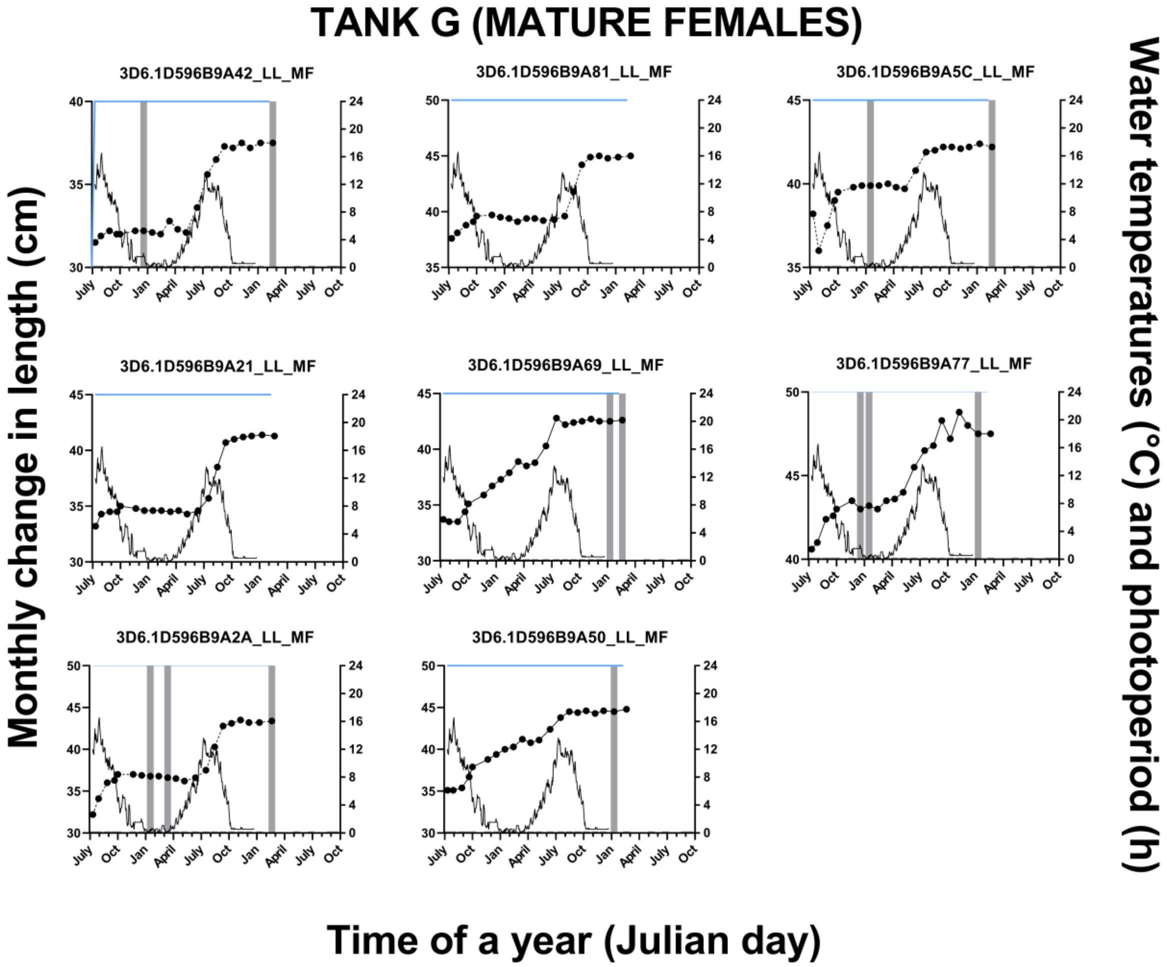

Figure 79: Monthly change in length in mature females of Arctic charr in Tank G

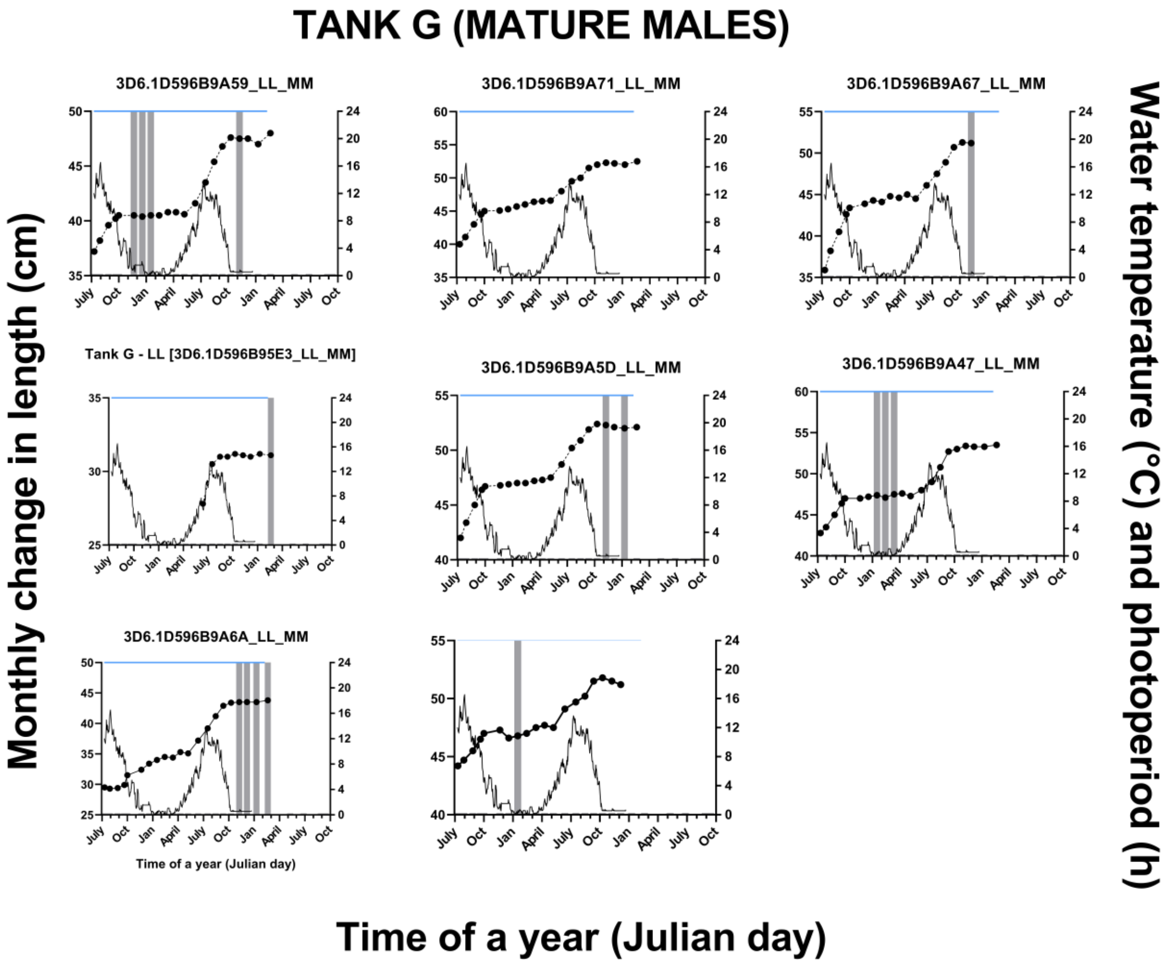

Figure 80: Monthly change in length in mature males Arctic charr in Tank G

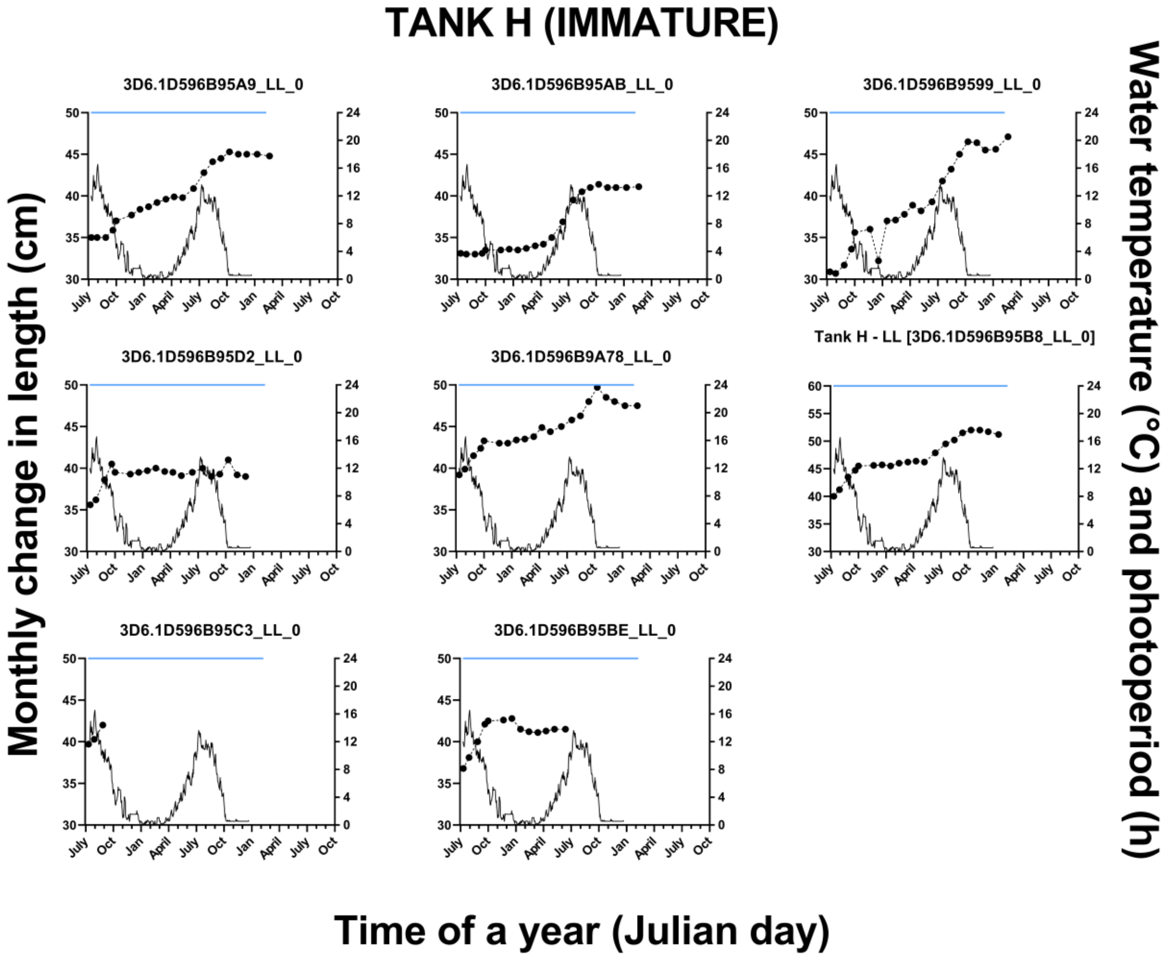

Figure 81: Monthly change in length in immature Arctic charr in Tank H

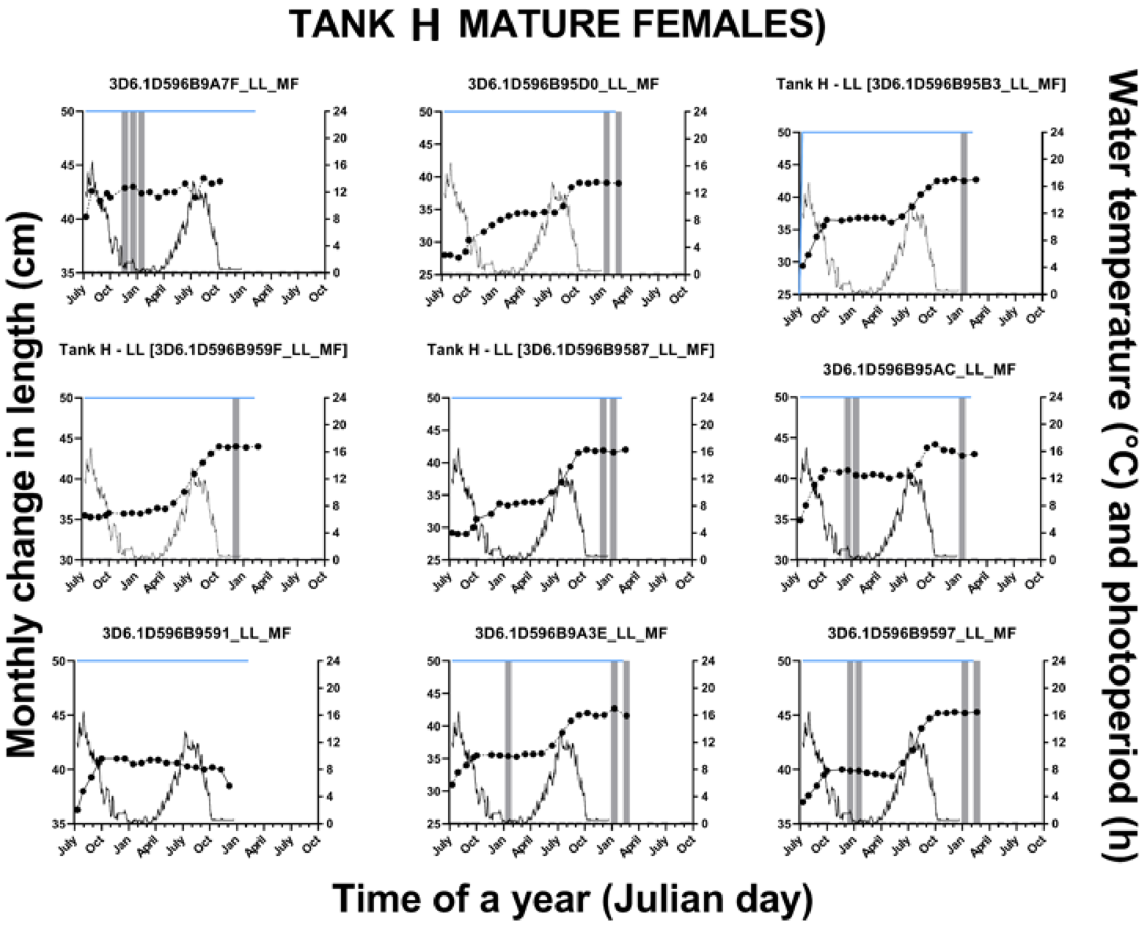

Figure 82: Monthly change in length in mature males of Arctic charr in Tank H

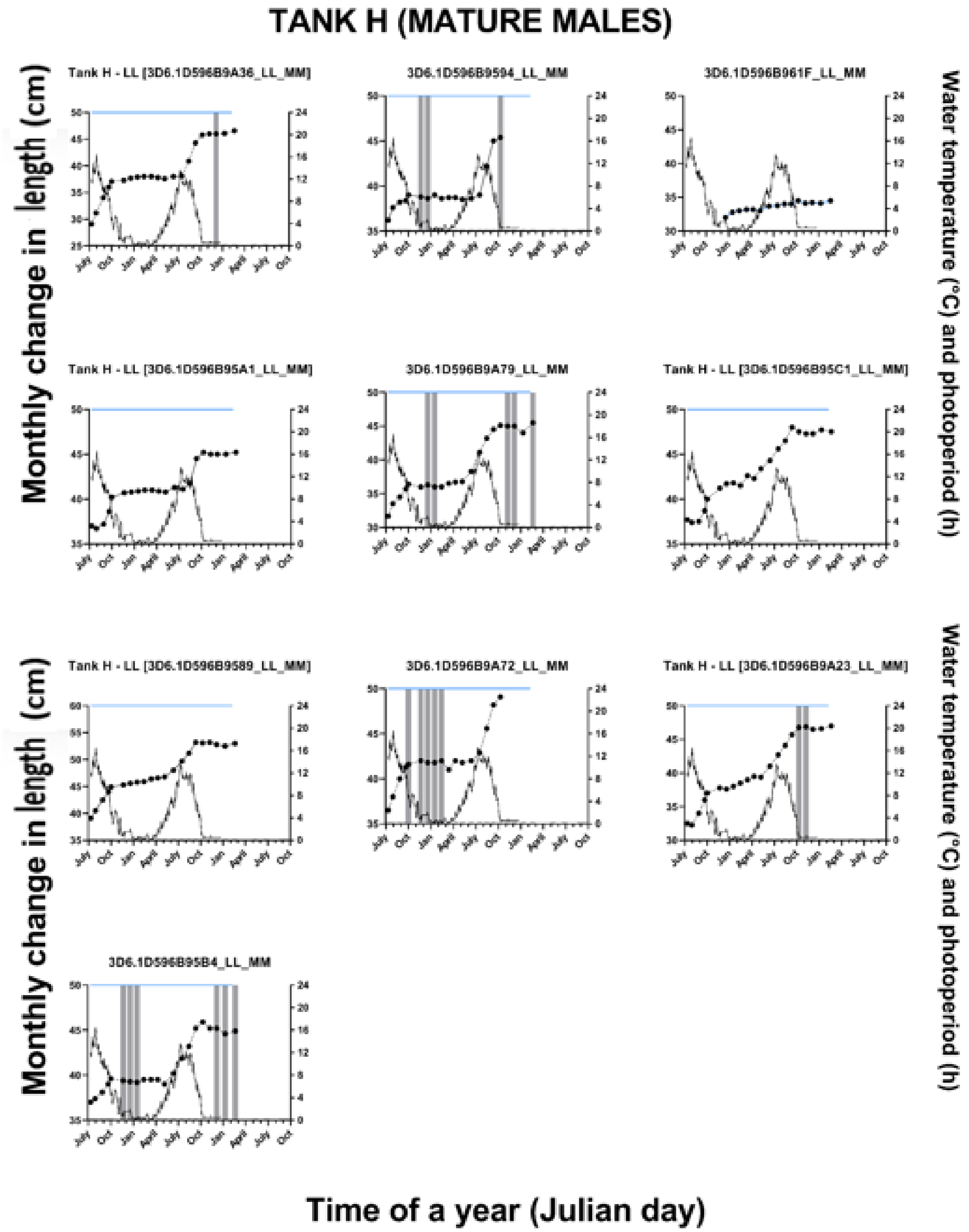

Figure 83: Monthly change in length in mature males of Arctic charr in Tank H