Comparative Biochemistry and Physiology, Part A 232 (2019) 60–66

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Termination of puberty in out-of-season male Atlantic salmon smolts☆

Thomas W.K. Fraser[a,*], Per Gunnar Fjelldal[a], Rüdiger W. Schulz[b,c], Birgitta Norberg[d], Tom J. Hansen[a]

[a] Reproduction and Developmental Biology Group, Institute of Marine research (IMR), Matre Aquaculture Research Station, 5984 Matredal, Norway
[b] Reproductive Biology Group, Division Developmental Biology, Institute of Biodynamics and Biocomplexity, Department of Biology, Faculty of Science, University of Utrecht, 3584, CH, Utrecht, the Netherlands
[c] Reproduction and Developmental Biology Group, Institute of Marine Research, P.O. Box 1870, Nordnes, 5817 Bergen, Norway
[d] Reproduction and Developmental Biology Group, Institute of Marine research (IMR), Austevoll Research Station, 5392 Storebø, Norway

ARTICLE INFO

Keywords:
Grilse
Jacks
Puberty
Testes

ABSTRACT

Environmental conditions are known to contribute to the phenotypic plasticity in the age of sexual maturation of Atlantic salmon (*Salmo salar*). Here, we report on an observation of out-of-season male Atlantic salmon initiating puberty as pre-smolts (jacks) but failing to complete maturation as post-smolts. Jacks were identified based on elevated plasma 11-ketotestosterone (range, 3–12 ng/ml) and the occurrence of type B spermatogonia in January 2017. However, these males failed to show running milt as post-smolts at the expected time in May 2017. Subsequently, 6 out of the 21 (32%) suspected "terminated jacks" went on to become grilse, whereas only 1 of the 22 (5%) males that showed no signs of initiating puberty in January became grilse in December 2017. Therefore, "terminated" jacks were more likely to mature as grilse than the males that remained immature. Why these pubertal pre-smolt males did not complete maturation is unclear but could be related to the transfer of fish from conditions of warm water and long days, risk factors for early maturation, to conditions of cold water and short days, which are expected to delay the age of maturation. We provide a description of the conditions under which male Atlantic salmon appear to have terminated the process of sexual maturation.

1. Introduction

There is a high degree of phenotypic plasticity in the age of sexual maturity in Atlantic salmon (*Salmo salar*). Wild males may enter puberty during the early freshwater life stage (parr) or as anadromous males following one (grilse) or more seawinters (Klemetsen et al., 2003). However, Atlantic salmon are also capable of undergoing multiple spawning cycles, including both consecutive and alternate year spawners (Erkinaro et al., 2019). Mature post-smolts, or "jacks", have also been observed in wild Atlantic salmon (Klemetsen et al., 2003), but is a rare phenotype. However, jacks are more frequently observed in domestic salmon populations (Imsland et al., 2014; Stefansson et al., 1993; Thrush et al., 1994). Here, males have been observed to enter puberty either directly before, or shortly after, seawater adaptation (Fjelldal et al., 2011; Melo et al., 2014). Jacks can occur during the natural spawning season (i.e. Stefansson et al., 1993) or out-of-season following photoperiod and/or temperature manipulation (i.e. Fjelldal et al., 2011).

Recent developments in salmonid husbandry, such as the manipulation of water temperature and photoperiod, are leading to an increased occurrence of jacking in domestic stocks (Fjelldal et al., 2011; Imsland et al., 2014). This is an economical and ethical issue, as mature individuals have reduced flesh quality and an increased risk of disease (Taranger et al., 2010). The high occurrence of jacking in modern day facilities is most likely related to improved growth during the juvenile phase and the use of large smolts. Here, the manipulation of the rearing environment through continuous light and elevated water temperature produces fast growing fish, but high-water temperature, photoperiod manipulation, and rapid growth are all risk factors for early maturation in salmon (Fjelldal et al., 2011; Imsland et al., 2014; McClure et al., 2007; Taranger et al., 2010). The exact mechanism that initiates puberty in salmon is still unknown, although it is theorised to be related to energy reserves at a given developmental threshold (Thorpe et al., 1998) and there is a genetic component (Ayllon et al., 2015; Barson et al., 2015; Lepais et al., 2017). To date, there are no reports on whether male Atlantic salmon can terminate puberty once it is initiated, as documented in females (Andersson et al., 2013). Furthermore, the long-term consequences of jacking on growth are unknown, as although

☆ This article is part of a special issue entitled: Aquaculture, edited by: Dr. Tillmann Benfey, Dr. Inna Sokolova and Dr. Mike Hedrick.

* Corresponding author.
E-mail address: thomas.fraser@hi.no (T.W.K. Fraser).

https://doi.org/10.1016/j.cbpa.2019.03.011
Received 28 September 2018; Received in revised form 2 February 2019; Accepted 12 March 2019
Available online 15 March 2019

jacking is known to lead to an initial growth spurt followed by a growth decline (Fjelldal et al., 2011; Imsland et al., 2014), no study has followed jacks up to market size.

Our initial objective was to determine the growth profiles of jacks up to market size. Therefore, we acquired fish from an experiment in which we expected a high prevalence of jacking based on external appearance. We confirmed the initiation of jacking prior to completion of sexual maturation using plasma 11-ketotestosterone (11-KT) levels, the main androgen in salmon (Idler et al., 1971), as using external appearance is not 100% reliable for determining jacking. However, although we observed a clear bimodal distribution in 11-KT, suggesting a number of males were indeed entering puberty, no males went on to produce running milt. Therefore, we suggest these males terminated puberty after undergoing the initial stages. We are unaware of any previous reports of male Atlantic salmon beginning, but not completing, sexual maturation. Therefore, we revised our initial objective, and instead describe the experimental setup and conditions in which we believe we observed terminated jacking. To aid our proposal, we also compare the growth profiles of the potential terminated jacks with mature jacks from the same genetic stock in a similar parallel study. Finally, we present possible hypothesises to explain the observation of male post-smolts terminating sexual maturation.

2. Materials and methods

The present experiment was approved by the Norwegian Animal Research Authority and performed according to prevailing animal welfare regulations (FOTS #10182).

2.1. Fish stock and experimental design

2.1.1. Experiment one

The fish originated from an Aquagen strain and were reared under the temperature and photoperiod conditions found in Fig. 1A at the Institute of Marine Research (IMR) facilities at Matre Research Station. On the 9th December 2016, parr were transferred to 8 tanks (475 L) with a density of approx. 5 kg/tank (fish weight 104-122 g) and for three weeks the fish were fed one daily meal (surplus, 2 h from 12:00). After this period, the fish were vaccinated (Aquavac PD7) on the 2nd of January. Food was withheld on the day of vaccination and until the first day after vaccination. The fish were weighed in bulk at the start of the experiment, at vaccination, and on the 22nd January 2017. On the 22nd January, all the fish in 3 tanks were killed by an overdose of anaesthetic (Finquel, 0.5 g/L), sexed, and measured for fork length, body mass, and gonad mass (males only). Of these, three males we suspected of being immature, and four suspected jacks based on external appearance, had their testis dissected and fixed in glutaraldehyde for histology (see below). All fish in the remaining 5 tanks were sedated (Finquel, 100 mg/L) and manually graded as immature or maturing based on their external phenotype. The maturing male phenotype was characterized based upon skin colouring and body condition. Immature fish are silvery with dark fins and a low body condition (i.e. skinny), while maturing males have a more yellowish colour with paler fins and a higher body condition (Fjelldal et al., 2011). In total, 25 fish with a phenotype typical for maturing males were identified. Based on this, the 25 fish with a maturing male smolt phenotype, along with 74 random fish, were individually tagged with a passive integrated transponder (PIT) tag, which allows for individual recognition, measured for fork length and body weight, and sampled for blood. The blood samples were centrifuged at 11228*g* for 2 min and plasma stored at −80 °C before measurement of 11-KT. Following this, the PIT-tagged fish were transferred to a 5 m tank and followed for one year up until the 4th December 2017, when all fish were killed, sexed, and measured for fork length, and body and gonad mass. During this time, fish were also examined in May and August 2017 for fork length, body mass, and signs of maturity (i.e. running milt). In total, 14 fish, all suspected of being immature, were found to have no PIT tag and were ejected from the study. A total of 7 fish were found to have vertebral deformities and were also ejected from the study.

2.1.2. Experiment two

These fish came from an experiment examining the effects of water temperature (12 vs 17 °C), oxygen saturation (60, 70, 80, and 100%), and vaccination (vaccinated vs unvaccinated), during smoltification on long-term growth. We present only the data from the control fish in this study, those that experienced 17 °C for a period of one-month post vaccination and 100% O_2 saturation throughout. Those males exposed to 17 °C were found to have 15% and 14% maturation (n = 165 and 174) in the unvaccinated and vaccinated fish, respectively, whereas no fish from the 12 °C treatment matured. The vaccinated fish were not available for the current dataset so only data from the saline injected control fish are presented.

Atlantic salmon from the same Aquagen strain as used in experiment one were reared under the temperature and photoperiod found in Fig. 1B at the IMR facilities at Matre Research Station. On the 28th August 2016 the fish (n = 2096, mean weight 50 g) were moved to ten 1 × 1 m tanks. Between the 9th and 12th September 2016 the fish were implanted with PIT tags and distributed among 16 tanks (1 × 1 × 0.43 m tanks, n = 131 fish/tank). Between the 3rd and 7th October 2016 the fish were acclimated to either 12 or 17 °C with one of four oxygen saturations, 60, 70, 80, and 100% (supplementary table 1). On the 10th October 2016, the fish were vaccinated with either Aquavac PD7 or a saline injection (i.e. unvaccinated). The water flow into the tanks was switched to seawater on the 2nd November 2016 for those fish reared at 17 °C, and on the 10th November 2016 for those fish reared at 12 °C. The difference in seawater timing is related to the degree days (temperature over time) required in order for fish to complete the parr-smolt transformation that physiologically adapts salmon for the switch from freshwater to seawater. On the 30th November 2016, all fish were transferred to a sea-cage (including the vaccinated fish and those that experienced reduced O_2 saturations) for common-garden rearing.

Body mass and fork length were collected at four-time points, the 10th October 2016 (vaccination), 30th November 2016 (transfer to seacage), 26th April 2017 (approx. End of seawinter), and the 19th October 2017 (market size). At each sampling time, fish were anaesthetized in 100 mg/L Finquel® (MS 222). On 19th October 2017, all fish were killed by an overdose of anaesthetic and the body length, body mass, sex and gonad weight were collected.

Body size data were used to calculate body condition (i.e. *K* factor) using the following equation; K = weight [g] / length^3 [cm] × 100. Growth was calculated as the % mass gain/day (i.e. specific growth rate [SGR]) as follows; $(e^q - 1)100$ (Houde and Scheckter, 1981), where $q = [\ln(W_2) - \ln(W_1)](t_2\text{-}t_1)^{-1}$ (Bagenal and Tesch, 1978), and W_2 and W_1 are average body mass at times t_2 and t_1, respectively.

2.2. Histology

After dissection and weighing, a testis tissue fragment was fixed in 4% buffered (PBS) glutaraldehyde at 4 °C overnight. The tissue was dehydrated in graded alcohol and embedded in 2-hydroxyethyl methacrylate according to conventional techniques. Testes sections of 3 μm thickness were stained with 1% toluidine blue and analysed qualitatively by light microscopy. The sections were scored for the presence of the most advanced stage of spermatogenesis, and the following stages were found: type A spermatogonia, type B spermatogonia, spermatocytes. The identification of the germ cell types was mainly based on the size, shape and staining of the nuclei of these germ cell types and followed the description given earlier (Melo et al., 2014).

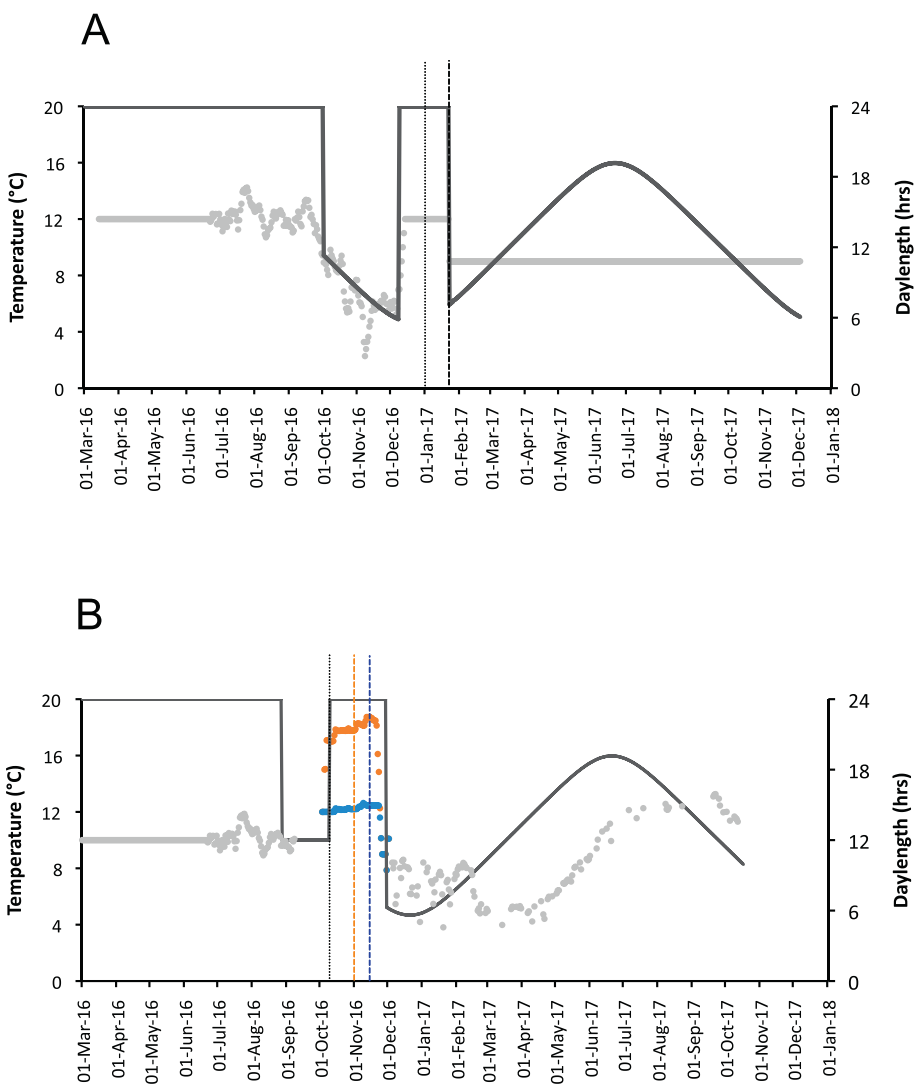

Fig. 1. Temperature and photoperiod from experiments one and two. (A) Experiment one. The photoperiod was simulated natural (60° N) between October and December 2016 and natural from mid-January 2017 onwards. The grey dots indicate the water temperature from the tank inflow. The timing of vaccination is indicated by a dotted line, whereas the dashed line indicates the timing of seawater transfer. (B) Experiment two. Two water temperatures were used between October and December 2016, the 17 °C treatment in orange, and the 12 °C treatment in blue. The photoperiod was natural from December 2016 onwards. The grey dots indicate the water temperature from the inflow when in tanks, and the average seawater temperature from 3 to 5 m depth when in sea-cages. The grey dotted line indicates the timing of vaccination, whereas the blue and orange lines indicate the date of seawater transfer, for the 12 and 17 °C reared fish, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. 11-ketotestosterone

Steroids were extracted from blood plasma by a method modified from Pankhurst and Carragher (1992). Briefly, plasma samples (100 µL) were mixed with 1 mL ethyl acetate, vortexed for 20 s and centrifuged for 3 min at 1800 rpm (700 rcf) and 4 °C. The organic phase was collected by a Pasteur pipette and the hydrophilic phase was extracted once more with 1 mL of ethyl acetate. The extracts were evaporated in a Speed Vac centrifuge (Savant 1000, USA), and dissolved in 1 mL buffer (phosphate 0.1 M pH 7.4, 0.4 M NaCl, 1 mM EDTA) by heating (60 °C for 10 min). The extracted and dissolved steroids were stored at −20 °C until analysis by enzyme-linked immunosorbent assay (ELISA, Cuisset et al., 1994). The ED80 and ED20 were 0.04 ng/ml and 1.00 ng/ml, and the detection limit of the assay was 0.005 ng/ml. Internal standards were prepared from mature male (11-KT) Atlantic salmon plasma extracted as described above. The accepted inter-assay coefficient of variation was 10%; assays with higher deviation of the internal standard were re-run. The intra-assay coefficient of variation was 6.2% (n = 10). Acetylcholine esterase-labelled tracers and microplates precoated with monoclonal mouse anti-rabbit IgG were supplied by Cayman Chemicals (USA). Anti-11-KT was a kind gift from Dr. David E. Kime, Sheffield University, UK, with details on cross-reactivity given by Cuisset et al. (1994). Standard steroids were purchased from Sigma Aldrich (Sigma reference standards).

2.4. Jacking classification

In experiment one, no fish produced running milt. Therefore, plasma 11-KT values were used to identify fish that began puberty as post-smolts (jacks), as previous research has identified 11-KT as the main sex steroid in salmon (Idler et al., 1971) with values > 1 ng/ml being reported in mature/maturing fish (Kjærner-Semb et al., 2018). A frequency distribution demonstrated a bimodal distribution, with fish either having a value < 1 ng/ml or > 3 ng/ml (Fig. 2A). No female had a value above the detection limit for 11-KT. All those males with an 11-KT value > 3 ng/ml were classified as jacks, all those below were considered immature. Grilsing was determined based on external appearance and confirmed by gonad weights at the final sampling in December 2017. Here, grilse had a GSI ≥ 3.8 whereas immature males had a GSI of ≤ 0.2 (Fig. 2B). Based on visual examination of the ovaries, no females were found to have matured. In experiment two, we classified fish as mature based on the occurrence of running milt in April 2017.

2.5. Statistical analysis

The data were transferred to R version 3.3.3 (R Development Core Team, http://www.r-project.org). Significance was assigned at $p < .05$. Model residuals were checked for linearity and normality following visual examination of plots (i.e. histograms/q-q plots/

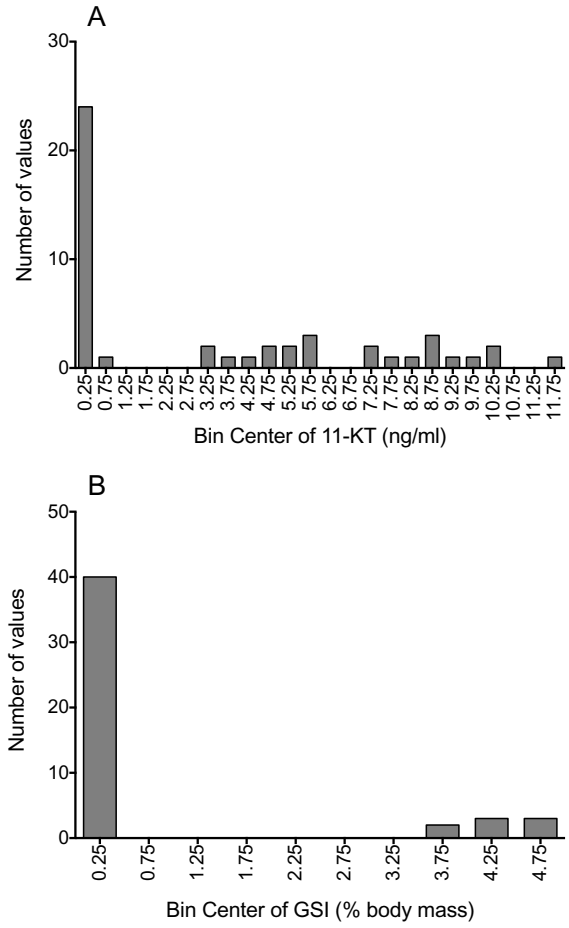

Fig. 2. Frequency distributions from experiment one. (A) Plasma 11-ketotestosterone (11-KT) in male Atlantic salmon from January 2017. (B) Gonadosomatic index (GSI, testes size as a % of body mass) in male Atlantic salmon from December 2017. For each histogram, the bin size is 0.5.

standardised residuals vs fitted values). We used the "nlme" library to perform linear mixed effect (lme) models to assess for the effect of explanatory variables on body size metrics.

We originally hypothesised jacks and immature males would have different growth profiles over time based on observations in post-smolts (Fjelldal et al., 2011; Imsland et al., 2014) and parr (Skilbrei, 1990), with an initial growth spurt during testis development followed by a growth decline. Therefore, we generated two global models, one 2-way interaction that includes an interaction between group (immature male, terminated jack, terminated jack to grilse) and time, and one without the interaction with time. The 2-way interaction demonstrates different growth trajectories in fish with high vs low 11-KT values, and evidence of initiated jacking, whereas the second provides no evidence for growth effects due to differing 11-KT values and thereby no evidence for the initiation of jacking. We used the Bayesian information criterion (BIC) to identify the model with the lowest BIC score and considered this the "true" model (Aho et al., 2014). We included fish as a random effect, to account for repeated sampling of the same individuals. For SGR, we included the body weight at the start of the investigated period as a main effect, as smaller fish are expected to have a higher SGR and we observed transient group differences in body mass (see results). Here, only one fish went from being immature to grilse, so this individual is not included.

For experiment two, we used the same models selected in experiment one for a direct comparison of results between experiments. We removed all fish that had externally visible vertebral deformities (i.e. short trunk or short tail, n = 1), as they are known to impact on growth (Hansen et al., 2010), and we removed those individuals for which data were not available from all time points (n = 6). Here, the one male that became a grilse was excluded from the analysis as it had an externally visible spinal deformity. Following this, group sizes were 30 and 5 individuals/timepoint for immature males and jacks, respectively.

The "Anova" command within the "car" library was used to extract the results for the main effects and type III sum of squares were used due to the presence of interactions. All body mass data were natural logged transformed prior to analysis to improve the distribution of the model residuals, and the SGR data from both experiments was transformed using the "weights = varPower" function due to heteroskedasticity. Post-hoc tests were done using least square means with a tukey adjustment from the "lsmeans" library, whereby means for groups are adjusted for means of other factors within the model (Lenth, 2016). To assess for differences in the prevalence of grilsing among males that had either high or low 11-KT in January, we used the exact binomial test using the "binom.test" command. All the raw data ("JacksSW.csv") and the R script (JacksSW.R) used to analyse the data can be found in the supplementary material.

3. Results

For all the main models in experiment one, those containing the interaction between group and time had a lower BIC score than the models without the interaction (Table 1) and these interactions were significant in both experiments (Table 2).

3.1. Effects of grouping fish on 11-KT values on body size and growth in experiment one

Those males with elevated 11-KT were initially heavier with a higher body condition than immature males with low 11-KT, but there was no group effect on body mass in May or August due to lower growth of those fish that had high 11-KT (Fig. 3A–C). In December, there was no effect of high or low 11-KT on growth or body size in males that did not become grilse, although those males that went on to become grilse showed decreased growth, lower body mass, and lower body condition, compared to all other groups.

3.2. Effects of jacking on body size and growth in experiment two

We found mixed support for our hypothesis that jacks would show an initial growth spurt, a growth disadvantage as post-smolts, and then catch-up growth (Fig. 4A–B). Although jacks did show a growth spurt and then a growth decline, jacks remained significantly smaller than immature males at the end of the experiment. This contrasted with experiment one, whereby males with high (suspected terminated jacking) and low (immature males) 11-KT only differed in body mass in January, but not at the expected time for completed sexual maturation in April/May. Jacks also showed an increase in body condition in Nov 2016 (Fig. 4C), which was similar to the increase in body condition seen in those males with elevated 11-KT in experiment one.

Table 1
Bayesian information criterion (BIC) scores for models with or without an interaction between group and time for body mass, specific growth rate (SGR), and body condition in each experiment.

Experiment	Model	BIC score		
		Body mass	SGR	Body condition
#1	Group × Time	−179	−216	−396
	Group + Time	−75	−169	−337

Table 2
Results from linear mixed effect (LME) models comparing the effects of group, time, and the interaction, for body mass, specific growth rate (SGR), and body condition in each experiment.

Experiment	Model variables	Body mass			SGR			Body condition		
		χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
#1	Intercept	15,644	1	< 0.001	3033	1	< 0.001	4067	1	< 0.001
	Group	36	2	< 0.001	54	2	< 0.001	40	2	< 0.001
	Time	20,802	3	< 0.001	168	2	< 0.001	1192	3	< 0.001
	Start mass	*na*	*na*	*na*	3	1	0.063	*na*	*na*	*na*
	Group × Time	240	6	< 0.001	95	4	< 0.001	132	6	< 0.001
#2	Intercept	15,301	1	< 0.001	790	1	< 0.001	3645	1	< 0.001
	Group	1	1	0.466	5	1	0.033	0.02	1	0.875
	Time	8426	3	< 0.001	101	2	< 0.001	34	3	< 0.001
	Start mass	*na*	*na*	*na*	14	1	< 0.001	*na*	*na*	*na*
	Group × Time	42	3	< 0.001	28	2	< 0.001	9	3	0.032

3.3. Histology

From experiment one, the three suspected immature males in December 2016 had a GSI < 0.07% and only type A spermatogonia, demonstrating puberty had yet to be initiated. In the four suspected jacks, showing a GSI range between 0.22 and 0.56%, type B spermatogonia were present in addition to type A spermatogonia, and in one case also the first spermatocytes were observed, demonstrating the initiation of puberty had taken place in these males.

4. Discussion

Our initial objective was to investigate the long-term performance of male salmon that mature as jacks. However, in experiment one none of the males fully matured even though we found strong evidence puberty had been initiated based on plasma hormones, testis histology, and growth profiles. Therefore, we revised our initial objective to instead describe the conditions under which we believe we observed terminated jacking, a phenomenon that has not been reported before in male Atlantic salmon. Its occurrence may be linked to alterations in environmental conditions after the initiation of puberty that were below optimal with regards to reproductive cues.

We found evidence of terminated male puberty in post-smolt Atlantic salmon. Initially, a fraction of males were found to have 11-KT within the range of pubertal male salmon (Kjærner-Semb et al., 2018). Unfortunately, we do not have testes data from the same fish, however, elevated 11-KT is known to coincide with testes development (i.e. Idler et al., 1971; Kjærner-Semb et al., 2018; Melo et al., 2014). The growth profiles of these males with elevated 11-KT also suggested they had initiated the early stages of puberty, as they were heavier with a higher body condition than those males with low 11-KT, the same pattern observed in the mature jacks from experiment two. In addition, an initial subsample in December 2016 also demonstrated those fish we expected to be pubertal jacks based on external appearance did indeed show elevated GSI and type B spermatogonia, clear signs of puberty (Schulz et al., 2010). However, no males produced running milt at the expected time, in this case May due to the use of out-of-season fish, suggesting they had all terminated maturation. Further evidence for this was that the growth reduction between November 2016 and April 2017 in these males with elevated 11-KT was not as severe as the mature jacks from experiment two, suggesting less energy had been deviated from growth. Here, we ruled out the possibility these males just stalled testes development at the initial stages in wait for the next spawning opportunity, as only 32% of those males identified as terminated jacks showed advanced testes development in December 2017, the next spawning opportunity. In addition, we feel it is unlikely these males were experiencing a “dummy run”, whereby activation of the brain-pituitary-gonad axis is observed one season before the expected time of sexual maturation (Amano et al., 1992; Prat et al., 1996) as dummy runs do not lead to the development of the gonads (Okuzawa, 2002), whereas we observed males with type B spermatogonia as well as one male that had the first spermatocytes.

The mechanism by which the high 11-KT males terminated puberty is unclear, but a change in environmental conditions may have halted maturation. Here, it is likely that high water temperature, or the water temperature increase following the winter photoperiod signal, 12 to 17 °C in experiment two and 5 to 12 °C in experiment one, triggered maturation (Fjelldal et al., 2011; Imsland et al., 2014; McClure et al., 2007). Following this, in both experiments, fish were then transferred to cold water, 17 to 9 °C in experiment two and 12 to 9 °C in experiment one, and short days (approx. 6:18 in both experiments). This drop in temperature at the same time as a winter photoperiod cue may have led to the decision to terminate maturation in those males from experiment one. Here, one may ask why males in experiment two did not terminate, given they received a larger drop in temperature? We suggest that as the fish from experiment two had experienced a greater number of degree days following the winter signal, but prior to transfer to cold water, 879 compared to 525 in experiment one, they may have already surpassed a threshold for terminating maturation. However, this hypothesis requires testing.

Due to the high energetic cost of sexual maturation (Jonsson et al., 1997), it would be an evolutionary advantage if one could stop the process due to unfavourable conditions post initiation of puberty. Indeed, in female salmon, Andersson et al. (2013) provided evidence of arrested gonadal development following photoperiod manipulation. Here, females were either kept under a natural photoperiod, or transferred to continuous light in winter, a technique known to suppress early maturation (Taranger et al., 1999). Those females given natural light all progressed to develop large oocytes in the tertiary stage of development. In contrast, of those females switched to continuous light, only half advanced to the same stage, whereas the others were found to have either oil droplets, a pre-vitellogeneic stage, or primary yolk vesicles and low plasma estradiol. The latter is considered a mismatch between oocyte maturation and plasma hormones (Andersson et al., 2013). Although photoperiod manipulation is known to suppress early male maturation (Hansen et al., 1992; Schulz et al., 2006; Taranger et al., 1999), we can find no records of male salmon terminating sexual maturation after initiating puberty.

In experiment two, we only found maturing males following the 17 °C treatment during smoltification, but not after the 12 °C treatment. However, in experiment one we found males with elevated 11-KT following a 12 °C treatment. This discrepancy could be explained by the initial starting size of the fish, as the larger the fish are following the winter signal the more likely they are to enter puberty during the smoltification period (T. Hansen, unpublished data). In experiment two, smoltification was induced in October when the fish were around 75 g, two months prior to experiment one where fish were around 90–95 g. Size dependent thresholds for the initiation of puberty also occur in

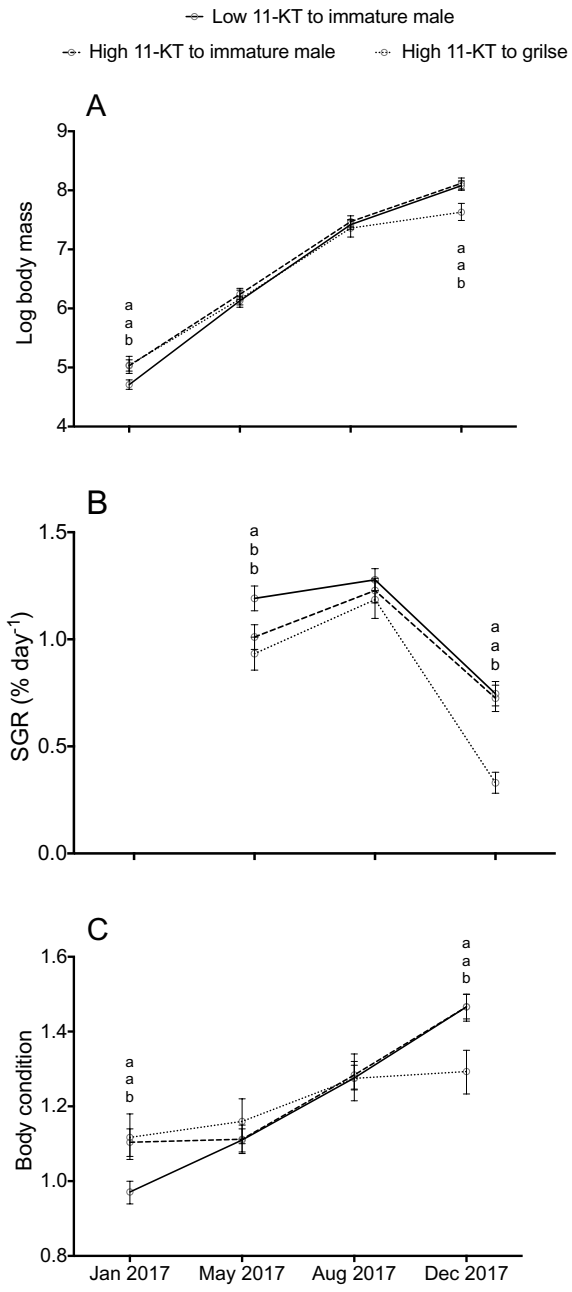

Fig. 3. Body mass, specific growth rates (SGR), and body condition, over time of Atlantic salmon from experiment one. Data are means, corrected for other significant model effects, ± 95% CI. Different letters indicate significant post-hoc effects within time point (lsmeans, $p < .05$).

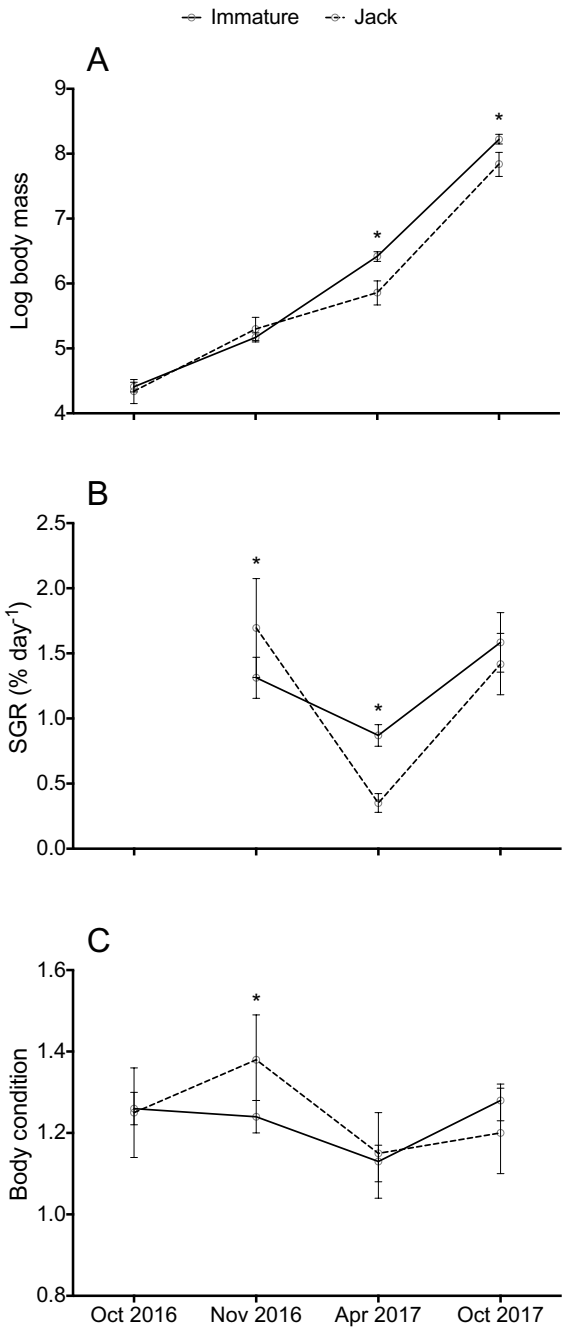

Fig. 4. Body mass, specific growth rates (SGR), and body condition over time of Atlantic salmon from experiment two and exposed to 17 °C prior to seawater transfer. Data are means, corrected for other significant model effects, ± 95% CI. An asterisk indicates significant post-hoc effects within time point (lsmeans, $p < .05$).

other teleost species such as European sea bass (*Dicentrarchus labrax*) (Carrillo et al., 2015). Alternatively, terminated jacking may have occurred in experiment two also, however we do not have the 11-KT data to explore this possibility.

In experiment one, there appeared to be an increased risk of grilsing in those males that had elevated 11-KT compared to those that had low 11-KT. Grilsing is particularly costly for the industry (McClure et al., 2007) due to the drop in flesh quality and increased risk of disease (Taranger et al., 2010). Therefore, even if one is able to induce the termination of puberty in males as jacks, these same males may be primed to initiate puberty at the next given opportunity. This point is further emphasised by the low level of grilsing in experiment two, whereby none of the fish that had previously fully matured as jacks became grilse.

We present data from experiment two to gain an understanding of the long-term effects of jacking on growth. This is primarily to provide evidence that the growth profile of those males that complete jacking, when compared to immature males, differs to those in experiment one in which the males are suspected of terminating jacking. Experiment two was not designed to look at the long-term effects of jacking, and the low numbers of jacks does not make for an ideal assessment. Nevertheless, the jacks in experiment two showed decreased growth as post-smolts and did not fully recover to the sizes of immature males. It is unclear whether this was related to the length of the experiment, or whether jacking results in lifelong reduced growth. Therefore, from an

industrial perspective, jacking may come at a cost of reduced harvest mass and requires more thorough investigation.

In conclusion, we find evidence that male salmon can terminate puberty as out-of-season smolts. However, those that terminate puberty appeared more likely to mature as grilse at the next opportunity.

Funding

This study is part of the project "Understanding postsmolt maturation in Atlantic salmon in the context of new, closed production systems" funded by the Research Council of Norway (project number 254870).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank the technical staff at Matre for helping with fish husbandry and sampling. Jon Egil Skjæraasen is thanked for help with statistics.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.03.011.

References

Aho, K., Derryberry, D., Peterson, T., 2014. Model selection for ecologists: the worldviews of AIC and BIC. Ecology 95, 631–636.

Amano, A., Aida, K., Okumoto, N., Hasegawa, Y., 1992. Changes in salmon GnRH and chicken GnRH-II contents in the brain and pituitary, and GTH contents in the pituitary in female masu salmon, *Oncorhynchuss masou*, from hatching through ovulation. Zool. Sci. 9, 375–386.

Andersson, E., Schulz, R.W., Male, R., Bogerd, J., Patiña, D., Bendet, S., Norberg, B., Taranger, G.L., 2013. Pituitary gonadotropin and ovarian gonadotropin receptor transcript levels: seasonal and photoperiod-induced changes in the reproductive physiology of female Atlantic salmon (*Salmo salar*). Gen. Comp. Endocrinol. 191, 247–258. https://doi.org/10.1016/j.ygcen.2013.07.001.

Ayllon, F., Kjærner-Semb, E., Furmanek, T., Wennevik, V., Solberg, M.F., Dahle, G., Taranger, G.L., Glover, K.A., Almén, S., Rubin, C.J., Edvardsen, R.B., Wargelius, A., 2015. The *vgll3* locus controls age at maturity in wild and domesticated Atlantic salmon (*Salmo salar* L.) males. PLoS Genet. 11, e1005628. https://doi.org/10.1371/journal.pgen.1005628.

Bagenal, T.B., Tesch, F.W., 1978. Age and growth. In: Bagenal, T.B. (Ed.), Methods for Assessment of Fish Production in Fresh Waters. Blackwell Scientific Publications, Oxford, pp. 101–136.

Barson, N.J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G.H., Fiske, P., Jacq, C., Jensen, A.J., Johnston, S.E., Karlsson, S., Kent, M., Moen, T., Niemelä, E., Nome, T., Næsje, T.F., Orell, P., Romakkaniemi, A., Sægrov, H., Urdal, K., Erkinaro, J., Lien, S., Primmer, C.R., 2015. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature 528, 405–408. https://doi.org/10.1038/nature16062.

Carrillo, M., Espigares, F., Felip, A., Escobar, S., Molés, G., Rodríguez, R., Alvarado, M.V., Gómez, A., Zanuy, S., 2015. Updating control of puberty in male European sea bass: a holistic approach. Gen. Comp. Endocrinol. 221, 42–53. https://doi.org/10.1016/j.ygcen.2015.06.019.

Cuisset, B., Pradelles, P., Kime, D.E., Kühn, E.R., Babin, P., Davail, S., Le Menn, F., 1994. Enzyme immunoassay for 11-ketotestosterone using acetylcholinesterase as laberl: application to the measurement of 11-ketotestosterone in plasma of Siberian sturgeon. Comp. Biochem. Physiol. C 108, 229–241.

Erkinaro, J., Czorlich, Y., Orell, P., Kuusela, J., Falkegård, M., Länsman, M., Pulkkinen, H., Primmer, C.R., Niemelä, E., 2019. Life history variation across four decades in a diverse population complex of Atlantic salmon in a large subarctic river. Can. J. Fish. Aquat. Sci. 76, 42–55. https://doi.org/10.1139/cjfas-2017-0343.

Fjelldal, P.G., Hansen, T., Huang, T.-S., 2011. Continuous light and elevated temperature can trigger maturation both during and immediately after smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture 321, 93–100. https://doi.org/10.1016/j.aquaculture.2011.08.017.

Hansen, T., Stefansson, S., Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, Salmon, *salar* L., reared in sea cages at two different light regimes. Aquac. Res. 23, 275–280. https://doi.org/10.1111/j.1365-2109.1992.tb00770.x.

Hansen, T., Fjelldal, P.G., Yurtseva, A., Berg, A., 2010. A possible relation between growth and number of deformed vertebrae in Atlantic salmon (*Salmo salar* L.). J. Appl. Ichthyol. 26, 355–359. https://doi.org/10.1111/j.1439-0426.2010.01434.x.

Houde, E.D., Scheckter, R.C., 1981. Growth rates, rations and cohort consumptions of marine fish larvae in relation to prey concentration. Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 178, 441–453.

Idler, D.R., Horne, D.A., Sangalang, G.B., 1971. Identification and quantification of the major androgens in testicular and peripheral plasma of Atlantic salmon (*Salmo salar*) during sexual maturation. Gen. Comp. Endocrinol. 16, 257–267. https://doi.org/10.1016/0016-6480(71)90037-2.

Imsland, A.K., Handeland, S.O., Stefansson, S.O., 2014. Photoperiod and temperature effects on growth and maturation of pre- and post-smolt Atlantic salmon. Aquac. Int. 22, 1331–1345. https://doi.org/10.1007/s10499-014-9750-1.

Jonsson, N., Jonsson, B., Hansen, L.P., 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic Salmon *Salmo salar*. J. Anim. Ecol. 66, 425–436. https://doi.org/10.2307/5987.

Kjærner-Semb, E., Ayllon, F., Kleppe, L., Sørhus, E., Skaftnesmo, K., Furmanek, T., Segafredo, F.T., Thorsen, A., Fjelldal, P.G., Hansen, T., Taranger, G.L., Andersson, E., Schulz, R.W., Wargelius, A., Edvardsen, R.B., 2018. Vgll3 and the hippo pathway are regulated in Sertoli cells upon entry and during puberty in Atlantic salmon testis. Sci. Rep. 8, 1912. https://doi.org/10.1038/s41598-018-20308-1.

Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F., Mortensen, E., 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecol. Freshw. Fish 12, 1–59. https://doi.org/10.1034/j.1600-0633.2003.00010.x.

Lenth, R.V., 2016. Least-square means: the R package lsmeans. J. Stat. Softw. 69, 1–33. https://doi.org/10.18637/jss.v069.i01.

Lepais, O., Manicki, A., Glise, S., Buoro, M., Bardonnet, A., 2017. Genetic architecture of threshold reaction norms for male alternative reproductive tactics in Atlantic salmon (*Salmo salar* L.). Sci. Rep. 7, 43552. https://doi.org/10.1038/srep43552.

McClure, C.A., Hammell, K.L., Moore, M., Dohoo, I.R., Burnley, H., 2007. Risk factors for early sexual maturation in Atlantic salmon in seawater farms in New Brunswick and Nova Scotia, Canada. Aquaculture 272, 370–379. https://doi.org/10.1016/j.aquaculture.2007.08.039.

Melo, M.C., Andersson, E., Fjelldal, P.G., Bogerd, J., França, L.R., Taranger, G.L., Schulz, R.W., 2014. Salinity and photoperiod modulate pubertal development in Atlantic salmon (*Salmo salar*). J. Endocrinol. 220, 319–332. https://doi.org/10.1530/JOE-13-0240.

Okuzawa, K., 2002. Puberty in teleosts. Fish Physiol. Biochem. 26, 31–41. https://doi.org/10.1023/A:1023395025374.

Pankhurst, N.W., Carragher, J.F., 1992. Oocyte maturation and changes in plasma steroid levels in snapper *Pagrus* (=*Chrysophrys*) *auratus* (Sparidae) following treatment with human chorionic gonadotropin. Aquaculture 101, 337–347. https://doi.org/10.1016/0044-8486(92)90036-K.

Prat, R., Sumpter, J.P., Tyler, C.R., 1996. Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod. 54, 1375–1382. https://doi.org/10.1095/biolreprod54.6.1375.

Schulz, R.W., Andersson, E., Taranger, G.L., 2006. Photoperiod manipulation can stimulate or inhibit pubertal testis maturation in Atlantic salmon (*Salmo salar*). Anim. Reprod. 3, 121–126.

Schulz, R.W., de França, L.R., Lareyre, J.J., Le Gac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. Gen. Comp. Endocrinol. 165, 390–411. https://doi.org/10.1016/j.ygcen.2009.02.013.

Skilbrei, O.T., 1990. Compensatory Sea growth of male Atlantic salmon, *Salmo salar* L., which previously mature as parr. J. Fish Biol. 37, 425–435. https://doi.org/10.1111/j.1095-8649.1990.tb05873.x.

Stefansson, S.O., Hansen, T.J., Taranger, G.L., 1993. Growth and parr-smolt transformation of Atlantic salmon (*Salmo salar* L.) under different light intensities and subsequent survival and growth in seawater. Aquac. Eng. 13, 231–243. https://doi.org/10.1016/0144-8609(93)90014-3.

Taranger, G.L., Haux, C., Hansen, T., Stefansson, S.O., Björnsson, B.T., Walther, B.Th., Kryvi, H., 1999. Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. Aquaculture 177, 47–60. https://doi.org/10.1016/S0044-8486(99)00068-X.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A., Dufour, S., Karlsen, Ø., Norberg, B., Andersson, E., Hansen, T., 2010. Control of puberty in fish. Gen. Comp. Endocrinol. 165, 483–515. https://doi.org/10.1016/j.ygcen.2009.05.004.

Thorpe, J.E., Mangel, M., Metcalfe, N.B., Huntingford, F.A., 1998. Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. Evol. Ecol. 12, 581–599.

Thrush, M.A., Duncan, N.J., Bromage, N.R., 1994. The use of photoperiod in the production of out-of-season Atlantic salmon (*Salmo salar*) smolts. Aquaculture 121, 29–44. https://doi.org/10.1016/0044-8486(94)90005-1.