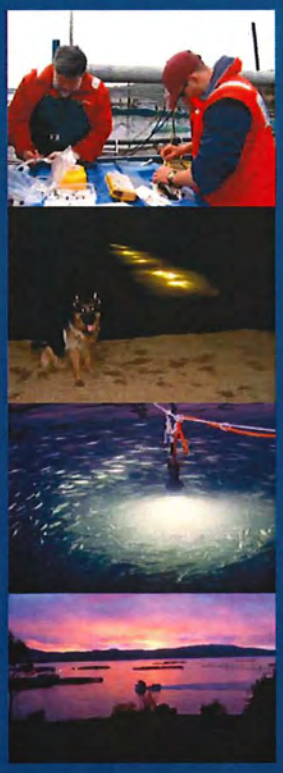
Final Report

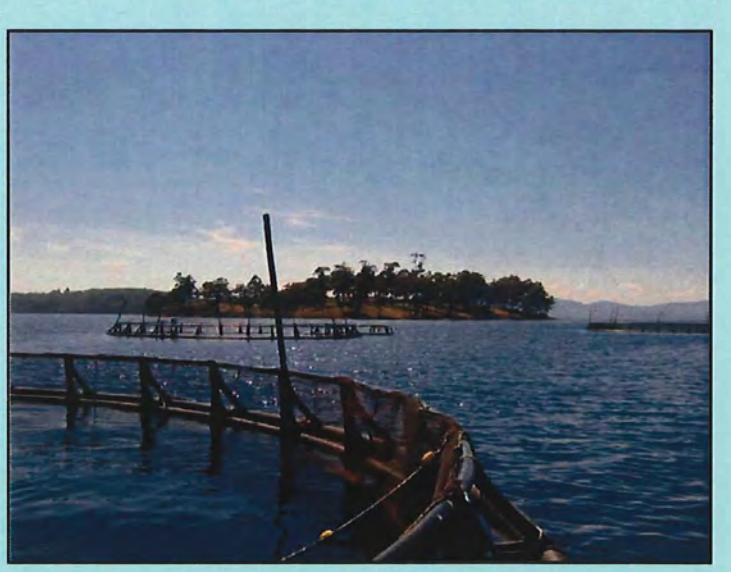
Aquafin CRC

Control of precocious maturation in Atlantic salmon (*Salmo salar*).

Mark Porter, Ryan Longland, Hannah Woolcott and Ned Pankhurst

August 2005
Aquafin CRC Project 2.5
(FRDC Project No. 2001/246)





National Library of Australia Cataloguing-in-Publication Entry

ISBN: 1 86295 352 X

Published by University of Tasmania, Launceston, 2005

Control of precocious maturation in Atlantic salmon (*Salmo salar*)

Mark Porter, Ryan Longland, Hannah Woolcott and Ned Pankhurst

August 2005

Aquafin CRC Project 2.5
(FRDC Project No.2001/246)

Australian Government
Fisheries Research and Development Corporation

Tasmanian Aquaculture & Fisheries Institute
University of Tasmania

Table of Contents

2001/246 Control of precocious maturation in Atlantic salmon (*Salmo salar*)

PRINCIPAL INVESTIGATOR: Dr M.J.R. Porter

ADDRESS:
School of Aquaculture
Tasmanian Aquaculture and Fisheries Institute
University of Tasmania
Launceston
Tasmania
Locked Bag 1-370
Telephone: 03 6324 3800 Fax: 03 6324 3804

Objectives

1. An improved understanding of the mechanisms of light regulated control of melatonin secretion in salmon.

2. An improved understanding of the association between melatonin levels and reproductive development in salmon (light mediated effects on melatonin synthesis and its control of the timing of maturation).

3. The capacity to rapidly and non-destructively assess the acute reproductive condition of caged salmon.

4. The development of commercial scale photomanipulation techniques for the retardation or prevention of precocious maturation in farmed Tasmanian salmon.

OUTCOMES ACHIEVED TO DATE

This project has significantly increased our knowledge of environmental influences on the timing and permissive effects of photoperiod and temperature on maturation in Atlantic salmon in Tasmania. It has provided the Tasmanian salmon industry with a range of production tools which have been adopted to control precocious maturation and to overcome the "harvest gap" thereby providing the processors and consumers with a fresh product of uniform size (3-5kg HOGG) year round. This has been achieved through the use of artificial illumination to delay maturation in farmed female Atlantic salmon; increase growth rates of mixed sex populations following seawater transfer; recondition female Atlantic salmon kelts and inhibit maturation in out-of-season smolts. Additionally, laboratory based trials have increased our understanding of the physiological response to the dark phase artificial illumination in comparison to the extremely high ambient daytime intensities observed in Tasmania.

NON TECHNICAL SUMMARY:

At present the culture of Atlantic salmon within Australia produces approximately 16,500t of fish per annum and is a direct employer of over 1000 workers with the majority of farmed fish sold nationally and only 15% exported yearly. One of the main restrictions in the export market is the cost of production within Tasmania compared to the traditional salmon producers such as Scotland and Norway, and more recently, Chile.

Environmental conditions, such as increased temperatures and high light intensities, found within Tasmania provide exceptional growing conditions for Atlantic salmon with growth rates far exceeding other salmon producing nations. However, the life history strategy of Atlantic salmon has evolved to ensure maximum opportunity for reproduction which means that both male and female fish are able to initiate maturation and reproduce at several times during their life cycle if environmental and nutritional factors are favourable (Thorpe *et al.,* 1990). Consequently salmon farmed within Tasmania have a far greater rate of early maturation resulting in a percentage of the stock being unsaleable due to poor flesh quality. Within the industry this produced a period known as the "harvest gap" where the farms were unable to provide the processors and consumers with a fresh product and had instead to rely on frozen fish which commands approximately 36% lower value than fresh product.

This project aimed to use artificial lighting to alter the timing of these reproductive events and thereby provide harvestable fish year-round. The initial studies focused on assessing the physiological response of the fish to both the ambient and artificial lighting. This required the measurement of plasma melatonin levels as a physiological indicator of light perception. These experiments, conducted at Saltas Ltd.'s Wayatinah hatchery site, revealed that Atlantic salmon reared in Tasmania respond to artificial lighting, however due to the elevated ambient intensities (200,000 lux compared to 12,000 in Norway) the number of lights required on sea cages was considerably greater than in Scotland or Norway.

Initial commercial trials aimed to delay the onset of maturation in a mixed sex population of Atlantic salmon. The work undertaken in this trial used photoperiods, originally developed for use on rainbow trout (Bromage et al., 1988), to artificially delay the onset of the winter decrease in daylength. The result of this was to phase shift reproductive development to a later date and thereby provide freshly harvested salmon throughout the traditional gap period. From this work it was clear that exposure to constant illumination following the summer solstice produced an eight week spawning delay in both male and female salmon compared to control populations maintained under ambient photoperiod saving Tassal, an estimated $2.3 million per annum (Pherose Jungawalla, Tassal press release 2003). However, a repeat experiment the following year highlighted the importance of water temperature, size of smolt and time of seawater transfer as only a five to six week delay in maturation in the females and two to three weeks delay in the males were observed under unfavourable conditions.

The use of constant artificial illumination is known to increase growth rates in a number of species (Taranger et al., 1991; Hansen et al., 1992; Porter et al., 1999; Oppedal et al., 2000). The increased growth is the result of a phase shift in the annual growth cycle. This was investigated on a mixed sex population of Atlantic salmon after seawater transfer in May. The effects of the additional lighting were apparent by the first sample point after transfer in August. By this time the lit group were significantly heavier than the

unlit control population and this trend continued throughout the remainder of the experiment with the lit population having an 18% growth advantage over the unlit group which equates to a 6 week advance to harvest time and a significant increase in growth. This provides the farmer with an option of reducing the length of the production cycle and achieving the 3kg market minimum weight 6 weeks earlier than in unlit cages.

Despite the best efforts of aquaculturists and the use of modified photoperiods it is inevitable that a percentage of the population will continue through to maturation. Traditionally these fish would represent a commercial loss to the farmer as the flesh quality and dark skin colouration makes them unsaleable, and therefore represents a significant loss in revenue to the Tasmanian aquaculture industry.

This work aimed to investigate whether previously mature female Atlantic salmon could be reconditioned using artificial lights, offering significant commercial advantages in terms of flesh quality and appearance at harvest. Using a modified photoperiod regime to advance reconditioning the oocyte reabsorption rate was significantly increased, as was flesh colour and body wall thickness. These factors together with increased size and a reversion from dark skin pigmentation typical of mature individuals to a silver sea going colouration contributed to a greater percentage of the harvest attaining top grade status.

Finally, the ultimate aim of this project from a commercial point of view was to develop commercial scale techniques to inhibit early maturation completely. The final trial aimed to assess the effects of additional dark phase illumination on reducing maturation rates in out of season smolts and to investigate the "gating" mechanism responsible for the decision to mature in adult Atlantic salmon. Part of this work investigated whether a critical size exists prior to maturation taking place and secondly, whether the application of a long-day or constant light photoperiod regime from seawater transfer can inhibit maturation.

For the purpose of this investigation, a thousand individuals were surgically implanted with passive integrated transponders (PIT tags) while still in freshwater. One of the key findings of this work was that a 5g difference in size prior to seawater transfer was sufficient to predispose an individual to mature during the production cycle 18 months later. This has significant implications for an industry that traditionally looks for large smolts from the hatcheries; and this practice may in fact be predisposing the fish to mature as the decision to mature in Tasmanian grown Atlantic salmon may by made in freshwater prior to seawater transfer or soon after transfer. When comparing maturation rates between treatments a reduction from 31% to 3.5% was achieved, thereby providing Tasmanian salmon growers with a method to alleviate one of their main production difficulties in a safe environmentally friendly manner and with an estimated production value of $900,000 for Huon Aquaculture alone (D. Mitchell pers comm.).

In conclusion, ambient environmental conditions mean that the Tasmanian salmon industry will always suffer from high maturation rates due to its high water temperatures and increased light intensity. However, additional artificial lighting in Tasmania has been shown to reduce maturation by up to 30%; increase growth rates significantly; and delay maturation by 8 weeks. These strategies have now been incorporated to improve seasonal production and have been estimated to be worth $8-16 million per year (TSGA report).

Acknowledgements

This work was a project of Aquafin CRC, and received funds from the Fisheries Research and Development Corporation and other CRC participants. These include members of the Tasmanian salmon industry, in particular Huon Aquaculture (Peter and Francis Bender, David Mitchell, Adrian Steinholt, Josh McKibben, Leanne Delaney, Inness Weir and Dr Dom O'Brien); Tassal Ltd. (Pheroze Jungawalla (formerly), Richard Taylor, David Cameron); SALTAS (Dr Harry King and Owen Ryan). We would like to thank Dr Peter Montague (Aquafin CRC), Ms Emily Downes (Aquafin CRC), Dr Patrick Hone (FRDC), Dr Steven Battaglene (FRDC, ASAS, TAFI), Dr Jenny Cobcroft (FRDC ASAS TAFI), Prof Colin Buxton (TAFI), Prof Chris Carter (TAFI), Pheroze Jungalwalla (formerly Tassal, ASAS TSGA) and Erika Laws (FRDC ASAS TAFI) for their continued support throughout the project.

BACKGROUND

Precocious sexual maturation of stock is a constraining factor in Atlantic salmon (*Salmo salar*) aquaculture world-wide (eg. Thorpe et al., 1990, Hansen et al., 2000). Deterioration in flesh quality (soft flesh, reduced flesh lipid and pigment levels) and the development of secondary sexual characters (changes in skin pigmentation in both sexes and the development of a hooked lower jaw (kype) in males) render stock unmarketable. This limits the harvest season or at the very least, results in significant seasonal fluctuations in product volumes, both of which adversely affect product value and producer cash-flows. The issue is of particular concern in Tasmania where precocious maturation of stock continues to hamper the industry goal of year-round harvests (Jungalwalla, 1991). This is in spite of developments such as production of out-of-season smolts (seedstock) and sterile stocks (all-female triploids) which have served to extend the harvest season from 6 months to 10-11 months.

In salmonids, sexual maturation is understood to occur largely in response to a range of photoperiod cues, with temperature exerting an accessory role. Maturation is initiated in response to the accumulation of energy reserves relative to a decision making "gate-open" point during the phase of increasing photoperiod (ie. spring), while completion of the process (culminating in ovulation in females, and spermiation in males) occurs in response to the phase of declining photoperiod (ie. autumn; Duston and Saunders, 1992, Randall et al., 2000). Armed with this knowledge, overseas workers have applied photoperiod manipulation techniques to alter the extent and/or timing of sexual maturation to extend commercial harvests (eg. Porter et al., 1999). As these manipulations occur in the field on sea-cage facilities where it is impossible to exclude ambient illumination, workers have been restricted to applying processes which increase rather than shorten ambient daylength. In this regard, long photoperiods are understood to advance maturation when applied during the phase of increasing photoperiod (Figure 1) and delay maturation when applied during the phase of declining photoperiod (reviewed by Bromage et al., 1993). Thus increased daylength may be applied after the summer solstice to extend harvests by desynchronizing completion of maturation. Importantly, however, this process simply retards the rate of processes in fish that are already maturing and has no effect on the numbers of fish or proportion of a population undergoing maturation.

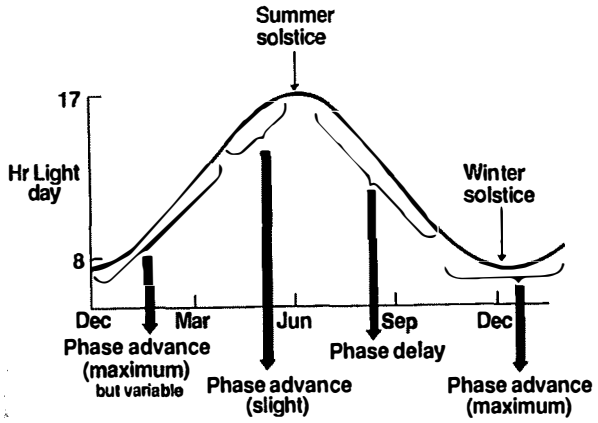


Figure 1. Schematic representation of the effects of long days on the phasing of the endogenous rhythm of salmonid sexual maturation (adapted from Bromage et al., 1993).

Increased daylengths have been applied prior to the summer solstice with the aim of inhibiting the entire maturational process rather than affecting its rate of progression (eg. Porter et al., 1999). In simple terms, the aim is to advance the maturational decision making "gate-open" point to such an extent that the fish 'judge' themselves to be inadequately resourced at that time and therefore postpone maturation for a photoperiod year. The physiological basis for the crosstalk between energetic status and the endocrine machinery that will regulate gonadal development is not known, but the process is suspected to have some relationship to the accumulation of lipid reserves (Thorpe 1994). In contrast, the mechanisms by which fish perceive changing daylengths are much better understood. Photoperiod is believed to entrain an endogenous rhythm of reproductive development via pineal melatonin secretion (Porter et al., 1999, 2000, 2001). Melatonin is synthesized by photoreceptors in the pineal body and its synthesis increases during the hours of darkness (scotophase) such that the melatonin profile reflects the light/dark cycle and acts as a zeitgeber for the entrainment of reproductive development (Porter, 2000). Thus the effectiveness of use of additional illumination to extend apparent daylength is dependent upon achievement of sufficient light intensity during the scotophase to reduce plasma melatonin below an as yet undefined threshold level (Porter et al., 1999, 2000). Taking this approach, use of artificial illumination prior to the summer solstice has been employed to achieve up to a 90% reduction in the levels of precocious sexual maturation in Atlantic salmon farmed in Scotland (Porter et al., 1999, 2000).

This project aims to asses the physiological response of salmonids in the Southern hemisphere to changes in photoperiod and artificial illumination in relation to ambient light levels and water temperatures. This information will then be used to design photoperiod regimes to alter the timing of maturation in Tasmanian grown Atlantic salmon in order to fill the harvest gap in fresh fish between April and August. Finally, artificial photoperiods will be developed to inhibit maturation in out of season Atlantic salmon prior to harvest.

Need

There are two major aspects:

1. Importance to industry of control of precocious sexual maturation
Tasmanian salmon typically mature after only one winter at sea, in contrast to northern-hemisphere populations where the majority take two 'sea-winters' to mature. The Tasmanian fish still reach 3 – 5 kg during this period due to the favourable effects of higher temperature on growth; however, the less desirable outcome of early maturation is the compression of the harvest season. Strategies designed to improve seasonal production have been estimated to be worth $8–16 million per year to the Tasmanian salmon industry (confidential industry estimate prepared for CRC for Aquaculture in 1998).

2. Requirements to conduct research in Tasmania
Functional photoperiod manipulation techniques have been developed overseas so why do it in Tasmania rather than simply import solutions? Overseas protocols have been trialled by the Tasmanian industry but have given negative or unpredictable results. Confounding factors which require consideration before overseas protocols can be applied successfully and predictably in Tasmania are:

A. Ambient light intensity.
Due to Tasmania's low latitude and high number of sunshine hours relative to the majority of northern hemisphere salmon farming areas, it is likely that salmon farmed in Tasmania are exposed to higher daytime light intensities, particularly at the equinoxes. Relative light intensity is a critical factor for the success of photoperiod manipulation practices and it is expected that higher, yet to be determined, levels of night-time illumination will be required under Tasmanian conditions.

B. Ambient temperature.
Overseas scientists report increased melatonin secretion (up to approx. 30%) at summer temperatures relative to winter temperatures in Atlantic salmon maintained under identical photoperiods. Tasmania's relatively high water temperatures suggest that a further increase in light intensity will be required to reduce plasma melatonin levels below the putative threshold required to ensure that the fish perceive any modification to photoperiod.

C. Individuals response to seasonal variations in environmental conditions.
Assuming that the preceding factors can be adequately clarified, it will be necessary to account for the possible effects of differences between seasons (both within and between years) and individual variation within fish populations in relation to the response of stocks to photoperiod manipulation. Relative to overseas salmon farming areas, Tasmania tends to have a short, mild winter, an early, warm spring and a long, hot summer. Thus timing of the critical "gate-open" decision period for maturation has yet to be determined under Tasmanian conditions.

Project Objectives

1. An improved understanding of the mechanisms of light regulated control of melatonin secretion in salmon.

2. An improved understanding of the association between melatonin levels and reproductive development in salmon (light mediated effects on melatonin synthesis and its control of the timing of maturation).

3. The capacity to rapidly and non-destructively assess the acute reproductive condition of caged salmon.

4. The development of commercial scale photomanipulation techniques for the retardation or prevention of precocious maturation in farmed Tasmanian salmon.

General Methods

Artificial light deployment and measurement

The submersible lights used throughout this work were either Aquabeam (Pisces 400 watt) or C&T Lighting (400 watt units). These were placed approximately mid way through the water column (i.e. at 5m in a 10m deep cage). The lights were deployed to create maximum coverage throughout the cage (number of lights are given in the text for each trial) and this was measured in micro-Einstein's (μE) and Lux using a LI-COR (Model LI-1400) submersible light meter in a three dimensional grid pattern. Light attenuation from a 400 watt submersible light in seawater with an 8m secci disc reading is given in Appendix 3

Husbandry & Anaesthesia

All commercial trials and tank experiments were conducted on either farm or hatchery sites. Consequently day-to-day husbandry, fish collection and sacrifice were conducted as per farm practice.

Morphometric measurements & calculations

Total body weight (g) and fork length (mm) of individual fish was determined in the field using a waterproof, motion-compensating scale (POLS, Iceland) fitted with a custom-made measuring cradle.

Throughout the project the body indices were calculated as follows:

$$\text{Condition Factor (\%)} = \left[\frac{\text{Total body weight (g)}}{[\text{Fork length (cm)}]^3} \right] \times 100$$

$$\text{Gonadosomatic Index (\%)} = \left[\frac{\text{Total gonad weight (g)}}{\text{Total body weight (g)}} \right] \times 100$$

$$\text{Hepatosomatic Index (\%)} = \left[\frac{\text{Total liver weight (g)}}{\text{Total body weight (g)}} \right] \times 100$$

Blood collection & hormone measurement

Blood samples were collected via caudal vein puncture of anaesthetised fish using either 1mL or 3mL heparinised syringes with either 25G or 22G needles. Blood samples were maintained on ice prior to centrifugation at 3000rpm for 15 minutes. The supernatant was then stored in 1.5mL eppendorf tubes at -80°C until analysis.

Sex Steroids

Measurement of plasma concentrations of the sex steroids testosterone (T) and estradiol (E_2) was carried out by radioimmunoassay (RIA) as described in Pankhurst & Carragher (1992).

Melatonin

Measurement of melatonin levels in plasma samples were also carried out by direct competitive binding radioimmunoassay (RIA) according to methods described in Porter et al., (1991).

Gonad tissue processing & histology

Gonad tissue fixed in 10% buffered neutral formalin was dehydrated for processing and storage by progressively transferring to 30% and 50% ethanol for a period of 24 hours each and finally into 70% ethanol. Gonads were then embedded in paraffin wax and transverse sections were cut at 5μm intervals. Sections were then stained with haematoxylin and eosin (Wood and Ellis 1994).

Flesh collection & Roche SalmoFan™ scoring

Samples of flesh sectioned from the dorsal musculature of individual fish (Norwegian Standard Cut: NSC) were collected and assigned a Roche SalmoFan (RSF) score. The Roche SalmoFan is an industry-accepted index of salmon flesh colour ranging from 20 – 36. The highest RSF score corresponds to a deep red colour that is highly valued at market. As Atlantic salmon mature, the colour of the flesh becomes washed out and consequently has a low RSF score (Figure 2).

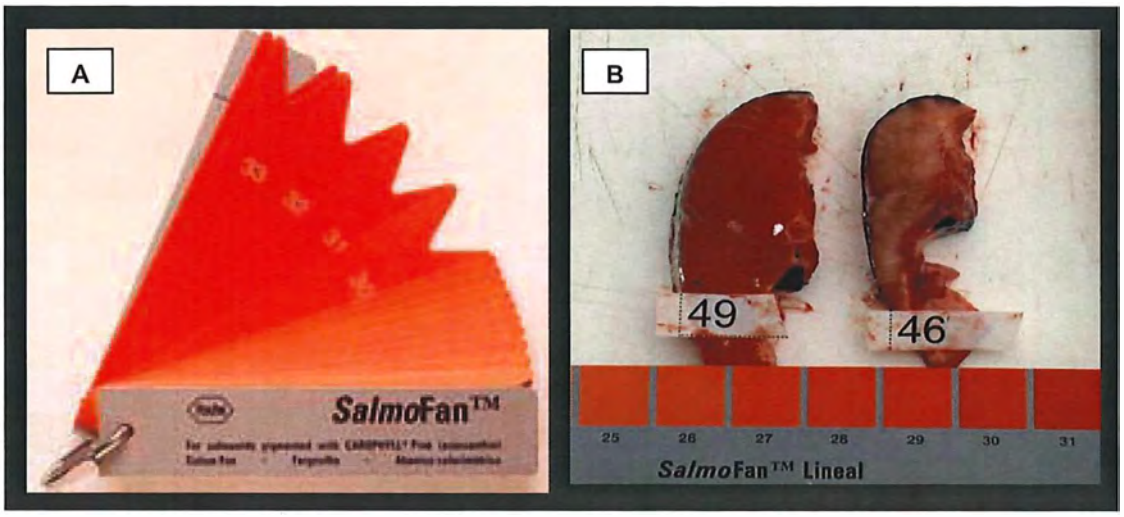


Figure 2: Roche SalmoFan (A). Examples of a Atlantic salmon flesh (NSC) with a high (#49) and low (#46) RSF scores (B).

Oocyte diameter

Mean diameter of oocytes from maturing female Atlantic salmon was measured as an indicator of gonad growth in selected commercial trials. Ten maturing females were sacrificed and an ovary was removed from the body cavity. Individual oocytes were carefully separated from surrounding connective tissue using fine tweezers and the diameter of a minimum of 20 oocytes per female was measured.

Statistical Analyses

All statistical analyses were conducted using the software program SPSS™. Where appropriate, means were compared using either Independent Sample T-tests or One-way fixed-model ANOVA. Assumptions of homogeneity of variances and data normality were assessed using Levene's statistic and residual plots respectively. Where necessary, non-normal data was $\log^{10}$ transformed. Significance level was set at alpha (0.05) and was two-tailed. Tukey's test was used for post hoc analyses to identify which groups were significantly different. Power of statistical analyses was set at 0.8 or greater and was satisfied for all significant results presented.

RESULTS & DISCUSSION

Commercial Trial 1 – Using Artificial Illumination to Delay Maturation in Atlantic Salmon.

This work aimed to investigate the ability of artificial illumination to delay the onset of maturation in a mixed sex population of Atlantic salmon to overcome the harvest gap. Photoperiods originally developed for use on rainbow trout (Bromage *et al.,* 1988) were used to artificially delay the onset of the winter decrease in daylength and hence phase shift reproductive development by six to eight weeks and thus provide freshly harvested salmon throughout the traditional gap period. Two commercial scale trials were conducted in consecutive years to enable comparison between year classes.

Year 1 - Methods

This trial was conducted in collaboration with Tassal Ltd. Initially 36 thousand Atlantic salmon from a mixed sex diploid population, reared from the 1999 year class, were transferred to sea as spring smolts. On 22nd December 2001, at the time of the summer solstice, two populations of 11 thousand fish were taken from the original pen, and placed in new pens. One pen was maintained under ambient conditions, while the other was supplied with a long day photoperiod (20L:4D). The additional artificial light was supplied by six (400 watt) underwater lights (dominant wavelength 535nm). On 15th March 2002 the group under ambient illumination was harvested and on the 21st May 2002 the lit group was also harvested.

At two weekly intervals from the 7th February 2002, 20 individuals from each group (10 males and 10 females) were sacrificed and weight, length, gonad weight, and oocyte diameters were measured and blood plasma collected. The plasma was later analysed for testosterone (T), estradiol (E_2) and vitellogenin (Vtg).

Year 1 – Results

The use of additional dark phase lighting produced an eight week delay in the maturation of both male and female Atlantic salmon. Figure 3A shows that male gonadosomatic index (GSI) increased rapidly in the control fish under ambient conditions from 0.7% to 5% before the fish were harvested. In comparison, the fish under artificial lighting (Lit) only initiated gonadal development in April and required a further eight weeks for the GSI in the Lit group to reach 5%. This equates to an eight week delay in maturation as a result of lighting the pens from December 2001.

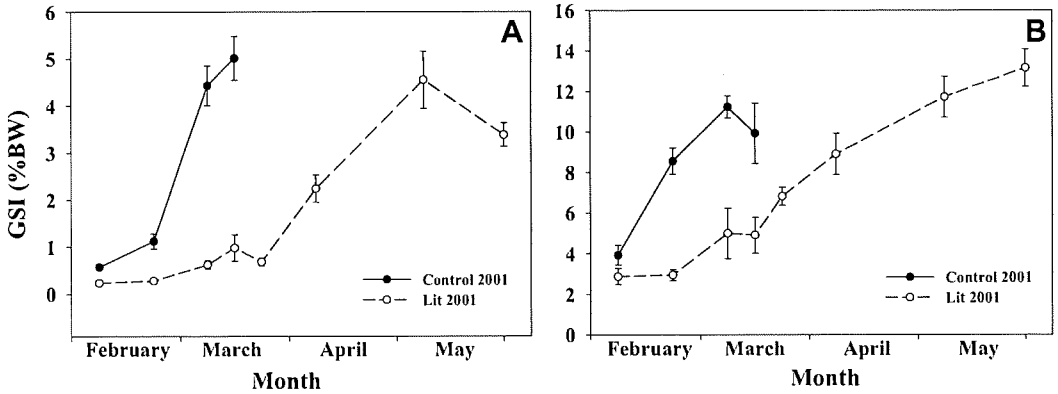


Figure 3 Change in gonadosomatic index (mean ±1SEM) in male (A) and female (B) Atlantic salmon maintained under either ambient photoperiod (Control) or additional artificial dark phase illumination (Lit) from 22nd December 2001. Error bars not shown were too small to be depicted.

Female GSI's also revealed an eight week delay in development under additional lighting (Figure 3B). On the 7th February 2002, no significant difference in the GSI's were observed ($P>0.05$). However, by the second sample point on the 21st February a significant increase in gonad development in the ambient photoperiod (Control) group was observed. This increase in GSI in the ambient (Control) group continued throughout March. It must be noted that at this point the industrial partner had to grade out some fish for harvest resulting in a drop in GSI on the 14th March, after which this group was harvested. The additional light (Lit) group took a further eight weeks to attain a mean GSI of greater than 10%.

The egg diameters in females from both groups were measured to give an indication of ovary developmental stage (Figure 4). From this work it is clear that ovarian development was delayed in the Lit fish and as with the GSI data, the group maintained under ambient conditions (Control) showed an advance of five weeks in comparison to the fish maintained under artificial light.

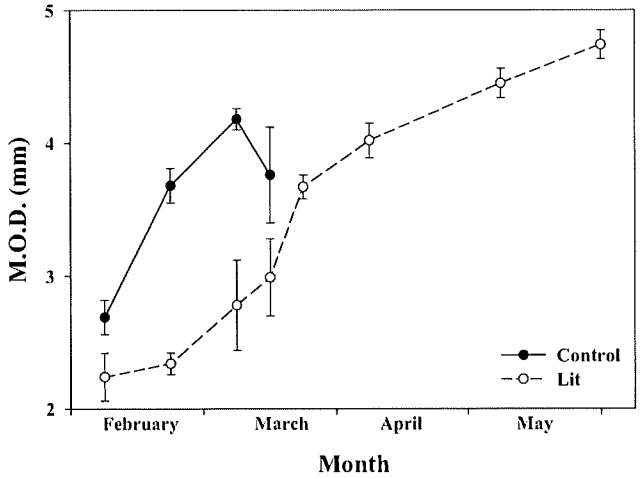


Figure 4 Change in oocyte diameter (mean ±1SEM) in female Atlantic salmon maintained under either ambient photoperiod (Control) or additional artificial dark phase illumination (Lit) from 22nd December 2001. Error bars not shown were too small to be depicted.

Plasma vitellogenin (Vtg) levels were also measured in females in the Control and Lit groups. Increased plasma Vtg levels were observed in the fish maintained under ambient conditions (Control) relative to Lit fish from the first sample in February, until harvest in March. This agrees with the data presented on oocyte diameter and corroborates the delay in gonadal development experienced by the additional light group. Unfortunately, commercial pressures meant that the ambient control group had to be harvested before a peak in Vtg production was observed.

Year 2 - Methods

In September 2001 a mixed sex diploid stock of Atlantic salmon (spring pre-smolts) were transferred into 4, 80m diameter sea cages (n~10,500; giving a harvest stocking density of approximately 12kg.m^{-3}) and fed to satiation using automated feeding systems (Aquasmart™) systems. At the end of 2002, two of the cages were then put under constant artificial light (8x400watt submersible light units spaced equidistant throughout the cage at 5m depth, see Appendix 3) from the summer solstice (December 22nd, 2002) and the remaining two groups were maintained under ambient conditions. Gonad samples, hormonal and morphometric measurements were collected at monthly intervals until harvest in April 2003.

Year 2 - Results/Discussion

As in the initial trial conducted in the summer of 2001-02, the additional light had very little effect on the size of the maturing fish; this is not surprising as the photoperiod employed was only designed to delay the onset of the winter reduction in daylength by approximately six weeks. The constant illumination following the summer solstice did produce the desired five to six week delay in maturation in the Lit females. However, only two to three weeks delay was observed in the Lit males compared to the Control populations maintained under ambient photoperiod. This is in contrast to the findings of the trial in the previous year which observed an eight week delay in both Lit male and female Atlantic salmon. On closer examination however it is clear that before comparisons between both trials could be made the timing of reproduction in both Control populations in relation to actual celestial dates had to be considered. Figure 5. shows mean GSI in male and female populations maintained under ambient and constant illumination in both trials.

It can be seen that the Control populations in 2002 actually matured earlier in the year compared to the Control groups in 2003. It is also apparent that in the 2003 gonad growth of the female Lit population was actually delayed to a greater extent than the group under illumination in 2002. To try to explain some of the variations the difference in water temperatures between the two seasons was examined. Figure 6 shows that although summer temperatures were comparable the average winter temperature in 2002 was almost 2^{o}C cooler than in 2003. The warmer temperatures over the winter period prior to spawning have the effect of delaying reproductive development (Watts et al., 2003) This is especially true in female salmonids which partition a greater percentage of their available energy into gonadal production over a longer period compared to males.

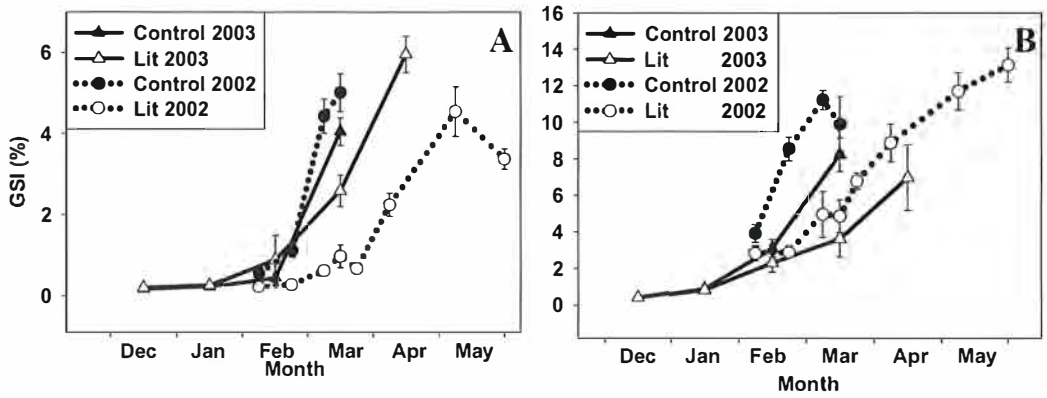


Figure 5 Gonadosomatic Indices of male (A) and female (B) Atlantic salmon maintained under either constant illumination from the summer solstice (Lit) or ambient photoperiod (Control) in the summers of early 2002 and 2003.

A second consideration in explaining the variation between the two trials is the timing and fish size at seawater transfer. For those fish used in the first trial seawater transfer in took place in November 2000 when the average size of the smolts were 103g whereas in the second trial, seawater transfer was in September 2001 when the fish were only 77g. Although the time of seawater transfer is almost two years prior to spawning, the fish size and date of transfer can have a large influence on the size of an individual at the point when the decision to mature is taken. The extra two months in the sea will always more than make up for the 26g difference at transfer. Therefore for the population that was sampled in early 2003, the fish were larger at the decision window.

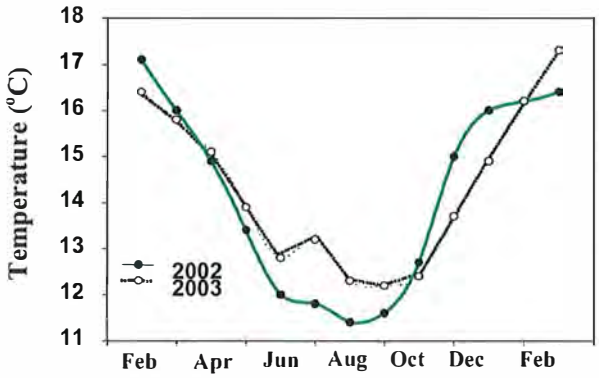


Figure 6 Annual average water temperatures in the Huon Estuary in 2002 and 2003.

Summary

This trial provides valuable evidence that additional dark phase illumination applied from the summer solstice can effectively delay maturation by approximately eight weeks in Atlantic salmon. However, it also highlights the specific nature of photoperiod manipulation in relation to the size of fish and time of seawater transfer together with the variability in ambient temperatures .

Trial 2 – The Effect of Constant Illumination on the Reconditioning of Mature Female Atlantic Salmon (Porter *et al.*, 2003)

This work aimed to investigate the reconditioning of mature female Atlantic salmon which represent a significant loss in revenue to the Tasmanian aquaculture industry due to characteristic poor flesh quality.

Methods

In April 2002 an all female stock of Atlantic salmon displaying pronounced secondary sexual characteristics were randomly divided into 4 sea cages (giving a harvest stocking density of approximately 12kg.m^{-3}) and fed to appetite using a smart feeding system i.e. Aquasmart™ systems. Three of the cages were then put under 24 hour artificial light (4x400 watt submersible light units) from May 23rd, June 6th or July 17th 2002. The remaining group was maintained under ambient conditions. Histological, flesh quality, hormonal and morphometric measurements were collected at monthly intervals until harvest in December 2002.

Results

From the histological data and the stages of ovarian development observed within the sacrificed individuals it was clear that the populations of "mature" fish selected at the outset of this trial included intact immature individuals. This reflects a condition known as a 'dummy run' when individuals undergo the initial stages of reproductive development and display all the secondary sexual characteristics associated with maturation, however, then terminate reproductive development prior to the latter stages of gonadal development (Porter *et al.,* 1999; Bromage *et al.,* 2001). Due to the darkened external appearance, visual grading of the fish is difficult.

Ovarian development within the initial populations ranged from; new season eggs/ovary i.e. small pale eggs with an ovary of approx 4-8cm long indicating immature fish to previously mature fish which contained developed ovaries and/or a body cavity filled with eggs i.e. the previous seasons eggs released into the peritoneal cavity having undergone atresia or if already ovulated, necrotic (Figure 7), and finally towards the end of the trial many of these individuals displayed ovaries with last seasons eggs embedded in a newly developing ovary.

Therefore, the treatment groups in the results sections were sub-divided into two cohorts depending upon the reproductive stage when the trial was initiated; these were classed as either previously mature or immature individuals. These cohorts within the population were clearly distinguishable when the endocrine and gonadal data was analysed. These cohorts will now be considered in turn.

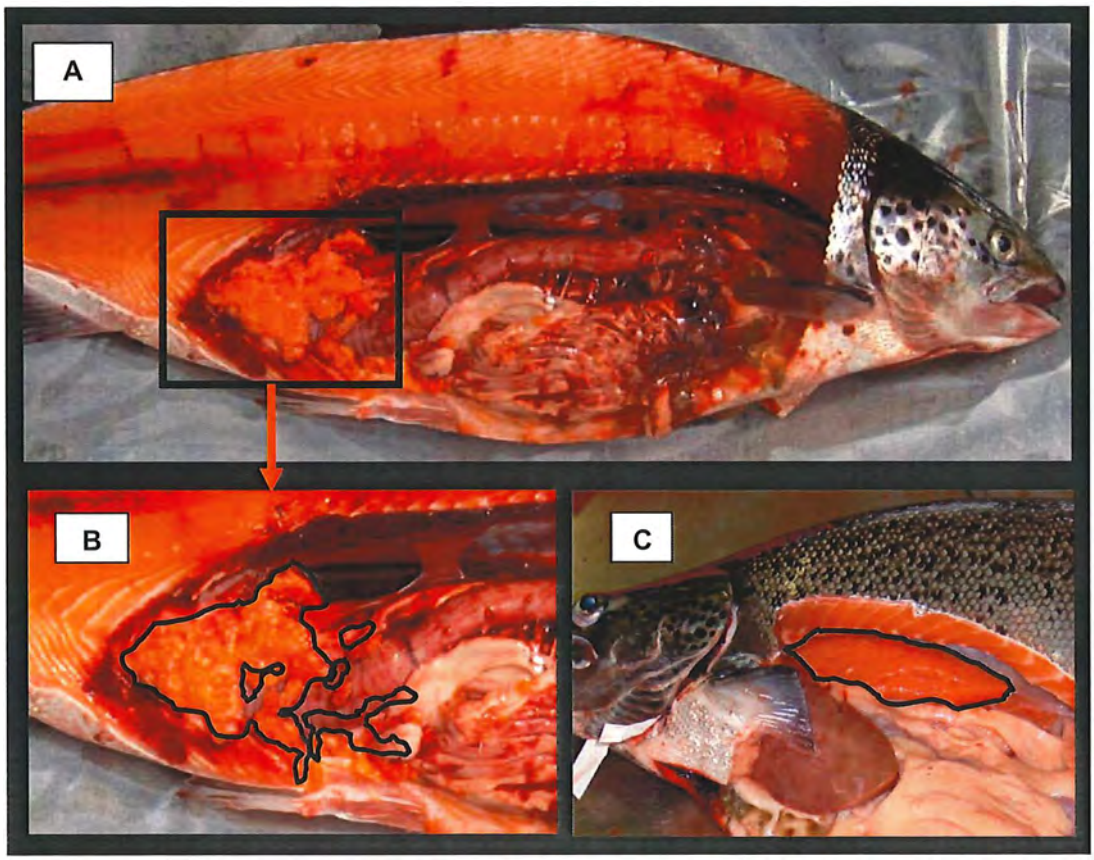


Figure 7 – Example of a previously mature female (A) that has released developed eggs into the peritoneal cavity, which subsequently undergo atresia and are slowly reabsorbed (B). The third image (C) is an example of a previously immature fish containing new season eggs embedded in the connective tissue of the ovary.

Previously Mature Individuals – Sex hormones

Determination of mean plasma estradiol concentrations for each group revealed that the May and June groups exhibited significantly higher ($P<0.05$) initial estradiol levels ($9.66\pm1.96 ng.ml^{-1}$ and $5.61\pm0.66 ng.ml^{-1}$ respectively) indicating these fish had been in a state of advanced maturity at the initiation of the trial. Interestingly, the plasma estradiol levels in all treatment groups were at basal levels by July suggesting that all groups had passed the spawning window and were beginning to recondition whether under lights or natural photoperiod. Estradiol levels in all groups then remained at basal levels until the October sample point when all groups showed a significant increase ($P<0.05$) in estradiol. This again increased in November and yet again in December in the June group. This second increase in plasma estradiol is the result of the development of the ovaries for a second reproductive cycle.

Plasma testosterone levels in previously mature fish closely follow the trends observed with estradiol with high levels measured in the May and June groups in May and June respectively. Testosterone levels then fell significantly ($P<0.05$) in both groups, and by the July sample point only the May group ($6.18\pm2.27 ng.ml^{-1}$) was significantly higher ($P<0.05$) than the June ($0.34\pm0.07 ng.ml^{-1}$) and July ($1.9\pm1.59 ng.ml^{-1}$) groups. All groups

then remained at basal levels until the final sample point in December when the June group had significantly higher plasma testosterone levels (5.56±0.83ng.ml^{-1}) than the control group (3.01±0.19 ng.ml^{-1}).

Immature individuals – GSI & Sex hormones

Immature fish were classed as any individual with no previous signs of maturation within the body cavity. These were typically fish with gonadosomatic indices (GSIs) of less than 1% at the initiation of the trial. The GSIs in the immature fish remained at base levels (<1%) through until August. September appeared to be the start of an increasing trend in the May group which showed an increased mean GSI of 1.65±0.58%. This may suggest an advance in reproductive development in the May population. In November the May group had significantly greater GSIs than the June and July group which in turn had significantly higher GSIs than the controls. This again suggests an advance in the May Lit population. Unfortunately, the May and July groups were harvested after this time however the June group again showed significantly higher ($P<0.05$) GSIs (4.57±0.83%) than the control fish (2.07±0.32%) at the last sample point in December.

Plasma testosterone and estradiol measurements corroborate the GSI findings in the immature populations and confirm that all groups contained maturing individuals within the previously immature fish. The May group showed the earliest signs of reproductive development in August compared to the control which had significantly higher ($P<0.05$) plasma testosterone levels than the June or July groups. No significant differences were then observed until the November sample when the control group had significantly lower ($P<0.05$) testosterone levels than all other groups. This was again observed between the June (7.65±0.85ng.ml^{-1}) and control group (3.57±0.29ng.ml^{-1}) in December.

In August and September plasma estradiol increased in the May group before the others. No significant difference was found between groups in October or November. However, at the last sample point in December the June group had significantly higher ($P<0.05$) estradiol levels (6.36±0.69ng.ml^{-1}) than the control group (2.94±0.37ng.ml^{-1}).

Weight

The use of additional dark phase illumination seemed to benefit the May lit group as the mean for this group was significantly greater ($P<0.05$) than the June group in July and both the June and control groups in August. After this point no significant differences ($P>0.05$) were recorded among the groups. When the population was sub-divided into previously mature or immature populations, there were no significant differences between the previously mature groups at any point throughout the trial. The significant differences could be attributed to the immature fish where again the May group was significantly heavier than either the June or July groups in July and significantly heavier than the June and control group at the August sample point.

ROCHE Salmofan™ Score

ROCHE Salmofan™ scores give a visual indication as to the colour of the flesh. It consists of a commercially accepted scale within the aquaculture industry ranging from a pale pink colour (20) up to a deep red (35). Figure 8A shows the effects of additional lighting on flesh colour scores of previously mature and immature fish. There was no significant difference between the lit groups ($P>0.05$) however from October onwards the

ambient control group had significantly lower (P<0.05) colour scores than the lit populations. Unfortunately due to commercial constraints the May and July groups had to be harvested before December, however, the remaining June lit group had significantly higher (P<0.05) colour scores (29.5±0.27) than the ambient controls (27.47±0.29).

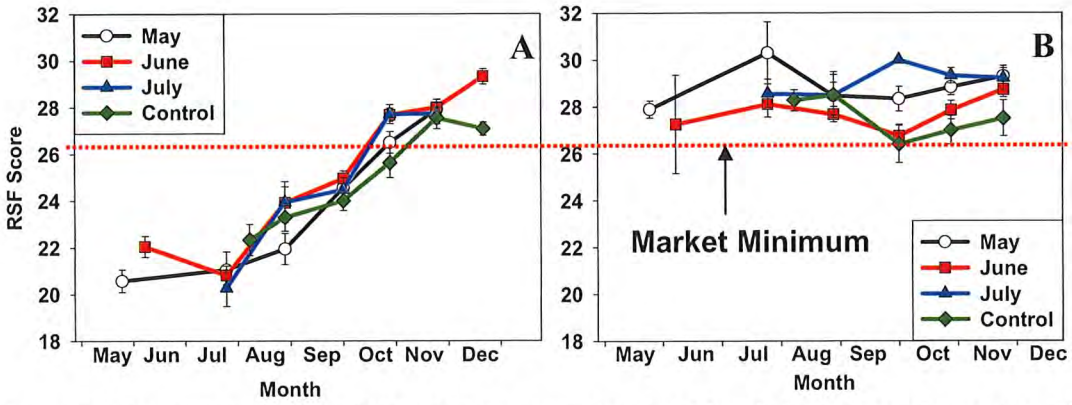


Figure 8 Change in ROCH Salmofan™ score (mean±1SEM) in previously mature (A) and immature (B) female Atlantic salmon maintained under either ambient photoperiod or additional dark phase illumination from May, June or July. Error bars not shown were too small to be depicted.

However, when the immature fish in each population were compared there was no significant difference (P>0.05) between groups (Figure 8B). Indeed all groups maintained acceptable levels of flesh colour (ranging from 18-32) throughout the trial with no obvious upward or downward trend over the experimental period.

Atresia score

To aid comparisons of recrudescence to be made between groups, atretic oocytes were scored against a visual scale as to the state of the ovaries in previously mature individuals. This scale awarded fish with new viable eggs as 0 through to fish that contained only the empty husks of last years eggs, which were rated as 10.

The data shown in Figure 9 shows all groups displayed increased atresia scores over time as the previous year's eggs were reabsorbed. However, from September the control group showed lower atresia scores compared to the other groups. In October and November the control group had significantly lower (P<0.05) atresia scores (5.0±0.39 and 6.3±0.49 respectively) than the July (7.2±0.4) and May (8.38±0.51) groups respectively. At the final sample point in December the eggs in the control group were larger and at an earlier stage of atresia than the June lit fish. This data shows that the lit populations reabsorbed the previous year's eggs faster than the unlit control group.

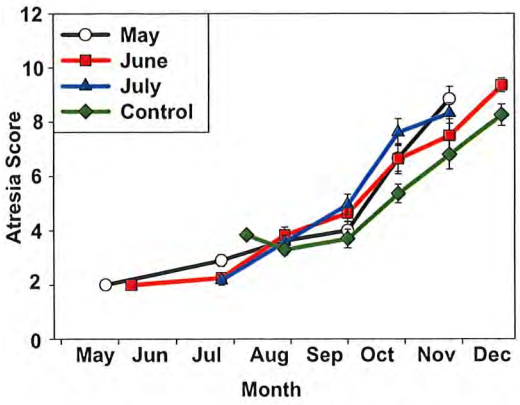


Figure 9 Change in atresia score (mean±1SEM) in previously mature female Atlantic salmon maintained under either ambient photoperiod or additional dark phase illumination from May, June or July). Error bars not shown were too small to be depicted

Body wall thickness

The measurement of body wall thickness was instigated by the management of Huon Aquaculture to assess the suitability of reconditioned fish for processing. Unfortunately this was in the latter stages of the trial and as such data was only obtained for the June and control groups on the December sample point. However, Figure 10 shows that the control group had a significantly thinner ($p<0.05$) mean body wall (7.08±0.3mm) compared to the June photoperiod group (7.78±0.17mm). It is suggested that this is mainly due to the presence of previous years eggs in the body cavity of the control fish which is corroborated by the atresia scores in this group compared to the June lit fish.

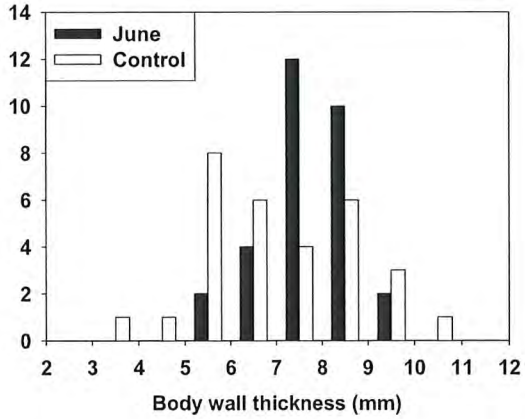


Figure 10 Frequency distribution of body wall thickness of female Atlantic salmon maintained on either additional lighting from June or ambient photoperiod.

Discussion

The results of this work answered a number of questions initially posed by the industry partners. Namely, can artificial illumination accelerate or aid reconditioning in female Atlantic salmon and why do reconditioned populations exhibit varied reproductive stages within the body cavity at harvest?

The first of these questions was answered very early in the trial and relates to the efficacy of the grading. Within all groups studied, there was a percentage of immature fish within the population, overall, 46.9% in the May group; 35.8% in the June group; 31.0% in the July group and 48.4% in the controls. Therefore, it was shown that the variation in individual response to the additional dark phase illumination is a result of the stage of maturation and size/energetic status when the lights were initiated. Fish that were in the latter stages of gonadal maturation when the lights were switched on, had by this time, proceeded too far in the reproductive cycle to terminate the spawning process and hence ignored the photoperiod cue and completed ovulation regardless. These fish were then identified as fish with old eggs many of which had been reabsorbed and the remainder undergoing atresia. However, in many cases new eggs in the early stages of maturation were also observed within the same fish as these had begun preparing for the next spawning cycle.

Salmon which were in the early stages of maturation when exposed to lights had their circannual rhythm of reproductive development advanced by the artificial light as they had already experienced a declining photoperiod followed by constant artificial light in May. These individuals had “decided” that they had missed their spawning season and therefore arrest gonadal development. This resulted in the ‘mixed’ ovary development described by the farm where last season’s small atretic oocytes are still visible and embedded in the ovary along side the new season’s developing eggs.

The remaining fish maintained under lights can be classified into two categories. One cohort consists of small individuals with low energetic status. These fish were stimulated into increasing somatic growth under the constant light (LL) regime. This resulted in immature females at the conclusion of the trial. This was characterized by the immature ovaries seen when these fish were opened at harvest. The second category were immature fish, but individuals with either greater size or energetic status, which, under the accelerated photoperiod had sufficient reserves to proceed with gonadal development and therefore resulted in the new season advanced population of first time maturing individuals.

The use of additional artificial lighting had clear effects on both the previously mature and immature cohorts. In the immature fish the additional lights from May resulted in a growth advantage over the other groups during July and August. However, this was lost as the natural day length and water temperatures increased from September onwards suggesting a possible overriding affect of the natural environmental conditions on an endogenous rhythm. Constant illumination did promote early maturation in the lit groups with the May group showing the greatest advance. This is confirmed by the gonadosomatic indices as well as plasma testosterone and estradiol measurements. If culture conditions, i.e. water temperatures and fish health, allow there would be a distinct advantage to grading off these larger fish and thereby allowing more time for the previously mature fish to continue reconditioning. The data suggests that greater

advantages could have been gained by maintaining all groups for a longer period of time as colour score and stage of atresia was still increasing at the final sample point.

The use of lights on the previously mature female Atlantic salmon has significant commercial advantages in terms of flesh quality at harvest. The atresia scores confirm that the lit groups showed significantly better reabsorption of the previous year's eggs when compared to the unlit controls. The flesh colour and body wall thickness were also improved by the use of lights. Both flesh colour and the thickness of the belly wall are key areas which merit downgrading of the harvest fish by the processors if they fall below standard. In the case of the ROCHE Salmofan™ score this is 25-26 with body wall thickness being at the processors discretion. This work has shown that body wall thickness was improved by the use of lights this will inevitably lead to a greater percentage of the harvest attaining top grade status.

In conclusion, fish maintained under lights attained a reversion from dark skin pigmentation typical of mature individuals to a silver sea going colouration more rapidly than the control group. In addition the ROCHE Salmofan™ score and body wall thickness was significantly greater than that of the control fish combining to produce a higher commercial quality fish. The results show that the use of artificial lights and photoperiods must be fine tuned account for Southern hemisphere conditions, however, used correctly, provides a useful tool with which to accelerate the reconditioning of previously mature Atlantic salmon in Tasmania.

Trial 3 – The Use of Artificial Lighting to Increase Growth Rates in Atlantic salmon post-smolts.

The use of constant artificial illumination is known to increase growth rates in a number of species (Porter *et al.,* 1999; Oppedal *et al.,* 2000). The increased growth is the result of a phase shift in the annual growth cycle. However, the danger in increased growth rates is the promotion of early maturation. Therefore, this trial investigated the use of additional lights to increase growth under Tasmanian environmental conditions and the consequences on maturation rates.

Methods

On 28th May 2002 a mixed sex population of 132,000 Atlantic salmon were transferred to sea (mean weight 98g). Half the population was maintained under ambient conditions while the rest of the fish were given constant additional illumination (4x400 watt submersible lights) in one 80m diameter pen until 5th November 2002. Both groups were fed a ration of 2.3% body weight per day. Females in each group (Lit & Control) were sampled at approximately 6 weekly intervals from August 2002 to May 2003. Weight, length data, gonads and blood plasma samples were collected each time.

Results

Maturation

Maturing fish were identified as individuals, that when sacrificed showed a gonadosomatic index (GSI) of greater than 1%. Neither treatment showed any increase in GSI until the March sample point (Figure 11). In the control group this only accounted for 3% of the sampled population whereas in the lit group 46% of sacrificed individuals had a GSI of greater than 1%. By May the number of maturing fish in the control population had increased to 26% and fallen to 32% in the lit population possibly due to 14% of the Lit population exhibiting a dummy run of maturation (Bromage *et al.,* 2001).

Figure 12A shows the rapid increase in GSIs in the maturing fish within both populations from March 2003 onwards. In March a significant increase ($P<0.05$) was observed between the immature and maturing individuals in both treatments. However, the lit group also revealed a significantly greater GSI than the control group (2.43% and 5.15% respectively). In May no difference ($P>0.05$) was observed between groups however there was a significant increase in the lit (18.31±0.74%) and unlit (18.49±0.8%) GSIs compared to the March values. However it must be stressed that at this point in time the GSIs and maturational status of the fish is not detrimental to harvest quality.

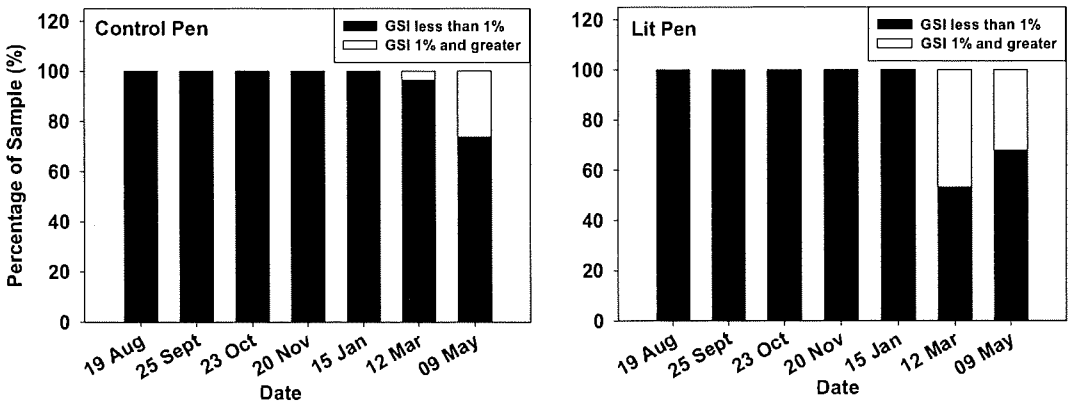


Figure 11 Maturation rates in Control and artificially illuminated (Lit) populations of Atlantic salmon, maintained under lights from seawater transfer in May 2002 until November 2002.

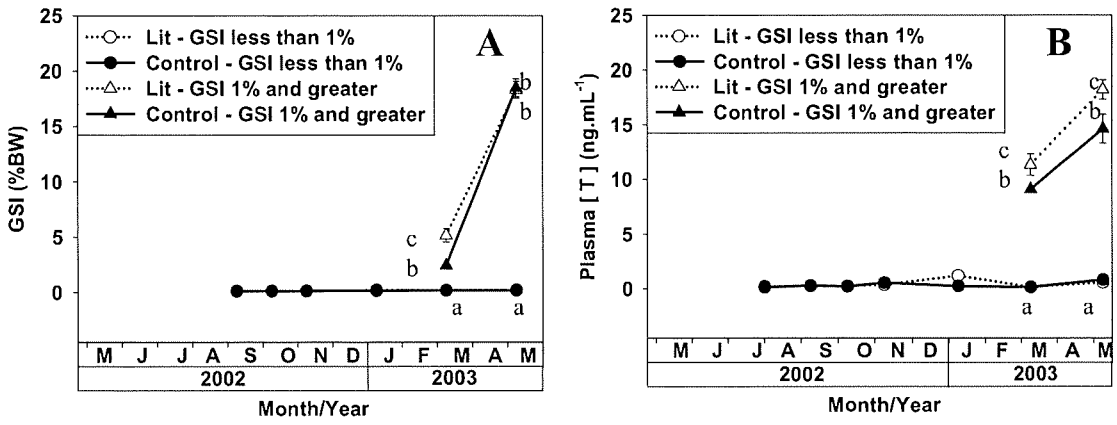


Figure 12 Gonadosomatic Index (A) and plasma testosterone levels (B) in a control and artificially illuminated population of Atlantic salmon (mean±1SEM), maintained under lights from seawater transfer in May until November. Similar letters indicate no significant differences within sample dates.

The GSI findings are corroborated by the plasma testosterone results (Figure 12B) that again show the initial increase to occur in March followed by another significant increase in May in both the lit and unlit populations. It is interesting to note that the lit population showed significantly higher testosterone levels than the maturing control population in both March and May samples. Plasma testosterone remains at basal levels in the immature individuals throughout the course of the trial.

Growth

The effects of the additional lighting were apparent by the first sample point after transfer in August. By this time the lit group were significantly ($P<0.05$) heavier than the unlit control population (Figure 13A) and this trend continued throughout the remainder of the experiment with the lit population having a mean weight of 2280±80g compared to 1880±70g in the unlit group which equates to a 5-6 week advance to harvest time and a significant increase in growth. The maturing individuals in the lit group were significantly ($P<0.05$) smaller (1820±90g) than the immature fish whereas the mature individuals from the control group although appearing smaller showed no significant difference when compared to the immature fish.

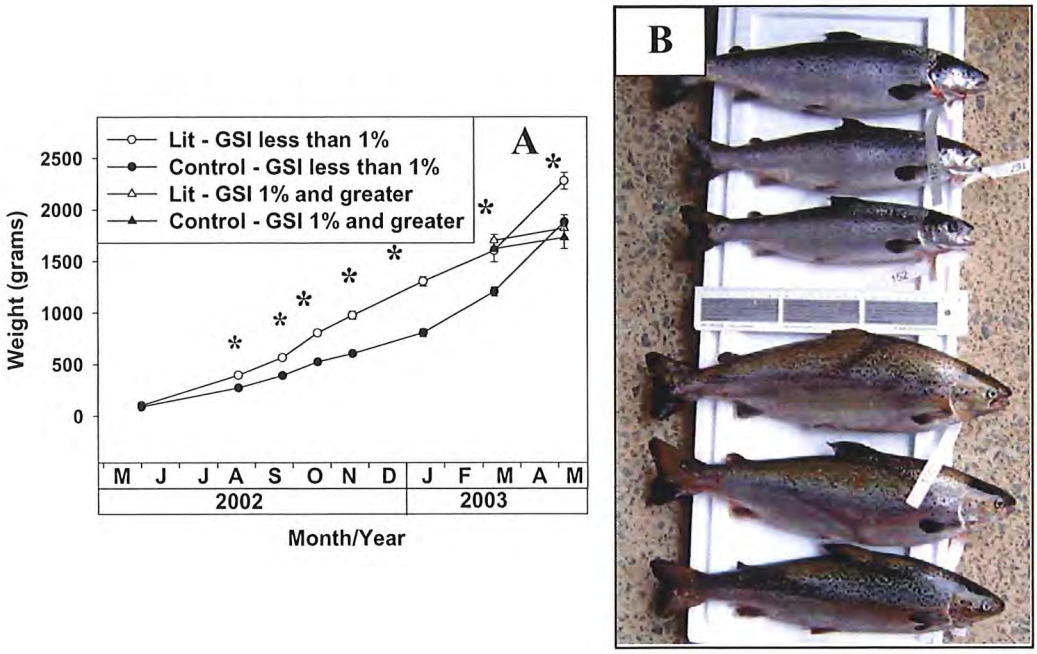


Figure 13 The change in weight of Atlantic salmon (mean±1SEM), maintained under lights from seawater transfer in May until November (A). Error bars not shown were too small to be depicted. * Indicates significantly different means within sample dates. Examples of fish collected from Control (top 3 fish) and Lit (bottom 3 fish) treatments in November (B).

With the increased growth rate there was also a clearly visible difference in condition factor in the two groups (Figure 13B). The lit group, which at the initiation of the trial, had a significantly ($P<0.05$) lower condition factor than the control population had increased rapidly after the onset of the lights and had a significantly greater condition factor than the controls by the 23 October (Figure 14). The condition factor then remained above 1.25 through to March after which it again increased to 1.45±0.03 in the final May sample point. In contrast the fish maintained on ambient photoperiod showed a sharp decline in condition factor from August (1.26±0.02) through to May (1.06±0.01) after which it began to rise again but still ended significantly lower than the lit group in May (1.26±0.02).

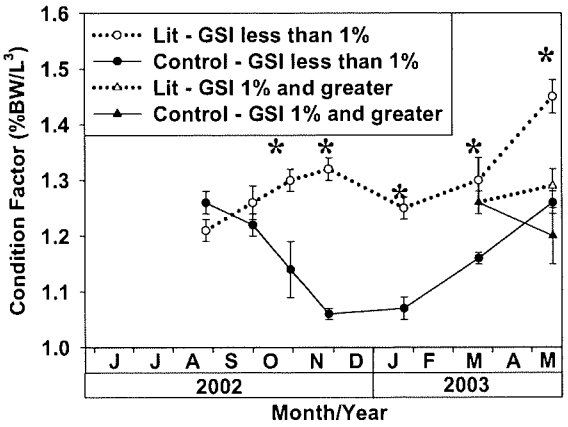


Figure 14 The change in condition factor of Atlantic salmon (mean±1SEM), maintained under lights from seawater transfer in May until November. Error bars not shown were too small to be depicted. * Indicates significantly different means within sample dates

Discussion

This trial conducted in collaboration with Tassal at their Killala site clearly demonstrated the efficacy of additional night time illumination in promoting increased growth rates in post smolt Atlantic salmon. In terms of production strategies it provides a tool with which farms can reduce the time required to grow stocks of fish to the market minimum of 3kg. In doing this they are then able to supply the processors and markets in advance of fish maintained under ambient illumination. This then reduces the gap period within the production cycle when fish the markets have traditionally been forced to accept frozen products.

Trial 4 – Mechanisms of Light Regulated Control of Melatonin Secretion in Atlantic salmon

Tank-based experiments at the Saltas P/L hatchery site at Wayatinah were conducted to assess melatonin production of Atlantic salmon juveniles and mature individuals in Tasmania. Previous work by Dr Porter documented melatonin profiles in Atlantic salmon adults and parr, and found melatonin levels, which are elevated in the dark phase, can be reduced to increasing degrees by additional dark phase lighting of increasing intensity (Porter *et al*, 1999). Due to Tasmania's closer proximity to the equator relative to Atlantic salmon-producing countries in the Northern hemisphere (42°S and 58°N respectively), ambient daylight intensity is in excess of 100-fold greater, and therefore it is suggested that higher additional lighting intensities are required during the dark phase to significantly depress melatonin production. This work is designed to assess the effects of varied day and night time light intensities on diel melatonin profiles of salmon in Tasmania.

Methods

Three distinct tank experiments were conducted over the 2002/2003 summer months at Saltas Ltd.'s Wayatinah Hatchery site. In each of these trials, blood samples were collected from groups of individual fish at various time points over a 24-hour period. Fish were not repeatedly sampled. Plasma from the blood samples was then assayed to determine melatonin levels in individuals, to provide a mean plasma melatonin concentration for fish sampled at each time point. Experimental set-up and results for each of these trials is described below.

Experiment 1 – Melatonin levels of adult Atlantic salmon maintained outdoors with additional artificial lighting of various intensities during the dark phase.

This experiment used adult Atlantic salmon reared in six outdoor brood stock tanks covered with shade cloth to limit daylight intensity to 10,000 lux (Lx). Three treatments in duplicate were maintained under either 10,000Lx light phase and 1Lx dark phase; 10,000Lx light phase and 10Lx dark phase; or 10,000Lx light phase and 100Lx dark phase. In December, blood samples were collected from 10 fish from each tank at midday (mid light) and midnight (mid dark).

Results

The mean plasma melatonin levels at midday and midnight of adult Atlantic salmon reared in outdoor tanks covered with shade cloth and provided with additional lighting of a range of intensities throughout the dark phase are presented in Figure 15. Mean plasma melatonin levels during the day were not significantly ($P>0.05$) different between tanks or treatments and averaged around 100 $pg.mL^{-1}$ regardless of the intensity of the additional lighting provided during the dark phase. Mean plasma melatonin levels were significantly elevated at midnight relative to those collected during the day in all treatments. Amongst the different light intensity treatments used during the dark phase, mean plasma melatonin levels were significantly ($P<0.05$) decreased in fish from those tanks that received 100 lux (342.2 $pg.mL^{-1}$) and 10 lux (373.7 $pg.mL^{-1}$), relative fish from those tanks that received 1 lux (446.1 $pg.mL^{-1}$) of additional lighting during the night. There was no significant difference in mean plasma melatonin levels at midnight between the 100 lux and 10 lux dark phase treatments.

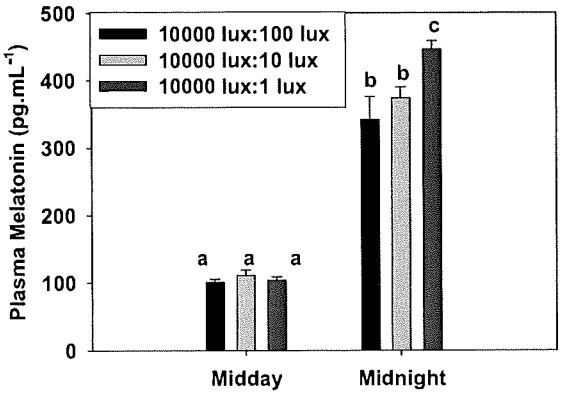


Figure 15 Mean plasma melatonin levels (±S.E.) of adult Atlantic salmon sampled at midday and midnight from 3 different light:dark intensity treatments in Experiment 1 (n=20). Bars with similar superscripts are not significantly different.

Experiment 2 – Plasma Melatonin levels in adult Atlantic salmon maintained indoors under various light intensities.

In this experiment, 60 adult Atlantic salmon which had been reared outdoors were moved inside and divided between 6 indoor research tanks (~1500L). Two of each of the tanks were located in one of 3 climate controlled rooms set with 12L:12D (hours light:dark) photoperiod. Light intensity treatments for the light and dark periods were different between rooms and are outlined in Table 1.

Table 1: Light and dark phase light intensity settings used as treatments in each research room and on each sampling date for Trials 2 and 3. Photoperiod was set at 12-hours light: 12-hours dark.

Date	25th November 2002		19th December 2002	
	Light phase	Dark phase	Light phase	Dark phase
Room 1	50 lx	0 lx	5 lx	0 lx
Room 2	50 lx	0.5 lx	5 lx	0.1 lx
Room 3	50 lx	5.0 lx	5 lx	0.5 lx

As indicated in the table, sampling was conducted on two separate dates following a 3 week acclimation period. Blood samples were collected from individual fish in all tanks at approximately midday (mid-light) and midnight (mid-dark).

Results

Figure 16 shows the mean plasma melatonin levels of adult Atlantic salmon sampled on the 25th November and 19th December 2002 in experiment 2. On the 25th November, following maintenance at 12L:12D photoperiod and light intensity treatments of 50 lx during the light phase and either 0, 0.5 or 5.0 lx during the dark phase, mean plasma melatonin levels obtained at midday and midnight were not significantly different either within or between treatments. Mean plasma melatonin levels at midday and midnight ranged between 300-400pg.mL^{-1}.

For the next sample on the 19th December, fish had been maintained on a 12L:12D photoperiod and light intensity treatments of 5.0 lx during the light phase and either 0, 0.1 or 0.5 lx during the dark phase. Mean plasma melatonin levels at midday and midnight were not significantly different within the 0 lux and 0.5 lux dark phase treatments, while in the tank exposed to 0.1 lux during the dark phase, the mean plasma melatonin level at midnight was significantly greater than that at midday. Within sample points, there were no significant differences between mean plasma melatonin levels amongst the fish sampled at midnight. However, at midday, fish exposed to 0.1 lux during the night had a significantly lower mean plasma melatonin level relative to those fish exposed to 0 lux during the dark phase. Mean plasma melatonin level at midday of fish exposed to 0.5 lux during the dark phase was not significantly different from the mean levels measured in either of the other treatments sampled at the same time point. Mean plasma melatonin levels at both midday and midnight ranged between 200-350pg.mL^{-1}.

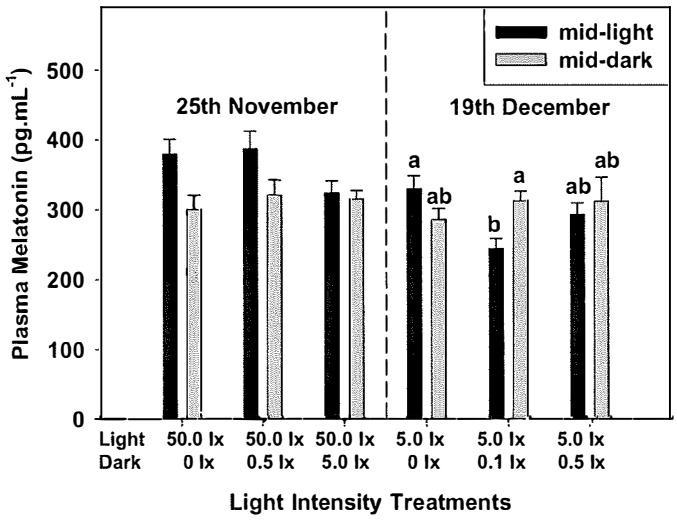


Figure 16 Mean plasma melatonin levels (±S.E.) at midday and midnight, of adult Atlantic salmon maintained indoors under 12L:12D photoperiod and various light intensity treatments (n=10). Bars with the same superscripts are not significantly different. No significant differences detected on the 25th November.

Experiment 3 – Melatonin levels of Atlantic salmon smolt maintained indoors under various light intensities.

Atlantic salmon smolt produced and reared at Saltas Ltd.'s Wayatinah hatchery site were used for this final experiments, which was conducted simultaneously with Experiment 2. An additional tank was placed in each of the 3 rooms listed in Table 1, and 60 smolt were placed in each of them (total fish for trial was 180). The smolt were thus exposed and acclimated to the same photoperiod and light intensity treatments used in Experiment 2 (refer to Table 1). On the same sampling dates as in Experiment 2, sampling was conducted at 6 time points during a 24 hour period. Each of these time points represented approximately midday, pre-dusk, post-dusk, midnight, pre-dawn and post-dawn. The purpose was to provide information on the circulating levels in melatonin in the smolt over a 24 hour period. At each sample point, blood was collected from 6 fish from each tank in each treatment room. Once sampled, fish were fin-clipped to preclude them from subsequent samples, and returned to their original treatment tank.

Results

Figure 17 shows the changes in mean plasma melatonin levels over a 24-hour period in Atlantic salmon smolt kept indoors on a 12L: 12D photoperiod. Light intensities were set at 50 lux during the light phase, and 5.0, 0.5 or 0 lux during the dark phase. Mean plasma melatonin levels changed significantly ($P<0.05$) over time in all three treatments, with elevations generally occurring at some point during the dark phase. In the 5.0 lux dark phase treatment, mean plasma melatonin rose late in the dark phase and peaked at pre-dawn. For the 0.5 lux dark phase treatment, mean plasma melatonin ranged around 160-180pg.mL^{-1} throughout the 24-hour period, with a brief increase to 251.07(±4.22) pg.mL^{-1} at post-dusk. Those fish exposed to 0 lux dark phase treatment displayed a similar pattern over the 24-hour period, peaking at 213.94(±17.60) pg.mL^{-1} early during the dark phase at post-dusk.

Between treatments at each time point, mean plasma melatonin levels of fish exposed to 0.5 and 0 lux during the dark phase, were not significantly different ($P>0.05$) at all time points except at post-dusk. Mean plasma melatonin levels of fish exposed to 5.0 lux during the dark phase were significantly different from the other two dark phase treatments at all time points with exceptions at pre-dusk and post-dawn.

Figure 18 shows the changes in mean plasma melatonin levels over a 24-hour period in Atlantic salmon smolt kept indoors on a 12L: 12D photoperiod with light intensities set at 5.0 lux during the light phase, and 0.5, 0.1 or 0 lux during the dark phase. Mean plasma melatonin levels changed significantly ($P<0.05$) over time in the 3 treatments, with gradual increases in means throughout the dark phase period.

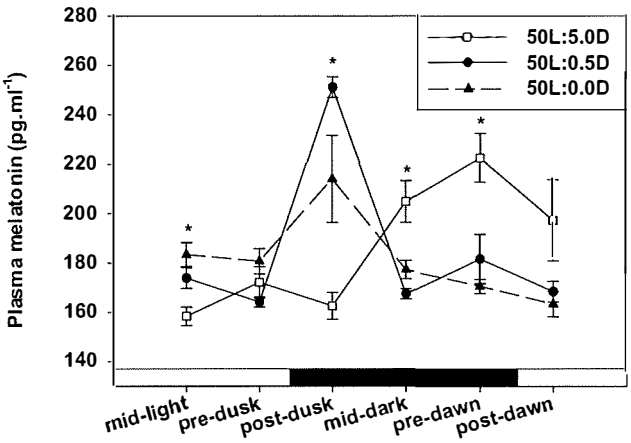


Figure 17 Mean plasma melatonin levels (±S.E.) over a 24 hour period on the 25th November 2002 in Atlantic salmon smolt held under 12L:12D photoperiod and various light intensities (n=6). Significant differences within sample points are indicated by *.

In the 0.5 lux dark phase treatment, mean plasma melatonin rose early in the dark phase and peaked at pre-dawn. For the 0.1 lux dark phase treatment, mean plasma melatonin over time exhibited a similar trend, rising early in the dark phase and peaking at pre-dawn, however the mean plasma melatonin levels were approximately 20-30ng.mL^{-1} lower than the mean levels recorded for the 0.5 lux dark phase treatment. Thus the fish in this treatment (0.1 lux) had significantly lower ($P<0.05$) mean plasma melatonin levels compared to fish in the 0.5 lux dark phase treatment at pre-dusk, mid-dark and post-dawn. Those fish exposed to 0 lux dark phase treatment displayed a similar pattern over the 24-hour period, peaking at 205.26(±9.32) ng.mL^{-1} at mid-dark. Mean plasma melatonin levels in this treatment started decreasing towards the end of the dark phase, at pre-dawn, unlike the other two treatments which both peaked at that time. Between

treatments at each time point, mean plasma melatonin levels of fish exposed to 0.5 and 0 lux during the dark phase, were not significantly different at all time points except at pre- and post-dawn.

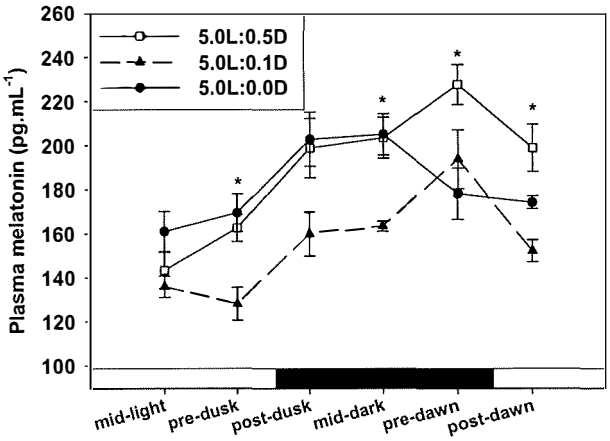


Figure 18 Mean plasma melatonin levels (±S.E.) over a 24 hour period on the 19th December 2002 in Atlantic salmon smolt held under 12L:12D photoperiod and various light intensities (n=6). Significant differences between treatments within sample points are indicated by *.

Discussion

Figure 15 shows the mean plasma melatonin levels of adults during the day are significantly lower than those collected during the dark phase. The mean plasma melatonin levels during the day are approximately 100ng.mL-1, which is comparable to mean levels in adult Atlantic salmon in Scotland (Porter *et al.,* 1999). Mean plasma melatonin levels during the dark phase ranged between 300-500ng.mL-1 and were significantly greater than day levels and are again comparable with dark phase levels in salmon and trout (Randall et al, 1995; Porter *et al.,* 1999; Bromage *et al.,* 2001). In this experiment the 10 and 100 lux additional night time illumination significantly reduced plasma melatonin levels compared to the fish under 1lux. This agrees with previous work on Atlantic salmon (Porter *et al.,* 2000) and shows a decrease in dark phase melatonin levels with increased light intensity.

The results of subsequent experiments indoors do not show expected melatonin profiles. The light phase levels in Experiment 2 (Figure 16) are far higher than would be expected under light phase conditions. It is suggested that despite the three week acclimation period the fish perceive the 50 and 5 lux light phase as a dark period due to the fact that prior to the trial they were maintained under far higher daylight intensities. This is illustrated in figure 17 where the 5 lux daylight intensities have higher melatonin levels than the dark phase intensities.

In both parts of Experiment 3 (Figures 17 and 18) melatonin 24 hour profiles exhibit increases in dark phase levels at some time points during the subjective dark phase. However, overall melatonin levels suggest poor acclimation to the indoor light levels, as described in Experiment 2, and the fact that dark phase melatonin levels decrease prior to daytime (light phase) suggests the presence of an endogenous rhythm of melatonin production remaining in the fish even after three weeks acclimation.

Trial 5 – The Development of Commercial Scale Techniques for the Inhibition of Precocious Maturation in farmed Tasmanian salmon

This trial was aimed at observing the effects of additional dark phase illumination on reducing maturation rates in out of season smolts and to investigate the gating mechanism responsible for the decision to mature in female adult Atlantic salmon. When Atlantic salmon begin maturing, energy is diverted away from growth for this purpose, and flesh quality deteriorates resulting in a substantial profit loss for the farmer. It has been suggested that the decision to mature is dependent on a critical amount of growth or energy reserve being attained by an individual before environmental cues such as photoperiod and temperature can initiate the start of the reproductive development. Part of this work aims to determine if such a critical size exists, so that final maturation rates can be predicted; and secondly, if the application of a long-day or constant light photoperiod regime from seawater transfer can inhibit maturation.

For the purpose of this investigation one thousand individuals were surgically implanted with passive integrated transponder (PIT) tags while still in freshwater. This has allowed morphometric data and hormone levels to be recorded from individually recognisable fish from parr in freshwater through seawater transfer in April 2003 up until harvest in June 2004. This then allowed a retrospective analysis of changes in size, condition and hormone status of individual fish that subsequently matured and allowed a comparison with those fish that remained immature. It was hoped this would then allow the detection of early morphometric indicators of maturation and allow commercial operations to select for fish that would not mature within the production cycle.

Methods

On February 17th 2003, one thousand pre-smolt from an all female stock of Atlantic salmon in freshwater at Saltas' Wayatinah Hatchery were surgically implanted with passive integrated transponder (PIT) tags. Morphometric measurements from the population were also collected on the 17th February and 7th April at Wayatinah. Tagged individuals were returned to the stock population following sampling and collectively transferred to sea on 21st April 2003 at Huon Aquaculture Company's Hideaway Bay site.

At seawater transfer the stock population was randomly divided amongst 4, 5m-research sea pens giving an initial stocking density of 2kgm^{-3}. Two pens were placed under constant artificial dark phase illumination (Lit Pens A and C). The two remaining pens were maintained under ambient light conditions as controls (Unlit Pens B and D). The two replicate pens of each treatment were held within a 40m-diameter production pen to prevent seal attacks and both treatments were maintained 400m apart to prevent any light contamination. Sampling was conducted at approximately 6-8 weekly intervals immediately prior to freshwater bathing for AGD from 16th June 2003 to harvest, in late June 2004. At each sample, all tagged fish were scanned, identified, and measurements of body weight and fork length were collected. The same thirty individuals from the tagged population from each pen were also repeatedly blood sampled on each date. Thirty untagged fish from each pen were also sacrificed on each date. Sacrificed individuals provided data and tissue samples for gonadosomatic (GSI) and hepatosomatic (HSI) indices, flesh pigment scores, and gonad histology, as well as additional morphometric data and blood plasma samples. At the conclusion of the

experiment, all tagged fish were sacrificed to obtain GSI's. This allowed tagged fish to be grouped according to treatment (Lit/Unlit) and maturity (mature/immature). Statistical analyses on tagged fish using one-way ANOVA have compared these groups at each date. Data obtained from untagged fish was also analysed using one-way ANOVAs to determine significant differences between lit and unlit treatments at each sample date.

Water Temperature

Mean monthly water temperature readings were recorded at Huon Aquaculture at a depth of three metres from June 2003 to June 2004. From June 2003 (12.96°C±0.17) a downward trend in water temperature was observed through winter into spring, reaching a low point in October 2003 (10.93°C±0.25). A marked increase followed in November (13.47°C±0.26) which continued into the summer months, peaking in February (16.84°C±0.13). With the onset of autumn, water temperatures gradually decreased, reaching 12.64°C±0.13 in May 2004.

Results

Recovery of PIT-tagged fish

Of the original 1000 fish PIT tagged in February 2003, a total of 723 survived through until harvest in early June 2004. The final number of PIT tagged fish in each pen at harvest is presented in Table 2 below.

Maturation at Harvest

In addition to the PIT tagged fish, 30 untagged females from each pen were also sacrificed at harvest. Gonads were collected from both PIT tagged and untagged fish, and weighed to determine gonadosomatic index for that individual. Those fish that had ovulated or had a GSI > 15% were classified as 'mature' females. The total number of females sampled and classified as mature are presented in Table 2 below, along with maturation percentages for each pen. Mean percent maturation for unlit treatment pens (B and D) was 31.0 and 30.0% respectively, while maturation in the lit treatment pens (A and C) were 0.35% and 7.4% respectively. Chi-square goodness-of-fit tests carried out on the observed maturation frequencies in pens A and C were compared to an expected frequency of 30.5 matures to 69.5 immatures. It was found that the observed frequency of maturation in these pens was significantly lower than expected if the light treatment had no effect (Pen A: Chi-square χ^2= 87.987, df=1, $p<0.01$; Pen C: Chi-square χ^2= 57.529, df=1, $p<0.01$). The percentage of mature fish within the untagged population was similar to the PIT tagged population. We can therefore conclude that the process of implanting the tags and subsequent implantation throughout the trial had no significant effect on the maturation.

Table 2: Total number of PIT tagged and untagged female Atlantic salmon recovered and sampled from each research pen and classified as mature at harvest in early June 2004.

Pen	Treatment	Number PIT tagged fish recovered	Total number of females sampled at harvest	Number of Mature females	Percent Maturation
A	Lit	177	207	1	0.35 %
B	Unlit	170	200	62	31.0 %
C	Lit	199	229	17	7.4 %
D	Unlit	177	207	62	30.0 %

Weight – PIT-tagged fish

Figure 19 shows the mean body weight of PIT tagged mature and immature fish from both lit and unlit pens throughout the experiment. A major finding of this work was that individual fish that matured were significantly ($P<0.05$) heavier than those that were immature at harvest (cf. 64.31±1.47g for unlit matures, and 59.49±0.99g for unlit immature fish in February 2003). Therefore, from this data it was possible to provide information on the fish that were predisposed to maturation up to two months before seawater transfer in April. The mean difference in weight between the unlit mature and unlit immature populations two weeks prior to transfer to sea was 86.87±1.5g and 81.30±1.1g respectively. After transfer to sea the unlit matures remained significantly heavier ($P<0.05$) than the unlit immature fish on all sample dates except at harvest. Between sampling in February 2004 and harvest in June 2004, maturing fish in unlit pens appeared to reach a growth plateau, and weighed on average 1092g less than their immature counterparts in the unlit pens at harvest.

Among the fish in the lit pens, there was no significant difference ($P>0.05$) in weight between those that matured and those that were immature at harvest on any sample date, with the exception of June 2004. As with the unlit treatment, the lit matures weighed significantly less at harvest than the lit immature fish (2315g and 3404g respectively).

Both lit matures and immatures were significantly heavier ($P<0.05$) than unlit matures from August 2003 to November 2003, and weighed significantly greater than unlit immatures for the duration of the trial prior to harvest. There was no significant difference in mean weight between lit immatures and unlit matures in February 2004. At harvest in June 2004 there was no significant differences between lit and unlit immatures (mean final weights of 3322.89±41.55g and 3360.84±53.33g respectively). There was also no significant difference in final weight between lit and unlit matures at harvest (2492.88±138.76g and 2267.88±64.94g respectively), which on average weighed 1029.85g less than immature fish in both treatments

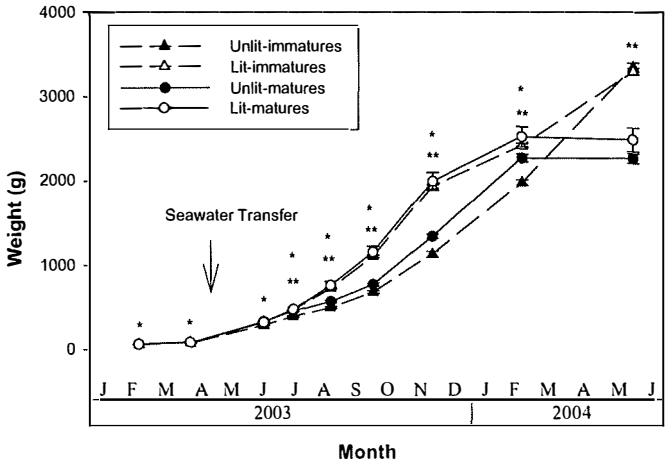


Figure 19 Mean body weight (grams) (±S.E.) of PIT tagged mature and immature fish from Lit and Unlit research pens prior to seawater transfer to harvest (February 2003 – June 2004). Significant differences between treatments denoted by *. Significant differences within treatments denoted by **.

Condition Factor – PIT-tagged fish

Mean condition factor of mature and immature PIT tagged fish in Lit and Unlit treatments were examined over the course of the experiment (Figure 20). Prior to seawater transfer in late April 2003, there were no significant differences between groups and mean condition factor of all fish significantly decreased from 1.34±0.02% in February to 1.14±0.00% in early April 2003 (Paired Sample t-test: $t=9.296$, $df=536$, $p<0.01$).

During grow out at sea, the condition factor of unlit matures and immatures were not significantly different from June through to September 2003. As a group, the condition factor of all unlit fish increased in June and July following transfer in April, which then decreased slightly in August, and then increased again to a peak of 1.43±0.01% in September. In November, mean condition factor of both Unlit groups decreased, particularly for the Unlit immatures, which had significantly lower condition factor than the Unlit Matures. In February 2004 it appeared that condition factor for the Unlit Matures remained relatively unchanged, while condition factor of the Unlit Immatures had improved and were no longer significantly different from their mature counterparts. At harvest in June 2004, condition factor of the Unlit Matures had decreased to 1.33±0.02%, while the condition factor of the Unlit Immatures further increased to be significantly greater than the matures, peaking at 1.54±0.01%.

Mean condition factor of Lit Matures and Immatures also increased slightly in June 2003 following seawater transfer; however not as rapidly as the Unlit fish. In June 2003, mean condition factor of Lit Matures and Immatures were significantly less than the unlit fish. This trend continued in July, however increased variation among Lit mature fish meant they were no longer significantly different from unlit immature fish. Condition factor of Lit immature fish remained significantly lower ($P<0.05$) than both unlit groups. In August 2003 there were no significant differences between any of the four groups, as condition factor of both Lit groups had increased. In September mean condition factor of both Lit groups had further increased and was significantly greater than the unlit groups. In November 2003 the mean condition factor of Lit Matures and Immatures peaked at 1.66±0.05% and 1.61±0.01% respectively and both were significantly greater ($P<0.05$) than the Unlit groups. In February 2004 there was a decline in mean condition factor

amongst Lit groups, with greater variation amongst Lit Matures which were no longer significantly different from the unlit fish. At harvest in June 2004, it appeared the mean condition factor of the Lit Matures had continued to decline and at 1.38±0.04% was not significantly different from the Unlit Matures, but were significantly less than both Immature groups. The mean condition factor of Lit Immatures at harvest had increased slightly since February 2004, and at 1.54±0.01% was not significantly different from the Unlit Immatures. With the exception at harvest, mean condition factor of Lit Matures and Immatures were not significantly different on any date throughout the experiment.

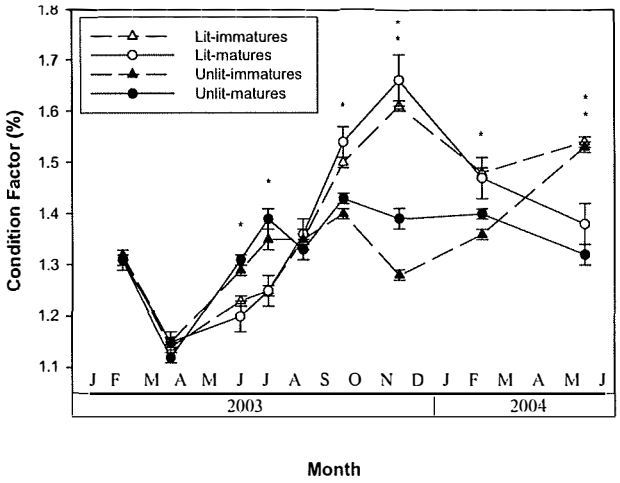


Figure 20 Mean Condition Factor (%) (±S.E.) of PIT tagged mature and immature fish from Lit and Unlit research pens prior to seawater transfer to harvest (February 2003 – June 2004). Significant differences between treatments denoted by *; significant differences within treatments denoted by **.

Overall the condition factors within the two treatments suggest that the lit population increased in weight following seawater transfer and the length of the fish did not increase at the same rate. However in the summer months following transfer the growth within the lit population reflects a rapid increase in both length and weight and hence this is apparent from the increased condition factor compared to the unlit fish. The similar condition factors observed between the mature and immature populations regardless of lighting regime suggests that the mature fish arrested somatic growth in favour of gonadal development while the unlit immature population showed increased growth rate compared to the lit population following the removal of the additional lighting in December 2003.

Gonadosomatic Index – Untagged fish

Mean GSI of both groups of fish did not exceed 0.5% from July to November 2003. During this period fish from Unlit pens had significantly greater ($P<0.05$) mean GSI's than Lit pens from August onwards suggesting that the decision to mature was made prior to this time point. In February 2004, mean GSI increased slightly in both groups with unlit pens (0.91±0.16%) still a having significantly greater ($P<0.05$) GSI than lit pens (0.35±0.08%). At harvest in June 2004, mature fish which had ovulated were noted but excluded from calculations of mean GSI's for each pen however fish with intact ovaries were included. In Unlit pens (7.31±1.57%) GSI had increased significantly from February, and were significantly greater than Lit pens (0.26±0.01%), which remained relatively unchanged for the duration of the experiment.

Hepatosomatic Index – Untagged fish

Collection of liver weight data for determination of hepatosomatic index (HSI) commenced in July 2003 (Figure 21). Mean HSI of fish in Unlit pens decreased from July to September, while Lit pens increased initially in August, they had dropped by September. HSI of Unlit pens were significantly less than Lit pens during this period. Unlit pens subsequently increase in November to 1.02±0.02%, and were not significantly higher than the lit pens, 1.00±0.02%. In February 2004, mean HSI continued to increase in unlit groups to 1.067± .03% and were significantly higher than lit pens which had dropped to 0.865±0.03%. Both groups exhibited a decrease in HSI by June 2004, with unlit fish remaining significantly greater than lit fish.

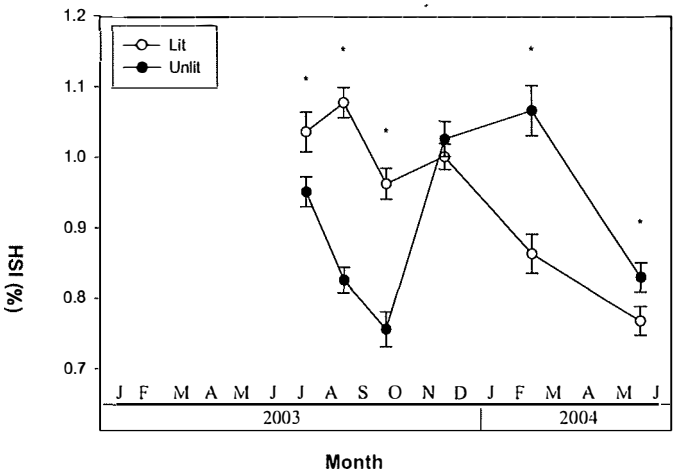


Figure 21 Mean Hepatosomatic index observed in female Atlantic salmon maintained under either constant light or ambient photoperiod from the summer solstice. Significant differences between treatments are denoted by (*).

Plasma Testosterone – Untagged fish

Plasma Testosterone (T) concentration of samples from untagged sacrificed fish were assayed from samples collected after sea transfer in June 2003 to harvest in June 2004. Plasma T levels were basal and at the limit of the RIA sensitivity from June 2003 through to September after which there was a slight increase in levels in November followed by a significant increase ($P<0.05$) in February and June 2004. In February 2004, unlit pens rose sharply to 8.17±3.6ng.mL^{-1}, while lit pens increased to 2.5±1.3ng.mL^{-1} However, due to the large variation of the samples, no significant differences were detected. By harvest in June 2004, plasma T in unlit pens decreased slightly (6.4±1.8ng.mL^{-1}) as lit pen levels increased (7.05±ng.mL^{-1}), with no significant differences detected.

Plasma Estradiol – Untagged fish

Mean plasma Estradiol (E_2) levels of untagged sacrificed fish were assayed from samples collected from June 2003 to June 2004. From June 2003 through to November 2003, very low plasma E_2 concentrations were detected as hormone levels were still at basal levels. It was not until February 2004, that an increase from basal levels was detected at which point plasma E_2 levels with unlit pens (2.34±0.31ng.mL^{-1}) were significantly higher than lit pens (1.36±0.28ng.mL^{-1}) although still very low. Plasma E_2 concentrations of the two groups converged in June 2004 with no statistical differences detected.

Histological Examination of Ovaries

Transverse sections of ovaries from fish in both Lit and Unlit pens were visually assessed under magnification and oocytes present were classified according to criteria described in Bromage & Cumaranatunga (1988). A brief summary of these classifications are described in Table 3.

Representative sections of ovaries from Lit and Unlit pens at each sample date are presented in the following pages as Figures 21 – 28. Figure 28 depicts each section in chronological order for ease of comparison of the rate of oocyte development and growth in both treatments throughout the trial.

Initially in June (Figure 22), the ovaries of both the lit and unlit groups display oocytes in the early and mid perinuclear stages of development (2a and 2b respectively). By July (Figure 23) the unlit treatment has significantly fewer stage 2a (early perinuclear oocytes) compared to the lit group suggesting that the decision to mature may have been made to mature by this point in time. Samples collected in August showed marked visual difference with the unlit fish now displaying oocytes in the late perinuclear (stage 3) and early vesicular (stage 4a) stages of development. In contrast the lit group were still showing stage 2a and 2b stages of development.

The gonadosomatic indices of fish within both groups had not yet shown a significant increase and therefore histological examination was the only method of detecting differences between treatments. By September there has still not been an increase in GSI's however the unlit treatment was now showing a large number of developing oocytes in the early vesicular stages. This was in contrast to the lit pens that remained at stage 2c (mid perinuclear).

The November sample point (Figure 26) coincided with a significant increase in GSI's of the unlit treatment. From the histology carried out on these fish it can be clearly seen that at this time point a significant increase in oocyte diameter was observed as the ovaries develop through the vesicular stage to late vesicle stage (4b). In comparison the lit group had some oocytes in the vesicle stage but interestingly also displayed signs of atretic oocytes. These are the result of early reproductive development being initiated but then being arrested before vitellogenesis occurs (Bromage et al., 2001). Figure 26 shows the clear size comparison between the two groups and again, a large number of atretic oocytes can be seen in the lit ovaries whereas the unlit oocytes have already initiated yolk absorption (vitellogenesis) prior to ovulation. Finally, the May sample point (Figure 28) displays histology from only the lit group as the unlit females had already ovulated and histology on the ovaries was not possible. Again within the lit ovaries there are some oocytes at the vesicle stages however there are still large numbers of atretic eggs visible.

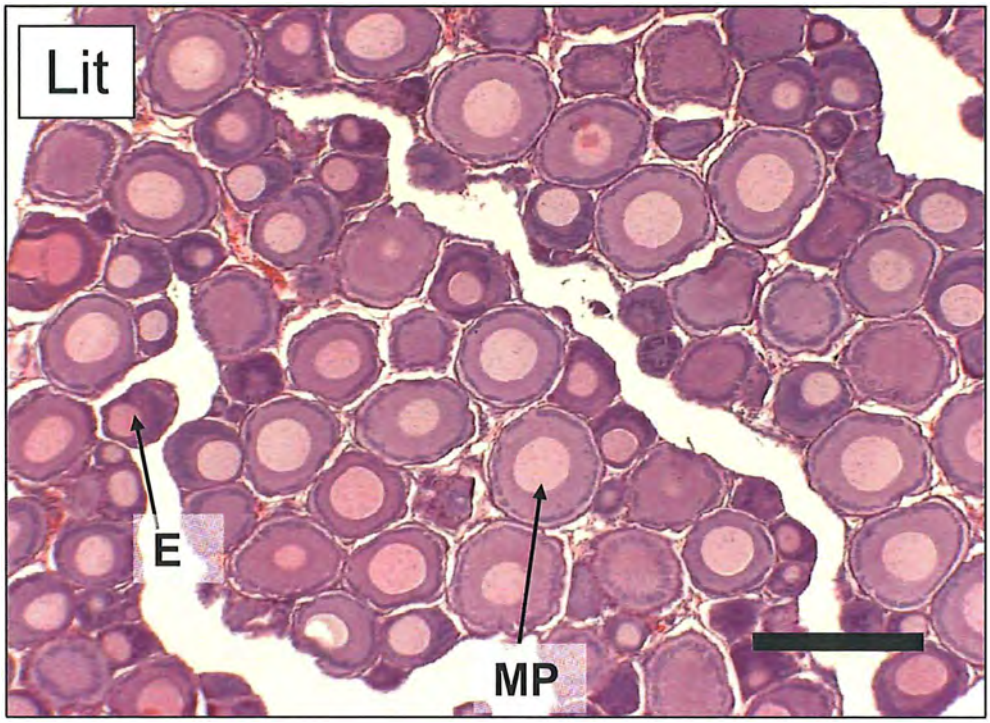
This work clearly emphasises the effects of additional illumination on the reproductive development in female Atlantic salmon (Figure 29). There is an initial reduction in the rate of development through to stage 3/4 prior to an inhibition prior to vitellogenesis (stages 5-7). Furthermore, when histology and the GSI's are compared it is evident that external appearance and size of the ovary is not a satisfactory indication of ovarian development and only by microscopic examination can the developmental stages be confirmed.

Table 3 Description of oocyte development and stage as reported by Bromage and Cumaranatunga (1988) in rainbow trout.

Stage	Descriptor	Characteristics
1	Chromatin nucleolar (C)	20-50μm diameter; narrow ring of basophilic (dark blue staining) cytoplasm.
2a	Early perinucleolar (EP)	Strongly basophilic cytoplasm; Prominent nucleus with one large and several small nucleoli.
2b	Mid-perinucleolar (MP)	Balbiani bodies appear close to nucleus; Basophilia restricted to Balbiani bodies.
2c		Balbiani bodies move towards periphery of oocyte; Basophilia becomes reduced.
3	Late perinucleolar (LP)	Balbiani bodies have disappeared; Cytoplasm has become acidophilic; 300 – 430μm diameter; Nuclei contain many nucleoli lying close to an undulated nuclear membrane.
4a	Early vesicular (EV)	Marked by appearance of vesicles ('air' pockets) at the periphery of cell; Gradually more are formed and area occupied extends towards nucleus.
4b	Late vesicular (LV)	Vesicles fill whole of cytoplasm; Characteristic net-like/ reticulate/ 'frothy' appearance to cytoplasm.
5,6,7	Exogenous yolk (EY)	Enormous increases in cytoplasmic size (Secondary growth stage); Uptake and incorporation of exogenous yolk (vitellogenin); Characterised by appearance of yolk granules (stain pink) adjacent to oocyte periphery.
Atretic	(AT)	Atresia can occur at any stage of development; characterised by 'collapsing' of oocyte wall, and separation of oocyte wall from surrounding connective tissue.

Characterised by presence of Balbiani bodies – densely packed, blue-staining organelles in the cytoplasm forming a ring around the oocyte nucleus.

Figure 22 Transverse sections of Atlantic salmon ovaries from Lit and Unlit pens sampled in June 2003, showing early perinucleolar (EP) and mid-perinucleolar (MP) oocytes. Scale bar represents 300µm (magnification x40).



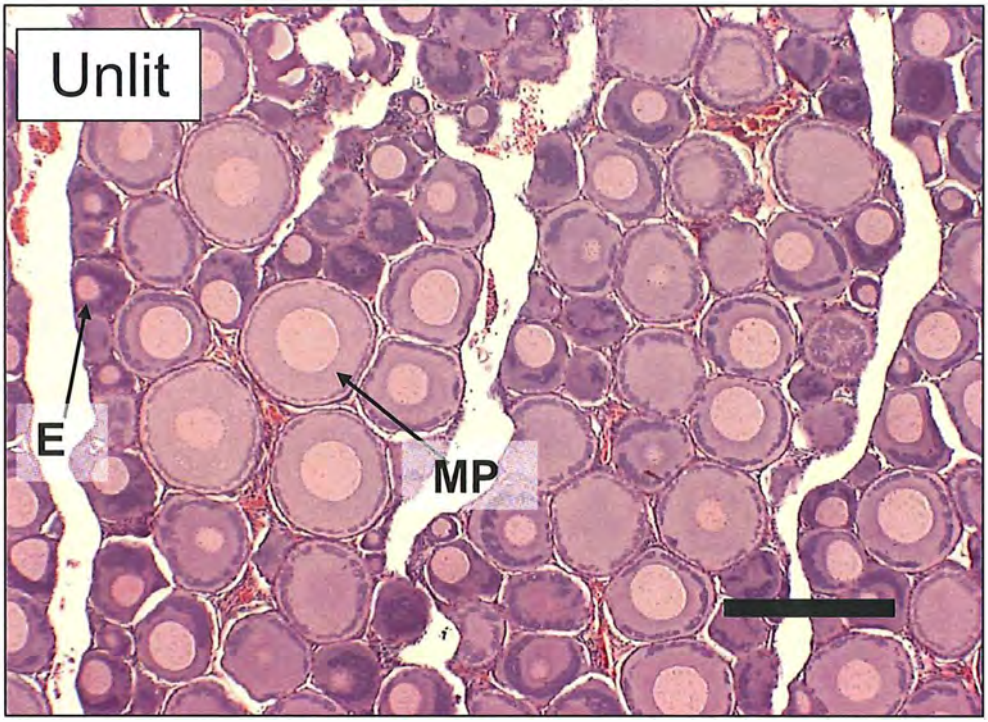


Figure 23 Transverse sections of Atlantic salmon ovaries from Lit and Unlit pens sampled in July 2003 showing early perinucleolar (EP) and mid-perinucleolar (MP) oocytes. Scale bar represents 300µm (magnification x40).

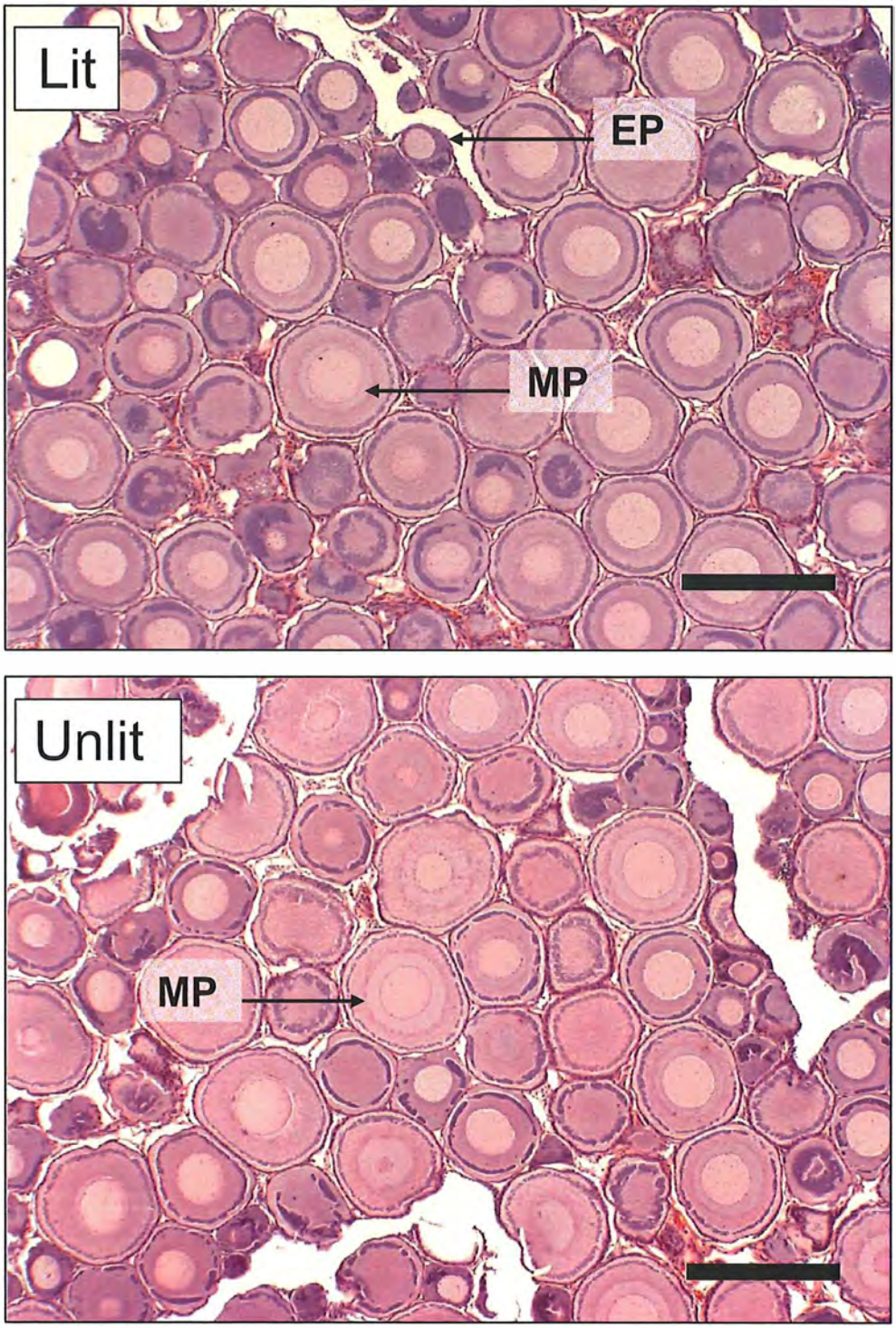


Figure 24 Transverse sections of Atlantic salmon ovaries from Lit and Unlit pens sampled in August 2003, showing late perinucleolar (LP) and early vesicular (EV) oocytes. Scale bar represents 300µm (magnification x40).

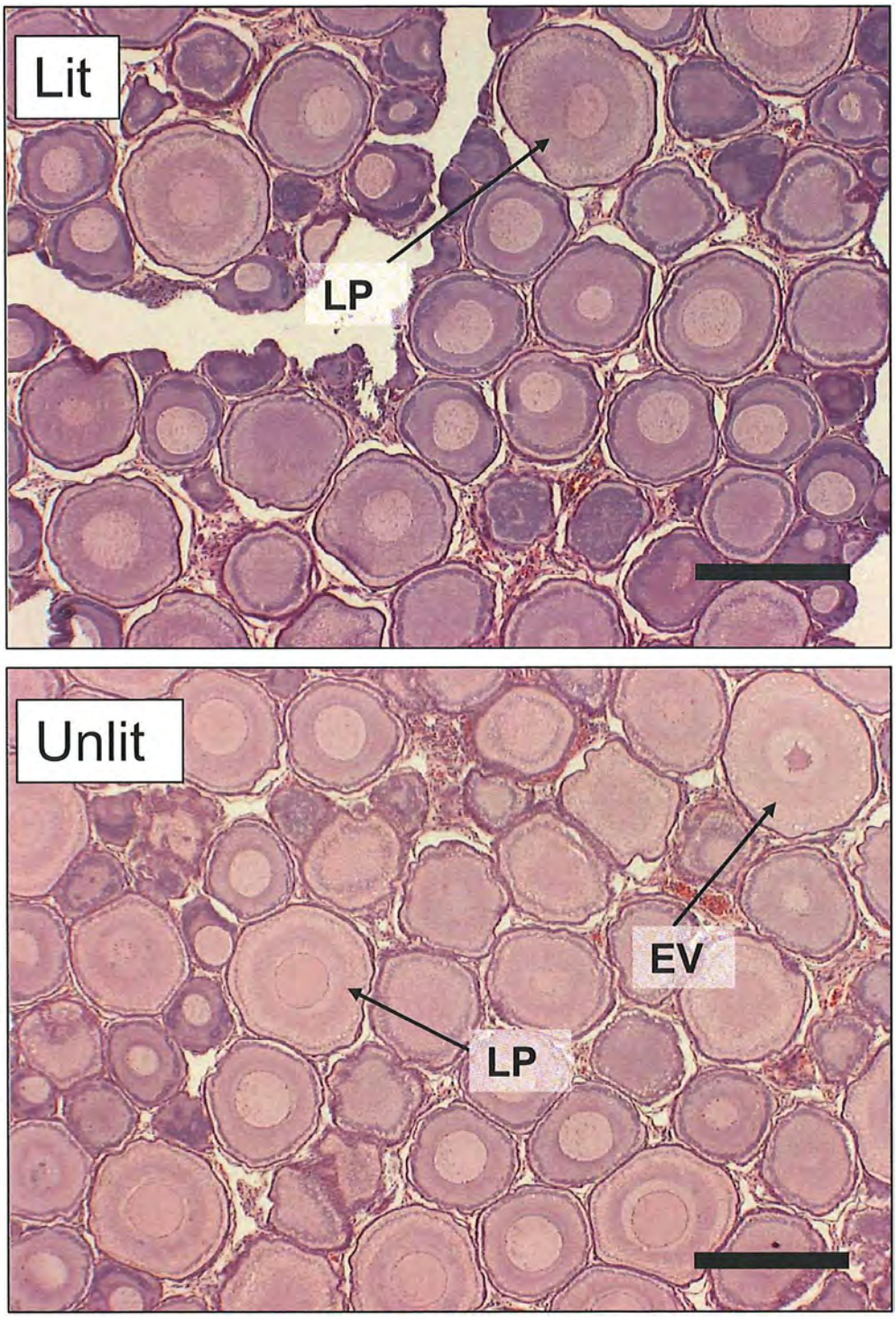


Figure 25 Transverse sections of Atlantic salmon ovaries from Lit and Unlit pens sampled in September 2003, showing mid-perinucleolar (MP) and early vesicular (EV) oocytes. Scale bar represents 300µm (magnification x40).

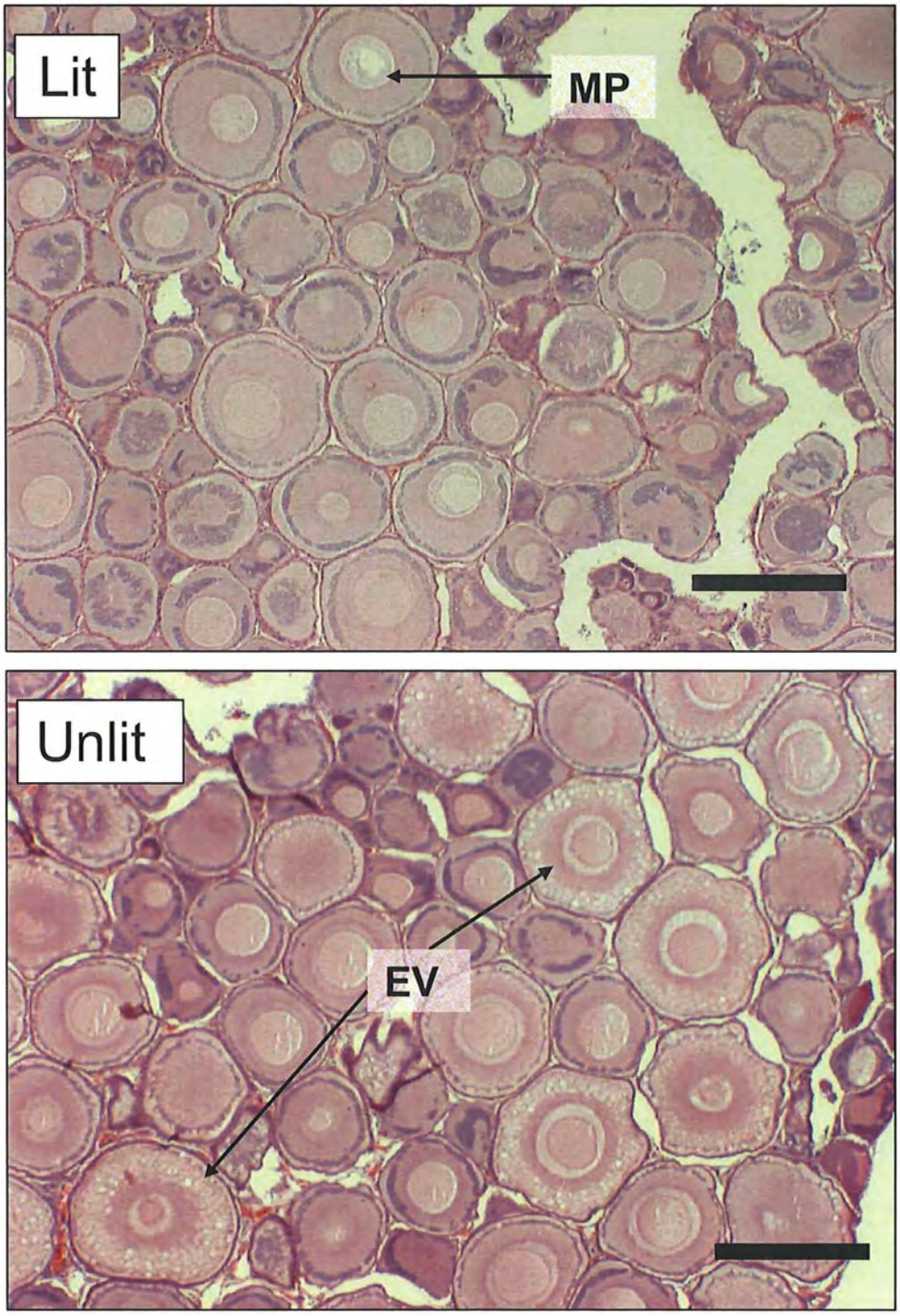


Figure 26 Transverse sections of Atlantic salmon ovaries from lit and unlit pens sampled in November 2003, showing early vesicular (EV), vesicular (VS), late vesicular (LS) and atretic (AT) oocytes. Scale bar represents 300µm (magnification x40).

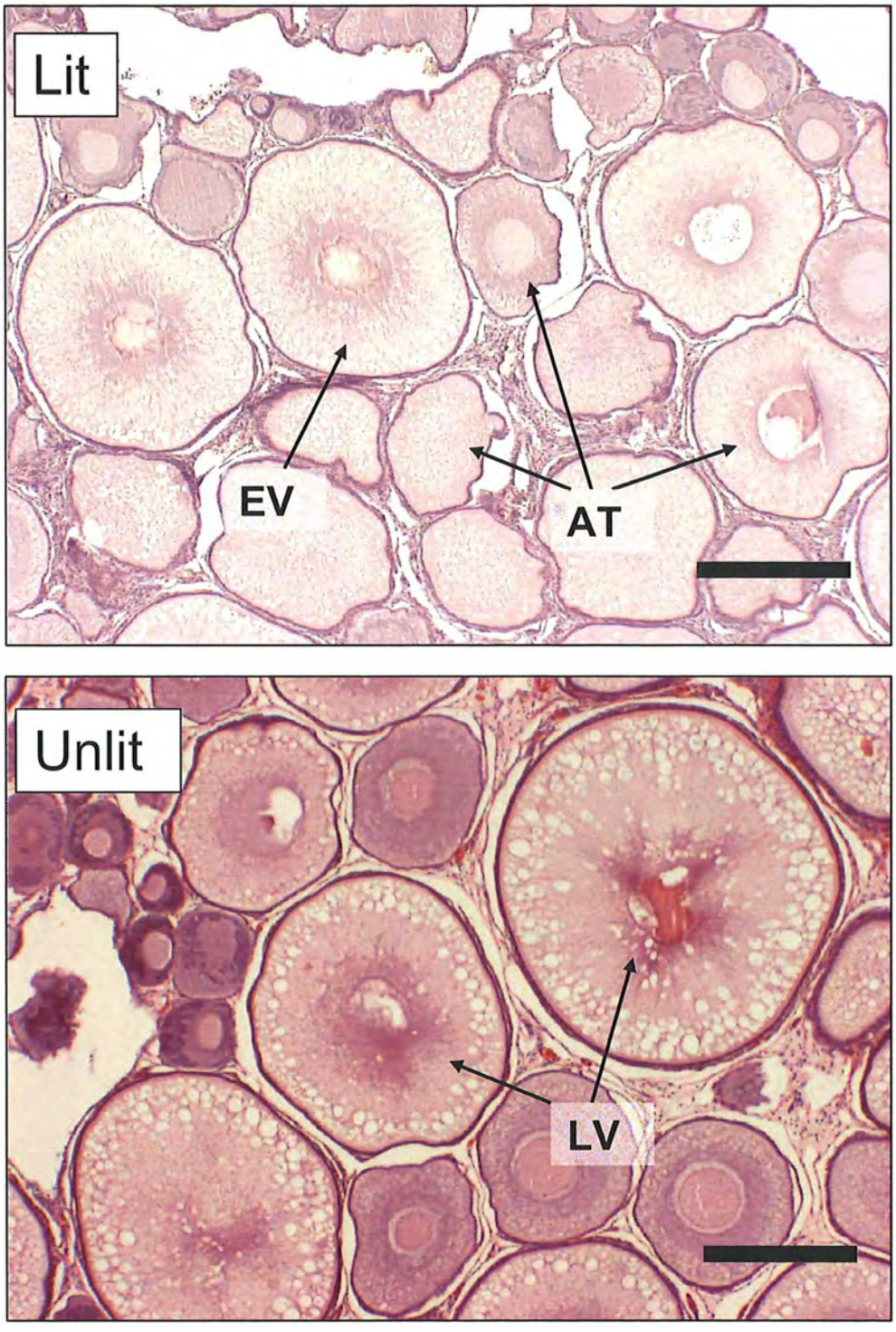


Figure 27 Transverse sections of Atlantic salmon ovaries from Lit and Unlit pens sampled in February 2004, showing atretic (AT) oocytes and exogenous yolk (EY) granules. Scale bar represents 1000µm (magnification x10).

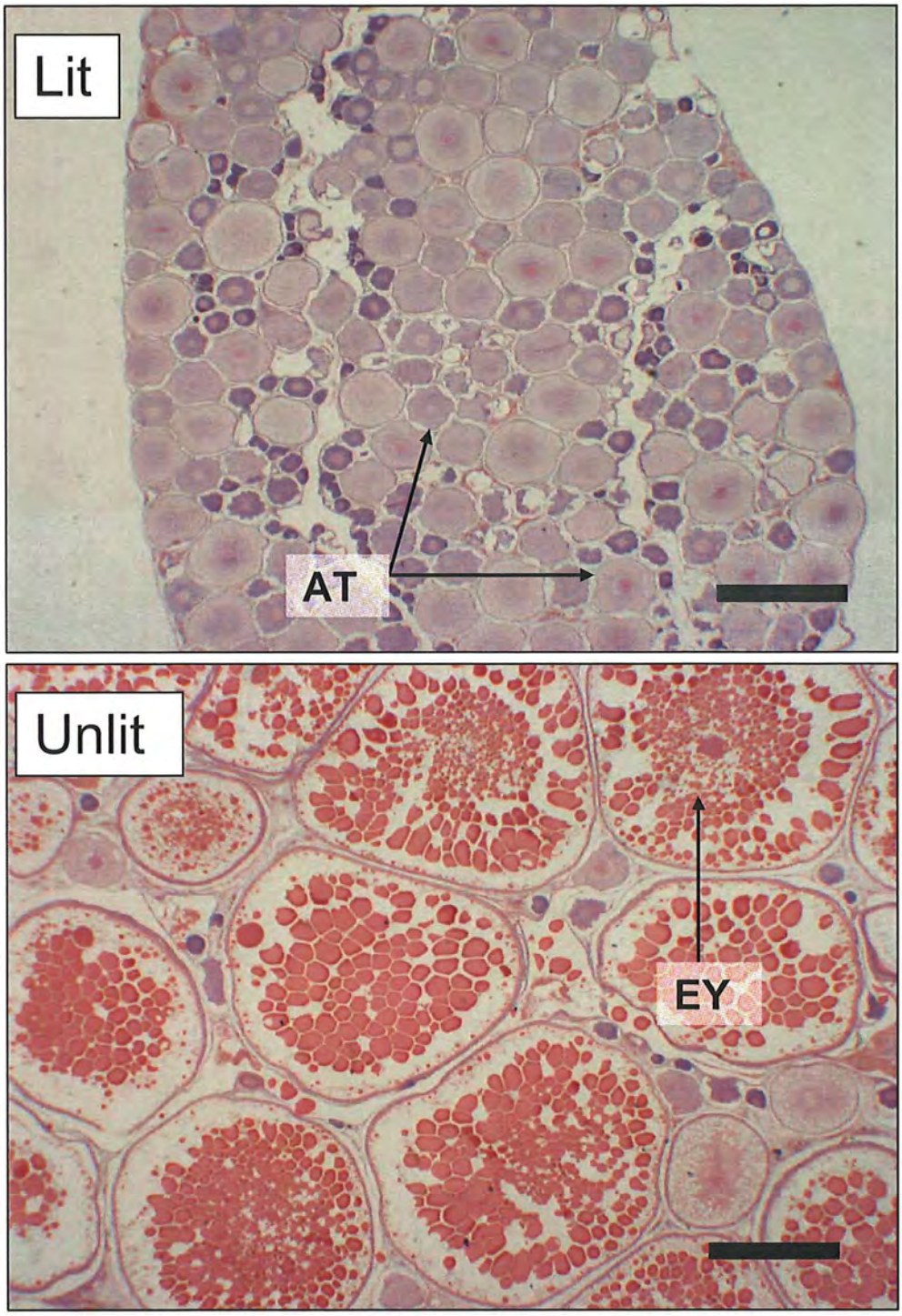


Figure 28 Transverse sections of Atlantic salmon ovary from a Lit pen sampled in May 2004, showing atretic (AT) and late vesicular (LV) oocytes. Scale bar represents 1000µm (magnification x10).

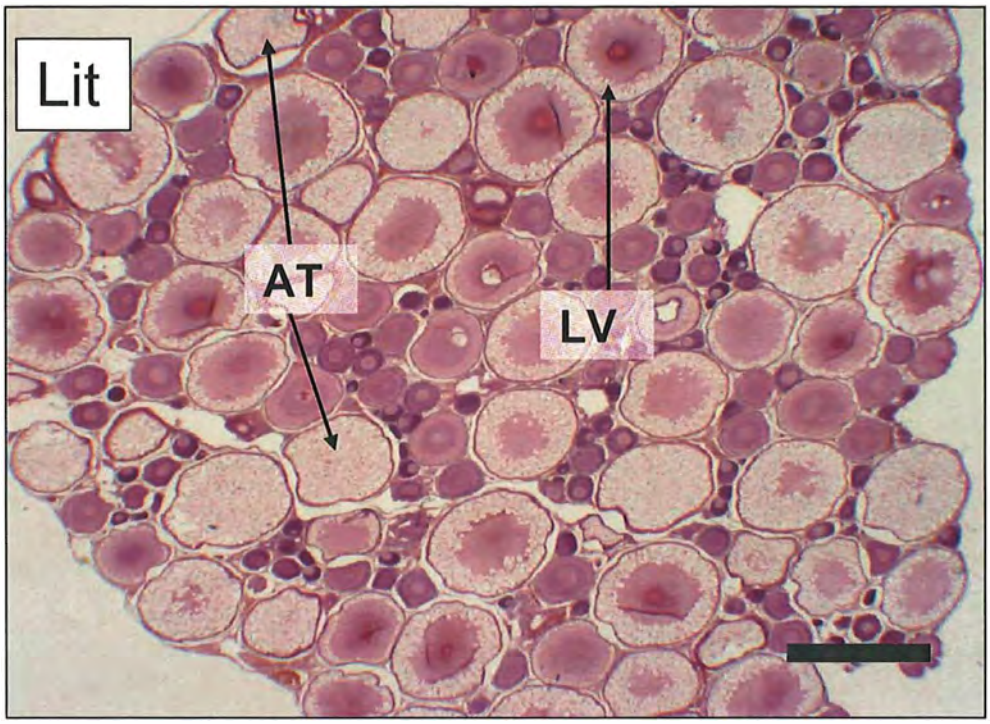
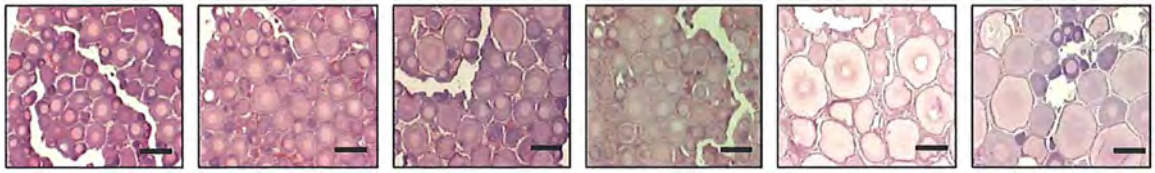


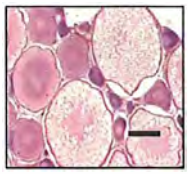
Figure 29 Transverse sections of Atlantic salmon ovaries from Lit and Unlit pens from in series from June 2003 to May 2004. All sections at x40 magnification and scale bars represent 300μm.

Lit pens

June 2003 July 2003 August 2003 September 2003 November 2003 February 2004

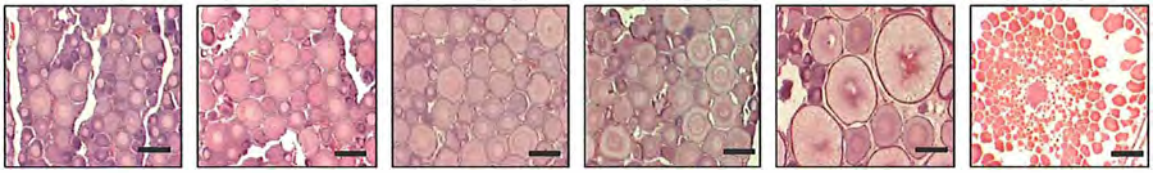


May 2004



Unlit pens

June 2003 July 2003 August 2003 September 2003 November 2003 February 2004



Discussion

One of the most interesting findings of this work was the fact that the size of an individual prior to seawater transfer was significantly different in fish that would subsequently mature or remain immature throughout the production cycle. This information was achieved through the PIT tagging of individuals while Atlantic salmon parr were still in the first freshwater phase of development. This allowed the retrospective assessment of weight throughout the experiment and also allowed the growth history of mature and immature fish at the final sample point to be calculated. Mature fish maintained under ambient (unlit) conditions were significantly heavier (64.31±1.47g) than those that were immature at harvest (59.49±0.99g) two months before seawater transfer in April. This had increased to 86.87±1.5g and 81.30±1.1g between the unlit mature and unlit immature populations respectively two weeks prior to transfer to sea. This average 6g difference between mature and immature populations has significant implications for an industry that traditionally looks for large smolts from the hatcheries and freshwater sites. By asking the hatcheries to produce larger smolt the on-growers may in fact be predisposing the fish to mature early as the gonadosomatic index results and hormone analysis suggest that the decision to mature in Tasmanian grown Atlantic salmon may by made in freshwater prior to seawater transfer or soon after transfer.

The unlit mature fish also maintained a higher condition factor than the unlit immature fish up to the final sample before harvest. Whilst this was certainly true when comparing unlit mature and immature fish data this was not observed in the lit treatment. A high condition factor is a typical indication in temperate water fish species that energy reserves within the fish are high and may well lead to the individual maturing if its size and environmental parameters allow. Mature and immature fish from the lit pens followed similar trends of weight gain and condition factor throughout their time at sea until harvest. Whereas unlit fish appeared to form into two groups early on, destined to become mature and immature fish, and continued to drift apart over the trial there did not appear to be any difference between the lit fish until harvest. It is suggested that the exposure to constant artificial illumination following seawater transfer synchronised the population in terms of growth and maturation. This means that even individuals that may have had sufficient size and energetic status before seawater transfer and therefore be predisposed to mature were recruited into the immature population due to the phase shifting of the seasonal light cycle. Again this has major implications for the on-growers as it means that even if larger smolt are transferred to sea with a predisposition to mature then the use of artificial lighting can be used to reduce the percentage of individuals that will mature before harvest. This then gives the salmon aquaculture industry within Tasmania two methods of reducing maturation in out of season smolts during the on-growing stage.

When comparing growth rates within the treatment groups it can be seen that there is a reduction in growth rate in the lit immature population from December 2004 onwards while in the unlit immature group the growth rate increases. The result of this change in growth rates is that the weight advantage observed in the lit group following seawater transfer is lost and there is no significant difference between the two treatment weights at the conclusion of the trial in June 2004. However, the reduction in growth rate in the lit group coincided with the lights being removed from the pens in December (summer solstice). This is when the natural day length is at its maximum and it was decided to remove the lights at this point to prevent any further recruitment from the immature to mature cohorts (Duston *et al.,* 1995). However, in Tasmania the summer solstice has 15.2 hours of daylight and when compared to the 24 hours the fish had been receiving appears as a short day winter photoperiod. The

result of this is that the fish then enter a winter growth pattern and the rate of growth slows even though the temperature remains constant or in some cases increases. In hindsight, after analysis of the current data, it is suggested that by maintaining the lights in the pens throughout the seawater phase of production right through to harvest would not in-fact increase maturation rates and would have maintained the growth advantage observed in the lit immature population.

Benefits and Adoption

There are clear benefits to the Tasmanian salmon industry from the results and information gained in this project. Initial trials into the physiological response of salmonids to additional dark phase illumination allowed the design and effective use of artificial lighting throughout the remainder of the project. This was evident in the first of the commercial scale trials into delaying maturation in mixed sex stocks of Atlantic salmon. The 8 week delay in maturation time was estimated by Pheroze Jungalwalla of Tassal Ltd. to save the company in the order of $2.3 million per annum following adoption into the Tassal production strategy (Tassal press release 2003).

All fish farmers strive to maximise production and the second of the commercial scale trials conducted at Tassal's Killala Bay provided the Tasmanian farms with a method to not only increase growth rates but also decrease the production time to harvest. The use of constant additional lighting immediately following seawater transfer resulted in an 18% increase in growth over twelve months, this relates to an estimated six week reduction in the production cycle. This technique is now being utilised by both main on-growers in Tasmania to boost growth following seawater transfer.

The work into reconditioning female Atlantic salmon was instigated by Huon Aquaculture in an attempt to recover previously mature fish that represent an economic loss to on-growers. The use of constant additional illumination to advance the reconditioning process significantly shortened the recovery time of these fish and is now incorporated into the production strategy of Huon Aquaculture to ensure these fish can now be reconditioned and sold for profit in addition to playing an important role in filling the "gap period".

However, the ultimate aim of the Tasmanian salmon farmer is to avoid maturation altogether. The final trial addressed the use of lights to inhibit maturation in out of season smolts, traditionally, the cohort that exhibits the greatest maturation rate. This work provided important information relating to the size of smolt at seawater transfer and as a result of this the on-growers are now requesting smaller fish at seawater transfer (D. Mitchell pers. comm.). In addition the reduced maturation observed in the lit population has meant that both Huon Aquaculture and Tassal Ltd have adopted this procedure in all out-of season production fish in 2005. This work has been estimated to have a direct saving for Huon Aquaculture of approximately $1 million per annum (D. Mitchell pers. comm.) and Tassal Ltd $1.5 million per annum (C. Selkirk pers. comm.).

These cost benefits are in addition to the saving to the farms in relation to reduced grading costs and increased health benefits observed by both companies due to the lower percentage of maturation in the stock. It is suggested that this may provide a gross financial benefit of over $4 million per annum.

The benefits to the Tasmanian salmon Industry can best be summarised in figure 30. This shows the supply of marketable fish over the course of a year in 2001 and the increased production in 2004. Therefore, by using photoperiod regimes developed during the course of this project the industry is now in a position to supply fresh 3-5kg fish throughout the year so reducing the need for frozen product.

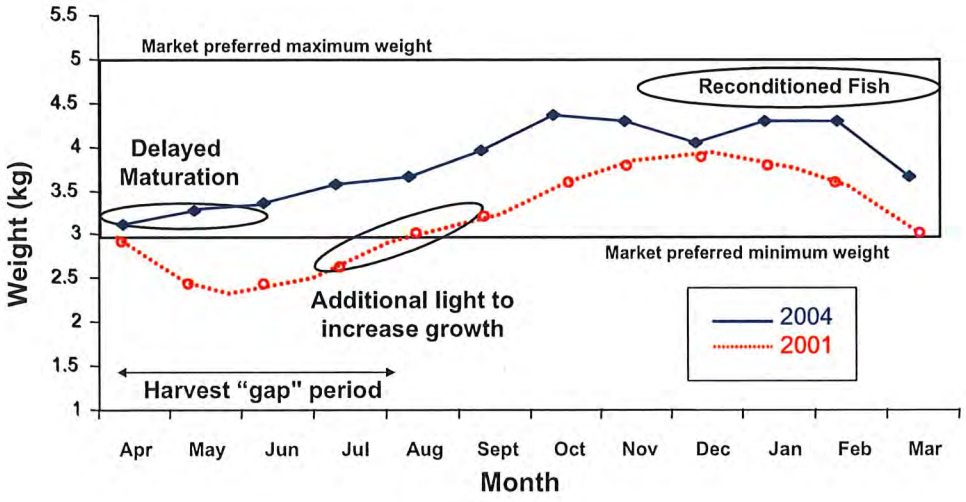


Figure 30. Mean monthly harvest weight (HOGG) of Atlantic salmon in 2001 and 2004. Data kindly supplied by Huon Aquaculture.

Finally, the information gained from this project is not only applicable to salmonids. In almost all sectors of finfish aquaculture there are production issues that lend themselves to photoperiod manipulation such as conditioning of Southern bluefin tuna or the winter reduction in growth observed in kingfish. After consultations with members of the Australian Barramundi Farmers Association photoperiod manipulation trials are now being conducted on juvenile barramundi. Work is also underway on Murray cod by members of Deakin University to control spawning times. It is suggested that now the basic problems experienced with the initial use of artificial lighting in Australia have been corrected that these techniques will become common practice in most forms of finfish culture within Australia over the next ten years.

Planned Outcomes

Only one main planned outcome was initially proposed for this project, and that was to develop commercially efficient strategies for managing the problem of precocious sexual maturation in farmed Tasmanian Atlantic salmon. In the process, techniques developed for assessing maturation will allow accurate non-destructive monitoring of stock sexual condition that will be of significance in other areas of stock management, for example assessment of broodstock.

In response to this, all four project objectives have been completed and all contributed to the final outcome.

1. An improved understanding of the mechanisms of light regulated control of melatonin secretion in salmon. The melatonin radioimmunoassay set up at the University of Tasmania allowed the accurate measurement of the fish's physiological response to light intensities while the tank trials at Saltas provided the background response of plasma melatonin synthesis in relation to varying light levels.

2. An improved understanding of the association between melatonin levels and reproductive development in salmon (light mediated effects on melatonin synthesis and its control of the timing of maturation). This output was crucial to the success of the planned outcome and it is now possible to accurately predict the minimum intensities and therefore number of lights required throughout a salmon cage to achieve the desired light mediated results. This has been shown to vary for growth and maturation responses.

3. The capacity to rapidly and non-destructively assess the acute reproductive condition of caged salmon. This output was hoped to provide a simple method to assess reproductive state using a mucus sample. Unfortunately although significant correlations were found between steroid levels and mean oocyte diameter these parameters were not measurable in external mucus samples.

4. The development of commercial scale photomanipulation techniques for the retardation or prevention of precocious maturation in farmed Tasmanian salmon. This output related directly to the planned outcome of the project and addresses the inhibition of maturation in out of season smolts together with developing photoperiod techniques to produce market size fish (3-5kg) year round. This was achieved though delaying the onset of maturation, increasing growth and reconditioning previously mature female Atlantic salmon (figure 30). It was also shown that the inhibition of maturation was possible through the use of constant illumination from seawater transfer and that the size of smolt had a direct impact on the likelihood of maturation prior to harvest.

Through output achievements the project was able to complete all planned outcomes. Salmon farmers in Tasmania now have the tools with which to supply fresh salmon year-round and significantly reduce early maturation in out-of-season smolt. This outcome has significant financial implications for the Tasmanian industry and increases its competitiveness in the global market.

Further Development

The use of artificial illumination to delay and inhibit maturation has now been adopted together with the use of additional lights to increase growth by the salmon industry in Tasmania because of the clear economic benefits. However, the variability of these effects were highlighted in both the use of lights to delay maturation and also to inhibit maturation. In the former of the two trials this was demonstrated when Tassal tried to reproduce the previous years findings using different seawater transfer dates, different mean weights at transfer combined with increased ambient winter water temperatures. Under such conditions the delay in maturation was restricted to six weeks in the females and only three weeks in the males compared to eight weeks in both sexes the previous year. Likewise, when additional lighting was used to inhibit maturation the two lit groups at Huon aquaculture showed a 4% disparity in results, with one replicate having 7% maturation and the second only 0.35% compared to 34% in the control cages. This variation between replicates equates to approximately $120,000 per annum and may be exacerbated by fluctuations in ambient environmental parameters. While the current project addressed several of the major industry questions on salmonid reproduction it is vital that further research increases our understanding of the mechanisms behind reproduction to provide industry with a reliable tool with which to predict production output under varying environmental and biological conditions.

While it is clear from the literature that photoperiod is the primary regulator of the timing of maturation in salmonids, there are several studies that indicate an important role of temperature (Reviewed by Van Der Kraak and Pankhurst 1996). Temperatures approaching the upper limit of the thermal range for salmonid production (18-22°C) have been shown to impact on oocyte steroidogenesis, ovulation and egg quality in both rainbow trout and Atlantic salmon (Pankhurst et al. 1996; Pankhurst and Thomas 1998; King et al. 2003; Watts et al. 2004). It is therefore important that a greater understanding of the interactions of between growth, temperature and photoperiod on the endocrine mechanisms involved in maturation is achieved to allow photoperiods to be altered to in relation to the environmental fluctuations.

One hormonal candidate for the transduction of information between growth and reproductive processes is insulin-like growth factor I (IGF-I). IGF-I is a polypeptide growth factor produced by the liver in response to growth hormone (GH). IGF-I mediates several of the effects of GH and also has a negative feedback action on GH production (Duan 1997). Together IGF-I and GH form the basis for a complex system that affects several physiological processes in teleosts, including primary regulation of somatic growth, protein synthesis, feed conversion, lipid catabolism, gluconeogenesis, behaviour, seawater tolerance and smoltification in salmonids (Bjornsson 1997).

In several teleost species, there is also evidence for the GH/IGF-I axis interacting with reproductive processes (Le Gac et al. 1993; Bjornsson 1997; Duan 1997). IGF-I has been shown to induce maturation of fully developed oocytes *in vitro* in several species including red seabream (*Pagrus major*) (Kagawa et al. 1994), striped Bass (*Morone saatilis*) (Weber and Sullivan 2000), and mummichog (*Fundulus heteroclitus*) (Negatu et al. 1998). There is also evidence for IGF-I modulating steroid production by preovulatory and mature oocytes from coho salmon and striped bass (Maestro et al. 1997; Weber and Sullivan 2000). Moriyama *et. al.* (1997) examined precociously maturing Amago salmon (*Oncorhynchus masou*) and observed that *in vivo* plasma IGF-I levels in the 9

months preceding the spawning period peaked and declined up to 2 months earlier in maturing males and females compared to individuals that did not mature.

While the field of research investigating the interactions between the GH/IGF system and maturation in teleosts encompasses a range of species, the majority of work to date has focussed on the latter stages of oocyte maturation i.e. secondary oocyte growth and development. Consequently there is a paucity of information on the impact of IGF-I on the initiation of oocyte maturation and primary oocyte growth and development. Determining the role of IGF-I at this stage would assist in our understanding of the interaction between growth and reproductive processes, and thus provide additional tools to control the timing of maturation in commercial operations.

It is suggested that increased knowledge of the zeitgebers (environmental cues) and timing of primary oocyte maturation would provide a greater understanding of the influence of external factors such as temperature and photoperiod while knowing when maturation is initiated would allow a more effective application of artificial photoperiod regimes.

These can be summarised as two basic needs:

1. To accurately predict maturation rates and optimize photoperiod regimes to prevent early maturation.

Ambient environmental conditions mean that the Tasmanian salmon industry will always suffer from high maturation rates due to its high water temperatures and increased light intensity. Trials to date have highlighted the need for increased light intensities both between sites and seasons depending on the results required. Therefore further work into the use of increased light intensity and plasma melatonin production is required.

2. To better understand the timing of oocyte maturation in relation to varied environmental conditions.

a) The development of oocytes within the gonads needs to be initiated well in advance of the fish spawning. At present it is unknown precisely when this occurs and what physiological parameters are required to allow maturation to proceed. The timing of this "gating" period needs to be determined as this is undoubtedly the most effective time with which to apply environmental manipulations to inhibit the maturation process to continue. The "gating mechanisms" i.e. size and energetic status will be investigated to more accurately determine the timing and duration of the application of artificial lights.

b) One hormonal candidate for the transduction of information between growth and reproductive processes is insulin-like growth factor I (IGF-I). The majority of research has investigated the interactions between the GH/IGF system and maturation during the latter stages of oocyte maturation i.e. secondary oocyte growth and development. Consequently, there is a paucity of information on the impact of IGF-I on the initiation of oocyte maturation and primary oocyte growth and development. Determining the role of IGF-I at this stage would assist in our understanding of the interaction between growth and reproductive processes, and thus provide additional tools to control the timing of maturation in commercial operations.

Conclusions

Objective 1. An improved understanding of the mechanisms of light regulated control of melatonin secretion in salmon.
A radioimmunoassay to measure plasma melatonin levels in teleosts was successfully set up at the University of Tasmania (UTAS). This assay, previously used by Dr Porter in the Northern Hemisphere, had not been performed in the Southern hemisphere and as such UTAS is now the only laboratory in the Southern hemisphere able to conduct this analysis at present.

The tank-based experiments were successfully completed at the Saltas P/L hatchery site at Wayatinah. These were set up to assess the effects of varied day and night time light intensities on diel melatonin profiles in Atlantic salmon juveniles and mature individuals. It was observed that the mean plasma melatonin levels of adults during the day are significantly lower than those collected during the dark phase and levels were in agreement of those reported in previous studies (Randall et al, 1995; Porter et al, 1999; Bromage et al, 2001). Additional dark phase illumination of 10 and 100 lux significantly reduced plasma melatonin levels compared to the fish under 1lux and shows a concomitant decrease in dark phase melatonin levels with increased light intensity. This work provided basic information regarding the fish's physiological response to ambient and artificial light levels which was then used in research and commercial scale field trials.

Objective 2. An improved understanding of the association between melatonin levels and reproductive development in salmon (Light mediated effects on melatonin synthesis and its control of the timing of maturation).
This project outcome was achieved from information gained in objectives 1 and 4. From this work it is clear that the intensity and number of watts of artificial lighting required per unit area in Tasmania is far greater than that used in the Northern hemisphere. This is due to the very high ambient intensities experienced in Tasmania (20,000+ lux) compared to 12-15,000 lux in Scotland or Norway. Through light levels developed in objective 4 it is now possible to accurately predict the minimum intensities required throughout a salmon cage to achieve the desired light mediated results.

It has also been shown that the intensity of light required to initiate a growth response in salmon is less than that needed to alter maturation. It is suggested that this is due to the energetic commitment made by males but especially females in the initiation of reproductive development in comparison to the daily process of growth. Therefore, if the zeitgeber responsible for maturation is found to be wrong this would severely compromise the survival of parent and offspring. Hence, only strong positive artificial light cues achieve a successful response in altering maturational processes.

Objective 3. The capacity to rapidly and non-destructively assess the acute reproductive condition of caged salmon.
Data and blood plasma samples collected from commercial scale trials were used to investigate the potential of using vitellogenin, testosterone and estradiol measurements correlated with mean oocyte diameters (MOD) in a range of reproductive stages to develop a model for predicting maturity status in individual fish. Additional data acquired from the reconditioning trial conducted at Huon Aquaculture Company P/L was incorporated into the model. The results of this work suggested that mean oocyte diameter when plotted against plasma testosterone produce a good indication of female reproductive status.

The final aim of this work was to use a mucus scrape instead of relying on blood plasma and analysis from a vitellogenin ELISA produced detectable levels of vitellogenin within mucus. Samples from the research scale pit tag trial run at Huon Aquaculture were analysed in parallel with known levels of plasma vitellogenin and validations performed on the initial ELISA samples. Unfortunately, the correlation of vitellogenin levels with mean oocyte diameter is poor as there is a large variation in levels between individual fish. Sex steroids, of which plasma testosterone produced the best correlation with (MOD), do not appear to be within the detectable range in mucus samples.

Objective 4. The development of commercial scale photomanipulation techniques for the retardation or prevention of precocious maturation in farmed Tasmanian salmon.

Initial commercial trials aimed to delay the onset of maturation in a mixed sex population of Atlantic salmon. This work produced an eight week delay in both male and female salmon compared to control populations maintained under ambient photoperiod. However, a repeat experiment the following year highlighted the importance of water temperature, size of smolt and time of seawater transfer as only a five to six week delay in maturation in the females and two to three weeks delay in the males were observed under unfavourable conditions.

The use of constant artificial illumination increased growth by 18% as the result of a phase shift in the annual growth cycle. In winter most temperate species show a reduction in growth rate due to temperature and photoperiod. The use of constant illumination provides a summer growth cue and hence reduces the effects of colder winter temperatures on a reduced weight gain. This equates to a 6 week advance to harvest time which provides the farmer with an option of reducing the length of the production cycle and achieving the 3kg market minimum weight 6 weeks prior to unlit cages.

The use of artificial lights on the previously mature female Atlantic salmon has significant commercial advantages in terms of flesh quality at harvest. Atresia scores confirmed that the lit groups showed significantly better reabsorption of the previous year's eggs when compared to the unlit controls. The flesh colour and body wall thickness were also improved by the use of lights combining to produce an increased rate of reconditioning and a higher quality product.

The final objective of the project was to inhibit early maturation in out-of season smolt. These fish traditionally have higher maturation rates due to increased time in the sea. By applying constant illumination from seawater transfer it was possible to artificially advance the summer photoperiod and the "gating mechanism" (Duston and Saunders, 1992) required to initiate maturation. The result is that the fish are too small at this point in time to mature and therefore cannot proceed with reproductive development. This successfully reduced maturation rates from 31% to 3.5% so providing a tool with which the fish farmer can control maturation. Furthermore, this work also revealed that the size of an individual prior to seawater transfer could be used to determine the likelihood of whether that fish would mature prior to harvest. This has significant implications for an industry that traditionally looks for large smolts from the hatcheries and this practice may in fact be predisposing the fish to mature early. The decision to mature in Tasmanian grown Atlantic salmon may by made in freshwater prior to seawater transfer or soon after transfer.

Appendix 1.

References

Bromage N.R. and Cumaranatunga R., 1988. Egg production in the rainbow trout. In: Muir JF, Roberts RJ, editors. Recent Advances in Aquaculture, Vol. III. Portland, Oregon: Timber Press. pp. 63-138.

Bromage, N.R., Randall, C., Duston, J., Thrush, M. and Jones, J., 1993. Environmental control of reproduction in salmonids. In: J.F. Muir and R.J. Roberts (Eds), Recent Advances in Aquaculture, Vol. IV. Blackwell Scientific Publications, Oxford. pp. 55-65.

Bromage N.R., Porter, M.J.R., Randall, C. 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197(1-4): 63-98.

Bjornsson, B.T., 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol Biochem 17(1-6):9-24.

Duan, C., 1997. The insulin-like growth factor system and its biological actions in fish. Am Zool 37(6):491-503.

Duston J. and Saunders, R. L. 1992. Effect of 6-,12-, and 18-month photoperiod cycles on smolting and sexual maturation in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 49: 2273-2280.

Duston, J. and Saunders, R.L., 1995. Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. Aquaculture 135(4):295-309.

Hansen, T., Stefansson, S. and Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquacul. Fish. Man., 23: 275-280.

Hansen, T., Stefansson, S., Taranger, G.L. and Norberg, B., 2000. Aquaculture in Norway. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson (Eds), Proceedings of the 6th international symposium on the reproductive physiology of fish. John Greig A/S, Bergen, pp. 408-411.

Jungalwalla, P.J., 1991a. The development of an integrated saltwater salmonid farming industry in Tasmania, Australia. Can. Tech. Rep. Fish. Aquat. Sci. 1831: 65-73.

Jungalwalla, P.J., 1991b. Production of non-maturing Atlantic salmon in Tasmania. Can. Tech. Rep. Fish. Aquat. Sci. 1739, 47-57.

Kagawa, H., Kobayashi, M., Hasegawa, Y., Aida, K., 1994. Insulin and Insulin-Like Growth-Factor-I and Growth-Factor-II Induce Final Maturation of Oocytes of Red Seabream, *Pagrus-Major*, in-Vitro. Gen Comp Endocrinol 95(2):293-300.

King, H.R., Pankhurst, N.W., Watts, M., Pankhurst, P.M., 2003. Effect of elevated summer temperatures on gondal steroid production, vitellogenesis and egg quality in female Atlantic salmon. J Fish Biol 63:153-167.

Le Gac, F., Blaise, O., Fostier, A., Le Bail, P.Y., Loir, M., Mourot, B., Weil, C., 1993. Growth Hormone (GH) and reproduction: a review. Fish Physiol Biochem 11(1-6):219-232.

Maestro, M.A., Planas, J.V., Moriyama, S., Gutierrez, J., Planas, J., Swanson, P., 1997. Ovarian receptors for insulin and insulin-like growth factor I (IGF-I) and effects of IGF-I on steroid production by isolated follicular layers of the preovlatory Coho salmon ovarian follicle. Gen Comp Endocrinol 106:189-201.

Moriyama, S., Shimma, H., Tagawa, M., Kagawa, H., 1997. Changes in plasma insulin-like growth factor-I levels in the precociously maturing amago salmon, *Oncorhynchus masou* ishikawai. Fish Physiol Biochem 17:1-6.

Negatu, Z., Hsiao, S.M., Wallace, R.A., 1998. Effects of insulin-like growth factor-I on final oocyte maturation and steroid production in *Fundulus heteroclitus.* Fish Physiol Biochem 19(1):13-21.

Oppedal, F; Taranger, GL; Juell, J-E; Hansen, T et al., Growth, osmoregulation and sexual maturation of underyearling Atlantic salmon smolt *Salmo salar* L. exposed to different intensities of continuous light in sea cages Aquaculture Research Vol. 30, no. 7, pp. 491-499. Jul 1999 2000

Pankhurst, N.W., and 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.

Pankhurst, N.W., Purser, G.J., Van Der Kraak, G., Thomas, P.M., Forteath, G.N.R., 1996. Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. Aquaculture 146:277-290.

Pankhurst, N.W. and Thomas, P.M., 1998. Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. Aquaculture 166:163-177.

Porter, M.J.R., Duncan, N.J., Mitchell, D. and Bromage, N.R., 1999. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176: 237-244.

Porter, M.J.R., Duncan, N.J., Roed, A.J., Oppedal, F., Taranger, G.L. and Bromage, N.R., 2000. Differential effects of light intensity on growth, maturation and plasma melatonin in Atlantic salmon and its importance in aquaculture. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson (Eds), Proceedings of the 6th international symposium on the reproductive physiology of fish. John Greig A/S, Bergen, pp. 321-324.

Porter, M.J.R., Duncan, N., Stefansson, S.O. and Bromage, N.R., 2001. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. J. Fish Biol., 58, 431-438.

Porter, M.J.R., Woolcott, H.M. and Pankhurst, N.W., 2003. The use of additional lighting and artificial photoperiods to recondition early maturing Atlantic salmon (*Salmo salar*) in Tasmania. Fish Phys. Biochem., 28: 391-393.

Randall, C.F., Bromage, N.R., Thorpe, J.E., Miles, M.S. and Muir, J.S., 1995. Melatonin rhythms in Atlantic salmon (*Salmo salar*) maintained under natural and out-of-phase photoperiods. Gen. Comp. Endocrinol., 98: 73-86.

Randall, C.F., Bromage, N.R., Porter, M.J.R., Gardener, J. and Auchinachie, N.A., 2000. Circannual rhythms of reproduction in rainbow trout. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson (Eds), Proceedings of the 6th international symposium on the reproductive physiology of fish. John Greig A/S, Bergen, pp. 325-327.

Sydney Fish Market 2005,

Taranger, G.L., Haux, C., Walther, B.T., Stefansson, S.O., Bjornsson, B.T. and Hansen, T., 1991. Photoperiodic control of growth, incidence of sexual maturation and ovulation in adult Atlantic salmon. In: Scott, A.P., Sumpter, J.P., Kime, D.E. and Rolfe, M.S. (Eds), Proceedings from the 4th International Symposium on Reproductive Physiology in Fish. Sheffield, U.K.: Fishsymp. 91. pp. 145-147.

Thorpe, J.E., Talbot., C., Miles, M.S. and Keay, D.S., 1990. Control of maturation in cultured Atlantic salmon, *Salmo salar*, in pumped seawater tanks, by restricting food intake. Aquaculture, 86: 315-326.

Thorpe, J.E., 1994. Reproductive strategies in Atlantic salmon, *Salmo salar* L. Aquacult. Fish. Man., 25: 77-87.

Van Der Kraak, G. and Pankhurst, N.W., 1996. Temperature effects on reproductive performance of fish. In: Wood CM, McDonald DG, editors. Global Warming: Implications for Freshwater and Marine Fish Society for Experimental Biology Seminar Series 61: Cambridge University Press. p 159-176.

Watts, M., Pankhurst, N.W. and King, H., 2003. Effects of dbcAMP on steroidogenesis in isolated ovarian follicles of Atlantic salmon. Fish Physiol. Biochem., 28(1-4): 375-376.

Watts M., Pankhurst, N.W., King, H.R., 2004. Maintenance of Atlantic salmon (*Salmo salar*) at elevated temperature inhibits cytochrome P450 aromatase activity in isolated ovarian follicles. Gen Comp Endocrinol 135:381-390.

Weber, G.M., Sullivan, C.V., 2000. Effects of Insulin-Like Growth Factor-I on In Vitro Final Oocyte Maturation and Ovarian Steroidogenesis in Striped Bass, *Morone saxatilis*. Biol. Reprod. 63(4):1049-1057.

Woods, A.E. and Ellis, R.C., 1994. Laboratory Histopathology: A Complete Reference. Edinburgh, Churchill Livingstone.

Appendix 2

Staff

School of Aquaculture, TAFI, University of Tasmania

Dr Mark Porter – Project Leader (PI)	100%
Ryan Longland – Technician	100%
Hannah Woolcott – PhD Student	100 %
Professor Ned Pankhurst – Dean of Science – (JCU)	5%
Brett Baker (Honours student)	In Kind
Kris Warrall (Honours student)	In Kind
Ben Fazioli – (Huon Aquaculture) (Honours student)	In Kind
Kristian Just - – (Tassal) (Honours student)	In Kind

Huon Aquaculture

David Mitchell – Operations Manager	10%
Peter Bender – Managing Director (Huon Aquaculture)	In Kind
Adrian Steenholdt – Technical Manage	In Kind
Lianne Delaney- Biological Assistant	In Kind
Josh McKibben- Research cage Manager	In Kind
Innes Weir – Technical Manager	In Kind

Saltas

Dr Harry King – Operations Manager (Saltas)	10%
Owen Ryan – Hatchery Manager	In Kind

Tasmanian Salmon Growers Association

Pheroze Jungalwalla – Executive Director	5%

Tassal

Richard Taylor – Operations Manager (Tassal)	In Kind
David Cameron-(Tassal)	In Kind
Craig Selkirk – Special Projects Manager (Aquatas)	In Kind
Guy Westbrook – (Tassal)	In Kind
Nick Murfit - (Aquatas)	In Kind
Simon Pitney - (Springfield Fisheries) -	In Kind

Appendix 3

Light attenuation from a 400 watt submersible light (Aquabeam Pisces or C&T Lighting units) in seawater with an 8m secci disc reading.

Vertical distance from light \ Horizontal distance from light	0	1	2	3	4	5	6	7	8
1		5570.2	216.5	85.8	42.2	44.7	37.1	37.9	33.6
2		1990.4	831.4	448.4	52.2	43.4	36.4	49.0	34.6
3		579.4	312.3	332.4	110.7	60.3	35.1	42.7	47.0
4		103.1	120.8	85.5	65.3	47.7	33.1	36.9	35.1
5		98.1	60.3	80.5	85.5	60.3	40.2	30.1	32.6
6		60.3	50.2	45.9	36.4	38.1	32.6	26.0	28.3