CHAPTER 5

PAPER V

SHORT-COMMUNICATION

THE USE OF CONTINUOUS LIGHT TO SUPPRESS PRE-HARVEST SEXUAL MATURATION IN SEA-REARED ATLANTIC SALMON (*SALMO SALAR* L.) CAN BE REDUCED TO A FOUR MONTH WINDOW.

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In Atlantic salmon *Salmo salar* L. sexual maturation is concomitant with a redistribution of the somatic resources and the development of nuptial colouration responsible for the low commercial value of mature fish (Michie, 2001). Maturing fish also exhibit an altered feeding activity (Kadri *et al.*, 1996; Kadri *et al.*, 1997a and b) and increased pathogen susceptibility (Bruno, 1989; St-Hilaire *et al.*, 1998; Currie and Woo, 2007) likely to compromise growth, health and welfare of the cohabiting immature cohort. The suppression of pre-harvest sexual maturation is therefore a priority in the salmon on-growing industry and is achieved by photoperiodic manipulation of the stock in the form of continuous artificial-light (LL) applied between the winter and summer solstice during the second year at sea. This 6-month period LL-regime is recognized as the most efficient by providing a key environmental signal that phase-advances the so-called "spring decision window" such that a reduced proportion of the stock meets the developmental/energetic thresholds required to proceed through maturation (Taranger *et al.*, 1998; Endal *et al.*, 2000; Oppedal *et al.*, 2006). Current knowledge on the photoperiodic entrainment of reproduction in Atlantic salmon suggests that terminating LL-exposure before the summer solstice could be equally efficient at suppressing sexual maturation. This study tested this hypothesis on a commercial scale with the objective of reducing energy usage and potential welfare impacts associated with the long-term use of powerful lighting systems in sea-pens (Migaud *et al.*, 2007a).

The trial was performed at a commercial Atlantic salmon sea-farm (56.41^{o}N, 5.42^{o}W, Marine Harvest (Scotland) L^{td}., Scotland) stocked in April 2007 with S1 smolts held under natural light conditions (NL) until the start of the trial. On the 3rd January 2008, six cages (24x24x12m) holding one sea-winter (1-SW) Atlantic salmon (n=26,493±779fish/pen) with a mean live body-weight (BW) of 1566±24g were exposed to LL using 4 metal-halide light-units per pen (Pisces 400, BGB Engineering,

Grantham, UK) placed in a standard set-up. Three photoperiodic treatments were tested in duplicate: two cages were returned to NL on the 20th April (LL-Apr), 20th May (LL-May) and 18th June (LL-Jun). Throughout the experiment fish were fed the same commercial diet according to manufacturer recommendations (Biomar, Grangemouth, UK) with water temperature at 6m depth ranging between 7.1°C and 14.7°C. Batch sample-weights were performed monthly (n=120 fish/pen/month) to calculate specific growth rate (SGR) and feed conversion ratio (FCR) (Taylor *et al.*, 2006). On June 20th, 60 fish/cage were randomly anaesthetized and measured for BW (±0.1g), fork length (FL, ±0.1cm) and Fulton condition factor (K) calculated as $K=(BWx100).FL^{-3}$. Blood was withdrawn for analysis of plasma testosterone (T) by radioimmunoassay with levels above 3ng.mL^{-1} indicating recruitment into maturation (Duston and Bromage, 1987; Taranger *et al.* 1998). Within these fish, 30 fish/cage were sacrificed, sexed, gonad-weight measured (GW) (±0.001g) and gonadosomatic index (GSI) calculated as $GSI=(GWx100).BW^{-1}$. Ovary samples were preserved in 10% buffered formalin for histological analysis and classified according to their leading oocyte stage using the primary yolk stage (the first stage of exogenous vitellogenesis) as an indicator of commitment toward maturation. Male maturity was determined based on the bimodal GSI frequency distribution in the population (Taranger *et al.*, 1998). Single pen harvest sampling allowed accurate estimation of maturation rate through external observation of 1000 fish/pen minimum using nuptial colouration as a reliable indicator of maturity (Leclercq *et al.*, 2010a). Additionally, a minimum of 80 apparently immature fish/pen were sexed, weight-lengthed and their sexual development determined through GSI and gonad histology. Due to commercial imperatives, one cage per treatment was harvested within a 7-day period both in October and November 2008 (Harvest group 1 and 2 respectively). BW, K and GSI were assessed in June by one-way nested ANOVA and

pooled per treatment where no significant differences occurred. The effect of treatment and time was then assessed independently for each harvest group by a two-way ANOVA manipulated by a General Linear Model. Datasets were transformed when required to meet the assumptions of normality and homogeneity of variance. A Tukey's post-hoc multiple comparisons test was applied where statistical differences occurred. Maturation rates at harvest were compared by a Chi-square test. Analyses were performed using SPSS v.15 and Minitab v.15 with a significance level of 5% ($P<0.05$). Data are expressed as mean ± SEM.

SGR and FCR were similar among treatments and averaged 0.36±0.02%.day^{-1} and 1.21±0.05 respectively from early January to late September. All experimental pens had the same BW and K in January (not shown) and June (Table 1). However, LL-Jun had a significantly higher BW than LL-May in October and a higher BW and K than both other treatments in November (Table 1a, 1b). Based on the low testosterone levels measured in all individuals (<1ng.mL^{-1}; not shown), the absence of exogenous vitellogenesis (Fig.1) and the unimodal GSI distribution in the male cohort, none of the fish assessed in June were sexually recruited at this time. Accordingly, maturation rates at harvest were consistently low (<1.2%) with no significant differences between treatments (Table 1c). In the immature cohort and for both genders, GSI-values were always significantly higher in October and November than in June (Table 1d, 1e). Differences in GSI between treatments occurred only in the female cohort and in November when it was significantly higher in LL-May and LL-Apr treatments (Table 1e). This was confirmed by ovarian histology with a higher rate of primary yolk stage observed in October and November than in June in all treatments (Fig.1). The prevalence rate of females in early exogenous vitellogenesis was circa 2.4±0.1% in all

treatments in October and highest in November reaching 10.5% in LL-Apr compared to 4.7% and 4.5% in LL-Jun and LL-May respectively.

This commercial trial reports firstly that the duration of LL-exposure could be reduced without compromising its efficiency at suppressing sexual maturation and secondly the observation of females initiating exogenous vitellogenesis under a short-day photoperiod. Although no ambient unlit treatment was available as a negative control due to commercial reasons, the low maturation rates achieved are consistent with those of previous studies using LL-Jun regime (Oppedal *et al.*, 1997; Oppedal *et al*,. 2006). Our data show that LL applied during the second year at sea for 3½, 4½ and 5½ months from early January was equally efficient at suppressing the occurrence of mature salmon in autumn with no effect of the increase in ambient day-length from April to the summer solstice (LL-Apr). Previous studies have shown that the switch from short-to-long days is the key photoperiodic signal regulating Atlantic salmon maturation. An arrestment of sexual development is indeed observed within 6 weeks of LL-exposure in fish remaining subsequently immature (Taranger *et al.*, 1998, 1999; Schulz *et al.*, 2006). This photo-inhibition would have occurred before mid-April in all regimes tested here such that timings of LL-offset had no effect on maturation rates at harvest. While LL-onset rapidly photo-inhibits the immature cohort, fish undergoing further sexual development can be regarded as photo-stimulated such that Atlantic salmon populations are consistently described as sexually bimodal over long-days (Schulz *et al.*, 2006). However, it remains unclear if long-days are required for recruitment into maturation to occur. Interestingly in this study, a proportion of 2-SW females were initiating exogenous vitellogenesis in the autumn when fully mature fish also occurred. Similarly in immature 1-SW salmonids, histological and physiological evidence of sexual development was reported under short-days prior to any photoperiod

treatment (Campbell *et al.*, 2003, 2006; Taranger *et al.*, 1998). Importantly, we observed a higher proportion of true vitellogenic females in pens returned earlier to NL. This suggests that the long-to-short day switch releases the photo-inhibition of the immature cohort toward a photo-sensitive or photo-neutral stage under short-days where sexual development progress, as described in the gating model (Duston and Bromage, 1988), and actual commitment toward maturation can also occur. Exposure to LL would close this open phase within weeks by inhibiting on-going sexual development, that is to say by promoting the annual decision *not* to mature, in Atlantic salmon falling below required developmental thresholds (Thorpe, 1986; Taranger *et al.*, 1998). Importantly, stocks exposed to a shorter LL-regime had a lower harvest weight which could be expected from the stimulating effect of light on growth and appetite in salmonids (Oppedal *et al.*, 1997; Endal *et al.*, 2000; Oppedal *et al.*, 2003, 2006; Taylor *et al.*, 2006; Taylor *et al.*, 2008). Further assessment of growth and maturation of Atlantic salmon stocks exposed to the different LL-window tested is required due to genetic, environmental and husbandry variations in commercial stocks. If confirmed, the duration of LL-exposure could be significantly reduced (~35%) without compromising its efficiency at suppressing sexual maturation but should be varied according to targeted harvest BW. These preliminary results highlight the potential for reducing the duration of photoperiod manipulation, its energy usage and potential welfare impact on the stock toward a greater ecological and economic sustainability of the salmon industry.

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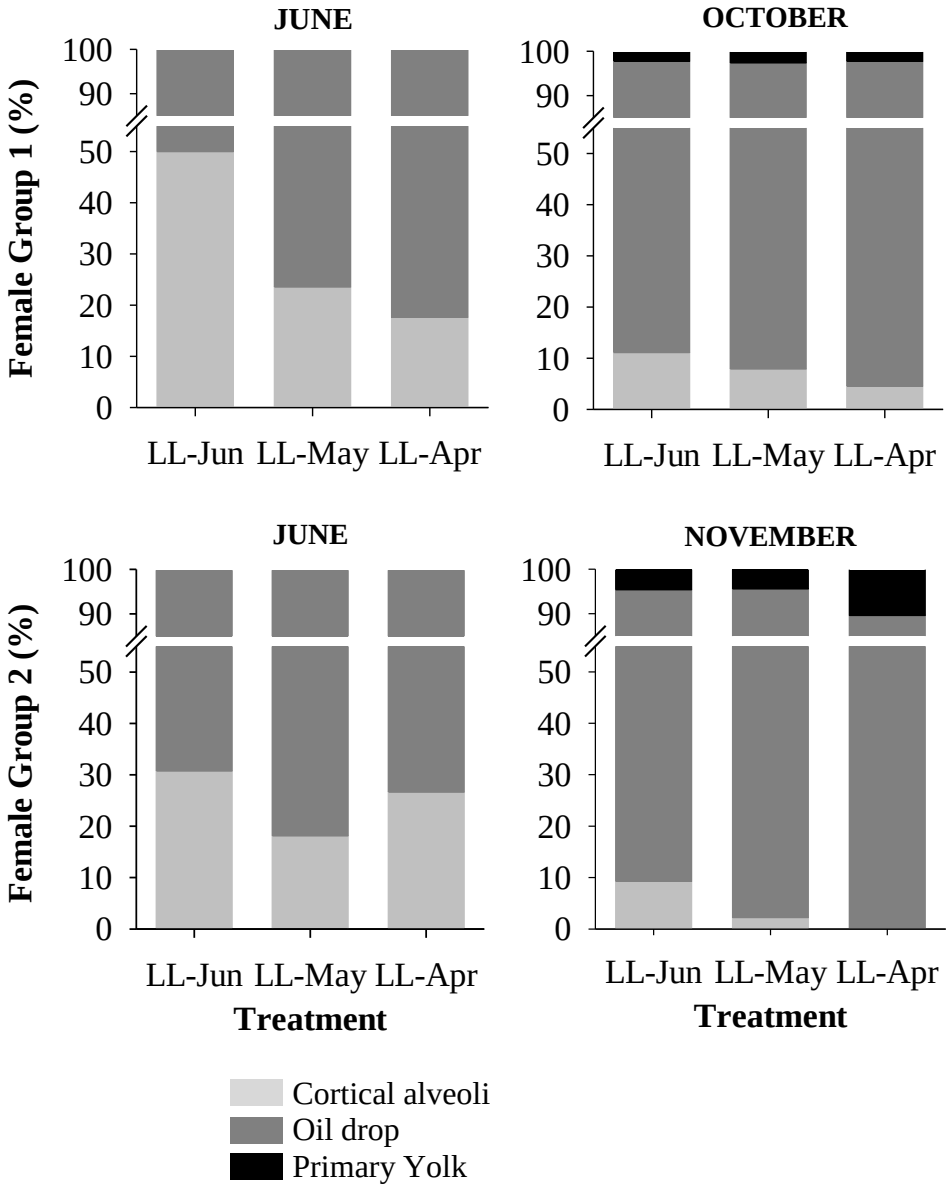

Figure 1. Proportion of females at the different oocyte leading stages in June and October and November harvests following rearing under continuous artificial-light from early January to mid-June (LL-Jun), mid-May (LL-May) and mid-April (LL-Apr) over the second year at sea. n=15 females/pen in June; n=40 females/pen at harvest.

Table 1 (**a.**) Live body-weight (BW), (**b.**) Fulton condition factor (K), (**c.**) maturation rate, (**d.**) male mean-GSI and (**e.**) female mean-GSI of Atlantic salmon *Salmo salar* L. reared under continuous artificial-light from early January to mid-June (LL-Jun), mid-May (LL-May) and mid-April (LL-Apr). Values are given as mean±SEM. Sexually recruited fish in June and fully mature fish at harvest (October and November) are not included in the dataset. Significant differences between replicates in June are shown in bold (ANOVA, $P<0.05$). Significant differences between treatments and month within each experimental group are shown by different superscript letter (GLM, $P<0.05$). Maturation rates were not significantly different (chi sqare test, $\chi^2=7.782$, $P=0.169$).

	June			**October**			**November**		
	LL-Jun	**LL-May**	**LL-Apr**	**LL-Jun**	**LL-May**	**LL-Apr**	**LL-Jun**	**LL-May**	**LL-Apr**
	(a) BW (g) *(n=60-80 fish/treatment/group/month)*								
Gp 1	3338±92^{a}	3384±125^{a}	3189±104^{a}	5139±108^{c}	4513±86^{b}	4773±81^{bc}			
Gp 2	3407±108^{a}	3075±104^{a}	3109±108^{a}				5529±110^{c}	4845±97^{b}	4850±93^{b}
	(b) K *(n=60-80 fish/treatment/group/month)*								
Gp 1	1.28±0.01^{a}	**1.26±0.02^{ab}**	1.25±0.02^{ab}	1.23±0.01^{ab}	1.22±0.01^{b}	1.23±0.01^{ab}			
Gp 2	1.24±0.01^{a}	**1.20±0.02^{a}**	1.19±0.02^{a}				1.28±0.01^{b}	1.21±0.01^{a}	1.21±0.01^{a}
	(c) Maturation rate (%) estimated based on skin colouration *(n=1000 fish/treatment/group/month)*								
Gp 1				0.38	0.66	0.47			
Gp 2							0.91	0.88	1.21
	(d) Male GSI (%) *(n=15 and n=40 fish/treatment/group/month in June and October-November respectively)*								
Gp 1	0.059±0.007^{a}	0.059±0.005^{a}	0.057±0.004^{a}	0.095±0.004^{b}	0.083±0.005^{b}	0.079±0.003^{b}			
Gp 2	0.058±0.003^{a}	0.055±0.004^{a}	0.049±0.004^{a}				0.086±0.003^{b}	0.078±0.002^{b}	0.082±0.002^{b}
	(e) Female GSI (%) *(n=15 and n=40fish/treatment/group/month in June and October-November respectively)*								
Gp 1	0.202±0.013^{a}	0.230±0.014^{a}	0.249±0.048^{a}	0.294±0.009^{b}	0.294±0.001^{b}	0.289±0.008^{b}			
Gp 2	0.191±0.007^{a}	0.204±0.010^{a}	0.211±0.012^{a}				0.308±0.012^{b}	0.361±0.013^{c}	0.379±0.016^{c}

BW: Live body-weight, K: Fulton condition factor, GSI: gonadosomatic index; Gp1 and Gp2: Harvest group 1 and 2 respectively; LL-Jun, LL-May and LL-Apr: Artificial continuous light applied from early January to mid-June, mid-May and mid-April respectively.