A review of factors influencing maturation of Atlantic salmon (*Salmo salar*) with focus on water recirculation aquaculture system environments

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EXECUTIVE SUMMARY

Maturation of Atlantic salmon *Salmo salar* is an extremely complex process, particularly in aquaculture systems, with many variables (known or otherwise) having the capacity to influence the timing and prevalence of maturation, and acting as promoters and/or inhibitors of sexual development. The vast majority of research carried out on salmon maturation in aquaculture has been in context of traditional culture; namely, land-based smolt production in freshwater systems, followed by transfer to sea cages for growout. However, very little research has specifically examined salmon maturation in land-based, closed containment water recirculation aquaculture systems (RAS), which have recently received attention as an alternative technology for the sustainable production of market-size Atlantic salmon. Given the high prevalence of grilsing observed in the nascent closed containment salmon industry, and the potential economic challenges facing its expansion, it is imperative that best management practices are developed to reduce economic losses and other deleterious outcomes resulting from maturation. This review provides a brief summary of published research on factors associated with early salmon maturation, as well as information on current practices applied in closed containment operations to minimize grilsing. In light of the novelty of raising salmon to market size in a closed containment environment, and the paucity of research on maturation in such environments, it is difficult at present to make specific recommendations to reduce grilsing in RAS; however, much-needed research can be directed, to an extent, based on findings made in traditional settings, as well as early observations made at closed containment operations producing market-size Atlantic salmon. Among the numerous environmental variables that reportedly influence salmon maturation, photoperiod and water temperature are foremost; while photoperiod generally influences the "decision" to undergo or delay

maturation (made months in advance), water temperature tends to control the magnitude of maturation in a given salmon population. Hence, the warmer water temperatures typically maintained in existing RAS growout operations could be contributing to increased grilsing; however, baseline research on water temperature, in conjunction with other variables such as photoperiod, salinity, and feed energy content / fish adiposity, needs to be carried out in order to make definitive recommendations that will guide best management practices for closed containment salmon operations. Research is also needed on other potential influencers of maturation that are specific to RAS, such as the degree of exercise provided in circular tanks, the accumulation and impact of waterborne steroid hormones, and other water quality elements including endocrine disrupting compounds. It must be noted that key variables that may promote maturation are also associated with improved growth performance, and therefore recommendations to reduce maturation could be at odds with current production practices. Given the complexity of the overall problem, and the potential conflict between reducing early maturation, while optimizing Atlantic salmon growth performance, the most expedient solution at present is for industry to partner with breeders to promote the availability of an all-female germplasm for closed containment operators. There is already a possibility that all-female eggs will be available from a commercial supplier in Iceland beginning in late 2015. If this becomes a reality (and all-female eggs are available to producers on a regular basis) it would avoid the significant research necessary to develop effective grilse reduction protocols for mixed sex populations. However, female grilsing, while not currently a major problem – but still present to a degree in specific operations – would also need to be investigated, and remedial best management practices developed.

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INTRODUCTION

The development of sexual maturation in Atlantic salmon *Salmo salar* is a complex, multifactorial process. The extreme variability of age and size at maturation observed for this species is considered the result of evolutionary adaptation within various river and ocean environments to maximize reproductive success. Although beneficial to the species in its natural environment, this variability in maturation timing can pose a significant problem to aquaculturists. Specifically, early maturing Atlantic salmon (i.e. "grilse") often exhibit decreased growth and feed conversion efficiency (McClure et al., 2007), reduced product quality (Aksnes et al., 1986), increased susceptibility to opportunistic microorganisms (St-Hilaire et al., 1998), and, overall, represent a major source of economic loss for farmers (Johnston et al., 2006; McClure et al., 2007). In the Canadian Maritime salmon farming industry (estimated gross revenue of $250 million CAD in 2002), grilsing was estimated to represent $11 - 24 million in lost revenue (McClure et al., 2007), with in-cage prevalence of grilsing estimated at 20-30% between 1998-2002 (Peterson et al., 2003). Over the years, the traditional salmon farming industry has adopted various strategies to reduce grilsing; these include photoperiod control (Bromage et al., 2001), selective breeding for late maturation (Gjedrem, 2000), and induced triploidy during egg incubation (Benfey, 1999). Overall, these efforts have been successful at reducing early salmon maturation; however, grilsing still remains a significant problem in certain regions of the world. With the development and implementation of next-generation technologies to produce salmon, i.e. land-based, closed containment operations utilizing water recirculation aquaculture systems (RAS), the issue of precocious maturation has returned to the forefront among factors affecting production and profitability of these new operations. During Atlantic salmon

growout trials conducted at The Conservation Fund's Freshwater Institute (TCFFI), precocious male maturation has been as high as 80%, with most grilse developing before the mean population weight reaches 2 kg. Anecdotally, early maturation has also been prevalent at other land-based, closed containment facilities raising Atlantic salmon to market size. Given the considerable up-front capital investment required to build and commission a closed containment growout facility, issues that affect profitability, such as early maturation, need to be investigated to improve the likelihood of economic success for these operations. Among the numerous benefits of closed containment technologies is the relatively high degree of control over the culture environment (Summerfelt and Vinci, 2008); therefore, it should be possible to control, refine, or eliminate environmental triggers of early maturation, once they have been identified. Another benefit of closed containment operations, however, is improved fish growth performance through environmental optimization (e.g. relatively high and constant water temperature, near-saturation dissolved oxygen levels, and 24-hour automated feeding regimes) which, as will be discussed, could also instigate early maturation. At this early stage in the development of a land-based, closed containment Atlantic salmon industry, it is imperative to address and solve issues such as early maturation in order to facilitate industry expansion and to meet the growing global demand for sustainable salmon.

The purpose of this paper is to review previous and ongoing scientific research investigating early sexual maturation in Atlantic salmon, with a focus on RAS technologies and the unique production environments that they provide. Current environmental manipulation protocols used in industry to reduce precocious maturation will be discussed, as will the status of other potential

solutions (e.g. all-female germplasm). Finally, based on these reviews, recommendations for specific areas of research will be provided.

LIFE HISTORY OF ATLANTIC SALMON

To better understand the phenomenon of early maturation in Atlantic salmon, as it relates to intrinsic and extrinsic variables, some background on the reproductive and evolutionary biology of the species is required. This information will provide insight into the natural behaviors and strategies for maturation that are ingrained in Atlantic salmon, and will serve as a basis for understanding the mechanisms controlling maturation in captive populations cultured in land-based, recirculation aquaculture systems.

Understanding the remarkably complex life cycle of Atlantic salmon begins with examination of the life history of this species. In context of this review, life history is defined as the sequence and timing of key life events, particularly those related to developmental biology, reproductive behavior, survivorship of offspring, and perpetuance of species, as influenced by the process of natural selection. Atlantic salmon exhibit a highly plastic and diverse range of life history forms that is unmatched by most vertebrates (Hutchings and Jones, 1998). Variation in life history traits, such as time of freshwater habitation and size/age at smoltification (Randall et al., 1987; Metcalfe and Thorpe, 1990; Økland et al., 1993), time of ocean residency and age at reproductive maturity (Scarnecchia, 1983; Saunders, 1986; Thorpe, 1986), and adult size at maturity (Hutchings and Morris, 1985; Saunders, 1986), among other variables (Youngson et al., 1983; Klementsen et al., 2003; Reid and Chaput, 2012), has been widely documented. Despite this diversity in life history tactics, most Atlantic salmon are anadromous and generally conform to a relatively similar

pattern of key life events. Therefore, a basic explanation of the typical Atlantic salmon life cycle is provided as the foundation for exploring the divergence of life history traits within this general strategy.

Adult Atlantic salmon typically spawn during the fall and early winter within freshwater tributaries of the Atlantic Ocean, depositing and burying eggs in a gravel nest or "redd". After a relatively long incubation period lasting into the spring the eggs hatch, and the emergent larvae or alevins rely on endogenous nutrition from a yolk sac for several months. When the alevins have exhausted their yolk reserves, the young fish (now known as fry) leave the redd to begin feeding. The juvenile salmon then develop into parr with laterally oriented vertical bars or stripes (parr marks) that provide camouflage. From the time of hatching, juvenile Atlantic salmon remain in the freshwater habitat for a period lasting one year or longer (Metcalfe and Thorpe, 1990; Økland et al., 1993). Prior to migrating to the ocean, salmon parr undergo a series of morphological and physiological changes that enable adaptation from freshwater to seawater, a process commonly known as smoltification (Hoar, 1976; Folmar and Dickoff, 1980; Wedemeyer et al., 1980; Stefansson et al., 2008). During this metamorphosis, parr marks fade, fin margins darken, and the body becomes more streamlined with a bright, silvery appearance (Folmar and Dickoff, 1980; Wedemeyer et al., 1980). Physiologically, Atlantic salmon smolts develop the ability for hypoosmotic regulation and associated ion regulation that in turn facilitates seawater adaptation (Folmar and Dickoff, 1980; Wedemyer, 1980; Stefansson et al., 2008). Smolts journey from their native rivers, usually in the spring, to specific locations in the Atlantic Ocean where they begin to feed on the rich marine food supply and grow rapidly as they advance towards reproductive maturity. When Atlantic salmon become sexually mature, the

parental migration pattern is repeated, with adults returning to the native streams and rivers from which they hatched to commence spawning. Unlike most Pacific salmonids, adult Atlantic salmon are iteroparous, meaning that they are capable of surviving the rigors of returning to the ocean and recommencing the spawning migration in subsequent year(s) (Ducharme, 1969).

While this general pattern of key life events is similar for most Atlantic salmon, many variations in life history traits exist within this strategy, both among and within populations, such as: the duration of freshwater occupancy and age at smoltification (Randall et al., 1987; Økland et al., 1993), the time of ocean residency and age at reproductive maturity (Scarnecchia, 1983; Saunders, 1986; Thorpe, 1986), and adult size at maturity (Hutchings and Jones, 1985; Saunders, 1986). Other documented variations in life history traits for Atlantic salmon include fecundity and egg size (Reid and Chaput, 2012), migratory behavior (Youngson et al., 1983), and non-anadromous versus anadromous forms (Berg, 1985). Marschall et al. (1998) and Klementsen et al. (2003) provide detailed reviews of the aforementioned life history characteristics, among others. For purposes of this report, several life history traits that distinctly relate to early maturation, including age/size at smoltification, as well as sea-age and size at reproductive maturity, will be summarized. Discussion of smoltification is relevant in the context of maturation, because this process represents a transition to the growth period of the life cycle and subsequent progression towards adulthood. For example, for most Atlantic salmon migrating to sea, important “decisions” are made regarding reproductive development during the months following smoltification (Mangel and Satterwaite, 2008).

Juvenile Atlantic salmon are known to remain in freshwater for a wide period of time ranging from 1-8 years (Metcalfe and Thorpe, 1990). Økland et al. (1993) found that smolt age varied from 2 to 6 years for Atlantic salmon populations in four Norwegian rivers. The duration of freshwater residency and commencement of the parr-smolt transformation has been correlated with achievement of a specific size and/or level of fitness that corresponds to increased marine survival (Stefansson et al., 2008). Numerous studies have described a bimodal length or size distribution that corresponds with smoltification, where faster growing Atlantic salmon parr become smolts after one year, while slower growing parr require an additional year or more to smolt (Thorpe, 1977; Thorpe et al., 1982; Kristinsson et al., 1985; Rowe and Thorpe, 1991; Økland et al., 1993). Thorpe (1977) determined that there was a strong genetic influence on the bimodal distribution of smoltification; however, other factors, particularly environmental cues such as photoperiod (McCormick et al., 1987, Björnsson et al., 1989; Solbakken et al., 1994), are also important. Eriksson and Lundqvist (1982) found evidence of an "innate timing system" for smoltification in Atlantic salmon and concluded that photoperiod acts to synchronize the parr-smolt transformation.

Of the anadromous populations, wide variation also exists relative to the duration of adult residency at sea and age at first maturity, with some salmon overwintering for just one year (typically referred to as grilse) and other groups spending 3-5 winters in the ocean before returning to their natal rivers to spawn (Saunders, 1986; Hutchings and Jones, 1998). Grilse commonly weigh 1-3 kg, while adult salmon spending multiple winters at sea can range from 3-12 kg (Saunders, 1986). Male parr maturation, which typically coincides with continued freshwater residency, is also utilized as a viable

reproductive strategy by a percentage of individuals within many Atlantic salmon populations (Saunders et al., 1982; Myers et al., 1986; Stefansson et al., 2008). Some male Atlantic salmon parr have been found to mature when they are only 10 cm in length (Fleming, 1996). It is important to note that both precocious parr and grilse maturation have been found to be at least partially heritable, as well as controlled by the environment (Naevdal, 1983; Myers et al., 1986; Herbinger and Friars, 1991; Marshcall et al., 1998).

The wide variation of reproductive tactics, particularly age at maturity, could be an evolutionary strategy designed to maintain biodiversity and genetic contribution of a cohort over a number of years (Saunders and Schom, 1985). Overall, the diverse range and plastic nature of life histories displayed by Atlantic salmon is likely an evolutionary adaptation to optimize reproductive success and to perpetuate the species (Fleming, 1996; Thorpe et al., 1998). In short, the life cycle of the Atlantic salmon is motivated by procreation and recruitment of successive generations. With this in mind, it is not surprising that reproductive investment occurs very early in the Atlantic salmon life cycle, with differentiation of germinal tissue occurring prior to first feeding during the embryo stage of development (Mangel and Satterthwaite, 2008). In this context, some scientists have described the general process of maturation as being controlled by inhibition during the juvenile life stages until a specific physiological threshold is reached that triggers a developmental switch (Thorpe, 1986; Thorpe et al., 1998; Mangel and Satterwaite, 2008). Proposed thresholds include: level of adipose tissue (Rowe and Thorpe, 1991; Simpson, 1992), size or weight of the fish (Skilbrei, 1989; Shearer et al., 2006 - chinook salmon, Oncorhynchus tshawytscha), condition factor (Herbinger and Friars, 1991; Peterson and Harmon, 2005), and energy/nutrient reserves (Kadri et al.,

1996), all of which provide information about optimal fitness, and the likelihood of successful survival and reproduction following a rigorous migration back to natal spawning waters (Mangel and Satterwaite, 2008). A number of literature sources indicate that the maturation trigger appears to depend on physiological or biochemical conditions (the aforementioned thresholds) that are to some degree genetically determined but also influenced by environmental factors (Naevdal, 1983; Gjerde, 1984; Saunders, 1986; Thorpe et al., 1998; Mangel and Satterwaite, 2008; Taranger, 2010). Saunders (1986) proposed that the genetic influence on maturation provides a basis for maturation but with "rather wide latitude," not necessarily preset for a specific time or age but instead expressed when the appropriate environmental and physiological/biochemical conditions are met. A similar synopsis was provided by Mangel and Satterwaite (2008) who described optimization of environmental conditions, such as water temperature, as creating an opportunity for growth along with other traits that typically parallel optimal growth performance, such as the accumulation of adipose tissue.

Although the exact mechanisms of the onset of maturation are not fully understood in Atlantic salmon, this literature review indicates that the process of maturation is likely triggered somewhere within the boundaries of a variety of heritable, physiological/biochemical, and environmental cues, and their interactions. This introductory review also emphasizes the high degree of plasticity of life history traits among and within Atlantic salmon populations, and demonstrates that the life cycle of the *Salmo salar* is highly motivated by reproduction. When considering the complexities of Atlantic salmon life history, it becomes clear that controlling maturation within aquaculture systems is a complex task. Possible solutions to early maturation, however,

likely begin within an understanding of the Atlantic salmon's inclination for reproductive advantage and subsequent identification and control of the environmental and physiological cues that trigger reproductive development.

FACTORS INFLUENCING MATURATION

Photoperiod

Photoperiod is considered an essential determinant for initiating sexual maturation in teleosts (Taranger et al., 2010). Its singular importance, which is rooted in the evolution of reproductive strategies of upper latitude fish species, is to ensure hatching of juveniles during periods of advantageous environmental conditions (Bromage et al., 2001). The effect of photoperiod on salmonid maturation has been extensively studied, and its interaction with water temperature has become an area of increased focus (i.e. Fjelidal et al., 2011; Imsland et al., 2014). Based on decades of research on salmonids and other species, annual physiological rhythms are thought to be entrained with seasonal changes that are sensed through periods of increasing or decreasing daylength, and sexual maturation is initiated or postponed during a "critical time window" based on numerous other factors, such as size, growth rate, nutritional status, and genetics (Duston and Saunders, 1992; Taranger et al., 1998; Taranger et al., 1999; Bromage et al., 2001; Taylor et al., 2008; Taranger et al., 2010). The direction of photoperiod change, as opposed to the specific day-length, is considered most important in orchestrating sexual maturation and the eventual seasonal timing of reproduction (Bromage and Duston, 1986; Bromage et al., 2001). In manipulating photoperiod to reduce the proportion of fish "switched on" to undergo puberty, artificial short days have been used during the early months of the year when natural photoperiod is otherwise increasing, followed by artificial long days after midsummer when natural

photoperiod declines (Randall et al., 1998; Bromage et al., 2001); however, prolonged exposure to long days, i.e. into the final month of the calendar year, could potentially increase the percentage of fish that sexually mature (Duncan et al., 1999). Towards harvest, however, continuous light is routinely applied in the net pen industry from winter to summer solstice to prevent maturation in salmon during their second year at sea (Leclercq et al., 2011).

Closed containment aquaculture allows for greater control of environmental conditions compared to open systems, and as such, photoperiod regimes can be easily applied throughout the production cycle, from hatch to harvest, while keeping other environmental variables (e.g. water temperature) relatively constant. The majority of research on developing photoperiod regimes to prevent early maturation, however, has been carried out in the context of early rearing in land-based, freshwater systems, followed by the transfer of smolts to sea cages. While photoperiods can be manipulated in both pre- and post-transfer rearing environments of the traditional culture scheme, other important variables (e.g. salinity) can obviously be widely different throughout the production cycle compared to land-based closed containment systems. Research strictly focused on photoperiod manipulation in land-based growout environments has been extremely limited, and much more work is needed in this regard to better understand fish physiology in closed containment systems in order to develop best management practices for reducing or eliminating early maturation. Consideration must also be given to photoperiod manipulation in the context of bioprogramming, given the potential simultaneous presence of multiple age classes and continuous production due to year-round eyed egg availability, which could present a new set of challenges beyond those faced in operations using a traditional salmon production cycle.

For purposes of this paper, photoperiod will be abbreviated in the form of LDX:Y, where LD stands for "light-dark" and X:Y is the amount of light (X) and dark (Y) hours over the course of a 24-hour period; for example, LD16:8 refers to a photoperiod in which fish are exposed to 16 hours of light followed by 8 hours of darkness. Good et al. (2015) demonstrated that Atlantic salmon exposed to a reduced photoperiod, i.e. LD18:6, from first feeding up to one-year post-hatch in freshwater RAS, exhibited a significantly higher proportion of mature males than those exposed to continuous light during their first year. The impetus for examining a reduced photoperiod, versus continuous light, and its effects on early maturation, was twofold: (i) Atlantic salmon cohorts raised to market size at TCFFI under continuous light (excepting a six-week LD12:12 "winter" to synchronize smoltification, beginning at 40 g average weight), have consistently produced a high percentage of maturing males, whereas the most affected group demonstrated approximately 80% male maturity before the mean population weight reached 2 kg; and (ii) previously published research suggests that a reduced photoperiod can reduce early maturation compared to continuous light. The latter research includes the study by Fjelldal et al. (2011), in which significantly higher proportions of mature males were observed in populations exposed to three months of early rearing continuous light, versus those exposed to a natural photoperiod; however, further analyses suggested that the continuous light treatment was acting as an "enabling factor", while elevated water temperature determined the actual degree of early maturation observed. The study by Good et al. (2015) found the opposite effect of reduced photoperiod during early rearing (but at 13 °C during this treatment period, versus 16 °C in the Fjelldal et al. (2011) study, and in fresh water as opposed to seawater). The findings by Good et al. (2015) resemble those of Berg et al. (1996), who found higher maturation in salmon exposed

to LD20:4 photoperiod from smolt to harvest size, versus those exposed to LD24:0, in marine net pen environments. Given the differences in treatments, photoperiod exposure times, and environment conditions, it is difficult to directly compare these (and other) experiments. The study by Good et al. (2015), however, was carried out under conditions similar to those provided in current closed containment salmon growout operations, and therefore the study results, although limited, can be considered of higher relevance to closed containment Atlantic salmon production, and can serve as a basis for further research.

Because the "decision" to mature is likely made during the first year post-hatch, when Atlantic salmon smolts in the traditional industry are often in freshwater recirculation systems, previous research within this context is relevant to closed containment producers. For example, Saunders and Henderson (1988) compared four photoperiod treatment groups, LD24:0, LD16:8, LD12:12, and LDN (simulated natural photoperiod), with fish exposed from first feeding (May) until the following January, to determine differences in precocious parr prevalence. Although precocious parr were prevalent in all groups (ranging from 43.9% to 66.7%), LDN fish had a significantly higher proportion of mature parr, while the LD16:8 had significantly less mature parr than the other treatment groups. While precocious parr have not tended to be a significant issue during the early stages of TCFFI growout trials, mature underyearling smolts were observed in very high numbers from a portion of one growout population that was transferred to six replicated experimental RAS for a parallel study. Whether this unusually high incidence of early maturation was due to sudden exposure to warmer water temperatures (approximately 2 °C increase), unintentional photoperiod change, or

other environmental cues, remains unknown. The results of Saunders and Henderson's (1988) study are interesting in that the authors found a significant decrease in mature salmon exposed to a reduced photoperiod during the first year, compared to a constant photoperiod, which is opposite to the relationship determined by Good et al. (2015); it is unfortunate that growth in freshwater up to market size was not a consideration for the study by Saunders and Henderson (1988), as a full comparison between studies cannot be carried out.

Thorpe (1986) proposed a unified model to study the interactions of salmon growth, smolting, and maturation rates based on observations of precocious parr in the Scottish salmon industry. This model was tested by Adams and Thorpe (1989) by comparing underyearling fish exposed to 2x2 factorial treatments of either elevated or ambient water temperatures, and either advanced (3-months ahead of natural phase) or natural, ambient photoperiods. As predicted by Thorpe (1986), conditions favoring growth (i.e. increased water temperature) during a February "maturation window" were associated with increased parr maturation. Enhanced parr maturation was avoided in other treatment groups, particularly for fish exposed to elevated water temperatures with an advanced photoperiod, such that they did not experience the "maturation window" of early-year ambient day-length increases. These findings emphasize the importance of photoperiod control during very early salmon development, in particular avoiding parr exposure to ambient photoperiod. Avoiding ambient photoperiod is relatively easy to achieve in closed containment aquaculture operations that use enclosed buildings; however, if exposure to ambient light during the maturation window cannot be guaranteed (e.g. when procuring young salmon from outside facilities using

assumed or unknown photoperiod conditions), other methods can be used to suppress early maturation. For example, parr maturation has been shown to be "switched off" by growth suppression during the spring months, such as by fasting fish in alternate weeks during this period (Rowe and Thorpe, 1990).

Beyond the parr stage, Atlantic salmon smolts have the capacity to sexually mature early as "grilse", which can be a major challenge to the traditional industry as these fish (unlike precocious parr) cannot be adequately identified and culled out prior to sea cage transfer. A variety of photoperiod treatments have been applied to sea cages, using underwater lighting, to reduce or eliminate grilsing. It must be noted that supplemental lighting in sea cages cannot be compared directly to lighting in closed containment growout conditions, as salmon in sea cages can still sense changes in ambient photoperiod beyond the artificial light being applied. Thus, "continuous" photoperiods employed in sea cages should be considered as "continuous additional lighting", versus a true LD24:0 photoperiod that can be applied in closed containment conditions. An interesting study by Taranger et al. (1998) compared the effects of nine different photoperiod regimes applied to immature Atlantic salmon in their final year of production (i.e. following 1.5 years at sea under natural photoperiod conditions, and a grilse cull prior to study initiation) on the incidence of early maturation. In this study, fish were raised in sea cages until mid-summer, and then transferred to land-based raceways using brackish water. Photoperiod treatment combinations included exposure to (i) natural light, (ii) continuous additional light beginning in January, or (iii) continuous additional light beginning in March, while in sea cages, and (i) natural light, (ii) LD24:0, or (iii) LD8:16 following transfer to raceways. All treatment groups receiving either natural light or continuous

additional light beginning in March while in sea cages exhibited high (>50%) grilsing rates, with the highest grilsing rate (78%) observed in the treatment group receiving natural photoperiods in both sea cages and raceways. By far, the lowest grilsing (5%) was observed in fish receiving continuous additional lighting beginning in January while at sea, and LD24:0 while in raceways. Again, while it is difficult to fully extrapolate these results to closed containment growout conditions, these findings suggest that continuous photoperiod during the final year of production can assist in decreasing overall grilsing rates. However, because LD24:0 photoperiods have been applied during TCFFI salmon growout trials, with correspondingly high, but variable grilsing rates, there are clearly other factors that could be influencing maturation in these trials, such as water temperature in the growout phase, photoperiod conditions during first-year rearing prior to growout, or other unknown risk factors.

Research on photoperiod manipulation immediately following the induction of smoltification, i.e. an artificial winter followed by a period of LD24:0, has been carried out by Duncan et al. (1999), who compared maturation rates in post-smolts transferred to seawater (in this case, land-based tanks with pumped-ashore ocean water) and monitored for a year under various photoperiod conditions. Surprisingly, first-year post-smolts receiving LD24:0 for one year starting in December, (whereas, photoperiod conditions did not change following the induction of smoltification), demonstrated the highest maturation rates, while salmon exposed to a simulated natural photoperiod demonstrated no maturation, based on a GSI cut-off of 3%. Fish in the LD24:0 group exhibited significantly greater growth during the study year, although specific growth rate (%/day) declined in comparison to the simulated natural

photoperiod group during the final months of the year. Given that the natural photoperiod during this timeframe represented a true calendar photoperiod (i.e., spring increase, followed by fall decrease), it would have been informative to have included a treatment group receiving natural photoperiod until summer solstice, followed by LD24:0 for the remainder of the year; this change to LD24:0 following midsummer could have switched off the development of puberty in the second half of the calendar year (Bromage et al., 2001). Nonetheless, the results of this study could have implications for closed containment production, in that a period of increasing daylength following induction of smoltification might decrease the incidence of early maturation observed during the remaining production period.

Berrill et al. (2003) investigated the timing of applying an artificial winter to induce smoltification on the subsequent incidence of precocious parr maturation, considering that faster growing fish would "decide" whether to devote energy to smoltification or sexual maturation. Fish were given LD24:0 photoperiod from first feeding onwards, and an S_0 winter either beginning in May, August, or September (or, in a fourth group, no S_0 winter was applied), followed by a return to LD24:0 photoperiod. Fish exposed to an early (May) winter had significantly higher precocity versus other groups, whereas late artificial winter groups (August and September) had relatively low maturity levels (although fish in the September group did not smoltify as completely as those in the August group). The S_0 winter is typically applied at TCFFI beginning in August; however, direct comparison between TCFFI maturation rates and those observed by Berrill et al. (2003) is difficult, due to (i) fish size differences (generally 40 g in average weight prior to S_0 winter at TCFFI, vs <10 g as reported by Berrill et al. (2003)), and (ii) water temperature

differences, whereas TCFFI fish are typically exposed to 12-13 °C water during first year rearing, versus Berrill et al. (2003) who exposed fish to ambient temperatures ranging from approximately 20 °C in midsummer to <5 °C in January. Therefore, in this study, fish exposed to different S0 winters were also exposed to different water temperatures, and different directions of water temperature change, during these winters. In a follow-up study under similar conditions, Berrill et al. (2006) determined that a longer (i.e. 12-week, versus 8-week) short-day period to induce smoltification, starting in June as opposed to May, significantly reduced the number of precocious parr observed later in the year. Again, while the results of these studies are ultimately difficult to compare to observations made during TCFFI growout trials, they clearly demonstrate the importance of early rearing environmental factors, i.e. photoperiod, temperature, or the interaction of these two parameters, on subsequent precocious maturation. Thus, emphasis in future research should be applied to first-year environmental conditions as maturation "decisions" are clearly made beginning at a very early age.

While much research has focused on the effects of various photoperiod regimes throughout the Atlantic salmon production cycle, a comparatively small volume of research has examined the quantity (light intensity) and, in particular, the quality (spectral composition) of artificial light that fish are exposed to within these photoperiod regimes. Both quantity and quality of light have been shown to affect growth, reproduction, and other performance variables in teleosts (Oppedal et al., 1997; Karakatsouli et al., 2007, 2008). Light intensity, in particular, appears to act in a threshold manner in regulating various physiological functions in fish (Porter et al., 1999; Taylor et al., 2005, 2006), and increasing light intensity beyond a specific threshold has been

shown to increase growth and decrease maturation in typical end-of-cycle constant-light photoperiods applied to Atlantic salmon (Stefansson et al., 1993; Oppedal et al., 1997, 1999); however, decreased welfare associated with high intensity lighting has been noted (Migaud et al., 2007; Vera and Migaud, 2009). Based on studies using the hormone melatonin as an indicator for light perception (i.e. with increased light levels, melatonin release by the pineal gland is reduced), the light intensity threshold for perception in Atlantic salmon appears to be around 0.016 W/m^2 (Migaud et al., 2006; Vera et al., 2010). In terms of light quality, studies have suggested that Atlantic salmon suppress melatonin production more efficiently in response to blue and green light (450nm and 550nm, respectively) compared to red light exposure (700nm) (Migaud et al., 2010; Vera et al., 2010); however, more research is needed on the spectral sensitivity of Atlantic salmon. In a recent study, Leclercq et al. (2011) examined different lighting strategies to determine, among other things, their respective efficacy at controlling sexual maturation in Atlantic salmon during sea cage growout. While the effects of spectral composition (blue, red, green, or broad spectrum) could not be distinguished from light intensity, the authors' data strongly suggest that light intensity is the major determinant affecting Atlantic salmon light perception (and hence, affecting suppression of sexual maturation), and that a mean intensity of 0.012 W/m2 appeared to be the threshold to reduce maturation (which coincides closely with the 0.016 W/m2 threshold intensity determined in laboratory studies, mentioned earlier). Given the differences in closed containment versus sea cage growout conditions (e.g. rearing unit volume, water clarity, rearing densities, etc.), baseline research is needed to establish lighting quantity and quality best management practices in order to reduce early maturation while not compromising welfare in Atlantic salmon closed containment production facilities.

Input from industry

TCFFI has, for the most part, employed a LD24:0 photoperiod from first feeding up to harvest at 4-6 kg in size using above-tank metal halide, fluorescent, and LED lights, excepting a pre-smolt LD12:12 6-week "winter", and has experienced varying but relatively high levels of grilsing in all Atlantic salmon growout cohorts. Several Atlantic salmon producers were contacted for the purposes of this technical paper, regarding their current photoperiod strategies and relative success at reducing grilsing. The following is a summary of anecdotal information generously provided by these individuals.

Danish Salmon (Hirtshals, Denmark) employs the same photoperiod strategy as TCFFI (i.e. LD24:0 throughout, aside from a LD12:12 winter to induce smoltification), although due to numerous water quality issues (as a consequence of chilling and discharge consents) over the past year, they are reluctant to make any conclusions regarding the impact of their photoperiod regime on maturation (Mark Russell, Danish Salmon, pers. comm.).

Sustainable Blue (Centre Burlington, Nova Scotia, Canada) has carried out in-house research to reduce maturation in their salmon, utilizing a LD16:8 photoperiod and focusing on feeding rates within this regime; Jeremy Lee provided the following description of this work:

"We performed an initial trial on our first batch of salmon [smolts from an off-site producer, 40-60 g in size]. These were St John River strain. The batch was split into two tanks on arrival from a local hatchery; roughly 50% per tank. One tank was held on 75% ration and the second on 100% ration for the first 100 days in saltwater. After 100 days both tanks were fed 100% planned

ration. Both tanks were at 15 °C and on the same water treatment system. Both tanks had a 16 hour daylight, 8 hour dark photoperiod. Total maturation was 8% at 9.5 months and an average weight of 2.8 kg. Importantly there was no significant difference in maturation between the tanks at the 95% confidence level. It is still too early to report on our latest batches. These are from our own hatchery and have been on controlled photoperiods throughout their lives. Those in the grow-out unit are currently on a 16 hour day, 8 hour night; the same that was used for the initial batch. The largest batch has now reached 1 kg after 3.5 months in 26 ppt. There is no sign of maturity so far and the water temperature is 14 °C. This stock is Mowi." (Jeremy Lee, pers. comm.)

Frode Mathisen, Director of Biological Performance and Planning for Grieg Seafood ASA (Bergen, Norway), provided the following information on photoperiods applied either through their freshwater or seawater regimes, prior to transferring smolts to sea cages:

"We haven't seen any maturation issues [no maturation in hatcheries and very low grilsing in sea cages]. We run two different light regimes, depending on the salinity the hatchery can run:

Freshwater regime

1. 0-40 gram (320 g) on 24 hour light
2. 40 gram (320 g) up to 3-4 weeks before delivery to sea: on 12 hour light (minimum time on 12 hour light is 4 weeks)
3. The last 3-4 weeks on 24 hour light until the fish finish smoltification and are delivered to seawater sites

In this regime it is important to turn the lights off before the fish go into a "size smoltification". There is some uncertainty about that process, but we have

kept fish up to 70 gram on 24 hour light without any significant smoltification issues. When the fish are on 12 hour light, we can hold it there as long as we want. With this process we have produced fish groups from 60 to 250 gram.

Seawater regime

1. 0-40 gram (320 g) on 24 hour light
2. 40 gram (320 g): 4-6 weeks on 12 hour light
3. Fish are moved into brackish RAS on 24 hour light (salinity 8-15 ‰) where the fish finish smoltification in 3-4 weeks.
    - Some fish are moved to seawater sites as soon as they have become smolts
    - Most fish are kept in the brackish RAS on 24 light until they reach 200-500 gram and are delivered to a seawater site

Lastly, through a 16-month production cycle Kuterra Land-Based Salmon (Port McNeill, BC, Canada) has generally employed a LD24:0 photoperiod from winter to summer solstice, and simulated natural photoperiod from summer to winter solstice, although this strategy has varied depending on the time of year that various smolt cohorts have arrived on-site. Grilsing (both male and female) has generally been high, and the percentage of grilse has varied among growout cohorts (Cathal Dineen, Kuterra, pers. comm.).

Water temperature

Water temperature is one of, if not the most important environmental parameter known to influence fish physiology. Fish are poikilothermic, meaning that their internal temperature is largely regulated by the ambient temperature of the environment; thus, water temperature has a profound influence on fish biology. Metabolic rate, growth rate, time of spawning and egg hatching,

the physiological development of eggs and larvae, and the reproductive development of fish are all related to water temperature of the natural environment (Piper et al., 1982; Jobling, 1995; Wedemeyer, 1996). The effect of water temperature on Atlantic salmon biology is no exception. Brett (1979) reported that salmonid growth increases linearly with temperature. Research provided by Austreng et al. (1987) supports these findings, demonstrating that young Atlantic salmon cultured in freshwater from 0.15-75 g at temperatures ranging from 2-16 °C, grew fastest at 16 °C and slowest at 2 °C. The same study reported maximum Atlantic salmon growth in marine net cages at 14 °C, when evaluating fish growing from 0.03 - 2.0 kg at temperatures ranging from 2-14 °C (Austreng et al., 1987). Handleland et al. (2008) reported that growth rate, feed intake, feed conversion efficiency, and stomach evacuation rate were significantly influenced by water temperature (6-18 °C) and size of post-smolt Atlantic salmon (70-300 g) cultured in seawater, with the fastest growth rates occurring at 14 °C and the slowest growth rates occurring at 6 °C. The importance of water temperature to Atlantic salmon biology is amplified when considering the seasonal migratory behavior of this species. Jonsson and Rudd-Hansen (1985) found that temperature acted as the primary parameter of influence for downstream smolt migration from the Imsa River in Norway, while McCormick et al. (2002) concluded that water temperature likely controlled the rate of juvenile development, but interacted with photoperiod relative to the timing of smoltification. Friedland et al. (2000) reported that Atlantic salmon smolts from the Figgio River in Norway and North Esk River in Scotland typically swim to sea during late April to early May when the marine water temperature is 8-10 °C. Furthermore, a wealth of studies (described in the subsequent section) have linked temperature to Atlantic salmon size/age at maturation, the timing of reproductive maturity, and the proportions of grilse versus multiple sea-winter salmon.

Saunders et al. (1983) cited substantial evidence of the water temperature of sea-cage sites acting as a determinant of the timing of Atlantic salmon maturation, where lower water temperatures often correlated with reduced maturation during the first sea-winter and a decreased rate of grilsing. In addition, Adams and Thorpe (1989) found that female Atlantic salmon exposed to increased water temperature, 5 °C above typical ambient temperature, showed higher reproductive investment (ooycyte size) than those under normal growing conditions, and also found that male parr exposed to temperature conditions consistent with an increased growth opportunity had a higher maturation rate. Several recent studies provide additional evidence that increased water temperature is at least partly related to early maturation of Atlantic salmon. During a long-term study comparing different combinations of light and water temperature, Imsland et al. (2014) observed an increased rate of early maturing male Atlantic salmon (i.e. 66% vs. 11%) when cultured at 12.7 °C vs. 8.3 °C, respectively. During this study, pre-smolt Atlantic salmon (initial weight = 15.9 g) were cultured in freshwater for 11 months, then relocated to seawater for 2 months. After the 2-month seawater period, the salmon were slaughtered to assess final weight (169-586 g) and maturity. Atlantic salmon cultured under continuous light at higher water temperature (12.7 °C) grew 70-330% faster than other groups, but also exhibited the highest degree of early male maturation (82%). Imsland et al. (2014) concluded that long term rearing of Atlantic salmon under continuous light, but lower water temperature (in this case 8.3 °C) led to a better balance of growth and reduced maturation. Based on these findings, Imsland et al. (2014) suggested that photoperiod was the primary directive for the onset of sexual maturation, but temperature likely controlled the magnitude of the photoperiod effect. Similarly, Fjelldal et al. (2011) demonstrated that a combination of elevated water temperature and

continuous light can trigger maturation of male Atlantic salmon during and immediately after smoltification. Early maturation of male Atlantic salmon was particularly pronounced for parr cultured at 16 °C with a 24-h photoperiod, as compared to culture at 5 and 10 °C in combination with various photoperiod regimes, whereas 47% of male salmon cultured at 16 °C began to mature (i.e. mean gonadosomatic index (GSI) > 1.5%) after only 6 weeks of exposure. After an additional 2 months of culture, the same population of maturing males was found to have a mean GSI of 7.3%. No maturing males were noted after the initial 6-wk trial for temperature treatments of 5 and 10 °C combined with any of the photoperiods (including 24-h continuous light) (Fjelldal et al., 2011).

With so many documented variables and combinations of parameters that could influence maturity, separating the most impactful factors is a complex task. Therefore, McClure et al. (2007) applied a multivariate approach to identify variables that were most associated with greater risk of grilsing in Atlantic salmon. Grilsing prevalence was evaluated at 266 commercial net cage sites in New Brunswick and Nova Scotia, at 24 different farms. The percentage of grilse within cages was variable, ranging from 0-64.1%; while the within-farm rate of grilsing ranged from 1.6-38.7%. The median within-cage and -farm grilsing rate was 6.6% (McClure et al., 2007). A variety of risk factors possibly contributing to increased grilsing were evaluated during the McClure et al. (2007) study including: smolt weight at time of transfer to net cage, cage type, use of moist feed and duration of feeding moist pellets, feeding rate, weight gain, average water temperature during the first February at sea, average water temperature during second September at sea, and the change in water temperature between the first February and second September at sea. The final statistical model identified two risk factors that were most associated

with increased grilsing rates: 1) salmon weight during the second August at sea, and 2) the difference in water temperature between the first February and the second September at sea. Surprisingly, weight gain was not significantly correlated to average monthly water temperature during this study. Overall, McClure et al. (2007) found that warmer water during summer months and colder water during winter months was generally associated with increased grilsing, but the most significant risk was related to the magnitude of the water temperature change between seasons.

Many physiological effects associated with increasing water temperature, such as increased growth, condition factor, and adipose content have been linked to expedited maturation in Atlantic salmon. For example, many of the aforementioned studies (Saunders et al., 1983; Adams and Thorpe, 1989; Imsland et al., 2014) discussed increased growth as correlating with early maturation. In addition, Herbinger (1987) identified relationships between patterns of growth and early maturation in Atlantic salmon, whereas grilse maturation was repeatedly associated with fast growth during the first winter, 6-12 months prior to maturation. Thorpe (1986) proposed a model that described Atlantic salmon maturation as governed by growth rate within a critical "maturation window" that occurs in the spring. In a review of factors influencing the onset of puberty in fish, Taranger (2010) identified growth as a key factor correlating with the initiation of reproductive development and stated that "the reproductive system [of fish] is usually silenced until an individual's somatic development has proceeded sufficiently to permit investment in pubertal development." Policansky (1983) encapsulated these concepts in the following statement, "Under stable conditions with abundant food, fishes should grow rapidly and mature as soon as they are developmen tally able to do so."

Other physiological variables that are typically concomitant with growth, such as adiposity, condition factor, and energy reserves, have also been associated with the onset of early maturation in Atlantic salmon. Simpson (1992) found that mature male Atlantic salmon parr (0+ age) were larger and had higher fat content than non-maturing salmon. Conversely, Rowe and Thorpe (1991) found evidence that maturation of male Atlantic salmon parr was suppressed when mesenteric fat failed to exceed an undefined level by May. Herbinger and Friars (1991) linked the initiation of grilsing with specific levels of lipid storage in the spring, along with a corresponding increase in condition factor. Similarly, Kadri et al. (1996) suggested that the spring/summer period of increased feeding and subsequent accelerated growth in maturing Atlantic salmon that had experienced one sea-winter resulted in a level of nutrient reserves that was critical for maintenance of the maturation process. In addition, Peterson and Harmon (2005) found that condition factor and GSI were correlated in post-smolt Atlantic salmon and a condition factor exceeding 1.3 was required by early summer for early maturation to develop. Furthermore, Mangel and Satterwaite (2008) proposed that if Atlantic salmon lipid stores are maintained at sufficient levels throughout the first sea-winter, the path to maturation will also be maintained and the individual salmon will mature by the following November. Conversely, if lipid stores are depleted during the first sea-winter and cannot be sufficiently maintained, further reproductive investment is postponed for another year (Mangel and Satterwaite, 2008). Similar studies evaluating other salmonid species have also drawn conclusions linking thresholds of adipose tissue or energy/nutrient reserves with advanced maturation (Silverstein et al., 1997 – amago salmon, Oncorhynchus masu ishiikawai; Silverstein et al., 1998; Shearer and Swanson, 2000; Shearer et al., 2006 – chinook salmon).

A brief review of information regarding water temperature utilized by the conventional salmon industry is critical for comparison to water temperatures that are used for Atlantic salmon culture in RAS. Kristensen et al. (2009) provided a detailed overview of inlet water quality at Atlantic salmon smolt facilities in Norway and Chile, and found that water temperature varied widely depending on season at most Norwegian smolt farms. Mean inlet water temperatures at Norwegian smolt farms ranged from as low as 3 °C during winter to as high as 14 °C during the summer (Kristensen et al., 2009). In Chile, mean annual water temperature for salmon smolt production facilities ranged from 9.4 - 13.1 °C with annual site variation of approximately 1 °C, based on a limited data set provided by the Chilean industry (Kristensen et al., 2009). While the variation in temperature for smolt production is primarily reflective of seasonal inlet water temperature (Marine Harvest ASA, 2014), the varied methods by which smolt are produced also has an influence. Bergheim et al. (2009) reported that more than 90% of European salmon smolt farms used land-based flow-through systems. Heating the water of flow-through systems can be energy intensive and costly; therefore, most smolt farms are subject to the inherent temperature of the inlet water. In contrast, Daalsgard et al. (2013) reported a mean water temperature of 12-14 °C for RAS-produced Atlantic salmon smolt in Nordic countries. Therefore, many Norwegian smolt farms are transitioning from flow-through to recirculation aquaculture technology in an effort to increase average water temperature (particularly during winter months), ultimately to enhance growth performance, grow larger smolts, and reduce time at sea, among other advantages (Bergheim et al., 2009; Kristensen et al., 2009; Daalsgard et al., 2013). It may be important to note that water temperatures reported for Norwegian smolt farms are typically below the range for optimal Atlantic salmon growth, i.e. 14-16 °C (Austreng et al.,

1987; Handleland et al., 2008). While Atlantic salmon cultured using traditional farming practices are subject to seasonal ambient water temperatures (Kristensen et al., 2009; Marine Harvest ASA, 2014), salmon cultured in recirculation aquaculture systems are generally reared at warmer temperatures due to the inherent control provided by this technology. Optimal temperatures for growth performance can often be targeted and maintained, depending on the recirculation system design. At TCFFI, water temperature of a commercial-scale RAS used for salmon growout is controlled by adding more or less cool (12-13 °C) spring water depending on the season, with greater volumes of spring water used during summer months to chill the culture water and less spring water added during winter months to retain heat (Summerfelt et al., 2013). This temperature control regimen generally allows TCFFI to maintain optimal and consistent temperatures for salmon growth of 15-16 °C year round. (For detailed summaries of the five growout trials conducted at TCFFI, with respect to photoperiod regimes, water temperature during each production phase, and estimates of male maturation during each trial, please refer to the Tables & Figures section at the end of this paper.) At the Kuterra Closed Containment salmon facility in British Columbia, Canada, water temperature is controlled through the use of heat pumps and heat exchangers and fine-tuned by varying the airflow exhaust of carbon dioxide stripping columns. Overhead heaters that circulate and heat the facility air within the enclosed building also provide a certain degree of temperature control seasonally (Cathal Dineen, Kuterra, pers. comm.). Other technologies such as heat exchangers, chillers, and in-line heaters are also available for heating and cooling the culture water of RAS. It should also be noted in the context of temperature control that some RAS are now being operated as nearly closed systems while employing denitrification technologies to limit nitrate accumulation (van Rijn et al., 2006), thereby allowing increased heat retention.

Sustainable Blue (Nova Scotia, Canada), a commercial venture culturing Atlantic salmon to market-size in land-based closed-containment systems, utilizes denitrification technology in the RAS recycle loop. The capital and operational (energy) costs of technologies associated with temperature control, as well as the farm location and the general climate, impact selection of water temperature control methods for RAS. When culturing Atlantic salmon in RAS, the natural inclination might be to utilize water temperatures that are known to optimize growth. However, as gleaned from this literature review, accelerated growth is generally associated with a faster track towards maturation, particularly for male Atlantic salmon. More research is needed to evaluate grilsing rates at various water temperatures, in order to identify a temperature range that provides acceptable growth performance while also resulting in diminished maturation rates. Assemblage of data from six Atlantic salmon production trials at the Kuterra land-based salmon facility indicates that Atlantic salmon production at a relatively constant water temperature of 13 °C has resulted in lower (initial) rates of early maturation compared to salmon cohorts cultured at average water temperatures >14 °C; however, this apparent benefit associated with lower temperature was not observed long-term (Cathal Dineen, Kuterra, pers. comm.). At Kuterra, numerous other factors could have been related to early maturation, including variation in water clarity and subsequent reduction of light penetration through the water column which in turn could have interrupted photoperiod cues (Cathal Dineen, Kuterra, pers. comm.). The exact influence of water temperature on early maturation remains unclear and controlled, replicated research is required for further understanding. Evidence indicates that sudden temperature increases should be avoided during land-based Atlantic salmon production. Fjelldal et al. (2011) and Melo et al. (2014) have suggested the importance of increased

water temperature (e.g., from 12 °C to 16 °C) for triggering male maturation immediately following the smoltification period. This has also been noted, anecdotally, at TCFFI, where an increased prevalence of early maturing male Atlantic salmon was observed on several occasions when recently smolted Atlantic salmon (70-110 g) were transferred from flow-through and partial reuse systems maintained at 12-13 °C into low exchange RAS with 14-16 °C water (see Figures 4 & 5). Likewise, anecdotal observations at Kuterra's closed containment facility indicated that a substantial increase in water temperature from 11.5 to 15 °C (caused by start-up of a water heating system) was one of several variables that could have instigated an apparently sudden increase in the prevalence of early maturing Atlantic salmon. In this case, the increased rate of maturation was noted during the later stages of an early growout trial when salmon were approximately 2 kg (Cathal Dineen, Kuterra, pers. comm.).

Atlantic salmon cultured in RAS can be subject to more rapid and sudden changes to water temperature if proper temperature control methods are not employed; therefore, variation in water temperature should not be ignored as a contributing factor to early maturation. Avoiding sudden increases in water temperature could have particular relevance when relocating salmon from juvenile (parr, pre-smolt, or even recent smolt) production systems to RAS. Younger life stage production of Atlantic salmon often takes place in flow-through, partial reuse systems, or RAS that use relatively high water exchange rates and cooler water, while large recirculation aquaculture systems for growout typically use substantially less water, thereby retaining more heat and providing increased water temperature. More research is needed to evaluate whether sudden changes in water temperature can instigate early maturation in RAS-produced salmon.

Feed

In previous sections, substantial evidence was provided indicating that early maturation in Atlantic salmon could be triggered by interactions between photoperiod, water temperature, enhanced growth, and other concomitant variables such as increased body lipids and condition factor. The following discussion describes early attempts and future considerations related to feeding RAS-produced Atlantic salmon with a goal to adjust growth and/or body lipid and thereby limit early maturation.

At TCFFI, a research trial was recently conducted to evaluate whether a restricted ration provided to post-smolt Atlantic salmon would lead to a reduction in early male maturation. The 3-month feeding trial began when the fish were 94 g and approximately 9.5 months old (post-hatch). The study was conducted using a partial-reuse system with water temperature ranging from 11-13.5 °C and 24-h overhead LED lighting. One tank of salmon was fed to satiation, while another tank of salmon was fed at approximately 65% of full ration; each tank began with 2,238 fish. At the conclusion of the trial, mean salmon weight was not remarkably different between feeding regimes; i.e. 242 g versus 203 g, for the full and restricted rations, respectively. Feed conversion ratios were significantly lower for the group of salmon fed the restricted ration. In order to evaluate subsequent effects of the respective rations on maturation, a representative number of salmon from each group were marked for identification using visible implant elastomer tags injected beneath the translucent tissue posterior to the eye. Subsequent observations of the tagged fish were made approximately 8 months later when all obvious early maturing males were culled from the population and mean weight was approximately 2 kg. The prevalence of maturation was assessed again when the salmon were harvested as food fish approximately 15-16 months after the ration trial had

taken place. No difference in grilsing rate was noted during either sampling event. The results from this research are possibly confounded by the less than dramatic difference in growth performance measured during the 3-month ration trial. It is suspected that the full ration feeding rate was in excess of what the fish required for growth, and that some of the feed was wasted and/or was consumed but not efficiently converted into biomass. In contrast, the feeding rate utilized in establishing the restricted ration treatment was likely closer to the optimal feeding rate, because relatively good growth resulted in combination with reduced FCR. Possible impacts on maturation would have been more interesting if observed in salmon that had substantially delayed growth performance, lower condition factor, and possibly lower adipose content resulting from reduced feeding, as thresholds for each of these traits have been described as possible triggers for early Atlantic salmon maturation (Thorpe, 1986; Herbinger, 1987; Adams and Thorpe, 1989; Herbinger and Friars, 1991; Peterson and Harmon, 2005; Shearer et al., 2006; Mangel and Satterwaite, 2008). Lipid content of the fish was not measured at the conclusion of the 3-month ration trial.

In addition to the impact of feeding regime on maturation, diet composition may be considered as a factor that could be adjusted to reduce grilsing. For example, several studies have suggested that adipose content or energy reserve thresholds at specific life stages could trigger the onset of reproductive maturation in Atlantic salmon (Rowe and Thorpe, 1991; Simpson, 1992; Kadri et al., 1996). With this in mind, it seems logical that consideration should be given to optimizing the fat content in Atlantic salmon diets to reduce the accumulation of excess body lipid. Jobling et al. (2002) proved that lipid composition in Atlantic salmon could be manipulated by adjusting the fat content of the feed. Two groups of Atlantic salmon parr fed diets containing

34 and 22% lipid were found to have percent body fat of 10-12% and 5-7%, respectively, after six months of feeding (Jobling et al., 2002). Therefore, it may be worthwhile to investigate whether there are potential benefits related to reduced lipid diets at specific life stages when RAS-produced salmon are expected to initiate maturation. Fine tuning diets for Atlantic salmon cultured throughout the production cycle in RAS might be of particular importance, because these systems provide the opportunity for rapid salmon growth at optimal water temperatures with automated feeding around-the-clock, a scenario that provides opportunity for satiation feeding and possible accumulation of excess energy reserves. However, research facilities and early commercial ventures culturing Atlantic salmon in RAS are primarily using Atlantic salmon diets designed for commercial net pen operations. These diets are often formulated to be fed during specific seasons; for example, EWOS recognizes the variation in Atlantic salmon feed intake and performance that occurs seasonally due to the changing ocean water temperatures that net cages are subject to, and therefore offers seasonal diets to accommodate varying fish metabolism (EWOS, 2014). The emerging salmon RAS industry could benefit from diets that are designed specifically for the rapid growth and high metabolism that is expected within these culture systems. Finally, baseline studies focusing on a range of nutrients in salmon diets, and their association with early maturation, still need to be carried out, such as work by Alne et al. (2009) who found reduced sexual maturation in male post-smolts fed supplemental tetradecylthioacetic acid.

Exercise

The use of circular rearing units in closed containment facilities provides aquaculturists with the opportunity to adjust rotational water velocity and,

hence, the current which fish are forced to swim against. Although not all species of fish benefit from swimming exercise, salmonids, which in general are athletic species, demonstrate significant improvement in growth performance when provided moderate, sustained exercise (Davison and Goldspink, 1977; East and Magnan, 1987; Totland et al., 1987; Davison, 1997). Increased growth in response to exercise has been considered mainly the result of hypertrophy of muscle fibers (Davison, 1997; Johnston, 1999). Exercised fish generally have improved fillet texture (Totland et al., 1987; Bugeon et al., 2003) and hence greater consumer appeal. Other benefits of sustained exercise in salmonids include less aggression, increased resistance to infectious organisms, and prevention of precocious maturation (Palstra and Planas, 2011). Whole body lipid content has been shown to influence early maturation (Shearer and Swanson, 2000; Shearer et al., 2006); however, there is little clear consensus in the scientific literature regarding the effects of exercise on overall energy deposition and whole body composition (fat, protein, etc.) in fish (Jobling et al., 1993; Rasmussen et al., 2011), and therefore the preventative effect of sustained exercise on sexual maturation is likely not via reduced adiposity but rather through other metabolic pathways. Studies investigating exercised versus unexercised Chinook salmon raised for enhancement stocking in the Pacific Northwest demonstrated a higher proportion of early maturing males (mini-jacks) in unexercised fish (Don Larsen, NOAA, pers. comm.), and unpublished research at TCFFI determined that exercised (i.e. >1.5 body-lengths per second, BL/s) first-year Atlantic salmon were significantly less likely to develop into precocious parr than salmon that were held under relatively static conditions (i.e. <0.5 BL/s). The inhibitory effect of exercise during early rearing on early maturation has thus been demonstrated; however, further research is needed to determine the effects of moderate sustained

exercise on the rate of grilsing during second-year growout, as there is currently no opportunity at TCFFI to maintain more than one swimming speed treatment group during this production phase. Berg et al. (1996) examined swimming speed and its effects on sexual maturation in adult Atlantic salmon, but no differences were determined between fish exposed to low versus medium swimming speeds, a likely reason being that there was actually a very small difference between the two treatment velocities. Therefore, more research is needed to examine the role of exercise, alone and in combination with other variables (e.g. photoperiod, water temperature), on growout Atlantic salmon, in order to develop best management practices for closed containment Atlantic salmon production.

Genetic strain

As with numerous other performance traits, the range of maturation timing for Atlantic salmon shows a degree of heritability between populations (Wolters, 2010), and various strains of Atlantic salmon are known for their "high grilsing" or "low grilsing" nature (e.g., as described by Berrill et al., 2003). Gjerde (1984) evaluated the heritability of age at sexual maturity in Atlantic salmon by using various crosses of fish from parents that matured as 1-year parr or precocious males, and at 4 and 5 years of age after two and three winters at sea, respectively. Gjerde (1984) found that parental age at maturity had a significant impact on offspring age at maturity and concluded that there is potential to alter the age at reproductive maturity in Atlantic salmon through genetic selection (although multiple generations were not assessed in this particular study). Scottish and Norwegian strains have been selected for late maturation; however, breeding for this trait has led to issues related to a decreased ability to induce spawning in captive broodstock (Rudi Seim,

SalmoBreed, pers. comm.). Strains that have been bred for late maturation may perform well in this regard in sea cages; however, as has been demonstrated at TCFFI, high levels of male grilsing can still occur in such "late maturing" strains under conditions of closed containment, recirculation aquaculture. Aside from genetic selection as a method to reduce early maturation in Atlantic salmon cultured in RAS, other genetic techniques could also be advantageous. The use of triploidy has been offered as a possible solution, because triploid fish are generally sterile; however, male triploid fish (including salmonids), still experience gonadal development (Benfey, 1999), and in turn the associated downgrades in product quality that are undesirable to the consumer (Aksnes et al., 1986). Development of an all-female strain of Atlantic salmon could solve the brunt of early maturation problems because most grilsing has been associated with male Atlantic salmon. While Norwegian and Icelandic breeding companies have been working on technologies to provide an all-female germplasm to customers (Rudi Seim, SalmoBreed, pers. comm.), these products are presently unavailable, although all-female eggs from Iceland might be available to producers as early as late 2015. A major reason for the slow development of all-female eggs is that sea cage producers generally prefer male salmon, provided that they do not mature early, due to their superior growth over females during the second year of growout. Therefore, there is little incentive for breeders to provide all-female eggs, although growth in the closed containment sector and the resultant potential demand for such products could increase incentive in the very near future. All-female salmon have been created previously in Chile (1990-2000), but poor performance and survival (for unknown reasons) led to the abandonment of this approach and a return to mixed-sex cohorts (Manuel Godoy, Recirculacionchile Ltda, pers. comm.). All-female Atlantic salmon eggs were also available, until relatively

recently, in the US Pacific Northwest, although the cessation of the particular company's domestic salmon broodstock program ended the availability of these eggs to producers. Presently, all-female salmon cohorts are only raised in Tasmania, Australia; however, these eggs are created in-house and are not commercially available for outside producers.

Water chemistry

A myriad of water quality parameters, both known and unknown, have the potential to directly or indirectly modify the developing endocrine system of teleosts under culture conditions, and hence affect the timing of sexual maturation onset in Atlantic salmon and other species raised in closed containment systems or in more traditional aquaculture environments. Water recirculation technologies are often operated with very low system water exchange rates; therefore, closed containment systems may create additional challenges in this regard due to the potential accumulation of metabolites and biologically active compounds in the recycled water (Davidson et al., 2009; Martins et al., 2009; Martins et al., 2011), which may in turn affect (either in a dose-response or threshold relationship) the timing of sexual maturation in cultured fish. Given the possibility that endocrine-influencing water quality parameters could act in concert with other environmental variables (e.g. light, water temperature) to exert their impact, it is nearly impossible, at present, to provide conclusive statements regarding any particular water quality variable and its direct influence on maturation, as a given parameter will most likely not work in isolation in a closed containment environment. With this in mind, this section will focus on a select group of relevant water quality parameters that have been evaluated, to one degree or another, on their role in fish maturation, as well as other biologically active compounds that need to be examined

further for their potential to accumulate in closed containment systems and/or influence salmon maturation in these state-of-the-art salmon culture environments.

Nitrate nitrogen (NO_3-N)

Nitrate has been identified as a possible endocrine disrupting compound, and maturation of aquatic species could be exacerbated with exposure to elevated nitrate concentrations (Hamlin, 2007; Hamlin et al., 2008). For example, female Siberian sturgeon Acipenser baerii cultured in water with 57 mg/L nitrate nitrogen (NO_3-N) exhibited a significant increase in sex steroids including plasma testosterone (T), 11-ketotestosterone (11-KT), and estradiol compared to females grown at 11.5 mg/L NO_3-N (Hamlin et al., 2008). Freitag et al. (2015) tested various NO_3-N levels (5.3, 10.3, and 101.8 mg/L) to evaluate the endocrine disrupting potential of nitrate in pre-smolt Atlantic salmon (102 g to begin). This 27-day study reported that plasma T concentrations were significantly elevated for Atlantic salmon exposed to 10.3 mg/L NO_3-N, but no significant difference in plasma T was detected at the other NO_3-N treatment levels. Other sex-steroids including plasma 11-KT did not vary between treatments. Freitag et al. (2015), unfortunately, did not evaluate the long-term effect of nitrate on the reproductive development (gonadosomatic index) of Atlantic salmon. Recent research at TCFFI has demonstrated that long-term effects of nitrate exposure (100 mg/L vs. 10 mg/L) in post-smolt Atlantic salmon does not appear to impact early maturation (although early male maturation was highly prevalent overall in this study, limiting the ability to fully assess the influence of NO_3-N concentration). Contrary to the results of the aforementioned studies, it should be noted that nitrate has also been found to cause inhibition of reproductive function in some fish (Folmar et al., 1996 -

common carp, Cyprinus carpio; Edwards et al., 2006 - mosquitofish, Gambusia holbrooki) and other aquatic and terrestrial species (Guillete and Edwards, 2005 – Review). More research is certainly needed to evaluate how moderately elevated nitrate-nitrogen, as often observed in RAS culture, impacts the endocrine function and reproductive development in Atlantic salmon.

Examination of nitrate as a toxicant and endocrine disruptor to fish and other aquatic species has only recently become an emerging topic. Much of the concern surrounding the effect of nitrate stems from increased concentrations within natural aquatic environments due to anthropogenic activity (Camargo, 2005), as well as from the trend to culture fish in recirculating aquaculture systems, where nitrate can accumulate as an end-product of nitrification (Camargo, 2005; Davidson et al., 2009; 2011). In nature, most fish, including Atlantic salmon, are not typically exposed to significantly elevated concentrations of nitrate, at least in comparison to levels that can be achieved in RAS. Low nitrate conditions are also common within smolt hatcheries that utilize flow through systems, as well as in commercial net pen facilities, where vast quantities of water are exchanged through the culture units. In recent years, an increased number of salmon smolt producers have begun to utilize recirculating aquaculture systems (Bergheim et al., 2009), and a trend towards culture of market-size Atlantic salmon in land-based RAS has also emerged (Summerfelt and Christianson, 2014). Very little information describing the effect of nitrate to Atlantic salmon is available, however, and a recommended threshold has not been fully established for Atlantic salmon culture in RAS.

A few studies describing the effects of nitrate, or lack thereof, on Atlantic salmon and other salmonids have been published. In reviewing the following

citations, it is important to recognize that nitrate toxicity is species-specific as well as life stage-specific (Camargo, 2005). In the case of life stage in fish, nitrate tolerance generally increases dramatically with increasing fish size. For example, Kincheloe et al. (1979) reported mortality of larval Chinook salmon, rainbow trout Oncorhynchus mykiss, and cutthroat trout Oncorhynchus clarkii at NO_3-N concentrations as low as 2.3-7.6 mg/L; while Westin (1974) reported a 7-day LC50 of 1,068 mg/L NO_3-N for rainbow trout fingerlings. Despite the high level of nitrate required to produce acute mortality in rainbow trout, Westin (1974) recommended a maximum allowable concentration of approximately 57 mg/L NO_3-N for chronic exposure. In addition, Davidson et al. (2014) observed chronic health and welfare impacts to juvenile rainbow trout cultured in low exchange RAS at NO_3-N levels of 80-100 mg/L. Kolarevic et al. (2014) reported nitrate nitrogen (NO_3-N) concentrations < 1 mg/L in flow through systems and 6-28 mg/L NO_3-N in experimental RAS during a study evaluating the performance and welfare of Atlantic salmon smolt, and did not observe any negative effects associated with the elevated NO3-N in RAS. Furthermore, Freitag et al. (2015) concluded that juvenile (pre-smolt) Atlantic salmon were relatively insensitive to NO_3-N concentrations as high as 101.8 mg/L, and therefore suggested that Atlantic salmon might be a good candidate for production in RAS. At TCFFI, Atlantic salmon have been cultured to market-size in RAS at maximum NO_3-N concentrations of approximately 60 mg/L with no apparent negative effects to growth, survival, or physiology, although low nitrate growout RAS were not available to provide comparison group(s). More research is needed to establish a definitive nitrate-nitrogen threshold for Atlantic salmon culture in RAS. As the upper limit of tolerance is established, however, care needs to be taken to ensure that the endocrine system of Atlantic salmon is not disrupted and reproductive development accelerated.

Other endocrine-disrupting compounds

Certain natural or anthropogenic chemicals in the environment have the capacity to impair normal endocrine function, and these are termed endocrine disrupting compounds (EDCs) (Colborn, 1993). The study of EDCs over several decades has shown that their effects can be quite different depending on fish life-stage, that these effects can be delayed (i.e. exposure in fry can lead to effects observed in adulthood), and that dose-response relationships can often be unusual, depending on the EDC and the characteristics of the target aquatic organism (Sumpter and Johnson, 2005). The disruption of the hypothalamic-pituitary-gonadal axis of fishes by EDCs is well known, but interspecies variation is considerable and only a relatively small number of fish species have so far been investigated (Hamlin, 2014). The presence and effects of EDCs in aquaculture environments have been poorly studied, and this area currently represents a frontier of much needed research. Recent investigation has focused on environmental EDCs and their impact on fish populations in natural settings (Blazer et al., 2007; Iwanowicz et al., 2009). For example, Blazer et al. (2012) examined intersex male smallmouth bass (i.e. male fish with testicular oocytes) in the Potomac River basin, and found a spatial-temporal relationship between intersex severity and potential sources of EDCs, such as wastewater treatment plants and areas of intensive agriculture, particularly poultry operations. While most closed containment aquaculture facilities use groundwater sources, as opposed to surface water, the possibility of surface influence on groundwater and the consequent presence of EDCs entering closed containment facilities via the source water avenue (or other avenues, such as through feed) has not been studied, and research is needed to investigate this possibility. Furthermore, potential sources of EDCs and other contaminants within RAS materials themselves have not been investigated. As

the industry continues to grow there may be a need for research in this area, not only in relation to precocious sexual maturation but also in the context of food safety (e.g. the possibility that polychlorinated biphenyls (PCBs), mercury, heavy metals, etc. that might contaminate aquaculture products originating from closed containment operations). Modern aquaculture systems incorporate a relatively large amount of fiberglass and polyvinyl chloride (PVC) in rearing tanks and piping, which has the potential to introduce anti-corrosion compounds, such as bisphenol A, a known endocrine disruptor of Atlantic salmon (Honkanen et al., 2004) and brown trout (Salmo trutta) (Lahnsteiner et al., 2005), a variety of flame retardants, and other compounds, into system water. In theory, these compounds have the potential to accumulate in recirculating water and, among other things, influence maturation timing in cultured fish. As mentioned, however, no research specific to aquaculture and materials used in RAS construction has been carried out, and as such this area remains fertile territory for scientific investigation.

Steroid hormones

All fish release steroid hormones into their surrounding environment, either through urine and feces in conjugated forms (Vermeirssen and Scott, 1996) or through the gills in unconjugated “free” forms (Sorensen et al., 2000; Ellis et al., 2005). Thus, in water recirculation systems there is potential for these compounds to gradually increase in concentration in the recirculated water; however, whether this accumulation can influence maturation has not been adequately assessed. Steroid hormones such as T, 11-KT, and estradiol (E2) have been shown to accumulate in RAS (Good et al., 2014; Mota et al., 2014), although more research is needed to determine the specific effects of these and other hormones on fish in recirculation system water. Good et

al. (2014) were not able to determine a relationship between waterborne hormone concentration and precocious maturation; however, this study was not definitive, and future research should focus on accurately quantifying waterborne hormones in RAS, understanding their impact on maturation, and determining whether removal of hormones (i.e. via unit processes) is feasible and/or necessary. Biofiltration in RAS may already remove waterborne steroid hormones through biodegradation or sorption to suspended solids (e.g. Rogers, 1996; Onda et al., 2003; Mansell and Drewes, 2004); hormones might also be removed through volatilization in stripping columns and/or low-head oxygenators. Unfortunately, the majority of published research on this particular subject has focused on estrogenic compounds (and other endocrine disrupting chemicals) and their potential removal following passage through wastewater or sewage treatment facilities (e.g. Onda et al., 2003; Chimchirian et al., 2007; Cicek et al., 2007). Such facilities cannot be directly compared to closed containment RAS, as they typically have far longer retention times (e.g. 10-15 days) than water being treated in RAS unit processes; therefore, further research is needed focusing on the fate of steroid hormones in closed containment water recirculation loops.

Although research has been carried out to establish and refine methodologies for measuring fish steroids in water (Scott and Ellis, 2007; Kidd et al., 2010), more studies are needed focusing on Atlantic salmon and the effects of waterborne hormones on the endocrine function and sexual maturation of this species. In similar research on other species, it has been demonstrated that sexually maturing male European eels (Anguilla anguilla) are able to influence maturation in cohabitating immature males, the likely route being waterborne chemical communication (Huertas et al., 2006; Huertas et al., 2007). In theory,

uptake of hormones through the gills, gastrointestinal tract, and/or other routes should influence maturation if provided in sufficient quantities. In male teleosts, 11-KT (a derivative of T) is the major androgen produced by the testes (Taranger et al., 2010) and triggers the onset of maturation in a variety of fish species (e.g. Cavaco et al., 2001; Schulz and Miura, 2002; Campbell et al., 2003; Rodriguez et al., 2005); likewise, rising E2 levels are associated with the onset of secondary oocyte growth in females (Chadwick et al., 1987; King and Pankhurst, 2003). A complicating factor is that male and female hormones can exert influence on either sex; for example, T has been shown to have a stimulatory effect on early oogenesis and female maturation in coho salmon (Forsgren and Young, 2012) and milkfish Chanos chanos (Marte et al., 1988). Given the multiple potential effects of hormones (and their potential for enzymatic transformation to other hormone forms) on maturation, or the inhibition of maturation, in both sexes, and the current lack of knowledge on how maturation is affected in closed containment RAS in response to hormone accumulation, baseline research is necessary to gain a better understanding of this area to guide technologies and/or best management practices in these production settings. Anecdotal evidence at TCFFI has suggested that cohabitating juvenile salmon with older fish (a portion of which was sexually mature, or maturing) could increase the level of subsequent grilsing in the younger population, suggesting an influence of waterborne hormones or other compounds. This same phenomenon has been repeatedly observed in Chilean salmon production settings (Claudio Garcia-Huidobro and Manuel Godoy, Recirculacionchile Ltda., pers. comm.).

Salinity

Existing closed containment Atlantic salmon facilities utilize source water

of varying salinities, from those using freshwater throughout the production cycle (e.g. TCFFI) to those using full strength seawater during growout. The adjustment of culture tank salinity to optimum levels for salmon performance is an area of ongoing research, and unfortunately at present very little information is available to indicate whether various salinity levels impact precocious maturation in Atlantic salmon. Melo at al. (2014) investigated the role of fresh- vs. saltwater, and LD24:0 vs. LD12:12, in a factorial study of 12-month old Atlantic salmon exposed to a 3-month "maturation regime" (i.e., a rise in water temperature from 12 °C to 16 °C at the onset of the post-smolt stage), and determined that, while the majority of males in all treatment groups matured, exposure to saltwater appeared to stimulate the onset of gamete development, while LD12:12 photoperiod appeared to influence the completion of spermatogenesis. Further, longer-term research is necessary to determine the relative impacts of salinity and photoperiod on male maturation as fish are grown to market size, without pubertal "induction" through temperature elevation at the end of the smoltification period. Ongoing research is being conducted at the University of British Columbia focusing on various salinities and their association with Atlantic and coho salmon (Oncorhynchus kisutch) growth performance and early maturation (Josh Emerman, UBC, pers. comm.); results will likely be available later in 2015 or 2016. Aside from the aforementioned studies, the role of salinity in early maturation has not been investigated; most research in this area has focused on salinity tolerance in the context of parr-smolt transformation (e.g. Saunders and Henderson, 1978; Bjerknes et al., 1992; Duston and Knox, 1992). Given that closed containment technologies offer the benefit of a controlled, optimized rearing environment, determining optimal salinity levels for Atlantic salmon is essential to inform best management practices for these facilities. Focus

needs to be placed on optimum salinities at different life stages; for example, although smoltification and parr maturation are not necessarily biologically opposite processes (Saunders et al., 1994), they are in developmental conflict (Thorpe, 1986), and therefore pre- and post-smoltification salinities should be optimized for smoltification in order to deter the development of precocious parr. Likewise, during growout, Atlantic salmon are at a stage in their life history when they are normally in the marine environment, and only return to freshwater when ready to spawn; whether second-year growout in freshwater closed containment systems instigates the drive to spawn is presently unknown. Overall, baseline research is needed to investigate the role of salinity in Atlantic salmon maturation at various life stages.

SUMMARY

Factors associated with the onset and prevalence of maturation in Atlantic salmon populations are numerous, and have the capacity to work in concert to exert their influence. This complex mixture of physical and biological factors, when existing in the novel environment of closed containment growout, represents a major challenge to this growing sector of the salmon aquaculture industry, as at present the high prevalence of grilsing in closed containment may be impacting its economic feasibility. Very little research has been carried out specifically examining salmon maturation in water recirculation systems; instead, past studies have focused on conditions within the traditional salmon production cycle of early rearing on land followed by sea cage growout. As the industry moves forward, if mixed sex salmon populations cannot be avoided, due to unavailability of all-female eggs from breeding companies, then it is essential to engage in focused research on maturation in closed containment to inform the development of effective best management practices to combat grilsing. First and foremost, this research should center on the environmental variables of photoperiod and water temperature, alone and in combination, as it has been strongly suggested through previous research that photoperiod influences the decision to commence or delay maturation, and that water temperature determines the magnitude of maturation observed in a particular salmon population. Current practices of utilizing water temperature near the upper ranges for this species in order to promote growth performance, may be inadvertently contributing to the problem of grilsing in closed containment systems. While a recommendation to reduce rearing temperatures may be in order, producers need to weigh the benefits of potentially reducing grilsing versus the potential for lost growth performance. In the meantime, baseline

research on photoperiod and water temperature, in combination with other variables including numerous water quality parameters, nutritional content of feeds and feeding strategies, and a range of husbandry conditions and practices, needs to be carried out to confidently provide recommendations for grilse reduction protocols for closed containment salmon operations. Other frontiers of research specific to salmon maturation in water recirculation systems include quantifying the accumulation and impact of waterborne steroid hormones, investigating the possibility of endocrine disrupting compounds existing and exerting an influence in closed containment settings, and examining a range of lighting technologies that provide different light quantities and qualities, to develop optimized in-tank photoperiod exposure techniques. However, due to the long production cycle and current deficiency of infrastructure to carry out long-term replicated research, focusing on the areas mentioned above, it is likely that the most expedient approach is to work with breeding companies to establish the availability of all-female eggs specifically for the growing closed containment industry. While female grilsing, as observed presently in specific closed containment operations, could still be a potential production issue, the comparatively major problem of male grilsing would be entirely circumvented with the availability of all-female eggs. Given that Icelandic all-female eggs could be available to the closed containment industry beginning late 2015, the major issue of early maturation in these production settings could quickly become a thing of the past.

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TABLES & FIGURES

Figures 1-5. Water temperature profiles throughout five Atlantic salmon growout trials conducted at The Freshwater Institute, specified by rearing system and indicating male maturation prevalence at grilse harvests.

*Nursery system uses flow-through spring water (15-20 min culture tank retention time).
*Partial reuse system reuses 85 % H2O on a flow basis. System hydraulic retention time (HRT) = 2-3h.
*Reuse Grow-out reuses 90-100 % H2O on a flow basis. Average system HRT = 2 days.
* Norwegian I salmon were cultured in 6 RAS at an average system HRT = 20 days.
* Norwegian II salmon were cultured in 6 RAS at an average system HRT = 1.3 days.

Figure 1. St. John River strain.

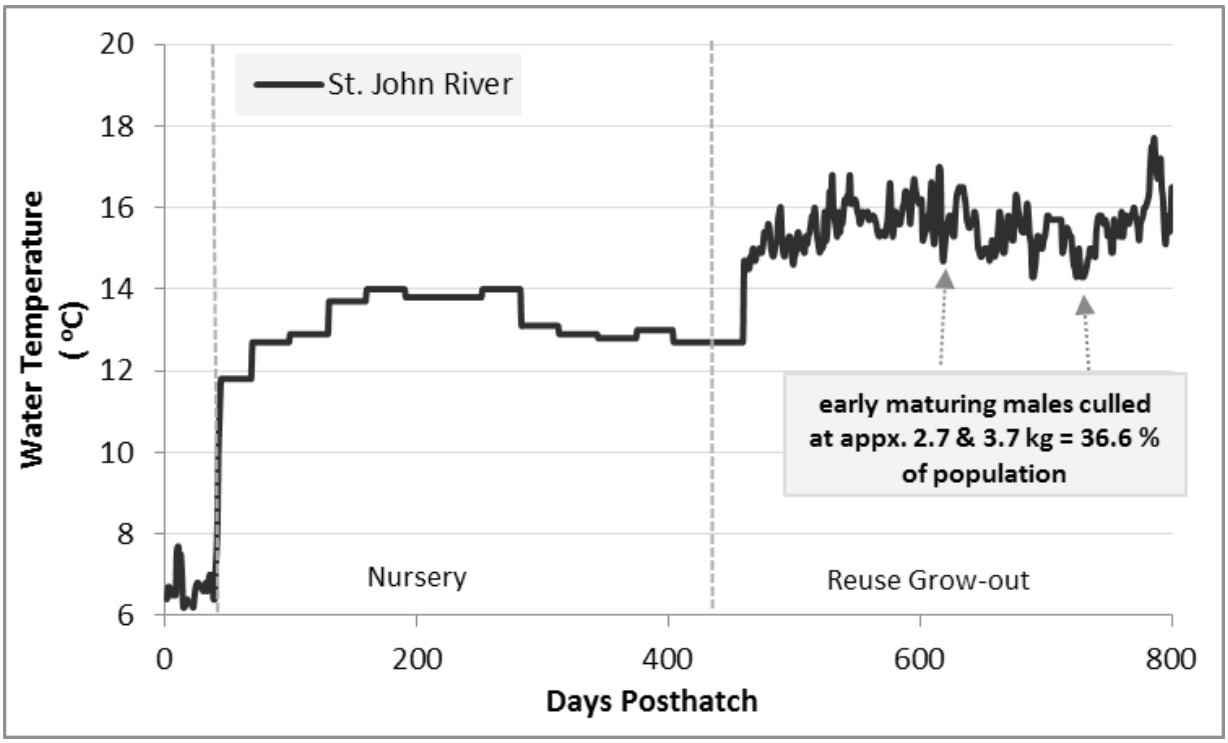

Figure 2. Cascade strain, first growout trial.

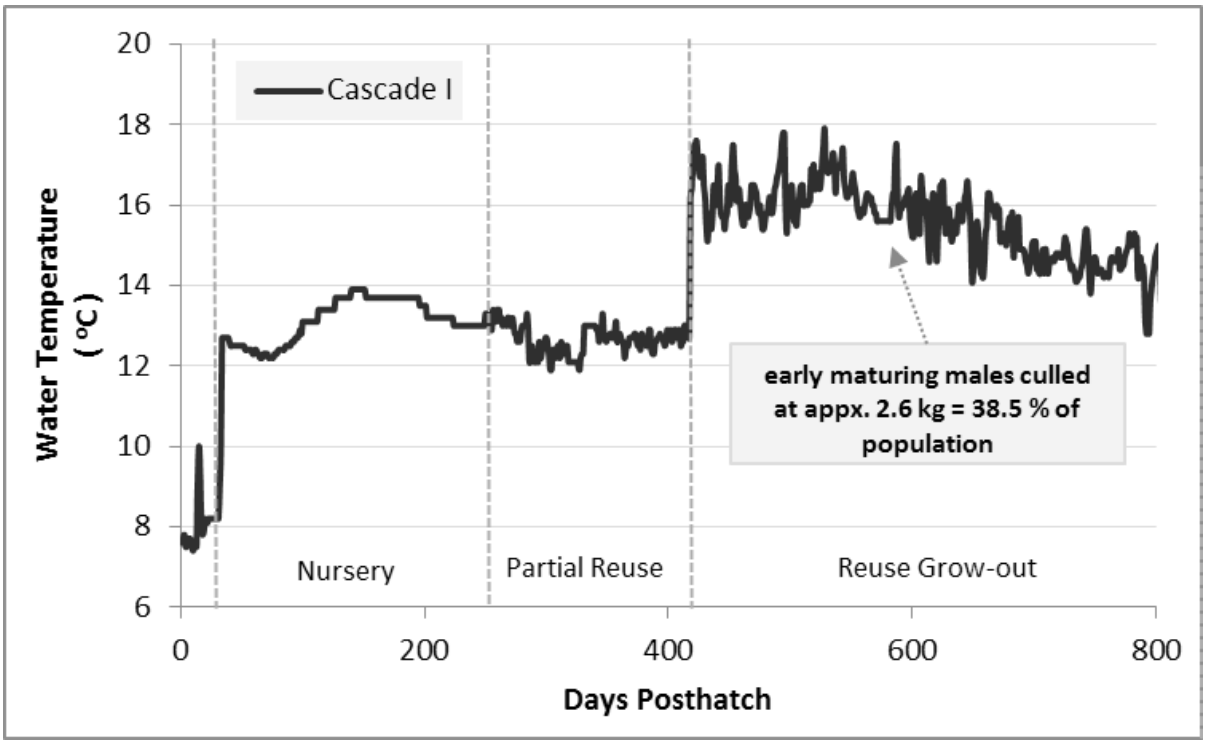

Figure 3. Cascade strain, second growout trial.

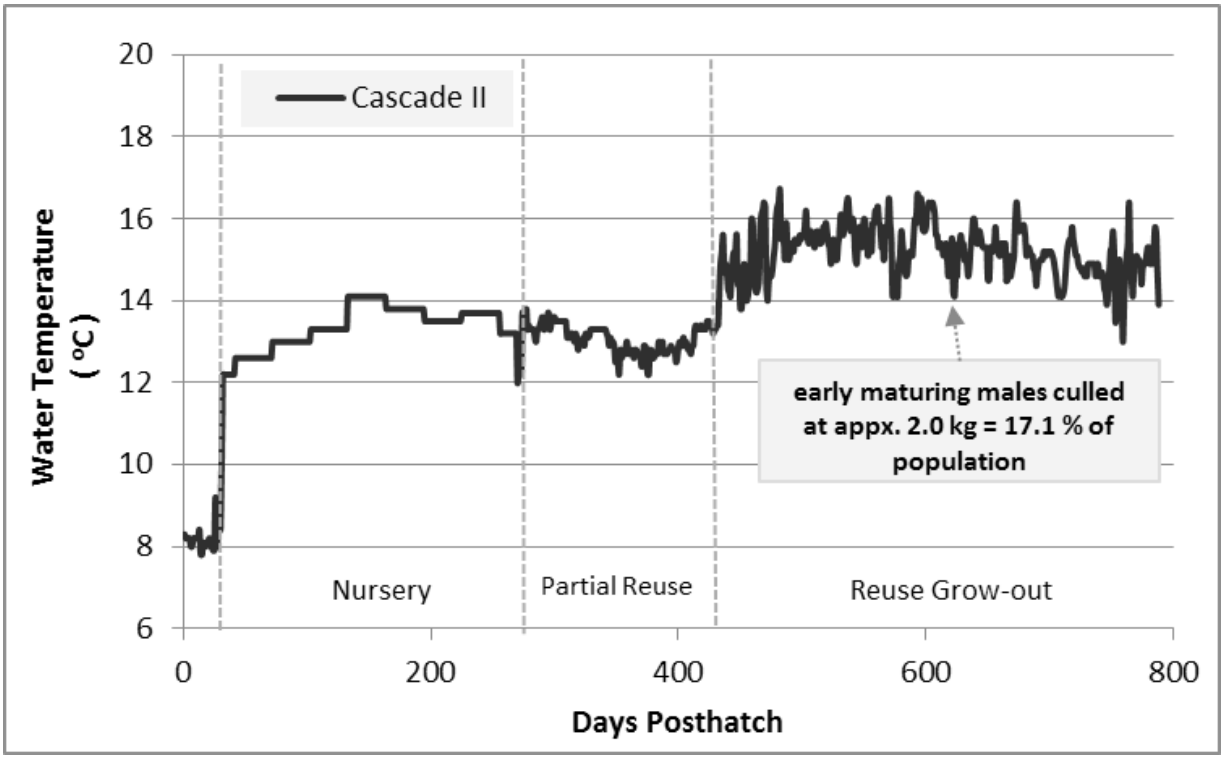

Figure 4. Norwegian strain, first growout trial.

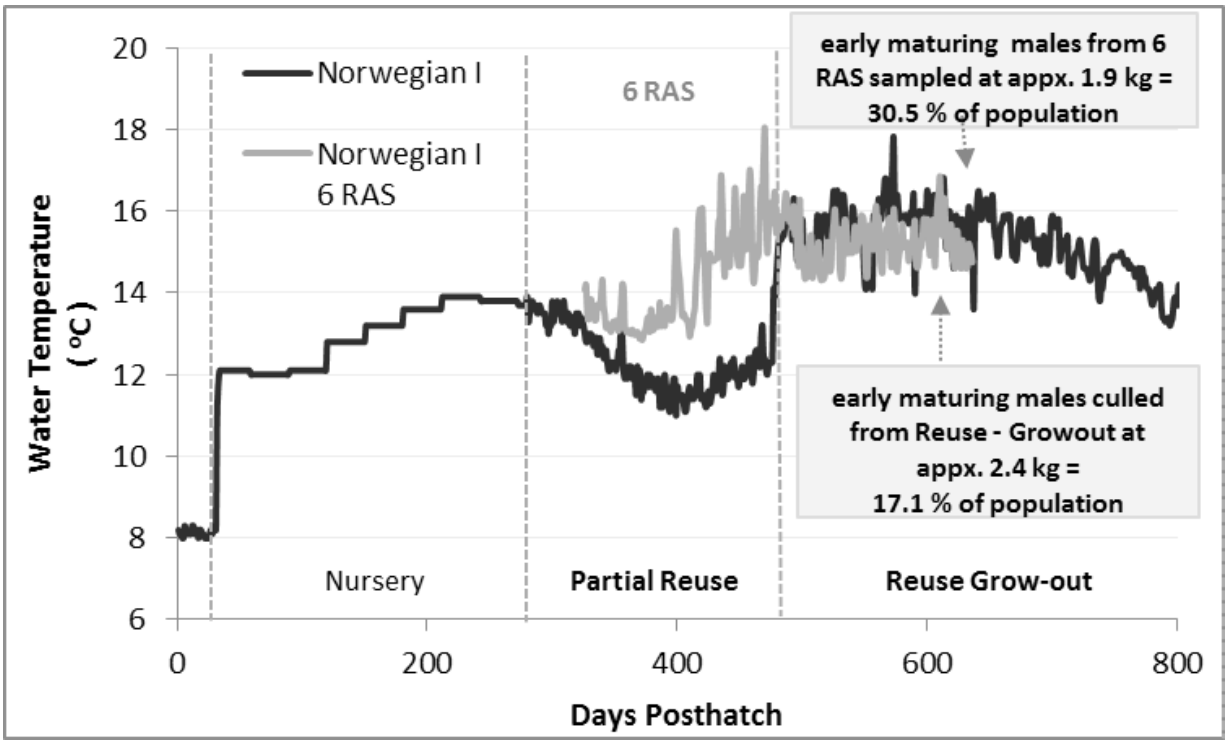

Figure 5. Norwegian strain, second growout trial (currently underway).