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A Review of Factors Influencing Maturation of Atlantic Salmon, *Salmo salar*, with Focus on Water Recirculation Aquaculture System Environments

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Abstract

Sexual maturation of Atlantic salmon, *Salmo salar*, is a complex process, with many variables having the capacity to influence the timing and prevalence of maturation and acting as promoters and/or inhibitors of sexual development. Precocious maturation has the capacity to seriously impact production in commercial aquaculture settings, and in response there has been a significant amount of research devoted to understanding this issue in order to develop remedial strategies. Very little research has been conducted specifically examining salmon maturation in land-based, closed containment water recirculation aquaculture systems, which have recently received attention as an alternative technology for the sustainable production of market-size Atlantic salmon. Unfortunately, the nascent closed containment salmon industry has thus far experienced high levels of precocious maturation, for reasons that are presently unclear. Given the economic challenges facing the closed containment industry's expansion, it is imperative that best management practices be developed to reduce economic losses from early maturation, in order to assist the sustainable growth of farmed Atlantic salmon production. This review provides a brief summary of published research on factors associated with early salmonid maturation, as well as information from research examining maturation and growout performance of Atlantic salmon in closed containment aquaculture systems.

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 in this species is considered the result of evolutionary adaptation to various river and ocean environments to maximize reproductive success. Although beneficial to the species in its natural environments, 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), and 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 within-cage prevalence of grilsing estimated at 20–30% between 1998 and 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; see Table 1 for example photoperiod regime), selective breeding for late maturation (Gjedrem 2000), and inducing triploidy during egg incubation (Benfey 1999). These efforts to reduce early maturation in production fish have led to mixed success overall, although grilsing remains a significant problem in certain regions of the world. With the development and implementation of next-generation technologies to produce salmon, specifically land-based, closed containment operations utilizing recirculation aquaculture system (RAS) technologies (Fig. 1), the

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TABLE 1. *Example photoperiod regimes[a] for land-based Atlantic salmon smolt production and sea-cage growout, employed to induce smoltification and to inhibit maturation up to harvest size (F. Mathisen, Grieg ASA, pers. comm.).*

Early rearing		Artificial winter		Smoltification induction	Sea-cage growout
Freshwater regime: LD24:0 Up to 40 g (±20 g)	⟶	LD12:12 Min. 4 wk	⟶	LD24:0 Applied 3–4 wk prior to sea transfer	Variable within-cage photoperiod regimes, depending on time of year and smolt age; continuous additional lighting typically applied winter/spring to reduce perception of increasing photoperiod, thereby delaying sexual development
Seawater regime: LD24:0 Up to 40 g (±20 g)	⟶	LD12:12 4–6 wk	⟶	LD24:0 Min. 3–4 wk in brackish[b] water Sea transfer up to 500 g in size	

[a]LDX:Y represents the ratio of light : dark hours over 1 d, that is LD12:12 represents 12 h light, 12 h dark over a 24-h period.

[b]8–15 ppt salinity.

FIGURE 1. *Market-sized Atlantic salmon being harvested from a land-based, closed containment facility. The tank pictured is 150 m^3 in volume; salmon are crowded and channeled through the tank side-wall box and down a chute into a purge system (to eliminate off-flavor compounds from fillets) prior to slaughter and processing. (Photo: K. Sharrer, The Conservation Fund Freshwater Institute).*

issue of precocious maturation has returned to the forefront among factors affecting production and profitability in these new operations. During Atlantic salmon growout trials conducted by the authors and colleagues (Davidson et al. in press), precocious male maturation has been as high as 80% by harvest time at 4–5 kg in average weight. Anecdotally, early maturation has also been prevalent at other land-based, closed containment facilities raising Atlantic salmon to market size (Table 2). Given the considerable upfront 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 of the culture tank environment (Summerfelt and Vinci 2008), and therefore it should be possible to control, refine, or eliminate environmental triggers of early maturation, once properly identified. The closed containment environment is typically optimized for growth (e.g., relatively high and constant water temperature, 24-h feeding regimes, etc.), which, as will be discussed, could represent significant instigation for early sexual 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 assist with industry expansion to meet global seafood product demands sustainably.

The purpose of this article 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.

TABLE 2. *Results of an informal survey conducted late 2015 on environmental conditions and observed maturation in seven currently or previously operational Atlantic salmon land-based, closed containment growout facilities. Note that some responses represent best estimates by producers, that variation exists as to how producers define and measure maturation, and that data on other important variables (e.g., thermal growth coefficient feeding rate and feed type, stocking densities, etc.) were not available across all seven facilities (Gary Robinson, GRV Inc., pers. comm.).*

Data source	Temperature (C)	Salinity (ppt)	Photoperiod[a]	Growth)	Maturation[b] (%)
Facility A[c]	14 (12–16)	24	LD24:0	Unknown	2 (est.)
Facility B	13 (12.5–16)	32	LD24:0	Unknown	5
Facility C	14	32	LD24:0	2.3	<5
Facility D	15	12 (variable)	LD24:0 (LD8:16 for 8 wk at 250–300 g)	2.2 (est.)	5–36
Facility E	15 (14–15)	26	LD16:8	2.4	2–12
Facility F[d]					
Cohorts 1 and 2	15	0	LD24:0	2.3	36–38
Cohorts 3 and 4	15	0	LD24:0	2.3	17–18
Facility G[e]					
Cohort 1	14.3 (13–15)	3.6	S_1 smolts: LD18:6 to Jun. 21; LDN to Dec. 21; LD24:0 to harvest	1.5	100
Cohort 2	13.9 (12–15)	3.6	S_0 smolts: LDN to Mar. 17; LD24:0 to Jun. 21; LDN to harvest	1.6–1.7	36
Cohort 3	14.4 (13–15)	3.6	S_1 smolts: LDN to Mar. 17; LD24:0 to Jun. 21; LDN to harvest	1.6–1.7	45
Cohort 4	13.4	3.6	S_1 smolts: LDN to Mar. 17; LD24:0 to Jun. 21; LDN to harvest	1.6	56

[a]LDN represents a simulated natural photoperiod.

[b]Total population maturation (males and females) observed up to harvest size.

[c]Partial water recirculation (i.e. no biofiltration); approximately 40% water exchange per day.

[d]Facility F – hard water (280 mg/L); drop in maturation between cohorts 1 and 2 and cohorts 3 and 4 believed to be related to change to low-grilsing genetic stocks.

[e]Facility G – cohort 1 raised under cloudy water conditions, with low light lux penetrating culture tanks.

Life History of Atlantic Salmon

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; Klemetsen et al. 2003; Reid and Chaput 2012), have 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 1 yr or longer (Metcalfe and Thorpe 1990; Økland et al. 1993). Prior to migrating to the ocean, salmon parr undergo a series of

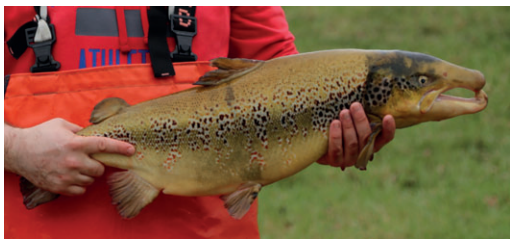

FIGURE 2. *A sexually mature male Atlantic salmon removed during final harvest at a land-based, closed containment facility. Note characteristic kype (i.e., hooked jaws) and overall bronze coloration. (Photo: K. Sharrer, The Conservation Fund Freshwater Institute).*

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 et al. 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 toward reproductive maturity. When Atlantic salmon become sexually mature (Fig. 2), 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 Morris 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 nonanadromous versus anadromous forms (Berg 1985). Marshcall et al. (1998) and Klemetsen et al. (2003) provide detailed reviews of the aforementioned life history characteristics, among others. For purposes of this review, 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 toward adulthood. For example, for most Atlantic salmon migrating to sea, important decisions are made regarding reproductive development during the months following smoltification (Mangel and Satterthwaite 2008). Tremendous diversity exists relative to the time of freshwater residency and age at which Atlantic salmon become smolts. Juvenile Atlantic salmon are known to remain in freshwater for a wide period of time ranging from 1 to 8 yr (Metcalfe and Thorpe 1990). Økland et al. (1993) found that smolt age varied from 2 to 6 yr for Atlantic salmon populations within 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 1 yr, while slower growing parr require an additional year or more to smolt (Thorpe 1977; Thorpe et al. 1982; Kristinsson et al. 1985; Rowe et al. 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 for parr-smolt transformation. 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 1 yr (typically referred to as grilse) and other groups spending three to five 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 to 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).

As with all living organisms, the life cycle of the Atlantic salmon is motivated by procreation and successful 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 Satterthwaite 2008). Proposed thresholds include: level of adipose tissue (Rowe et al. 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 Satterthwaite 2008). A number of literature sources indicate that the trigger for initiation of maturation appears to depend on physiological or biochemical conditions (the aforementioned thresholds) that are to some degree genetically determined and also influenced by environmental factors (Naevdal 1983; Gjerde 1984; Saunders 1986; Thorpe et al. 1998; Mangel and Satterthwaite 2008; Taranger et al. 2010). Recently, Barson et al. (2015) identified a single genome region in Atlantic salmon that is associated with the age at which fish mature; further research is needed to determine how this finding can be exploited by breeders to produce late-maturing fish. Previously, 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 Satterthwaite (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, the process of maturation is clearly governed by a variety of heritable, physiological/biochemical, and environmental cues and their interactions. When considering the

complexities of Atlantic salmon life history, it becomes apparent that controlling maturation within aquaculture systems is a complex task. Possible solutions for early maturation, however, must begin within an understanding of the Atlantic salmon's inclination for reproductive advantage, with the ultimate goal being the identification and control of environmental and physiological cues that trigger reproductive development.

Factors Influencing Atlantic Salmon Maturation

Photoperiod

Photoperiod is considered an essential determinant for initiating sexual maturation in teleosts (Taranger et al. 2010); its singular importance is rooted in the evolution of upper latitude fish species' reproductive strategies to ensure hatching of juveniles during periods of advantageous environmental conditions (Bromage et al. 2001). The effect of photoperiod on the maturation of salmonids has been extensively studied, and its interaction with water temperature has become an area of increased focus (e.g., Fjelldal 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 day length, 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 (Duston and Bromage 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, that is, into the final month of the calendar year, could potentially increase the percentage of fish that sexually mature (Duncan et al. 1999). Toward 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 (Leclerqc et al. 2011).

Closed containment aquaculture allows for greater control of environmental conditions compared with 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; therefore, while photoperiods can be manipulated in both pre- and posttransfer rearing environments, other important variables (e.g., salinity) can obviously be widely different throughout the production cycle. 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 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 with the more traditional salmon production cycle.

Good et al. (2015) demonstrated that Atlantic salmon exposed to a reduced photoperiod, from first feeding up to 1 yr posthatch in freshwater RAS, showed a significantly higher proportion of mature males than those exposed to continuous light during their first year. The specific reduced photoperiod utilized in this study

was LD16:8 (i.e., fish exposed to 16 h of light followed by 8 h of darkness). 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 in closed containment growout trials under continuous light have consistently produced a high percentage of maturing males, the most affected group demonstrating approximately 80% mature males by harvest at 24–26 mo posthatch (Davidson et al. in press), and (ii) previously published research has suggested that a reduced photoperiod can reduce early maturation when compared with 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 3 mo of early rearing continuous light, versus those exposed to a natural photoperiod during this period; however, further analyses suggested that the continuous light treatment was acting as an "enabling factor," whereas elevated water temperature determined the actual 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 water temperature in the Fjelldal et al. (2011) study, and in freshwater as opposed to seawater. The findings by Good et al. (2015) resemble those obtained by Berg et al. (1996), who found higher maturation in salmon exposed to LD20:4 photoperiod from smolt size to harvest size, versus those exposed to LD24:0, in marine net pen environments. Given the differences in treatments, photoperiod exposure times, and environmental 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 relevant to closed containment production of Atlantic salmon and a basis for further research.

Because the decision to mature is likely made during the first year posthatch, when smolts in the traditional industry are often in freshwater recirculation systems, previous research within this context can still be 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) up 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 closed containment growout trials (Davidson et al. in press), mature underyearling smolts have been observed in very high numbers subsequent to being transferred to experimental-scale RAS, compared with those remaining for commercial-scale RAS growout (authors' unpublished data). Whether this unusually high incidence of early maturation was related to the sudden exposure to warmer water temperatures (ca. 2 C increase) upon entering the experimental-scale RAS, to unintentional photoperiod change, or to 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 at a reduced photoperiod during the first year, compared with 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 at the time of the study by Saunders and Henderson (1988); as such, it remains difficult to fully compare these two studies.

Thorpe (1986) proposed a unified model that incorporates salmon growth, smoltification, and maturation rates based on observations of precocious parr in the Scottish salmon industry, and this was tested by Adams and Thorpe (1989) by comparing underyearling fish exposed to 2×2 factorial treatments of either elevated or ambient water temperatures and either advanced or ambient photoperiods. As predicted by Thorpe (1986), conditions favoring growth (i.e.,

increased water temperature) during the February maturation window were also associated with increased parr maturation; this was avoided in the other 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 and, in particular, to avoid exposing parr at this stage to ambient photoperiod. Avoiding ambient photoperiod is relatively easy to achieve in closed containment aquaculture; however, if exposure to ambient light during the maturation window cannot be guaranteed (e.g., when procuring young salmon from outside facilities employing assumed or unknown photoperiod conditions), then maturation of parr 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 in ocean conditions. 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 lighting being applied, for example, "continuous" photoperiods 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 yr 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 being exposed 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 in the sea cage stage 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, it does suggest that continuous photoperiod during the final year of production can assist in decreasing overall grilsing rates. In our experience, however, maintaining an LD24:0 photoperiod during the second year of closed containment growout has been correlated with variable but generally high grilsing rates; therefore, there are clearly other factors influencing maturation in these growout trials, such as water temperature in the growout phase, or photoperiod conditions during first-year rearing prior to growout transfer.

Research on photoperiod manipulation directly following the induction of smoltification, that is, an artificial 6-wk LD12:12 winter following by a period of LD24:0, has been carried out by Duncan et al. (1999), who compared maturation rates in postsmolts 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 postsmolts receiving LD24:0 from December to the following December (i.e., photoperiod conditions did not change following the induction of smoltification) demonstrated the highest maturation rates, while salmon exposed to a simulated natural photoperiod during this time frame demonstrated no maturation, based on a gonadosomatic index (GSI) cutoff 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 time frame 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 might have implications for closed containment production, in that a period of increasing day length following induction of smoltification might decrease the incidence of early maturation observed in 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 maturation in parr, 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 provided 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 levels of precocity versus the other groups, whereas late artificial winter groups (August and September) had relatively low levels of maturity (although fish in the September group did not smoltify as completely as those in the August group). In a follow-up study under similar conditions, Berrill et al. (2006) determined that a longer (i.e., 12-wk, versus 8-wk) short-day period to induce smoltification, begun in June as opposed to May, significantly reduced precocious parr observed later in the year. While the results of these studies are ultimately difficult to compare to more recent experimental closed containment growout trials (Davidson et al. in press), they clearly demonstrate the importance of early rearing environmental factors, that is, photoperiod, temperature, or the interaction of these two parameters, on subsequent precocious maturation, and emphasis in future research should be applied to first-year environmental conditions as maturation decisions are clearly made in the 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 been conducted examining the quantity (light intensity) and, in particular, the quality (spectral composition) of artificial light to which fish are exposed within these photoperiod regimes. This is particularly important for the closed containment industry, in that the RAS culture tank environment can present a unique set of challenges (e.g., comparatively high stocking densities and sometimes fluctuating water turbidity levels) for providing fish populations with optimal quantity and quality of light in a uniform and well-distributed manner. 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 (λ 450 nm and λ 550 nm, respectively) compared with red light

exposure (λ 700 nm) (Migaud et al. 2010; Vera et al. 2010); however, more research is needed on the spectral sensitivity of Atlantic salmon. In a recent study, Leclerqc et al. (2011) examined different lighting strategies to determine, among other things, their respective efficacy at controlling sexual maturation in Atlantic salmon during growout in sea cages. 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/m^2 appeared to be the threshold to reduce maturation (which coincides with the 0.016 W/m^2 threshold intensity determined in laboratory studies, as 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.

Water Temperature

The effect of water temperature on Atlantic salmon biology has been well studied. Brett (1979) reported that salmonid growth increases linearly with temperature, and Austreng et al. (1987) demonstrated that young Atlantic salmon cultured in freshwater from 0.15 to 75 g at temperatures ranging from 2 to 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 performance of fish growing from 0.03 to 2.0 kg at temperatures ranging from 2 to 14 C (Austreng et al. 1987). Handeland 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 postsmolt 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 Ruud-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 subsequently) have linked temperature to Atlantic salmon size and 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 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 versus 8.3 C, respectively. During this study, presmolt Atlantic salmon (initial weight = 15.9 g) were cultured in freshwater for 11 mo, then relocated to seawater for 2 mo. After the 2-mo 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 and also demonstrated 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 with 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 GSI $\geq$ 1.5%) after only 6 wk of exposure. After an additional 2 mo 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 directly or indirectly influence maturity, separating the most impactful factors is a complex task. 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 within 266 commercial net cage sites in New Brunswick and Nova Scotia, Canada, at 24 different farms. The percentage of grilse within cages was variable, ranging from 0 to 64.1%, while the within-farm rate of grilsing ranged from 1.6 to 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: (i) salmon weight during the second August at sea and (ii) the difference in water temperature between the first February and the second September at sea. Overall, McClure et al. (2007) found that warmer water during summer months and colder water during the colder 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 mo 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 et al. (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 developmentally 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 nonmaturing salmon. Conversely, Rowe et al. (1991) found evidence that maturation of male Atlantic salmon parr is 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 postsmolt Atlantic salmon and a condition factor exceeding 1.3 was required by early summer for early maturation to develop. Furthermore, Mangel and Satterthwaite (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 Satterthwaite 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 the winter months to as high as 14 C during the summer months (Kristensen et al. 2009). In Chile, mean annual water temperature for salmon smolt production facilities ranged from 9.4 to 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 have 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, Dalsgaard 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 the winter months) to ultimately enhance growth performance, grow larger smolts, and reduce time at sea, among other advantages (Bergheim et al. 2009; Kristensen et al. 2009; Dalsgaard 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, that is, 14–16 C (Austreng et al. 1987; Handeland 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 land-based RAS are typically exposed to constant, albeit usually warmer, water temperatures that are targeted and maintained through various means of environmental control. Water temperature in an experimental

near-commercial-scale RAS used for salmon growout by the authors and colleagues has been controlled by adding more or less cool (12–13 C) spring water depending on the season, with greater volumes of spring water used during the summer months to chill the culture water and less spring water added during the winter months to retain heat (Summerfelt et al. 2013; Davidson et al. in press). This temperature control regime has been applied to maintain optimal and consistent temperatures for salmon growth of 15–16 C year round. At a commercial 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 (C. Dineen, Kuterra, pers. comm.). Other technologies such as heat exchangers, chillers, and in-line heaters are also available for heating and cooling RAS culture water. 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 nitrogen accumulation (van Rijn et al. 2006), thereby allowing increased heat retention. Depending on geographical location, RAS operated in this manner may require a greater investment in water cooling technologies to maintain optimal temperatures for Atlantic salmon production.

When culturing Atlantic salmon in RAS, the natural inclination might be to utilize water temperatures that are known to optimize growth; however, as discussed, accelerated growth is generally associated with expedited maturation, particularly for male Atlantic salmon. More research is needed to evaluate grilsing rates at various water temperatures, in order to identify a water temperature range that provides acceptable growth performance while also resulting in diminished maturation rates. 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 to 16 C) in triggering male maturation immediately following the smoltification period. We have also noted this (unpublished data), 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.

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, presmolt, 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 growout within recirculating aquaculture systems typically uses substantially less water, thereby retaining more heat and providing increased water temperature. More research is needed to evaluate whether sudden changes, particularly increases in water temperature, can instigate early maturation for RAS-produced salmon.

Feed

In previous sections, substantial evidence was provided indicating that early maturation in Atlantic salmon could be triggered by interactions of 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.

A recent, unpublished study of ours aimed to assess whether a restricted ration provided to postsmolt Atlantic salmon would lead to a reduction in early male maturation. The 3-mo

feeding trial began when the fish were 94 g and approximately 9.5 mo old (posthatch), and was conducted within a partial-reuse system with water temperature ranging from 11 to 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 2238 salmon. At the conclusion of the trial, mean salmon weight was not remarkably different between feeding regimes, that is, 242 versus 203 g, for the full and restricted rations, respectively. Feed conversion ratios (FCRs) 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 mo 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 mo 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-mo 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 feed conversion ratio (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 maturation in Atlantic salmon (Thorpe 1986; Herbinger 1987; Adams and Thorpe 1989; Herbinger and Friars 1991; Peterson and Harmon 2005; Shearer et al. 2006; Mangel and Satterthwaite 2008). Lipid content of the fish was not measured at the conclusion of the 3-mo 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 1990; Simpson 1992; Kadri et al. 1996). As such, 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) demonstrated 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 6 mo 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. Low-level dietary phosphorus has been shown to increase whole body adiposity in Atlantic salmon (Albrektsen et al. 2009), and therefore modifying phosphorus levels in feeds might indirectly affect maturation through alteration of whole body lipid content. Indeed, Fjelldal et al. (2012) demonstrated that increasing dietary phosphorus from 0.6 to 0.9% significantly reduced the prevalence of mature males in their study population. In the same experiment, Fjelldal et al. (2012) reported at study's end a significantly higher percentage of immature fish among those that had been vaccinated (versus unvaccinated fish); however, the authors speculate that this finding is likely due to the vaccinated fish having a reduced growth rate (a consequence of postvaccination inappetence) during the decision window for maturation. This could have particular relevance for Atlantic salmon producers using RAS, as these systems provide the opportunity for rapid growth, at (typically) higher water temperatures, through

automated around-the-clock feeding, a scenario that likely leads to the accumulation of excess energy reserves.

Research facilities and early commercial ventures culturing Atlantic salmon in RAS primarily use salmon diets that are designed for commercial net pen operations. These diets are often formulated to be fed during specific seasons, taking into account the variation in salmon feed intake and performance that occurs seasonally due to changing ocean water temperatures and related changes in 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 postsmolts 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 against which fish can swim. 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, and this has been demonstrated in numerous studies (Davison and Goldspink 1977; East and Magnan 1987; Totland et al. 1987; Davison 1997). This increased growth in response to exercise has been considered mainly the result of hypertrophy of muscle fibers (Davison 1997; Johnston 1999), and exercised fish have improved fillet texture (Totland et al. 1987; Bugeon et al. 2003) and hence greater consumer appeal. Other reported benefits of sustained exercise in salmonids include less aggression, increased resistance to infectious organisms, and prevention of precocious maturation (Castro et al. 2011; 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 avenues. 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 (D. Larsen, NOAA, pers. comm.), and our unpublished data 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 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 in closed containment systems. 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 very little difference between the two treatment velocities. More research is therefore 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 closed containment Atlantic salmon aquaculture best management practices.

Genetic Strain

As with numerous other performance traits, the range of maturation timing of 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-yr parr or precocious males, and at 4 and 5 yr 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 of reproductive maturity in Atlantic salmon through genetic selection (although multiple generations were not assessed in this particular study). The recent discovery of a single region of the Atlantic salmon genome that governs age at maturation (Barson et al. 2015) will undoubtedly impact breeders' future approaches to selecting for late maturation. At present, late maturation is selected for in Scottish and Norwegian strains; however, in our experience high levels of male grilsing can still occur in such late-maturing strains under conditions of closed containment, water 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). Further development and broader commercial availability of all-female Atlantic salmon eggs, a direction which is currently being undertaken by breeders in Iceland and Australia, could solve the brunt of early maturation problems, as most grilsing has been associated with male Atlantic salmon. 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 while in second-year 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 provide this incentive in the near future. Previous attempts at developing all-female salmon have been carried out 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 (M. Godoy, Recirculacion-Chile Ltda, pers. comm.). All-female Atlantic salmon eggs were also available, up 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.

Water Chemistry

There are numerous water quality parameters, both known and unknown, which have the potential to directly or indirectly modify the developing endocrine system of teleosts under culture conditions, and consequently affect the timing of sexual maturation onset in Atlantic salmon. Water recirculation technologies are often operated with very low system water exchange rates; therefore, closed containment systems may be additionally challenging 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 and 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 focuses on a select group of relevant water quality parameters that have been evaluated, to varying extents, 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 their potential to influence salmon maturation in these culture environments.

Nitrate Nitrogen (NO_3-N)

Nitrate has been identified as a possible endocrine disrupting compound (EDC), and

maturation of aquatic species could be exacerbated when cultured under concentrations of elevated nitrate (Hamlin 2007; Hamlin et al. 2008). Nitrate enters fish primarily through the gills, and it is thought that this compound, through *in vivo* conversion to nitric oxide, can influence steroid hormone synthesis (Meyer 1995; DelPunta et al. 1996), which in turn can accelerate or delay sexual maturation. 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 with 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 presmolt Atlantic salmon (102 g to begin). This 27-d 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) did not evaluate the long-term effect of nitrate on the reproductive development (e.g., GSI) of Atlantic salmon, unfortunately. Recent research of ours at present in development for publication, has demonstrated that long-term effects of nitrate exposure (100 vs. 10 mg/L) in postsmolt 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 as reviewed by Guillette and Edwards (2005). More research is certainly needed to evaluate how moderately elevated NO_3-N, as often observed in RAS culture, impacts the endocrine function and reproductive development in Atlantic salmon.

Examination of NO_3-N 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 NO_3-N stems from increased concentrations within natural aquatic environments due to anthropogenic activity (Camargo et al. 2005), as well as from the trend to culture fish in recirculating aquaculture systems, where NO_3-N can accumulate as an end-product of nitrification (Camargo et al. 2005; Davidson et al. 2009, 2011). In nature, most fish, including Atlantic salmon, are not typically exposed to significantly elevated concentrations of NO_3-N, at least in comparison to levels that can be achieved in RAS. Low NO_3-N 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 toward culture of market-size Atlantic salmon in land-based RAS has also emerged (Summerfelt and Christianson 2014). Very little information describing the effect of NO_3-N on 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 NO_3-N, or lack thereof, on Atlantic salmon and other salmonids have been published. It is important to recognize that NO_3-N toxicity is species specific as well as life stage specific (Camargo et al. 2005). In the case of life stage in fish, NO_3-N 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 clarkia*, at NO_3-N concentrations as low as 2.3–7.6 mg/L, while Westin (1974) reported a 7-d LC_{50} of 1068 mg/L NO_3-N for rainbow trout fingerlings. Despite the high level of NO_3-N 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 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 NO_3-N in RAS. Furthermore, Freitag et al. (2015) concluded that juvenile (presmolt) 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. In our experience, 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) (Davidson et al. in press).

More research is needed to establish a definitive NO_3-N 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 EDCS

EDCs are natural or anthropogenic chemicals in the environment have the capacity to impair normal endocrine function (Colborn et al. 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). 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 the possibility. Furthermore, potential sources of EDCs and other contaminants within RAS materials themselves have not been investigated, and as the industry continues to grow there is a strong 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, mercury, heavy metals, etc. might contaminate aquaculture products originating from closed containment operations). Modern aquaculture systems incorporate a relatively large amount of fiberglass and polyvinyl chloride in rearing tanks and piping, which has the potential to introduce anti-corrosion compounds, such as bisphenol A, a variety of flame retardants, and other compounds into system water. Bisphenol A, in particular, has been identified as an EDC of Atlantic salmon (Honkanen et al. 2004) and brown trout, *Salmo trutta* (Lahnsteiner et al. 2005). In theory, these compounds have the potential to accumulate over time 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. 2005; Ellis et al. 2005). Thus, in RAS 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 much 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 (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 EDCs) and their potential removal following passage through wastewater or sewage treatment facilities (Onda et al. 2003; Chimchirian et al. 2007; Cicek et al. 2007). Such facilities cannot be directly compared with closed containment RAS, as they typically have far longer retention times (e.g., 10–15 d) 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. Similar research with other species has 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, *Oncorhynchus kisutch* (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 forms of hormones) 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. Unpublished observations we have made suggest 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 (C. Garcia-Huidobro and M. 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 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 there is very little information available to indicate whether salinity at various levels impacts precocious maturation in Atlantic salmon. Melo et al. (2014) investigated the role of freshwater versus saltwater, and LD24:0 versus LD12:12, in a factorial study of 12-mo-old Atlantic salmon exposed to a 3-mo "maturation regime" (i.e., a rise in water temperature from 12 to 16 C at the onset of the postsmolt 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. Aside from the aforementioned studies, the role of salinity in early maturation has not been the focus of published research; most studies in this area have 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 postsmoltification 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, in order to assist the development of closed containment best management practices.

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 lack of, or limited, availability of all-female eggs, then it is essential to engage in focused research on maturation in closed containment to inform the development of effective 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 under such conditions. With current practices of water temperature being in the upper ranges for this species in order to promote growth performance, these practices may be inadvertently contributing to the problem of grilsing in closed containment. 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 EDCs existing and exerting an influence in closed containment settings, and examining a range of lighting technologies, providing different light quantities and qualities, to develop optimized within-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 at present is to work with breeding companies to establish the widespread 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 consistent availability of all-female eggs.

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