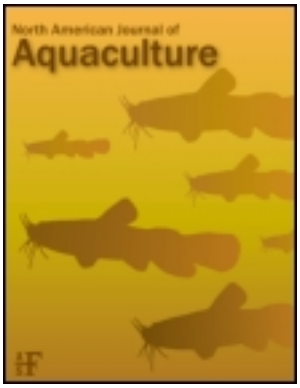

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[Article]

Effect of Photoperiod Advancement of Atlantic Cod Spawning on Egg Size and Biochemistry

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Abstract.—We report on the proximate composition, lipid class, and fatty acid content of fertilized eggs during the course of one spawning season from two broodstocks of Atlantic cod *Gadus morhua*: one group was maintained under ambient photoperiod (PP), whereas for the second group the natural photoperiod cycle was compressed by approximately 4 months by PP advancement from ambient. We tested the hypothesis that PP compression, which reduces the time between successive spawning seasons and therefore reduces the time available for vitellogenesis and maturation, might also impair nutrient transfer to the eggs, causing reduced egg quality as measured by biochemical indices. Advanced PP eggs were significantly larger than ambient control eggs (absolute mean values for dry weight = 109.2 and 97.9 μg, respectively). Advanced PP eggs also had significantly greater mean total protein (60.0 versus 49.4 μg), mean total lipid (15.1 versus 9.7 μg), and greater mean values for several major lipid classes (hydrocarbons, triacylglycerols, sterols, diacylglycerols, and phospholipids) than ambient controls. Proportionally (percent of dry weight), mean total lipid, ash, triacylglycerols, total saturated fatty acids, and total polyunsaturated fatty acids, including total ω-3, total ω-6, docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid, were all significantly greater in eggs of the advanced PP group than in ambient controls. The latter contained proportionally more monounsaturated fatty acids. Mean dry weight of eggs declined over time from onset of first spawning in the ambient PP group but not in the advanced PP group, and none of the measured biochemical indices varied significantly over time from onset of first spawning in either PP group. We conclude that with the PP advancement technique used here, compression of time between successive spawnings by up to 4 months in Atlantic cod does not negatively affect biochemical indices of egg quality associated with normal development or survival performance.

Most wild stocks of teleost fishes inhabiting temperate latitudes use natural patterns of seasonally changing day length modulated by temperature to synchronize their reproductive cycles. In most cases, this results in mass spawning within a single discrete period of days or a few weeks. In commercial finfish hatcheries, manipulation of the natural photoperiod (PP) cycle has come into widespread use as the method of choice to alter reproductive cycles of hatchery broodstocks (Bromage 1995). Typically, a hatchery broodstock is induced to spawn before its normal season by imposition of an artificial light regime, which compresses the ambient PP cycle (advanced PP broodstock). Conversely, onset of spawning may be delayed beyond the normal spawning time by lengthening the PP cycle (delayed PP broodstock). Photoperiod manipulations to alternatively compress or delay spawning cycles have been used with various degrees of success in many marine species, including Atlantic salmon *Salmo salar* (Taranger et al. 1998), rainbow trout *Oncorhynchus mykiss* (Bromage et al. 1984), Atlantic halibut *Hippoglossus hippoglossus* (Björnsson et al. 1998), turbot *Scophthalmus maximus* (Devauchelle et al. 1988), common soles *Solea solea* (Devauchelle et al. 1987), European seabass *Dicentrarchus labrax* (Carrillo et al. 1989), and Atlantic cod *Gadus morhua* (Hansen et al. 2001; Norberg et al. 2004). The resulting PP-manipulated broodstocks allow egg production for much longer periods throughout the year, which facilitates optimum utilization of hatchery space along with reduced production costs.

Production of the highest-quality eggs (high egg survival rates, high hatching success rates, and larvae with enhanced growth and survival attributes) is of great importance to industry. Studies to determine the effect of PP manipulation on egg quality have yielded equivocal results (Brooks et al. 1997). However, it is known that PP manipulation can reduce the size of

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Atlantic cod eggs (Hansen et al. 2001; Penney et al. 2006a), although evidence of correlation between egg size and survival or hatching success in Atlantic cod under laboratory or hatchery conditions has been either conflicting (Zhao et al. 2001) or lacking altogether (Ouellet et al. 2001; Penney et al. 2006b). In some stocks at least, hatching success is significantly related to levels of specific fatty acids (docosahexaenoic acid [DHA], eicosapentaenoic acid [EPA], and arachidonic acid [AA]) and their ratios (Pickova et al. 1997), although evidence of such relationships is contradictory (Penney at al. 2006b). Lipid content, fatty acids, and especially fatty acid ratios are known to be important for larval survival and growth (Sargent 1995; Bell and Sargent 2003). However, the effect of PP manipulations on Atlantic cod egg lipids and fatty acids is poorly understood.

The eggs of oviparous teleosts such as Atlantic cod are endogenous systems; in other words, embryos are dependent on nutrients accumulated during prespawn oocyte development for their biochemical content. The maturational cycle—beginning with endocrine-controlled secretion of estradiol (Wallace 1985), the synthesis of vitellogenin, the uptake and incorporation of lipids and protein from vitellogenin into the developing oocytes (Tyler 1993; Hyllner et al. 1994), ovulation, and spawning—normally encompasses several months in most teleosts from northern latitudes. In Atlantic cod, vitellogenesis normally is initiated during September–November (Kjesbu 1994), commencing with vitellogenin synthesis induced by estradiol-17β (Silversand et al. 1993). In Atlantic cod stocks adjacent to Newfoundland, spawning subsequently occurs during April–June and the major spawning period is in May (Brander 1993). In teleost eggs, vitellogenin is believed to be the major source of lipid and protein (Wiegand 1982; Wallace 1985; Nagler and Idler 1990; Silversand and Haux 1995). In Atlantic cod, this has been substantiated by comparing the fatty acid composition of vitellogenin (Silversand and Haux 1995) with that of the eggs (Ulvund and Grahl-Nielsen 1988; Klungsøyr et al. 1989), which indicates that the fatty acid composition of eggs bears strong similarity with the fatty acid composition of vitellogenin (Silversand and Haux 1995). Compressing the period between successive spawnings reduces the time available to complete this cycle and for accumulation and incorporation of nutrients into the eggs. Photoperiod compression is known to produce changes in several biochemical indicators associated with progression of the maturational cycle; these indicators include plasma calcium, sex steroid hormones, and thyroid hormones in Atlantic cod (Norberg et al. 2004) and estradiol and vitellogenin in European seabass (Mañanós et al. 1997). It would seem a reasonable hypothesis that compressing the period between successive spawnings by an environmental manipulation such as PP advancement might negatively affect the accumulation and incorporation of protein and lipid nutrients into the eggs. Furthermore, since hatching success in Atlantic cod eggs derived from at least some wild stocks is significantly related to egg biochemical composition (e.g., the fatty acids DHA, EPA, and AA; Pickova et al. 1997), it is reasonable to presume that PP advancement might in turn negatively affect egg development, fertilization, or hatching success or other performance measures.

Photoperiod compression and delay manipulations are routinely applied mainly during the first year of stock domestication when wild stocks are first introduced to the hatchery or to expand the seasonal availability of eggs from an existing hatchery broodstock that has been modified by another treatment, such as selective breeding. Once successfully applied, PP cycles are typically returned to 12-month periods in subsequent years but remain time-shifted out of phase with ambient PP. In the present work, we describe the effect of a PP compression technique on wild Atlantic cod broodfish in the first year of hatchery domestication. In a previous paper, Penney et al. (2006a) observed that the advanced PP fish used in the current analyses produced a lower total volume of eggs over the entire spawning season, had lower mean daily batch volumes of eggs, and produced eggs of smaller mean diameter, although rates of fertilization success between groups and rates of developmental abnormalities were unaffected. In the current analyses, we compare the biochemistry of Atlantic cod eggs derived from advanced PP broodfish with that of eggs from ambient PP (control) broodfish of similar age and size to determine whether compression of the period between successive spawnings by approximately 4 months affects the uptake of lipid and protein nutrients in the resulting eggs.

Methods

During February–March 2002, 50 Atlantic cod broodfish were placed in each of two 35-m^3, flow-through tanks equipped with supplemental oxygen infusion. Broodfish were selected from populations of wild Atlantic cod held since the previous autumn in sea cages and fed a diet of chopped mixed raw baitfish (e.g., Atlantic herring *Clupea harengus*, capelin *Mallotus villosus*, and northern short-finned squid *Illex illecebrosus*). Individual fish were selected on the basis of visual inspection of overall appearance as well as size to ensure that (1) all fish were probably in good condition and of reproductive age and (2) the size

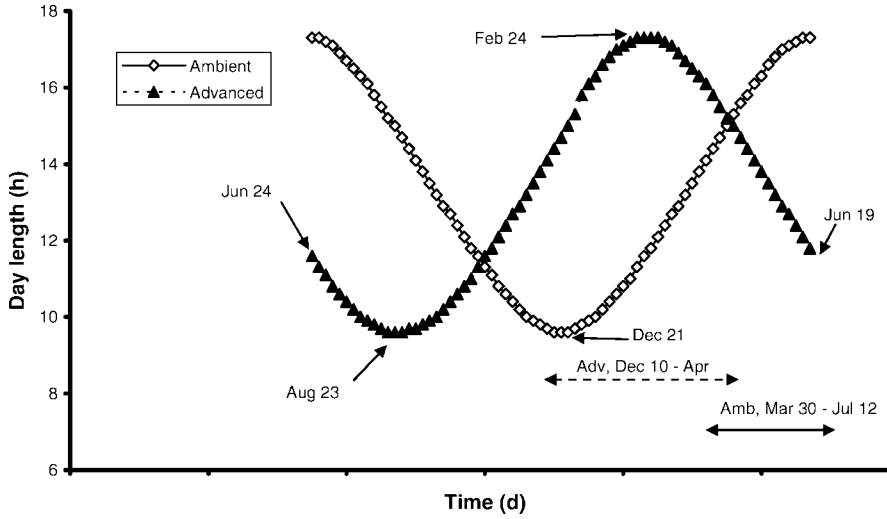

FIGURE 1.—Schematic diagram showing differences between photoperiod (PP) regimes (Amb = ambient; Adv = advanced) imposed on two groups of Atlantic cod broodfish after they had completed spawning under ambient conditions in 2002 (June 24). Lines with arrows indicate start and end dates of the successive spawning period for each group in relation to the calendar date and PP cycle.

range of fish in each broodstock group was similar. Both groups were maintained under similar conditions with water temperatures of approximately 5–6°C and were fed to satiation on a diet of mixed baitfish supplemented with a vitamin and mineral premix three times weekly throughout the year during the nonspawning period and at least twice weekly during the spawning season. Commencing in March and continuing through May 2002, both groups spawned under ambient PP. After this spawning was completed, overhead artificial lights at 1–2 lx were set to computerized timers that mimicked the ambient PP for one group and a 4-month phase-shifted PP in the other group (advanced PP). For the latter group, this compressed the natural 12-month seasonal PP cycle into 8 months by advancing the time period between successive spawning seasons by about 4 months (Figure 1). After the 4-month phase shift was imposed, PP was adjusted gradually in equal steps every 5 d to maintain the desired PP compression and to continue the annual light cycle at the set regime for both groups.

Both groups subsequently spawned naturally; spawning of the advanced PP group began 105 d in advance of the ambient PP group (Penney et al. 2006a). Buoyant eggs were passively collected daily in Nitex bags connected to the tank water overflow lines. Tank drains were monitored with collector bags to intercept nonbuoyant eggs. Fertilization success was estimated by counting the proportion of eggs that had commenced cell division. Dead or unfertilized eggs were not buoyant and quickly settled out of suspension. The number of daily egg batches collected totaled 96 from the advanced PP group and 97 from the ambient PP group. Only 33 egg batches from the advanced PP group and 34 egg batches from the ambient PP group contained sufficient material for subsequent biochemical analyses. A 20-egg subsample from each daily egg batch was rinsed with distilled water and dried at room temperature on glass fiber filters for dry weight determination on a Mettler microbalance. Subsamples from each daily batch were divided into three groups. Two replicates used for lipid chemistry were weighed in pre-tared glass vials (cleaned to remove all lipids); these were stored at −20°C with 2 mL of chloroform and were covered with nitrogen gas in capped vials. The remaining replicate was lyophilized and kept in glass vials in a desiccator for protein and ash determination. Lipids were extracted using a modified Folch method (Parrish 1999). Total lipid was determined gravimetrically. Lipid classes were separated by thin-layer chromatography on rods coated with silica gel (Chromarods-III; Shell-USA, Fredericksburg, Virginia) and were quantified by flame ionization detection using an Iatroscan MK VI (Mitsubishi Kagaku Iatron, Tokyo, Japan; Parrish 1987). Identified lipid classes were hydrocarbons, steryl and wax esters, methyl esters, ethyl ketones, methyl ketones, glycerol ethers, triacylglycerols, free fatty acids, alcohols, sterols, diacylglycerols, acetone mobile polar lipids, and phospholipids. A subsample of the lipid extract was derivatized using a fatty acid methyl ester procedure and analyzed on an HP5890 gas chromato-

graph equipped with a flame ionization detector. Lipid classes and fatty acids were identified using procedures previously described (Parrish 1999; Copeman and Parrish 2003). A Perkin Elmer Model 2400 Series II CHN analyzer was used to determine carbon and nitrogen content and C:N ratios, quantified by reference to acetanilide standards. Protein content was calculated from the resulting N values using the protein conversion factor of 5.8 (Gnaiger and Bitterlich 1984). Ash content was determined from replicate lyophilized egg samples in pre-ashed, pre-weighed glass vials combusted in a muffle furnace at 500°C for 5 h.

Data analyses were conducted using analysis of variance (ANOVA) or analysis of covariance (ANCOVA) procedures in the Statistical Analysis System (SAS Institute 1988). All values used for statistical analyses were the means of replicate subsamples. Values for proximate composition and lipid class variables for individual eggs used in the ANCOVA are estimates obtained by multiplication of the mean dry weight of individual eggs in each daily egg batch by the corresponding percent data. Differences between PP group means for all variables were tested for significance using ANCOVA with elapsed time from onset of first spawning as the covariate (Sokal and Rohlf 1995). All data initially recorded as rates or percentages were arcsine–square root transformed before statistical analysis (Snedecor and Cochran 1967). The resulting transformed data were checked for unequal variances and nonnormality using the Folded F' and Kolmogorov–Smirnov D test statistics, respectively (SAS Institute 1988). In all cases where the data failed to pass these tests, nonparametric Kruskal–Wallis tests were used to compare PP groups. In those cases, the Kruskal–Wallis test and the corresponding ANCOVA F-statistic agreed (no significant differences were found), so all such comparisons were recorded as not significant. Significance levels were adjusted using the Bonferroni method (Sokal and Rohlf 1995) to minimize the likelihood of experimentwise type I error when conducting multiple comparisons of the same data set. Tukey's multiple range test was used to determine significant differences among PP group means for all ANCOVAs with significant F-values after Bonferroni adjustment.

Results

Mean dry weight of eggs varied significantly with both PP group and elapsed time from onset of first spawning (ANCOVA; Table 1). Expressed as a mean value for all daily egg batches over the entire spawning season, dry weight of individual eggs in daily batches was significantly greater in the advanced PP group (109.20 ± 2.73 μg [mean ± SE]) than in the ambient PP group (97.86 ± 6.35 μg). Mean egg dry weight declined from the start to the end of the spawning season in the ambient PP group (ANOVA: slope $\neq 0$, $P < 0.05$) but not in the advanced PP group. This resulted in significantly smaller egg dry weights in the ambient PP group compared with the advanced PP group towards the end of their respective spawning seasons (ANCOVA homogeneity of slopes test: $P < 0.05$; Figure 2).

The C:N ratios, protein, total lipid, and ash content did not vary significantly with elapsed time from the onset of spawning (ANCOVA; Table 1), but both protein and total lipid content of individual eggs varied significantly with PP. Mean protein and total lipid content of individual eggs were both significantly greater in the advanced PP group. Proportionally (i.e., expressed as a percent of dry weight), total lipid and ash were both significantly greater in the advanced PP group than in the ambient PP group.

Most egg lipid was in the form of phospholipid (~80% of total lipid), followed by sterols (~9%) and triacylglycerols (~4%). Analysis of covariance of the lipid class data indicated that none of the individual lipid classes varied significantly with elapsed time and that none of the interaction terms (PP × time) were significant (Table 2). This was true whether expressed as absolute lipid class content of individual eggs or as a percentage of total lipid. Hydrocarbon, triacylglycerol, sterol, diacylglycerol, and phospholipid content of individual eggs varied significantly with PP. The mean of each of these lipid classes was greater in the advanced PP group compared than in the ambient PP group. Proportionally, however, only percent triacylglycerol varied significantly with PP. Mean percent triacylglycerol was greater in advanced PP fish than in ambient PP fish.

Proportionally, percent fatty acids were dominated by polyunsaturated fatty acids (PUFAs), followed by saturates and monounsaturated fatty acids (MUFAs) in the approximate ratio of 2:1:1 (Table 3). The ω-3 PUFAs (mainly DHA and EPA) predominated, and the DHA:EPA ratio was 1.9:1. The ω-6 PUFAs were in relatively minor concentration (1% or less), and AA was the numerically largest single ω-6 fatty acid present. The major MUFAs were 18:1(ω-9) and 18:1(ω-7), while the single major saturated fatty acid (SFA) was 16:0. Variability in individual fatty acids, fatty acid group sums, or selected fatty acid ratios was not significantly related to time from onset of spawning (ANCOVA; Table 3). The interaction term for 14:0 was significant. However, many of the individual fatty acids, group sums, and ratios varied significantly with PP. Mean percent PUFA and SFA and several individual PUFAs and saturates were greater in the

TABLE 1.—Results of analysis of covariance examining variation in dry weight, carbon : nitrogen (C:N) ratio, and proximate composition (protein, lipid, and ash) of individual eggs and the proximate composition expressed as a percentage of dry weight for eggs from Atlantic cod broodfish subjected to an ambient photoperiod (Amb PP) or an advanced photoperiod (Adv PP; phase shifted by 4 months). Time is elapsed time (d) since onset of first spawning within each group. Asterisks denote significant *F*-values after Bonferroni adjustment of probability levels. Different letters next to mean values denote significant differences between groups (Tukey's multiple range test: $P < 0.05$). All percentage data were arcsine–square root transformed prior to analysis.

	Individual egg (μg)					% of dry weight				
Variable	PP *F*	Time *F*	PP × time *F*	Adv PP mean	Amb PP mean	PP *F*	Time *F*	PP × time *F*	Adv PP mean	Amb PP mean
Dry weight	26.31*	26.68*	20.02*	109.20 z	97.86 y					
C:N ratio	NS	NS	NS	3.88 z	3.83 z					
Protein	4.72*	NS	NS	60.00 z	49.39 y	NS	NS	NS	52.40 z	52.00 z
Lipid	19.12*	NS	NS	15.14 z	9.68 y	15.73*	NS	NS	13.92 z	9.73 y
Ash	NS	NS	NS	24.86 z	22.19 z	5.33*	NS	NS	24.11 z	22.57 y

advanced PP group, while percent MUFA was greater in the ambient PP group. The latter was largely due to significant differences between PP groups in two MUFAs, 18:1(ω-9) and 18:1(ω-7). Overall, the advanced PP group may be characterized as having greater percent PUFA, including total ω-3 and ω-6 fatty acids, DHA, EPA, and AA, and greater percent saturates than the ambient PP group, whereas the ambient PP group had relatively more total MUFA than the advanced PP group. Comparing both groups on their major PUFA content, the DHA:EPA and EPA:AA ratios were not significantly different between PP groups.

Discussion

The purpose of this work along with that described previously in a companion paper (Penney et al. 2006a) is to determine the effect, if any, of PP compression between successive spawning seasons on Atlantic cod egg production and egg quality. In this paper, we report the effect of a PP advancement technique that compressed the time between successive spawnings in an Atlantic cod broodstock by approximately 4 months. Adoption of PP manipulation techniques on a commercial scale could have a profound effect on efforts to commercialize culture of new finfish species. Thus, it is critical that such manipulations do not

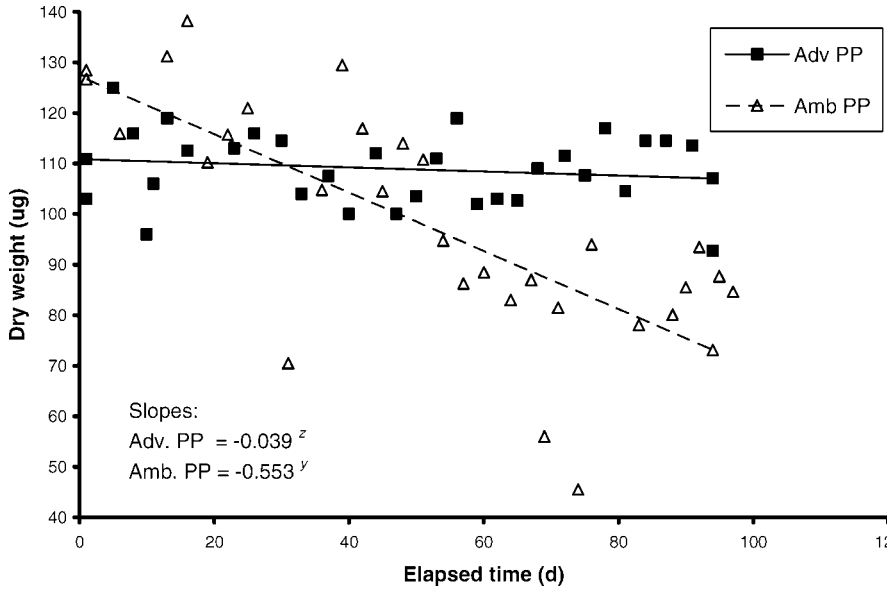

FIGURE 2.—Relationship between mean dry weight (μg) of individual Atlantic cod eggs in each daily batch and elapsed time (d) from onset of first spawning for broodfish subjected to advanced (Adv) and ambient (Amb) photoperiod (PP) regimes. Letters next to slope values denote significant differences between slopes (analysis of covariance homogeneity of slopes test: $P < 0.05$).

TABLE 2.—Results of analysis of covariance examining variation in lipid class weight of individual eggs and expressed as a percentage of total lipid in eggs from Atlantic cod broodfish subjected to an ambient photoperiod (Amb PP) or an advanced photoperiod (Adv PP; phase shifted by 4 months). Time is elapsed time (d) since onset of first spawning within each group. Asterisks denote significant *F*-values after Bonferroni adjustment of probability levels. Different letters next to mean values denote significant differences between groups (Tukey's multiple range test: $P < 0.05$). All percentage data were arcsine–square root transformed prior to analysis.

	Individual egg (μg)					% of total lipid				
Lipid class[a]	PP *F*	Time *F*	PP × time *F*	Adv PP mean	Amb PP mean	PP *F*	Time *F*	PP × time *F*	Adv PP mean	Amb PP mean
HC	9.49*	NS	NS	0.17 z	0.09 y	NS	NS	NS	1.17 z	0.86 z
ST–WE	NS	NS	NS	0.05 z	0.03 z	NS	NS	NS	0.30 z	0.33 z
ME	NS	NS	NS	0.06 z	0.05 z	NS	NS	NS	0.40 z	0.54 z
EK	NS	NS	NS	0.03 z	0.02 z	NS	NS	NS	0.18 z	0.20 z
MK	NS	NS	NS	0.04 z	0.03 z	NS	NS	NS	0.27 z	0.35 z
GE	NS	NS	NS	0.04 z	0.03 z	NS	NS	NS	0.25 z	0.26 z
TAG	28.18*	NS	NS	0.64 z	0.32 y	10.47*	NS	NS	4.28 z	3.43 y
FFA	NS	NS	NS	0.06 z	0.04 z	NS	NS	NS	0.40 z	0.47 z
ALC	NS	NS	NS	0.06 z	0.04 z	NS	NS	NS	0.40 z	0.46 z
ST	18.13*	NS	NS	1.34 z	0.89 y	NS	NS	NS	9.14 z	9.56 z
DAG	8.09*	NS	NS	0.03 z	0.01 y	NS	NS	NS	0.18 z	0.13 z
AMPL	NS	NS	NS	0.43 z	0.29 z	NS	NS	NS	3.06 z	2.85 z
PL	15.91*	NS	NS	12.03 z	7.82 y	NS	NS	NS	79.98 z	80.51 z

[a] HC = hydrocarbons, ST–WE = steryl and wax esters, ME = methyl esters, EK = ethyl ketones, MK = methyl ketones, GE = glycerol ethers, TAG = triacylglycerols, FFA = free fatty acids, ALC = alcohols, ST = sterols, DAG = diacylglycerols, AMPL = acetone mobile polar lipids, and PL = phospholipids.

TABLE 3.—Results of analysis of covariance examining variation in fatty acid proportions (means > 1% in at least one group; fatty acid notation is of the form 16:1[ω-7], where 16 is the number of carbon atoms, 1 is the number of double bonds, and 7 is the position of the first double bond from the methyl end) and selected fatty acid ratios in eggs from Atlantic cod broodfish subjected to an ambient photoperiod (Amb PP) or an advanced photoperiod (Adv PP; phase shifted by 4 months). Fatty acids include docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA), polyunsaturated fatty acids (PUFAs), and monounsaturated fatty acids (MUFAs). Time is elapsed time (d) since onset of first spawning within each group. Asterisks denote significant *F*-values after Bonferroni adjustment of probability levels. Different letters next to mean values denote significant differences between groups (Tukey's multiple range test: $P < 0.05$). All percentage data were arcsine–square root transformed prior to analysis.

Fatty acid	PP *F*	Time *F*	PP × time *F*	Adv PP mean	Amb PP mean
14:0	33.78*	NS	9.40*	1.60 z	1.37 y
16:0	31.13*	NS	NS	20.86 z	18.08 y
16:1(ω-7)	94.99*	NS	NS	2.80 z	0.79 y
18:0	NS	NS	NS	2.45 z	2.11 z
18:1(ω-9)	39.28*	NS	NS	11.20 z	7.66 y
18:1(ω-7)	30.39*	NS	NS	2.05 z	11.58 y
18:4(ω-3)	83.65*	NS	NS	1.17 z	3.90 y
18:4(ω-1)	50.87*	NS	NS	0.15 z	1.58 y
20:1(ω-9)	21.73*	NS	NS	3.36 z	2.83 y
20:4(ω-6) (AA)	15.46*	NS	NS	0.83 z	0.66 y
20:5(ω-3) (EPA)	35.42*	NS	NS	16.51 z	14.39 y
22:1(ω-11)	NS	NS	NS	1.17 z	1.05 z
22:5(ω-3)	73.46*	NS	NS	2.03 z	1.21 y
22:6(ω-3) (DHA)	59.60*	NS	NS	31.21 z	27.23 y
DHA : EPA	NS	NS	NS	1.89 z	1.91 z
EPA : AA	NS	NS	NS	21.39 z	22.12 z
Σ saturates	42.12*	NS	NS	25.22 z	21.68 y
Unsaturates : saturates	23.99*	NS	NS	2.95 z	3.56 y
Σ PUFAs	11.81*	NS	NS	52.87 z	49.86 y
Σ MUFAs	11.04*	NS	NS	21.48 z	25.53 y
Σ(ω-3)	29.40*	NS	NS	51.54 z	47.28 y
Σ(ω-6)	31.59*	NS	NS	1.00 z	0.71 y

compromise future performance of the progeny. Since the commercial objective of broodstock management is to maximize production of high-quality eggs with the best possible future production potential, understanding the effect of PP manipulations of broodstocks on egg production and indices of egg quality is of significant interest.

Atlantic cod respond to starvation by reducing fecundity (the number of oocytes that mature; Kjesbu et al. 1991) or by failing to spawn altogether in wild populations (Rideout et al. 2005, 2006). The latter may occur through failure to start vitellogenesis or through mass atresia of vitellogenic oocytes, both of which are linked to poor nutritional condition of the parental population (Rideout et al. 2005). Similarly, for captive Atlantic cod broodstock populations, the nutritional status also significantly affects the number of previtellogenic oocytes produced (Waiwood 1982; Kjesbu et al. 1991), the proportion of fish that undergo maturation and ovulation, and the extent of atresia (Kjesbu et al. 1991). This implies that compressed PP, which gives the broodfish reduced time to both acquire nutrients and to transfer these nutrients to the eggs, might also result in reduced fecundity and perhaps reduced nutrient levels in the eggs. Individual Atlantic cod broodfish may produce 20 or more egg batches over 6–8 weeks in a single spawning season (Kjesbu 1989; Kjesbu et al. 1996). The decrease observed in egg size of successive egg batches produced by individual females during the spawning season (Kjorsvik et al. 1990; Kjesbu et al. 1991) has been related to the exhaustion of reserves for vitellogenesis, and the rate of decrease has been related to the nutritional state of the broodfish (Kjesbu et al. 1991). The size of previtellogenic oocytes remains small before spawning but rises sharply before hydration (Kjesbu and Kryvi 1993). Thus, a large number of previtellogenic oocytes can be produced at relatively low energetic cost, suggesting that the energy-intensive process of transferring nutrients to maturing eggs before spawning can be regulated (Kjesbu et al. 1991).

Since neither the mean dry weight nor any of the measured biochemical features known to be indicative of egg quality (protein, total lipid, lipid classes, and fatty acids) significantly declined over the course of the spawning season in the advanced PP group, there is no evidence of exhaustion of nutritional reserves over time in the parental broodfish subjected to advanced PP. The conflicting observations that mean dry weight of ambient PP eggs declined over time while that of advanced PP eggs did not is unsurprising because while mean egg size in successive egg batches from individual female Atlantic cod broodfish is often observed to decline over time (Kjesbu et al. 1991; Ouellet et al. 2001), seasonal changes in egg size among eggs pooled from all females in a communal broodstock may not be apparent (Chambers and Waiwood 1996). Most of the variation in egg size within Atlantic cod broodstocks has been related to individual parental effects (Chambers and Waiwood 1996; Ouellet et al. 2001). In communal broodstocks, the parental origin of daily egg batches may therefore change unpredictably on a daily basis and thus will either obscure or enhance egg size variation attributable to individual females over time.

Atlantic cod respond to PP compression by reducing the number of oocytes that mature (Hansen et al. 2001) rather than skipping spawning altogether as occurs in striped bass *Morone saxatilis* (Blythe et al. 1994) and Atlantic halibut (Björnsson et al. 1998). In the broodstock populations studied in the present work, the PP advanced group initiated spawning approximately 4 months before the ambient controls but had a longer spawning season, had lower mean daily egg batch volumes, and produced a lower total volume of eggs over the entire spawning season (Penney et al. 2006a). These observations support the conclusion that PP compression has reduced the number of maturing oocytes in Atlantic cod. However, in the present work, we report that individual eggs from advanced PP broodfish were of greater mean dry weight and had greater mean protein, total lipid, triacylglycerol, and phospholipid levels than did eggs from ambient control broodfish. The greater mean dry weight, protein, and lipid levels of eggs from advanced PP broodfish seem to indicate that relatively more resources are being put into each egg, but since these eggs are also of smaller mean diameter than those from ambient PP broodfish (Penney et al. 2006a) apparently less water is incorporated during hydration. On a percentage basis, total lipid and triacylglycerol were also both higher in eggs from the advanced PP broodfish, while percent protein and remaining lipid class levels were not significantly different. Furthermore, the mean percentages of selected fatty acids known to be critical for future egg and larval development (e.g., PUFAs including DHA, EPA, and AA; Sargent 1995; Bell and Sargent 2003) were all higher in eggs from advanced PP broodfish. Thus, we conclude that there is certainly no evidence of reduced nutritional status in individual eggs from advanced PP broodfish and therefore no evidence to suggest that future performance potential of the progeny would be compromised as a result of PP compression in Atlantic cod. We propose that the observed reduction in total and individual batch egg volumes in advanced PP Atlantic cod broodfish populations is an energy-sparing mechanism that is supportive of maintaining critical nutrient

levels in spawned eggs and thus becomes a strategy to ensure that potential survivability of progeny is maintained.

In a previous paper, Penney et al. (2006a) noted that both fertilization success and the rate of cell developmental abnormalities did not differ between the advanced and ambient PP groups in this experiment. Fertilization success was high (>90% for both PP groups), while the rates of cell developmental abnormalities were low (<10%) in both groups. Both of these variables have been considered direct indicators of egg quality in Atlantic cod (Kjorsvik et al. 1990). These data, coupled with the present biochemical analyses, strongly support the conclusion that egg quality is not compromised by subjecting parental Atlantic cod broodstocks to PP compression. Furthermore, since none of the measured biochemical indices varied with elapsed time over the entire spawning season, this supports the conclusion that continued feeding of broodfish during the spawning season effectively maintains egg quality over successive egg batches within a spawning season. Therefore, we recommend that the husbandry protocols employed (~4-month PP compression combined with continued feeding during the spawning period) constitute an effective technique for maintaining the condition of Atlantic cod broodfish such that egg quality can be maintained in successive egg batches over the entire spawning period. However, reduced egg volumes obtained from advanced PP populations may necessitate adjustment of total broodstock numbers needed in commercial hatcheries to effectively maintain target egg production levels.

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