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ARTICLE

Seasonal Variations in Skin Pigmentation and Flesh Quality of Atlantic Salmon (*Salmo salar* L.): Implications for Quality Management

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The external coloration of fish is a key driver in consumer buying decisions and is typically altered during sexual maturation in salmonids. Farmed Atlantic salmon (*Salmo salar* L.) exhibiting distinct phenotypes from the typical silver and nuptial coloration were described in terms of sexual development, flesh quality, and skin pigment profiles. Reconditioning of skin coloration during storage was also tested (CIE[1976]$L^*a^*b^*$) with the overall view to optimize quality management. The intermediary phenotype never reflected significant deteriorations of flesh quality. It originated from a lack of purine pigments (guanine and hypoxanthine), revealing the carotenoid compounds dominated by the yellow-orange β-carotene. The resulting distinctive lightness and yellowness were reduced by direct ice contact at a post-mortem stage. Storage conditions can be optimized to improve and standardize the coloration of whole-fish, yielding superior flesh quality parameters. This would facilitate product quality grading during primary processing and also increase product acceptance and attractiveness.

KEYWORDS: Atlantic salmon; skin pigmentation; coloration; flesh quality; sexual maturation; reconditioning

INTRODUCTION

The external coloration of fish is an instinctive indicator of product freshness and quality and hence constitutes a key driver in consumer buying decisions (*1*). This is true for both skin and flesh color in many aquaculture species that are sold whole or processed. From Atlantic salmon (*Salmo salar* L.), the consumer expects a deep pink-red flesh and a silvery-blue skin characteristic of the sea-running immature life stage. Fish skin coloration is a complex trait generated by four main types of pigment stored in interacting chromatophores. Melanin, carotenoids, and pteridines are true light-absorbing pigments found primarily in black-brown melanophore, red-orange erythrophore, and ocher-yellow xantophore, respectively. In contrast, iridophore and leucophore are light-reflecting chromatophores generating blue-green iridescent and white-creamy colors, respectively, due to variations in the structural organization of their purine-based crystalline organelles (*2*). Alterations in the distribution, morphology, or density of chromatophores and/or in the concentration of their pigment granules define morphological skin color changes. They are slow and long-lasting phenomena elicited in response to environmental (e.g., surrounding light conditions) and social (e.g., subordination; territoriality) factors but also observed during life-stage transitions such as smoltification and nuptial metamorphosis in salmonids (*3*). In contrast, physiological skin color changes occur from the migration, that is, dispersion or aggregation, of pigment vesicles within their cells. They are acute and transient events stimulated by neuronal and endocrine factors (*2*) and can occur at a post-mortem stage (*4*, *5*). The coloration of salmonid muscle originates from carotenoid pigments fed in the form of astaxanthin (Ax) and canthaxanthin (Cx) through well-defined pigmentation feeding regimens during growth (*6*). Although a variety of nutritional factors affect flesh carotenoid deposition and visualization (*7*), sexual maturation has the most extensive effect under standard farming practices. Carotenoid pigments are in fact accumulated in the flesh at the immature stage and redistributed to both the skin and gonads over the course of sexual maturation (*6*−*9*). The development of nuptial coloration is therefore used as an indicator of not only reduced flesh carotenoid but also lipid and protein levels, also due to the reproductive effort (*10*), during product quality grading.

Holding both ecological and economical interests, nuptial metamorphosis is the best studied phenomenon of skin color change in salmonids but only with regard to carotenoid compounds. Melanin pigments could confer nuptial salmonids their typical dark gray appearance, whereas purine compounds are likely to be altered during desmoltification associated with sexual maturation. Skin silvering during smoltification positively correlated with purine concentration and alteration in osmoregulatory parameters (*11*, *12*). The focus given to carotenoid pigments is likely to restrict understanding of color expression in Atlantic salmon. With virtually no other factors described as recurrently

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affecting the skin coloration of farmed Atlantic salmon, sexual maturation and altered flesh quality are typically presumed when deviations from the desirable silver phenotype occur. This results in product downgrading toward a lower value market channel at primary processing and reduces the economic sustainability of the industry.

Sea-reared Atlantic salmon exhibiting an intermediary skin coloration between the typical silver and nuptial phenotypes, of immature and mature salmon, respectively, are repeatedly observed at harvest. These fish display a pearly white belly characteristic of immature fish but a pale-greenish back resembling the instigation of the nuptial metamorphosis. This phenotype could be expected in spring/early summer during the recognized window for initiation of sexual maturation but is observed over the whole reproductive season. The present study was therefore undertaken to confirm, quantify, and characterize such altered phenotype in terms of sexual development, flesh quality, and skin pigment profile in comparison to the typical silver-immature and nuptial-mature skin coloration. Feasibility of their postharvest reconditioning was also assessed. The aim of this study was to improve understanding of color expression in Atlantic salmon in relation to flesh quality with the view to optimize postharvest quality management.

MATERIALS AND METHODS

Animals and Sampling. Atlantic salmon were reared under standard commercial management up to harvest size in one Scottish sea site (latitutde 56° 39′ 19″; longitude −5° 19′ 56″). The stock was fed a commercial diet according to the manufacturer's recommendations (35% protein, 32% lipids, 16.5% carbohydrates, 10 ppm Ax, and 5 ppm Cx; Skretting, Invergordon, U.K.). Sampling was performed once a month from June to December 2007 at the processing plant, 8−12 h post-mortem. Up to three distinctive phenotypes were subjectively observed: silver, intermediary (white belly and pale-green back), and nuptial. Their prevalence was determined by a single observer with a minimum of 600 observations from at least three counting sessions over the processing period. Within each skin color category, 30 fish/gender/skin color category were randomly collected and measured for whole body weight (BW) (±0.1 g), fork length (FL) (±0.1 cm), and gonad weight (GW) (±0.01 g). Fulton condition factor (K) and the gonado-somatic index (GSI) were calculated as follows: $K=(100\,\mathrm{BW})\,\mathrm{FL}^{-3}$ and $\mathrm{GSI}\,(\%)=(100\,\mathrm{GW})\,\mathrm{BW}^{-1}$. Female ovaries were preserved in Bouin's fixative for 24 h and then in 70% ethanol before processing for histological observation. Among these fish, the left-hand side flesh Scottish quality cut (SQC), representing the steak taken immediately below the dorsal fin, were excised from 10 females/skin color category and stored at −20 °C until quality analysis. In December, a further 10 fish/skin color (1:1 sex ratio) were collected for flesh, skin, and gonad pigment analysis following assessment of morphological and gonadal parameters. The left-hand side SQC was carefully skinned using a scalpel. Skin samples from standardized areas of the dorsal and ventral SQC were excised and divided vertically in two for carotenoids and melanin and purine content analysis, respectively. The skinned SQC, skin, and gonad samples were frozen at −20 °C until carotenoid analysis. Finally, postharvest skin color reconditioning was assessed using five silver and five intermediary colored fish randomly sampled in December. Each fillet from each fish was cut into three transverse sections (flesh quality cut, SQC, Norwegian quality cut (*13*)) and then rinsed in distilled water before testing for reconditioning.

Sexual Development and Flesh Quality Analysis. Males were classified as immature or sexually recruited on the basis of their bimodal GSI frequency distribution in the population with a threshold value of GSI = 0.20% (*14*). Female ovary samples were classified according to their leading oocyte stage with onset of true exogeneous vitellogenesis used as indicator of recruitment into sexual maturation (*15*). Flesh SQCs were thawed overnight, skinned, and deboned prior to analysis. Flesh color was scored by two independent assessors under standard light condition using the Roche *Salmo*Fan lineal color card for salmonids, scale 20−34 (Hoffman-LaRoche Ltd., Basel, Switzerland) in the dorsal, midline, and belly regions and then averaged. Flesh color composition (CIE[1976]-$L^*a^*b^*$) was measured in the same areas using a tristimulus colorimeter (Minolta Chroma Meter, CR-310, Minolta Corp., Osaka, Japan) against a standard calibration tile (*1*). Flesh samples were then homogenized for analysis of total carotenoids, total lipid, and lipid proximate composition by near-infrared reflectance spectroscopy (NIR) (FOSS 6500 NIR analyzer, Foss NIR Systems, Foss UK Ltd., Didcot, U,K,) (*16*). The NIR analyzer was calibrated daily prior to analysis using a calibration check cell and twice over the analysis period against wet chemistry (HPLC).

Pigment Extraction. For carotenoid analysis (December samples), skin samples were weighed (±0.001 g) and their surface areas determined by image analyses (Image ProPlus 4.5, Media Cybernetics, Bethesda, MD) using a stereomicroscope and then homogenized for carotenoid extraction. Skinned SQCs and gonads were thawed overnight, homogenized (immature male gonads were pooled per skin color category due to low tissue weight and expected carotenoid concentration), and 1 g of tissue was separated for extraction of carotenoids as follows (*17*, *18*). Tissue samples were placed in a stoppered glass tube containing 10 mL of absolute ethanol/ethyl acetate mixture (1:1; v/v) and homogenized using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). The homogenate was centrifuged (1000*g*, 5 min) and the supernatant removed to a clean stoppered glass tube. The pellet was rehomogenized in 5 mL of ethyl acetate and then in 10 mL of isohexane, and the supernatants were pooled following centrifugation (1000*g*, 5 min). The pooled supernatant was evaporated to dryness under a nitrogen (N_2) flow and vacuum desiccated in total darkness overnight. The residue was finally redissolved in 5−20 mL of isohexane containing 0.2% (w/v) butylated hydroxytoluene (BHT) before injection into the HPLC system. Skin melanin was extracted from the carotenoid-stripped skin pellet, which was immediately resuspended in 10 mL of 0.2% NaOH and stored overnight at −4 °C. The sample was then boiled in 0.2% NaOH for 1 h with regular mixing. The solution was centrifuged (2100*g*; 5 min), the supernatant removed to a stopper glass tube, and the skin pellet re-extracted as previously described. Supernatants were combined, topped up to 20 mL precisely with 0.2% NaOH, and mixed. A 2 mL fraction was centrifuged (6100*g*; 5 min) and measured by spectrophotometry at 340 nm against a synthetic melanin standard (Sigma-Aldrich Ltd., Poole, U.K.) presolubilized in 1 mL of 1 M NaOH and 10 μL of 3% H_2O_2 by heating in a boiling water bath for 30 min (*19*). Purine extraction protocol was modified from ref *20* as follows. Surface area and weight of the skin samples were determined as previously described and then homogenized with an Ultra-Turrax tissue disrupter (Fisher Scientific) in 10 mL of 0.1 M NaOH. The stoppered glass tube was placed in an ultrasonic bath for 20 min and regularly mixed for another 40 min, and the supernatant was separated by centrifugation (220*g*, 5 min). The skin pellet was re-extracted twice in 5 mL of 0.1 M NaOH and a final time in 10 mL of 0.1 M NaOH following overnight storage at −4 °C. The final supernatant was translucent, and the supernatants were combined for a total extract volume of 30 mL. A 2 mL fraction was diluted in 2 mL of distilled H_2O providing a total dilution factor of 60 and a 0.05 M NaOH extract. This aliquot was mixed and centrifuged (6100*g*; 5 min) before injection into the HPLC system.

HPLC Systems. The chromatographic system used for carotenoid analysis was previously described (*18*). It consisted of a 5 μm ODS2 column (4.6 × 150 mm, Phenomenex, Macclesfield, U.K.) equipped with a Waters model 501 pump and a Waters 490E multiwavelength UV−vis detector (Millipore, Watford, U.K.) for detection at 450 nm. An isocratic solvent system containing ethyl acetate/methanol/water (20:72:8; v/v/v) at a flow rate of 1 mL min^{-1} was used, and carotenoid compounds were quantified using as external standards Ax, Cx, and β-carotene (DSM Nutritional Products, Basle, Switzerland). Purine (guanine; hypoxanthine; adenine; Sigma-Aldrich Ltd., Poole, U.K.) and pteridine standards (xanthopterin, sepiapterine, leucopterin, xanthine, biopterin, isoxanthopterin, lumazine; Schircks Laboratories, Jona, Switzerland) were dissolved in 0.05 M NaOH at concentrations ranging from 0.2 to 1 mg mL^{-1} according to the manufacturer's recommendations. Serial dilutions to concentrations of 1, 2, or 5 $\mu\mathrm{g\,mL}^{-1}$ were performed prior to injection into the HPLC system. For analysis of purine compounds, the chromatographic system consisted of a constaMetric 4100 pump equipped with a UV 3000 detector, and peak areas were calculated using ChromQuest software (ThermoFisher, Hemel Hempstead, U.K.). The mobile phase consisted of a mixture of 0.01 mM potassium phosphate buffer, pH 3.2,

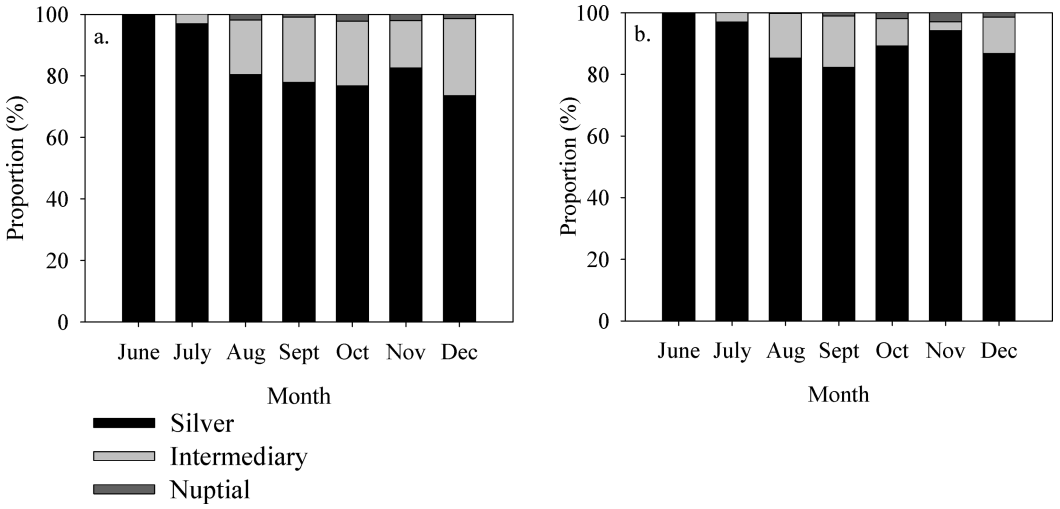

Figure 1. Proportion of the three skin color categories observed at harvest in (**a**) female cohort and (**b**) male cohort (n = 30 fish/sex/color group/sampling point).

using phosphoric acid, with 4% methanol. Pigments were eluted isocratically at a flow rate of 1 mL mn^{-1} and detected at 254 nm after elution from a Partisil-10 ODS-1 column (250 × 4.6 mm; Phenomenex, Macclesfiled, U.K.; modified from refs *21* and *22*). This method for purine analysis was expected to be equally effective for pteridine analyses, as described by these authors in other tissues. Pteridine standards were revealed, but no pteridine compounds were identified in our samples. This was possibly due to the low concentration of pteridine compounds in teleost integuments along with the high sample dilution factor required for analysis of purines found at high concentration in teleost skin.

Skin Color Reconditioning. Five cuts from the same fish were randomly placed skinned side down in direct contact with ice of different composition (freshwater (FW); FW + 0.25 M Na^+; FW + 0.5 M Na^+; FW + 0.75 M Na^+; FW + 5 mM caffeine), and the last cut was placed in direct contact with skin by juxtaposing another sample. Caffeine is known to induce melanophore dispersion at this concentration (*23*). These treatments are later referred to as FW, Na1, Na2, Na3, CAF, and skin contact, respectively. Each treatment comprised five samples from five different fish/color categories except for skin contact treatment, which received four samples. The different ice baths were placed in a Styrofoam box to maintain a constant ice temperature throughout the experiment. Skin color composition was measured immediately before treatment application (T0) and then at T0 + 6 h (T1), T0 + 24 h (T2), and T0 + 30 h (T3) by means of a tristimulus colorimeter as described previously for flesh color analysis. Skin color was assessed in triplicate in both the dorsal and belly regions and then averaged per body area. With a^* values (red-greenness) and b^* values (yellow-blueness) close to achromatic in the dorsal skin, minor changes in those parameters led to high and irrelevant changes in hue (H_{ab}) and chroma (C_{ab}) (*4*) such that focus was given to primary $L^*a^*b^*$ parameters of color composition.

Statistics. Prior to statistical analysis using Minitab v.15, data sets were tested for normality (Kolmogorov−Smirnov test) and homogeneity of variance (Bartlett's test, examination of residual plots), log-transformed and proportions arcsin transformed when required. Differences in morphological, GSI, and flesh quality parameters between color groups were tested at each sampling point by one-way analysis of variance (ANOVA). For December samples for pigment analysis, parameters were analyzed by three-way ANOVA to test the effect of skin color, body area, and sex on skin pigment concentration and compound proportions and by two-way ANOVA to test the effect of skin color and sex on morphological parameters, skin dorso-ventral pigment ratio, and flesh and gonad carotenoid content. Parameters of skin color composition assessed in the post-mortem reconditioning challenge were analyzed using a three-way repeated measure ANOVA manipulated by a General Linear Model (GLM) to test the effect of treatment, storage time, and skin color category in each body area. In each color group, parameters were never statistically different at T0, that is, between on-coming treatments. Samples were pooled at this time point to characterize, for each body area, differences in color composition between color groups (one-way ANOVA). Furthermore, for each color group, there were no statistical differences between treatments in the ventral area or between the five ice quality tested in the dorsal area (FW, Na1, Na2, Na3, CAF). Data were pooled accordingly to compare, in particular, the effect of ice and skin contact in the dorsal area. When statistical differences were found ($P < 0.05$), post hoc multiple comparisons were applied using Tukey's test. All data are expressed as mean values ± standard error of mean (SE).

RESULTS

Seasonal Occurrence, Sexual Development, and Flesh Quality of Observed Phenotypes. The proportions of the different color groups observed at harvest are presented in **Figure 1**. Intermediary fish were observed from July until December. Their incidence increased over the summer, accounting for 3% in both the male and female cohorts in July but for 17 and 21% in those respective genders in September. Comparatively less frequent in October and November, 12 and 25% of harvested males and females exhibited a distinctive intermediary coloration in December. Nuptial fish were observed from August, a month later than the first occurring intermediary fish, to December with a monthly prevalence rate always below 3%.

Histological evidence of recruitment into sexual maturation were first observed in July in fish exhibiting an intermediary skin coloration (male-GSI, 0.2−0.9%; female-GSI, 0.72−2.0% at the primary and secondary yolk stage). Intermediary males and females had a significantly higher GSI than their silver siblings in July (**Figure 2a**,**b**), although not all but 77 and 63% of the intermediary males and females were sexually recruited (data not shown). Silver and intermediary males were systematically immature from August to December as shown by a maximum GSI of 0.16% (**Figure 2a**). Similarly, there were no significant differences in GSI between silver and intermediary females over this period (**Figure 2b**). However, whereas silver females were always immature from August to October, 6.7 and 17% of the intermediary females were at an exogenous vitellogenic stage in August and September, respectively (data not shown). Later in November and December, females at an early maturational stage (primary yolk stage, GSI < 0.72%) occurred in both the silver and intermediary cohorts and at a rate of 23 and 33%, respectively, in December (data not shown). In comparison, the GSI of nuptial males and females were always significantly higher than both their silver and intermediary siblings. Over this period, GSI of

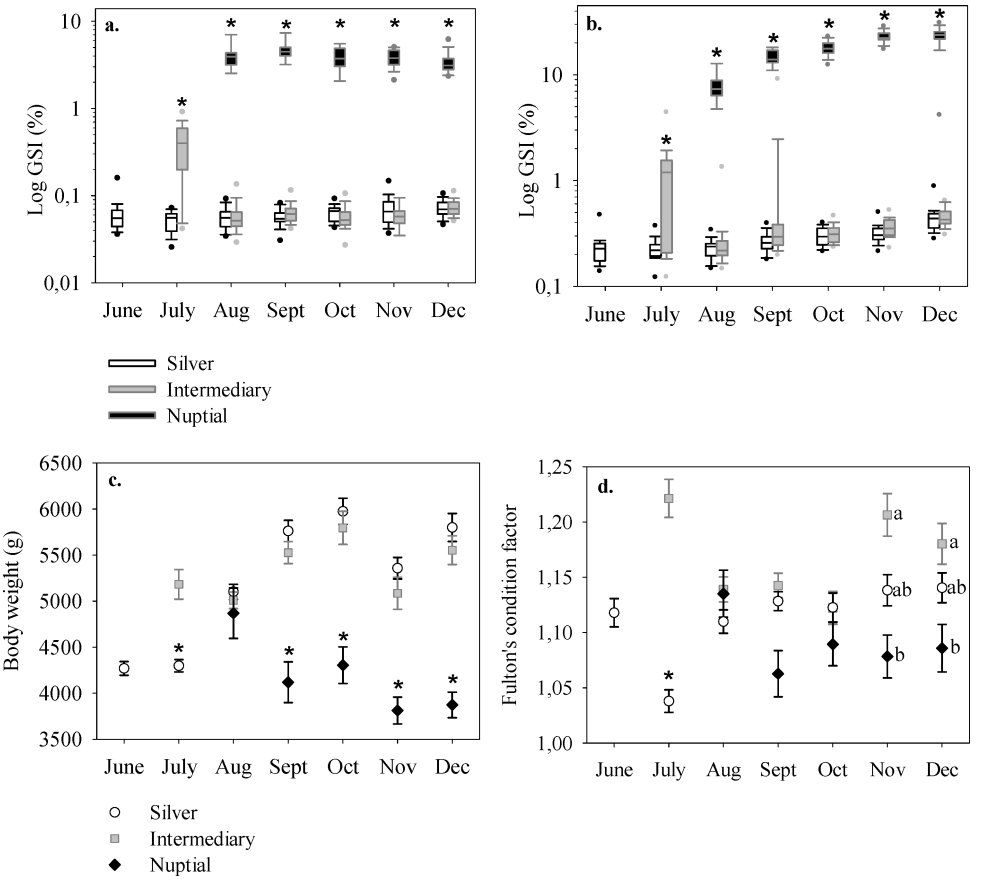

Figure 2. GSI box-plot distribution of (**a**) male cohort and (**b**) female cohort within each skin color group ($n = 30$ fish/sex/color group/sampling point). Asterisks (∗) or different letters indicate significant differences between skin color groups within each month (ANOVA, $P < 0.05$).

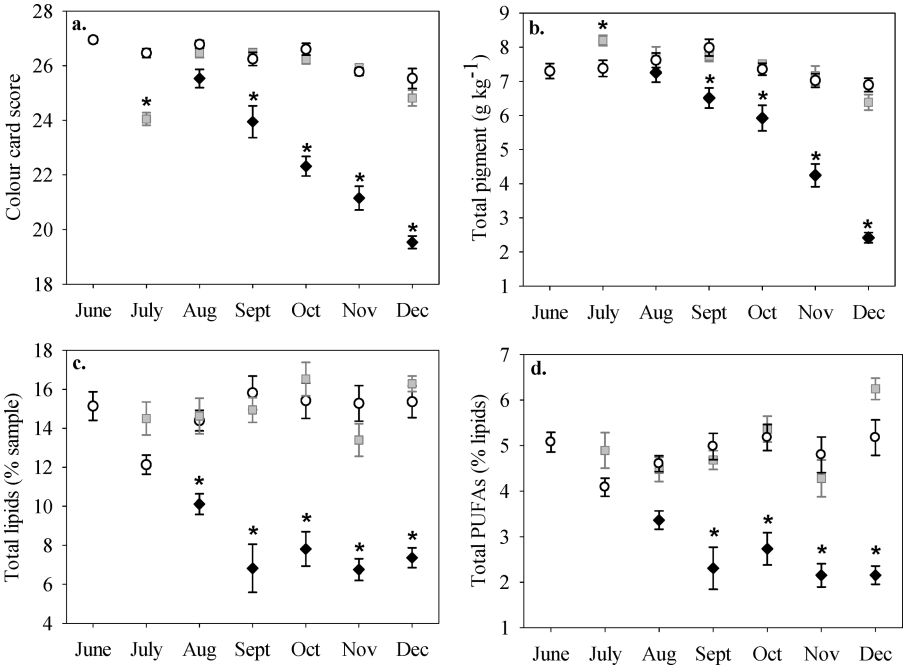

Figure 3. Flesh quality parameters within each skin color group: (**a**) Roche color card rating score; (**b**) total pigment (mg kg^{-1}); (**c**) total lipids (percent sample); (**d**) total PUFAs (percent lipids). Values are expressed as mean ± SE ($n = 10$ female/color group/sampling point). Asterisks (∗) indicate significant differences between skin color groups within each month (ANOVA, $P < 0.05$).

nuptial males ranged from 2.3 to 7.3% and GSI of nuptial females, always at the tertiary yolk stage, between 4.7 and 31.4% (**Figure 2a**,**b**).

Differences in morphological parameters between the silver and intermediary fish occurred in July only when the former cohort was significantly heavier (+884 g, +21%) with a higher *K*

Table 1. Morphological Parameters of Individuals Sampled in December for Pigment Analysis[a]

	female			male		
	silver	intermediary	nuptial	silver	intermediary	nuptial
body weight (g)	4425 ± 167 ab	4483 ± 208 ab	3803 ± 92 a	4750 ± 181 ab	5021 ± 255b	4182 ± 245 ab
condition factor	0.96 ± 0.07 a	1.08 ± 0.02 ab	1.16 ± 0.02 b	1.00 ± 0.03 ab	1.01 ± 0.01 ab	0.94 ± 0.03 a
GSI (%)	0.30 ± 0.04 a	0.39 ± 0.07 a	24.01 ± 2.00 b	0.07 ± 0.01 y	0.07 ± 0.01 y	3.42 ± 0.23 z

[a] Values are expressed as mean ± SE (n = 5 fish/sex/color group). Different letters indicate significant differences between experimental groups (ANOVA, $P < 0.05$). GSI were analyzed separately for each sex.

Table 2. Skin Melanin and Purine Pigment Concentrations and Profiles[a]

	female			male		
	silver	intermediary	nuptial	silver	intermediary	nuptial
Total Melanin Concentration (μg cm^{-2})						
dorsal	288.0 ± 20.4 ab	219.7 ± 28.5 a	272.6 ± 16.4 ab	286.9 ± 22.2 ab	269.6 ± 3.1 ab	311.5 ± 9.6 b
ventral	*179.2 ± 17.8* a	*80.3 ± 18.8* b	*154.4 ± 13.1* ab	*172.6 ± 8.7* a	*149.0 ± 3.4* ab	*213.8 ± 16.1* a
D/V	1.7 ± 0.2 a	3.1 ± 0.5 b	1.8 ± 0.1 ab	1.7 ± 0.2 a	1.8 ± 0.0 ab	1.5 ± 0.1 a
Total Purine Concentration (μg cm^{-2})						
dorsal	298.9 ± 37.1 a	218.6 ± 15.7 ab	181.3 ± 28.0 ab	307.8 ± 18.6 a	237.2 ± 25.7 ab	156.2 ± 14.7 b
ventral	*1338.1 ± 84.0* a	*844.5 ± 114.6* ab	*565.1 ± 61.2* b	*1398.5 ± 96.6* a	*857.4 ± 175.6* ab	*700.1 ± 44.4* b
D/V	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0
Purine Profile (%)						
dorsal						
guanine	73.4 ± 0.8 a	69.5 ± 1.6 ab	67.3 ± 1.4 bc	73.3 ± 1.4 a	70.6 ± 1.3 ab	61.4 ± 2.4 bc
hypoxanthine	26.6 ± 0.8 a	30.5 ± 1.6 ab	32.8 ± 1.4 b	26.7 ± 1.4 a	29.5 ± 1.3 ab	38.6 ± 2.4 c
G/H	2.8 ± 0.1 a	2.3 ± 0.2 ab	2.1 ± 0.1 bc	2.8 ± 0.2 a	2.4 ± 0.2 ab	1.6 ± 0.2 c
ventral						
guanine	*80.5 ± 0.6*	*79.9 ± 0.7*	*80.5 ± 0.3*	*80.9 ± 0.2*	*80.5 ± 0.3*	*80.8 ± 0.7*
hypoxanthine	*19.5 ± 0.6*	*20.1 ± 0.7*	*20.3 ± 0.6*	*19.1 ± 0.2*	*19.5 ± 0.3*	*19.2 ± 0.7*
G/H	*4.1 ± 0.2*	*4.0 ± 0.2*	*4.0 ± 0.1*	*4.2 ± 0.1*	*4.1 ± 0.1*	*4.0 ± 0.1*

[a] Values are expressed as mean ± SE (n = 5 fish/sex/color group). Different letters indicate significant differences between experimental groups. Italics denote significant differences between body areas within each experimental group for each parameter (ANOVA, $P < 0.05$). D, dorsal skin; V, ventral skin; G, guanine; H, hypoxanthine.

(+0.18, +18%) (**Figure 2c**,**d**). In comparison, nuptial fish were significantly lighter from September onward and their K lower than that of intermediary fish in November and December. Such differences in body morphology were concomitant with differences in flesh quality parameters assessed in females only. In July, intermediary compared to silver females had a higher total flesh lipid (+2.4 g 100 g^{-1}, +20%) and carotenoid content (+0.8 mg kg^{-1}, +11%), but their flesh color score was significantly reduced (−2.4 points, −9%; **Figure 3**). There were no further differences in flesh quality between both cohorts over the remainder of the harvest season. In contrast, nuptial females always had significantly altered flesh quality parameters. They had in August a significantly lower total flesh lipid content (10.1 ± 0.5%) when compared to the silver (14.4 ± 0.5%) and intermediary cohorts (14.6 ± 0.9%). This was followed from September onward by a significant reduction in all flesh quality parameters assessed (**Figure 3**).

Pigment Concentration and Profiles. Morphological and gonadal parameters of fish sampled in December for pigment analyses are presented in **Table 1**. No differences in BW, K, and GSI between silver and intermediary fish were observed at this time as in the seasonal sampling. Melanin pigments were more concentrated in the dorsal than ventral regions in all experimental groups (**Table 2**). This was particularly marked in intermediary females, for which the melanin dorso-ventral ratio was significantly higher, reaching 3.1 ± 0.5 compared to an average of 1.7 ± 0.1 in the other cohorts. Melanin concentrations were significantly lower in intermediary females than in silver males, in silver females and nuptial males in the ventral area, than in nuptial males dorsally. The highest skin melanin concentrations were measured in nuptial males in both body areas. Purine pigments were always more concentrated ventrally than dorsally with a steady dorso-ventral ratio among experimental cohorts averaging 0.3 ± 0.0 (**Table 2**). Nuptial fish had significantly less purines in their integument than silver fish. Differences were significant for both genders in the ventral area (male, −699 μg cm^{-2}, −50%; female, −773 μg cm^{-2}, −58%) and for males only in the dorsal area (male, −152 μg cm^{-2}, −49%; female, −118 μg cm^{-2}, −39%). In comparison, intermediary fish had an intermediate purine concentration, which was never different from any other cohort despite being reduced by 24.9% (−76 μg cm^{-2}) dorsally and by 37.8% (−518 μg cm^{-2}) ventrally when compared to their silver siblings (both gender averaged). Two purine compounds were identified, guanine (G) and hypoxanthine (H), with a strong positive linear correlation between their concentration expressed per skin surface area (G = 4.74 H − 142.13, $r^2 = 0.98$) (data not shown). Purine profiles were never different between cohorts in the ventral area ,where the G/H ratio averaged 4.1 ± 0.1. The proportion of G, and hence the G/H ratio, was significantly lower dorsally than ventrally in all experimental groups and in the dorsal skin of nuptial compared to silver fish (male, −12%; female, −6%) (**Table 2**). The last class of pigment analyzed, carotenoid compounds, was always at a higher concentration dorsally with a steady dorso-ventral ratio averaging 1.9 ± 0.1 among cohorts. They were found at similar levels in silver and intermediary fish of both genders. In males, carotenoids were

Table 3. Carotenoid Concentrations and Profiles in the Skin, Flesh, and Gonads[a]

		female			male		
		silver	intermediary	nuptial	silver	intermediary	nuptial
Total Carotenoid Concentration							
dorsal skin	μg cm^{-2}	3.3 ± 0.1 bc	3.2 ± 0.3 bc	4.7 ± 0.2 ab	3.0 ± 0.3 c	3.1 ± 0.3 c	5.6 ± 0.5 a
ventral skin	μg cm^{-2}	*1.8 ± 0.1* a	*1.8 ± 0.1* a	*2.1 ± 0.2* a	*1.7 ± 0.2* a	*1.7 ± 0.2* a	*3.3 ± 0.4* b
D/V		1.8 ± 0.2	1.9 ± 0.3	2.3 ± 0.3	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
flesh	mg kg^{-1}	6.0 ± 0.2 a	5.9 ± 0.4 a	3.3 ± 0.6 bc	4.7 ± 0.1 ab	5.7 ± 0.2 a	2.4 ± 0.3 c
gonads	mg kg^{-1}	32.4 ± 2.7 a	36.8 ± 2.4 a	12.2 ± 0.3 b	3.2	2.9	2.0 ± 0.1 c
Carotenoid Profile (%)							
dorsal skin	β-carotene	73.7 ± 2.8	72.4 ± 1.6	67.1 ± 3.8	72.3 ± 1.3	68.6 ± 3.7	65.1 ± 1.6
	Ax esters	24.0 ± 2.6	25.5 ± 1.7	30.7 ± 3.9	25.3 ± 1.4	29.4 ± 3.9	27.7 ± 1.0
	Cx	0.5 ± 0.0 a	1.1 ± 0.1 a	1.7 ± 0.4 a	0.7 ± 0.2 a	0.9 ± 0.2 a	5.7 ± 1.1 b
	Ax	0.5 ± 0.2	0.5 ± 0.0	0.3 ± 0.1	0.7 ± 0.2	0.8 ± 0.3	0.5 ± 0.1
	unidentified	1.3 ± 0.3	0.6 ± 0.1	0.2 ± 0.0	1.0 ± 0.4	0.3 ± 0.2	1.0 ± 0.2
ventral skin	β-carotene	67.4 ± 3.9	71.3 ± 2.5	69.9 ± 1.6	64.4 ± 2.3	63.3 ± 7.1	63.1 ± 3.0
	Ax esters	28.1 ± 4.1	25.3 ± 2.6	27.7 ± 1.5	30.4 ± 2.7	32.7 ± 7.9	31.9 ± 3.5
	Cx	1.1 ± 0.2 ab	1.2 ± 0.2 ab	1.4 ± 0.1 ab	0.7 ± 0.1 a	1.3 ± 0.2 ab	*2.7 ± 1.2* b
	Ax	1.0 ± 0.3	0.9 ± 0.1	0.9 ± 0.1	1.6 ± 0.4	2.1 ± 0.9	0.8 ± 0.1
	unidentified	2.5 ± 0.4	1.3 ± 0.4	0.2 ± 0.0	2.9 ± 0.8	0.8 ± 0.6	1.5 ± 0.4
flesh	Ax	61.4 ± 1.0 a	59.3 ± 1.1 a	50.0 ± 1.0 b	61.5 ± 0.7 a	61.5 ± 1.3 a	40.4 ± 2.0 c
	Cx	38.6 ± 1.0 a	40.7 ± 1.1 a	50.0 ± 1.0 b	38.5 ± 0.7 a	38.5 ± 1.3 a	59.6 ± 2.0 c
gonad	β-carotene	1.9 ± 0.3 a	1.8 ± 0.3 a	5.9 ± 1.0 b	21.6	20.5	11.1 ± 2.4 c
	Ax esters	3.7 ± 0.8 a	4.3 ± 0.9 a	6.5 ± 1.0 a	49.4	44.5	40.5 ± 2.4 b
	Cx	53.3 ± 4.2 a	57.3 ± 2.1 a	43.8 ± 0.9 b	16.5	17.0	24.8 ± 1.5 c
	Ax	33.2 ± 3.6 a	29.2 ± 2.7 a	35.0 ± 0.6 a	11.6	16.2	21.4 ± 2.1 b
	unidentified	3.9 ± 1.2 a	3.7 ± 0.9 a	4.4 ± 1.4 a	0.9	1.8	2.1 ± 0.1 b

[a] Values are expressed as mean ± SE (n = 5 fish/sex/color group). Different letters indicate significant differences between experimental groups. Italics denote significant differences between body areas within each experimental group for each parameter (ANOVA, $P < 0.05$). Note: Testes of silver and intermediary male were pooled per color group and could not be statistically analyzed. Ax, astaxanthin; Cx, canthaxanthin, D, dorsal skin; V, ventral skin.

significantly more concentrated in nuptial fish both dorsally (+2.6 μg cm^{-2}, +85.2%) and ventrally (+1.6 μg cm^{-2}, +89.6%). A similar trend was observed in females, but their higher carotenoid content at the nuptial stage was not significant dorsally (+1.4 μg cm^{-2}, +43.0%) or ventrally (+0.4 μg cm^{-2}, +19.2%). At this life stage, males accumulated significantly more carotenoids than females ventrally (+1.1 μg cm^{-2}, +53.3%) but not dorsally (+1.0 μg cm^{-2}, +20.6%). The prime carotenoid compound in the integument was β-carotene (69.9 ± 1.4%) followed by a number of uncharacterized Ax esters (27.1 ± 1.1%), whereas Ax and Cx accounted for a reduced proportion of the carotenoid pool. Carotenoid profiles were consistent among experimental cohorts with the exception of Cx present at a significantly higher level in nuptial male dorsally (5.7 ± 1.1%) when compared to any other cohort (1.0 ± 0.2%) and ventrally (2.7 ± 1.2%) when compared to silver males (0.7 ± 0.1%). The proportion of Cx in nuptial males was also significantly higher in their dorsal than ventral area (**Table 3**) and in their red marks (19.7 ± 0.8%; data not shown).

Flesh and gonad carotenoids were also analyzed. Flesh carotenoid concentration was significantly lower in nuptial than in silver and intermediary fish (male, −2.8 mg kg^{-1}, −54.6%; female, −2.7 mg kg^{-1}, −44.9%). This was concomitant with an altered carotenoid profile in the form of a reduced Ax and increased Cx level in nuptial fish (**Table 3**). Carotenoid concentrations were significantly reduced in mature gonads but, having a developed GSI at this stage, total gonad carotenoid content was 20.7-fold higher in nuptial female (111.3 μg against 5.4 μg in silver and intermediary ovaries) and 27.2-fold higher in nuptial male (2.9 μg against 0.1 μg in silver and intermediary testes) (data not shown). The prime ovarian carotenoid compound was always Cx followed by Ax with, at the mature stage, a reduction in the proportion of Cx and an increased level of β-carotene. Significantly different, the testicular carotenoid pool was dominated by Ax esters with a higher proportion of β-carotene and lower levels of Ax and Cx.

Table 4. Tristimulus Color Composition of Silver and Intermediary Phenotype in the Dorsal and Ventral Skin Areas before Post-mortem Treatment (T0)[a]

	dorsal skin		ventral skin	
	silver	intermediary	silver	intermediary
L^*	29.5 ± 0.8 a	36.7 ± 0.7 b	84.4 ± 0.6	83.3 ± 0.6
a^*	−0.5 ± 0.1	−0.4 ± 0.1	3.0 ± 0.3	3.2 ± 0.3
b^*	−0.5 ± 0.3 a	6.9 ± 0.6 b	6.4 ± 0.4 z	8.3 ± 0.5 y

[a] Values are expressed as mean ± SE (n = 30 sample/color group from 5 fish/color group). Different letters indicate significant differences between color groups; analyzed separately for each body area (ANOVA, $P < 0.05$). L^*, lightness; a^*, red-greeness; b^*, yellow-blueness.

Post-mortem Skin Color Reconditioning. Within each skin color category and body area, there were no significant differences in tristimulus $L^*a^*b^*$ parameters between on-coming treatment (T0). Data sets were pooled in accordance to establish that, before treatment application, intermediary fish had a significantly higher b^* value in both body areas and a higher L^* value dorsally than silver fish (**Table 4**). Color parameters of the ventral skin varied in the same fashion regardless of the treatment and skin color group with a systematic reduction in both a^* and b^* values observed from T0 to T1. The drop in b^* value was particularly marked in intermediary fish such that no differences between cohorts remained from T1 onward in the ventral area (**Figure 4a−c**). All ice treatments had the same effects on $L^*a^*b^*$ parameters of the dorsal area, and they were pooled before analysis (**Figure 4d−f**). In direct ice contact, intermediary fish underwent

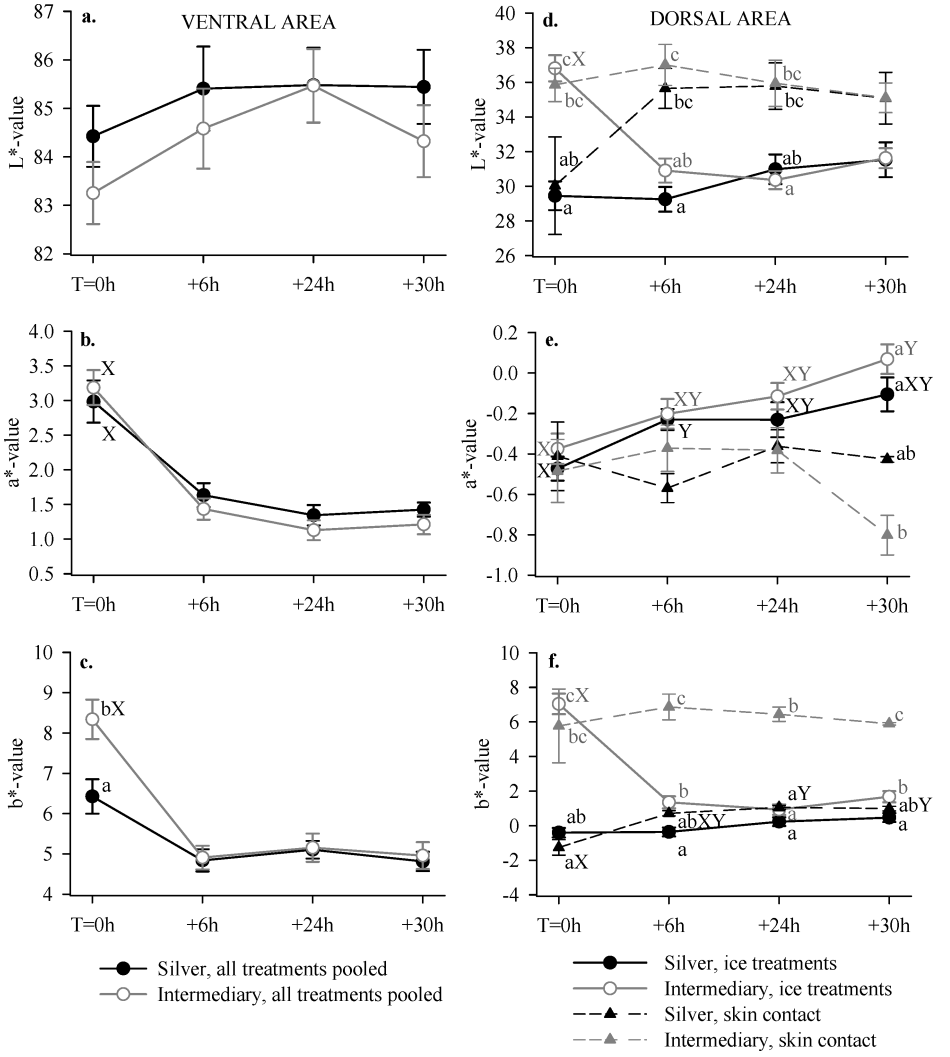

Figure 4. Tristimulus color parameters (L^*, lightness; a^*, red-greeness; b^*, yellow-blueness) of silver and intermediary fish in the ventral and dorsal areas before treatment ($T = 0$ h) and the following 6 h (T1), 24 h (T2), and 30 h (T3) of treatment (ice treatments: freshwater (FW) ; FW + 0.25 M Na^+; FW + 0.5 M Na^+; FW + 0.75 M Na^+; FW + 5 mM caffeine and direct contact with skin). Values are expressed as mean ± SE ($n = 5$ samples/treatment/color group except direct skin contact, where $n = 4$ samples/color group. Different lower case letters indicate significant differences between skin color group within each month. Different capital letters indicate significant differences between sampling points within each treatment and color group (ANOVA, $P < 0.05$).

a significant decrease in L^* parameter from T0 to T1 to values measured in silver fish. This parameter was thereafter stable and never different between color groups. Similarly, the b^* value of intermediary but not silver fish decreased significantly from T0 to T1 but remained significantly higher than in silver fish at T1 and T3. Finally, both color categories underwent a significant and parallel increase in a^* value. Skin contact had a distinct effect on skin color composition. In particular, L^* and b^* values of intermediary fish remained unchanged, but the L^* parameter of silver fish increased from T0 to T1 to values measured in the intermediary cohort. This parameter did not differ between color classes but between treatments at T1 and T2. The b^* value of silver fish in contact with skin increased from T0 to T3 but always remained significantly lower than in intermediary fish under the same conditions and never different from both color categories exposed to ice contact. Finally, the a^* parameter, which did not vary between color groups at T0, was significantly lower at T3 in intermediary fish in contact with skin than in both color classes stored on ice.

DISCUSSION

Significant skin color variations occur in immature Atlantic salmon that do not reflect deteriorations in flesh quality and can be minimized by direct ice contact. The seasonal occurrence and flesh quality of the three observed phenotypes (silver, intermediary, and nuptial) are first discussed in relation to sexual maturation. Their pigmentation origin is then described with emphasis on carotenoid dynamics toward the expression of a sexually dichromatic nuptial color and on the involvement of purine compounds. Finally, the possibility to improve at the postharvest stage the appearance of immature Atlantic salmon exhibiting a distinctive nonsilver phenotype is discussed.

Relationship between Phenotypes, Sexual Development, and Flesh Quality. Initiation of gonadal development was observed in intermediary colored fish in July followed by the occurrence of nuptial fish at an advanced stage of sexual maturation from August onward. This is in agreement with the well reported window of sexual maturation in Atlantic salmon (*15*, *24*) and suggests that intermediary coloration in July represented the initiation of nuptial display for a majority of the stock exhibiting this phenotype. Their higher body size, flesh lipid, and also carotenoid content could thus be expected as recruitment into maturation is inherent to a feeding surge defining the so-called anabolic window of Atlantic salmon maturation that is reported in late spring in Scotland (*10*, *25*, *26*). Furthermore, in a previous study, total flesh

carotenoid positively correlated with body size and dietary lipid level (*27*). Apparently contradictory, it is of interest to report a reduced flesh color score in July intermediary fish characterized by a higher flesh carotenoid and lipid content when compared to silver-immature fish. Flesh lipids, particularly in the form of intermuscular fat, were previously shown to negatively affect the perceived color of salmonid fillet (*28*). Accordingly in this study, the higher flesh lipid content of July intermediary fish, although not significant, was likely responsible for their lower visual flesh redness despite a higher flesh carotenoid concentration. Flesh color score was the only parameter negatively affected in July intermediary fish (−2.4 points at 24.1 points), but it remained above the commercially acceptable standard of 16 reported when using the Roche color card scale (*29*). Fish exhibiting an intermediary skin coloration were also observed from August to December, that is, over the species reproductive window, at a prevalence rate ranging from 10.1 to 19.4%. During this period it correlated poorly with sexual recruitment, which occurred only in a few intermediary females (≤17%) in August and September and in both silver and intermediary females in November and December. Flesh quality showed no discrepancies between silver and intermediary cohorts from August to December. Overall, intermediary skin coloration was never associated with poor flesh quality and constituted a good indicator of recruitment into sexual maturation in July only, that is, not over the full duration of the species reproductive window. This was in contrast to nuptial coloration, which was always associated with advanced sexual maturation and flesh quality deterioration as reported in previous salmonid studies (*10*, *24*).

Carotenoid Dynamic during Sexual Maturation. Sexual maturation in salmonids is concomitant with a redistribution of the flesh carotenoid pool to both the skin and gonads (*6*−*8*). In this study, total skin carotenoid increased in the nuptial compared to silver phenotype by an average of 89 and 29% in the male and female, respectively. It appeared sexually dimorphic at the nuptial stage with a significant carotenoid excess in males ventrally (+53%) but not dorsally (+21%) in this study. No such sexual dichromatism has been previously described in Atlantic salmon, unlike in other salmonids. At the nuptial stage and compared to females, male skin carotenoid levels were 91% higher in rainbow trout (*Oncorhynchus mykiss* Walbaum (*6*)) and 42% higher in wild chum salmon (*Oncorhynchus keta* Walbaum (*30*)). Similar dimorphism was also observed in Artic charr (*Salvelinus alpines* L. (*31*)), where 17β-estradiol implants significantly increased skin carotenoid concentration. Skin carotenoid profiles were largely constant in all experimental groups being dominated by β-carotene, a yellow-orange metabolite of Cx (*32*), followed by a variety of Ax esters. It is noteworthy that the proportion of orange Cx was significantly higher on the back of nuptial males and preferentially accumulated in their red marks. Of note, the proportion of Cx in the flesh also increased at the nuptial stage and to a higher extent in males. Similarly in gonads, Cx was at a higher proportion in mature than in immature testes (+51%) but inversely reduced in ripe compared to immature ovaries, where it remained the principal carotenoid compound. Gonad carotenoids have received much less attention in the male than in the female. They are present at a much lower concentration in testes than in ovaries at all developmental stages, but total carotenoid content nonetheless increased 27-fold in testes and 21-fold in ovaries when mature.

Carotenoids have an array of beneficial properties, including acting as antioxidants, vitamin A precursors, and free radical scavengers against peroxide chain reactions of fatty acids and UV-light-induced photooxidation (*3*). They were previously shown to be a critical factor of sperm quality (*33*) as shown in male three-spine stickleback (*Gasterosteus aculeatus* L.), where functional fertility and testes antioxidant capacity significantly increased when fed a higher carotenoid level (*34*).

The present results show that the dynamics of carotenoid redistribution and biotransformation during Atlantic salmon maturation is to a degree sex-specific, which is likely due to differential sex-steroid hormone profiles at this stage. This could serve the development of gonad-specific carotenoid profiles according to gamete needs and the expression of sexual dichromatism in nuptial Atlantic salmon. In particular, our data highlight a specific role of Cx in male signaling, which could reflect milt quality toward mate selection.

Role of Purine Pigments in the Expression of Colors. Purine and melanin are key pigments in the expression of teleost color but remain poorly characterized in adult Atlantic salmon skin. Purine concentrations were reduced by about 50% in both the dorsal and ventral regions of nuptial fish compared to their silver-immature siblings. This was expected from the visible loss of silver-coating in nuptial fish and previous work showing that skin purine concentration increases and correlates with skin reflectance during salmonid smoltification (*12*, *35*). Only G and H were identified as previously reported in immature Atlantic salmon using chromatography and spectrophotometry (*11*). These are also the main purine compounds in other salmonids and teleosts (*35*−*37*). The reduced purine level in nuptial compared to immature integument was concomitant with a lower G/H ratio as found in parr compared to smolt, where similar ratios are reported (*11*). Although not assessed in this study, the distribution of purine pigments within the different layers of the integument is also known to vary with life stage in teleosts. In European eel, *Anguilla anguilla*, the transition from yellow to silver phenotype during the spawning migration arises solely from a redistribution of purines from the inner skin to the outer scale layer of the integument (*38*). Similarly in smolting Atlantic salmon, the parr becomes a silvery-parr from the accumulation of purine in the inner skin layer and then a silver smolt from purine deposition in the outer scale layer. The decrease in purine pigment measured in this study in nuptial salmon is likely to occur preferentially in the outer scale layer toward an apparent absence of silver material on the back and a creamy-yellow belly area. This would also further expose dermal melanophores, which are located directly below the purine-based reflecting cells resulting in a darkening of nuptial salmon (*24*) without an actual increase in melanin content as measured here. Reduced purine levels would equally reveal the carotenoid-filled chromatophores present in the dermis (*3*) and dominated by the yellow-orange β-carotene. This would favor the expression of the carotenoid-based nuptial cover but also explain the higher skin yellowness (b^*) measured in intermediary fish characterized by a reduced purine level (−31%) when compared to the silver phenotype.

Reconditioning of the Intermediary Phenotype. Deviation from the desirable silver phenotype was confirmed instrumentally and related to altered pigment concentrations. Although this phenotype did not reflect altered flesh quality, it is likely to cause product rejection, hence the desire to recondition it. The intermediary coloration remained when in contact with skin but not when stored on ice, which induced a strong decrease in both skin lightness (L^*) and yellowness (b^*) to values measured in the silver phenotype. By contrast in silver fish, those color parameters remained constant when stored on ice, whereas skin lightness but not yellowness increased when in contact with skin. Such rapid color changes arose from the migration of pigment vesicles within their chromatosomes, mainly melanophores, which are recognized as the prime factor of physiological color change and related alterations in teleost skin lightness (*2*, *39*). They reflect

variations in purine pigment concentrations and melanosome physiological state (aggregated or dispersed) in relation to the exposition of carotenoid compounds as previously addressed. Skin contacts would induce melanosome aggregation, increasing skin lightness in silver but not in intermediary fish, where melanosomes were initially aggregated. Conversely, ice contact induced melanosome dispersion and darkening of intermediary fish only, highlighting that melanosomes were initially dispersed in silver fish. Color differences between phenotypes are concealed by melanosome dispersion and revealed by melanosome aggregation (brought about by skin contact) when silver fish maintain their blueness due to the light-scattering effect of the overlaying purine structures. Storage condition had a significant impact on the external appearance of whole Atlantic salmon. Direct ice contact did not alter the desirable silver phenotype but significantly enhanced the appearance of initially pale-green intermediary fish through melanophore dispersion. Greatly improved, intermediary fish were nonetheless dull-dark dorsally compared to a metallic dark-blue in the silver phenotype from differences in purine levels.

This study improves understanding of the expression of skin color in Atlantic salmon. Results show that immature Atlantic salmon can exhibit significant variations in skin coloration that do not necessarily reflect alterations in flesh quality. Deviations from the desirable silver phenotype originate from a lack of purine pigments, revealing the carotenoid compounds dominated by the yellow-orange β-carotene. The distinctive lightness and yellowness of these fish are exacerbated by aggregation of the melanophore induced by skin contact and conversely reduced by direct ice contact at a post-mortem stage. Maximizing ice contact of the final product at a postharvest stage has the potential to improve and standardize whole-fish external coloration. This would facilitate optimum biomass quality grading during primary processing, which is also based on external skin coloration, and also the satisfaction of the consumer, for whom coloration is an important quality indicator. Purine pigments were found at the lowest level in nuptial fish and can be considered as critical for revealing the sexually dimorphic nuptial cover. Further research is required to assess the significance of Cx in male nuptial cover in relation to gamete quality and to elucidate the environmental and physiological factors lowering purine levels in teleost skin. Skin reflectance related to purine level in adult Atlantic salmon could constitute a relevant welfare indicator related to desmoltification.

ABBREVIATIONS USED

Ax, astaxanthin; Cx, canthaxanthin; BW, whole body weight; FL, fork length; GW, gonad weight; K, Fulton condition factor; GSI, gonado-somatic index; SQC, Scottish quality cut; NIR, near-infrared reflectance spectroscopy; G, guanine; H, hypoxanthine.

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