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Review

Epigenetics—Potential for Programming Fish for Aquaculture?

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Abstract: Epigenetic marks affecting the expression of genes are triggered by environmental stimuli, can persist throughout life or across multiple generations and can affect an individuals phenotype. In recent years there has been a revival of interest about the possible role of epigenetics in affecting complex or quantitative traits. This growing interest is partly driven by the increasing affordability of ultra-high throughput sequencing methods for studying the epigenome. In this review we focus on some of the possible applications of epigenetic knowledge to the improvement of aquaculture. DNA methylation, in which a methyl group is added to the C5 carbon residue of a cytosine by DNA methyltransferase, has been the most widely studied epigenetic mechanism to date, and methods used to obtain and analyse genome-wide DNA methylation data are outlined. The influence of epigenetic processes on the estimation of breeding values and accuracy of genomic selection for genetic improvement of aquatic species is explored. The possibility of tightly controlling nutritional stimuli found to affect epigenetic processes in order to tailor the development of fish for aquaculture is also discussed. Complex experiments will be required in order to gain a better understanding of the role of epigenetics in affecting quantitative traits in fish.

Keywords: epigenetics; aquaculture; DNA methylation; high-throughput sequencing; quantitative genetics; nutritional programming

1. Introduction

Traditionally we have viewed the influence of a given gene on an animal's phenotype as a functional consequence of the order in which its nucleotide bases are arranged. However, over the past few years, data mainly from model organisms and a few non-model species have demonstrated the importance of additional genomic modifications that can affect an individual's phenotype. These modifications, generally referred to as "epigenetics" (Greek for "upon" genetics), might persist during mitosis and meiosis and can impact transcriptional regulation of a gene across various somatic cell types as well as germ cells. Epigenetic marks such as DNA methylation, histone modifications and chromatin conformational changes can be triggered by environmental stimuli and can persist throughout life or across multiple generations. However, since DNA methylation is the most widely investigated epigenetic mechanism and since the majority of the data and literature are associated with this particular epigenetic type, in this short review, we will mainly focus on the methylation impacts on gene expression and phenotype.

Methylation of DNA is a process in which a methyl group ($-CH_3$) is added to the carbon 5 position of a cytosine residue, mostly within a CpG dinucleotide, although the mechanism has also been reported outside of the CpG context [1]. Studies in mammals suggest that approximately 70%–80% of such dinucleotide sites remain methylated in non-embryonic cells [2]. Interestingly, the distribution of CpG dinucleotides seem to be underrepresented throughout the length of the genome, possibly due to high mutability of the methylated cytosine bases and much higher frequency at which the deamination of a methylated cytosine can create thymine [3–5]. This process has therefore been suggested as a major evolutionary force in shaping the vertebrate genome. On the other hand, dense repeats of CpG dinucleotides also exist within the genome, referred to as CpG islands. These islands are an important feature of the vertebrate genome, are usually found at the 5′ ends of almost all housekeeping genes as well as a number of tissue specific genes [6], are mainly associated with the promoter region [7], generally remain unmethylated throughout an individual's life, and as a result are less prone to spontaneous deamination [4,6].

The enzymatic activities of DNA methyltransferases (DNMTs), a large enzyme family which are evolutionary conserved in animals and plants (e.g., [8–10]), regulate methylation processes, either through *de novo* marking of a cytosine nucleotide or by the maintenance of existing methylation patterns. Through conformational changes to DNA and histone structures, the level of methylation at the promoter region is usually inversely correlated with the transcriptional activity of a gene [11]. Although CpG islands, particularly the ones that are in close proximity to promoters, are rarely methylated, methylation of these sites can significantly reduce or completely silence the expression of a gene by blocking the binding of transcription factors to DNA and recruiting methyl binding proteins [12,13]. However, the exact mechanisms of such processes are still not very well understood [12,14]. We also lack a sufficient understanding of the spatio-temporal alterations of a gene's methylation status, even in well-studied model species, although such mechanisms have been suggested as one of the key components affecting development, genomic imprinting, sex chromosome inactivation and transposable element silencing [15]. Although DNA methylation is generally considered as a stable genomic modification, particularly for imprinted genes, a number of investigations have revealed that genome-wide DNA demethylation, either

through active or passive processes, is also a common feature of some cells during certain developmental stages [16].

Recent findings from different studies have also started to converge on the view that the environment in which an individual experiences during early life stages, even prior to its birth, is an important determinant for its future health and metabolism. Through epigenetic marks, environmental cues can be transmitted to the genomic machinery of a cell and regulate patterns of gene transcription. Such exposures have been shown to mark their effects in a locus-specific manner throughout the length of the genome. For instance it was shown that the increased stress experienced by rat pups with reduced grooming and licking by their mother can affect the expression of the glucocorticoid receptor gene (GR), and that this is associated with differences in DNA methylation at GR promoter, which is thought to affect behavioural and hypothalamic–pituitary axis responses to stress later in life [17]. However, in addition to environmental factors, an individual's genotype also influences the methylation landscape and the transcriptional patterns of associated genes. Recent findings suggest that the genetic background of an organism may affect how it responds to particular environmental stimuli. For example it was shown that the methylome profile of neonatal humans is a function of the health of the uterine environment and its interaction with genetic variation (G × E) [18]. Teh *et al.* (2014) [18] showed that the variability of 75% of differentially methylated regions could best be explained by G × E interactions. Such findings reveal the importance of analysing DNA methylation along with genetic variation and gene expression through integrative approaches. Considering the heritable nature of many epigenetic marks and their impacts on gene expression, in future breeding plans we expect that it will not only become important to understand how an epigenetic mark might influence a phenotype through regulation of a target gene, but also how genetic variation within a gene might interact with a specific methylation site [19].

2. Obtaining and Analyzing DNA Methylation Data

Recent advances in genomic technologies and bioinformatics have made it possible to obtain genome-wide signatures of DNA methylation data, with a low cost per sequenced base, high yield and in a short time span. These types of studies can be done on a large number of samples, and depending on the method, can generate data with resolution in base pairs [20]. The methylation profile of a genome can be studied with microarray or high-throughput sequencing using methods such as DNA methylation enrichment and capture [14,21,22], bisulfite treatment [23] or methylation sensitive digestion [24,25]. Recent comparative studies suggest that all of these methods generate accurate methylation data [26,27], albeit with various resolution and sensitivity.

Below we will briefly introduce some of the most popular of these methods and some of their associated challenges. We also focus on high-throughput sequencing approaches, as for some important aquaculture species a draft sequence genome assembly is already available, and for many others we expect that such resources will become available in the near future. Using a sequencing approach for methylation studies is advantageous because such data can also be used for identifying variations including single-nucleotide polymorphisms (SNPs) and insertion/deletion at the genome level. High Performance Capillary Electrophoresis (e.g., [28]), 5-Methylcytosine Immunolocation (e.g., [29]) and Methylation-sensitive Amplified Polymorphism (e.g., [30]) are other potentially useful methods that could be considered for non-model species where fully sequenced genomes are unavailable.

2.1. Methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq)

MeDIP-Seq relies on the purification and enrichment of fragmented DNA using specific antibodies with high affinity against 5-methylcytosine [14]. DNA fragments are usually about 300–1000 bp in size and one of the main advantages of this method is that the constructed libraries, unlike restriction enzyme based methods, are not biased towards any specific sequence pattern, as the DNA fragmentation process is completely random. The fragments are treated with proteinase K and the purified fractions are then sequenced and aligned to the reference genome. The depth and the pattern of the read coverage can be used as a proxy to infer methylated regions of the genome. To obtain absolute methylation levels, Down *et al.* [21] developed an algorithm known as the Bayesian tool for methylation analysis (BATMAN), allowing more accurate assessment of the sequenced fragments and methylation profile. In human embryonic stem cells, this method was able to identify about 80% of CpGs with maximum resolution of 150 bp [27]. For a more comprehensive coverage, MeDIP-Seq can also be complemented by other approaches, e.g., by treating DNA with methyl-sensitive restriction enzymes such as HpaII, Hin6I and AciI (to obtain unmethylated CpG sites), followed by high-throughput sequencing of DNA libraries [31].

2.2. Methylated DNA Binding Domain Sequencing (MBD-Seq)

The MBD-Seq enrichment method is similar to the immunoprecipitation approach, as sequence fragments are enriched based on their methylation content. Unlike the immunoprecipitation methods, however, the fragment precipitation and enrichment is achieved using methyl binding proteins such as MBD2 [32], which have high affinity against methylcytosine. Compared to MeDIP-Seq, MBD-Seq results in a greater enrichment of DNA fragments with higher CpG density [27]. This discrepancy might in part be a function of salt concentration during elution of methyl-CpG containing DNA fragments [33].

2.3. Whole Genome Shotgun Bisulfite Sequencing (BS-Seq)

This method is regarded as the current gold standard for detecting methylated cytosine bases throughout the genome. It involves shotgun sequencing of DNA following treatment of DNA fragments with sodium bisulfite. Bisulfite treatment of the DNA sequences selectively converts the unmethylated cytosyine residues to uracils while leaving the vast majority of the methylated bases intact. Since DNA polymerase reads uracils as thymines, the unmethylated Cs appear as Ts in the sequence data. The methylation profile of the sequenced loci can then be inferred by aligning these reads to a reference genome and assessing the relative percentage of methylation at individual CpG sites. This is a powerful approach for investigating the methylome as it provides resolution at a single nucleotide level and has long been used for targeted analysis of candidate loci through either direct Sanger sequencing [34] or more recently using pyrosequencing [35]. However, sequencing the entire genome using a BS-Seq approach is still expensive, particularly for large sample sizes. Other limitations of this method include the conversion efficiency which can be incomplete and unstable [36] resulting in false methylation assignments, potentially high levels of DNA degradation [37–39] and an inability to distinguish between 5-methylcytosine and 5-hydroxymethylcytosine, a putatively important intermediate in the DNA demethylation pathway [40], in the absence of a complementary Tet-assisted bisulfite sequencing [41].

2.4. Reduced Representation Bisulfite Sequencing (RRBS)

Reduced representation bisulfite sequencing (RRBS) provides an alternative, cost-effective approach to BS-Seq. Still providing bp resolution, this method targets and enriches regions of the genome such as promoters, which have moderate to high levels of CpG [42]. RRBS relies on DNA digestion using a methylation-insensitive restriction enzyme such as MspI (C|CGG) followed by repair to the sticky ends of the double stranded digested DNA and addition of an A base to the ends of both strands. Sequencing adapters are then ligated to DNA fragments and desired sizes (40–250 bp) are separated using gel-electrophoresis, excised and purified. Purified DNA is subjected to bisulfite treatment, PCR amplification, purification and sequencing [42]. Compared to BS-Seq, RRBS's coverage of the genome-wide CpG sites is much lower (about 12%) except for the CpG islands [27]. However, both of these methods allow accurate estimation of genome-wide methylation at the nucleotide level.

2.5. Sequence Assessment and Analysis

Prior to sequence alignment, it is important to assess the quality of the reads and to identify and correct for any irregular pattern that might exist. For instance, there might be contamination from PCR primers, sequencing adapters and barcodes, which need to be either trimmed or filtered out from the sequence data. Low quality bases, which can mostly be found at the ends of the reads, should also be trimmed, or sometimes the entire read should be removed. FastQC [43] can be used to detect contamination, low quality scores or other types of biases. Trimming or filtering can be done using tools such as TRIMMOMATIC [44] or PRINSEQ [45].

In immunoprecipitation-based methods, the assessment of the methylation status of a site is generally deduced based on the mapping patterns of sequenced reads for any particular locus across different replicates and conditions. The mapping can be done using any short-read aligner such as BOWTIE [46] or BWA [47]. Immunoprecipitation-based methods are expected to yield higher mapping efficiency compared to sequence reads generated following bisulfite treatment, as nucleotides are not modified by the library preparation process. The number of mapped reads for any pre-defined regions of the genome such as gene, promoter, genomic bin, etc. is obtained and scored on a binary scale [27]. Although locus-specific coverage can be interpreted as methylation level, the pattern is heavily biased and dependent on the CpG content of that genomic region [21]. This is perhaps one of the main limitations in the enrichment-based methods, as methylation levels cannot be easily determined. To mitigate this bias and to normalize the data, algorithms such as BATMAN [21], MEDME [48] and MEDIPS [49,50] have been developed. The normalization algorithms can also be applied to other enrichment-based methods including MBD-Seq [20]. Regions with different coverage between the treatment groups can then be identified using tools like MEDIPS [50].

On the other hand through BS based methods, the frequencies of Cs and Ts at each position of bisulfite treated sequences can be used to obtain the level of DNA methylation for a particular site. However, nucleotide conversion poses a unique challenge for efficient mapping of such reads to the genome. Overall, there are two common approaches, implemented in different softwares, for handling bisulfite sequence alignment. Aligners such as BSMAP [51] and GSNAP [52] use the concept of a wildcard where they either change the alignment scoring matrix or convert all the genomic Cs to Ys, so they can

match all the Cs and Ts in the sequenced data. Alternatively, aligners like Bismark [53] and BS-Seeker [54] operate by replacing all the Cs in the sequence reads as well as both strands of the genomic DNA into Ts. In this way, these tools reduce the complexity of the sequence data and facilitate the mapping process. Due to incomplete bisulfite conversion, sequencing errors or sample heterogeneity, the methylation status of any nucleotide is generally reported as a percentage. Tools such as methylKit [55] can then use these percentages as well as the read coverage to normalized data across samples and to identify differentially methylated bases between different conditions.

By scanning the alignment sequence data, in addition to the methylated regions, one can also identify genomic variation such as single nucleotide changes (SNPs) and insertion/deletions (INDELS). There are a number of established variant calling tools including GATK [56] and FreeBayes [57], which are ideal for processing non-bisulfite reads. For bisulfite treated sequences on the other hand, the correct identification and distinction of C to T SNPs from those of the bisulfite conversion can be made using tools such as Bis-SNP [58].

3. Influence on Phenotypic Variance, Estimation of Breeding Value and Accuracy of Genomic Selection

This topic has been recently reviewed in relation to sheep and cattle genetic improvement [59], but because of the different biology and breeding systems of aquatic species, epigenetics could be of greater influence for aquaculture species, and could have greater ramifications for the estimation of breeding values and for the implementation of genomic selection. Therefore, we will elaborate on the topics discussed by [59] and add our thoughts in relation to genetic improvement for aquaculture.

The phenotypic variance of any trait is composed of heritable genetic effects and environmental effects. Heritable effects can be influenced by additive and non-additive (dominant, interactive and epigenetic) genetic components [60]. The causative variation affecting phenotype could be due to changes that result in structural modification of particular proteins (amino acid sequence, alternative splicing etc.) or due to the variations in the relative expression of genes affecting the level of production of transcripts or proteins in response to particular external or internal stimuli or at different stages of development and in different tissues, cell types, spatial locations in the body etc. Most traits of interest in aquaculture are quantitative by nature, that is the phenotypes of animals in a population show a typical Gaussian distribution which is thought to arise due to the additive effects of many genes and environmental effects. Non-heritable effects on phenotype are influenced by the environmental factors that can modify cellular processes such as gene expression, transcript splicing, half-life of enzymes and other proteins, etc.

To make genetic improvement we need accurate estimates of the additive genetic effect. This is the main component of the genetic variance causing the similarity observed between close relatives, and is the main component determining the response of the population to selection. The additive genetic effect is estimated from the degree of resemblance between close relatives, particularly the resemblance between sibs, or between parents and their offspring. Because epigenetic effects might also be vertically transmitted from parent to offspring and affect the resemblance between sibs (as is the case for the control of expression of the mouse agouti locus [61] which affects fur colour, weight and likelihood of developing cancer), epigenetics could in some cases affect the estimation of the additive effect

component. Because epigenetic programming can be inherited, and because epigenetic programming can be reversed, or wiped, in subsequent generations, the influence of epigenetics could therefore cause inaccuracies when estimating breeding values.

The level of epigenetic influence will depend on population structure, on the size of the epigenetic component relevant to additive genetic component and on the persistence of the epigenetic markings. If the epigenetic effects are stable over many generations then we could consider them as part of the true additive genetic effect.

For the typical situation in aquaculture where there are simple pedigree structures consisting of either large full- or half-sibling families, the potential for epigenetics to bias the estimation of the additive genetic component is greater than for more complex pedigree structures. It is conceivable that for some traits, all offspring will inherit epigenetic marks from their parents, so that some offspring groups that appear to have inherited superior additive genetic variation (additive genetic variation causing permanent and accumulative effects on the phenotype of subsequent generations), might actually have inherited temporary epigenetic marks that have a strong influence on the trait. The Gaussian type distribution of phenotypes for a trait in a population might also be caused or influenced by epigenetic effects. This is because the epigenetic effects (e.g., degree of DNA methylation and consequent effect on gene expression) are quantitative in nature [19]. Unlike a single gene mutation effect on a trait, the degree of methylation at a single locus could potentially have a quantitative effect on a trait and lead to a Gaussian distribution of trait phenotypes.

Epigenetics could play an important role in influencing disease resistance traits. A consideration of epigenetic effects might help explain the age-dependant development of some diseases (those not well explained by accumulated mutation) and the mechanisms by which the environment might modulate genetic predisposition to disease [19]. When the mother encounters disease, she builds immunity and transmits immunity to offspring so that they are better able to resist the disease. The environment encountered during early development is also likely to affect latter predisposition of adult fish to disease. These influences may be less important for some aquatic species where the adaptive immune system is less well developed. The diet of the mother could also play an important role, or nutrition of the developing fish could be important. In the case of aquatic species, the window for a maternal dietary influence is short (*i.e.*, during gamete development in the ovary or testis), but epigenetic marking could be occurring as the gamete matures or might be inherited by the gamete at the time it is formed, and this might affect predisposition to the disease. Bacteria are known to directly alter epigenetic marks and to alter the machinery for epigenetic regulation, and this could also negatively or positively affect the ability of the host, or its offspring, to resist disease [62].

It has been suggested that epigenetic modification could directly influence expression and genetic disease predisposition or that the epigenetic marking may interact with specific DNA-sequence variants so that it affects the penetrance of disease-causing variants [19]. This type of interaction has been demonstrated in the case of the "masking" or "unmasking" of a Hsp90 gene mutation in *Drosophila melanogaster* [63], and is also the way that imprinting affects the expression of genes originating from one parent in mammals [64]. If such mechanisms were involved in controlling disease resistance then the association between the disease phenotype and QTL controlling the trait would be "buffered" and dependent on the state of the epigenetic marking. Therefore, for some complex traits, measurement of the epigenome (e.g., using DNA methylation sequencing) could be especially informative.

Estimation of the Epigenomic Variance Component and Calculation of GenEomic EBVs

In cases where there is a significant component explained by epigenetic effects and the pedigree structure affects the estimation of true additive genetic effects (as is possible for fish pedigrees containing many full-sibling offspring), correction of genetic solutions accounting for epigenetic effects would result in a more accurate prediction of breeding value and therefore result in more rapid genetic gain. In these cases it will be necessary to estimate the "true" additive genetic component (V_{Atrue}), where,

$$V_{Atrue} = V_{Auncorrected} - (V_{EpiMeth} + V_{EpisRNA} \ldots..)$$

where $V_{Auncorrected}$ is the uncorrected additive genetic component estimate, $V_{EpiMeth}$ is the epigenetic component caused by methylation markings and $V_{EpisRNA}$ is the epigenetic component caused by small RNA molecules etc.

Future genomic selection could estimate and account for the effects of epigenetics by using the solutions for V_{Atrue} at every marker position across the genome (we will refer to such selection as "GenEomic Selection" here). The most efficient option for finding genome-wide epigenetic markings associated with phenotype (which ultimately would allow estimation of the epigenetic variance component) would be to utilise information from the same training families that are used for estimating genome-wide marker associations for the purpose of within-family genomic selection. Technologies for measuring epigenetic markings throughout the genome were discussed above. As well as following the inheritance of allelic variation (used for the estimation of the additive genetic component), experiments should be designed so that it is possible to search for differential methylation or other epigenetic marks between families and within families at loci spread over the whole genome. This could allow discrimination between the two main random effect components on the phenotype, one due to the additive genetic effects, and one due to the epigenetic effect components (*i.e.*, $y = \mu + V_A + V_{EpiMeth} + V_{EpiSrna}$ etc. $+V_R$). To discriminate between these effects it will be important to use genotype information from across the genome (genomic relationships) to estimate the actual genetic relatedness between full-sibs (*i.e.*, normally the relationship between full-sibs is assumed to be 0.5, which is the theoretical average relationship). Genes contributing to the additive genetic variance component should be inherited in a Mendelian fashion, and therefore, those individuals with a higher genomic relationship should have a more similar additive genetic component, whereas this will not hold for the epigenetic component.

The epigenetic component itself might consist of paternal and/or maternally inherited effects, sex specific effects on offspring and/or interactive effects (where the epigenome interacts with specific gene mutations). In some cases, the allele inherited from one parent might be silenced (imprinted) so that only the allele from the other parent is expressed. In order to be able to distinguish between these types of epigenetic effects the sex of all animals would need to be recorded and included as a fixed effect in the model for analysis and the changes to the epigenome would need to be mapped relative to QTL found to affect the trait.

It may be that the influence of epigenetics is small and that V_{Atrue} is almost the same as $V_{Auncorrected}$, or there may be a significant epigenetic component for some traits. The potential for epigenetics to influence quantitative traits, has been recently demonstrated in lines of Arabidopsis where several experimentally-induced DNA methylation changes have been shown to effectively act as epigenetic quantitative trait loci affecting flowering time and primary root length, accounting for 60%–90% of the heritability for the two traits [65]. Many of these inducible DNA-methylated regions were also found to

be variable in natural populations of Arabidopsis. For this review we were unable to find any attempt to measure the epigenetic variance component for traits in an aquaculture species, probably because techniques such as whole genome methylation sequencing are new and expensive to apply across many animals. We simply do not have good estimates of the extent of the epigenetic variance component for traits in aquaculture species and the importance of this component is therefore uncertain.

On-the-other-hand it would be much easier to identify epigenetic effects and to quantify this variance component, if we could reduce the noise caused by additive genetic effects [59]. Reduction of the additive genetic effects might be possible in fish by creating inbred experimental lines [66], or completely homozygous double haploid fish [67], so long as these fish can be bred with other inbred or completely homozygous double haploid fish to produce a new generation for tracing the inherited epigenetic effects. Because it is often easier and faster to create these inbred or double haploid lines for fish than it is for livestock or other experimental model organisms, fish could be useful model species for studying epigenetics and estimating the epigenetic variance components affecting certain traits. In addition, it could be possible to create epigenetic recombinant inbred lines of fish, and to use these animals to try to map epigenetic-based quantitative trait loci affecting particular traits, in much the same way as has been done to for Arabidopsis [65].

4. Nutrigenomics—Programming the Fish through Nutrition?

The more we understand about epigenetics and how it influences phenotype, the more accurately we may be able to program the development of the fish by tightly controlling various components of the environment (including the diet of the fish) at different stages of development. Nutrients and bioactive food components can influence epigenetic phenomena either by directly inhibiting enzymes that catalyze DNA methylation or histone modifications, or by altering the availability of substrates necessary for those enzymatic reactions [68], and this is known as nutritional programming. There have been few studies demonstrating nutritional programming in fish [69,70], but if this phenomena does occur in fish it might be possible to manipulate programming to improve performance in the aquaculture environment. As development occurs in the water column this would allow very simple and controlled manipulation of groups of larvae or fingerlings for programming. Nutrients in the diet or other environmental influences could have a direct effect fuelling development, or they could be changing the epigenome in such a way that particular genes are more highly or lowly expressed after exposure, with some changes to the epigenome being inherited by subsequent sons and/or daughters through paternal and/or maternal lines.

Production efficiency and fish health have large effects on the profitability of aquaculture, and it would therefore be highly desirable to be able to control the development of these traits using nutrigenomic programming. Variation in fillet quality is also important, in particular soft texture and deviating appearance (colour and deformities) are quality problems that cause negative market responses. Fish with shortening of the body due to fusion of the vertebrae cause problems during machine filleting and are considered as second grade fish by the processing industry. Additionally, fish deformities have a detrimental effect on the consumers' image of aquaculture and, therefore, also on the market value of the juvenile fish [71]. Research into the factors influencing performance, health and flesh quality have identified important contributing factors, but may have overlooked major root

causes of the problems, such as the possibility of epigenetic effects controlling cellular differentiation and development.

DNA methylation and other epigenetic controls of gene expression are known to be important in affecting cellular differentiation during development [72]. There are a number of nutritional stimuli that are known to affect the differentiation of fish cells during development and possibly do so by changing the epigenetic markings in particular cells. A lack of vitamins (A, D, C) in sea bass has been shown to disrupt the temporal sequence and coordination of growth factor gene expression which affects the differentiation of osteoblasts so that some are converted into adipocytes resulting in deformities [73]. The conversion of osteoblasts into adipocytes can also occur when dietary highly unsaturated fatty acid (HUFA) levels are too high during the early stages of the development of marine fish [71]. Salmon pre-adipocytes seem to possess the ability to differentiate into cells of the immune lineage (most probably macrophage-like cells) depending on environmental conditions [74].However, epigenetic cues affecting cellular differentiation during the development of fish have not yet been investigated.

Growth of skeletal muscle in fish is a plastic process that involves a combination of enlargement of already existing muscle fibres (hypertrophy) and the recruitment of new fibres (hyperplasia). The balance between these mechanisms determines the fiber density, which in turn affects muscle firmness and robustness to handling and processing [75,76]. The hypertrophy–hyperplasia balance depends on strain genetic differences and extrinsic factors such as early nutritional history [77]. The cellular development of fish muscle in general appears to be strongly influenced by environmental conditions faced by the egg, larvae and fry during development [78]. For instance, temperature during embryonic stages is known to affect muscle fibre recruitment in zebra fish (*Danio rerio*) and Atlantic cod (*Gadus morhua*) [79,80] and is also known to affect the development of deformities, deviant heart development and the expression of stress related genes in Atlantic salmon [81–83]. Although fry nutrition is assumed to be important, there are relatively few studies following the effect of fry nutrition on meat quality or health at harvest [84]. On the other hand, most research on optimising fillet quality has focused on the impact of dietary composition and feeding practices for larger fish. The expression of a number of genes in the adult salmon fillet has been correlated with fillet softness [85], but we know little about the expression of these genes during early development stages, what factors affect their expression and the precise role, if any, that these genes may play in determining fillet quality.

There are emerging findings that suggest that fatty acids, in particular polyunsaturated fatty acids (*n*-3 HUFA's), can modify the epigenome [86]. The *n*-3 HUFA's are important as precursors of eicosanoids, which are particularly essential for the development and growth of fish [87], for providing metabolic energy to the fish in the form of ATP [88] and for the formation of membrane structure [87]. Changes to the dietary lipid source of female salmon during gonadal development affect egg fatty acid composition, and as a consequence, can result in abnormal embryonic development [89]. Supplementation of broodstock diets with higher proportions of fish oil has been shown to influence survival rate of 3 day post-hatching larvae, egg diameter, length of fry, oil globule diameter of hatchlings, egg protein content, lipid content and the morphology and metabolism of adipocytes in sea bream [90,91]. Research in humans has shown that perinatal manipulation of alpha-linolenic acid uptake induces epigenetic changes in the liver of offspring [92] and that some fatty acids act as hormones and control the activity of transcription factors [93]. With the production of salmon for harvesting at 3–5 kg, fish have been shown to develop typical life-style related symptoms, such as arteriosclerosis, abnormal

fat deposition in liver and heart which results in impaired organ function and various circulation problems [94,95]. Health problems such as these have been recently documented to affect flesh softness and ability to cope with stress of handling [96,97]. Early life exposures can, through epigenetic programming, determine the response to nutritional signals in later life [98], and high-fat exposure during early life phases could therefore program the fish and lead to a higher incidence of obesity, and related symptoms, in response to adult diets and decreased resilience to pathogen exposure and other stresses.

Therefore, the nutrition and health of the parents, egg, larvae and/or fry, and consequent programming prior to conception in the parents or during early development of the individual itself, could influence the type, number and distribution of osteoblast, adipocyte and myocyte precursor cells, gene expression and subsequent development of muscle and meat quality [77], but this needs to be further explored.

5. Key Challenges and Knowledge Gaps

Understanding the epigenome and the changes affecting particular phenotypes will involve complex experiments using family material, like the experiments needed for finding quantitative trait loci. The cost of measuring epigenetic changes across the genome is becoming cheaper, but there are additional experimental costs and considerations to be made when studying the epigenome. For instance, at what stage(s) of development should the epigenome be assessed? To see if the effects are inherited it would be necessary to study the epigenome of mature parents and offspring at an early stage of development (egg or larvae). In some cases it may be necessary to follow epigenetic markings as the fish develops which would also necessitate repeated testing of fish.

There is currently very little knowledge about the genes that are programmed by nutritional treatments in early life stages (e.g., fry) and of epigenetic processes in early life stages affecting robustness and meat quality. Knowledge of these processes could drive changes in the formulation of new diets, could result in greater selection accuracy for the genetic improvement of important traits and could result in improved robustness and in greater consistency and quality at harvest. If the programming persists until maturation, it may result in heritable changes, and could affect the early stage development of subsequent generations. Histone methylation may be an epigenetic regulator in some molluscs [99]. We therefore need to determine whether programming persists until harvest and how programming is regulated in fish and shellfish.

We need to be able to discriminate between true additive effects and epigenetic effects to give greater accuracy for selective breeding to make genetic improvement. We also need to develop biomarkers for early life stages that can enable us to predict negative consequences (phenotypes) that only become apparent later in life. Epigenetic marks in the genome could be useful biomarkers for this purpose. Regular biomarker testing could provide an alert about variation in nutritional or environmental factors affecting fry and subsequent meat quality in the adult fish. Active surveillance of this type is commonly used to warn about the spread of agricultural diseases. If nutritional driven epigenetic effects are important, this type of surveillance could also help ensure consistent quality of aquaculture products from year to year, which in turn would have large effects on the industry in terms of reduced wastage and reduced production of low quality grade product, improved confidence of consumers and export markets for salmon products and higher profitability for producers, processors and exporters.

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Conflicts of interest

The authors declare no conflict of interest

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