Title: Epigenetic considerations in aquaculture

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Key Words: epigenetics, DNA methylation, aquaculture, shellfish

Abstract:
Epigenetics has garnered considerable attention in many aspects of agricultural production, particularly where environmental conditions can be manipulated or natural variation exists. In the past decade, technology and our fundamental knowledge of transcriptional regulation has dramatically increased to where we are now able to characterize numerous aspects of epigenetic variation in aquaculture species. This review will introduce key concepts and definitions of epigenetic mechanisms including DNA methylation, histone modifications and non-coding RNA, review the current understanding of epigenetics in both fish and shellfish and propose key areas of aquaculture where epigenetic knowledge could be applied. Based on aspects of life history and husbandry practices in aquaculture species, application of epigenetic knowledge could have a significant impact on productivity and sustainability of aquaculture practices. Conversely, there is the possibility that elucidating the role of epigenetic mechanisms in aquaculture species may upend traditional assumptions about selection practice. Ultimately, there are still many unanswered questions regarding how epigenetic mechanisms might be leveraged in aquaculture.

Introduction

Maintaining and improving aquaculture production requires an understanding of genetic and physiological mechanisms that control desired traits. An understanding of these mechanisms has led to the development of pioneering biotechnological methods that have important applications. For example, molecular markers are used in broodstock selection and transcriptomic studies have been used to improve environmental conditions to decrease physiological stress in animals. Recently, interest in epigenetics has surged within the agricultural community as it becomes more clear that epigenetic mechanisms can provide a measurable link between environment and phenotype.

Epigenetics refers to heritable processes that alter gene activity without manipulating the underlying DNA sequence (Jablonka & Lamb 2002). Epigenetic mechanisms (or ‘marks’), including DNA methylation, histone modifications and non-coding RNA activity, influence gene expression primarily through local modification of chromatin. Unlike DNA, epigenetic marks can be directly influenced by the environment, and because of this, have been shown to be important mediators of phenotypic responses to environmental signals (Figure 1). For example, in mammals, nutrition (Weaver *et al.* 2004), exposure to toxins (Dolinoy *et al.* 2006), and photoperiod (Azzi *et al.* 2014) have all been associated with changes in DNA methylation and concomitant changes in phenotype. Fish, while less studied, show similar environmental sensitivity in DNA methylation patterns (Wang *et al.* 2009, Strömqvist *et al.* 2010, Campos *et al.* 2013). Many environmentally-induced epigenetic changes are transient, while some may persist over the course of an organism's lifetime (Weaver *et al.* 2004, Dolinoy *et al.* 2006, Heijmans *et al.* 2008). In certain cases, epigenetic changes can be transgenerationally inherited (Guerrero-Bosagna *et al.* 2010, Manikkam *et al.* 2012). As such, it is important to understand the nature and function of these mechanisms and their influence on phenotype in fish and shellfish.

Interest in epigenetics has been gaining ground in agricultural science for crops (Ong-Abdullah et al. 2015, Álvarez-Venegas & De-la-Peña 2016) and, more recently, livestock (Goddard & Whitelaw 2014,González-Recio 2015), but less is known about epigenetic mechanisms in economically valuable aquaculture species. Considering that a majority of aquaculture takes place in open or natural systems subject to environmental change, it is important to consider the role of epigenetics. This is particularly the case now that tools and resources are available to study these important molecular mechanisms. In recent years, studies in species ranging from salmonids to sea bass to oysters and mussels, are providing the first evidence that epigenetics is associated with commercially important traits in aquaculture species. In sea bass and half smooth tongue sole, temperature induced sex-determination has been associated with changes in DNA methylation (Navarro-Martin 2011, Shao et al 2014). In salmonids there is some evidence that changes in DNA methylation are associated with variation in life history phenotypes including early male maturation (Morán *et al.* 2011), smoltification (Morán *et al.* 2013), and anadromy (Baerwald *et al.* 2016). Recent studies in European sea bass and rainbow trout examined the role of epigenetics in mediating phenotypic responses to various aspects of diet (Terova et al 2016, Marandel et al 2016, Panserat et al 2017). In Pacific oysters, the role of epigenetics in mediating effects of temperature on oyster physiology has been investigated (Fellous et al. 2015). As is described below, the relationship between epigenetics and phenotype is less clear in shellfish as opposed to finfish.

This mini-review will introduce key concepts and definitions of epigenetic mechanisms, briefly review the literature as it pertains to the nascent field of epigenetics in aquatic species and highlight key aspects of aquaculture that would benefit from a deeper understanding of the role of epigenetics. There have been excellent reviews published recently regarding epigenetics, primarily DNA methylation, and various aspects of finfish aquaculture (e.g. Li & Leatherland 2013, Moghadam 2016) and those will be highlighted where appropriate.

What is epigenetics?

The following section will briefly describe specific epigenetic marks and review where we stand in terms of understanding (or lack of understanding) the relationship between epigenetics, environment and phenotypes in aquaculture species.

DNA methylation

DNA methylation refers to the enzymatic addition of a methyl group to a cytosine residue in DNA, which occurs almost exclusively at CpG dinucleotides (i.e. a cytosine located 5' of a guanine) in animals. The enzymatic machinery supporting DNA methylation includes a family of DNA methyltransferases (DNMTs) including the maintenance methyltransferase DNMT1 (responsible for copying pre-existing DNA methylation patterns to the new strand during mitosis) and the *de novo* methyltransferases DNMT3A/3B. DNA methylation is known to be repressive when located in promoters of genes through associations with other DNA binding proteins or through physical blocking of transcription factors (Bell & Felsenfeld 2000), however DNA methylation located in gene bodies is associated with high levels of expression (Jones 1999). Therefore, although typically associated with silencing, the regulatory role of DNA methylation is specific to the genomic context. In mammals, DNA methylation plays important roles in providing genomic stability through repression of transposable elements (Maloisel & Rossignol), genomic imprinting (Bell & Felsenfeld 2000), and dosage compensation (Csankovszki et al. 2001). DNA methylation is also important for cell-type differentiation and embryonic development (Li et al. 1992). DNA methylation is the most well studied epigenetic mechanism, where a majority of studies have been done in plants and mammals. For instance, in these systems it has been shown DNA methylation is sensitive to external factors including nutrition (Weaver *et al.* 2004), exposure to toxins (Dolinoy *et al.* 2006), and photoperiod (Azzi *et al.* 2014). It is important to note that the meiotic transmission of DNA methylation patterns, thus the opportunity for transgenerational epigenetic inheritance through DNA methylation, is rare in mammals, which undergo extensive DNA methylation reprogramming in early embryos (Daxinger and Whitelaw 2012). As you will see in the following sections, it is unclear if and to what extent DNA methylation resetting occurs in fish and shellfish.

Histone Modifications

Chromatin is a dynamic structure that supports both the packaging of the genome into the nucleus, and importantly, the regulation of genes and other genomic regions via changes in DNA accessibility (Cheung et al. 2000). The basic repeating structure of chromatin is the nucleosome which consists of DNA wrapped around histone proteins. These histones can be post-translationally modified at their N-terminal tails altering the degree of which DNA can be wrapped around them resulting in either euchromatin (referring to open chromatin that is accessible to transcription factors, RNA polymerase II (Pol II) and other DNA binding proteins that support gene expression) or heterochromatin (referring to tightly packed DNA associated with transcriptional silencing). These states are dependent on the type (i.e. acetylation, methylation, phosphorylation, ubiquitylation) and location (e.g. various lysine or arginine residues) of the modification (see review by Lawrence et al 2016 for a complete list of modifications). These various modifications can exist in bivalent and multivalent states with each other and have led to the much debated existence of the 'histone code' (Jenuwein & Allis 2001). These modifications are enabled by various families of enzymes including histone acetylases (HATs), histone deacetylases (HDACs), histone methyltransferases (e.g. HMT) and histone demethylases (e.g. Jumonji and Lys-specific demethylase). Modifications are important for regulation of gene activity but also have roles in DNA repair, replication, and cell fate/determination (see reviews by Lawrence et al 2016, Martin and Zhang 2005, Eberharter and Becker 2002). The enzymatic machinery responsible for these modifications are highly regulated during embryonic development (Lin & Dent 2006), and like DNA methylation, can be altered by various environmental conditions (Chinnusamy & Zhu 2009). Less is known about the mitotic and meiotic persistence of histone modifications, but interestingly, it has been shown in both mammals and zebrafish that certain modified histones are non-randomly retained during spermatogenesis when the majority of these proteins are replaced with protamines, suggesting that these marks may have a role transferring epigenetic information to the embryo (Brykczynska et al. 2010, Wu et al. 2011).

Non-coding RNA

Although a large majority of the genome is transcribed, only a small portion of these transcripts code for protein. These non-coding transcripts, originally regarded as 'junk', are now recognized for their role in modulating gene expression and are categorized broadly as non-coding RNA (ncRNA). There are two major classes of ncRNA: long ncRNA (> 200 nt) and small ncRNA (< 200 nt), which includes micro RNA (miRNA), short interfering RNA (siRNA), and piwi-interacting RNA (piRNA). Small ncRNAs are highly conserved and their major mechanism of action is to inhibit protein synthesis by blocking or degrading primary transcripts (see review by Castel & Martienssen 2013). Long ncRNAs (lncRNA) by contrast are less conserved and have complex mechanisms of action that may work either in *cis* or *trans* (see review by Wang & Chang 2011). Non-coding RNAs have important functions in gene expression and have demonstrated to be important regulators of genome stability, environmental plasticity and embryonic development (Mercer et al. 2009, Bizuayehu et al. 2014). Generally speaking, ncRNA molecules are considered 'epigenetic' in the traditional sense because they interact with other epigenetic mechanisms such as DNA methylation and histone modifications to silence or activate various parts of the genome (Peschansky & Wahlestedt 2014).

Taxa specific Patterns

Epigenetic mechanisms and particularly DNA methylation have been the focus of numerous studies in both fish and shellfish in recent years. However, a majority of what we know about epigenetics in animals comes from studies done in mammals and care should be taken when generalizing functions from mammals to fish and shellfish. Although there are certainly similarities (e.g. DNA methylation patterns are very similar across all vertebrates) there are also important differences (e.g. invertebrate DNA methylation patterns are very different from vertebrates). This section will focus on foundational information about epigenetic marks in fish and shellfish and highlight both significant gaps in our understanding as well as noting differences from well-studied mammalian systems.

DNA methylation in fish and shellfish

DNA methylation is the most well-studied epigenetic mark among fish and shellfish. Both fish and shellfish have genes present that encode basic methylation machinery (e.g. DNMTs and MBDs) and DNA methylation is present in all species examined to date. However, there are striking differences in DNA methylation patterns between vertebrates and invertebrates as well as significant unknowns in terms of DNA methylation resetting in both fish and shellfish.

Considerable work has been done on understanding patterns and functions of DNA methylation in model fish species such as zebrafish and medaka, with increasing information on DNA methylation in non-model species. For example, a recent review by Metzger & Schulte (2016) extensively covers the current state of knowledge of DNA methylation patterns and functions in marine fish. Generally speaking, DNA methylation patterns are similar across all vertebrates which exhibit a 'global' DNA methylation pattern, meaning majority of CpGs are methylated with the exception of regions of DNA with high CpG content referred to as CpG islands. In comparison to mammals, however, global DNA methylation levels are higher in fish though the significance of this remains unclear (Jabbari 1997, Zhang et al. 2016). The function of DNA methylation also appears to be similar across vertebrates with the exception of a role in genomic imprinting which is unique to mammals (Potok et al. 2013). One outstanding question is regarding the extent of DNA methylation resetting in fish. While mammals undergo extensive DNA methylation reprogramming in the early embryo (Daxinger and Whitelaw 2012), it is unclear to what extent DNA methylation reprogramming occurs in fish (Potok *et al.* 2013, Jiang *et al.* 2013). A recent study, discussed in more detail in the following section, shows clear evidence of transgenerational inheritance of environmentally-induced DNA methylation patterns in a fish, suggesting at least some of the genome escapes putative resetting between generations (Shao et al. 2014). Certainly there is a need for more detailed studies on the extent of DNA methylation resetting in fish, particularly in aquacultured species. In addition, more studies should examine the potential meiotic inheritance of environmentally-induced epigenetic changes.

Invertebrate DNA methylation patterns are strikingly different from vertebrates. Whereas vertebrates exhibit a global pattern of DNA methylation, invertebrates show a 'mosaic' pattern with stretches of methylated DNA punctuating regions of unmethylated DNA (Tweedie et al. 1997, Simmen et al. 1999). DNA methylation was examined genome-wide in the Pacific oyster where it was reported that 15% of CpGs were methylated in a somatic tissue, whereas 60-70% of CpGs are methylated in mammals (Gavery and Roberts 2013). In oysters, as in other invertebrates, the methylated fraction tends to consist of gene bodies, while other genomic regions exhibit less methylation. Unlike vertebrate species, transposable elements show surprisingly little methylation in oysters and other invertebrate species (Simmen et al 1999, Feng et al. 2010, Zemach et al. 2010). Functionally, DNA methylation does appear to be associated with gene regulation in shellfish. In the Pacific oyster, high levels of methylation in gene bodies (and putative promoter regions) were associated with high levels of expression (Gavery & Roberts 2013; Olson & Roberts 2014). Interestingly, genes with limited methylation in oysters have variability in exon-specific expression across tissue types, indicating that hypomethylation allows increased plasticity (Gavery & Roberts 2013). While more studies are needed to quantify this relationship, there are significant implications for improving resilience in shellfish - particularly if DNA methylation patterns are heritable. There are few studies on heritability of DNA methylation patterns in shellfish, however, a small study looking at methylation states in parents and larvae found significant clustering of methylation patterns within families, indicating that methylation patterns differ significantly depending on the male parent (Olson and Roberts 2015). More recently, Rondon et al. (2017) have shown parental herbicide exposure influences progeny DNA methylation patterns in oysters.

Histone modifications in fish and shellfish

Histone modifications and their dynamics have been studied in zebrafish where evidence indicates modifications are conserved among vertebrates. Functional analysis of histone acetylation in zebrafish confirms its role in embryogenesis (Vastenhouw & Schier 2012) and in tissue regeneration (Stewart et al 2009). In terms of meiotic inheritance, zebrafish show multivalent modified histone retention in sperm similar to mammals (Wu et al. 2011). Studies examining histone modification in non-model fish are rare, though recent studies in rainbow trout and European sea bass indicate that diet influences bulk histone modification levels and can regulate the expression of associated enzymes (Marandel et al. 2016, Terova et al. 2016, Panserat et al. 2017).

Histone modifications are less studied in shellfish, but work by Fellous et al. (2014) identified homologs of Jumonji histone demethylase genes (Jmj) in Pacific oysters that, similar to vertebrates, were regulated during embryonic development. A subsequent study showed that both bulk histone methylation levels and the expression of histone demethylases were responsive to temperature during development, suggesting a role for histone modifications in mediating the physiological responses of oysters to temperature (Fellous et al., 2015). Although histone modifications have not been studied directly in sperm from bivalves, it is interesting to note that some bivalves, e.g. Pacific oysters, do not replace histones with canonical protamines (Sellos 1985). This leaves open the possibility that environmentally-induced modified histones may be passed on through the male germline in these species.

Non-coding RNA in fish and shellfish

A majority of studies on non-coding RNAs in fish and shellfish focused on miRNAs, including important aquaculture species (e.g. Atlantic salmon (Bekaert et al. 2013, Andreassen et al. 2013) and rainbow trout (Juanchich et al. 2016)). There are several examples of examining miRNAs in a physiological context including: maternal transcripts in the egg (e.g. Ma et al. 2012), immune function (e.g Andreassen et al. 2017) and embryonic development (Bizuayehu et al. 2015). There is less information about other types of small ncRNA, except in zebrafish where, for example, piRNA have been shown to silence transposable elements in gametes, functioning similarly as in mammals (Houwing et al 2007). Recently there have been several descriptions of long non-coding RNAs in salmonids including associations between lncRNA expression and disease in both Atlantic salmon and rainbow trout (Boltaña et al. 2016, Valenzuela-Miranda et al. 2016, Paneru et al. 2016).

Predicably, non-coding RNAs are less investigated in shellfish, though generally speaking, miRNAs and their biogenesis are highly conserved over evolutionary scales (Wheeler et al 2009). As such, genes for miRNA biogenesis have been detected in available bivalves genomes (Rosani et al. 2016). With respect to long non-coding RNAs, researchers have reported an association with larval development in the Pacific oyster (Yu et al. 2016).

Potential Aquaculture Applications

Environmental Manipulation

Given what we know about environmental influences on epigenetic mechanisms in fish and shellfish and the relationship with phenotype, one avenue where epigenetics and aquaculture could intersect is environmental manipulation. In addition to mechanisms described in the previous section, there is also the fundamental concept of “developmental programming”. Developmental programming suggests that environmental conditions experienced in early-life influences phenotypes later in life and has gained momentum in human research (e.g. Gluckman et al. 2008). In other words, developmental programming offers an environmental memory that could be beneficial in controlled aquaculture settings. However, in some cases embryos and juveniles are not raised in the same environmental conditions as the adults. For example, hatchery-reared salmon or hatchery-reared bivalve juveniles placed into a natural setting for grow out. Identifying sensitive periods for environmental memory could offer a “programming window” that could be leveraged in husbandry practices.

There are several lines of evidence for developmental programming in fish, and for an excellent review see Jonnson and Jonnson (2014). Traits associated with early environmental conditions include metabolism, growth, sex determination, fecundity, and behavior (Jonnson and Jonnson 2014). There are also several examples where environmental memory has been described in shellfish. In some instances this occurs within a generation and in other cases there is transmission of information from parents to offspring. Within generation environmental memory has been described in Olympia oysters where early larval exposure to ocean acidification impacted juvenile traits (Hettinger et al 2013). Adult Manila clams exposed to low pH during gonadal maturation have faster growing offspring compared to controls (Zhao et al., 2017). In the Sydney rock oyster, larvae produced by parents incubated under low pH conditions are larger and develop faster in low pH conditions and also have higher fitness as adults (Parker *et al.* 2012, 2015). In addition to water chemistry, disease is another significant concern in shellfish aquaculture. There is increasing evidence to suggest prior exposure to immune challenge can increase response later in life and that this environmental memory can be transmitted to offspring. Green et al. (2016) demonstrated offspring of Pacific oyster parents treated with poly(I:C) possess enhanced protection against Ostreid herpesvirus type I infection. The mechanism(s) responsible for providing this memory are not fully understood in cultured species, and arguably not required to improve aquaculture production, though we would suggest that elucidating the epigenetic mechanisms involved could increase the degree and breadth of improvement.

Two relevant cases for aquaculture where the epigenetic mechanisms have been described involve sex determination in fish. In European sea bass (*Dicentrarchus labrax*), exposure to high temperature in early development is associated with a higher proportion of phenotypic males (Navarro-Martin et al 2009). In work done by Navarro-Martin et al. (2011) this early high temperature exposure was associated with increased DNA methylation in the promoter of the aromatase gene (*cyp19a1a*) in adults. Furthermore, the authors showed that *in vitro* methylation of the aromatase promoter was sufficient to suppress transcription of the gene. More recently, the commercially important half-smooth tongue sole (*Cynoglossus semilaevis*) was used as a model to investigate the role of epigenetic regulation in environmental sex determination. Using genome-wide DNA methylation profiling, authors showed that pseudomales (generated by exposing genetic females to high temperature during a sensitive developmental window) exhibit methylation patterns consistent with genetic males, both of which differ from the methylome of normal females. Excitingly, it was reported that global methylation patterns are inherited by F1 pseudomale offspring generated by crosses between pseudomales and normal females, suggesting transgenerational epigenetic inheritance of environmentally-induced sex reversal in this species (Shao et al 2014). Controlling sex in fish broodstock is certainly a priority for aquaculture and these studies shed light on the epigenetic mechanisms that could be leveraged in future work. More work would be needed to establish the degree of mechanism conservation across species exhibiting environmental sex determination.

Nutrition and feeding are important aspects of aquaculture production, and research has shown memory of early-life nutritional conditions influences key phenotypic traits later in life. In mammals, nutritional status of the mother can predispose offspring to adult onset metabolic disease and mounting evidence suggests that epigenetic mechanisms are involved (reviewed by Vickers et al. 2014). In fish, rainbow trout fry fed a plant based diet for 3 weeks starting as swim-up fry showed higher growth rates, feed intakes, and feed efficiencies when challenged again with a plant based diet after 7 months of grow out on a fishmeal/fish oil diet (Geurden et al 2013). Interestingly, in a follow up study, transcriptomic analyses suggest that epigenetic mechanisms may be involved in this response (Balasubramanian et al 2016). In addition, a study looking at vitamin supplementation at first feeding in rainbow trout identified changes in global methylation and histone modification 7 months after the supplementation had ended, despite no observed phenotypic responses (Panserat et al. 2017). These studies provide the first link between early-environmental exposures and epigenetic mechanisms in aquaculture species.

Epigenetic Selection

Beyond understanding how early environmental exposure can be used for improving aquaculture, there is the possibility epigenetic markers could be integrated into broodstock selection. This is plausible as in one important agriculture commodity, oil palms, it has been shown that a critical trait, oil content, can be epigenetically selected for (Ong-Abdullah et al. 2015). While there is much more we need to learn with regard to desired phenotypes and epialleles, work such as this demonstrates value in including epigenetics in association studies. Furthermore, Patel et al. (2013) have shown in a clinical study that integrating both genetic (SNP) and epigenetic (DNA methylation) markers in genome wide association studies improved associations with a phenotype (i.e. diabetes). The influence of epigenetics, specifically DNA methylation, on estimating breeding values for quantitative traits has been considered recently for finfish aquaculture in a review by Moghadam et al. (2015).

It should also be noted that epigenetics might make genetic selection more challenging. Many organisms have the potential to generate new genetic variation in response to stressful conditions through modulation of epigenetic marks associated with transposable elements (Dowen et al. 2012, Yu et al. 2013, reviewed by Rey et al. 2016). Transposable elements, or “jumping genes”, are regions of repetitive DNA that can move and amplify their copy number in the host genome. In the model plant *Arabidopsis* the genomic response to bacterial challenge was a global reduction of DNA methylation and reactivation of previously silent TEs associated with defense genes (Yu et al. 2013). It is interesting to consider that in invertebrates, shown specifically in *C. gigas,* that TEs are not preferentially methylated (Gavery & Roberts 2013, Olson and Roberts 2015). It has been hypothesized that the lack of TE silencing by DNA methylation may indicate pressure to generate and maintain genetic diversity in a species that inhabits heterogeneous environments (Gavery & Roberts 2014). This means that in theory, if culture conditions become stressful, shellfish could respond by modulating transposable element expression to create new genetic variation (Rey et al., 2016), thereby having the unintended consequences of “erasing” phenotypic gains made through selective breeding.

Conclusions

Epigenetics has the potential to change the way we think about how a phenotype is generated and maintained. Through a greater understanding of DNA methylation, histone modifications and ncRNAs we can functionally annotate genomes, better predict phenotypic outcomes of early environmental exposures, and possibly select on epigenetic markers. With careful experimental design and special considerations for epigenetic differences between taxa (see Lea et al. 2016), the aquaculture community is primed to begin to integrate epigenetics into husbandry practices. The concepts and ideas of epigenetics provide an attractive lens through which to consider manipulation of traits through environmental memory or selection of beneficial traits based on epigenetic markers. It is also important to consider that epigenetics may also function to disrupt predictable, robust phenotypes through the creation of new, unexpected variation.

References

Andreassen, R. & Høyheim, B. miRNAs associated with immune response in teleost fish. *Dev. Comp. Immunol.* (2017). doi:10.1016/j.dci.2017.02.023

Andreassen, R., Worren, M. M. & Høyheim, B. Discovery and characterization of miRNA genes in atlantic salmon (Salmo salar) by use of a deep sequencing approach. *BMC Genomics* **14,** 482 (2013).

Azzi, A. *et al.* Circadian behavior is light-reprogrammed by plastic DNA methylation. *Nat. Neurosci.* **17,** 377–382 (2014).

Baerwald, M. R. *et al.* Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. *Mol. Ecol.* **25,** 1785–1800 (2016).

Balasubramanian, M. N. *et al.* Molecular pathways associated with the nutritional programming of plant-based diet acceptance in rainbow trout following an early feeding exposure. *BMC Genomics* **17,** 449 (2016).

Bekaert, M. *et al.* Sequencing and Characterisation of an Extensive Atlantic Salmon (Salmo salar L.) MicroRNA Repertoire. *PLoS One* **8,** e70136 (2013).

Bell, A. C. & Felsenfeld, G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature* **405,** 482–485 (2000).

Bell, J. T. *et al.* DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. *Genome Biol.* **12,** R10 (2011).

Bizuayehu, T. T. & Babiak, I. MicroRNA in teleost fish. *Genome Biol. Evol.* **6,** 1911–1937 (2014).

Bizuayehu, T. T., Johansen, S. D., Puvanendran, V., Toften, H. & Babiak, I. Temperature during early development has long-term effects on microRNA expression in Atlantic cod. *BMC Genomics* **16,** 305 (2015).

Boltaña, S., Valenzuela-Miranda, D., Aguilar, A., Mackenzie, S. & Gallardo-Escárate, C. Long noncoding RNAs (lncRNAs) dynamics evidence immunomodulation during ISAV-Infected Atlantic salmon (Salmo salar). *Sci. Rep.* **6,** 22698 (2016).

Brykczynska, U. *et al.* Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nat. Struct. Mol. Biol.* **17,** 679–687 (2010).

Campos, C., Valente, L. M. P., Conceição, L. E. C., Engrola, S. & Fernandes, J. M. O. Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. *Epigenetics* **8,** 389–397 (2013).

Castel, S. E. & Martienssen, R. A. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nat. Rev. Genet.* **14,** 100–112 (2013).

Cheung, P., Allis, C. D. & Sassone-Corsi, P. Signaling to chromatin through histone modifications. *Cell* **103,** 263–271 (2000).

Chinnusamy, V. & Zhu, J.-K. Epigenetic regulation of stress responses in plants. *Curr. Opin. Plant Biol.* **12,** 133–139 (2009).

Csankovszki, G., Nagy, A. & Jaenisch, R. Synergism of Xist RNA, DNA methylation, and histone hypoacetylation in maintaining X chromosome inactivation. *J. Cell Biol.* **153,** 773–784 (2001).

Daxinger, L. & Whitelaw, E. Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nat. Rev. Genet.* **13,** 153–162 (2012).

Dolinoy, D. C., Weidman, J. R., Waterland, R. A. & Jirtle, R. L. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.* **114,** 567–572 (2006).

Dowen, R. H. *et al.* Widespread dynamic DNA methylation in response to biotic stress. *Proc. Natl. Acad. Sci. U. S. A.* **109,** E2183–91 (2012).

Eberharter, A. & Becker, P. B. Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. *EMBO Rep.* **3,** 224–229 (2002).

Fellous, A., Favrel, P. & Riviere, G. Temperature influences histone methylation and mRNA expression of the Jmj-C histone-demethylase orthologues during the early development of the oyster Crassostrea gigas. *Mar. Genomics* **19,** 23–30 (2015).

Fellous, A., Favrel, P., Guo, X. & Riviere, G. The Jumonji gene family in Crassostrea gigas suggests evolutionary conservation of Jmj-C histone demethylases orthologues in the oyster gametogenesis and development. *Gene* **538,** 164–175 (2014).

Feng, S. *et al.* Conservation and divergence of methylation patterning in plants and animals. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 8689–8694 (2010).

Gavery, M. R. & Roberts, S. B. A context dependent role for DNA methylation in bivalves. *Brief. Funct. Genomics* **13,** 217–222 (2014).

Gavery, M. R. & Roberts, S. B. Predominant intragenic methylation is associated with gene expression characteristics in a bivalve mollusc. *PeerJ* **1,** e215 (2013).

Geurden, I. *et al.* The Positive Impact of the Early-Feeding of a Plant-Based Diet on Its Future Acceptance and Utilisation in Rainbow Trout. *PLoS One* **8,** e83162 (2013).

Gluckman, P. D., Hanson, M. A., Cooper, C. & Thornburg, K. L. Effect of in utero and early-life conditions on adult health and disease. *N. Engl. J. Med.* **359,** 61–73 (2008).

Goddard, M. E. & Whitelaw, E. The use of epigenetic phenomena for the improvement of sheep and cattle. *Front. Genet.* **5,** 247 (2014).

González-Recio, O., Toro, M. A. & Bach, A. Past, present, and future of epigenetics applied to livestock breeding. *Front. Genet.* **6,** 305 (2015).

Green, T. J., Helbig, K., Speck, P. & Raftos, D. A. Primed for success: Oyster parents treated with poly(I:C) produce offspring with enhanced protection against Ostreid herpesvirus type I infection. *Mol. Immunol.* **78,** 113–120 (2016).

Guerrero-Bosagna, C., Settles, M., Lucker, B. & Skinner, M. K. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS One* **5,** (2010).

Heijmans, B. T. *et al.* Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 17046–17049 (2008).

Hettinger, A. *et al.* Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. *Ecology* **93,** 2758–2768 (2012).

Houwing, S. *et al.* A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. *Cell* **129,** 69–82 (2007).

Jabbari, K., Cacciò, S., Païs de Barros, J. P., Desgrès, J. & Bernardi, G. Evolutionary changes in CpG and methylation levels in the genome of vertebrates. *Gene* **205,** 109–118 (1997).

Jablonka, E. & Lamb, M. J. The changing concept of epigenetics. *Ann. N. Y. Acad. Sci.* **981,** 82–96 (2002).

Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293,** 1074–1080 (2001).

Jiang, Q., Li, Q., Yu, H. & Kong, L.-F. Genetic and epigenetic variation in mass selection populations of Pacific oyster Crassostrea gigas. *Genes Genomics* **35,** 641–647 (2013).

Jones, P. A. The DNA methylation paradox. *Trends Genet.* **15,** 34–37 (1999).

Jonsson B. and Jonsson N. 2014 Early environment influences later performance in fishes. *J Fish Biol.* **85**: 151-188.

Juanchich, A. *et al.* Characterization of an extensive rainbow trout miRNA transcriptome by next generation sequencing. *BMC Genomics* **17,** 164 (2016).

Kevin C. Wang and Howard Y. Chang. Molecular mechanisms of long noncoding RNAs, Mol Cell. 2011 Sep 16; 43(6): 904–914.

Lawrence, Daujat, Schneider. January 2016, vol 32: Pages 42–56 Lateral Thinking: How Histone Modifications Regulate Gene Expression.

Lea, A., Vilgalys, T., Durst, P. & Tung, J. Maximizing ecological and evolutionary insight from bisulfite sequencing data sets. *bioRxiv* 091488 (2016). doi:10.1101/091488

Li, E., Bestor, T. H. & Jaenisch, R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69,** 915–926 (1992).

Li, M. & Leatherland, J. F. The implications for aquaculture practice of epigenomic programming of components of the endocrine system of teleostean embryos: lessons learned from mammalian studies. *Fish Fish* **14,** 528–553 (2013).

Lin, W. & Dent, S.Y. Functions of histone-modifying enzymes in development. *Curr. Opin. Genet. Dev.* **16**, 137–142 (2006).

Ma, H., Hostuttler, M., Wei, H., Rexroad, C. E., 3rd & Yao, J. Characterization of the rainbow trout egg microRNA transcriptome. *PLoS One* **7,** e39649 (2012).

Maloisel, L. & Rossignol, J. L. Suppression of crossing-over by DNA methylation in Ascobolus. *Genes Dev.* **12,** 1381–1389 (1998).

Manikkam, M., Guerrero-Bosagna, C., Tracey, R., Haque, M. M. & Skinner, M. K. Transgenerational Actions of Environmental Compounds on Reproductive Disease and Identification of Epigenetic Biomarkers of Ancestral Exposures. *PLoS One* **7,** e31901 (2012).

Marandel, L. *et al.* Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (Oncorhynchus mykiss) by nutritional status and dietary carbohydrates. *Sci. Rep.* **6,** 32187 (2016).

Martin, C. & Zhang, Y. The diverse functions of histone lysine methylation. *Nat. Rev. Mol. Cell Biol.* **6,** 838–849 (2005).

Mercer, Tim R., Marcel E. Dinger, and John S. Mattick. 2009. “Long Non-Coding RNAs: Insights into Functions.” *Nature Reviews. Genetics* 10 (3): 155–59.

Metzger, D. C. H. & Schulte, P. M. Epigenomics in marine fishes. *Mar. Genomics* **30,** 43–54 (2016).

Moghadam, H., Mørkøre, T. & Robinson, N. Epigenetics—Potential for Programming Fish for Aquaculture? *J. Mar. Sci. Eng.* **3,** 175–192 (2015).

Morán, P. & Pérez-Figueroa, A. Methylation changes associated with early maturation stages in the Atlantic salmon. *BMC Genet.* **12,** 86 (2011).

Morán, P., Marco-Rius, F., Megías, M., Covelo-Soto, L. & Pérez-Figueroa, A. Environmental induced methylation changes associated with seawater adaptation in brown trout. *Aquaculture* **392-395,** (2013).

Navarro-Martín, L. *et al.* DNA Methylation of the Gonadal Aromatase (cyp19a) Promoter Is Involved in Temperature-Dependent Sex Ratio Shifts in the European Sea Bass. *PLoS Genet.* **7,** e1002447 (2011).

Navarro-Martín, L., Blázquez, M., Viñas, J., Joly, S. & Piferrer, F. Balancing the effects of rearing at low temperature during early development on sex ratios, growth and maturation in the European sea bass (Dicentrarchus labrax).: Limitations and opportunities for the production of highly female-biased stocks. *Aquaculture* **296,** 347–358 (2009).

Olson, C. E. & Roberts, S. B. Genome-wide profiling of DNA methylation and gene expression in Crassostrea gigas male gametes. *Front. Physiol.* **5,** 224 (2014).

Olson, C. E. & Roberts, S. B. Indication of family-specific DNA methylation patterns in developing oysters. *bioRxiv* 012831 (2015). doi:10.1101/012831

Ong-Abdullah, M. *et al.* Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm. *Nature* **525,** 533–537 (2015).

Paneru, B., Al-Tobasei, R., Palti, Y., Wiens, G. D. & Salem, M. Differential expression of long non-coding RNAs in three genetic lines of rainbow trout in response to infection with Flavobacterium psychrophilum. *Sci. Rep.* **6,** 36032 (2016).

Panserat, S. *et al.* Muscle catabolic capacities and global hepatic epigenome are modified in juvenile rainbow trout fed different vitamin levels at first feeding. *Aquaculture* **468, Part 1,** 515–523 (2017).

Parker, L. M. *et al.* Adult exposure influences offspring response to ocean acidification in oysters. *Glob. Chang. Biol.* **18,** 82–92 (2012).

Parker, L. M., O'Connor, W. A., Raftos, D. A., Pörtner, H.-O. & Ross, P. M. Persistence of Positive Carryover Effects in the Oyster, Saccostrea glomerata, following Transgenerational Exposure to Ocean Acidification. *PLoS One* **10,** e0132276 (2015).

Patel, C. J., Chen, R., Kodama, K., Ioannidis, J. P. A. & Butte, A. J. Systematic identification of interaction effects between genome- and environment-wide associations in type 2 diabetes mellitus. *Hum. Genet.* **132,** 495–508 (2013).

Peschansky, V. J. & Wahlestedt, C. Non-coding RNAs as direct and indirect modulators of epigenetic regulation. *Epigenetics* **9,** 3–12 (2014).

Potok, M. E., Nix, D. A., Parnell, T. J. & Cairns, B. R. Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell* **153,** 759–772 (2013).

Rey, O., Danchin, E., Mirouze, M., Loot, C. & Blanchet, S. Adaptation to Global Change: A Transposable Element–Epigenetics Perspective. *Trends Ecol. Evol.* **31,** 514–526 (2016).

Rondon, R. *et al.* Effects of a parental exposure to diuron on Pacific oyster spat methylome. (2017). doi:10.1093/eep/dvx004

Rosani, U., Pallavicini, A. & Venier, P. The miRNA biogenesis in marine bivalves. *PeerJ* **4,** e1763 (2016).

Sellos, D. The histones isolated from the sperm of the oyster Crassostrea gigas. *Cell Differ.* **17,** 183–192 (1985).

Shao, C. *et al.* Epigenetic modification and inheritance in sexual reversal of fish. *Genome Res.* **24,** 604–615 (2014).

Simmen, M. W. *et al.* Nonmethylated transposable elements and methylated genes in a chordate genome. *Science* **283,** 1164–1167 (1999).

Stewart, S., Tsun, Z.-Y. & Izpisua Belmonte, J. C. A histone demethylase is necessary for regeneration in zebrafish. *Proc. Natl. Acad. Sci. U. S. A.* **106,** 19889–19894 (2009).

Strömqvist, M., Tooke, N. & Brunström, B. DNA methylation levels in the 5' flanking region of the vitellogenin I gene in liver and brain of adult zebrafish (Danio rerio)--sex and tissue differences and effects of 17alpha-ethinylestradiol exposure. *Aquat. Toxicol.* **98,** 275–281 (2010).

Terova, G. *et al.* Effects of Sodium Butyrate Treatment on Histone Modifications and the Expression of Genes Related to Epigenetic Regulatory Mechanisms and Immune Response in European Sea Bass (Dicentrarchus Labrax) Fed a Plant-Based Diet. *PLoS One* **11,** e0160332 (2016).

Tweedie, S., Charlton, J., Clark, V. & Bird, A. Methylation of genomes and genes at the invertebrate-vertebrate boundary. *Mol. Cell. Biol.* **17,** 1469–1475 (1997).

Valenzuela-Miranda, D. & Gallardo-Escárate, C. Novel insights into the response of Atlantic salmon (Salmo salar) to Piscirickettsia salmonis: Interplay of coding genes and lncRNAs during bacterial infection. *Fish Shellfish Immunol.* **59,** 427–438 (2016).

Vastenhouw, N. L. & Schier, A. F. Bivalent histone modifications in early embryogenesis. *Curr. Opin. Cell Biol.* **24,** 374–386 (2012).

Vickers, M. H. Early life nutrition, epigenetics and programming of later life disease. *Nutrients* **6,** 2165–2178 (2014).

Wang, Y., Wang, C., Zhang, J., Chen, Y. & Zuo, Z. DNA hypomethylation induced by tributyltin, triphenyltin, and a mixture of these in Sebastiscus marmoratus liver. *Aquat. Toxicol.* **95,** 93–98 (2009).

Wang, Y., Wang, C., Zhang, J., Chen, Y. & Zuo, Z. DNA hypomethylation induced by tributyltin, triphenyltin, and a mixture of these in Sebastiscus marmoratus liver. *Aquat. Toxicol.* **95,** 93–98 (2009).

Weaver, I. C. G. *et al.* Epigenetic programming by maternal behavior. *Nat. Neurosci.* **7,** 847–854 (2004).

Wheeler, B. M. *et al.* The deep evolution of metazoan microRNAs. *Evol. Dev.* **11,** 50–68 (2009).

Wu, S.-F., Zhang, H. & Cairns, B. R. Genes for embryo development are packaged in blocks of multivalent chromatin in zebrafish sperm. *Genome Res.* **21,** 578–589 (2011).

Wu, S.-F., Zhang, H. & Cairns, B. R. Genes for embryo development are packaged in blocks of multivalent chromatin in zebrafish sperm. *Genome Res.* **21,** 578–589 (2011).

Yu, A. *et al.* Dynamics and biological relevance of DNA demethylation in Arabidopsis antibacterial defense. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 2389–2394 (2013).

Yu, H., Zhao, X. & Li, Q. Genome-wide identification and characterization of long intergenic noncoding RNAs and their potential association with larval development in the Pacific oyster. *Sci. Rep.* **6,** 20796 (2016).

Zemach, A., McDaniel, I. E., Silva, P. & Zilberman, D. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* **328,** 916–919 (2010).

Zhang, C., Hoshida, Y. & Sadler, K. C. Comparative Epigenomic Profiling of the DNA Methylome in Mouse and Zebrafish Uncovers High Interspecies Divergence. *Front. Genet.* **7,** 110 (2016).

Tables and Figures

Figure 1. Schematic representation of epigenetic influence on phenotype. Markers used for characterizing organismal variation are listed.

<<Figure 1 is included as a separate attachment: Gavery_Roberts_Fig1.jpg>>