PACIFIC SALMON FOUNDATION

GENOMICS IN HATCHERIES

Hatchery Effectiveness Review

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Photo by Mitch Miller

Currently, more than six billion juvenile salmon are released from hatcheries each year around the Pacific Rim. The resulting adults provide fishing opportunities, help preserve endangered populations, and promote awareness of salmon conservation. Despite these beneficial outcomes, hatchery fish have lower rates of survival and may cause unintended consequences to their wild counterparts. Additionally, different hatcheries can vary significantly in their performance. With the conservation and preservation of wild salmon populations being of the highest priority (Wild Salmon Policy (DFO 2005)), it is critical we have a thorough understanding of the complex and difficult-to-quantify implications of hatcheries and manage them carefully.

Tools to help increase hatchery salmon survival, as well as explain differences in hatchery performance, and ensure conservation goals are being met would be valuable to managers. The application of the latest scientific 'omics' technologies and cutting-edge research can help us unlock the information needed to improve hatchery performance and minimize the risks to wild salmon.

WHAT IS MEANT BY 'OMICS' TOOLS AND HOW CAN THEY BE USED BY HATCHERIES?

Named after their common suffix, 'omics' refers to the related fields of genomics, transcriptomics, proteomics, and metabolomics, each of which is defined below. Omics, generally speaking, is the study of the molecules within a cell and how they interact with the environment and the traits that result (Figure 1). These fields of study have undergone a rapid evolution resulting in the development of a number of new tools that can increase our understanding of what contributes to the fitness of an individual salmon. Also, importantly, the cost of using these technologies has dropped over the years, and it is now feasible to routinely apply the techniques that previously would have been out of reach. Together, these technologies could be a game-changer for the survival of hatchery-reared fish, and future generations of salmon.

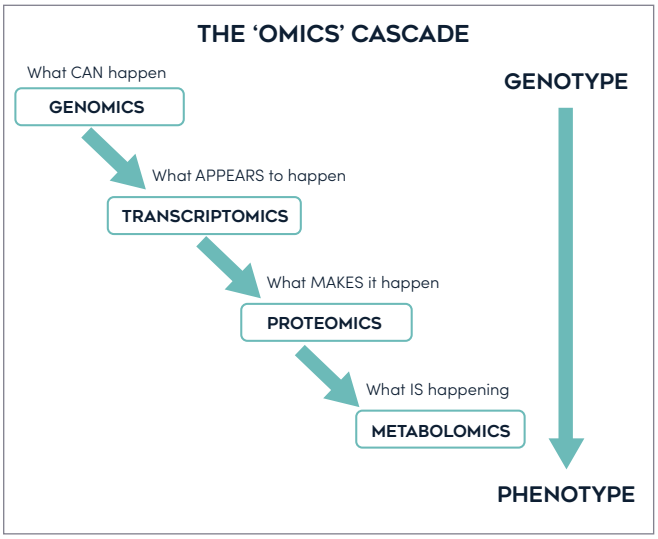


Figure 1: *Four 'omics' technologies showing how they relate along the gradient of genotype — the genetic code of an individual or population to phenotype — the traits that result from the interaction of genes with the environment. Since genomics considers genes, it can be used to understand the instructions available to an organism, it's potential — what can happen, so to speak. Transcriptomics can provide an indication of how these genes or instructions appear to be used at a given time. Proteomics and metabolomics are more direct and sensitive ways to detect responses to the environment. Of the 'omics' technologies, genomics and transcriptomics have been in use longer and offer more tried and tested applications. However, scientists see great potential in proteomics and metabolomics. Figure from Genetics and 'Omics' Technologies Review for Salmon Hatcheries.*

GENOMICS — the study of the entire DNA genetic code that makes up an individual.

Using genomics, a researcher can investigate how different genes relate to one another, the environment, and the traits of the organism being studied.

Photo by Mitch Miller

HOW IS IT DIFFERENT FROM GENETICS?

Genetics refers to the study of specific genes and the way that certain conditions or traits are passed down from one generation to the next, whereas genomics studies ALL of the genes. The greater amount of information that can be gained from genomics provides more robust results and increased insights.

Genomic approaches can help us understand the genetic basis of a salmon's characteristics, allow us to compare the marine survival of the offspring of different families reared in hatcheries, and understand to what degree hatchery fish are mixing with wild fish genetically (introgression). Genomics is also useful to help define a smaller subset of genetic markers that can be used to answer a particular question, to understand how populations adapt to climate change or to monitor variations in the environment.

EXAMPLES OF GENOMICS APPROACHES

PARENTAGE-BASED TAGGING (PBT)

Photo by Collin Middleton

One widely-applied example of a genomics approach is parentage-based tagging (PBT). The original incentive for developing PBT was to meet the increased demand from fisheries managers for precise information on the contributions of different hatchery stocks to fishery harvests. PBT is increasingly being used in the hatchery setting. A tissue sample (usually a fin clip) is collected from each parent fish by hatchery staff and is genotyped using hundreds to thousands of identifiers known as single nucleotide polymorphisms[1] (or SNP markers). With genotype information collected for all the hatchery parents, all of the resulting offspring are genetically tagged — which can include millions of juveniles. Later, when offspring are captured they can then be assigned back to their parents from another tissue sample. This enables high accuracy in stock identification: the hatchery assignment accuracy is near perfect (> 99%) for steelhead trout using > 100 SNP markers, coho salmon using 304 SNP markers and Chinook salmon using 321 SNP markers.

How can we use this information? PBT can provide an estimation of specific hatchery contributions of different families to fisheries and escapement (i.e. those fish that successfully survived to make it back to the rivers in which they were released). The former may have higher relevance to hatcheries that have an objective of maximizing fishing opportunities while the latter may be more relevant to hatcheries with conservation objectives (e.g. supplementation and captive breeding hatcheries). PBT allows the performance of different hatchery fish to be studied as well as assessment of the impacts of various hatchery practices. For example, a hatchery may test different brooding practices, rearing conditions, or release strategies and work out which resulted in better returns; and then adapt their practices accordingly. Hatcheries can use PBT to identify the genes associated with higher survival of different offspring, to assess genetic interactions between wild and hatchery fish, to reduce domestication and improve the fitness of hatchery fish in the wild, to determine the genes associated with age at maturity and other important traits, as well as to assess impacts of climate change on hatchery fish. PBT information can also be used for quantifying the amount of straying by hatchery fish to other river systems. Hatchery salmon straying is generally considered undesirable because interbreeding may decrease the fitness of locally adapted wild populations. PBT information has shown that early maturing (i.e. jack) hatchery coho salmon commonly stray to non-local rivers on Vancouver Island and southern BC.

1. A Single Nucleotide Polymorphism is a DNA sequence variation that occurs when a single nucleotide (adenine, thymine, cytosine, or guanine) in the genome sequence is altered

Photo by Danny Swainson

USING PBT TO UNDERSTAND PROPORTIONATE NATURAL INFLUENCE (PNI)

The British Columbia Salmon Enhancement Program must adhere to the Wild Salmon Policy principle that "conservation of wild Pacific salmon and their habitats is the highest priority in resource management decision making"[2]. Therefore, knowledge is required to understand the relationship between the impacts of hatchery rearing on hatchery-influenced and surrounding wild populations. One recently adopted approach is the use of PNI — proportionate natural influence — as an index of gene flow between the natural and hatchery environment. The PNI is a value between 0 and 1 that is calculated from the estimated proportion of wild-origin fish in the hatchery brood and hatchery-origin fish on the spawning grounds. Knowledge of the PNI can lead to focused action to minimize genetic risks through management practices that minimize the size of the hatchery program, manipulate the composition of the broodstock (i.e. increase use of natural-origin spawners), or prevent hatchery-origin fish from spawning in the river.

To measure PNI we need to accurately identify wild- and hatchery-origin adults prior to spawning, and PBT can be useful to identify origin of spawning adults.

2. DFO (Fisheries and Oceans Canada), 2005. Canada's policy for conservation of wild Pacific salmon. http://www- comm.pac.dfo-mpo.gc.ca/publications/wsp/default_e.htm.

SPOTLIGHT ON EPIGENETICS

UNDERSTANDING EPIGENETIC EFFECTS

Investigating for differences between hatchery and wild populations is revealing that genetic codes (e.g. genotype) are not drastically changing even after multiple generations of hatchery-origin parents. Then why are scientists observing differences in the survival and traits (e.g. phenotype) of hatchery-origin fish? It turns out there are other mechanisms at play called epigenetic effects.

Epigenetic changes are shifts in gene activity/expression that occur due to alterations in how certain genes are accessed. The DNA code may be folded differently or may have promotor or inhibitor molecules that attach to the sequences. These changes arise during development in response to environmental conditions or behavioural differences and can result in different traits from the same genetic code. It is another way that an organism can adapt to the environment it experiences. However, when the adaptation is favourable to artificial rearing conditions in hatcheries, it will not necessarily serve these salmon well once they are in the natural environment.

A way to investigate epigenetic effects is by using genomic techniques (e.g. bisulfite sequencing or chromatin immunoprecipitation (ChIP)) to scan for differences in promotor or inhibitor molecules along the genetic sequence.

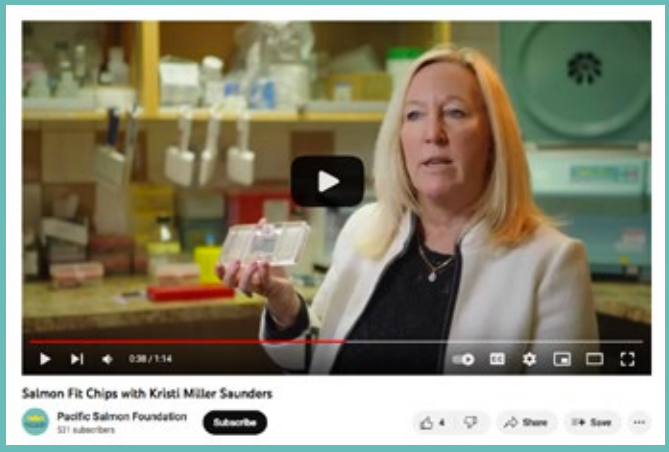
Photo by Niclole Christiansen

These approaches could be used to examine whether certain aspects of the hatchery environment, such as rearing density, water temperature, or rearing surroundings, are causing epigenetic changes. With this knowledge, managers can work to address the conditions accordingly.

Interestingly, epigenetic effects, although not part of the genetic code itself, can be inherited and persist for generations or they can also be reversible, so understanding the impacts and ways to reduce epigenetic effects may be significant for improving hatchery-origin fish outcomes.

TRANSCRIPTOMICS — similar to genomics but studies the transcriptome, which, in simple terms, means all the genes that are being used. Rather than DNA, transcriptomics looks at RNA 'transcripts', which are genes that have been copied for use in a cell — e.g. to build proteins and carry out functions of the cell.

With transcriptomics, the use, or 'expression', of particular genes can be measured. Depending on what the genes of interest are, their expression could indicate that a fish is stressed, diseased, or reaching a particular maturity state (e.g. the "smoltification window", when their bodies are best prepared to adjust from freshwater to saltwater). Hatcheries can use this information to reduce stress for their fish or optimize release timing. Another application could be investigating differences between hatchery and wild salmon using gene expression.



EXAMPLE OF TRANSCRIPTOMIC APPROACHES

SALMON FIT-CHIPS

Some genes may be expressed differently in response to a certain stressor. Changes in the expression of particular genes can be used as 'biomarkers' to identify various conditions. For example, gene expression biomarkers have been developed for smoltification, thermal stress, salinity stress, hypoxic stress, general stress, imminent mortality, and viral disease development in Pacific salmon. DFO and the PSF Salmon Health team have developed Salmon "Fit-Chips" to examine the physiological condition of many salmon at once. They use a high-throughput Fluidigm BioMarkTM platform to examine 96 gene expression biomarkers for 96 salmon samples at a time. The types of biomarkers and samples examined are fully customizable. They can also examine pathogen loads using the same platform. These can provide valuable predictive screening information, such as the health and condition of hatchery salmon prior to release. Together with salmon return rates, this information may be used to explain changes in hatchery performance over time or differences among hatcheries.


Photo by Danny Swainson


Photo by Benjamin Fortini

SPOTLIGHT ON ENVIRONMENTAL SAMPLING

A great feature of omics methods is that they can be used on tissue samples (fin or gill clips, and blood) that can be collected without causing significant harm to the fish. In some cases, the methods can be applied to samples collected from the water when the fish are swimming in (**e.g. environmental DNA or eDNA**) and therefore do not involve handling the salmon at all.

What is eDNA?

Environmental DNA (eDNA) is the genetic material shed by organisms in the environment such as the water column. By collecting samples of water that include mucus, feces, or tissue particles that have been shed, scientists can process eDNA to tell us what species are in the area and their relative abundance. In general, there are positive relationships between water eDNA content and salmon counts or biomass although these relationships can be affected by different environmental factors e.g. the relationship is more precise when water is warmer than cooler, possibly because of increased salmon eDNA release, e.g. shedding of skin, mucus, feces, and urine, in warmer waters. There may also be spatial and temporal limits to salmon detectability.

It's also worth noting that environmental sampling can be applied to RNA (eRNA) as well. Similar to the differences in information that can be obtained from genomics versus transcriptomics, tracking RNA in the environment gives an indication of what genes are being used. Due to the nature of RNA, which breaks down more rapidly than DNA, eRNA can be used to improve interpretation of eDNA data.

Photo by Warren Umoh, Unsplash

How can we use eDNA?

Hatcheries may use eDNA information to address questions such as what is the distribution of a salmon species across a landscape? Which habitats have been colonized by different species of salmon, or is an endangered species found in a particular area? Such knowledge can help guide the release sites that might be best for a particular hatchery to use.

eDNA may also be used to assist with pathogen screening at hatchery release sites, assessing food availability for determining optimal release timing, assessing the diversity of fish in an area, or monitoring for invasive species. The relative amounts of eRNA and eDNA can give an indication of the ratio of living to dead components. eRNA can also be useful for detecting virus pathogens, some of which only have RNA for their genetic material.

Generally, eDNA analysis holds a lot of potential for addressing questions related to aquaculture and fisheries management but does require further development before it can be used reliably.

PROTEOMICS — rather than studying genes (DNA and RNA), proteomics looks directly at the proteins, specifically the entire protein pool of a tissue. Changes in the amount of or type of protein produced can reflect a response to a change in the environment.

Still a relatively new field, there are fewer applications of proteomics in use. Potential uses under development include disease diagnosis and the development of vaccines by studying certain proteins that may increase during stress. Such applications can lead to healthier fish. Proteomics techniques may be able to help us better understand the reasons for the lower performance by hatchery salmon as compared to wild salmon.

METABOLOMICS — the study of small molecules or metabolites and enzymes within a tissue. Changes in these may provide the best direct indication of what is happening with an organism physiologically.

Again, there is great potential with metabolomics as it is a more direct measure and highly responsive to environmental conditions. However, this is still a relatively new and evolving field. An example of a potential use of metabolomics in hatcheries includes developing metabolite markers for diseases of concern to help detect them. This can help hatcheries monitor the health of their fish.

Cover photos by Mitch Miller (top and right) Danny Swainson (left), and Collin Middleton (centre)

THE PSF HATCHERY EFFECTIVENESS REVIEW

While there is a desire among hatchery managers to incorporate the latest technologies into their operations, the rapid development and the complex nature of the methods pose a challenge.

Therefore, PSF set out to review the recent developments in these technologies and highlight how they could be used to support BC's Salmon Enhancement Program (SEP). We have recently released two documents as part of PSF's Hatchery Effectiveness Review:

> Genetics and 'Omics' Technologies Review for Salmon Hatcheries and

> Applications of 'Omics' Technologies in the Salmonid Enhancement Program.

THE REVIEW

Genetics and 'Omics' Technologies Review for Salmon Hatcheries, by Aimee Houde (PhD), provides a hatchery specific review of how the 'omics' technologies may be used to better manage and achieve the goals of hatcheries. To the best of our knowledge, this is the first review with an emphasis on hatchery applications.

The document contains:

> Contextual and background information
- the overlapping and unique objectives of different types of hatcheries (e.g. conservation or fisheries enhancement).
- genetic lessons learned over the years and how hatchery practices have changed as a result.

> Review of recent scientific literature of the 'omics' techniques
- each technology is reviewed within its own section (genomics, transcriptomics, proteomics, and metabolomics).
- the tools within each technology that have been developed, the questions they can address, and areas of potential development.

> Discussion of knowledge gaps and a summary of conclusions.

APPLICATIONS OF OMICS TECHNOLOGIES

Applications of 'Omics' Technologies in the Salmonid Enhancement Program, authored by Wendy Vandersteen (PhD), complements Houde's review. It summarizes the challenging subject matter and presents it in a way that is approachable and supportive for incorporating 'omics' technologies into hatchery operations. A number of handy resources are included like visual decision trees (Figure 2), illustrations to convey concepts, and a glossary of terms.

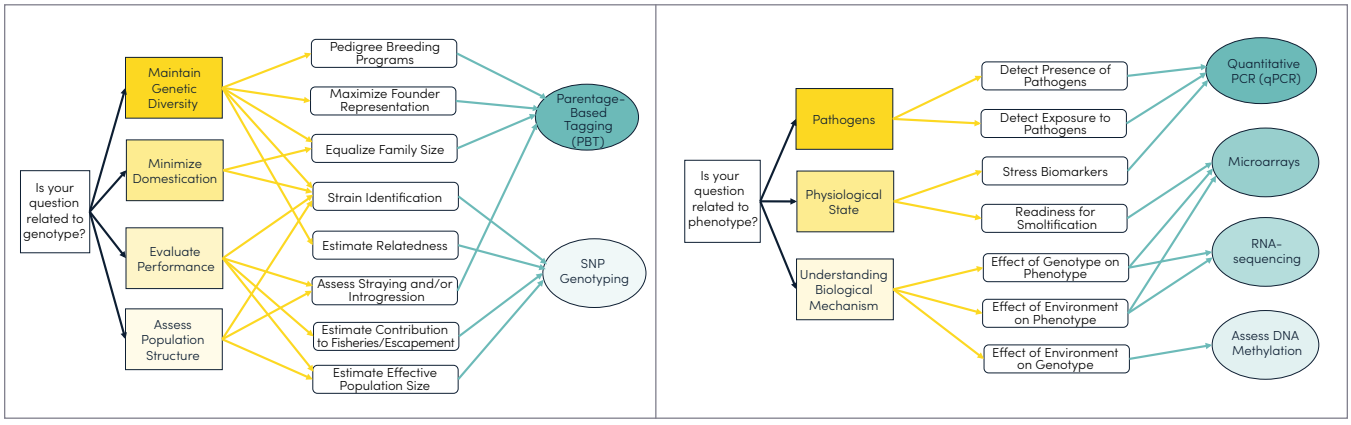


Figure 2: *Decision trees included in the Applications of 'Omics' Technologies in the Salmonid Enhancement Program. Users of the guide can consider their question or concern and move through the options to determine what techniques would be best suited to address it.*

The document, which is designed as a user-friendly guide to support SEP's hatchery managers, contains:

- An overview of genetics versus genomics
  - the benefit of using more comprehensive genomic approaches for addressing hatchery objectives.
- Summary of the SEP risk management framework
  - suggested opportunities to incorporate 'omics' approaches into routine operations that fit within the framework.
- A discussion of the challenges and limitations
  - technical: e.g. applying techniques, data handling, and analysis of the information.
  - non-technical: e.g. time and staffing constraints.
- Recommendations, for example
  - foster collaborations and lines of communication between hatchery managers, SEP facilities, and geneticists.
  - certain facilities could be developed into research facilities with additional resources.
- Additional informative resources
  - table of techniques, their applications, and considerations.
  - list of resources.
  - glossary of terms.
  - a series of 'Deep Dives' into the details for each technique.

Photo by Eiko Jones

TO SUM UP

There is great potential from the evolving field of molecular genetics and 'omics' techniques for application within hatcheries. The review and handbook presented here serve as a critical link between the current state of knowledge and application in BC's SEP hatcheries. Given the high level of investment in hatcheries, we hope that empowering hatchery managers with information about these cutting edge tools will help optimize hatchery operations and reduce impacts to wild Pacific salmon.

Photo by Niclole Christiansen

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