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# Innovating transcriptomics for practitioners in freshwater fish management and conservation: best practices across diverse resource-sector users

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REVIEWS

# Innovating transcriptomics for practitioners in freshwater fish management and conservation: best practices across diverse resource-sector users

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**Abstract** Thriving freshwater fish populations contribute to people's economic prosperity and well-being. Yet, freshwater fish populations are in critical condition around the globe. Most stressors to freshwater fishes, fisheries, and culture stem from habitat impacts, water-quality issues, and aquatic invasive species. Logistical difficulties of monitoring fish health are compounded by the limitations of conventional (capture-based) sampling methods, which provide only a temporal "snapshot" and generate data lacking in sensitivity and prognostic ability. Here, we propose an innovative genomics approach to develop a health toolkit that will allow resource-sector users to determine the health status of freshwater fishes, including their coping capacity, to environmental stressors. The stress-response transcription profile (STP)-chip is a suite of quantitative gene transcription assays that represents key gene pathways broadly associated with fish functional responses

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to environmental stress; therefore, the differential expression of well-selected genes can provide sensitive fish-health status indicators. Despite the scientific achievement of using genomics tools, actualizing the toolkit in practice is only successful if resource-sector users have full buy-in. We present seven case studies representing different practitioners and resource users – Indigenous rightsholders, environmental consultants (industry), commercial aquaculture, environmental charities (ENGO), and fishery commissions and managers (government) where each explores the benefits and risks associated with the adoption of a genomics fish-health toolkit. Using a co-production approach, wherein practitioners and resource users are engaged from the outset, these case studies reveal translational pathways that would be needed to overcome barriers to technological adoption and, hence, accelerate the responsible uptake of genomics-based applications in fisheries assessment, management, and conservation.

**Keywords** Gene-expression · Fish health · Genomics toolkit · Adaptive capacity · Innovative technology · Case study

## Introduction

Freshwater ecosystems are among the most threatened and degraded on the planet (Dudgeon et al. 2006) as evidenced by freshwater biodiversity declining by greater than 80% since 1970 according to the World Wildlife Fund Living Planet Index (Harrison et al. 2019). Not surprisingly, freshwater fishes have been impacted by human activities with extinction rates that exceed those of nearly every other taxa (Sisk et al. 1994). Unlike the marine realm, where the primary driver of fish population declines is often overharvest, there are many interacting stressors in fresh waters, often external to the fisheries sector, that influence fish populations (Adamus 2001; Cooke et al. 2016). For example, hydropower facilities impede connectivity and change flows in rivers; pollution acts on fishes and their food supplies; habitats needed for key life-history processes are altered; and invasive species (including pathogens) compete with native species and alter ecosystem structure and function (reviewed in Dudgeon et al. 2006; Arthington et al. 2016; Ives et al. 2018; Reid et al. 2019). Overharvest does occur in inland waters (Allan et al. 2005), but tends to be not as readily detectable as in marine environments (Post et al. 2002). Fishes reared in culture facilities face their own suite of stressors, both within the hatchery and after release (for conservation or management purposes); and the ability to assess the health and adaptability of cultured fish can be critical for maximizing production in culture facilities (Moreira et al. 2021). Exacerbating those stressors is climate change, which can have dramatic effects on fish populations given the manifold effects of water temperature on the biology of fishes (Myers et al. 2017).

Knowledge of the health status and adaptive coping capacity of a population is therefore particularly important as fish face the need for rapid adaptation to increasing and varied stressors (Bernos et al. 2020). The diversity of tools to assess the health and condition of fishes and fish populations has expanded greatly in the last few decades (Sopinka et al. 2016), but most approaches are still limited and rely directly on standard assessments of fish necropsies, body-condition indices, collection of blood to run basic assays (e.g., cortisol, glucose), or indirectly on community- and population-based indices such as Index of Biotic Integrity (Hughes et al. 1998). Genomics-based approaches can contribute to addressing the pressures facing freshwater fishes (Su et al. 2021). For example, they can be used to determine whether freshwater fish populations, individuals, or both, possess the scope to adjust, acclimate, and evolve in response to contemporary stressors including climate change, aquatic invasive species, and pollution across a gradient of environmental impacts (Bahamonde et al. 2016). Genomic methods can provide remarkable opportunities for transformative fish and fisheries assessment technologies that can potentially overcome the logistical difficulties of conventional methods. In particular,

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transcriptional profiling—the quantification of transcribed variation at multiple gene loci with the goal of detecting a coordinated (and adaptive) response to specific stimuli – can prove a valuable tool for identifying and ultimately reducing abiotic and biotic stress in animals (He et al. 2016). Such an approach would improve fish health through increased resilience to disease and more accurate diagnostic tests, and adapting production to accelerated climate change (He et al. 2016; Connon et al. 2018). This method can be more sensitive, prognostic, and accurate than conventional assessment methods of fish health, and the data gathered would be more robust and comprehensive. Nonetheless, to improve the sustainability of freshwater fish resources, even transformative genomic tools are not enough if they are not widely adopted outside of academia. The comfort level with genomics technologies, such as transcriptomics, is generally low within the fisheries and aquaculture sectors (Millar 2013; Kadykalo et al. 2021), and regional users may be more receptive should a marketable transcriptomics 'toolkit' be developed expressly for their use. However, because government agencies, non-governmental organizations (ENGOs), and private enterprises are acutely aware of the public scrutiny given any decisions impacting freshwater fish resources, they need strong evidence that new technologies will provide returns on investments of time and money with a measurable improvement in fisheries management.

Here, we explore ways to actualize the use of transcriptomics in fisheries for sectors directly involved in their assessment, management and/or conservation. We begin by reviewing the benefits and limitations of conventional methods employed for fish-health monitoring. We then discuss the more recent incorporation of adaptive capacity as an indicator of a fish population's status, and introduce the role of transcriptomics as a way to assess the ability of species and populations to respond and adapt to environmental change. While the call for the use of transcriptomics in fish management and conservation is not new (Connon et al. 2018), its adoption by non-academics has yet to be fully realized, and we explore the development of a transcriptomics toolkit for use by agencies, organizations, industries and communities firsthand. In particular, we describe the process of creating a user-friendly tool—its development, calibration, and validation—and propose features that would be required to overcome barriers to its adoption and facilitate its use in best practices. To deepen this discussion, we present case studies from five different Canadian sectors that detail the advantages and potential risks of incorporating transcriptomic technologies in their own suite of tools when addressing threats to Canada's freshwater fisheries resources. We conclude with a brief description of a research program funded by Genome Canada that is currently developing a 'universal' transcriptomics profile array with the goal of enabling effective management interventions to advance the sustainability, productive capacity, and resulting competitive position of the Canadian fisheries and aquaculture sectors, and protect livelihoods and ways of life.

## Conventional methods of fish health monitoring

Often missing in fisheries assessment and monitoring are data on the health and physiological status of individual fish, which can serve to link observations of fish declines with changes in habitat or other stressors (Jeffrey et al. 2015). Although focusing on individuals is not intuitive, stressors act on individuals, which then cascade up to influence population-level processes (Callow and Forbes 1998). Conventional fish-health assessment sampling often involves conducting laborious fish health assessments using necropsies (Adams et al. 1993) or sampling tissues, such as blood and muscle, to evaluate physiological status (e.g., energy density, stress hormones such as cortisol, oxidative stress biomarkers, osmoregulatory status; Adams and Ham 2011). Both of these approaches are laborious and may fail to detect potential issues given that efforts tend to focus on one or several biomarkers rather than using a broader panel of biomarkers, not unlike those used in veterinary or human medicine. Moreover, they tend to fail to test for key pathogens (including viruses)(Chapman et al. 2021). In addition, many of the conventional measures of stress (such as cortisol) can be influenced by method of capture/handling and only yield information on the status of the animal in very recent history (minutes to hours; Sopinka et al. 2016). Finally, the volume of tissue (e.g., blood) or type of tissue (e.g., whole body for proximate body analysis) may require that animals be lethally sampled, which may be undesirable if working with rare species.

Genetic and genomic tools would overcome many of these shortcomings of conventional sampling (Leese et al. 2016). Indeed, 'omics' tools tend to rely on very small amounts of tissue (e.g., a non-lethal gill biopsy) and provide longer-term integrated measures of fish health and condition that span organismal systems and functions (Jeffries et al. 2021). Great opportunities exist for incorporating such tools into routine fisheries assessment and monitoring to provide information on immune function, pathogen presence, stress, reproductive state, and metabolic state and, thus, allow linking observed trends in fish populations to specific mechanistic drivers allowing targeted management interventions (Cruz et al. 2012; Miller et al. 2014). There is also much opportunity to use omics to address pressing conservation issues for imperiled fishes (Castañeda et al. 2021). Ultimately, these omic tools reveal the capacity of fishes to respond to different forms of environmental stress (Hamdoun and Epel 2007).

## Genomic variation and its effects on adaptive capacity

Whether individuals, populations, or species are able to cope with the stressors they face is determined by their adaptive capacity – that is, the ability to move when conditions change (individual behavioural flexibility), ability to acclimate to changes (plasticity in response, tolerance), and the ability to evolve as a population or species (adaptive potential and standing genetic variation) (Houde et al. 2015; Beever et al. 2016; Hare et al. 2016). Determining a species' (non) adaptive capacity must first begin with identifying if variation exists in response to environmental change, and whether this variation occurs at the species-, population-, or individual-level (Seaborn et al. 2021). Recent advances in genomics technologies have demonstrated that such genomic variation gives rise to a multitude of behavioural and physiological phenotypes that interact with varying degrees of success with changing environmental conditions (Kültz et al. 2013). Reflecting both genetic and environmental factors (López-Maury et al. 2008), the up- or down- regulation of certain functional genes either in isolation or in epigenetic coordination with others is an adaptive process that mediates an organism's response to both biotic and abiotic environmental change/stress (Schulte 2004). Indeed, patterns of differential expression of selected (or candidate) genes are interpreted as putatively representing adaptive variation among natural populations (Whitehead and Crawford 2006; He et al. 2015).

Changes in gene expression are a result of a cellular response that leads to the transcription of DNA into RNA, and the translation of mRNA into proteins that contribute to the functional response of individuals to an abiotic or biotic stressor (Stanford and Rogers 2018). Quantifying the first step in gene expression—i.e., transcription—by measuring mRNA transcript abundance provides a method of identifying the intermediate step between the genetic makeup of an individual and the functional response to stressors (Jeffries et al. 2021). Measuring the transcriptomic response at multiple selected gene loci with the goal of detecting a coordinated (and potentially adaptive) response to specific stimuli has applications in assessing health and adaptive capacity in wild and captive fish populations (De Groot et al. 2002; Connon et al. 2018). In general, organisms with transcriptional profile patterns reflecting plastic- or specifically adapted phenotypes have the greatest capacity to cope with stress (He et al. 2016). This transcriptome response is both sensitive to and reflective of an individual's health status and affects population dynamics, species interactions, community ecology, and, ultimately, ecosystem-level processes (De Groot et al. 2002). As a result, targeting specific types of genes (i.e., transcripts) that respond can allow researchers to distinguish between different types of stressors (DeBiasse and Kelly 2016; Schwartz 2020).

## Use of transcriptomics—from researchers to practitioners

Another advantage of targeting transcripts is that laboratory-based approaches that quantify mRNA are the same across fish species, and therefore there is the ability to screen numerous species within a common facility with relatively rapid turnaround times (Qian et al. 2014). Two of the more common contemporary methods for assessing the transcriptome stress response in fishes is to use RNA sequencing technology to examine whole transcriptome patterns (e.g., reviewed in Oomen and Hutchings 2017) or to use a more targeted candidate gene approach such as

high-throughput qPCR to examine suites of genes that are indicative of specific responses (e.g., Jeffries et al. 2014; Miller et al. 2014; Wellband et al. 2018; Toews et al. 2019). While RNA sequencing is ideal for characterizing transcriptome responses of species with little or no molecular genetic information available, it is still relatively expensive and computationally challenging to perform, the data are complex, and there may be a longer turnaround time from sample collection through data interpretation than is appropriate for rapid (i.e., "real time") monitoring of fishes (Logan & Buckley 2015). Further, environmental researchers using RNA sequencing often lack the sample sizes necessary to address the full range of phenotypic plasticity that may occur within and among populations (Todd et al. 2016). The alternative is to generate transcriptome resources or mine existing publicly available resources to obtain molecular genetic sequence information and develop assays that target specific genes of interest or 'biomarkers' of a response (e.g., Akbarzadeh et al. 2018; Swirplies et al. 2019; Houde et al. 2019). An important consideration for either of these approaches is that the researchers must recognize that the methods of collecting fish prior to sampling can influence the transcriptome patterns of highly inducible genes (Jeffry et al. 2020). For example, a fish can be dip-netted from a tank or collected using electrofishing and sampled much quicker than fish captured by using gill nets, beach seines or trap nets. While no formal assessment of the effects of different capture methods on the transcriptome have been reported, the time it takes to capture and handle the fish can influence the transcriptome patterns, especially when considering stress-inducible genes (Jeffries et al. 2021). One way to address this issue is to develop transcriptome assays that reflect slow-responding processes (e.g., acclimatization or the adaptive immune response) that would not be greatly influenced by the acutely stressful capture experience (discussed in Jeffrey et al. 2020). Further, samples must be collected from live animals and immediately preserved (i.e., in RNA stabilizing solution or flash frozen in liquid nitrogen) and stored correctly (i.e., at −80 °C) to avoid RNA degradation (Jeffries et al. 2021). Any degradation of the RNA will hinder this approach; therefore, application may be limited in remote locations.

The above discussion clarifies a need to rapidly quantify the capacity for fishes to adapt and acclimate to stressors, with large numbers of individuals for genome-specific, large-effect markers of anthropogenic stress, all at relatively low cost. One way this can be achieved is through a partnership of universities, users, and biotech companies to work together to develop a set of transcriptional profile arrays with a multitude (e.g., 70–100) of genes known to play a role in environmental stress response (including endogenous control genes). While gene sequence variation is common among fish species, small conserved regions of DNA should exist within genes associated with fitness for closely related species (Cooper and Brown 2008; Akbarzadeh et al. 2018; Moura et al. 2019), and one can target those regions to produce "universal" transcriptional assays. Using existing gene sequence data (e.g., GenBank) for diverse fish species, one can identify short (60–120 bp) regions of highly conserved DNA sequence to design primers and probes for quantitative real-time PCR (qPCR) for selected gene loci, capable of discriminating among gene homologs. Selected loci should include known function genes from fitness-related gene categories: immune-pathogen/parasite resistance; growth and metabolism; stress response; neural plasticity; osmoregulation; apoptosis; hypoxia; contaminant response/detoxification; and circadian rhythm (Hook 2010). By including multiple genes with related function, one can avoid bias due to incorrect gene function assumptions in non-model fish species, as well as more accurately identify the specific stressor leading to a response, both primary goals of transcriptional profiling.

After calibrating qPCR assays for the candidate genes, assays are then tested using tissues collected from representative individuals from the target fish species with the goal of determining tissue specificity and assay sensitivity (minimal copy number detection for at-rest fish; Shahraki et al. 2019). Further validation can incorporate lab- and field-based tests using fish undergoing known stressors in captive and wild environments. Many candidate genes are expressed in tissues available for non-lethal biopsy sampling (e.g., gill, red blood cells, muscle tissue; Jeffries et al. 2021); however, some key genes are expressed in tissues that can only be collected terminally (e.g., brain, spleen, liver). Ultimately, qPCR assays must be transitioned to high throughput nanofluidic qRT-PCR platforms (such as Thermo Fisher OpenArray® or the Fluidigm Biomark™ HD systems) that have

the potential to provide assays capable of simultaneous qPCR at multiple gene loci. For example, the OpenArray® 'gene chips' (Fig. 1) can contain over 100 candidate gene transcription assays (in duplicate) for use with cDNA from RNA that has been derived from multiple tissues, and provide low-cost (e.g., ~ 0.30 ($CDN) per Taqman™ qPCR assay on OpenArray®), high throughput (single chip can assess 12 fish at 112 genes on an OpenArray®) assessment of fish health. The idea is for users to collect tissues from targeted fish, fix the tissues in a stabilizing storage solution (e.g. RNAlater™), and send the samples for RNA extraction and gene-transcription analysis (although users with suitable facilities could perform the full analysis in house). Using gene chips composed of pre-determined stress-response transcription profile (STP) markers, a full gene transcriptional profile would be available rapidly and at relatively low cost.

Although many gene transcription chip platforms are available, the methodology for gene transcription profiling for assessing fish health would be similar across platforms. The key component to such 'STP-chips' would be the suite of qPCR assays designed to have high PCR efficiency across a broad range of fish species of interest. For example, Genome Canada—a non-profit organization that supports the use of genomics-based technologies for improved Canadian living, awarded a multi-year research project grant in 2019 for the development, testing, and verification of the first universal gene expression panel for fish health (Genome Canada 2019). Spearheaded by the University of Windsor, researchers from the Genomic Network for Fish Identification, Stress and Health (GEN-FISH; https://gen-fish.ca) from 13 universities are working to produce and validate STP-chips for approximately 42 key Canadian freshwater fishes (Supplementary Materials Table S1). The first task was to select 112 candidate genes of known function to assay physiological stress responses at the mRNA level, as well as internal control genes (e.g., Jeffries et al. 2021) – the list of preliminary candidate genes includes a diverse range of array of putative gene function (Supplementary Materials Table S2). A subset of Canadian university labs directly involved with GEN-FISH (as principle investigators) are working towards the development and initial testing of those qPCR assays. Other collaborator university labs, in collaboration with partners representing various resource-use sectors across Canada, are performing validation assays of the STP-chip using controlled

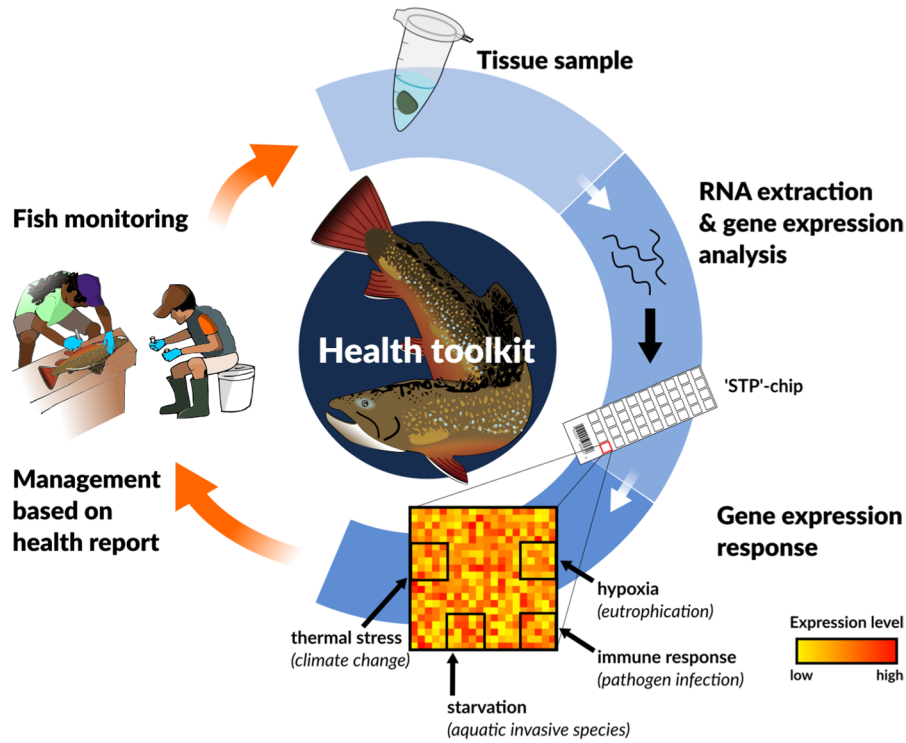


**Fig. 1** Genomics health toolkit in action. Resource-sector users sample fish tissue (fins, gill, liver, muscle) as part of their routine monitoring program. Tissue is sent to laboratories where samples are processed, and RNA extracted and prepared for transcriptomic analysis. Expression levels of a set of universal-health genes are measured from the Stress-response Transcription Profile (STP)-chip; and values interpreted and translated into a health report. Results are then delivered to resource users for integration into their assessment, conservation and management programs

challenges in the lab and in the field. While still a work in progress (e.g., the final list of genes included on the STP-chip will depend on the outcome of the validation trials), the individual qPCR assay details will be made public at https://gen-fish.ca. Although the GEN-FISH Network will initially provide data analyses and interpretation, ultimately individual research-, government-, and commercial (consultant) labs will provide formal health reports that would include reliability estimates (based on repeatability analyses), to be used in the assessment, management, and conservation of their fish(eries) resources. STP-chips, whether developed by GEN-FISH or not, have the potential to minimize the technological and resource limitations of conventional fish health assessment (e.g. necropsies, disease and stress challenges) and foster better supplementation of commercial, recreational, and at-risk fish stocks. The chips also have important applications in aquaculture for determining the transcriptomic profile of important performance and production traits under various culture practices (Canario et al. 2008).

## Benefits and costs of the STP-chip toolkit for practitioners

Whether resource-sector users adopt novel technologies for health assessment, such as the STP-chip toolkit, depends on the perceived benefits and costs of the tool, which can be unique to each user. The following section provides seven case studies from the perspectives of an Indigenous-use rights holder and three Canadian resource-use sectors: industry (i.e., environmental consultants, commercial aquaculture), environmental NGO, and government. Each contribution represents a case study specific to a resource-sector user; and highlights the potential advantages of using the STP-chip within the field, as well as identifies unique potential barriers to its adoption. Collectively, these case studies suggest a suite of best-practices needed to facilitate widespread use of the transcriptomic toolkit.

### Case study 1: indigenous perspectives and best practices

There are many ways in which Indigenous Nations and organizations are engaging with fisheries in terms of research, commercial, traditional, and recreational use (Reid et al. 2021). Increased knowledge of the mechanisms of harm to fish populations could support both Indigenous goals and priorities, as well as reinforce communities' concerns regarding negative impacts to a highly valued resource. Knowledge gained from the use of transcriptomics could support calls for further study into cumulative stresses or sublethal responses in fish populations as a result of stressors related to industrial, agricultural, commercial, or residential development. Important stressors include increases in water temperature, entrainment and impingement, pollution, loss of or destruction of critical habitat(s), introduction of invasive species and accumulation of microplastics (Desforges et al. 2022).

Another potential benefit of a transcriptomics toolkit could be as an early warning system for climate-related stress responses in certain species (e.g., to increasing water temperatures, increased intensity of storm events, introduced and/or invasive species and damage to or loss of habitat)(Brosset et al. 2021). The ability to identify species-specific threats, especially to species of concern or value (cultural and/or economic) to the community could be extremely beneficial. This knowledge could provide communities with the potential to support early adoption of protective measures for key species and aid in recovery actions by providing baseline data on several health variables (e.g., chronic stress, immune function, aerobic scope; Brosset et al. 2021). Comparing baseline data to emerging trends has the potential to support calls for higher environmental protections and standards, as well as the implementation of adaptive strategies.

Data collected from the use of transcriptomics could be compared or added to the wealth of Traditional Ecological Knowledge (TEK) and community-based observations of fish stressors. This could add important detail to interviews with local fishers about changes to species and fish communities over time. A transcriptomics toolkit could also provide valuable data on (further) loss of critical habitat for key species such as the Lake sturgeon (*Acipenser fulvescens*), which could help communities create and implement strategic resource-management plans. This would be especially critical for those who stand to lose livelihoods and to prevent the loss of species of cultural importance (e.g., white sturgeon (*Acipenser transmontanus*).

In the actualization of the transcriptomics tool in the form of an STP-chip, simply sharing a handbook or toolkit would do little to support communities in benefiting from this technology without first engaging with communities to understand needs and concerns, as well as working to understand and address barriers. Care should be taken to ensure that the adoption of this technology supports precautionary approaches and is not used to negatively impact treaty rights in any way. It is important to gain an understanding of Indigenous worldviews, cultural differences, and potential structural barriers at the outset of any project, partnership or collaboration with Indigenous groups (e.g. language considerations, protocols, difference in timelines, differing priorities and priority species). It is also important to understand that each Indigenous Nation represents its own government, and people. Work done with one community cannot necessarily be transferred to, and adopted by, another. Consequently, time, energy, and commitment are needed to understand the needs and concerns of each community. However, it is possible that developing strong relations with one nation will, in turn, strengthen processes and aid in the development of best practices for other groups.

One best-practices approach may be to consider how STP-chip processes and procedures could be incorporated into a training program for Indigenous users. There may be hesitation to adopt a new technology where the data are stored outside of the community or have the potential to impact not only economic livelihoods, but treaty rights. In general, the first step of connecting with a community would be to approach Chief and Council and/or the Environment Office. After consideration and review of a project proposal, depending on the nature of what is being decided, the community may wish to consult with elders, youth, and different community groups. It is likely that there would need to resolve infrastructure, training, buy in, education and data management issues before adopting any toolbox that is created without community input.

Case study 2: toxicity thresholds and effects—science versus practice, environmental consulting

In many environmental contaminant situations such as coal mining, selenium has become a primary element of concern because of its bioaccumulative nature in food webs (Schneider et al. 2015). Studies have raised concerns about selenium effects on aquatic resources in both the US (e.g., Southeastern Idaho) and western Canada (e.g., British Columbia; Hamilton 2004), with fishes generally considered one of the most sensitive taxa to selenium in aquatic systems (Khan et al. 2017). The scientific community agrees that tissue selenium level is the most reliable indicator of toxic effects in the field due to its bioaccumulation from diet-borne exposures (Janz et al. 2010; DeForest and Adams 2011). In recent years, non-governmental experts (consulting scientists) have proposed higher site- and species-specific selenium thresholds in diet and tissue relative to those broadly defined by government. This difference in opinion is due, in part, to the selection of datasets and caveats from various scientific literature sources used to develop adequate guidelines (Lemly and Skorupa 2007; McDonald and Chapman 2009; Ohlendorf et al. 2008; Janz et al. 2010; DeForest and Adams 2011). This illustrates the critical importance of considering other factors, such as environmental and cellular, when investigating potential selenium toxicity in fishes.

Relative species sensitivities are not well understood, but established linkages between the molecular or cellular mechanisms of selenium toxicity (i.e., oxidative stress), effects on individuals (i.e., teratogenic deformities), and acute adverse effects on populations and community structure provide clear examples of ecotoxicological cause-effect relationships between exposure and altered population dynamics (Janz et al. 2010). Given the ongoing debate surrounding the application of a generic versus site- or species-specific selenium tissue benchmark, a next logical step is to develop field and associated lab studies that directly relate selenium toxicity effects to the internal selenium concentration in the organism. Transcriptomics offers the potential to improve scientific understanding of selenium toxicity in real-world scenarios by revealing toxicant-responsive genes and identifying unique expression profiles. For example, Janz et al. (2010) described the toxicodynamic process of selenium incorporation into vitellgenin (VTG; via vitellogenesis), a phospholipid glycoprotein incorporated into developing ovarian follicles (Kime 1998), that is then enzymatically cleaved into primary yolk proteins (Arukwe and Goksoyr 2003) that bind selenium in fishes (Kroll and Doroshov 1991). Monitoring the expression response of genes associated with

VTG could offer a valuable tool for early detection of selenium transfer to eggs or ovary, where potential risk of population-level effects, including recruitment failure, could be detected early.

From a regulatory standpoint, the effective application of transcriptomics could bring added certainty to decision-making when considering more liberal, site-specific aqueous selenium benchmarks. Recent studies (e.g., Pacitti et al. 2016) have indeed applied transcriptomic technology to evaluate rainbow trout (*Oncorhynchus mykiss*) antiviral response to selenium supplementation in an aquaculture setting. Thus, the application in natural aquatic environments holds real promise. Regulators can leverage the "precautionary principle" in contrasting ways as a means to hold steady on applying an industry-wide standard or blocking it even in the presence of strong science-supporting alternatives (e.g., Saltelli et al. 2022; Viatori 2019). Transcriptomics has the potential to break those barriers of uncertainty and offer regulators more confidence to make key industry decisions. As with other recent 'innovative tools' (e.g., environmental DNA), decision-makers appear open to new approaches to assessing large infrastructure projects, as long as the method is backed by sound science and validation (Jerde 2021).

### Case study 3: qualification vs. quantification of habitat alteration effects, environmental consulting

Characterization of aquatic ecosystems by private-sector consultants as part of environmental assessments for infrastructure development projects are typically subjective and rely on varied qualitative information and data (e.g. substrate composition, macrophyte abundance, flow morphology, etc.) to determine current functioning and, by extension, the sensitivity of a particular fishery (Dufour, pers. obs.). This evaluation process relies on information that may be outdated and lacks specificity to enable an accurate understanding of the fishery; namely, environmental pressures and stressor-related effects on fish species. The ability to integrate a scientific process as a part of this evaluation through use of transcriptomics technologies can provide end-users with the ability to understand and identify unique system stressors (e.g. turbidity, temperature, pollutants, etc.), direct project planning and design, and implement effective mitigation strategies during construction to break project cause-stressor linkages and thus minimize impacts.

The identification of unique system stressors can also challenge conventional methods employed as part of compliance inspections during active project construction. Typical inspections focus on visual observations of control/protection measures and often focus solely on controlling potential sediment intrusion into the system (B. Dufour, pers. obs.). The use of transcriptomic technologies can provide an extension to these inspections by providing a quantitative evaluation of fish responses to active construction stressors (e.g. changes in turbidity, temperature and flow). The use of transcriptomic technologies during active construction can also increase our understanding of construction impacts on fishes and aquatic ecosystems, particularly indirect impacts not currently well understood and where minimal existing information exists (e.g., vibration, noise, water quality). Characterizing indirect stressors and responses can increase knowledge of these relationship impacts and contribute to more effective management and mitigation of effects on fishes and fish habitat during construction projects.

In a competitive marketplace, the cost of service is often a significant contributing factor in successful procurement of consultant work, in both public and private sectors. The feasibility of adopting any new technology requires an evaluation of cost of implementation versus pursuit success, while still ensuring meaningful and purposeful data can be generated. Ensuring that transcriptomic technologies are not cost prohibitive is paramount to their success and early adoption by private sector end-users. Conversely, in a marketplace not solely driven by cost, highlighting new technologies as innovative approaches and value-added services can be advantageous in the proposal evaluation process and enable proponents the ability to leverage these new technologies by supporting and complementing their fisheries management and watershed policies and initiatives.

Limitations to the use of data generated through transcriptomic technologies may be a barrier to adoption and negatively affect marketability for private sector end-users. The ability to incorporate these data into larger watershed protection and management plans likely does not currently exist. The integration of transcriptomic data by fisheries and watershed managers requires a cooperative effort and acceptance

across multiple jurisdictions, including consultants, proponents, provincial regulatory agencies, and municipal level regulators.

The acceptance of transcriptomic technologies by end-user consultants has the potential to be transformative to private-sector industry that, in the past, has relied on practices that are static and subjective. Leveraging the peer-reviewed, scientific approach employed by academia can provide valid and reliable data to increase our understanding and protection of fisheries in a landscape where development stressors are only becoming more complex (e.g., Lacaze et al. 2019).

### Case study 4: freshwater-adapted pacific salmon strains, commercial aquaculture

Canada is ideally situated for a vibrant and fast-growing finfish aquaculture industry, yet production has plateaued (Chopin 2015). The vast majority of that production is marine culture based and, indeed, Yellow Island Aquaculture Ltd. ("YIAL") primarily markets sea-cage reared Chinook salmon (*Oncorhynchus tshawytscha*). However, as regulations and restrictions on marine open-cage culture become more stringent due to concerns over escapement (Bjørndal and Tusvik 2019), land-based culture of freshwater and marine finfish species is being actively explored (Davidson et al. 2016; Bjørndal and Tusvik 2019). The marine finfish industry is thus actively pursuing the development of normally marine-production species best suited to freshwater culture for full life-cycle rearing.

Of specific concern is the performance of the obligate anadromous Chinook salmon under full life-cycle fresh water. Unlike Atlantic salmon (*Salmo salar*) and many other salmonids, Chinook salmon do not have naturally occurring land-locked populations or life histories within anadromous populations (Healey 1991). YIAL is thus experimenting with family-level breeding trials in full freshwater recirculating rearing conditions. While these trials are promising, using transcriptional profiling to accelerate the process of developing a recirculated freshwater adapted strain of Chinook salmon is an exciting prospect and would open up the commercial freshwater culture of this iconic species. Barriers to the adoption of transcriptional profiling can be categorized into: (1) cost; (2) turn-around time for results; (3) accuracy /reliability; and, (4) acceptance by government regulators.

The two primary applications of the STP-chip for companies such as YIAL would be to: (1) select appropriate mature fish for breeding to improve performance under recirculating freshwater rearing conditions, and (2) routinely monitor fish health and performance (e.g., Føre et al. 2018). Logistically, producers would need to have access to transcriptional profiling at costs low enough for relatively large numbers of fish to be tested, plus the process should be non-lethal. Furthermore, the turn-around time for the profiling results would have to be less than 30 days for the data to be of maximal value since fish grow quickly and the relevance of transcriptional data would become increasingly weak especially early in the growth cycle (J. Heath, pers. obs.). Of perhaps greater concern are the related issues of accuracy/reliability and acceptance of the outcomes to government regulators. Transcriptional profiling for salmon health and performance would have to provide results that would be closely correlated with health and performance (e.g. growth, feed conversion). The specific challenge would thus be to "validate" a gene panel that would be appropriate to reflect Chinook salmon status. While conventional fish-health technologies (e.g. microbial assays, biopsies, necropsies) have limitations, they are well-accepted by fish-health personnel, whereas gene transcription profiles are not (J.W. Heath, pers. obs.). Thus, not only would the STP-chip have to be validated in peer-reviewed literature, a concerted effort to educate through clear communication—free from vested interest—would be essential.

### Case study 5: assessing restoration success, environmental charities

Globally, environmental non-government organizations (ENGOs) and charities lead, or collaborate on, many applied fish conservation projects. These practitioners turn private financial contributions and government grants into on-the-ground action using the best available knowledge to address local threats to fishes and fish habitat. Increasingly, ENGO groups are seeking to assess the efficacy of the restoration and mitigation techniques being applied, which requires high-quality monitoring data.

A challenge faced by ENGOs is that the collection of monitoring data to assess conservation projects

is labour intensive, requires a professional capacity lacking in smaller organizations and, in the case of population-level data, is obtainable only after several years of data collection, which rarely matches funding and reporting timelines. To this end, fish conservation practitioners stand to benefit from the development of easy-to-use transcriptomic tools capable of assessing species' response to stressors, or conversely, their response to the restoration of habitats or the mitigation of stressors. Additionally, the ability to assess adaptive potential of populations may highlight those populations with the greatest potential to respond to recovery actions.

Another challenge facing conservation practitioners is identifying the most impactful stressor when multiple stressors influence populations (e.g. Hale et al. 2017; O'Brien et al. 2019). Determining the relative importance of each stressor may dictate which remedial actions should be applied and how resources should be allocated. Transcriptomics may be a valuable tool in this regard as there is evidence that gene expression biomarkers can highlight specific stressors after fish have been exposed to a suite of stressors (Houde et al. 2019), potentially including multifactorial effects such as simultaneous exposure to multiple pathogens (Krol et al. 2020).

Arguably, one of the most attractive promises of transcriptomic tools in general, and the STP-chip specifically, is the potential to support the adaptive management of conservation projects (Flanagan et al. 2018). Rather than waiting for populations to respond to conservation action as an indication of how recovery actions mitigate presumed stressors, ENGOs will be able to rapidly assess the adaptive potential of populations to face habitat change (Connon et al. 2018) or, conversely, respond to habitat restoration. Users will then be able to refine their approach to recovery actions appropriately.

How ENGOs will manage the large datasets sure to arise from transcriptomic tools warrants consideration. Making these datasets available to the broader community will be important for the development of transcriptomic tools and the downstream conservation ramifications. Many ENGOs face high turnover in staff and management and, thus, secure data warehousing is imperative (E. Halfyard, pers. obs.). It may be prudent to learn from data managers in other big data fields, such as biotelemetry (e.g. Iverson et al. 2019).

Case study 6: fisheries management, governmental commissions

Fishery managers have long recognized the value and potential application of genetics to Great Lakes fisheries management (Billingsley 1981). "Omics" tools continue to evolve in scope and application, becoming more powerful discriminators with greater resolution (Bernatchez et al. 2017; Casey et al. 2016). Current management challenges that could be informed by a 'transcriptomics tool kit,' include: (1) native fish restoration; (2) responses of populations to changing environments; and, (3) effects of invasive species on population health and sustainability.

*Restoration*

Fishery managers have been engaged for more than a half-century to re-establish economically and ecologically important freshwater fishes (e.g., lake charr *Salvelinus namaycush* (Muir et al. 2012) and ciscoes *Coregonus* spp. (Eshenroder and Krueger 2002)). New transcriptomics tools could help address outstanding issues such as differential strain performance of stocked lake charr (Larson et al. 2021; Scribner et al. 2018). Indeed, rapid assessment techniques for strain performance and adaptation could strengthen stocking decisions (e.g., He et al. 2015), inform development of superior captive- breeding approaches (Bernatchez et al. 2017) and, ultimately, contribute to economical use of hatchery resources. Fishery managers are also interested in how and where shifts in growth or maturation rates affect recruitment in response to fishing pressure (Dunlop et al. 2015; 2018), which is another potential area that could be informed by a transcriptomics tool kit (He et al. 2015).

*Changing environments*

Native deep-water species such as those in the Laurentian Great Lakes have narrow metabolic requirements for cold, well-oxygenated water, which limits their physiological scope for activity (Evans 2007). Changing aquatic thermal regimes, as is occurring rapidly in Lake Superior (Cline et al. 2013) and projected to occur in all Great Lakes (Collingsworth et al. 2017) have implications for dispersal, habitat availability, growth, niche partitioning, and

ultimately, recruitment for Great Lakes fish communities. Transcriptomics could help identify the ability of populations to adapt to such change or where habitat conservation or restoration activities are required. For example, the Lake Erie Committee (GLFC 1981) is currently evaluating the feasibility of cisco *Coregonus artedi* restoration given a changing environment. An understanding of the adaptive capacity of cisco could help inform the Lake Erie Committee's decision to pursue restoration objectives.

### *Invasive species*

The sea lamprey (*Petromyzon marinus*), which is partially responsible for the decimation of some Great Lakes fish populations (Muir et al. 2012), has been controlled by pesticides for 60 years (Siefkes et al. 2012), but remains a threat. A transcriptomics toolkit could potentially help identify adaptive responses by invasive species to control measures. For example, of 336 genes differentially expressed in lampricide-treated sea lamprey populations compared to native non-treated populations and experimental controls, many of the up-regulated genes were functionally linked to uncoupling oxidative phosphorylation, the pesticide's mode of action (Yin et al. 2021). A transcriptomics toolkit could potentially test the efficacy of lampricides by identifying which sea lamprey populations are more robust to lampricide exposure.

### *Implementation*

While advancements in omics have revolutionized the science, several important challenges remain to the integration of new omics methods, results, and guidance into Great Lakes fishery management. A standardized and repeatable means of interpreting transcriptomics results could strengthen commitment to implementation by mangers. A technical transcriptomics toolkit would likewise require a well-conceived plan for transferring the science in plain language to the management community, such that new knowledge can be implemented and transcriptomics-based management decisions effectively communicated to stakeholders. A final challenge to adoption of a transcriptomics toolkit by fishery managers is that results must be interpreted in appropriate spatio-temporal, ecological, and geological contexts and presented in a relevant social context recognizing diverse stakeholder values.

### Case study 7: recovery potential assessment of species at risk, federal government

Within the Government of Canada, Fisheries and Oceans (DFO) is the lead for managing fisheries, oceans and freshwater resources. This mandate includes a commitment to ensuring healthy and sustainable aquatic ecosystems, including the recovery of at-risk aquatic species, which is supported by decision-making processes based on sound science. After an aquatic species is assessed as Threatened, Endangered or Extirpated, DFO undertakes a number of actions to support the implementation of the *Species at Risk Act* (SARA). Many of these actions require scientific information on the current status of the species, threats to its survival and recovery, and the feasibility of recovery. Usually, this information is incorporated into a Recovery Potential Assessment (RPA) (DFO 2007) and then into advice for the design of long-term monitoring programs to inform recovery and management decisions. These programs need to not only fill gaps in the knowledge of the species abundance and distribution, but they would also ideally be able to contribute information for predictions on how the species may respond to changes in their environment.

Transcriptomic tools offer great potential for monitoring programs designed for assessing baseline information and ecological responses in aquatic species at risk (Connon et al. 2018; Bernos et al. 2020), especially as most of these species have limited genomic resources (Veldhoen et al. 2012). Many of the at-risk designated units of freshwater fish in central Canada, such as River Darter (*Percina shumardi;* DFO 2019), Bull Trout (*Salvelinus confluentus*; DFO 2017), and Lake Sturgeon (*Acipenser fulvescens*, DFO 2010a, b), have major threats to their recovery that include: habitat alteration/degradation/fragmentation; mortality, injury or reduced survival related to fishing activities; water quality changes; and climate change. It has been shown that variation in expression of ecologically important genes can provide information about the potential of small, at-risk populations of freshwater fishes to respond to rapid climate change and habitat degradation (Brauer et al. 2017). Furthermore, differences in water quality across sites

is an important factor influencing expression variation potentially related to metabolic and reproductive traits within and across populations (Grummer et al. 2019). Therefore, inclusion of this type of gene expression response in a health toolkit could also provide useful information on the adaptive potential of aquatic species at risk of interest to DFO. Another advantage to monitoring at-risk species is that transcriptomic data can be collected from a small tissue biopsy using non-lethal methods (Veldhoen et al. 2012, 2014). Examples for how transcriptomic data may be used in a predictive fashion already exist for commercial (e.g. Pacific salmons—*Oncorhynchus* spp. (Miller et al. 2011)) and subsistence (e.g. Arctic grayling -*Thymallus arcticus* (Veldhoen et al. 2014)) fisheries of importance to DFO.

Incorporating transcriptomics results into formal science advice for RPAs, risk assessments and management decisions remains challenging. Connon et al. (2018) highlighted the main issues; specifically, translating complex information into clear and understandable advice (especially when involving Indigenous community partners), establishing a measure of confidence in the results, and defining thresholds that would trigger a management or policy change. Furthermore, managers often want to see a period of verification of results over time and validation of the "cause and effect" using both laboratory and field experiments. These needs can be very time consuming and costly, and are often impeded by the availability of stable funding.

## Overcoming barriers to adoption

Barriers between knowledge and action are common with the so-called "knowledge-action" gap (Cooke et al. 2021), and is even more apparent when dealing with knowledge generated using novel technology (Nguyen et al. 2021). The decision of whether users adopt novel technologies is often complex and, therefore, the study of adoption must draw upon multidisciplinary knowledge generated through natural science, social science, ethics, and policy analyses. In economic theory, rational users' decisions are determined by a cost–benefit framework such that, one chooses to adopt a new practice as long as the benefit of adoption outweighs the cost. (Mas-Colell et al. 1995). However, the real world has witnessed numerous advantageous innovations that failed to be widely adopted (Li et al. 2021). This intractability becomes especially problematic when the innovation process requires large-scale human capital and technological inputs, and the end products are scientifically proven to be beneficial. Adoption decisions of a new technology can be affected by lack of scientific understanding, and influenced by communication and social learning that, in turn, can change users' risk perception and preferences. The innovation-adoption lifecyle reveals that innovations spread in a predictable manner—first being embraced by early adopters before wider acceptance and use (Rogers 1962). Anticipated market response, as well as various behavioral, economic and policy instruments, also play important roles in facilitating or obstructing pathways to technology adoption. Other key drivers of adoption and rejection (Streletskaya et al. 2020) include emerging risks for which information is not readily available (e.g., AIS, climate change) and decisions that must be made despite uncertainty associated with diverse data-gathering approaches (Huang et al. 2011).

Robust selection of technologies must, therefore, consider multiple criteria. These criteria can influence the decision-makers' goals and often reflect priorities that vary among users who represent different fish(ery) resource-use sectors. Investigating how best to achieve efficient adoption by users can be informed by social-science studies and methodologies, such as incentive-compatible economic experiments, individual- and focus-group surveys, and empirical analysis. For example, behavioural instruments—such as social norm nudging, anchoring, and information framing—can be effective in increasing the likelihood of adoption by end-users (Li et al. 2021). In addition, survey and focus-group studies, community consultation, and trust-building can help identify users' decision-making factors regarding technology acceptance and adoption preferences. These methods can also identify users who are likely to be early adopters—allowing one to then model the effectiveness of subsidizing early-stage adoption. Such multi-criteria decision-making encourages users to evaluate the potential value of the transcriptomic toolkit by juxtaposing economic costs of adoption alongside the expected allocation of effort, level of data certainty, social acceptance, and known expertise with conventional approaches. These studies provide critical

information that collectively assist users in assessing the utility of adopting genomic toolkits for their own objectives, reveal the barriers to potential adoption and other concerns, and help promote ex-ante mitigation strategies. By facilitating partnership and dynamical communication between genomicists, social scientists, and users from the outset using a knowledge co-production framework (Cooke et al. 2020), this process can ultimately allow users to better orientate both their current and future benefit–cost priorities based on a bio-socioeconomic representation of freshwater fish(eries) and fish culture sustainability.

## Conclusion

In this paper we presented an innovative genomics approach to develop transcriptomic profile arrays that will allow resource-sector to rapidly and economically measure gene expression responses of individual fish to a number of important biological and environmental stressors such as water-quality stressors, aquatic invasive species, and climate change. While the genomic technology behind the STP-chip concept is exciting and promising, there are certainly risks associated with its commercial actualization, as conveyed through the case studies. We highlight the need for: (i) reliability of universal transcriptional profiling across spatio-temporal gradients; (ii) quantifiable evidence for return on investments of time and money with measurable improvements in fisheries and fish culture yields; (iii) general acceptance across multiple jurisdictions and knowledge-holders; (iv) big-data management and accessibility requirements; and, (v) integration and incorporation into training, infrastructure, and best-practices. By co-engaging with multiple resource-sector users from the outset to address the costs associated with the adoption of genomics tools, one can facilitate cost-effective management of natural resources for Indigenous governments and communities, ENGOs, fishery managers, environmental consultants and captive-breeding facility operators; and ultimately protect the livelihoods and ways of life for all. This paper describes the costs and benefits of genomics-based technology in fisheries applications; however, future work is needed to broaden the scope of such innovation. This paper represents the first step.

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**Declarations**

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