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SYMPOSIUM

Linking Landscape-Scale Disturbances to Stress and Condition of Fish: Implications for Restoration and Conservation

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Synopsis Humans have dramatically altered landscapes as a result of urban and agricultural development, which has led to decreases in the quality and quantity of habitats for animals. This is particularly the case for freshwater fish that reside in fluvial systems, given that changes to adjacent lands have direct impacts on the structure and function of watersheds. Because choices of habitat have physiological consequences for organisms, animals that occupy sub-optimal habitats may experience increased expenditure of energy or homeostatic overload that can cause negative outcomes for individuals and populations. With the imperiled and threatened status of many freshwater fish, there is a critical need to define relationships between land use, quality of the habitat, and physiological performance for resident fish as an aid to restoration and management. Here, we synthesize existing literature to relate variation in land use at the scale of watersheds to the physiological status of resident fish. This examination revealed that landscape-level disturbances can influence a host of physiological properties of resident fishes, ranging from cellular and genomic levels to the hormonal and whole-animal levels. More importantly, these physiological responses have been integrated into traditional field-based monitoring protocols to provide a mechanistic understanding of how organisms interact with their environment, and to enhance restoration. We also generated a conceptual model that provides a basis for relating landscape-level changes to physiological responses in fish. We conclude that physiological sampling of resident fish has the potential to assess the effects of landscape-scale disturbances on freshwater fish and to enhance restoration and conservation.

Introduction

As a result of a demand for resources and associated development, humans have altered landscapes dramatically (Vitousek et al. 1997), which has led to loss of biodiversity and ecosystem services (Turner et al. 2007). Some of the more insidious alterations of landscapes have occurred in the form of extraction of resources, urbanization, construction of dams, drainage of wetlands, and the conversion of natural areas to agricultural lands, which collectively influence composition and connectivity across landscapes (Dale et al. 2000; Foley et al. 2005). In addition, pollution from a range of sources degrades the quality of air, land, and water (e.g., Nriagu and Pacyna 1988; Carpenter et al. 1998), while climatic change has further altered temperature, precipitation, pH, and other environmental factors (IPCC 2014). Few (if any) ecosystems have been left untouched by the need to harvest and harness resources for human development (Sala et al. 2000), and the amount and quality of natural landscapes is expected to decline further with continued growth of the human population, multiple stressors (Foley et al. 2005), and consumption of resources (Rees 1999).

Traditionally, ecosystem health has been investigated by broad metrics at the scales of the

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population and community (Attrill and Depledge 1997; Rapport et al. 1998). Although these methods are useful in understanding how disturbances of landscapes affect species' distributions, these broad-scope metrics can be slow to respond to stressors, and do not provide information on causal mechanisms. As such, there has been growing interest in utilizing physiological tools to understand how ecosystem health is affected in the short-term, as well as to understand the mechanisms driving changes in the population by landscape-scale disturbances. Physiological tools are sensitive compared with most population-level tools, as they can be sublethal and graded, rather than binary (presence/absence). Physiological tools also allow for more immediate, short-term assessments of individual health or condition, as opposed to longer-term population or community metrics such as changes in abundance or lowered reproductive output (e.g., Adams et al. 2002; Adams and Ham 2011). In addition, the field of conservation physiology specifically addresses how conservation issues relate to organismal physiology, particularly with respect to stress (Wikelski and Cooke 2006; Cooke et al. 2013). The use of physiological metrics to assist in restoring habitat recently has gained attention from conservation practitioners and managers (Cooke and Suski 2008; Cooke and O'Connor 2010).

Based on this background, the overall goal of this review was to identify how physiological tools could be used to study the status of freshwater fish in relation to disturbances of landscapes. Specifically, this review aimed to: (1) understand how landscape-scale disturbances affect freshwater systems and the traditional, broad-scale methods being used to assess ecosystem health, (2) define how changes to landscapes have the potential to influence physiological properties of resident freshwater fish, (3) provide an overview of past studies that have quantified landscape-scale impacts on the physiological properties of fish, (4) generate a conceptual model linking landscape-level disturbances to physiological processes in fish, and finally (5) explore the potential for linking physiological properties of fish to "traditional" assessments of changes in individuals and populations due to disturbances of landscapes.

Resident freshwater fish are at particular risk

The freshwater biome, which is habitat for 40% of the globe's fish biodiversity (Lundberg et al. 2000), is particularly sensitive to landscape-level changes (Ricciardi and Rasmussen 1999; Sala et al. 2000; Meybeck 2004). Indeed, analyses have shown that freshwater ecosystems are degrading at a faster rate than terrestrial ecosystems (Ricciardi and Rasmussen 1999). Because freshwater habitats are so closely linked to the surrounding watershed, land cover, underlying geology, topography, land use, and climatic variables, they all can dictate the quality of water and habitat (Hynes 1975; Allan 2004). Freshwater systems, and the organisms that inhabit them, are particularly vulnerable to perturbations in the landscape.

Globally, freshwater fishes are among the taxa most at risk, due in part to the landscape-level changes described above. Karr et al. (1985) described a decline of between 43% and 67% of fish species in two North American Rivers (Illinois and Maumee Rivers). More recent studies suggest that 39% of North American fish species are imperiled (Jelks et al. 2006), 25% of evaluated global freshwater fish are at risk of extinction (Vié et al. 2009), and an estimated four North American freshwater species of fish will be lost every decade (Ricciardi and Rasmussen 1999). Degradation of the habitat appears to be the primary cause of the decline of freshwater species of fish, and Miller et al. (1989) found that, in 73% of observed declines, physical alteration of the habitat was the most frequently cited causal factor. Additional studies have listed several other landscape-related variables as the main reasons freshwater fish are threatened globally, including (but not limited to) hydrologic alterations, poor quality of water, climatic change, changes in the landscape (e.g., draining of wetlands), and modification of flow (Carpenter et al. 1992; Dudgeon et al. 2006). Several reviews on the conservation of freshwater fish have identified landscape-level changes as a primary factor for declines in populations and hence in losses of species (e.g., Moyle and Leidy 1992; Maitland 1995; Richter et al. 1997; Collares-Pereira and Cowx 2004). Clearly, understanding the responses of fish to changes in the landscape (i.e., landscape ecology) can assist with predictions of how species and populations will be impacted.

Landscape-level disturbances to freshwater systems

Anthropogenic developments are changing the physics, chemistry, and hydrology of aquatic ecosystems and their surrounding landscapes (Hynes 1975; Dudgeon et al. 2006). Arguably the most common large-scale change to landscapes is deforestation, as it occurs during virtually all development projects. Removal of trees from riparian zones in particular has been linked to poor quality of the habitat,

including increases in temperature of the water, run-off, sedimentation rates, and input of nutrients (Allan 2004; Sweeney et al. 2004; Dudgeon et al. 2006). Depending on the extent of deforestation and resiliency of the affected system, these impacts can alter the quality of the habitat, change floral and faunal biodiversity, and shift trophic dynamics (Rahel 2002; Sutherland et al. 2002). Fragmentation of habitats and alterations in the flow of water can have equally damaging results; the construction of dams, urbanization (roads, commercial and residential developments) (Walsh et al. 2005), and agriculture not only create physical barriers between habitats, but also can alter hydrological regimes along the entire watercourse (Poff et al. 1997; Bunn and Arthington 2002). Dykes and channelization of watercourses used to protect developments and provide irrigation have disconnected many rivers from their flood plains, thereby eliminating natural nutrient cycles and removing access to habitats important for foraging and reproduction (Aarts et al. 2004). Urbanization has further been implicated as one of the most damaging uses of land because of increased run-off, alteration of habitats, and point-source pollution from industry and urban effluent (Gergel et al. 2002; Miltner et al. 2004). To compound all of these factors, climatic change is predicted to increase not only global temperatures, but also the frequency and severity of extreme weather events (Easterling et al. 2000). Predicted flash flooding, drought, and extreme temperatures may push aquatic biota beyond physiological tolerances, particularly those species that are already living on the edge of their distributions. Collectively, these anthropogenic alterations to freshwater systems can degrade the quality of habitats, reduce connectivity, and limit resources (Allan 2004), thereby highlighting the importance of understanding the implications for fish residing in these freshwater systems.

Traditional methods used to assess the status of freshwater ecosystems

In general, landscape ecology investigates habitat-quality, connectivity, and organismal biology across spatial scales so as to understand how species are distributed (Wiens 1989, 1992). Typically, landscape ecologists relate landscape-patterns, often across a disturbance and/or natural gradient, to species' distributions and abundances (Mazerolle and Villard 1999; Elith and Leathwick 2009). Landscape ecology is transferable to freshwater ecosystems and fauna (Schlosser 1991; Robinson et al. 2002; Wiens 2002), and fish ecologists have demonstrated that the distributions and assemblages of fish are related to a range of watershed characteristics and land-use patterns (e.g., Barton et al. 1985; Tonn et al. 1990; Jackson et al. 2001; Fausch et al. 2002; Allan 2004; Chu et al. 2015). Traditionally, studies have relied on field-derived presence/absence data to assess species' abundance, richness, and evenness (see previously referenced papers above), and/or biotic integrity (Karr et al. 1986; Karr 1991; Wang et al. 1997), an index that combines several attributes of fish assemblages (e.g., number of native species; percent omnivores). Other traditional methods include measuring size-distributions and size-at-age (Summerfelt and Hall 1987). However, these traditional metrics are normally broad in spatial and temporal scale, and comparisons often are made by relating coarsely collected biological data to even coarser landscape data that may or may not be paired (i.e., biological samples collected at one place at one time, and landscape data collected elsewhere or at another time). Furthermore, sampling effort is typically minimal across both space and time, resulting in datasets that can be difficult to interpret. Such traditional sampling methods have been useful for broad-scale understanding of how species are distributed across landscapes, and how distributions are influenced by watershed characteristics and land use. Unfortunately, the interpretation of results from these studies can be misleading, slow to respond to changes in system function, and provide no definitive information on causal mechanisms, should declines in species be identified. Therefore, using broad indices of fish assemblages and populations to understand how landscape-level changes relate to declines of freshwater fish is inherently difficult and not always informative.

In addition, the use of community-level and population-level metrics for understanding the landscape's effects on fish populations has been criticized for a number of reasons. Broadly, Rose (2000) outlined six issues relating to population-level outcomes with environmental quality, including detectability, complexity, reliance on predictions, community interactions, and sublethal and cumulative effects. Van Horne (1983) suggested that simple positive correlations are not sufficient for understanding the link between habitat-quality and species' density, as demographic data, such as survivorship and fecundity, are needed to fully understand the relationship. In studies on freshwater fish, a decoupling of metrics was observed in degraded habitats, as changes in species richness occurred but abundances were not altered (Lenat and Crawford

1994), and biotic responses cannot be fully explained by ''generalized stressor gradients'' (Davies and Jackson 2006). Changes in fish species' diversity and taxonomic distinctness do not always reflect anthropogenic disturbances (Heino et al. 2007), and models that rely on presence/absence data may be inaccurate because the absence of a species is potentially biased by sampling methods, e.g., species may not be truly absent from a habitat (MacKenzie 2005). Other issues related to using community/population-level metrics for understanding landscape-level effects are that detection occurs after the impact has occurred, and pre-impact (or historical) data are lacking in most studies (Ellis et al. 2012a). Clearly, attempting to relate landscape variables to data on species' abundances is challenging, making it difficult to understand how fish are impacted by degraded landscapes using ''traditional'' field-based monitoring. It would therefore be beneficial to develop novel tools and techniques, beyond coarse community-level and population-level metrics, that can be used to define how landscape-level changes impact freshwater fish.

Physiological tools

Recently, due to the challenges of relating broad-scale variables to data on species, populations, and communities, biologists have begun to focus on the processes or ''chain of effects'' that predict species' distributions and abundances (Helmuth 2009; Ellis et al. 2012a), essentially using physiological tools to provide metrics for assessing landscape-level changes to populations. Previously, Huey (1991) argued the importance of incorporating thermal habitats into the understanding of species' ecology, which, in the case of ectotherms (i.e., fish), is a strong driver of growth, metabolism, and reproduction. Habitat-use also influences stress and choice of food, along with interspecific and intraspecific interactions (Wiens et al. 1993), which, in turn, can influence metrics of reproduction and performance (Wingfield et al. 1997, 1998). Furthermore, physiological indicators across a range of taxa have now been related to temporal and spatial patterns in the environment (Porter et al. 2002; Somero 2005; Acevedo Seaman et al. 2006), and these relationships have resulted in the development of ''macrophysiology'' (the investigation and ecological implications of variation in physiological traits over large geographical and temporal scales; Chown et al. 2004). More importantly, variation in the characteristics of habitats have been shown to influence phenotypes and performance of organisms, which can drive reproductive success and genotypes, providing a link between habitats and fitness (physiology/life-history nexus; Ricklefs and Wikelski 2002). As such, to fully understand how landscape-level changes influence fish populations, there is a need to quantify how those changes influence the physiological responses of resident organisms. Development of physiological tools that relate variables to fish populations, particularly those under stress, can serve as an important link between landscape-scale disturbances and population-level declines prominent in many freshwater fish.

Expected physiological responses of fishes to landscape-scale disturbances

Typically, if fish perceive landscape-linked stressors (unpredictable and/or uncontrollable stimuli; Levine and Ursin 1991) that disturb homeostasis (McEwen and Wingfield 2003), fish enter a state of reactive homeostasis to return a (or multiple) physiological mediator(s) to a ''normal'' level (Romero et al. 2009). In response to short-term stressors, fish transiently increase levels of catecholamine and glucocorticoid (cortisol in teleost fish) in the plasma; increases in these hormones allow fish to maintain homeostasis in the face of challenges by increasing energy metabolism, facilitating movement by fueling muscles with oxygen and energy stores, and preventing damage to tissues (Wendelaar Bonga 1997; Barton 2002; Romero 2004). However, if stressors are severe or prolonged (i.e., upper end of the reactive homeostatic range), the fish enters a pathological state, which is also known as homeostatic overload (Romero et al. 2009). Homeostatic overload may negatively impact reproduction, immune-function, and growth, and can impair a fish's response to subsequent or additional stressors (reviewed by Wendelaar Bonga 1997; Romero 2004). Landscape-scale changes can be abrupt and fleeting (e.g., flash flooding from changes in land use), or gradual and long-term (e.g., summer anoxic zones from agricultural practices; exposure to contaminants). Therefore, landscape-level stressors may only cause physiological mediators to be within the reactive homeostatic range for a brief period of time, or may result in homeostatic overload. Ideally, linking the magnitude and suite of landscape stressors that lead to homeostatic overload would provide managers and restoration biologists with biomarkers and ultimately enable them to assess goals for conservation.

Landscape-scale disturbances result in three key changes to freshwater systems that can have negative

physiological consequences and potentially lead to homeostatic overload: reductions in quality of the habitat, decreased connectivity, and fewer resources (Fig. 1). For the purpose of this review, "habitat" is defined as the physical environment from the focal point of individual fish that is required to carry out its entire life cycle. Reductions in the parameters of habitat quality that previously have been found to influence the physiology of fish include changes in important abiotic factors such as temperature, pH, and dissolved oxygen, as well as exposure to contaminants and toxins (Barton et al. 2002). Decreased connectivity and/or increased fragmentation can lead to isolation (Morita and Yamamoto 2002), crowding (McCormick 2006), and limited habitat access (Junge et al. 2014). These outcomes have been shown to reduce growth rates (isolation; Morita et al. 2000), decrease feeding (crowding;

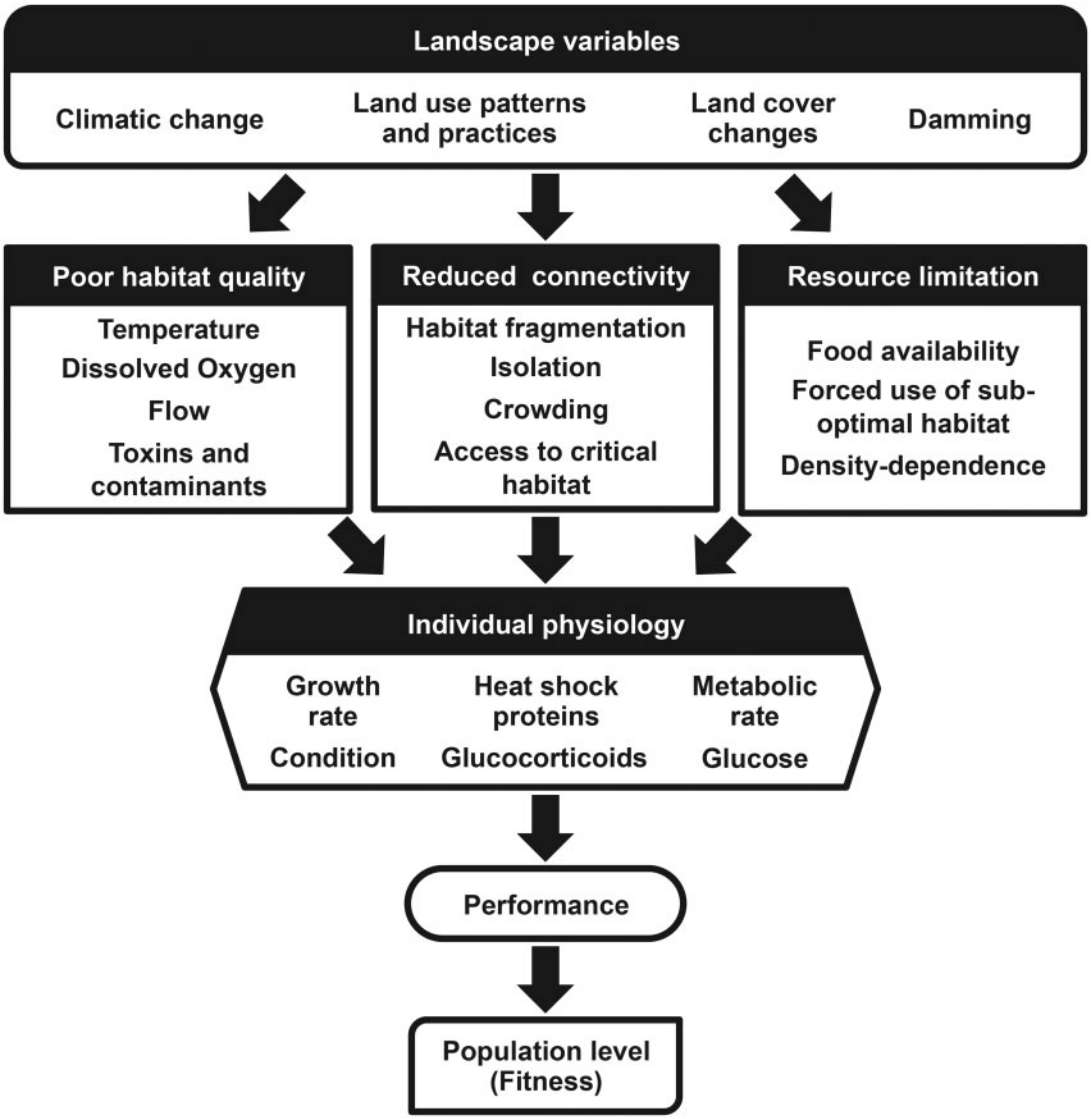

Fig. 1 A conceptual model of the link between landscape-level changes and physiological responses in fish. Landscape-level disturbances due to changes in land cover (e.g., deforestation), land-use patterns and practices (e.g., urbanization and agriculture), damming, and climatic change affect a number of variables in aquatic systems. Broadly, these landscape-scale changes lower quality of the habitat, reduce connectivity, and limit the availability of resources. Although the ultimate consequences for the population or community are of great importance (i.e., fitness and reproductive success), these broad-scale metrics may be insensitive, require a long-term dataset, and generally supply information only after a system has been significantly affected. Accordingly, an attractive level to investigate is the physiological responses of the individual (e.g., growth rate, condition, heat shock proteins, glucocorticoids, metabolic rate, and glucose), in which responses of fishes are directly linked to individual performance, and consequently are tied to reproductive success, fitness, and thus to population-level responses.

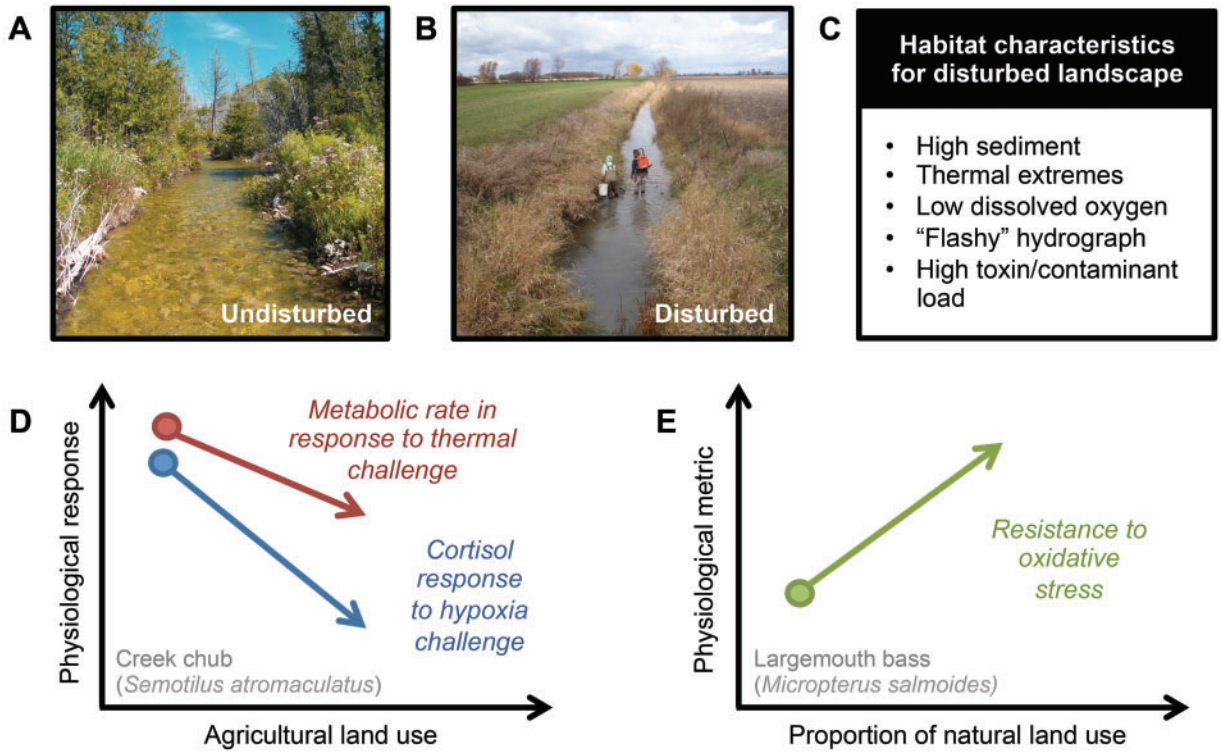

Fig. 2 Physiological consequences of landscape-scale disturbances in freshwater systems. Panels A and B represent freshwater streams within undisturbed and disturbed watersheds, respectively. Landscape-level disturbances disrupt a number of characteristics (C) that influence several physiological factors of resident fish. Two examples of altered physiological responses due to landscape-level disturbances are illustrated in panels D and E. Creek chub (*Semotilus atromaculatus*) sampled from streams within watersheds dominated by agriculture showed lower metabolic rates and an attenuated cortisol response following hypoxia and thermal challenges, relative to fish collected from watersheds dominated by forests (D; Blevins et al. 2013). In largemouth bass (*Micropterus salmoides*) sampled from watersheds spanning a range of land uses, a greater proportion of natural land in the watershed (forests, wetlands) resulted in an increased resistance to oxidative stress, thereby highlighting the importance of natural land uses in driving oxidative resistance in resident fish (E; King 2014). Photograph for panel A by J. Imhof and for panel B by G. King.

Wedemeyer 1976), increase expression of heat-shock proteins (crowding; Iwama et al. 1999), increase levels of cortisol and glucose in the plasma (crowding; Yin et al. 1995) and may force fish into sub-optimal environmental gradients, which promote non-optimal growth and alter metabolic rate (Rahel et al. 1996). Fewer resources can result in increased interspecific and intraspecific competition, as well as limited food, increased threat of predation, and, again, forced-use of sub-optimal habitat (Schlosser 1991). Physiologically, the ecological outcomes of fewer resources are known to increase starvation (Niimi 1972), expenditure of energy (Li and Brocksen 1977; Metcalfe 1986), and injury (Adams et al. 1998; MacLean et al. 2000) in fish. The degree to which all of these outcomes of landscape-level change influence physiological responses of fishes and the potential for homeostatic overload varies widely and depends on perception (i.e., spatial-scale at which an animal interacts with the environment; Olden et al. 2004). Few studies have explicitly quantified the relationship of landscape changes to fishes' physiology, and therefore, our understanding of how fish respond to a suite of changes (i.e., as opposed to studies performed in controlled settings where many variables can be controlled) that result from alterations of the landscape is limited.

Links between changes in the landscape and the physiological properties of fish

As previously outlined, physiological responses are useful when quantifying the impacts of stressors on individuals (Fig. 2), and can provide a sensitive and mechanistic basis for understanding such responses (Adams and Ham 2011). This concept of assessing physiological responses to landscape-level disturbances, however, has been relatively underutilized until recently (Young et al. 2006). For example, Adams and Ham (2011) evaluated the possibility of using physiological indicators (representing five

different functional responses and levels of biological organization) to assess the health status of resident fish in a polluted stream and the subsequent influence of a number of remedial actions. Overall, results from that study suggested that biochemical, physiological, bioenergetic, and nutritional indicators of redbreast sunfish (*Lepomis auritus*) in a previously disturbed stream began to resemble those indicators of fish from a reference stream following restoration of the habitat (Adams and Ham 2011). Interestingly, a set of bioindicators responded more readily to restoration, while other metrics were relatively unaffected by either disturbance or mediation, thereby highlighting the importance of assessing a variety of bioindicators at multiple levels of biological organization (Adams and Ham 2011). Similarly, Blevins et al. (2013) showed that creek chub (*Semotilus atromaculatus*) residing in forested watersheds did not differ in baseline physiological properties relative to creek chub from watersheds dominated by agriculture. However, upon being presented with thermal and hypoxic challenges, creek chub from forested landscapes displayed increasing sensitivity and elevated physiological responses relative to individuals from agricultural landscapes (Blevins et al. 2013). Adams et al. (2002) highlighted the importance of using an integrative approach that incorporates analyses of bioindicators across different temporal and spatial scales. Kim et al. (2014) exemplified this integrative approach, establishing the integrated health response (IHR_s) model. The IHR_s model uses a star-plot approach, combined with a comprehensive suite of physiological variables, to assess the state of an ecosystem, including low-level physiological parameters (biomarkers), high-level bioindicators, dose–response toxicity tests, and variables of the health of habitats. Using the IHR_s, Kim et al. (2014) showed that biomarkers and bioindicators of fish residing in the downstream reaches of a stream, and representing organizations from the cellular/biochemical to community levels, were negatively influenced by urban land use relative to headwater regions. This conclusion would not have been possible without incorporating molecular/cellular physiology with ''traditional'' sampling to evaluate disturbance in fish. Together, physiological data have been shown to provide useful information on resident fishes that can be predictive of organismal responses to challenges, and can be integrated with population-level monitoring for a valuable overview of how fish populations respond to landscape-level challenges.

It is not always feasible, or necessary, to generate long-term, population-level datasets if the goal is to assess short-term responses to landscape-level changes (e.g., determining whether recovery measures are effective). In such cases, physiological assessments may be highly valuable due to their fine temporal scale and their sensitive nature. In addition, as previously mentioned, long-term measurements (e.g., mortality, growth, and reproduction) are not necessarily ideal metrics to define the condition of populations because these effects are typically observed in already severely impacted ecosystems. Recent work by Nagrodski et al. (2013), Blevins et al. (2013, 2014), and King (2014) have all assessed the impact of different land-use practices (e.g., urban and agricultural use of land adjacent to streams) on fishes' physiological responses (e.g., responsiveness to environmental challenge; see Fig. 2). Collectively, these studies indicated that localized physiological acclimatization may play a role in allowing generalist species of fish to prosper in altered environments, and lack of ability to acclimatize in such ways may be a mechanism causing extirpation of more sensitive species. The above authors emphasized that understanding how landscape-level processes affect performance of individual fish can aid in the understanding of how land use impacts stream communities as a whole. Mierzejewski et al. (2014) used biomarkers to determine site-specific point-sources along the Saluda River, South Carolina, by measuring physiological indicators of centrachid species at multiple sites along this river. Studies by Corsi et al. (2011) and Crago et al. (2011) used *in situ* chambers and laboratory-reared fathead minnows (*Pimephales promelas*) to assess both spawning (Corsi et al. 2011) and the molecular indicators of reproductive status (Crago et al. 2011) as a function of a watershed's quality. Taken together, these studies exemplify successful use of physiological tools to link the health of freshwater fish to landscape-scale disturbances.

To further assess links between physiological responses and landscape-level stressors, a number of studies have examined the effects of complex mixtures (e.g., wastewater effluents) on aquatic biota. These studies have largely used laboratory-reared animals (e.g., fathead minnows) that were exposed to ''effluent water'' either in a laboratory setting (e.g., Garcia-Reyero et al. 2011; Vidal-Dorsch et al. 2013) or in *in situ* cages (e.g., Garcia-Reyero et al. 2009; Ings et al. 2011; Sellin Jeffries et al. 2012; Kahl et al. 2014). Because sampling of wild fish can pose certain difficulties (e.g., species of interest either absent, or present only as transients), *in situ* cages containing laboratory-reared fish provide a useful alternative when monitoring the effects of complex effluents. Additionally, molecular tools are generally more

readily available for laboratory-reared animals, making molecular analysis of gene expression, for instance, more straightforward. Additional studies have taken this strategy one step further by comparing *in situ,* caged, laboratory-reared fish with resident fish (Schultz et al. 2013; Minarik et al. 2014), thereby allowing for assessment of physiological responses over different temporal as well as spatial (i.e., multi-site sampling) scales.

When environments are complex, such as in the case of exposure to complex mixtures from wastewater effluents, it becomes difficult to predict effect outcomes because unknown chemicals may be present; in this case, genome-wide or ''omics'' approaches are useful. Transcriptomics, proteomics, and metabolomics are strategies that allow a large variety of biological pathways to be examined in a tissue-specific manner. Three recent and complementary studies employed such approaches to assess exposure of complex effluents on the ovarian transcriptome (Berninger et al. 2014), as well as the liver transcriptome (Martinovic-Weigelt et al. 2014) and metabolome (Skelton et al. 2014) of laboratory-reared fathead minnows. In particular, these authors point out that metabolomics is not hampered by the need for a sequenced genome and could thus be useful as a tool for non-model species. Together, these studies provide evidence for the successful use of omics-based approaches to assess the link between landscape-level disturbances and fishes' physiological responses on a broad-scale, i.e., approaches that allow for specific molecular targets to be identified for more focused, hypothesis-driven studies.

Using physiological responses to address landscape-level changes does have limitations, however. For example, many physiological responses are sensitive to external factors such as the stress from handling, sampling technique, life stage, reproduction, and time of day that may confound physiological measurements (e.g., Hanson et al. 2008; Ellis et al. 2012b). Careful choice of control sites (i.e., nearby, but non-impacted systems) as well as sampling techniques that reduce the possibility for additional stressors (e.g., angling and the drawing of blood within minutes; the placement of fish in sensory-deprived ''black boxes'' for a period prior to sampling; e.g., Gingerich et al. 2010; Blevins et al. 2013) can help to establish reliable baseline/control values of physiological measures. In addition, the complexities that come with measuring responses of fish in ''uncontrolled'' field-settings (i.e., multiple possible stressors within a single landscape) can make untangling physiological responses difficult. Understanding how fish respond to a complex environment can provide information about their responses to concurrent stressors, a situation that may better represent the health of fish overall. However, a more targeted approach may be necessary for conservation biologists to isolate primary stressors and facilitate restoration of habitats; in this case transporting fish to a controlled laboratory setting (e.g., Blevins et al. 2013) can aid in reducing the influence of confounding factors and additional stressors. When possible, using a Before-After Control-Impacted (BACI) design may additionally strengthen studies that assess changes in a site due to a potential impact. The BACI approach involves comparing data collected prior to an impact, to data collected after an impact, from control as well as impacted sites (Smith 2002). Conquest (2000) discussed the value, but also the drawbacks, of the BACI design, cautioning researchers to use replicate control and impacted sites in their analyses to strengthen the interpretation of their results (for further discussion of BACI designs see Conquest [2000] and Smith [2002]). Together, although assessing physiological parameters to examine landscape-level effects carries certain limitations, a strong experimental design and careful interpretation of the results, will help to strengthen such studies.

Directions for future studies

With human populations projected to continue growing into the next century, anthropogenically-driven stressors will continue to negatively impact natural environments through processes such as destruction and fragmentation of habitats, climatic change, and removal of water. It is therefore critical that our monitoring of the responses of fish to broad-scale challenges increases to help conservation efforts reverse the alarming declines in fish populations. Lake et al. (2007) called for integrating ecological theory within stream restoration projects, along with inputs from hydrologists, biogeochemists, managers, and other stakeholders to improve the effectiveness of habitat-restoration projects and improve the framework for generating data on the effectiveness of restoration. Ideally, scientists can build upon this concept, and integrate physiological metrics into traditional field-based studies of abundance, distribution, and restoration to improve management, and to provide ''early warning'' indicators of problems at the individual level that can help protect populations at risk. Specifically, blood-based physiological monitoring has two additional strengths that contribute to traditional field-based monitoring. First, blood samples provide a wealth of information on an

individual's health and condition, and can be drawn non-lethally, even from small fish, thereby permitting sampling of rare or threatened species without immediate negative consequences. Second, the red blood cells of fish are nucleated and thus permit the use of emergent technologies that focus on gene expression or mRNA production as endpoints to be used in non-lethal blood sampling. Thus, future studies should strive to integrate physiological monitoring with traditional field-based monitoring across a range of species and a range of habitats, where possible. This approach not only will provide an opportunity to test theories and hypotheses about drivers of individual health and condition across landscapes, but also will improve outcomes and provide novel insights into how organisms are interacting with their surroundings in the face of future challenges.

Future studies should also aim to better understand the expected range of physiological profiles from the breadth of fish that dwell in different natural habitats. Most physiological studies performed to date have focused on few species of fish (e.g., salmonids) and on few habitats (e.g., temperate rivers and streams, or aquaculture and laboratories). Thus, when making comparisons of physiological responses to landscape-level disturbances across studies, comparisons often are limited to a narrow range of species over a narrow range of environmental conditions. Therefore, to better understand the physiological responses observed across different landscapes, effort should be made to understand the range of physiological responses in a wide variety of fish and habitats. This current gap in knowledge highlights the importance of choosing reference sites that closely mimic treatment/disturbed sites in all but a few desired traits, and that interpretation of individual physiological responses should be carried out with caution (i.e., functional ranges of physiological responses can vary among individuals, populations, and species).

Finally, linking physiological responses to population size and other population-level attributes, such as persistence, fecundity, and overall ecosystem health (for a review see Adams 2002) will undoubtedly assist conservationists and managers (Fefferman and Romero 2013). By understanding the mechanisms by which changes in the landscape influence populations and ecosystems *via* measurable physiological parameters, practitioners will become empowered and better able to identify species and habitats that are at risk (Cooke and O'Connor 2010), ideally well before populations decline.

Summary and conclusions

While underutilized in the past, researchers have recently embraced physiological indices as a measurement of the health of aquatic ecosystems with respect to landscape-level changes, and have demonstrated the benefit of measuring responses at multiple hierarchal levels. Such integrative approaches have provided evidence for links between fishes' physiology and population-level and community-level responses to characteristics of the habitat. Under circumstances in which higher-level, long-term responses are insensitive to sub-lethal stressors, short-term physiological responses can provide valuable information to fill gaps in knowledge. In addition, the use of omics-based approaches can help reveal genome-wide responses to landscape-scale changes, approaches that will be particularly useful when the effects of changes in the landscape may not be easy to predict. Together, these studies act as the precursors for future work in utilizing a multitude of physiological techniques to determine the effects of landscape-scale disturbances on freshwater species of fish.

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