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Validation de l'utilisation d'indicateurs physiologiques de stress
comme indicateurs de qualité des habitats

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Résumé

Le développement de modèles de qualité des habitats est souvent limité par notre incapacité à lier les processus à l'échelle des écosystèmes au succès écologique des individus. Les indicateurs physiologiques de stress ont été proposés comme une méthode complémentaire aux approches classiques de modélisation de la qualité des habitats basées sur des indicateurs d'utilisation des habitats. Néanmoins, l'utilisation d'indicateurs physiologiques associés au stress comme outil de modélisation de qualité des habitats n'a pas encore été rigoureusement validée. Les échelles temporelles pour lesquelles les indicateurs de stress constituent potentiellement le lien entre processus écosystémiques et le succès écologique sont encore inconnues. Dans le but de contribuer à la validation de ces indicateurs, les niveaux de bases et réponses du cortisol, du glucose et du lactate ont été mesurés chez 323 bec-de-lièvres (*Exoglossum maxillingua*) situés dans 3 différentes rivières des Laurentides (Québec, Canada) durant l'été 2016. Ces indicateurs de stress ont par la suite été liés aux caractéristiques de l'environnement et à deux facteurs de conditions utilisés pour évaluer le succès écologique des individus. Les résultats obtenus démontrent qu'il serait possible d'utiliser les niveaux réponses de glucose pour modéliser la qualité des habitats à de courtes échelles temporelles.

Mots-clés : Indicateurs de stress, Cortisol, Glucose, Lactate, Qualité des habitats, Conservation

Abstract

Stress-related physiological indicators have been proposed as an alternative approach to model habitat quality due to their relation to an individual's fitness. However, the validation of this approach is far from complete since the temporal scales at which physiological indicators are related to local environmental characteristics and are a predictor of fish fitness has not been assessed. Thus, the goal of this study was to further explore the potential of physiological indicators in facilitating the development of habitat quality models. To achieve that, the basal and response levels of cortisol, glucose and lactate in the blood were assessed in cutlip minnows (*Exoglossum maxillingua*) located in three different Laurentian rivers (Quebec, Canada) from July to August 2016. Those indicators were then linked to the assessed environmental characteristics and to two condition factors used to represent fish fitness (whole-body lipid concentration and LeCren condition factor) with models built through a two-stage hybrid variable selection procedure using ridge and LASSO type penalties. Our results suggest that response levels of blood glucose depend strongly on the local environmental characteristics and are good predictors of the fish's lipid concentration validating their potential use at the daily temporal scale as habitat quality indicators.

Keywords : Stress indicators, Cortisol, Glucose, Lactate, Habitat quality, Conservation

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Liste des sigles et abréviations

ANOVA : Analysis of variance (Analyse de variance)

ELISA : Enzyme-linked immuno-sorbent assay (Dosage d'immunoabsorption par enzyme liée)

HPI : Axe hypothalamo-hypophysaire-interrénal.

HSC : Axe hypothalamo-sympathique-chromaffine.

OLS : Ordinary least square (Méthode des moindres carrés ordinaire)

NADH et NAD⁺: Nicotinamide adenine dinucleotide (Nicotinamide adénine dinucleotide)

P.val : Probability value (Valeur de probabilité)

R².cv : Cross-validation R-squared (R carré de validation croisée)

À Aldo Leopold

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Introduction générale

Les approches de modélisation de la qualité des habitats

La destruction, la fragmentation et l'altération des habitats font partie des menaces les plus importantes à la biodiversité mondiale (Brooks, 2006; Hanski, 2011; Mantyka-Pringle, 2012). Les actions de conservation et de protection des populations menacées sont souvent limitées par la difficulté de modéliser la capacité d'un habitat à assurer la survie et la reproduction des populations le fréquentant (Cooke, 2008). Cette capacité, appelée qualité des habitats, est le plus souvent modélisée chez les poissons à l'aide d'approches se basant sur l'utilisation des habitats en raison de leur faible coût et leur relative simplicité (Johnson, 2007). Ces approches supposent une relation positive entre les indicateurs d'utilisation comme la présence, l'abondance, la biomasse ou le mouvement des organismes (Johnson, 2007; Van Horne, 1983) et la qualité de l'habitat. Cette relation peut être causée par le déplacement volontaire des poissons d'un habitat de faible qualité vers des habitats de plus grande qualité ou par un plus grand succès écologique des individus fréquentant les habitats de meilleure qualité (Chalfoun, 2007; Morris, 2003; Remes, 2000). Cependant, ces modèles semblent échouer dans plusieurs contextes à identifier les paramètres environnementaux déterminant la qualité des habitats. Une des raisons avancées pour expliquer ces échecs est qu'ils ne prennent pas en compte les processus se déroulant à l'échelle des individus (Horodysky, 2015). Dans un contexte de changements globaux, l'identification de ces processus s'avère primordiale pour soutenir les efforts de conservation (Seebacher, 2012).

Ces dernières années, un nombre important d'études (Cooke, 2010; Horodysky, 2015; Lennox, 2018; Mckenzie, 2016; Seebacher, 2012; Wikelski, 2006; Young, 2006) avancent que des modèles de qualité des habitats incluant la physiologie des individus permettraient de pallier aux faiblesses des modèles d'utilisation des habitats. Ces modèles seraient en mesure d'offrir des mécanismes concrets liant environnement, individus et population à travers le succès écologique ou « fitness » des organismes étant donné que les processus physiologiques sont, d'une certaine manière, la courroie de transmission entre l'environnement et le succès écologique des individus (Feder, 2000; Horodysky, 2015; Huey, 1991). De récentes études suggèrent que des indicateurs physiologiques associés à la réponse au stress seraient potentiellement de bons indicateurs de la qualité des habitats chez les poissons puisque les voies métaboliques associées au stress jouent le rôle d'intermédiaires entre l'environnement et la physiologie interne de l'organisme (Belanger, 2017; Blevins, 2013; Lennox, 2018; O'Connor, 2010; Pottinger, 2011).

La réponse au stress chez les poissons

Lorsqu'un organisme fait face à un évènement menaçant son équilibre interne appelée homéostasie (Cannon, 1929), il doit dépenser une certaine quantité d'énergie pour conserver cet équilibre. L'énergie que l'organisme doit dépenser pour conserver son homéostasie dans un environnement donné est appelée charge allostasique (Romero, 2009). Pour rétablir l'équilibre interne, une série de réactions physiologiques appelée réponse au stress est sollicitée. Cette réponse, dont les mécanismes ont fortement été conservés au cours de l'évolution (Bonier, 2009; Denver, 2009), peut être divisée en trois différentes étapes chez les vertébrés : la réponse primaire, secondaire et tertiaire (Barton, 2002).

La réponse primaire au stress implique deux voies métaboliques différentes: la voie adrénergique associée aux hormones catécholamines et la voie glucocorticoidienne associée aux hormones corticostéroïdiennes (Barton, 2002). Elles sont contrôlées respectivement chez les poissons par l'axe hypothalamo-sympatique-chromaffine (HSC) et l'axe hypothalamo-hypophysaire-interrénalien (HHI).

La réponse primaire au stress pour l'axe HSC commence avec le relâchement dans le sang des hormones catécholamines, soit l'épinéphrine et la norépinephrine aussi appelées adrénaline et noradrénaline. Elles sont relâchées à partir des cellules chromaffines situées dans les tissus interréniaux des poissons aussitôt que le stress est perçu. Contrairement aux autres vertébrés, les poissons et amphibiens n'ont pas de glandes surrénales à proprement parler. Ainsi, les cellules chromaffines sont distribuées dans le rein au lieu de former une glande distincte (Barton, 1998). Le relâchement des hormones catécholamines est régulé par stimuli nerveux provenant des fibres cholinergiques préganglionnaires du système nerveux sympathique, par des facteurs hormonaux comme la concentration de catécholamines ou de cortisol et par des facteurs non hormonaux comme la concentration dans le plasma d'ions potassium, de CO₂ et d'O₂ (Randall, 1992). Les principaux effets de cette famille d'hormones sont l'augmentation de la glycémie dans le sang, de la ventilation, de la circulation sanguine et de l'apport en oxygène aux tissus (Bonga, 1997; Fabbri, 2016). Toutefois, l'utilisation des hormones catécholamines est assez rare en modélisation physiologique de la qualité des habitats puisque leurs niveaux augmentent rapidement (<1min) après la perception d'un stress comme la capture ou un prélèvement sanguin et leurs effets directs sur la réponse au stress sont de courte durée (Bonga, 1997; Koolhaas, 2011).

Le cortisol, principale hormone glucocorticoidienne chez les poissons, est relâché par les tissus interrénaux comme les hormones catécholamines. Cette libération est régulée par deux différentes hormones issues de l'hypophyse; la corticotropine (CRH) et l'adrénocorticotropine (ACTH) (Barton, 2002; Bonga, 1997). Le rôle du cortisol dépend de sa concentration. À faible concentration, il est impliqué chez les poissons dans l'attribution des acides aminés à différentes voies métaboliques non protéiques, l'osmorégulation, la croissance et la reproduction (Mommsen, 1999). À forte concentration, le rôle principal du cortisol est l'activation de la glycogénolyse dans le foie permettant la libération de glucose dans le sang, mais participe aussi à la modulation du rythme cardiaque et de la ventilation (Martinez-Porchas, 2009; Pankhurst, 2011). Suite à un stress, l'augmentation de la concentration du cortisol dans le sang est observée à partir de trois minutes (Romero, 2005). Il est donc possible d'évaluer, à l'aide d'un protocole de prélèvement approprié, les niveaux de cortisol avant et après un stress.

La réponse secondaire correspond à des changements métaboliques induits par la réponse primaire. Elle est caractérisée par des changements au niveau de métabolites comme le glucose et le lactate, la production de protéines de choc thermique et des changements relatifs à l'osmorégulation (Barton, 2002). Plus spécifiquement, le glucose est le principal responsable de l'apport en énergie dans les tissus de l'organisme et les deux voies associées à la réponse primaire au stress mènent à sa libération (Barton, 2002). Cette libération semble plus être contrôlée par la voie adrénergique que par la voie glucocorticoidienne (Pankhurst, 2011). De plus, il existe un système de rétroaction négative où l'augmentation de la glycémie sanguine a tendance à réduire la glycogénolyse dans le foie (Polakof, 2008a).

Le lactate est aussi un produit de la réponse secondaire au stress chez les poissons. Lors d'un stress, leur consommation en oxygène a tendance à être plus grande que leur capacité d'en capter de l'environnement. Face à une importante demande énergétique et une concentration d'oxygène limitante, le pyruvate, au lieu d'être transformé en acétyl-CoA, va être réduit en lactate. Normalement, le NADH est oxydé dans la mitochondrie à l'aide de l'oxygène, mais en absence de celui-ci, cette réaction est impossible. À la place, c'est le pyruvate qui va jouer le rôle d'accepteur d'électron. Celui-ci va accepter l'électron du NADH qui va devenir NAD^+ . Cette réaction permet, en absence d'oxygène, la régénération du NAD^+ , molécule essentielle à la production d'ATP (Facey, 2013). Toutefois, le lactate n'est pas seulement un déchet métabolique. Il peut aussi être utilisé comme source d'énergie et comme précurseur à la synthèse de glycogène (Chatham, 2002; Omlin, 2014; Polakof, 2008a).

Enfin, la réponse tertiaire correspond à des changements au niveau de l'organisme comme la perte de masse musculaire, la réduction de la croissance, une réduction au niveau du système immunitaire et des changements dans le comportement. Ces changements peuvent avoir des conséquences importantes sur l'organisme et peuvent réduire fortement son succès écologique (Barton, 2002, 1987; Schreck, 2001).

Les indicateurs physiologiques, le succès écologique et la qualité des habitats

L'information apportée par ces différents indicateurs physiologiques associés à la réponse au stress dépend du moment du prélèvement. Ainsi, pour le cortisol, les niveaux de base (ou niveaux naturels), mesurés moins de trois minutes après la capture, reflètent généralement la charge allostatique actuelle de l'environnement sur l'organisme (Bonier, 2009). Les niveaux réponses, définis comme le niveau maximal atteint après un stress aigu (généralement après 30min), reflètent la capacité d'un organisme à faire face à un stress (Barton, 2002). Ainsi, il est généralement supposé que la relation entre les niveaux de base du cortisol et le succès écologique des individus est négative. Plus la charge allostatique sur l'individu est grande, moins celui-ci sera en mesure de contribuer à la génération suivante (Bonier, 2009). Pour les niveaux réponses de cortisol, il est généralement supposé que leur relation avec le succès écologique des individus est positive. Plus un organisme est en mesure d'activer les axes associés à la réponse au stress lors d'un évènement stressant, plus il est probable que celui-ci soit capable de faire face à la charge allostatique de son environnement et ainsi être en mesure de contribuer à la génération suivante (Barton, 2002; Breuner, 2008). De plus, une activation fréquente de la réponse au stress causée entre autres par une grande charge allostatique entraîne l'habituation au stress. L'habituation est un mécanisme adaptatif caractérisé par une réduction de l'intensité de la réponse (Barton, 1987; Koolhaas, 2011; Rich, 2005). Elle permettrait de réduire les effets néfastes d'une constante activation de la réponse au stress tel que la suppression du système immunitaire, du système reproducteur et de la croissance (Rich, 2005). Il n'existe donc pas de bonne ou de mauvaise réponse au stress, mais plutôt des réponses typiques d'environnements associés à de petites ou de grandes charges allostatiques.

Pour les niveaux de glucose dépendent en partie de la concentration en cortisol. Il serait donc possible de supposer que les niveaux de bases, comme ceux du cortisol, sont associés négativement au succès écologique et que les niveaux réponses y soient associés positivement. Similairement, des niveaux de bases de lactates élevés suggéreraient une charge allostatique élevée donc une relation négative avec le succès écologique. Des niveaux réponses élevés suggéraient une bonne capacité à répondre à un stress et donc un meilleur succès écologique. Certaines méta-analyses ont néanmoins soulevé des incohérences entre les niveaux de cortisol et le succès écologique (Bonier, 2009; Breuner, 2008). Il semblerait ainsi que la réponse au stress est modulée par d'autres facteurs que la concentration de cortisol dans le sang tel que la concentration de récepteurs cellulaires associés à la réponse au stress ou à la concentration de globulines liant le cortisol (Breuner, 2008, 2006).

Pour être en mesure d'utiliser les niveaux de base et réponses du cortisol, du glucose et du lactate pour modéliser la qualité des habitats, certaines informations manquent encore. En effet, les échelles temporelles auxquelles les indicateurs physiologiques de stress sont associés aux caractéristiques de l'environnement et auxquels les indicateurs physiologiques de stress prédisent le succès reproducteur des individus sont encore inconnues. Dans l'éventualité où cette échelle temporelle est très grande (ex. années), il serait impossible d'utiliser les indicateurs physiologiques de stress pour modéliser des variations de qualité des habitats à des échelles plus petites (ex. semaines). Inversement, si cette échelle temporelle est petite (ex. jours), il serait possible d'utiliser les indicateurs de stress pour modéliser des variations de qualité des habitats à de petites (ex. jours) et grandes échelles (ex années).

La validation de l'utilisation d'indicateurs physiologiques de stress pour modéliser la qualité des habitats

Quelques études ont été en mesure d'associer des indicateurs physiologiques de stress avec des caractéristiques environnementales en milieu naturel à travers le temps, mais seulement à de longues et moyennes échelles temporelles. Par exemple, une étude Pottinger, 2011 a réussi à détecter des différences entre les niveaux réponses de cortisol et de lactate selon un gradient de pollution chez l'épinoche à trois épines à travers plusieurs années. Une autre étude, Liss, Sass, & Suski, 2014 a réussi à modéliser la variation des niveaux de bases de cortisol et de glucose à l'aide des caractéristiques de l'environnement chez la carpe argentée (*Hypophthalmichthys molitrix*, Valenciennes 1844) entre différents mois d'une même saison. Au niveau du lien entre les indicateurs physiologiques de stress et succès écologique, une étude O'Connor, 2010 a démontré que l'implantation d'une capsule contenant du cortisol dans des achigans à grande bouche (*Micropterus salmoides*, Lacépède, 1802) réduisait leur capacité à survivre à des conditions d'anoxie hivernale.

Il existe plusieurs lacunes au niveau de la validation de l'utilisation des indicateurs physiologiques de stress comme outil de modélisation de qualité des habitats. L'ensemble des études recensées liant l'environnement aux indicateurs physiologiques associés à la réponse au stress se déroulent à de longues échelles temporelles et n'incluent pas des mesures de succès écologiques. De comprendre comment ces indicateurs interagissent à de courtes échelles temporelles avec l'environnement et d'évaluer s'ils sont effectivement liés au succès écologique des individus est nécessaire avant de pouvoir les utiliser comme outil de

modélisation de qualité des habitats. Conséquemment, l'objectif de la présente étude vise à contribuer à la validation de l'utilisation d'indicateurs associés à la réponse au stress comme indicateurs de qualité des habitats dans le but de mieux comprendre la relation entre la physiologie des organismes et leur habitat afin de contribuer au développement de nouveaux outils en conservation. Plus précisément, notre étude vise à évaluer l'existence d'un lien à l'échelle du jour entre les caractéristiques environnementales, les niveaux de bases et réponses de cortisol, de glucose et de lactate et deux facteurs de conditions utilisés pour évaluer le succès écologique des organismes soit le pourcentage de gras total et le facteur de condition de LeCren chez le bec-de-lièvre (*Exoglossum maxillina*, Lesueur 1817).

Contributions des différents auteurs à l'article

La conception et la réalisation de la présente étude sont un travail original de Cédric Lejeune sous la supervision des coauteurs de l'article soit Daniel Boisclair et Steven J. Cooke. L'analyse des données a été faite par Cédric Lejeune. L'écriture de l'article a été faite par Cédric Lejeune avec révisions et contributions de Daniel Boisclair.

Validation of Indicators of Organismal Physiological Status to Model Fish Habitat Quality

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Abstract

Stress-related physiological indicators have been proposed as an alternative approach to model habitat quality due to their relation to an individual's fitness. However, the validation of this approach is far from complete since the temporal scales at which physiological indicators are related to local environmental characteristics and are a predictor of fish fitness has not been assessed. Thus, the goal of my study was to further explore the potential of physiological indicators in facilitating the development of habitat quality models. To achieve that, the basal and response levels of cortisol, glucose and lactate in the blood were assessed in cutlip minnows (*Exoglossum maxillingua*) located in three different Laurentian rivers (Quebec, Canada) from July to August 2016. Those indicators were then linked to the assessed environmental characteristics and to two condition factors used to represent fish fitness (whole-body lipid concentration and LeCren condition factor) with models built through a two-stage hybrid variable selection procedure using ridge and LASSO type penalties. Our results suggest that response levels of blood glucose depend strongly on the local environmental characteristics and are good predictors of the fish's lipid concentration validating their potential use at the daily temporal scale as habitat quality indicators.

Keywords : Physiological indicators, Cortisol, Glucose, Lactate, Habitat quality, Conservation

Introduction

The destruction and modification of habitats have been identified as major threats to the persistence of many species around the globe (Brooks, 2006). The capacity of conservation scientists to quantify and predict the consequences of habitat alteration is impeded by the lack of understanding of the linkage between the determinants of species perpetuation and environmental characteristics (Horodysky, 2015; Ricklefs, 2002). Habitat-based conservation, which hinges on relationships between metrics of species perpetuation (fitness metrics such as reproduction, growth, and survival rates) and environmental characteristics is one of the most common approaches used to quantify the consequences of habitat alteration on species (Huey, 1991; Primack, 2012). The development of such relationships, hereafter referred to as “habitat quality models” (Hall, 1997) is hampered by the mismatch between the spatial and temporal scales at which fitness metrics are expressed and environmental characteristics change (Cooke, 2008).

In fish ecology, “habitat-use models” are often taken as a substitute for “habitat quality models”. Habitat-use models consist of relationships between indicators of the extent to which habitats are used by species (preference indices, habitat suitability indices, probability of presence, numerical abundance, biomass; Beutel, Beeton, & Baxter, 1999; Brind’Amour, Boisclair, Legendre, & Borcard, 2005; Guay et al., 2000; Souchon & Capra, 2004) and environmental characteristics found in these habitats. The substitution of “habitat-use models” for “habitat-quality models” may be a result of the relative ease and rapidity of estimating indices of habitat use by species under spatially and temporally changing environmental characteristics. However, the conceptual and practical validity of habitat-use models has been

the subject of a number of criticisms (e.g. habitat use may not be related to fitness: (Amarasekare, 2001; Cassini, 2011; Guisan, 2005; Rose, 2000; Van Horne, 1983). As such, numerical abundance or biomass of species in a habitat may only provide a fraction of what may be taken as “habitat quality”.

Ecophysiology has been proposed as an alternate strategy to develop habitat quality models (Horodysky, 2015). This strategy is based on the expectation that the homeostasis (Cannon, 1929) or balance of physiological metrics such as energy reserves, body fluids, blood electrolytes, hormone concentrations, etc. may constitute a reliable linkage between environmental characteristics and fitness metrics (Cooke, 2008; Seebacher, 2012). When an organism’s physiological balance is threatened, a physiological response ensues to restore homeostasis. This response is defined as the “stress response” and the condition, internal or external to the organism, which threatens homeostasis is defined as a “stressor” (Barton, 2002; Selye, 1950). The cumulative energetic demand exerted by the environment on the organism is referred as “allostatic load”. Fitness metrics such as reproduction, growth, and survival rates are thought to depend on the capacity of the organism to respond adequately to stressors (for a review, see (Breuner, 2008). In this context, it may be hypothesized that the study of the relationship between stress and environmental characteristics may facilitate the development of habitat quality models.

Stress is generally assessed using various physiological indicators taken to represent the state of organisms under particular environmental characteristics (Belanger, 2015; Hontela,

1992; Johnson, 1992; King, 2015; Marra, 1998; O'Connor, 2011; Romero, 2010). In fish, two physiological axes are involved in the stress response: the hypothalamus-sympathetic-chromaffin axis (HSC) and the hypothalamus-pituitary-interrenal (HPI) axis. The catecholamines such as epinephrine or norepinephrine are the main hormones of the HSC axis. They increase plasma glucose concentration, blood circulation and oxygen intake (Bonga, 1997; Fabbri, 2016). However, their levels increase almost immediately when facing stress. Measuring natural levels of catecholamines is difficult and needs special apparatus. Their use in habitat quality modelling is limited (Barton, 1998). Cortisol is one the main hormones of the HPI axis and is commonly used as a fish stress indicator (Martinez-Porchas, 2009; Mommsen, 1999; Sopinka, 2016).

When a stressor is sensed by the central nervous system, the anterior pituitary gland releases adrenocorticotrophic hormone, which activates the release of cortisol from the interrenal tissues (Barton, 2002). Cortisol, in turn, induces the release of glucose in the blood stream through the activation of the breakdown of glycogen in glucose in the liver (Pankhurst, 2011; Reid, 1998). Glucose levels increase during the stress response to facilitate the return to homeostasis by providing energy to the different tissue of the organism and is used as a fish stress indicator (Jiang, 2017; Martinez-Porchas, 2009; Mommsen, 1999; Polakof, 2012). Stress increases metabolic requirements in oxygen which can cause an imbalance between the fish oxygen requirements and acquisition. Muscles, in order to contract in low oxygen concentration will produce lactate through pyruvate reduction (Facey, 2013). As a result, lactate has been commonly used as a stress indicator in fish (Barton, 2002; Grutter, 2000).

Levels of physiological indicators estimated in natural situations before the imposition of an additional stressor are referred to as basal levels. They are thought to reflect the degree of challenge also called allostatic load perceived by an organism under a specific combination of environmental characteristics (Bonier, 2009). However, such relationship between basal levels and allostatic load is not always present, possibly as a result of the modulation of receptors expression, change in corticosteroid-binding globulins concentration or interaction with feeding (Breuner, 2006; Mommsen, 1999; Ramsay, 2006). Higher basal levels of cortisol are generally considered as symptomatic of an organism facing challenging environmental characteristics and may thus associated with individuals with lower fitness (Bonier, 2009). Nonetheless, there is inherent variation in cortisol levels among individual independent of stress.

Maximum levels of stress indicators estimated after the imposition of an additional stressor are hereafter referred to as response levels (Wikelski, 2006). Those levels depend on the intensity of the stressor and can be modulated through habituation. Habituation or acclimation consists in lower response levels of stress indicators as a result of repeated exposure to stressors from various environmental challenges (Barton, 1998). Habituation is considered as an adaptive mechanism that protects fish from the long-term detrimental effects of high levels of cortisol such as impaired immune response, growth, reproduction and alteration of behaviour (Barton, 2002; Busch, 2009; Mommsen, 1999; Romero, 2004). This attenuation is usually considered as indicative of organisms under high allostatic load. As such, it is usually associated with individuals in habitat of lower quality (Busch, 2009). However, as with basal levels, such relationship between response levels of stress indicators

and allostatic load is not always present since stress-related receptor expression and corticosteroid-binding globulin concentration can modulate response levels of cortisol (Breuner, 2008, 2006).

Cortisol, glucose and lactate have other roles outside of the stress response in fish. Cortisol helps regulate osmoregulation, glucose levels are associated with feeding and lactate is produced during activity. Referring to them as stress indicators does not reflect the complex dynamics associated with each of them. As a result, they will be hereafter referred as physiological indicators. To our knowledge, the capacity of basal and response levels of physiological indicators to act as fitness related metrics of habitat quality has not yet been thoroughly assessed. Jackson, Kurtz, & Fisher, 2001 described four phases in the evaluation of an indicator: 1) the conceptual relevance (can the indicator be conceptually related to the process of interest?); 2) the feasibility of implementation (can the indicator be estimated?); 3) the spatio-temporal variability of the response (is the natural variability of an indicator sufficiently small to be related to the process of interest?), and; 4) the interpretation and utility (can the indicator be used to make adequate interpretations and relevant decisions about the process of interest?).

A number of studies have conceptually linked physiological indicators to fitness metrics (Bonier, 2009; Horodysky, 2015; Wikelski, 2006). In addition, the feasibility of estimating fish physiological indicators in the field has repeatedly been demonstrated (Hontela, 1992; Rich, 2005). Spatial variations of fish physiological indicators have been documented on a number of occasions (e.g. rivers located in a forested vs deforested

landscape: Blevins, Wahl, & Suski, 2014; King, Chapman, Cooke, & Suski, 2016). Temporal variations of fish physiological indicators in the field have been noted among years (Pottinger, 2010) and among seasons (Belanger, 2015). Variations of in fish physiological indicators have been observed across two consecutive seasons (Liss, 2014). However, variations of in fish physiological indicators between days or weeks, and their potential linkage to fitness metrics, remain to be assessed.

Identifying the temporal scales at which physiological indicators are related to environmental characteristics and predict fitness is necessary for further development of physiological indicators as habitat quality modelling tools. In the eventuality that physiological indicators are related to environmental characteristics and predict fitness only at temporal scale of great magnitude (i.e. years), physiological indicators would only model habitat quality at equal or greater temporal scales, limiting the usefulness of stress indicators as habitat quality modelling tools. Being limited to only big temporal scales would greatly reduce physiological indicators utility as a habitat quality modelling tools. But, if physiological indicators are related to environmental characteristics and predict fitness at small temporal scales (i.e. days), physiological indicators would model habitat quality at scales relevant for conservation efforts (Wikelski, 2006).

The general objective of the present study was to further explore the potential of physiological indicators in the development of habitat quality models. The specific objectives of this study were, at small temporal scale (i.e. days): 1) to test for a relationship between six physiological indicators associated to the stress response (basal and response levels of cortisol,

glucose and lactate) and environmental characteristics; 2) to test for a relationship among two fish condition factors representing fish fitness metrics (the whole-body lipid concentration and the LeCren condition factor), physiological indicators and environmental characteristics, and; 3) to quantify the relative contribution of environmental characteristics, and physiological indicators in explaining the two condition factors taken to represent fish fitness. Taken together, these specific objectives permitted us to test the hypothesis that physiological indicators constitute a reliable linkage between environmental characteristics and fitness metrics at short temporal scales. To do so, we sampled for fish and environmental characteristics at the spatial scale of mesohabitats (habitat patches possessing relatively homogenous environmental characteristics) found in rivers and over consecutive days. This permitted us to better represent the potential interaction between both the dependent (physiological indicators or fitness metrics) and independent variables (depending on the objective: environmental characteristics and/or physiological indicators) on a day-to-day basis.

Material and Methods

Study Sites

We achieved our objectives by sampling fish and surveying environmental characteristics in three 2000 m² mesohabitats (100 m alongshore x 20 m habitat patches of river possessing relatively similar environmental characteristics; Table I) located in three different tributaries of Rivière du Nord, Québec, Canada (Aux Mulets, Bellefeuille, and Simon Rivers; Figure 1). These sites were selected for study because they were suitable to implement the sampling techniques required to achieve our objectives (Lanthier, 2013; Macnaughton, 2015). Sampling took place from July 4th to August 5th 2016 between 8:00 and 17:00. One or two mesohabitats was sampled daily. The mesohabitats were sampled during two (Bellefeuille) or three (Aux Mulets and Simon) groups of two to four consecutive days for a total of nine (Aux Mulets), five (Bellefeuille), and ten (Simon) sampling days per mesohabitats (see table XII, appendix). Groups of consecutive sampling days were separated by nine to twelve days to minimize the effects of sampling on abiotic and biotic characteristics.

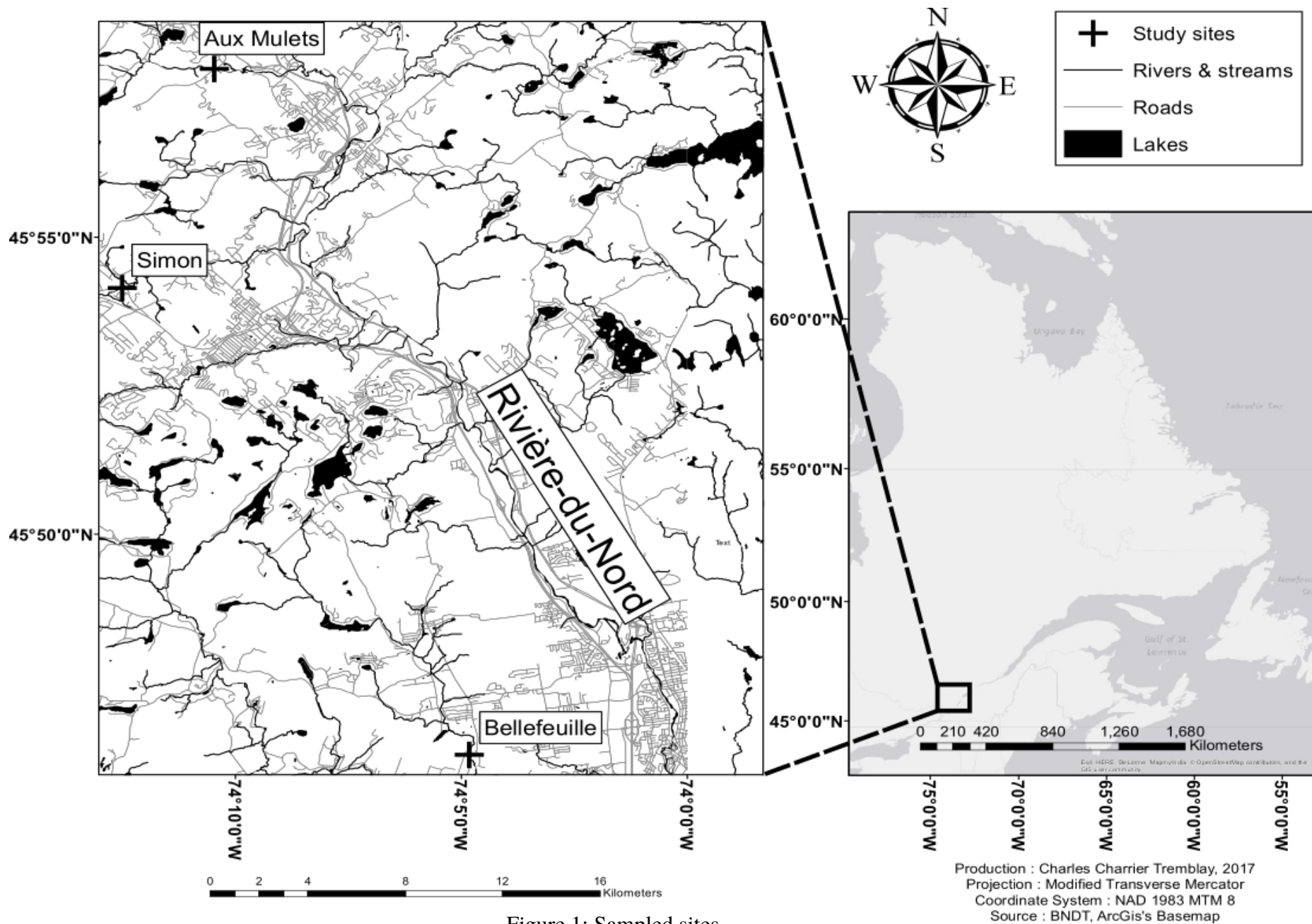


Figure 1: Sampled sites

Study Species

The studied species was a cyprinid, the cutlip minnow (*Exoglossum maxilingua*, Lesueur 1817) because, in the study sites, it was highly abundant and had the tendency to be solitary (no shoaling). This minimized the probability that fish sampling would cause stressful conditions to fish that avoided capture, and consequently, would affect their physiological indicators if captured later on. Adult cutlip minnow total length is about 10cm. They prefer rocky and slow moving clear water streams. Cutlip minnow diet is composed invertebrates and molluscs. They are known to be vulnerable to degradation of their natural habitat (Scott, 1973). For the cutlip minnow, the study sites correspond to the northernmost part of its distribution range which span from the north shore of the St-Lawrence river, Canada down to North Carolina, USA. Although little is known about the physiological ecology of the cutlip minnow, cyprinids are commonly used in environmental monitoring and have been the subject of laboratory and field studies.

Fish Sampling

Fish sampling generally followed the methods used by (King, 2015) and was conducted in accordance with the guidelines of the animal care committee of Université de Montréal. Fish were captured by a team of three operators using an LR-24 backpack electrofishing unit (Smith-Root®, Vancouver, WA). These operators sampled fish by zigzagging from the downstream to the upstream limits of the mesohabitats. The power of the electrofishing unit was set at 150 watts to minimize mortality while keeping an adequate sampling effectiveness. A total of 16 cutlip minnows measuring from 8 to 12 cm (total length)

was collected on each sampling day. Half of these fish (8) were euthanized by cerebral percussion followed by brain tissue destruction within 1 minute of being stunned by electrofishing to estimate the baseline levels of the physiological indicators. The other fish were placed in a 15-liter container filled with fresh river water and euthanized in a similar fashion after 30 minutes to estimate the acute response levels of stress-related physiological indicators. The strategy used to estimate basal and the response levels is consequent with published studies indicating that it takes at least 3 minutes for fish to show an elevation of cortisol, glucose, and lactate after stress (Barton, 2002; Blevins, 2013; Lawrence, 2018; Romero, 2005) and that maximum levels of stress-related physiological indicators in a number of teleost fish may be observed 30 minutes later (Acerete, 2004; Barton, 2002; O'Connor, 2011)

Each fish was measured for total length total length (± 0.1 cm) and wet blotted mass (± 0.1 g) after euthanasia. Blood samples were obtained from each individual fish within one minute after euthanasia by cutting the ventral artery using a scalpel. Blood glucose (± 1 mg/dL) and blood lactate (± 0.1 mmol/L) concentrations were respectively measured using an Accu-Chek Aviva® glucose meter (Roche Canada, Laval, QC, Canada) and a Lactate Pro® lactate meter (Arkray, Edina, MN, USA), devices previously validated for use on fish (Stoot, 2014) Cadavers were put on ice immediately after sampling for blood and preserved in liquid nitrogen individually within 3 h for further laboratory analyses. A total of 323 fish were captured throughout sampling.

Surveys of Environmental Characteristics

Seven environmental characteristics were assessed on each time a mesohabitat was sampled (Table 1). These environmental characteristics were selected for their potential to vary among days, to affect fish behaviour and/or habitat selection, and to influence fish fitness metrics. Adjusted water conductivity, water temperature, oxygen saturation, the presence of rain and cloud cover was assessed at the downstream end of the mesohabitat immediately before sampling. Water transparency was evaluated using a Secchi disk after sampling. The measurement was taken horizontally at the downstream limit of the mesohabitat. Water depth and velocity were assessed by dividing the mesohabitat in 10 equal sections along its length. One measure was taken in each section alternating between taking the measure at the rightmost, middle and leftmost part of the section. The 10 measures were then averaged for each mesohabitat. Water velocity was measured at 40% of the depth.

Table I : Surveyed environmental characteristics used to study their effect on fish physiological indicators. For each environmental characteristic, the instrument or method used is given, as well as a general hypothesis of how it could affect fish stress.

Variable	Instrument	Hypothesized role	Reference
Adjusted water Conductivity (uS)	YSI [®] 30	River productivity	(Copp, 2003; Dennis, 1995)
Water Temperature (°C)		Metabolism	(Enders, 2006)
O² water saturation (%)	YSI [®] 55	Metabolism	(Enders, 2006; Oligny-Hébert, 2015)
Rain	Visual observations	Modification of patterns of activity	(Payne, 2013)
Cloud cover (%)	Visual observations	Vulnerability to avian predators	(Girard, 2003)
Transparency (m)	Secchi disc	Capacity to see prey and predators	(Boisclair, 1996; Turesson, 2007)
Depth (cm)	Gauge	Vulnerability to wading/diving predators	(Harvey, 1991; Hughes, 1990)
Velocity (m/s)	Flo-mate [®] 2000 (Hach, Loveland, CO, USA)	Metabolism (Swimming cost)	(Enders, 2003)

Laboratory Analysis

The cortisol and lipid concentrations of individual fish were quantified in the laboratories of the Département de sciences biologiques, Université de Montréal, Canada, during the winter of 2017. Whole body instead of plasma cortisol concentrations were used due to the small fish size (Belanger, 2015; Yeh, 2013). Cortisol extraction procedures were adapted from Canavello et al., (2011). Frozen fish cadavers were taken out of the liquid nitrogen and directly homogenized using a tabletop grinder (Cuisinart® SG-10C, 1min). For this operation, 5 g of dry ice was added to the fish to prevent heating of the sample and to increase the volume of the sample allowing a better grinding efficiency. Homogenates of each

individual fish were placed in 5 ml eppendorf after sublimation of the dry ice. Homogenates were then individually lyophilized overnight, weighted to obtain the dry weight and then stored at -20C°.

Cortisol concentration was then analyzed using Enzyme-linked Immunosorbent Assay (ELISA; Engvall & Perlmann, 1971). Cortisol was extracted by adding 1 ml of ether to each homogenate, mixing for 15s with a vortex mixer, centrifuging for 10 min at 1500 g and by pipetting the ether fraction containing lipid and cortisol. The ether fraction was allowed to evaporate overnight and the remaining fraction (the lipid) was weighted in order to measure whole-body lipid concentration (g of lipid/g of dry fish) The remaining fraction was then suspended in phosphate buffered saline (PBS, pH 7.4, 1X) and analyzed following the instructions provided by the manufacturer of the ELISA plate (EIA-1887,DRG, Springfield, NJ, USA). The levels of cortisol were reported as ng per g of dried fish. Aux Mulets' response levels of cortisol had to be excluded from the analysis due to poor sample quality.

Computations and Statistical Analysis

All statistical analyses were performed with the computing environment R. For each sampling day, environmental characteristics, physiological indicators and condition factors were averaged and then transformed so as all averages were equal to zero and standard-deviations equal to 1 in order to be able to compare their coefficients in subsequent analysis. The LeCren condition factors were computed by determination of the log(length)-log(weight) curve for all the sampled fish. Then the weight of each fish was then divided by the predicted

value given by the log(length)-log(weight) curve yielding the LeCren condition factor (LeCren, 1951). A fish with a greater mass than the value predicted by the curve are attributed a greater LeCren condition factor value.

Variation in environmental conditions among mesohabitats and among groups of sampling days, and variation in physiological indicators and condition factors among mesohabitats, among groups of sampling days and among sampling days were tested using ANOVA models. Since each sampling day was assigned to only one group and each group was assigned to only one mesohabitat, nested ANOVA models were used. Days were nested in groups of consecutive sampling days and in mesohabitats and groups of consecutive sampling days were nested in mesohabitats. Effect size was estimated using the maximum variation factors ($100 * (\text{maximum} - \text{minimum}) / ((\text{maximum} + \text{minimum}) / 2)$) and was computed for each variable (mesohabitats, groups of consecutive sampling days and days) of the ANOVA models. The computation of variation factors respected the nested nature of the ANOVA models meaning that variation factor was only computed within their respective mesohabitats and group of consecutive sampling days. For example, the variation factor for days was computed by finding the maximum variation factor between all days in the same group of consecutive sampling days in the same mesohabitats. For this study, a P. value smaller than 0.05 was considered as statistically significant. Correlations between physiological indicators were computed using the Pearson correlation coefficient.

To achieve the first and the second objective we 1) tested for the existence of a relationship between six physiological indicators (basal and response levels of cortisol,

glucose and lactate) and environmental characteristics and 2) tested for the existence of a relationship among the two fish condition factors representing fish fitness metrics (the whole-body lipid concentration and the LeCren condition factor), physiological indicators and environmental characteristics. To do so, we used a regularized model building method. Regularized model building consists in adding a penalty for complex models to reduce overfitting. Regularization is suited for situations where the number of predictors is of similar magnitude or greater than the number of observations (Tibshirani, 1995). Moreover, regularization usually yields models with better variable selection and predictive power than classical model building methods like stepwise selection (Tibshirani, 1997).

However, some regularization methods can lead to models that are hard to interpret and are prone to false positive (when a variable is selected when it should not). A two-stage hybrid variable selection procedure was proposed by (Guo, 2015) to overcome those limitations. The model building method used in this study was adapted from their work. We applied sequentially to the objective function F (the function that the regression tries to minimize) of the ordinary least square regression OLS (equation 1), the penalty used in ridge regression (equation 2; Tikhonov, 1963) and the penalty used in the Least Absolute Shrinkage Selection Operator (LASSO) regression (equation 3; Tibshirani, 1995). The procedure starts by fitting a ridge regression model by minimizing the ridge regression objective function (equation 2) where y_i and \hat{y}_i are the observed and predicted values of physiological indicators and condition factor, b the coefficients of the regression, c the number of coefficients, n the number of data points and λ a tuning parameter for the amount of regularization that varies between 0 (regular multiple linear regression) and ∞ (all coefficients = 0).

$$\text{Equation 1 (OLS)} : F = \sum_{i=1}^n (y_i - \hat{y}_i)^2$$

$$\text{Equation 2 (Ridge)} : F_{\lambda} = \sum_{i=1}^n (y_i - \hat{y}_i)^2 + \lambda \sum_{l=1}^{c-1} b_l^2$$

$$\text{Equation 3 (Weights)} : w = |b_l|^{-2}$$

$$\text{Equation 4 (LASSO)} : F_{\lambda} = \sum_{i=1}^n (y_i - \hat{y}_i)^2 + \lambda \sum_{m=1}^{c-1} w |b_m|$$

Selection of the optimal λ was done through leave one out cross-validation. For the second step, w , the inverse of the absolute value of the squared coefficients computed by the ridge regression (equation 3) was used as a penalty factor for each coefficient in the LASSO regression as shown in equation 4. The ridge and LASSO regression were performed with the glmnet package for R (Friedman, 2010). Six separate LASSO models were needed to achieve the first objective since each LASSO model linked one of the six physiological indicators used in the study (basal and response levels of cortisol, glucose and lactate) with environmental characteristics. Fourteen different LASSO models were built for the second objective. Environmental characteristics alone and each of the six physiological indicators with environmental characteristics were used to explain one of the two condition factor (whole-body lipid concentration or LeCren condition factor). The same regularization method was used to assess the effects of confounding variables such as length and mass on the six physiological indicators and on the two condition factors.

Finally, to achieve third objective that consisted of quantifying the relative contribution of environmental conditions and physiological indicators in explaining the two fish conditions factors, the variation explained by each of the fourteen models used for the second objective were partitioned (Peres-Neto, 2006). Since we wanted to measure and compare the contribution of two different sets of variables, we needed to compute three different fractions: A, B, C. The A and C fractions correspond respectively to the unique contribution of the physiological indicators and environmental characteristics in explaining the condition factors while B corresponds to the contribution shared by both sets of variables.

To compute those fractions, intermediate LASSO regression model (equation 3) relating the condition factor with only the physiological indicator was used with the λ and the respective weight selected by the global model. This yielded all the variation explained by the physiological indicator corresponding to the sum of fractions A and fraction B (A+B). Afterwards, another intermediate LASSO regression model relating the condition factor and all environmental characteristics was computed using the λ and the respective weights selected by the global model yielding all the variation explained by the environmental characteristics corresponding to the sum of fraction B and fraction C (B+C). To obtain the values of fractions A and C, the variation explained by each intermediate model was subtracted from the global model. ($A+B+C - A+B = C$; $A+B+C - B+C = A$) Finally, to compute fraction B, the variation explained only by each model was subtracted from the intermediate models ($A+B - A = B$ or $B+C - C = B$).

Results

Variation in Environmental Characteristics

All environmental characteristics (Table II and Table XII, appendix) varied significantly among mesohabitats except cloud cover. Differences between mesohabitats ranged from 9% for adjusted water conductivity to 110% for water velocity. Only adjusted water conductivity and oxygen saturation varied significantly among groups of consecutive sampling days. Differences of 16% were found for both variables. Differences within groups of consecutive sampling days were greater than 20% except for water temperature and oxygen saturation (Table III). Rain was observed on three sampling days.

Variation in Physiological Indicators and Condition Factor

Statistically significant variations among mesohabitats were found for all physiological indicators except for basal levels cortisol and for both condition factor (Table III). Differences among mesohabitats ranged from 7% for LeCren condition factor to 62% for whole-body lipid concentration. Statistically significant variation in physiological indicators among groups of sampling days was found for basal and response levels of glucose and lactate and whole-body lipid concentration. Difference among groups of sampling day ranged from 17% for response levels of lactate to 34% for whole-body lipid concentration. Variation among sampling days was found for response levels of cortisol, basal levels of lactate and both condition factor (Table V). Differences between days ranged from 21% for LeCren condition

factor up to 140% for response levels of cortisol. Basal levels of glucose were strongly (i.e. >0.50) and significantly correlated with response levels of glucose and basal and response levels of lactate ($0.50 < r < 0.65$; Table IV) at the sampling day level. Response levels of lactate were strongly and significantly correlated ($0.50 < r < 0.65$) with all physiological indicators except basal levels of cortisol (Table VI) at the sampling day level. Whole-body lipid concentration and LeCren condition factors were significantly correlated at 0.53 at the sampling day level.

Table II : Mean standard deviation (SD), minimum value and maximum value of environmental characteristics distribution by sampling day. Since the presence of rain was observed only 3 times during sampling, it was omitted from the table.

Environmental Characteristics	Summary statistics			
	Mean	SD	Min	Max
Adj. Conductivity (uS)	140.0	50.2	79.0	226.0
Water Temperature (°C)	22.4	1.6	19.8	25.6
O₂ saturation (%)	96	12	72	110
Cloud cover (%)	46	36	0	100
Transparency (m)	2.61	1	0.90	4.2
Depth (cm)	28.3	6.4	15.5	39.8
Velocity (m/s)	0.183	0.097	0.036	0.375

Table III : ANOVA results (F statistic, P.values and Variation factor in %) for variation across mesohabitats and groups of consecutive sampling days of environmental characteristics. Statistically significant P.values are marked with an *.

Environmental Characteristics	ANOVA results						
	Mesohabitats			Groups			Days
	F	P.val	VF (%)	F	P.val	VF (%)	VF (%)
Adj. Conductivity (uS)	1220	<0.001*	86	14.2	<0.001*	16	14
Water Temperature (°C)	4.0	0.040*	9	1.0	0.45	10	25
O ₂ saturation (%)	66.3	<0.001*	30	4.4	0.01*	16	15
Cloud cover (%)	0.9	0.43	46	25	0.08	148	200
Transparency (m)	24.7	<0.001*	100	0.6	0.72	19	63
Depth (cm)	12.7	<0.001*	37	0.8	0.59	20	46
Velocity (m/s)	8.96	0.002*	110	1.62	0.211	70	99

Table IV : Mean, standard deviation (SD), minimum value and maximum value of physiological indicators and condition factors of all fish by sampling day.

Variable	Summary statistics			
	Mean	SD	Min	Max
Basal Cortisol (ng/g)	257	282	1.5	1670
Response Cortisol (ng/g)	5830	4570	306	34300
Basal Glucose (mg/dl)	49.7	15.1	16	123
Response Glucose (mg/dl)	70.6	23.3	20	153
Basal Lactate (mmol/L)	4.21	1.75	1.1	10.4
Response Lactate (mmol/L)	7.58	1.61	4.2	13.4
Whole-body lipid (%)	14.1	7.4	1.3	44.1
LeCren	1	0.08	0.72	1.33

Table V : ANOVA results (F statistic, P.val. and Variation factor in %) for variation across mesohabitats and groups of consecutive sampling days of physiological indicators and condition factors using means per sampling day. Statistically significant P.values are marked with an *.

Variable	ANOVA results								
	Mesohabitats			Groups			Days		
	F	P.val	VF (%)	F	P.val	VF (%)	F	P.val	VF (%)
Basal Cortisol (ng/g)	0.23	0.8	16	1.1	0.38	64	1.6	0.082	143
Response Cortisol (ng/g)	9.8	<0.001*	61	0.5	0.77	23	1.8	0.035*	140
Basal Glucose (mg/dl)	11	<0.001*	22	2.5	0.04*	24	1.6	0.092	44
Response Glucose (mg/dl)	40	<0.001*	38	4.3	0.001*	24	1.3	0.229	40
Basal Lactate (mmol/L)	8.7	<0.001*	27	3.5	0.005*	34	2.7	0.002*	55
Response Lactate (mmol/L)	20	<0.001*	20	3.3	0.008*	17	1.7	0.054	23
Whole-body lipid (%)	88	<0.001*	62	3.7	0.003*	29	2.7	<0.001*	95
LeCren	33	<0.001*	7	1.3	0.29	3	2.2	0.004*	21

Table VI : Pearson's correlation coefficients for each physiological indicator using the means of each sampling days. Significant correlations ($p < 0.05$) are marked with an asterisk (*).

Physiological indicators	Response Cortisol (ng/ml)	Basal Glucose (mg/dl)	Response Glucose (mg/dl)	Basal Lactate (mmol/L)	Response Lactate (mmol/L)
Basal Cortisol (ng/ml)	-0.07	0.31	-0.04	0.56*	0.36
Response Cortisol (ng/ml)		0.08	0.37	0.05	0.56*
Basal Glucose (mg/dl)			0.65*	0.57*	0.51*
Response Glucose (mg/dl)				0.30	0.65*
Basal Lactate (mmol/L)					0.50*

Relationship between Physiological Status and Environmental Characteristics

Statistically significant relationships between physiological indicators and environmental characteristics (Objective 1) were found for response levels of cortisol and basal and response levels of glucose and lactate (Table VII). No such relationships were found for basal levels of cortisol. Environmental characteristics explained 28% (basal levels) and 65% (response levels) of the observed variance in glucose levels. Corresponding values for lactate were 21% and 63%. Adjusted water conductivity and water temperature were the two environmental characteristics most commonly selected as explanatory variables in the LASSO models. Basal and response levels of glucose and lactate increased with adjusted water conductivity and water temperature. The LASSO model developed for basal levels of glucose comprised only water temperature and O₂ saturation. In contrast, the LASSO model obtained for response levels of glucose comprised six explanatory variables (Table VII). In this model, the presence of rain was the only environmental characteristic that had a negative effect on response levels of glucose. The LASSO model developed for basal levels of lactate indicated that this physiological indicator was significantly affected only by adjusted water conductivity and water temperature. The observed variance in response levels of lactate was explained by three environmental characteristics (Table VII).

Table VII : Selected coefficients and cross-validated R^2 for the LASSO models relating scaled physiological indicators with scaled environmental characteristics. Only values greater than zero are shown.

Environmental Characteristics	Selected coefficient					
	Cortisol		Glucose		Lactate	
	Basal	Response	Basal	Response	Basal	Response
Adj.Cond (uS)		0.38		0.50	0.093	0.64
Temp (°C)			0.51	0.27	0.55	0.12
O ₂ (%)			0.26	0.35		0.75
Rain				-0.41		
Clouds (%)						
Transparency (m)				0.27		
Depth (m)		0.23		0.39		
Velocity (m/s)						
R ² .cv		28%	28%	65%	21%	63%

Relationship between Conditions Factors, Physiological Status and Environmental Characteristics

Statistically significant relationships between fish conditions factors representing fitness metrics, physiological indicators, and environmental characteristics (Objective 2) were found for both whole-body lipid concentration and LeCren condition factor. LASSO models explained 60% up to 69% of the variations in whole-body lipid concentration (Table VIII; Total). Mass and length had no effect on the six physiological indicators or on the two condition factors. Although various environmental characteristics always contributed in explaining the variance of whole-body lipid concentration, adjusted water conductivity and oxygen saturation were selected in all models and water temperature was selected in three models (Table VIII). When used without physiological indicators, adjusted water conductivity, water temperature and oxygen saturation could explain 61% of the variation observed in whole-body lipid concentration. Basal and response levels of glucose and lactate were selected

as explanatory variables for whole body-lipid concentration models. The variance explained by physiological indicators and environmental characteristics varied less for whole-body lipid concentration than for LeCren condition factor. It ranged from 61% up to 69% (Table VIII) for whole-body lipid concentration and from 10% up to 47% (Table IX) for LeCren condition factor. In contrast with basal levels, which were selected as explanatory variable in only one LASSO model, response levels of glucose and lactate contributed in explaining the variance of LeCren condition factor. Among the environmental conditions, water temperature (in three of the six LASSO models) and water depth (in five of the six LASSO models) had a significant effect on LeCren condition factor (Table IX). Water temperature and depth used together could explain 10% of the observed variation when used without any physiological indicators.

In whole-body lipid concentration models and LeCren condition factor models, water temperature was selected when using only environmental characteristics but was not when using basal or response levels of glucose (Table VI and VIII). Similar patterns were observed for the adjusted water conductivity and oxygen saturation in the whole-body lipid concentration models. Both environmental characteristics were selected with smaller coefficient when paired with basal and response levels of glucose and lactate. In a similar fashion, water depth was not selected in the LeCren condition factor when paired with response glucose levels.

Table VIII: Coefficients of the LASSO models relating whole-body lipid concentration with scaled physiological indicators and scaled environmental characteristics. Only values greater than zero are shown. No physiological indicators were used to predict whole-body lipid concentration in the environment only model represented in the first column. In the other six models, environmental characteristics and each physiological indicator were used as explanatory variables conjointly.

Variable	Coefficients of the whole-body lipid concentration LASSO models						
	Environment	Cortisol		Glucose		Lactate	
	Only	Basal Response	Basal Response	Basal Response	Basal Response	Basal Response	
Adj.Cond (uS)	0.64	0.64	0.61	0.56	0.47	0.53	0.48
Temp (°C)	0.12	0.13	0.13				
O ₂ (%)	0.74	0.73	0.72	0.57	0.58	0.68	0.72
Rain							
Clouds (%)							
Transparency (m)							0.0062
Depth (m)							
Velocity (m/s)							-0.16
Physiological indicator				0.35	0.37	0.31	0.26
R ² .cv	61%	60%	60%	67%	69%	68%	65%

Table IX : Coefficients of the LASSO models relating scaled LeCren condition factor with scaled physiological indicators and scaled environmental characteristics. Only values greater than zero are shown. No physiological indicators were used to predict whole-body lipid concentration in the environment only model represented in the first column. In the other six models, environmental characteristics and each physiological indicator were used as explanatory variables conjointly.

Variable	Coefficients of the LeCren condition factor LASSO models						
	Environment	Cortisol		Glucose		Lactate	
	Only	Basal Response		Basal Response		Basal Response	
Adj.Cond (uS)							
Temp (°C)	0.3	0.3	0.3			0.21	
O ₂ (%)							
Rain							
Clouds (%)							
Transparency (m)							
Depth (m)	0.25	0.25	0.25	0.36		0.28	0.15
Velocity (m/s)							
Physiological indicator				0.61	0.43		0.38
R ² .cv	10%	10%	10%	47%	21%	9%	15%

Quantifying the Relative Contribution of Physiological Indicators and Environmental Characteristics in Explaining Condition Factors

LASSO models that incorporated the combined effects of environmental characteristics and physiological indicators explained a total of 60% up to 69% of the variation in whole-body lipid concentration (Table X). The unique contribution of environmental characteristics to the explanatory capacity of LASSO models developed for whole-body lipid concentration ranged from 16% up to 59% whereas the unique contribution of physiological indicators in these models ranged from 0% up to 11%. The unique contribution of environmental characteristics and physiological indicators in explaining whole-body lipid concentration varied with the physiological indicator included in the LASSO models and ranged from 16%

(response levels of glucose) to 59% (basal levels of cortisol). The distinction between the LASSO models developed using different physiological indicators was also evidenced by the fraction of the explanatory capacity shared by environmental characteristics and physiological indicators. For models developed using cortisol, the shared contribution of environmental characteristics was 1% when combined with basal levels of cortisol and 7% when combined with response levels of cortisol. However, the shared contribution of environmental characteristics for models developed using glucose was 33% when combined with basal levels of glucose and 44% when combined with response levels of glucose. Corresponding values for LASSO models developed using lactate were respectively 16% (response levels of lactate) and 25% (basal levels of lactate).

LASSO models developed for LeCren condition factor using the combined effects of environmental characteristics and physiological indicators generally had lower total explanatory capacities (9 to 47%; Table XII) than LASSO models developed for whole-body lipid concentration). The unique contribution of environmental characteristics to the explanatory capacity of LASSO models developed for LeCren condition factor ranged from -21% up to 9% whereas the unique contribution of physiological indicators in these models ranged from -2% up to 45%. It is useful to note that, as suggested by (Legendre, 2012) negative shared fractions in variation partitioning can be observed when the variation explained by both sets of variable is greater than the sum of the unique contributions. In a similar fashion, negative unique contributions can be observed when the contribution of a variable to the model is greater with the presence of the other variable. As observed with whole-body lipid concentration, the unique contributions of environmental characteristics and

physiological indicators in explaining LeCren condition factor varied with the physiological indicator included in the LASSO models. Environmental characteristics had the highest unique contributions in LASSO models developed to explain variations in LeCren condition factors when cortisol was used as a physiological indicator (9% for both basal and response levels of cortisol). Conversely, physiological indicators had the highest unique contributions in these models when glucose was used as a physiological indicator (16% for response levels of glucose; 45% for basal levels of glucose). The explanatory capacity shared by environmental characteristics and physiological indicators tended to be particularly low for LASSO models using cortisol as a physiological indicator to explain variations in LeCren condition factors (1% for both basal and response levels of cortisol) and relatively high when these models used response levels of glucose (21%) or lactate (32%) as physiological indicators.

Table X: Variation partitioning for the LASSO models relating scaled whole-body lipid concentration with scaled environmental characteristics and physiological indicators. The A fraction corresponds to the variation explained exclusively by each physiological indicator, the B fraction corresponds to the variation explained conjointly by environmental characteristics and physiological indicators and the C fraction corresponds to the variation explained exclusively by environmental characteristics.

Fractions	Variation partitioning (R2.cv %) for whole-body lipid concentration					
	Cortisol		Glucose		Lactate	
	Basal	Response	Basal	Response	Basal	Response
AB (Stress)	1	7	41	53	27	34
BC (Env.)	60	60	59	60	57	56
A (Stress only)	0	0	8	9	11	9
B (Shared)	1	7	33	44	16	25
C (Env. only)	59	53	26	16	41	31
ABC (Total)	60	60	67	69	68	65

Table XI : Variation partitioning for the LASSO models relating scaled LeCren condition factor with scaled environmental characteristics and scaled physiological indicators. The A fraction corresponds to the variation explained exclusively by each physiological indicator, the B fraction corresponds to the variation explained conjointly by environmental characteristics and physiological indicators and the C fraction corresponds to the variation explained exclusively by environmental characteristics.

Fractions	Variation partitioning (R2.cv %) for LeCren condition factor					
	Cortisol		Glucose		Lactate	
	Basal	Response	Basal	Response	Basal	Response
AB (Stress)	1	1	40	37	14	36
BC (Env.)	10	10	2	5	11	11
A (Stress only)	0	0	45	16	-2	4
B (Shared)	1	1	-5	21	16	32
C (Env. only)	9	9	7	-16	-5	-21
ABC (Total)	10	10	47	21	9	15

Discussion

Relationship between Physiological Status and Environmental Characteristics

The results obtained during the present study support the existence of a relationship between five of the six physiological indicators (response levels of cortisol and basal and response levels of glucose and lactate) and environmental characteristics and at the mesohabitat scale and day scales (Objective 1) for cutlip minnows. Since the LASSO models explained more variation in response levels of cortisol, glucose and lactate than in basal levels, it would be safe to say that their response levels are more strongly linked to environmental characteristics than their basal levels.

The capacity of environmental characteristics to explain more variations in response levels of cortisol than basal levels of cortisol may be related to fish habituation to stress. Habituation consists in the attenuation of the stress response in order to reduce detrimental effects of chronic high levels of cortisol such as suppression of the immune system (Barton, 2002). Many studies suggest that mechanisms for habituation are located upstream and downstream of cortisol release (Barton, 1998; Breuner, 2002; Flodmark, 2002; Hontela, 1992; Jentoft, 2005; Magel, 2017; Rich, 2005; Shrimpton, 1994). Those mechanisms have not been completely uncovered yet but potentially include difference expression of cortisol receptors and changes in concentration of corticosteroid-binding globulins (Breuner, 2006; Mommsen, 1999; Pottinger, 2001; Ramsay, 2006). Habituation allows fish to keep basal levels of cortisol

near physiological optimum under different stressor levels but reduce stress-related physiological indicators expression when facing acute stressors such as capture by electrofishing (Rey, 2016; Shrimpton, 1994). As such, detrimental environmental characteristics like hypoxia or limited food availability can impact response levels without any change in the basal levels. Thus, habituation could explain why we observed a stronger linkage between response levels of cortisol and environmental characteristics than with basal levels. Since liver glycogenesis activity depends partly on levels of cortisol, the previous explanation could also be relevant for plasma glucose levels. On the other hand, levels of lactate don't depend directly on glucose levels (Polakof, 2008b). As a result, it is not possible to affirm that response levels of lactate are more linked to environmental characteristics than basal levels for the same reason as cortisol or glucose.

Adjusted water conductivity and water temperature were some of the most important environmental characteristics selected in the LASSO models relating physiological indicators to environmental characteristics. Adjusted water conductivity varied strongly between mesohabitats, thus could possibly represent differences in streams productivity through increase in nutrient concentration (Copp, 2003; Dennis, 1995). It can be hypothesized that cutlip minnows in more productive streams have access to more food thus are better at mobilizing their metabolism through an increase in levels of cortisol, glucose and lactate when facing an acute stressor like capture (Davis, 2011).

As for water temperature, it was selected with a strong coefficient in basal and response glucose and lactate models. Although the thermal niche of cutlip minnows has not yet been defined, it should be noted that the study sites were located at the northern limit of cutlip minnow's distributional range (Scott, 1973). This situation, together with the observed effect of water temperature on response levels of glucose and lactate, may be taken as an indication that at these sites, water temperature represents an ecologically significant limiting factor. The results of the present study therefore suggest that cutlip minnows in warmer waters were in better physiological conditions. However, for aquatic ectotherms like cutlip minnows, shifts in water temperature of few degrees can result in significant changes in metabolism (Claireaux, 2000; Enders, 2006) and in behaviour such as feeding (Stoner, 2004) which in turn changes plasma glucose concentration (Lermen, 2004; Liss, 2014; Polakof, 2012). Thereby, it is plausible that the positive relationship observed between basal and response levels of glucose and water temperature are due to the increases of the cutlip minnow's metabolism at higher water temperature or change in feeding behaviour.

Relationship Between Conditions Factors, Physiological Indicators and Environmental Characteristics

The second objective aimed to test for the existence of a relationship among two fish condition factors representing fish fitness metrics (the whole-body lipid concentration and the LeCren condition factor), physiological indicators and environmental characteristics. Our results suggest a connection between condition factors (whole-body lipid concentration and LeCren condition factor), environmental characteristics and physiological indicators. All whole-body lipid models selected adjusted water conductivity with a positive coefficient. This

support the hypothesis that fish in mesohabitats with higher adjusted water conductivity had access to more food since whole-body lipid concentration is a proxy of nutritional status (Neff, 2004).

Oxygen saturation was selected with a positive coefficient in all whole-body lipid models. Well-oxygenated waters allow a greater scope for activity which is defined as the energy available for all metabolic activities at the exclusion of basal metabolism (e.g. growth, reproduction and movement; Horodysky, 2015). A habitat providing a greater scope for activity has been hypothesized to represent a habitat that also provides higher survival (higher habitat quality; Claireaux, 2000; Huey, 1991). The present study supports this hypothesis. In our case, it is plausible that higher oxygen saturation provided ecophysiological opportunities that allowed fish to spend more energy foraging, accumulate more lipid reserve, and thereby increase their whole-body lipid concentration (Claireaux, 2000; Neff, 2004).

LeCren condition factor was higher in deeper mesohabitats. This observation is consistent with the fact that LeCren condition factor is strongly linked to fish shape (LeCren, 1951) and that fish shape is related to environmental conditions used by fish (Senay, 2015). The present study does not permit to formally discriminate the hypothesis that fish that use deeper habitats acquire a larger LeCren condition factor from the hypothesis that fish that acquire a larger LeCren condition factor select deeper habitats. It is our contention that the later hypothesis may be more plausible given that none of the physiological indicators per se were related to water depth.

Basal and response levels of glucose and lactate were all selected as predictors of whole-body lipid concentration with positive coefficients. This suggests positive relationships between fitness and basal and response levels of glucose and lactate. Adverse condition such as fasting and hypoxia are known to decrease basal levels of glucose (Polakof, 2012). It could be hypothesized that fish with higher fitness were more active thus had higher basal levels of lactate when captured (Omlin, 2014; Polakof, 2008b). Adverse conditions are known to reduce fish fitness and cause habituation through reduction of stress-related physiological indicators (Barton, 1998; Jentoft, 2005). As a result, habituation could explain why high response levels of glucose and lactate were associated with higher levels of whole-body lipid concentration.

Our results also suggest that basal and response levels of glucose and lactate provided a functional linkage between whole-body lipid concentration and specific environmental characteristics namely adjusted water conductivity, water temperature and oxygen concentration. Coefficients for those aforementioned environmental characteristics were smaller or null when basal and response levels of glucose and lactate were used with environmental characteristics in the whole-body lipid models. In a similar fashion, our results suggest that response levels of glucose provided a functional linkage between LeCren condition factor and water temperature and water depth. The presence of such functional linkage suggests that stress-related physiological indicators can help identify which environmental characteristics are linked to greater habitat quality and but can also provide potential mechanism for such linkage.

Basal and response levels of cortisol could not provide linkage between environmental characteristics and both conditions factors due possibly to the important inter-individual variation in cortisol levels (Table IV). However, it could also be possible that modulation of the HPI axis that does not involve cortisol could explain the absence of linkage. Modulation of the HPI axis in fish can occur outside of levels of cortisol regulation through change in cortisol receptor expression or change in corticosteroid-binding globulins concentration (Breuner, 2006; Mommsen, 1999; Ramsay, 2006). Compared to glucose, cortisol is one of many hormones responsible for the stress response in fish, whereas glucose is the end product of the two main stress response pathways (HPI axis and HSC axis). This could explain why the use of an intermediate hormone of the HPI axis like cortisol was not as successful as using an end product like glucose in linking environmental characteristics to both condition factors whereas using glucose, an end product of the stress response was more successful.

Quantifying the Relative Contribution of Physiological Indicators and Environmental Characteristics in Explaining Conditions Factors

The third objective of the study was to quantify the relative contribution of environmental characteristics, and physiological indicators in explaining the two fish condition factors used to represent fish fitness metrics. For this objective, our results suggest that response levels of glucose provide strong linkage between whole-body lipid concentration and environmental characteristics. On the other hand, basal and response levels of glucose and lactate could only provide a weak linkage between environmental characteristics and LeCren condition factor. The weakness of this linkage is not surprising since environmental characteristics could explain little variation in LeCren condition factor. It is possible that basal levels provide linkage between environmental characteristics and LeCren condition factor at scales that were not assessed during our study (seasons or years).

Habitat Modelling Using Physiological Indicators

In summary, response level of glucose seems to be the best of the assessed physiological indicators for habitat quality modelling since it provided strong linkage between whole-body lipid concentration and environmental characteristics. Basal levels of glucose and levels of lactate behaved in similar ways but did not perform better than response levels of glucose. Cortisol levels performed poorly since they failed to explain variation in both condition factors and only response levels were linked to environmental characteristics. Response levels of glucose showed the greatest potential as a habitat quality modelling. They were positively associated to condition factors and provided linkage between specific environmental characteristics namely adjusted water conductivity, water temperature and

condition factor. Thus it provided an indication of which habitats were of greater quality, but also provided potential mechanisms for the link between environmental characteristics and condition factor.

The present study revealed the existence of statistically significant, and at times strong (e.g. whole-body lipid concentration/response levels of glucose/environmental characteristics), relationships between condition factors, physiological indicators and environmental conditions for cutlip minnows living in different mesohabitats surveyed over consecutive summer days. These results suggest that physiological indicators may represent useful tools to facilitate the development of habitat quality models. Indeed, the present study suggests that physiological indicators, like most indices that are part of habitat-use models (preference indices, habitat suitability indices, probability of presence, numerical abundance, biomass. etc.), can be forecasted at spatial (mesohabitats) and temporal (days) scales that are amenable to model the effects of spatially heterogeneous environmental conditions that can vary over short temporal scales. As such, models based on physiological indicators may permit to forecast the effects on fish of modifications of flow or thermal regimes, whether these are related to local (hydropower facilities) or global (climate change) scenarios. As shown by the present study, physiological indicators may possess an added value compared with indices of habitat use by virtue of their relationship with fitness metrics.

Despite this potential, much remains to be done to assess the utility of physiological indicators on habitat quality. Future studies should focus on different species and on the long-

term/cumulative relationships between the temporal fluctuations of stress indicators and fish growth, survival and reproduction. Irrespective of the findings of future studies aimed at filling these knowledge gaps, it is expected that physiological indicators will complement, and not replace, indices of habitat use. Habitat productivity ($P = B \cdot G$; Ricker, 1946) could potentially provide such complimentary framework since it is defined as the product of biomass (B), a measure of habitat use and growth rate (G) a measure of fitness. As a result, habitat productivity may be the ultimate metric of habitat quality (Boisclair, 2001).

Discussion générale

Les résultats obtenus nous ont permis de démontrer le potentiel de certains indicateurs de stress comme outils de modélisation de la qualité des habitats à l'échelle temporelle des jours. À notre connaissance, cette étude est la première à le faire chez des poissons à une courte échelle temporelle. Nos résultats suggèrent que dans notre système d'étude, les niveaux réponses de glucose étaient l'indicateur de stress ayant le plus de potentiel puisqu'ils étaient fortement liés aux caractéristiques de l'environnement et au % de gras total des poissons (un indicateur de succès écologique). Notre étude suggère de plus que les niveaux de lactate sont aussi liés à l'environnement et au % de gras total. Par contre, ces relations sont moins fortes que lorsque les niveaux de glucose sont utilisés. Au regard du cortisol, notre étude ne permet pas d'appuyer son utilisation pour la modélisation de la qualité des habitats étant donné que seuls les niveaux réponses de cortisol sont liés à l'environnement et aucun niveau de cortisol n'est lié aux facteurs de conditions.

Les résultats obtenus par cette étude suggèrent que les indicateurs physiologiques de stress pourraient effectivement contribuer à la modélisation de la qualité des habitats. Il est possible de prédire les niveaux de certains de ces indicateurs à l'aide des caractéristiques environnementales aux échelles spatiales (mésohabitats) et temporelles (jours) où se déroulent les processus déterminant la qualité des habitats. Des variations à de courtes échelles temporelles des caractéristiques environnementales peuvent avoir des conséquences importantes sur le succès écologique des individus. Ces effets sont difficiles à caractériser à

l'aide de méthodes basées sur l'utilisation de l'habitat, particulièrement lorsque ces effets ne sont pas létaux. Notre étude suggère ainsi que certains indicateurs physiologiques sont appropriés pour évaluer les effets de variations environnementales non létales à courtes échelles temporelles. (Wikelski, 2006).

Néanmoins, le travail de validation de l'utilisation des indicateurs de stress pour modéliser la qualité des habitats n'est pas encore terminé. Il est nécessaire que de futures études identifient les mécanismes physiologiques sous-jacents à la relation entre les indicateurs physiologiques, les caractéristiques de l'environnement et le succès écologique chez différentes espèces de poisson. De plus, il serait important d'utiliser des mesures à long terme de succès écologique tel que la survie ou le succès reproducteur des individus. Les facteurs de conditions sont limités dans leur capacité à représenter le succès écologique des individus puisqu'ils ne donnent qu'une indication de la probabilité que l'organisme soit en mesure de contribuer à la génération suivante.

Les indicateurs physiologiques ne seront pas en mesure à eux seuls d'offrir un portrait complet de la qualité des habitats. Boisclair, 2001 argumente que d'une certaine manière, la productivité serait l'ultime indicateur de qualité des habitats. En effet, le concept de productivité intègre dans sa définition ($P = B \cdot C$; Ricker, 1946) une composante liée à l'utilisation des habitats soit la biomasse (B) et une composante liée au succès écologique des organismes fréquentant l'habitat soit la croissance (C). Cette intégration des métriques traditionnelles d'utilisation des habitats avec des métriques de succès écologiques permettrait

potentiellement de mieux guider les actions de conservation et de protection des populations menacées. Malheureusement, ces efforts de modélisation ne seront pas en mesure d'endiguer seuls l'extinction massive causée par les activités humaines. Afin d'assurer une protection à long terme des environnements naturels, il sera nécessaire, en plus développer notre compréhension des organismes et des écosystèmes, de reconnecter les humains à leurs communautés humaines et biotiques (Leopold, 1989; Sauvé, 1997).

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Annexes

Table XII : Adjusted water conductivity (uS), water temperature (°C), O² saturation (%), presence of rain (0 = no rain, 1 = rain) , cloud cover (%), water transparency (m) values for each sampling day and mean of 10 random stratified measurement of water column depth (cm) and velocity (m/s) for each sampling days.

River	Date (mm/dd)	Adj.Cond (uS)	Temp (°C)	O ² (%)	Rain	Clouds (%)	Transparency (m)	Depth (cm)	Velocity (m/s)
Aux Mulets	07/04	84.2	21.4	76	0	20	2.2	23.0	0.29
Aux Mulets	07/05	80.1	21.4	79	0	60	4.2	15.5	0.28
Aux Mulets	07/06	80.5	22.2	73	0	0	3.6	20.6	0.11
Aux Mulets	07/17	80.0	22.4	81	0	35	2.7	25.8	0.33
Aux Mulets	07/18	80.6	22.1	72	0	100	3.0	26.1	0.25
Aux Mulets	07/19	79.0	20.9	109	0	0	2.6	20.2	0.34
Aux Mulets	07/30	83.6	21.0	115	0	30	2.3	22.7	0.23
Aux Mulets	07/31	81.8	22.6	99	0	30	3.4	22.7	0.23
Aux Mulets	08/01	83.0	22.8	102	0	40	3.5	23.6	0.14
Bellefeuille	07/15	185	21.7	94	0	50	1.0	35.6	0.08
Bellefeuille	07/16	191	21.4	97	0	95	1.1	24.6	0.15
Bellefeuille	07/17	197	20.7	91	0	30	1.0	30.6	0.05
Bellefeuille	07/27	220	22.1	92	1	15	1.0	32.4	0.06
Bellefeuille	07/28	226	21.1	92	0	90	0.9	32.8	0.04
Simon	07/06	146	25.4	101	0	60	3.6	25.0	0.21
Simon	07/07	153	22.3	106	1	100	3.4	37.9	0.17
Simon	07/08	153	21.1	107	0	100	3.3	39.8	0.13
Simon	07/09	168	19.8	109	1	100	1.9	34.3	0.38
Simon	07/22	162	25.6	104	0	20	3.1	31.6	0.21
Simon	07/23	164	23.5	106	0	20	2.3	33.6	0.12
Simon	07/24	163	24.0	96	0	60	2.8	33.1	0.26
Simon	08/03	167	21.8	103	0	0	3.1	24.1	0.08
Simon	08/04	168	25.6	101	0	40	3.6	36.9	0.13
Simon	08/05	172	23.9	103	0	0	3.1	26.8	0.13

Table XIII : Mean per sampling day of basal and response levels of cortisol, glucose and lactate, whole-body lipid concentration and LeCren condition factor. Due to unforeseen shipping issue, lactate values are not available for one sampling days and are represented by empty cells.

River	Date (mm/dd)	Sampled fish		Cortisol (ng/g)		Glucose (mg/dL)		Lactate (mmol/L)		Whole- body Lipid (%)	LeCren
		n	Mean length (cm)	Basal	Response	Basal	Response	Basal	Response		
Aux Mulets	07/04	14	9.4	309	3360	48.2	55.8	3.15	7.24	11.9	0.96
Aux Mulets	07/05	10	9.2	443	3120	52.2	60.2	4.22	6.32	14.1	0.93
Aux Mulets	07/06	14	9.3	409	5010	47.2	57.5	2.80	6.45	12.9	1.02
Aux Mulets	07/17	10	9.8	70.6	7730	49.5	56.0	3.58	6.90	07.4	0.95
Aux Mulets	07/18	14	9.2	423	3680	46.4	60.7	3.89	6.79	11.1	0.97
Aux Mulets	07/19	14	9.3	196	2430	40.3	56.0	3.31	7.20	7.1	0.96
Aux Mulets	07/30	14	9.7	285	7680	34.8	62.4	3.85	7.85	11.9	0.96
Aux Mulets	07/31	13	9.1	160	1370	50.3	67.0	4.28	6.24	5.9	0.95
Aux Mulets	08/01	12	9.2	144	1810	45.6	52.0	3.66	6.53	8.8	1.01
Bellefeuille	07/15	15	10	225	7030	47.1	66.0			10.6	0.97
Bellefeuille	07/16	16	10	117	6830	47.5	69.6	3.39	7.12	13.5	0.99
Bellefeuille	07/17	15	9.7	321	6310	48.1	65.0	5.48	8.13	10.2	1.00
Bellefeuille	07/27	13	10	353	5540	44.7	51.1	4.23	7.09	12.6	0.99
Bellefeuille	07/28	14	9.9	132	10600	28.6	56.9	3.54	6.85	8.1	0.94
Simon	07/06	14	9.5	476	5780	62.1	78.6	4.89	8.69	17.7	1.08
Simon	07/07	15	9.8	147	6160	48.8	77.7	3.92	7.11	19.2	1.05
Simon	07/08	11	9.6	84.5	6160	46.3	86.5	3.40	7.15	17.8	1.02
Simon	07/09	11	10	241	6350	42.5	57.7	2.78	7.24	17.0	1.03
Simon	07/22	15	9.3	147	10300	63.4	88.0	7.24	9.03	22.1	1.04
Simon	07/23	16	9.2	156	6390	57.3	95.8	4.19	8.35	19.1	1.03
Simon	07/24	15	9.5	400	6880	61.1	92.4	4.78	9.71	17.7	1.08
Simon	08/03	15	9.4	212	3660	64.5	83.1	5.78	7.80	25.7	1.06
Simon	08/04	15	9.5	392	8340	47	109	5.23	9.84	11.6	1.00
Simon	08/05	14	10	267	7650	51.7	87.9	4.1	8.88	18.3	1.00