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IMPORTANCE DES TRANSITIONS ALIMENTAIRES SUR L'ACTIVITÉ ENZYMATIQUE DES POISSONS

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AVANT-PROPOS

Ce mémoire comprend deux chapitres. Le premier présente une revue de littérature et un survol des résultats préliminaires découlant de l'exploration des données qui ont mené au projet de maîtrise tel qu'il est aujourd'hui. Le second est un article en anglais qui sera soumis pour publication dans le journal « Functional Ecology » et qui présente les résultats de mon projet de maîtrise.

RÉSUMÉ

L'activité maximale du lactate déshydrogénase (LDH) dans la musculature axiale des poissons serait reliée aux activités de nages lors de quêtes alimentaire et associée aux coûts énergétiques encourus. La relation de l'activité de la LDH selon la taille est généralement positive durant l'ontogénie des poissons. On a toutefois noté un ralentissement temporaire de l'activité de la LDH suite à une transition alimentaire dans le contenu stomacal des poissons. Un changement de taille de proie ou du type de proie devrait modifier suffisamment l'activité natatoire du poisson lors de la quête alimentaire pour induire une réponse enzymatique de la LDH au niveau des muscles blancs. Plus de 1800 poissons, représentant 16 espèces, ont été échantillonnés dans un lac fluvial du système du fleuve St-Laurent. Comme résultats préliminaires, une variation de l'activité de la LDH a été observé à la taille où se situe une transition alimentaire plus ou moins ponctuelle dans le contenu stomacal de certaines espèces, notamment chez la perchaude (espèce la plus représentée dans les captures).

Mots-clés : Lactate déshydrogénase (LDH), transition alimentaire, activité de nage, enzyme, ontogénie, quête alimentaire.

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CHAPITRE I

IMPORTANCE DES TRANSITIONS ALIMENTAIRES SUR L'ACTIVITÉ ENZYMATIQUE DES POISSONS

1.1 Introduction

Une quête alimentaire est considérée optimale lorsqu'un animal maximise la quantité nette d'énergie consommée par unité de temps relié à la recherche de nourriture (Henderson *et al.* 2004). Plusieurs facteurs influencent le succès d'une quête de nourriture : les temps de recherche, de capture et de digestion ainsi que le risque de prédation, la taille de la proie, son apport énergétique et la complexité de l'habitat (Werner et Hall, 1979; Abrams, 1993). Les bénéfices nets de la nutrition augmentent lorsque l'apport énergétique des proies est supérieur aux coûts métaboliques encourus (activité natatoire, digestion et métabolisme de base). Les coûts métaboliques (aérobiques et anaérobiques) reliés aux activités natatoires augmentent avec la taille des poissons par individu; plus un poisson est gros, plus les forces de friction sont élevées (Webb, 1977; Henderson et Anderson, 2002). Au cours de l'ontogénie, le métabolisme aérobique augmente positivement mais n'est pas directement proportionnel avec la taille de l'animal par unité de masse (Schmidt-Nielsen, 1984). Ceci implique que le métabolisme aérobique ne peut être suffisant pour combler les activités de nage. Il a été proposé que le métabolisme anaérobique (glycolytique) servirait à compenser les besoins énergétiques de l'activité natatoire (Childress et Somero, 1990).

Les différentes activités de nage impliquent une utilisation distincte de l'énergie. Une accélération brusque, par exemple, est requise afin d'attaquer une proie ou échapper à un prédateur. Une telle accélération rapide (angl : burst) implique l'utilisation des muscles blancs qui ont un métabolisme glycolytique anaérobique. En contraste, une nage régulière et prolongée, rencontrée lors des déplacements, est soutenue par les muscles rouges qui ont un métabolisme oxydatif aérobique.

Il y a deux transitions alimentaires possibles chez les poissons, la première entre la planctivorie et la benthivorie, et la deuxième entre la benthivorie et la piscivorie. Les transitions s'effectuent en général progressivement, c'est-à-dire les poissons ingèrent des proies de plus en plus grosses dans un même mode d'alimentation (ex. un poisson benthique pourrait manger des chironomes, des trichoptères et ensuite des odonates avant d'effectuer une transition). La transition alimentaire, vers des proies plus grosses chez les poissons de plus grande taille, sert à maintenir une rentabilité énergétique des gains envers les pertes afin de profiter d'une croissance constante (Sherwood *et al.*, 2002a). Dans Sherwood *et al.* (2002b) on note que lorsque les coûts métaboliques dépassent le budget énergétique total du poisson, il est possible de se retrouver en présence d'un ralentissement ou bien d'un arrêt de la croissance. Un tel phénomène apparaît lorsque le poisson se retrouve dans un environnement où la chaîne trophique est trop simplifiée pour permettre une transition alimentaire (Vander Zanden *et al.*, 1999; Pazzia *et al.*, 2002; Sherwood *et al.*, 2002a; Sherwood *et al.*, 2002b). De ce fait, un poisson se trouvant en absence de proies intermédiaires entre deux transitions alimentaires (ex. l'absence de grosses larves d'odonates entre la benthivorie et la piscivorie) se voit contraint à manger davantage de petites proies, ce qui implique plus de dépenses d'énergie reliées à la quête de nourriture et donc une diminution de la croissance (Pazzia *et al.*, 2002).

L'utilisation d'enzymes métaboliques est une approche permettant de quantifier le niveau d'activité natatoire individuelle à court terme. Afin d'évaluer l'importance de l'apport énergétique des proies chez les poissons prédateurs selon leur taille (Sherwood *et al.*, 2002a). L'activité maximale de certaines enzymes glycolytiques, incluant celle de la lactate déshydrogénase (LDH) dans les muscles blancs des poissons, serait reliée aux comportements de quête de nourriture et associée aux coûts énergétiques occasionnés (Sullivan et Somero 1980; Childress et Somero 1990). De plus, la quantification de l'activité enzymatique de la LDH permet d'avoir une image des besoins énergétiques récents, car les poissons répondent aux variations des demandes en énergie des derniers jours ou des dernières semaines (Nathanailides, 1996; Schulte *et al.*, 2000).

1.2 Revue de la littérature

Cette section présente la revue de littérature sur laquelle s'est basé le fond de cette étude.

Types de muscles

Chez les poissons, les muscles occupent une partie importante de la masse totale, environ 60 %. La musculature est divisée en trois types, muscle rouge, blanc et rose (Sanger et Stoiber, 2001). Les deux types principaux de muscles, blancs et rouges, sont distribués dans des régions homogènes distinctes qui sont facilement séparables anatomiquement, tandis que les muscles roses occupent les régions intermédiaires entre les deux. Les muscles blancs occupent près de 70 % de la masse musculaire des poissons, les rouges entre 5 et 30 % et les roses la proportion restante, soit entre 0 et 25 % (Sanger et Stoiber, 2001).

Les muscles blancs des poissons sont fortement composés de fibre de type IIb. Ces fibres sont spécialisées pour donner des contractions rapides et fortes mais se fatiguent très rapidement. Elles utilisent la respiration anaérobique qui est peu efficace pour fournir de l'ATP soit 2 molécules d'ATP par molécule de glucose. Les muscles blancs sont associés aux accélérations rapides servant, entre autres, à capturer des proies ou à fuire un prédateur (Sullivan et Somero, 1980). Les muscles rouges chez les poissons sont en grande majorité composés de fibres de type I. Ces fibres effectuent des contractions lentes et soutenues et se fatiguent peu. Elles utilisent la respiration aérobique qui est très efficace pour fournir de l'ATP soit 36 molécules d'ATP par molécule de glucose ce qui procure l'énergie nécessaire à une nage soutenue (Sullivan et Somero, 1980). Le haut taux d'hémoglobine dans leurs nombreux vaisseaux sanguins ainsi que la grande quantité de myoglobine présente dans la musculature procurent la couleur rougeâtre aux muscles rouges. Certaines espèces de poissons ont des muscles roses qui sont en fait un type intermédiaire entre les muscles blancs et rouges. Ces muscles sont caractérisés par un mélange de fibres de type I et IIB.

Chaque type de muscles a un profil métabolique qui lui est propre. Le changement de régime alimentaire et de niche induisent une réponse enzymatique de la LDH dans les muscles blancs, ceux-ci sont donc intéressants afin d'étudier l'évolution d'une enzyme au cours de l'ontogénie.

Conditions aérobiques vs anaérobiques

Les muscles rouges fonctionnent en conditions aérobiques lors de déplacements prolongés et de nages soutenues demandant de l'endurance musculaire. La glycolyse en présence d'oxygène produit bien plus de molécules d'ATP qu'en absence d'oxygène. Le pyruvate produit par la glycolyse peut être transformé en acétyle-CoA et ainsi entrer dans le cycle de Krebs et produire davantage d'ATP. La dégradation d'une molécule de glucose en conditions aérobiques produit 36 molécules d'ATP. La présence de la LDH est limitée dans les muscles rouges, car il est peu utilisé lors d'activités aérobiques comme des déplacements prolongés ou de la nage soutenue.

Les muscles blancs fonctionnent surtout en conditions anaérobiques pour de courts déplacements rapides demandant une accélération soudaine. La glycolyse en absence d'oxygène ne produit que très peu d'ATP et le pyruvate produit est transformé en lactate plutôt qu'en acétyle-CoA. La LDH agit comme catalyseur enzymatique afin d'initialiser la réaction dégradant le pyruvate en lactate. Lors de cette réaction, une molécule de NADH s'oxyde en NAD+, le pyruvate accepte l'atome d'hydrogène et devient une molécule de lactate (Figure 1.1). La dégradation d'une molécule de glucose en absence d'oxygène ne produit que deux molécules d'ATP ainsi que deux de lactate. La réaction initialisée par la LDH est réversible. Au repos, suite à un effort produit en condition anaérobique, le surplus de lactate dans les muscles blancs peut être reconverti in situ en pyruvate ou en glycogène, transporté dans le sang vers d'autres organes (cœur et muscles rouges) pour être oxydé ou vers le foie pour reformer du glucose par gluconéogenèse (Milligan et Girard, 1993).

L'activité enzymatique de la LDH est fonction des demandes récentes en énergie (Nathanailides, 1996; Schulte *et al.*, 2000). Étant donné que la LDH est fortement utilisé dans les muscles blancs lors des accélérations rapides reliées aux attaques sur une proie ou l'évitement d'un prédateur, l'étude de l'activité enzymatique du lactate déshydrogénase permet d'avoir une image fiable des demandes énergétiques récentes et requises lors des activités de quête de nourriture.

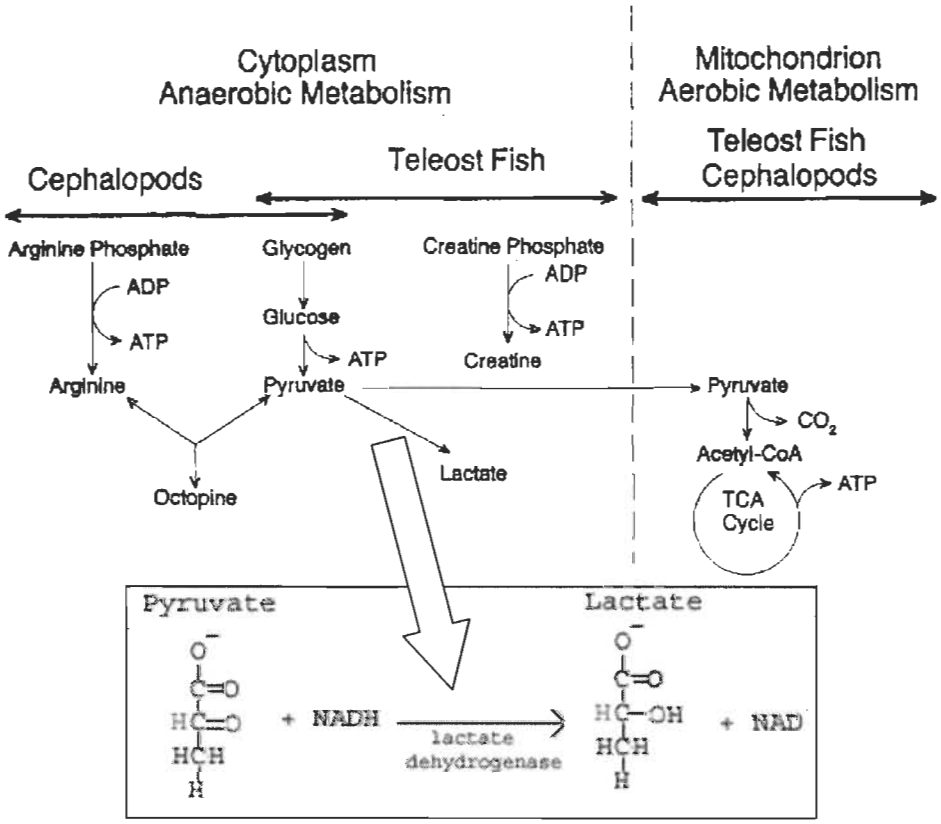

Figure 1.1 : À gauche, métabolisme anaérobique; à droite, métabolisme aérobique; en bas, détail de la réaction impliquant la LDH (Tiré de Huss, 1995).

Ontogénie alimentaire

Au début de leur ontogénie, les poissons larvaires sont trop petits pour se nourrir d'autres poissons. De ce fait, lors de la croissance, les espèces piscivores subissent des changements qui influencent leurs contraintes structurelles (ex. ouverture buccale maximale (DeVries *et al.*, 1998), leur acuité visuelle et leur vitesse de nage (Morote *et al.*, 2008). Les jeunes de l'année se nourrissent habituellement de zooplancton, les

juvéniles de benthos et les adultes de poissons. Certaines espèces, notamment les piscivores spécialistes, deviennent piscivores très tôt dans leur développement, tandis que d'autres, les piscivores secondaires, prennent des années avant de devenir piscivores (Mittelbach et Persson, 1998). Pour les espèces non-piscivores, il peut n'y avoir aucune transition alimentaire ou n'y avoir qu'une seule.

Ces transitions alimentaires ontogéniques sont souvent reliées aux stratégies de quête de nourriture. En effet, le bénéfice énergétique dépend de la taille des proies et de leur qualité nutritionnelle ainsi que de la capacité du prédateur à repérer, capturer et ingérer ses proies (Mittelbach, 1981). Le temps de recherche et le nombre d'attaques requis pour rassasier un prédateur augmentent lorsque la taille relative de ses proies diminue (Kerr, 1971; Kerr et Ryder, 1977). Afin de maintenir sa croissance constante, un individu doit optimiser sa quête de nourriture (Sherwood, 2002a) en visant des proies ayant un meilleur rapport entre gains et coûts d'énergie. Les juvéniles d'espèces piscivores semblent maximiser leur gain d'énergie en favorisant la piscivorie dès que les contraintes physiques sont disparues (Galarowicz *et al.* 2006).

En absence de transition alimentaire, il arrive que les gains en énergie de la nourriture soient égaux ou moindres aux pertes encourues, le poisson se retrouve alors dans une impasse (goulot d'étranglement énergétique : en anglais « energetic bottleneck »), où la croissance peut ralentir ou arrêter (Figure 1.2). Une telle situation peut se produire lorsque il y a un changement d'habitat ou lorsque la chaîne trophique est trop simplifiée, c'est-à-dire qu'il manque d'éléments transitoires entre deux diètes afin qu'un prédateur puisse effectuer une transition alimentaire. (Sherwood *et al.*, 2002a; Sherwood *et al.*, 2002b). Une simplification de la chaîne trophique peut être occasionnée par la pollution d'un plan d'eau (Sherwood *et al.*, 2002b), l'introduction d'une espèce invasive ou d'un compétiteur pour une ressource ou une niche écologique particulière. (Vander Zanden *et al.*, 1999; Pazzia *et al.*, 2002).

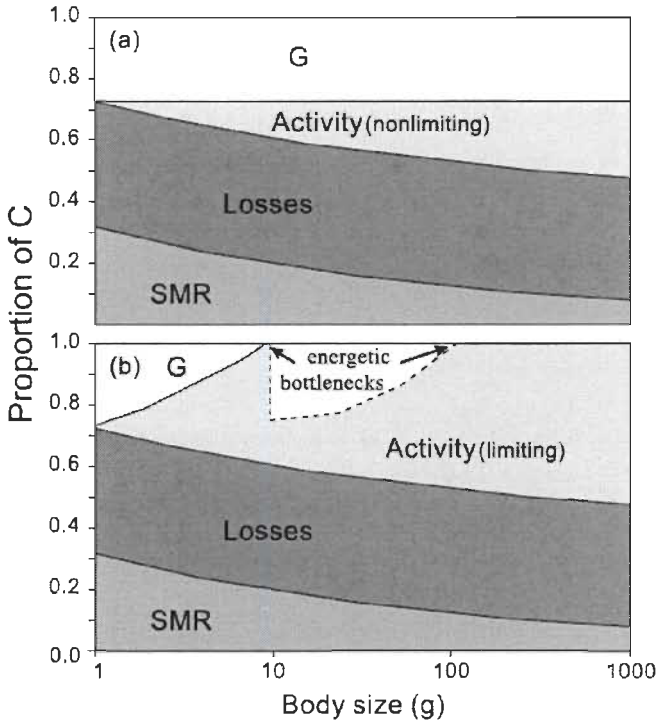

Figure 1.2 : Relation entre les coûts métaboliques et la taille des poissons. En a) les coûts métaboliques ne sont pas limitants. En b) ralentissement ou arrêt de la croissance dus aux activités de quête de nourriture limitantes. (SMR : Standard Metabolic Rate (Taux métabolique standard), Losses : pertes, Activity : activité de quête de nourriture, G : Growth (Croissance)) (Tiré de Sherwood *et al.*, 2002a.).

En présence d'une transition alimentaire vers des proies plus grosses, on rencontre en général une augmentation du taux de croissance (Werner and Gilliam 1984). Cela peut se traduire par une augmentation de la biomasse consommée, par une diminution des dépenses énergétiques pour la quête de nourriture (Kerr, 1971; Kerr et Ryder, 1977) ou une combinaison des deux. Pazzia *et al.* (2002) ont examiné la croissance du touladi (*Salvelinus namaycush*) entre des populations effectuant ou non une transition alimentaire vers une piscivorie stricte. Ils démontrent que les populations piscivores montrent un meilleur taux de croissance que les populations se trouvant dans une impasse alimentaire (Figure 1.3).

La présence ou l'absence de transition alimentaire sont donc importantes dans l'ontogénie des poissons pour la croissance. Afin de détecter les transitions alimentaires,

l'examen des contenus stomacaux permet d'avoir une image récente de la diète d'un individu. Dans ce projet nous étudions les contenus stomacaux de plusieurs individus de différentes tailles d'une même espèce, pour une vingtaine d'espèces, représentant ainsi l'évolution ontogénique de l'alimentation de chacune.

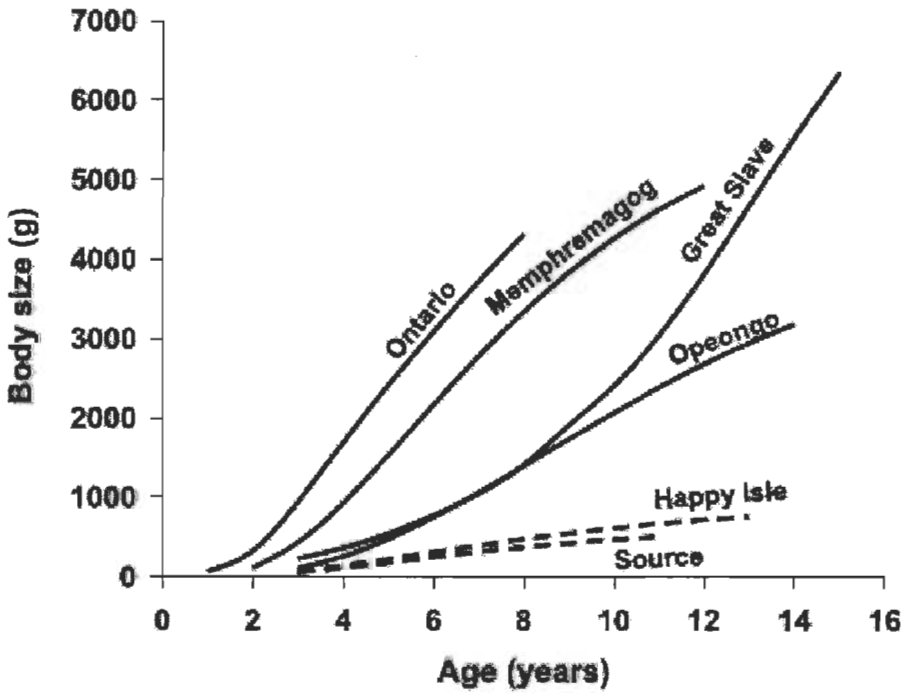

Figure 1.3 : Évolution de la taille de touladi en fonction de leur âge pour des populations piscivores (trait plein) et non-piscivores (trait hachuré) (Tiré de Martin, 1966).

Hypothèse

Selon la littérature consultée, un changement au niveau de l'activité de la LDH serait induit par la présence d'une transition alimentaire à une taille donnée. Il y a en général deux approches qui permettent de mettre en lien la relation de l'activité d'une enzyme et la présence de transitions alimentaires. La première consiste à déterminer s'il y a la présence d'une transition alimentaire durant l'ontogénie pour ensuite vérifier si cette transition a un effet sur l'activité enzymatique. Cette approche infère qu'un bris dans la relation ne peut découler que d'une transition alimentaire. La deuxième approche consiste plutôt à déterminer s'il y a la présence d'un bris dans la relation de l'activité de

la LDH pour ensuite vérifier si la position de ce bris correspond à une transition alimentaire. Cette étude arbore la seconde approche qui est moins restrictive, car celle-ci infère qu'il est possible d'avoir un bris dans la relation en absence d'une transition alimentaire.

1.3 Matériel et méthodes

1.3.1 Site de l'étude

Le lac Saint-Pierre (LSP; 46° 12' N, 72° 49' O) est le plus grand lac fluvial dans le système du fleuve St-Laurent. La surface du LSP fluctue entre 387 et 501 km² selon la saison, ayant un maximum en avril et un minimum en septembre (Hudon 1997). Le LSP a une profondeur moyenne de 3,1 m; le chenal maritime atteint cependant des profondeurs de plus de 13 m. La perchaude (*Perca flavescens*, Mitchill) est l'espèce de poisson dominante dans le LSP qui accueille plus de 80 des 116 espèces de poissons retrouvés au Québec (La Violette *et al.* 2003). Plusieurs tributaires se retrouvent sur la rive nord (rivières du Loup et Yamachiche) et la rive sud (rivières Yamaska, Saint-François et Nicolet). Les rives nord et sud sont caractérisées par des masses d'eau distinctes qui se différencient horizontalement par leurs propriétés optiques et chimiques correspondant à leurs tributaires d'origines (Frenette, Arts et Morin 2003). La position et la forme de ces masses d'eau diffèrent grandement dans le temps (Frenette *et al.* 2006).

1.3.2 Échantillonnage des poissons

L'échantillonnage s'est effectué à l'aide d'un bateau pêche à l'électricité, le Narke, du 21 juin au 24 août 2006, sur des transects de 50 mètres parallèle à la rive localisés, dans la zone littorale des rives nord et sud à des profondeurs inférieures à 2,5 mètres (Figure 1, page 44). Deux autres transects ont été échantillonnés près du chenal maritime du LSP.

Les poissons capturés ont été mesurés (longueur totale (mm)), pesés (masse humide (g)) et gardés sur de la glace sèche avant d'être transférés à la fin de la journée dans un

congélateur (-80°C) au laboratoire. Un total de 1916 poissons ont été échantillonnés, représentant plus de 30 espèces; cependant, les analyses quantitatives n'ont pu être effectuées que sur 1804 individus des 16 espèces suivantes qui étaient présentes en nombre suffisant : barbotte brune (*Ameiurus nebulosus (Lesueur))*, crapet soleil (*Lepomis gibbosus*, Linne), doré jaune (*Sander vitreus*, (Mitchill)), fondule barré (*Fundulus diaphanus*, LeSueur), gaspareau (*Alosa pseudoharengus*, Wilson), grand brochet (*Esox lucius*, Linne), laquaiche argentée (*Hiodon tergisus*, LeSueur), méné d'argent (*Hybognathus regius*, Girard), méné émeraude (*Notropis atherinoides*, Rafinesque), méné jaune (*Notemigonus crysoleucas*, (Mitchill)), méné pâle (*Notropis volucellus*, (Cope)), meunier noir (*Catastomus commersoni*, (Lacepede)), museau noir (*Notropis heterolepis*, Eigenmann et Eigenmann), omisco (*Percopsis omiscomaycus*, (Walbaum in Artedi)), perchaude et queue à tache noire (*Notropis hudsonius*, (Clinton)).

1.3.3 Activité enzymatique de la LDH

Afin de déterminer la présence d'un bris de linéarité dans l'activité enzymatique de la LDH durant l'ontogénie, nous avons quantifié l'activité maximale (U = µMol min-1 g-1) de la LDH du muscle blanc de chaque individu. Un échantillon de muscle blanc (~ 100 mg) a été prélevé sur le flanc gauche des poissons, derrière la nageoire dorsale bien au-dessus de la ligne latérale. Les échantillons de muscle furent homogénéisés dans une solution tampon d'imidazole (50 mM, pH de 7,2) (dilution 5 m/v) et centrifugés à 2000 g pour cinq minutes. Les concentrations finales de la solution permettant d'évaluer l'activité maximale de la LDH étaient les suivantes :

Lactate déshydrogénase (EC 1.1.1.27); 50 mM imidazole, pH ajusté à 20°C, 0,2 mM NADH et 100 mM d'acide pyruvique (omis lors du contrôle).

L'activité maximale fut déterminée à 20°C en suivant la diminution d'absorbance du NADH à une longueur d'onde de 340 nm à l'aide d'un spectrophotomètre (CARY 100 UV-Visible) thermorégulé.

1.3.4 Transitions alimentaires

Le contenu stomacal de chaque individu a été déterminé en utilisant un microscope à dissection (1x to 60x). Les proies ont été identifiées à l'ordre ou à la famille lorsque possible, dénombrées, mesurées (largeur de la tête/capsule céphalique (mm)) et classées en six catégories : benthos (petit < 1 mm et grand > 1 mm), zooplancton (cladocère et copépode), végétation (chenille), neuston, poisson proie, restes d'insectes (surtout des capsules céphaliques et des ailes) et débris (sable, végétation, etc.). Le stade de développement (œuf, larve, pupe ou adulte) a été noté pour les insectes. Afin de comparer les proies complètes et incomplètes, la largeur de la tête/ capsule céphalique, qui semble mieux résister à la digestion que d'autres parties du corps, a été utilisée au lieu de la longueur totale des proies.

1.3.5 Analyses exploratoires

Cette section présente un survol des analyses exploratoires qui ont mené au choix des analyses quantitatives de cette étude.

1.3.5.1 Activité de la LDH

Afin de déterminer si l'activité de la LDH était positive un modèle linéaire a été utilisé. Par la suite nous avons appliqué une courbe de lissage au modèle afin de détecter tout changement d'activité de la LDH durant l'ontogénie des poissons.

1.3.5.2 Transition alimentaire

Lorsqu'un bris est présent dans la relation de l'activité de la LDH chez une espèce, la taille et le type de proies de chaque contenu stomacal de cette espèce ont été mis en relation avec la taille des poissons à l'aide d'un modèle linéaire. En plus de la courbe de lissage de la relation de l'activité de la LDH, une courbe de lissage a été ajoutée au modèle afin de vérifier visuellement si une transition alimentaire était présente à une taille semblable que pour le bris dans l'activité de la LDH.

1.4 Résultats préliminaires

L'activité de la LDH était positive pour la majorité des espèces; seule l'activité de la LDH de l'omisco semble négative (Figure 2, page 45). On remarque chez certaines espèces que l'activité de la LDH semble ralentir ou même diminuer à une certaine taille durant l'ontogénie. C'est le cas de l'activité de la LDH de la perchaude où un point de cassure dans l'activité de la LDH est présent à une taille variant entre 90 et 120 mm. La courbe de lissage utilisée pour illustrer la progression de la relation de l'activité de la LDH selon la taille a été transposée sur le graphique présentant le contenu stomacal des perchaudes (Figure 1.4).

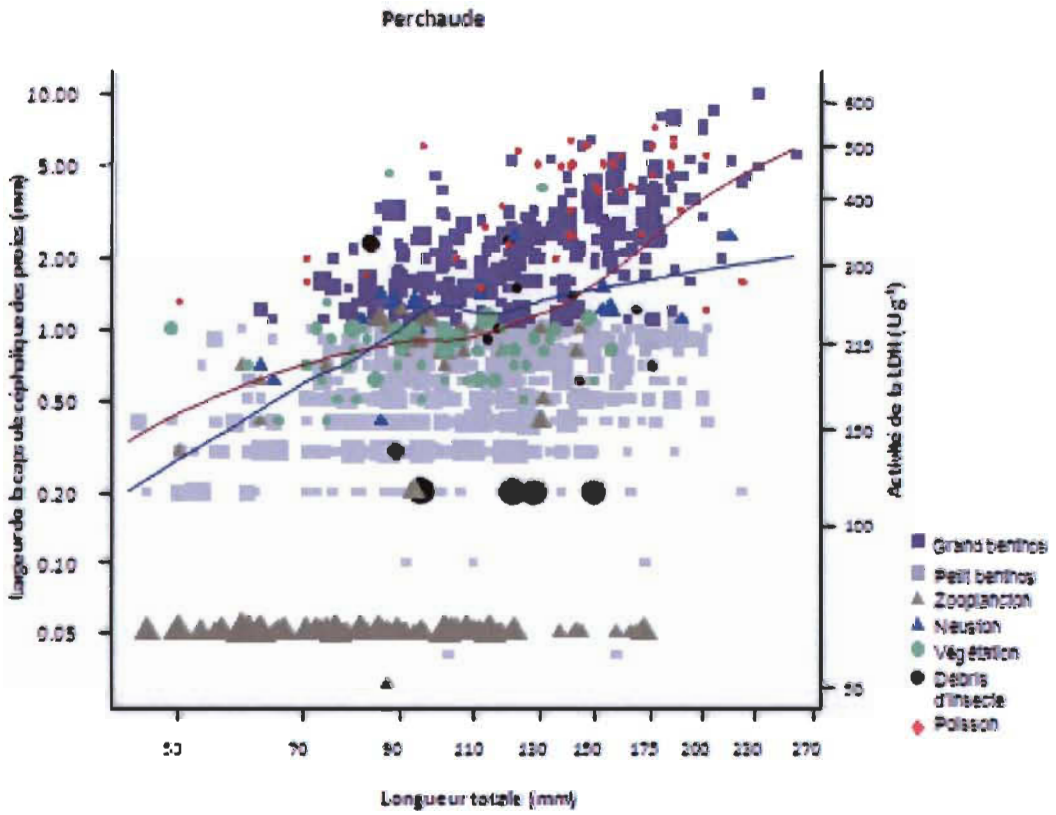

Figure 1.4 : Relation entre l'activité de la LDH et la taille des poissons (courbe en bleu) et relation entre la taille et le type de proie avec la taille des poissons (courbe en rouge).

On remarque que le bris dans la relation de la LDH semble coïncider avec un plus grand nombre de proies tels que le grand benthos et les poissons au détriment du zooplancton. L'examen des contenus stomacaux révèle la présence d'un chevauchement des régimes alimentaires. Ceci ne permet pas de statuer qu'une transition alimentaire ponctuelle et bien définie est présente, c'est-à-dire que les poissons, ayant atteint une certaine taille durant leur ontogénie, n'ont pas modifié radicalement leur régime alimentaire. On observe plutôt une transition alimentaire partielle, c'est-à-dire que la proportion de la fréquence des types de proies consommées diffère lorsque les poissons ont atteint une certaine taille durant leur ontogénie.

1.4.1 Prélude à l'article

Deux tests ont été nécessaires afin de quantifier d'une part la présence et la position d'un bris de linéarité dans la relation entre l'activité de la LDH et la tailles des poissons, et d'autre part la présence et la position d'une transition alimentaire.

Premièrement, la présence d'un bris de linéarité dans l'allométrie de l'activité de la LDH a été déterminée en effectuant un test de Davies et une régression par morceau « piecewise regression ». Le modèle de régression par morceau calculé se défini comme suit :

$$ln(\text{activité LDH}) = \beta_0 + \beta_1 ln(\text{masse humide})_i + \beta_2 (ln(\text{masse humide})_i - \psi) \qquad (1)$$

où chaque poisson est indexé par i, β_0 est l'ordonnée à l'origine, β_1 et β_2 sont respectivement les coefficients quantifiant les effets de la masse humide et la différence entre les pentes et ψ représente la position du bris de linéarité. Les données d'activité de la LDH et la masse humide ont été transformées en log afin d'éviter de donner trop de poids aux valeurs extrêmes.

Deuxièmement, les deux transitions alimentaires principales chez les poissons, qui sont définies dans d'autres études (Sherwood *et al.* 2002a; Iles et Rasmussen 2005), sont soit une transition entre la planctivorie et la benthivorie et une autre entre la benthivorie et la

piscivorie. Dans cette étude, nous définissons une transition alimentaire comme étant un changement dans la fréquence de la présence des proies à une taille donnée, sans nécessairement le passage à une nouvelle diète (benthivorie ou piscivorie). Par conséquent, afin de déterminer la présence d'une transition alimentaire durant l'ontogénie chez les espèces ayant une relation bilinéaire de l'activité de la LDH selon la taille, un arbre de classification a été réalisé sur la fréquence des proies de chaque catégorie en fonction de la masse des poissons (Gning, Vidy et Thiom Thiaw 2008). L'arbre de classification ayant la plus petite erreur a été retenu, comme le suggèrent De'ath et Fabricius (2000). La taille des poissons au premier nœud est notée comme étant la taille spécifique où un changement de fréquence de la présence d'un certain type de proie diffère significativement durant l'ontogénie. La proportion des proies avant et après le premier nœud de l'arbre de classification a été estimée à l'aide de l'intervalle de confiance Bayésien à 95 % afin de déterminer la position de la transition alimentaire.

Au meilleur de notre connaissance, cette étude serait la première à quantifier l'allométrie d'une enzyme glycolytique à l'aide d'un modèle bilinéaire. Alors que d'autres études (Sherwood *et al.* 2002; Iles et Rasmussen 2005) ont observés une diminution de l'activité de la LDH suivant des transitions alimentaire ponctuelles et bien définies de la planctivorie à la benthivorie et de la benthivorie à la piscivorie; nos résultats suggéreraient plutôt qu'une diminution de l'activité de la LDH peut se produire en présence d'une transition alimentaire partielle et progressive.

1.5 Remerciements

Nous remercions M. Léveillé, J.-F. Déry, O. S. Soumana et G. Trottier pour leur aide sur le terrain et en laboratoire. Nous remercions aussi A. Bertolo, G. Cabana et P. Massicotte pour leur aide précieuse et leurs commentaires tout au long de cette étude. Cette étude a été possible grâce à une subvention d'équipe du Fond québécois de la recherche sur la nature et les technologies (FQRNT) à M.A.R. et H.G. ainsi qu'aux subventions individuelles du CRSNG de M.A.R. et H. G.

CHAPITRE II

EFFECT OF DIET SHIFT ON THE ALLOMETRY OF ENZYMATIC ACTIVITY IN FISHES FROM A SHALLOW FLUVIAL LAKE

Effect of diet shift on the allometry of enzymatic activity in fishes from a shallow fluvial lake

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Summary

1. Maximal activity of lactate dehydrogenase (LDH) in the axial musculature of fish has been suggested to be related to foraging behaviour and associated activity costs. LDH activity has mostly a positive linear relationship with body size during ontogeny; however, following a diet shift, LDH activity has been noted to decline, creating a break in the linear relationship.

2. The objective of this study was to quantify the presence and the position of any breakpoint in the relationship of LDH activity with body size during ontogeny and then verify if that breakpoint corresponds to a diet shift.

3. White muscle LDH activity and stomach content analyses were determined for 1804 fish, representing 16 species, from shallow fluvial, Lake St.-Pierre in the St. Lawrence River system. Davies test and piecewise regression were used to determine respectively the presence and position of a breakpoint. Classification tree analyses were generated to identify changes in prey proportions leading to a diet shift.

4. Piecewise regressions reveal the presence of a breakpoint for blacknose shiner, brown bullhead, emerald shiner, mooneye, northern pike and yellow perch. Classification tree gut content analyses of these fish confirmed that a diet shift occurred within the confidence interval of the body size associated with the breakpoint.

5. Our results suggest that bilinearity as shown by the presence of a breakpoint in the allometry of LDH activity can arise from a diet shift. The reduction in slope of LDH activity is likely related to changes in swimming activities during foraging following a diet shift. Feeding on larger or different prey types might lead to a reduction in the time spent searching and the number of attacks which are reflected by a reduction in LDH activity.

Key-words: Bilinear, classification tree, lactate dehydrogenase (LDH), ontogeny, piecewise regression

Introduction

Larvae of most fish species are exclusively zooplanktivores due to morphological constraints such as gape-size limitation (DeVries, Bremigan and Stein 1998) or swimming speed (Morote *et al.* 2008). In order to sustain continual growth during ontogeny, fish must undergo diet shifts (Townsend and Winfield 1985). For example, larvae of piscivorous species start by feeding on zooplankton, and progressively feed on larger prey to eventually switch to foraging on fish (Mittelbach and Persson 1998). It is easily conceivable that foraging activity and associated cost will not be the same for a zooplanktivorous versus a benthivorous and a piscivorous fish. Consequently, foraging activities might change following an ontogenic diet shift and implicate different swimming activities, which in turn modulate the distinct use of available energy.

Metabolic enzymatic activity in muscle represents a potentially useful approach for quantifying recent activity levels of individual fish (Sherwood *et al.* 2002a). Enzymatic activity in red and white muscle provides different information on recent swimming activity. Aerobic red muscle produces steady propulsion to sustain long periods of swimming whereas anaerobic white muscle produces short bouts of fast acceleration which is used to attack prey and flee predators. White muscle lactate dehydrogenase (LDH) activity has been related to foraging behaviour and associated activity costs (Somero and Childress 1990; Childress and Somero 1980). LDH activity in white muscle may provide a snapshot of recent energetic requirements for swimming, for individual fish, in the order of two to seven days (Schulte *et al.* 2000). LDH activity has been shown to mainly scale positively in a linear relationship with body size (Somero and Childress 1990; Childress and Somero 1980). However, following an ontogenic diet shift, LDH activity may slow down (Sherwood *et al.* 2002a; Iles and Rasmussen 2005). This phenomenon has been described by Sherwood *et al.* (2002a) as a "saw tooth" distribution as LDH activity seemed to reset to lower values in presence of a discrete diet shift (Fig. 1.). A slowdown in LDH activity has also been noted to occur when fishes undergo an ontogenic change in habitat from pelagic toward benthic habitat (Bailey *et al.* 2005; Siebenaller 1984; Selch and Chipps 2007). Previous studies (Sherwood *et al.* 2002a; Iles and Rasmussen 2005) have observed the relationship

between diet shift and LDH allometry during ontogeny, however there have been no statistical quantification of the presence or the position of a breakpoint in the relation. Moreover, most studies had either relatively small sample size (Gibb and Dickson 2002; Sherwood *et al.* 2002a; Selch and Chipps 2007) and (or) a small number of species studied (Norton, Eppley and Sidell 2000; Gibb and Dickson 2002; Bailey *et al.* 2005).

There are two main approaches when relating the allometry of LDH activity and the presence of a diet shift. Firstly, one can detect the presence of a diet shift and afterward verify if diet shift has an effect on the allometry of LDH activity. Secondly, one can verify the presence of a break in the relationship of LDH activity with body size and then verify if the break corresponds to a diet shift. This study will use the later approach as it considers the possibility of a break in the relationship of LDH activity with body size in the absence of a diet shift. The goal of this study is to quantify the presence and the position of a breakpoint at a particular body size (g) in LDH activity during ontogeny and then verify if an ontogenic diet shift occurs at that same body size. We determine this relationship for LDH activity with diet shift for 1804 fish, representing 16 species, from a shallow fluvial lake.

We hypothesise that a slowdown in LDH activity following a breakpoint in the bilinear allometry will be related to a diet shift. A change in size or prey type will likely influence swimming activity during foraging leading to a response in LDH activity.

Materials and methods

Study site

Lake St-Pierre (LSP, 46° 12' N, 72° 49' W) is the largest fluvial lake in the St. Lawrence River system. LSP is shallow (mean depth < 5 m), with the exception of a central navigation channel that reaches depths of over 13 m. The surface area fluctuates between 387 and 501 km^2 depending on water level, attaining a maximum in April and minimum in September (Hudon 1997). Inflowing tributaries are found on both the north and south shores of LSP. The north and south shores are associated with distinct water

masses that are differentiated horizontally by optical and chemical properties corresponding to their watersheds of origin (Frenette, Arts and Morin 2003). The littoral zone of LSP offers an highly heterogeneous environment in regards to water transparency (Frenette *et al.* 2003) and macrophyte cover (Vis, Hudon and Carignan 2003) thus providing different feeding opportunities. LSP harbours more than 80 species of the 116 freshwater fish species found in Quebec (La Violette 2003) and was therefore designated ecological biosphere reserve by UNESCO in 2001.

Fish sampling

Fish were collected in the shallow littoral zone of LSP (<2·5 m depth) by electrofishing (Smith-Root Cataraft 17 boat) mostly along the north and south shore, as well as in the navigational channel, in the summer of 2006 (26 June – 24 August) (Fig. 2). Fish were measured (total length (mm)), weighed (wet mass (g)), and kept on ice in the field, and then transferred at the end of the day to an ultra low temperature freezer (-80°C) in the laboratory. A total of 1916 fishes were sampled representing more than 30 species; however, quantitative analyses were performed on 1804 fishes for the following 16 species present in sufficient number: Alewife (*Alosa pseudoharengus*, Wilson), banded killifish (*Fundulus diaphanus*, LeSueur), blacknose shiner (*Notropis heterolepis*, Eigenmann and Eigenmann), brown bullhead (*Ameiurus nebulosus*, (LeSueur)), emerald shiner (*Notropis atherinoides*, Rafinesque), golden shiner (*Notemigonus crysoleucas*, (Mitchill)), mimic shiner (*Notropis volucellus*, (Cope)), mooneye (*Hiodon tergisus*, LeSueur), northern pike (*Esox lucius*, Linne), pumpkinseed sunfish (*Lepomis gibbosus*, Linne), silvery minnow (*Hybognathus regius*, Agassiz), spottail shiner (*Notropis hudsonius*, (Clinton)), trout-perch (*Percopsis omiscomaycus*, (Walbaum in Artedi)) walleye (*Sander vitreus*, (Mitchill)), white sucker (*Catastomus commersoni*, (Lacepede)) and yellow perch (*Perca flavescens*, Mitchill.

Enzymatic activity of LDH

In order to determine the presence of a breakpoint in LDH activity during ontogeny, we assessed maximal activity (U = μMol min^{-1} g^{-1}) in white muscle of individual fish. White muscle samples were dissected in the laboratory. Samples were taken on the left side of the frozen carcass of fish behind dorsal fin and well above the lateral line. Muscle samples (~100 mg) were homogenized in imidazole buffer (50 mM, pH 7·2) (5 w/v) and centrifuged at 2000 g for 5 minutes. Assay conditions were optimized with respect to substrate concentration and were as follow:

Lactate dehydrogenase (EC 1.1.1.27); 50 mM imidazole, pH was adjusted at 20°C, 0·2 mM NADH and 100 mM pyruvic acid (omitted for control).

Maximal activity was determined at 20°C by following the decrease in absorbance of NADH at 340 nm with a spectrophotometer (CARY 100 UV-Visible) equipped with thermostated cell changer.

Diet shift

Stomach contents of individual fish were determined using a dissection microscope (0x to 60x). Prey were identified to order or family level when possible, counted, measured (head/cephalic capsule width (mm)), and classified accordingly: small benthos (insect larvae like Trichoptera and Odonata; cephalic capsule width < 1mm), large benthos (mainly large insect larvae, bivalva and gasteropoda; cephalic capsule width or total width > 1 mm), zooplankton (Cladocera, Copepoda, etc.), vegetation (Lepidoptera), neuston, prey fish, insect remains (unidentified insects remains, mainly head/cephalic capsule and wings) and debris (sand, vegetation, etc.). The developmental stage (egg, larva, pupae or adult) were also noted for insects. In order to compare complete and incomplete prey width of head/cephalic capsule which seem to resist digestion longer than other body parts, were used instead of total length of prey.

Quantitative analysis

LDH activity relationship with body size

We first assessed a linear model in order to determine if the relation of LDH activity with body size was positive, then tested the three following models: quadratic model of level two, quadratic model of level three and a bilinear model. The best fitting model among the four tested was selected using the Akaike's information criterion (AIC).

In order to determine the presence and location of a breakpoint in the relationship of LDH activity with body size, a Davies test and a piecewise regression were performed. The Davies test is appropriate for testing the presence of a breakpoint despite being slightly conservative and tends to somewhat overestimate the p-value (Muggeo 2008). On the other hand piecewise regressions seems to be an overlooked, objective technique for estimating ecological thresholds (Toms and Lesperance 2003; Betts, Forbes and Diamond 2007). The method is suitable for any regression model with a linear predictor, therefore classical general linear models (GLMs) as well as generalized additive models (GAMs), survival models, ordinal response models having segmented relationships with some explanatory variables, may be fitted (Muggeo 2003). The piecewise regression was performed by fitting the following bilinear relationship to LDH activity allometry:

$$\ln(\text{LDH activity}) = \beta_0 + \beta_1 \ln(\text{total length})_i + \beta_2(\ln(\text{total length})_i - \psi) \qquad (1)$$

where individual fish are indexed by i, β_0 is the intercept, β_1 and β_2 are coefficients quantifying the effects of total length and difference between slopes respectively and ψ represents the position of the breakpoint. LDH activity and body mass were log-transformed to avoid giving undue weight to extreme observations.

Diet shift

Different studies (Sherwood *et al.* 2002a; Iles and Rasmussen 2005) have defined two principal and complete diet shifts, the first occurring between zooplanktivory and benthivory and the second between benthivory and piscivory. In this study, we define a

diet shiftas being a change in prey occurrence at a given body size without necessarily shifting to a completely new diet (benthivory or piscivory). In order to determine the presence of a diet shift during ontogeny, a classification tree was performed on the prey occurrence of each category (Gning, Vidy and Thiom Thiaw 2008), for each species having a bilinear allometry in LDH activity[1]. The tree having the smallest error was retained since, according to De'ath and Fabricius (2000), it is the best estimated predictive single tree. The body mass of fish at the first node (cutpoint) was noted as the specific size at which the occurrence of a particular prey type changes significantly during their ontogeny. In order to determine the position of a diet shift, we assessed the proportion of prey before and after the cutpoint given by the classification tree, with a Bayesian confidence interval of 95%.

All quantitative analyses were performed using R version 2·10·1 with the packages segmented 0·2-6, mvpart 1·2-6 and binom 1·0-5.

Results

LDH activity relation with body size

LDH activity relation was positive for 15 out of 16 species (Fig. 3); only trout-perch had a negative relation during ontogeny. LDH activity was isometric for alewife, banded killifish, golden shiner, mimic shiner, walleye and white sucker. Pumpkinseed sunfish, silvery minnow, spottail shiner and trout-perch LDH activity relationship with body size were quadratic of second degree for the first two species and quadratic of third degree for the last two species respectively. The relation of LDH activity of six species (blacknose shiner, brown bullhead, emerald shiner, mooneye, northern pike and yellow perch) were bilinear (best AIC score for bilinear model) and were therefore tested whether the breakpoint was significant. All six species, blacknose shiner ($p = 0{\cdot}04$), brown bullhead ($p = 0{\cdot}005$), emerald shiner ($p < 0{\cdot}001$), mooneye ($p = 0{\cdot}003$), northern pike ($p = 0{\cdot}001$) and yellow perch ($p = 0{\cdot}04$) had a significant breakpoint in their

[1] As the chosen approach dictate, diet shift is assessed only when a breakpoint occurs in the LDH activity allometry.

allometry during ontogeny (Table 1). For mooneye, even in the presence of an outlier the LDH activity allometry was significant (Fig. 3). Furthermore, the presence of a breakpoint was corroborated by a diet shift occurring at a similar body size. Following the breakpoint, all species show a slowdown in LDH activity except for northern pike where LDH activity increases (Fig. 3).

Diet shift

After assessing a breakpoint in the allometry of LDH activity for six species, we determined whether the breakpoint in LDH activity relation was induced by a diet shift. Stomach content analyses revealed diet overlap between different type of prey and provided no evidence of a complete diet shift which occur when a fish species having reached a particular size during its ontogeny changes its diet completely. In contrast, we observe partial diet shifts where at a particular body size, the percentage of prey occurrence consumed differs. The occurrence of zooplankton decreased in the stomach contents of blacknose shiner, brown bullhead and yellow perch, as does the occurrence of insect remains for emerald shiner and mooneye during their ontogeny (Table 2). In addition, for mooneye, the occurrence of large benthos and neuston prey in stomachs increases. Those changes in prey occurence coincide with the breakpoint in the relationship of LDH activity with body size for each species. However, a change in prey occurrence in the diet did not coincide with the breakpoint for some species. This was the case for blacknose shiner and emerald shiner where small benthos increased, and decreased for yellow perch, with no coincidence with the breakpoint. Even though there was the presence of a breakpoint in the relationship of LDH activity with body size for northern pike (Table 1), there was no apparent diet shift as they were already feeding on fish (Table 2).

Discussion

LDH activity relationship with body size

White muscle LDH activity of fishes from LSP has a highly variable and mostly positive relationship with body size during ontogeny. This result is consistent with previous studies that showed positive relationship of LDH activity with body size in marine (Childress and Somero 1990; Sullivan and Somero 1980) and freshwater fish (Sherwood *et al.* 2002a; Iles and Rasmussen 2005; Kaufman *et al.* 2009). This relationship between LDH activity and body size may reflect an increase of anaerobic power requirements in larger fish to overcome higher drag forces while burst swimming (Sullivan and Somero 1980; Childress and Somero 1990).

Trout-perch is the only species in this study that showed a negative relationship of LDH activity with body size. This result does not appear to be related to the anaerobic power requirements of fish. Rather, the observed negative allometry for trout-perch might LDH activity slowing down after a recent diet shift and (or) habitat shift (Sherwood *et al.* 2002a). Other studies (Hanke 1996; Coker, Portt and Minns 2001) reported that trout-perch, of similar size as the ones in this study, were already feeding on benthos while still feeding on zooplankton. Since in this study, trout-perch have already begun feeding on benthos, it is likely that a partial diet shift from zooplankton to benthos has already occurred and influences foraging activity (see below).

Different studies (Sherwood *et al.* 2002a; Sherwood *et al.* 2002b; Iles and Rasmussen 2005) observed that LDH activity decreases sharply at well-defined diet shifts from planktivory to benthivory and from benthivory to piscivory using line-fitting techniques such as analysis of covariance (ANCOVA). They observed LDH activity decreases for pumpkinseed sunfish and yellow perch following well-defined diet shifts from one type of prey to another. In this study, instead of defining the presence of a diet shift before investigating the bilinearity of LDH activity, we first assessed the presence and the position of a breakpoint in the allometry of LDH activity. Afterward, we assessed the presence and position of a diet shift and verified if the diet shift corresponded to the

breakpoint in the relationship of LDH activity with body size. We observe a bilinear relationship for blacknose shiner, brown bullhead, emerald shiner, mooneye, northern pike and yellow perch for which we were able to quantitatively identify the presence and the position of a breakpoint. A decrease in LDH activity following the breakpoint in the bilinear relationship occurs for five of the six species while an increase in LDH activity occurs for northern pike following the breakpoint (Table 1). Northern pike switches to piscivory at sizes varying from 40 to 100 mm (Mittlebach and Perrson 1998). Northern pike sampled in this study, of size varying from 90 to over 300 mm, were already feeding on fishes, consequently the negative slope before the breakpoint might be due to a missed diet shift similar to trout-perch.

Diet shift

As pointed earlier, the approach of this study is to assess the presence of a breakpoint in the LDH activity relationship with body size and then verify if this breakpoint is related to a diet shift. Therefore, after assessing a breakpoint in the allometry of LDH activity for six species, we determined whether the breakpoint in LDH activity allometry correspond to a diet shift.

Stomach content analyses in this study revealed intraspecific diet overlap during ontogeny. Rezsu and Specziár (2006) showed similar diet overlapping for many fish species including yellow perch of similar size to those in this study. Diet overlap does implicate that there is no complete diet shift, from one prey type to another, at a specific fish body size.

A partial diet shift from zooplankton to benthos was observed for blacknose shiner, brown bullhead and yellow perch evidenced by a decrease in the occurrence of zooplankton in their diet as they continue to feed mainly on benthos. These diet shifts correspond to the 95% interval of confidence of the LDH activity breakpoint in the allometry and a reduction in LDH activity (Table 2). Moreover, an increase in occurrence of insect remains for emerald shiner, in large benthos and neuston for

mooneye also resulted in the 95% interval of confidence of a breakpoint and a slowdown in LDH activity (Table 2).

Partial diet shift might be responsible for the reduction in the slope in the allometry of white muscle LDH activity of blacknose shiner, brown bullhead, emerald shiner, mooneye and yellow perch. Changes in muscle enzyme activity observed following a diet or habitat shift have been related to muscle use (Bailey *et al.* 2005). By switching from a prey type to another during ontogeny, foraging may implicate different muscle use like swimming activity and (or) swimming time. Reduction of occurrence of zooplankton in the diet of blacknose shiner, brown bullhead and yellow perch, and a higher occurrence of larger preys in the diet of emerald shiner and mooneye probably also has an effect on their swimming activity during foraging.

Childress and Somero (1990) pointed out that scaling of glycolytic enzymes resulted from different patterns of selection acting on the different species living in different habitats and having different habits. At the same time, they stressed on the fact that the largest variable appears to be benthic versus pelagic lifestyles. Pelagic species usually have a higher LDH activity than benthic species (Childress and Somero 1990). Bailey *et al.* (1995) observed a decrease in LDH activity in a deep-sea eel (*Synaphobranchus kaupii, Johnson*) when switching from a pelagic habitat to a benthic habitat. In analogy, a small freshwater fish feeding on zooplankton has a lifestyle close to a pelagic species by swimming in the water column, with little to no refuge opportunities. Later, when feeding on benthos, it has a lifestyle close to a benthic species with more refuge opportunities thus having a different swimming activity than a zooplanktivore. In consequence, freshwater fish might show a slowdown in LDH activity following a change from a pelagic to benthic diet/habitat as Bailey *et al.* (1995) observed for a marine species. Subsequently, the partial diet shifts from zooplankton to benthos observed for blacknose shiner, brown bullhead and yellow perch correspond likely to the decrease in LDH activity. The two occur at the same size as the breakpoint in the allometry of LDH activity.

By feeding on relatively larger preys emerald shiner and mooneye will likely reduce the number of attacks to reach satiation, thus spending less time searching for preys and being exposed to predators (Kerr 1971; Kerr and Ryder 1977). As a result, numbers of burst swimming will decrease as preys become larger, thus leading to a decrease in white muscle use. The resulting decrease in glycolytic power requirement in white muscle is thus reflected by a reduction in LDH activity. Subsequently, the partial diet shifts from small to larger prey observed for emerald shiner and mooneye is probably related to the decrease in LDH activity as it occurs at the same size as the breakpoint in the allometry of LDH activity.

In some cases, a diet shift seemed to occur without a change in LDH activity. A shift in occurrence of small benthos in the stomach contents of blacknose shiner, emerald shiner and yellow perch did not correspond to a breakpoint in LDH allometry. The occurrence of small benthos dropped for yellow perch later in the ontogeny than when the breakpoint occurs as they continue to feed on larger benthos and fish. Similar results have been observed by Iles and Rasmussen (2005) where benthos became gradually more abundant in the fish diet with no clear LDH response. Given that the decrease in LDH activity following a diet shift is a result of different swimming activity related to foraging, there seems to be no difference in swimming as yellow perch gradually feed on larger benthos.

Small benthos were already present in stomach content of blacknose and emerald shiner; it became almost omnipresent in stomach content at a body size not corresponding to the breakpoint in LDH allometry (Table 2). As stated earlier, a decrease in LDH activity following a diet or habitat shift is a result of different swimming activity during foraging. While the occurrence of small benthos gradually increase in stomach content of blacknose and emerald shiners, even though it becomes almost dominant, their swimming activity may not change enough to be detectable (Iles and Rasmussen 2005).

Missed diet shift

The first diet shift, when most species start feeding on benthos following an exclusively zooplankton diet, was not observed for any of the species. Due to electrofishing limitations, fish sampled were all longer than 45 mm; consequently, all non-piscivorous species had already started feeding on benthos or larger prey and piscivorous species were already feeding on fish. It would have been interesting to catch smaller individuals since the first diet shift is easily detectable (Rezsu and Specziár 2006) and there is a strong response in LDH activity following this diet shift (Joseph B. Rasmussen, personal communication). Nonetheless, breakpoints in LDH activity allometry were present for six species even though the strongest response in LDH activity following the first diet shift has been missed.

As stated earlier, the negative relationship of LDH activity with body size for trout-perch and the increase in LDH activity after the breakpoint in the enzymatic allometry for northern pike might be due to a missed diet shift. The former being a missed diet shift from zooplankton to benthos leading to a negative relationship in LDH activity with body size, whereas the latter being a missed diet shift to a larger type of preys resulting in a negative slope before the breakpoint.

An increase in LDH activity following the breakpoint might be due to the fact that as northern pike grows, they feed on constant prey type and size in this study. While feeding on constant prey size, northern pike should likely increase the number of attacks to reach satiation, thus spending more time searching for preys and being exposed to predators (Kerr 1971; Kerr and Ryder 1977), resulting in a highly positive scaling of LDH activity (Sherwood *et al.* 2002a).

Conclusion

To the best of our knowledge, this study is the first to assess allometry of glycolytic enzyme using a bilinear model. While other studies (Sherwood *et al.* 2002; Iles and Rasmussen 2005) have observed that LDH activity decreases sharply at well-defined

diet shifts from planktivory to benthivory and from benthivory to piscivory; our results suggest that decreases in LDH activity, observed as bilinear allometric model, can arise from partial diet shifts. Piecewise regression model appear to be robust enough to detect slight LDH decreases occurring from different swimming activities following a partial diet shift.

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Table 1. Linear regression model and piecewise regression model when Davies test *p* value is lower than 0.05.

		Linear regression				Piecewise regression				
Species	Number of fish	Intercept	Slope	*p* value	R^2	Intercept	Base slope	Change in slope	R^2	Davies test *p* value
Alewife	59	-0.55 ± 0.10	0.91 ± 0.10	<0.001	0.61					0.37
Banded killifish	134	-0.27 ± 0.11	0.79 ± 0.20	<0.001	0.11					1
Blacknose shiner	76	-0.18 ± 0.10	1.42 ± 0.23	<0.001	0.34	0.02 ± 0.15	2.85 ± 0.84	-2.36 ± 0.84	0.40	0.04
Brown bullhead	100	-1.61 ± 0.42	0.42 ± 0.11	<0.001	0.15	-2.30 ± 0.55	0.63 ± 0.15	-1.93 ± 0.67	0.23	0.02

Table 1 (continued)

Emerald shiner	131	-0.30 ± 0.18	0.35 ± 0.14	0.02	0.04	-1.27 ± 0.24	1.39 ± 0.24	-2.38 ± 0.45	0.26	<0.001
Golden shiner	246	-0.27 ± 0.12	0.25 ± 0.08	0.004	0.03					0.10
Mimic shiner	55	-0.03 ± 0.14	0.69 ± 0.39	0.08	0.04					0.97
Mooneye	54	-1.01 ± 0.10	0.50 ± 0.04	<0.001	0.79	-1.09 ± 0.09	0.56 ± 0.05	-0.63 ± 0.24	0.82	0.01
Northern pike	31	-2.17 ± 0.96	0.67 ± 0.29	0.03	0.17	0.27 ± 1.62	-0.19 ± 0.57	1.88 ± 0.83	0.32	0.04

Table 1 (continued an concluded)

Pumpkinseed sunfish	153	-1.11 ± 0.28	0.44 ± 0.11	<0.001	0.10					0.12
Silvery minnow	59	-1.17 ± 0.30	0.79 ± 0.19	<0.001	0.24					0.22
Spottail shiner	90	-0.92 ± 0.52	0.57 ± 0.31	0.03	0.03					0.20
Trout perch	30	0.24 ± 0.17	-0.06 ± 0.15	0.71	0.01					0.17
Walleye	111	-1.29 ± 0.16	0.52 ± 0.06	<0.001	0.46					0.80
White sucker	30	-1.17 ± 0.27	0.50 ± 0.10	<0.001	0.48					1
Yellow perch	445	-0.68 ± 0.09	0.28 ± 0.03	<0.001	0.17	-1.05 ± 0.16	0.49 ± 0.08	-0.32 ± 0.11	0.18	0.02

Table 2. Position of breakpoint in LDH activity allometry given by piecewise regression model. Diet shift represented by a change in percentage of prey presence in stomach contents below and above cutpoint given by classification tree (CT).

				Percentage presence in stomach contents (95% confidence interval)	
Species (number of fish stomachs)	Regression breakpoint in LDH activity (g) (95% confidence interval)	Prey category	Significant Cutpoint in CT (g)	Below cutpoint (number of fish)	Above cutpoint (number of fish)
Blacknose shiner (73)	1.2 (0.9-1.5)	Insect remains and detritus	None		
		Large benthos	None		
		Small benthos	2.1	0.51 (0.39-0.62) (67)	0.79 (0.44-0.98) (6)
		Neuston	None		
		Vegetation	None		
		Zooplankton	0.9	0.74 (0.56-0.89) (24)	0.43 (0.30-0.57) (49)

Table 2. (continued)

Brown bullhead	83.5 (64.7-107.8)	Insect remains and detritus	None		
(92)		Large benthos	None		
		Small benthos	None		
		Neuston	None		
		Vegetation	None		
		Zooplankton	65.6	0.51 (0.37-0.65)	0.10 (0.03-0.21)
				(49)	(43)
Emerald shiner	3.7 (3.2-4.3)	Insect remains and detritus	3.2	0.07 (0.02-0.15)	0.33 (0.21-0.46)
				(60)	(52)
(112)		Large benthos	None		
		Small benthos	5.3	0.71 (0.62-0.79)	0.96 (0.84-1.00)
				(101)	(11)
		Neuston	None		

Table 2. (continued)

		Vegetation	None		
		Zooplankton	None		
Mooneye	37.2 (20.6-67.5)	Insect remains and detritus	47.53	0.42 (0.28-0.57)	0.07 (0.00-0.26)
				(43)	(6)
(49)		Large benthos	39.3	0.17 (0.07-0.30)	0.54 (0.27-0.80)
				(38)	(11)
		Small benthos	None		
		Neuston	25.23	0.48 (0.31-0.66)	0.79 (0.59-0.93)
				(29)	(20)
		Vegetation	None		
		Zooplankton	None		

0

Table 2. (continued and concluded)

Northern pike (28)	38.4 (27.4-53.8)	Insect remains and detritus	None		
		Large benthos	None		
		Small benthos	None		
		Neuston	None		
		Vegetation	None		
		Zooplankton	None		
Yellow perch (409)	10.6 (5.2-21.8)	Insect remains and detritus	None		
		Large benthos	None		
		Small benthos	69.9	0.87 (0.83-0.90) (369)	0.45 (0.30-0.60) (40)
		Neuston	None		
		Vegetation	None		
		Zooplankton	14.4	0.41 (0.35-0.48) (204)	0.10 (0.07-0.15) (205)

Figure captions

Figure 1. Simplified "saw-tooth" pattern in LDH activity relation with body size as observed by other studies.

Figure 2. Map of Lake St. Pierre Quebec, Canada. Black circles represents the sampling sites.

Figure 3. Relationship of LDH activity with body size during ontogeny. Best fitting curve are in black. Linear regression curves are in grey when not the best fitting curve. LDH activity has been centered and reduced by species.

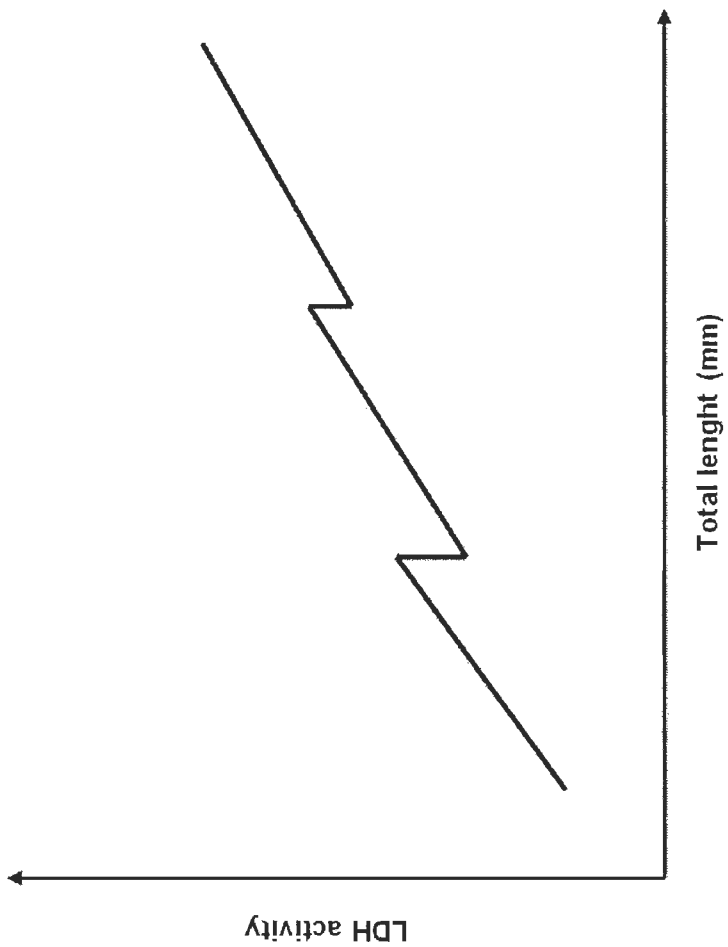

Figure 1.

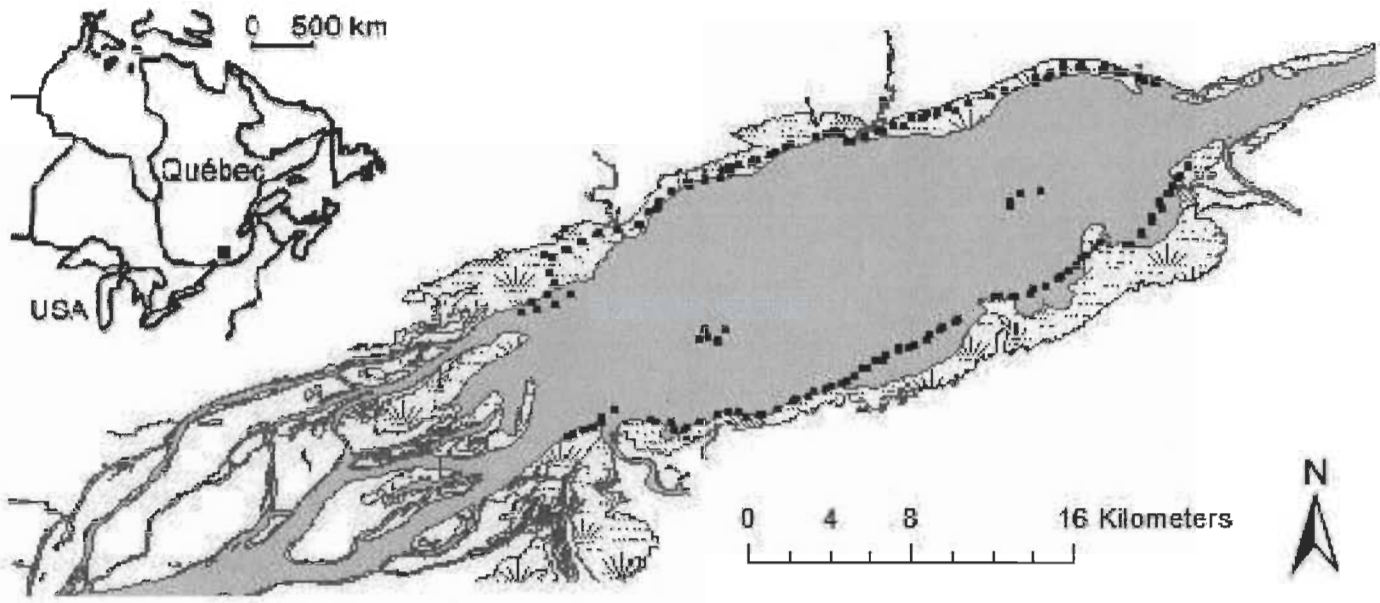

Figure 2.

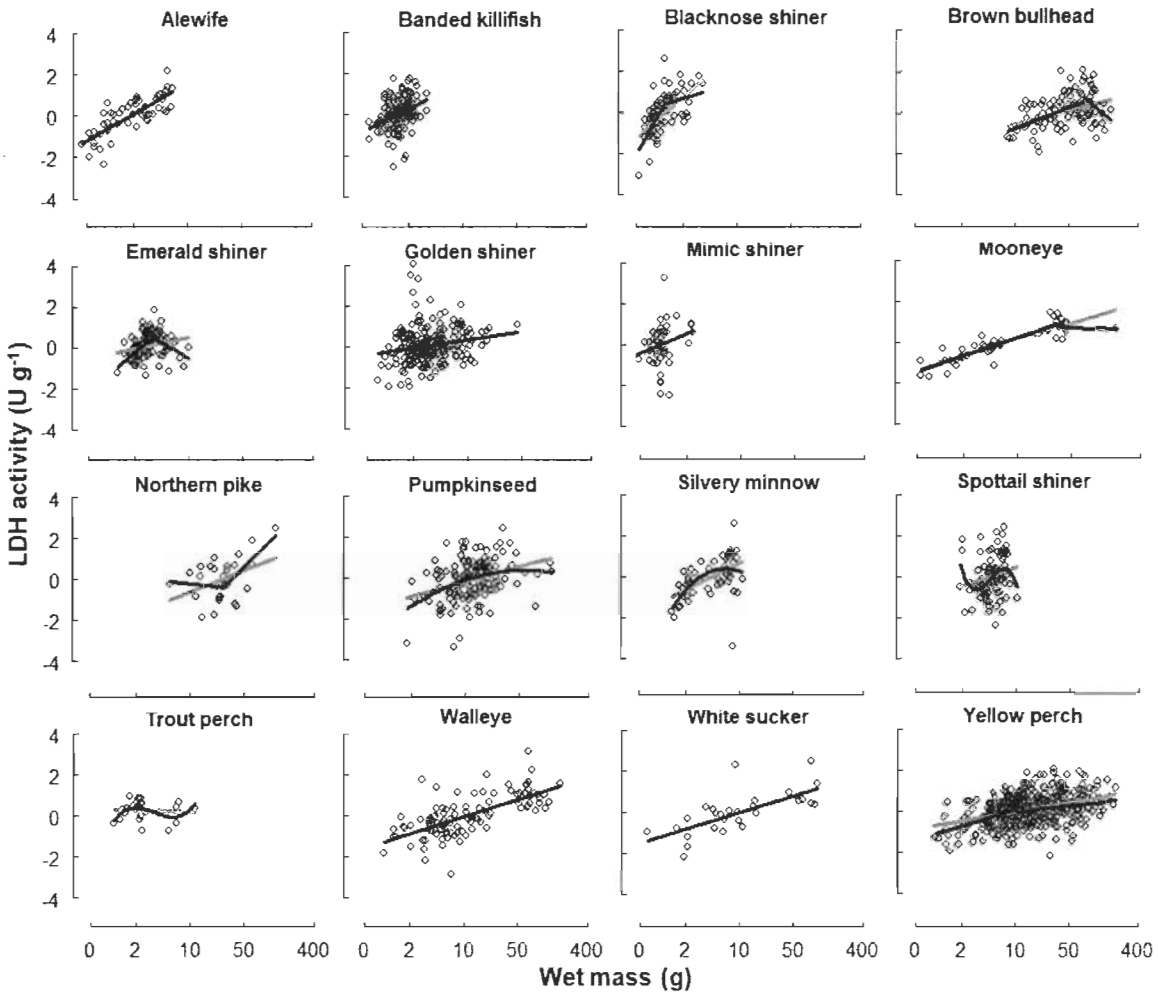
Alewife
Banded killifish
Blacknose shiner
Brown bullhead
Emerald shiner
Golden shiner
Mimic shiner
Mooneye
Northern pike
Pumpkinseed
Silvery minnow
Spottail shiner
Trout perch
Walleye
White sucker
Yellow perch
LDH activity (U g-1)
4
2
0
-2
-4
0
2
10
50
400
Wet mass (g)

Figure 3.

ANNEXE 1

Instructions aux auteurs

Author guidelines for Functional Ecology

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