Université du Québec
à Rimouski

Aspects évolutifs et environnementaux de la plasticité phénotypique chez deux Moronidés, le bar européen (*Dicentrarchus labrax*) et le bar rayé (*Morone saxatilis*)

Thèse présentée

dans le cadre du programme de doctorat en océanographie

en cotutelle avec l'université de Bretagne Occidentale (France)

en vue de l'obtention du grade de Philosophiæ Doctor (Ph.D.)

PAR

© CLÉMENCE GOURTAY

Février 2018

Composition du jury :

Céline Audet, directrice de recherche, Université du Québec à Rimouski (Canada)

José Zambonino, directeur de recherche, Institut français de recherche pour l'exploitation de la mer (France)

Jean Laroche, membre du jury, Université de Bretagne Occidentale (France)

Christel Lefrançois, examinateur externe, Université de La Rochelle (France)

Stéphane Panserat, membre du jury, Institut national de la recherche agronomique (France)

Gesche Winkler, examinateur interne, Université du Québec à Rimouski (Canada)

Dépôt initial le 26 octobre 2018 Dépôt final le 18 février 2019

UNIVERSITÉ DU QUÉBEC À RIMOUSKI
Service de la bibliothèque

Avertissement

La diffusion de ce mémoire ou de cette thèse se fait dans le respect des droits de son auteur, qui a signé le formulaire « *Autorisation de reproduire et de diffuser un rapport, un mémoire ou une thèse* ». En signant ce formulaire, l'auteur concède à l'Université du Québec à Rimouski une licence non exclusive d'utilisation et de publication de la totalité ou d'une partie importante de son travail de recherche pour des fins pédagogiques et non commerciales. Plus précisément, l'auteur autorise l'Université du Québec à Rimouski à reproduire, diffuser, prêter, distribuer ou vendre des copies de son travail de recherche à des fins non commerciales sur quelque support que ce soit, y compris l'Internet. Cette licence et cette autorisation n'entraînent pas une renonciation de la part de l'auteur à ses droits moraux ni à ses droits de propriété intellectuelle. Sauf entente contraire, l'auteur conserve la liberté de diffuser et de commercialiser ou non ce travail dont il possède un exemplaire.

À mon grand-père, ma famille, et mon chum

REMERCIEMENTS

Mes remerciements vont à mes directeurs de thèse Céline Audet et José Zambonino, qui se sont toujours montrés à l'écoute, disponibles et qui ont su m'apporter de précieux conseils et enseignements tout au long de ces trois années. Merci pour cette belle collaboration outre-Atlantique. Je souhaite également remercier mes co-encadrants Denis Chabot et Guy Claireaux, pour les discussions, leur soutien et leur aide précieuse lors des tests de performance physiologiques. Sans leur apport professionnel, plusieurs aspects de cette thèse n'auraient pu être réalisés.

Je tiens à remercier Gesche Winkler, Christel Lefrançois, Stéphane Panserat et Jean Laroche d'avoir accepté de faire partie du jury de thèse. Merci pour vos commentaires, vos suggestions et votre bienveillance. Mes remerciements vont également à Marie Vagner, David Mazurais, et Patrick Prunet pour leur implication dans le comité de suivi individuel.

Merci à l'entreprise Aquastream et à la station de piscicole de Baldwin, nos approvisionneurs de poissons. Je souhaite également remercier Nathalie Morin de la station aquicole de Pointe-au Père, pour son accueil et son soutien technique tout au long de l'élevage du bar rayé. J'exprime toute ma gratitude à Patrick Quazuguel, avec qui j'ai appris tous les secrets de l'élevage du bar européen.

Je remercie chaleureusement toute l'équipe du laboratoire d'Adaptation, Reproduction et Nutrition (ARN) à Brest, pour leur accueil, leur aide et leur soutien. Je remercie particulièrement David Mazurais, pour son apport professionnel concernant la biologie moléculaire. Merci également à Lauriane Madec, Christine Huelvan et Hervé Le Delliou pour leur aide au laboratoire. Merci à Laura Frohn et Loïck Ducros pour leur aide et leur implication dans le projet.

Je remercie tout aussi chaleureusement l'équipe de l'Institut des sciences de la mer de Rimouski (ISMER). J'adresse mes remerciements à Renée Gagné, pour son aide et sa bonne

humeur lors des longues heures de dissection ou de laboratoire. Je remercie également Réjean Tremblay pour son expertise concernant les analyses lipidiques, ainsi que Jean-Bruno Nadalini et Mathieu Babin pour leur soutien technique. Merci à Stéphanie Coté et Emeline Romelus pour leur aide et leur implication dans le projet.

J'adresse un chaleureux merci également à toutes les personnes des différents laboratoires que j'ai côtoyées durant ces trois dernières années. Je remercie vivement tous les membres de l'équipe d'administration à l'EDSML et l'ISMER, qui ont toujours été présents, et avec le sourire, pour leur aide précieuse.

Ce travail a été possible grâce au support financier de l'Institut français de recherche pour l'exploitation de la mer (IFREMER), des Fonds de recherche Nature et technologies (FRQNT), du Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), du regroupement « Ressources Aquatiques Québec », et de l'ISMER.

J'adresse mes remerciements et ma gratitude aux amis d'ici et d'ailleurs qui se reconnaîtront : Louise, Chloé, Sarah, Lola, Mélina, Laélien, Margaux, Léopold, Camille, Martin, Noëllie, Myriam, Florian, Alexandra, Efflam, Aurélien, Anaïs, Aurélie, Emilien, Ghassen, Sonia, Antoine, Bastien, Carole, Guillhem… Mention spéciale pour tous les courageux qui m'ont rendu visite !

C'est bien plus que des remerciements que je souhaite adresser à ma famille et surtout à mes parents pour leur soutien sans faille. Maman, papa, merci pour vos encouragements tout au long de la poursuite de mes études, merci aussi pour votre aide et votre compréhension au quotidien. Je remercie également Louis et Noémie pour leur soutien et leur visite.

Enfin, je remercie Thibault, pour son soutien indéfectible et ses encouragements quotidiens, son enthousiasme contagieux à l'égard de mes travaux comme de la vie en général. Merci pour ces belles années, où tu as su faire preuve de patience, d'amour et d'altruisme. C'est le début d'une nouvelle aventure au Québec qui nous attend !

Je remercie tous ceux que j'ai pu oublier par mégarde et qui ont contribué d'une façon ou d'une autre au bon déroulement de cette thèse.

AVANT-PROPOS

Ce projet de thèse de trois ans a été réalisé dans le cadre d'une convention de cotutelle entre le laboratoire d'adaptation, reproduction et nutrition des poisons (unité mixte de recherche 6539 Laboratoire des sciences de l'environnement marin) à l'institut français pour l'exploitation de la mer (Ifremer) à Brest (France), et le laboratoire d'écophysiologie des poissons de Céline Audet à l'institut des sciences de la mer à Rimouski (ISMER) à Rimouski (Canada).

Le projet est né d'une collaboration entre Céline Audet, Denis Chabot, Guy Claireaux et José Zambonino et a été mené à bien par Clémence Gourtay. Cette thèse a été cofinancée par l'Ifremer et le conseil de recherches en sciences naturelles et en génie du Canada (subvention découverte accordée à Céline Audet). Les avancées du projet ont été examinées par un comité de suivi individuel auquel les encadrants ont participé composé de Denis Chabot (Pêches et Océans Canada), Guy Claireaux (Université de Bretagne Occidentale), David Mazurais (Ifremer), Patrick Prunet (Institut National de la recherche Agronomique), et Marie Vagner (Centre National de la Recherche Scientifique).

La majorité des travaux rédigés dans ce manuscrit concernent le bar européen, car cette espèce est domestiquée et son élevage est maitrisé depuis plusieurs années notamment au sein du laboratoire de l'Ifremer Brest. Aucun problème majeur n'a donc été rencontré lors de l'élevage et des différentes expérimentations, ce qui a permis une acquisition rapide de nouvelles données originales. Contrairement au bar européen, très peu de données existent concernant la domestication du bar rayé et une seule pisciculture (la station piscicole de Baldwin, Coaticook, Canada) ayant pour unique vocation le réensemencement de l'espèce est présente au Québec. L'élevage des juvéniles de bar rayé fut donc un défi majeur, d'autant plus que l'espèce s'est révélée très sensible aux manipulations et au stress d'élevage. Les données obtenues sur cette espèce sont donc totalement inédites et

contribuent à une meilleure connaissance de sa biologie. Pour les raisons citées précédemment, le manuscrit de thèse est composé de trois articles sur le bar européen, et d'un article sur le bar rayé. Les différentes expériences réalisées ont permis d'étudier l'effet des acides gras nutritionnels (via deux aliments expérimentaux) sur la plasticité phénotypique des deux espèces. Les deux aliments utilisés sont caractérisés par leur richesse en acides gras à longue chaîne polyinsaturée n-3 (voir Annexe I page 149 pour la composition des aliments). Pour des raisons pratiques, dans le texte en français AL est l'aliment le moins riche, AL++ le plus riche en acide gras à longue chaîne polyinsaturée n-3. Dans les articles le nom des aliments a été determiné en fonction des spécificités des journaux scientifiques visés, ainisi qu'en fonction des besoins de l'espèce étudiée (se référerer à la liste des abréviations pour trouver les correspondances). Quelques points de comparaison entre les espèces sont abordés dans la discussion générale. D'autres analyses sont actuellement en cours parallèlement au projet de thèse afin d'enrichir cette comparaison.

Cette thèse débute par une mise en contexte portant sur l'impact des changements climatiques globaux sur les écosystèmes marins induisant une raréfaction des acides gras à longues chaîne polyinsaturée de type n-3 (oméga 3) à la base des réseaux trophiques aquatiques. L'état de l'art est composé d'une revue de littérature définissant ce que sont les lipides et leurs rôles chez les poissons. Cette partie est suivie par une synthèse des connaissances concernant l'adaptation homéovisqueuse et l'impact des lipides sur les performances physiologiques des poissons. Enfin, les modèles biologiques sont présentés et la justification de leur utilisation est faite dans une dernière partie. Les chapitres de cette thèse sont rédigés sous forme d'articles scientifiques.

Article 1: « *Will global warming affect the functional need for essential fatty acids in juvenile sea bass (*Dicentrarchus labrax*)? A first overview of the consequences of lower availability of nutritional fatty acids on growth performance* » soumis le 22 avril 2018 dans Marine Biology, accepté le 10 août 2018.

Article 2: « *Effect of thermal and nutritional conditions on fatty acid metabolism and oxidative stress response in juvenile European sea bass (*Dicentrarchus labrax*)* » soumis le 2 septembre 2018 dans Journal of experimental Biology.

Article 3: « *Effects of dietary fatty acids on the plasticity of critical swimming speed of european sea bass (*Dicentrarchus labrax*) juveniles* » en préparation.

Article 4: « *Seasonal response to salinity and nutritional fatty acids in juvenile striped bass (*Morone saxatilis*) »* en préparation.

La discussion générale est présentée dans le dernier chapitre de cette thèse. Elle se compose d'une première partie consistant en la mise en perspective de certains résultats, et d'une seconde partie qui présente les conclusions de cette étude.

RÉSUMÉ

Une des questions cruciales dans le débat écologique actuel est de déterminer si la plasticité phénotypique pourra permettre aux espèces de répondre au rythme rapide des changements environnementaux en cours. L'objectif général de cette thèse était d'étudier les effets d'un appauvrissement en acide gras à longue chaîne polyinsaturée (AGLPI) du type n-3 sur la plasticité (tissulaire, moléculaire et individuelle) de deux espèces, le bar européen (*Dicentrarchus labrax*) et le bar rayé (*Morone saxatilis*). L'effet combiné d'une augmentation de la température et d'une réduction en disponibilité des AGLPI n-3 nutritionnels chez les juvéniles de bar européen a entrainé une modification importante des acides gras neutres musculaires ainsi qu'un taux de croissance et une masse hépatique plus faibles. À température élevée, une croissance accrue a été observée avec les deux régimes, suggérant une absence de carence. En revanche, l'aliment n'a pas eu d'effet sur les facteurs transcriptionnels hépatiques liés à la régulation de la bioconversion des AG. Les juvéniles nourris avec le régime le plus faible en AGLPI n-3 présentaient une vitesse critique de nage accrue en présence de contraintes hypoxiques et hypo-osmotiques. Chez le bar rayé, le régime alimentaire modifie les profils en AG du muscle (fraction neutre) et du foie. Le régime faible en AGLPI n-3 a été associé à une augmentation de la masse cardiaque, sans effet sur la croissance en eau froide. Un niveau de stress plus élevé associé à des mortalités a été observé en eau douce. Ces résultats contribuent à une meilleure compréhension de l'impact des changements globaux sur les organismes aquatiques et ouvrent la voie à de nouvelles perspectives de recherche.

Mots clés: besoin nutritionnel, acides gras, allométrie des organes, nage, croissance, physiologie

ABSTRACT

One of the major questions in the current ecological debate about global change is whether phenotypic plasticity will enable species to respond to the rapid pace of ongoing environmental change. The main objective of this thesis was to study the effects of n-3 polyunsaturated fatty acid (PUFA) depletion on the plasticity (molecular, tissular and individual) of two species, the European sea bass (*Dicentrarchus labrax*) and the striped bass (*Morone saxatilis*). In juvenile European sea bass, the combined effects of an increase in temperature and of a reduction in availability of n-3 nutritional PUFA resulted in marked modifications of the profile in neutral muscular fatty acids and a smaller liver mass. At high temperature, growth was increased with the two diets which suggests an absence of nutritional deficiency. Conversely, diet did not affect the hepatic transcriptional factors involved in the regulation of FA bioconversion. Juveniles fed with the low n-3 PUFA diet had a higher critical swimming speed in presence of hypoxic or hypo-osmotic constraints. In striped bass juveniles, n-3 nutritional PUFA availability brought modifications of lipid profiles both in muscle (neutral fraction) and in liver. Diet with low n-3 PUFA was associated with a larger cardiac mass, but had no effect on growth in cold water. A higher stress level was observed in fresh water which was associated to higher mortality. These results contribute to a better understanding of the impact of global changes on marine organisms and pave the way for new research perspectives.

Keywords: nutritional need, fatty acids, organ allometry, swimming, growth, physiology

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

	Français		**English**
ARA	acide arachidonique	**ARA**	arachidonic acid
DHA	acide docosahexaénoique	**DHA**	docosahexaenoic acid
EPA	acide eicosapentaénoique	**EPA**	eicosapentaenoic acid
AG	acide gras	**FA**	fatty acid
AGLPI	acide gras à longue chaîne polyinsaturée		long-chain polyunsaturated fatty acid
AGE	acide gras essentiel	**EFA**	essential fatty acid
	acide gras monoinsaturé	**MUFA**	monounsaturated acid
	acide gras polyinsaturé	**PUFA**	polyunsaturated acid
	acide gras saturé	**SFA**	saturated fatty acid
	acide gras synthase	***fas***	fatty acid syntase
LIN	acide linoléique		linoleic acid
AO	acide oleique	**OA**	oleic acid
ALA	acide α-linolénique	**LA**	linoleic acid
AL	aliment faible en AGLPI n-3	**LD** (chap 1, 2)	low n-3 PUFA diet
		IP (chap.3)	inland profile diet
		RD (chap.4)	reference diet
AL++	aliment riche en AGLPI n-3	**RD** (chap 1, 2)	reference diet
		SP (chap.3)	sea profile diet
		MD (chap.4)	marine diet
	catalase	**CAT**	catalase
	citrate synthase	**CS**	citrate synthase
	coefficient thermique de croissance	**TGC**	thermal growth coefficient
	cycle de quantification	**Cq**	quantification cycle
DD	degré-jours	**dd**	degree-days
	degré-jour de croissance	**GDD**	growing degree-day
ED	eau douce	**FW**	fresh water
ES	eau saumâtre	**BW**	brackish water

	Français		**English**
	erreur standard	**SD**	standard deviation
	espèce réactive à l'oxygène	**ROS**	reactive oxygen species
	esters méthyliques d'acides gras	**FAMEs**	fatty acid methyl esters
GES	gaz à effet de serre		greenhouse gas
GIEC	groupe d'experts intergouvernemental sur l'évolution du climat		intergovernmental panel on climate change
	indice de peroxydation	**PI**	peroxidation index
INE	indice nutritionnel environnemental	**ENI**	environmental nutritional index
	jeune de l'année	**YOY**	young-of-the-year
	jour post-éclosion	**d-**	day post hatching
	longueur corporelle	**BL**	body length
	longueur standard	**SL**	standard length
	malondialdehyde	**MDA**	malondialdehyde
	masse corporelle	**BM**	body mass
MS	matière sèche	**DM**	dry matter
n-3, ω3	oméga 3	**n-3, ω3**	omega 3
n-6, ω6	oméga 6	**n-6, ω6**	omega 6
n-9, ω9	oméga 9	**n-9, ω9**	omega 9
	oxygène dissous	**DO**	dissolved oxygen
PO	pression osmotique		osmolality
	protéine se liant à l'élément régulateur du stérol	***srebp***	sterol regulatory element binding protein
	ratio du contenu en FA tissulaire/alimentaire	**TD**	tissue/dietary FA ratio
	récepteur activé par les prolifératrices des péroxysomes	***ppar***	peroxisome proliferator activated receptor
	substances ractives à l'acide thiobarbiturique	**TBARS**	thiobarbituric acid reactive substances
	superoxide dismutase	**SOD**	superoxide dismutase
	taux de croissance spécifique	**SGR**	specific growth rate
	transcritpion inverse	**RT**	reverse transcription
	triacylglycérol	**TAG**	triacylglycerol
	vitesse de nage critique	**Ucrit**	maximum swimming speed

INTRODUCTION GÉNÉRALE

CONTEXTE

Les changements climatiques

Les écosystèmes marins ont une grande valeur socio-économique (Rogers et al. 2014). Ils forment la première source de protéines pour une personne sur sept parmi la population mondiale (FAO 2012) et régulent le climat de la Terre. Les variations climatiques sont des éléments clés dans l'histoire de la Terre depuis sa formation (Huggett 2012). Ainsi, l'évolution des espèces et l'extinction de certaines d'entre elles ont été en partie guidées par l'équilibre et les déséquilibres que le système climatique a connu. Aujourd'hui, l'origine anthropique du changement climatique a été établie de manière consensuelle (Cook et al. 2016). En effet, avec l'explosion démographique de la population mondiale en cours et les révolutions industrielles successives, les émissions anthropiques de gaz à effet de serre (GES) n'ont cessé d'augmenter et sont actuellement les plus élevées jamais observées (Groupe d'experts intergouvernemental sur l'évolution du climat ; GIEC 2014). Les changements climatiques globaux ont été définis par la convention cadre des Nations Unies sur les changements climatiques (Nations Unies 1992) comme étant les changements du climat qui sont attribuables directement ou indirectement à une activité humaine et qui viennent s'ajouter à la variabilité naturelle du climat observée au cours de périodes comparables. Depuis les 30 dernières années, la hausse de concentration en GES a conduit à une augmentation de la température moyenne globale de ~0.2°C par décennie (Hansen et al. 2006) et en 2013, c'est 10 gigatonnes de carbone qui ont été émises dans l'atmosphère (Jones et al. 2008). Les océans du monde représentent 71% de la surface de la Terre et ils jouent donc un rôle primordial dans notre système climatique via l'absorption et le stockage de dioxyde de carbone atmosphérique (Parekh et al. 2006, Kwon et al. 2009).

Le réchauffement océanique constitue l'essentiel de la hausse de la quantité d'énergie (chaleur) emmagasinée au sein du système climatique. Il représente plus de 90% de la chaleur accumulée entre 1971 et 2010, tandis que le réchauffement atmosphérique représente environ 1% (GIEC 2014). Quatre principaux facteurs du climat ont été déterminés par le GIEC comme affectant la structure, le fonctionnement et la capacité d'adaptation des écosystèmes marins: le pH, la température, la concentration en oxygène et la disponibilité en ressources alimentaires. Les futurs scénarios climatiques projettent d'importantes perturbations de ces quatre facteurs (Gattuso et al. 2015, Sumaila et al. 2011).

De l'individu à l'écosystème, les réponses face aux changements climatiques globaux - *survival of the fittest*

Au-delà des projections, nombreuses sont les études qui relatent déjà les effets des changements actuels sur les écosystèmes marins (Harley et al. 2006, Lehodey et al. 2006, Brander 2007, Rijnsdorp et al. 2009, Gattuso et al. 2015) notamment concernant l'augmentation de la température (Genner et al. 2010, Johansen et al. 2014), l'acidification (Wittman et Pörtner 2013, Gaylord et al. 2015) et l'eutrophisation des eaux marines (Rijnsdorp et van Leeuwen 1996, Wasmund et al. 1998). Des changements pour tous les niveaux d'organisation des espèces (individuel à écosystémique) sont attendus en réponse à ces variations (Cheung et al. 2008, Bellard et al. 2012, Cheung et al. 2013). L'ensemble de ces réponses est résumé dans un schéma conceptuel simple proposé par Bellard et al. (2012; Figure 1).

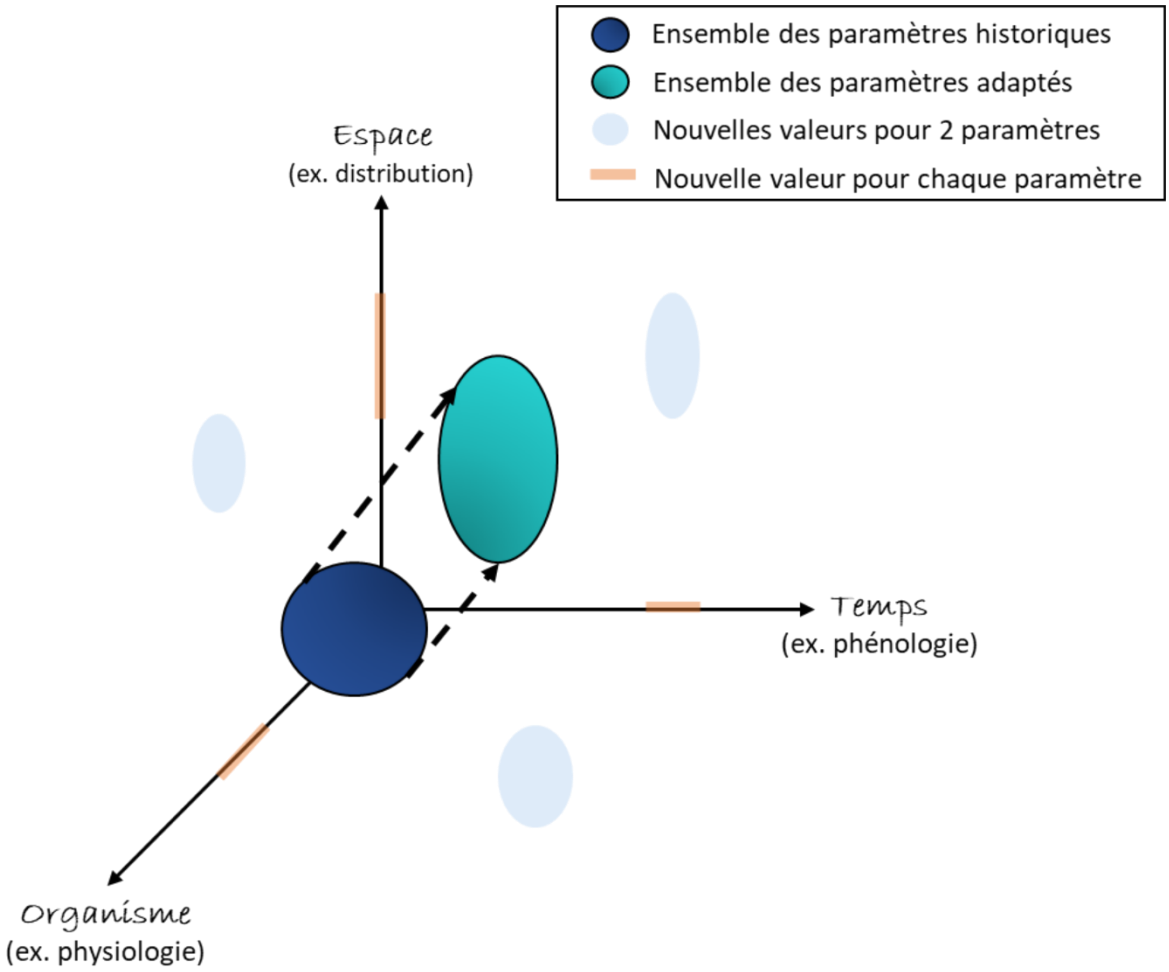


Figure 1: Réponse des espèces face aux changements globaux (adaptée de Bellard et al. 2012). Trois directions possibles en réponse aux changements globaux à travers la plasticité phénotypique ou les réponses évolutives : déplacement dans l'espace (déplacement avec la niche écologique), évolution des traits d'histoire de vie dans le temps (ajustement du cycle de vie pour s'adapter aux nouvelles conditions climatiques), ou modification des traits d'histoire de vie au niveau physiologique (plasticité) pour répondre aux nouvelles conditions climatiques. Les espèces, pour faire face aux changements globaux peuvent se déplacer le long d'un ou plusieurs de ces axes.

Trois grands types de réponses sont mis en place par les individus/espèces/populations: le déplacement, l'acclimatation (plasticité phénotypique), ou l'adaptation à travers le processus de sélection naturelle. Si aucune de ces réponses n'est mise en place, l'individu/l'espèce/la population est voué à disparaitre. Alors que l'évolution est un processus lent se déroulant sur plusieurs générations, la plasticité permet à un

organisme d'utiliser son patrimoine génétique et d'exprimer différents phénotypes dans des environnements contrastés. Des décennies de recherches en écologie et en physiologie ont établi que les variables climatiques étaient les principaux facteurs déterminant les distributions (axe « espace ») et la dynamique (axe « temps ») des espèces pélagiques (Roessig et al. 2004, Hays et al. 2005). Les données planctoniques récoltées à l'échelle mondiales montrent des changements importants dans les communautés de phyto et de zooplancton de concert avec les changements de régimes climatiques régionaux, ainsi que dans leur calendrier d'efflorescence (Beaugrand et al. 2002, de Young et al. 2004, Edwards et Richardson 2004, Richardson et Schoeman 2004, Hays et al. 2005). Suite à l'augmentation de la température constatée en Mer du Nord, plus d'une soixantaine d'espèces de poissons ont changé d'aire de répartition en latitude et/ou en profondeur (Perry et al. 2005), alors que certaines communautés de copépodes ont migré jusqu'à 1000 km vers le nord (Beaugrand et al. 2002). Aujourd'hui un nombre croissant d'études illustre l'ampleur de la plasticité (axe « organisme ») des organismes marins en termes de comportement, de physiologie ou de morphologie (Schulte 2007) en réponse aux changements climatiques globaux. Les traits d'histoire de vie tels que le taux de croissance (Sandblom et al. 2016), l'âge à la première maturité sexuelle (Auer et al. 2012), ou encore les performances de nage (Johansen et Jones 2011) peuvent être modifiés. Une des questions cruciales dans le débat écologique concernant les espèces face aux changements globaux est de déterminer si la plasticité phénotypique pourra permettre aux espèces de répondre au rythme rapide du changement climatique (Lavergne et al. 2010, Salamin et al. 2010). C'est pourquoi l'étude des relations trait-environnement, notamment via les stratégies de vie des espèces, est une approche puissante pour révéler des mécanismes de réponse généraux et améliorer notre capacité à prédire les réponses des communautés face aux changements environnementaux (McGill et al. 2006, Mouillot et al. 2013).

Les stratégies de vie des espèces – *Live fast, die young*

L'histoire de vie d'une espèce est caractérisée par une série de traits phénotypiques (taille, âge, fécondité) et comportementaux (âge à la maturité, calendrier des migrations

saisonnières), plus ou moins plastiques, qui, collectivement, guident un individu durant l'accomplissement de son cycle de vie (Waples et Audzijonyte 2016). Bien que le nombre de combinaisons possibles de traits de vie au sein d'une espèce donnée soit illimité, seule une fraction de ces combinaisons se trouve dans la nature (Waples et Audzijonyte 2016). En effet, des compromis inhérents sont déterminés par la quantité finie d'énergie disponible pour la croissance, le maintien et la reproduction. Dans ce jeu à somme nulle, la quantité d'énergie allouée à la croissance ou au maintien réduit l'énergie disponible pour la reproduction, et vice versa. L'existence de ces compromis a conduit à la théorie des invariants d'histoire de vie, affirmant que seules certaines combinaisons de traits produisent un organisme ayant une bonne valeur adaptative [fitness] (Charnov 1993). Le concept de stratégie de vie a d'abord été développé en 1967 par MacArthur et Wilson (2001) qui ont élaboré une première définition des traits caractérisant les stratégies de reproduction r et K. Aujourd'hui, basé sur le même concept, les espèces peuvent être placées sur le continuum lent-rapide de l'histoire de vie (Gaillard et al. 1989, Jones et al. 2008). Les espèces caractérisées par un rythme de vie lent (stratégie K), investissent plus d'énergie dans leur maintien, leur maturité est tardive, leur longévité est importante. A l'inverse, les espèces au rythme de vie rapide (stratégie r) investissent plus dans la reproduction, elles sont caractérisées par une reproduction précoce et une longévité courte (Figure 2).

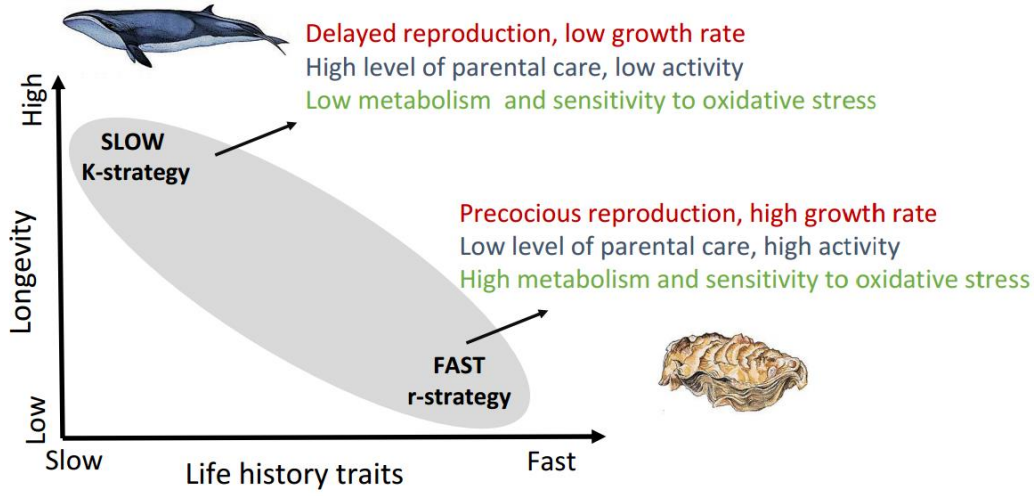


Figure 2: Schéma intégrant les grands concepts de stratégie de vie r et K, et rythme de vie rapide-lent (adaptée de Réale et al. 2010). En rouge: traits d'histoire de vie, en bleu: traits de comportement et en vert: traits physiologiques.

Ce compromis fondamental entre la mortalité et les paramètres définissant la taille maximale et le taux de croissance des poissons marins a déjà été illustré chez les poissons (Figure 3A ; Gislason et al. 2010). Par exemple, dans le continuum lent-rapide de l'histoire de vie, les sébastes (*rockfish*), et les morues (*cod*) ont une maturité tardive et une longévité importante, alors que les espèces ayant un rythme de vie rapide (anchois *anchoveta* et maquereaux *mackrel*) ont une maturité précoce et une longévité faible. Un cycle de vie rapide est essentiel si la mortalité naturelle est importante, car l'organisme doit pour perpétuer l'espèce, se reproduire au moins une fois avant de mourir. Lorsque la mortalité naturelle est faible, il est rentable d'investir plus d'énergie dans la croissance. Les individus suivant cette stratégie d'évolution sont favorisés car étant plus gros lorsqu'ils atteignent leur maturité, ils ont une meilleure capacité de reproduction. Le coût de reproduction est alors plus faible chez les individus de grande taille que chez les petits individus, ce qui en retour, permet d'accroitre la longévité.

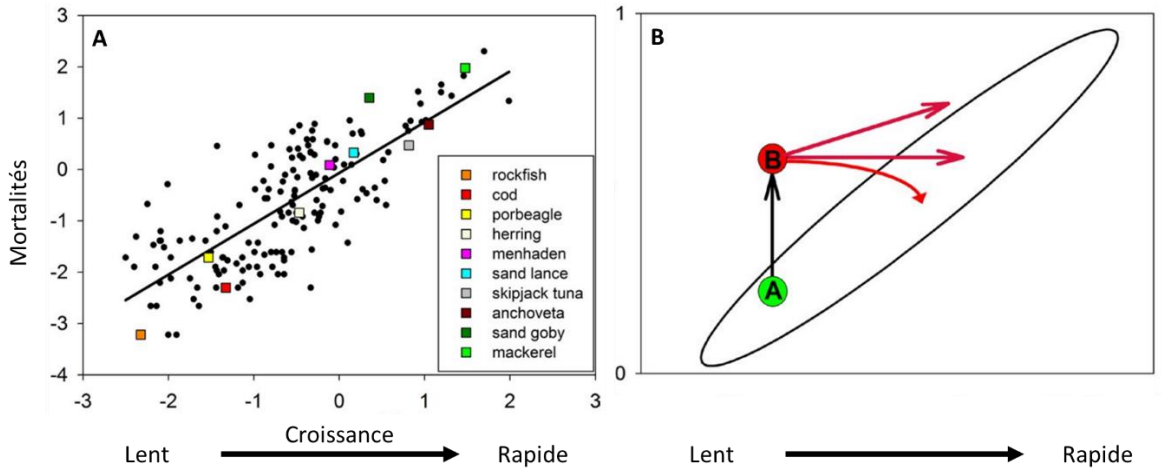


Figure 3: Mortalité et rythme de vie de plusieurs espèces marines (adaptée de Waples et Audzijonyte 2016). A) Relation entre la mortalité naturelle associée à une longueur et les paramètres de croissance de Van Bertalanffy pour des espèces de poissons marines et estuariennes. Les espèces sont positionnées le long du continuum lent-rapide de l'histoire de vie. B) Représentation schématique de la figure A). L'ellipse noire représente la régression de la mortalité naturelle sur les paramètres de croissance de Van Bertalanffy. La flèche noire représente la mortalité globale d'adultes appartenant à une population due a une augmentation de la pression anthropique (changement climatique ou exploitation intensive des ressources), causant un déplacement de la population dans la partie supérieure gauche (état A à B), perturbant sa viabilité. Les flèches rouges représentent les trajectoires possibles de réponse. Dans tous les cas, la population, pour retrouver sa viabilité se doit d'évoluer vers une histoire de vie « plus rapide ».

Un ensemble donné de conditions écologiques va privilégier une stratégie de vie particulière (rapide vs. lente), en affectant toute une série de traits d'histoire de vie caractéristiques (Réale et al. 2010). En étudiant l'évolution de l'histoire de vie d'espèces de poissons à forte valeur économique face à la pression croissante de la pêche depuis les années 70, l'hypothèse que l'impact de la pêche pourrait être comparable à celui du changement climatique a ainsi été émise (Waples et Audzijonyte 2016). En effet, dans les deux cas une pression forte est exercée sur les individus de grande taille. La pression de pêche se concentre sur les espèces de poissons de grande taille (Pauly et al. 1998, Jackson et al. 2001), ces dernières sont également les plus sensibles au changement climatique. Les individus de grande taille ont une sensibilité importante à l'augmentation de la température à cause d'un rapport surface-volume réduit limitant l'apport d'oxygène, et des taux cataboliques versus anaboliques élevés (Kozlowski et al. 2004). Aujourd'hui, à cause des pressions anthropiques, les taux de mortalités actuels dépassent les taux de mortalités naturels (Darimont et al. 2009; Figure 3B). La mortalité causée par l'Homme ne joue pas sur le taux de mortalité naturelle (ellipse noire), elle s'additionne. La population passe d'un état A (taux de mortalité naturel) à un état B lorsque l'on ajoute les mortalités causées par l'homme. La viabilité de la population est alors menacée. Afin de retrouver sa viabilité, la population doit évoluer (flèche rouge) vers une histoire de vie « plus rapide » qui donne à chaque individu une plus grande chance de se reproduire au moins une fois avant de mourir (Waples et Audzijonyte 2016). Cette « accélération » du rythme de vie a également été montrée chez d'autres espèces ectothermes et a souvent été mise en corrélation avec l'augmentation de la température (Houde 1989, Blaxter 1992, Benoît et al. 2000, Bestion et al. 2015). En effet, la température corporelle des espèces ectothermes, et donc leurs fonctions physiologiques de bases, sont directement dépendantes des conditions environnementales (Gillooly et al. 2001). Question de cinétique, si la température extérieure augmente, cela va généralement de pair avec l'augmentation du métabolisme de ces espèces. Les stratégies de vie et leurs traits associés sont donc susceptibles d'avoir une forte incidence sur la façon dont les espèces vont répondre aux gradients abiotiques. De ce

fait, le rythme de vie contient beaucoup d'informations sur la capacité que vont avoir les espèces à répondre aux changements environnementaux (Stoffels 2015).

Vers une raréfaction globale en oméga 3: le rôle du phytoplancton

Au cours des vingt dernières années, plusieurs études ont montré que la modification des paramètres physico-chimiques de l'océan liée aux changements globaux (augmentation de la température, diminution du pH, diminution de la saturation en oxygène) avait des répercussions directes sur la base des réseaux trophiques marins, notamment au niveau de la physiologie des espèces phytoplactoniques et de leur assemblage (Gomez et Souissi 2008, Guschina et Harwood 2006, Pahl et al. 2010, Chen 2012). Le phytoplancton est constitué de nombreuses espèces considérées comme producteurs primaires (production de matière organique à partir de matière minérale). Il constitue un gisement majeur de lipides et d'acides gras (AG) représentant une source d'énergie importante pour les organismes de niveau trophique supérieur (Sargent et al. 2002). Les AG sont également d'une importance vitale pour les espèces puisqu'ils sont les constituants majoritaires des membranes cellulaires (Sargent et al. 2002). La composition précise en AG des membranes est déterminante pour la structure ainsi que la fonction des cellules et des tissus, et peut avoir des effets importants à différents niveaux écologiques et biologiques (Arts et al. 2009, Parrish 2013). Les espèces phytoplanctoniques produisent la majeure partie des AG à longue chaîne polyinsaturée du type n-3 (AGLPI n-3, aussi appelés omégas 3) présents dans la biosphère, car les plantes ne sont pas capables de synthétiser ces molécules, notamment les acides eicosapentaenoïque (C20:5n-3, EPA) et docosahexaenoïque (C22:6n-3, DHA ; Arts et al. 2009).

Plusieurs études ont montré une corrélation négative entre température et richesse en AGLPI du type n-3 des espèces phytoplanctoniques (Ackman et Tocher 1968, Thompson et al. 1992, Renaud et al. 2002, Guschina et Harwood 2006). Par exemple, il a été montré que le taux de croissance des diatomées et leur contenu en AGLPI n-3 diminuait avec l'augmentation de la température de l'eau (Gomez et Souissi 2008, Guschina et

Harwood 2006, Pahl et al. 2010, Chen 2012). Cette raréfaction des AGLPI n-3 a été démontrée à une échelle globale par Hixson et Arts (2016), lors de l'étude de 6 groupes majeurs d'espèces phytoplanctoniques marines et dulcicoles. Plus particulièrement, cette étude montre que la température est positivement corrélée aux contenus relatifs en oméga 6 (notamment en acide arachidonique, C20:4n-6) et acides gras saturés, et inversement corrélée avec les contenus relatifs en EPA et DHA. Les mêmes tendances ont été rapportées dans une étude plus complète où plus de 3000 profils d'acide gras d'organismes marins et terrestres ont été analysés (Colombo et al. 2016). Au-delà de la modification de l'assemblage des espèces phytoplanctoniques, cette raréfaction des AGLPI n-3 peut être expliquée à travers le concept d'adaptation homéovisqueuse (cf partie « Lipides et température: l'adaptation homéovisqueuse », page 15). Parmi les AGLPI n-3, le DHA et l'EPA sont des AGLPI essentiels chez les espèces qui ne sont pas capables de les synthétiser et qui dépendent de l'apport alimentaire pour satisfaire leurs besoins (cf partie « Lipides nutritionnels et synthèse *de novo* », page 12). Un appauvrissement en AGLPI n-3 à la base des réseaux trophiques pourrait se propager aux niveaux trophiques supérieurs puisqu'une partie de la composition en AG des organismes est dépendante de l'apport alimentaire (Bell et al. 1996, Leu et al. 2006), et avoir des conséquences sur la physiologie et les performances de nombreuses espèces.

ÉTAT DE L'ART

Les lipides: définitions et rôles

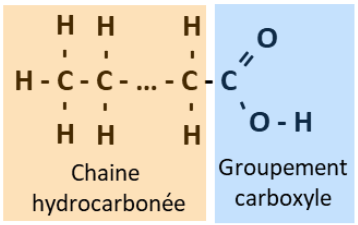
Les lipides sont constitués de molécules organiques hétérogènes et peuvent être classés en fonction de leur solubilité dans les solvants organiques ainsi qu'avec la structure de leur squelette carboné (Fahy et al. 2005). Chez les organismes marins, ils peuvent contribuer pour plus de 20% du carbone organique (Rullkötter 2000) et jusqu'à 60% dans certaines cellules phytoplanctoniques (Fernandez et al. 1994). Ils sont utilisés par les organismes comme source ou réserve de carbone et d'énergie, comme composants des membranes cellulaires (Sargent et al. 1989) et comme précurseurs d'activité biologique (eicosanoïdes; Tocher 2003). Les AG sont

présents dans différents groupes de lipides (sphingolipides, glycérides, phospholipides) et se définissent comme des molécules comportant une chaîne aliphatique de 4 à 28 atomes de carbone (généralement en nombre pair) et se terminent par un groupement acide (Figure 4A). Les AG à longue chaîne sont composés d'une chaîne carbonée comportant de 14 à 22 atomes de carbone. Lorsque plus de 22 atomes de carbone sont présents, ils sont appelés AG à très longue chaîne. Les AG peuvent être saturés, c'est-à-dire qu'ils ne possèdent pas de double liaison, leurs atomes de carbone étant liés entre eux par des liaisons covalentes simples; ou insaturés (Figure 4A). Dans ce cas, il y présence d'une (AG monoinsaturé) ou plusieurs doubles liaisons (AG polyinsaturés) sur le squelette carboné. La nomenclature des acides gras utilisée dans le manuscrit est la suivante: C:Xn-Y, où C est le nombre d'atomes de carbone, X est le nombre de doubles liaisons (aussi appelé insaturation), et où n-Y représente la position du premier carbone portant la première insaturation en partant du CH3 terminal. Il existe trois grandes familles parmi les AG: les omégas 3, 6 et 9 (notés aussi n-3, n-6, n-9 ou ω3, ω6 et ω9).

A Les acides gras

Position de la 1ére insaturation à partir du méthyl terminal

Nombre de carbones → C : X n-Y ← Nombre d'insaturations

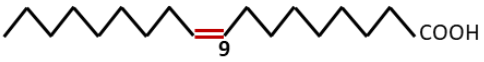


Acides gras saturés

Acide palmitique C16:0

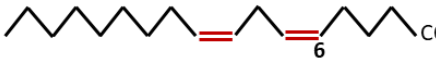
Acides gras monoinsaturés

Acide oléique C18:1n-9 ω9



Acides gras polyinsaturés

Acide linolénique C18:2n-6 ω6



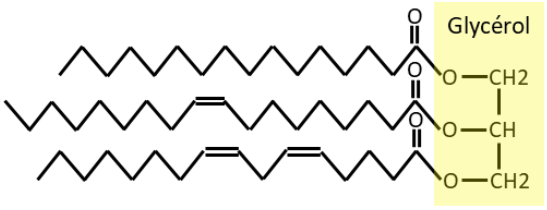
Insaturation

Acide docosahexaenoique C22:6n-3 ω3

B Les lipides

Neutres

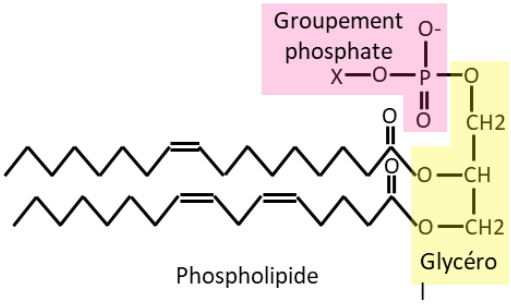
Utilisés comme source et réserve d'énergie



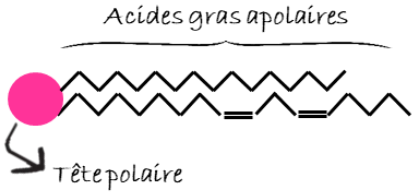
Triglycéride

Polaires

Constituants principaux des membranes cellulaires



Phospholipide



C Les membranes

Adaptation homéovisqueuse: modulation de la perméabilité des membranes par remodelage des lipides

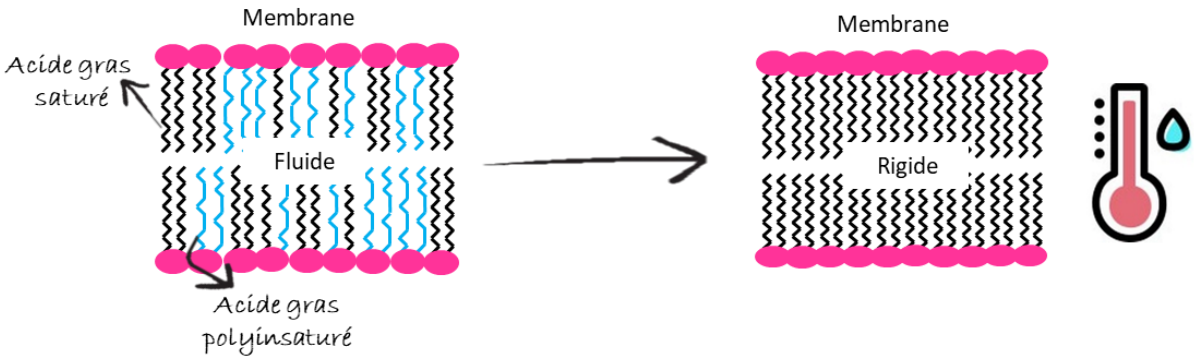


Figure 4: Les lipides. Représentations schématiques, définitions et nomenclatures des acides gras (A), des lipides (B), et des membranes avec une illustration du processus d'adaptation homéovisqueuse (C).

L'ensemble de ces molécules peut être subdivisé en lipides neutres et polaires, en référence aux méthodes chromatographiques de séparation des classes (Figure 4B). Généralement, dans un organisme, la composition en AG des lipides neutres de réserve reflète assez bien celle de l'alimentation (Regost et al. 2003, Montero et al. 2005, Skalli et al. 2006) alors que la composition en lipides polaires membranaires fait l'objet d'une régulation plus stricte. Les lipides polaires sont caractérisés par une tête polaire hydrophile qui permet la constitution de bicouches lipidiques dans les membranes cellulaires (Figure 4C). La nature des têtes polaires et les caractéristiques des AG (longueur de chaîne, position et nombre de doubles liaisons) qui composent ces molécules vont être déterminantes pour la structure membranaire et vont directement influencer leurs propriétés et fonctionnalités. Les phospholipides constituants les membranes cellulaires sont généralement riches en AG à longue chaîne polyinsaturée de type n-3 (Vaskovsky 1989) tels que l'EPA (20:5n-3) et le DHA (22:6n-3) qui permettent d'augmenter la fluidité membranaire et procurent un environnement favorable à l'activité des protéines transmembranaires (Eldho et al. 2003). L'acide arachidonique (ARA, 20:4n-6) est également un composant important des phospholipides membranaires. Chez les poissons, EPA, DHA et ARA ont des rôles déterminants dans l'ontogenèse, le développement et la fonctionnalité du cerveau, de la vision et du système nerveux, la croissance, la survie, la pigmentation et la résistance au stress et aux maladies (pour revue voir Sargent et al. 2002).

Lipides nutritionnels et synthèse *de novo*

Chez les poissons, les AG fournis par l'alimentation sont stockés sous forme de lipides neutres (majoritairement comme triglycérides) ou insérés dans les membranes cellulaires comme phosphoglycérides polaires (Sargent et al. 1999, Tocher 2003). Plus exactement, 85% de leurs réserves énergétiques sont stockées sous forme de triglycérides car les lipides constituent la source d'ATP la plus efficace par gramme comparé aux autres

macronutriments (Weber 2011). En effet, les lipides apportent 39,5 kJ g^{-1} par rapport aux protéines et aux glucides qui fournissent respectivement 23,7 et 17,2 kJ g^{-1} (National Research Council 1993). Selon les espèces de poissons, cette réserve d'énergie peut être stockée dans le foie, le muscle, le tissu adipeux péri-viscéral ou sous-cutané (Ando et al. 1993).

La lipogenèse est le terme utilisé pour décrire les réactions de biosynthèses de novo des lipides. Les acides gras polyinsaturés du type n-9 peuvent être synthétisés à base d'AG apportés par l'alimentation ou de novo (lipogénèse) à partir d'acides aminés et de glucides. A l'opposé, les AG de la série n-6 et n-3 ne peuvent être synthétisés qu'à partir d'AG précurseurs, aussi appelés acides gras essentiels (AGE) fournis uniquement par voie alimentaire. Les biotransformations (élongation, désaturation et β-oxydation) interviennent uniquement au sein d'une même famille d'acide gras (Figure 5). Deux familles d'enzymes sont importantes dans la biosynthèse des AG : les élongases qui permettent une élongation des chaînes carbonées par l'ajout de 2 carbones et les désaturases qui ont pour rôle d'introduire une double liaison entre deux carbones par déshydrogénation (perte d'atomes d'hydrogène). Les poissons, comme les vertébrés, ne possèdent pas les désaturases nécessaires pour former l'acide linoléique (LIN, 18:2n-6) et α-linolénique (ALA, 18:3n-3) à partir de l'acide oléique (AO, 18:1n-9), c'est pourquoi les AGLPI sont essentiellement d'origine nutritionnelle (revu par Arts et al. 2009). Toutefois, LIN et ALA peuvent être désaturés et élongués, avec des rendements variables en fonction des espèces, pour former des AG à plus longue chaîne y compris le DHA, EPA et ARA. L'efficacité de cette synthèse dépend de l'activité des acyl désaturases et des élongases, qui elle-même peut dépendre de la disponibilité en AGLPI nutritionnels.

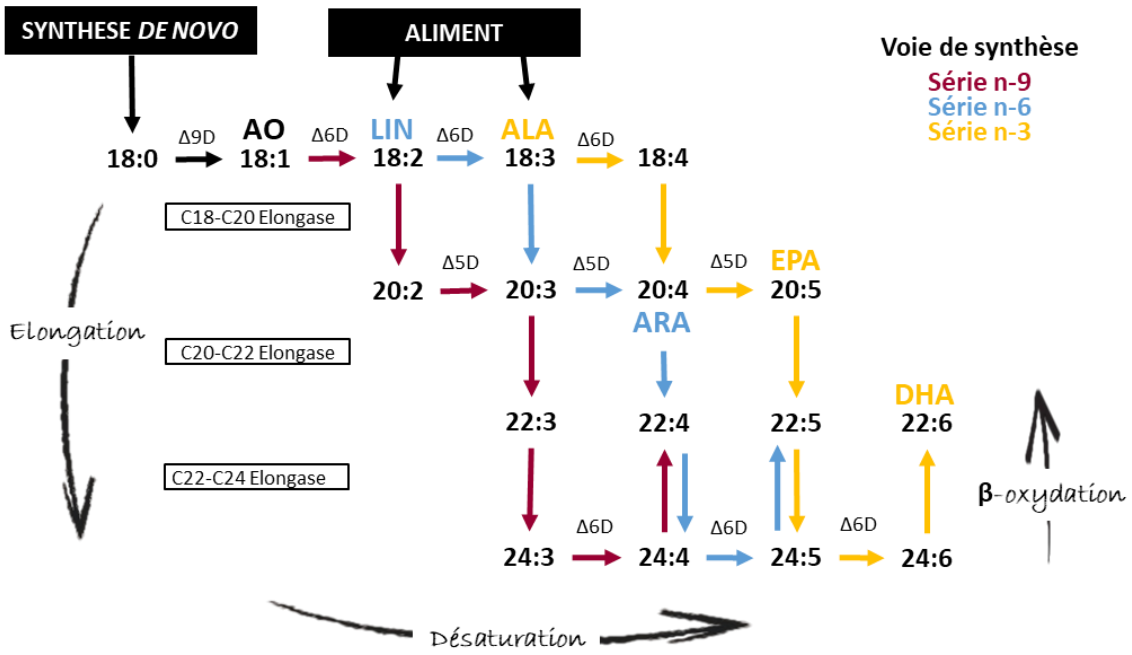


Figure 5: Représentation schématique de la voie métabolique des acides gras à longue chaîne polyinsaturée et des processus enzymatiques associés à chaque étape. La voie de synthèse des acides gras de chaque série, n-9 (magenta), n-6 (bleu) et n-3 (jaune), est représentée. AO: acide oléique, LIN: acide linoléique, ALA: acide α-linolénique, ARA: acide arachidonique, EPA: acide eicosapentaénoique, DHA: acide docosahexaenoique. D: désaturase.

De grandes disparités existent entre les besoins en apport d'AG nutritionnels entre espèces marines et dulcicoles (pour revue: Tocher 2010). Les poissons marins ont accès à des quantités importantes d'EPA et de DHA via leur alimentation avec des proies riches en ces 2 AG. A l'opposé, les proies naturelles de nombreux poissons d'eau douce sont bien moins riches en AGLPI n-3, notamment en DHA (Arts et al. 2009). L'importante disponibilité des AGLPI n-3 fournis par les espèces phytoplanctoniques dans les réseaux trophiques marins, pourrait expliquer l'absence de pression de sélection sur cette voie de biosynthèse chez plusieurs espèces de poissons marins, et notamment l'inefficacité de cette voie de synthèse de novo chez certaines espèces piscivores (Tocher et al. 2006). Avec un régime plus pauvre en AGLPI du type n-3, les espèces d'eau douce possèdent généralement une voie de biosynthèse de ces AG plus efficace. C'est en étudiant des hépatocytes de

poissons dulcicoles tels que la truite arc-en-ciel (*Oncorhynchus mykiss*, Buzzi et al. 1996, 1997), la truite commune (*Salmo trutta*, Tocher et al. 2001a), le tilapia (*Oreochromis niloticus*) et le poisson-zèbre (*Danio rerio*, Tocher et al. 2001b) qu'il a été mis en évidence que l'EPA et le DHA étaient produits à partir d'ALA. Les hépatocytes des espèces marines possèdent une faible activité de désaturation de l'ALA, qui ne permet pas d'aboutir à la production d'EPA ou de DHA (Sargent et al. 2002, Tocher 2003). Ainsi, le turbot (*Scophtalamus maximus*), le bar européen (*Dicentrarchus labrax*) et la daurade royale (*Sparus aurata*) ne sont pas capables de produire du DHA à partir d'ALA en quantité suffisante pour permettre de couvrir les besoins nutritionnels (Owen et al. 1975, Sargent et al. 2002, Geay et al. 2010, Geay et al. 2012) à cause de déficiences dans une ou plusieurs étapes de la voie de biosynthèse (Ghioni et al. 1999, Tocher et Ghioni 1999, Santigosa et al. 2010). C'est pourquoi, EPA, DHA et ARA sont considérés comme des AGE chez les espèces marines. Ces capacités de biosynthèse ont notamment été beaucoup étudiées à des fins aquacole afin d'optimiser les apports nutritionnels en huiles végétales et de poissons pour des espèces commerciales dulcicoles et marines.

Lipides et température: l'adaptation homéovisqueuse

Dans le contexte des changements globaux et notamment de l'augmentation globale des températures, les espèces aquatiques sont exposées à des conditions environnementales variables et parfois extrêmes qui peuvent induire des effets marqués sur leur physiologie, notamment chez les espèces poïkilothermes qui sont totalement dépendantes de la température de leur milieu. Dans certaines circonstances, les poissons sont capables de modifier leur comportement (par exemple en passant d'une eau plus chaude à une eau plus froide), ou de mettre en place des modifications biochimiques et physiologiques, en particulier au niveau des membranes cellulaires. L'ajustement des propriétés physico-chimiques des membranes est une réponse plus durable aux changements de température, ce processus est aussi appelé adaptation homéovisqueuse (Sinensky 1974; Figure 4C). Ce changement implique un remodelage des lipides membranaires entrainant une modification de la longueur des chaînes d'AG et de leur niveau d'insaturation (Figure 4C), dans le but de

maintenir la fluidité souhaitée dans les membranes cellulaires (Sinensky 1974, Lande et al. 1995, Guschina et Harwood 2006). Les doubles liaisons des AGLPI améliorent la capacité des AG à « se plier » et permettent plus de flexibilité, ce qui entraine une augmentation de la fluidité membranaire (Eldho et al. 2003; Figure 4C). Chez les espèces poïkilothermes, y compris les poissons, il a été montré qu'une augmentation de la proportion en AGLPI dans les lipides membranaires permet de maintenir la membrane fluide sous des températures froides (Hazel et al. 1992, Calabretti et al. 2003, Los and Murata 2004). A l'inverse, lors d'une augmentation de température, les AGLPI sont remplacés par des AG saturés (Skalli et al. 2006). Les lipides ont donc un rôle primordial dans l'adaptation des organismes aux changements de température, mais l'impact d'une raréfaction globale en AGLPI n-3 nutritionnels sur la capacité de réponse de ces organismes n'a été que très peu étudiée.

Lipides et performances: croissance et nage

Un aspect fondamental de l'histoire de vie et de l'écologie d'une espèce réside dans sa capacité à convertir les ressources disponibles en nutriments et en énergie pour les utiliser pour son maintien, ses activités, et sa reproduction (Karasov et Martinez del Rio 2007). C'est pourquoi l'influence des lipides sur l'ontogénèse d'espèces modèles, notamment à caractère aquacole, a été largement étudiée dans des conditions contrôlées en laboratoire (McKenzie 2001, Koven et al. 2003, Vagner et al. 2007b, Fountoulaki et al 2009, Torrecillas et al. 2017b). L'étude de la substitution des huiles de poissons (riches en AGLPI n-3) par des huiles végétales (pauvres en AGLPI n-3) pour la nutrition des poissons a également fait l'objet de nombreuses études. La plupart de ces études portent sur des salmonidés (McKenzie et al. 1998, Tocher et al. 2000, Torstensen 2000, Caballero et al. 2002), des espèces d'eau douce (Martino et al. 2002, Ng et al. 2003) et quelques espèces marines incluant le bar européen (*D. labrax*; Yildiz et Sener 1997, Montero et al. 2005, Chatelier et al. 2006), le turbot (*P. maxima*; Regost et al. 2003), la dorade (*S. aurata*; Kalogeropoulos et al. 1992, Caballero et al. 2003, Montero et al. 2003) et le pagre (*Pagrus auratus;* Glencross et al. 2003). Ainsi chez le saumon atlantique (*Salmo salar*), la substitution de 100% de l'huile de poisson par de l'huile de colza dans l'alimentation n'a

pas eu d'effet sur la croissance sur une période de 17 semaines (Bell et al. 2001). De la même façon, une substitution totale, pendant 62 semaines, de l'huile de poisson par un mélange d'huiles végétales (55% colza, 30% palme, 15% lin), n'a pas affecté la croissance de la truite arc-en-ciel (*Oncorhynchus mykiss*, Drew et al. 2007). Chez le bar, selon les études réalisées, l'incorporation de lipides marins dans l'aliment à hauteur de 10 à 15% (par rapport au % de matière sèche [MS]) permet d'optimiser la croissance. Chez les juvéniles de bar européen (15 g), on estime le seuil minimal d'apports en AGLPI du type n-3 à 0.7% par rapport à la matière sèche de l'aliment (Skalli et Robin 2004, Skalli et al. 2006). En dessous de ce seuil, la croissance diminue significativement car les besoins en AGLPI ne sont pas satisfaits. Pour les poissons marins, les AGLPI sont donc déterminants pour la croissance qui est un trait de performance très recherché en aquaculture, mais également essentiels pour la survie de l'espèce en milieux naturel. La croissance est un trait biologique d'intérêt car elle est considérée comme un trait intégrateur, c'est-à-dire qu'elle dépend d'un ensemble de fonctions physiologiques. Qu'en est-il de l'effet des AGLPI sur un autre trait de performance intégrateur, la nage ?

La nage est d'une importance écologique vitale chez les poisons (par exemple: éviter la prédation, capture de proies, migrations, etc.) et, chez de nombreuses espèces, elle est considérée comme un facteur déterminant de la survie (Videler 1993, Reidy et al. 2000, Armsworth 2001, Johnston et al. 2001, Plaut 2001, Fisher et Wilson 2004, Green et Fisher, 2004, Claireaux et al. 2006). De plus, la nage est un trait intégrateur de performance puisqu'elle est tributaire d'un ensemble de processus et de fonctions physiologiques (par exemple: la ventilation, le transport de l'oxygène, le travail musculaire et l'homéostasie de manière générale). C'est la raison pour laquelle elle est souvent utilisée comme un indice intégré de l'état physiologique et de santé chez les poissons (Nelson 1989, Randall et Brauner 1991, Plaut 2001). La quantité et la qualité des apports nutritionnels apparaissent comme une source importante de diversité physiologique chez les poissons, notamment en termes de performance de nage, en particulier via l'accumulation tissulaire de certains AG (McKenzie 2001, Tocher 2003, Chatelier et al. 2006). En effet, la performance musculaire dépend de l'approvisionnement en carburants métaboliques. Lorsqu'un poisson nage, il

utilise ses réserves énergétiques principalement stockées sous forme de triglycérides (lipides neutres; Richards et al. 2002a, Magnoni et al. 2006, Weber 2011). Les poissons ont différents modes de nage classés en fonction de la durée de l'effort fourni, des muscles impliqués et des substrats énergétiques utilisés (Beamish 1978, Webb 1993, Shadwick et al. 1998). La performance de nage prolongée est estimée par la vitesse de nage que les poissons peuvent maintenir avant d'atteindre l'épuisement (Beamish 1978). Lors d'une nage prolongée, lorsque la demande métabolique aérobie des muscles rouges dépasse la capacité en oxygène et en nutriments des systèmes respiratoire et cardiovasculaire, les muscles blancs qui fonctionnent en anaérobie sont sollicités. Par conséquent, à mesure que la vitesse de nage augmente, il en est de même pour le métabolisme anaérobie (Peake et Farrell 2004). Afin de déterminer la performance de nage prolongée, la vitesse de nage critique est calculée (Brett 1964, Beamish 1978, Hammer 1995, Plaut 2001). Seules quelques études ont été réalisées sur les effets des lipides nutritionnels sur la vitesse de nage critique. Les différences de formulation/composition des aliments et de protocoles de nage rendent la comparaison directe de ces études difficile. Pour certains auteurs la vitesse critique de nage est diminuée suite à la substitution partielle des huiles de poissons (riches en AGLPI n-3) de l'aliment par des huiles végétales (pauvres en AGLPI n-3). C'est ce qui a été observé chez l'omble chevalier (*Salvelinus alpinus*; Petterson et al. 2010) et le saumon atlantique (*S. salar*; Wagner et al. 2004). A l'opposé, d'autre auteurs ont trouvé qu'un régime pauvre en AGLPI n-3 favorisait de meilleures performances de nage, notamment chez le saumon (*S. salar*; McKenzie et al. 1998), le bar (*D. labrax*; Chatelier et al. 2006) et le mulet (*Liza aurata*; Vagner et al. 2014). Ces dernières études ont montré que de faibles niveaux nutritionnels en AGLPI n-3 (remplacés par une teneur élevée en AG monoinsaturés) résulteraient en une utilisation préférentielle des AG monoinsaturés comme source d'énergie pour soutenir l'effort de nage. Ces résultats contrastés révèlent l'importance d'une meilleure compréhension de l'effet de la teneur en AGLPI n-3 nutritionnels sur les performances de nage des poissons.

LES MODELES BIOLOGIQUES

Des deux côtés de l'Atlantique Nord, les bars sont des espèces économiquement importantes et à forte valeur patrimoniale. Bien que le bar européen et le bar rayé ne soient pas du même genre, ces espèces appartiennent toutes les deux à la famille des *Morinidae* (Tableau 1). Les genres *Morone* et *Dicentrarchus* formeraient une lignée monophylétique, c'est-à-dire qu'ils seraient issus d'un même ancêtre commun (Williams et al. 2012). L'hypothèse de la présence d'un ancêtre commun anadrome, dont *M. saxatilis*, serait une radiation évolutive plus récente que *D. labrax* a été avancée par différents auteurs (Leclerc et al. 1999, Williams et al. 2012).

Tableau 1: Nomenclature des espèces

	Bar européen	Bar rayé
		
Classe	*Actinopterygii*	
Ordre	*Perciformes*	
Famille	*Moronidae*	
Genre	*Dicentrarchus*	*Morone*
Espèce	*D. labrax*	*M. saxatilis*

Au cours des 2 dernières décennies, et à l'image de nombreuses espèces de l'Atlantique Nord-Est, l'aire de répartition du bar européen s'est très largement étendue vers le nord (Figure 6). Alors que dans les années 90, la limite septentrionale de son aire de distribution était le sud de la Mer du Nord (Pickett et Pawson 1994), il est aujourd'hui très couramment pêché dans les fjords norvégiens, d'Oslo à Tromsø. On retrouve le bar européen dans les eaux côtières mais aussi dans les eaux saumâtres des zones estuariennes, les lagunes côtières, et parfois en rivières. Cette espèce est considérée comme eurytherme et euryhaline, capable de tolérer une large gamme de températures (de 2 à 32°C; Hidalgo et

Alliot 1988) et de salinités (de 0.5‰ à 40‰; Eroldoğan et al. 2004). Depuis 2005, les stocks de bars européens sont menacés par la surpêche et la pollution qui induisent un déclin des populations (ICES 2018).

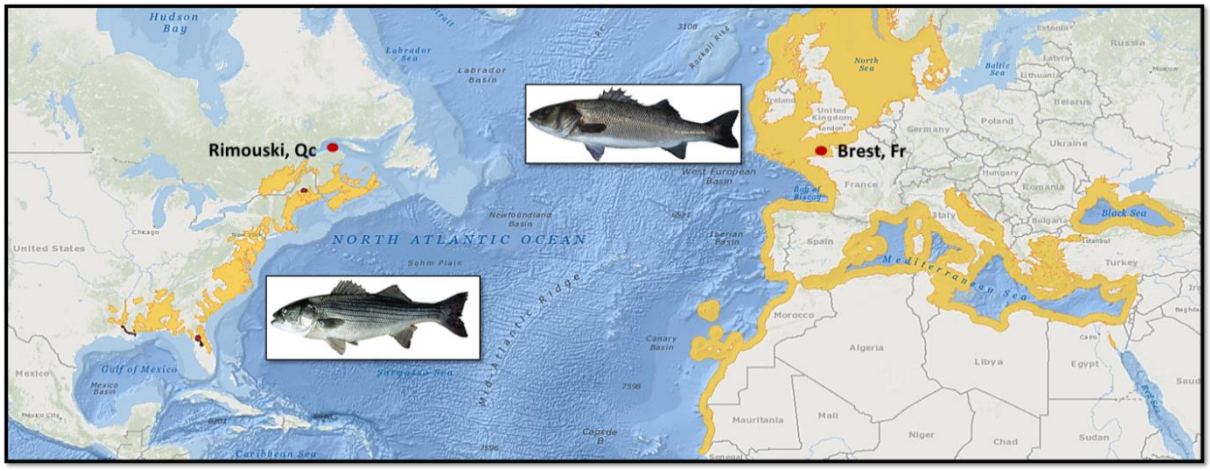


Figure 6: Carte de distribution du bar rayé (*Morone saxatilis*) et du bar européen (*Dicentrarchus labrax*). L'aire en jaune représente l'aire géographique où est présente l'espèce (fonds de carte modifiés de Freytof et Kottelat 2008, NatureServe 2013).

Le bar rayé (*M. saxatilis*) est un poisson typique des estuaires et du littoral de la côte Est de l'Amérique du Nord (Scott et Scott 1988). Son aire de distribution naturelle couvre la côte Est de l'Amérique du Nord, de l'estuaire du Saint-Laurent à la rivière St. Johns, dans le nord de la Floride (Figure 6). Poisson anadrome, le bar rayé doit se déplacer, pour compléter son cycle vital, entre un habitat de reproduction en eau douce et des aires d'alimentation en eau saumâtre ou salée, en estuaire ou le long des côtes. Aux stades d'immature et d'adulte, on retrouve ponctuellement le bar rayé dans les habitats côtiers et les milieux estuariens (Bain et Bain 1982). En revanche, les populations canadiennes de bar rayé sont caractérisées par une remontée automnale et un hivernage en eau douce ou saumâtre. Dans le passé, on dénombrait au Canada trois populations distinctes de bars rayés, dont une au Québec occupant une partie du fleuve et de l'estuaire du Saint-Laurent (Figure 6). La population de bars rayés du fleuve du Saint-Laurent a longtemps été exploitée dans le cadre de pêches commerciales et sportives, mais a disparu vers le milieu des années 1960 (Pelletier et al. 2011). La surexploitation par la pêche, les activités de

dragage et d'entretien de la voie maritime, ainsi que le braconnage seraient les causes probables de sa disparition (Beaulieu 1985, Beaulieu et al. 1990). Depuis lors, les travaux de réintroduction de l'espèce ont débuté et sont toujours en cours pour essayer de rétablir une nouvelle population dans le corridor fluvial du Saint-Laurent.

Le bar européen et le bar rayése situent à un niveau trophique similaire. Considérés comme superprédateurs, le bar européen et le bar rayé sont adaptables et opportunistes, ce qui leur permet de se nourrir avec les proies les plus abondantes (Boulineau-Coatanea 1969, Kennedy et Fitzmaurice 1972, Hartman et Brandt 1995, Sánchez Vázquez et Muñoz-Cueto 2014). Les jeunes bars, de petite taille, se nourrissent d'invertébrés aquatiques et deviennent majoritairement piscivores avec l'âge et l'augmentation de leur taille (Boynton et al.1981, Gardinier et Hoff 1982, Rulifson et McKenna 1987, Sánchez Vázquez et Muñoz-Cueto 2014). En revanche, leur écologie trophique, notamment la nature des proies consommées diffère en fonction de l'accomplissement de leur cycle de vie, et de l'habitat qu'ils occupent (Hartman et Brandt 1995). Issues d'un ancêtre commun, et morphologiquement proches, ces deux espèces se différencient largement lorsque l'on étudie leurs traits d'histoire de vie (Secor 2002, Tableau 2). Les deux espèces n'évoluent pas dans le même habitat, leur écologie est différente: *D. labrax* se reproduit en mer alors que son cycle de vie se déroule principalement dans les eaux côtières et parfois en estuaires; *M. saxatilis* possède un cycle de vie majoritairement estuarien et fraye en eau douce. A la lumière des traits d'histoire de vie de chacune des espèces (Tableau 2), on observe que *D. labrax* et *M. saxatilis* possèdent globalement une stratégie de reproduction différente. En effet, la taille des œufs (rayé > européen), le taux de croissance larvaire (rayé > européen), l'âge de maturité des femelles (rayé > européen), la longévité des femelles (rayé > européen), le temps de génération des femelles (rayé > européen) ou encore la taille maximale des femelles (rayé > européen) soulignent des différences marquées. Nous avons donc choisi ces deux espèces, car elles semblent avoir un rythme de vie différent (voir partie « Les stratégies de vie des espèces », page 4): *D. labrax* possèderait un rythme de vie rapide tandis *M. saxatilis* aurait un rythme de vie plus lent.

Tableau 2: Traits d'histoire de vie du bar européen (*Dicentrarchus labrax*) et du bar rayé (*Morone saxatilis*, inspiré de Secor 2002)

Traits	**Bar européen** *D. labrax*	**Bar rayé** *M. saxatilis*	
Ecologie de la reproduction	Gonochorique Plusieurs pontes par an Saison de ponte : 2-3 mois	Gonochorique Ponte unique par an Saison de ponte : 1-2 mois	
Habitats de ponte	Zones côtières	Eaux douces Estuaires	
Taille des œufs	200 µg	1000 µg	× 5
Habitats larvaire	Zones côtières Estuaires	Estuaires	
Taux de croissance larvaire	0,13mm.j^{-1}	0,20mm.j^{-1}	× 1,5
Age à la maturité des femelles	2-3 ans	5-8 ans	× 2 à 3
Longévité des femelles	15 ans	31 ans	× 2
Fécondité maximale des femelles	2,5 10^{6} ovocytes	8,0 10^{6} ovocytes	× 3
Temps de génération des femelles	3 ans	10 ans	× 3
Masse maximale des femelles	12kg	57kg	× 5

Le bar européen est une espèce dont la domestication est bien maitrisée en Europe, car elle est depuis longtemps élevée en aquaculture et utilisée comme espèce modèle pour la science. En revanche, il existe très peu de données concernant le bar rayé. La plupart des publications datent des années 70-90, où un engouement certain s'est développé aux Etats-Unis pour l'aquaculture du bar rayé hybride (croisement entre un bar rayé [*M. saxatilis*] et un bar blanc [*M. chrysops*]), aussi appelé bar américain, *palmetto bass* (♀ *saxatilis* × ♂ *chrysops*) ou *sunshine bass* (♀ *chrysops* × ♂ *saxatilis*; Hodson 1990). Le bar rayé hybride présente une meilleure croissance que le bar rayé (Smith et al. 1985), ce qui explique l'engouement pour sa production et les différentes études scientifiques portant sur cet hybride. Par le passé, le bar rayé a suscité un intérêt limité parmi la communauté scientifique, mais les défis de protection et de réintroduction actuels nécessitent l'acquisition de nouveaux savoirs scientifiques sur cette espèce.

OBJECTIFS DU PROJET

Ce projet doctoral s'intègre dans un vaste projet qui propose d'examiner les déterminants de la capacité de réponse de deux espèces issues d'un ancêtre commun mais ayant une histoire de vie divergente, face aux changements climatiques globaux. Au cours de leur histoire évolutive ces espèces ont été soumises à des pressions de sélection différentes, elles ont aujourd'hui une écologie qui leur est propre et il est donc probable qu'elles répondront de manière différente aux changements climatiques contemporains. De nombreuses disciplines comme la physiologie, la génomique ou encore la nutrition ont été mises à contribution dans le cadre de ce projet mené en collaboration par différents laboratoires. L'objectif global de ce projet doctoral était d'étudier les effets de différents niveaux en AGLPI n-3 sur la plasticité de ces deux espèces modèles, à plusieurs niveaux fonctionnels. Dans le cadre de cette thèse, quatre objectifs spécifiques ont été identifiés.

Premier objectif de recherche

Le premier objectif de recherche était d'étudier l'effet combiné entre une augmentation de la température de l'eau et une réduction en disponibilité des AGLPI n-3 nutritionnels chez les juvéniles de bar européen. Nous avons testé l'hypothèse que 1) les juvéniles ayant reçu un apport moindre en AGLPI possèdent un taux de rétention des AGE plus important que ceux ayant reçu un régime riche, 2) que les poissons élevés à une température élevée présentent un contenu tissulaire moindre en AGE en lien avec le concept d'adaptation homéovisqueuse et à cause de besoins énergétiques plus élevés.

Deuxième objectif de recherche

Un autre objectif de recherche était de caractériser les effets de l'augmentation de la température de l'eau et de la réduction en disponibilité des AGLPI n-3 alimentaires sur le métabolisme des lipides et le stress oxydatif chez les juvéniles de bar européen. Les hypothèses testées étaient que 1) un aliment pauvre en AGLPI stimule la biosynthèse des

AG, 2) un aliment riche en AGLPI peut engendrer un stress oxydatif aggravé lorsque la température est élevée.

Troisième objectif de recherche

Un troisième objectif de recherche était d'établir de quelle manière les acides gras et la température pouvaient affecter la plasticité phénotypique chez les juvéniles de bar européen. La plasticité a été étudiée via les capacités de nage en utilisant un protocole de vitesse de nage critique dans différentes conditions environnementales contraignantes sur le plan physiologique (salinité et hypoxie). Nous avons testé l'hypothèse que 1) la richesse en AG du muscle influence les performances de nage et 2) que les composés plasmatiques (lactate, glucose et osmolarité) permettent d'expliquer les performances de nage.

Quatrième objectif de recherche

Le dernier objectif de recherche était d'étudier l'effet combiné entre deux salinités (eau douce vs. eau saumâtre) et deux niveaux en AGLPI n-3 nutritionnels chez les juvéniles de bar rayé en conditions hivernales. Les hypothèses étaient 1) qu'une croissance plus importante serait observée avec l'aliment riche en AGLPI n-3, 2) que les juvéniles en eau saumâtre auraient besoin de plus grandes réserves énergétiques pour faire face à des besoins plus élevés.

CHAPITRE 1

LE RECHAUFFEMENT CLIMATIQUE AFFECTERA-T-IL LE BESOIN FONCTIONNEL EN ACIDES GRAS ESSENTIELS CHEZ LES JUVENILES DE BAR EUROPEEN (*DICENTRARCHUS LABRAX*)? UN PREMIER APPERÇU DES CONSEQUENCES DE LA FAIBLE DISPONIBILITE EN ACIDES GRAS NUTRITIONNELS SUR LES PERFORMANCES DE CROISSANCE

Ce premier article intitulé « *Will global warming affect the functional need for essential fatty acids in juvenile sea bass (Dicentrarchus labrax)? A first overview of the consequences of lower availability of nutritional fatty acids on growth performance* » a été publié dans *Marine Biology* (doi.org/10.1007/s00227-018-3402-3).

Les larves de bar européen ont été fournies par l'entreprise Aquastream (Ploemeur, France). L'élevage a été réalisé par Clémence Gourtay en étroite collaboration avec Patrick Quazuguel (Ifremer) dans les locaux de l'Ifremer (Brest, France) et supervisé par Guy Claireaux et José Zambonino. Les aliments expérimentaux ont été formulés par José Zambonino et fabriqués par Frédéric Terrier (INRA) et Frédéric Vallée (INRA). Les analyses biométriques et les échantillonnages ont été effectués par Clémence Gourtay avec l'aide des permanents, vacataires et stagiaires : Cassandre Aimon, Laura Cadiz, Guy Claireaux, Louise Cominassi, Loïck Ducros, Laura Frohn, Sarah Howald, Christine Huelvan, Nicolas Le Bayon, Lauriane Madec, David Mazurais, Ariana Servilli, Marie Vagner, Dorothée Vincent, Patrick Quazuguel, José Zambonino.

La rédaction ainsi que les analyses statistiques, les figures et tableaux ont été réalisés par Clémence Gourtay, qui a rédigé les premières versions et intégré au fur et à mesure les corrections apportées par les co-auteurs. Elle a réalisé la soumission des articles et proposé une première version du manuscrit révisé ainsi qu'une réponse aux arbitres. Cette dernière a été amendée par les co-auteurs avant l'envoi final. Pour cet article la préparation des

échantillons pour l'analyse des acides gras a été réalisée par Clémence Gourtay en collaboration avec Hervé Le Delliou. L'analyse des chromatogrammes et l'intégration des analyses de lipides a été menée par Hervé Le Delliou sous la supervision de José Zambonino.

Différents éléments de cet article ont été présentés au congrès international de la « *Society for Experimental Biology* » (Brighton, Angleterre, juillet 2016), à la réunion annuelle de Ressources Aquatiques Québec (Québec, Canada, novembre 2017), et lors de la journée de l'école doctorale des sciences de la mer (Brest, France, février 2018).

1.1 RESUME

Les changements climatiques mondiaux ont entraîné une diminution des acides gras à longue chaîne polyinsaturée (AGLPI) du type oméga 3 contenus dans le phytoplancton marin qui, suite aux transferts au sein de la chaîne alimentaire, pourrait avoir un impact négatif sur les performances des poissons. Le but de cette étude était d'évaluer l'effet d'une réduction de la disponibilité alimentaire des AGLPI n-3 sur la performance de croissance, l'allométrie des organes et la composition en AG chez les juvéniles de bar européen (*Dicentrarchus labrax*) élevés à deux températures différentes: 15°C (condition naturelle) et 20°C (scénario de réchauffement global). Les poissons ont été nourris pendant cinq mois avec deux régimes isoénergétiques et isoprotéiques: un régime de référence (AL++; AGLPI n-3 = 1,65% de la matière sèche, MS) utilisé comme indicateur des réseaux trophiques où les AGLPI n-3 seraient abondants et un régime alimentaire contenant moins d'AGLPI n-3 (AL; AGLPI n-3 = 0,73% DM) conçu pour simuler la diminution attendue en AGLPI n-3 dans les sources alimentaires résultant des changements climatiques globaux. Les résultats ont montré une diminution des taux de croissance et de légères modifications du profil des lipides polaires dans le muscle des juvéniles de bar nourris avec AL, alors que les lipides neutres ont été plus affectés à long terme. Les masses relatives du cœur et du système gastro-intestinal étaient plus élevées à 20°C, tandis que la masse hépatique était plus élevée à 15°C chez les juvéniles nourris avec l'aliment AL. Cependant, la masse de gras mésentérique des juvéniles nourris avec l'aliment AL++ était plus élevée à 15°C. Globalement, les résultats suggèrent que les juvéniles de bar sont capables de mettre en place des mécanismes physiologiques pour faire face à une diminution en AGLPI n-3 alimentaires et sont capables d'améliorer leur croissance lorsque la température est élevée, même lorsque la disponibilité des AGLPI n-3 est réduite. Une augmentation de la croissance à température plus élevée a également été observée avec le régime alimentaire restreint en AGLPI n-3, accompagnée d'effets significatifs sur l'allométrie des organes et les profils en acides gras. Ces observations peuvent indiquer la présence de certains coûts métaboliques qui restent à évaluer, mais qui montrent que l'augmentation de la température

combinée à la déplétion en AGLPI n-3 a des effets significatifs sur les traits d'histoire de vie de cette espèce.

Mots clés : acides gras essentiels, croissance, allométrie des organes, température, physiologie des poissons

1.2 WILL GLOBAL WARMING AFFECT THE FUNCTIONAL NEED FOR ESSENTIAL FATTY ACIDS IN JUVENILE SEA BASS (*DICENTRARCHUS LABRAX*)? A FIRST OVERVIEW OF THE CONSEQUENCES OF LOWER AVAILABILITY OF NUTRITIONAL FATTY ACIDS ON GROWTH PERFORMANCE

Authors:

Clémence Gourtay[1,2], Denis Chabot[3], Céline Audet[1], Hervé Le Delliou[2], Patrick Quazuguel[2], Guy Claireaux[4], José-Luis Zambonino-Infante[2]

Institutions:

[1]Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski, 310 des Ursulines, Rimouski, QC, G5L 3A1, Canada

[2] Institut Français de Recherche pour l'Exploitation de la Mer, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

[3]Institut Maurice-Lamontagne, Pêches et Océans Canada, C.P. 1000, Mont-Joli, QC, G5H 3Z4, Canada

[4]Université de Bretagne Occidentale, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

1.3 ABSTRACT

Global climate changes have led to a depletion in omega-3 polyunsaturated fatty acids (n-3 PUFA) in marine phytoplankton that—with food web transfers—could negatively impact fish performance. The aim of this study was to assess the effect of a reduction in the dietary availability of n-3 PUFA on growth performance, organ allometry, and fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) raised at two different temperatures: 15°C (natural conditions) and 20°C (global warming scenario). Fish were fed for five months with two isoenergetic and isoproteic diets: a reference diet (RD; 1.65% n-3 PUFA on a dry matter basis, DM) used as a proxy of trophic networks where n-3 PUFA were plentiful, and a lower n-3 PUFA diet (LD; 0.73% n-3 PUFA on DM) designed to mimic the expected decrease in n-3 PUFA sources resulting from global climate changes. Results showed decreasing growth rates and slight changes in the muscle polar lipid profile in LD-fed sea bass juveniles, whereas neutral lipids were more affected over the long term. The relative masses of the heart and gastrointestinal system were higher at 20°C, while liver mass was higher at 15°C in LD-fed juveniles. However, the mesenteric fat of RD-fed juveniles was higher at 15°C. Altogether, the results suggest that sea bass juveniles are able to implement physiological mechanisms to cope with a decrease in dietary n-3 PUFA and are able to improve growth at the higher temperature, even with a decreased availability of n-3 PUFA. The temperature-driven increase in growth is also observed under the restricted n-3 PUFA diet, and this is accompanied by significant effects on organ allometry and FA profiles. This may indicate the presence of some metabolic costs that remain to be evaluated, but which illustrate that the combination of warming temperatures and n-3 PUFA depletion have significant effects on life history traits.

Key words: essential fatty acids, growth, organ allometry, temperature, fish physiology

1.4 INTRODUCTION

Oceans, which cover 71% of Earth's surface, play a major role in regulating the global climate (Reid et al. 2009). Over the past 30 years, rising atmospheric greenhouse gas concentrations have increased global average temperatures by ~0.2°C per decade (Hansen et al. 2006), with most of this added energy being absorbed by the oceans. The resulting global climate changes have already had a large impact on ecosystems, and especially on marine ecosystems (Harley et al. 2006, Lehodey et al. 2006, Brander 2007, Rijnsdorp et al. 2009, Gattuso et al. 2015) through increasing temperatures (Genner et al. 2010, Johansen et al. 2014), acidification (Wittman and Pörtner 2013, Gaylord et al. 2015), and eutrophication (Rijnsdorp and Van Leeuwen 1996, Wasmund et al. 1998).

Phytoplankton form the base of most marine food webs. They are a major source of lipids, including fatty acids (FA) that represent an important source of energy for higher trophic levels (e.g. Sargent et al. 2002). FA are crucial constituents of biological membranes (e.g. Sargent et al. 2002). The lipid composition of cell membranes is critical for the structure and function of cells and tissues, and thus has important effects at different biological/ecological levels (Arts et al. 2009, Parrish 2013). It has been shown that water temperature affects FA composition in phytoplankton species and that the omega-3 polyunsaturated fatty acid (n-3 PUFA) content generally decreases with warming (Ackman and Tocher 1968, Thompson et al. 1992, Renaud et al. 2002, Guschina and Harwood 2006). This conclusion has been confirmed for six major groups of marine and freshwater phytoplankton (Hixson and Arts 2016): temperature is positively correlated with the relative contents of omega-6 FA (n-6; notably arachidonic acid, 20:4n-6, ARA) and saturated fatty acids (SFA), but inversely correlated with the relative contents of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). The same trends were observed by Colombo et al. (2016) in a more comprehensive study, where more than 3000 FA profiles from marine and terrestrial organisms were analyzed. The relationship between water temperature and cell membrane FA content is generally explained through the concept of homeoviscous adaptation: ectotherms are said to adjust

membrane lipid and FA composition to preserve its viscosity, fluidity, and function when faced with changes in ambient temperature.

DHA, EPA, and ARA are identified as essential fatty acids (EFA) in species that are not able to synthesize them and depend on food intake to fulfill their needs (Sargent 1976). Interestingly, phytoplankton produce most of the DHA and EPA present in the biosphere since land plants are unable to synthesize these molecules (Arts et al. 2009). These two n-3 PUFA are of particular interest because they are biochemically important but scarce in nature. They are largely produced by diatoms, cryptophytes, and dinoflagellates (Brett and Muller-Navarra 1997) and are consumed and selectively retained while moving to higher trophic levels (Kainz et al. 2004, Hixson and Arts 2016). Piscivorous marine fish consume prey likely to be situated at lower trophic levels and, therefore, potentially richer in EPA and DHA; this may explain why they have lost the capacity for de novo synthesis of n-3 PUFA (Tocher et al. 2006). In most other fish species, pathways of de novo EFA synthesis are present but their efficiency is reduced, making these species also highly dependent upon dietary sources of n-3 PUFA (Ghioni et al. 1999, Tocher and Ghioni 1999). Despite their scarcity in marine food webs, n-3 PUFA are key compounds involved in fish growth, reproduction, behaviour, vision, osmoregulation, cell membrane structure (thermal adaptation), and immune function (Higgs and Dong 2000, for reviews see Sargent et al. 2002, Glencross 2009, Tocher 2010, Kiron et al. 2011, Tian et al. 2014).

The European sea bass (*Dicentrarchus labrax*, Linnaeus 1758) is commercially important along European coasts. It is typically a marine species that spends most of its life in coastal and estuarine areas, although it is occasionally observed in rivers, particularly at early life stages. Until now, the consequences of nutritional n-3 PUFA depletion have been studied under aquaculture conditions and rarely in the field, with an ecological perspective. For example, the substitution of dietary fish oil with vegetable oils (which lack n-3 PUFA) has been extensively investigated in farmed fish. Most studies have concentrated on salmonids (McKenzie et al. 1998, Tocher et al. 2000, Torstensen 2000, Caballero et al. 2002), freshwater fishes (Martino et al. 2002, Ng et al. 2003), and some marine fish species

such as European sea bass (Yildiz and Sener 1997, Montero et al. 2005, Chatelier et al. 2006), turbot (*Psetta maxima*) (Regost et al. 2003), gilthead sea bream (*Sparus aurata*) (Kalogeropoulos et al. 1992, Caballero et al. 2003, Montero et al. 2003), and red sea bream (*Pagrus auratus*) (Glencross et al. 2003). In European sea bass juveniles, Skalli and Robin (2004) showed that low dietary n-3 PUFA (0.2% of the diet on a dry matter [DM] basis) significantly lowered growth compared to diets with at least 0.7% n-3 PUFA. Moreover, the level of dietary n-3 PUFA modified FA composition in muscle neutral lipids, while muscle polar lipid composition was less affected. Skalli et al. (2006) tested a crossed factorial design combining two diets (0.4 and 2.2% DM n-3 PUFA) and two temperatures (22°C and 29°C). One of the main outcomes of this study was that 0.7% DM was found to be the minimal n-3 PUFA level necessary to sustain juvenile sea bass growth.

Studies that combine the effects of n-3 PUFA and temperature on fish growth and physiology are scarce. The aim of this study, therefore, was to test the effect of a reduction in n-3 PUFA dietary content on growth performance, organ allometry, and the FA profile in juvenile European sea bass raised at two different temperatures, 15°C and 20°C. Two experimental diets were tested: a reference diet (RD) that mimicked a trophic network where n-3 PUFA are plentiful, and a lower n-3 PUFA diet (LD) that simulated the expected decrease in phytoplankton sources resulting from ocean warming (Colombo et al. 2016, Hixson and Arts 2016). We hypothesized that (1) juveniles fed the depleted diet will show higher EFA tissue retention than those fed RD, and (2) that fish raised at the higher temperature will retain a lower amount of EFA in tissues in accordance with the homeoviscous adaptation concept and also because of higher energetic needs.

1.5 MATERIALS AND METHODS

1.5.1 Fish origin and maintenance

Adult European sea bass were captured in winter 2013 by fishermen in the Gulf of Morbihan (Plomeur, France) and brought to the Aquastream hatchery (Lorient, France).

After three years in captivity, four females and 10 males were bred in the facility. At day 2 post hatching (d-2), sea bass larvae were transferred to the Ifremer rearing facility in Brest (France), where experiments were conducted. Larvae were divided among three conical tanks (230 L, 10 µm filtered seawater, UV, salinity 35‰, initial density 10000 larvae $tank^{-1}$). Water temperature in the tanks was progressively increased from 14°C to 20°C within six days. Larvae were fed with Artemia from mouth opening (d-8) to d-39. To condition the larvae to more readily accept the manufactured diet at the end of the live-feed period, they were co-fed with both Artemia and a microparticulate diet (Marinstart, Le Gouessant, Lamballe, France) for four days starting at d-40. Larvae were then fed exclusively with the microparticulate diet until d-74. After d-74, juveniles were fed with larger pellets for ornamental fish (EPA + DHA = 1.5%; Le Gouessant, Lamballe, France) until the beginning of the experiment at d-93

1.5.2 Environmental and nutritional conditioning

At d-93, juveniles (mass = 0.75 ± 0.02 g; standard length = 3.57 ± 0.02 cm; mean ± SD) were divided among 12 indoor 500 L tanks supplied with filtered and aerated natural seawater, six of which were maintained at 15°C and the other six at 20°C. Each tank contained 300 fish, representing a mean biomass of 263.93 ± 0.28 g. During the following 150 days, fish were fed one of two experimental diets: a reference n-3 PUFA diet (RD; EPA+DHA = 1.65% DM) and a low n-3 PUFA diet (LD; EPA+ DHA = 0.73% DM). Feeding took place for 7 h during daytime (08:00 to 15:00) using an automatic distributor (2 cm h^{-1}). Each diet × temperature combination was replicated in three tanks.

1.5.3 Experimental diets

The two diets tested were identical except for the FA source. LD contained only colza oil as a source of FA (essentially oleic acid [18:1n-9], linoleic acid [18:2n-6], and linolenic acid [18:3n-3]), while RD contained 50% colza oil and 50% fish oil, the latter being richer

in EPA and DHA (20:5n-3, 22:6n-3). Diets were isoenergetic and contained the same percentages of proteins and lipids (Table 3).

Table 3: Composition of experimental diets

	LD	RD
	Mean	Mean
	% of dry mass	
Dry matter	94.84	95.12
Proteins	50.48	50.23
Total lipids	21.98	21.63
Triglycerides	16.99	17.05
Phospholipids	4.70	4.71
	% of total lipids in diet	
18:1n-9	5.69	4.65
18:2n-6	2.50	2.16
18:3n-3	0.97	0.77
18:3n-6	0.00	0.01
18:4n-3	0.08	0.14
20:4n-6 (ARA)	0.03	0.07
20:5n-3 (EPA)	0.28	0.94
22:5n-3	0.03	0.07
22:6n-3 (DHA)	0.45	0.71
SFA	2.18	2.97
MUFA	7.32	6.48
n-3	1.93	2.68
n-6	2.65	2.28
n-9	6.56	5.46
EPA+DHA	0.73	1.65

For dry matter, proteins, total lipids, triglycerides, and phospholipids, data are presented as % of dry mass. Data for specific fatty acid (FA) categories are presented as % of total lipids. LD: low n-3 polyunsaturated fatty acid (PUFA) diet, RD: reference n-3 PUFA diet, SFA: saturated FA, MUFA: monounsaturated FA, ARA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

1.5.4 Experimental time line

For the growth survey, seven samplings were done at intervals of 200 degree-days (dd, day × exposed temperature). Fish were not fed 24 h prior to sampling. A total of 30 fish, randomly sampled in each tank (n = 90 per diet–temperature treatment), were lightly anaesthetized with tricaine methanesulfonate (MS-222; dose adapted to water temperature and fish body mass [BM]), weighed, and their standard length (SL) measured. After recovery, individuals were returned to their original tanks.

Mass gain (Δmass, eq.1), specific growth rate (SGR, eq.2), and thermal growth coefficient (TGC, eq.3 which is a thermal unit approach; for reviews see Dumas et al. 2010) were calculated as follows:

$$\Delta mass\ (g) = BM_{final} - BM_{initial} \quad \text{eq.1}$$

$$SGR\ (\%BM\ days^{-1}) = \frac{\ln(BM_{final}) - \ln(BM_{initial})}{days\ of\ feeding} \times 100 \quad \text{eq.2}$$

$$TGC\ (g\ degree\ days^{-1}) = \ 1000 \times (BM_{final}^{0.33} - BM_{initial}^{0.33}) \ / \ \sum(degree\ days) \quad \text{eq.3}$$

At 720 dd (d-129 and d-141 at 20°C and 15°C, respectively) and 1660 dd (d-176 and d-204 at 20°C and 15°C, respectively), eight fish per tank were randomly sampled, euthanized with an overdose of MS-222 (200 mg L^{-1}), and a piece of white epaxial muscle located under the first dorsal fin was dissected out. Muscle samples obtained from the same tank were pooled and stored at −80°C until analyses (n = 3s per experimental treatment).

At the end of the experiment (d-243), 10 fish per tank (30 per diet–temperature combination) were euthanized as described above. After fish were weighed and measured, heart, liver, gastrointestinal system, and mesenteric fat were sampled and weighed for the organ allometry study.

1.5.5 Fatty acids

White muscle samples were ground in liquid nitrogen using a mixer (MM 400, Retsch). Lipids were then extracted following a procedure derived from that of Folch et al. (1957). Muscle powder was homogenized in Folch solution and stored at −20°C until polar and neutral lipids were separated.

Samples were sonicated for 5 min at 4°C then centrifuged for 2 min (4°C, 1482 g). Lipids were fractionated into neutral lipids (including triglycerides, free FA, and sterols) and polar lipids (including phospholipids and glycolipids). An aliquot of total lipid extract (1 mL) was evaporated to dryness under nitrogen, recovered with three (0.5 mL) washings of chloroform:methanol (98:2, v:v), and deposited at the top of a silica gel micro-column (40 mm × 5 mm i.d. Pasteur pipette plugged with glass wool and filled with silica gel 60 that had been heated for 6 h at 450°C and deactivated with 6% water by weight). Neutral lipids were eluted with 10 mL of chloroform:methanol (98:2, v:v), and polar lipids were eluted with 15 mL of methanol. Tricosanoic acid (23:0, 200 μg) was added as an internal standard.

Each lipid fraction was vacuum dried and directly transesterified under nitrogen using 0.5 mL of 2M methanolic potash (KOH-MeOH) for 3 min at 90°C. A total of 0.5 mL of 6N hydrochloric acid was added and vortex mixed. Before gas chromatography analysis, 2 mL of hexane was added and centrifuged for 10 min at 630 g to collect the organic phase containing fatty acid methyl esters (FAMEs). FAMEs were washed three times with 1 mL of hexane. The organic phase was finally transferred to tapered vials and stored at −20°C.

FAMEs were analyzed in a CLARUS 500 gas chromatograph (Perkin-Elmer) equipped with a split/splitless injector and a flame ionization detector. FAMEs were identified using two different capillary columns (BPX70 30 m × 0.25 mm i.d., 0.25 μm thickness; SGE Analytical Science) using a standard 37 component FAME mix (Sigma) and other known standard mixtures (i.e. 18919-1AMP FAME Mix, C4-C24 and 18918-

1AMP FAME Mix, C8-C24; Sigma). The FA were expressed as the molar percentage of the total FA content.

1.5.6 Statistical and data analysis

Data normality and homoscedasticity were tested using Shapiro-Wilk and Levene tests, respectively. Growth rate in BM and SL was regressed against time. The effects of diet and temperature were tested by comparing regression slopes; when slopes were homogeneous, an ANCOVA was run to compare intercepts. Two-way ANOVAs were used to test for significant differences among diets and temperatures for Δmass, SGR, and TGC. Scatter plots between organ mass and BM for each temperature did not overlap due to a large difference in BM because of the temperature differences. For this reason, the effect of diet on organ allometry was tested separately for each temperature by comparing slopes and using ANCOVA if required. In order to meet normality, BM, SL, and organ mass were $\log_{10}$ transformed. Because the response at 1660 dd could be considered as dependent on the response at 720 dd, two-way ANOVAs were performed for each sampling period to assess the effects of diet and temperature on muscle FA content and the muscle/dietary FA ratio. When required, pairwise comparisons (Tukey tests) were performed (the homoscedasticity condition was respected). Differences were considered significant at $\alpha = 0.05$. Statistical analyses were conducted in R (ver.3.3.3; R Development Core Team).

1.6 RESULTS

Only a few mortalities occurred during the experiment (less than 0.2%), therefore this effect was not considered.

1.6.1 Growth performance and organ allometry

BM and SL differed according to time and temperature (higher slopes at 20°C than at 15°C, Figure 7; BM: $F_{[1,76]} = 222.23$, $P < 0.001$; SL: $F_{[1,76]} = 211.46$, $P < 0.001$;

Supplementary Table 16 in Annexe II). Diet composition also had a significant effect on growth, which was similar at both temperatures: growth was faster in RD-fed fish than in LD-fed fish (BM: $F_{[1,76]} = 19.40$, $P < 0.001$; SL $F_{[1,76]} = 27.48$, $P < 0.001$). In addition, the Δmass was about 20% higher in the juveniles fed RD than in those fed LD independent of temperature, while Δmass was about twice as high at 20°C than at 15°C (Table 4). The same pattern was observed for TGC, whereas only temperature affected SGR, which was 35% higher at 20°C (Table 4). Linear regressions showed that fish fed RD at 20°C were 14.5% heavier (Figure 7A) and 34.1% longer (Figure 7B) at the end of the experiment compared to fish fed LD. The difference was less pronounced at 15°C, with RD-fed fish being 12.8% heavier (Figure 7A) and 5.7% longer (Figure 7B) than LD-fed fish.

Table 4: Effect of temperature and diet on growth indices

	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
Δmass (g)	2.5 ± 0.51	1.9 ± 0.07	5.6 ± 0.11	4.7 ± 0.49	P < 0.001	P < 0.01	—
TGC (g degree-days^{-1})	0.36 ± 0.06	0.30 ± 0.01	0.61 ± 0.02	0.55 ± 0.04	P < 0.001	P < 0.01	—
SGR (%BM d^{-1})	1.3 ± 0.18	1.1 ± 0.02	1.9 ± 0.07	1.8 ± 0.10	P < 0.001	—	—

Δmass: mass gain, TGC: thermal growth coefficient, SGR: specific growth rate, RD: reference n-3 polyunsaturated fatty acid (PUFA) diet, LD: low n-3 PUFA diet; BM: body mass, d: day. Values are means ± standard deviations.

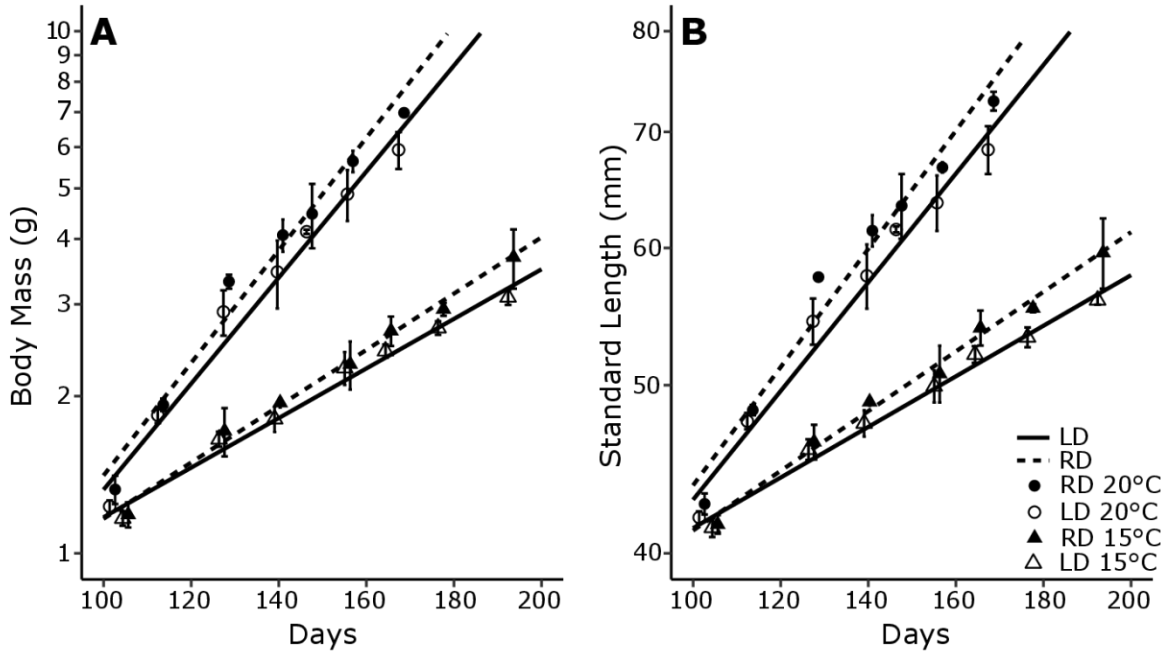


Figure 7: Effect of diet and temperature on A) body mass (g) and B) standard length (mm) in logarithmic scale. LD: low omega-3 polyunsaturated fatty acid (n-3 PUFA) diet; RD: reference n-3 PUFA diet. Values are means ± standard deviations

Differences in the relationship between organ masses and BM were examined at each rearing temperature. At 20°C, RD-fed fish had lower heart (Figure 8A; ANCOVA: $F_{[1,54]} = 6.82$, $P < 0.05$) and gastrointestinal (Figure 8B; ANCOVA: $F_{[1,54]} = 4.52$, $P < 0.05$) masses, but no differences for liver or mesenteric fat were observed. At 15°C, liver mass was lower in RD-fed juveniles (Figure 8C; ANCOVA: $F_{[1,54]} = 8.73$, $P < 0.01$), while the quantity of mesenteric fat relative to BM increased more in juveniles fed RD (Figure 8D; slopes significantly different, $\log_{10}$ [BM] × diet: $F_{[1,54]} = 9.90$, $P < 0.01$). There were no differences for heart or gastrointestinal mass. Allometric regression parameters are available in Supplementary Table 17 (Annexe II, pages 150-51).

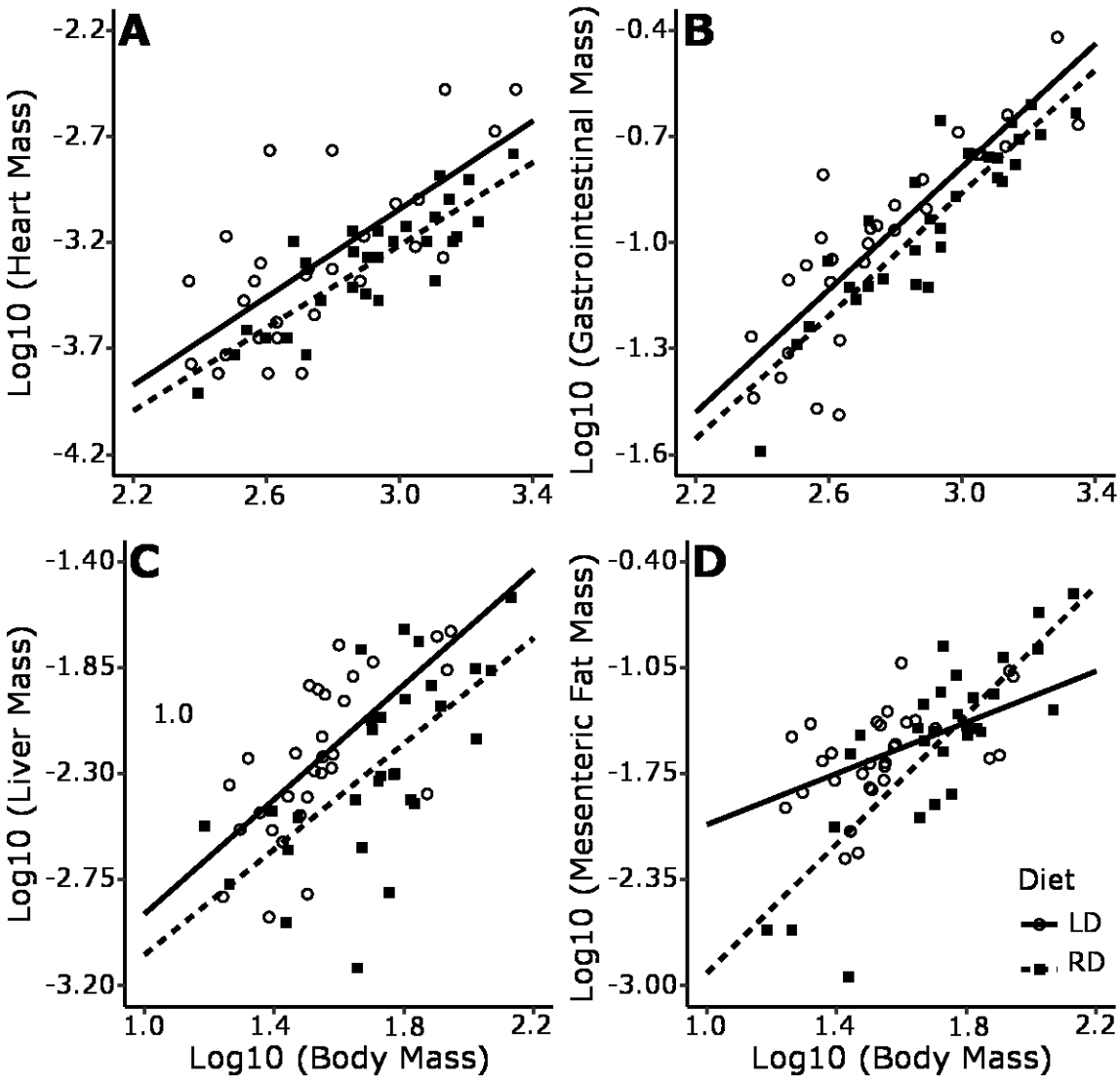


Figure 8: Effect of diet (LD, low omega-3 polyunsaturated fatty acid [n-3 PUFA] diet; RD, reference n-3 PUFA diet) on organ allometry of fish raised at 20°C: A) heart; B) gastrointestinal system; and for fish raised at 15°C: C) liver; D) mesenteric fat

1.6.2 Fatty acids

At 720 dd, significant effects of both temperature and diet were observed in the white muscle FA composition. Both temperature and diet affected polar ΣSFA levels and proportions, which were significantly higher at 20°C than at 15°C and with RD compared to LD (Table 5A). Temperature (20°C > 15°C) but not diet significantly affected white

muscle polar ΣMUFA (monounsaturated FA) and ΣPUFA. The ΣPUFA to ΣSFA ratio was significantly higher in fish fed LD and raised at 15°C. Polar Σn-3 and Σn-6 both significantly increased with temperature. However, independently of the temperature conditions, Σn-3 was higher in juveniles fed RD while Σn-6 dominated in juveniles fed LD in the polar fraction (Table 5A). Consequently, polar Σn-3/Σn-6 ratios were higher in RD-fed juveniles. In LD-fed juveniles, the ratio was higher at 15°C than in those reared at 20°C (Table 5A). At both temperatures, the percent content of 18:2n-6 was significantly higher in juveniles fed LD, but the difference between the two diets was larger at 20°C than at 15°C. On the contrary, the 18:3n-3 content was higher in juveniles fed RD, although juveniles contained overall more 18:3n-3 at 20°C than at 15°C. The ARA and DHA levels were significantly higher in juveniles reared at 20°C, while there was no effect of temperature on DHA present in the polar fraction. A significant diet effect was only present for ARA and EPA, with higher levels in juveniles fed RD than in those fed LD. The lowest DHA/EPA ratios were observed in fish fed RD at both temperatures. Juveniles reared at 20°C and fed LD had a higher DHA/EPA ratio than those raised at 15°C (Table 5A). Changes in the neutral fraction were clearly less pronounced at 720 dd. No effect of either temperature or diet was observed for Σn-3, Σn-6, Σn-3/Σn-6, ΣSFA, ΣMUFA, ΣPUFA, or the percent content of 18:2n-6 and 18:3n-3. The ARA and EPA contents were significantly lower in juveniles fed LD, while the DHA/EPA ratio was significantly lower in juveniles fed RD. The ratio was also generally lower at 15°C than at 20°C.

Table 5: Effect of temperature and diet on muscle fatty acid (FA) profiles

A	POLAR						
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	$1.29^{a} \pm 0.06$	$1.82^{b} \pm 0.06$	$1.44^{a} \pm 0.09$	$2.19^{c} \pm 0.05$	—	—	P < 0.05
ARA	0.29 ± 0.02	0.2 ± 0.01	0.34 ± 0.03	0.21 ± 0.01	P < 0.05 *15°C < 20°C*	P < 0.001 *LD < RD*	—
18:3n-3	0.33 ± 0.01	0.49 ± 0.02	0.38 ± 0.02	0.6 ± 0.02	P < 0.001 *15°C < 20°C*	P < 0.001 *RD < LD*	—
EPA	1.94 ± 0.12	1.41 ± 0.04	2.03 ± 0.1	1.3 ± 0.04	—	P < 0.001 *LD < RD*	—
DHA	3.13 ± 0.26	2.99 ± 0.19	3.55 ± 0.3	3.34 ± 0.11	P < 0.05 *15°C < 20°C*	—	—
DHA/EPA	$1.61^{a} \pm 0.05$	$2.12^{b} \pm 0.11$	$1.75^{a} \pm 0.09$	$2.57^{c} \pm 0.1$	—	—	P < 0.05
Σn-3	5.76 ± 0.41	5.22 ± 0.24	6.34 ± 0.44	5.56 ± 0.14	P < 0.05 *15°C < 20°C*	P < 0.01 *LD < RD*	—
Σn-6	1.68 ± 0.09	2.13 ± 0.06	1.89 ± 0.13	2.55 ± 0.06	P < 0.001 *15°C < 20°C*	P < 0.001 *RD < LD*	—
Σn-3/Σn-6	$3.42^{c} \pm 0.06$	$2.45^{b} \pm 0.05$	$3.34^{c} \pm 0$	$2.18^{a} \pm 0.03$	—	—	P < 0.01
ΣSFA	3.33 ± 0.16	3.07 ± 0.09	3.73 ± 0.27	3.62 ± 0.11	P < 0.001 *15°C < 20°C*	P < 0.001 *LD < RD*	—
ΣMUFA	3.33 ± 0.21	3.7 ± 0.02	4.12 ± 0.28	4.88 ± 0.05	P < 0.05 *15°C < 20°C*	—	—
ΣPUFA	7.44 ± 0.5	7.34 ± 0.29	8.23 ± 0.56	8.11 ± 0.2	P < 0.01 *15°C < 20°C*	—	—
ΣPUFA/ΣSFA	2.23 ± 0.12	2.39 ± 0.11	2 .21 ± 0.01	2.24 ± 0.02	P < 0.001 *20°C < 15°C*	P < 0.001 *RD < LD*	—
ΣPUFA/ΣMUFA	2.23 ± 0.03	1.98 ± 0.07	2.00 ± 0.03	1.66 ± 0.02	—	—	—
ΣTotal	14.1 ± 0.82	14.12 ± 0.34	16.07 ± 1.11	16.62 ± 0.36	P < 0.001 *15°C < 20°C*	—	—

	NEUTRAL						
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n6	2.14 ± 1.62	2.03 ± 1.34	2.99 ± 0.51	2.99 ± 1.14	—	—	—
ARA	0.05 ± 0.04	0.02 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	—	P < 0.01 *LD < RD*	—
18:3n3	0.69 ± 0.52	0.7 ± 0.47	0.97 ± 0.17	1.06 ± 0.42	—	—	—
EPA	0.6 ± 0.44	0.22 ± 0.13	0.84 ± 0.15	0.27 ± 0.11	—	P < 0.05 *LD < RD*	—
DHA	0.65 ± 0.46	0.33 ± 0.14	0.99 ± 0.16	0.57 ± 0.26	—	—	—
DHA/EPA	1.09 ± 0.04	1.6 ± 0.31	1.18 ± 0.04	2.12 ± 0.16	P < 0.05 *15°C < 20°C*	P < 0.001 *RD < LD*	—
Σn-3	2.16 ± 1.56	1.39 ± 0.81	3.1 ± 0.53	2.08 ± 0.85	—	—	—
Σn-6	2.3 ± 1.75	2.12 ± 1.4	3.2 ± 0.55	3.11 ± 1.18	—	—	—
Σn-3/Σn-6	0.96 ± 0.04	0.68 ± 0.05	0.97 ± 0.01	0.66 ± 0.02	—	P < 0.001 *LD <RD*	—
ΣSFA	3.58 ± 2.6	2.4 ± 1.39	5.16 ± 0.77	3.7 ± 1.71	—	—	—
ΣMUFA	7.43 ± 5.57	6.53 ± 4.18	10.56 ± 1.52	9.85 ± 4.16	—	—	—
ΣPUFA	4.46 ± 3.31	3.52 ± 2.21	6.3 ± 1.07	5.18 ± 2.03	—	—	—
ΣPUFA/ΣSFA	1.23 ± 0.06	1.44 ± 0.08	1.22 ± 0.05	1.43 ± 0.10	P < 0.001 *20°C < 15°C*	P < 0.001 *RD < LD*	—
ΣPUFA/ΣMUFA	0.60 ± 0.02	0.54 ± 0.00	0.66 ± 0.03	0.53 ± 0.00	—	—	—
ΣTotal	15.47 ± 11.47	12.44 ± 7.77	22.02 ± 3.35	18.73 ± 7.9	—	—	—

B	POLAR						
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	1.61 ^{ab} ± 0.02	1.83 ^{ab} ± 0.51	1.22 ^{a} ± 0.1	2.27 ^{b} ± 0.31	—	—	P < 0.05
ARA	0.38 ^{b} ± 0.01	0.18 ^{a} ± 0.05	0.3 ^{b} ± 0.03	0.19 ^{a} ± 0.03	—	—	P < 0.05
18:3n-3	0.43 ^{a} ± 0	0.5 ^{ab} ± 0.12	0.34 ^{a} ± 0.01	0.62 ^{b} ± 0.08	—	—	P < 0.05
EPA	2.04 ± 0.07	0.97 ± 0.33	1.54 ± 0.34	1.07 ± 0.13	—	P < 0.001 *LD < RD*	—
DHA	4.41 ± 0.23	3.38 ± 0.9	3.12 ± 0.61	3.15 ± 0.53	—	—	—
DHA/EPA	2.17 ± 0.12	3.55 ± 0.41	2.05 ± 0.26	3.05 ± 0.28	—	—	—
Σn-3	7.3 ± 0.27	5.14 ± 1.4	5.31 ± 0.95	5.05 ± 0.84	—	—	—
Σn-6	2.14 ± 0.03	2.16 ± 0.58	1.61 ± 0.14	2.46 ± 0.49	—	—	—
Σn-3/Σn-6	3.42 ± 0.07	2.37 ± 0.02	3.28 ± 0.31	2.06 ± 0.13	P < 0.05 *20°C < 15°C*	P < 0.001 *LD < RD*	—
ΣSFA	4.6 ± 0.29	3.23 ± 0.92	3.45 ± 0.4	3.77 ± 0.75	—	—	—
ΣMUFA	4.29 ± 0.02	3.91 ± 0.8	3.62 ± 0.2	4.7 ± 0.85	—	—	—
ΣPUFA	9.44 ± 0.3	7.3 ± 1.98	6.92 ± 1.09	7.51 ± 1.32	—	—	—
ΣPUFA/ΣSFA	2.06 ± 0.17	2.27 ± 0.07	2.00 ± 0.08	2.00 ± 0.12	P < 0.01 *20°C < 15°C*	P < 0.01 *RD <LD*	—
ΣPUFA/ΣMUFA	2.20 ±0.08	1.85 ± 0.14	1.91 ± 0.20	1.60 ± 0.09	P < 0.05 *20°C < 15°C*	—	—
ΣTotal	18.33 ± 0.26	14.43 ± 3.69	13.99 ± 1.68	15.98 ± 2.89	—	—	—
	NEUTRAL						
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	2.76 ± 0.47	5.38 ± 2.74	3.97 ± 0.54	5.99 ± 0.5	—	P < 0.05 *RD < LD*	—
ARA	0.07 ^{b} ± 0.01	0.04 ^{a} ± 0.02	0.11 ^{c} ± 0.01	0.0 ^{a} ± 0	—	—	P < 0.05
18:3n-3	0.91 ± 0.16	1.87 ± 1	1.44 ± 0.07	2.18 ± 0.17	—	P < 0.05 *RD < LD*	—
EPA	0.68 ^{a} ± 0.14	0.42 ^{a} ± 0.2	1.14 ^{b} ± 0.08	0.48 ^{a} ± 0.05	—	—	P < 0.05
DHA	1.06 ± 0.2	0.63 ± 0.24	1.34 ± 0.09	1.13 ± 0.15	P < 0.01 *15°C < 20°C*	P < 0.05 *LD < RD*	—
DHA/EPA	20.09 ^{b} ± 3.56	32.41 ^{b} ± 14.36	29.87 ^{a} ± 1.72	35.95 ^{c} ± 5.53	—	—	P < 0.001
Σn-3	2.88 ± 0.51	3.19 ± 1.56	4.25 ± 0.12	3.92 ± 0.69	—	—	—
Σn-6	2.97 ± 0.5	5.62 ± 2.81	4.25 ± 0.56	5.91 ± 0.88	—	P < 0.05 *RD < LD*	—
Σn-3/Σn-6	0.97 ± 0.01	0.57 ± 0.01	1.01 ± 0.11	0.66 ± 0.02	—	P < 0.001 *LD < RD*	—
ΣSFA	4.28 ± 0.82	5.9 ± 1.7	6.96 ± 0.03	6.83 ± 1.14	P < 0.05 *15°C < 20°C*	—	—
ΣMUFA	9.95 ± 1.74	17.7 ± 8.29	14.41 ± 1.03	19.29 ± 2.88	—	P < 0.05 *RD < LD*	—
ΣPUFA	5.86 ± 1.02	8.81 ± 4.37	8.5 ± 0.67	9.82 ± 1.57	—	—	—
ΣPUFA/ΣSFA	1.37 ± 0.03	1.42 ± 0.40	1.22 ± 0.09	1.44 ± 0.09	P < 0.01 *20°C < 15°C*	P < 0.01 *RD < LD*	—
ΣPUFA/ΣMUFA	0.59 ± 0.01	0.49 ± 0.02	0.59 ±0.01	0.51 ± 0.02	P < 0.05 *15°C < 20°C*	—	—
ΣTotal	2.88 ± 0.51	3.19 ± 1.56	4.25 ± 0.12	3.92 ± 0.69	—	—	—

Values are given as % of dry matter (DM) in the neutral and polar lipid fractions at A) 720 degree-days (DD) and B) 1660 DD. LD: low n-3 polyunsaturated FA (PUFA) diet, RD: reference n-3 PUFA diet. When factor interactions were significant, groups were compared with a posteriori tests (α = 0.05). For temperature × diet interactions, significantly different groups were assigned different letters. Σn-3 includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3; Σn-6 includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6; ΣSFA (saturated FA) includes 20:0s, 22:0s, 24:0s; ΣMUFA (monounsaturated FA) includes 14:1n-9, 18:1n-11, 18:1n-7, 20:1n-7, 22:1n-9, 24:1n-9; ΣPUFA includes 18:3n-6, 18:4n-3, 20:2n-6, 20:3n-3, 20:3n-6, 20:4n-3. Values are means ± standard deviations.

At 1660 dd, polar ΣSFA, ΣMUFA, ΣPUFA, Σn-3, and Σn-6 percentages were similar regardless of the rearing conditions (Table 5B). However, the Σn-3/Σn-6 ratio was 8.4% lower when temperature increased and 51.2% lower in juveniles fed LD compared to those fed RD (Table 5B). As observed at 720 dd, the 18:2n-6 content was the highest in juveniles fed LD, but only at 20°C. A similar response was observed for the 18:3n-3 content. The EPA content was significantly higher in juveniles fed RD, whereas DHA and the DHA/EPA ratio were similar among treatments. However, the ARA content was lowest in juveniles fed LD at both temperatures, while those fed RD at 20°C had the highest proportion (Table 5B). In contrast to what was observed at 720 dd, more pronounced effects of diet and temperature were observed in the neutral fraction at 1600 dd. Indeed, the ΣSFA content was 26.2% higher at 20°C than at 15°C, while the ΣMUFA was 34.1% higher in juveniles fed LD than in those fed RD (Table 5B). The ΣPUFA to ΣSFA ratio was significantly higher in fish fed LD and raised at 15°C, while the ΣPUFA to ΣMUFA ratio was higher at 20°C. The Σn-6 was significantly higher in juveniles fed LD and, consequently, the Σn-3/Σn-6 ratio was significantly higher in juveniles fed RD compared to those fed LD (Table 5A, B). Both 18:2n 6 and 18:3n-3 contents were lower in juveniles fed RD. Interestingly, the ARA content was extremely low in juveniles fed LD at both 15°C and 20°C. EPA was significantly higher in juveniles fed RD at 20°C compared to the three other treatments, while DHA was overall significantly lower at 15°C and in juveniles fed LD. At 20°C, RD-fed fish had the lowest DHA/EPA ratios, while LD-fed fish had the highest.

The muscle/dietary FA ratios suggest that the retention of EFA was more pronounced in the polar fraction than in the neutral fraction (Figure 9). A stronger selective retention of polar ARA, DHA, and EPA was observed in juveniles fed LD compared to those fed RD. Retention of ARA, DHA, and EPA was significantly higher at 20°C than at 15°C, but only at 720 dd (Figure 9A). At 1660 dd, the temperature effect was no longer evident. It should be noted that even though the muscle/dietary 18:2n-6 ratio was higher than one in the neutral fraction, it decreased below one in polar lipids (Figure 9B). No

effect of diet, except for DHA at 1660 dd, and no effect of diet, except for EPA at 1600 dd, was observed in neutral fraction (Figure 9B).

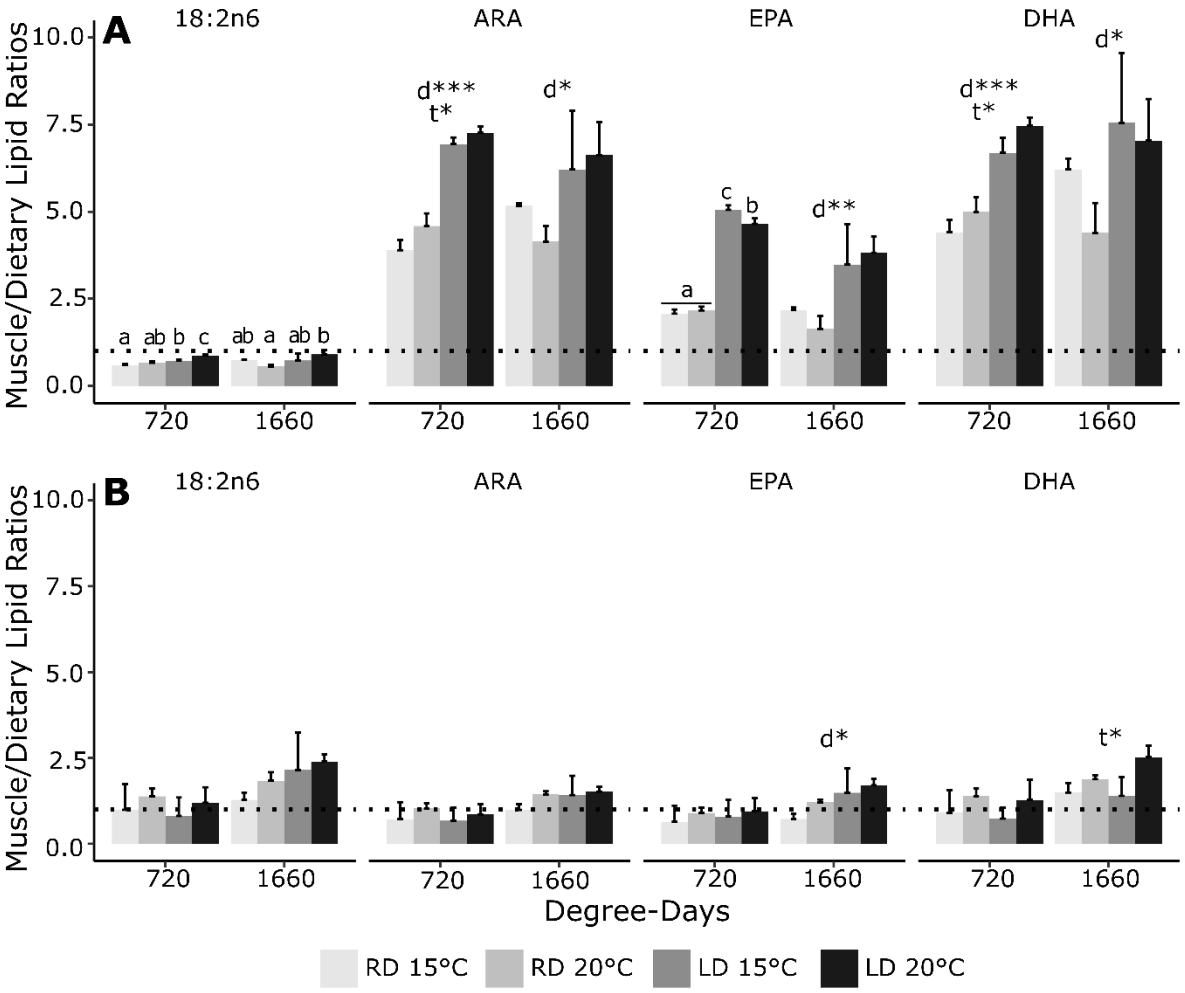


Figure 9: Effect of diet on muscle/dietary lipid ratios for linoleic acid (18:2n-6), arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosapentenoic acid (DHA, 22:6n-3) in A) the polar fraction and B) the neutral fraction. When factor interactions were significant, groups were compared with a posteriori tests ($\alpha = 0.05$). For temperature × diet interactions, significantly different groups were assigned different letters. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. LD: low omega-3 polyunsaturated fatty acid (n-3 PUFA) diet, RD: reference n-3 PUFA diet, d: diet, t: temperature. Values are means ± standard deviations

1.7 DISCUSSION

Diet and temperature both induced different growth trajectories. After 720 dd, diet clearly modified the FA composition of muscle; this effect was modulated by temperature for ARA, linolenic acid, and DHA, whereas the FA profiles were more alike at 1660 dd. Diet also influenced organ allometry at both 20°C and 15°C, although with some variations.

1.7.1 Growth performance

As expected, increased water temperature significantly improved the growth of juvenile sea bass fed both diets, with the differences in body length and mass increasing with time. Both Δmass and TGC were higher at 20°C than at 15°C, and higher in RD-fed than in LD-fed fish, which is consistent with the growth trajectories obtained by a linear model. Several indices have been developed to determine the daily growth increment in fish. The most commonly used is SGR (Ricker 1979). SGR may be affected by both the fish's body size (Jobling 1983, Iwama et al. 1997) and environmental temperature (Tidwell et al. 1999, Person-Le Ruyet et al. 2004); to avoid such bias, the thermal unit growth coefficient was proposed by Iwama and Tautz (1981). Even though this estimate is thought to be less sensitive to body size and temperature (Azevedo et al. 1998, Cho and Bureau 1998, Kaushik 1998), TGC and SGR of sea bass were affected by temperature in a similar way in our study. Such a result was also reported for the Eurasian perch (*Perca fluviatilis*; Strand et al. 2011).

Skalli and Robin (2004) defined the minimal n-3 PUFA levels necessary to sustain juvenile sea bass growth at 0.7% DM, and they observed no improvement with higher n-3 PUFA content. However, we observed better growth with 1.65% DM (RD), indicating that n-3 PUFA in excess of 0.7% DM may increase growth further under certain circumstances. This different requirement may also result from the fact that our fish were younger than those used by Skalli and Robin (2004). RD allowed better growth than LD, and this diet-

related effect was observed at both temperatures. Consequently, the low dietary levels of n-3 PUFA did not impair the temperature-related growth-promoting effect.

1.7.2 Organ allometry

From the perspective of evolutionary biology, the functional capacity of an organ should match the demands imposed upon it (Starck 1999). Therefore, individuals ought to respond to changes in actual demands by adjusting their functional capacities (Diamond and Hammond 1992, Elia 1992, Diamond 1998, Weibel 1998), including organ size. In ectotherms, temperature has a direct effect on metabolism. In the sea bass, Claireaux and Lagardère (1999) showed that when temperature was increased from 15°C to 20°C, standard metabolism and active metabolism increased by 37% and 125%, respectively. This implies that more oxygen and nutrients are needed to cover the energetic demand. In sea bass, energy costs related to digestion mobilize a great proportion of the cardiac output (Farrell et al. 2001, Axelsson et al. 2002, Altimiras et al. 2008). In our study, the LD-fed fish reared at 20°C had higher heart and gastrointestinal masses. The change in fatty acids may have induced a thickening or an elongation of the gastrointestinal tract. Our hypothesis, is that this increase in heart mass relative to BM may result from a greater energetic demand due to the simultaneous increase in gastrointestinal mass. It has also been established in vitro that tissues and organs have mass-specific metabolism (Krebs 1950, Schmidt-Nielsen 1984). An evaluation of the heart's working capacity—and, for instance, of the stroke volume—would be necessary to know if the increased heart mass also implied better performance.

Why did the gastrointestinal mass increase more in juveniles fed LD? One explanation could be that offering a diet that minimally meets nutritional needs of juveniles may require an optimization of the nutrient assimilation processes, thus increasing the overall energy demand. Indeed, previous studies have shown that size and activity of the gastrointestinal tract are phenotypically plastic and respond strongly to consumption and food availability (Starck 1999, Armstrong and Bond 2013). Here, we showed that the type

of FA provided in the diet affects the gastrointestinal mass. It seems reasonable to speculate that the higher values of gastrointestinal mass could be explained either by an elongation of the gut or by modifications of the brush border epithelium of the small intestine induced by the necessity to improve FA assimilation. In addition, Torrecillas et al. (2017a) showed that fish oil replacement by vegetable oil increases the lipid deposition in anterior gut lamina propria in sea bass, which could also explain the largest viscera mass obtained with LD.

Knowing that the optimum temperature for European sea bass growth was reported to be about 22°C to 25°C (Barnabé 1991), colder temperatures represent an additional constraint on juvenile metabolism. The liver has a major role in energy storage, and it is the first site for lipid storage in a number of benthic and demersal species (Drazen 2002, Hoffmayer et al. 2006, Lloret et al. 2008). Another important storage site is the mesenteric fat that surrounds the gastrointestinal tract. It is much more labile than other fat stores, such as muscular fat, and therefore mesenteric fat is likely to be the first fat store to be mobilized. In our study, liver mass was higher in fish fed LD than RD at 15°C. This result corroborates the findings of Mourente and Bell (2006), who found that the liver mass of juvenile sea bass fed vegetable oil was higher than that of fish fed fish oil. As n-3 PUFA were scarcer in LD, further experiments will be needed to assess if the higher liver mass could be explained by greater FA storage.

1.7.3 Fatty acids

Muscle/diet ratios greater than 1 mean that the muscle is richer in FA than the diet is, suggesting that retention occurred. After 720 dd, fish were already showing the effects of their diet, with muscle/dietary ratios > 1. At 720 dd, both temperature and diet had significant effects on most of the FA in polar lipids. At 1660 dd, however, the differences in the FA profiles among dietary treatments were smaller in the polar than in the neutral lipid fraction. This suggests that, over time, fish regulated their phospholipid composition, possibly membrane phospholipids, in order to maintain tissue functionality; this agrees with previous work (Sargent 1976, Skalli and Robin 2004). It should be noted that a high

retention rate, approximately six times higher than the diet content, was observed for polar EFA (EPA, DHA, and ARA) in LD-fed fish, but this high retention rate did not compensate for the low EFA contents induced by this diet.

The main representatives of the n-3 and n-6 FA in the dietary lipids were linolenic (18:3n-3) and linoleic (18:2n-6) acids, respectively. In fish, these FA are accumulated without transformation due to the reduced capacity of these species for chain elongation and desaturation (Bell et al. 1986, 1994). However, n-3 intermediates in the desaturation elongation pathway such as 20:5n-3 (EPA), 22:5n-3, and 22:6n-3 (DHA) were found in both lipid fractions at higher values than those present in the diets. This may indicate a certain biochemical capacity to elongate or to selectively preserve specific EFA, even though the conversion rates are probably extremely low (Mourente and Dick 2002, Mourente et al. 2005). It should be noted that polar EPA and DHA were high in juveniles fed RD; these two FA are eicosanoid precursors involved in several physiological functions such as stress response or osmoregulation (Sargent et al. 2002). One could then wonder whether low dietary EPA and DHA levels could impair stress response capacity, and this would justify examination of fish response to specific challenge tests. As previously reported, a significant decrease in n-3 PUFA content in fish tissues was observed when fish oil was replaced by vegetable oil (Bell et al. 2001, Mourente et al. 2005, Torstensen et al. 2005, Pettersson et al. 2009, Sanden et al. 2011). This was confirmed in the present study, where we observed the same overall tendencies.

Fish, as ectothermic species, do not control their body temperature. The relationship between water temperature and cell membrane FA content is generally explained through the concept of homeoviscous adaptation (Sinensky 1974). For instance, the proportion of unsaturated acyl chains in membrane lipids is generally increased under cold conditions to maintain membrane fluidity (Los and Murata 2004). Changes in the proportions of polar ΣPUFA to ΣSFA and/or ΣMUFA were already present at 720 dd but were more pronounced at 1660 dd. At 720 dd, ΣSFA, ΣPUFA, and ΣMUFA polar contents were higher at 20°C than at 15°C. However, the ΣPUFA/ΣSFA ratio was similar at both 15°C and 20°C

(respectively 2.31 and 2.22), while a higher proportion of ΣPUFA to ΣMUFA was observed at 15°C than at 20°C (respectively 2.10 and 1.83). Such a change in the ΣPUFA/ΣMUFA ratio would be consistent with adjustments related to homeoviscous adaptation. At 1660 dd, both polar ΣPUFA/ΣSFA and ΣPUFA/ΣMUFA ratios were higher at 15°C than at 20°C. Ratios remained very stable in the neutral lipid fraction over time and between temperature conditions. The ΣPUFA/ΣSFA ratio was about 1.34, while the ΣPUFA/ΣMUFA ratio was around 0.56. These results clearly indicate remodelling in polar lipids (mostly represented by membrane phospholipids) with temperature changes, while storage lipids remained stable regardless of temperature or time. In marine fishes, n-3 PUFA (EPA + DHA) tend to decrease with increasing temperature, while n-6 PUFA (ARA and linolenic acid) and SFA increase (Hixson and Arts 2016). In salmonids, a classical thermal response is a higher proportion of PUFA at low temperatures (Hazel et al. 1992, Calabretti et al. 2003). Similar effects of temperature were observed in European sea bass, with lower SFA and conversely higher n-3 PUFA contents at 22°C than at 29°C (Skalli et al. 2006). In our study, temperature affected both relative FA contents and the muscle/dietary FA ratio of most polar FA at 720 dd, whereas temperature only affected polar Σn-3/Σn-6 at 1660 dd. The fact that n-3 and n-6 PUFA showed an inverse relationship with regard to temperature is coherent from a biosynthesis perspective, since synthesis depends on the activities of the same enzymes (desaturases and elongases). Competition for enzymes, in the context of increasing ambient water temperature, tends to favour n-6 over n-3 production (Hixson and Arts 2016). This hypothesis remains to be tested.

1.8 CONCLUSIONS

Depletion of n-3 PUFA and a decreased temperature contributed to the decrease in sea bass growth rate while only slightly altering the muscle polar lipid profile. Neutral lipid profiles were more affected than polar ones. However, regarding the PUFA/SFA and PUFA/MUFA ratios, a higher proportion of PUFA at low temperature was present in polar lipids. This effect increased with time. These results are consistent with the homeoviscious adaptation theory. Higher contents of SFA and MUFA compared to PUFA were present in

the storage lipids, but ratios remained stable regardless temperature and time. A depleted n-3 PUFA diet induced low EFA contents in muscle even though a higher retention of EFA was noted in fish fed this diet. For the first time in fish, the allometry of several organs has been shown to respond to the type of dietary FA acid provided. This topic has been little investigated in fish even though it is easily achievable and inexpensive, and can reveal valuable information on key organs like the heart, liver, and gastrointestinal system. We also showed that the allometric organ response depends on temperature conditions. Dietary n-3 PUFA affected organ allometry of the heart and gastrointestinal system at the higher temperature (20°C), while liver and mesenteric fat were affected at the lower temperature (15°C). Juvenile sea bass are able to implement rapid phenotypic change in response to dietary FA. This is interesting from the point of view of global warming, where fish species are faced with rapid changes in mean temperature. More broadly, these results open up new perspectives in the study of seasonal adaptations. Altogether, these results suggest that juvenile sea bass are able to implement compensatory mechanisms to cope with a reduced availability of dietary n-3 PUFA. Because of this, the temperature-driven increase in growth is still observed under a restricted diet. However, the fact that this was accompanied by significant effects on organ allometry and FA profiles may indicate the presence of some metabolic cost, although this physiological adjustment remains to be evaluated. Further studies should be conducted to assess the impacts of these modifications of FA profiles on sea bass life history traits.

CHAPITRE 2

EFFET DES CONDITIONS THERMIQUES ET NUTRITIONNELLES SUR LE METABOLISME DES ACIDES GRAS ET LA REPONSE AU STRESS OXYDATIF CHEZ LES JUVENILES DE BAR EUROPEEN (*DICENTRARCHUS LABRAX*)

Ce second article, intitulé « *Effect of thermal and nutritional conditions on fatty acid metabolism and oxidative stress response in juvenile European sea bass (Dicentrarchus labrax)* » a été soumis dans *Journal of Experimental Biology*.

Tous les co-auteurs ont participé au processus de rédaction selon le processus décrit dans le chapitre précédent. Pour cet article les analyses d'expression de gènes ont été réalisées par Lauriane Madec sous la supervision de David Mazurais. Les analyses enzymatiques ont été réalisées par Clémence Gourtay en étroite collaboration avec Loïck Ducros et avec l'aide de Christine Huelvan.

2.1 RESUME

Les zones de nourricerie en milieu côtier sont soumises à une grande variété de facteurs de stress d'origines anthropique et naturelle, incluant le réchauffement climatique, qui influence indirectement les réseaux trophiques. Une raréfaction globale des acides gras à longue chaîne polyinsaturée (AGLPI) du type n-3 dans les réseaux trophiques est en cours. Le but de cette étude était d'évaluer l'effet d'une réduction de la disponibilité en AGLPI n-3 sur le métabolisme des lipides et la réponse au stress oxydatif chez des juvéniles de bar européen (*Dicentrarchus labrax*) élevés à deux températures (15°C et 20°C). Les poissons ont été nourris pendant cinq mois avec un aliment de référence (AL++; AGLPI n-3: 1.65% de la matière sèche, MS) utilisé comme le proxy d'un réseau trophique où les AGLPI n-3 sont abondants, et un aliment avec un niveau plus bas d'AGLPI n-3 (AL; n-3 AGLPI: 0.73% MS) destiné à mimer un déclin en AGLPI n-3, résultant des changements globaux. Les résultats ont montré que l'aliment n'affectait pas les facteurs transcriptionnels hépatiques impliqués dans la régulation des voies métaboliques en lien avec la bioconversion des acides gras. Ces résultats suggèrent la présence d'un seuil nutritionnel d'AGLPI au dessus duquel ces voies métaboliques ne sont pas actives. En revanche, la température a induit une augmentation de l'expression génique de la plupart des gènes testés. Malgré un indice de peroxydation élevé chez les poissons nourris avec l'aliment AL++, de très faibles modifications de la réponse au stress oxydatif ont été associées à l'effet de l'aliment. La température a augmenté la réponse des enzymes antioxydantes, sans qu'elle puisse être corrélée à l'indice de peroxydation ou à la malondialdéhyde.

Mots clés : temperature, nutrition, acides gras, expression de gène, métabolisme des acides gras, indice de peroxydation

2.2 EFFECT OF THERMAL AND NUTRITIONAL CONDITIONS ON FATTY ACID METABOLISM AND OXIDATIVE STRESS RESPONSE IN JUVENILE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

Authors:

Clémence Gourtay[1,2], Denis Chabot[3], Céline Audet[1], Lauriane Madec[2], Christine Huelvan[2], Loïck Ducros[2], Guy Claireaux[4], David Mazurais[2], José-Luis Zambonino-Infante[2]

Institutions :

[1]Institut des Sciences de la Mer de Rimouski, Université du Québec à Rimouski, 310 des Ursulines, Rimouski, QC, G5L 3A1, Canada

[2]Institut Français de Recherche pour l'Exploitation de la Mer, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

[3]Institut Maurice-Lamontagne, Pêches et Océans Canada, C.P. 1000, Mont-Joli, QC, G5H 3Z4, Canada

[4]Université de Bretagne Occidentale, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

2.3 ABSTRACT

Coastal nursery areas are subjected to a wide range of anthropogenic and natural stressors, including global warming, which indirectly influences trophic food webs. A global rarefaction of n-3 polyunsaturated fatty acids (PUFA) in trophic networks is in progress. The aim of this study was to assess the effect of a reduction in the dietary availability of n-3 PUFA on lipid metabolism and the oxidative stress response in juvenile European sea bass (*Dicentrarchus labrax*) raised at two temperatures (15°C and 20°C). Fish were fed for five months with a reference diet (RD; 1.65% n-3 PUFA on a dry matter basis, DM) used as a proxy of trophic networks where n-3 PUFA is plentiful, and a lower n-3 PUFA diet (LD; 0.73% n-3 PUFA DM) designed to mimic a decrease in n-3 PUFA, resulting from global changes. Results showed that diet did not affect hepatic transcriptional factors involved in the regulation of the metabolic pathways related to fatty acid bioconversion. This suggests the presence of a threshold in nutritional supply of PUFA above which the activation of these pathways does not occur. However, temperature induced an increase of gene expression for most of the genes tested. Despite the high peroxidation index in fish fed RD, very few modifications of the oxidative stress response were associated with diet. Temperature also increased the enzymatic antioxidant response, but there was no correlation with the PI index or malondialdehyde products.

Key words: temperature, nutrition, fatty acids, gene expression, fatty acid metabolism, peroxidation index

2.4 INTRODUCTION

Many fish species have life history strategies in which successive ontogenetic stages occupy different habitats. In those characterized by the production of large amounts of small pelagic eggs and the lack of parental care, the survival of eggs, larvae, and juveniles strongly depend on abiotic and biotic environmental factors acting on their habitats (Cushing 1995, Sogard 1992, 1997, Juanes 2007, Houde 2008). Coastal zones and estuaries are productive nursery grounds for a large number of marine species because they provide refuge from predators together with high food availability and enhancement of survival, development, and growth of early stages (Beck et al. 2001, Able et al. 2013). However, coastal and estuarine nursery areas are subjected to a wide range of anthropogenic and natural stressors. Coastal habitats particularly face anthropogenic activities including global warming, which indirectly influences trophic food webs (Harley et al. 2006, Lehodey et al. 2006, Brander 2007, Rijnsdorp et al. 2009, Gattuso et al. 2015, Hixson and Arts 2016).

Lipids represent an important supply of energy and play a crucial role as major components of cell membranes (Sargent 2002). In marine trophic networks, polyunsaturated fatty acids (PUFA) are mainly supplied by the dominant phytoplankton species, such as diatoms and dinoflagellates (Hu et al. 2008, Lang et al. 2011). The n-3 PUFA (also known as omega 3), particularly eicosapentenoic acid (EPA) and docosahexaenoic acid (DHA), are naturally abundant for primary consumers. However, it has been shown that phytoplankton growth and biochemical composition, including fatty acids (FA), are affected by temperature (e.g., Arts et al. 2009). An increase of water temperature results in a decrease in the omega 3 content of phytoplankton species (Ackman and Tocher 1968, Thompson et al. 1992, Renaud et al. 2002, Guschina and Harwood 2006). At least, two recent studies have shown a global decline of n-3 PUFA in trophic networks while n-6 tends to be more abundant (Colombo et al. 2016, Hixson and Arts 2016). The high availability of n-3 PUFA in phytoplankton may explain the lack of selection pressure on its biosynthesis pathway in several marine fish species and thus why piscivorous fish have lost the capacity for de novo synthesis of n-3 PUFA (Tocher et al. 2006). In most

other fish species, the n-3 PUFA biosynthesis pathway is present but its efficiency is reduced, making these fishes highly dependent upon dietary sources of n-3 PUFA (Ghioni et al. 1999, Tocher and Ghioni 1999). To what extent n-3 PUFA decline induced by global climate change may impact species that depend on phytoplankton to fulfill their needs whether as primary or secondary consumers is not yet known.

Saturated short-chain fatty acids (SFA) are biosynthesized by the fatty acid synthetase complex (*fas*). This enzyme catalyzes the key lipogenesis pathway in fishes (Sargent et al. 2002). The main products of *fas* are the saturated 16:0 (palmitic acid) and 18:0 (stearic acid) FA. In the carnivorous fishes blackspot seabream (*Pagellus bogaraveo*; Figueiredo-Silva et al. 2009), sea bass (*Dicentrarchus labrax*; Dias et al. 1998), and gilthead sea bream (*Sparus aurata*; Gomez-Requeni et al. 2003), dietary fat intake inhibits *fas* activity. Because of their low capacity to bioconvert 18-carbon-atom fatty acids (linoleic 18:2n-6 and alpha-linolenic 18:3n-3) into PUFA with 20 or 22 carbon atoms (arachidonic 20:4n-6; EPA, and DHA), marine fishes require the presence of preformed PUFA in their diet (Mourente and Tocher 1994). The extent to which fish species can convert C18 PUFA to C20/22 PUFA varies according to their complement of FA desaturase and elongase enzymes. Marine fish species such as turbot (*Scophtalamus maximus*), sea bass (*D. labrax*), and gilthead seabream (*S. aurata*) are unable to produce DHA from 18:3n-3 to fulfill their nutritional needs (Owen et al. 1975, Sargent et al. 2002, Geay et al. 2010, 2012) due to apparent deficiencies in one or more steps in the pathway (Ghioni et al. 1999, Tocher and Ghioni 1999, Santigosa et al 2010). The first step of this bioconversion pathway requires the presence of the delta 6-desaturase gene (*fads2*; Sprecher, 2000). PUFA are known to bind and directly influence the activity of a variety of transcription factors including peroxisome proliferator activated receptors (ppars), which in turn have been shown to be regulators of many genes involved in lipid homeostatic processes (Jump 2002). In mammals, ppars and sterol regulatory element binding protein-1 (*srebp-1*) are the main transcription factors regulating the gene expression of FA elongase and desaturase (Nakamura and Nara 2002, 2003), and their expression is regulated by

dietary FA (Lindi et al. 2003, Juliano et al. 2004, MacLaren et al. 2006). Similar results have been reported by Geay et al. (2010, 2012) in sea bass.

Biological membranes rich in PUFA are highly sensitive to oxidation due to their high level of unsaturation (Pamplona et al. 2000, Hulbert 2005); this oxidation can lead to the production of reactive oxygen species (ROS). ROS are generated as by-products of cellular metabolism, primarily in mitochondria (Barja 2007). The effects of ROS are dose dependent (Gechev et al. 2006, Quan et al. 2008): at low levels, they act as important signaling molecules (Finkel 1998, Rhee 1999, Thannickal and Fanburg 2000); when ROS production overwhelms antioxidant capacity, damage to cellular macromolecules such as lipids, proteins, and DNA may occur (Chen et al. 2007, Halliwell and Gutteridge 2007, Hulbert et al. 2007). In addition, warming may promote ROS production especially in organisms rich in membrane PUFA. Indeed, owing to the presence of PUFA double bonds, cellular membranes are putative targets for free radical propagation causing peroxidative damage that may induce several cellular and tissular dysfunctions (Kawatsu 1969, Watanabe et al. 1970, Murai and Andrews 1974, Sakai et al. 1998). The in vitro rate of oxygen consumption during peroxidation has been measured for DHA, and the damage rate was eight times higher than for linoleic acid and 320 times higher than oleic acid (Holman, 1954). These relative rates of peroxidation of different acyl chains have been used to convert the fatty acid composition of a particular membrane into the peroxidation index (PI; see Hulbert et al. 2007, for details). The PI indicates the theoretical susceptibility of membrane lipid composition to lipid peroxidation, and it appears to be homeostatically regulated with respect to the dietary PI. In practice, malondialdehyde (MDA) is one lipid peroxidation product that can be measured (Frankel 2005). Under most physiological states, ROS production is closely matched by the antioxidant response. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidases, form an important part of the antioxidant response (Lesser 2006).

European sea bass migrate offshore to spawn, and the developing larvae drift to sheltered coastal or estuarine nurseries where they will spend their first few years of life

(Holden and Williams 1974, Kelley 1988, Vinagre et al. 2009). During that period, juvenile sea bass will exhibit rapid growth and will be faced with a broad spectrum of environmental constraints and feeding conditions, making it an attractive model organism to examine the regulatory response of the PUFA biosynthesis pathway. The aim of this study was therefore to test the effects of two levels of n-3 PUFA dietary contents on FA metabolism in European sea bass juveniles raised at two different temperatures: 15°C and 20°C. We hypothesized that a diet poor in n-3 PUFA would stimulate lipid metabolism while a diet richer in n-3 PUFA would lead to oxidative stress and that this would worsen under higher temperature conditions.

2.5 MATERIALS AND METHODS

2.5.1 Feeding experiment

The protocols for fish maintenance, diet composition, and nutritional conditioning are detailed in Gourtay et al. (2018). They are summarized here to facilitate understanding of the experimental protocol. Adult European sea bass were captured in winter 2013 by fishermen in the Gulf of Morbihan (Plomeur, France) and brought to the Aquastream hatchery (Lorient, France). After three years in captivity, a batch of four females and 10 males were bred in the facility. At day 2 post hatching (d-2), sea bass larvae were transferred to the Ifremer rearing facility in Brest (France), where experiments were conducted. Larvae were divided among three conical tanks (230 L, 10 µm filtered seawater, UV, salinity 35, initial density 10000 larvae tank–1). Water temperature in the tanks was progressively increased from 14°C to 20°C within six days. Larvae were fed with Artemia from mouth opening (d-8) to d-39. Larvae were fed with microparticulate (Marinstart, Le Gouessant, Lamballe, France) until d-74. After d-74, juveniles were fed with larger pellets for ornamental fish (EPA + DHA = 1.5%; Le Gouessant, Lamballe, France) until the beginning of the experiment at d-93.

The two diets tested were identical except for the FA source. The low n-3 PUFA diet (LD) contained only colza oil as a source of FA (essentially oleic acid [18:1n-9], linoleic acid [18:2n-6], and linolenic acid [18:3n-3]; EPA + DHA = 0.73% DM), while the reference diet (RD) contained 50% colza oil and 50% fish oil, the latter being richer in EPA and DHA (EPA + DHA = 1.65% DM). Diets were isoenergetic and contained the same percentages of proteins and lipids (Table 6).

At d-93, juveniles (0.75 ± 0.02 g; 3.57 ± 0.02 cm; mean ± SD) were divided among 12 indoor 500 L tanks supplied with filtered and aerated natural seawater, six of which were maintained at 15°C and the other six at 20°C. Each tank contained 300 fish, representing a mean biomass of 263.93 ± 0.28 g. During the following 150 days, fish were fed one of the two experimental diets. Feeding took place for 7 h during daytime (8 PM to 3 AM) using an automatic distributor (2 cm h^{-1}). Each diet × temperature combination was replicated in three tanks.

Table 6: Composition of experimental diets

	LD	RD
	Mean	Mean
	% of dry mass	
Dry matter	94.84	95.12
Proteins	50.48	50.23
Total lipids	21.98	21.63
Triglycerides	16.99	17.05
Phospholipids	4.70	4.71
	% of total lipids in diet	
18:1n-9	5.69	4.65
18:2n-6	2.50	2.16
18:3n-3	0.97	0.77
18:3n-6	0.00	0.01
18:4n-3	0.08	0.14
20:4n-6 (ARA)	0.03	0.07
20:5n-3 (EPA)	0.28	0.94
22:5n-3	0.03	0.07
22:6n-3 (DHA)	0.45	0.71
SFA	2.18	2.97
MUFA	7.32	6.48
n-3	1.93	2.68
n-6	2.65	2.28
n-9	6.56	5.46
EPA+DHA	0.73	1.65

For dry matter, proteins, total lipids, triglycerides, and phospholipids, data are presented as % of dry mass. Data for specific fatty acid (FA) categories are presented as % of total lipids. LD: low n-3 polyunsaturated fatty acid (PUFA) diet, RD: reference n-3 PUFA diet, SFA: saturated FA, MUFA: monounsaturated FA, ARA: arachidonic acid; EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

2.5.2 Tissue sampling

At 720 degree-days (dd, d-129 and d-141 at 15°C and 20°C, respectively) and at 1660 dd (d-176 and d-204 at 15°C and 20°C respectively), 32 fish were randomly sampled from each tank (three tanks per treatment, N = 96 per treatment), anesthetized (lethal dose of MS-222, 200mg L^{-1}), and their livers excised. Because livers were small, the livers of eight juveniles were pooled for a total of four pools per tank, 12 per experimental condition. Pools were stored at –80°C. Two pools per tank were used for molecular biology analysis (N = 6 per experimental treatment for a total of 24), one pool was used for enzyme activity, and the fourth was used for malondialdehyde (MDA) analysis (N = 3 per experimental condition).

2.5.3 Peroxidation index, enzyme activity, and malondialdehyde assay

The peroxidation index (PI) was calculated (eq.4) for total dietary lipids and the polar fraction of muscle lipids (see Gourtay et al. [2018] for details of fatty acid content) following the equation provided by Hulbert et al. (2007):

$$PI = (0.025 \times \% \, monoenoics) + (1 \times \% \, dieonics) + (2 \times \% \, trienoics) + (4 \times \% \, tetraenoics) + (6 \times \% \, pentaenoics) + (8 \times \% \, hexaenoics) \quad \text{eq.4}$$

Liver samples were reduced to powder using a mixer mill (MM 400, Retsch, 30 Hz, 30 s) with liquid nitrogen. To extract total proteins, 100 mg of liver powder was diluted in 1 mL lysis buffer (150 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X100, 0.5% Igepal, 1% phosphatase inhibitor cocktail II, 2% NaPPi, and 1 tablet EDTA-free protease inhibitor cocktail, pH adjusted to 7). Samples were then homogenized on ice using a polytron (PT-MR 2100, BioBlock). For protein extraction, samples were left on ice for 40 min and then centrifuged (3-30K Sigma, 6000 g, 1 h, 4°C). The middle phase containing the protein extract was recovered with a Pasteur pipette and centrifuged (3-30K Sigma, 10000 g, 45 min, 4°C). The middle phase containing the protein extract was

recovered with a Pasteur pipette, separated into aliquots, and stored at –80°C for the enzyme activity assay.

Total protein concentrations of the liver homogenates were assayed in triplicate according to the Bio-Rad DC Protein Assay (5000116, Bio-Rad), based on the Lowry assay (Lowry et al., 1951).

Specific CAT activity was measured in triplicate according to Aebi (1984) by observing the decomposition of H2O2 into oxygen and water. The reaction mixture consisted of 20 mM phosphate, pH 7, and 10 mM H2O2. We used a Multiskan GO (Thermo Scientific) plate reader with GREINER UV-Star 96 (ref: M3812, Sigma). The kinetics of the colouring solutions were monitored at 22°C by readings of the absorbance at 240 nm every 10 s for 4 min.

The specific enzyme activity of superoxide dismutase (SOD, combined Cu, Zn-SOD, and Mn-SOD) was determined in triplicate using a commercial kit (ref: 19160, Sigma), using a NUNC F-bottom 96-well (Thermo Scientific) with Multiskan GO (Thermo Scientific) plate reader. Final absorbances were read at 450 nm after 20 min incubations at 37°C.

The specific enzyme activity of cytochrome c oxidase (COX) was measured in triplicate using a commercial kit (ref: CYTOCOX1, Sigma) combined with Evolution 21 UV-Visible Spectrometer (Thermo Scientific). The kinetics of colouring solutions were monitored at 25°C by readings absorbance at 550 nm every 0.25 s for 1 min.

The specific enzyme activity of citrate synthase (CS) was measured in triplicate according to Srere (1969) and Bergmeyer et al. (1974). The assay medium contained 100 mM Tris-HCl (pH 8), 10 mM DTNB, and 2 mM acetyl-CoA. Liver homogenates and assay media were protected from light and incubated for 5 min in NUNC F-bottom 96-well plates (Thermo Scientific); 5 mM oxaloacetate solution was added to initiate the reaction. The kinetics of colouring solutions were monitored at 25°C by reading absorbance at 412 nm every 10 s for 4 min.

The lipid peroxide assay was adapted from the thiobarbituric acid reactive substances (TBARS) protocol (Uchiyama and Mihara, 1978). Liver samples were ground on ice using a polytron (PT-MR 2100, BioBlock) with distilled water containing 1% BHT in methanol, 50 mg of liver for 1 mL of grinding solution. A total of 500 µL of 1% TBA, and 1.5 mL of 1% phosphoric acid were added to 500 µL of liver homogenate. This mixture was heated for 30 min at 100°C and cooled for 30 min on ice, at which point 2 mL of butanol were added and the mixture vortexed. Protein precipitates were removed by centrifugation at 800 g. The absorbance of the supernatant was read in NUNC F-bottom 96-well plates (Thermo Scientific) with a Multiskan GO spectrophotometer (Thermo Scientific) at 532 nm.

2.5.4 Gene expression analysis

Liver samples were reduced to powder using a mixer mill (MM 400, Retsch, 30 Hz, 30 s) with liquid nitrogen. Samples (50–100 mg) were placed in 1 mL of extractall reagent and homogenized using a polytron (PT-MR 2100, BioBlock) tissue disruptor for 30 s. Potential contaminating DNA was removed using an RTS DNase™ Kit (MoBio Laboratories Inc.) according to the manufacturer's recommendations. Total RNA was extracted following the manufacturer's instructions. The quantity and purity of RNA were assessed using a NanoDrop spectrophotometer (ND-1000, Thermo Scientific Inc.). The RNA integrity was determined by electrophoresis using an Agilent Bioanalyser 2100 (Agilent Technologies Inc.). All samples had an RNA integrity number higher than seven and could thus be used for real-time quantitative polymerase chain reaction analysis. RNA samples were stored at −80°C until use.

After extraction, total RNA was reverse transcribed into cDNA using an iScript™ cDNA Synthesis kit (Bio-Rad Laboratories Inc.) following the manufacturer's instructions. Briefly, 500 ng total RNA was reverse transcribed into cDNA in a volume of 20 µL that was composed of 15 µL of sample; 4 µL 5 × iScript™ Reaction Mix containing oligo, random primers, and RNaseA inhibitor; and 1 µL of iScript™ reverse transcriptase. Reverse transcription (RT) negative controls were also performed on each sample using the

same reaction mix except for the reverse transcriptase (substituted by water).The cDNA synthesis reaction was incubated for 5 min at 25°C followed by 30 min at 42°C and terminated by incubation for 5 min at 85°C to inactivate the enzyme. RT was performed using a T 100 Thermal-cycler (Bio-Rad Laboratories Inc.). cDNA samples were stored at −20°C until use.

The analysis of gene expression in liver tissue of European sea bass was carried out by qPCR using the primers listed in Table 7. Primers were designed using Primer3plus (http://primer3plus.com/) based on sequences available from the Genbank database (https://www.ncbi.nlm.nih.gov/genbank/). Gene expression was quantified using a C1000 touch thermal cycler (CFX96 system, Bio-Rad Laboratories Inc.). For each primer pair, qPCR efficiencies were estimated through standard curves performed using serial dilutions (from 1/10 to 1/270) of a pool of cDNA. In the present study, qPCR efficiencies for each primer pair ranged from 95 to 100%, with $R^2 > 0.99$. Samples were then analyzed in triplicate.

Table 7: Specific primers used for quantitative PCR with Genbank accession numbers

Gene name (abbreviation)	Accession number	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)
28S ribosomal RNA (*28S*)	CBXY010007006	TTTCCCATGAGAGAGCAGGT	TCAGATGCGCTTCTTAGGATGT
Elongation factor 1 a (*ef1a*) *	AJ866727.1	GCTTCGAGGAAATCACCAAG	CAACCTTCCATCCCTTGAAC
Fatty acid synthase (*fas*)	MF566098.1	CCTCTGAACCTGGTCTGGTG	ATTGGAGAGAGCCTCCACGA
Delta-6-desaturase (*fads2*)	EU439924.1	AGCATCACGCTAAACCCAAC	CAAGCCAGATCCACCCAGTC
Peroxisome proliferator activated receptors alpha (*ppar α*)	AJ880087.1	ACCTCAGCGATCAGGTGACT	AACTTCGGCTCCATCATGTC
Peroxisome proliferator activated receptors beta (*ppar β*)	AJ880088.1	GCTCGGATCTGAAGACCTTG	TGGCTCCATACCAAACCACT
Peroxisome proliferator activated receptors gamma (*ppar γ*)	AY590303	CAGATCTGAGGGCTCTGTCC	CCTGGGTGGGTATCTGCTTA
Sterol regulatory element binding protein 1 (*srebp-1*)	FN677951	CTGGAGCCAAAACAGAGGAG	GACAGGAAGGAGGGAGGAAG

*Used as housekeeping gene

The final well volume was 15 µL, containing 5 µL cDNA (1/30 dilution) and 10 µL of reaction mix (0.5 µL of each primer [10 µM]), 1.5 µL RNase/DNase-free water, 7.5 µL iQ™ SYBR® Green Supermix (Bio-Rad Laboratories Inc.) containing antibody-mediated hot-started iTaq DNA polymerase, dNTPs, MgCl2, SYBR® Green I dye, fluorescein, stabilizers, and enhancers. In each plate, negative controls (RT negative controls) were systematically included to ensure the absence of residual genomic DNA contamination. Non-template controls (H_2O) were also analyzed. The qPCR thermal cycling contained an initial activation step at 95°C for 2 min, followed by 39 cycles of 5 s at 95°C and 20 s at 60°C. A melting curve was performed at the end of the amplification phase to confirm the amplification of a single product in each reaction.

The corresponding Cq (quantification cycle) value was determined automatically for each sample using the Gene Expression Module of CFX Manager software (Bio-Rad Laboratories Inc.). Cq is the number of cycles required to yield a detectable fluorescence signal. CFX Manager Software was used to normalize the relative quantity of messenger with the ΔΔCt method. The elongation factor 1 (*ef1a*) and ribosomal (28S) genes were tested to correct for loading differences or other sampling variations present in each sample. The *ef1a* gene was used as a reference gene in the liver tissue of juvenile fish since it did not show any significant variation of expression among experimental conditions ($P > 0.05$).

2.5.5 Statistical and data analysis

Relative gene expressions and enzyme activity data were log10 transformed to obtain normal distributions. The effects of time, temperature, and diet were tested for peroxidation index, relative genes expression (*pparα*, *pparβ*, *pparγ*, *fas*, *srebp-1*, *fads2*), enzyme activity (CAT, SOD, CS, COX), and MDA content using three-way ANOVAs. Normality and homoscedasticity of data were tested using Shapiro-Wilk and Levene tests, respectively. When appropriate, Tukey mean comparison tests were done because homoscedasticity was respected. Differences were considered significant at $\alpha = 0.05$. Statistical analyses were conducted in R (ver.3.3.3; R Development Core Team).

2.6 RESULTS

2.6.1 Liver mass and protein content

After 720 dd of feeding, liver mass was significantly higher (+48%) in juveniles reared at 20°C than at 15°C, and diet had no significant effect (Table 8, Figure 10A). At 1660 dd, the increase in liver mass was higher for juveniles reared at 20°C (+67%) than for those reared at 15°C (+59%; Table 8, Figure 10A). Again, no diet effect was detected (Figure 10A). Liver protein concentration decreased significantly over time (0.12 ± 0.01 mg proteins mg liver^{-1} at 720 dd vs. 0.10 ± 0.00 mg proteins mg liver^{-1} at 1660 dd; Table 8), and no significant effect of diet or of temperature was observed.

Table 8: Effect of diet, temperature, and time on liver lipid metabolism (N = 6) and oxidative stress (N = 3)

	Three-way ANOVA			
	Diet	Temperature	Time	Interactions
Liver mass	–	–	–	Diet × Time × Temperature: P < 0.05
Liver protein content	–	–	P < 0.001 *1660dd < 720dd*	–
	Lipid metabolism			
fads2	–	–	–	Diet × Temperature × Time: P < 0.05
fas	–	P < 0.001 *15°C < 20°C*	P < 0.001 *720dd < 1660dd*	–
pparα	–	–	–	Temperature × Time: P < 0.01
pparβ	–	–	–	–
pparγ	–	P < 0.05 *15°C < 20°C*	P < 0.001 *720dd < 1660dd*	–
srebp-1	–	P < 0.05 *15°C < 20°C*	P < 0.001 *720dd < 1660dd*	–
	Oxidative metabolism and antioxidant response*			
CAT	–	–	–	Diet × Time × Temperature: P < 0.01
SOD	P < 0.05 *RD < LD*	P < 0.01 *15°C < 20°C*	P < 0.01 *1660dd < 720dd*	–
CS	–	–	–	–
COX	–	–	–	Time × Temperature: P < 0.01 Diet × Time: P < 0.001
COX / CS	P < 0.05 *RD < LD*	–	–	Time × Temperature: P < 0.001
MDA	–	–	P < 0.05 *720dd < 1660dd*	–
PI	P < 0.01 *LD < RD*	–	–	Time × Temperature: P < 0.05

*: per mg prot (specific enzyme activity)

Italics indicate the presence of significant differences, RD: reference n-3 polyunsaturated fatty acid (PUFA) diet, LD: low n-3 PUFA, dd: degree-days.

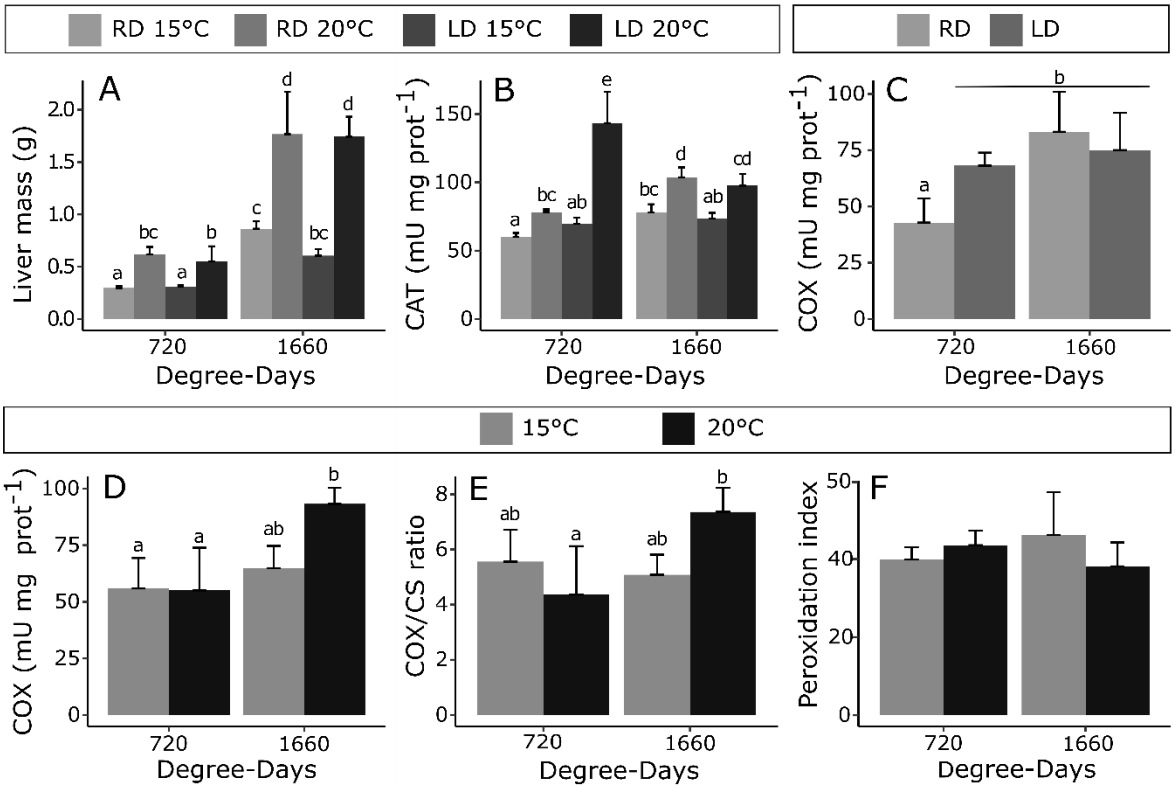


Figure 10: Effect of diet, time (in degree-days [dd]), and temperature on A) liver mass and B) specific activity of catalase (CAT). Results of two-way ANOVAs: effect of diet and time on C) specific activity of cytochrome c oxidase (COX). Results of two-way ANOVAs: effect of temperature and time on D) specific COX activity, E) COX/citrate synthase (CS) ratio, and f) peroxidation index. Significantly different groups were assigned different letters following Tukey post-hoc tests ($\alpha = 0.05$). Values are means ± standard deviations, N = 3.

2.6.2 Peroxidation index and MDA

The PI of the RD diet was 34% higher than the LD diet (15.86 ± 1.78 vs. 10.52 ± 0.50). Accordingly, PI in the polar lipids of juveniles fed RD was significantly higher (+18%) compared to fish fed LD (45.36 ± 6.96 vs. 38.50 ± 5.45; Table 8). The temperature × diet interaction was significant (Table 8), while no difference was detected with the post-hoc test (Figure 10F). Surprisingly, MDA content was not affected by diet or temperature (Table 8). However, MDA content increased with time (2.06 ± 0.48 vs. 2.77 ± 0.45 nmol mg protein^{-1}; Table 8).

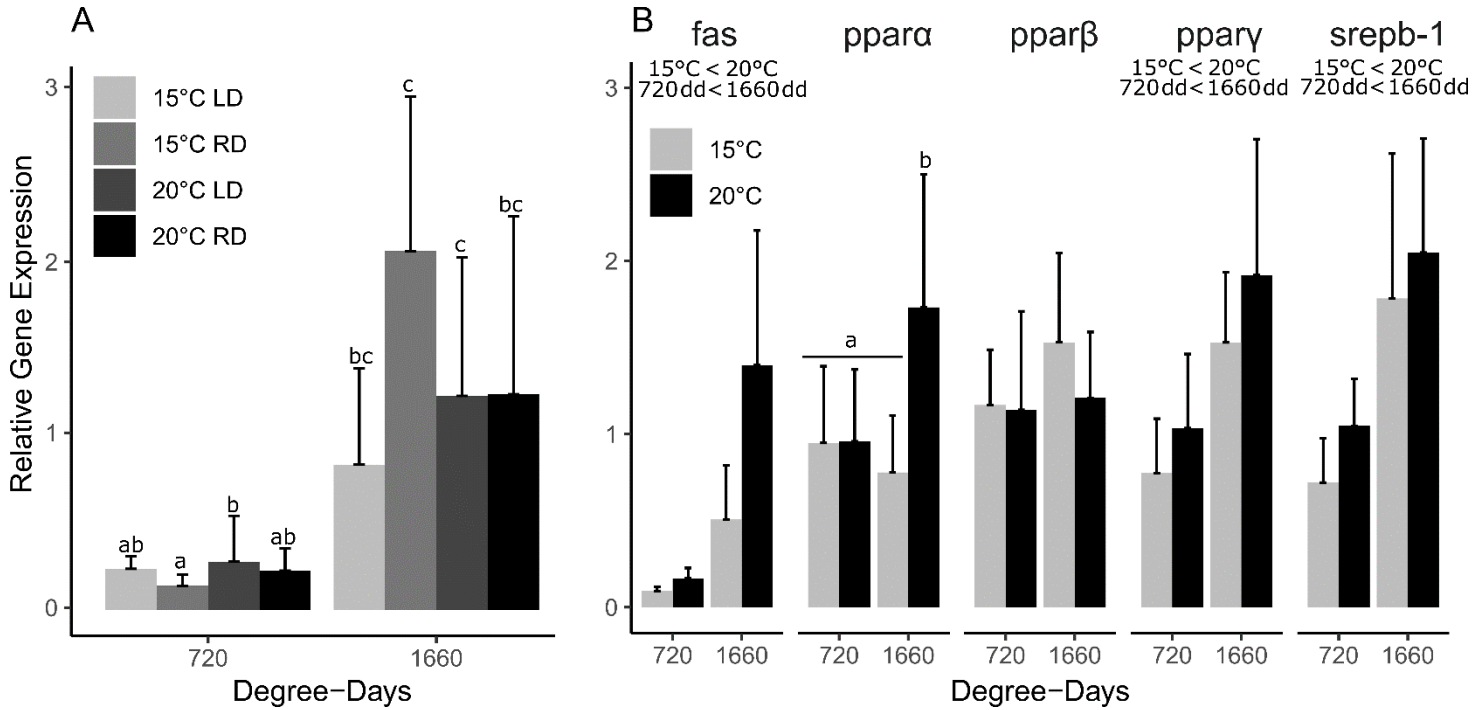
2.6.3 Oxidative metabolism and antioxidant response

CS activity remained stable regardless of the experimental treatment, while COX activity varied depending on diet × temperature and degree-days × temperature interactions (Table 8, Figure 10C, D). There was no temperature effect on COX activity at 720 dd, while fish raised at 20°C exhibited a higher COX activity at 1660 dd than fish reared at 15°C (Table 8, Figure 10D). The COX activity was significantly lower in fish fed RD at 720 dd while the diet effect was no longer present at 1660 dd (Table 8, Figure 10C). The COX/CS ratio was higher (+15%) in juveniles fed LD than in those fed RD (6.01 ± 1.21 vs. 5.09 ± 1.80; Table 8). In addition, the COX/CS ratio remained stable over time for fish raised at 15°C, while it increased at 20°C (Figure 10E, Table 8). CAT activity increased slightly but significantly over time both at 15°C and 20°C (70 ± 7.93 vs. 105 ± 27.01 mU mg proteins^{-1}; Table 8, Figure 10B). At 20°C, however, juveniles fed the RD diet displayed CAT activity that was more than twice the activity measured in LD-fed fish. At 1660 dd, the diet effect was no longer present. The SOD activity was significantly lower (8%) in juveniles fed RD compared to those fed LD (3.94 ± 0.36 U mg proteins^{-1} vs. 4.25 ± 0.43 U mg proteins^{-1}, Table 8). SOD activity significantly decreased (–8%) with time (4.25 ± 0.31 U mg proteins^{-1} at 1660 dd vs. 3.95 ± 0.47 U mg proteins^{-1} 720 dd; Table 8). There was also a significant increase in SOD activity (+10%) with the rise in temperature (3.91 ± 0.43 U mg proteins^{-1} at 15 °C vs. 4.29 ± 0.33 U mg proteins^{-1} at 20°C; Table 8).

2.6.4 Relative gene expression

At 720 dd, the relative expression of *fads2* was highest at 20°C in juveniles fed RD, while it was lowest at 15°C in juveniles fed LD (Table 8, Figure 11A). After 1660 dd of feeding, there was no significant increase of *fads2* in juveniles fed RD at 15°C or LD at 20°C; however, the expression was approximately 1.5 times higher in juveniles fed LD at

Figure 11: Effect of diet, temperature and time on lipid metabolism. A) Interaction between diet, time (in degree-days [dd]), and temperature for *fads2*. B) Effect of time (dd) and temperature on relative gene expression for *fas*, *pparα*, *pparβ*, *pparγ*, and *srebp-1*. When factor interactions were significant, groups were compared with Tukey post-hoc tests ($\alpha = 0.05$); significantly different groups were assigned different letters. Values are means ± standard deviations, N = 6.



15°C and RD at 20°C (Table 8, Figure 11A). The expression of other genes showed no difference according to diet. Expressions of *fas*, *pparγ*, and *srebp-1* were significantly lower (158%, 29%, and 24%, respectively) for fish raised at 15°C than at 20°C (Table 8, Figure 11B). These expressions were respectively 630%, 91%, and 118% lower at 720 dd than at 1660 dd (Table 8, Figure 11B). The highest relative gene expression for *pparα* was observed at 1600 dd for fish raised at 20°C (Table 8, Figure 11B), while no treatment effect was observed for the expression of *pparβ* (Table 8).

2.7 DISCUSSION

In a previous study, we showed that the depletion of n-3 PUFA and a low temperature (15°C) contributed to a decreased growth rate in sea bass: the depleted n-3 PUFA diet resulted in lower contents of essential fatty acids (EFA; ARA, DHA, EPA) in muscle even though a higher retention of those FA was noted in fish fed this diet (Gourtay et al. 2018). The aim of the present work was to examine to what extent these diets and temperature can affect lipid metabolism and antioxidant response in liver. Diet had no effect on lipid metabolism, but development and temperature effects were observed: mRNA levels for *pparα*, *pparγ*, *fas*, *fads2*, and *srebp-1* increased over time, and we showed—for the first time—that temperature affects mRNA levels of *pparα*, *pparγ*, *fas*, and srepb-1. Surprisingly, however, diet marginally increased the expression of *fads2* in the LD treatment. No clear pattern of oxidative stress response occurred in relation to diet based on the specific activity of the studied enzymes. MDA, which is an indicator of lipid peroxidation, remained stable while the activity of antioxidant enzymes increased with higher temperature conditions.

2.7.1 Lipid metabolism

Because the n-3 PUFA-depleted diet induces lower growth and low EFA muscle content despite a higher retention of EFA (Gourtay et al. 2018), modifications within the lipid bioconversion pathway were expected relative to the diet. Indeed, it has been reported

that a nutritional regulation of several actors of this pathway (notably of *fads2*) occurred in several fish species, some of which were strictly marine (for review see Vagner and Santigosa 2011). Considering the greater EFA muscle content in LD juveniles, we hypothesized that the biosynthesis pathway was activated in LD juveniles based on the greater EFA muscle content. This hypothesis was not verified at the molecular level because no effect of diet on *fas*, *pparα*, *pparβ*, *pparγ*, or *srebp-1* expression was observed, suggesting that no regulation of these transcriptional factors occurred at the dietary PUFA levels tested here. Comparable conclusions were drawn by Yilmaz et al. (2016) in juvenile sea bass and by Tocher et al. (2006) in cod. However, our results contrast with those obtained on sea bass by Vagner et al. (2007a, b, 2009), who showed that *fads2* activity was higher in larvae fed the low PUFA diet (0.5% or 0.7% EPA + DHA, % DM) compared to those fed a high PUFA diet (1.7% or 3.7% EPA + DHA, % DM). González-Rovira et al. (2009) also showed a significantly higher *fads2* mRNA level in the liver of sea bass fed with linseed and rapeseed oils compared to those fed fish oil, while it remained stable in individuals fed a diet based on olive oil. Because sea bass studies were based on different dietary formulations and different developmental stages, it is difficult to pinpoint why our results would differ from theirs. As mentioned above, previous studies reporting modulation of the elongation and desaturation pathways used diets that were highly deficient in EPA + DHA content (Vagner et al. 2007a, b, 2009); in consequence, one may hypothesize that it possibly exists a nutritional supply threshold in PUFA above which the activation of these pathways does not occur.

The activities of *fas*, *pparγ*, and *srebp-1* increased over time and with temperature. The *fads2* expression tended to increase over time, but not significantly due to the high inter-individual variability within experimental treatments at 1660 dd. This may indicate changes in expression of the lipid regulation pathway with age or with temperature conditions. Generally, and within the limits of thermal tolerance of ectotherm species, increasing temperature accelerates all biochemical processes, including growth performance. Indeed, a significantly higher growth was observed in juveniles fed the same experimental diets at 20°C compared to 15°C (Gourtay et al. 2018). It then seems

reasonable to speculate that a higher growth rate should be accompanied by a higher metabolic demand, especially of FA, leading to an increase in the expression of involved genes. Despite a temperature-driven stimulation of the PUFA bioconversion pathway, only polar DHA and ARA contents were higher at 720 dd for fish reared at 20°C compared to those reared at 15°C, and at 1660 dd only the neutral DHA content was higher at 20°C (Gourtay et al. 2018). Temperature may simply accelerate a process occurring through time and resulting to a rise in polar lipids followed by a rise in the neutral fraction. Obviously, the results showed more than just a general metabolic increase with temperature rise since a selective effect of the different fatty acids is also present. However, we do not have the data to investigate further on this aspect.

2.7.2 Aerobic metabolism and oxidative stress response

There is growing evidence that inclusion of high levels of PUFA in fish diet, due to their high degree of unsaturation, can induce lipid oxidative damage (Álvarez et al. 1998; Luo et al. 2012). The RD-fed juveniles had the highest PI, which is coherent with the PUFA content in the two diets. However, MDA content remained stable regardless of the diet fish were fed, which does not match an enhanced level of lipid peroxidation. In addition, the antioxidant enzyme activities also did not vary according to diet except for SOD, which was higher in juveniles fed LD than in those fed RD. This seems to indicate that the PI did not provide a good evaluation of the need to regulate ROS via the diet. PI is calculated with *in vitro* oxidation rate values (Holman 1954, Cosgrove et al. 1987, Hulbert et al. 2007). It does not take into account all the *in vivo* processes that occur to deal with ROS production. It seems that MDA was a better indicator of lipid peroxidation.

Increased environmental temperature has been associated with oxidative stress in fish (Parihar and Dubey 1995, Heise et al. 2006a, b, Lushchak and Bagnyukova 2006, Bagnyukova et al. 2006, 2007). Vinagre et al. (2012) studied the impact of temperature on oxidative stress in sea bass juveniles. They found that lipid peroxidation and catalase activity were very sensitive to environmental temperature. Accordingly, the SOD activity

was higher at 20°C than at 15°C, and the highest CAT activity was observed at 720 dd in fish fed LD at 20°C. In several organisms including fish, it has been shown that COX and CS activities are adjusted to maximum aerobic metabolism capacity (Childress and Somero 1979, Thuesen and Childress 1993, 1994). Elevated temperature stimulates metabolic processes in ectotherms, increasing ATP demand and, therefore, electron transfers along the mitochondrial respiratory chain, possibly explaining the highest COX activity and COX/CS ratio recorded at 20°C. Such circumstances are also likely to enhance ROS production as a side product, resulting in oxidative stress. It should be noted that we analyzed the activity of specific enzymes, and that liver mass increased with time and temperature. With the increase in liver mass, total enzyme activity (per whole liver) should be higher and thus enable fish to cope with oxidant stress. The absence of change with temperature in the MDA content, which is one of the products of lipid peroxidation (Frankel 2005), again did not match the original hypothesis. Knowing that the effects of ROS are dose dependent (Gechev et al. 2006, Quan et al. 2008), the ROS production in our study probably remained under the trigger point of antioxidant defense.

2.8 CONCLUSIONS

Dietary depletion PUFA did not affect hepatic transcriptional factors (except *fads2*) involved in the regulation of metabolic pathways related to FA bioconversion at the molecular level. This suggests that the n-3 PUFA level in juvenile sea bass cannot be modified through an increased capacity of liver fatty acid bioconversion. Despite the high peroxidation index in fish fed RD, very few modifications in the oxidative stress response were associated with diet, suggesting that PI did not represent a status perceived as stressful from a physiological point of view. Temperature affected the fatty acid bioconversion capacity, possibly through metabolic processes already present in fish. However, we were not able to match this increase with specific outcomes regarding the biosynthesis pathway. Temperature also increased the enzymatic antioxidant response, but there was no correlation with the PI index or MDA products.

CHAPITRE 3
EFFETS DES ACIDES GRAS NUTRITIONELS SUR LA PLASTICITE DE VITESSE DE NAGE CRITIQUE DE JUVENILES DE BAR EUROPEEN (*DICENTRARCHUS LABRAX*)

Ce troisième article, intitulé « *Effects of dietary fatty acids on the plasticity of critical swimming speed of European sea bass (*Dicentrarchus labrax*) juveniles* » est actuellement en cours d'échange avec les co-auteurs.

Tous les co-auteurs ont participé au processus de rédaction selon le processus décrit dans le premier chapitre. Pour cet article les expériences de nage ont été réalisées par Clémence Gourtay sous la supervision de Guy Claireaux, les prélèvements sanguins ont été réalisés par Clémence Gourtay en collaboration avec Cassandre Aimon, Nicolas Le Bayon et Guy Claireaux.

Différents éléments de cet article ont été présentés au congrès de la Société canadienne de zoologie (Terre-Neuve, Canada, mai 2018).

3.1 RESUME

Dans le contexte des changements globaux, comprendre comment les organismes réagiront aux nouvelles conditions environnementales est d'une grande importance. Le but de cette étude était d'établir de quelle manière les acides gras et la température pouvaient affecter la plasticité phénotypique chez le bar juvénile (*Dicentrarchus labrax*). Les poissons ont été élevés à deux températures (15 et 20° C) et ont été nourris pendant cinq mois avec des régimes isoénergétiques et isoprotéiques dont le profil en acides gras diffère: un régime de type marin (AL++: 1,65% d'acides gras polyinsaturés [AGLPI] n-3 sur une base de matière sèche [MS]) et un régime alimentaire de type estuarien (AL: 0,73% d'AGLPI n-3 de MS). Nous avons émis l'hypothèse que 4 phénotypes différents avaient été créés suite à l'élevage, et que leur plasticité et leur abilités régulatoires pourraient être distinguées avec un protocole de vitesse de nage critique (Ucrit). Les capacités homéostasiques de ces phénotypes ont été testées en exposant les juvéniles à plusieurs combinaisons d'oxygène (de 100% à 40% de saturation en oxygène) et de salinités (S = 30 à 55) contraignantes sur le plan physiologique. Les résultats ont montré que les poissons nourris avec AL présentaient de meilleures performances que ceux nourris avec AL++ et que l'hypersalinité était plus contraignante que l'hypoxie. Les juvéniles élevés à 20°C avaient une plasticité intra-phénotypique plus importante, alors que la plasticité inter-phénotypes était clairement affectée par les régimes alimentaires. De manière surprenante, lactate, glucose et osmolarité plasmatique ont peu contribué à expliquer les différences d'Ucrit observées. Cette étude révèle l'importance du régime alimentaire et de la température comme facteurs environnementaux capables de générer une diversité phénotypique significative, avec des impacts potentiels sur les traits d'histoire de vie des espèces.

Mots clés : plasticité, Ucrit, salinité, hypoxie, lactate, glucose, osmolarité

3.2 EFFECTS OF DIETARY FATTY ACIDS ON THE PLASTICITY OF CRITICAL SWIMMING SPEED OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*) JUVENILES

Authors:

Clémence Gourtay[1,2], Denis Chabot[3], Céline Audet[1], José-Luis Zambonino-Infante[2], Guy Claireaux[4].

Institutions:

[1]Institut des Sciences de la Mer de Rimouski, Université du Québec à Rimouski, 310 des Ursulines, Rimouski, QC, G5L 3A1, Canada

[2] Institut Français de Recherche pour l'Exploitation de la Mer, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

[3]Institut Maurice-Lamontagne, Pêches et Océans Canada, C.P. 1000, Mont-Joli, QC, G5H 3Z4, Canada

[4]Université de Bretagne Occidentale, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

3.3 ABSTRACT

In the context of global changes, understanding how organisms will respond to new environmental conditions is of great importance. The aim of this study was to establish how fatty acids and temperature may affect phenotypic plasticity in juvenile sea bass (Dicentrarchus labrax). Fish were raised at two temperatures (15°C, 20°C) and fed for five months with isoenergetic and isoproteic diets that differed in their fatty acid profiles: a sea-profile diet (SP: 1.65% n-3 polyunsaturated fatty acid [PUFA] on a dry matter basis [DM]) and an inland-profile diet (IP: 0.73% n-3 PUFA on DM). We hypothesized that four different phenotypes would be produced, and that their plasticity and integrated regulatory abilities could be distinguished with a critical swimming speed (Ucrit) protocol. The homeostatic ability of these phenotypes was tested by acutely exposing juveniles to combinations of oxygen (ranging from 100 to 40% air saturation) and salinity levels (S = 30 to 55). The results showed that IP-fed fish performed better than SP fish, and that hypersalinity was more constraining than hypoxia. Fish reared at 20°C had higher intra-phenotype plasticity while inter-phenotype plasticity was clearly affected by dietary regimes. Surprisingly, lactate, glucose, and plasma osmolality contributed little to explaining differences in Ucrit results. This study reveals the importance of dietary regimes and temperature as crucial environmental factors that may generate significant phenotypic diversity, with potential impacts on the life history traits of species.

Key words : plasticity, Ucrit, salinity, hypoxia, lactate, glucose, osmolality

3.4 INTRODUCTION

Swimming capacity is of high ecological relevance in fishes (e.g., predator avoidance, prey capture, distribution, and migration), and it is considered a determining factor of individual fitness in numerous species (Videler 1993, Reidy et al. 2000, Armsworth 2001, Johnston et al. 2001, Plaut 2001, Fisher and Wilson 2004, Green and Fisher 2004, Claireaux et al. 2006). Because it combines the performance of a number of physiological processes and functions (e.g., ventilation, respiration, oxygen transport, muscular work, and general homeostasis), swimming ability has been used as an integrated index of physiological performance and health (Nelson 1989, Randall and Brauner 1991, Plaut 2001, Mauduit et al. 2016, Zhang et al. 2016).

Individuals are developmentally, functionally, and phenotypically complex units (Olson and Miller 1958, Schlichting and Pigliucci 1998, Pigliucci and Preston 2004, Valladares et al. 2007, Piersma and van Gils 2011). Depending on environmental conditions, a single genotype may become one of many possible phenotypes, which is called phenotypic plasticity. This has long been recognized as a key strategy enabling organisms to respond to varying environments (Bradshaw 1965). In the context of global changes, understanding the adaptation potential of organisms to their environment is crucial, and studying the effects of environment on swimming performance becomes a key tool to reach that goal.

One environmental factor that is emerging as a significant source of phenotypic plasticity in fish is diet quality, and particularly the relative intake and subsequent tissue accumulation of certain fatty acids (FA; McKenzie 2001, Tocher 2003, Chatelier et al. 2006). Dietary FA are either stored as neutral triacylglycerol (TAG) or inserted into membranes as polar phosphoglycerides (Sargent et al. 1999, Tocher 2003). More than 85% of fish energy reserves are stored as TAG, mainly because lipids can yield more ATP per gram than any other fuel source (Weber 2011). Fish make predominant use of these large reserves to support swimming (Magnoni et al. 2006, Richards et al. 2002a). However, fats

are scarce in nature and their quality can be highly variable since considerable fluctuations in FA composition of natural food sources occur in both space and time. Consequently, available dietary FA can heavily influence the nature of FA being stored by consumers (Ayre and Hulbert 1996, Huang et al. 2005, Pierce and McWilliams 2005), with consequences on disease resistance, aging, general health, and physiological performance (Pond 1998, Hulbert et al. 2005, Simopoulos 2007, Weber 2009, Valencak and Azzu 2014).

Only a few studies have examined the effects of dietary lipids on maximum swimming speed (Ucrit). McKenzie et al. (1998) found that salmon fed a diet rich in n-3 polyunsaturated FA (PUFA) (29.5% on a total lipids basis) had a significantly lower Ucrit than those fed a diet enriched with canola oil, and therefore poorer in n-3 PUFA. These authors also found a negative correlation between dietary n-3 PUFA content and Ucrit, and a positive correlation between muscle n-3/n-6 ratio and Ucrit. In seabass (*Dicentrarchus labrax*), swimming performance was found to be correlated with a high level of oleic acid (OA) and linoleic acid (LA) in muscle tissues (Chatelier et al., 2006), and in the golden grey mullet (*L. aurata*), increased swimming efficiency was observed when fish were fed a diet poor in n-3 PUFA (0.2% on a dry matter [DM] basis) (Vagner et al., 2014). On the contrary, Arctic charr (*Salvelinus alpinus*) fed a diet in which 75% of the fish oil was replaced by vegetable oil had lower swimming performance than individuals fed with fish oil (Petterson et al. 2010). A similar pattern was observed by Wagner et al. (2004) in Atlantic salmon (*Salmo salar*): maximum swimming speed (Ucrit) of salmon was higher in fish fed a diet rich in polyunsaturated FA of the n-3 series (n-3 PUFA, 0.2%), namely eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. On the contrary, swimming performance was lower in fish fed a diet with low n-3 PUFA / saturated FA (SFA) and n-3 PUFA/ arachidonic acid ratios. These contrasting results reveal the importance of better understanding the effects of n-3 PUFA dietary content on the swimming performance of fish.

The European sea bass (*D. labrax*), a marine species that spends most of its life in coastal areas and estuaries, is commercially important along European coasts. Coastal

environments are characterized by high environmental variability, which in turn induces variability in phytoplankton assemblages and the biochemical composition of phytoplankton, and thus directly affects FA quality and availability patterns (Pohl and Zurheide 1979). It has been shown that the fatty acid composition of muscle in European sea bass is altered by temperature and diet (Chatelier et al. 2006, Skalli et al. 2006, Gourtay et al. 2018).

The aim of the present study was to establish how the FA composition of food can affect phenotypic plasticity in juvenile sea bass. Using a crossed factorial design combining two dietary regimes and two temperatures, four phenotypes were constructed and their integrated regulatory ability was compared using a Ucrit protocol. The homeostatic abilities of the phenotypes were tested by acute exposition of juveniles to combinations of oxygen (ranging from 100 to 40% air saturation; air sat. hereafter) and salinity levels (S = 30 to 55). Acclimation temperature (15°C versus 20°C) was used as a co-factor. Two sets of experiments were conducted. In the first set, fish swimming capacity (Ucrit) was determined; in the second set, blood samples were collected at predetermined times during the trials and plasma glucose, lactate, and osmolality were examined in relation with the homeostatic abilities of phenotypes. The experimental diets had similar compositions except that one contained 1.65% of EPA+DHA (sea profile, SP) while the other contained only 0.73% (inland profile, IP) on a DM basis.

3.5 MATERIALS AND METHODS

3.5.1 Fish acclimation and diet

Details on fish origin, rearing, and nutritional conditioning are given in Gourtay et al. (2018). Briefly, sea bass juveniles (mean mass ± SD: 0.75 ± 0.02 g; standard length: 3.57 ± 0.02 cm) were assigned to one of 12 rearing tanks supplied with natural seawater (500 L; > 95% air sat.; n = 300 per tank). Fish were fed one of two experimental diets: SP (EPA+DHA = 1.65% DM) and IP (EPA+ DHA = 0.73% DM), and were raised at either

15°C or 20°C. Each diet × temperature combination was tested in triplicate tanks for a total of 12 experimental tanks. The two diets were isoenergetic and contained the same amounts of proteins and lipids (Gourtay et al., 2018). The IP diet contained only colza oil as an FA source (essentially OA, 18:1n-9; LA, 18:2n-6; and linolenic acid, 18:3n-3), while the SP diet contained half colza oil and half fish oil, the latter being richer in EPA (20:5n-3) and DHA (22:6n-3).

3.5.2 Muscle fatty acid composition

To assess muscle fatty acid profiles, eight fish per tank were randomly sampled at the end of the feeding experiment (day 176 and 204 post hatching at 20°C and 15°C, respectively, 1660 degree days at both temperatures). They were euthanized with an overdose of MS-222 (200 mg L^{-1}), and a piece of white epaxial muscle located under the first dorsal fin was dissected out. Muscle samples obtained from the same tank were pooled and stored at −80°C until fatty acid analyses (n = 3 per experimental treatment). FA were expressed as percentage of DM. The diet effect on FA content was assessed with a Student T-test at each temperature since the Ucrit swimming protocol differed for fish held at the two temperatures (Table 9).

Table 9: Effects of diet (sea profile [SP] or inland profile [IP]) on muscle fatty acid (FA) profiles of experimental groups prior to challenges.

Neutral lipids						
	15°C			20°C		
	SP	IP	T-test	SP	IP	T-test
14:0s	0.43 ± 0.05	0.52 ± 0.08	–	0.79 ± 0.01	0.55 ± 0.04	P < 0.01
16:0s	3.02 ± 0.34	4.13 ± 0.69	–	4.85 ± 0.02	5.07 ± 0.23	–
18:0s	0.64 ± 0.06	0.95 ± 0.13	–	1.02 ± 0.03	1.18 ± 0.07	–
SFA	4.28 ± 0.47	5.9 ± 0.98	–	6.96 ± 0.02	6.83 ± 0.66	–
16:1n9	0.65 ± 0.08	0.73 ± 0.09	–	1.01 ± 0.03	0.7 ± 0.04	P < 0.01
18:1n7	0.63 ± 0.07	0.94 ± 0.23	–	0.89 ± 0.05	1.03 ± 0.05	–
18:1n9	7.84 ± 0.81	14.41 ± 4.01	–	11.41 ± 0.46	16.99 ± 0.73	P < 0.01
20:1n9	0.63 ± 0.06	1.01 ± 0.27	–	0.84 ± 0.06	1.12 ± 0.06	P < 0.05
MUFA	9.95 ± 1.00	17.7 ± 4.78	–	14.41 ± 0.6	19.29 ± 1.66	–
18:2n6	2.77 ± 0.27	5.38 ± 1.58	–	3.97 ± 0.31	5.99 ± 0.29	P < 0.01
18:3n3	0.91 ± 0.09	1.87 ± 0.58	–	1.44 ± 0.04	2.18 ± 0.10	P < 0.01
20:4n6	0.07 ± 0.01	0.04 ± 0.01	–	0.11 ± 0	0.04 ± 0.00	P < 0.001
20:5n3	0.68 ± 0.08	0.42 ± 0.12	–	1.14 ± 0.05	0.48 ± 0.03	P < 0.001
22:6n3	1.06 ± 0.11	0.63 ± 0.14	–	1.34 ± 0.05	1.13 ± 0.09	–
PUFA	5.86 ± 0.59	8.81 ± 2.52	–	8.5 ± 0.39	9.82 ± 0.90	–
n-3/n-6	0.97 ± 0.01	0.57 ± 0.01	–	1.01 ± 0.06	0.66 ± 0.01	–

Values are given as percent of dry matter in the neutral lipids at 15°C and 20°C. Values are means ± s.e., N=3. Only significant results are reported.

3.5.3 Set 1: Critical swimming speed

Swimming tests were conducted after 160 days of acclimation. Because fish reared at 15°C and 20°C differed significantly in body size (Gourtay et al. 2018), the swimming protocol used for these two groups was slightly different in terms of the rate at which water velocity was increased during the first 10 min of the swimming challenge tests. As a result, direct comparison between temperature treatments was not possible.

Swimming performance was assessed using a 30 L swimming tunnel (Loligo system, Tjele, Denmark). The section of the tunnel where fish were held was 55 cm × 14 cm × 14 cm (length, width, height). The section was placed in an outer tank that was supplied with temperature- (cooling/heating system, Tr10, TECO, Italy) and oxygen-controlled seawater. The oxygen level was regulated manually by bubbling nitrogen or air in a water tank situated upstream of the swim tunnel. Water oxygen level was monitored

continuously in the swimming tunnel using an oxymeter (Firesting, PyroScience, Germany). Water salinity was measured using a salinometer (WTW LF 325, Bioblock Scientific, Germany) and adjusted using aquarium marine sea salt (Tetra).

To establish the influence of dietary conditioning on the integrated performance of the four phenotypes, fish critical swimming speed (Ucrit) was measured in six combinations of dissolved oxygen (DO) and salinity conditions. Three DO levels (100, 60, and 40% air sat.) were combined with two salinity levels (30 and 55). For each DO × salinity combination, two groups of 15 fish were tested. These fish were selected at random from the rearing tank and were starved 24 h before the swimming trials. Fish were transferred from the rearing tank to the swim tunnel without emersion, and water temperature in the tunnel was identical to that in the rearing tank. Following transfer, fish were allowed to habituate at the minimum speed of 7 cm s^{-1} for 1 h when exposed to oxygen depletion and for 2 h when exposed to the salinity treatments. Following the habituation period, water velocity in the swim chamber was gradually increased (over 10 min) to 4.5 body lengths (BL) s^{-1}, i.e., 25 cm s^{-1}, at 15°C (fish BL: 6.16 ± 0.03 cm) and to 6 BL s^{-1}, i.e., 47 cm s^{-1}, at 20°C (8.04 ± 0.04 cm). At that time, water velocity was incrementally increased by 2.78 cm s^{-1} every 5 min until fish were exhausted. When exhausted, fish were removed from the swim tunnel, measured (SL), weighed, and returned to their rearing tank. Critical swimming speed was calculated following the equation of Brett (1964; eq.5):

$$Ucrit = \frac{u_i + (\frac{t}{t_i} u_{ii})}{SL} \qquad \text{eq.5}$$

Ucrit is the critical swimming speed (body lengths s^{-1}),
ui is the highest velocity at which the fish swam the during the time period (cm s^{-1}),
uii is the incremental speed increase (cm s^{-1}),
t is the time spent swimming at the final velocity increment (s),
ti is the time interval (s), and
SL is the standard length (cm).

3.5.4 Set 2: Plasma glucose, lactate and osmolality

Another set of swimming challenge tests was performed using 20°C-acclimated fish to reveal the metabolic basis of the homeostasis ability of two phenotypes. Two environmental conditions were tested, i.e., a moderate hypoxic condition (60% air sat.) combined with normal salinity (30) and a moderate hypersaline condition (45) in normoxia (100% air sat.).

The same habituation and swimming protocol as described above were followed. Four samplings were done (nine fish per sampling): the first sampling took place directly in the rearing tank (T0) while the next samplings occurred in the swimming chamber at mid-habituation (T1), at the end of habituation (T2), and after the swimming challenge (T3). The swimming protocol was not interrupted until the sampling time, so that means that the swimming protocol was repeated for each sampling time with nine different fish each time.

To collect blood samples, fish were removed from the swimming chamber and anaesthetized with MS-222 (200 mg L^{-1}). Blood was drawn via the caudal vein using heparinized syringes. Four operators performed the operation simultaneously and blood samplings were completed within 7 min. Blood samples were pooled (three pools of three fish each), centrifuged (1000 g, 4 min, 4°C), and the plasma was stored at –80°C until assayed. Osmolarity was measured with a Milliosmol Osmometer (Roebling). Lactate and glucose were respectively measured with a colorimetric L-lactate assay kit (13815 AmpliteTM, AAT Bioquest, USA) and a colorimetric glucose quantitation kit (40004 AmpliteTM, AAT Bioquest, USA).

3.5.5 Statistical and data analysis

Data normality and homoscedasticity were tested using Shapiro-Wilk and Levene tests, respectively. Three-way ANOVAs were performed to assess significant differences among diet, oxygen, and salinity for Ucrit independently at 15°C and 20°C. Glucose, lactate, and osmolality were analyzed with two-way ANOVAs to test for significant

differences between diets and times in fish raised at 20°C for each scenario (moderate hypoxia and moderate hypersaline condition). In order to meet normality, Ucrit and lactate concentration data were log10 transformed. When appropriate, Tukey pairwise comparison tests were done since the assumption of homoscedasticity was met. Differences were considered significant at $\alpha = 0.05$. Statistical analyses were conducted in R (ver. 3.3.3; R Development Core Team).

3.6 RESULTS

3.6.1 Effects of dietary FA, DO, and salinity on Ucrit

At 20°C, diet as well as water oxygen and salinity levels significantly influenced the swimming ability of phenotypes (Ucrit) independently (interactions between factors were not significant, $P > 0.05$). No significant interaction between these factors was found. Under normoxia (100% air sat.) and normal salinity (30), IP-fed fish had 8% higher Ucrit than those fed the SP diet ($F_{[1,348]} = 40.79$, $P < 0.001$; Figure 12A). With depletion of oxygen, Ucrit decreased significantly in all groups ($F_{[1,348]} = 104.04$ $P < 0.001$, Figure 12A). On average, compared to normoxia, Ucrit decreased by about 10% and 22% at 60% and 40% air sat., respectively (Figure 12B). When swimming under normal salinity, fish displayed 13% higher Ucrit than when swimming under hypersaline conditions ($F_{[1, 348]} = 98.44$, $P < 0.001$).

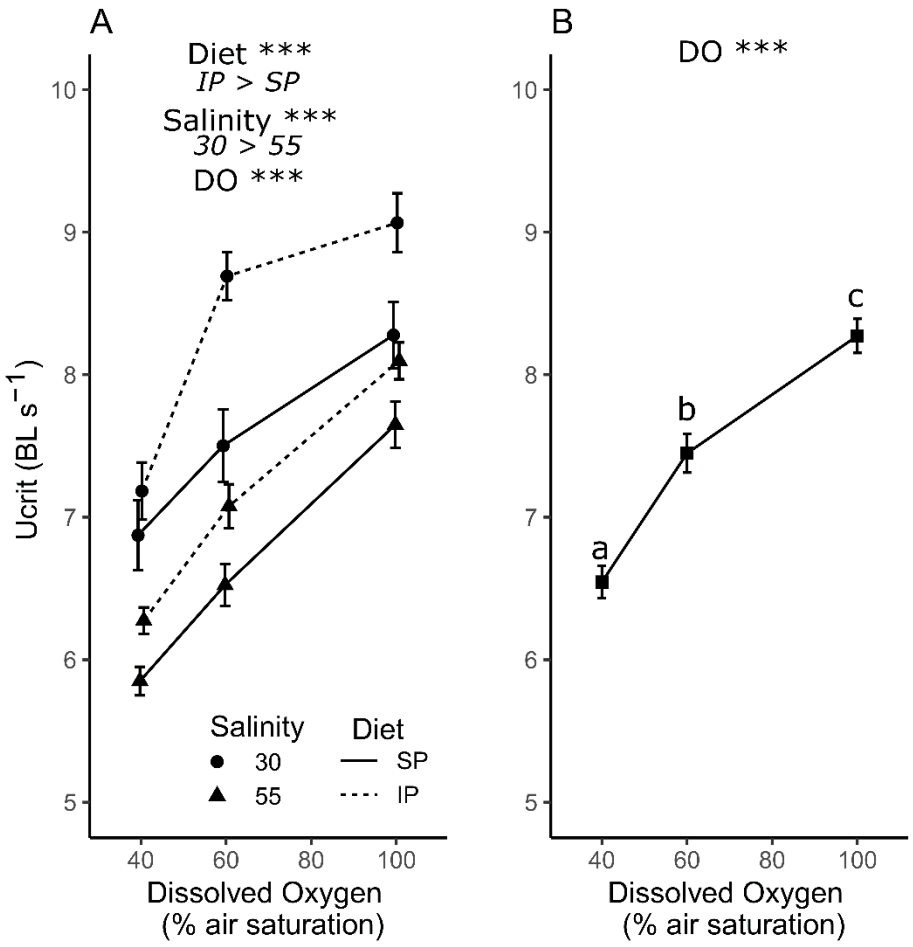


Figure 12: A) Diet (IP: inland profile diet, SP: sea profile diet), salinity and dissolved oxygen (DO) effects on critical swimming speed (Ucrit) in body length (BL) s^{-1} for fish raised at 20°C. B) Oxygen effect on mean Ucrit. Different letters indicate significant differences among means (Tukey test, $\alpha = 0.05$).*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. N = 30. Values are means ± standard errors.

The same pattern was observed in 15°C-acclimated fish. Ucrit was again significantly higher (8%) in IP-fed than in SP-fed fish ($F_{[1,347]} = 59.15$, $P < 0.001$, Figure 13A) while there was a significant interaction between oxygen and salinity ($F_{[2,347]} = 5.22$, $P < 0.01$; Figure 13B). In normoxia, salinity had no significant effect on Ucrit. At S = 55, Ucrit decreased significantly, by 8%, in the presence of mild hypoxia (60% air sat.), and by a further 12% at 40% air sat. At S = 30, Ucrit was only significantly different from normoxia at 40% air sat. (–11%).

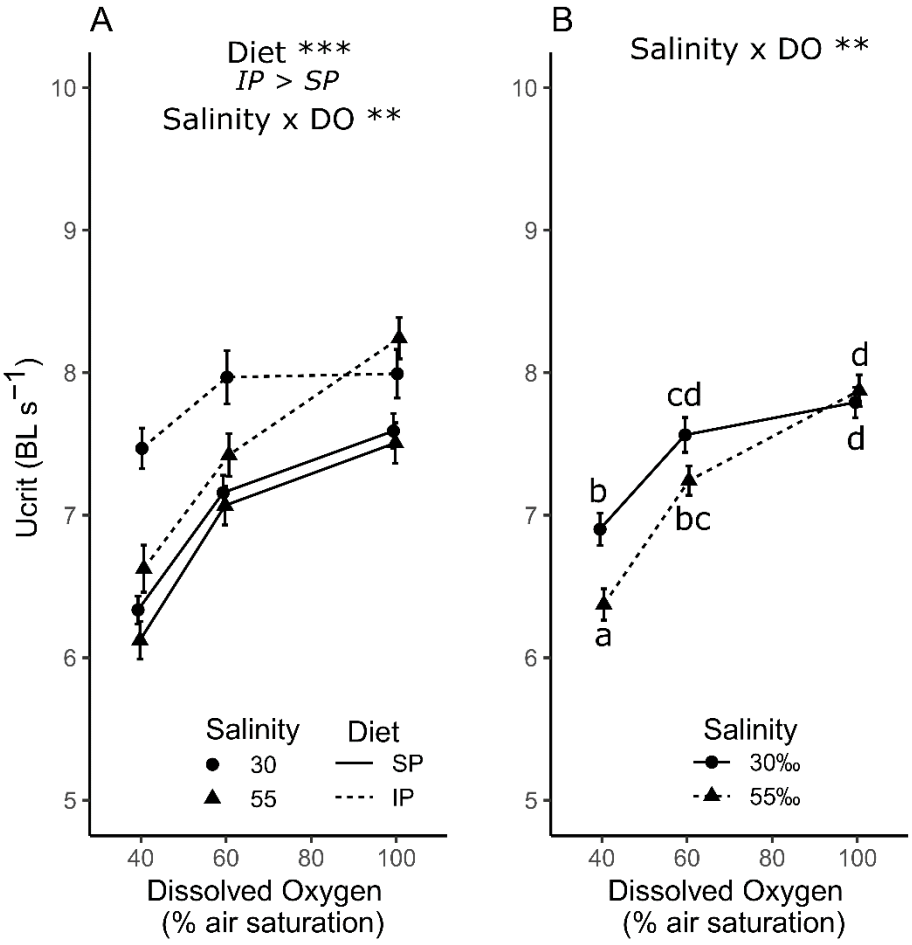


Figure 13: A) Diet (IP: inland profile diet, SP: sea profile diet), salinity and dissolved oxygen (DO) effects on critical swimming speed (Ucrit) in body length (BL) s^{-1} for fish raised at 15°C. B) Interaction between dissolved oxygen (DO) and salinity effect on mean Ucrit. Different letters indicate significant differences among means (Tukey test, $\alpha = 0.05$). *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. N = 30. Values are means ± standard errors.

3.6.2 Plasma glucose, lactate, and osmolality under moderate hypoxia and hypersaline conditions

In the moderate hypoxia scenario, no significant change in plasma glucose was observed during the acclimation period or swimming trials (Figure 14A, upper). However, a significant increase in plasma lactate ($F_{[3,16]} = 1.54$, $P < 0.05$, Figure 14A, middle) and osmolality ($F_{[3,16]} = 3.99$, $P < 0.05$, Figure 14A, lower) compared to the control (measured

in tank) was observed 30 min after fish had been introduced into the mild hypoxic swimming chamber. This difference was no longer significant half an hour later. Fish diet had no significant effect on these responses. No significant interaction between time and diet was present ($P > 0.05$).

In the moderate hypersaline scenario, plasma glucose did not change significantly during the acclimation and swimming trials (Figure 14B, upper). Lactate significantly increased ($F_{[3,16]} = 3.90$, $P < 0.05$; Figure 14B, middle) after 1 h in the swimming tunnel while osmolality reached a peak after 2 h ($F_{[3,16]} = 7.43$, $P < 0.05$, Figure 14B, lower). Fish diet had no significant effect on the glucose or lactate responses. However, SP-fed fish had a higher plasma osmolality (+3%) than the IP-fed fish ($F_{[3,16]} = 5.06$, $P < 0.05$, Figure 14B, lower). At the end of swimming, the osmolality of IP-fed fish was similar to that of fish in the rearing tank, while the osmolality of SP-fed fish was 8% higher than in IP-fed fish. Again, the observed diet and time effects were independent since no significant interaction was present ($P > 0.05$).

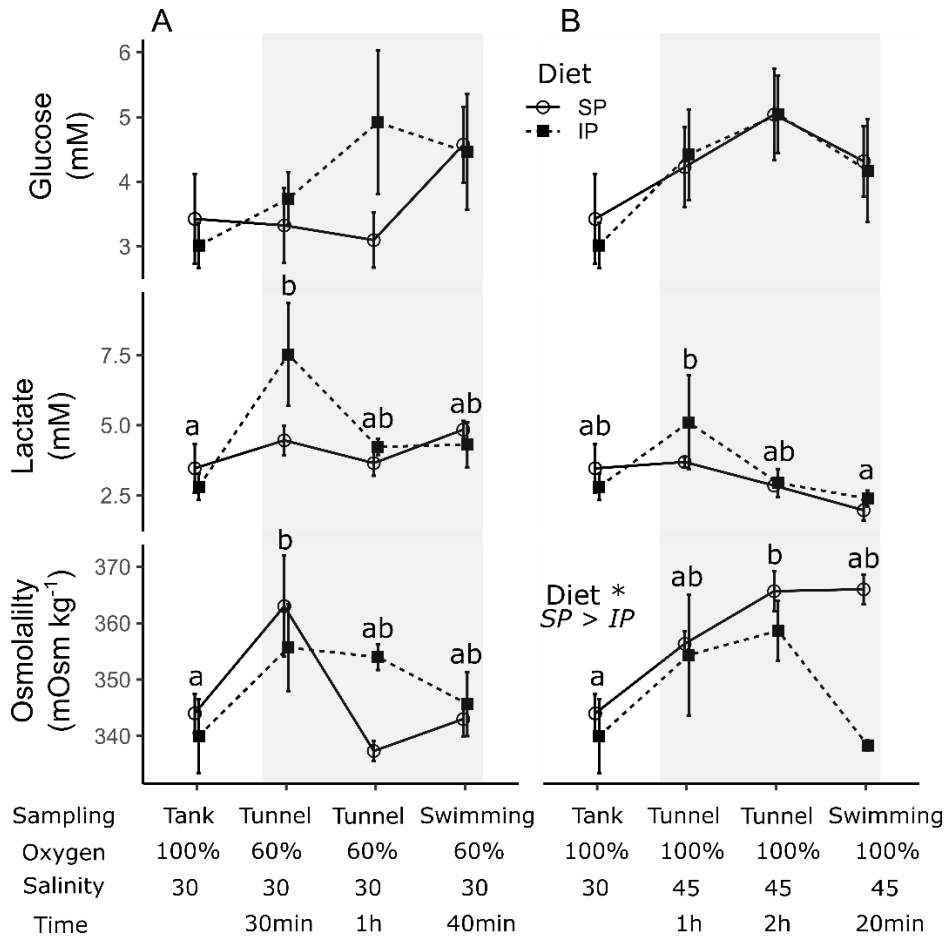


Figure 14: Diet effect on plasma glucose, lactate, and osmolality in A) the moderate hypoxia scenario and B) the moderate hypersaline scenario. When the time effect was significant, groups were compared with a posteriori tests ($\alpha = 0.05$); significantly different groups were assigned different letters. When there is one letter for two points, the letter applies to the average of those points. In the shaded areas, fish were in the swimming chamber. Values are means ± s.e., N=3.

3.7 DISCUSSION

We used swimming capacity as an integrated gauge of homeostatic condition, to establish how dietary FA and temperature acclimation may affect the physiological performance of European sea bass faced with different combinations of oxygen and salinity conditions. In a first set of experiments, we showed that fish fed an inland-like diet (IP; low n-3 PUFA) displayed higher swimming capacity (Ucrit) than fish fed a sea-like diet (SP;

high n-3 PUFA). This difference was observed at both experimental temperatures. Although hypoxia and hypersaline conditions tended to reduce fish swimming performance, this diet effect remained discernable. In the second set of swimming trials, we found that diet did not affect plasma glucose or lactate responses. However, SP-fed fish exhibited a higher osmolality under hypersaline condition.

3.7.1 Swimming plasticity

In fish, lipid oxidation is the primary fuel used to power locomotion (Richards et al. 2002b). Aerobic activities are primarily fueled by neutral FA released by lipolysis through mitochondrial β-oxidation (Richards et al. 2002a, Tocher 2003, Magnoni et al. 2006, Frayn 2010). It has been reported in several species that the rate of FA oxidation decreased from 12-carbon to 22-carbon molecules (Hryb and Hogg 1979, Neat et al. 1981, Bronfman et al. 1979), including fish (Henderson and Sargent 1985). Dietary regimes induced clear phenotype divergences, significantly affecting the swimming capacity of sea bass, with IP-fed fish having a better swimming capacity than SP-fed fish. Since the muscle of IP-fed fish contained higher levels of oleic acid, one could expect that this fatty acid would be selectively β-oxidized in vivo since in vitro experiments showed that oleic and linoleic acids are preferred substrates for β-oxidation over other FA, especially PUFA (Sidell and Driedzic 1985, Henderson and Sargent 1985, Egginton 1996, Henderson 1996). Higher levels of these preferred substrates in the tissues might allow the animal to achieve higher rates of aerobic work (McKenzie 2001, Vagner et al. 2014).

Different phenotypes induced by dietary regimes were observed at both acclimation temperatures with higher Ucrit in SP-fed fish compared to IP-ones; however, a stronger effect on Ucrit was observed in fish reared at 20°C than at 15°C. This corroborates the presence of a diet effect seen in the muscle FA profile of 20°C-reared fish whereas no diet effect was seen in 15°C-reared fish. Larger differences in neutral 18-carbon FA content were observed in fish reared at 20°C independent of their diet, indicating that the metabolic substrate was high enough to sustain the energy demand. It has been shown in sea bass that

improved swimming performance was accompanied by a higher maximum cardiac output and net cardiac scope as well as a higher active metabolic rate and aerobic scope (Chatelier et al. 2006). IP-fed juveniles raised at 20°C exhibited a higher heart mass than the SP-fed fish (Gourtay et al. 2018). Assuming that a larger heart provides greater cardiac output, this could partially explain why these fish had higher Ucrit. It is important to note that in 15°C-acclimated fish, no difference in heart mass was observed between the two diets (Gourtay et al. 2018). Yet, IP-fed fish performed better than SP-fed ones, suggesting that cardiac mass alone does not explain the enhanced swimming performance of IP-fed fish.

Acclimation to both temperature and dietary regimes induced phenotypic responses. To find out if these phenotypes have the same capacity, Ucrit was tested under oxygen and salinity conditions. As expected, Ucrit decreased with the depletion of oxygen saturation, regardless of diet or salinity. This result is consistent with the limiting oxygen level curve theory (Neill and Bryan 1991, Neill et al. 1994), which describes the gradual limitation imposed by progressive oxygen depletion upon the capacity of a fish for aerobic activities. In experimental conditions, several studies showed that hypoxic conditions resulted in a significant decrease in aerobic scope, i.e., the active metabolic rate that is correlated to a lower critical swimming speed for several fish species (Fitzgibbon et al. 2007, Petersen and Gamperl 2010, Pang et al. 2015). Hypersalinity was used as an additional constraint on aerobic scope to bring juvenile sea bass to their physiological limits, even if the conditions tested were not ecologically relevant. When facing changes in environmental conditions, fish make regulatory adjustments allowing them to perform better under the new conditions. The two levels of salinity tested here did succeed in decreasing swimming performance in normoxia in 20°C-aclimated fish, while a significant difference was seen only from 60% air sat. in 15°C-acclimated fish. It has been shown that the Ucrit of several species decreased in hyperosmotic conditions (Kolok and Sharkey 1997, Randall and Brauner 1991). In this case, the increase in acclimation temperature made fish more sensitive to salinity.

When salinity levels were combined with DO levels, our results suggested that the diet effect was still present even in the most constraining case. When fish swam in hypersaline conditions and at the lowest DO level, the IP-fed fish performed better than SP-fed fish; however, the Ucrit pattern observed was modulated by the temperature acclimation. In 20°C-aclimated fish at normal salinity, Ucrit was higher than their counterparts performing in the hypersaline condition. The advantage of the IP diet observed in normoxia was still present at 60% air sat., but Ucrit decreased and was similar for both diets at 40% air sat., indicating that the advantage associated with FA profile was not sufficient to account for higher resistance in these conditions. However, when these fish swam in the hypersaline condition, the difference between SP and IP diets was similar among DO levels. In 15°C-aclimated fish, differences in Ucrit between fish swimming at normal salinity vs. the hypersaline condition was less evident, with the salinity effect appearing significant from 60% air sat. Nevertheless, the diet effect was present at each salinity treatment and among DO levels. These results suggest that such hypersaline conditions combined with hypoxia was more deleterious to the aerobic scope of juvenile sea bass acclimated at 20°C than at 15°C.

3.7.2 Intra and inter-phenotype plasticity

The violin plot representations enable a more specific visualization of how environmental conditions may affect intra- and inter-phenotypic plasticity. Regarding intra-phenotypic plasticity, i.e., the range between good and poor swimmers in phenotypes, our results indicate that 20°C-acclimated fish at S = 30 exhibited higher intra-phenotypic plasticity than 15°C-acclimated fish (Figure 15A, B). In the hypersaline condition, this higher intra-specific plasticity was clearly reduced for 20°C-acclimated fish, dropping to near that of the 15°C-acclimated fish. However, DO levels did not seem to have any effect on intra-phenotypic plasticity. Concerning inter-phenotypic plasticity, the SP-phenotypes performed better than IP-phenotypes at S = 30, although this advantage was lost in the hypersaline condition. These results confirm that there is an added energetic cost for hypersaline conditions, and that the cost appears high relative to the energetic demands of

swimming since it affects both intra-phenotypic and inter-phenotypic plasticity in 20°C-acclimated fish. The SP-phenotype advantage was lost in the hypersaline condition.

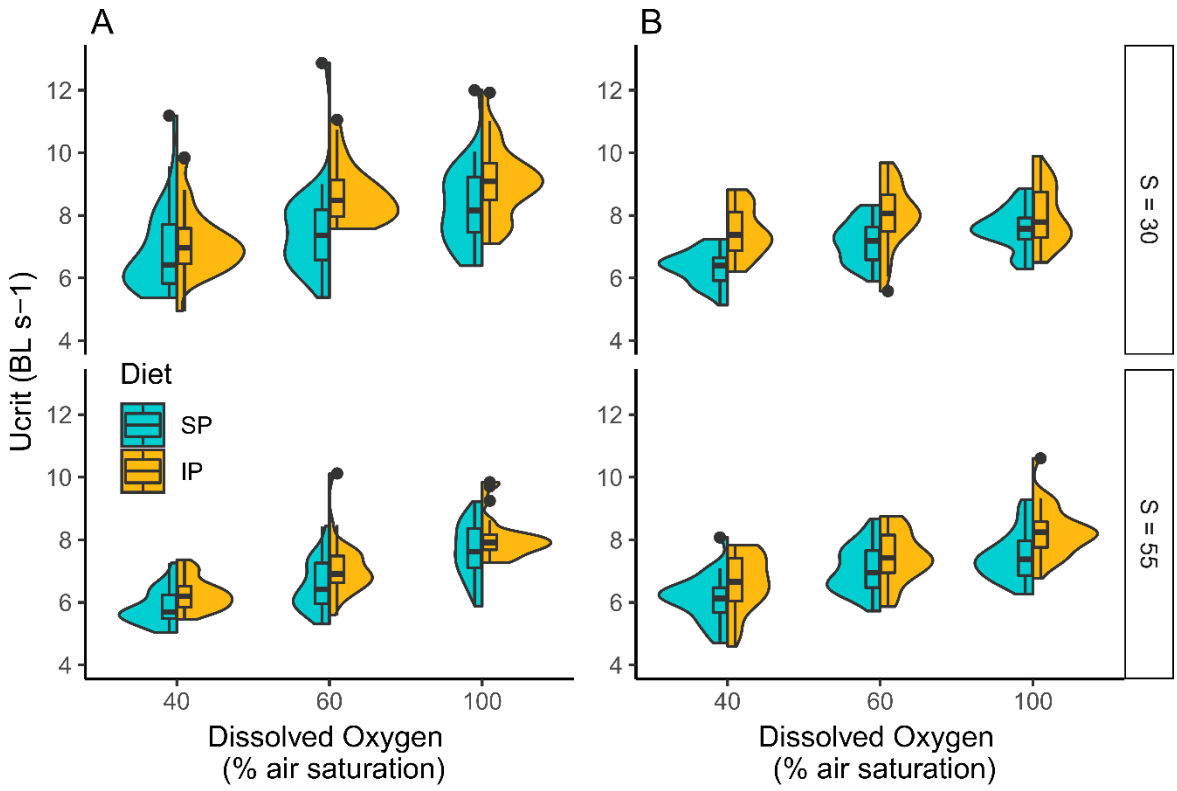


Figure 15: Box plots and violin plots of the distribution of critical swimming speed (Ucrit; body lengths [BL] s^{-1}) at salinities of 30 or 55 in fish raised at A) 20°C or B) 15°C. The box plots show the median (solid horizontal bands), the first through the third interquartile range (open vertical bands), the outlier (solid dots), and the density estimator (coloured vertical curves) of Ucrit in each group. N=30.

3.7.3 Plasma glucose, lactate, and osmolality

Globally, the plasma indicators that were measured in this study were not affected by diet. Only a time effect was observed most of the time, with a similar pattern in the moderate hypoxia and moderate hypersaline scenarios. However, in the moderate hypersaline scenario, diet significantly affected fish osmolality: IP-fed fish were able to maintain a lower osmolality than the SP ones. At the end of the trial, IP-fed juveniles were back to normal osmolality levels, while a plateau was maintained in the SP fish.

Considering that plasma osmolality is a good secondary stress indicator (Mugnier et al. 1998, Lowe and Davison 2005, Vargas-Chacoff et al. 2009), these results indicate a higher level of stress in SP-fed juveniles.

3.8 CONCLUSIONS

Different temperature and feeding regimes allowed us to produce four different phenotypes of juvenile European sea bass that respond to environmental constraints in different ways. The IP-fed fish characterized by higher contents in oleic and linoleic acids had better swimming capacity compared to those fed SP when submitted to hypersaline and hypoxia constraints. The plasma indicators investigated in this study showed that these factors were not responsible for the observed responses. The fatty acid profile of the diet was found to have significant effects on the ability of sea bass juveniles to cope with environmental modifications, but further study is needed to try to find the mechanism explaining this plasticity.

CHAPITRE 4
REPONSE SAISONNIERE A LA SALINITE ET AUX ACIDES GRAS NUTRITIONNELS CHEZ LES JUVENILES DE BAR RAYE (*MORONE SAXATILIS*)

Ce quatrième article, intitulé « *Seasonal response to salinity and nutritional fatty acids in juvenile striped bass (*Morone saxatilis*) »* est actuellement en discussion avec les co-auteurs.

La station piscicole de Baldwin (Coaticook, Canada) a fourni les juvéniles de bar rayé. L'élevage a été réalisé à la station aquicole de Pointe-au-Père (Rimouski, Canada) avec le soutien technique de Nathalie Morin (Université du Québec à Rimouski, UQAR), sous la supervision de Céline Audet. Les analyses biométriques et les échantillonnages ont été effectués par Clémence Gourtay avec l'aide des permanents, vacataires et stagiaires : Céline Audet, Denis Chabot, Stéphanie Coté, Renée Gagné et Emeline Romelus.

Tous les co-auteurs ont participé au processus de rédaction selon le processus décrit dans le premier chapitre. Pour cet article, la préparation des échantillons pour l'analyse des lipides a été réalisée par Clémence Gourtay en collaboration avec Jean-Bruno Nadalini. L'analyse des chromatogrammes et leur intégration été menée par Clémence Gourtay en étroite collaboration avec Mathieu Babin sous la supervision de Réjean Tremblay. Les statistiques réalisées sous PRIMER ont été menées par Réjean Tremblay.

4.1 RESUME

Les zones etuariennes sont des nourriceries productives pour un grand nombre d'espèces, elles offrent un refuge contre les prédateurs, une disponibilité alimentaire élevée et des conditions favorables à la survie, au développement et à la croissance des stades précoces. Depuis 2002, le gouvernement du Québec a lancé un important programme de réintroduction visant à rétablir les bars rayés disparus dans le fleuve Saint-Laurent. Afin de relever les défis associés aux efforts de réintroduction, une meilleure connaissance de la biologie de cette espèce est nécessaire. Nous avons testé l'hypothèse selon laquelle la variabilité de la salinité et l'origine des apports alimentaires (source terrestre ou marine) auraient une incidence majeure sur la physiologie des juvéniles de bar rayé. Les juvéniles ont été élevés en conditions hivernales à deux salinités (salinité [S] = 0 et 14) et nourris avec un aliment de référence (AL, 0.73% an acides gras à longue chaîne polyinsaturé [AGLPI] sur base de la matière sèche [MS]) utilisé comme proxy d'un réseau trophique dulcicole et un aliment plus riche en n-3 AGLPI (AL++, 1.65% n-3 AGLPI MS) proxy d'un réseau trophique marin. Les résultats montrent que les effets de la salinité étaient moins prononcés comparés à ceux des régimes alimentaires sur les profils en acides gras (AG) totaux du foie des lipides neutres du muscle, alors que les AG polaires du muscle étaient faiblement affectés. Nous proposons un nouvel indice — l'indice nutritionnel environnemental (INE) — pour estimer l'impact de conditions environnementales spécifiques sur les besoins en AG, indépendamment des régimes alimentaires utilisés. L'utilisation de cet indice a révélé que les besoins en AG essentiels (acides eicosapentaénoique et docosahexaénoique) étaient plus importants en eau douce. Globallement, les résultats suggèrent que l'eau douce représentait une condition stressante pour les juvéniles de bar rayés, cela a été corroboré par une valeur plus élevée d'hématocrite et une survie plus faible.

Mots clés: salinité, survie, acides gras, allométrie des organes, l'indice nutritionnel environnemental

4.2 SEASONAL RESPONSE TO SALINITY AND NUTRITIONAL FATTY ACIDS IN JUVENILE STRIPED BASS (*MORONE SAXATILIS*)

Authors:

Clémence Gourtay[1,2], Denis Chabot[3], Réjean Tremblay[1], José-Luis Zambonino-Infante[2], Guy Claireaux[4], Céline Audet[1].

Institutions:

[1]Institut des Sciences de la Mer de Rimouski, Université du Québec à Rimouski, 310 des Ursulines, Rimouski, QC, G5L 3A1, Canada

[2]Institut Français de Recherche pour l'Exploitation de la Mer, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

[3]Institut Maurice-Lamontagne, Pêches et Océans Canada, C.P. 1000, Mont-Joli, QC, G5H 3Z4, Canada

[4]Université de Bretagne Occidentale, LEMAR (UMR6539), Centre Ifremer de Bretagne, 29280 Plouzané, France

4.3 ABSTRACT

Estuarine zones are productive nursery grounds for a large number of species; they provide refuge from predators, high food availability, and conditions favourable for

enhanced survival, development, and growth of early stages. Since 2002, the Québec government has undertaken a major reintroduction program to re-establish striped bass, which have disappeared from the St. Lawrence River. In order to meet the challenges posed by restocking efforts, a better knowledge of the biology of this species is needed. We tested the hypothesis that variable salinity and food source (terrestrial vs marine source) would markedly impact the physiology of striped bass juveniles. Juveniles were raised in winter conditions at two salinities (salinity [S] = 0 and 14) and fed with a reference diet (RD; 0.73% n-3 polyunsaturated fatty acid [PUFA] on a dry matter [DM] basis) used as a proxy of an estuarine trophic network, and a lower n-3 PUFA diet (MD; 1.65% n-3 PUFA DM) used as a proxy of a marine trophic network. Results showed that salinity effects were less pronounced compared to those of dietary regimes on total liver and muscle neutral fatty acid (FA) profiles, while muscle polar FA were slightly affected. We proposed a new index—the environmental nutritional index (ENI)—to estimate the impact of specific environmental conditions on specific FA requirements regardless of the dietary regime used. The use of this index highlighted that nutritional needs in essential FA (eicosapentanoic and docosahexaenoic acid, EPA and DHA respectively) were higher when fish were in fresh water (FW). These results suggested that FW represented a stressful condition for juvenile striped bass, and this was corroborated by the higher hematocrit value and the lower survival observed in FW.

Key words : salinity, survival, fatty acids, organ allometry, environmental nutritional index

4.4 INTRODUCTION

The striped bass (*Morone saxatilis*, Walbaum 1792) is an anadromous fish typical of estuaries and shorelines of the east coast of North America (Scott and Scott 1988), with a natural range from the St. Lawrence Estuary (Québec, Canada) to the St. Johns River in northern Florida. In the past, there were three distinct populations of striped bass off the east coast of Canada: Bay of Fundy, the southern Gulf of St Lawrence, and the upper estuary of the St. Lawrence River between Sorel and Kamouraska (COSEPAC 2012). Due to habitat alterations and heavy exploitation by sport and commercial fishing, the upper estuary population was extirpated in the late 1960s (Beaulieu 1985, Beaulieu et al. 1990). Starting in 2002, the Québec government launched a major reintroduction program aiming to re-establish striped bass in this area. Breeders originating from the Southern Gulf of St. Lawrence population (Miramichi River, New Brunswick, Canada) were used, considering the proximity and the nordicity of this population, with the hope of eventually obtaining a population in the St. Lawrence River that will perpetuate itself in a sustainable fashion (Comité aviseur sur la réintroduction du bar rayé 2001).

Little is known about these three populations and more generally about this species in North America. Most of the research efforts were made in the 1970s to 1990s, when a growing interest for the aquaculture of reciprocal hybrids between striped bass (*M. saxatilis*) and white bass (*M. chrysops*), such as the American bass, also known as the palmetto bass (♀ *saxatilis* × ♂ *chrysops*) or the sunshine bass (♀*chrysops* × ♂ *saxatilis*; Hodson 1990). These hybrids showed better growth than native species (Smith et al. 1985), which explains the large amount of scientific literature related to their production. However, ecological and physiological studies on striped bass remain scarce.

To meet the challenges associated with reintroduction efforts, better knowledge of the biology of this species is required. The survival of juvenile fish strongly depends on the biotic and abiotic characteristics of their habitats (Cushing 1995, Sogard 1992, 1997, Juanes 2007, Houde, 2008). It is known that estuarine zones are productive nursery grounds

for a large number of species because they provide refuge from predators together with high food availability, which enhances the survival, development, and growth of early stages (Beck et al. 2001, Able et al. 2013). Since the beginning of these reintroduction efforts, the Rivière du Sud and the harbour of Québec City were identified as breeding sites (Valiquette et al. 2018). Young-of-the-year (YOY) fish were found between Lévis and Rivière-du-Loup on the south shore of the St. Lawrence system, and between Neuville and Petite-Rivière-Saint-François on the North Shore (all locations in Québec, Canada) (Valiquette et al. 2018). During the first years of their life, striped bass juveniles are therefore exposed to a wide range of salinity (0–15) and trophic sources. The possible physiological consequences of such natural changes in salinity for striped bass juveniles have not yet been studied. Furthermore, there is no information on the specific nutritional needs, especially lipid requirements, to cope with these environments and to allow growth and survival (see Sargent et al. 2002 for a review, Tocher et al. 2010). Thus, we tested the hypothesis that variable salinity and food source (terrestrial vs marine source) strongly impact the physiology of striped bass juveniles. Nutritional needs, notably in lipids, are crucial for growth and survival (see Sargent et al. 2002 for a review, Tocher et al. 2010). Dietary lipids determine the lipid composition of cell membranes, which are critical for the structure and function of cells and tissues. Thus the availability of dietary lipids can have marked effects at different biological/ecological levels (Arts et al. 2009, Parrish 2013). The aim of this study was therefore to examine the effects of two dietary regimes characterized by different fatty acid (FA) contents on survival, growth, organ allometry, hematocrit level, lipid accumulation, and membrane FA profile in striped bass exposed to two salinities (0 and 14).

4.5 MATERIALS AND METHODS

4.5.1 Fish

Adult striped bass were captured in the Miramichi River (New Brunswick, Canada) and brought to the Baldwin-Coaticook Fish Station (Coaticook, Québec, Canada). The F2

generation was produced in this facility: 11 females were crossed with 33 males. At day 105 post-hatching (September), striped bass juveniles were transferred to the ISMER rearing facilities in Rimouski (Québec, Canada), where experiments were conducted. Juveniles were divided among four tanks (230 L, initial density 4 kg $tank^{-1}$) filled with brackish water ([BW], i.e., fresh water [FW] mixed with filtered St. Lawrence water, S = 5, temperature 15°C) for stress and disease prevention. After a 10-day period, salinity was lowered by 1 per day until S = 0. Fish were then split among four tanks (70 L, 192 fish per freshwater tank). Water temperature and photoperiod followed natural seasonal variations (September to February) at this latitude and were monitored daily throughout the trial.

4.5.2 Feeding regimes

The two diets tested were identical except for the FA source. A marine diet (MD) contained 50% colza oil and 50% fish oil, the latter being richer in eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids, whereas a diet low in essential FA (RD) contained only colza oil as a source of FA (essentially oleic acid [18:1n-9], linoleic acid [18:2n-6], and linolenic acid [18:3n-3]). The two diets were isoenergetic and contained the same percentages of proteins and lipids (Table 10).

Table 10: Composition of experimental diets

	RD Mean	MD Mean
	% of dry mass	
Dry matter	94.84	95.12
Proteins	50.48	50.23
Total lipids	21.98	21.63
Triglycerides	16.99	17.05
Phospholipids	4.70	4.71
	% of total lipids in diet	
SFA	2.18	2.97
MUFA	7.32	6.48
n-3	1.93	2.68
n-6	2.65	2.28
n-9	6.56	5.46
EPA+DHA	0.73	1.65
18:1n-9	5.69	4.65
18:2n-6	2.50	2.16
18:3n-3	0.97	0.77
18:3n-6	0.00	0.01
18:4n-3	0.08	0.14
20:4n-6 (ARA)	0.03	0.07
20:5n-3 (EPA)	0.28	0.94
22:5n-3	0.03	0.07
22:6n-3 (DHA)	0.45	0.71

For dry matter, proteins, total lipids, triglycerides, and phospholipids, data are presented as percent of dry mass. Data for specific fatty acid (FA) categories are presented as percent of total lipids. RD: reference n-3 polyunsaturated fatty acid (PUFA) diet, MD: marine n-3 PUFA diet; SFA: saturated FA; MUFA: monounsaturated FA; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Upon their arrival at the ISMER rearing facilities, juveniles were fed with Nutra XP (Skretting) for two days and then with a mixture of Nutra XP and the low n-3 PUFA experimental diet (RD; EPA+ DHA = 0.73% DM) for the next week. From that time on, juveniles were exclusively fed with the RD diet for 10 days. This diet was chosen as a reference diet because it is more representative of a freshwater food source. At the end of this 10-day period, two of the four tanks were switched to the high DHA and EPA diet (MD; EPA+DHA = 1.65% DM) while the other two were maintained on the RD diet. Fish

were fed ad libitum each morning (manual distribution in three meals), survival was monitored, and dead fish were removed.

4.5.3 Salinity acclimation

After two weeks of diet acclimation, fish from each tank were randomly divided among two replicate tanks (70 L). There were four FW tanks and the other four tanks were progressively supplied with increasing proportions of estuarine salt water to increase the salinity by 2 per day, up to S = 14 (each diet × salinity combination was duplicated). The initial density varied from 68 to 72 fish $tank^{-1}$, respectively, for RD and MD. The average initial body mass (BM) was similar among treatments (BM = 5.54 ± 019 g). However, there was a small difference in initial standard length (SL) between the two salinity treatments ($F_{[1,4]}$ = 8.30, P < 0.05; FW: 69.70 ± 0.86 mm; BW: 65.73 ± 1.01 mm), even though juveniles were randomly distributed among the experimental tanks. The experiment lasted 102 days, including 20 days of acclimation. Water temperature followed natural variations (Table 11).

Table 11: Timeline analysis summary

Experimental day	Date	Temperature	Analysis
Day 0	16 October 2017	FW: 13°C BW: 13°C	Biometry
Day 25	10 November 2017	FW: 11°C BW: 10°C	Hematocrit HSI
Day 56	11 December 2017	FW: 8°C BW: 7°C	Biometry
Day 102	26 January 2018	FW: 5°C BW: 4°C	Biometry Hematocrit HSI Fatty acids Organ allometry

FW: fresh water, BW: brackish water, HSI: hepato-somatic index.

4.5.4 Biometry

Growth rate was determined with three growth measurements done on day 0 (N = 30 for all conditions), day 56 (N = 60 for all conditions, except for RD-fed fish N = 58), and day 102 (N = 57 for FW fish and N = 60 for BW fish; Table 11). Fish were not fed 24 h prior to sampling. Fish were randomly sampled in each tank (about half of the total sample size per combination of salinity and diet), lightly anaesthetized with tricaïne methanesulfonate (MS-222; dose adapted to BM and temperature), weighed, and measured (SL). After recovery, individuals were returned to their original tanks. The mass gain / loss (Δmass, eq.6), length increment (Δlength, eq.7), and the Fulton index (eq.8) were calculated as follows:

$$\Delta mass\ (g) = BM_{final} - BM_{initial} \quad \text{eq.6}$$

$$\Delta length\ (mm) = SL_{final} - SL_{initial} \quad \text{eq.7}$$

$$Fulton\ index = \frac{BM}{SL^3} \quad \text{eq.8}$$

4.5.5 Samplings

At days 25 and 102 (Table 11), eight fish per tank were randomly sampled, euthanized with an overdose of MS-222, and a blood sample taken. Hematocrit (red blood cell volume/total serum) was determined after centrifugation (Fisher Scientific, 3000 g, 3 min). The liver was also dissected out for hepato-somatic index (HSI, eq.9) determination as follows:

$$Hepato - somatic\ index = 100 \times Liver\ Mass \times BM^{-1} \quad \text{eq.9}$$

At the end of the experiment (day 102; Table 11), four fish per tank were randomly sampled, euthanized with an overdose of MS-222, and a piece of white epaxial muscle located under the first dorsal fin was dissected out and stored at −80°C until analyses (N = 8 per experimental treatment). Fifteen additional fish per tank (30 per diet−salinity

combination) were euthanized as described above and used for organ allometry measurements. Fish were weighed and measured, then heart, liver, gastrointestinal system, and mesenteric fat were sampled and weighed.

4.5.6 Fatty acids

Total lipids were extracted following a modification of the procedure of Folch et al. (1957) proposed by Parish (1987). White muscle samples were ground in a conical tissue grinder with a glass pestle (CanadaWide) in 4 ml of dichloromethane–methanol (2:1, v:v). Lipids from muscle tissue were fractionated into neutral lipids (including triglycerides, free FA, and sterols) and polar lipids (including phospholipids and glycolipids) using a silica gel micro-column (40 mm × 5 mm i.d. Pasteur pipette plugged with glass wool and filled with silica gel that had been heated for 2.5 h at 450°C and deactivated with 6% water by weight). Neutral lipids were eluted with 10 ml of chloroform:methanol (98:2) and polar lipids with 10 ml of methanol (Marty et al. 1992). Total lipids for liver tissues and each lipid fraction for muscle was methylated under nitrogen by the addition of 2 ml of sulfuric acid:methanol (2:98) and 0.8 ml of toluene. Samples were heated for 10 min at 100°C, then 4 ml of distilled water and 0.8 ml of hexane were added to recover the organic phase containing fatty acid methyl esters (FAMEs). Finally, samples were purified to remove remaining sterols. FAMEs were analyzed using a full scan mode (ionic range: 50–650 m/z) on a Polaris Q ion trap coupled to a Trace GC ultra gas chromatograph (Thermo Scientific) equipped with an autosampler (Triplus), a PTV injector, and a mass detector model ITQ900 (Thermo-Scientific). FAMEs were identified using capillary columns (DB-23 60 m × 0.25 mm i.d., 0.25 μm thickness; Agilent Technologies) with a standard 37 component FAME mix (Supelco 37 Component FAME Mix, Supelco). Data were analyzed using Xcalibur v2.1 software (Thermo Scientific). Total FA were expressed as the percentage of tissue dry matter, while relative percentages were used for specific FA composition. Retention ratios were calculated by dividing the relative FA percentage of tissue by the relative FA percentage of diet.

4.5.7 Statistical and data analysis

Data normality and homoscedasticity were tested using Shapiro-Wilk and Levene tests, respectively. Survival analysis started from day 0, when salinity 14 was reached. Survival curve comparisons were performed with a Kaplan-Meier analysis followed by a log-rank test (Kaplan and Meier, 1958; Mantel, 1966) for diet (RD vs MD) and salinity (0 vs 14). Because temperature was season dependent and the different samplings were not truly independent (same tank), two-way ANOVA were performed for each sampling day to assess the effects of diet and salinity on BM, SL, and Fulton index. Two-way ANOVA were also used to test for differences among diets and salinities for Δmass and Δlength.

For lipid analysis, two-way permutational multivariate ANOVA (PERMANOVA) were used on the relative percentage of FA to assess the effects of diet and salinity on FA composition in liver (total FA) and muscle (polar and neutral). The homogeneity of multivariate dispersions was evaluated for each factor using the permutation analysis of multivariate dispersion (PERMDISP) routine before each PERMANOVA (Anderson, 2001). SIMPER analysis was performed to determine the contribution of FA responsible for the dissimilarities between treatments. For FA categories accounting > 2% of dissimilarities, a T-test was run to assess differences between salinities (FW vs BW) or between diets (RD vs MD). T-tests were also performed on ΣSFA, ΣMUFA, ΣPUFA, Σn3, Σn6, Σn3/Σn6, ΣPUFA/ΣMUFA, ΣPUFA/ΣSFA, and on total FA concentration (%DM) as well as on FA retention ratios.

Diet and salinity effects on organ allometry were assessed by comparing slopes. When slopes were homogeneous, an ANCOVA was run to compare intercepts. In order to meet normality, BM and organ mass were log10 transformed. Differences were considered significant at $\alpha = 0.05$. Statistical analyses were conducted in R (ver. 3.5.0; R Development Core Team) or with PRIMER software (ver. 7.0.11; PRIRD-E Ltd).

4.6 RESULTS

4.6.1 Survival

Diet did not affect survival (MD: 0.86 survival probability vs RD: 0.89 survival probability; Figure 16A). A significantly higher survival probability was observed for fish exposed to BW (97%) than for those exposed to FW (78%; Figure 16B).

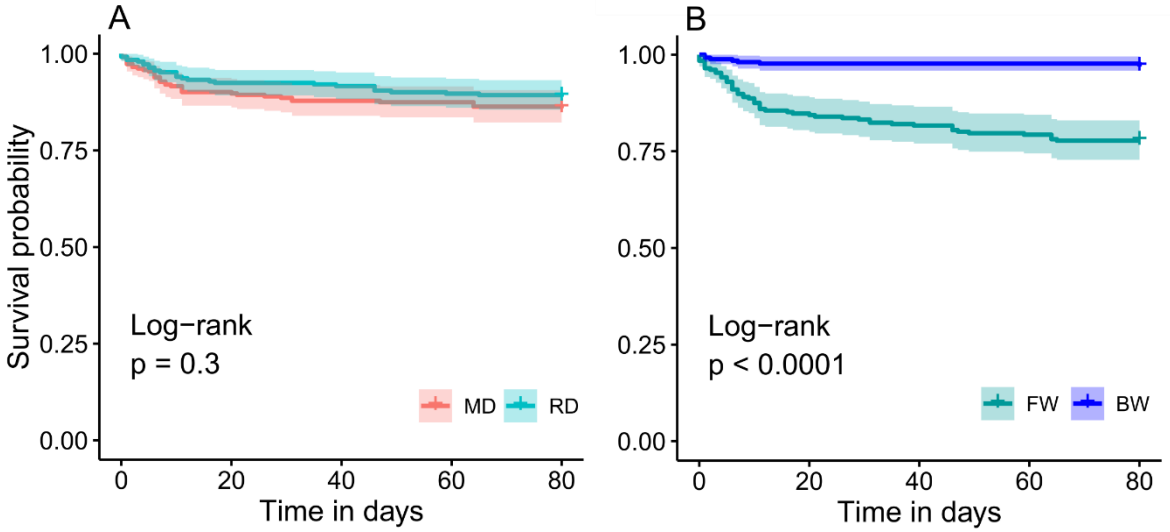


Figure 16: Kaplan–Meier plots showing 95% confidence intervals for the log-rank test on A) diet effect and B) salinity effect. RD: reference n-3 polyunsaturated fatty acid (n-3 PUFA); MD: marine n-3 PUFA diet; FW: fresh water, S = 0; BW: brackish water, S = 14.

4.6.2 Growth and condition index

Dietary regimes and salinity did not significantly affect BM, SL, or Fulton index at day 0 (except for SL when fish were randomly split into experimental groups), 56, or 102 ($P > 0.05$), and no interaction effect was present. Hereafter, the result is not mentioned when the interaction effect was not significant. During the fall, day 0 (13°C) until day 56 (8°C), the increments in mass (Δmass) and length (Δlength) did not differ among treatments (1.29 ± 0.25 g and 2.40 ± 1.47 mm, respectively). During winter, day 56 (8°C) until day 102 (5°C), fish lost mass similarly in every treatment ($P > 0.05$). Between days 56 and 102,

length increment was greater in FW than in BW juveniles (3.54 ± 1.76 mm vs –1.33 ± 1.06 mm; $F_{[1,4]} = 13.38$; $P < 0.05$) and greater in RD than in MD juveniles (3.21 ± 1.76 mm vs –1.00 ± 1.47 mm; $F_{[1,4]} = 10.00$, $P < 0.05$). The mean Fulton index increased in fall from 1.35 ± 0.44 to 1.63 ± 0.46, while a slight decrease was observed in winter (1.53 ± 0.43).

The hepato-somatic index significantly decreased by 28.4% over time in fish reared in BW, while it remained stable for those reared in FW (time × salinity, $F_{[1,8]} = 6.48$, $P < 0.05$; Figure 17A). At day 25, hematocrit was significantly higher in juveniles raised in FW than in those raised in BW. At day 102, the hematocrit had significantly increased by 12.6% in fish raised in BW, while it remained stable in FW juveniles (time × salinity, $F_{[1,8]} = 10.88$, $P < 0.05$; Figure 17B).

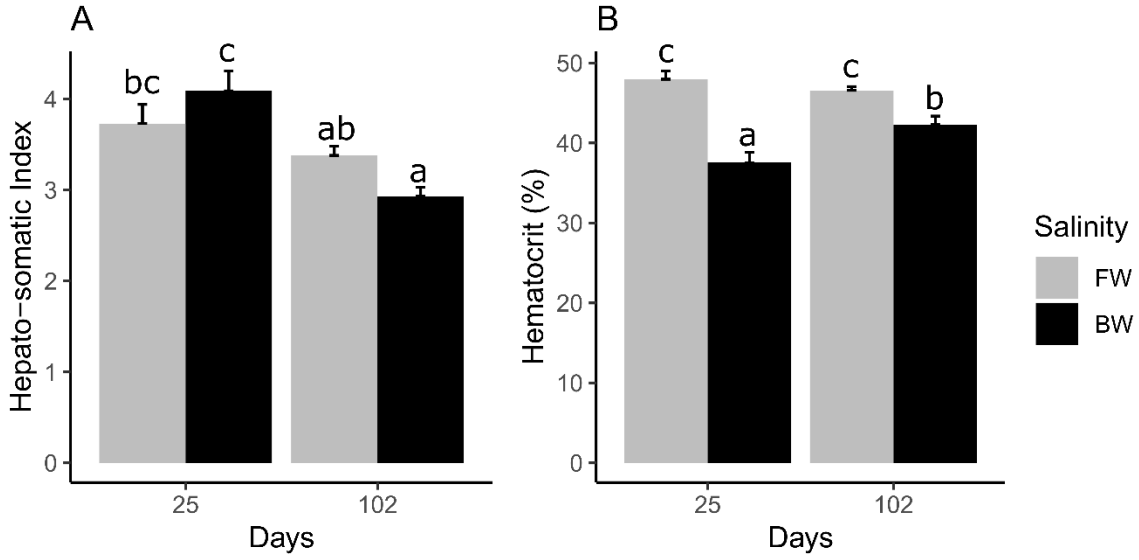


Figure 17: Interaction effect of time and salinity on A) hepato-somatic index and B) hematocrit (%). Significantly different groups were assigned different letters following Tukey post-hoc tests ($\alpha = 0.05$). FW: fresh water, S = 0; BW: brackish water, S = 14. Values are mean ± s.e.

4.6.3 Organ allometry

Heart mass was significantly affected by diet, with RD-fed fish having a lower heart mass than those fed MD (ANCOVA: $F_{[1,115]} = 6.93$, $P < 0.0$; Figure 18A). No diet effect was observed for the liver mass, but juveniles raised in FW had higher liver masses than those raised in BW (ANCOVA: $F_{[1,115]} = 8.44$, $P < 0.01$, Figure 18B). No effect of diet or salinity was observed for visceral and mesenteric fat masses.

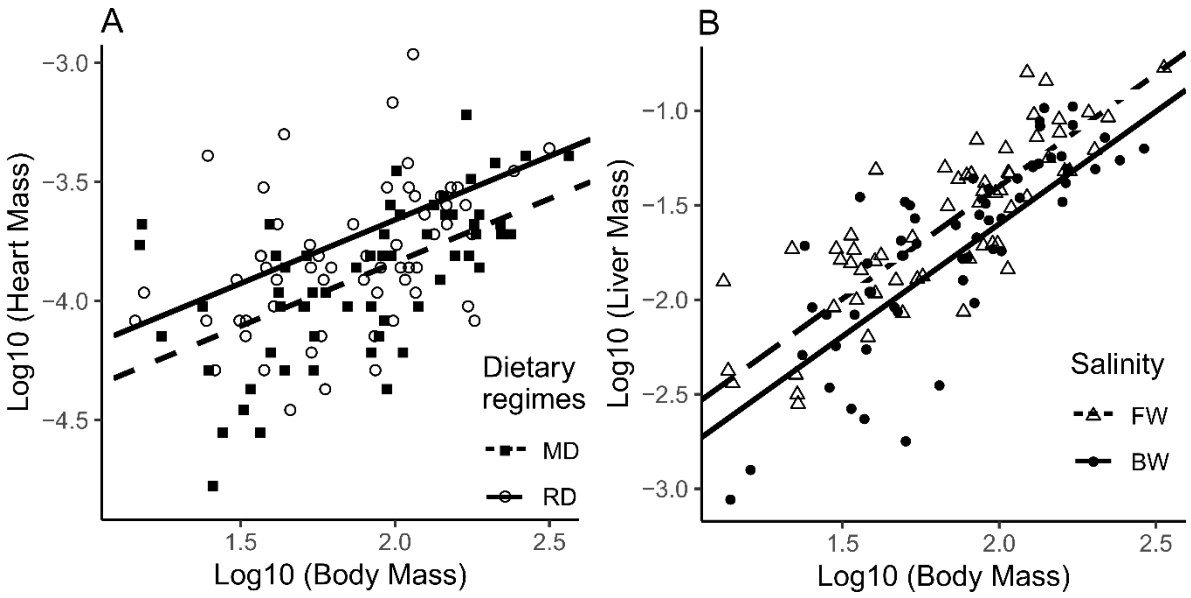


Figure 18: Effect of dietary regime (RD: reference n-3 polyunsaturated fatty acid [n-3 PUFA] diet; MD: marine n-3 PUFA diet) on organ allometry of A) heart, and effect of salinity (FW: fresh water, S = 0; BW: brackish water, S = 14) on B) liver.

4.6.4 Fatty acid profiles

Liver total FA composition was significantly affected by diet (PERMANOVA: F (1,28), $P < 0.05$) and salinity (PERMANOVA: F (1,28); $P < 0.01$) without significant interaction. Palmitic (C16:0) and oleic (C18:1n9) acids were dominant in liver FA profiles (Table 12), with stearic (C18:0) and linoleic (C18:2n6) acids accounting for 94% of the dissimilarities observed for both diet and salinity. The content of saturated FA (ΣSFA) in liver, including palmitic and stearic acids, was significantly higher in MD-fed fish, and

there were significantly lower levels of ΣMUFA, ΣPUFA, Σn6, ΣPUFA/ ΣSFA, oleic, and linoleic acids in MD- than in RD-fed fish (Table 12). The same pattern was observed for salinity effect, with significantly higher liver contents of ΣSFA (plamitic, stearic) and lower levels of oleic and linoleic acids in FW compared to BW juveniles (Table 12). However, BW juveniles also had higher ΣMUFA, ΣPUFA, Σn3, Σn6, and ΣPUFA/ΣMUFA and ΣPUFA/ΣSFA ratios than FW juveniles (Table 12).

In white muscle, neutral and polar FA were both significantly affected by diet (PERMANOVA: respectively $F_{[1,28]} = 4.42$, $P < 0.01$; $F_{[1,28]} = 2.76$, $P < 0.05$), but not by salinity. No significant interactions were obtained for any lipid fraction analysis. Overall, 93% of the difference between muscle neutral FA composition was explained by eight FA, particularly by oleic acid, DHA, and palmitic acid, which accounted for 74% of the dissimilarities (Table 13A). T-tests revealed that MD-fed fish had higher relative contents of ΣSFA (mostly represented by palmitic acid), EPA, and DHA than RD-fed fish. Σn6 were significantly higher in RD-fed fish, explaining why the Σn3/Σn6 ratio was greater in MD-fed fish. However, this difference was only related to the variability of 18:2n6. The RD-fed fish were also characterized by higher contents of linoleic acids (C18:3n6 and C18:3n3) as well as a higher ΣPUFA/ΣSFA ratio than MD-fed fish.

Almost all—98%—of the differences in the polar FA composition of the muscle tissue were explained by six FA, including palmitic acid and DHA (Table 13B). Significant differences between diets were observed for oleic, linoleic, ΣMUFA, and Σn6, which were significantly higher in RD-fed fish (Table 13B).

Table 12: Effect of diet and salinity on liver total fatty acid (FA) composition

Total fatty acids in liver						
FA (%)	FW		BW		T-test	
	MD	RD	MD	RD	Diet	Salinity
C14:0	3.68 ± 0.24	3.38 ± 0.34	4.12 ± 0.47	2.84 ± 0.34		
C15:0	0.86 ± 0.06	0.84 ± 0.08	0.84 ± 0.12	0.55 ± 0.09		
C16:0	33.88 ± 2.26	24.24 ± 2.37	25.49 ± 2.09	18.73 ± 2.3	(44%) P < 0.01 *MD > RD*	(44%) P < 0.05 *FW > BW*
C17:0	0.76 ± 0.05	0.75 ± 0.07	0.72 ± 0.11	0.46 ± 0.08		
C18:0	12.46 ± 0.68	9.92 ± 0.69	8.53 ± 0.97	6.31 ± 0.8	(6%) P < 0.05 *MD > RD*	(7%) P < 0.001 *FW > BW*
C20:0	1.25 ± 0.32	0.91 ± 0.36	0.68 ± 0.35	0.72 ± 0.16		
C22:0	0.54 ± 0.21	0.12 ± 0.12	0.4 ± 0.22	0.33 ± 0.16		
ΣSFA	53.42 ± 2.89	40.16 ± 3.24	40.77 ± 3.17	29.93 ± 3.29	P < 0.01 *MD > RD*	P < 0.01 *FW > BW*
C16:1	5.98 ± 0.55	5.48 ± 0.43	7.35 ± 0.55	6.34 ± 0.27		
C18:1n9	26.9 ± 1.35	34.77 ± 2.41	32.24 ± 2.04	40.28 ± 2.4	(38%) P < 0.01 *RD > MD*	(35%) P < 0.05 *BW > FW*
C20:1n9	7.54 ± 0.61	7.89 ± 0.45	7.08 ± 0.8	6.78 ± 0.39		
C22:1n9	0.11 ± 0.11	0.61 ± 0.31	0.17 ± 0.17	0.63 ± 0.19		
C24:1n9	0.29 ± 0.2	0.13 ± 0.13	0.49 ± 0.22	0.62 ± 0.15		
ΣMUFA	40.82 ± 2.04	48.88 ± 2.56	47.32 ± 1.66	54.65 ± 2.25	P < 0.01 *RD > MD*	P < 0.05 *BW > FW*
C18:2n6	2.73 ± 0.47	5.34 ± 0.62	6.14 ± 0.98	9.16 ± 1.05	(6%) P < 0.05 *RD > MD*	(8%) P < 0.001 *BW > FW*
C18:3n3	0.45 ± 0.25	1.38 ± 0.34	1.15 ± 0.32	1.61 ± 0.28		
C20:2n6	2.04 ± 0.39	2.85 ± 0.27	2.24 ± 0.47	2.2 ± 0.21		
C20:4n6	0.47 ± 0.2	0.72 ± 0.24	0.77 ± 0.2	0.68 ± 0.12		
C20:5n3	0.09 ± 0.09	0.69 ± 0.23	0.73 ± 0.25	0.82 ± 0.15		
C22:6n3	0 ± 0	0 ± 0	0.88 ± 0.38	0.95 ± 0.23		
ΣPUFA	5.77 ± 1.1	10.97 ± 1.35	11.91 ± 2.06	15.42 ± 1.66	P < 0.05 *RD > MD*	P < 0.01 *BW > FW*
Σn3	0.53 ± 0.29	2.07 ± 0.51	2.76 ± 0.86	3.38 ± 0.63	NS	P < 0.01 *BW > FW*
Σn6	5.23 ± 0.89	8.9 ± 0.92	9.15 ± 1.3	12.04 ± 1.05	P < 0.05 *RD > MD*	P < 0.01 *BW > FW*
Σn3/Σn6	0.08 ± 0.04	0.22 ± 0.06	0.25 ± 0.07	0.26 ± 0.04	NS	NS
ΣPUFA/ΣMUFA	0.14 ± 0.02	0.23 ± 0.03	0.25 ± 0.04	0.28 ± 0.03	NS	P < 0.05 *BW > FW*
ΣPUFA/ΣSFA	0.12 ± 0.03	0.3 ± 0.05	0.33 ± 0.07	0.58 ± 0.09	P < 0.01 *RD > MD*	P < 0.01 *BW > FW*
Total FA % DM	3.81 ± 0.86	7.71 ± 1.34	16.13 ± 9.59	14.56 ± 2.52	NS	NS

FA in unshaded rows accounted for the main dissimilarities observed with the SIMPER test, and dissimilarity percentages are indicated in parentheses. FA in shaded rows explained < 2% of the dissimilarity. FA values are given in relative percent except for total FA, which is expressed in percent of dry matter (DM). MD: marine n-3 polyunsaturated FA (n-3 PUFA) diet; RD: reference n-3 PUFA diet; FW: fresh water, S = 0; BW: brackish water, S = 14; NS: not significant. Values are presented as mean ± s.e.

Table 13: Effects of diet on the muscle fatty acid (FA) composition in the A) neutral and B) polar lipid fractions

A	Neutral fatty acids in muscle				
FA (%)	FW		BW		T-test
	MD	RD	MD	RD	Diet
C14:0	2.74 ± 0.16	2.63 ± 0.13	3.1 ± 0.17	2.27 ± 0.09	
C15:0	0.37 ± 0.07	0.57 ± 0.04	0.49 ± 0.07	0.34 ± 0.06	
C16:0	14.58 ± 0.37	13.23 ± 0.42	14.73 ± 0.52	12.74 ± 0.32	(7%) P < 0.001 *MD > RD*
C17:0	0.34 ± 0.06	0.37 ± 0.06	0.35 ± 0.06	0.19 ± 0.04	
C18:0	2.9 ± 0.38	2.83 ± 0.09	2.53 ± 0.18	2.47 ± 0.13	
C20:0	0.71 ± 0.18	0.91 ± 0.3	0.68 ± 0.16	0.66 ± 0.17	
ΣSFA	21.63 ± 0.73	20.53 ± 0.58	21.88 ± 0.88	18.67 ± 0.44	P < 0.01 *MD > RD*
C16:1n7	5.35 ± 0.36	4.95 ± 0.26	6.05 ± 0.35	4.51 ± 0.16	(3%) P < 0.01 *MD > RD*
C18:1n9	39.84 ± 1.9	39.76 ± 1.19	38.38 ± 1.84	43.79 ± 0.88	(62%) NS
C20:1n9	5.29 ± 0.28	5.5 ± 0.25	4.82 ± 0.36	4.36 ± 0.65	(4%) NS
C22:1n9	1.04 ± 0.16	1.32 ± 0.22	1.09 ± 0.2	1.16 ± 0.17	
C24:1n9	1.28 ± 0.29	1.37 ± 0.3	1.07 ± 0.33	1.15 ± 0.18	
ΣMUFA	52.79 ± 1.24	52.89 ± 0.87	51.39 ± 1.22	54.97 ± 1	NS
C18:2n6	12.27 ± 0.31	13.04 ± 0.45	12.54 ± 0.54	14.11 ± 0.17	(5%) P < 0.01 *RD > MD*
C18:3n3	3.02 ± 0.16	3.61 ± 0.24	2.88 ± 0.28	4.02 ± 0.15	(2%) P < 0.001 *RD > MD*
C20:2n6	1.38 ± 0.19	1.84 ± 0.14	1.23 ± 0.28	1.5 ± 0.18	
C20:4n6	0.92 ± 0.1	1.1 ± 0.07	1.09 ± 0.14	0.84 ± 0.1	
C20:5n3	3 ± 0.28	2.81 ± 0.31	3.43 ± 0.33	2.33 ± 0.14	(2%) P < 0.05 *MD > RD*
C22:6n3	5 ± 0.39	4.19 ± 0.56	5.57 ± 0.46	3.56 ± 0.3	(7%) P < 0.01 *MD > RD*
ΣPUFA	25.59 ± 0.58	26.58 ± 0.51	26.74 ± 0.52	26.36 ± 0.7	NS
Σn3	11.01 ± 0.62	10.61 ± 0.72	11.88 ± 0.69	9.91 ± 0.54	NS
Σn6	14.57 ± 0.3	15.97 ± 0.45	14.85 ± 0.26	16.45 ± 0.25	P < 0.001 *RD > MD*
Σn3/Σn6	0.76 ± 0.05	0.68 ± 0.07	0.81 ± 0.06	0.6 ± 0.03	P < 0.05 *MD > RD*
ΣPUFA/ΣMUFA	0.49 ± 0.02	0.51 ± 0.02	0.52 ± 0.02	0.48 ± 0.02	NS
ΣPUFA/ΣSFA	1.19 ± 0.03	1.3 ± 0.04	1.23 ± 0.04	1.41 ± 0.03	P < 0.001 *RD > MD*
Total FA % DM	10.56 ± 3.2	8.34 ± 2.3	5.62 ± 1.6	8.44 ± 2.7	NS

B	Polar fatty acids in muscle				
FA (%)	FW		BW		T-test
	MD	RD	MD	RD	Diet
C14:0	1.04 ± 0.1	0.89 ± 0.03	1.03 ± 0.1	1.08 ± 0.11	
C15:0	0.38 ± 0.04	0.31 ± 0.02	0.38 ± 0.04	0.41 ± 0.05	
C16:0	35.25 ± 2.9	26.4 ± 1.57	31.77 ± 3.84	30.26 ± 2.45	(47%) NS
C17:0	0.37 ± 0.04	0.28 ± 0.01	0.37 ± 0.03	0.39 ± 0.04	
C18:0	9.58 ± 0.73	7.3 ± 0.32	9.91 ± 1.11	9.32 ± 0.62	(4%) NS
C20:0	0.81 ± 0.11	0.72 ± 0.07	0.86 ± 0.11	1.09 ± 0.14	
C22:0	1.12 ± 0.2	1.51 ± 0.1	1.22 ± 0.24	1.26 ± 0.19	
ΣSFA	48.54 ± 3.76	37.42 ± 1.61	45.55 ± 4.87	43.8 ± 3.07	NS
C16:1	1.23 ± 0.07	1.07 ± 0.02	1.21 ± 0.06	1.24 ± 0.1	
C18:1n9	11.1 ± 0.93	11.99 ± 0.95	11.36 ± 0.64	14.75 ± 1.04	(6%) P < 0.05 *RD > MD*
C20:1n9	3.14 ± 0.26	2.88 ± 0.23	3.15 ± 0.15	4.1 ± 0.33	
C24:1n9	2.78 ± 0.5	3.21 ± 0.24	2.9 ± 0.44	2.39 ± 0.4	
ΣMUFA	18.24 ± 0.82	19.15 ± 1.03	18.61 ± 0.66	22.49 ± 0.99	P < 0.05 *RD > MD*
C18:2n6	8.92 ± 0.66	12.89 ± 0.89	8.37 ± 0.94	11.37 ± 1.15	(8%) P < 0.001 *RD > MD*
C18:3n3	1.39 ± 0.08	2.07 ± 0.22	1.31 ± 0.16	2.05 ± 0.17	
C20:2n6	1.25 ± 0.11	1.23 ± 0.1	1.23 ± 0.12	1.57 ± 0.12	
C20:4n6	2.25 ± 0.28	2.61 ± 0.17	2.91 ± 0.41	2.23 ± 0.23	
C20:5n3	5.1 ± 0.94	6.51 ± 0.62	5.55 ± 0.79	4.48 ± 0.63	(3%) NS
C22:6n3	14.32 ± 3.13	18.11 ± 1.42	16.47 ± 2.55	12.01 ± 2.15	(30%) NS
PUFA	33.22 ± 4.14	43.43 ± 1.61	35.84 ± 4.56	33.71 ± 3.65	NS
Σn3	20.8 ± 4.08	26.7 ± 1.93	23.34 ± 3.41	18.55 ± 2.86	NS
Σn6	12.42 ± 0.58	16.74 ± 0.86	12.51 ± 1.29	15.17 ± 1.16	P < 0.01 *RD > MD*
Σn3/Σn6	1.7 ± 0.36	1.67 ± 0.23	1.76 ± 0.23	1.19 ± 0.19	NS
ΣPUFA/ΣMUFA	1.89 ± 0.29	2.34 ± 0.23	1.92 ± 0.25	1.55 ± 0.2	NS
ΣPUFA/ΣSFA	0.76 ± 0.15	1.18 ± 0.08	0.89 ± 0.14	0.83 ± 0.13	NS
Total FA % DM	5.57 ± 0.84	5.61 ± 0.45	6.34 ± 0.72	5.17 ± 0.54	NS

FA in unshaded rows accounted for the main dissimilarities observed with the SIMPER test, and dissimilarity percentages are indicated in parentheses. FA in shaded rows explained < 2% of the dissimilarity. FA values are given in relative percentage except for total FA, which is expressed in percent of dry matter (DM). MD: marine n-3 polyunsaturated FA (n-3 PUFA) diet; RD: reference n-3 PUFA diet; FW: fresh water, S = 0; BW: brackish water, S = 14; NS: not significant. Values are presented as mean ± s.e.

In liver, the liver/dietary lipid ratios were not significantly affected by diet in contrast to salinity. Stearic ($P < 0.001$), oleic ($P < 0.01$), and linoleic ($P < 0.001$) ratios significantly differed among juveniles reared in FW and BW (Figure 19A). FW juveniles retained 1.5 times more stearic acid than those reared in BW. However, their oleic and linoleic ratios were respectively 1.2 and 2 times lower (Figure 19B). In muscle tissue, the muscle/dietary lipid ratio of palmitic acid (C16:0) was twice as high in RD-fed fish ($P < 0.001$; Figure 19B). Those fish also had a significantly higher muscle/dietary lipid ratio in EPA (2.6 times; $P < 0.001$), while MD-fed fish exhibited a higher ratio in oleic (2 times; $P < 0.001$) and linoleic (1.2 times; $P < 0.01$) acids (Figure 19B). In the polar fraction, retention of EPA ($P < 0.001$) and DHA ($P < 0.05$) was significantly higher, by 3.3 and 1.4 times, respectively, in RD-fed fish (Figure 19C).

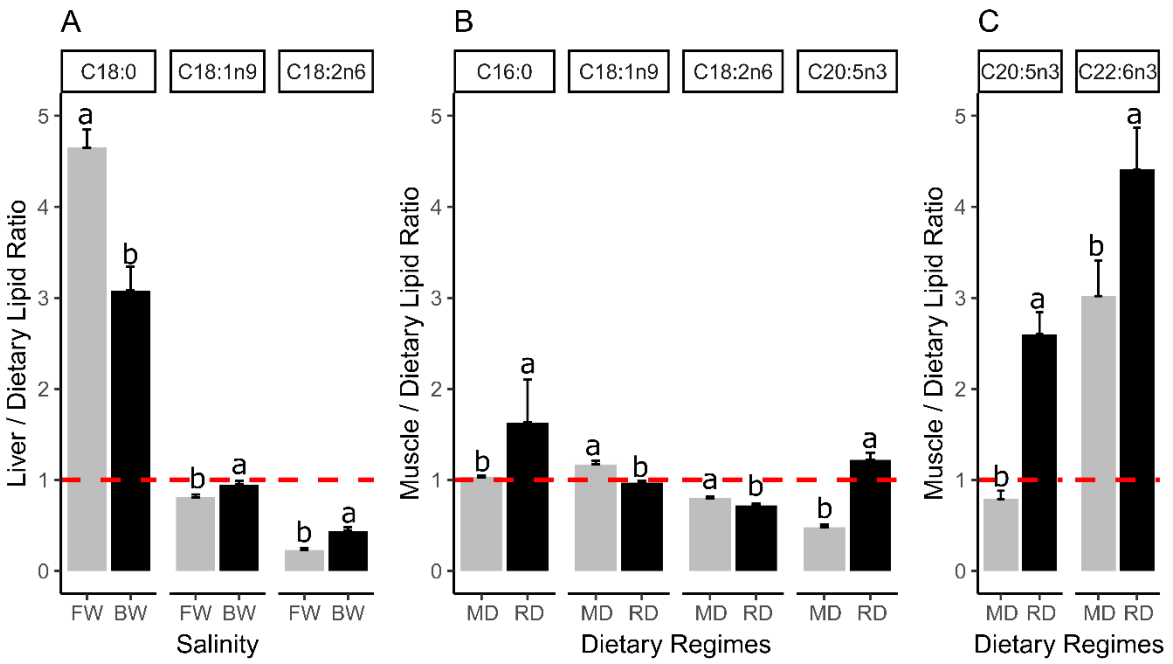


Figure 19: Tissue/dietary lipid ratios in A) liver total fatty acids (FA), B) muscle neutral FA, and C) muscle polar FA. Significantly different groups were assigned different letters. MD: marine n-3 polyunsaturated fatty acid (n-3 PUFA) diet; RD: reference n-3 PUFA diet; FW: fresh water, S = 0; BW: brackish water, S = 14.

4.7 DISCUSSION

Striped bass juveniles showed a better survival rate in BW than in FW. During winter conditions, neither dietary regime nor salinity affected growth or loss of mass. This higher survival rate could be related to the nutritional need induced by environmental conditions, which showed that the nutritional needs of BW fish were fulfilled for overall dominant neutral muscle FA, while those of FW fish were not. Salinity was found to influence liver allometry, with FW-reared juveniles having a higher liver mass than BW juveniles. Our results suggested that FW represents a stressful condition for this species since fish in this environment exhibited higher hematocrit levels, which is a secondary stress indicator.

4.7.1 Diet effects

Liver total FA and neutral muscle FA compositions were closely related to diet composition while differences in polar muscle FA were smaller. The liver provides a short-term picture of lipid storage since this organ is the main site of FA, triglyceride, and phospholipid synthesis, while muscle lipid composition results from a longer physiological pathway. Palmitic and oleic acids, in addition to being the dominant FA in liver, explained the most dissimilarities between dietary regimes and salinities, and contributed to the high levels of SFA and MUFA that were observed. Storage lipids can act as a reserve of metabolic energy that may be used for ATP production (Sargent et al., 2002). For this reason, the increased presence of 18:1n-9 in liver may be related to energy storage since this FA is the preferred substrate for β-oxidation in fish (Henderson and Sargent, 1985; Torstensen et al., 2000). Interestingly, significant accumulations of stearic acid were indicated by the liver tissue/dietary lipid (TD) ratio: it was greater in FW fish, suggesting higher energy needs for those fish. In muscle neutral FA, only oleic acid remained dominant while SFA content decreased. Polar muscle FA were less affected, suggesting that regulation occurred in phospholipid composition, possibly in membrane phospholipids, to maintain tissue functionality; this agrees with previous work (Sargent, 1976; Skalli and

Robin, 2004; Gourtay et al., 2018). EPA and DHA were dominant in polar muscle, although no effect of diet was observed, suggesting that these FA are preserved because of their functional role.

In fish nutrition, it has been widely recognized that EPA, DHA, and ARA requirements are essential considering that these essential FA (EFA) are required for a number of crucial physiological pathways (see Sargent et al., 2002, for review; Tocher, 2010). In striped bass and its hybrids, several studies have shown that diets deficient in n-3 PUFA resulted in high mortality or low growth rates at the larval stage (Webster and Lovell, 1990; Tuncer and Harrel, 1992). Although striped bass tolerate a wide range of salinities, it has been demonstrated that larval FA requirements were similar to those of marine species (Webster and Lovell, 1990; Tuncer and Harrell, 1992). EPA was found to be essential for striped bass larvae at levels higher than 5% of total fatty acids (Webster and Lovell, 1990). It has been shown that in juvenile (4 g) sunshine bass (M. saxatilis hybrid with saxatilis male), n-3 highly unsaturated FA such as EPA and DHA were essential for maximum growth, feeding efficiency, and survival (Nematipour and Gatlin, 1993). Hybrid striped bass are also unable to synthesize de novo adequate amounts of long-chain PUFA (Nematipour and Gatlin, 1993; Trushenski, 2009), even when fed substantial amounts of 18:3 n-3 or 18:4 n-3 as biosynthetic precursors (Bharadwaj et al., 2010); this result appears to be true for other Moronidae taxa (Tuncer and Harrell, 1992; Harel and Place, 2003; Geay et al., 2010, 2012) and more broadly for marine fish species (Sargent et al., 2002). Surprisingly, EPA and DHA did not account for much of the dissimilarities observed between FA profiles in our study. Despite higher contents in EPA and DHA in the MD diet, no difference was seen in liver total FA composition, while DHA and EPA respectively accounted for 7% and 2% of the dissimilarity in neutral muscle FA, with MD-fed fish having the higher content. However, DHA played a major role in muscle polar FA, accounting for 30% of the dissimilarity observed while EPA accounted only for 3%. Considering that the tissue FA composition reflected that of the dietary regime, we calculated the tissue/dietary lipid ratio (TD ratio) for the main FA as revealed by the PERMANOVA analysis. A TD ratio greater than 1 for one FA indicates that the tissue is

richer in this FA than the diet, demonstrating that a specific retention occurred. By examining the TD ratio for EPA and DHA in muscle polar FA, we observed that RD- and MD-fed fish selectively retained DHA (ratio > 1), but only RD-fed fish selectively retained EPA. Interestingly, the RD-fed fish had higher ratios than the MD fish. This seemed to allow them to compensate for the low EPA and DHA dietary content since no difference was observed in relative muscle polar FA composition. This was not the case in muscle neutral FA: even though RD-fed fish had a higher ratio of EPA than MD-fed fish, this was enough to compensate for the low level of EPA in their diet, and a lower content in EPA was observed in the muscle neutral FA of those fish.

In some cases, it is necessary to analyze the concept of nutritional requirements considering a specific abiotic environmental condition. In such a specific situation, the nature and composition of the feed will directly influence the retention ratio, which in turn can complicate the determination of the specific nutritional need induced by the environment. This type of determination could be made by standardizing the effect of the nature and composition of the dietary regime by calculating the new index that we propose here—the environmental nutritional index (ENI).

$$ENI = \frac{FA_i\ TD\ ratio\ Diet\ B\ /FA_i\ TD\ ratio\ Diet\ A \qquad eq.10}{FA_i\ Diet\ A\ content/FA_i\ Diet\ B\ \ content \qquad eq.11}$$

In the conceptual scheme, fish are fed two diets: diet A with a higher content in FAi than diet B (Figure 20A). The difference in this FA between the two dietary regimes (dashed arrows) can be estimated by calculating the diet ratio (Figure 20B; FA_i Diet A / A_i Diet B; eq. 11). In this example, we fix the TD ratio of diet A at 1 and consider three different scenarios for the TD ratio of diet B (Figure 20B), the latter always remaining higher than the TD ratio of diet A. By calculating eq. 10 (FA_i TD Diet B / FA_i TD Diet A) and dividing it by eq. 10, (Figure 20C), we can estimate whether the initial proportion of FAi between the two dietary regimes (eq.10) has been preserved between their two corresponding TD ratios. In the first case, the difference in FAi diet composition was higher than in TD FA ratio, so ENI is < 1 and fish nutritional needs are probably not met. In

the second case, the same range was observed between the two ratios, so ENI = 1 and nutritional needs are met. In scenario 3, ENI > 1, so the nutritional needs are exceeded considering the specific needs related to the environment.

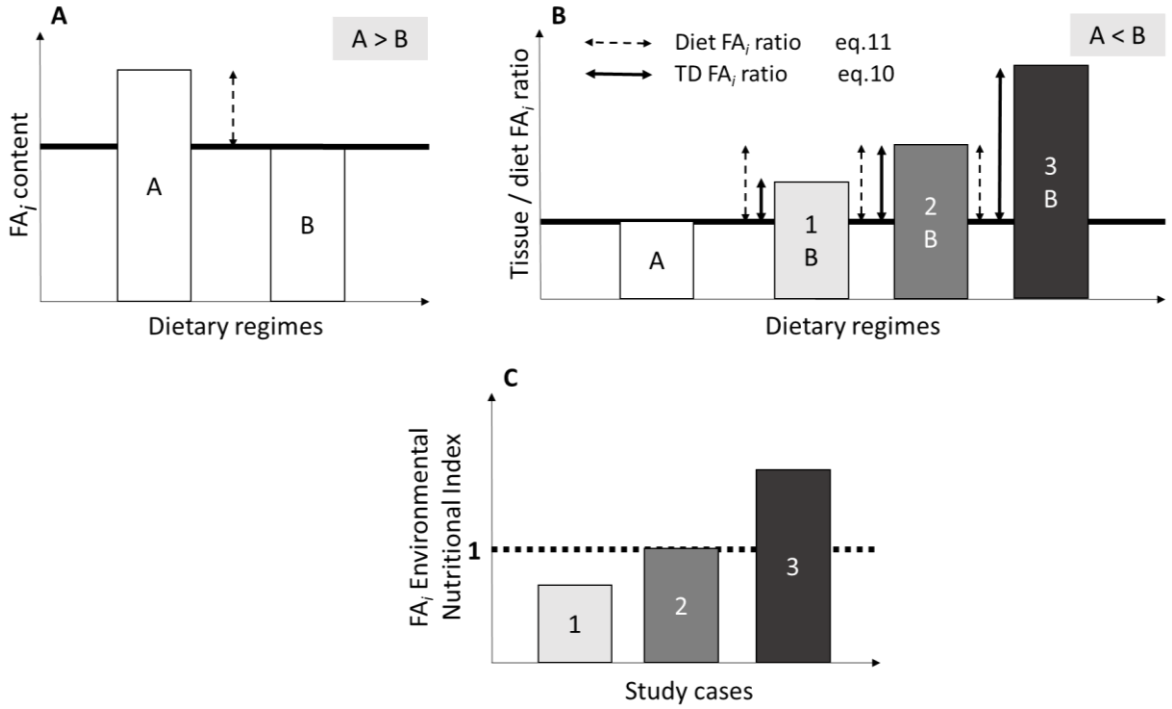


Figure 20: Calculation of the environmental nutritional index. A: content in fatty acids (FA) of two dietary regimes; B: tissue / dietary (TD) FA$_i$ ratio provided by the dietary regimes according to three case studies; C: environmental nutritional index according to the same scenarios. Dashed arrows represent the difference between diets in FA$_i$ content (eq. 11), solid arrows indicate the difference between TD FA$_i$ content.cases (eq.10).

We calculated the ENI for the dominant FA as revealed by PERMANOVA in muscle energy reserves (neutral lipids) for each salinity condition (Figure 21). The nutritional needs for EFA (EPA and DHA) were fulfilled (ENI > 1); this was also the case for palmitic acid for BW fish, while adequate quantities of oleic, linoleic, and linolenic acids were not provided (ENI < 1). Interestingly, the ENI indicated that the EFA needs of BW fish were better met than were those of FW fish. The greatest difference was between palmitic acid, which was far below the minimum requirements of FW fish. This difference likely reflected a strong involvement of this FA in energy metabolism. Once again, this

shows that the FW condition was stressful for this species since they need to invest more energy to satisfy their nutritional needs.

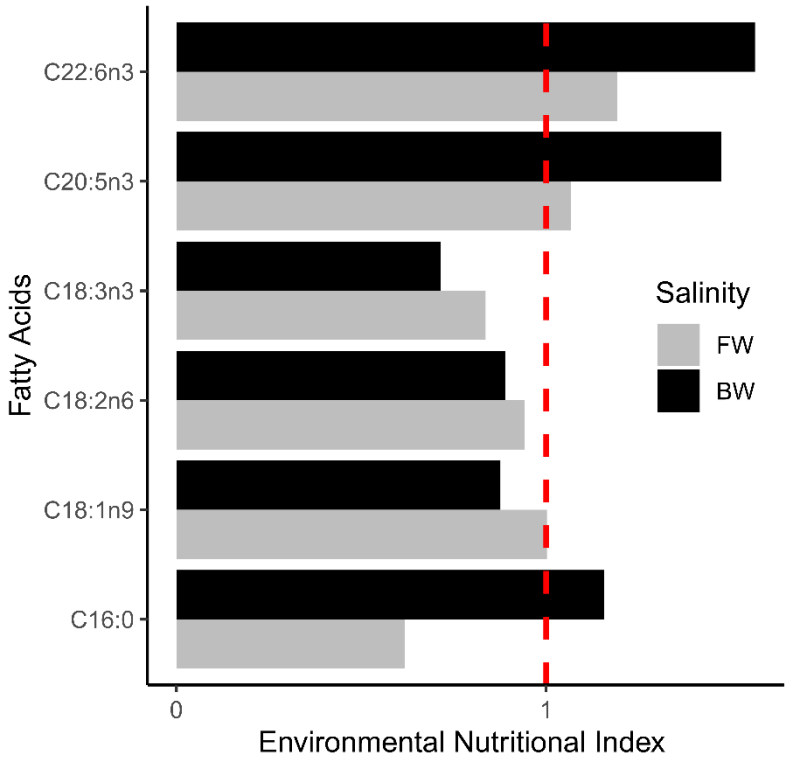


Figure 21: Effect of salinity (FW: fresh water, S = 0; BW: brackish water, S = 14) on the environmental nutritional index in muscle neutral fatty acids.

This is the second study showing that environmental parameters can greatly influence organ allometry. It has been shown that relative organ masses were affected by diet and temperature in juvenile European sea bass (Gourtay et al., 2018). In that study, when fish were reared at 20°C, the RD-fed fish exhibited lower heart and gastrointestinal masses while RD-fed at 15°C had higher liver mass. In our study, even if temperature was not fixed, the RD dietary regime also induced higher heart mass. Contrary to Gourtay et al. (2018), we were not able to correlate this increased of heart mass with an increased gastrointestinal mass.

4.7.2 Salinity effects

The liver plays a major role in energy storage, and it is the first site for fat storage in a number of benthic and demersal species (Drazen, 2002; Hoffmayer et al., 2006; Lloret et al., 2008). HSI is associated with liver energetic reserves, and this index indicates differences according to the salinity of the rearing environment that may indicate a greater use of energy reserves in BW (Eliassen and Vahl, 1982). However, we were not able to confirm that it was solely due to salinity or if the prolonged exposition to a temperature difference of 1°C between FW and BW mattered.

The optimal temperature for juveniles has been reported to be about 24–28°C (Kellogg and Gift, 1983; Secor, 2000; Duston et al., 2004; Cook et al., 2010), so colder temperatures represent an additional constraint on juvenile metabolism. This constraint seemed to be accentuated in FW fish, which exhibited a higher relative liver mass compared to BW fish. Survival and hematocrit data indicated that FW was a stressful condition for striped bass, and this could explain the increased lipid storage in liver to try to compensate stress. However, the trend (not significant) observed for total lipid content showed that liver lipid content in FW fish was lower than in BW fish. The higher liver mass observed in FW fish could be explained by higher glycogen accumulation while BW fish could exhibit enhanced glycogenolysis.

Survival data obtained in the present study are consistent with those already published on YOY striped bass. In the past decades, it was reported that abundances peaked near fresh–brackish water interfaces, indicating better physical (Albrecht, 1964; Otwell and Merriner, 1975; Morgan et al., 1981; Winger and Lasier, 1994) and biological (Jassby et al., 1995) conditions for survival. Indeed, BW (S = 14) seemed more suitable since BW juveniles had a higher and stable survival rate compared to observations in FW, while dietary regime had no effect. These results corroborate the findings of Hurst and Conover (2002), who studied the effects of winter temperature and salinity conditions in YOY striped bass. These authors monitored survival rates at salinities of 15 and 30 in

overwintering conditions (temperature declined from 13 to 2°C) and found a better survival rate (87 to 100%) for fish exposed to S = 15 than for fish at S = 30 (0 to ¬90% survival). In additional experiments, they showed that fish were intolerant of low temperatures in FW, dying at significantly higher temperatures than fish held at intermediate salinity.

The effects of temperature for Morone saxatilis have been documented by several studies, for example, small striped bass juveniles (< 5–40 g) of several strains grew optimally at 24–28°C (Kellogg and Gift, 1983; Secor, 2000; Duston et al., 2004; Cook et al. 2010) while larger juveniles and sub-adults preferred temperatures closer to 20°C (Cheek et al., 1985; Duston et al., 2004; Lapointe et al. 2014). In our study, fish were reared below the thermal optimum for growth, and this could explain why neither salinity nor dietary regime affected growth. Better growth was observed with MD regimes in juvenile sea bass (Dicentrachus labrax), a related species, when reared at fixed temperatures of 15°C and 20°C (Gourtay et al., 2018). Our study showed that the minimal growth temperature was not reached during fall because growth was still observed, but that this temperature must be between 8°C to 5°C since we did observe weight loss during winter.

4.8 CONCLUSIONS

For future research concerning juvenile striped bass, salinity and dietary regimes can be addressed separately since no interaction was observed in this cross-factorial design on physiological parameters measured in winter conditions. Salinity effects were less pronounced compared to those of dietary regimes on total liver and neutral muscle FA profiles, while polar muscle FA were slightly affected. We developed a new index to estimate the impact of environmental conditions on specific FA requirements: the ENI. The use of this index has highlighted that nutritional needs in EFA were higher when fish were in FW, and that requirements for palmitic acid were not fulfilled. The ENI results agreed with the higher hematocrit value and the lower survival observed in FW.

DISCUSSION GENERALE

L'objectif principal de ce projet doctoral était d'étudier l'effet des AGLPI n-3 sur la plasticité de deux espèces de Moronidés: le bar européen (*D. labrax*) et le bar rayé (*M. saxatilis*) dans des contextes différents. Afin de réaliser cet objectif, des juvéniles de ces deux espèces ont reçu deux aliments expérimentaux isoénergétiques et isoprotéiques caractérisés par leur quantité relative en AG. Le premier aliment avait un contenu en AGLPI n-3 égal aux besoins nutritionnels connus pour le bar européen (AL; EPA + DHA = 0.73% MS) et les acides oléique (18:1n-9), linoléique (18:2n-6), et linolénique (18:3n-3) représentaient une part importante des AG composant l'aliment; le second aliment était caractérisé par un contenu relatif élevé en EPA et DHA (AL++; EPA + DHA = 1.65% MS), environ deux fois supérieur aux besoins nutritionnels du bar européen. L'étude du bar européen a été réalisée dans un contexte de changement climatique, les poissons ont été élevés à 15°C (température naturelle de l'espèce) et 20°C (augmentation attendue à la fin du siècle). En milieu marin, la hausse des températures pourrait être associée à une raréfaction des AGLPI n-3 impliquant une modification des lipides des espèces de phytoplancton, premier niveau de la chaîne trophique. L'aliment AL++ correspond aux niveaux actuels présents dans les farines et les huiles de poissons sauvages, alors que l'aliment AL représente un scénario de raréfaction. L'étude du bar rayé a été réalisée dans un contexte d'écophysiologie, les poissons ont été élevés selon les variations thermiques saisonnières et en eau douce et saumâtre, salinités représentant les limites de l'aire de répartition des juvéniles de la population du Saint-Laurent. Le bar rayé étant une espece anadrome, l'aliment AL a été utilisé comme aliment de réference. Dans cette expérience, les aliments ont permis d'acquérir de nouvelles connaissances sur les besoins nutritionnels et la physiologie de cette espèce qui sont encore aujourd'hui mal connus.

La thèse étant présentée sous forme d'articles, chaque chapitre comprend déjà un résumé et une discussion ciblée sur les résultats présentés dans ce chapitre. Cette discussion générale a surtout pour objectif de mettre en évidence la portée de l'étude ainsi que de tisser le fil conducteur existant entre les différents chapitres. Elle se subdivise ainsi : nouvelles connaissances acquises sur le bar européen, comparaison des deux modèles biologiques utilisés, l'intérêt d'utiliser l'allométrie des organes dans ce type d'études, une remise en perspective des besoins nutritionnels définis en aquaculture et se termine par les perspectives mises en lumière par cette thèse.

ACQUISITION DE NOUVELLES CONNAISSANCES SUR LE BAR EUROPEEN

Croissance: de nouvelles données pour une nouvelle approche

Il existe de nombreuses manières d'étudier la croissance d'un organisme. Elle peut être analysée via le gain de masse ou de longueur, ces données permettent alors de construire des modèles de croissance (par exemple: Von Bertalanffy 1938 et ses variantes), d'estimer leurs paramètres, de calculer des taux de croissance ou encore des indices de croissance (delta masse, coefficient thermique de croissance, taux spécifique de croissance ; revus par Dumas et al. 2010). Généralement ces méthodes sont basées sur les jours calendaires. Les expériences de longue durée sont souvent coûteuses en matériel, en temps et en main d'œuvre, ce qui rend difficile l'obtention de séries temporelles de qualité. Lorsque les données sont disponibles, les plans expérimentaux et les méthodes utilisées divergent de telle manière que toute comparaison directe est rendue difficile. Il existe une méthode peu usitée dans l'élevage de poisson et qui pourtant est utilisée depuis plusieurs centaines d'années en agriculture et plusieurs dizaines en entomologie : les degrés-jour de croissance (DJC ou « *growing degree-day* »; Gilmore et Rogers 1958, Cross et Zuber 1972, Klepper et al. 1984, Russelle et al. 1984, McMaster et Smika 1988, McMaster 1993). Le calcul de cette méthode nécessite une bonne connaissance de la gamme thermique de croissance de l'espèce étudiée afin d'appliquer l'équation suivante (eq.12):

$$GDD = \frac{T_{max} - T_{min}}{2} - T_{base} \quad \text{eq.12}$$

T_{max} : température maximum journalière

T_{min} : température minimale journalière

T_{base} : température de base en dessous de laquelle le processus d'intérêt ne progresse pas

Les DJC sont représentatifs du temps thermique et sont fréquemment utilisés pour déterminer l'apparition d'évènements phénologiques clés pendant la croissance et le développement. Chaque stade de développement se déroule en fonction d'une accumulation d'énergie sous forme de chaleur, on peut alors estimer leur durée en sommant les degrés jour accumulés dans la gamme thermique de croissance de l'organisme. Cette méthode de calcul est basée sur le fait que l'ensemble des processus physiologiques déterminant la croissance (taux métabolique, échanges gazeux, demande en oxygène, etc.) sont tributaires de la température extérieure (Atkinson 1994, van der Have et De jong 1996) chez les espèces ectothermes (Claireaux et Lagardère 1999, Person-Le Ruyet et al. 2004). Ainsi, en agriculture, l'émergence (cotylédons complètement dépliés) du blé a lieu entre 125-160°C jour, celle de l'avoine entre 760-947°C jour, alors que celle du lin à lieu entre 104-154°C jour (Miller et al. 2001). Ce concept a également été appliqué aux poissons (Lange et Greve 1997, Neuheimer et Taggart 2007, Venturelli et al. 2010, Neuheimer et Grønkjær 2012, Chezik et al. 2013). Chez le saumon quinnat (*Oncorhynchus tshawytscha*), 516 ± 40°C jour sont nécessaires à l'éclosion des œufs, les œufs de truite fario (*Salmo fario*) ont besoin de 493 ± 48°C jour, alors que ceux de la limande à queue jaune (*Limanda ferruginea*) éclosent à 52 ± 3°C jour (Neuheimer et Taggart 2007). Dans l'étude de Neuheimer et Taggart (2007), 41 séries de données obtenues sur le terrain et en laboratoire, incluant 9 espèces de poissons dulcicoles et marines, vivants dans des climats tempérés et tropicaux, exposés à des régimes de températures constants et variables ont été analysées. La fonction linéaire consistant à exprimer la longueur en fonction des DJC explique plus de 92% des données. Les auteurs ont également montré que la méthode des DJC était applicable pour différents stocks et différentes populations d'une même espèce, ce qui ne peut être fait lorsque les

données sont exprimées en fonction des jours calendaires. Les DJC apparaissent comme une estimation fiable et essentielle permettant de comparer des variations ontogéniques à travers une constante thermique.

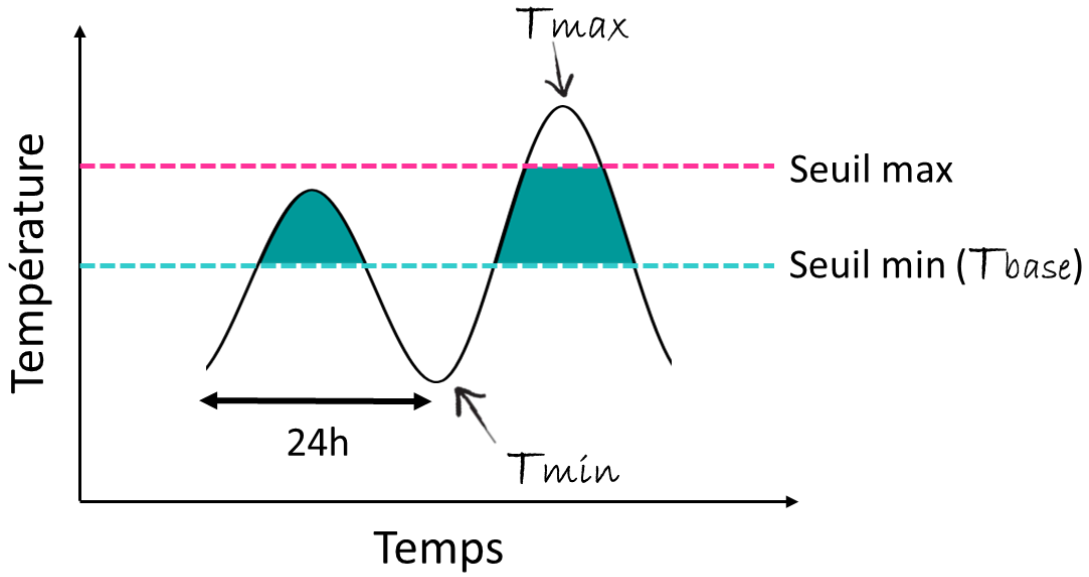


Figure 22: Accumulation des degrés-jour en fonction des seuils thermiques (minimum et maximum) de croissance. La ligne noire représente la variation de température temporelle, la droite en pointillée bleue est le seuil minimal thermique au-dessus duquel la croissance est possible, la droite magenta est le seuil maximal au-dessus duquel la croissance n'est plus possible. L'aire en bleu représente l'intégration d'énergie disponible pour la croissance en °C.

Même si la température est un facteur qui a été largement étudié chez le bar européen (Hidalgo et Alliot 1988, Claireaux et Lagardère 1999, Person-Le Ruyet et al. 2004, Besson et al. 2016, Gourtay et al. 2018), la température de base (T_{base}, température minimale à laquelle on observe de la croissance) de cette espèce n'a jamais été déterminée et aucune étude n'a été faite en utilisant les DJC de croissance. Grâce aux nombreuses mesures de croissance réalisées lors de ce projet, la T_{base} du bar européen a été estimée à 11°C (pour voir la méthode en détail, voir Annexe III: Figure supplémentaire 26, pages 152-53). En analysant les données en fonction des jours calendaires, on observe un taux de croissance (pente) différent entre chacun des quatre traitements (AL++ 20°C > AL 20°C >

AL++ 15°C > AL 15°C, Figure 23A). En revanche, lorsque l'on représente la longueur standard en fonction des DJC (Figure 23B), une réponse différente est mise en évidence (Figure 23A). En effet, l'utilisation des DJC permet de normaliser l'effet de la température et d'expliquer la variation longueur standard-âge observée avec les jours calendaires. Cette représentation met en évidence, seulement deux taux de croissance (pentes) différents induits par la disponibilité en n-3 AGLPI (Figure 23B). Cette méthode permet de s'affranchir de l'effet de la température et de mettre en évidence l'effet d'autres variables. On observe que pour une même quantité d'énergie emmagasinée (DJC), les juvéniles élevés à 15°C et 20°C possèdent une longueur équivalente. En revanche, cette longueur dépend de l'aliment reçu et la différence entre les deux aliments augmente plus la quantité d'énergie emmagasinée est grande.

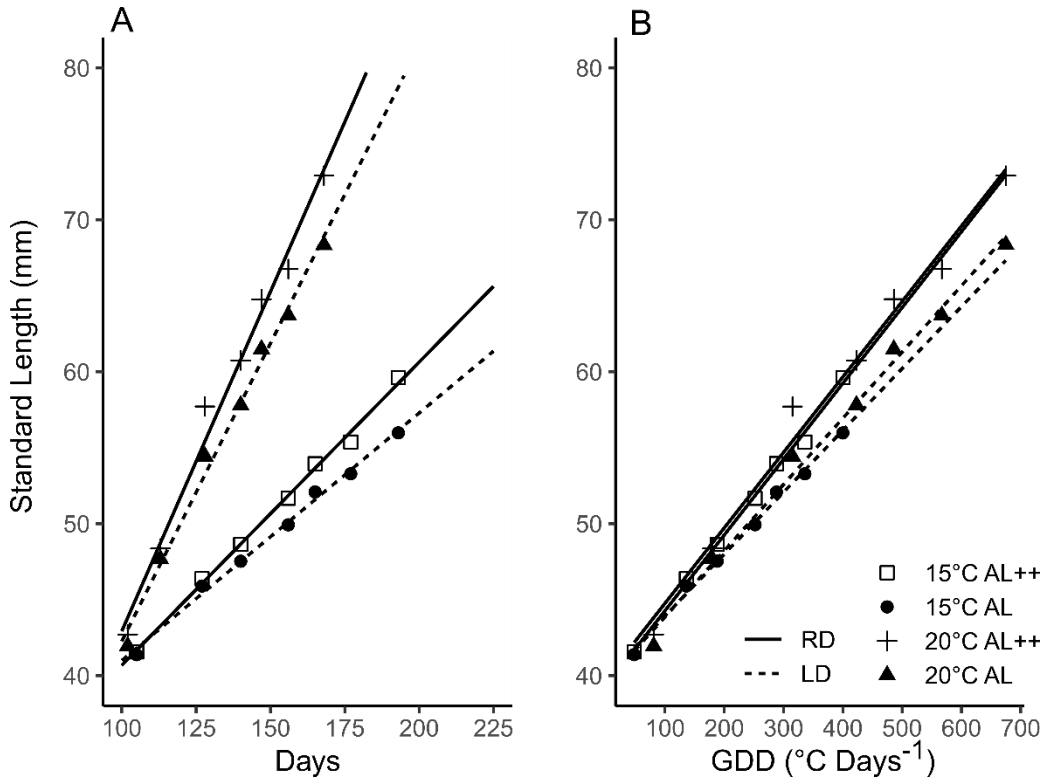


Figure 23: Effet de l'apport nutritionnel en acides gras à longue chaîne polyinsaturée (AGLPI) sur la longueur standard en fonction a) des jours calendaires, b) des degrés-jours de croissance. GDD: growing degree-days, AL: aliment faible en AGLPI n-3, AL++: aliment riche en AGLPI n-3.

Une capacité osmorégulatoire à toute épreuve

Le bar européen est généralement retrouvé dans les eaux côtières, il peut également pénétrer dans les eaux saumâtres des zones estuariennes, des lagunes côtières et également occasionnellement dans les rivières (Pickett et Pawson, 1994). Il a donc été considéré comme un poisson euryhalin capable de tolérer une large gamme de salinités (0.5‰ à 40‰ ; Eroldoğan et al. 2004). Plusieurs travaux ont d'ailleurs confirmé la grande capacité de cette espèce à maintenir son homéostasie sur le plan de son osmolarité plasmatique et de son équilibre hydrique tissulaire suite à des modifications majeures de la salinité de son milieu (Varsamos et al. 2001, Eroldoğan et al. 2004, Chatelier et al. 2005, Sinha et al. 2015). Par exemple, aucune différence de pression osmotique n'a été observée lorsque des juvéniles ont été exposés à une salinité de 39.5‰ ou 5.3‰, celle-ci demeurant stable aux environs de 350 mosm (Varsamos et al. 2001). Dans le cadre de cette étude, des juvéniles de bar européen ont été exposés à une salinité de 55‰ pendant plusieurs heures et soumis à des mesures de vitesse de nage donc à une dépense énergétique importante. Aucune mortalité n'a été observée pendant ce challenge de nage, ni durant les jours qui ont suivi. Ces résultats corroborent l'idée que les juvéniles de bar européen possèdent une grande capacité osmorégulatoire. En conditions normales, soit dans les bacs d'élevage, la pression osmotique (PO) des juvéniles était environ d'environ 350 mosm. A la fin de l'acclimatation à une salinité de 45‰ dans le tunnel de nage, une augmentation de 20 mosm a été mesurée. De plus, cette expérience a montré un effet significatif de l'aliment sur la PO, les poissons nourris avec AL, ont conservé une PO inférieure à ceux ayant reçu AL++. Des études plus poussées demeurent cependant nécessaires pour essayer de mieux comprendre les implications mécanistiques et métaboliques de tels résultats.

Les téléostéens, qui habitent des environnements aux salinités diverses, possèdent des mécanismes complexes et sophistiqués d'osmorégulation permettant de maintenir l'homéostasie ionique et osmotique interne, afin d'assurer un fonctionnement normal des processus cellulaires et physiologiques assurant la survie (Evans et al. 2005, Hwang and Lee 2007). L'osmorégulation est un champ d'étude controversé, car des résultats très

contradictoires ont été publiés (pour revue: Tseng et Hwang 2008) c'est pourquoi les mécanismes osmorégulatoires sont encore mal définis. Des mesures de consommation d'oxygène auraient pu être réalisées afin de caractériser les coûts énergétiques associés à l'exposition aux conditions d'hypersalinité. En effet, plusieurs travaux ont reporté un changement de cette consommation chez plusieurs espèces lors d'un changement de salinité (Plaut 2000, Sardella et al. 2004, Gracia-Lopez et al. 2006, Wagner et al. 2005). Le métabolisme des carbohydrates semble jouer un rôle majeur dans l'approvisionnement en énergie dédiée à l'osmorégulation, et le foie semble être l'organe source permettant de fournir ces carbohydrates (Tseng et Hwang 2008). Dans le cadre de notre étude, le contenu du foie en glycogène aurait pu être un autre élément explicatif des capacités osmorégulatoires observées. Les auteurs de la revue dédiée au métabolisme énergétique de l'osmorégulation chez les poissons, soulignent que le rôle des lipides et des protéines comparé aux carbohydrates reste encore ambigu (Tseng et Hwang 2008).

DIFFERENCES ET SIMILITUDES CHEZ LES MORONIDES

Élevage et prophylaxie

L'élevage du bar européen a été réalisé au début du projet et celui-ci s'est déroulé sans problème majeur. En effet, cette espèce est domestiquée en Europe et les conditions d'élevage sont maitrisées depuis plusieurs années au laboratoire ARN (Ifremer, Brest). Bien que les juvéniles utilisés pour les expérimentations étaient issus de parents sauvages reproduits dans les locaux d'Aquastream (Lorient, France), les juvéniles se sont montrés résistants aux manipulations et aux expérimentations et très peu de mortalités ont été observées. Avant le début du nourrissage avec les deux aliments expérimentaux, les poissons ont été triés à deux reprises. Un premier tri dans une eau sursalée a permis d'éliminer les poissons qui n'avaient pas développé de vessie natatoire, un second tri par classes de taille a été réalisé afin de former des lots expérimentaux homogènes.

À l'inverse, les données existantes concernant la domestication du bar rayé sont rares et cette espèce n'est pas produite en aquaculture. Les juvéniles obtenus pour nos travaux, ont été fournis par la station piscicole de Baldwin (Coaticook, Canada), une station de production gouvernementale québécoise. Cette production de bar rayé est essentiellement destinée au réensemencement de cette espèce dans le corridor fluvial du Saint-Laurent dont elle avait complètement disparu depuis quelques décennies. Actuellement de nombreux tests sont menés de front à la pisciculture afin de déterminer les conditions optimales d'élevage de cette espèce (besoins nutritionnels des différents stades de l'espèce, stratégie de sevrage, niveau optimum de salinité en fonction des stades de développement). Nous n'avons reçu confirmation que nous pourrions avoir des juvéniles qu'à la fin de l'été, car en 2017 les mortalités ont été telles que les autorités de la pisciculture devaient être certains que les quotas nécessaires aux activités d'ensemencement seraient atteints avant de pouvoir nous donner des surplus. Nous avons donc hérité de survivants d'une année difficile sur le plan de la production. Lors de l'élevage des juvéniles à la station piscicole de l'ISMER (Pointe-au-Père, Canada), un ajustement rapide a été nécessaire, car les juvéniles étaient extrêmement fragiles et sensibles aux manipulations, ces dernières induisant des mortalités importantes. De ce fait le schéma expérimental a été ajusté afin de minimiser au maximum les manipulations et le stress chez les poissons. Un tri visuel rapide a été réalisé et les poissons ayant des malformations ont été éliminés avant de débuter la période expérimentale (Figure 24).

Figure 24: Juvéniles de bar rayé présentant des difformités. A) Déformations du corps, B) individu ne présentant pas de signe de déformation, C) l'individu du haut a une déformation de la tête, celui du bas présente bas une déformation de la colonne.

Il est possible que les morts subites observées lors de l'élevage soient en lien avec ce qui a été décrit comme le « *Stress Shock Syndrome* » par Castell et al. (1972a). Ce syndrome pourrait également avoir un lien avec l'observation de myopathie qui a déjà été faite sur les animaux de la pisciculture de Baldwin (communication personnelle). La myopathie est un terme générique désignant toutes les maladies affectant les fibres musculaires. Un syndrome de myopathie cardiaque, aussi appelé « insuffisance cardiaque aiguë » ou « rupture cardiaque » a été reporté à plusieurs reprises chez le saumon Atlantique (*S. salar*; Amin et Trasti 1988, Ferguson et al. 1990, Rodger et al. 1991, Bruno et Poppe 1996, Rodger et Turnbull 2000). Cette maladie a également été rapportée chez le mérou (SP; Nagasawa et Cruz-Lacierda 2004), cette fois la myopathie concernait les muscles squelettiques. Il a été démontré que l'origine de certaines des myopathies et du « *Stress Shock Syndrome* » était liée à l'alimentation, particulièrement à une déficience en

vitamine E et sélénium (Poston et al. 1976), ou encore en acides gras essentiels (Castell et al. 1972a; Bell et al. 1991). Les signes de cette déficience sont aigus, les poissons deviennent hypersensibles au stress et aux manipulations ce qui peut aboutir à une perte d'équilibre et causer jusqu'à la mort de l'organisme (Castell et al. 1972a, b, Millikin 1982, Watanabe 1982). Chez le mérou les poissons atteints présentaient un assombrissement de la couleur du corps, des pétéchies à la base de l'opercule et une déformation occasionnelle de la moelle épinière (Nagasawa et Cruz-Lacierda 2004). Des signes cliniques similaires ont été observés lors de l'élevage du bar rayé. La période larvaire est une période critique qui est fortement dépendante de la qualité de la nutrition (Izquierdo 1996, Cahu et al. 2003, Kolkovski et al. 2009). D'après les informations obtenues, les aliments utilisés par la station gouvernementale sont tout à fait équilibrés en termes de composition (Nutra XP et Gemma micro de Skretting, Aglonorse de Tromsø Fiskeindustri AS) et auraient dû satisfaire les besoins nutritionnels de l'espèce. Toutefois l'Aglonorse (Tromsø Fiskeindustri AS) possède un taux d'humidité élevé et requiert donc une conservation rigoureuse (environnement sec et frais). Que les causes soient nutritionnelles ou environnementales, les animaux utilisés présentaient une grande sensibilité au stress, dont nous avons dû tenir compte. Cependant, compte-tenu du fort taux de mortalité observé en élevage larvaire, du stress de transport (plus de 500 Km) et d'acclimatation, on peut aussi supposer que les animaux utilisés pour nos expériences étaient parmi les plus résistants de cette cohorte.

Des réponses similaires interspécifiques: allométrie des organes et performances physiologiques

Une comparaison entre les deux espèces de Moronidés peut être réalisée à la lumière des chapitres 1 et 4. Bien que les schémas expérimentaux aient été différents, des réponses similaires ont été observées chez les deux espèces. C'est le cas de l'allométrie des organes, qui s'est révélée être un trait plastique. Dans le chapitre 1, il a été mis en évidence que la masse cardiaque des juvéniles de bar européen était plus élevée chez les animaux ayant reçu AL. La même réponse a été observée chez le bar rayé. Il semble donc que la quantité relative en AG soit capable d'induire une réponse plastique de la masse cardiaque

chez ces deux espèces de Moronidés. Ce résultat soulève un grand nombre de questions. Est-ce que cette réponse est propre aux Moronidés? Est-ce une réponse qui concerne un plus grand nombre d'espèces? Comment l'alimentation peut agir sur la taille des organes? Par quels mécanismes? Quelques pistes de réflexion sont proposées dans la section suivante « L'allométrie des organes, un domaine de recherche prometteur », page 140.

L'alimentation a également eu un impact important sur les performances de nage chez le bar européen (chapitre 3). Une plus grande vitesse de nage critique a été observée chez les animaux ayant reçu AL (chapitre 3). Des résultats similaires ont également été observés chez la même espèce (Chatelier et al. 2006), chez le saumon atlantique (*S. salar*, McKenzie et al. 1998) et chez le mulet (*Liza aurata*, Vagner et al. 2014). En parrallèle du projet doctoral, un test de performance a également été réalisé sur les juvéniles de bar rayé élevés en eau douce afin d'examiner l'effet de l'aliment sur la résistance à un stress thermique aigu. Cette étude est menée dans le cadre des travaux de recherche de maîtrise d'Émeline Durand. C'est pourquoi les résultats préliminaires de cette étude sont présentés en annexe. Brièvement, l'expérience a consisté à exposer les juvéniles à une augmentation progressive de la température de l'eau (Annexe IV: Figure Supplémentaire 27A, page 154). Ce challenge permet de séparer les individus sensibles et résistants au stress thermique, ainsi que d'observer la cinétique à laquelle les poissons perdent leur équilibre (proxy de la mort). Malgré que ce test de performance soit différent du test de nage, la même réponse a été observée: les juvéniles de bar rayé nourris avec AL ont montré une meilleure performance que ceux nourris avec AL++ (Test du Log Rank sur les courbes de Kaplan Meier, Annexe IV: Figure Supplémentaire 27B, page 154). La performance est considérée comme un trait intégrateur d'une espèce, c'est-à-dire que la réponse de ce trait dépend de nombreuses fonctions physiologiques (osmorégulation, ventilation, etc.). Une fois encore ces résultats soulèvent les mêmes questions que celles posées dans le paragraphe précédent.

L'ALLOMETRIE DES ORGANES, UN DOMAINE DE RECHERCHE PROMETTEUR

L'allométrie des organes reste un domaine peu étudié chez les organismes vivants alors qu'elle peut apporter de nombreuses informations sans obligation de procéder à des analyses complexes. Elle peut être un indice utile pouvant guider les décisions analytiques et orienter les travaux vers une étude plus approfondie sur certains organes clés, certaines voies métaboliques ou encore certains éléments de physiologie.

L'allométrie, dans son sens le plus large, décrit comment les caractéristiques des êtres vivants changent avec la taille. Pour les caractéristiques morphologiques, la variation de la taille de chaque organe/partie de l'organisme relative à sa taille globale peut être étudiée (Huxley et Tessier 1936). Dans la littérature, il existe trois types d'allométries: ontogénique, évolutive et statique (Cheverud 1982, Schlichting et Pigliucci 1998). Dans le cadre des chapitres 1 et 4, l'étude des organes se limite au domaine de l'allométrie statique. Cette dernière décrit la taille relative de différents organes (ou traits) chez des individus au même stade de développement et au sein d'une même espèce. La masse relative des organes a été mesurée pour chacune des espèces à la fin de leur élevage. Les juvéniles utilisés étaient issus des mêmes parents, ils ont donc la même information génétique. Pour chacune des deux espèces, l'ensemble des juvéniles partagent une « même histoire », c'est-à-dire qu'ils ont subi la même procédure d'élevage que leurs congénères avant d'être soumis aux procédures expérimentales. On peut donc conclure que les différences dans l'allométrie des organes observées au sein de chaque espèce ont été induites par les conditions expérimentales. La présence de plusieurs phénotypiques en regard de ces traits allométriques indique que certains organes présentaient un caractère plastique. Cette plasticité a notamment été révélée par l'aliment et la salinité (ordonnées à l'origine différentes, pentes similaires) en fonction des conditions expérimentales auxquelles ont été exposées chacune des espèces (Tableau 14).

Tableau 14: Effet des conditions environnementales sur l'allométrie des organes du bar européen et du bar rayé

Organes	Bar européen	Bar rayé
Cœur	AL > AL++ (20°C)	AL > AL++
Foie	AL > AL++ (15°C)	ED > ES
Viscères	AL > AL++ (20°C)	_
Gras mésentérique	_	_

AL: aliment avec faible en acides gras longue chaîne polyinsaturée (AGLPI) n-3, AL++: aliment riche en AGLPI n-3, ED: eau douce, ES: eau saumâtre.

Plusieurs hypothèses peuvent être émises pour expliquer la relation allométrique observée chez les bars européens élevés à 20°C entre le cœur et les viscères. Cette dernière peut être la conséquence d'une réponse indépendante de chaque organe à l'aliment/salinité. De manière alternative, l'aliment/salinité n'a pu influencer uniquement la réponse au niveau des viscères, alors que la variation de la masse du cœur pourrait être une conséquence secondaire, soit une réponse mécanistique à la variation de la masse des viscères. L'inverse est aussi possible. Plusieurs travaux portent sur l'étude de la corrélation entre les normes de réactions (Schlichting 1986, Schlichting et Pigliucci 1998, Scheiner et al. 1991). En revanche, les mécanismes génétiques et physiologiques sous-jacents qui sont à l'origine de ces « corrélations plastiques » restent à ce jour toujours mal connus. Chez les insectes, il semblerait que les hormones jouent un rôle important dans la régulation de l'allométrie des organes, ainsi que dans la réponse spécifique des organes à ces hormones (Shingleton et al. 2007). L'allométrie des organes est un vaste champ d'études, comprendre comment l'environnement peut la modifier reste un enjeu majeur, car cela pourrait permettre de mieux comprendre comment les espèces ont pu évoluer au cours du temps et au fil des contraintes environnementales qu'elles ont rencontrées (allométrie évolutive).

D'un point de vue mécanistique, l'allométrie des organes est souvent reliée au taux métabolique standard. En effet, le taux métabolique standard reflète les coûts de maintien nécessaires à la machinerie métabolique (tissus et organes) afin d'accomplir un mode de vie

particulier (Killen et. al 2010, Piersma et Van Gils 2011). Les tissus et organes diffèrent grandement dans leur activité métabolique via leur rôle et donc également leurs coûts de maintenance (Gallagher et al. 1998). Les organes associés à la croissance (par exemple le foie, le pancréas, les reins, le tube digestif) ou la respiration (cœur) ont des taux métaboliques spécifiques à la masse très élevés par rapport au muscle squelettique ou aux tissus conjonctifs (Oikawa et Itazawa 1984, 1993, Elia 1992, Wang et al. 2001, 2012). Plusieurs études ont montré que la proportion de tissus ayant un coût énergétique important vs. ceux ayant un faible coût pouvait entrainer des changements dans le taux métabolique standard de l'organisme entier (Itazawa et Oikawa 1986, Mueller et Diamond 2001, Muller et al. 2011, Wang et al. 2012). D'autres travaux ont montré que l'intensité métabolique des tissus varierait en fonction de plusieurs paramètres incluant le nombre de doubles liaisons présentes dans les acides gras composant les membranes cellulaires (Brzek et al. 2007, Hulbert et al. 2005). Notre étude a révélé chez le bar européen une augmentation de la masse relative du cœur, des viscères et du foie lorsque moins d'AGLPI n-3 étaient présents dans l'aliment (Tableau 14). Chez le bar rayé, le même résultat a été observé sur la masse relative du cœur, alors que la masse relative du foie était plus élevée en ED (Tableau 14). En revanche, les coûts métaboliques et physiologiques associés à ces changements restent cependant étudier.

BESOINS NUTRITIONNELS EN ACIDES GRAS: AQUACULTURE VS NATURE

Selon les projections, la population mondiale devrait atteindre 9.3 milliards d'habitants en 2050 (United Nation 2017). La pêche à elle seule, ne parviendra pas à subvenir au besoin de cette population croissante. D'ici 2050, il est prévu que la majorité des produits de la mer consommés proviennent de l'aquaculture (Diana 2009, Tacon et Metian 2013). Les bénéfices d'une alimentation riche en poissons sont basés sur l'abondance des omégas 3 à longue chaîne, ainsi que de la qualité des protéines, des acides aminés, des vitamines et des minéraux caractéristiques aux poissons (Tacon et Metian 2013). En effet, le poisson représente la première source d'oméga-3 à longue chaîne (EPA et DHA) pour l'Homme (Arts et al. 2009). Les bienfaits de ces acides gras sur la santé

humaine ont été démontrés par de nombreuses études (pour revue: Riediger et al 2009). C'est pourquoi les autorités sanitaires de plusieurs pays recommandent une augmentation de la consommation des omégas-3 à longue chaîne par la population (pour revue voir: Salem et Eggersdorfer 2015). Dans un contexte où un déclin d'oméga 3 est en cours au plus bas des réseaux trophiques aquatiques (phytoplancton), où la majorité des stocks de poissons sont surexploités (Jackson et al. 2001, Pauly et al. 2002), la nutrition des poissons est un enjeu crucial pour relever les défis de l'aquaculture de demain et valoriser au mieux l'utilisation des omégas 3 à longue chaîne EPA et DHA. A noter qu'en parallèle la recherche sur des sources alternatives d'EPA et DHA durables est en pleine expansion (AGLPI n-3 d'origine algale [Ryckebosch et al. 2014], végétaux génétiquement modifiés [Petrie et al. 2012, QI et al. 2004, Venegas- Calerón et al. 2010], huile de krill [Olsen et al. 2006]).

Ces dernières années, d'énormes progrès ont été réalisés dans le domaine aquacole concernant la nutrition des poissons. En aquaculture, le but est d'obtenir un taux de croissance important pour assurer une certaine rentabilité, tout en s'assurant que l'animal est en santé et qu'il ait une bonne qualité nutritionnelle finale. C'est pourquoi une partie importante des recherches s'est concentrée sur la détermination du besoin nutritionnel en AG, plus particulièrement AGLPI n-3 et n-6 et de leurs impacts sur la physiologie des poissons. Chez plusieurs espèces il a été démontré qu'une déficience en AGLPI ou un déséquilibre entre les voies n-3 et n-6, pouvait affecter les fonctions immunitaires et ainsi induire une réponse inflammatoire (Henderson et al. 1985, Sheldon Jr. et al. 1991, Lie et al. 1992, Ashton et al. 1994, Kiron et al. 1995, Bell et al. 1996, Montero et al. 2003, Ganga et al. 2005). Les AGLPI sont également impliqués dans la résistance au stress et aux maladies (pour revue, voir Sargent et al. 2002). Ces AG ont un rôle essentiel en tant que précurseurs des eicosanoïdes qui sont des médiateurs cellulaires agissant sur le système nerveux, l'excrétion rénale et branchiale et l'osmorégulation (Bell et al. 1996, Tocher 2003). Très peu d'informations sont disponibles concernant les besoins nutritionnels du bar rayé. En revanche pour le bar européen le besoin en EPA + DHA, en condition d'élevage piscicole, a été établi à 0.7% n-3 AGLPI de la MS (Skalli et Robin 2004).

En étudiant la composition lipidique des aliments commerciaux couramment utilisés pour le bar européen, notamment leur contenu en EPA et DHA on peut observer que ces contenus sont 4 à 5 fois supérieurs aux besoins qui ont été définis par Skalli et Robin (2004 ; cf la gamme Néo Start, Le Gouessant). Ces données expliquent pourquoi les bars issus d'élevage sont souvent plus gras et plus riches en EPA et DHA que les bars sauvages (Bhouri et al. 2010, Orban et al. 2003). Notre étude sur le bar européen montre qu'avec AL++ (richesse équivalente aux aliments commerciaux), la croissance est améliorée par rapport à AL (richesse équivalente au besoin de l'espèce). Pour rappel, la croissance est un trait intégrateur, c'est-à-dire qu'elle est dépendante de nombreuses fonctions physiologiques. C'est le trait le plus recherché et donc étudié en aquaculture. En revanche, ce bénéfice peut ne pas être profitable à l'ensemble des traits d'une espèce. Comme l'a montré notre étude, les poissons nourris avec AL présentaient de meilleures performances de nage (bar européen) et de résistance au stress thermique (bar rayé) sans pour autant montrer de signe clinique physiologique (bar européen). Ces résultats montrent donc l'importance d'avoir une approche multi-traits lors de la définition du besoin chez une espèce particulièrement lorsque les études se situent dans un contexte écologique, avec des conditions environnementales pas toujours très favorables contrairement au contexte piscicole. Cette observation pose clairement la question d'une redéfinition du besoin tel que celui établi actuellement.

Un nouvel indice a été calculé dans le cadre de cette thèse et pourrait permettre de mieux appréhender le besoin nutritionnel des espèces en lien avec les conditions d'élevage ou environnementales: l'indice nutritionnel environnemental (INE). Cet indice a été construit en se basant sur le postulat que la composition en AG des tissus (notamment muscle et foie) chez les poissons reflète celle de l'alimentation (Person-Le Ruyet et al. 2004, Gourtay et al. 2018). L'INE permet de recalculer les besoins nutritionnels en AG en fonction d'une condition environnementale, afin d'évaluer comment cette condition impacte le besoin en lui-même. Non seulement cet indice permet de s'affranchir de la composition en AG des aliments utilisés, des conditions expérimentales/ environnementales testées mais permet également une comparaison inter-espèces. Dans le cadre des travaux

réalisés sur le bar rayé, la condition physiologique des poissons a pu être reliée à l'INE. Pour ce qui est de l'étude du bar européen, on peut observer que les besoins des poissons élevés à 15°C et ayant reçu AL, étaient les moins bien couverts en AG, excepté en EPA (ENI > 1, Figure 25), ce qui pourrait peut-être expliquer le faible taux de croissance observé. De manière surprenante, au second temps d'échantillonnage, les poissons ont dû mettre en place des mécanismes compensatoires car leur ENI était semblable aux juvéniles élevés à 20°C. L'ENI des poissons à 20°C est resté stable, en revanche seuls les besoins en AGE étaient couverts. L'ENI corrobore l'idée que ces poissons, élevés à 15°C étaient à un stade physiologique plus précoce, dans ce cas on observe une « dynamique » du besoin nutritionnel. Pour ces poissons, l'indice souligne également que le DHA est fortement préservé, voire synthétisé (ce qui pourrait confirmer la tendance [non significative] observée pour l'expression du gène *fads2* dans le chapitre 2, expression la plus élevée chez les RD 15°C à la fin de l'expérimentation). Chez nos deux espèces, cet indice met en évidence l'importance de l'acide palmitique comme indicateur d'une situation physiologique inconfortable, surement du fait de son rôle énergétique.

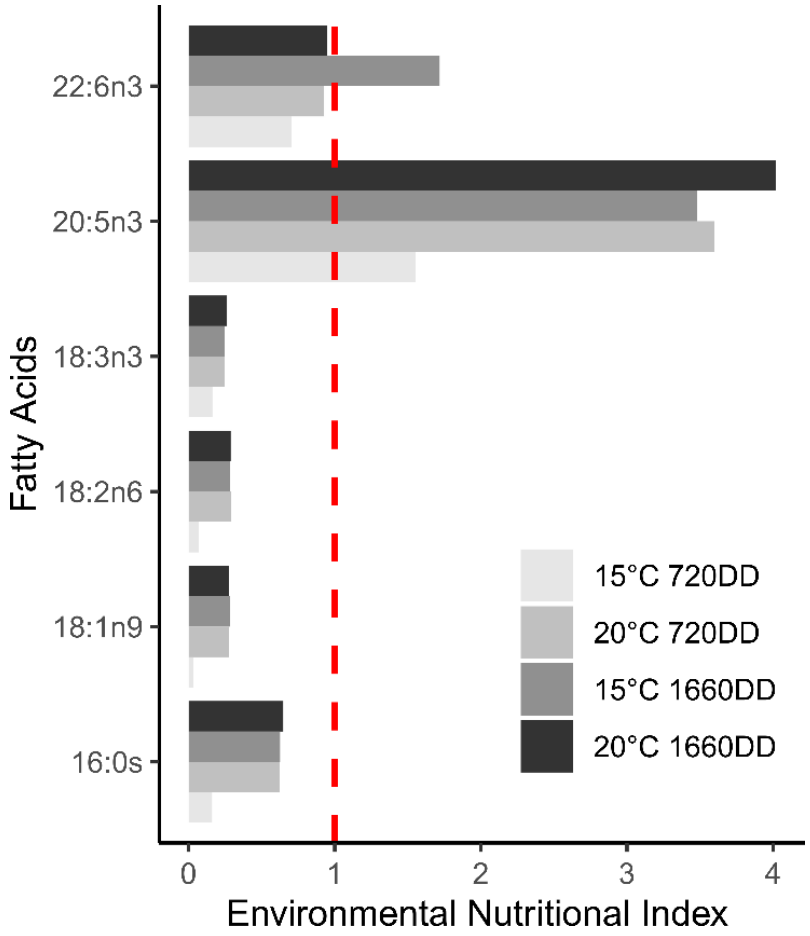


Figure 25: Effet du temps et de la température sur l'indice environnemental nutritionnel (ENI) chez les juvéniles de bar européens. DD : degrés-jours.

CONCLUSIONS GENERALES ET PERSPECTIVES

Cette thèse a mis en évidence que les facteurs environnementaux, respectifs au plan expérimental mis en place pour chaque espèce, étaient capables d'induire une plasticité phénotypique et physiologique. Il a été montré que les AG jouaient un rôle considérable dans cette plasticité. En revanche, une approche mécanistique plus approfondie est nécessaire afin d'observer comment l'aliment est capable de générer cette plasticité. Quelques perspectives de recherches sont proposées ci-après, elles pourraient permettre de mieux comprendre et d'expliquer les résultats obtenus dans le cadre de ce projet.

L'étude du bar européen a permis de déterminer la température de base de l'espèce, et ouvre donc la voie de l'utilisation des DJC pour de prochaines études. Le principe fondateur justifiant cette méthode est que la température est le facteur qui influence le plus l'ensemble des processus physiologiques d'un organisme. Dans le cadre de notre étude, il aurait été intéressant d'étudier les paramètres physiologiques des poissons en fonction des DJC. Il aurait alors été possible de déterminer si les individus d'une même condition (même aliment) mais exposés à des températures différentes présentaient des caractéristiques physiologiques similaires (effet température en fonction des DJC), ainsi que de déterminer l'effet d'autres paramètres environnementaux en fonction des DJC (par exemple l'aliment). De plus, si le même schéma expérimental avait été utilisé pour le bar rayé, les DJC auraient peut-être présentés des avantages permettant une comparaison originale des deux espèces. Finalement les DJC peuvent être un outil utile dans un contexte aquacole comme écologique, permettant de prédire, plutôt que de décrire, des réponses physiologiques/écologiques.

L'expérience réalisée sur les juvéniles de bar rayé a été réalisée sur les survivants d'une génération extrêmement sensible. Réaliser une nouvelle expérience à partir

d'individus issus d'une cohorte en meilleure santé pourrait permettre d'avoir une vision plus réaliste des capacités de l'espèce. Un tri préalable des poissons permettrait de travailler sur des lots de poissons homogènes, ce qui pourrait peut-être permettre d'obtenir des réponses plus contrastées que celles que nous avons observées. En temps normal, cette espèce aurait été capable d'être élevée dans les mêmes conditions expérimentales que celles qui ont été utilisées pour le bar européen. Une comparaison interspécifique plus aisée pourrait alors être réalisée et la dimension de rythme de vie de ces deux espèces serait alors pertinente.

Finalement, pour compléter les résultats obtenus dans le cadre de ce projet doctoral, il serait pertinent de faire des recherches concernant l'aspect énergétique de nos animaux. En effet la plasticité observée pourrait s'expliquer par une gestion et/ou une sélection de réserves énergétiques divergente. En utilisant des techniques comme la respirométrie, il serait possible de déterminer la consommation d'oxygène de nos animaux, et de déterminer leur taux métabolique. Le lien entre le taux métabolique de nos animaux et l'allométrie des organes, ou encore les performances (nage et résistance thermique) observées pourrait être caractérisé. De plus, il pourrait également être intéressant d'examiner le fonctionnement mitochondrial des deux espèces afin d'obtenir une indication plus fondamentale de la production énergétique de nos espèces. Dans ce cas-ci, la dimension de rythme de vie des deux espèces pourrait également prendre tout son sens.

ANNEXES

ANNEXE I

Tableau 15: Composition des aliments expérimentaux

BASE ALIMENT		
Farine de Poisson	**32,0g**	
Protéines %	21,3	
Lipides %	3,8	
Dont EPA+DHA %	0,7	
Farine lupin	34,0g	
Protéines %	12,8	
Lipides %	3,2	
EPA+DHA %	0,0	
CPSP 90	12,0g	
Protéines %	9,7	
Lipides %	0,8	
EPA+DHA %	0,1	
ADDITIFS		
Lécithine de colza	3,0g	
Amidon	8,0g	
Vitamines	1,0g	
Minéraux	1,0g	
Bétaïne	1,0g	
FORMULATION		
	Aliment mer	Aliment terre
Huile de poisson	**4,0g**	0,0g
EPA+DHA %	**1,0**	0,0
Huile de colza	4,0g	**8,0g**
EPA+DHA %	0,0	0,0
Total pour 100g d'aliment		
Protéines %	43,749	
Lipides %	17,563	
EPA+DHA %	**1,854**	0,854

ANNEXE II

Supplementary Table 16: Regressions for the logarithm 10 of body mass (BM, g) and standard length (SL, mm) on time (days, D), n = 3

Temperature	Diet	Regression	R^2
20°C	RD	BM = 0.025 D – 2.136	0.96
		SL = 0.008 D + 2.994	0.96
	LD	BM = 0.023 D – 2.055	0.96
		SL = 0.007 D + 3.039	0.96
15°C	RD	BM = 0.012 D –1.091	0.95
		SL = 0.004 D + 3.321	0.95
	LD	BM = 0.011 D – 0.935	0.97
		SL = 0.003 D + 3.386	0.97

LD: low n-3 polyunsaturated fatty acid (PUFA) diet, RD: reference n-3 PUFA diet.

Supplementary Table 17: Allometric regressions and R^2 values

Temperature	Diet	Allometric regression	R^2
20°C	RD	Log_{10} (Heart Mass) = 0.9757 log_{10} (BM) – 6.1386	0.73
		Log_{10} (Liver Mass) = 0.9861 log_{10} (BM) – 3.8864	0.48
		Log_{10} (Gastrointestinal Mass) = 0.8708 log_{10} (BM) – 3.4709	0.81
		Log_{10} (Mesenteric Fat Mass) = 0.9144 log_{10} (BM) – 2.6372	0.52
	LD	Log_{10} (Heart Mass) = 0.9305 log_{10} (BM) – 5.8454	0.46
		Log_{10} (Liver Mass) = 1.1802 log_{10} (BM) – 4.3872	0.66
		Log_{10} (Gastrointestinal Mass) = 0.8198 log_{10} (BM) – 3.2503	0.71
		Log_{10} (Mesenteric Fat Mass) = 1.1400 log_{10} (BM) – 3.4005	0.58
15°C	RD	Log_{10} (Heart Mass) = 0.9593 log10 (BM) – 5.9868	0.56
		Log_{10} (Liver Mass) =1.1203 log_{10} (BM) – 4.1891	0.42
		Log_{10} (Gastrointestinal Mass) = 1.0066 log_{10} (BM) – 3.7108	0.54
		Log_{10} (Mesenteric Fat Mass) fat = 1.9780 log_{10} (BM) – 4.9006	0.66
	LD	Log_{10} (Heart Mass) = 0.9304 log_{10} (BM) – 5.8888	0.32
		Log_{10} (Liver Mass) = 1.2170 log_{10} (BM) – 4.1127	0.46
		Log_{10} (Gastrointestinal Mass) = 0.8037 log_{10} (BM) – 3.2768	0.47
		Log_{10} (Mesenteric Fat Mass) = 0.7833 log_{10} (BM) – 2.7958	0.25

LD: low n-3 polyunsaturated fatty acid (PUFA) diet, RD: reference n-3 PUFA diet, BM: body mass. n = 30 for each allometric regression.

ANNEXE III

Afin de determiner la température seuil de croisance (T_{base}) du bar européen, les données de croissance acquises aux différentes temperatures ont été utilisées. Les températures journalières ont été sommées en fonction du nombre de jour d'exposition (par exemple 10 jours à 20°C, réprésentent 200 °C jours) pour chaque prélèvement et pour chaque aliment (Figure Supplémentaire 26A). Ensuite, les températures journalières (15°C ou 20°C) ont été soustraite à une T_{base} (eq.12) avant d'être sommées. De manière exploratoire, la gamme de T_{base} testée était de 0°C à 14°C. Dans la Figure Supplémentaire 26B, 10°C représente une T_{base} trop basse; dans la Figure Supplémentaire 26D, T_{base} est trop élevée car les pentes des taux de croissance (représentés par les regressions linéaires) ne sont pas similaires. La T_{base} du bar européen a été déterminée graphiquement à 11°C car les poisons élevés à 15 et 20°C possèdaient des taux de croissance (pentes) similaires (Figure Supplémentaire 26C).

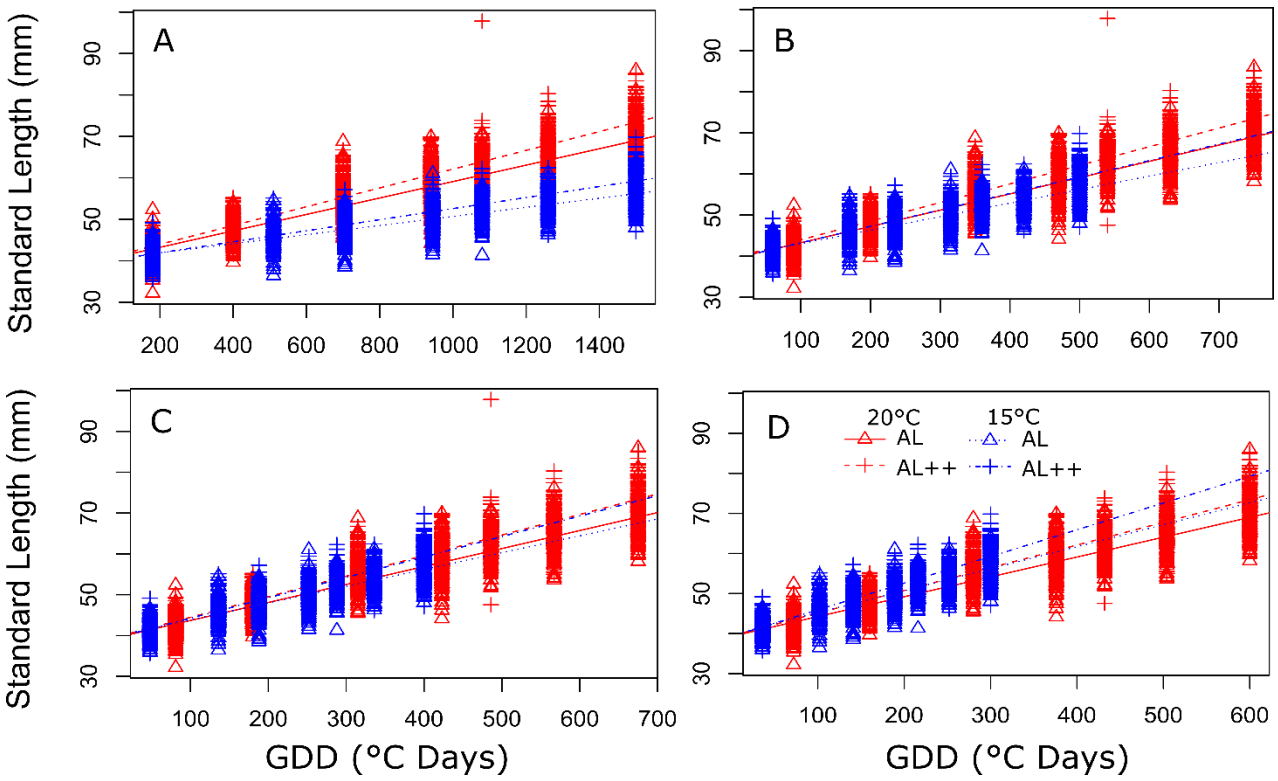


Figure Supplémentaire 26: Recherche de la température seuil de croissance (T_{base}) pour le bar européen. Température seuil testée A) 0°C, B) 10°C, C) 11°C, D) 12°C. GDD: *growing degree-days*, AL: aliment faible en acides gras à longue chaîne polyinsaturée (AGLPI) n-3, AL++: aliment riche en AGLPI n-3.

ANNEXE IV

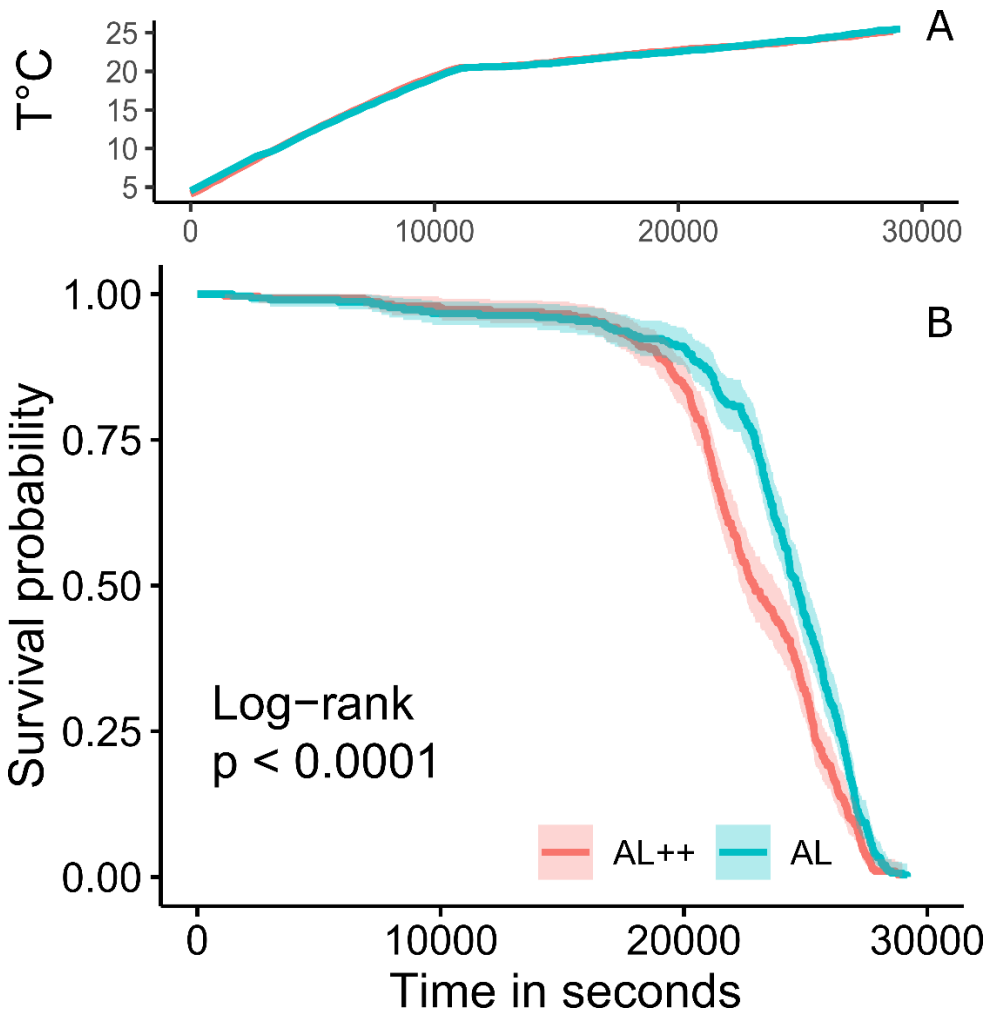


Figure Supplémentaire 27: Challenge thermique. A) Cinétique thermique (T°C: temperature en °C), B) Effet de l'aliment (AL++: aliment riche en acides gras longue chaîne polyinsaturée [AGLPI] n-3, AL: aliment pauvre en AGLPI n-3) sur la résistance à un stress thermique aigu chez les juvéniles de bar rayé.

RÉFÉRENCES BIBLIOGRAPHIQUES

Able K.W., Wuenschel M.J., Grothues J., Vasslides T.M., Rowe P. (2013) Do surf zones in New Jersey provide “nursery” habitat for southern fishes? Environ. Biol. Fishes 96, 661–675.

Ackman R.G., Tocher C.S. (1968) Marine phytoplankter fatty acids. J. Fish. Res. Board. Can. 25, 1603–1620.

Aebi H. (1984) Catalase in vitro. Methods Enzymol. 105, 121–126.

Albrecht A.B. (1964) Some observations on factors associated with survival of striped bass eggs and larvae. Cal. Fish Game 50, 100–113.

Altimiras J., Claireaux G., Sandblom E., Farrell A.P., McKenzie D.J., Axelsson M. (2008) Gastrointestinal blood flow and postprandial metabolism in swimming sea bass *Dicentrarchus labrax*. Physiol. Biochem. Zoo. 81, 663–672.

Álvarez M.J., López-Bote C.J., Diez A., Corraze G., Ariel J., Dias J., Kaushik S.J., Bautista J.M. (1998) Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). Br. J. Nutr. 80, 281–289.

Amin A.B., Trasti J. (1988) Endomyocarditis in Atlantic salmon in Norwegian seafarms. Bull. Eur. Assoc. Fish Pathol. 8, 70–71.

Anderson M.J. (2001) A new method for non–parametric multivariate analysis of variances. Aust. Ecol. 26, 32–46.

Ando S., Mori Y., Nakamura K., Sugawara A. (1993). Characteristics of lipid accumulation types in five species of fish. Nippon Suisan Gakk. 59, 1559–1564.

Armstrong J.B., Bond M.H. (2013) Phenotype flexibility in wild fish: Dolly Varden regulate assimilative capacity to capitalize on annual pulsed subsidies. J. Anim. Eco. 82, 966–975.

Armsworth P.R. (2001) Directed motion in the sea: efficient swimming by reef fish larvae. J. Theor. Biol. 210, 81–91.

Arts M., Brett M., Kainz M. (2009) Lipids in aquatic ecosystems. New York, USA: Springer.

Ashton I., Clements K., Barrow S.E., Secombes C.J., Rowley A.F. (1994) Effects of dietary fatty acids on eicosanoid-generating capacity, fatty acid composition and chemotactic activity of Rainbow trout (*Oncorhynchus mykiss)* leukocytes. Biochim. Biophys. Acta, Lipids Lipid Metab. 1214, 253–262.

Atkinson D. (1994) Temperature and organism size: a biological law for ectotherms? Adv. Ecol. Res. 25, l–58.

Auer S.K., Lopez-Sepulcre A., Heatherly T., Kohler T.J., Bassar R.D., Thomas S.A., Reznick D.N. (2012) Life histories have a history: effects of past and present conditions on adult somatic growth rates in wild Trinidadian guppies. J. Anim. Ecol. 81, 818–826.

Axelsson M., Altimiras J., Claireaux G. (2002) Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. J Exp. Biol. 205, 2891–2896.

Ayre K.J., Hulbert A.J. (1996) Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. J. Nutr. 126, 653–62.

Azevedo P.A., Cho C.Y., Leeson S., Bureau D.P. (1998) Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquat. Living Resour. 11, 227–238.

Bagnyukova T.V., Chahrak O.I., Lushchak V.I. (2006) Coordinated response of goldfish antioxidant defenses to environmental stress. Aquat. Toxicol. 78, 325–331.

Bagnyukova T.V., Luzhna L.I., Pogribny I.P., Lushchak V.I. (2007) Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. Environ. Mol. Mutagen. 48, 658–665.

Bain M.B., Bain. J.L. (1982) Habitat suitability index models: coastal stocks of striped bass. U.S. Fish and Wildlife Service, Office of Biological Services. Report FWS/OBS– 82/10.1., Washington, D.C., 29 p.

Barja G. (2007) Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuvenation Res. 10, 215–223.

Barnabé G. (1991) Grossissement des poissons en élevage intensif. Dans : Barnabé G. (ed) Bases biologiques et écologiques de l'aquaculture. Lavoisier-Tec et Doc, Paris, pp 422–451.

Beamish F.W.H. (1978) Swimming capacity. In : Hoar W.S. and Randall D.J. (eds) Fish Physiology. Vol. VII, Academic Press, New York, pp 101–187.

Beaugrand G., Reid P.C., Ibanez F., Lindley J.A., Edwards M. (2002) Reorganization of North Atlantic marine copepod biodiversity and climate. Science 296, 1692–94.

Beaulieu H. (1985) Rapport sur la situation du bar rayé (*Morone saxatilis*). Faune et flore à protéger au Québec, Association des biologistes du Québec. Publication n˚ 7, Québec, 53 p.

Beaulieu H., Trépanier S., Robitaille J.A. (1990) Statut des populations indigènes de bar rayé (*Morone saxatilis*) au canada. Ministère du Loisir, de la chasse et de la Pêche, direction générale des espèces et des habitats, Québec. Rapport technique, Québec, CA, 50 p.

Beck M.W., Heck K.L., Able K.W., Childers D.L., Egglston D.B., Gillanders B.M., Halpern B.S., Hayes C.G., Hoshino K., Minello T.J., Orth, R.J., Sheridan P., Weinstein M. (2001) The role of nearshore ecosystems as fish and shellfish nurseries. BioScience 51, 633–641.

Bell J.G., Ashton I., Secombes C.J., Weitzel B.R., Dick J.R., Sargent J.R. (1996) Dietary lipid affects phospholipid fatty acid compositions, ecosanoid production and immune function in Atlantic salmon (*Salmo salar*). Prostaglandin Leukot. Essent. Fat. Acids 54, 173–182.

Bell J.G., McEvoy J., Tocher D.R., McGhee F., Campbell P.J., Sargent J.R. (2001) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535–1543.

Bell J.G., Mcvicar A.H., Park M.T., Sargent J.R. (1991) High dietary linoleic acid affects the fatty acid compositions of individual phospholipids from tissues of Atlantic salmon (*Salmo salar*): association with stress susceptibility and cardiac lesion. J. Nutr. 121, 1163–1172.

Bell J.G., Tocher D.R., McDonald F.M., Sargent J.R. (1994) Effects of diets rich in linoleic (18:2n–6) and a-linolenic (18:3n–3) acids on the growth, lipid class and fatty acid composition and eicosaenoid production in juvenile turbot (*Scophthalmus maximus*). Fish Physiol. Biochem. 13, 105–118.

Bell M.V., Henderson J.R., Sargent J.R. (1986) The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol. B 83, 711–719.

Bellard C., Bertelsmeier C., Leadley P., Thuiller W., Courchamp F. (2012) Impacts of climate change on the future of biodiversity. Ecol. Lett. 15, 365–377.

Benoît H.P., Pepin P., Brown J.A. (2000) Patterns of metamorphic age and length in marine fishes, from individuals to taxa. Can. J. Fish. Aquat. Sci. 57, 856–869.

Bergmeyer H.U., Gawehn K., Grassi M. (1974) Enzymes as biochemical reagents. In : Bergmeyer H.U. (ed) Methods of Enzymatic Analysis. Academic Press, New York, pp 425–522.

Besson M., Vandeputte M., Van Arendonk J.A.M., Aubin J., De Boer I.J.M., Quillet E., Komen H. (2016) Influence of water temperature on the economic value of growth rate in fish farming: the case of sea bass (*Dicentrarchus labrax*) cage farming in the Mediterranean. Aquacult. 462, 47–55.

Bestion E., Teyssier A., Richard M., Clobert J., Cote, J. (2015) Live fast, die young: experimental evidence of population extinction risk due to climate change. PLoS Biol. 13, e1002281.

Bharadwaj A.S., Hard S.D., Brown B.J., Li Y., Watkins W.A., Brown P.B. (2010) Dietary source of stearidonic acid promotes higher muscle DHA concentrations than linolenic acid in hybrid striped bass. Lipids 45, 21–27.

Bhouri A.M., Bouhlel I., Chouba L., Hammami M., El Cafsi M., Chaouch A. (2010) Total lipid content, fatty acid and mineral compositions of muscles and liver in wild and farmed sea bass (*Dicentrarchus labrax*). Afr. J. Food Sci. 4, 522–530.

Blaxter J.H.S. (1992) The effect of temperature on larval fishes. Neth. J. Zool. 42, 336–357.

Boulineau-Coatanea F. (1969) Régime alimentaire du bar (*Dicentrarchus labrax*, *Serranidae*)-Sur la côte atlantique bretonne. Bull. Mus. Hist. Nat 2, 1106–1122.

Boynton W.R., Zion H.H., Polgar T.T. (1981) Importance of juvenile striped bass food habits in the Potomac estuary. Trans. Am. Fish. Soc. 110, 56–63.

Bradshaw A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13, 115–155.

Brander K.M. (2007) Global fish production and climate change. PNAS 104, 19709–19714.

Brett J.R. (1964) The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Board Canada 21, 1183–1226.

Brett M.T., Muller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biol. 38, 483–499

Bronfman M., Inestrosa N.C., Leighton F. (1979) Fatty acid oxidation by human liver peroxisomes. Biochem. biophys. Res. Commun. 88, 1030–1036.

Bruno D.W., Poppe T.T. (1996) A colour atlas of salmonid diseases. London : Academic Press.

Brzek P., Bielawska K., Ksiazek A., Konarzewski M. (2007) Anatomic and molecular correlates of divergent selection for basal metabolic rate in laboratory mice. Physiol. Biochem. Zool. 80, 491–9.

Buzzi M., Henderson R.J., Sargent J.R. (1996) The desaturation and elongation of linolenic acid and ecosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. Biochem. Biophys. Acta 1299, 235–244.

Buzzi M., Henderson R.J., Sargent J.R. (1997) Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24–carbon intermediates. Comp. Biochem. Physiol. 116B, 263–267.

Caballero M.J., Izquierdo M.S., Kjørsvik E., Montero D., Socorro J., Fernández A.J., Rosenlund G. (2003) Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. Aquacult. 225, 325–340.

Caballero M.J., Obach A., Rosenlund G., Montero D., Gisvold M., Izquierdo M.S. (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquacult. 214, 253–271.

Cahu C., Infante J.-Z., Takeuchi T. (2003) Nutritional components affecting skeletal development in fish larvae. Aquacult. 227, 245–258.

Calabretti A., Cateni F., Procida G., Favretto L.G. (2003) Influence of environmental temperature on composition of lipids in edible flesh of rainbow trout (*Oncorhynchus mykiss*). J. Sci. Food Agric. 83, 1497–1498.

Castell J.D., Sinnhuber R.O., Wales J.H., Lee J.D. (1972a) Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. J. Nutr. 102, 77–86.

Castell J.D., Sinnhuber R.O., Wales J.H., Lee J.D. (1972b) Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): physiological symptoms of EFA deficiency. J. Nutr. 102, 87–92.

Charnov E.L. (1993) Life history invariants: some explorations of symmetry in evolutionary ecology (Vol. 6). USA : Oxford University Press.

Chatelier A., McKenzie D.J., Claireaux G. (2005) Effects of changes in water salinity upon exercise and cardiac performance in the European seabass (*Dicentrarchus labrax*). Mar. Biol. 147, 855–862.

Chatelier A., McKenzie D.J., Prinet A., Galois R., Robin J., Zambonino J., Claireaux G. (2006) Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (*Dicentrarchus labrax*). J. Exp. Biol. 209, 3429–3439.

Cheek T.E., Van Den Avyle M.J., Coutant C.C. (1985) Influences of water quality on distribution of striped bass in a Tennessee River impoundment. Trans. Am. Fish. Soc. 114, 67–76.

Chen J.H, Hales C.N, Ozanne S.E. (2007) DNA damage, cellular senescence and organismal ageing: causal or correlative? Nucleic Acids Res. 35, 7417–7428.

Chen Y-C. (2012) The biomass and total lipid content and composition of twelve species of marine diatoms cultured under various environments. Food Chem. 131, 211–219.

Cheung W.W.L., Lam V.W.Y., Pauly D. (2008) Modelling present and climate-shifted distribution of marine fishes and invertebrates. Fisheries Centre Research Report 16, University of British Columbia, Vancouver, CA, 76 p.

Cheung W.W.L., Pauly D., Sarmiento J.L. (2013) How to make progress in projecting climate change impacts. ICES J. Mar. Sci. 70, 1069–1074.

Cheverud J.M. (1982) Relationships among ontogenetic, static, and evolutionary allometry. Am. J. Phys. Anthr. 59, 139–149.

Chezik K.A., Lester N.P., Venturelli P.A. (2013) Fish growth and degree-days I: selecting a base temperature for a within–population study. Can. J. Fish. Aquat. Sci. 71, 47–55.

Childress J.J., Somero G.N. (1979) Depth-related enzymatic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Mar. Biol. 52, 273–283.

Cho C.Y., Bureau D.P. (1998) Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. Aquat. Living Resour. 11, 199–210.

Claireaux G., Couturier C. Groison A.-L. (2006) Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). J. Exp. Biol. 209, 3420–3428.

Claireaux G., Lagardère J.P. (1999) Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J. Sea Res. 42, 157–168.

Colombo S.M., Wacker A., Parrish C.C., Kainz M.J., Arts M.T. (2016) A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. Environ. Rev. 25, 163–174.

Comité aviseur sur la réintroduction du bar rayé (2001) Plan d'action pour la réintroduction du bar rayé (*Morone saxatilis*) dans l'estuaire du Saint-Laurent. Société de la faune et des parcs du Québec, Direction du développement de la faune, Document de travail 41, Québec, CA, 7 p.

Cook A.M., Duston J., Bradford R.G. (2010) Temperature and salinity effects on survival and growth of early life stage Shubenacadie River striped bass. Trans. Am. Fish. Soc. 139, 749–757.

Cook J., Oreskes N., Doran P.T., Anderegg W.R., Verheggen B., Maibach E.W., Carlton J.S., Lewandowsky S., Skuce A.G., Green S.A., Nuccitelli D., Jacobs P., Richardson M., Winkler B., Painting R., Rice K. (2016) Consensus on consensus: a synthesis of consensus estimates on human-caused global warming. Environ. Res. Lett. 11, 048002.

COSEPAC (2012) Évaluation et Rapport de situation du COSEPAC sur le bar rayé (*Morone saxatilis*) au Canada. Comité sur la situation des espèces en péril au Canada, Ottawa, 86 p.

Cosgrove J.P., Church D.F., Pryor W.A. (1987) The kinetics of the autoxidation of polyunsaturated fatty acids. Lipids 22, 299–304.

Cross H.Z., Zuber M.S. (1972) Prediction of flowering dates in maize based on different methods of estimating thermal units. Agron. J. 64, 351–355.

Cushing D. (1995) Population Production and Regulation in the Sea: A Fishery Perspective. Cambridge, UK: Cambridge University Press.

Darimont C.T., Carlson S.M., Kinnison M.T., Paquet P.C., Reimchen T.E., Wilmers C.C. (2009) Human predators outpace other agents of trait change in the wild. PNAS 106, 952–954.

deYoung B., Harris R., Alheit J., Beaugrand G., Mantua N., Shannon L. (2004) Detecting regime shifts in the ocean: data considerations. Prog. Oceanogr. 60, 143–64.

Diamond J., Hammond K.A. (1992) The matches, achieved by natural selection, between biological capacities and their natural loads. Experientia 48, 551–557.

Diamond J.M. (1998) Evolution of biologic safety factors: a cost/benefit analysis. In : Weibel E., Taylor C.R., Bolis L. (eds) Principles of animal design. Cambridge University Press, Cambridge, pp 21–27.

Diana J.S. (2009). Aquaculture production and biodiversity conservation. Bioscience 59, 27–38.

Dias J., Alvarez M.J., Diez A., Arzel J., Corraze G., Bautista J.M., Kaushik S.J. (1998) Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European seabass (*Dicentrarchus labrax*). Aquacult. 161, 169–186.

Drazen J.C. (2002) A seasonal analysis of the nutritional condition of deep-sea macrourids fishes in the north–east Pacific. J. Fish Biol. 60, 1280–1295.

Drew M.D., Ogunkoya A.E., Janz D.M., Van Kessel A.G. (2007) Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout (*Oncorhynchus mykiss*). Aquacult. 267, 260–268.

Dumas A., France J., Bureau D. (2010) Modelling growth and body composition in fish nutrition: where have we been and where are we going? Aquacult. Res. 41, 161–181.

Duston J., Astatkie T., MacIsaac P.F. (2004) Effect of body size on growth and food conversion of juvenile striped bass reared at 16-28 C in freshwater and seawater. Aquacult. 234, 589–600.

Edwards M., Richardson A.J. (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430, 881–884.

Egginton S. (1996) Effect of temperature on optimal substrate for β-oxidation. J. Fish Biol. 49, 753–758.

Eldho N.V., Feller S.E., Tristram-Nagle S., Polozov I. V., Gawrisch K. (2003) Polyunsaturated docosahexaenoic vs docosapentaenoic acid-differences in lipid matrix properties from the loss of one double bond. J Am. Chem. Soc. 125, 6409 – 6421.

Elia M. (1992) Organ and Tissue contribution to metabolic rate. In : Kinney J. and Tucker H. (eds) Energy Metabolism: Tissues Determinants and Cellular Corollaries. Raven Press, New York, pp 61–79.

Eliassen J.E., Vahl O. (1982). Seasonal variations in biochemical composition and energy content of liver, gonad and muscle of mature and immature cod, *Gadus morhua* (L.) from Balsfjorden, northern Norway. J. Fish Biol. 20, 707–716.

Eroldoğan O.T., Kumlu M., Aktaş M. (2004) Optimum feeding rates for European sea bass *Dicentrarchus labrax* L. reared in seawater and freshwater. Aquacult. 231, 501–515.

Evans D.H., Piermarini P.M., Choe K.P. (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.

Fahy E., Subramaniam S., Brown H.A., Glass C.K., Merrill A.H. Jr., Murphy R.C., Raetz C.R., Russell D.W., Seyama Y., Shaw W., Shimizu T., Spener F., van Meer G., VanNieuwenhze M.S., White S.H., Witztum J.L., Dennis E.A. (2005) A comprehensive classification system for lipids. J. Lipid Res. 46, 839–861.

FAO (Food and gricultural organisation) (2012) The State of World Fisheries and Aquaculture Fisheries and Aquaculture. Report I2727, FAO fisheries and aquaculture department, Rome, 230 p.

Farrell A.P., Thorarensen H., Axelsson M., Crocker C.E., Gamperl A.K., Cech J.J. (2001) Gut blood flow in fish during exercise and severe hypercapnia. Comp. Biochem. Physiol. Part A 128, 551–563.

Ferguson H.W., Poppe T., Speare D.J. (1990) Cardiomyopathy in farmed Norwegian salmon. Dis. Aquat. Org. 8, 225–231.

Fernandez E., Balch W.B., Maranon E., Holligan P.M. (1994) High rates of lipid biosynthesis in cultured, mesocosm and coastal populations of the coccolitophore *Emiliana huxleyi*. Mar. Ecol. Prog. Ser. 114, 13–22.

Figueiredo-Silva A.C., Corraze G., Rema P., Sanchez-Gurmaches J., Gutiérrez J., Valente L.M.P. (2009) Blackspot seabream (*Pagellus bogaraveo*) lipogenic and glycolytic pathways appear to be more related to dietary protein level than dietary starch type. Aquacult. 291, 101–110.

Finkel T. (1998) Oxygen radicals and signaling. Curr. Opin. Cell Biol. 10, 248–253.

Fisher R., Wilson S. (2004) Maximum sustainable swimming speeds of late-stage larvae of nine species of reef fishes. J. Exp. Mar. Biol. Ecol. 312, 171–186.

Fisher R., Wilson S. (2004) Maximum sustainable swimming speeds of late-stage larvae of nine species of reef fishes. J. Exp. Mar. Bio. Ecol. 312, 171–186.

Fitzgibbon Q.P., Strawbridge A., Seymour R.S. (2007) Metabolic scope, swimming performance and the effects of hypoxia in the mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae). Aquacult. 270, 358–368.

Folch J., Lees M., Sloane-Standley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 276, 497–507.

Fountoulaki E., Vasilaki A., Hurtado R., Grigorakis K., Karacostas I., Nengas I., Rigos G., Kotzamanis Y., Venou B., Alexis M.N. (2009) Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile: recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquacult. 289, 317–326.

Frankel E.N. (2005) Lipid oxidation. Bridgewater, UK: Elsevier.

Frayn K.N. (2010) Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. Acta Physiol. 199, 509–518.

Freyhof J., Kottelat M. (2008) *Dicentrarchus labrax*. The IUCN Red List of Threatened Species 2008: e.T135606A4159287. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T135606A4159287.en. Downloaded on 26 October 2015.

Gaillard J.M., Pontier D., Allaine D., Lebreton J.D., Trouvilliez J., Clobert J. (1989) An analysis of demographic tactics in birds and mammals. Oikos, 59–76.

Gallagher D., Belmonte D., Deurenberg P., Wang Z., Krasnow N., Pi-Sunyer F.X. et al. (1998) Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. Am. J. Physiol. 275, E249–E258.

Ganga R., Bell J.G., Montero D., Robaina L., Caballero M.J., Izquierdo M.S. (2005) Effect of dietary lipids on plasma fatty acid profiles and prostaglandin and leptin production in gilthead seabream (*Sparus aurata*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 142, 410–8.

Gardinier M.N., Hoff T.B. (1982) Diet of striped bass in the Hudson River Estuary [*Morone saxatilis*, New York]. New York Fish and Game J.

Gattuso J.P., Magnan A., Billé R., Cheung W.W.L., Howes E.L., Joos F., Allemand D., Bopp L., Cooley S.R., Eakin C.M., Hoegh-Guldberg O., Kelly R.P., Pörtner H.-O., Rogers A.D., Baxter J.M., Laffoley D., Osborn D., Rankovic A., Rochette J., Sumaila U.R., Treyer S., Turley C. (2015) Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. Science 349, aac4722.

Gaylord B., Kroeker K.J., Sunday J.M., Anderson K.M., Barry J.P., Brown N.E., Connell S.D., Dupont S., Fabricius K.E., Hall-Spencer J.M., Klinger T., Milazzo M., Munday P.L., Russell B.D., Sanford E., Schreiber S.J., Thiyagarajan V., Vaughan M.L.H., Widdicombe S., Harley C.D.G. (2015). Ocean acidification through the lens of ecological theory. Ecology 96, 3–15.

Geay F., Culi E.S.I., Corporeau C., Boudry P., Dreano Y., Corcos L., Bodin N., Vandeputte M., Zambonino-Infante J.-L, Mazurais D., et al. (2010) Regulation of FADS2 expression and activity in European sea bass (*Dicentrarchus labrax*, L.) fed a vegetable diet. Comp. Biochem. Physiolog. B 156, 237–243.

Geay F., Zambonino-Infante J.-L., Reinhardt R., Kuhl H., Santigosa E., Cahu C., Mazurais D. (2012) Characteristics of fads2 gene expression and putative promoter in European sea bass (*Dicentrarchus labrax*): Comparison with salmonid species and analysis of CpG methylation. Mar. Genomics 5, 7–13.

Gechev T.S., Van Breusegem F., Stone J.M., Denev, I., Laloi C. (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssay 28, 1091–1101.

Genner M.J., Halliday N.C., Simpson S.D., Southward A.J., Hawkins S.J., Sims D.W. (2010) Temperature-driven phenological changes within a marine larval fish assemblage. J. Plankton Res. 32, 699–708.

Ghioni C., Tocher D.R., Bell M.V., Dick J.R., Sargent J.R. (1999) Low C18 to C20 fatty acid elongase activity and limited conversion of stearidonic acid, 18:4n-3, to eicosapentaenoic acid, 20:5n-3, in a cell line from the turbot, *Scophthalmus maximus*. Biochim. Biophys. Acta 1437, 170–181.

GIEC (Groupe d'experts intergouvernemental sur l'évolution du climat) (2014) Changements climatiques 2014: Rapport de synthèse. Contribution des Groupes de travail I, II et III au cinquième Rapport d'évaluation du Groupe d'experts intergouvernemental sur l'évolution du climat [Sous la direction de l'équipe de rédaction principale, R.K. Pachauri et L.A. Meyer]. Genève, Suisse, 161 p.

Gillooly J.F., Brown J.H., West G.B., Savage V.M., Charnov E.L. (2001) Effects of size and temperature on metabolic rate. Science 293, 2248–2251.

Gilmore EC. Jr., Rogers J.S. (1958) Heat units as a method of measuring maturity in corn. Agron. J. 50, 611–615.

Gislason H., Daan N., Rice J.C., Pope J.G. (2010) Size, growth, temperature and the natural mortality of marine fish. Fish and Fish. 11, 149–158.

Glencross B.D. (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquacult. 1, 71–124.

Glencross B.D., Hawkins W.E., Curnow J.G. (2003) Restoration of the fatty acid composition of red seabream (*Pagrus auratus*) using a fish oil finishing diet after grow-out on plant oil based diets. Aquacult. Nutr. 9, 409–418.

Gómez F., Souissi S. (2008) The impact of the 2003 summer heat wave and the 2005 late cold wave on the phytoplankton in the north-eastern English Channel. C. R. Biol. 331, 678–685.

Gomez-Requeni P., Mingarro M., Kirchner S., Calduch-Giner J.A., Medale F., Corraze G., Panserat S., Martin S.A.M., Houlihan D.F., Kaushik S.J., et al. (2003) Effects of dietary amino acid profile on growth performance, key metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream (*Sparus aurata*). Aquacult. 220, 749–767.

González-Rovira A., Mourente G., Zheng X., Tocher D.R., Pendón C. (2009) Molecular and functional characterization and expression analysis of a Δ6 fatty acyl desaturase cDNA of European sea bass (*Dicentrarchus labrax* L.). Aquacult. 298, 90–100.

Gourtay C., Chabot D., Audet C., Le Delliou H., Quazuguel P., Claireaux G., Zambonino-Infante J.-L. (2018) Will global warming affect the functional need for essential fatty acids in juvenile sea bass (*Dicentrarchus labrax*)? A first overview of the consequences of lower availability of nutritional fatty acids on growth performance. Mar. Biol. 165, https://doi.org/10.1007/s00227–018–3402–3.

Gracia-Lopez V., Rosas-Vazquez C., Brito-Perez R. (2006) Effects of salinity on physiological conditions in juvenile common snook *Centropomus undecimalis*. Com. Biochem. Physiol. A Mol. Integr. Physiol. 145, 340–345.

Green B.S., Fisher R. (2004) Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. J. Exp. Mar. Biol. Ecol. 299, 115–132.

Guschina I.A., Harwood J.L. (2006) Mechanisms of temperature adaptation in poikilotherms. FEBS Lett. 580, 5477–5483.

Halliwell B., Gutteridge J.M.C. (2007) Free Radicals in Biology and Medicine (4th ed). Oxford, UK: Clarendon Press.

Hammer C. (1995) Fatigue and exercise tests with fish. Comp. Biochem. Physiol. A Physiol. 112, 1–20.

Hansen J., Sato M., Ruedy R., Lo K., Lea D.W., Medina-Elizade M. (2006) Global temperature change. PNAS 103, 14288–14293.

Harel M., Place A.R. (2003) Tissue essential fatty acid composition and competitive response to dietary manipulations in white bass (*Morone chrysops*), striped bass (*M. saxatilis*), and hybrid striped bass (*M. chrysops* × *M. saxatilis*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 135, 83–94.

Harfmart K.J., Brandt S.B. (1995) Predatory demand and impact of striped bass, bluefish, and weakfish in the Chesapeake Bay: applications of bioenergetics models. Can. J. Fish. Aqua. Sci. 52, 1667–1687.

Harley C.D.G., Hughes A.R., Hultgren K.M., Miner B.G., Sorte C.J.B., Thornber C.S., Rodriguez L.F., Tomanek L., Williams S.L. (2006) The impacts of climate change in coastal marine systems. Ecol. Letters 9, 228–241.

Hays G.C., Richardson A.J., Robinson C. (2005) Climate change and marine plankton. Trends Ecol. Evol. 20, 337–44.

Hazel J.R., McKinley S.J., Williams E.E. (1992) Thermal adaptation in biological membranes: interacting effects of temperature and pH. J. Comp. Physiol. 162B, 593–601.

Heise K., Puntarulo S., Nikinmaa M., Abele D., Pörtner H.O. (2006a) Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparous*. L. J. Exp. Biol. 209, 353–363.

Heise K., Puntarulo S., Nikinmaa M., Lucassen M., Pörtner H.O., Abele D. (2006b) Oxidative stress and HIF–1 DNA binding during stressful cold exposure and recovery in the North Sea eelpout (*Zoarces viviparus*). Comp. Biochem. Physiol. A 143, 494–503.

Henderson R.J. (1996) Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5–22.

Henderson R.J., Sargent J.R. (1985) Chain-length specificities of mitonchondrial and peroxisomal β-oxidation of fatty acids in livers of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 82B, 79–85.

Hidalgo F., Alliot E. (1988) Influence of water temperature on protein requirement and protein utilization in juvenile sea bass, *Dicentrarchus labrax*. Aquacult. 72, 115–129.

Higgs D.A., Dong F.M. (2000) Lipids and fatty acids. In : Stickney R.R. (ed) Aquaculture Encyclopedia. Wiley, New York, pp 476–496.

Hixson S.M., Arts M.T. (2016) Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. Global Change Biol. 22, 2744–2755.

Hodson R.G. (1990) Hybrid striped bass biology and life history. Leaflet n^{o} 2416, Texas agricultural extension service. Texas, USA, 1 p.

Hoffmayer E.R., Parsons G.R., Horton J. (2006) Seasonal and interannual variation in the energetic condition of adult male Atlantic sharpnose shark *Rhizoprionodon terraenovae* in the northern Gulf of Mexico. J. Fish Biol. 68, 645–653.

Holden M.J., Williams T. (1974) The biology, movements and populations dynamics of bass, *Dicentrarchus labrax*, in English waters. J. Mar. Biol. Assoc. UK 54, 91–107.

Holman R.T. (1954) Autoxidation of fats and related substances. Prog. Chem. Fats Other Lipids 2, 51–98.

Houde E.D. (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish. Bull. 87, 471–495.

Houde E.D. (2008) Emerging from Hjort's shadow. J. Northwest Atl. Fish. Sci. 41, 53–70.

Hryb D.J., Hogg J.F. (1979) Chain, length specificities of peroxisomal and mitochondrial β-oxidation in rat liver. Biochem. biophys. Res. Commun. 87, 1200–1206.

Hu Q., Sommerfeld M., Jarvis E., Ghirardi M., Posewitz M., Seibert M., Darzins A. (2008) Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. Plant J. 54, 621–639.

Huang C.-H., Lin W.-L., Chu J.-H. (2005) Dietary lipid level influences fatty acid profiles, tissue composition, and lipid peroxidation of soft-shelled turtle, *Pelodiscus sinensis*. Comp. Biochem. Physiol. A 142, 383–8.

Huggett R.J. (2012) Climate, earth processes and earth history. Berlin : Springer–Verlag.

Hulbert A.J. (2005) On the importance of fatty acid composition of membranes for aging. J. Theor. Biol. 234, 277–288.

Hulbert A.J., Else P.L. (2005) Membranes and the setting of energy demand. J. Exp. Biol. 208, 1593–9.

Hulbert A.J., Pamplona R., Buffenstein R., Buttemer W.A. (2007) Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87, 1175–1213.

Hulbert A.J., Turner N., Storlien L.H., Else P.L. (2005) Dietary fats and membrane function: implications for metabolism and disease. Biol. Rev. 80, 155–69.

Hurst T.P., Conover D.O. (2001) Diet and consumption rates of overwintering YOY striped bass, *Morone saxatilis*, in the Hudson River. Fish. Bull. 99, 545–554.

Huxley J.S., Tessier G. (1936) Terminology of relative growth. Nature 137, 780–781.

Hwang P.P., Lee T.H. (2007) New insights into fish ion regulation and mitochondrionrich cells. Comp. Biochem. Physiol. A 148, 479–497.

ICES 2018. WGCSE Report, Section 10 :1, Sea bass in IVbc and VIIa,d– h (North Sea, Channel, Celtic Sea and Irish Sea). ICES, UK, pp 1256–1337.

Itazawa Y., Oikawa S. (1986) A quantitative interpretation of the metabolism-size relationship in animals. Experientia 42,1985–1986.

Iwama G.K., Pickering A.D., Sumpter J.P., Schreck C.B. (1997) Fish stress and health in aquaculture. Cambridge : Cambridge University Press.

Iwama G.K., Tautz A.F. (1981) A simple growth model for salmonids in hatcheries. Can. J. Fish Aquat. Sci. 38, 649–656.

Izquierdo M.S. (1996) Essential fatty acid requirements of cultured marine fish larvae. Aquacult. Nutr. 2, 183–191.

Jackson J.B.C., Kirby M.X., Berger W.H., Bjorndal K.A., Botsford L.W., Bourque B.J., Bradbury R.H., Cooke R., Erlandson J., Estes J.A., Hughes T.P., Kidwell S., Lange C.B., Lenihan H.S., Pandolfi J.M., Peterson C.H., Steneck R.S., Tegner M.J., Warner R.R. (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293, 629–637.

Jassby A.D., Kimmerer W.J., Monismith S.G., Armor C., Cloern J.E., Powell T.M., Schubel J.R., Vendlinski T.J. (1995) Isohaline position as a habitat indicator for estuarine populations. Ecol. Appl. 5, 272–289.

Jobling M. (1983) Growth studies with fish-overcoming the problem of size variation. J. Fish Biol. 22, 153–157.

Johansen J.L., Jones G.P. (2011) Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. Global Change Biol. 17, 2971–2979.

Johansen J.L., Messmer V., Coker D.J., Hoey A.S., Pratchett M.S. (2014) Increasing ocean temperatures reduce activity patterns of a large commercially important coral reef fish. Global Change Biol. 20, 1067–1074.

Johnston I.A., Vieira V.L.A., Temple G.K. (2001) Functional consequences population differences in the developmental plasticity of muscle to temperature in Atlantic herring *Clupea harengus*. Mar. Ecol. Prog. Ser. 213, 285–300.

Jones O.R., Gaillard J.M., Tuljapurkar S., Alho J.S., Armitage K.B., Becker P.H., Bize P., Brommer J., Charmantier A., Charpentier M., Clutton-Brock T., Dobson F.S., Festa–Bianchet M., Gustafsson L., Jensen H., Jones C.G., Bo-Goran L., McCleery L., Merila J., Neuhaus P., Nicoll M.A.C., Norris K., Oli M.K.,Pemberton J., Pietia H., Petiainen H., Ringsby T.H., Roulin A., Saether B.E., Setchell J.M., Sheldon B.C., Thompson P.M., Weimerskirch H., Wickings E.J., Coulson T. (2008) Senescence rates are determined by ranking on the fast-slow life-history continuum. Ecol. Lett. 11, 664–673.

Juanes, F. (2007) Role of habitat in mediating mortality during the post–settlement transition phase of temperate marine fishes. J. Fish Biol. 70, 661–677.

Juliano R.A., Deckelbaum R.J., Worgall T.S. (2004) Fatty acids, cholesterol, and sphingolipids: interactive regulators and targets for SREBP processing. Int. Congr. 1262, 55–58.

Jump D.B. (2002) The biochemistry of n–3 polyunsaturated fatty acids. J. Biol. Chem. 277, 8755–8758.

Kainz M., Arts M.T., Mazumder A. (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol. Oceanogr. 49, 1784–1793.

Kalogeropoulos N., Alexis M.N., Henderson R.J. (1992) Effects of dietary soybean and cod–liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). Aquacult. 104, 293–308.

Kaplan E.L., Meier P. (1958) Nonparametric–estimation from incomplete observations. J. Am. Stat. Assoc. 53, 457–481.

Karasov W.H., Martinez del Rio C. (2007) Physiological ecology: how animals process energy, nutrients and toxins. USA : Princeton University Press.

Kaushik S.J. (1998) Nutritional bioenergetics and estimation of waste production in non–salmonids. Aquat. Living Resour. 11, 211–217.

Kawatsu H. (1969) Studies on the anemia of fish – III. An example of macrocytic anemia found in brook trout, *Salvelinus fontinalis*. Bull. Freshwater Res. Lab. 19, 161–167.

Kelley D.F. (1988) The importance of estuaries for sea bass *Dicentrarchus labrax* (L.). J. Fish Biol. 33, 25–33.

Kellogg R.L., Gift J.J. (1983) Relationship between optimum temperatures for growth and preferred temperatures for the young of four fish species. Trans. Am. Fish. Soc. 112, 424–430.

Kennedy M., Fitzmaurice P. (1972) The biology of bass, *Dicentrarchus labrax*, in Irish waters. J. Mar. Biol. Assoc. UK 52, 557–597.

Killen S.S., Atkinson D., Glazier D.S. (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecol. Lett. 13, 184–193.

Kiron V., Fukuda H., Fukuda H., Takeuchi T., Watanabe T. (1995) Essential fatty acid nutrition and defense mechanisms in rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol. 111, 361–367.

Kiron V., Thawonsuwan J., Panigrahi A., Scharsack J.P., Satoh S. (2011) Antioxidant and immune defences of rainbow trout (*Oncorhynchus mykiss*) offered plant oils differing in fatty acid profiles from early stages. Aquacult. Nutr. 17, 130–140.

Klepper B., Belford R.K., Rickman R.W. (1984) Root and shoot development in winter wheat. Agron. J. 76, 117–122.

Kolkovski S., Lazo J., Leclercq D., Izquierdo M. (2009) Fish larvae nutrition and diet: new developments. In : New Technologies in Aquaculture, pp 315–369.

Kolok A.S., Sharkey D. (1997) Effect of freshwater acclimation on the swimming performance and plasma osmolarity of the euryhaline gulf killifish. Trans. Am. Fish. Soc. 126, 866–870.

Koven W., van Anholt R., Lutzky S., Atia I.B., Nixon O., Ron B., Tandler A. (2003) The effect of dietary arachidonic acid on growth, survival, and cortisol levels in different-age gilthead seabream larvae (*Sparus auratus*) exposed to handling or daily salinity change. Aquacult. 228, 307–320.

Kozłowski J., Czarnołęski M., Dańko M. (2004) Can optimal resource allocation models explain why ectotherms grow larger in cold? Integr. Comp. Biol. 44, 480–493.

Krebs H.A. (1950) Body size and tissue respiration. Biochim. Biophys. Acta 4, 249–269.

Kwon E.Y., Primeau F., Sarmiento J.L. (2009) The impact of remineralization depth on the air-sea carbon balance. Nat. Geosci. 2, 630–635.

Lande M.B., Donovan J.M., Zeidel M.L. (1995) The relationship between membrane fluidity and permeabilities to water, solutes, ammonia, and protons. J. Gen. Physiol. 106, 67–84.

Lang I., Hodac L., Friedl T., Feussner I. (2011) Fatty acid profiles and their distribution patterns in microalgae: A comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biol. 11, 1–16.

Lange U., Greve W. (1997) Does temperature influence the spawning time, recruitment and distribution of flatfish via its influence on the rate of gonadal maturation? Dt. Hydrogr. Z. 49, 251–263.

Lapointe D., Vogelbein W.K., Fabrizio M.C., Gauthier D.T., Brill R.W. (2014) Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass *Morone saxatilis* metabolic performance. Dis. Aquat. Organ. 108, 113–127.

Lavergne S., Mouquet N., Thuiller W., Ronce O. (2010) Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. Annu. Rev. Ecol. Evol. Syst. 41, 321–350.

Leclerc G.M., Han K., Leclerc G.J., Ely B. (1999) Characterization of a highly repetitive sequence conserved among the North American Morone species. Mar. Biotech. 1, 122–130.

Lehodey P., Alheit J, Barange M., Baumgartner T., Beaugrand G., Drinkwater K., Fromentin J.M., Hare S.R., Ottersen G., Perry R.I., Roy C., Van der Lingen C.D., Werner F. (2006) Climate variability, fish, and fisheries. J. Climate 19, 5009–5030.

Lesser M.P. (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol. 68, 253–278.

Leu E., Wängberg S.Å., Wulff A., Falk-Petersen S., Ørbæk J.B., Hessen D.O. (2006) Effects of changes in ambient PAR and UV radiation on the nutritional quality of an Arctic diatom (*Thalassiosira antarctica* var. *borealis*). J. Exp. Mar. Biol. Ecol. 337, 65–81.

Lie O., Hemre G.I., Lambertsen G. (1992) Influence of dietary fatty acids on the glycerophospholipid composition in organs of cod (*Gadus morhua*). Lipids 27, 770–775.

Lindi V., Schwab U., Louheranta A., Laakso M., Vessby B., Hermansen K., Storlien L., Riccardi G., Rivellese A.A., Matti I.J. (2003) Impact of the Pro12Ala polymorphism of the PPAR–γ2 gene on serum triacylglycerol response to n-3 fatty acid supplementation. Mol. Genet. Metab.79, 52–60.

Lloret J., Demestre M., Sánchez-Pardo J. (2008) Lipid (energy) reserves of European hake (*Merluccius merluccius*) in the North-western Mediterranean. Vie et Milieu 58, 75–85.

Los D.A., Murata N. (2004) Membrane fluidity and its roles in the perception of environmental signals. Biochim. Biophys. Acta 1666, 142 – 157.

Lowe C.J., Davison W. (2005) Plasma osmolarity, glucose concentration and erythrocyte responses of two Antarctic nototheniid fishes to acute and chronic thermal change. J. Fish Biol. 67, 752–766.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luo Z., Tan X.Y., Li X.D., Yin G.J. (2012) Effect of dietary arachidonic acid levels on growth performance, hepatic fatty acid profile, intermediary metabolism and antioxidant responses for juvenile *Synechogobius hasta*. Aquacult. Nutr. 18, 340–348.

Lushchak V.I., Bagnyukova T.V. (2006) Effects of different environmental oxygen levels on free radical processes in fish. Comp. Biochem. Physiol. B 144, 283–289.

MacArthur R.H., Wilson E.O. (2001) The theory of island biogeography (Vol. 1). USA : Princeton University Press.

MacLaren L.A., Guzeloglu A., Michel F., Thatcher W.W. (2006) Peroxisome proliferator–activated receptor (PPAR) expression in cultured bovine endometrial cells and response to omega-3 fatty acid, growth hormone and agonist stimulation in relation to series 2 prostaglandin production. Domest. Anim. Endocrin. 30, 155–169.

Magnoni L.J., Patterson D.A., Farrell A.P., Weber J.-M. (2006) Effects of long-distance migration on circulating lipids of sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 63, 1822–1829.

Mantel, N. (1966). Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer. Chemother. Rep. 50, 163–170.

Martino R.C., Cyrino J.E.P., Portz L., Trugo L.C. (2002) Performance and fatty acid composition of surubin (*Pseudoplatystoma coruscans*) fed diets with animal and plant lipids. Aquacult. 209, 233–246.

Marty Y., Delaunay F., Moal J., Samain J.-F. (1992) Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. J. Exp. Mar. Biol. Ecol. 163, 221–234

Mauduit F., Domenici P., Farrell A.P., Lacroix C., Le Floch S., Lemaire P., Nicolas-Kopec A., Whittington M., Zambonino-Infante J.-L., Claireaux G. (2016). Assessing chronic fish health: An application to a case of an acute exposure to chemically treated crude oil. Aqua. Toxicol. 178, 197–208.

McGill B.J., Enquist B.J., Weiher E., Westoby M. (2006) Rebuilding community ecology from functional traits. Trends Ecol. Evol. 21, 178–185.

McKenzie D.J. (2001) Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. Comp. Biochem. Physiol. A 128, 607–621.

McKenzie D.J., Higgs D.A., Dosanjh B.S., Deacon G., Randall D.J. (1998) Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. Fish Physiol. Biochem. 19, 111–122.

McMaster G.S. (1993) Another wheat (*Triticum* spp.) model? Progress and applications in crop modeling. Riv. Agron. 27, 264–272.

McMaster G.S., Smika D.E., (1988) Estimation and evaluation of winter wheat phenology in the central Great Plains. Agric. For. Meteorol. 43, 1–18.

Miller P., Lanier W., Brandt S. (2001) Using growing degree days to predict plant stages. Extension communications coordinator, communications Services, Montana State University–Bozeman, 6 p.

Millikin M.R. (1982) Qualitative and quantitative requirements of fishes: a review. Fish. Bull. 80, 655–686.

Montero D., Kalinowski T., Obach A., Robaina L., Tort L., Caballero M.J., Izquierdo M.S. (2003) Vegetable lipid sources for gilthead sea bream (*Sparus autata*): effects on fish health. Aquacult. 225, 353–370.

Montero D., Robaina L., Caballero M.J., Gines R., Izquierdo M.S. (2005) Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time course study on the effect of a re–feeding period with 100% fish oil diet. Aquacult. 248, 121–134.

Morgan R.P., Rasin Jr. V.J., Copp R.L. (1981) Temperature and salinity effects on development of striped bass eggs and larvae. Trans. Am. Fish. Soc. 110, 95–99.

Mouillot D., Graham N.A., Villéger S., Mason N.W., Bellwood D.R. (2013) A functional approach reveals community responses to disturbances. Trends Ecol. Evol. 28, 167–177.

Mourente G., Bell J.G. (2006) Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. Comp. Biochem. Physiol. B 145, 389–399.

Mourente G., Dick J.R. (2002) Influence of partial substitution of dietary fish oil by vegetable oils on the metabolism of [1-14C] 18:3n-3 in isolated hepatocytes of European sea bass (*Dicentrarchus labrax* L.). Fish Physiol. Biochem. 26, 297–308.

Mourente G., Dick J.R., Bell J.G., Tocher D.R. (2005) Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and beta–oxidation of [1-14C] 18:3n-3 (LNA) and [1-14C] 20:5n-3 (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.). Aquacult. 248, 173–186.

Mourente G., Tocher D.R. (1994) In vivo metabolism of [1-14C] linolenic acid (18:3n-3) and [1-14C] eicosapentaenoic acid (20:5n-3) in marine fish: time-course of the desaturation / elongation pathway. Biochim. Biophys. Acta 1212, 109–118.

Mueller P., Diamond J. (2001) Metabolic rate and environmental productivity: well-provisioned animals evolved to run and idle fast. PNAS 98, 12550–12554.

Mugnier C., Fostier A., Guezou S., Gaignon J.L., Quemener L. (1998) Effect of some repetitive factors on turbot stress response. Aquacult. Int. 6, 33–45.

Muller M.J., Langemann D., Gehrke I., Later W., Heller M., Glüer C.C., Heymsfield S.B., Bosy-Westphal A. (2011) Effect of constitution on mass of individual organs and their association with metabolic rate in humans – a detailed view on allometric scaling. PLoS One 6, e22732.

Murai T., Andrews J.W. (1974) Interaction of dietary α-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (*Ictalurus punctatus*). J. Nutr. 104, 1416–1431.

Nagasawa K., Cruz–Lacierda E.R. (2004). Diseases of cultured groupers. Aquaculture department, southeast asian fisheries development center. Technical Report. Iloilo, Philippines, 93 p.

Nakamura M., Nara T. (2002) Gene regulation of mammalian dasaturases. Biochem. Soc. Trans. 30, 1076–1079.

Nakamura M., Nara T. (2003) Essential fatty acid synthesis and its regulation in mammals. Prostag. Leukotr. Ess. 68, 145–150.

National Research Council (1993) Nutrient requirements of fish. Washington, DC : National Academy Press.

Nations Unies (1992) Convention cadre des Nations Unies sur les changements climatiques, Rapport FCCC/INFORMAL/84, GE.05-62221 (F) 180705. Rome, Italie, 25 p.

NatureServe (2013) *Morone saxatilis*. The IUCN Red List of Threatened Species 2013: e.T192942A18230574. http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T192942A18230574.en. Downloaded on 26 October 2015.

Neat C.E., Thomassen M.S., Osmundsen H. (1981) Effects of high-fat diets on hepatic fatty acid oxidation in the rat. Biochem. Biophys. Res. Commun. 196, 149–159.

Neill W.H., Bryan J.D. (1991) Responses of fish to temperature and oxygen, and response integration through metabolic scope. In: Brune D.E., Tomasso J.R. (eds) Aquaculture and Water Quality, Advances in World Aquaculture. The World Aquaculture Society, Baton Rouge, pp 30–57.

Neill W.H., Miller J.M., Van der Veer H.W., Winemiller K.O. (1994) Ecophysiology of marine fish recruitment: a conceptual framework for understanding interannual variability. Neth. J. Sea Res. 32, 135–152.

Nelson J.A. (1989) Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. J. Exp. Biol. 145, 239–254.

Nematipour G.R., Gatlin III D.M. (1993) Effects of different kinds of dietary lipid on growth and fatty acid composition of juvenile sunshine bass, *Morone chrysops* ♀ × *M. saxatilis* ♂. Aquacult. 114, 141–154.

Neuheimer A.B., Grønkjær P. (2012) Climate effects on size-at-age: growth in warming waters compensates for earlier maturity in an exploited marine fish. Global Change Biol. 18, 1812–1822.

Neuheimer A.B., Taggart C.T. (2007) The growing degree-day and fish size-at-age: the overlooked metric. Can. J. Fish. Aquat. Sci. 64, 375–385.

Ng W.K., Lim P.K., Boey P.L. (2003). Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. Aquacult. 215, 229–243.

Oikawa S., Itazawa Y. (1984) Allometric relationship between tissue respiration and body mass in the carp. Comp. Biochem. Physiol. 1984, 415–418.

Oikawa S., Itazawa Y. (1993) Allometric relationship between tissue respiration and body mass in a marine teleost, *Porgy Pagrus*. Major. Comp. Biochem. Physiol. 105, 129–133.

Olsen R.E., Suontama J., Langmyhr E., Mundheim H., Ringø E., Melle W., Malde M.K., Hemre G.-I. (2006) The replacement of fish meal with Antarctic krill, Euphausia superba in diets for Atlantic salmon, *Salmo salar*. Aquacult. Nutr. 12, 280–290.

Olson E.C., Miller R.L. (1958) Morphological Integration. Chicago : University of Chicago Press.

Orban E., Nevigato T., Lena G.D., Casini I., Marzetti A. (2003) Differentiation in the lipid quality of wild and farmed seabass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). J. Food Sci. 68, 128–132.

Otwell W.S., Merriner J.V. (1975) Survival and growth of juvenile striped bass, *Morone saxatilis*, in a factorial experiment with temperature, salinity and age. Trans. Am. Fish. Soc. 104, 560–566.

Owen J.M., Adron J.W., Middleton C., Cowey C.B. (1975) Elongation and desaturation of dietary fatty acids in turbot (*Scophtalamus maximus* L.), and rainbow trout (*Salmo gairdnerii* Rich.). Lipids 10, 528–531.

Pahl S.L., Lewis D.M., Chen F., King K.D. (2010) Heterotrophic growth and nutritional aspects of the diatom *Cyclotella cryptica* (*Bacillariophyceae*): Effect of some environmental factors. J. Biosci. Bioeng. 109, 235–239.

Pamplona R., Portero-Otin M., Ruiz C., Gredilla R., Herrero A., Barja G. (2000) Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals. Mech. Ageing Dev. 112, 169–183.

Pang X., Fu S.J., Zhang Y.G. (2015) Individual variation in metabolism and swimming performance in juvenile black carp (*Mylopharyngodon piceus*) and the effects of hypoxia. Mar. Freshw. Behav. Physiol. 48, 431–443.

Parekh P., Dutkiewicz S., Follows M.J., Ito T. (2006) Atmospheric carbon dioxide in a less dusty world. Geophys. Res. Lett. 33, L03610.

Parihar M.S., Dubey A.K. (1995) Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase. Comp. Biochem. Physiol. C 112, 309–313.

Parrish C.C. (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In : Arts M.T., Wainman B.C. (eds) Lipids in freshwater ecosystems. New York, Springer, pp 4–20.

Parrish C.C. (2013) Lipids in marine ecosystems. ISRN Oceanogr. 1–17.

Pauly D., Christensen V., Dalsgaard J., Froese R., Torres F. (1998) Fishing down marine food webs. Science 279, 860–863.

Pauly D., Christensen V., Guénette S., Pitcher T.J., Sumaila U. R., Walters C. J., Watson R., Zeller, D. (2002) Towards sustainability in world fisheries. Nature 418, 689.

Peake S.J., Farrell A.P. (2004) Locomotory behaviour and post-exercise physiology in relation to swimming speed, gait transition and metabolism in free-swimming smallmouth bass (*Micropterus dolomieu*). J. Exp. Biol. 207, 1563–1575.

Pelletier A.M., Bourget G., Legault M., Verreault G. (2011) Réintroduction du bar rayé (*Morone saxatilis*) dans le fleuve Saint-Laurent: bilan du rétablissement de l'espèce. Nat. Can. 135, 79–85.

Perry A.L., Low P.J., Ellis J.R., Reynolds, J.D. (2005) Climate change and distribution shifts in marine fishes. Science 308, 1912–1915.

Person–Le Ruyet J., Mahé K., Le Bayon N., Le Delliou H. (2004) Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. Aquacult. 237, 269–280.

Petersen L.H., Gamperl A.K. (2010) Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). J. Exp. Biol. 213, 808–819.

Petersson A., Pickova J., Brännäs E. (2010) Swimming performance at different temperatures and fatty acid composition of Arctic charr (*Salvelinus alpinus*) fed palm and rapeseed oils. Aquacult.300, 176–181.

Petrie J.R., Shrestha P., Zhou X.R., Mansour M.P., Liu Q., Belide S., Nichols P.D., Singh S.P. (2012) Metabolic engineering plant seeds with fish oil-like levels of DHA. PLoS One 7, e49165.

Pettersson A., Johnsson L., Brannas E., Pickova J. (2009) Effects of rapeseed oil replacement in fish feed on lipid composition and self-selection by rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. 15, 577–586.

Pettersson A., Pickova J., Brännäs E. (2010) Swimming performance at different temperatures and fatty acid composition of Arctic charr (*Salvelinus alpinus*) fed palm and rapeseed oils. Aquacult. 300, 176–181.

Pickett G.D., Pawson M.G. (1994) Sea Bass: Biology (Vol. 12). Springer Science & Business Media, New York, 433 p.

Pierce B.J., McWilliams S.R. (2005) Seasonal changes in composition of lipid stores in migratory birds: causes and consequences. Condor 107, 271–81.

Piersma T., van Gils J.A. (2011) The Flexible Phenotype: A Body–Centred Integration of Ecology, Physiology, and Behaviour. UK: Oxford University Press.

Pigliucci M., Preston K. (2004) Phenotypic Integration: Studying the Ecology andEvolution of Complex Phenotypes. Oxford, UK : Oxford University Press.

Plaut I. (2000) Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities. Physiol. Biochem. Zool. 73, 590–596.

Plaut I. (2001) Critical swimming speed: its ecological relevance. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 131, 41–50.

Pohl P., Zurheide F. (1979) Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Hoppe H.A., Levring T., Tanaka Y. (eds) Marine algae in pharmaceutical science. Walter de Gruyter, Berlin, pp 473–523.

Pond C.M. (1998) The fats of life. Cambridge, UK: Cambridge University Press.

Poston H.A., Combs Jr. G.F., Leibovitz L. (1976) Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): gross, histological and biochemical deficiency signs. J. Nutr. 106, 892–904.

Qi B., Fraser T., Mugford S., Dobson G., Sayanova O., Butler J., Napier J.A, Stobart A.K., Lazarus C.M. (2004) Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nature Biotech. 22, 739.

Quan L.J, Zhang B., Shi W.W., Li H.Y. (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J. Integr. Plant Biol. 50, 2–18.

Randall D., Brauner C. (1991) Effects of environmental factors on exercise in fish. J. Exp. Biol. 160, 113–126.

Réale D., Garant D., Humphries M.M., Bergeron P., Careau V., Montiglio P.O. (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos. Trans. R. Soc. B: Biol. Sci. 365, 4051–4063.

Regost C., Arzel J., Robin J., Roselund G., Kaushik S.J. (2003) Total replacement of fish oil by soybean or linseed oil with return to fish oil in turbot (*Psetta maxima*) 1. Growth performance, flesh fatty acid profile, and lipid metabolism. Aquacult. 217, 465–482.

Reid P.C., Fischer A.C., Lewis-Brown E., Meredith M.P., Sparrow M., Andersson A.J., Antia A., Bathmann U., Beaugrand G., Brix H., Dye S., Edwards M., Furevik T., Gangstø R., Hátún H., Hopcroft R.R., Kendall M., Kasten S., Keeling R., Le Quéré C., Mackenzie F.T., Malin G., Mauritzen C., Ólafsson J., Paull C., Rignot E., Shimada K., Vogt M., Wallace G., Wang Z., Washington R. (2009) Impacts of the oceans on climate change. Adv. Mar. Biol. 56, 1–150.

Reidy S.P., Kerr S.R., Nelson J.A. (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. J. Exp. Biol. 203, 347–357.

Renaud S.M., Thinh L.V., Lambrinidis G., Parry D.L. (2002) Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquacult. 211, 195–214.

Rhee S.G. (1999) Redox signaling: hydrogen peroxide as intracellular messenger. Exp. Mol. Med. 31, 53.

Richards J.G., Heigenhauser G.J.F., Wood C.M. (2002a) Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. Am. J. Physiol. 282, R89–R99.

Richards J.G., Mercado A.J., Clayton C.A., Heigenhauser G.J., Wood C.M. (2002b) Substrate utilization during graded aerobic exercise in rainbow trout. J. Exp. Biol. 205, 2067–2077.

Richardson A.J., Schoeman D.S. (2004) Climate impact on plankton ecosystems in the Northeast Atlantic. Science 305, 1609–12.

Ricker W.E. (1979) Growth rates and models. In: Hoar W.S., Randall D.J., Brett J.R. (eds) Fish physiology, Vol. 8. Academic Press, New York, pp 677–743.

Riediger N.D., Othman R.A., Suh M., Moghadasian M.H. (2009) A systemic review of the roles of n–3 fatty acids in health and disease. J. Am. Diet. Assoc. 109, 668–679.

Rijnsdorp A.D., Peck M.A., Engelhard G.H., Möllmann C., Pinnegar J.K. (2009) Resolving the effect of climate change on fish populations. ICES J. Mar. Sci. 66, 1570–1583.

Rijnsdorp A.D., Van Leeuwen P.I. (1996) Changes in growth of North Sea plaice since 1950 in relation to density, eutrophication, beam-trawl effort, and temperature. ICES J. Mar. Sci. 53, 1199 –1213.

Rodger H., Turnbull T. (2000) Cardiomyopathy syndrome in farmed Scottish salmon. Vet. Rec. 146, 500–501

Rodger H.D., Murphy T.M., Drinan E.M., Rice D.A. (1991) Acute skeletal myopathy in farmed Atlantic salmon *Salmo salar*. Dis. Aquat. Organ. 12, 17–23.

Roessig J.M., Woodley C.M., Cech J.J., Hansen L.J. (2004) Effects of global climate change on marine and estuarine fishes. Rev. Fish Biol. Fish. 14, 215–75.

Rogers A., Sumaila U., Hussain S., Baulcomb, C. (2014) The high seas and us: understanding the value of high-seas ecosystems. Report, Global ocean commission, Somerville College, Oxford, 23 pp.

Rowley A.F., Knight J., Lloyd–Evans P., Holland J.W., Vickers P.J. (1995) Eicosanoids and their role in immune modulation in fish – a brief overview. Fish Shellfish Immunol. 5, 549–567.

Rulifson R.A., McKenna S.A. (1987) Food of striped bass in the upper Bay of Fundy, Canada. Trans. Am. Fish. Soc. 116, 119–122.

Rullkötter J. (2000) Organic matter: the driving force for early diagenesis. In: Schulz H.D., Zabel M. (eds) Marine Geochemistry. Springer, Berlin, pp. 129–172.

Russelle M.P., Wilhelm W.W., Olson R.A., Power J.F. (1984) Growth analysis based on degree days. Crop Sci. 24, 28–32.

Ryckebosch E., Bruneel C., Termote-Verhalle R., Goiris K., Muylaert K., Foubert I. (2014) Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food Chem. 160, 393–400.

Sakai T., Murata H., Endo M., Shimomura T., Yamauchi K., Ito T., Yamaguchi T., Nakajima H., Fukudome M. (1998) Severe oxidative stress is thought to be a principal cause of jaundice of yellowtail *Seriola quinqueradiata*. Aquacult. 160, 205–214.

Salamin N., Wüest R.O., Lavergne S., Thuiller W., Pearman P.B. (2010) Assessing rapid evolution in a changing environment. Trends Ecol. Evol. 25, 692–698.

Salem Jr. N., Eggersdorfer M. (2015) Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? Curr. Op. Clin. Nutr. Met. Care 18, 147–154.

Sánchez Vázquez F.J.; Muñoz-Cueto J.A. (2014) Biology of European sea bass. In : Sánchez Vázquez F.J.; Muñoz-Cueto J.A. (eds) CRC press, pp 1–436

Sandblom E., Clark T.D., Gräns A., Ekström A., Brijs J., Sundström L.F., Odelström A., Adill A., Aho T., Jutfelt F. (2016) Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nature Commun. 7, 11447.

Sanden M., Stubhaug I., Berntssen M.H.G., Lie Ø., Torstensen B.E. (2011) Atlantic salmon (*Salmo salar* L.) as a net producer of long–chain marine ω-3 fatty acids. J. Agri. Food Chem. 59; 12697–12706.

Santigosa E., Saénz de Rodrigáñez M.Á., Rodiles A., Barroso F.G., Alarcón, F.J. (2010) Effect of diets containing a purified soybean trypsin inhibitor on growth performance, digestive proteases and intestinal histology in juvenile sea bream (*Sparus aurata* L.). Aquacult. Res. 41, e187–e198.

Sardella B.A., Cooper J., Gonzalez R.J., Brauner C.J. (2004) The effect of temperature on juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* × *O. urolepis hornorum*) exposed to full-strength and hypersaline seawater. Comp. Biochem. Physiol. A Mol. Int. Physiol. 137, 621–629.

Sargent J., Bell G., McEvoy L., Tocher D., Estevez A. (1999) Recent developments in the essential fatty acid nutrition of fish. Aquacult. 177, 191–199.

Sargent J.R. (1976) The structure, metabolism and function of lipids in marine organisms. In : Malins D.C., Sargent J.R. (eds) Biochemical and Biophysical Perspectives in Marine Biology, Vol. 3., Academic Press, London, pp 149–212.

Sargent J.R., Bell M.V., Henderson R.J., Tocher D.R. (1989) The lipids. In: Halver J (eds) Fish nutrition. New York, Academic press, pp 153–218.

Sargent J.R., Tocher D.R., Bell J.G. (2002) The lipids, third edition. In: Halver, J.E., Hardy, R.W. (eds), Fish Nutrition. Academic Press, London, pp 182–259.

Scheiner S. M., Caplan R.L., Lyman R.F. (1991) The genetics of phenotypic plasticity. III. Genetic correlations and fluctuating asymmetries. J. Evol. Biol. 4, 51–68.

Schlichting C.D. (1986) The evolution of phenotypic plasticity in plants. Annu. Rev. Ecol. Syst. 17, 667–693.

Schlichting C.D., Pigliucci M. (1998). Phenotypic evolution: a reaction norm perspective. Sunderland, MA : Sinauer Associates.

Schmidt–Nielsen K. (1984) Scaling: Why is animal size so important? Cambridge, UK : University Press.

Schulte P.M. (2007) Responses to environmental stressors in an estuarine fish: interacting stressors and the impacts of local adaptation. J. Thermal Biol. 32, 152–161.

Scott W.B., Scott M.G. (1988) Atlantic fishes of Canada. Can. Bull. Fish. Aquatic Sci. 219, 731.

Secor D.H. (2002) Estuarine dependency and life history evolution in temperate sea basses. Fish. Sci. 68, 178–181.

Secor D.H., Gunderson T.E., Karlsson K. (2000) Effect of temperature and salinity on growth performance in anadromous (Chesapeake Bay) and nonanadromous (Santee-Cooper) strains of striped bass *Morone saxatilis*. Copeia 1, 291–296.

Shadwick R.E., Steffensen J.F., Katz S.L., Knower T. (1998) Muscle dynamics in fish during steady swimming. Am. Zool. 38, 755–770.

Sheldon Jr. W.M., Blazer V.S. (1991) Influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages. J. Aquat. Anim. Health 3, 87–93.

Shingleton A.W. (2005) Body-size regulation: combining genetics and physiology. Curr. Biol. 15, R825–R827.

Sidell B.D., Driedzic W.R. (1985) Relationship between cardiac energy metabolism and cardiac work demand in fishes. In : Gilles G. (ed) Circulation, Respiration and Metabolism. Springer–Verlag, Berlin, pp. 381–401.

Simopoulos A.P. (2007) Omega-3 fatty acids and athletics. Curr. Sports Med. Rep. 6, 230–6.

Sinensky M. (1974) Homeoviscous adaptation – a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. PNAS 71, 522–525.

Sinha A.K., Dasan A.F., Rasoloniriana R., Pipralia N., Blust R., De Boeck G. (2015) Hypo-osmotic stress-induced physiological and ion-osmoregulatory responses in European sea bass (*Dicentrarchus labrax*) are modulated differentially by nutritional status. Comp. Biochem. Physiol. A Mol. Int. Physiol. 181, 87–99.

Skalli A., Robin J.H. (2004) Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid composition. Aquacult. 240, 399–415.

Skalli A., Robin J.H., Le Bayon N., Le Delliou H., Person-Le Ruyet J. (2006) Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*). Aquacult. 255, 223–232.

Smith T.I., Jenkins W.E., Snevel J.F. (1985) Production characteristics of striped bass (*Morone saxatilis*) and f1, f2 hybrids (*M. saxatilis* and *M. chrysops*) reared in intensive tank systems. J. World Mar. Soc. 16, 57–70.

Sogard S.M. (1992) Variability in growth rates of juvenile fishes in different estuarine habitats. Mar. Ecol. Prog. Series 85, 35–53.

Sogard S.M. (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. B. Mar. Sci. 60, 1129–1157.

Sprecher H. (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. BBA Mol. Cell Biol. L. 1486, 219–231.

Srere P.A. (1969) Citrate synthase. Methods Enzymol. 13, 3–11.

Starck J. (1999) Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. J. Exp. Biol. 202, 3171–3179.

Stoffels R.J. (2015) Physiological trade-offs along a fast-slow lifestyle continuum in fishes: what do they tell us about resistance and resilience to hypoxia? PloS One 10, e0130303.

Strand Å., Magnhagen C., Alanärä A. (2011) Growth and energy expenditures of Eurasian perch *Perca fluviatilis* (Linnaeus) in different temperatures and of different body sizes. J. Aquacult. Res. Dev. 2, 114.

Sumaila U.R., Cheung W.W., Lam V.W., Pauly D., Herrick S. (2011) Climate change impacts on the biophysics and economics of world fisheries. Nat. Clim. Change 1, 449.

Tacon A.G., Metian M. (2013) Fish matters: importance of aquatic foods in human nutrition and global food supply. Rev. Fish. Sci. 21, 22–38.

Thannickal V.J., Fanburg B.L. (2000) Reactive oxygen species in cell signaling. Am. J. Physiol. Lung Cell Mol. Physiol. 279, 1005–1028.

Thompson P.A., Guo M.X., Harrison P.J., Whyte J.N.C. (1992) Effects of variation in temperature on the fatty-acid composition of 8 species of marine-phytoplankton. J. Phycol. 28, 488–497.

Thuesen E.V., Childress J.J. (1993) Metabolic rates, enzyme activities and chemical compositions of some deep-sea pelagic worms, particularly *Nectonemertes mirabilis* (Nemertea; Hoplonemerinea) and *Poeobius meseres* (Annelida; Polychaeta). Deep-Sea Res. I 40, 937–951.

Thuesen E.V., Childress J.J. (1994) Oxygen consumption rates and metabolic enzyme activities of oceanic California medusae in relation to body size and habitat depth. Biol. Bull. 187, 84–98.

Tian J., Ji H., Oku H., Zhou J. (2014) Effects of dietary arachidonic acid (ARA) on lipid metabolism and health status of juvenile grass carp, *Ctenopharyngodon idellus*. Aquacult. 430, 57–65.

Tidwell J.H., Coyle S.D., Evans J., Weibel C., McKinnley J., Dodson K., Jones H. (1999) Effect of temperature on growth, survival, and biochemical composition of yellow perch *Perca flavescens*. J. World Aqua. Soc. 30, 324–330.

Tocher D., Zheng X., Christian S., Hastings N., Dick J.R., Teale A.J. (2006) Highly unsaturated fatty acid synthesis in marine fish: Cloning, functional characterization, and nutritional regulation of fatty acyl Δ6 desaturase of Atlantic cod (*Gadus morhua* L.). Lipids 41, 1003–1016.

Tocher D.R. (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.

Tocher D.R. (2010) Fatty acid requirements in ontogeny of marine and freshwater fish. Aquacult. Res. 41, 717–732.

Tocher D.R., Agaba M., Hastings N., Bell J.G., Dick J.R., Teale A.J. (2001b) Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis nilotica*). Fish Physiol. Biochem. 24, 309–320.

Tocher D.R., Bell J.G., Dick J.R., Henderson R.J., McGhee F., Michell D., Morris P.C. (2000) Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. 23, 59–73.

Tocher D.R., Bell J.G., MacGlaughlin P., McGhee F., Dick J.R. (2001a) Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil. Comp. Biochem. Physiol. 130, 257–270.

Tocher D.R., Ghioni C. (1999) Fatty acid metabolism in marine fish: low activity of Δ5 desaturation in gilthead sea bream (*Sparus aurata*) cells. Lipids 34, 433–440.

Torrecillas S., Mompel D., Caballero M.J., Montero D., Merrifield D., Rodiles A., Robaina L., Zamorano M.J., Karalazos V., Kaushik S., Izquierdo M. (2017a) Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). Aquacult. 468, 386–398.

Torrecillas S., Román L., Rivero–Ramírez F., Caballero M.J., Pascual C., Robaina L., Izquierdo M.S., Acosta F., Montero D. (2017b) Supplementation of arachidonic acid rich oil in European sea bass juveniles (*Dicentrarchus labrax*) diets: Effects on leucocytes and plasma fatty acid profiles, selected immune parameters and circulating prostaglandins levels. Fish Shellfish Immuno. 64, 437–445.

Torstensen B.E. (2000) Transport and metabolism of lipids in Atlantic salmon *Salmo salar* L. Thesis for the degree of doctor Scientiarum, University of Bergen, Norway, pp 5–43.

Torstensen B.E., Bell J.G., Rosenlund G., Henderson R.J., Graff I.E., Tocher D.R., Lie O., Sargent J.R. (2005) Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. J. Agri. Food Chem. 53, 10166–10178.

Torstensen B.E., Lie Ø., Frøyland L. (2000) Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) – effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 35, 653–664.

Trushenski J.T. (2009) Saturated lipid sources in feeds for sunshine bass: Alterations in production performance and tissue fatty acid composition. N. Am. J. Aquac. 71, 363–373.

Tseng Y.C., Hwang P.P. (2008) Some insights into energy metabolism for osmoregulation in fish. Comp. Bioch. Physiol. C Tox. Pharmacol. 148, 419–429.

Tuncer H., Harrell R.M. (1992) Essential fatty acid nutrition of larval striped bass (*Morone saxatilis*) and palmetto bass (*M. saxatilis* × *M. chrysops*). Aquacult. 10, 105–121.

Uchiyama M., Mihara M. (1978) Determination of malonaldehyde in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271–278.

Vagner M., Robin J.H., Zambonino–Infante J.L., Person–Le Ryuet J. (2007a) Combined effect of dietary HUFA level and temperature on sea bass (*Dicentrarchus labrax*) larvae development. Aquacult. 266, 179–190.

Vagner M., Robin J.H., Zambonino–Infante J.L., Tocher D. R., Person–Le Ruyet J. (2009) Ontogenic effects of early feeding of sea bass (*Dicentrarchus labrax*) larvae with a range of dietary n–3 highly unsaturated fatty acid levels on the functioning of polyunsaturated fatty acid desaturation pathways. Br. J. Nutr. 101, 1452–1462.

Vagner M., Santigosa E. (2011) Characterization and modulation of gene expression and enzymatic activity of delta–6 desaturase in teleosts: A review. Aquacult. 315, 131–143.

Vagner M., Zambonino–Infante J.L., Mazurais D., Imbert–Auvray N., Ouillon N., Dubillot E., Le Delliou H., Akbar D., Lefrançois C. (2014) Reduced n–3 highly unsaturated fatty acids dietary content expected with global change reduces the metabolic capacity of the golden grey mullet. Mar. Biol. 161, 2547–2562.

Vagner M., Zambonino-Infante J.-L., Robin J.H., Person-Le Ruyet J. (2007b) Is it possible to influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a nutritional conditioning during larval stage? Aquacult. 267, 165–174.

Valencak T.G., Azzu V. (2014) Making heads or tails of mitochondrial membranes in longevity and aging: a role for comparative studies. Longev. Healthspan 3, 3.

Valiquette E., Legault M., Mainguy J., Bujold V., Pelletier A.-M. (2018) Répartition du bar rayé au Québec – mise à jour des connaissances, Ministère des Forêts, de la Faune et des Parcs. Québec, CA, 17 p.

Valladares F., Gianoli E., Gómez J.M. (2007) Ecological limits to plant phenotypic plasticity. New Phytologist 176, 749–763.

Van der Have T.M., De Jong G. (1996) Adult size in ectotherms: temperature effects on growth and differentiation. J. Theor. Biol. 183, 329–340.

Vargas-Chacoff L., Arjona F.J., Polakof S., Martın del Rıo M.P., Soengas J.L. Mancera J.M. (2009) Interactive effects of environmental salinity and temperature on metabolic responses of gilthead sea bream *Sparus aurata*. Comp. Biochem. Physiol. A 154, 417–424.

Varsamos S., Connes R., Diaz J.P., Barnabé G., Charmantier G. (2001) Ontogeny of osmoregulation in the European seabass *Dicentrarchus labrax* L. Mar. Biol. 138, 909–915.

Vaskovsky V.E. (1989) Phospholipids. In: Ackman R.G. (ed), Marine Biogenic Lipids, Fats, and Oils. CRC Press, Boca Raton, Florida, pp 199–242.

Venegas-Calerón M., Sayanova O., Napier J.A. (2010) An alternative to fish oils: metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids. Progress Lipid Res. 49, 108–119.

Venturelli P.A., Lester N.P., Marshall T.R., Shuter B.J. (2010) Consistent patterns of maturity and density-dependent growth among populations of walleye (*Sander vitreus*): application of the growing degree-day metric. Can. J. Fish. Aquat. Sci. 67, 1057–1067.

Videler J.J. (1993). Fish Swimming. London: Chapman and Hall.

Vinagre C., Ferreira T., Matos L., Costa M.J., Cabral H.N. (2009) Latitudinal gradients in growth and spawning of sea bass *Dicentrarchus labrax* (L.) and their relationship with temperature and photoperiod. Estuar. Coast. Shelf Sci. 81, 375–380.

Vinagre C., Madeira D., Narciso L., Cabral H., Diniz M. (2012) Impact of climate change on coastal versus estuarine nursery areas: cellular and whole-animal indicators in juvenile seabass *Dicentrarchus labrax*. Mar. Ecol. Prog. Ser. 464, 237–243.

Von Bertalanffy L. (1938) A quantitative theory of organic growth (inquiries on growth laws. II). Human Biol. 10, 181–213.

Wagner G.N., Balfry S.K., Higgs D.A., Lall S.P., Farrell A.P. (2004) Dietary fatty acid composition affects the repeat swimming performance of Atlantic salmon in seawater. Comp. Biochem. Physiol. A 137, 567–576.

Wagner G.N., Kuchel L.J., Lotto A., Patterson D.A., Shrimpton J.M., Hinch S.G., Farrell A.P. (2005) Routine and active metabolic rates of migrating adult wild sockeye salmon (*Oncorhynchus nerka* Walbaum) in seawater and freshwater. Physiol. Biochem. Zool. 79, 100–108.

Wang Z., O'Connor T.P., Heshka S., Heymsfield S.B. (2001) The reconstruction of Kleiber's law at the organ–tissue level. J. Nutr. 1, 2967–2970.

Wang Z., Zhang J., Ying Z., Heymsfield S.B. (2012) Organ-tissue level model of resting energy expenditure across mammals: new insights into Kleiber's law. ISRN Zool. 2012, 1–9.

Waples R.S., Audzijonyte A. (2016) Fishery-induced evolution provides insights into adaptive responses of marine species to climate change. Front. Ecol. Envir. 14, 217–224.

Wasmund N., Nausch G., Matthäus W. (1998) Phytoplankton spring blooms in the southern Baltic Sea – spatio-temporal development and long-term trends. J. Plankton Res. 20, 1099 –1117.

Watanabe T. (1982) Lipid nutrition in fish. Comp. Biochem. Physiol. B 73, 3–15.

Watanabe T., Takashima F., Ogino C., Hibiya T. (1970) Effect of α-tocopherol on carp. Bull. Japan. Soc. Sci. Fish 36, 623–630.

Webb P.W. (1993) Is tilting behaviour at low swimming speeds unique to negatively buoyant fish? Observations on steelhead trout, *Oncorhynchus mykiss*, and bluegill, *Lepomis macrochirus*. J. Fish Biol. 43, 687–694.

Weber J.-M. (2009) The physiology of long-distance migration: extending the limits of endurance metabolism. J. Exp. Biol. 212, 593–7.

Weber J.-M. (2011) Metabolic fuels: regulating fluxes to select mix. J. Exp. Biol. 214, 286–294.

Webster C.D., Lovell R.T. (1990) Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid composition. Aquacult. 90, 49–6 1.

Weibel E.R. (1998) Symmorphosis and optimization of biological design: introduction and questions. In : Weibel E.R., Taylor C.R., Bolis L. (eds) Principles of animal design. Cambridge University Press, Cambridge, pp 1–10.

Williams E.P., Peer A.C., Miller T.J., Secor D.H., Place A.R. (2012) A phylogeny of the temperate seabasses (Moronidae) characterized by a translocation of the mt-nd6 gene. J. Fish Biol. 80, 110–130.

Winger P.V., Lasier P.J. (1994) Effects of salinity on striped bass eggs and larvae from the Savannah River, Georgia. Trans. Am. Fish. Soc. 123, 904–912.

Wittmann A.C., Pörtner H.O. (2013) Sensitivities of extant animal taxa to ocean acidification. Nat. Clim. Change 3, 995–1001.

Yildiz M., Sener E. (1997) Effect of dietary supplementation with soybean oil, sunflower oil or fish oil on the growth of seabass (*Dicentrarchus labrax* L. 1758). Cah. Options Mediterr. 22, 225–233.

Yılmaz H.A., Corraze G., Panserat S., Eroldoğan O.T. (2016). Effects of alternate feeding with different lipid sources on fatty acid composition and bioconversion in European sea bass (*Dicentrarchus labrax*). Aquacult. 464, 28–36.

Zhang Y., Timmerhaus G., Anttila K., Mauduit F., Jørgensen S. M., Kristensen T., Claireaux G., Tackel H., Farrell, A.P. (2016) Domestication compromises athleticism and respiratory plasticity in response to aerobic exercise training in Atlantic salmon (*Salmo salar*). Aquacult. 463, 79–88.