The relationship between migration behavior and energetic status in the European glass eel (Anguilla anguilla)

Hengtong Liu

THÈSE

UNIVERSITE DE PAU ET DES PAYS DE L'ADOUR

École doctorale 211-SCIENCES EXACTES ET LEURS APPLICATIONS

Présentée et soutenue le 29 September 2020

par **Hengtong LIU**

pour obtenir le grade de docteur
de l'Université de Pau et des Pays de l'Adour
Spécialité : Biologie et Physiologie des Organismes

The relationship between migration behavior and energetic status in the European glass eel (*Anguilla anguilla*)

****

Relation entre le comportement migratoire et le statut énergétique de la civelle d'anguille européenne (*Anguilla anguilla*)

MEMBRES DU JURY

RAPPORTEURS

• Patrice GONZALEZ	Directeur de recherche CNRS / UMR EPOC CNRS - Université de Bordeaux
• David MAZURAIS	Chargé de recherche/ Ifremer, Plouzané

EXAMINATEURS

• Françoise DAVERAT	Ingénieur de l'agriculture et de l'environnement INRAE/ UMR Ecobiop INRAE- UPPA
• Christine MOISAN	Professeur / Université de Brest

DIRECTEURS

• Valérie BOLLIET	Professeur UPPA/ UMR Ecobiop INRAE- UPPA
• Iban SEILIEZ	Directeur de recherche INRAE / UMR NuMeA INRAE- UPPA

INRAE

UNIVERSITÉ DE PAU ET DES PAYS DE L'ADOUR

This thesis is dedicated to all those who are devoted to eel career

--------Hengtong LIU

ACKNOWLEDGEMENT

Time always flies quickly, my four-year PhD life is coming to an end without knowing it. Four years ago, I couldn’t imagine that I am sitting in front of a computer 9,000 kilometers away from my hometown at this moment and writing down these words. This place, called Saint-Pée-sur-Nivelle, does not have the French romanticism of Paris or the enthusiasm of Barcelona, but exists in its unique calmness and peace. To quote from the novelist Milan Kundera: It is unobtrusively charming, and charmingly unobtrusive. The place where I work is called INRA, and in 2020 it has a new name INRAE. The story between INRAE and me goes back to 2016, a year full of color in my life. In that year, I graduated with a master's degree, experienced parting from my friends and embarked on the road of studying abroad. This benefited from the encouragement of my master mentor, the support of my family and the funding from the China Scholarship Council. During this process, I would like to thank Dr. Weiwei Dai who introduced INRAE institute to me. I would also like to thank Prof. Agnès Bardonnet for forwarding my PhD application email to my current mentors. Without them, it would be another very different story to be told today.

On October 16, 2016, my PhD life began. During my four years here, I would like to thank my two mentors, Valérie Bolliet and Iban Seiliez, who help me grow up from an outsider of fish physiology research step by step to an independent phd who can complete this thesis as scheduled. They not only play a role of mentors, but are more like sharers for me. Whenever I encounter any academic difficulties, they can always here to share their experiences and ideas with me. Valérie's great insight into scientific issues, Iban's academic enthusiasm and sharp mind have benefited me a lot. Their selfless sharing and gradual guidance made me progress in my work and also made understand how to be a researcher. Of course I have to admit that I am not a perfect student, and there is still a gap with their expectations. But the future way of my research career is still long, I hope that one day in the future I can work with my mentors again as a qualified researcher. Too much gratitude is beyond words, maybe becoming a better self is the best feedback for them.

In addition, I would like to thank all the technicians in INRAE. Without their help, my PhD experiment could not be completed smoothly. In particular, I would like to thank Jacques Rives and Emmanuel Huchet for helping me obtain glass eels in the field. This is an important basis for my experiments. I have personally experienced this fishing work and know how difficult it is, so I want to express many many thanks to them again for their selfless help. In addition, I would also like to thank Pascale Coste, who has participated in almost all of my experiments in the past four years and has made great contributions to my work. I would like to thank Elisabeth Plagnes-Juan, Karine Dias and Vincent Veron in NuMeA unit. They not only provided me with technical support, but more importantly, I learned

from them how to manage the laboratory to run in an orderly manner. Sincere thanks for their patience and tolerance for me. Moreover, I would like to thank numerous scientists, especially those who participated in my PhD report each year, including my two mentors Valérie Bolliet and Iban Seiliez, as well as Jacques Labonne, Stephane Panserat, and Patrice Gonzalez. I would like to thank them very much for their willingness to join my PhD committee in form of live or video, and many thanks for their valuable comments on my doctoral work, which has really benefited me a lot. Among them, I would like to thank Jacques Labonne in particular. His unique humor and gentle personality broke my stereotypes about scientists. During the writing of my academic articles, he provided a lot of meticulous reference opinions from the perspective of data scientist. The smooth publication of my academic papers was largely under his help.

I also want to thank my friends who have given me warmful company during these years. Many thanks to Dr. Jingwei Liu, who is always here with me and help me during my most difficult time. I hope her enjoy the life in U.S., and have a smooth and fruitful postdoctoral period. Thanks to Dr. Luis-Paulo Araujo-Lage, Ms. Pauline Wischhusen, Dr. Araya Jangprai, Dr. Yhou Suksan, Dr. Yan Song, Dr. Huihua Hu, Dr. Xuerong Song, Ms. Hongyan Li, Dr. Lokesh Jep and all the friends I met here. Because of your company, I never feel lonely.

I would like to thank my grandfather, my dear mom, dad and younger brother for their unconditional support and concerns. I especially thank my mother, who always uses her wisdom to understand and encourage me whenever I feel confused in life. I think this wisdom has nothing about education, work or age, it is all about love and trust. I am so sorry that I have spend too many years on my study, and neglected family time. I hope to be a competent daughter and sister in the years to come. I also want to thank my silent partner Flik. Even though she is just a doll, I am not so lonely for more than 700 days and nights with her. In addition, I would like to thank my idol singer ☆Hua, whose songs have brought a lot of color to my amateur life, though I have never successfully grabbed a concert ticket. In the end, I have not forgotten to thank myself, although a little ashamed, I would like to thank myself for the decision I made four years ago to come to France, thank myself for my hard work and not giving up, and thank myself for persevering till today to sit here and write this story.

Hengtong Liu

July 19th, 2020, Saint-Pée-sur-Nivelle, France

Table of contents

LIST OF ABREVIATIONS

Abbreviations	Full name
A. anguilla	*Anguilla anguilla*
A. japonica	*Anguilla japonica*
A. rostrata	*Anguilla rostrata*
AANAT	arylalkylamine N-acetyltransferase
AAs	amino acids
ADP	adenosine diphosphate
AMPK	AMP-activated protein kinase
ARNTL/BMAL1	aryl hydrocarbon receptor nuclear translocator- like protein 1
AS	aerobic scope
ATGL	adipose triglyceride lipase
ATGs	autophagy-related genes
ATP	adenosine triphosphate
BNIP3L/NIX	BCL2 interacting protein 3 like
Ca	calcium
Cd	cadmium
CMA	chaperone-mediated autophagy
CNS	central nervous system
COX	cytochrome c oxidase
CRY	cryptochrome
Cu	copper
DAGs	diacylglycerols
Drp1	dynamin-related protein 1
E1	ubiquitin-activating enzyme,
E2	ubiquitin-conjugating enzyme
E3	ubiquitin-ligase enzyme
EQS	environmental quality standard
FFAs	free fatty acids

FKBP5	FK506 binding protein 51
FOXO	Forkhead box protein O
FUNDC1	FUN14 domain containing 1
GCs	glucocorticoids
GPx	glutathione peroxidase
GR	glucocorticoid receptor
GSH	glutathione
GST	GSH s-transferases
HAT	histone acetyltransferase
HDAC	histone deacetylase
Hg	mercury
Hg(II), Hg^{2+}	inorganic mercury
Hg^0	elementary mercury
HPA axis	hypothalamic-pituitary-adrenal axis
HPI axis	hypothalamic-pituitary-interrenal axis
HSPA8/HSC70	heat shock protein family A [Hsp70] member 8
LAL	lysosomal acid lipase
LAMP2A	lysosomal associated membrane protein 2A
LC3	microtubule-associated protein 1A/1B-light chain 3
LDs	lipid droplets
LIR	LC3-interacting region
MAFbx	muscle atrophy F-box
MAGs	monoacylglycerols
MAPKs	mitogen-activated protein kinases
MeHg, CH_3Hg^+	methylmercury
Mfn1	mitochondrial fusion mediators mitofusin 1
Mfn2	mitochondrial fusion mediators mitofusin 2
MGL	monoacylglycerol lipase
MMR	maximal aerobic metabolic rate
mTOR	mechanistic target of rapamycin

MTs	metallothioneins
MuRF-1	muscle RING finger-1
NADH	reduced nicotinamide adenine dinucleotide
NFR	nuclear respiratory factor
NPAS2	neuronal PAS domain protein 2
Opa1	optic atrophy protein 1
Pb	lead
PER	period
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1 α
PINK1	PTEN-induced kinase 1
PLINs	perilipin family members
PNPLA2	patatin-like phospholipase domain-containing protein 2
PRX	peroxiredoxin
ROS	reactive oxidative species
SIRT1	silent mating type information regulation 2 homolog 1
SMR	standard metabolic rate
SNPs	single nucleotide polymorphisms
Sr	strontium
STST	selective tidal stream transport
TAGs	triacylglycerides
THg	total Hg
Trx	thioredoxin
TrxR	thioredoxin reductase
UPS	ubiquitin proteasome system

LIST OF FIGURES

INTRODUCTION

Over the last half-century, the European eel stock declined by about 5% per year to an unprecedented level (Dekker, 2019). Today, the population has exceeded safe biological limits, and has been listed in the IUCN Red List as a critically endangered species (IUCN Red List, 2019). Therefore, gaining knowledge about the complex life cycle of European eels is of paramount importance and a prerequisite for the conservation of the population.

European eel is a catadromous species with a long ocean migration route. They spawn in Sargasso Sea and the larvae (known as leptocephalus) migrate passively towards European and North African coastal areas (Tsukamoto et al., 2003; Schmidt, 1923). Once on the continental shelf, the European eel goes through successive stages of glass eel, yellow eel and silver eel, as well as a habitat transition and a series of physiological and behavioral adaptations. Catadromy should consist of an estuarine migration of glass eels to freshwater for a period of growth before silver eels migrate back to natal site to reproduce and die. However, some European eels have never entered freshwater and at least three ecotypes have been characterized, namely freshwater eels, marine eels, and nomadic eels (Daverat et al., 2005, 2006). Therefore, the migration of glass eel to fresh water is highly flexible, which has been defined as a facultative migration (Tzeng et al., 2000; Arai et al., 2006). However, these different patterns of migration can have a strong impact on the fate of the population. Indeed, sex determination in European eel is environmental: individuals remaining downstream mostly develop in males and return earlier to the Sargasso Sea, whereas individuals colonizing upstream develop mainly into females and stay longer on the continent (Davey and Jellyman, 2005; Geffroy and Bardonnet, 2016). Therefore, variations in propensity to migrate upstream can have major consequences on the phenotypic structure (sex and size distribution) and thus on the population dynamics.

Glass eels almost cease feeding during their estuarine migration, where individual's swimming activity highly depends on its energy condition. A theory of conditional strategy based on individual energy stores has been proposed to explain the facultative migration in European glass eels, according to which fish with a higher energy stores should have a higher migratory activity (Edeline, 2007; Edeline et al., 2006). However, this energy-based theory has been conflicted by data from both European eel (Bolliet et al., 2017) and American eel (Boivin et al., 2015; Gaillard et al., 2015). Thus, the role of energy in shaping the divergent migratory patterns in European glass eels needs to be further characterized in term of energy stores but also of abilities to mobilize the energy stores. Fish ability to mobilize energy can include metabolic rate representing the speed at which energy reserves are consumed, as well as metabolic pathways such as lipid/protein cytosolic catabolism, autophagy, mitochondrial activity, and antioxidant system. Characterizing a glass eel's energetic status by these comprehensive indicators should help to bring forward our understandings about the conditional strategy. In addition, glass eel's energy condition is also associated

with environmental stress. Once entering estuaries, which represent a highly stressful habitat due to a high variation of environmental conditions (including salinity, temperature, current, and contaminants), fish physiological responses to the stress factors should produce additional energy cost and possibly drive the occurrence of settlement in estuaries. For example, contaminant, as a remarkable stressor in estuarine environment, can induce energy consumption through a series of cellular responses such as detoxification process.

In this context, this thesis is aiming to investigate the complex relationship between the energetic status and the migratory behavior during the estuarine migration in European glass eels in the framework of energy-based conditional strategy.

Chapter 1 - LITERATURE REVIEW

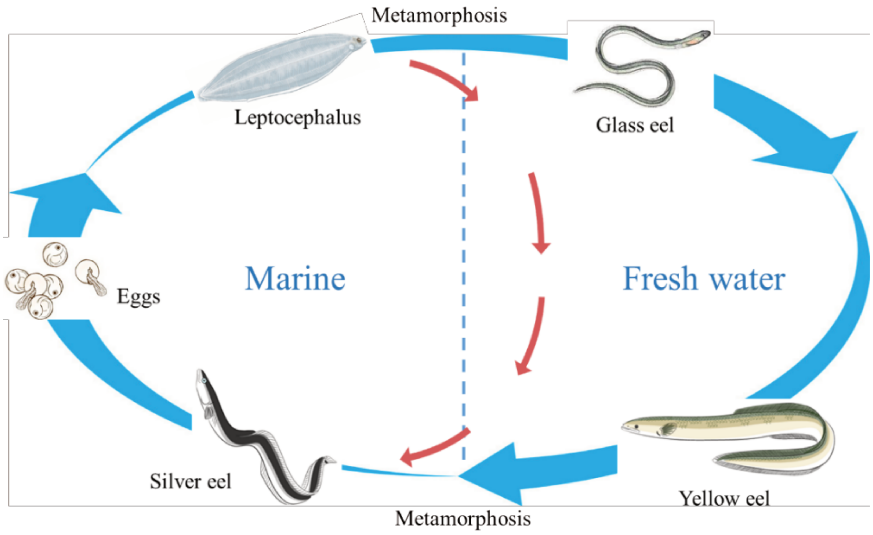


Figure 1-1. Schematic diagram of the European eel life cycle, showing facultative migration during continental colonization. Life history of 'freshwater residents' is shown in blue arrows, while the life history of 'marine residents' is shown in red arrows (modified from Cresci et al., 2019).

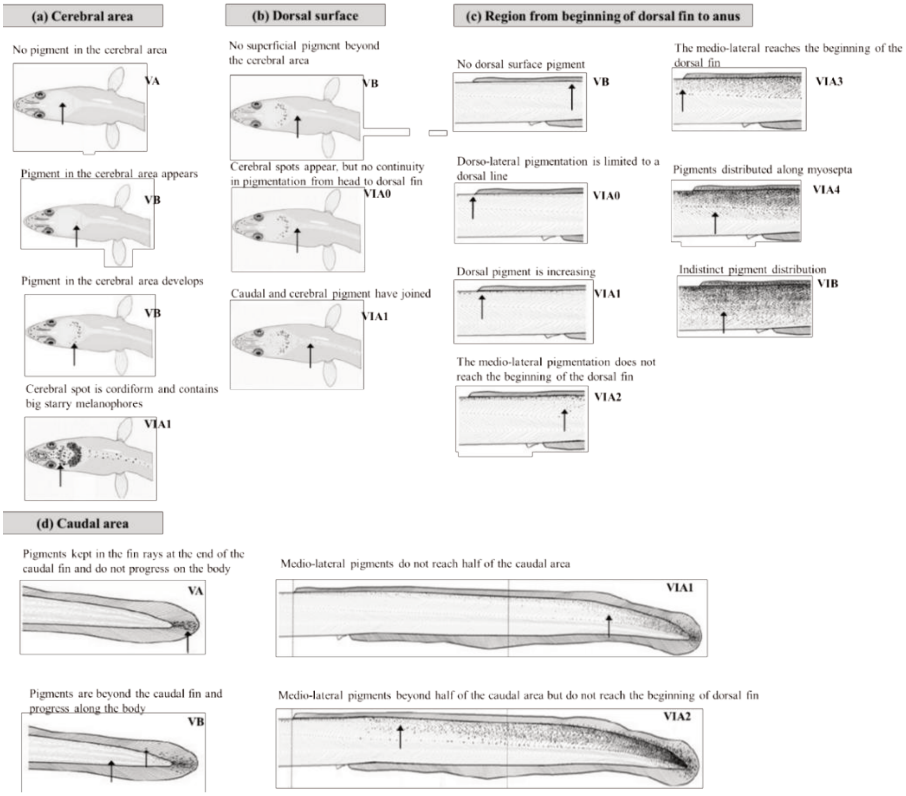


Figure 1-2. Pigmentation stages from VA to VIB in *A. anguilla*: (a) Cerebral area; (b) Dorsal surface; (c) Region from beginning of dorsal fin to anus; (d) Caudal area. Arrows show the pigmentation character locations to discriminate each stage (modified from Grellier et al., 1991; Strubberg, 1913; Elie et al., 1982; Lecomte-Finiger, 1982).

1.1 European eel ecology and glass eel migration behavior

The eel is a bony fish belonging to the superorder of elopomorphs and to the order of Anguilliformes (Forey et al., 1996). Nowadays, 19 species/subspecies of freshwater eels have been reported worldwide (Arai, 2016; Castle and Williamson, 1974; Ege, 1939; Watanabe, 2003). They are mainly found in tropical and temperate waters, except in the Eastern Pacific and South Atlantic (Arai, 2016). The European eel (*Anguilla anguilla* Linnaeus 1758) can be found from the North Cape in Northern Norway, along the coasts of Europe, to the Mediterranean coasts and even the coasts of North Africa (Dekker, 2003; Schmidt, 1909).

1.1.1 European eel life cycle

The European eel is a catadromous fish with a complex life cycle between oceanic spawning sites and inland feeding grounds (Tsukamoto, 1998). In its remarkable life history, European eels experience two trans-Atlantic migrations, develop through four major stages (leptocephalus, glass eel, yellow eel and silver eel stages) and present two metamorphoses (Figure 1-1).

1.1.1.1 Leptocephalus stage

Based on the size distribution of newly hatched leptocephali, the spawning area of European eel has been traced back to frontal zone region of the southern Sargasso Sea (Schmidt, 1922, 1923). The leptocephalus larvae cross the Atlantic Ocean using the Gulf Stream during 6-10 months (Arai et al., 2000; Bishop and Torres, 1999, 2001;Lecomte-Finiger, 1994; Miller, 2009; Tsukamoto, 2009) or 21 months (Bonhommeau et al., 2009) depending on the author. During the oceanic migration, the leptocephalus larvae feed on plankton or 'marine snow' (Riemann et al., 2010; Tsukamoto, 2009) and accumulate energy reserves.

1.1.1.2 Glass eel stage

The metamorphosis of leptocephalus larvae into transparent glass eels occurs at the slope of the continental shelf and is accompanied by significant morphological and physiological changes that allow them to adapt to their new habitat and life style (Schmidt, 1909; Tesch, 1977). This stage is characterized by body shape change from willow-leaf to eel-shape (Tesch, 2008), reduction in length, weight, and body moisture content (Bertin, 1951; Otake, 2003), and change of the brain structure (Tomoda and Uematsu, 1996). Glass eels enter estuaries and then migrate up to reach rivers. Most individuals fast during estuarine migration (Bardonnet and Riera, 2005; Jegstrup and Rosenkilde, 2003) and the ageing process is accompanied by an increase in pigmentation: VA, VB, VIA0, VIA1, VIA2, VIA3, VIA4 and VIB stages, as defined by Elie et al. (1982) (Figure 1-2). Complete feeding resumption occurs around the pigment stage VIA3 (Charlon and Blanc, 1982; Elie, 1979).

1.1.1.3 Yellow eel stage

Following migration to continental waters, glass eels turn into yellow eels via elver stages marked by full pigmentation (stage VII, according to Elie et al., 1982). The yellow eel stage corresponds to a growth phase of 6-8 years for male and 10-13 years for females to reach an average length of about 0.4 m and 0.7 m respectively (Moriarty and Dekker, 1997; Rossi and Villani, 1980). During this growth phase, eel feeding activity present seasonality: they feed voraciously in summer and become less active in winter, often lying dormant and half-buried in the muddy bottoms of the waters (Horne and Birnie, 1978).

1.1.1.4 Silver eel stage

When eels approach maturity, they become silver eels and begin their migration back to the Sargasso Sea to reproduce (Miller et al., 2015; Righton et al., 2016; Schmidt, 1923; Tesch, 1980, 2003). The silvering metamorphosis is signed by a change in belly colour (Pankhurst and Lythgoe, 1982; Tesch 2003), proliferation of the gonads (Pankhurst, 1982), enlargement of the eyes (Pankhurst, 1982; Pankhurst and Lythgoe, 1983), a change in visual sensitivity of the retina pigments from green-sensitive to blue-sensitive (Archer et al., 1995; Wood and Partridge, 1993) and a stop of food intake (Piper, 2007). All these changes have been documented as physiological and morphological adaptations to migrate to the Sargasso Sea, 6000 km away from Europe (van Ginneken et al., 2007). The spawning migration may last one year or more and eels exhibit diel vertical migrations, moving from deeper water during the day into shallower water at night, with a range of migration speeds of 3 to 47 km day^{-1} (Righton et al., 2016). Reproduction in the Sargasso Sea likely begins in December, peaks in February and then eels probably die (Righton et al., 2016).

1.1.2 An endangered species

Today, many Anguillid eels are of conservation concern, including the European eel (*A. Anguilla*), the American eel (*A. rostrata*), the Japanese eel (*A. japonica*), the New Zealand Longfin eel (*A. dieffenbachii*), and the Indonesian longfinned eel (*A. borneensis*) (IUCN Red List, 2020). The European eel has been listed in the IUCN Red List as well as in CITES Appendix II in September 2008 and then in 2014, as a critically endangered species. The last three decades have witnessed a dramatic drop in eel recruitment to the European continent, which has fallen to as little as 1% of their previous levels (1960-1979) according to some estimates (Figure 1-3).

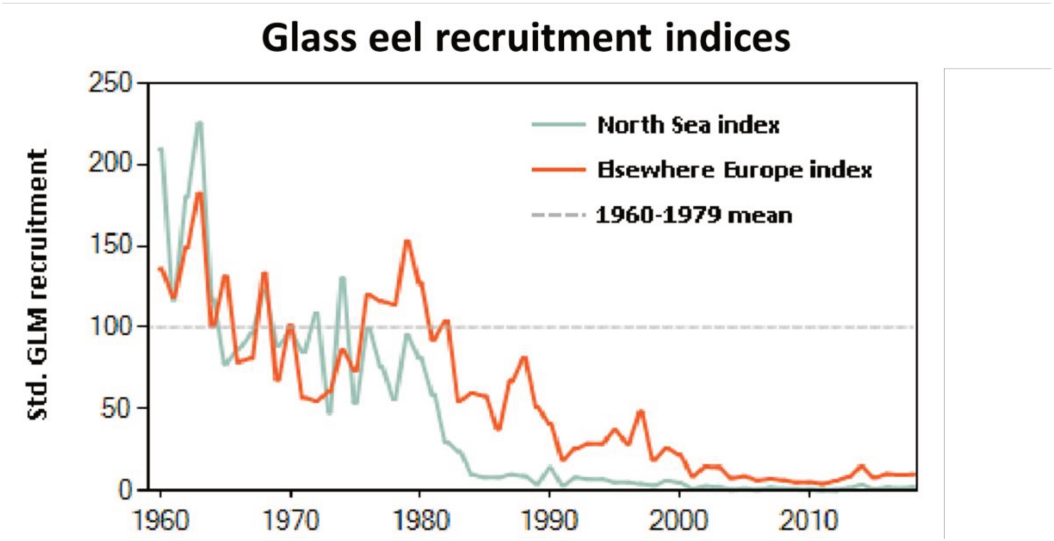


Figure 1-3. European glass eel recruitment indices, showing the geometric mean of estimated (Generalised Linear Model-GLM) glass eel recruitment for the continental "North Sea" and "Elsewhere Europe" series. The GLM was fitted to 46 time-series comprising either pure glass eel or a mixture of glass + yellow eels. The predictions were then scaled to the 1960-1979 average $P_{1960\text{-}1979}$. In the Baltic area, recruitment occurs at the yellow eel stage only. The "North Sea" series are from Norway, Sweden, Germany, Denmark, the Netherlands, and Belgium. The "Elsewhere" series are from UK, Ireland, France, Spain, Portugal, and Italy (ICES, 2018).

The main factors proposed to explain the decline include both natural and anthropogenic sources. Baltazar-Soares et al. (2014) suggested that regional atmospherically driven ocean current variations in the Sargasso Sea may be the major driver of the onset of the sharp decline in eel recruitment in early 1980s, affecting the survival and migration success of the leptocephalus larvae. In addition, climate-driven changes may also reduce the oceanic productivity in the spawning area, limit food availability for larvae during the oceanic migration and increase larval mortality (Friedland et al., 2007; Knights, 2003; McCleave and Edeline, 2009). Anthropogenic causes mainly include overfishing for both glass eels and silver eels located on rivers and estuaries (Castonguay et al., 1994; Dekker, 2003, 2004), habitat fragmentation by manmade obstructions (Drouineau et al., 2017; Podgorniak et al., 2017; Verhelst et al., 2018), as well as turbine smash by pumping stations and hydro-power for downstream migrating silver eels (Durif et al., 2003; McCleave, 2001). On the other hand, infection with the swimbladder parasite *Anguillicola crassus* has been repeatedly blamed for the dramatic drop in European eel (Haenen, 1995; Sauvaget et al. 2003; Wurtz et al., 1996). Furthermore, one of the most highlighted threats in eel is the continuous degradation of water quality by chemical pollutants. Persistent toxic contaminants that are uptaken by eel, such as heavy metals, pesticides and PCBs, can interfere with energy metabolism during eel migration (Ginneken et al., 2009; Maes et al., 2005) but also maternally transferred to their eggs (Ginneken et al., 2009) affecting the survival period of

eel embryos (Palstra et al., 2006). Moreover, contaminants with cytotoxicity and neurotoxicity may also disrupts complex migration behavior of glass eels through various endocrine and metabolic pathways (Claveau et al., 2015a; Robinet and Feunteun, 2002; Scott and Sloman, 2004). These factors may affect eels at all stages concerning their recruitment level, fecundity and survival (Dekker, 2008; Dorow et al., 2010; Kirk, 2003; Knights, 2003; Winter et al., 2007).

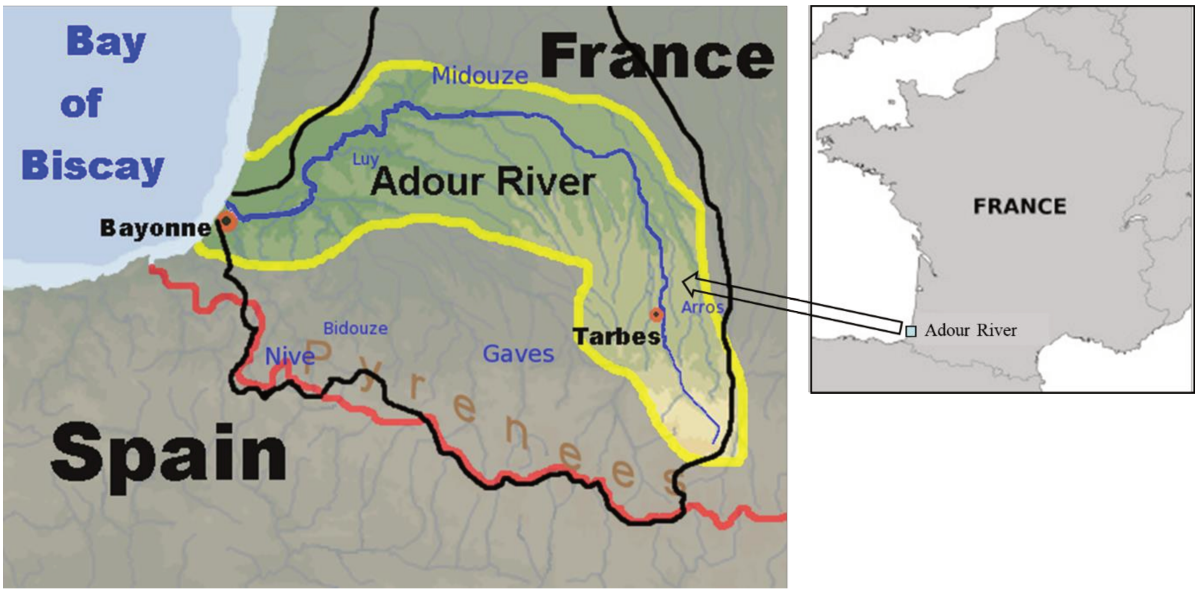


Figure 1-4. Map of the Adour River.

1.1.3 Management actions

In order to prevent further decline of the eel stock to extinction, the European Commission has introduced national eel management plans for conservation purpose, such as restocking (Righton and Walker, 2013; Stacey et al., 2015). Restocking consists in transferring young eels (mostly glass eels), from estuaries to reception habitats that are most favorable to their growth and development, including the translocation of wild glass eels from crowded areas with high recruitment (donator) to sparse areas experiencing recruitment declines (recipient). As a recovery tool, restocking aims for a higher level of benefit than natural colonization, with an ultimate goal to contribute a higher number of spawning adults to the sea. The Bay of Biscay area receives approximately ninety per cent of all European glass eel recruitment, with the most abundance along the Atlantic coast of France (Dekker and Beaulaton, 2016). France, as an essential actor in restocking implementation, is the main provider of glass eels on the European scale, where 60% of all caught glass eels are reserved for restocking programs set up by Members States, and between 5 and 10% of the national production in each year is stocked in several suitable areas of various watersheds of the French Atlantic coast.

In this management framework, the conservation stocking depends on the supply of wild glass eels and concerns the process of introducing glass eels from their natural sites to another new environment, which raise two concerns. Firstly, the changes of ambient conditions and life-history characteristics, including changes in growing salinity, temperature, density as well as inter/intra-specific interactions, represent stressful challenges on glass eels. Thus, the individual vulnerability to stressors and adaptive ability should be fundamentally relevant to the outcomes of restocking actions. Secondly, restocking is used as recovery tool but it is at the same time costing natural source of young eels, whereby a close monitoring on the risk, feasibility and achievement of restocking is needed.

1.1.4 Glass eel migration behavior

In the main areas of European eel distribution, recruitment occurs during the whole year with a peak period, which depends on latitude and oceanic factors (Harrison et al., 2014). Glass eels appear on French and Spanish coasts as early as September with the highest densities occurring between late autumn and spring (Arribas et al., 2012; Gascuel et al., 1995). In the Adour River in the south of France (Figure 1-4), the main migration season of glass eel lasts from November to March (Charlon and Blanc, 1982).

1.1.4.1 A migration synchronized to the tide: Selective tidal stream transport

Species that migrate through estuaries are exposed to the alternation of ebb and flood and can present semidiurnal and vertical migrations in phase with tidal cycle (Forward and Tankersley, 2001; Shanks, 1995). This rhythmic swimming activity consists of animals moving up in the water column during one tide and remaining on or near the bottom during the other one (Gibson, 1992; Neilson and Perry 1990). It has been called selective tidal-stream transport (STST) by Walker et al. (1978), and described for a variety of taxa and life stages, from invertebrates to fish and from juveniles to adults (De Veen, 1967, 1978; Forward and Tankersley, 2001; Harden Jones et al., 1979; Walker et al., 1978, 1980).

When European glass eels enter estuary, they are exposed to tidal cycles and STST is widely accepted to be the main mechanism allowing them to migrate up estuaries (Elie and Rochard, 1994; McCleave and Kleckner, 1982; Sheldon and McCleave, 1985). They rise in the water column during the flood to swim with the current and go down near the substrate during the ebb (Figure 1-5). STST reduce energy expenditure and in comparison with a continuous migration, the energy spared in flatfishes using STST was estimated as much as 90% (Metcalfe et al., 1990; Weihs, 1978). This innate tendency to orient and swim with a current is called negative rheotaxis. However, both field and laboratory observations showed that glass eels can also respond to a current flow by using positive rheotaxis (swimming against the current). *In situ* observations evidenced that glass eels could swim against the current when the water flow was lower than 0.3 m s^{-1} (Adam et al., 2008; Prouzet et al., 2003). Creutzberg (1961) experimentally demonstrated that glass eels expressed a positive rheotaxis for ebb currents of 0.2 m s^{-1}, and a negative one for currents higher than 0.36 m s^{-1}, swimming close to or burying into the bottom. Experimental studies also provided evidences that European glass eels subjected to a change in water current direction every 6.2 h (current velocity of about 0.13 m s^{-1}) could alternate swimming with and against the current at each water current reversal (Bolliet et al., 2007, 2008). The authors also showed that positive rheotaxis decreased with decreasing body weight likely because of the high energy cost of this behavior. The use of both positive and negative rheotaxis, may allow a sustained swimming activity to migrate up estuary when energy reserves are not limited and the tidal current is not too fast.

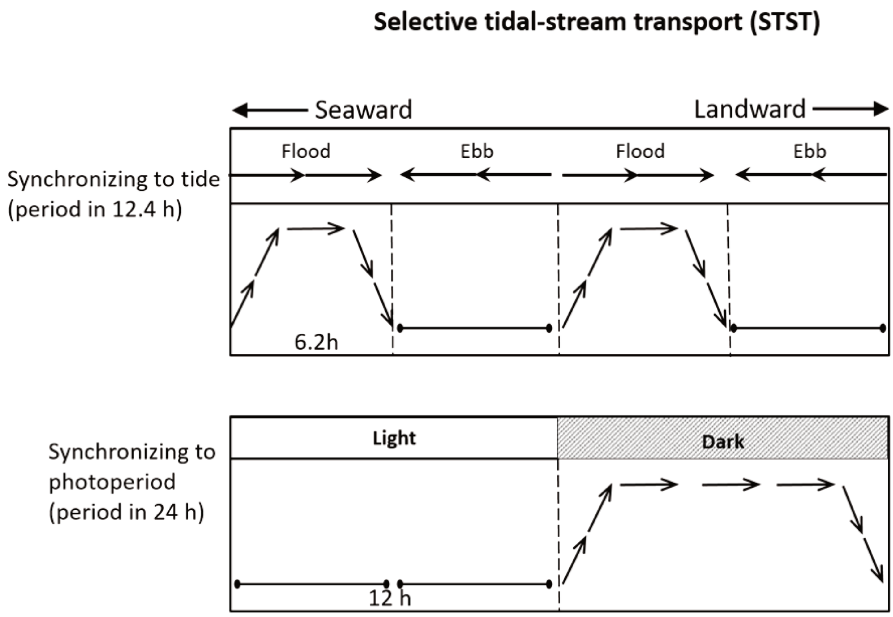


Figure 1-5. Selective tidal-stream transport of glass eel. Glass eels synchronize their swimming activity to tidal current reversal with a period of 12.4 h: during flood tide, glass eels move up in the water column and migrate with the current while they go down and remain on or in the substratum during ebb tide. Glass eels also avoid swimming during day time in clear water (modified from Forward and Tankersley, 2001).

1.1.4.2 Synchronization to the photoperiod

In the wild, the rhythmic swimming activity of glass eels is mostly observed during the night and glass eels have been reported to begin their estuarine migration when the light intensity gradually decreases from 120 to 9 lux (Tesch, 2003). In *A. japonica*, glass eels kept in experimental conditions were found to hide in the sand during daytime (Dou and Tsukamoto, 2003), but in very turbid water as in the Gironde Estuary of France 30% of European glass eels can migrate during the day (Lambert et al., 2007). During the night, most glass eels also recruit to coastal areas during the new moon phase when the light intensity is low (Harrison et al., 2014; Sugeha et al., 2001; Tesch, 2003; Tzeng, 1985). Altogether, these studies provide evidences that glass eels, as adult eels (Tesch, 1978, 1989, 1995; Tesch et al., 1991; Westerberg, 1979), are negatively phototaxic and migrate up estuary during the night mainly at the new moon, at least in clear water.

1.1.4.3 A migration driven by endogenous clocks

Experimental studies provided evidence that the activity rhythms synchronized to tidal cycle are under the control of endogenous clocks, which have not yet been located. Indeed, when exposed to constant water current direction and very dim light after synchronization to the change in water current direction, American

(Wippelhauser and McCleave, 1987, 1988) and European glass eels (Bolliet et al., 2007, 2008) sustained a swimming activity rhythm with a period close to 12.4 h. As for tidal activity, a clock also likely drives the nocturnal rhythmic swimming activity in glass eels. In both the studies of Wippelhauser and McCleave (1988) and Bolliet et al. (2007) a circadian periodicity of activity was detected (22.4 h~30 h) when fish were previously synchronized to a light dark cycle. Thus, two clock systems, one circatidal and the other circadian, probably interact to drive glass eel's swimming activity during estuarine migration.

If the main synchronizer of the circadian clock is known to be the photoperiod, tidal synchronizers are more complex. Indeed, to synchronize the tidal clock, several exogenous cues related to the tide have been hypothesized, such as current speed and turbulence (McCleave and Kleckner, 1982), odors (Creutzberg, 1959, 1961), temperature and salinity gradients (Edeline et al., 2005; McCleave and Edeline, 2009; Tosi et al., 1990), water current reversal (Bolliet et al., 2007; Wippelhauser and McCleave, 1987) or the electrical fields, which may reflect the presence and direction of water currents moving through the earth's magnetic field (Cresci et al., 2017, 2019; Deelder, 1952; McCleave and Kleckner, 1982). In the shore crab (*Carcinus moenas*), artificial tidal cycles of salinity, temperature and pressure applied 120° out of phase with each other, all synchronized locomotor activity (Warman and Naylor, 1995). The activity rhythm persisted in constant conditions with three peaks corresponding to each cue. Such observations provide evidence that different cues might synchronize glass eel locomotor activity but the interaction between them and the underlying mechanisms of synchronization remain to be elucidated. In experimental conditions, glass eels have been submitted to change in odors or water current direction every 6.2 h (Bolliet et al., 2007; Creutzberg, 1961; Wippelhauser and McCleave, 1987). Both factors individually synchronized the swimming activity of some glass eels but we cannot exclude the fact that using more cues at the same time would allow more fish to synchronize.

1.1.4.4 Swimming activity level

Colonizing continent also needs glass eels to sustain swimming activity. Since glass eels fast during their estuarine migration, they can only rely on their endogenous reserves to provide the energy necessary for swimming activity, vital functions and osmoregulation (Wilson et al., 2004). Therefore, swimming activity level may differ between individuals because of different energetic status (including energy reserves, standard metabolic rate and/or their ability to mobilize energy reserves) but also of their swimming tactics (negative and/or positive rheotaxis).

1.1.5 Facultative migration

Since 1980s, the development of microchemical techniques quantifying the concentrations of strontium (Sr) and calcium (Ca) in fish otoliths allowed to identify the history of individual fish movement and time spent in diverse habitats with different salinities (low Sr concentration in fresh water organisms and higher concentration in the marine ones; Arai et al., 2002; Campana, 1999). The direct link between Sr/Ca ratios in otoliths and ambient salinity has also been demonstrated in eels (Arai et al., 2003a, b, 2004, 2006, 2008, 2009; Tzeng, 1996; Tzeng et al., 1997, 2000), and applied to trace their migratory history and past habitat use (Arai et al., 2006; Daverat et al., 2005; Tzeng, 1996; Tzeng et al., 2000). In the Japanese eel, Tsukamoto et al. (1998, 2001, 2002) provided the first evidence that some individuals never entered freshwater and settled in marine or brackish waters. Then, more and more data in the European eel (Arai et al., 2006; Daverat et al., 2005, 2006; Tzeng et al., 2000) and the American eel (Lamson et al., 2006), showed that glass eel's migration into freshwater was also facultative. Therefore, based on the different habitat use, glass eels can be classified by four patterns of migratory history: freshwater residents, brackish residents, marine residents, and nomadic eels (Daverat et al., 2006; Figure 1-6).

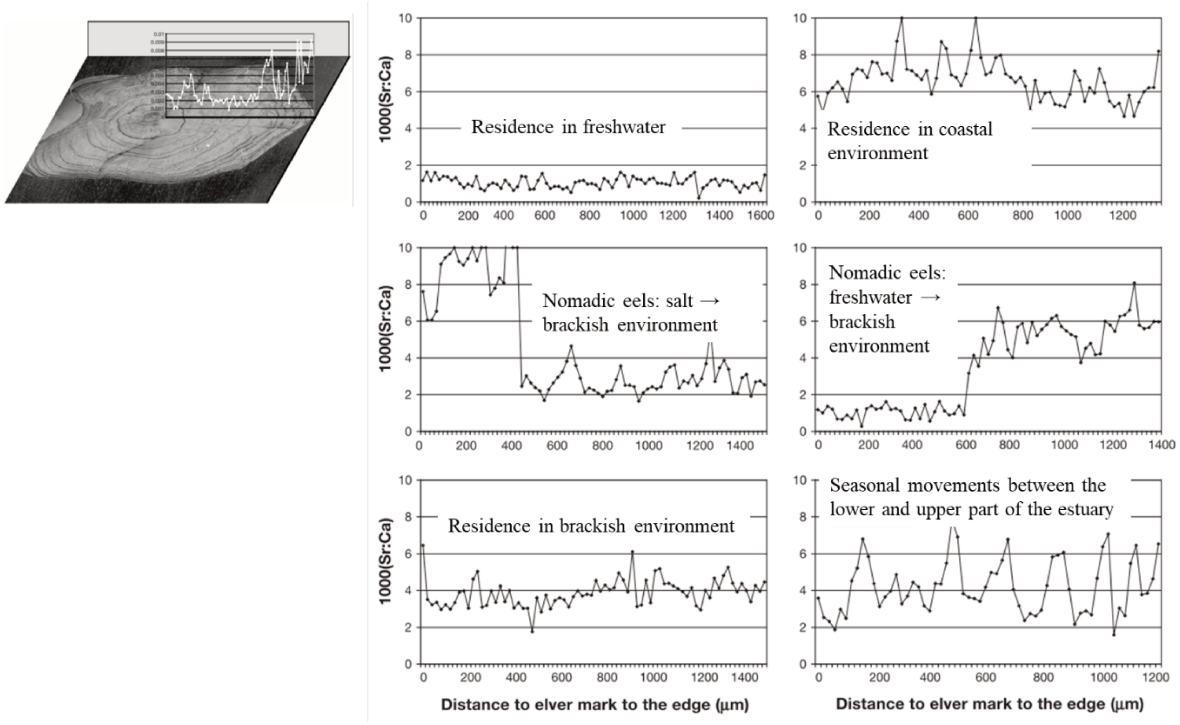


Figure 1-6. Otolith Sr:Ca ratio indicating patterns of European eel habitat use in the Gironde watershed (coast, estuary and river), France. The otolith Sr:Ca ratio variations along transects from first annulus (elver check or glass eel mark) to the otolith edge represent 6 different patterns of habitat use (Daverat et al., 2005).

Implications of glass eel's highly plastic migration pattern for the demography of eel population have been outlined by some studies (Davey and Jellyman, 2005; Geffroy and Bardonnet, 2016; Geffroy et al., 2016). Indeed, eels colonize continent as sexually undifferentiated glass eels and then develop into males and females possibly at the yellow eel stage. Although it is not yet known to what extent genetic factors contribute to the final sexual phenotype, both field and laboratory observations have highlighted that the sex determinism in eels is mostly environmental (Davey and Jellyman, 2005; Egusa, 1979; Geffroy and Bardonnet, 2016; Roncarati et al., 1997; Wiberg, 1983). Population density in the habitat used by early stage eels has been identified as one factor being positively correlated to the male production (Colombo and Grandi, 1996; Colombo et al., 1984; Beullens et al., 1997; Davey and Jellyman, 2005; Geffroy and Bardonnet, 2016; Huertas and Cerda, 2006; Krueger and Oliveira, 1999). As described, males tend to predominate in crowded environment, often associated with estuarine or lower river reaches, whereas females have been documented to be more predominant in upstream locations where densities are much lower (Adam et al., 2008; Davey and Jellyman, 2005; Harrison et al., 2014; Krueger and Oliveira, 1999; Laffaille et al., 2006; Oliveira and McCleave, 2000; Parsons et al., 1977). Thus, glass eels migrating into freshwater habitat should mostly differentiate into female while fish that settle in the estuary should mostly developed into males. To explain these different migration strategies, some studies proposed a conditional strategy based on individual's energetic status in the European eels (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006). In the framework of conditional strategy, higher propensity to migrate in glass eels is associated to higher body weight.

1.2 Glass eel energetic status and metabolism

1.2.1 Energy-based conditional strategy

The underlying mechanisms of European glass eel's facultative migration are far from being elucidated but the main hypothesis to date concerned an energy-based conditional strategy (Edeline, 2007). In this theory, a higher propensity to migrate up estuary in glass eels should be closely related to higher energy stores (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006).

What is energy-based 'conditional strategy'?

'Conditional strategy', a term first defined by Dawkins (1980) and then refined by Gross (1984, 1996), is a theoretical concept that postulates the existence within populations of individuals that express different behavioral, physical or life history tactics (phenotypes) (Gross and Repka, 1998; Hazel et al., 1990, 2004; Roff, 1996). The conditional traits, which cue the phenotypic tactics, could be the environment or individual status (Edeline, 2005; McCleave and Edeline, 2009). Conditional strategies are widespread in nature and reflect an important evolutionary force generating individual variation within a population. Especially in

populations displaying migration, which usually encounter habitat choice and environmental challenges, the phenotypic tactics of migratory patterns involve a fitness maximizing decision by the individual.

To explain the facultative migration in glass eels, some studies proposed a conditional strategy based on energy. Edeline et al. (2006) first reported that European glass eels switched from a freshwater- to a saltwater-preference as their energetic status decreased. Similarly, in the estuary of the Vilaine river, glass eels caught on the bottom of the estuary during flood tide were smaller than upstream migrants caught climbing the eel ladder (Edeline et al., 2004). Another experiment conducted in an annular flow-through flume showed that glass eels that synchronized to the photoperiod, swimming with the current at dusk (called migrant), had a higher energetic content than those remaining hidden in the shelters (non-migrant) (Bureau du Colombier et al., 2007). However, in this last study, migrant glass eels were bigger than non-migrant ones in February but not in November suggesting a seasonal variation in energy-based 'conditional strategy'. In addition, neither experimental test nor field observation could support the conditional theory in American glass eels, results showing that salinity preferences were not influenced by body condition (Boivin et al., 2015) or lipid contents (Gaillard et al., 2015). Finally, in a recent study, Bolliet et al. (2017) did not observed any significant difference in the wet weight and length of European glass eels between fish that synchronized their swimming activity to the tide (change in water current direction every 6.2 h in experimental conditions) and glass eels that remained in the substratum.

Altogether, these contradictory results suggest that a conditional strategy based on energy cannot fully explain the facultative migration in glass eels. However, it is noteworthy that to express glass eels energetic status, studies mostly used a measure of wet weight/ dry weight (Bolliet et al., 2017; Bureau du Colombier et al. 2007), body condition (Boivin et al., 2015; Edeline et al., 2006) and lipid contents (Gaillard et al., 2015) which may be not sufficient markers of the individual energetic status in glass eels.

1.2.2 Glass eels energetic status

Glass eels do not feed during their upstream migration and energy reserves must be sufficient to sustain their swimming activity in addition to vital functions and other physiological processes such as osmoregulation. However, for a fasting fish, indicators of its energetic status may be more complex than their energy content and may also concern the rate at which energy is expended for vital functions (expressed by the standard metabolic rate: SMR) and the ability to mobilize and/or use the energy stores via efficient coordination of various cellular pathways.

1.2.2.1 Energy stores

Body lipids represent the main energy source that fish can mobilize to produce energy in prior to proteins, which can also be metabolized if lipid pool is not sufficient to sustain metabolic homeostasis and exercise (Cassidy et al., 2016). *A. anguilla* leptocephali acquire food and store energy in the Sargasso Sea (Riemann et al., 2010). Glycosaminoglycans and lipids make up most of their organic mass, with the former being metabolized when leptocephali approach the time of metamorphosis to the glass eel stage and the late form providing most of the energy required to undertake this crucial life history transformation (Lecomte-Finiger et al., 2004). It has been suggested in *Anguilla japonica* that the endogenous energy reserves of the subsequent glass eel stage are provided by the leptocephali depot lipids, which remain following metamorphosis (Kawakami et al., 1999). Furthermore, considerably higher quantities of energy reserves are observed in glass eels recruiting in autumn than the ones in spring, probably due to the seasonal changes in oceanic ecosystems productivity affecting the growth of leptocephalus larvae during transatlantic transport (Désaunay and Guerault, 1997).

1.2.2.2 Standard metabolic rate

As defined by Aschoff and Pohl (1970), the standard metabolic rate (SMR) of fish is the minimal maintenance metabolic rate representing the energy expenditure of an animal during the circadian rest phase. SMR is estimated through measuring the whole-organism oxygen consumption rate (Brett, 1962; Fry and Hart, 1948) and represents an integrative measure of the physiological energy expenditures involved in the anabolism and catabolism of tissues and organism homeostasis (Metcalfe et al., 2016).

Inter-individual variation in SMR relates to fish behavioral performance

SMR presents a high variability in fish and a positive link between SMR and behavioral performance has been established in a broad range of fish species and particularly in salmonids (for review see Metcalfe et al., 2016). The most studied behavior include foraging, predator avoidance, risk-taking, mating, aggression and dominance (Alvarez and Nicieza, 2005; Biro and Stamps, 2010; Cutts et al., 1998; Eliason and Farrell, 2016; Huntingford et al., 2010; Killen et al., 2011; Metcalfe et al., 1995; 2016 for reviews), while studies investigating the propensity to migrate in fish remain scarce. In their review, Eliason and Farrell (2016) suggested in salmonids that energy reserves and energy depletion were important factors determining successful upriver migration. However, in glass eels, Bolliet et al. (2017) did not observed any significant differences between SMR of migrant and non-migrant marine and estuarine glass eels sampled in April.

The underlying mechanisms of the relationship between SMR and behavior are not fully understood in fish but a higher SMR may induce a higher efficiency to obtain food or territory as well as a better digestion or use of energy resources, maximizing the growth rate and fitness of individuals (see Metcalfe et al., 2016). On the other hand, high SMR and aerobic activities should also increase the rate at which energy substrates are oxidized and thus increase the oxidative stress. However, in brown trout (*Salmo trutta*), individual fish with higher SMR were evidenced to have lower *in vivo* content of reactive oxidative species (ROS) (Salin et al., 2015). This ability to reduce the accumulation of oxidative damage may be a selective advantage in situations when aerobic activity is intensively required, as during migration.

On the other hand, when energy stores decrease as in fasting migratory fish, the ability to migrate might also be directly related to the maximum capacity of the fish to increase oxygen consumption reflected by the maximal aerobic metabolic rate (MMR) that can be reached by the organism, setting the threshold towards which the animal can perform aerobically in a given environmental context (Norin and Malte, 2012). MMR has been found to correlate positively with SMR in some species (Norin and Malte, 2012; Priede, 1985; Zhang et al., 2014), but not in others (see Metcalfe et al., 2016).

SMR, food availability and starvation

As mentioned above, a high SMR may promote dominance and growth in fish but individuals may lose their advantage in a low food environment. Similarly, in fasting fish, advantages in behavioral performance related to a high SMR could be expected to diminish quickly even leading to a negative selective pressure on individual. Indeed, fish with a high SMR mobilize energy stores more quickly when compared to low SMR individuals (Cook et al., 2000; O'Connor et al., 2000) and a rapid depletion of energy reserves, caused by a high metabolic demand under starvation, has been observed in the crucian carp (*Carassius auratus*) (Zeng et al., 2017), the juvenile European sea bass (*Dicentrarchus labrax*) (Dupont-Prinet et al., 2010; Killen et al., 2011; McKenzie et al., 2014), and the brown trout (*Salmo trutta*) (Auer et al., 2016). Accordingly, it could be expected that in diadromous fish species which can undergo naturally fasting periods in their life cycle (Bardonnet and Riera, 2005; Jørgensen et al., 2013), active swimming during migration might be considered as risky when energy reserves are too low (Chabot et al., 2016).

One adaptative mechanism to spare energy during starvation is to reduce SMR. Indeed, although SMR is generally repeatable over time when measured under constant condition (Nespolo and Franco, 2007), e.g. in salmon (O'Connor et al., 2000) and European eel (Boldsen et al., 2013) there are increasing evidence suggesting the flexibility of fish SMR in response to food availability (Guppy and Withers, 1999; McCue, 2010; McKechnie, 2008; Metcalfe et al., 2016 for review; Rescan et al., 2007). Studies on juvenile Atlantic

salmon (O'Connor et al., 2000), brown trout (*Salmo trutta*) (Auer et al., 2015) and crucian carp (*Carassius auratus*) (Zeng et al., 2017) have shown that SMR decreased in starved fish and increased again once food was supplied, although a high variability was observed. Through reducing SMR, fish attempt to minimize energy investment in self-maintainance and spare energy stores (Armstrong et al., 1992). This flexible metabolic rate has mostly been considered as an adaptive mechanism to maximize fitness under the challenges of energy distress (Auer et al., 2015). Altogether, these studies suggest that SMR and its inter-individual variability may be an important parameter to explore for a better understanding of the ability of glass eels to migrate.

1.2.2.3 Energy mobilization ability

In addition to their energy reserves and the speed at which they use them, fasting fish have also to own the ability to closely coordinate various cellular pathways to mobilize internal stores to sustain metabolism and activity.

Main forms of energy reserves mobilized in migration

Fish store energy primarily as triacylglycerides (TAGs) in adipose tissues and largely mobilize them during fasting to support energy requirements (Eaton, 2002; Finn and Dice, 2006; Kerner and Hoppel, 2000). Once lipid depletion reaches a critical threshold, animals enter in a depleted phase in which proteins can also be used as a fuel source to survive prolonged fasting (Bower et al., 2009).

European glass eels arriving along European coast depend on the energy reserves accumulated during the larval stage to reach fresh water for growth. In the Adour estuary, glass eels would restart feeding at spring when individuals develop to the VIA3 stage (Charlon and Blanc, 1982). However, before refeeding, glass eels have to allocate a substantial part of their somatic and visceral energy reserves to meet the costs of migration. Thus, their ability to efficiently mobilize their energy stores may directly affect their swimming ability and migratory strategies. This implies the existence of tightly controlled mechanisms to mobilize the various energy reserves, which are still very little studied in glass eels.

Mechanisms involved in substrate catabolism and energy production

Organisms can survive starvation by inducing the breakdown of lipid stores and even those of proteins when the period of starvation is prolonged. Different routes exist for the degradation of energy reserves. Lipids and proteins breakdown via cytosolic lipases and the ubiquitin-proteasome system have been largely described, and most factors involved in these processes have been identified in mammals and teleosts (Collins and Goldberg, 2017; Mashek, 2013; Zechner et al., 2012). However, increasing evidences demonstrate the existence of an alternative pathway known as autophagy, which involved the lysosomal

degradative system (Bustamante et al., 2018; Zechner et al., 2017). In the sections below, we will present the main factors involved in the degradation of lipids and proteins. We will then provide an overview of the factors and processes involved in mitochondrial oxidation and turnover.

A. Lipid degradation

The degradation of lipid stores involve different processes of TAG breakdown as well as the β-oxidation of free fatty acids (FFAs). Indeed, FFAs can be released from lipid droplets (LDs) by two primary mechanisms: Cytosolic lipolysis and lipophagy, the latter process corresponding to a subtype of autophagy during which portions of cytosolic LDs are engulfed by autophagosomes and transported to lysosomes, where TAG and other lipids undergo acid lipolysis by lysosomal acid lipase (Singh et al., 2009; Zechner et al., 2012).

Cytosolic lipolysis

Cytosolic lipolysis is a highly regulated process by which cytoplasmic neutral lipases directly hydrolyze TAGs at the surface of LDs (Wang et al., 2008; Zechner et al., 2012). Three important enzymes have been identified participating in this process: first, the adipose triglyceride lipase (ATGL, also known as patatin-like phospholipase domain-containing protein 2 (PNPLA2)), selectively hydrolyzes TAGs to generate diacylglycerols (DAGs) and non-esterified FFAs (Zimmermann et al., 2004); then, the hormone-sensitive lipase (HSL) and the monoacylglycerol lipase (MGL) complete the process by consecutively hydrolysing DAGs into monoacylglycerols (MAGs) and FFAs and hydrolysing MAGs into glycerol and FFAs (Vaughan et al., 1964; Zechner et al., 2017). These proteins together constitute the basis of 'lipolytic machinery' (Figure 1-7; Bolsoni-Lopes and Alonso-Vale, 2015).

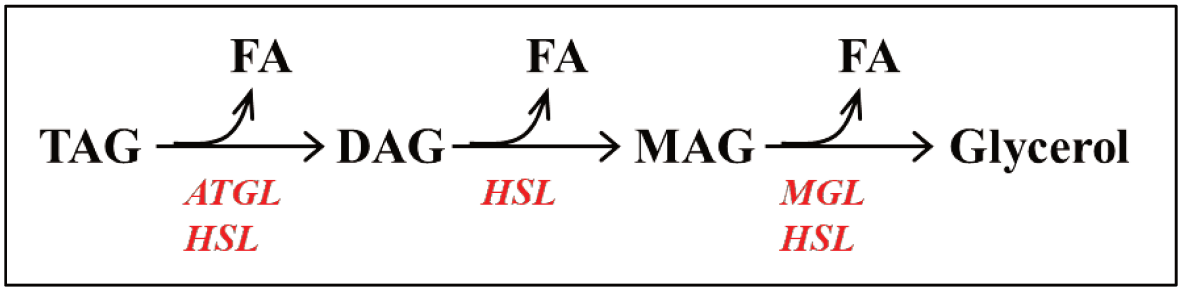


Figure 1-7. Sequential hydrolysis of triacylglycerol. Lipolysis consists of the sequential hydrolysis of TAG to its constituent molecules glycerol and three fatty acids, catalyzed by three different enzymes (Bolsoni-Lopes and Alonso-Vale, 2015).

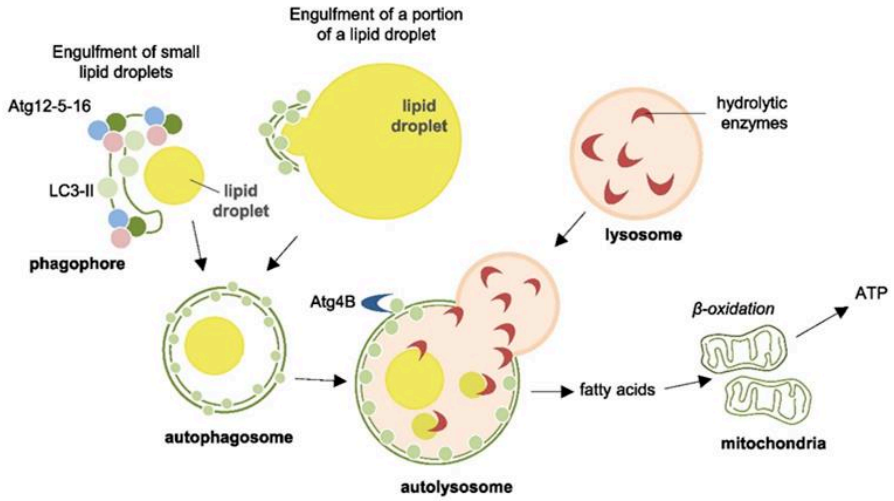


Figure 1-8. Lipophagy. Degradation of LDs through lipophagy involves the classical autophagosome-mediated pathway of budding off and sequestering LDs for their subsequent delivery to autolysosomes (Ward et al., 2016).

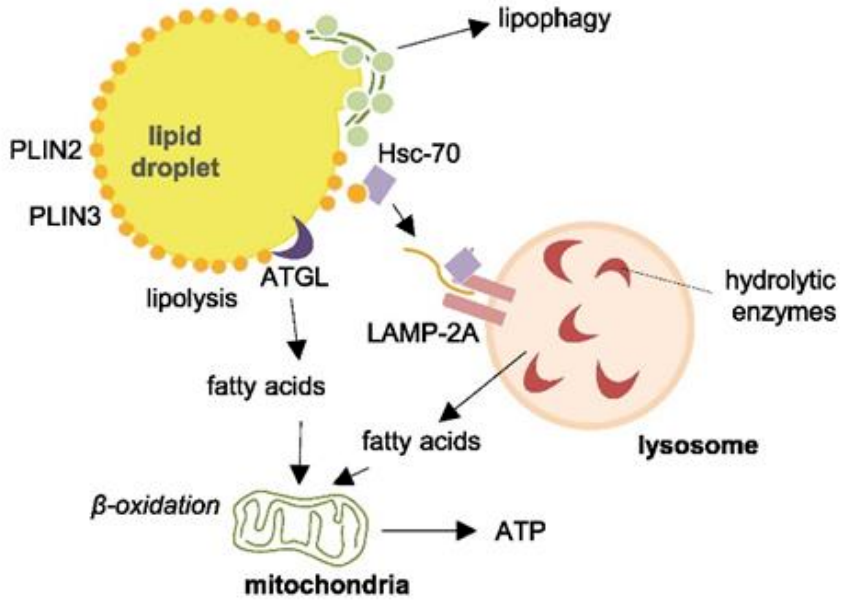


Figure 1-9. Lipid droplet lipolysis through CMA-dependent degradation. PLINs, perilipin family members, are the best characterized LD-associated proteins. CMA promotes LD catabolism via the degradation of PLIN LD proteins, thus, allowing access for lipases and lipophagic organelles (Ward et al., 2016).

Lipophagy

Autophagy is a highly conserved homeostatic mechanism that involves degradation and recycling of cellular constituents in lysosomes (Mizushima and Komatsu, 2011). Defective autophagy is associated with numerous diseases, including neurological disorders, cancer, cardiomyopathies and metabolic disorders (Levine and Kroemer, 2008). The major variants of autophagy include macroautophagy, chaperone-mediated autophagy (CMA) and microautophagy (Mizushima and Komatsu, 2011). One form of macroautophagy, known as 'lipophagy', was discovered in 2009 and was shown to contribute to the hydrolysis of TAG stored in cytosolic LDs (Singh et al., 2009). As the general mechanisms of macroautophagy, lipophagy is also tightly regulated by signals of cellular energy status during fasting. Briefly, during this process, autophagosomes engulf small or portion of LDs and, through the action of several autophagy-related factors, fuse with late-endosomes or lysosomes to form autolysosomes (Figure 1-8; Itakur et al., 2012; Saftig et al., 2008). Within autolysosomes, lysosomal acid lipase (LAL) finally hydrolyses TAGs to release FFAs (Dubland and Francis, 2015).

A substantial contribution of lipophagy to the catabolism of cellular TAGs and cholesteryl esters has been demonstrated in various cell types, including hepatocytes, enterocytes, macrophages, brown adipocytes and neurons (Cingolani and Czaja, 2016). Both autophagy inhibitors and genetic invalidation substantiated the crucial role of lipophagy in the breakdown of hepatic TAGs (Singh et al., 2009). Inhibition of neutral lipases or autophagy showed that both processes are activated in hepatocytes during fasting (Singh et al., 2009). The quantitative contribution of each of the lipolytic pathways (cytosolic lipolysis and lipophagy) to overall lipid catabolism is unknown and may vary considerably between different cell types, such as hepatocytes, macrophages and adipocytes.

Cytosolic lipolysis–lipophagy crosstalk

The recent findings that the key autophagy factor LC3-II binds ATGL on cytosolic LDs in brown adipocytes after cold exposure to promote TAG hydrolysis (Martinez-Lopez et al., 2016) strengthened the possibility of a functional link between autophagy and cytosolic lipolysis.

Further evidence for a functional link between autophagy and lipolysis came from the discovery that CMA degrades cytosolic LD-associated proteins and thereby regulates neutral lipolysis (Figure 1-9; Kaushik and Cuervo, 2015; Ward et al., 2016). During CMA, cytosolic proteins are first recognized by HSPA8/HSC70 (heat shock protein family A [Hsp70] member 8) and co-chaperones. The substrate-chaperone complex then docks at the lysosomal membrane through specific binding to the cytosolic tail of LAMP2A (lysosomal associated membrane protein 2A). LAMP2A then organizes into a multimeric complex that allows the substrate to translocate across the lysosomal membrane where it is degraded by acid hydrolases. Perilipin

2 and perilipin 3, which are abundant cytosolic LD-associated proteins that shield LD from lipases and lipolysis, are CMA targets (Kaushik and Cuervo, 2015). Consequently, the removal of perilipin 2 and perilipin 3 by CMA enables ATGL to efficiently access the LD surface, thereby increasing lipolytic rates.

Interestingly, neutral lipolysis may also be directly involved in the regulation of autophagy, specifically through the effect of ATGL on the hepatic function of PPARα and SIRT1, both of which are well-established activators of autophagy in the liver (Efeyan et al., 2015; Lee et al.. 2014). Together with the co-regulation of lipophagy and neutral lipolysis by the major metabolic hormones and their associated regulatory hubs (mTOR, AMPK and the FOXO transcription factors), these data support the view that neutral lipolysis and lipophagy should not be considered distinct, but instead should be seen as two sides of the same coin.

B. Protein degradation

Eukaryotic cells mainly use two distinct mechanisms for the degradation of most proteins during nutrient stress: the ubiquitin-proteasome pathway and autophagy-lysosome system (Ciechanover et al., 1984; Mommsen, 2004).

Ubiquitin-proteasome system

The ubiquitin proteasome system (UPS) is responsible for the degradation of most cytosolic and nuclear proteins, including short- and long-lived proteins (Collins and Goldberg, 2017), as well as aberrant or misfolded proteins. The UPS mediates the proteolysis of target proteins by the conjugation of ubiquitin molecules in an ATP-requiring reaction (Ciechanover et al., 1984; Hershko and Heller, 1985; Murton et al., 2008). Three enzymes control the processes of ubiquitin transfer and conjugation to substrate proteins: the E1 ubiquitin-activating enzyme first binds ubiquitin, then transfers it to the E2 ubiquitin-conjugating enzyme, and finally the ubiquitin molecule is transferred from the E2 to a lysine residue of the target protein. This last step is regulated by the E3 ubiquitin-ligase enzyme, which has a central role in achieving the selectivity and specificity of the UPS by recognizing and binding to specific substrate sequences (Figure 1-10; Campello et al., 2013; Haas et al., 1982; Hershko and Ciechanover, 1998; Lecker et al., 1999; Passmore and Barford, 2004). Two most identified muscle-specific E3s are muscle RING finger-1 (MuRF-1) and muscle atrophy F-box (MAFbx) (Bodine et al., 2001; Gomes et al., 2001;), up-regulations of which were found to produce muscle loss and atrophy in both mammals and teleosts (Cleveland and Evenhuis, 2010; Lokireddy et al., 2011). The polyubiquitinated protein substrates are recognized and degraded by a protease complex, the 26S proteasome (Cassidy et al., 2016; Ciechanover et al., 1984).

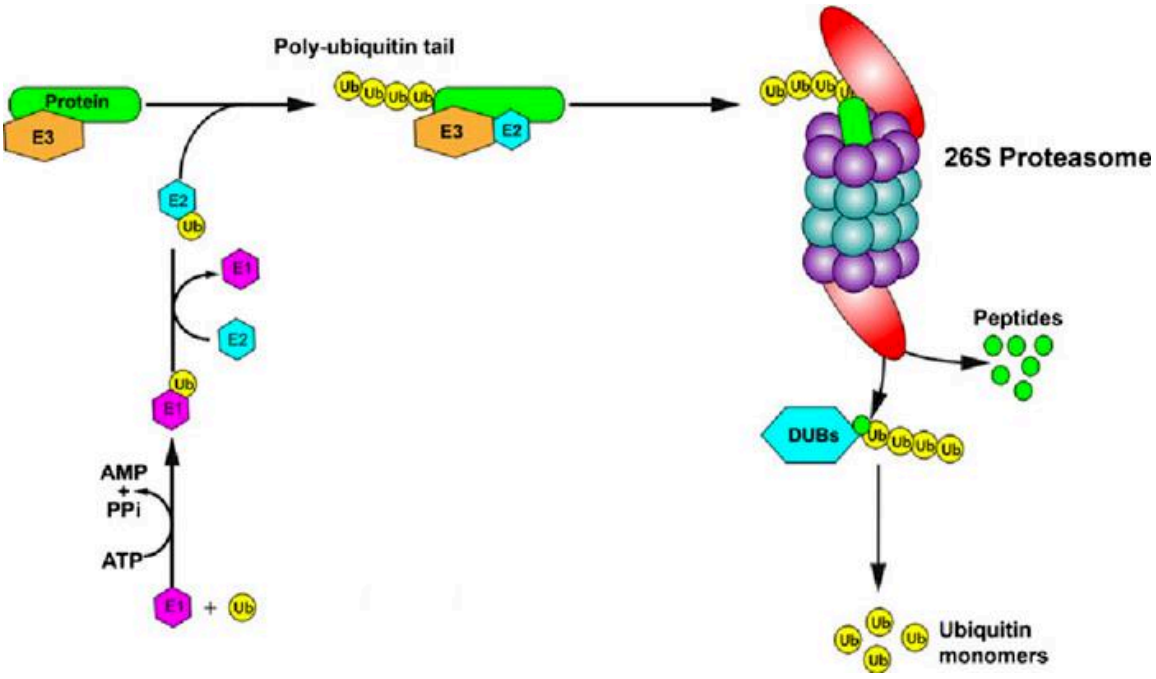


Figure 1-10. Ubiquitin-proteasome system (UPS). Intracellular misfolded, damaged, and obsolete proteins are degraded by the UPS in a process in which major enzymatic components (E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, E3 ubiquitin-ligase enzyme) poly-ubiquitinate the target proteins and allow a degradation by 26S proteasome (Campello et al., 2013).

Autophagy

In contrast to the UPS, autophagy-lysosome degradation is restricted to the cytoplasm, and tend to target long-lived proteins. This system has been shown to degrade a wide spectrum of substrates, including functional or misfolded soluble proteins, protein complexes, oligomers and aggregates (Korolchuk et al., 2009). Under stress stimuli, autophagy-lysosome pathway is highly inducible in muscle cells to induce protein catabolism, for instance in starvation (Bustamante et al., 2018; Mammucari et al., 2007; Mizushima et al., 2004) as well as endurance exercise (Grumati et al., 2011; Jamart et al., 2012). The autophagic protein degradation can be both non-selective and selective. In selective pathway, lysosomal degradation is also ubiquitin-dependent for protein targeting (Figure 1-11; Rabinowitz and White, 2010). Briefly, during selective autophagy, ubiquitin moieties added to the targeted proteins are recognized and bound by autophagy receptors, such as p62 or NBR1, which interact with LC3 to deliver cargo to autophagosomes (Kuma and Mizushima, 2010; Shaid et al., 2013). Enclosed proteins in autophogosome are then degraded in lysosome by cathepsins which have a wide range of specificities (De Duve and Wattiaux, 1966). Finally, amino acids produced are released into the cytoplasm and will be used for energy production and/or the synthesis of new molecules essential for survival.

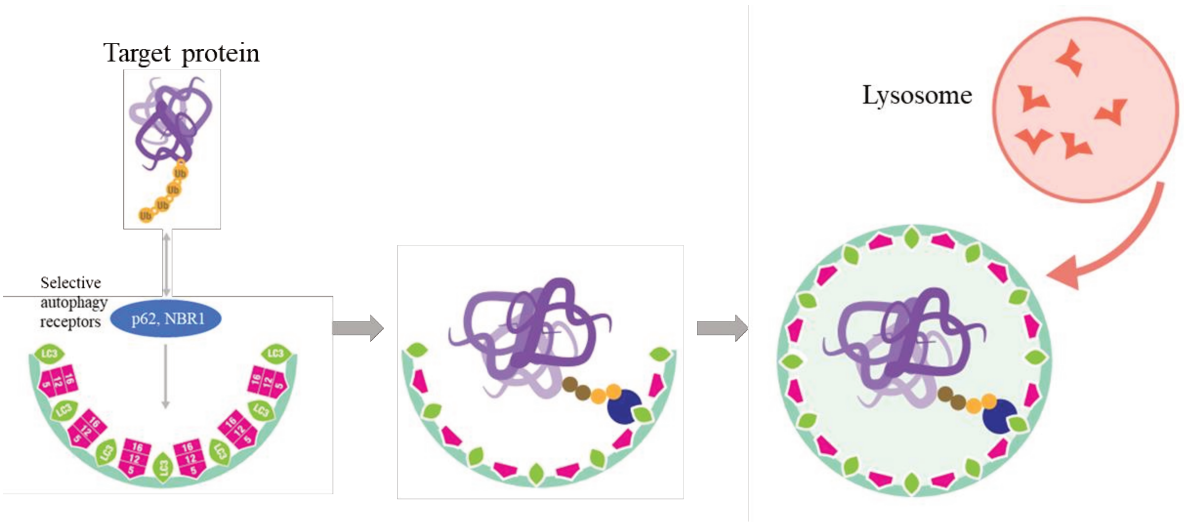


Figure 1-11. Selective autophagy for protein degradation. Particular protein aggregates are targeted into the autophagosome by selective autophagy receptors (Shaid et al., 2013).

The UPS-Autophagy Connection

The UPS and autophagy are the two major and evolutionarily conserved degradation and recycling systems in eukaryotes. Although their mode of action and their requirements for substrate recognition are different, recent studies show several interconnections between them (reviewed by Kocaturk and Gozuacik, 2018). Compensation mechanisms exist between the two systems (Demishtein et al., 2017; Fan et al., 2018; Selimovic et al., 2013; Wu et al., 2008). Indeed, following proteasome inhibition, several autophagy-related factors was shown to be activated, such as Beclin1, LC3, p62 or GABARAPL1 (Ge et al., 2009; Sha et al., 2018; Zhu et al., 2010). The proteasome inhibition can also been sensed by the major regulators of autophagy - i.e. AMPK (Jiang et al., 2015; Xu et al., 2012) and mTORC1 (Zhao and Goldberg, 2016; Zhao et al., 2015), which in turn activate autophagy system. Similarly, impairment of autophagy has been evidenced to increase UPS activity (Wang et al., 2013). Yet in several cases in mammal models, autophagy inhibition via ATGs (autophagy-related genes) knockout resulted in the impairment of UPS, accumulation of ubiquitylated proteins as well as other important UPS substrates (Hara et al., 2006; Komatsu et al., 2005, 2006; Riley et al., 2010). In the compensatory connection between UPS and autophagy, ubiquitylation was proposed to be a common component that directs substrates to the proper degradation system and even contribute to the UPS-autophagy crosstalk (Dikic, 2017; Korolchuk et al., 2010). In this regard, K48-linked ubiquitylation was proposed to be a signal for the UPS, whereas K63-linked ubiquitylation directed proteins for autophagosomal degradation (Herhaus and Dikic, 2015). Another important component of the UPS-autophagy switches concerns E3 ligases. Accordingly, Cullin-3 (Pintard et al., 2004), SMURF1 (Ebisawa

et al., 2001), MDM2 (Shi and Gu, 2012) E3 ligases directed proteins to degradation by the UPS, while Parkin (Chan et al., 2011), LRSAM1 (Huett et al., 2012), CHIP (Shin et al., 2005) E3 ligases primed proteins for autophagic degradation. Therefore, the reciprocal regulation and coordination mechanisms between UPS and autophagy (existing at various levels) allow remodeling of the cellular proteome under different conditions to maintain cellular and organismal homeostasis.

The degradative pathways presented above allow the release of FFAs and amino acids (AAs) to supply mitochondria (the primary site for oxidation) and generate energy. During these processes, remodeling of mitochondria into highly connected networks usually occurs to enhance the respiratory functions (Gomes et al., 2011; Rambold et al., 2011).

C. Mitochondrial oxidation and turnover

Mitochondria are considered as the powerhouse of the cell, and are responsible to sustain cellular energy levels. During mitochondrial respiration, oxidation of substrates (FFAs and AAs) results in electron transfer to molecular oxygen coupled to electrochemical proton translocation across the mitochondrial inner membrane. The sequential reduction/oxidation (redox) reactions in the electron transport chain generate an electrochemical gradient that drives ATP synthase to phosphorylate adenosine diphosphate (ADP) to adenosine triphosphate (ATP), the major form of cellular energy (Mitchell, 2011).

In order to fulfil their function, mitochondria must constantly adapt to their environment. In this respect, the mitochondria are organized in a dynamic network that is in permanent evolution. The mitochondria undergo continual biogenesis, fusion/fission and degradation to maintain their mass, morphology and functions in response to changes in their environment (Figure 1-12; Seo et al., 2010). These mitochondrial turnover processes efficiently control the organelle quantity and quality.

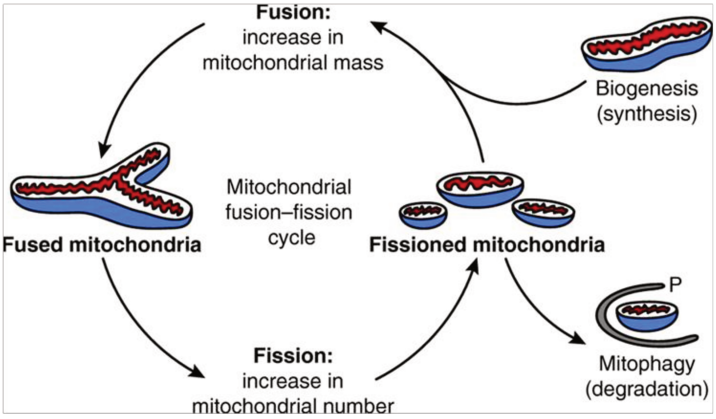


Figure 1-12. Mitochondrial fusion, fission, biogenesis and degradation (Seo et al., 2010).

Mitochondrial biogenesis consists of the addition of new proteins and/or lipids to the pre-existing mitochondrial reticulum, through the action of both mitochondrial and nuclear factors (Figure 1-13). Peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1α) is a major regulator of mitochondrial biogenesis (Baar et al., 2002). It is now well established that it activates different transcription factors, including nuclear respiratory factors 1 and 2 (NRF1, NRF2), that promote the expression of the mitochondrial transcription factor Tfam (Bruni et al., 2010), required for the transcription of mitochondrially encoded proteins and mtDNA replication (Campbell et al., 2012; Dhar and Wong-Riley, 2009; Ongwijitwat et al., 2006).

As stated above, mitochondria are highly dynamic cellular organelles, with the ability to change size, shape and position to adapt to their environment. Many of these changes are related to the ability of mitochondria to undergo the highly co-ordinated processes of fission (division of a single organelle into two or more independent structures) or fusion (the opposing reaction). These actions occur simultaneously and continuously in many cell types, and the balance between them regulates the overall morphology of mitochondria within any given cell. Fission and fusion are active processes which require many specialized proteins, including mechanical enzymes that physically alter mitochondrial membranes, and adaptor proteins that regulate the interaction of these mechanical proteins with organelles (Gomes et al., 2011; Rambold et al., 2011). Many of the proteins involved in mitochondrial fission/fusion dynamics have been identified in yeast, and most are conserved in mammals, including the fusion mediators mitofusins 1 and 2 (Mfn1 and Mfn2) (on the outer mitochondrial membrane) and the optic atrophy protein 1 (Opa1) (on the inner mitochondrial membrane), and the fission mediators dynamin-related protein 1 (Drp1) (Hoppins and Nunnari, 2009; Hoppins et al., 2007; Westermann, 2008).

In some circumstances, damaged mitochondria or parts of the mitochondrial network can also be digested through mitophagy (autophagy dependent mitochondria degradation; Figure 1-13). Several types of mitophagy (depending on the factors involved) have been described in mammals, two of which have been particularly studied: the NIX-dependent mitophagy and the PINK1/PARKIN-dependent mitophagy. The first mechanism involves mitochondrial receptors NIX (also known as BNIP3L) and FUNDC1 (mostly induced in hypoxia). During this process, the receptors are anchored in the outer mitochondrial membrane and contain an LC3-interacting region (LIR). By recognizing the autophagy-related protein LC3 at the phagophore surface, they facilitate the aggregation of autophogosomes around the target mitochondria (Melser et al., 2015). Different to the NIX-dependent mitophagy, the second mechanism is activated by PINK1 kinase at the outer mitochondrial membrane. PINK1 kinase recruits PARKIN to the mitochondria, which mediates the ubiquitination of mitochondrial substrates (Narendra et al., 2008, 2010). Organelles

recognized by p62 and LC3 are then enclosed into the autophagosome to be digested in lysosome (Geisler et al., 2010).

Above all, the metabolic rate and molecular regulation of cellular catabolism can characterize glass eel's energetic status in term of energy mobilization ability. However, once entering estuaries which is a stressful ecosystem, glass eel's energy condition will be also associated with environmental stressors, such as mercury contaminant, especially its organic form methylmercury.

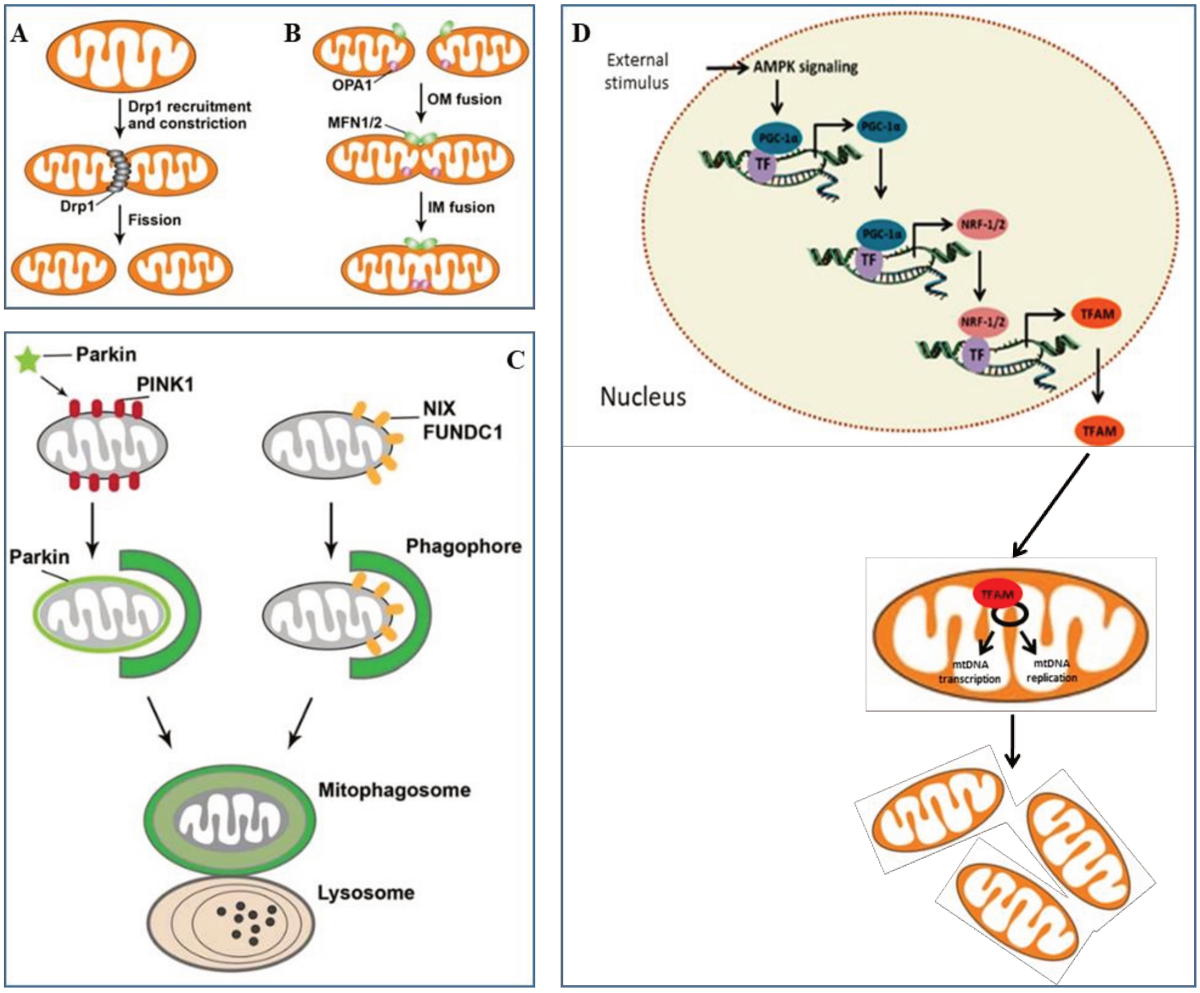


Figure 1-13. Schematic illustration depicting the core proteins of the molecular machinery that mediate mitochondrial turnover (modified from Cai and Tammineni, 2016; Picca et al., 2017). (A) Mitochondrial fission; (B) Mitochondrial fusion; (C) Mitophagy; (D) Mitochondrial biogenesis.

1.3 Toxicity of methylmercury (MeHg) contaminant

1.3.1 Estuary: a stressful environment

Estuaries have long been regarded as environmentally stressed areas because of the high degree of variability in their physico-chemical characteristics, for example hydraulic conditions, oxygen, temperature and salinity in the water column, bed sediment dynamics, as well as pollution related to anthropogenic activity. Due to the proximity to urbanized and industrial areas, estuaries represent the major receptacle of a wide variety of potentially toxic chemical substances coming from upstream.

Most reported pollutants include pesticides from agriculture waste (Leong et al., 2007), organic chemicals PAHs and PCBs (Vane et al., 2007), and heavy metals such as cadmium (Cd), copper (Cu), and lead (Pb) (Milenkovic et al., 2005; Vieira et al., 2009). Mercury (Hg) is one of the most hazardous pollutants present in aquatic environments (Grilo et al., 2015; Pereira et al., 2009). The presence of Hg in the estuaries raised many concerns about its influence on aquatic communities in these environments (Lawson and Mason, 1998; Sunderland et al., 2004). Despite the strict Hg restrictions, anthropogenic release and historically contaminated sediments still act as sources of Hg to the aquatic environment, especially in areas requiring maintenance dredging or where sediments may be disturbed and resuspended into the water column (Alonso et al., 2000; De Marco et al., 2006; Meybeck et al., 2007; Sun et al., 2012). Hg compounds present in several estuaries across Europe have been shown to exceed the EU environmental quality standard (EQS) levels (Nguetseng et al., 2015) and research focused on Hg pollution has still been a hotspot in estuary during the last decade.

1.3.2 Mercury occurrence in aquatic systems

Hg is considered a devastating environmental pollutant, mainly due to its toxicity, persistence and biomagnification along the food webs and risk for human health (Cardoso et al., 2014; Mathews and Fisher, 2008). Hg is released into environment from both anthropogenic and natural sources, such as industrial uses, the combustion of fossil fuels, the weathering of Hg-bearing rocks and ores (e.g. cinnabar), the fallout of atmospheric gases from volcanoes and geothermal vents and the emissions of deep-sea hydrothermal vents (Kennish, 1997; Wren et al., 1995). This metal compound occurs in diverse chemical forms, including inorganic (Hg(II)) and methylated (MeHg), which can be interconverted in a global Hg cycle among the aquatic systems, the atmosphere and the sediment (Figure 1-14; Clarkson, 1993; Pereira et al., 2019). Concentrated literature have presented the toxicological background of Hg with a lot of concerns over the organometal MeHg, often considered as a more toxic form than Hg(II). It is widely found in the aquatic environment (Ullrich et al., 2001) with the majority present in nature being derived from the biomethylation of Hg(II) by sulfate-reducing bacteria (Jensen and Jernelov, 1969; Compeau and Bartha, 1985).

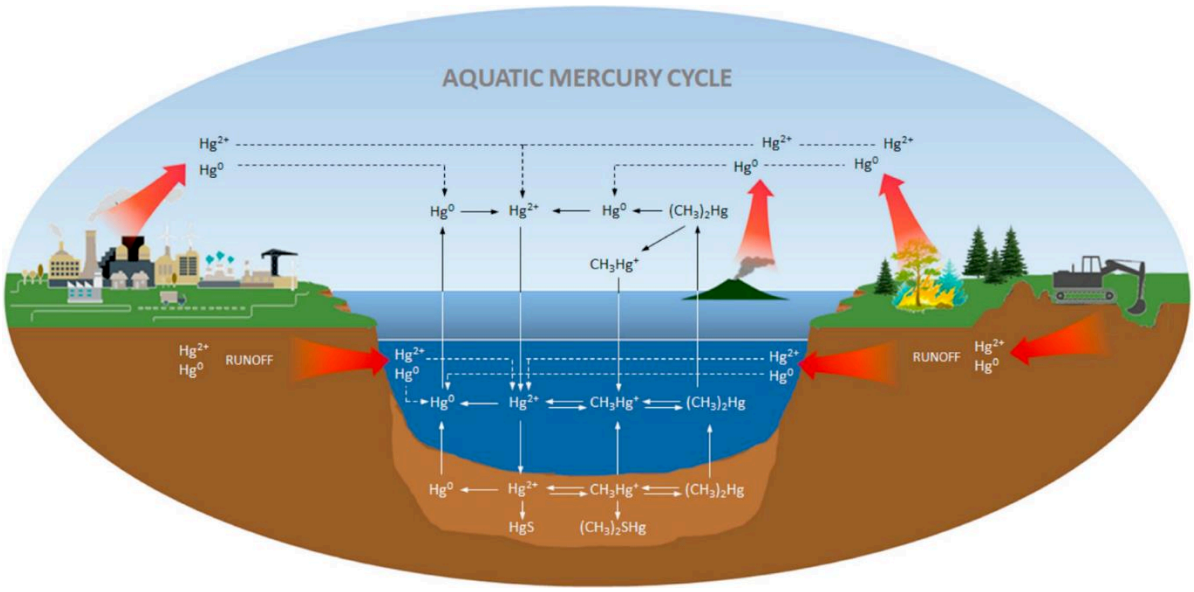


Figure 1-14. Summary of the major transformations of mercury (Hg) in the environmental compartments (atmosphere, aquatic systems and sediment). Total Hg in the aquatic systems includes dissolved species of divalent mercury (Hg^{2+}), dissolved elemental mercury (Hg^0) and methylated forms (MeHg, CH_3Hg^+) in water-air surface. Hg^0 is relatively volatile, supersaturated in water surface, and is the main form of Hg found in the atmosphere. Hg^{2+} is the generally predominant form found in water. The quantity of MeHg form is relatively large at greater depths in the water and sediment. Cycling pathways involve biogeochemical processes within each compartment and inter-compartmental movements (e.g. deposition, runoff, volatilization, sedimentation and sediment diffusion/advection/resuspension). Natural (e.g. volcanoes and forest fires) and anthropogenic (e.g. hydroelectric, pulp/paper and mining industries, incineration of municipal waste) sources of Hg are depicted by the red arrows (Pereira et al., 2019).

1.3.3 MeHg effects on fish

1.3.3.1 MeHg absorption by fish and tissue-specific toxicity

Wild fish are exposed to MeHg by both food and water. Uptake of this toxin from diet accounts for approximately 80% to 90% of total uptake, with the remaining fraction coming from drinking water, gill intake, and skin absorption (Erickson et al., 2008; Hall et al., 1997; Hrenchuk et al., 2011; Sindayigaya et al., 1994).

The ability of MeHg to distribute throughout the body is often attributed to its presumed lipid solubility. However, this explanation is untenable, given the physicochemical properties of MeHg (Clarkson, 1972). As a consequence of its high affinity for –SH groups, most of the MeHg in tissues is normally conjugated with water-soluble sulfhydryl-containing molecules, primarily L-cysteine, glutathione (GSH), hemoglobin, albumin and other thiol-containing polypeptides (Carty and Malone, 1979; Hughes, 1957). Hence, dietary

MeHg is first delivered to the liver through a portal system after uptake in the intestine (Bridges and Zalups, 2010; Mela et al., 2014), then crosses the hepatocyte plasma membrane and bind to thiol groups before being eliminated by the bile or redistributed by blood stream to other target tissues as muscles (Harris et al., 2003; Leaner and Mason, 2004; Morcillo et al., 2017; Ribeyre and Boudou, 1984; Sweet and Zelikoff, 2001).

When fish cease eating and do not have to drink to maintain their osmolarity (as in glass eels during their migration in fresh water), the primary pathway of MeHg entering fish body is through gills. In Monperrus et al. (2020), the pathway of the uptake, transport, and accumulation over time of different Hg species in European glass eels have been imaged. This study suggested a preferential uptake of the MeHg than Hg(II) and a dynamic transport of MeHg within different organs: after rapid uptake through the gills, MeHg was first transported in the heart, the liver, and the brain, and finally transferred to the muscle. Metabolic and histopathological changes in MeHg-targeted tissues are of particular interest because these changes can trigger fish loss of endogenous homeostasis, abnormal motor coordination, altered behavior, and disrupted energy metabolism (Roos et al., 2012; Wolfe et al., 1998).

Nervous system

As in mammals, fish central nervous system (CNS) has been demonstrated as a primary target for MeHg (Pereira et al., 2019), especially in the developing stage (Yadetie et al., 2013). MeHg easily goes through the blood-brain barrier via a cysteine-facilitated transport, and reaches the CNS, particularly the astrocytes (Aschner, 1989; Cariccio et al., 2018; Pletz et al., 2016). In contrast to liver and muscle, which can regulate multiple genes to help detoxify MeHg, the brain is considered defenseless against this chemical (Cambier et al., 2012; Thacker, 2005). In fish brain, MeHg has been seen to alter several functions such as cell structural degeneration, Ca^{2+} homeostasis, oxidative system, metabolic markers, and the visual/sensory pathway (Berg et al., 2010; Berntssen et al., 2003; Cambier et al., 2012; Pereira et al., 2019; Weber et al., 2008).

Liver and skeletal muscle

After MeHg crosses the epithelia, it is first carried with the bloodstream to the liver, which is an organ primarily involved in the storage, redistribution, detoxification, and transformation of this toxin (Bradley et al., 2017; Maulvault et al., 2016). Then, MeHg is redistributed by bloodstream to other target tissues, including muscle, the largest pool and final storage tissue of MeHg in fish (Guardiola et al., 2016; Monperrus et al., 2020). Induced oxidative stress and hampered energy metabolic activity in liver and skeletal muscle after MeHg exposure have been shown in different fish species, such as Atlantic cod (*Gadus morhua*) (Olsvik et al., 2015; Yadetie et al., 2016), rainbow trout (Mozhdeganloo et al., 2015), zebrafish

(Cambier et al., 2009; Gentes et al., 2015), and fathead minnow (*Pimephales promelas*) (Klaper et al., 2008). Morphological and ultrastructural injuries after MeHg contaminant in these tissues were also reported by some histopathological studies, describing malformed hepatocytes, and disorganized myofibrils (Gentes et al., 2015; Guardiola et al., 2016; Lee et al., 2012; Muller et al., 2015), as well as injured mitochondria and endoplasmic reticulum (Oliveira Ribeiro et al., 2008).

Gill

Gill is directly and constantly exposed to external environment (Pereira et al., 2019). As a pivot in various physiological functions, such as osmoregulation and respiration process, gill's sensitivity to Hg has attracted some attentions. Indeed, there is increasing evidence suggesting a respiratory damage in mosquitofish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*) induced by MeHg in gills (Pickhardt et al., 2006). The damage, i.e., disruption in gill epithelium, can potentially result in compensatory changes in ventilation frequency, increased energy demands, or altered gas exchange efficiency, and possibly increased metabolic rate and decreased swimming performance (Morcillo et al., 2016; Tatara et al., 2001).

Other tissues

In addition to the tissues mentioned above, lesions and injuries elicited by MeHg were also recorded in other fish tissues, such as kidney (Lee et al., 2012; Oliveira Ribeiro et al., 2002, 2006), gut (Mela et al., 2014), skin (Guardiola et al., 2016), spleen (Skak and Baatrup, 1993), and olfactory organs (Baatrup and Døving, 1990; Ribeiro et al., 2002; Skak and Baatrup, 1993).

1.3.3.2 Behavioral effects in fish

Effects of MeHg exposure on fish behavior have been largely documented, most of which focused on the locomotor activity (Pereira et al., 2019). For example, Atlantic salmon (*Salmo salar*) exposed to dietary MeHg (10 μg g^{-1}) for 4 months displayed lower swimming activity (Berntssen et al, 2003); white seabream (*Diplodus sargus*) swam for a shorter time after 7 days of exposure to dietary MeHg (8.7 μg g^{-1}) (Puga et al., 2016) and zebrafish exposed to waterborne MeHg (15 μg L^{-1}) for 32 h displayed a reduced swimming distance and speed (Strungaru et al., 2018). Acute MeHg exposure (5.0 μg g^{-1} by injection) was also shown to trigger an anxiety-like status in adult zebrafish, accompanied with transient hyper-locomotion (Maximino et al., 2011). Some other studies have described the behavioral impairment induced by MeHg, concerning prey capture ability (Weis and Khan, 1990; Zhou and Weis, 1998), predator avoidance (Webber and Haines, 2003), reproduction (Sandheinrich and Miller, 2006), habitat selection (Sampaio et al., 2016) and more recently on fish memory and aggressiveness (Strungaru et al., 2018). The disturbed swimming performance

documented in these cases was largely linked to neurophysiological and brain structural mechanisms, such as cellular damages in the brain (Berntssen et al, 2003) and brain morphometric alterations in some regions (Puga et al., 2016).

1.3.3.3 Mechanims of MeHg toxicity

MeHg is a soft electrophile that preferentially interacts with nucleophilic groups (mainly thiols and selenols) contained in biomolecules to form MeHgCys complexes (Bradley et al., 2017; Farina et al., 2011; Franco et al., 2006; Kaur et al., 2006, 2007). Such interactions occur in a stable way to form complexes of MeHg and targeted molecules, which severely block molecular function, induce accelerated formation of free radicals, such as ROS, and generate oxidative stress leading to cell damages (Farina et al., 2011; Franco et al., 2006; Kaur et al., 2006).

Inactivation of antioxidant defence system: decreased ROS elimination

Many important enzymatic and non-enzymatic compounds with antioxidant activities such as the glutaredoxin and thioredoxin enzyme systems are rich in thiol and selenol groups, which give primary access to MeHg affinity resulting in a depletion of antioxidant capacity (Branco et al., 2011; Carvalho et al., 2008; Franco et al., 2009). For example, GSH (a major thiol antioxidant) and thiol- or selenol-containing enzymes belonging to the GSH antioxidant system, such as glutathione peroxidase (GPx), glutathione reductase (GR) and GSH s-transferases (GST), represent key molecular targets involved in MeHg-toxicity (Figure 1-15; Farina and Aschner, 2019; Farina et al., 2011). Depletion of intracellular GSH levels via MeHg complexation and increased ROS have been observed in MeHg-exposed rainbow trout liver (Mozhdeganloo et al., 2015), in human neurons and astrocytes (Kaur et al., 2006; Shanker et al., 2005), and in mouse brain (Franco et al., 2010). MeHg was found to hamper the activities of GR and GPx in rodent CNS during the early postnatal period (Stringari et al., 2008), and also in adult animals studied using both *in vivo* and *in vitro* approaches (Farina et al., 2003; Franco et al., 2009). In addition, several studies have shown that MeHg exposure may inhibit the thioredoxin system (Figure 1-16), for instance, reduced activities of thioredoxin reductase (TrxR) and thioredoxin (Trx) following MeHg comtamination were observed in the liver of Atlantic cod (*Gadus morhua*) (Yadetie et al., 2016), the brain and liver of zebra-seabream (Branco et al., 2011), the cultured human Hela cells (Carvalho et al., 2008), and the kidney and liver of mice (Wagner et al., 2010).

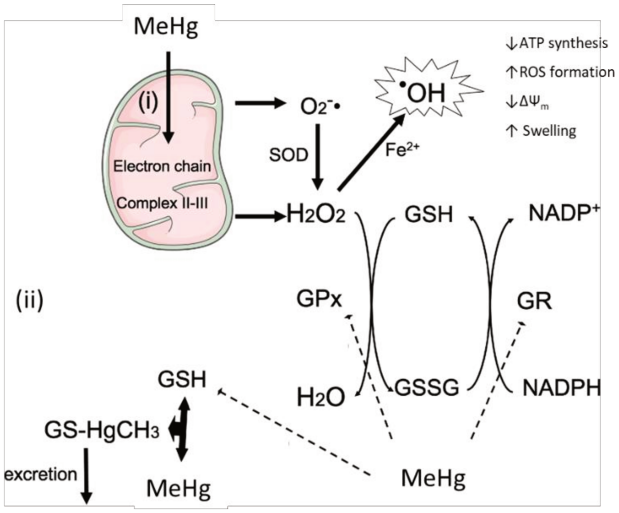


Figure 1-15. Effects of MeHg on mitochondrial function and GSH antioxidant system. (i) MeHg disrupts mitochondrial electron transport chain, leading to decreased ATP synthesis, decreased membrane potential, and increased formation of ROS, such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-•). (ii) MeHg reacts with reduced glutathione (GSH), leading to GSH depletion due to the formation of a MeHg-GSH ($GS\text{-}HgCH_3$) complex, which is excreted from the body. MeHg hampers the physiological increase in glutathione reductase (GR) and glutathione peroxidase (GPx) activities. Events (i) and (ii) lead to increased ROS concentration and oxidative stress (modified from Farina et al., 2011).

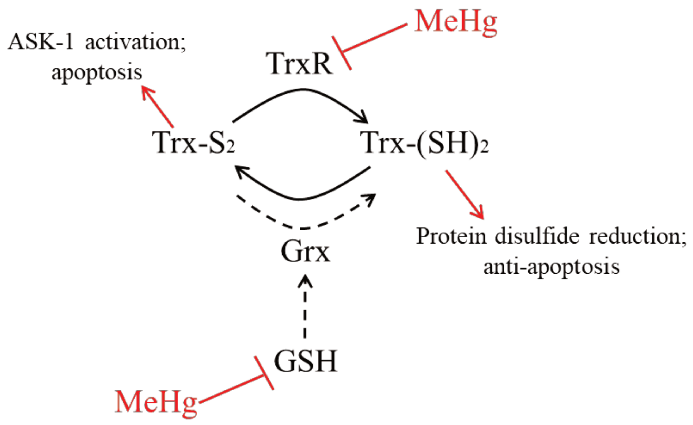


Figure 1-16. Effects of MeHg on the thioredoxin system. The thioredoxin system includes thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH as the electron donor, and is responsible for maintaining a reduced intracellular environment preventing oxidative and nitrosative stress. MeHg targets TrxR inhibiting its activity. When TrxR activity is hampered an alternative mechanism involving GSH and Grx keeps Trx reduction. While as MeHg concentration rises, this GSH/Grx backup mechanism is inhibited, and oxidized Trx activates ASK-1 leading to apoptosis. ASK-1 (apoptosis signal-regulating kinase 1) is a component in mitogen-activated protein kinase pathway (modified from Branco and Carvalho, 2019).

Impairment of mitochondrial metabolism and structure: increased ROS production

Another primary route for MeHg-mediated oxidation and ROS production is to interact with thiol-containing proteins located in cellular particulate fractions, e.g., mitochondria. MeHg can directly disrupt mitochondrial function by targeting mitochondrial respiratory chain complexes (Atchison and Hare, 1994; Shanker et al., 2005; Dreiem and Seegal, 2007; Yin et al., 2007; Usuki et al., 2008; Cambier et al., 2009; Glaser et al., 2010). The modifications of these complexes or enzymes can cause mitochondrial depolarization and swelling, leading to ROS overproduction (Figure 1-15; Farina et al., 2011; Mori et al., 2007; Roos et al., 2011). Mitochondrial sensitivity to MeHg and dysfunction in metabolic organs (i.e. skeletal muscle and liver) are likely to result in a decreased respiration efficiency and an altered energy metabolism (Cambier et al., 2009). Accordingly, 49-day exposure of adult zebrafish to MeHg at environmentally relevant dose exerted a strong inhibition of muscle fibers mitochondrial activity, reflected by a decrease in both the cytochrome c oxidase (COX) activity and rate of ATP release (Bourdineaud et al., 2013; Cambier et al., 2009). Similarly, integrated transcriptomic and proteomic analyses in Atlantic cod (*Gadus morhua*) liver after MeHg exposure have indicated severe effects of MeHg on major energy pathways, in particular the mitochondrial fatty acid metabolism (Karlsen et al., 2014; Yadetie et al., 2013, 2016). In addition, MeHg was found to mediate mitochondrial structural abnormalities, which present a pattern of cristae disorganization, outer membrane bubbling and a decrease of total area (Cambier et al., 2009; Gentes et al., 2015; Oliveira Ribeiro et al., 2008). Furthermore, these MeHg-induced damages provoke the release of cytochrome c from the mitochondria to the cytosol, subsequently leading to cell death by apoptosis, remarkably in sensitive organ like fish brain (Carratù and Signorile, 2015).

Notably, MeHg-toxicity has also been linked to increased Na^+ and Ca^{2+} efflux, resulting from MeHg-induced glutamate dyshomeostasis (Choi, 1992). Increased intracellular Ca^{2+} levels are associated with ROS generation, consequently contributing to mitochondrial dysfunction (Franco et al., 2009; Lafon-Cazal et al., 1993; Stringari et al., 2008). The Ca^{2+} and mitochondrial dysregulation elicited by MeHg have been frequently highlighted in the event of neuronal death in animals (Limke et al., 2004; Roos et al., 2012).

1.3.3.4 Cellular detoxification and repair

Above all, MeHg exposure *in vivo* (Guardiola et al., 2016) or *in vitro* (Morcillo et al., 2015, 2017; Voccia et al., 1994) can induce a severe imbalance between ROS production and its clearance by the antioxidant system, leading to a remarkable increase of cellular ROS concentration. Indeed, oxidative stress is one of the best-studied causative factors associated to MeHg toxicity in fish, as demonstrated in other species (Antunes et al., 2018), which generates different types of cell damage such as lipid peroxidation, DNA

double-strand breaks, and even apoptosis (Farina et al., 2011; Roos et al., 2012). The cell tends to react to these aggressions by activating different detoxification mechanisms.

First, given the substantial fraction of MeHg toxicity due to its avidity for thiol/selenol groups, a known mechanism of MeHg detoxification is to increase the cellular levels of different sulfur-containing protective molecules, mitigating the risk of MeHg bioaccumulation and toxicity (Navarro et al., 2009). This type of molecules commonly include metallothioneins (MTs, cysteine-rich, low-molecularweight proteins with high affinity for metals) and Se species (Dang et al., 2019; Stringari et al., 2008; Toyama et al., 2011; West et al., 2008). In particular the efficient protection by MTs faced to MeHg toxicity has been largely reported in several *in vivo* (Gentes et al., 2015; Gonzalez et al., 2005) and *in vitro* studies (Morcillo et al., 2016, 2017), where increased MTs level and enhanced *mt* gene expression after MeHg exposure were found. These scavenger compounds interact with MeHg to form adducts of MeHg-MT and MeHg-Se, which are then transported to the lumen of digestive tract via bile (Loumbourdis and Danscher, 2004) and excreted by the urine (Gailer, 2007).

Second, vitamin C and vitamin E can effectively neutralize free radicals via donating electrons to ROS and ultimately quenching their reactivity (Badgujar et al., 2014). They are considered as the most effective antioxidants to prevent lipid peroxidation, maintain the integrity of membrane, and thus buffer the MeHg-elicited oxidation (Do Nascimento et al., 2008; Mozhdeganloo et al., 2015; Ricciarelli et al., 2001; Usuki et al., 2001).

Third, there are numerous reports on the protective role of autophagy against MeHg-induced cytotoxicity: an *in vitro* study in rat primary astrocytes indicated that MeHg-induced neurotoxicity was partly reduced through the activation of autophagy (Yuntao et al., 2016); Takanezawa et al. (2016) explicated the protection of Atg5-dependent autophagy on mouse embryonic fibroblast cells from MeHg cytotoxicity.

Finally, after incorporation into tissues, some MeHg can be transformed mainly in liver to Hg(II), which is less toxic and easier to be eliminated from body (Gonzalez et al., 2005; Yasutake and Hirayama 1990). Although this demethylation process is protective against MeHg toxicity, the ability to metabolize MeHg to Hg(II) varies from one species to another, and the participating enzymes or underlying mechanisms are largely unknown (Takanezawa et al., 2019).

1.3.3.5 MeHg in estuarine glass eels

Catadromous life cycle of European eels exposes them at different stages to different pollutants. Glass eels colonize different ecosystem types, from marine to estuaries and rivers/lakes, to further develop. Studies by Navarro et al. (2013) and Claveau et al. (2015b) showed a significant accumulation of MeHg in European

glass eels. Furthermore, the adverse interactions of Hg on glass eels' estuarine migration have been identified in term of its metabolic and genotoxic pressures. For example, study of Claveau et al. (2015a) showed that MeHg exposure at ecologically relevant concentrations affected mitochondrial structure and metabolism, with a stronger effect in non-migrant glass eels. The authors propose that MeHg-induced oxidative stress and mitochondrial dysfunction could impair the energetic metabolism of glass eels and thus their migratory propensity. Similarly, *in situ* study by Castro et al. (2018) found an increased impact on genome integrity with increase of total Hg (THg) body burden in European glass eels, suggesting a harmful impact of Hg on glass eels' condition and ultimately the population sustainability. In addition, the adaptive responses to combat MeHg toxicity, such as detoxification/elimination actions, and repair of DNA damage and lipid peroxidation, have metabolic costs and require an increased expenditure of energy (Lock and Wendelaar Bonga, 2008). Taken together, all these studies suggest that environmental MeHg does exert an influence on the metabolic status in glass eels.

1.4 Objectives

European glass eels arriving from the sea use different migratory tactics that can lead to the colonization of rivers or to an early settlement in marine or estuarine habitats. According to previous studies, the migratory behavior could be dependent on the body condition. Indeed, Edeline et al. (2005) proposed a theory of conditional strategy suggesting that individuals with higher energy stores should have a higher propensity to migrate up estuary. However, some studies do not support this hypothesis, suggesting that energy stores alone cannot explain the observed behavioral differences. In this context, the main objective of this thesis was to investigate the conditional strategy in the European glass eels, not only based on energy stores but also on the individual ability to mobilize these stores (energetic status). In addition, several studies provided evidence that energy stores of European glass eels fluctuated depending on the season, likely because of the fluctuation of food resources during the oceanic migration at the leptocephalus stage. Such different levels of energy reserves suggest that energetic strategies to migrate up estuary may also fluctuate depending on these reserves and to investigate this question, this study focused on both autumn and spring individuals.

So, the first objective of this thesis was to:

Characterize the energetic status in relation to the migration behavior of European marine glass eels arriving near the European coasts both in autumn and spring.

Glass eels sampled in both seasons were submitted in experimental conditions to a change in water current direction every 6.2 h that mimic tides. Individuals were tagged so that their swimming behavior can be monitored individually and the swimming test allowed to distinguish fish with a high ability to migrate (synchronized to the change in water current direction), or a low ability to migrate (non-synchronized). Individual behaviors were then related to the energetic status of glass eels in terms of energy stores, metabolism and energy-related genes expression.

Once marine fish were characterized depending on the season, the second objective was to better understand the underlying mechanisms of settling processes in the estuary in relation to the conditional strategy based on energy. In accordance to the facultative migration, estuarine glass eels were considered as a subsample of marine ones, some individuals stopping migration before reaching the river, possibly because of too low energy reserves.

Thus in the second objective, we aimed to:

Compare marine and estuarine glass eels migration behavior in relation to their energetic status in terms of energy stores, standard metabolism and energy-related gene expression.

For this purpose, marine and estuarine glass eels were sampled in autumn and spring and their migration behavior analyzed as described in the first objective. Then, behaviors were individually related to energetic status.

Finally, since glass eels entering estuaries are exposed to different stressful factors that may increase energy expenditure and affect migration in glass eels, **we tested in a third objective one of these factors and investigated:**

The effect of methylmercury on marine glass eels migration behavior and their energetic status.

To do so, we exposed marine glass eels to MeHg (100 ng L^{-1}) for 7 days, and then contaminated and non-contaminated fish were submitted to a swimming test as described above. The energetic status was then analyzed in relation to the migration behavior.

Chapter 2 - RESULTS

Publication 1

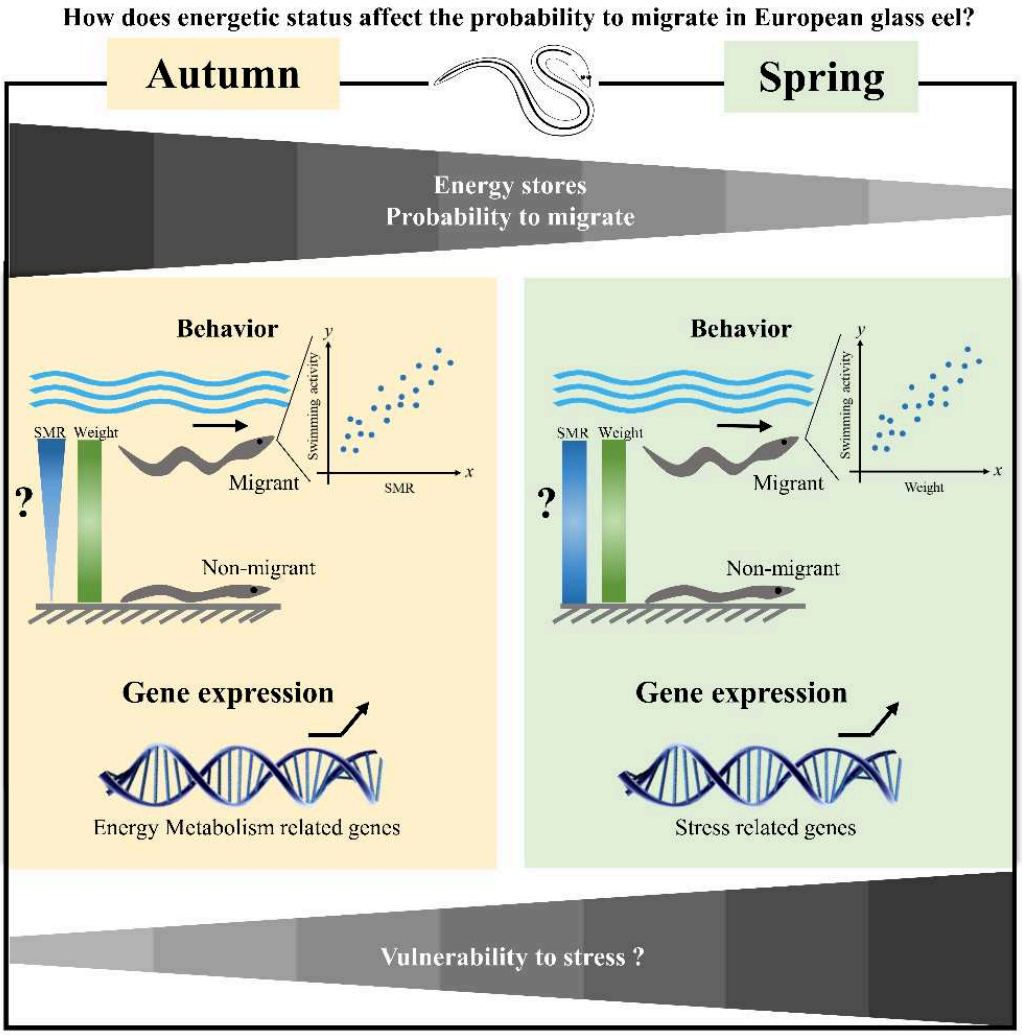


Figure 2-1. Graphical abstract and highlights from Publication 1- Relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (*Anguilla anguilla*).

Highlights

1. Autumn glass eels are more active and bigger than spring ones.

2. Mitochondrial fission and autophagy are triggered in spring glass eels

3. Swimming activity is related to SMR in autumn fish and weight in spring ones.

4. Migration could be linked to energy in spring glass eels but not in the autumn ones

2.1 Characterization of the energetic status in terms of energy stores, standard metabolism and energy-related genes expression in European marine glass eels in autumn and spring

Presentation of Publication 1

Looking at the complex relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (*Anguilla anguilla*)

Objective and methods

In the studies up to date, energy store was often used as the single proxy for energetic status. We hypothesize that it may be too limited to fully explain glass eel's diverse migratory patterns and that other factors as energy mobilization, may also be involved. In addition, glass eels energy profiles markedly differ depending on the season and individuals that arrive in autumn present higher energy stores than those reaching the coasts in spring. Therefore, the first objective was to characterize the energetic status of glass eels arriving in autumn and spring and to relate this status to their migration behavior. To do so, marine glass eels were successively sampled in autumn (November) and spring (April). Initial wet weight, as a proxy for energy stores, was compared between the two groups, then individual swimming behavior was observed in laboratory installation (as described in last chapter). The individual metabolism (expressed as oxygen consumption) and expression of energy metabolism-related genes were determined after swimming test. Finally, these energetic status indicators (energy stores, metabolism, gene expression) were linked to glass eel's behavioral pattern.

Results and Conclusions

Autumn glass eels presented higher energy stores than spring individuals. Molecular data also showed that expression level of genes related to energy metabolism was higher in autumn glass eels than their spring conspecifics, while the stress related genes overexpressed in spring glass eels. These results suggest that autumn glass eels presented a higher ability to produce energy while the spring ones displayed an energy distress. This confirm that autumn and spring glass eels present strong differences in their energetic status and that they have to be studied separately (Figure 2-1).

Concerning their migratory behavior, we observed a higher number of synchronized individuals and a higher swimming activity level in autumn glass eels than in spring ones which may support the conditional strategy based on energy. However, regardless of the season, no difference of energy stores or energy metabolism-related gene expression was observed between synchronized and non-synchronized glass eels. Interestingly, individual swimming activity was positively correlated to energy stores in spring glass eels, but not in the autumn group suggesting an energy-based conditional strategy in the former ecotype but not

in the latter one. We hypothesize that low energy stores may become limiting for swimming activity and force individuals to stop migration. However, when energy reserves are high as observed in autumn, this factor, and the gene expression levels of energy-related genes cannot explain our behavioral results (Figure 2-1).

Science of the Total Environment 696 (2019) 134039

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Looking at the complex relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (*Anguilla anguilla*)

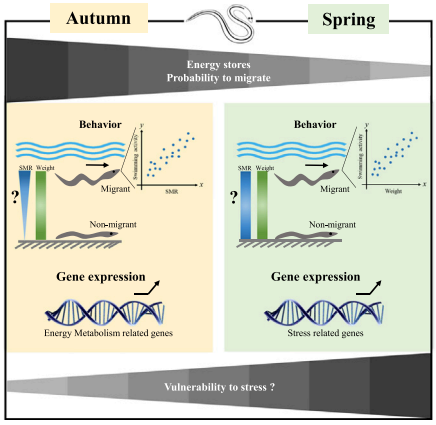
Hengtong Liu [a,c], Jacques Labonne [a,c], Pascale Coste [a,c], Emmanuel Huchet [a,c], Elisabeth Plagnes-Juan [b,c], Jacques Rives [a,c], Vincent Veron [b,c], Iban Seiliez [b,c,1], Valérie Bolliet [a,c,*,1]

[a] INRA, UMR 1224 Ecobiop, Aquapôle, F-64310 Saint Pée sur Nivelle, France
[b] INRA, UMR 1419 Nutrition Metabolisme Aquaculture, F-64310 Saint Pée sur Nivelle, France
[c] Univ Pau & Pays Adour, Collège STEE Côte Basque, E2S, MIRA, Anglet, France

HIGHLIGHTS

- Autumn glass eels are more active and bigger than spring ones.
- Migration behavior is not related to the weight in autumn or spring glass eels.
- Swimming activity is related to metabolism in autumn fish and weight in spring ones.
- Mitochondrial fission and autophagy are triggered in spring glass eels.
- Spring glass eels might have limited ability to adapt to variation in environmental conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 June 2019
Received in revised form 19 August 2019
Accepted 20 August 2019
Available online 21 August 2019

Editor: Henner Hollert

Keywords:
Glass eel
Anguilla anguilla
Migration behavior
Energetic status

ABSTRACT

Diadromy in eel is facultative and the diverse propensities in glass eel estuarine migration may lead to a large spatial dispersion, which may has profound influences on the species sex determination. In this study, we sought to clarify the relationship between European glass eel energetics and their pattern of migration behavior, in the framework of the conditional strategy. Marine glass eels were sampled in autumn and spring, stratifying high and low energetic status, respectively. Their migration behavior was determined in experimental installations that mimic tides using a change in water current direction every 6.2 h. When synchronized to the current reversal, glass eels were called active while individuals hiding in the substratum were considered as non-active. Then, for each active fish, a level of swimming activity was determined and both migration behavior and level of swimming activity were correlated to the individual wet weight (used as a proxy of energy stores), standard metabolic rate (relative SMR) and transcriptomic profile of metabolism related genes. Results showed that spring glass eels presented a lower probability to migrate, a lower wet weight and a higher expression of genes involved in energy stress resistance than autumn ones, supporting a conditional strategy based on individual's energy status.

* Corresponding author at: UMR 1224 Ecobiop, Aquapôle, 64310 Saint Pée sur Nivelle, France.
E-mail address: valerie.bolliet@inra.fr (V. Bolliet).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scitotenv.2019.134039

Metabolism
Autophagy

However, within each season, no wet weight difference was observed between active and non-active fish. In autumn glass eels, migration behavior was weakly related to relative SMR while in spring, none of the parameters analyzed allows discriminating active and non-active glass eels. The level of swimming activity in active fish was related to their relative SMR in autumn and to their wet weight in spring. Altogether, our results could not validate a conditional strategy but spring glass eels displayed some signs of energy distress and a lower level of swimming activity than autumn ones.

1. Introduction

The life cycle of the European eels has been described as a catadromous life history during which they migrate between oceanic spawning areas and continental rearing habitats (Tesch, 2003). The leptocephalus larvae drift with the Gulf Stream to join the continental shelf where they metamorphose and are then referred to as glass eels. Then, glass eels migrate up estuaries to join rivers using selective flood transport: during flood tide, glass eels move up in the water column and migrate with the current while they go down and remain on or in the substratum during ebb tide (Forward and Tankersley, 2001; Gascuel, 1986; Jellyman, 1979). However, a high degree of geographical dispersion crossing marine and riverine water has been documented regarding to different migratory patterns of settlement and river colonization (Daverat et al., 2006; Secor et al., 1995; Tsukamoto and Arai, 2001; Tsukamoto et al., 1998; Tzeng, 1996). These different patterns of migration could have a strong impact on the fate of the population because of the sex determinism in eels, which is environmental (Geffroy and Bardonnet, 2016; Krueger and Oliveira, 1999). Briefly, in European eels, males are generally observed to dominate in high-density environments, often associated with estuarine or lower river reaches, whereas females tend to become increasingly dominant with increasing distance from the sea (Adam et al., 2008; Davey and Jellyman, 2005; Harrison et al., 2014; Laffaille et al., 2006; Parsons et al., 1977).

The underlying mechanisms of this facultative migration are far from being elucidated but some studies proposed a conditional strategy based on individual's energetic status in the European eels (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006). Indeed, most glass eels do not feed during migration (Bardonnet and Riera, 2005) and energy reserves accumulated by the leptocephalus larvae during oceanic migration are used to sustain activity and reach fresh water (Kawakami et al., 1999; Tesch, 2003). According to the conditional strategy, glass eels presenting high wet weight (Edeline, 2007; Edeline et al., 2006) or high dry weight (Bureau du Colombier et al., 2007) should have a higher propensity to migrate than those showing low energy stores. However, some results appear to contradict the conditional strategy hypothesis, either in experimental conditions (Bolliet et al., 2017a) or in natural environments (Gaillard et al., 2015). The European eel is listed in the IUCN red list as 'critically endangered' and while the reasons for this tremendous decline of the eel population are still not fully understood, anthropogenic causes are often pointed out. In glass eels, global changes (pollution, increase in water temperature) may lead to an increase in energy expenditure because of a higher metabolism or detoxication processes. The role of the energetic status of glass eels on their ability to migrate has to be elucidated, particularly in a context where managers do not account for variation in energetic condition in their population exploitation and conservation policy.

It is noteworthy that in most of the experiments supporting the conditional strategy, the energetic status of individual was mainly evaluated using integrative proxies like body mass and size. However, in fasting and migrating fish, energetic budgeting is a dynamic process mediated not only by energy reserves but also associated with a highly interplaying network of energy metabolisms. For example, we recently reported the existence of a non-random fluctuating expression dynamics of autophagy- and lysosome-related genes during long term fasting in *A. anguilla* glass eel and demonstrated a significant contribution of these transcripts production over time to weight loss (Bolliet et al., 2017b). Besides its function in the removal of altered or dysfunctional proteins and organelles, autophagy plays also a critical role in energy supply by allowing starved cells to mobilize their own constituents including lipid and glycogen stores (Singh and Cuervo, 2012). Another well-documented cellular energetic pivot is mitochondrial metabolic system mediated through ATP production and antioxidant activity (Bermejo-Nogales et al., 2015), but the detailed role of mitochondrial functions as autophagy processes on mediating glass eel's locomotion has been seldom studied.

Here, we sought to clarify the relationship between European glass eel energetics and their pattern of migration behavior, in the framework of the conditional strategy. For this purpose, we characterized the individual's energetic status of glass eels in terms of wet weight (as a proxy of energy stores), standard metabolic rate (SMR) and transcriptomic profile of metabolism-related genes and determined the relationship of these energy- and metabolism-related factors with the propensity to migrate of glass eels, as evaluated in experimental condition. In order to strengthen the possible link between energetic status of glass eels and their propensity to migrate, we used glass eels arriving at the mouth estuary in autumn and spring known to present high and low energetic stores, respectively (Charlon and Blanc, 1982; Claveau et al., 2015; De Casamajor et al., 2000; Elie, 1979).

2. Materials and methods

2.1. Ethics

Procedures used in this study have been validated by the ethics committee N°073 (ref: 2017012015086652). The experiment was carried out in strict accordance with the EU legal frameworks, specifically those relating to the protection of animals used for scientific purposes (i.e., Directive 2010/63/EU), and under the French legislation governing the ethical treatment of animals (Decret no. 2013-118, February 1st, 2013).

2.2. Origin and handling of fish

The main migration season of glass eel in South-West France coastal area lasts from November to April. In this study, one group of 72 marine glass eels was sampled using a dip-net at night and during flood tide on November 3rd 2016 and the same number of glass eels were sampled in the same way on November 17th (hereafter autumn glass eels). Similarly, 72 glass eels were sampled on April 13th 2017 and the same number of glass eels were sampled again on April 28th (hereafter spring glass eels). The sampling marine site is located at Moliets (43° 55′N, 1° 23′W, located 40 km north of the mouth of the Adour estuary, Fig. 1). After each collection, glass eels were transferred to the laboratory and maintained at 12 °C overnight in a tank containing aerated water from the fishing site. In the next morning, all glass eels were anesthetized and individually measured for initial wet weight (Sartorius CP 153 balance, ±1.0 mg) and length (±1.0 mm). Measurements of triglycerides (TG) at the end of the experiment allowed us to highlight a significant correlation between the final wet weight of individuals and their TG levels in both seasons (Pearson's correlation test, $p = 0.0002$ and $p = 0.00001$, respectively). According to these results, wet weight was

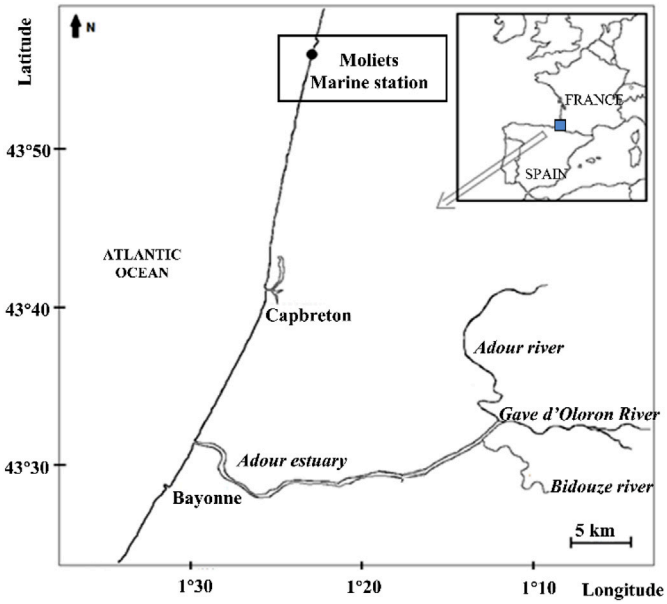


Fig. 1. Sampling station of glass eels.

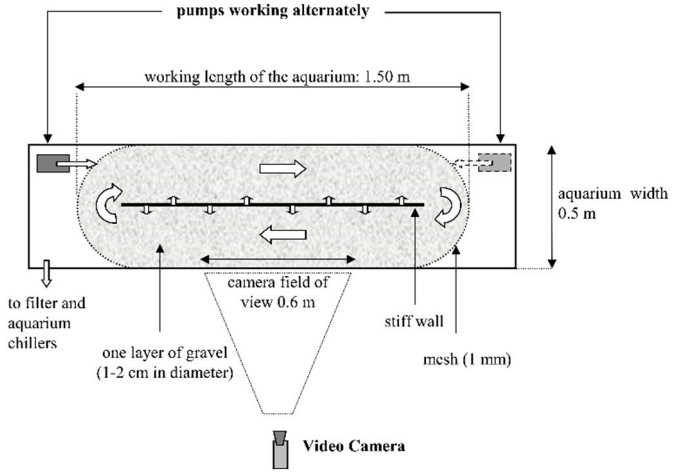


Fig. 2. Diagrammatic top view of the annular flume.

considered in this study as a good proxy of energy stores. Pigment stages were determined according to the criteria of Elie et al. (1982), who described eight stages: VA, VB, VIA0, VIA1, VIA2, VIA3, VIA4 and VIB, in order of increasing pigmentation. 100% of marine glass eels presented a VB stage, regardless of the season.

36 of the 72 individuals were randomly selected and tagged using Visible Implant Elastomer (VIE Tag) (combinations of one or two hypodermic spots of different colors as described by Imbert et al., 2008) in order to follow the swimming activity individually. Once tagged, glass eels were released to wake up in the water from fishing site with the untagged fish. During the next 48 h, the water was continuously aerated and progressively diluted with fresh water. Water temperature (12 ± 0.5 °C) was regulated using an air conditioner.

2.3. Experimental design and protocol

For each experiment, 36 tagged animals were mixed with the same number of untagged ones to facilitate synchronization of swimming activity to the change in water current direction by increasing density (Bolliet and Labonne, 2008; Bolliet et al., 2007). This group of 72 glass eels was released to an annular tank (Fig. 2) installed in a temperature-controlled room. To mimic the tides, the tank was equipped with two pumps - located at its opposite ends - that alternately generated clockwise or counterclockwise water flow every 6.2 h as described in Bolliet et al. (2007). The room was maintained under a photoperiod of 12 L/12 D with a very low light intensity during the photophase (0.2–0.3 μW/cm^2) and a constant UV light (0.6 μW/cm^2). The water temperature was kept at 12 ± 0.5 °C and continuously recorded by thermistors placed in the tank.

The swimming activity of glass eels was traced individually during seven days by a camera programed to record 15 s every 40 min. The UV light allowed the identification of each glass eel during the light and dark phases by its elastomer mark. The sampling session duration was chosen to allow fish to pass once through the camera's field of view when swimming in the water column (see Bolliet and Labonne, 2008). A total of 177 sessions of 15 s were obtained for autumn glass eels and the same number of sessions for spring ones.

To migrate up estuary, glass eels must synchronize their swimming activity to the tide to use selective flood transport but they also have to sustain swimming activity. So, to evaluate the propensity of glass eels to migrate in our experimental conditions, we first analyzed the synchronization of their swimming activity to the change in water current direction (every 6.2 h). When the swimming activity was synchronized to the water current reversal with a period of 12.4 h, glass eels were considered as having a high propensity to migrate and were called 'active'. In contrast, fish that did not synchronize and stayed in the substratum most of the time were called 'non-active'. Then, the level of swimming activity in active glass eels was quantitatively analyzed by counting the total number of observations of each elastomer mark for the 177 sessions of 15 s recorded in each experiment.

After the swimming test, we obtained 35 active glass eels in autumn (19 and 16 in the two experiments, respectively) and 12 active glass eels in spring (5 and 7 in the two experiments, respectively). Active and non active glass eels were pooled per season for analyses.

2.4. Standard metabolic rate (SMR) assay

22 active and 13 non-active glass eels and 12 active and 24 non-active glass eels in autumn and spring, respectively, were randomly retrieved for oxygen consumption determination. Briefly, glass eels were acclimated in a same tank with still water for three days to stay at rest and relative quiet before analysis. Then, they were transferred to 12 respirometry chambers (diameter: 11.2 mm, length: 90 mm) of an intermittent flow respirometer as described by Régnier et al. (2010), where oxygen consumption was measured individually (one fish per chamber). Tagged glass eels were introduced in the chambers at 3 p.m. and oxygen consumption was recorded continuously every minute until 10 a.m. the next day. The closed/open phase of the system was 20 min/20 min and the duration of the closed phase was determined in order that oxygen level in the chamber was always kept above 80% O_2 saturation. After termination of the SMR measurements, the background respiration, i.e. oxygen consumption within the respirometer due to microbial respiration, was estimated by measuring the oxygen consumption rate in the respirometer without a fish (i.e. blank run for 2 h). Temperature and photoperiod used for the acclimatization phase and oxygen measurement were similar to those used for the behavioral test. The first 15 h were considered as a period of acclimatization and the average oxygen consumption per eel was calculated using the last 4 h of recording. Measurements were conducted for three days in autumn and spring. SMR (mm^3 O_2/h) was expressed in mm^3 O_2 consumed per hour. We then regressed the logarithm of SMR on the logarithm of wet weight, and used the residuals of this model (i.e., relative SMR) for further analyses. Following oxygen consumption measurement, glass eels were anesthetized, individually measured for wet weight (Sartorius CP 153 balance, ±1.0 mg) and length (±1.0 mm) and stocked at −80 °C after immersion in liquid nitrogen.

2.5. High throughput quantitative RT-PCR

The extraction of total RNA from each glass eel analyzed for its oxygen consumption was performed using TRIzol reagent (Invitrogen, 15596018) according to the manufacturer's recommendations. One microgram of the resulting total RNA was reverse transcribed into cDNA using the SuperScript III Reverse Transcriptase kit (Invitrogen, 18,080,085) with random primers (Promega, Charbonniéres, France) according to the manufacturer's instructions.

Primers specific to 59 genes involved in either energy metabolism or oxidative stress (Table 1) were newly designed using Primer3 software (version 4.1.0) and based on the available genomic resources for the European eel (GenBank assembly accession GCA_000695075.1). Primers were validated on a Roche LightCycler 480 System (Roche Diagnostics, Neuilly sur Seine, France). The assays were performed using a reaction mix of 6 μl per sample, each of which contained 2 μl of diluted cDNA template, 0.24 μl of each primer (10 μM), 3 μl Light Cycler 480 SY Green Master mix (Roche Diagnostics, 4887352001) and 0.52 μl DNAse/RNAse-free water (5 Prime GmbH, 2500020). The PCR protocol was initiated at 95 °C for 10 min for initial denaturation of the cDNA and hot-start Taq-polymerase activation, followed by 45 cycles of a 3-step amplification program (15 s at 95 °C; 10 s at 60 °C and 15 s at 72 °C). Melting

Table 1
Genes involved in each metabolism-related function and sequences of the primer pairs used for real-time quantitative RT-PCR.

Function	Sub-function	Gene *abb.*	5′/3′ forward primer	5′/3′ reverse primer
Autophagy-lysosome system	Macroautophagy	*atg5*	AGGGTCAGGTGGTCAATGAG	CTGTCGCTCATCGTCTGGTA
		atg7	CCTGAGCTCTCCCTGAACAC	CAGATCCAAGGAAGGAACCA
		atg12	GCAGTAGGGGACACTCCTAT	CACTGCCAAAACATTCAAATAAC
	Lysosome	*lc3*	TACAGGACATAGGCCGCTAA	ACTCGCTGTTCAAATGTCCT
		ulk1	GGACCTGTGGAGCATAGGAA	GGAAAAACTCATCGAAGTCCAT
		catha	GGGAACAAGCACCTGCATTA	CGCCATCATCCTGAATTAGA
		cathd	TCCAGGGAGAGTACATGGTTG	ACATCTCCCAGAATCCACAG
		cathf	GGGATATGGACATCGTAATGG	GCAGATGGGCGTTGTTTAAT
		cathl	TCAGTTCTACCAATCTGGAATCTAC	CTTCCTGTCTTTGGCCATGT
		tfeb	TGTCCAGCAGTCACATGGAT	CTTCCGACAGCTCCTTCTTGA
		lipa	GTGTGCGTTTGCTTGTGTCT	TTTTCACAGTGGCTTCATGC
	CMA	*lamp2a*	CTGAGGAATGCCAAGCTGAT	TGAAATAGGCCACCAACACA
		phlpp1	GAGGAGGTGAAGAGGCACAG	CAGACTGCAGCATGACAGGT
		hsp90	GAAGGCAGAGGCTGACAA	ATCAATTCCCAGGCCAAGTT
		hsc70a	ATGTCAAAGGGACCAGCAG	ATCAGCCTCTCGGTGTCAGT
		hsc70b	AGCAGTTGGGATTGATCTGG	CAACTTGATTCTTCGCTGCAT
	Mitophagy	*fundc1*	TGGTGTGCAGGATATCTTTTTCA	GCTTTGTTCACGTCCTTCTC
		pink1	TAGCTGCCAACATTCTGCAC	CCAGGAAGCACCTCTGTAGC
		parkin	CTGGACTGCTTCCACACGTA	CGTACTGCTCCTCTCCAAGG
		bnip3a	GGGAAAATGAGTTGCACGTT	CCCTTGCATTTTGGGTAGAA
		bnip3b	TAGCCAGGGTAAATGCAAGG	TCCCAGCAGCAGGTCTATTT
Mitochondrial turnover	Mito biogenesis	*pgc1α1*	GATGAGAGACGGGTGGTGTA	GTGTAGCGGTAGGTGATGAAG
		pgc1α2	AGGTGGCAAGACACAAAACC	CCGGACGTGAGGTACTTGAG
		tfam	CACAATGCCGGCAAAGTTGA	CACAACGTCCTCTCGTCCAA
		hsp60	TGGACGCTGGAGATCTTCTT	TCACTGTGAAGGATGGCAAA
	Mito Fusion	*mfn1*	GCTGAGAGACGACCTGGTTC	AAAGTGTTTCTCCGTGTTCATC
		mfn2	CCTGGTGCAATGTCTCTGGT	TTTGATGTAGGCCGCCAACT
		opa1	CCACTACTACCAGAGGGGCT	CCAGGACCTCCTTCACGTTC
	Mito Fission	*drp1*	AAACTGGACCTGATGGATGC	GGGAGGGGTACTTCTTCTGC
Mitochondrial metabolism	Fatty acid catabolism	*cpt1α1*	AGAGACCTGTGACCCCACAC	TGAAGACATGTACCCCACCA
		cpt1α2a	TGATGTTCAGGAGCTTGTGC	GTTCTGGCAAGTGCAACTCA
		cpt1α2b	AGGCAGGTGTACCTCTCTGG	GCGCACTGAGAGACTGACTG
		cpt1β	CCAGGCTGTGGATGAATCTT	GCCGAGTGCTTTTTGAAGTC
		hadh	CCTTCGATGCTCTCCTTGAC	GGCCACATCAATGTCTTCCT
	Amino acid catabolism	*glud1*	CGAGGATCCGAACTTCTTCA	GACAGGCTCAAAACGTGGTT
		got1	CAACAGATCGGCATGTTCAG	TTATTGGACCTTGGTGACAGC
		got2	GGAGTGTTACCGTCCAGAGC	GTGTAGGCCTTGAGCTGCAT
		gpt2a	TTAAGAAGCCATTCACTGAGGT	TGGGCATGTGCAGAGAGC
		gpt2b	ACTCCACATCCAAGTGCTACAT	CGACACCAGTTTGGTGAGC
	Mitochondrial respiratory chain complexes	*mt-ATP6*	CTAGGCCTGCTCCCATACAC	CTGGTGTTCCTTCTGGCAGT
		mt-nd5	CCTCTGTTCAGGCTCCAT	GGGCTCAGGCGTTAAGGTA
	Mito 12S rRNA	*cox1*	TAGAGGCCGGAGCTGG	GGGGAGTTTGGTACTGTGTAAT
		12s rRNA	ACCCGTGAGAATGCCCT	GTGGCTGGCACGAGTT
Antioxidant system		*sod1*	TCAAGGACAGAATGCTCACG	TTTTCAGGCTTTCCTCGTTG
		sod2	CACCACGCCACATATGTCAA	AGCTCCCCTTGAGGTTCC
		catalase	AGCAACCGATCAAATGAAATTATGG	CAGCTCCCTTGGCGTG
		mtl	TGCACTACGTGTAAGAAAAGCTG	TTCATGTTCAGGCAGGAATG
		gstp	CACTGGGGATGTAGGCTGTT	GCCAGACTGATCAACTGCAA
		gsr	GCCCTATCGCTCTCAGTGAC	GAGGTCACAGACCCCAGTGT
		gpx1	CAAGTGCATCATCTGGAGCC	GGGACGTTTTACTTCGCCAG
		gfap	TGGAGATCGAGAGGGACAAC	CCTCTCCAACTGGACACGAT
Cytosol catabolism	Lipid catabolism	*hsla*	GGTCACCTCCGGCATAAGTA	AATGACCAACAGCAATGCAA
		hslb	CTGTCAGAGACGGAGCTGAT	AGATGAGGCGCATGTTGAC
		mgl	TGCAGCACATAGACCAGATCA	AACACCTTGACGGGTGAGAC
		atgl1	CACCAACACCAGCATTCAGT	CTCGATATTGCAGCAGTCCA
		atgl2	GGTGATGGCAGAGATGTGTC	CTTCTCAGGCACGGGTTG
		atgl3	GCGCGCAAAATTTAGTGTTT	TCCTCTTCACTCCCTTCAGC
	Protein catabolism	*fbxo32*	TCCTCTTCTGGAAGGACACA	TCCAGGAATCCATTGCACTA
		murf1	CTGTGAAGTCTCGCCCCTAC	CAACTGCTTCTGACGCTGAC
Reference gene		*luciferase*	CATTCTTCGCCAAAAGCACTCTG	AGCCCATATCCTTGTCGTATCCC

curves were systematically monitored (temperature gradient at 1.1 °C/10 s from 65 to 94 °C) at the end of the last amplification cycle to confirm the specificity of the amplification reaction. Each PCR assay included negative controls (reverse transcriptase- and cDNA template-free samples, respectively). Finally, to confirm specificity of the designed primers, the amplicons were purified and sequenced (Beckman-Coulter Genomics, Takeley, UK). The validated primers are listed in Table 1.

High-throughput qRT–PCR was performed by the Genotoul service (https://get.genotoul.fr) in Toulouse (France) using the BioMark 96:96 Dynamic Array integrated fluidic circuits (Fluidigm Corporation, San Francisco, USA) described in Cassan-Wang et al. (2012). The specificity of the PCR products was confirmed by analyzing melting curves. Only primers that produced a linear amplification and qPCR products with a single-peak melting curves were used for further analyses. The efficiency of each pair of primers was determined from the data of amplification Ct value plot with a serial dilution of mixture cDNA. $2_T^{-\Delta\Delta C}$ method was used to calculate relative mRNA fold change using formula $2^{\Delta Ct\_target\ (control-sample)}/2^{\Delta Ct\_reference\ (control-sample)}$ (Livak and Schmittgen, 2001). The relative expression of Luciferase was used for data normalization as described previously (Marandel et al., 2016).

2.6. Statistical analyses

To characterize the propensity of glass eels to migrate, we first investigate the synchronization of glass eel's swimming activity to the change in water current direction (active/non-active). We assumed that the swimming activity of an individual i at time t $AC(t,i)$ followed a Bernoulli distribution of probability $P(t,i)$ such as:

$$AC(t,i) \sim dbern(P(t,i))$$

We assumed that $P(t,i)$ was a periodic function of time, since it has been previously shown that glass eels display rhythmic swimming activity in response to current reversal (Bolliet and Labonne, 2008):

$$logit(P(t,i)) = a(i) \times \sin(t \times b(i) + c(i)) + d(i)$$

where $a(i)$ was the strength of the synchronized component of activity, $b(i)$ was related to period, $c(i)$ was related to the trigonometric function phase, $d(i)$ was the non-synchronized component of activity. Fish having a P value above the mean of P meanwhile having an activity periodicity close to 12.4 h were considered synchronized and active, others were considered non-active.

A Markov-chain Monte-Carlo (MCMC) sampling approach with Gibbs algorithm in the Bayesian framework (Spiegelhalter et al., 2000, Openbugs software, Version 3.2.3) was used to estimate parameters a, b, c, d. Convergence of estimates was reached during a first set of 10,000 iterations. Another consecutive set of 5000 iterations was run to approximate the posterior distribution of parameter estimates (see Supplementary material 1).

The second parameter used to evaluate the propensity of glass eels to migrate was the level of swimming activity in active glass eels, expressed as the mean number of times each individual was seen swimming in the water column during 7 days.

All other statistical analyses were performed using the R software (v.3.3.1)/R Commander Package. Data were presented as means ± standard deviation. The comparison of number of active fish in the two seasons was analyzed by chi-square test based on the counts of active/non-active fish and the average swimming activity in the two seasons was compared by Student's t-test. Normalized genes expressions were first analyzed using a hierarchical clustering analysis based on correlation between genes (R Pvclust package). We used the Approximately Unbiased bootstrapping approach to detect clusters of genes (with an accuracy p-value >0.95) that displayed the same overall patterns throughout the experimental conditions (see Supplementary material 2). Two-way ANOVA was used to analyze the varying wet weight, length and relative SMR in response to season and migration behavior. The interactions in the responses were also evaluated. Differences were considered statistically significant at $p < 0.05$. For synchronized fish, the relationships of swimming activity to wet weight, length and relative SMR were estimated by simple linear regression model. The regression model was considered significant at $p < 0.05$ level. Finally, we evaluated whether genes expression level was correlated with swimming activity (for synchronized fish) for both seasons. To do so, we first grouped genes into five metabolism-related functions: autophagy-lysosome system, mitochondrial turnover, mitochondrial metabolism, antioxidant system and cytosol catabolism (see Table 1). For each group of genes, we ran a Principal Component Analysis (PCA), using a table providing the gene expression levels for all individuals. We then retrieved the score of individuals on the first axis of the PCA, and used these coordinates as a synthetic indicator of the individual level of expression for the genomic function. We then assessed the effects of the swimming activity, the season, and the interaction of both on this synthetic indicator of the genomic function, using a linear regression model. Bonferroni's adjustment for multiple comparisons (here five) lowered the statistical significance to $p < 0.01$ level.

3. Results

3.1. Seasonal variation in glass eel swimming activity

One dead glass eel was found in autumn glass eels and two in springtime glass eels after the swimming test, which left a total of 71 and 70 glass eels in autumn and spring, respectively. The propensity of glass eels to migrate was investigated by their ability to synchronize their swimming activity to the change in water current direction every 6.2 h. Glass eels presenting a swimming activity with a period of

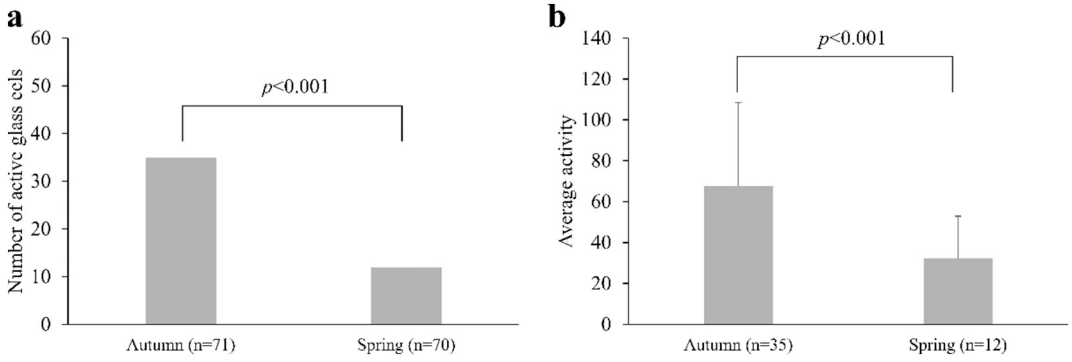


Fig. 3. Swimming behavior of autumn and spring glass eels. (a) Number of active glass eels, (b) level of swimming activity of active glass eels expressed as the mean number of times each individual was seen swimming in the water column during 7 days. Data is presented by means ± standard deviation. Statistical significance p-values are indicated at 0.05% level. Numbers in the x-axis text represent sample sizes for each group.

12.4 h were considered as active and the others as non-active. Results showed that the number of active glass eels was higher in autumn than in spring (chi-square test, $X^2 = 15.0$, $p < 0.001$; Fig. 3a). Then, the level of swimming activity was analyzed in active glass eels using the mean number of times each individual was seen swimming in the water column during 7 days. Swimming activity level was higher in autumn than in spring with a mean of 68 ± 41 and 32 ± 21 observations in autumn and spring, respectively (Student's *t*-test, $p < 0.001$; Fig. 3b). The mean number of observations in inactive glass eels (not synchronized to the change in water current direction) was 3 ± 4 in autumn and 2 ± 3 in spring.

3.2. Energetic status of glass eels depending on season and migration behavior

The energetic status (wet weight, length, standard metabolic rate (relative SMR) and transcriptomic profile of metabolism-related genes) was investigated in 35 autumn glass eels (22 active and 13 non-active) and in 36 spring glass eels (12 active and 24 non-active).

3.2.1. Wet weight, length and relative SMR

The initial wet weight of autumn glass eels measured before the swimming test ranged from 315 to 398 mg and was higher than that measured in spring glass eels (124 to 285 mg, Fig. 4a, two-way ANOVA, $p < 0.001$). Similar results were observed for length ranging from 70 to 76 mm in autumn and from 60 to 72 mm in spring (Fig. 4b, two-way ANOVA, $p < 0.001$). No significant difference in length or weight was observed between active and non-active fish in autumn or spring (Fig. 4a, b, two-way ANOVA, $p = 0.902$ in wet weight, $p = 0.565$ in length). There was also no interaction between migration behavior and season for these two parameters. Relative SMR, showed no significant difference depending on season (Fig. 4c, two-way ANOVA, $p = 0.666$) or migration behavior ($p = 0.441$). However, a weakly significant interaction between these two factors was observed ($p = 0.048$), wherein active glass eels presented a slightly higher relative SMR than non-active ones in autumn but not in spring.

3.2.2. Expressions of genes involved in energetic metabolism

We analyzed mRNA levels of 59 genes and showed the results by a heat map in a red-white scale (from lower to higher mRNA-expression level, Fig. 5). Some of these genes code for proteins involved in the autophagy/lysosome-related functions, including Macroautophagy (the best characterized sub-class of autophagy involving the formation of double-membrane organelles, or autophagosomes, which engulf portions of cytoplasm for subsequent degradation via lysosomes), Mitophagy (a macroautophagy-dependent specific degradation of mitochondria) and Chaperone-mediated autophagy (a specific autophagic route, known as CMA, that involves the direct delivery of cytosolic proteins targeted for degradation to the lysosomes). The other genes code for proteins involved in mitochondrial turnover and metabolism, the cytosol catabolism and finally the antioxidant system. mRNA levels of these genes were compared in response to season and migration behavior. Using clustering analyses and bootstrapping on normalized individual gene expression data we detected two clusters of genes (and some genes outside) that displayed the same overall patterns throughout the experimental conditions. Overall, the genes involved in energy stress resistance (macroautophagy and mitophagy) clustered in the group of genes overexpressed in spring glass eels compared to autumn ones, while genes involved in energy production and use (mitochondrial metabolism and CMA) showed an opposite trend. In detail, four macroautophagy genes (*atg5*, *atg7*, *atg12*, *ulk1*) and four mitophagy receptors (*fundc1*, *pink1*, *bnip3a*, *bnip3b*), showed significantly higher transcript levels in spring than in autumn, reflecting advanced energy distress in spring glass eels. In this regard, higher expression was also evidenced in spring glass eels for the gene *drp1*, known as the main player in the process of mitochondrial fragmentation, and three genes involved in mitochondrial biogenesis (*pgc1α1*, *pgc1α2*, *hsp60*), suggesting high mitochondrial turnover to compensate to the loss of mitochondria through mitophagy. In contrast, two genes (*mfn1* and *mfn2*) involved in mitochondrial fusion and cooperation were significantly more expressed in autumn than in spring. Likewise, most studied mitochondrial metabolism related genes, i.e., two genes coding for subunits of the mitochondrial membrane respiratory chain (*mt-ATP6*, *mt-nd5*), two genes involved in fatty acid catabolism (*cpt1α1*, *hadh*) and

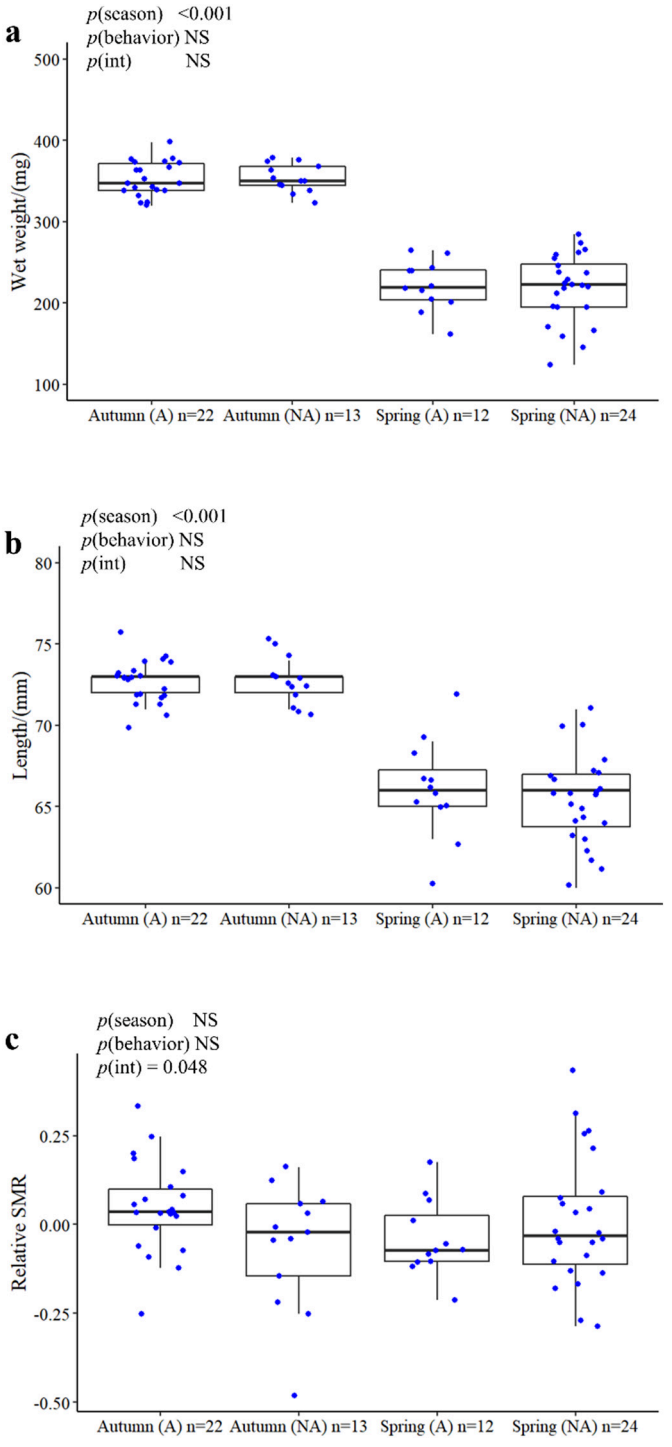


Fig. 4. Box-plots (median, 25–75% CI, min-max) of the wet weight (a), length (b) and relative SMR (c) of glass eels in response to season (autumn and spring) and differentiated migration behavior (A = active, NA = non-active). Statistical significance *p*-values and the interactions in the responses are indicated. Numbers in the x-axis text represent sample sizes for each group.

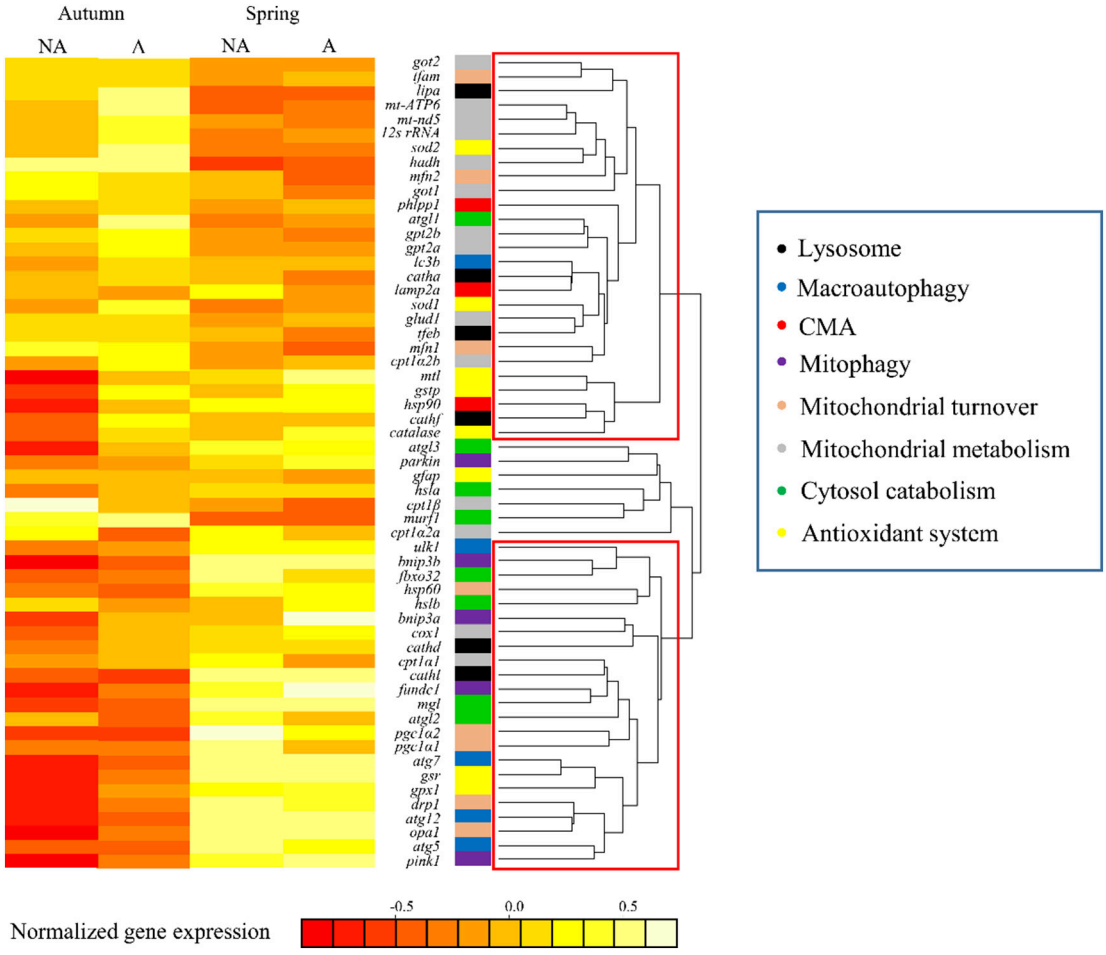


Fig. 5. Heatmap of mean gene expressions calculated per season and migration behavior (A = active, NA = non-active). The heatmap is organized through hierarchical clustering based on correlation of expression between genes among individuals. Two clusters were detected using bootstrapping resampling to estimate approximated unbiased values. They were statistically supported with accuracy *p*-values >0.95. Six other genes did not fit in any of the two clusters.

five genes involved in amino acid catabolism (*glud1*, *got1*, *got2*, *gpt2a*, *gpt2b*), exhibited higher expression in autumn than in spring. Interestingly, a similar trend was also observed for the genes related to CMA (*hsc70a*, *hsc70b*, *lamp2a*, *hsp90*, *phlpp1*), which has been demonstrated to play a major role in the regulation of the hepatic intermediary metabolism. Similarly, five genes involved in the oxidative stress defense (*sod1*, *sod2*, *mtl*, *gstp* and *catalase*) clustered in the group of genes overexpressed in autumn glass eels, possibly as a consequence of higher mitochondrial activity in these animals compared to their spring counterparts. Altogether, these results highlighted the existence of strong differences of the metabolic status between autumn and spring glass eels. However, no clear difference was evidenced between active and non-active glass eels whatever the season considered.

3.3. Relationships of the swimming activity levels of each active glass eel with its energetic status

The individual swimming activity level, expressed as the number of observations for each active glass eel swimming in the water column, ranged from 11 to 161 in autumn and from 11 to 79 in spring.

Linear regression models showing the correlations between the level of swimming activity in active glass eels and the individual wet weight, length and relative SMR are presented in Fig. 6. In autumn glass eels, activity levels were positively correlated to relative SMR (Fig. 6c) but not with wet weight or length (Fig. 6a, b), while spring glass eels displayed a positive correlation between activity and body weight and length, but not with relative SMR (Fig. 6a, b, c).

Among the five genomic functions of interest (autophagy-lysosome system, mitochondrial metabolism, mitochondrial turnover, antioxidant system and cytosol catabolism), the expression levels of mitochondrial metabolism genes and antioxidant system genes were significantly related to swimming activity (Fig. 7). We found a positive relationship between the first axis of the PCA for both of the functions and swimming activity level (two-way ANOVA, $p = 0.008$ for mitochondrial metabolism and $p = 0.007$ for antioxidant system), with no effect of season ($p = 0.772$ and 0.074, respectively) and no significant interaction ($p = 0.560$ and 0.384, respectively; See Supplementary material 3). For both functions, the first axis of the PCA explained 51% of the total gene expression variance. For mitochondrial metabolism function, this first axis was mainly correlated to genes of fatty acid catabolism (*cpt1α2b* and *hadh*), amino acid catabolism (*got1*, *got2*, *gpt2a*, *gpt2b* and *glud1*), mitochondrial respiratory chain complexes (*mt-nd5* and *mt-ATP6*) and *12s rRNA*, while for antioxidant system, this first axis was mainly correlated to *sod1*, *sod2*, *catalase*, *gstp* and *mtl* genes (see Supplementary material 4).

4. Discussion

The main objective of this study was to investigate the relationship between European glass eel energetics and their pattern of migration behavior, in the framework of the conditional strategy. For this purpose,

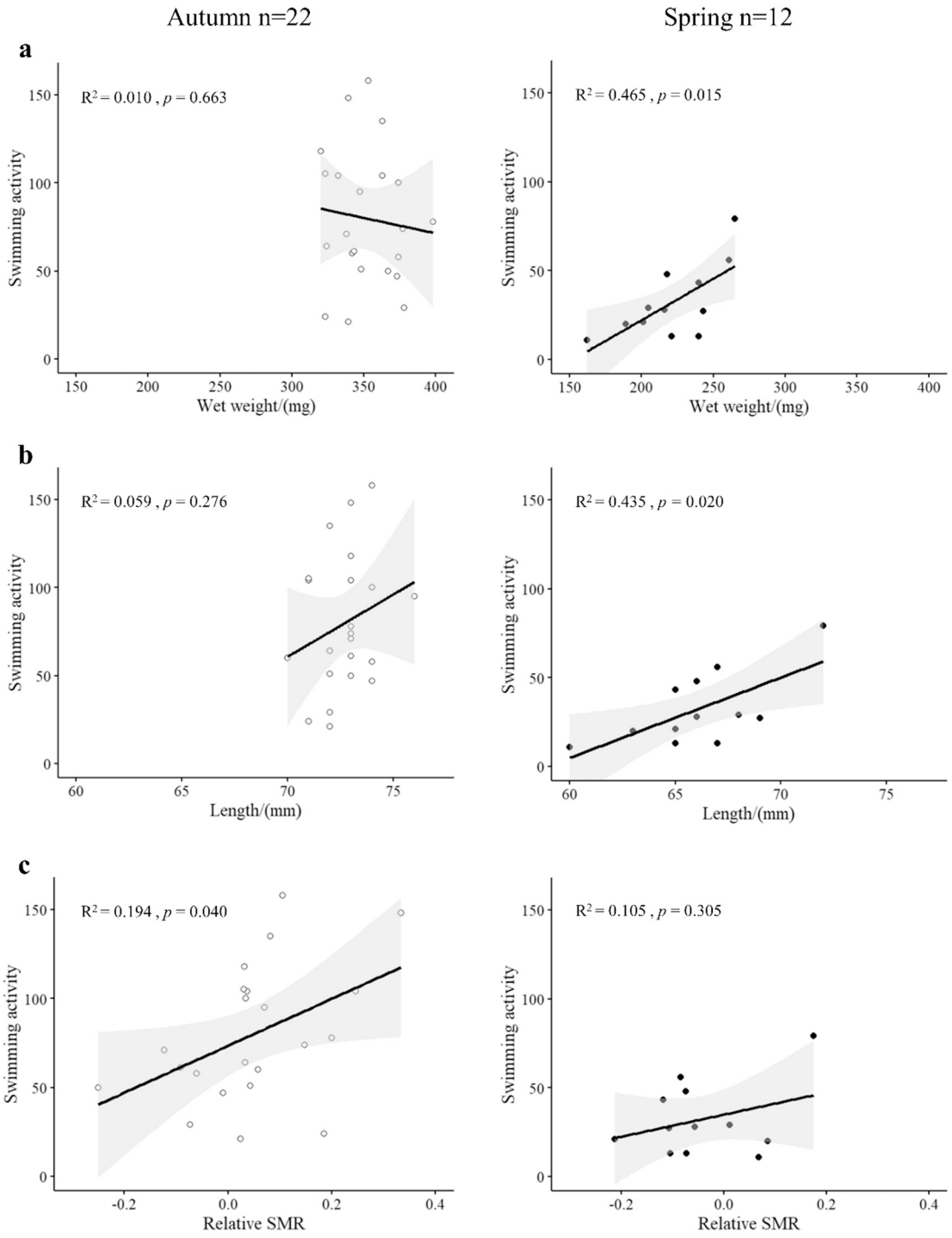


Fig. 6. Scatter plot for the observed individual swimming activity of active autumn and spring glass eels against the wet weight (a), length (b) and relative SMR (c). Empty circle = autumn, full circle = spring.

we used autumn and spring marine glass eels known to present high and low energetic stores, respectively. We did observe a higher propensity to migrate in autumn as predicted by the conditional strategy, and individual swimming activity level was also higher in autumn. However, when we compared the energy status of active and non-active glass eels within each seasonal group, we found no support for the conditional strategy: both behavioral groups showed similar wet weight and length. At the individual level, we also found little support for the conditional strategy: energy stores were correlated to the level of swimming activity in active glass eels only in spring, not in autumn. These results are further explained partly by variation in metabolic rates between individuals, and partly by the transcriptome analyses, that indicate striking contrasts in the metabolic states of autumn versus spring eels.

4.1. Autumn and spring glass eels: highly contrasting metabolic states

Final wet weight was highly correlated to triglycerides (see Section 2.2) and wet weight was considered in this study as a good proxy of energy stores. Here we show that autumn glass eels displayed

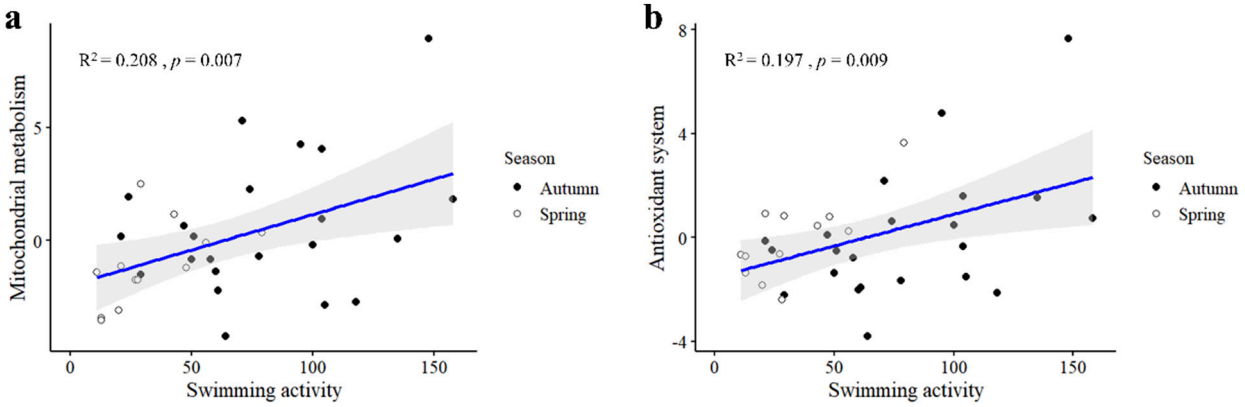


Fig. 7. Relationship between the first axis of the PCA for genes related to mitochondrial metabolism (a) and antioxidant system (b) and the swimming activity level. Empty circle: autumn, full circle: spring. The prediction plotted on the figure corresponds to a simple linear regression between the first PCA axis and the swimming activity level (considering no effect of season was detected) with associated R^2 and *p*-value.

higher wet weight than spring ones, which is in close agreement to previous observations made in *A. anguilla* glass eels (Charlon and Blanc, 1982; Claveau et al., 2015; De Casamajor et al., 2000; Elie, 1979). Such seasonal differences in biomass and energetic stores have been suggested to be due to the seasonal variations in oceanic ecosystems productivity affecting the growth of leptocephalus larvae during transatlantic transport (Désaunay and Guerault, 1997).

To further characterize the glass eels studied here, we measured their relative standard metabolism but no significant difference was observed between seasons. A high-throughput qRT-PCR analysis was then performed allowing the simultaneous assessment of the expression of 59 genes involved in energy metabolism and oxidative stress. They include genes of the autophagy-related pathways (including macroautophagy, mitophagy and CMA), genes involved in mitochondrial turnover and metabolism and genes of the cellular antioxidant system. Our results show that most of the monitored mitochondrial metabolism-related genes clustered in the group of genes overexpressed in glass eels caught in autumn compared to those sampled in spring. Similarly, the two genes *mfn1* and *mfn2*, known to promote mitochondrial fusion and therefore the cooperation between these organelles (Chen et al., 2003) also grouped to this cluster. These data strongly support that autumn glass eels displayed higher energy production capacity and use than the spring ones. Interestingly, autumn glass eels also presented higher expression of genes related to CMA, known as a master regulator of intermediary metabolism (Tasset and Cuervo, 2016), which support this idea.

In contrast, genes involved in energy stress resistance (macroautophagy and mitophagy) grouped in the cluster of genes overexpressed in spring glass eels compared to autumn glass eels. Macroautophagy is now widely recognized to play a major role in mobilizing diverse cellular energy and nutrient stores (including proteins, carbohydrates and lipids) as well as cellular organelles (including mitochondria, peroxisome, endoplasmic reticulum, the nucleus and ribosomes) during starvation in a large panel of taxa (Kaur and Debnath, 2015). This process is described as a key adaptive response to modulate the metabolism and provides energy when nutrients are scarce. Macroautophagy increases during periods of cellular stress in many eukaryotic species (from yeast to mammals) in order to conserve energy and promote survival. In this regard, we recently reported a significant contribution of macroautophagy-related transcripts production during long-term fasting to weight loss in glass eels (Bolliet et al., 2017b). The over-representation of genes involved in macroautophagy but also in mitophagy in the cluster of genes highly expressed in spring glass eels compared to their autumn counterpart would therefore reflect a higher catabolic state and an energy distress in spring glass eels.

Overall, the data clearly show that autumn and spring marine glass eels exhibit strong differences in term of both energetic stores and transcriptomic profile of metabolism-related genes, making them relevant models for a better understanding of the relationship between glass eel's organism energetics and their pattern of migration behavior.

4.2. Active vs non-active glass eels

To migrate up estuary, glass eels synchronize to the flood and mobilize energy stores accumulated by the leptocephalus stage to sustain activity (Forward and Tankersley, 2001; Gascuel, 1986; Jellyman, 1979; McCleave and Kleckner, 1982; Wippelhauser and McCleave, 1987). In our experimental conditions, fish that synchronized their swimming activity to the change in water current direction with a period close to 12.4 h were considered as having a high probability to migrate. They were called active glass eels. In contrast, non-active glass eels did not synchronized to the water current reversal and were considered as having a low propensity to migrate.

As most glass eels do not feed during migration (Bardonnet and Riera, 2005), energy stores have been considered as a physiological driver in fueling the landward colonization (Edeline, 2007; Edeline et al., 2006). Using salinity preference experiment, it has been suggested that European glass eels choosing fresh water should present a higher energetic status and migration activity than those choosing salt water (Edeline et al., 2005). According to these studies, it was expected here that active glass eels would have a higher wet weight that non-active ones. However, although the higher number of active glass eels and activity level observed in autumn glass eels were associated to a higher initial wet weight when compared to spring fish, active and non-active glass eels did not show any difference in wet weight in autumn nor in spring. This suggests that energy stores might possibly enhance migration but also that a conditional strategy based on an individual's energetic status cannot fully explain the European glass eel migration behavior observed in our experimental conditions.

Another factor that may be involved in the underlying mechanism of migration concerns relative SMR that represents an integrative measure of the energy expenditure and the celerity at which energy stores are consumed. In salmonids, it is known to fluctuate among individuals in correlation to dominance, aggression, growth rate or starvation (Finstad et al., 2007; Eliason and Farrell, 2016; Metcalfe et al., 2016 for reviews). Studies investigating the relationships between SMR and propensity to migrate in fish remain scarce but there is some evidence that low energy condition and excessive energy depletion are important factors determining successful upriver migration in salmonids (Eliason and Farrell, 2016 and references herein). In the present study, active glass

eels presented a slightly higher relative SMR than non-active ones in autumn but not in spring. Links between metabolic rate and fish behavior show a high intra-specific plasticity and are far from being understood (Auer et al., 2016; Careau et al., 2008; Metcalfe et al., 2016). During fasting, fish with a high SMR may have less energy available to allocate to activity but SMR may also reflect their capacity to perform activity (Biro and Stamps, 2010; Careau et al., 2008). In addition, fasting fish with a high SMR will deplete their energy stores more quickly that could decide them to migrate in order to switch their habitat and reach growth area. On the other hand, relative SMR was measured after the swimming test and although fish were kept in a tank during three days before oxygen measurement, one cannot exclude the fact that it was not long enough for active fish to fully recover to a resting metabolic rate after 7 days of activity. Zhang et al. (2018) evidenced that exercise training increased excess post-exercise oxygen consumption in exhausted *Salmo salar* when compared to control exhausted fish. Duration of recovering reached 15 h which was longer than previously found in the literature (Zhang et al., 2018) but still below the resting period fixed in our experiment before oxygen measurement. Further experiment comparing oxygen consumption before and after behavioral test are now required to validate the link between relative SMR and decision to synchronize to the change in water current direction in glass eels.

To further investigate the difference between migration patterns, we then monitored the transcriptomic profile of active and non-active glass eels within and between seasonal groups. However, no clear difference was observed between active and non-active glass eels whatever the considered season. Altogether, our data challenged the conditional strategy based on individual's energetic status for the European glass eels. The relationship between relative SMR and migration behavior remains to be elucidated and although no clear molecular evidence was observed between active and non-active fish, it is not excluded that the genes analyzed and the associated metabolic pathways may be involved in the different behavior observed. Indeed, we focused on gene expression at a single time point that does not give a real picture of the dynamic aspect of the different events at play during complex and integrative metabolic processes such as energy expenditure at rest, which include not only gene transcription but also protein translation and enzyme activity.

4.3. Variability in swimming activity level

Although the swimming activity of all active glass eels was synchronized to the change in water current direction, we observed a high inter-individual variability in activity level as expressed by the total number of observations of each individual swimming in the water column. When the activity level was plotted to the relative SMR, a positive correlation was found in autumn glass eels but not in spring ones, which may support hypothesis proposed in the previous section concerning SMR in active and non-active autumn fish. In contrast, when the activity level was individually plotted to the weight, a positive correlation was found in spring but not in autumn. Interestingly, the wet weight was higher in autumn glass eels than in spring ones and no overlapping was observed in their distributions. This could suggests a threshold in energy stores below which energy becomes a limiting factor for swimming efficiency. In regarding to gene expression analysis, whatever the season, the genes related to mitochondrial metabolism and antioxidant system positively correlated to swimming activity level. During endurance exercise, the increase of mitochondrial respiration induces the production of reactive oxygen species (ROS), which can lead to the accumulation of cellular damage, unless it can be counterbalanced by antioxidants that act to quench ROS and prevent the oxidation of other important biological molecules (Powers et al., 2011). This finding is also supported by a recent report on birds, where the authors summarized an upregulated antioxidant system coping with the oxidative challenges associated with migratory flight (Cooper-Mullin and McWilliams, 2016).

4.4. Consequences for eel management

As we demonstrated, there is strong seasonal effect on both condition of eels and their behavior. Whereas autumn eels displayed high wet weight and activity, spring eels were strongly constrained energy wise, and displayed reduced migration activity. Population monitorings and management actions should therefore be evaluated in the light of these results. For instance, because of being in energy distress, spring glass eels might be more vulnerable to stress, whereas autumn glass eels could provide a higher plasticity to cope with variation in environmental conditions. In the context of global change, wherein fluctuations in temperatures, hydraulic conditions or pollutants may increase energy expenditure, the spring component of populations should be carefully monitored. Measures for the recovery of the stock of European eel as presented by the EU (Council Regulation (EC) 1100/2007) propose stocking of eels i.e. a transfer of glass eels from their fishing area to another place more suitable to their survival and/or growth. Such management actions should however be planned by carefully balancing their costs and benefits: the spring eels should probably be avoided, for they might have limited ability to adapt to the new environments and also because they represent a distinct yet threatened part of the phenotypic variation in the population. Autumn glass eels might adapt with more efficiency to new environments. In any case, if any restocking action is to be implemented, the managers would be well inspired to track the effect of glass eels origins (i.e., season) on the success of their action.

5. Conclusion

Autumn and spring glass eels displayed different energy status that may affect migration behavior in different ways. Autumn fish displayed high energy reserves and capacity for energy production that may have triggered migration behavior in our experimental conditions. However, in both seasons, a conditional strategy based on individual's energy status could not explain why some fish decided to synchronize to the water current direction while some others stayed sheltering in the substratum. The relative SMR may be a possible candidate to explain the different patterns of behavior in autumn glass eels but further studies are now needed to clearly elucidate this point. Spring glass eels presented a higher energy distress than the autumn ones and molecular results also evidenced a higher expression of genes involved in fission, macroautophagy and mitophagy. These processes may have helped glass eels to maintain standard metabolism for vital functions but may be not sufficient to allow a high swimming activity. Indeed, migration requires energy in addition to others physiological tasks and when energy stores dwindle, the ability to migrate may be directly related to the fish's maximum capacity to increase oxygen consumption. Investigating the metabolic scope in active and non-active fish could be another interesting avenue to explore. Finally, it is also paramount for stocks and biodiversity managers to recognize that the physiological status of glass eels arriving on the oceanic shelf change drastically throughout the seasons, making them nonrandom parts of the whole phenotypic variation at the species level.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We wish to thank the IE ECP for the technical support and the China Scholarship Council for the grant to H.L.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134039.

References

Adam, G., Feunteun, E., Prouzet, P., Rigaud, C., 2008. L'anguille EUROPÉENNE: indicateurs d'abondance et de colonisation. 1st ed. Coll. Savoir-faire, Versailles.

Auer, S.K., Bassar, R.D., Salin, K., Metcalfe, N.B., 2016. Repeatability of metabolic rate is lower for animals living under field versus laboratory conditions. J. Exp. Biol. 219 (5), 631–634. https://doi.org/10.1242/jeb.133678.

Bardonnet, A., Riera, P., 2005. Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: new insights from stable isotope analysis. Estuar. Coast. Shelf Sci. 63 (1–2), 201–209. https://doi.org/10.1016/j.ecss.2004.11.009.

Bermejo-Nogales, A., Calduch-Giner, J.A., Perez-Sanchez, J., 2015. Unraveling the molecular signatures of oxidative phosphorylation to cope with the nutritionally changing metabolic capabilities of liver and muscle tissues in farmed fish. PLoS One 10 (4). https://doi.org/10.1371/journal.pone.0122889.

Biro, P.A., Stamps, J.A., 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol. Evol. 25 (11), 653–659. https://doi.org/10.1016/j.tree.2010.08.003.

Bolliet, V., Labonne, J., 2008. Individual patterns of rhythmic swimming activity in *Anguilla anguilla* glass eels synchronised to water current reversal. J. Exp. Mar. Biol. Ecol. 362 (2), 125–130. https://doi.org/10.1016/j.jembe.2008.06.017.

Bolliet, V., Lambert, P., Rives, J., Bardonnet, A., 2007. Rhythmic swimming activity in *Anguilla anguilla* glass eels: synchronisation to water current reversal under laboratory conditions. J. Exp. Mar. Biol. Ecol. 344 (1), 54–66. https://doi.org/10.1016/j.jembe.2006.12.027.

Bolliet, V., Claveau, J., Jarry, M., Gonzalez, P., Beaudrimont, M., Monperrus, M., 2017a. Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European eels (*Anguilla Anguilla*). Physiol. Behav. 169, 33–40. https://doi.org/10.1016/physbeh.2016.11.008.

Bolliet, V., Labonne, J., Olazcuaga, L., Panserat, S., Seiliez, I., 2017b. Modeling of autophagy-related gene expression dynamics during long term fasting in European eel (*Anguilla anguilla*). Sci. Rep. 7 (1), 17896. https://doi.org/10.1038/s41598-017-18164-6.

Bureau du Colombier, S.B., Bolliet, V., Lambert, P., Bardonnet, A., 2007. Energy and migratory behavior in glass eels (*Anguilla anguilla*). Physiol. Behav. 92 (4), 684–690. https://doi.org/10.1016/j.physbeh.2007.05.013.

Careau, V., Thomas, D., Humphries, M.M., Reale, D., 2008. Energy metabolism and animal personality. Oikos 117 (5), 641–653. https://doi.org/10.1111/j.0030-1299.2008.16513.x.

Cassan-Wang, H., Soler, M., Yu, H., Camargo, E.L.O., Carocha, V., Ladouce, N., Grima-Pettenati, J., 2012. Reference genes for high-throughput quantitative reverse transcription-PCR analysis of gene expression in organs and tissues of eucalyptus grown in various environmental conditions. Plant Cell Physiol. 53 (12), 2101–2116. https://doi.org/10.1093/pcp/pcs152.

Charlon, N., Blanc, J.M., 1982. Etude des civelles d'*Anguilla anguilla* L. dans la région du bassin de l'Adour. 1. Caractéristiques biométriques de longueur et de poids en fonction de la pigmentation. Arch. Hydrobiol. 93 (2), 238–255.

Chen, H.C., Detmer, S.A., Ewald, A.J., Griffin, E.E., Fraser, S.E., Chan, D.C., 2003. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J. Cell Biol. 160 (2), 189–200. https://doi.org/10.1083/jcb.200211046.

Claveau, J., Monperrus, M., Jarry, M., Pinaly, H., Baudrimont, M., Gonzalez, P., Bolliet, V., 2015. Spatial and seasonal variations of methylmercury in European glass eels (*Anguilla anguilla*) in the Adour estuary (France) and relation to their migratory behaviour. Environ. Sci. Pollut. Res. 22 (14), 10721–10732. https://doi.org/10.1007/s11356-015-4303-3.

Cooper-Mullin, C., McWilliams, S.R., 2016. The role of the antioxidant system during intense endurance exercise: lessons from migrating birds. J. Exp. Biol. 219, 3684–3695. https://doi.org/10.1242/jeb.123992.

Daverat, F., Limburg, K.E., Thibault, I., Shiao, J.C., Dodson, J.J., Caron, F.O., Wickstrom, H., 2006. Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A-japonica* and *A-rostrata*. Mar. Ecol. Prog. Ser. 308, 231–241. https://doi.org/10.3354/meps308231.

Davey, A.J.H., Jellyman, D.J., 2005. Sex determination in freshwater eels and management options for manipulation of sex. Rev. Fish Biol. Fish. 15 (1–2), 37–52. https://doi.org/10.1007/s11160-005-7431-x.

De Casamajor, M., Prouzet, P., Lazure, P., 2000. Identification des flux de civelles (*Anguilla anguilla*) à partir des relations d'allométrie en fonction des conditions hydrodynamiques de l'estuaire de l'Adour. Aquat. Living Resour. 13 (6), 411–420. https://doi.org/10.1016/S0990-7440(00)01092-5.

Désaunay, Y., Guerault, D., 1997. Seasonal and long-term changes in biometrics of eel larvae: a possible relationship between recruitment variation and North Atlantic ecosystem productivity. J. Fish Biol. 51, 317–339. https://doi.org/10.1111/j.1095-8649.1997.tb06106.x.

Edeline, E., 2007. Adaptive phenotypic plasticity of eel diadromy. Mar. Ecol. Prog. Ser. 341, 229–232. https://doi.org/10.3354/meps341229.

Edeline, E., Dufour, S., Elie, P., 2005. Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. Mar. Ecol. Prog. Ser. 304, 191–199. https://doi.org/10.3354/meps304191.

Edeline, E., Lambert, P., Rigaud, C., Elie, P., 2006. Effects of body condition and water temperature on *Anguilla anguilla* glass eel migratory behavior. J. Exp. Mar. Biol. Ecol. 331 (2), 217–225. https://doi.org/10.1016/j.jembe.2005.10.011.

Eliason, E.J., Farrell, A.P., 2016. Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. J. Fish Biol. 88 (1), 359–388. https://doi.org/10.1111/jfb.12790.

Elie, P., 1979. Contribution à l'étude des montées de civelles d'*Anguilla Anguilla* L., dans l'estuaire de la Loire: Pêche écophysiologie et élevage. Ph.D. thesis. University of Rennes I (383 p).

Elie, P., Lecomte-Finiger, R., Cantrelle, I., Charlon, N., 1982. Définition des limites des différents stades pigmentaires durant la phase civelle *d'Anguilla anguilla* L. (Poisson Téléostéen Anguilliforme). Vie et Milieu 32 (3), 149–157. https://prodinra.inra.fr/record/78575.

Finstad, A.G., Forseth, T., Ugedal, O., Naesje, T.F., 2007. Metabolic rate, behaviour and winter performance in juvenile Atlantic salmon. Funct. Ecol. 21 (5), 905–912. https://doi.org/10.1111/j.1365-2435.2007.01291.x.

Forward, R.B., Tankersley, R.A., 2001. Selective tidal-stream transport of marine animals. Oceanogr. Mar. Biol. 39 (39), 305–353.

Gaillard, M., Bernatchez, L., Tremblay, R., Audet, C., 2015. Regional variation in energy storage strategies in American glass eels from Eastern Canada. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 188, 87–95. https://doi.org/10.1016/j.cbpa.2015.06.019.

Gascuel, D., 1986. Flow-carried and active swimming migration of the glass eel (*Anguilla-anguilla*) in the tidal area of a small estuary on the French Atlantic coast. Helgoländer Meeresun. 40 (3), 321–326. https://doi.org/10.1007/bf01983739.

Geffroy, B., Bardonnet, A., 2016. Sex differentiation and sex determination in eels: consequences for management. Fish Fish. 17 (2), 375–398. https://doi.org/10.1111/faf.12113.

Harrison, A.J., Walker, A.M., Pinder, A.C., Briand, C., Aprahamian, M.W., 2014. A review of glass eel migratory behaviour, sampling techniques and abundance estimates in estuaries: implications for assessing recruitment, local production and exploitation. Rev. Fish Biol. Fish. 24 (4), 967–983. https://doi.org/10.1007/s11160-014-9356-8.

Imbert, H., Arrowsmith, R., Dufour, S., Elie, P., 2008. Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage-dependent mechanisms in European eel upstream migration. Horm. Behav. 53 (1), 69–81. https://doi.org/10.1016/j.yhbeh.2007.06.011.

Jellyman, D.J., 1979. Upstream migration of glass-eels (*Anguilla* spp) in the Waikato River. N. Z. J. Mar. Freshw. Res. 13 (1), 13–22. https://doi.org/10.1080/00288330.1979.9515776.

Kaur, J., Debnath, J., 2015. Autophagy at the crossroads of catabolism and anabolism. Nat. Rev. Mol. Cell Biol. 16 (8), 461–472. https://doi.org/10.1038/nrm4024.

Kawakami, Y., Mochioka, N., Nakazono, A., 1999. Immigration patterns of glass-eels *Anguilla japonica* entering river in northern Kyushu, Japan. Bull. Mar. Sci. 64 (2), 315–327.

Krueger, W.H., Oliveira, K., 1999. Evidence for environmental sex determination in the American eel, *Anguilla rostrata*. Environ. Biol. Fish 55 (4), 381–389. https://doi.org/10.1023/A:1007575600789.

Laffaille, P., Acou, A., Guioullet, J., Mounaix, B., Legault, A., 2006. Patterns of silver eel (*Anguilla anguilla* L.) sex ratio in a catchment. Ecol. Freshw. Fish 15 (4), 583–588. https://doi.org/10.1111/j.1600-0633.2006.00195.x.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(−Delta Delta C) method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Marandel, L., Lepais, O., Arbenoits, E., Veron, V., Dias, K., Zion, M., Panserat, S., 2016. Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (*Oncorhynchus mykiss*) by nutritional status and dietary carbohydrates. Sci. Rep. 6. https://doi.org/10.1038/srep32187.

McCleave, J.D., Kleckner, R.C., 1982. Selective tidal stream transport in the estuarine migration of glass eels of the american eel (*Anguilla-rostrata*). J. Conseil. 40 (3), 262–271.

Metcalfe, N.B., Van Leeuwen, T.E., Killen, S.S., 2016. Does individual variation in metabolic phenotype predict fish behaviour and performance. J. Fish Biol. 88 (1), 298–321. https://doi.org/10.1111/jfb.126.

Parsons, J., Vickers, K.U., Warden, Y., 1977. Relationship between elver recruitment and changes in sex-ratio of silver eels *Anguilla-anguilla*-L migrating from Lough Neagh, northern-Ireland. J. Fish Biol. 10 (3), 211–229. https://doi.org/10.1111/j.1095-8649.1977.tb05127.x.

Powers, S.K., Nelson, W.B., Hudson, M.B., 2011. Exercise-induced oxidative stress in humans: cause and consequences. Free Radic. Biol. Med. 51, 942–950. https://doi.org/10.1016/j.freeradbiomed.2010.12.009.

Régnier, T., Bolliet, V., Labonne, J., Gaudin, P., 2010. Assessing maternal effects on metabolic rate dynamics along early development in brown trout (*Salmo trutta*): an individual-based approach. J. Comp. Physiol. B. 180 (1), 25–31. https://doi.org/10.1007/s00360-009-0385-x.

Secor, D.H., Hendersonarzapalo, A., Piccoli, P.M., 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes. J. Exp. Mar. Biol. Ecol. 192 (1), 15–33. https://doi.org/10.1016/0022-0981(95)00054-u.

Singh, R., Cuervo, A.M., 2012. Lipophagy: connecting autophagy and lipid metabolism. Int. J. Cell Biol. 2012, 282041. https://doi.org/10.1155/2012/282041.

Spiegelhalter, D.J., Myles, J.P., Jones, D.R., Abrams, K.R., 2000. Bayesian methods in health technology assessment: a review. Health Technol. Assess. 4 (38), 1–130.

Tasset, I., Cuervo, A.M., 2016. Role of chaperone-mediated autophagy in metabolism. FEBS J. 283, 2403–2413. https://doi.org/10.1111/febs.13677.

Tesch, F.W., 2003. The Eel. fifth ed. Wiley, New York.

Tsukamoto, K., Arai, T., 2001. Facultative catadromy of the eel Anguilla japonica between freshwater and seawater habitats. Mar. Ecol. Prog. Ser. 220, 265–276. https://doi.org/10.3354/meps220265.

Tsukamoto, K., Nakai, I., Tesch, W.V., 1998. Do all freshwater eels migrate? Nature 396 (6712), 635–636. https://doi.org/10.1038/25264.

Tzeng, W.N., 1996. Effects of salinity and ontogenetic movements on strontium: calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. J. Exp. Mar. Biol. Ecol. 199 (1), 111–122. https://doi.org/10.1016/0022-0981(95)00185-9.

Wippelhauser, G.S., McCleave, J.D., 1987. Precision of behavior of migrating juvenile American eels (*Anguilla-rostrata*) utilizing selective tidal stream transport. J. Conseil. 44 (1), 80–89.

Zhang, Y., Claireaux, G., Takle, H., Jorgensen, S.M., Farrell, A.P., 2018. A three-phase excess post-exercise oxygen consumption in Atlantic salmon *Salmo salar* and its response to exercise training. J. Fish Biol. 92 (5), 1385–1403. https://doi.org/10.1111/jfb.13593.

Publication 2

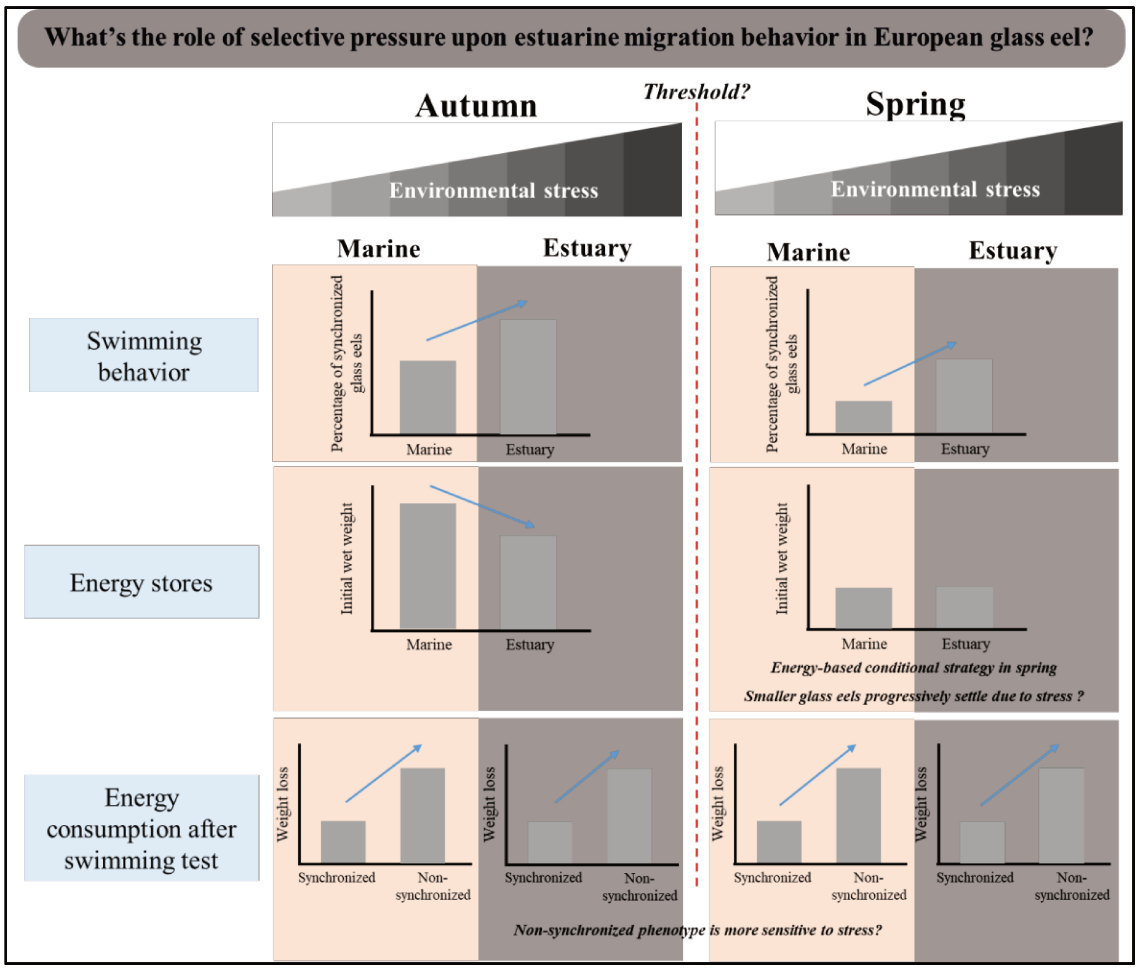


Figure 2-2. Graphical abstract and highlights from Publication 2- The role of selective pressure upon estuarine migration behavior in European glass eel (*Anguilla anguilla*).

Highlights

1. Energy-based conditional strategy was supported in spring glass eels, not in autumn ones.

2. A higher proportion of estuarine glass eels synchronized their swimming behavior to the rhythmic environmental cues than marine glass eels.

3.Molecular and metabolism analyses suggested that the estuarine glass eels were more stressed than the marine ones.

4. The weight loss corrected by swimming activity level was higher in non-synchronized glass eels than their synchronized conspecifics, suggesting a higher stress or sensitivity to stress in the former.

2.2 Estuarine migration behavior in relation to the energetic status of glass eels in terms of energy stores, standard metabolism and energy-related gene expression

Presentation of Publication 2

Rethinking the role of selective pressure upon estuarine migration behavior in European glass eel (*Anguilla anguilla*)

Objective and methods

The conditional strategy based on energy suggests that once entering estuaries, glass eels will migrate up estuary if they have enough energy reserves. Accordingly, estuarine glass eels should represent a subsample of marine fish. In order to test this hypothesis, we compared marine and estuarine glass eels in terms of swimming behavior and energy stores. We also hypothesize that the ability to mobilize energy could be involved in the migration propensity and thus investigated individual metabolism and energy related genes expression. In addition, as glass eels energy stores strongly differ between autumn and spring individuals, both seasons were investigated.

The propensity to migrate of glass eels and the relationships with their energetic status were individually investigated in each groups (autumn and spring marine and estuarine glass eels) as described in the precedent chapter.

Results and Conclusions

Autumn glass eels lost weight during upstream migration reflected by a decrease in wet weight, probably because of the 22 km covered without feeding between the sea and the estuarine site of sampling. In contrast, spring estuarine glass eels presented no different weight to the marine ones, which may indicate a progressive process of settlement or death of some smallest individuals during migration. These results confirm those obtained in the first chapter and are in accordance with an energy-based conditional strategy in this ecotype, characterized by very low energy reserves (Figure 2-2).

Behavioral results showed that estuarine glass eels presented a higher number of synchronized individuals than marine glass eels, suggesting that their ability to synchronize their swimming behavior to the rhythmic environmental cues may represent another factor involved in the facultative migration of glass eels (Figure 2-2).

In estuary, glass eels are challenged by a combination of changed temperature, salinity, hydraulic conditions, or pollutants which may induce a stress. Stress should increase metabolism which was supported in both seasons by the higher energy expenditure and metabolism observed in estuarine glass eels than in

marine ones. Genes related to stressful catabolism processes, including lysosomal catabolism and CMA, were also overexpressed in estuarine fish than in marine ones while the expression of genes related to mitochondrial turnover was lower in the former.

Interestingly, in both seasons and sites, and regardless of the swimming activity levels, non-synchronized glass eels lost more weight than synchronized ones suggesting a higher stress or vulnerability to stress. Stress should increase energy expenditure but may also affect rhythmic function and endogenous clock(s). We hypothesize that glass eels may present a variability in their ability to cope with stress and during estuarine migration, the most sensitive to stress may not be able to achieve migration and would settle in the estuary (Figure 2-2).

Rethinking the role of selective pressure upon estuarine migration behavior in European glass eel (*Anguilla anguilla*)

(Publication submitted to Physiology & Behavior)

Hengtong Liu[1,2], Jacques Labonne[1], Pascale Coste[1], Karine Dias[2], Emmanuel Huchet[1], Elisabeth Plagnes-Juan[2], Jacques Rives[1], Vincent Veron[2], Iban Seiliez[2,†] and Valérie Bolliet[1†,*]

[1] *Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, ECOBIOP, Aquapôle INRAE, MIRA, F64310, Saint-Pée-sur-Nivelle, France*

[2] *INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, NuMéA, F64310, Saint-Pée-sur-Nivelle, France*

[†]These authors contributed equally to this work

∗ Corresponding author:
valerie.bolliet@inrae.fr
UMR 1224 Ecobiop, Aquapôle, 64310 Saint Pée sur Nivelle,
France. Tel.: +33 5 59 51 59 78

KEYWORDS

conditional strategy, energy metabolism, facultative migration, stress, tidal rhythm

ABSTRACT

To investigate the facultative estuarine migration of the European glass eel, marine and estuarine individuals were collected in autumn and spring and their migration behavior was assessed in facilities mimicking the tide by a change in water current direction every 6.2 h. For each glass eel, migration behavior was expressed by its swimming synchronization to the water current reversal, its probability of swimming, and swimming rheotaxis. We then analyzed their energetic status expressed as weight, standard metabolism (SMR) and transcriptomic profiles of metabolism-related genes. Spring glass eels presented lower energy condition than autumn ones. Estuarine individuals displayed lower weight than marine ones in autumn but not in spring suggesting that a conditional strategy based on energy may explain facultative migration when energy reserves become a limiting factor. We observed a higher percentage of individuals synchronized to the current direction in estuarine fish than in marine ones suggesting that the selection may also target their ability to synchronize swimming activity to the tide. Weight loss, SMR and the expression of metabolism-related genes suggested that estuarine glass eels were more stressed and had a lower capacity of energy production than marine fish. The non-synchronized glass eels also presented a higher energy expenditure than synchronized individuals possibly reflecting a higher stress and/or vulnerability to stress in the former. These results provide new insights on the facultative estuarine migration in glass eels and suggest that stress might represent an important factor to consider in the settlement process.

1 INTRODUCTION

Threatened by a markedly dropped population size since the 1980s (ICES, 2018; Jacoby & Gollock, 2014), the European eel is currently classified as critically endangered on the red list of the International Union for Conservation of Nature (IUCN). European eels display a catadromous life history involving oceanic spawning in the Sargasso Sea and continental growth (Tesch, 2003). After hatching, Leptocephalus larvae use ocean currents (mainly the Gulf Stream) to migrate to the European coasts. Then, larvae metamorphose into glass eels at the slope of the continental shelf and migrate up estuaries to reach rivers for growth (Tesch, 2003). However, it is now well accepted that some glass eels do not migrate to freshwater and complete their life cycle between marine and brackish waters (Daverat et al., 2006; Tsukamoto, Aoyama, & Miller, 2002; Tsukamoto, Nakai, & Tesch, 1998; Tzeng, Wang, Wickstrom, & Reizenstein, 2000). Demographic studies suggest an environmental sex determinism in the European eel closely associating the colonization of freshwater habitats to female production while settlement in marine or estuarine areas is mostly associated to males (Geffroy & Bardonnet, 2016; Tesch, 2003). Thus, the propensity to colonize rivers in glass eels may have strong effects on the sex ratio of the population and it is therefore crucial to understand both ecosystems occupation by the species and the evolutionary consequences on the population structure.

Most glass eels fast during estuarine migration (Bardonnet & Riera, 2005) and depend upon energy stores accumulated by the Leptocephalus larvae to sustain swimming activity. Accordingly, a theoretical model of energy-based conditional strategy has been proposed as a major mechanism of the facultative migration in the European glass eels (Edeline, 2007). In this theory, migrant glass eels should present higher energy reserves than non-migrant ones but some studies, performed in experimental conditions, failed to confirm such difference (Bureau Du Colombier, Bolliet, Lambert, & Bardonnet, 2007; Liu et al., 2019). In addition, neither experimental tests nor field observations could support the conditional theory in American glass eels (Boivin et al., 2015; Gaillard, Bernatchez, Tremblay, & Audet, 2015). These contradictory results suggest that a conditional strategy based on energy, at least with the energy markers used to date, cannot fully explain the facultative migration in glass eels. In addition, we recently evidenced strong seasonal variations in the wet weight and the expression of energy-related genes in marine glass eels, spring individuals showing an high energy distress when compared to autumn ones (Liu et al., 2019). Such variations may also suggest different energy-related strategies in glass eels during estuarine migration that remain to be elucidated.

Besides the energy needed to sustain swimming activity during migration, glass eels also require a good synchronization of activity with the environmental cues. Indeed, glass eels migrate up estuaries using selective flood transport: during flood tide, they move up in the water column and migrate with the current while they go down and remain on or in the substratum during ebb tide (Forward & Tankersley, 2001;

Gascuel, 1986; Jellyman, 1979). Glass eels are also synchronized to the photoperiod, avoiding light and swimming mainly during the night (Tesch, 2003). Both tidal and photoperiodic cues synchronize swimming activity through endogenous clock(s) which remain to be identified for the tidal one(s) (Bolliet & Labonne, 2008; Bolliet, Lambert, Rives, & Bardonnet, 2007; Wippelhauser & McCleave, 1987, 1988). In a recent study, it has been reported that estuarine glass eels submitted to a light/dark cycle in experimental conditions presented a higher proportion of synchronized individuals (glass eels ascending in the water column at dusk to swim with the water current) than marine glass eels (Bolliet et al., 2017). As estuarine glass eels represent a naturally subsampled population of the total marine arrivals, the increased proportion of synchronized fish between the two sites may reflect a selection on their ability to synchronize their swimming activity with the environmental cues. Selection here is defined as the product of decision of individuals to settle in a given environment, and their subsequent success (i.e., survival) in doing so.

To better understand the processes of selection during estuarine migration in the European glass eels, marine and estuarine individuals were collected both in autumn and spring and introduced in an experimental facility mimicking the alternation of tides with a change in water current direction every 6.2 h. Their migration behaviors, mainly expressed by the swimming synchronization to the water current reversal and the level of swimming activity, were analyzed in relation to their energetic status including energy reserves, rate of oxygen consumption and expression of energy-related genes.

2 MATERIALS AND METHODS

2.1 Ethics

Procedures used in this study have been validated by the ethics committee N°073 (ref: 2017012015086652). The experiment was carried out in strict accordance with the EU legal frameworks, specifically those relating to the protection of animals used for scientific purposes (i.e., Directive 2010/63/EU), and under the French legislation governing the ethical treatment of animals (Decret no. 2013-118, February 1st, 2013).

2.2 Fish collection and tagging

The peak of glass eel migration to the coastal area of South-West France occurs from November to April. In this study, four samplings were carried out respectively in marine and estuarine sites in November 2016 (hereafter autumn glass eels) and April 2017 (hereafter spring glass eels). The marine site is located at Moliets (43° 55′N, 1° 23′W, located 40 km north of the mouth of the Adour estuary) and the estuarine site is located at Urt (43° 28′N, 1° 17′W, located 22 km from the mouth of the Adour estuary, Supplementary Figure S1). The samplings were operated using a dip-net at night and during flood tide. Once collected, fish were transferred to the laboratory and maintained at 12 ± 0.5°C overnight in a tank containing aerated water

from the fishing site. In the next morning, all glass eels were anesthetized (Benzocaine, 0.01 mg L^{-1}) and individually measured for initial wet weight (± 1.0 mg) and length (± 0.5 mm). In both autumn and spring experiments, groups of 72 and 36 glass eels (collected from marine and estuarine sites, respectively) were tagged using Visible Implant Elastomer (VIE Tag) in order to follow the swimming activity individually under UV light (combinations of one or two hypodermic spots of different colors as described by Imbert, Arrowsmith, Dufour, and Elie, 2008). Previous data showed more active glass eels in estuary than marine (Bolliet et al., 2017) and we tested more fish in marine site than estuarine site to get sufficient sample size for the comparative analyses between synchronized and non-synchronized fish. Once tagged, glass eels were released to wake up in the water from fishing site. During the next 48 h, the water was continuously aerated and progressively diluted with fresh water.

2.3 Swimming test

In both seasons, two swimming tests were conducted for marine glass eels and one for estuarine ones. They were performed in an annular tank installed in a temperature-controlled room as described in Liu et al. (2019). The room was maintained under a photoperiod of 12 L/ 12 D with a very low light intensity during the photophase (0.2-0.3 $\mu W/cm^2$) and a constant UV light (0.6 $\mu W/cm^2$) to see the VIE Tag. The water temperature was kept at 12 ± 0.5°C and continuously recorded by thermistors placed in the tank.

After acclimatization, glass eels were transferred into the annular tank and exposed to a change in water current direction every 6.2 h (alternately clockwise and counterclockwise water flow projected by two pumps fixed on the opposite ends of the tank). For each experiment, tagged animals were mixed with the same number of untagged ones to facilitate synchronization of swimming activity to the change in water current direction by increasing density (Bolliet et al., 2007). The swimming behaviour of glass eels was traced individually during 7 days by a camera programed to record 15 s every 40 min. The duration of 15 s was chosen so that a same glass eel swimming in the water column can only be observed once during a session. A total of 177 sessions of 15 s were obtained for each glass eel.

In similar experimental conditions than in the present study, we previously showed that glass eels could synchronize to the change in water current direction by swimming with the flow (negative rheotaxis), against the flow (positive rheotaxis) or by alternating both behavior at each water current reversal (Bolliet et al., 2007; Bolliet & Labonne, 2008). Thus, the propensity to migrate of each glass eel was described in the present study by: (i) its level of swimming activity, (ii) its ability to synchronize its swimming activity to a tidal rhythm with a period of 12.4 h (hereafter called synchronized glass eels); (iii) for a synchronized fish, its rheotaxis (swimming with and/or against the current).

After the swimming test, a subsample of synchronized and non-synchronized fish were kept for oxygen consumption measurement (see below) and all others glass eels were anaesthetized, killed using a lethal bath of anaesthesia (Benzocaine, 0.05 mg L^{-1}), individually measured for wet weight (± 1.0 mg) and length (± 0.5 mm) and then flash-frozen in liquid nitrogen, and stored at -80°C.

2.4 Standard metabolic rate (SMR) assay

After 7 days of swimming test, subsamples of 35 and 20 tagged glass eels in autumn and 36 and 24 tagged glass eels in spring, originated from marine and estuarine site respectively, were retrieved to determine resting oxygen consumption as described in Liu et al. (2019). Briefly, an intermittent flow respirometer with 16 respirometry chambers (diameter: 11.2 mm, length: 90 mm) was used. Measurements were conducted over four days under temperature and photoperiod conditions similar to those used for the swimming test. Tagged glass eels were introduced in the chambers at 3 p.m. and oxygen consumption was recorded continuously every minute until 10 a.m. the next day. After the first 15-hours of acclimatization in the chambers, the average oxygen consumption per eel was calculated using the last 4 h of recording. SMR was expressed in mm^3 O_2 consumed per hour. We then regressed the logarithm of SMR on the logarithm of wet weight and used the residuals of this model (i.e., relative SMR) for further analyses. Following the measurement of oxygen consumption, glass eels were anesthetized as described above and stocked at -80 °C after immersion in liquid nitrogen until RNA extraction.

2.5 Gene expression analysis by high-throughput RT-qPCR

The protocol conditions for sample preparation and the high-throughput RT-qPCR have been previously published (Liu et al., 2019). The primers used have been previously described (Liu et al., 2019) and targeted 59 energy-related genes involved in cytosol catabolism (including lipid and protein degradation), mitochondria-related functions (including mitochondrial metabolism and turnover), autophagy and antioxidant system. For the expression analysis, relative quantification of target gene expression was done using the ΔCT method described by Pfaffl, Horgan, and Dempfle (2002). The relative expression of Luciferase was used for data normalization as described previously (Marandel et al., 2016).

2.6 Statistical analyses

After the 7-day swimming test, one marine glass eel died in autumn experiment, and two marine ones in spring, which left a total of 71 and 70 marine glass eels in autumn and spring, respectively. No death was recorded in estuarine glass eels.

2.6.1 Propensity of glass eels to migrate

To characterize the propensity of glass eels to migrate, we first assessed their swimming activity. To do so, we fitted a General Linear Model (GLM) based on binomial distribution, using as successes the number of observations where the eel was seen swimming in the water column, and as failures the number of observations where the eel was not observed in the water column and likely hidden in the substratum. For each season, the effect of site (Marine or Estuary) on swimming activity was included in the GLM as a categorical factor and its effect was assessed using Chi² test applied on deviance analysis.

We then investigated the synchronization of glass eel's swimming activity by a change in water current direction every 6.2 h (synchronized / non-synchronized glass eels). Based on the 177 sessions of 15 seconds of video recording in each experiment, we categorized all tagged glass eels into synchronized ones and non-synchronized ones by a modeling method, as previously described by Liu et al. (2019) (See Supplementary Text S1). From this model, two parameters identifying individual's tidal activity rhythm were derived: the probability of being swimming of an individual i at time t, $P(t,i)$ and the periodicity of swimming occurrence of an individual i, $per(i)$. Fish having a P value above the mean of P meanwhile having an activity periodicity close to 12.4 h were considered synchronized, others were considered non-synchronized. The comparison of number of synchronized fish between marine and estuarine groups was analyzed by Fisher's exact test for count data in both seasons.

Finally, the probability of negative rheotaxis used by synchronized glass eels was analyzed. Again, we fitted a GLM based on binomial distribution, using as successes the number of observations where the eel was seen active and swimming *with* the current, and as failures the number of observations where the eel was seen active and swimming *against* the current. For each season, the effect of site (Marine or Estuary) on negative rheotaxis probability was included in the GLM as a categorical factor and its effect was assessed using Chi² test applied on deviance analysis.

2.6.2 Energy reserves, weight loss and SMR

Two-way Anova was used to analyze the effect of site (Marine and Estuary) and synchronization behavior (synchronized and non-synchronized) on energy reserves, weight loss and SMR, followed by Tukey HSD post-hoc test.

Equations:

Weight loss and weight loss efficiency were calculated as follows:

Weight loss (%) = (Initial wet weight – Final wet weight) * 100 / Initial wet weight

Weight loss efficiency = Weight loss (%) / Swimming activity

2.6.3. Principal Component Analysis (PCA) of gene transcriptional profiles

To determine the transcriptional profiles of studied genes, PCA was used as a multivariate statistical approach to reduce the number of the variables considered. We ran a PCA analysis for each genetic function considered to evaluate the global transcriptional response of the genes involved in this function. Supplementary Figure S2 (A, B, C, D) shows the relevance of all the genes involved in each function to the first axis of PCA. The score of individuals on the first axis of the PCA was retrieved as a synthetic indicator of the individual level of expression for each genetic function. Supplementary Table S1 listed the percentage of explained variance on the first axis of PCA. All the statistical analyses relevant to gene transcriptions were examined using the first axis of PCA for each genomic function. Two-way Anova was used to analyze the varying gene transcriptional profiles in response to site and synchronization behavior. The interactions in the responses were also evaluated. To test whether each single gene is differently expressed between the two sites and/or the two synchronization behavioral patterns, we performed a two-way Anova (Supplementary Table S2 and Figure S3).

All the statistical analyses and modeling were carried out using the statistical software R (v.3.3.1) and OpenBUGS (v.3.2.3). Differences were considered significant at $p < 0.05$.

3 RESULTS

3.1 Swimming test

Glass eels propensity to migrate was first assessed by their swimming activity. Regardless of the season, estuarine glass eels displayed a higher probability of swimming than the marine fish (P(swimming) = 0.26 for estuarine eels and 0.20 for marine eels in autumn; P(swimming) = 0.12 for estuarine eels and 0.04 for marine eels in spring; Chi^2 test, $p < 0.001$; Figure 1A and 1B; Supplementary Table S3A). It is noteworthy that despite being significant, the site effect explained only 1.2% of the total variation in swimming activity in autumn against 13% in spring. Then, using ability of eels to synchronize swimming activity to the change in water current direction with a period close to 12.4 h, we determined the percentage of synchronized eels: regardless of the season, we observed a trend that the percentage of synchronized fish was higher in the estuarine group than in the marine one (66% and 49%, respectively in autumn; 33% and 17%, respectively in spring; Figure 1C and 1D), although such trend cannot be statistically supported because low sample numbers in each season (Fisher's test, $p = 0.22$ in autumn, $p = 0.10$ in spring). These results altogether suggest that a higher proportion of estuarine glass eels than marine ones present a good ability to migrate, this observation being more pronounced in spring.

Finally, the swimming tactic displayed by the synchronized glass eels was compared between marine and estuarine sites (Figure 1E and 1F). Most of the fish alternated negative rheotaxis (swimming with the current) and positive rheotaxis (swimming against the current) at each change in water current direction, although some individuals used only one of the two tactics. In autumn, marine fish had a higher probability to use negative rheotaxis than the estuarine ones (P(negative rheotaxis) = 0.42 and 0.35, respectively; Chi^2 test, $p < 0.001$; Figure 1E; Supplementary Table S3B) while in spring, marine fish had a lower probability to use negative rheotaxis than the estuarine ones (P(negative rheotaxis) = 0.29 and 0.67, respectively; Chi^2 test, $p < 0.001$; Figure 1F; Supplementary Table S3B).

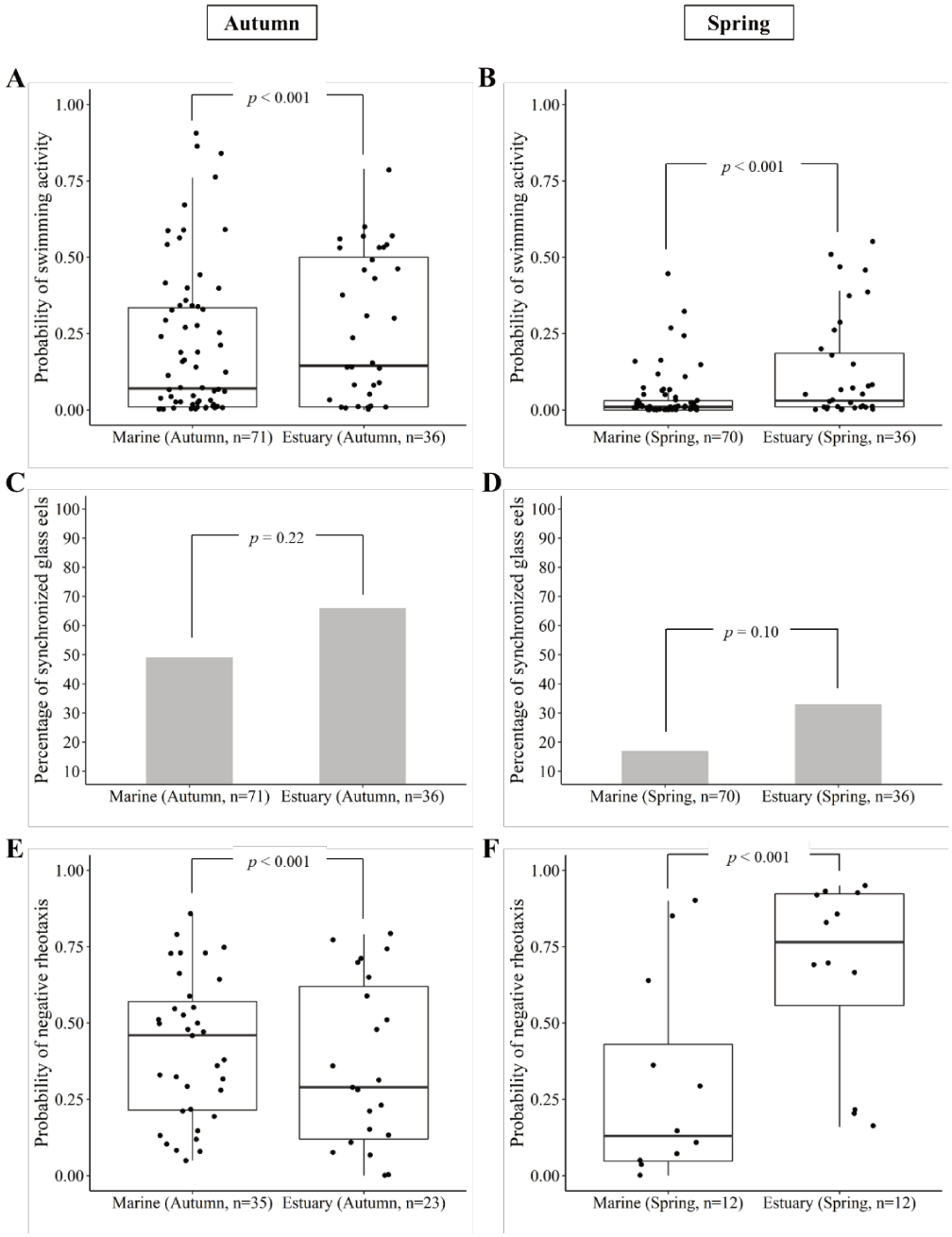


Figure 1. Comparisons of swimming behavior between marine and estuarine glass eels in autumn and spring. (A, B) Level of swimming activity (expressed as the ratio between the number of observations where the fish was active in the water column on the total number of observations for each fish); (C, D) Percentage of synchronized glass eels as determined by the model; (E, F) Swimming rheotaxis of synchronized glass eels (expressed as the number of negative rheotaxis observations on the total number of active observations). For each behavior, results from the associated statistical analysis are provided: exact Fisher's test *p*-values for percentage of synchronized individuals, and *p*-values obtained from Chi² tests on GLM deviance tables for swimming activity and swimming rheotaxis (see Supplementary Table S3).

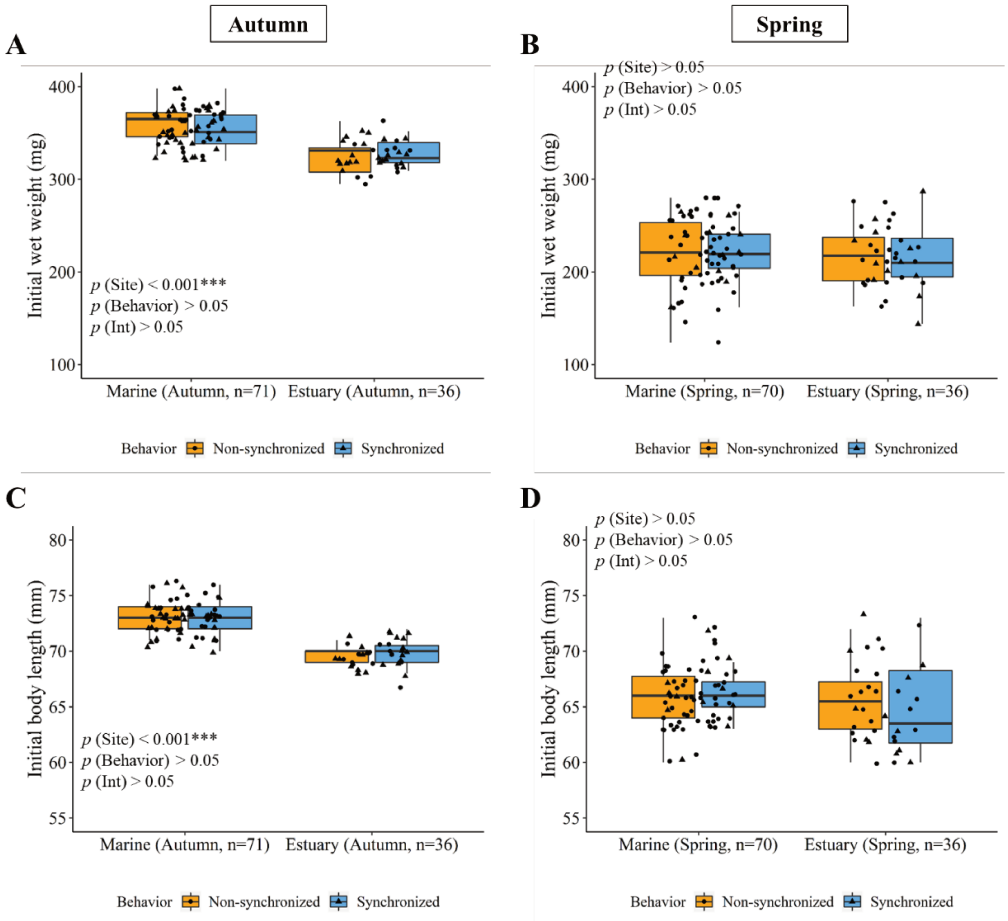


Figure 2. Comparisons of (A, B) initial wet weight and (C, D) initial body length between marine and estuarine glass eels in autumn and spring. Statistical significance *p*-values and the interactions in the responses are indicated.

3.2 Energy reserves and expenditure measures

As the ability of glass eels to migrate could depend on their energy reserves, we first measured their wet weight and body length as a proxy of energy content (see Liu et al., 2019). The initial wet weight was higher in autumn glass eels than in spring ones (345 mg ± 23 and 220 mg ± 35, respectively, Figure 2A and 2B). In autumn, the wet weight and body length were significantly lower in estuarine glass eels than in marine glass eels ($p < 0.001$), while no difference was observed in spring (Figure 2A-D). These results suggest that different metabolic processes may occur during the migration depending on the season and the energy conditions of glass eels.

In order to investigate the use of energy stores in marine and estuarine glass eels, we then measured the percentage of weight loss after the 7-day swimming test. Regardless of the season or the behavior, estuarine

glass eels lost more wet weight than their marine counterparts ($p < 0.001$, Figure 3A and 3B). Results also showed that non-synchronized fish lost less weight than synchronized fish, with the exception of estuarine glass eels collected in spring and displaying a similar weight loss in non-synchronized and synchronized fish (Figure 3A and 3B). However, regardless of the site or the season, when the weight loss was corrected by the swimming activity, non-synchronized glass eels lost more wet weight than the synchronized ones (for a same scale of activity, $p < 0.001$; Figure 3C and 3D). An interacting effect of the site and the behavioral pattern was also observed in autumn fish, showing that weight loss was higher in estuarine non-synchronized glass eels than in their marine counterpart (Tukey's test, $p < 0.001$; Figure 3C).

Rate of oxygen consumption was recorded as an integrative measure of the individual energy expenditure. Interestingly, no significant effect of the site nor of the behavior could be observed on the relative SMR in autumn (Figure 3E). In contrast, a higher individual SMR was observed in estuarine glass eels than in marine ones in spring ($p = 0.013$, Figure 3F) while the behavioral effect was not significant.

Overall, these results provide evidences that estuarine glass eels expended more energy than marine glass eels. After correction for their activity level, non-synchronized fish also used more energy than synchronized ones.

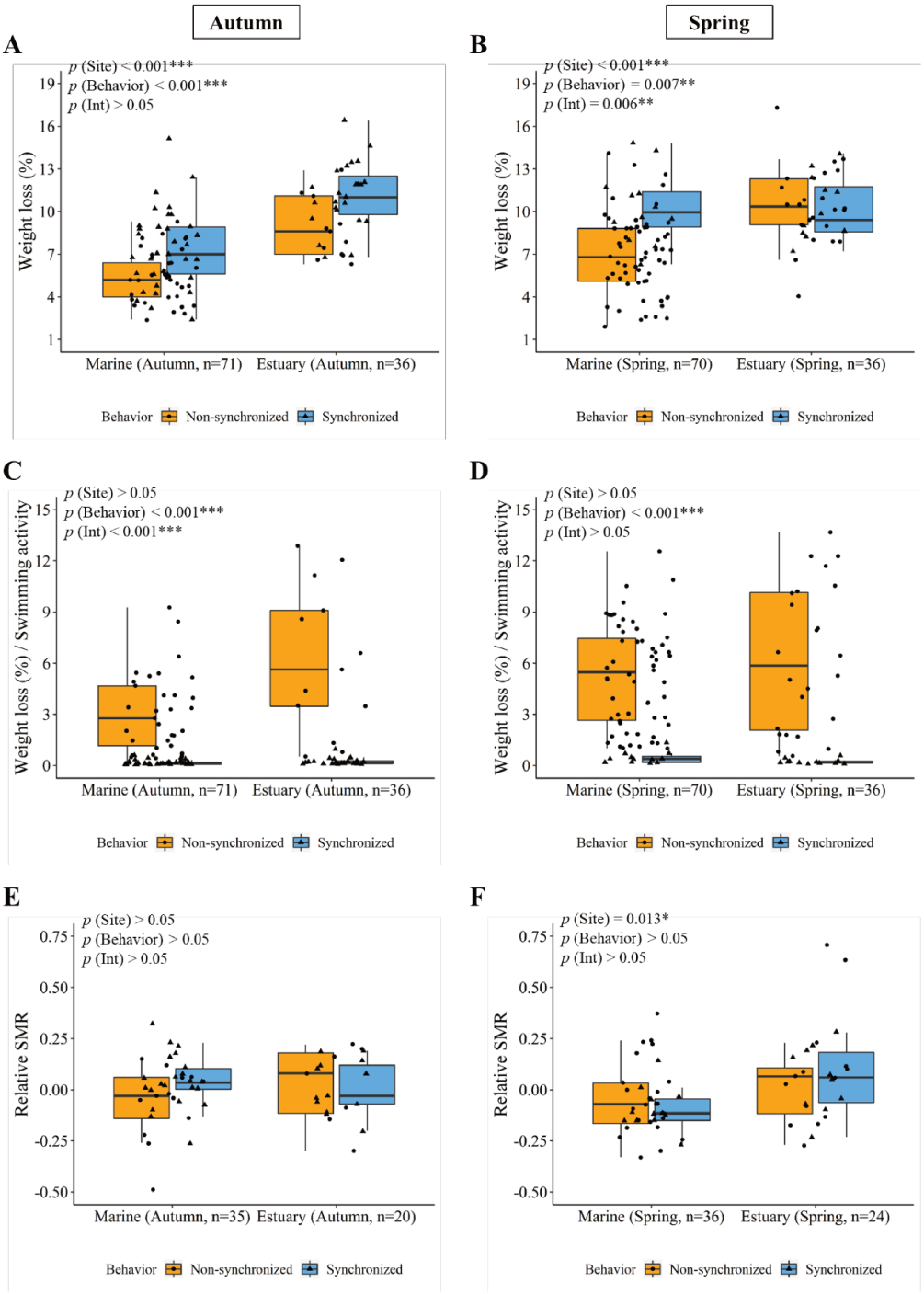
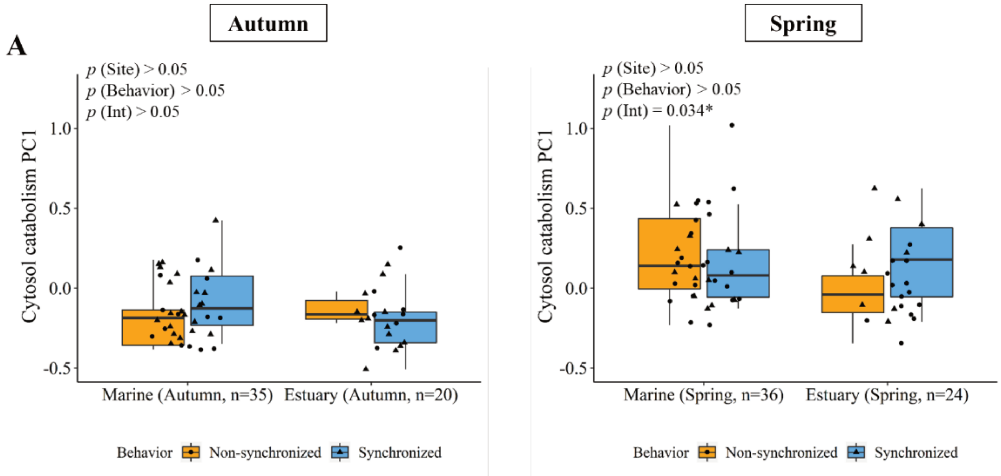


Figure 3. Comparisons of (A, B) weight loss (%) after behavioral test, (C, D) weight loss efficiency in relation to activity and (E, F) relative standard metabolic rate (SMR) between marine and estuarine glass eels in autumn and spring. Statistical significance *p*-values and the interactions in the responses are indicated.

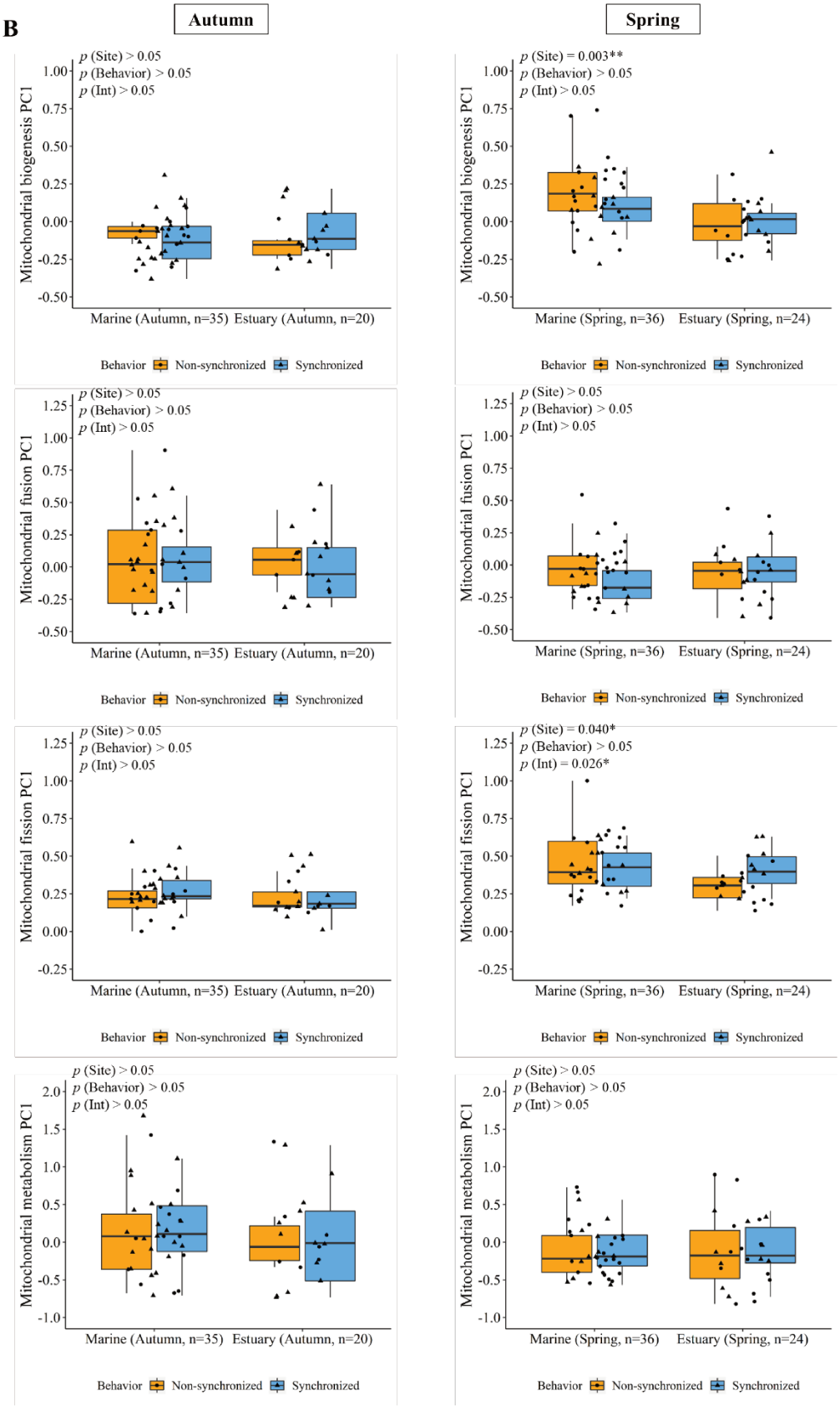
3.3 Transcriptional profiles of genes involved in the use of energy stores

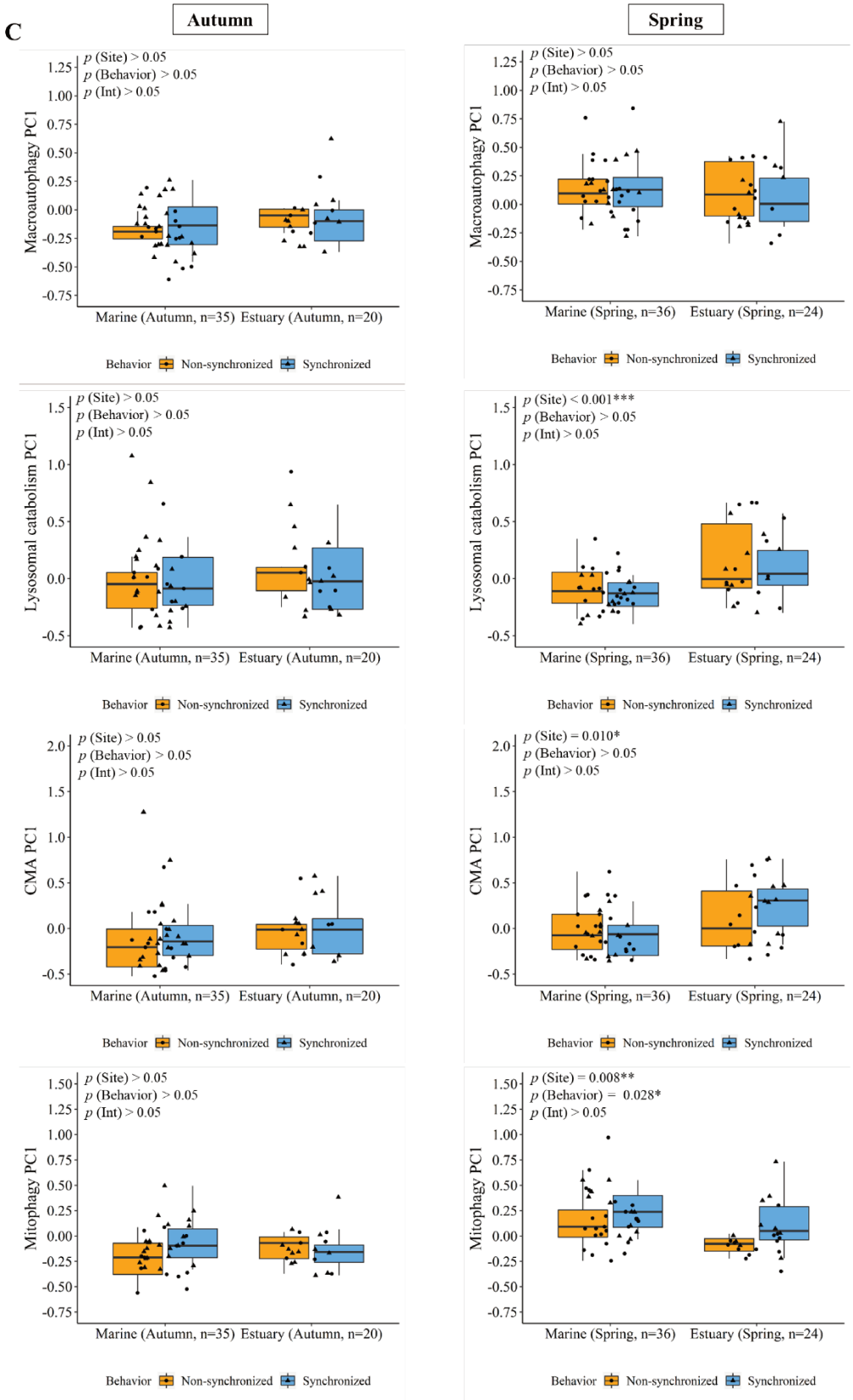
In order to investigate at molecular level the mechanisms at play in the use of energy stores during glass eel migration, the transcriptional profiles of 59 genes involved in four energy-related functions were analyzed. These genes code for proteins involved in: (A) cytosol catabolism, including lipid and protein breakdown; (B) mitochondria-related functions, including mitochondrial turnover (biogenesis, fusion and fission) and metabolism; (C) autophagy, including macroautophagy (the best-characterized autophagy subclass), chaperone-mediated autophagy (a specific autophagic route, known as CMA, that involves the direct delivery of cytosolic proteins targeted for degradation to the lysosomes), mitophagy (a macroautophagy-dependent specific degradation of mitochondria) and lysosomal catabolism; (D) antioxidant system (See Supplementary Table S2).

Results show that the behavior has little or no effect on the expression of the genes studied. Only genes involved in mitophagy fluctuated in relation to behavior and only in spring (Figure 4C). Our data also reveal a difference in the expression of some genes between estuarine and marine glass eels in spring but not in autumn. In this regard, the expression of genes involved in mitophagy, mitochondrial biogenesis and fission were lower in spring estuarine glass eels than in their marine counterparts (Figure 4B, 4C and confirmed at single gene level in Table S2 and Figure S3), while the expression of genes related to CMA and lysosomal catabolism were higher in estuarine glass eels than the marine fish (Figure 4C and confirmed at single gene level in Table S2 and Figure S3). These results suggest that estuarine glass eels may present a lower capacity to produce energy and a stronger energy distress than marine fish in spring.



B


Autumn
Spring
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
p (Site) = 0.003**
p (Behavior) > 0.05
p (Int) > 0.05
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) = 0.026*
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
Mitochondrial biogenesis PC1
Mitochondrial fusion PC1
Mitochondrial fission PC1
Mitochondrial metabolism PC1
Marine (Autumn, n=35)
Estuary (Autumn, n=20)
Marine (Spring, n=36)
Estuary (Spring, n=24)
Behavior
Non-synchronized
Synchronized


C
Autumn
Spring
Macroautophagy PC1
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
Marine (Autumn, n=35) Estuary (Autumn, n=20)
Marine (Spring, n=36) Estuary (Spring, n=24)
Behavior Non-synchronized Synchronized
Lysosomal catabolism PC1
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
p (Site) < 0.001***
p (Behavior) > 0.05
p (Int) > 0.05
CMA PC1
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
p (Site) = 0.010*
p (Behavior) > 0.05
p (Int) > 0.05
Mitophagy PC1
p (Site) > 0.05
p (Behavior) > 0.05
p (Int) > 0.05
p (Site) = 0.008**
p (Behavior) = 0.028*
p (Int) > 0.05

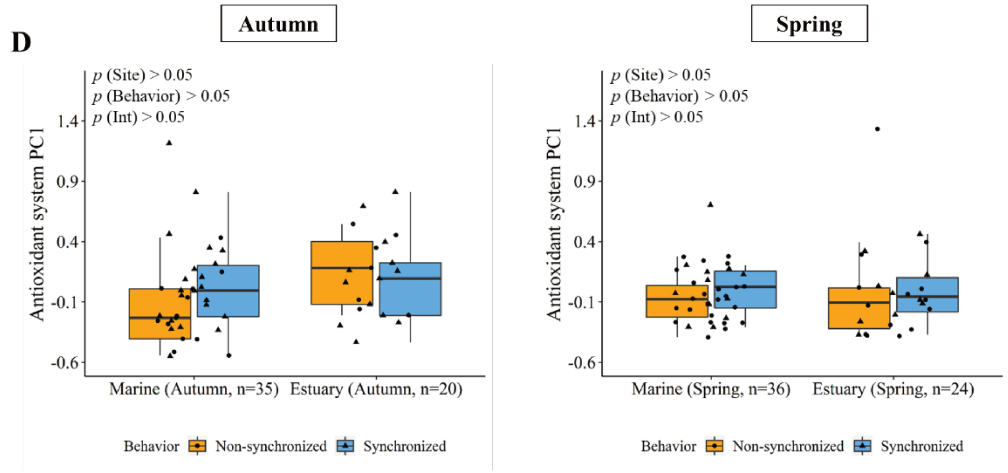


Figure 4. Comparisons of the transcriptional profiles of energy-related genes, expressed as the first axis of PCA for each cellular function, between marine and estuarine glass eels in autumn and spring. Plots show targeted genes involved in (A) Cytosol catabolism; (B) Mitochondria-related functions; (C) Autophagy; (D) Antioxidant system. Statistical significance *p*-values and the interactions in the responses are indicated.

4 DISCUSSION

Once recruited to the coast, European glass eels are known to migrate up estuary using a selective tidal stream transport (Forward & Tankersley, 2001). However, it is also now well accepted that this behavior is facultative, some individuals stopping their migration and settling in the estuary or the sea (Daverat et al., 2006; Tsukamoto et al., 1998). In order to better understand this process of settlement and the processes of selection during estuarine migration, the propensity to migrate in marine and estuarine glass eels was assessed in experimental conditions in relation to their energetic status.

Conditional strategy based on energy

In the energy-based conditional strategy Edeline (2007) proposes that a higher propensity to migrate up estuary should be related to a higher energetic status in glass eels. In the American eel, several studies provided contradictory arguments on this link (Boivin et al., 2015; Gaillard et al., 2015) and seasonal variations have been proposed in the European species. Indeed, Bureau du Colombier et al. (2007) first advocated this hypothesis by showing in experimental conditions that migrant glass eels presented a higher energy content than non-migrant ones in February but not in November. Liu et al. (2019) also showed a positive correlation between glass eel swimming activity and energy reserves in spring but not in autumn. In the present study, autumn estuarine glass eels were smaller than marine ones, probably as a result of the 22 km covered without eating. However, in spring, no difference in weight nor length could be observed between estuarine and marine individuals. Glass eels restart feeding in spring (Charlon & Blanc, 1983) and

as proposed by Bolliet et al. (2017), the resumption of feeding behavior may allow estuarine fish to compensate the energy expenditure related to migration. However, in this last study, 71% of glass eels presented food in the digestive tract after catching while no food residual was observed in the present one. Thus, in accordance to the conditional strategy, the similar weight observed in spring estuarine and marine glass eels may rather result from a selection process, the smallest glass eels progressively settling or dying in the estuary before reaching the estuarine site of collection. We already shown in marine glass eels (Liu et al., 2019), and confirm in the present study in estuarine fish, that spring glass eels displayed an energy distress which was not observed in autumn. These results support the idea that the role of energy in glass eels migration may depend on the season and suggest that energy may represent a limiting factor for migration under a given threshold of reserves.

In addition, estuaries are considered as stressful environments with a combination of different stressors including large variations of temperature, hydraulic conditions, salinities, oxygen availability and/or contaminants. Stress responses such as detoxification and cellular repairment to avoid cellular and organismal damages may lead to energy over-consumption in addition to the energy require for other physiological processes (Riahi et al., 2019). Our results support this idea, suggesting that estuarine glass eels were more stressed than marine ones, as evidenced in the former by the greater weight loss and SMR after swimming test and by the higher expression of genes involved in CMA and lysosomal catabolism (two pathways known to be activated by stress) (Dash, Aydin, & Moroz, 2019; Kagedal, Johansson, & Ollinger, 2001; Kiffin, Christian, Knecht, & Cuervo, 2004). Results also showed lower expression levels of genes involved in mitochondrial turnover and mitophagy in estuarine glass eels than in the marine ones, possibly reflecting a lower capacity of energy production and use in estuarine fish. However, although a higher weight loss was observed in both autumn and spring estuarine glass eels when compared to their marine counterpart, difference in transcriptional profiles and SMR were only observed in spring. This suggests that the higher energy distress reported in this season (Liu et al., 2019), in addition to stressful conditions of migration in estuary, might drive stronger responses and trigger a selection based on energy condition.

Interestingly, the behavioral results of swimming rheotaxis, which could be both negative (swimming with the current) and positive (swimming against the current), showed that spring glass eels changed their swimming tactic between marine and estuarine sites. Estuarine fish mainly shift from a positive to a negative swimming rheotaxis which may represent an adaptive mechanism to spare energy and migrate up estuary in this season.

Altogether, these results strengthened the idea that a conditional strategy based on energy may contribute to explain the facultative migration in glass eels but likely when energy reserves become a limiting factor to sustain activity. Surprisingly, in both seasons, synchronized glass eels, considered to present a high

probability to migrate did not display higher wet weight than non-synchronized ones in our experimental conditions. This may first suggest that the initial variability in wet weight inside each group was not strong enough to separate migrant phenotypes from non-migrant ones on the basis of energy content in our experimental conditions. But it also suggests that other factors may be involved in settlement processes.

Selection on the ability to synchronize the swimming activity to the tide

Regardless of the migratory season, estuarine glass eels were more numerous to synchronize to the change in water current direction than marine glass eels, a result previously reported in a study using photoperiod as a synchronizer (Bolliet et al., 2017). The rhythmic swimming behavior of glass eels during estuarine migration is known to be controlled by endogenous clock(s) which synchronize fish activity with environmental cues related to the tide and the photoperiod (Bolliet et al., 2007; Forward & Tankersley, 2001). Several exogenous cues related to the tide have been proposed, such as odour (Creutzberg, 1961), turbulence (McCleave & Kleckner, 1982), salinity (Edeline, Dufour, & Elie, 2005), electrical fields (Cresci et al., 2019) and water current reversal (Bolliet et al., 2007; Wippelhauser & McCleave, 1987). In the shore crab (*Carcinus maenas*), artificial tidal cycles of salinity, temperature and pressure applied 120° out of phase with each other, all synchronized locomotor activity (Warman & Naylor, 1995). The activity rhythm persisted in free-running conditions with three peaks corresponding to each cue which supported the idea that different cues might synchronize tidal activity. In the present study, only one cue was used as synchronizer (water current reversal every 6.2 h) and it cannot be ruled out that this synchronizer may be more efficient in estuarine glass eels than in marine ones.

On the other hand, we can also hypothesize that glass eels could be selected based on their ability to synchronize their swimming activity to the tide during estuarine migration. As explain above, estuaries represent stressful environment and several studies have reported that stress may cause arhythmicity and loss of oscillation in clock networks in both mammals (Tahara et al., 2015) and fish (Prokkola & Nikinmaa, 2018). In fish, the circatidal system has not yet been identified but the main circadian clock(s) as well as their molecular mechanism involving transcriptional/translational loops of several clock genes (*per*, *clock*, *bmal*, *cry*, *ror*, and *reverb*) and their rhythmic secretion of melatonin are well documented (Falcón, 1999; Falcón, Besseau, Sauzet, & Boeuf, 2007; Steindal & Whitmore, 2019; Vatine, Vallone, Gothilf, & Foulkes, 2011; Zhdanova & Reebs, 2006). Decreased amplitude and mean expression levels for most of the clock genes have been reported in stressed rainbow trout (*Oncorhynchus mykiss*) coupled with an increase in cortisol production, a stress hormone (Hernández-Pérez et al., 2019; Naderi et al., 2018). Naderi et al. (2018) suggested that cortisol may not directly modulate clock gene expression in the trout but this hormone may have a key role in mediating stress-effects on pineal melatoninergic system, thus on rhythmicity persistence (López-Patiño, Gesto, Conde-Sieira, Soengas, & Miguez, 2014; see Sánchez-Vázquez et al., 2019 for

review). All these studies were conducted on circadian systems but it cannot be excluded that circatidal systems may present similar stress-clock interactions. In such a case, it would suggest that the higher proportion of synchronized fish observed in estuarine than in marine glass eels may reflect a selection based on stress, targeting the ability of glass eels to synchronize their swimming activity to the tide during the migration process. Interestingly, our results showed that non-synchronized glass eels lost more weight than their synchronized conspecifics suggesting that they could be more stressed or affected by stress. This assumption is in accordance with the studies of Claveau et al. (2015) and Liu et al. (2020), showing that non-synchronized glass eels may be more vulnerable to stress as they reacted more markedly to stressors in experimental conditions. Altogether, these results suggest that glass eels may have varying degrees of sensitivity to stress and that in a stressful environment such as estuaries, the most vulnerable individuals may stop migration and settle. Underlying mechanisms such as direct effect of stress on the internal clock(s) remain to be demonstrated.

5 CONCLUSION

Our results provide new insights on the facultative estuarine migration in the European glass eels. Regardless of the season, a selection based on stress may be an interesting avenue to explore in future research. Stress could be involved in both the decision to settle in an environment, and on the odds of surviving in the chosen environment (Crowley & Labonne, submitted). In the American eel, it has been suggested that a divergent natural selection of phenotypes and/or genotype-dependent habitat choice by individuals may results in genetic differences between fresh water and marine habitats (Gagnaire, Normandeau, Cote, Hansen, & Bernatchez, 2012; Gaillard et al., 2016; Pavey et al., 2015). Whether the ability to deal with stress may differ between individuals on a genetic basis in the European glass eels remains to be clarified. On the other hand, a conditional strategy based on energy may also contribute to the selection, possibly depending on a threshold in energy reserves below which energy may become a limiting factor for migration.

REFERENCES

Bardonnet, A., & Riera, P. (2005). Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: New insights from stable isotope analysis. *Estuarine Coastal and Shelf Science*, *63*, 201-209. https://doi.org/10.1016/j.ecss.2004.11.009

Boivin, B., Castonguay, M., Audet, C., Pavey, S. A., Dionne, M., & Bernatchez, L. (2015). How does salinity influence habitat selection and growth in juvenile American eels *Anguilla rostrata*? *Journal of Fish Biology*, *86*, 765-784. https://doi.org/10.1111/jfb.12604

Bolliet, V., & Labonne, J. (2008). Individual patterns of rhythmic swimming activity in *Anguilla anguilla* glass eels synchronised to water current reversal. *Journal of Experimental Marine Biology and Ecology*, *362*, 125-130. https://doi.org/10.1016/j.jembe.2008.06.017

Bolliet, V., Claveau, J., Jarry, M., Gonzalez, P., Baudrimont, M., & Monperrus, M. (2017). Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European glass eels (*Anguilla anguilla*). *Physiology & Behavior*, *169*, 33-40. https://doi.org/10.1016/j.physbeh.2016.11.008

Bolliet, V., Lambert, P., Rives, J., & Bardonnet, A. (2007). Rhythmic swimming activity in *Anguilla anguilla* glass eels: Synchronisation to water current reversal under laboratory conditions. *Journal of Experimental Marine Biology and Ecology*, *344*, 54-66. https://doi.org/10.1016/j.jembe.2006.12.027

Bureau Du Colombier, S., Bolliet, V., Lambert, P., & Bardonnet, A. (2007). Energy and migratory behavior in glass eels (*Anguilla anguilla*). *Physiology & Behavior*, *92*, 684-690. https://doi.org/10.1016/j.physbeh.2007.05.013

Charlon, N., & Blanc, J. M. (1983). Etude des civelles d'*Anguilla anguilla* L. dans la région du bassin de l'Adour. 2. Régime alimentaire et variations de quelques caracté ristiques biochimiques en cours de migration. *Archiv fur Hydrobiologie*, *98*, 240-249.

Claveau, J., Monperrus, M., Jarry, M., Baudrimont, M., Gonzalez, P., Cavalheiro, J., … Bolliet, V. (2015). Methylmercury effects on migratory behaviour in glass eels (*Anguilla anguilla*): An experimental study using isotopic tracers. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, *171*, 15-27. https://doi.org/10.1016/j.cbpc.2015.03.003

Cresci, A., Durif, C. M., Paris, C. B., Thompson, C. R. S., Shema, S., Skiftesvik, A. B., & Browman, H. I. (2019). The relationship between the moon cycle and the orientation of glass eels (*Anguilla anguilla*) at sea. *Royal Society Open Science*, *6*, 190812. https://doi.org/10.1098/rsos.190812

Creutzberg, F. (1961). On the orientation of migrating elvers (*Anguilla vulgaris* Turt.) in a tidal area. *Netherlands Journal of Sea Research*, *1*, 257-338. https://doi.org/10.1016/0077-7579(61)90007-2

Dash, S., Aydin, Y., & Moroz, K. (2019). Chaperone-mediated autophagy in the liver: Good or bad? *Cells*, *8*, 1308. https://doi.org/10.3390/cells8111308

Daverat, F., Limburg, K. E., Thibault, I., Shiao, J. C., Dodson, J. J., Caron, F. O., … Wickstrom, H. (2006). Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A. japonica* and *A. rostrata*. *Marine Ecology Progress Series*, *308*, 231-241. https://doi.org/10.3354/meps308231

Edeline, E. (2007). Adaptive phenotypic plasticity of eel diadromy. *Marine Ecology Progress Series*, *341*, 229-232. https://doi.org/10.3354/meps341229

Edeline, E., Dufour, S., & Elie, P. (2005). Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. *Marine Ecology Progress Series*, *304*, 191-199. https://doi.org/10.3354/meps304191

Falcón, J. (1999). Cellular circadian clocks in the pineal. *Progress in Neurobiology*, *58*, 121-162. https://doi.org/10.1016/s0301-0082(98)00078-1

Falcón, J., Besseau, L., Sauzet, S., & Boeuf, G. (2007). Melatonin effects on the hypothalamo-pituitary axis in fish. *Trends in Endocrinology and Metabolism*, *18*, 81-88. https://doi.org/10.1016/j.tem.2007.01.002

Forward, R. B., & Tankersley, R. A. (2001). Selective tidal-stream transport of marine animals. *Oceanography and Marine Biology*, *39*, 305-353.

Gagnaire, P. A., Normandeau, E., Cote, C., Hansen, M. M., & Bernatchez, L. (2012). The genetic consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*). *Genetics*, *190*, 725-U703. https://doi.org/10.1534/genetics.111.134825

Gaillard, M., Bernatchez, L., Tremblay, R., & Audet, C. (2015). Regional variation in energy storage strategies in American glass eels from Eastern Canada. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, *188*, 87-95. https://doi.org/10.1016/j.cbpa.2015.06.019

Gaillard, M., Pavey, S. A., Cote, C. L., Tremblay, R., Bernatchez, L., & Audet, C. (2016). Regional variation of gene regulation associated with storage lipid metabolism in American glass eels (*Anguilla rostrata*). *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, *196*, 30-37. https://doi.org/10.1016/j.cbpa.2016.02.013

Gascuel, D. (1986). Flow-carried and active swimming migration of the glass eel (*Anguilla anguilla*) in the tidal area of a small estuary on the French Atlantic coast. *Helgolander Meeresuntersuchungen*, *40*, 321-326. https://doi.org/10.1007/bf01983739

Geffroy, B., & Bardonnet, A. (2016). Sex differentiation and sex determination in eels: Consequences for management. *Fish and Fisheries*, *17*, 375-398. https://doi.org/10.1111/faf.12113

Hernández-Pérez, J., Naderi, F., Chivite, M., Soengas, J. L., Miguez, J. M., & López-Patiño, M. A. (2019). Influence of stress on liver circadian physiology. A study in rainbow trout, *Oncorhynchus mykiss*, as fish model. *Frontiers in Physiology*, *10*, 611. https://doi.org/10.3389/fphys.2019.00611

ICES (2018). European eel (*Anguilla anguilla*) throughout its natural range. https://doi.org/10.17895/ices.pub.4601

Imbert, H., Arrowsmith, R., Dufour, S., & Elie, P. (2008). Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage-dependent mechanisms in European eel upstream migration. *Hormones and Behavior*, *53*, 69-81. https://doi.org/10.1016/j.yhbeh.2007.06.011

Jacoby, D., & Gollock, M. (2014). *Anguilla anguilla*. The IUCN Red List of Threatened Species. https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T60344A45833138.en

Jellyman, D. J. (1979). Upstream migration of glass-eels (*Anguilla* spp) in the Waikato River. *New Zealand Journal of Marine and Freshwater Research*, *13*, 13-22. https://doi.org/10.1080/00288330.1979.9515776

Kagedal, K., Johansson, U., & Ollinger, K. (2001). The lysosomal protease cathepsin D mediates apoptosis induced by oxidative stress. *Faseb Journal*, *15*, 1592-+. https://doi.org/10.1096/fj.00-0708fje

Kiffin, R., Christian, C., Knecht, E., & Cuervo, A. M. (2004). Activation of chaperone-mediated autophagy during oxidative stress. *Molecular Biology of the Cell*, *15*, 4829-4840. https://doi.org/10.1091/mbc.E04-06-0477

Liu, H. T., Labonne, J., Coste, P., Huchet, E., Plagnes-Juan, E., Rives, J., … Bolliet, V. (2019). Looking at the complex relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (*Anguilla anguilla*). *Science of the Total Environment*, *696*, 134039. https://doi.org/10.1016/j.scitotenv.2019.134039

Liu, H. T., Lamarins, A., Labonne, J., Monperrus, M., Coste, P., Huchet, E., … Bolliet, V. (2020). New insights into methylmercury induced behavioral and energy-related gene transcriptional responses in

European glass eel (*Anguilla anguilla*). *Chemosphere*, *255*, 127020. https://doi.org/10.1016/j.chemosphere.2020.127020

López-Patiño, M. A., Gesto, M., Conde-Sieira, M., Soengas, J. L., & Miguez, J. M. (2014). Stress inhibition of melatonin synthesis in the pineal organ of rainbow trout (*Oncorhynchus mykiss*) is mediated by cortisol. *Journal of Experimental Biology*, *217*, 1407-1416. https://doi.org/10.1242/jeb.087916

Marandel, L., Lepais, O., Arbenoits, E., Veron, V., Dias, K., Zion, M., & Panserat, S. (2016). Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (*Oncorhynchus mykiss*) by nutritional status and dietary carbohydrates. *Scientific Reports*, *6*, 32187. https://doi.org/10.1038/srep32187

McCleave, J. D., & Kleckner, R. C. (1982). Selective tidal stream transport in the estuarine migration of glass eels of the American eel (*Anguilla rostrata*). *Journal Du Conseil*, *40*, 262-271.

Naderi, F., Hernández-Pérez, J., Chivite, M., Soengas, J. L., Miguez, J. M., & López-Patiño, M. A. (2018). Involvement of cortisol and sirtuin1 during the response to stress of hypothalamic circadian system and food intake-related peptides in rainbow trout, *Oncorhynchus mykiss*. *Chronobiology International*, *35*, 1122-1141. https://doi.org/10.1080/07420528.2018.1461110

Pavey, S. A., Gaudin, J., Normandeau, E., Dionne, M., Castonguay, M., Audet, C., & Bernatchez, L. (2015). RAD sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic American eel. *Current Biology*, *25*, 1666-1671. https://doi.org/10.1016/j.cub.2015.04.062

Pfaffl, M. W., Horgan, G. W., & Dempfle, L. (2002). Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research*, *30*, e36. https://doi.org/10.1093/nar/30.9.e36

Prokkola, J. M., & Nikinmaa, M. (2018). Circadian rhythms and environmental disturbances - underexplored interactions. *Journal of Experimental Biology*, *221*, jeb179267. https://doi.org/10.1242/jeb.179267

Riahi, H., Brekelmans, C., Foriel, S., Merkling, S. H., Lyons, T. A., Itskov, P. M., … Schenck, A. (2019). The histone methyltransferase G9a regulates tolerance to oxidative stress-induced energy consumption. *Plos Biology*, *17*, e2006146. https://doi.org/10.1371/journal.pbio.2006146

Sánchez-Vázquez, F. J., Lopez-Olmeda, J. F., Vera, L. M., Migaud, H., López-Patiño, M. A., & Miguez, J. M. (2019). Environmental cycles, melatonin, and circadian control of stress response in fish. *Frontiers in Endocrinology*, *10*, 279. https://doi.org/10.3389/fendo.2019.00279

Steindal, I. A. F., & Whitmore, D. (2019). Circadian clocks in fish-What have we learned so far? *Biology*, *8*, 17. https://doi.org/10.3390/biology8010017

Tahara, Y., Shiraishi, T., Kikuchi, Y., Haraguchi, A., Kuriki, D., Sasaki, H., … Shibata, S. (2015). Entrainment of the mouse circadian clock by sub-acute physical and psychological stress. *Scientific Reports*, *5*, 11417. https://doi.org/10.1038/srep11417

Tesch, F. W. (2003). The eel (fifth ed.). Wiley, New York.

Tsukamoto, K., Aoyama, J., & Miller, M. J. (2002). Migration, speciation, and the evolution of diadromy in anguillid eels. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*, 1989-1998. https://doi.org/10.1139/f02-165

Tsukamoto, K., Nakai, I., & Tesch, W. V. (1998). Do all freshwater eels migrate? *Nature*, *396*, 635-636. https://doi.org/10.1038/25264

Tzeng, W. N., Wang, C. H., Wickstrom, H., & Reizenstein, M. (2000). Occurrence of the semi-catadromous European eel *Anguilla anguilla* in the Baltic Sea. *Marine Biology*, *137*, 93-98. https://doi.org/10.1007/s002270000330

Vatine, G., Vallone, D., Gothilf, Y., & Foulkes, N. S. (2011). It's time to swim! Zebrafish and the circadian clock. *Febs Letters*, *585*, 1485-1494. https://doi.org/10.1016/j.febslet.2011.04.007

Warman, C. G., & Naylor, E. (1995). Evidence for multiple, cue-specific circatidal clocks in the shore crab *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology*, *189*, 93-101. https://doi.org/10.1016/0022-0981(95)00014-i

Wippelhauser, G. S., & McCleave, J. D. (1987). Precision of behavior of migrating juvenile American eels (*Anguilla rostrata*) utilizing selective tidal stream transport. *Journal Du Conseil*, *44*, 80-89.

Wippelhauser, G. S., & McCleave, J. D. (1988). Rhythmic activity of migrating juvenile American eels *Anguilla rostrata*. *Journal of the Marine Biological Association of the United Kingdom*, *68*, 81-91. https://doi.org/10.1017/s0025315400050116

Zhdanova, I. V., & Reebs, S. G. (2006). Circadian rhythms in fish. In K. A. Sloman, R. W. Wilson & S. Balshine (Eds.), *Behaviour and Physiology of Fish*, (pp. 197-238). https://doi.org/10.1016/S1546-5098(05)24006-2

Supplementary data

Rethinking the role of selective pressure upon estuarine migration behavior in European glass eel (*Anguilla anguilla*)

Number of pages: 11; Number of Text: 1; Number of Tables: 3; Number of Figures: 3

Text S1. Modeling analysis of swimming behavior

We assumed that the swimming activity of an individual i at time t $AC(t,i)$ followed a Bernoulli distribution of probability $P(t,i)$ such as:

$$AC(t,i) \sim dbern\big(P(t,i)\big)$$

We assumed that $P(t,i)$ was a periodic function of time, since it has been previously shown that glass eels display rhythmic swimming activity in response to current reversal (Bolliet & Labonne, 2008):

$$logit\big(P(t,i)\big) = a(i) \times \sin\big(t \times b(i) + c(i)\big) + d(i)$$

where $a(i)$ was the strength of the synchronized component of activity, $b(i)$ was related to period, $c(i)$ was related to the trigonometric function phase, $d(i)$ was the non-synchronized component of activity. Fish having a P value above the mean of P meanwhile having an activity periodicity close to 12.4 h were considered synchronized, others were considered non-synchronized.

A Markov-chain Monte-Carlo (MCMC) sampling approach with Gibbs algorithm in the Bayesian framework (Spiegelhalter et al., 2000, Openbugs software, Version 3.2.3) was used to estimate parameters a, b, c, d. Convergence of estimates was reached during a first set of 10 000 iterations. Another consecutive set of 5000 iterations was run to approximate the posterior distribution of parameter estimates.

```
##################
### MODEL ######
################

#
model {
#likelihood
#building synchronized swimming activity model
        for( t in 1 : T ) {
            for (i in 1:N) {
               AC[t,i] ~ dbern(P[t,i])
               logit(P[t,i])<-a[i] *sin(t*b[i]+c[i]) +d[i]
                           }
                }
            for (i in 1:N) {
                 per[i]<-(2*3.1415927/b[i])*(2/3)
                 mP[i]<-mean(P[,i])
                 sdP[i]<-sd(P[,i])
                }
```

Table S1. Proportion of variance of PCA first axis.

Genomic function	Sub-function	PC1 variances%
Cytosol catabolism	Lipid and protein catabolism	27.2
Mitochondria-related functions	Mitochondrial biogenesis	38.7
	Mitochondrial fusion	51.8
	Mitochondrial metabolism	46.6
Autophagy	Macroautophagy	46.7
	Lysosomal catabolism	43.2
	CMA	66.2
	Mitophagy	55.7
Antioxidant system	Antioxidant system	45.3

Table S2. List of genes involved in each metabolism-related function; effects of synchronization behavior and sampling site on the expression level of each gene are assessed using two-way Anova analyses in autumn and spring, *p* values indicating the effects of synchronization behavior and sampling site and the interaction of both are listed on the table. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

			Autumn			Spring		
Genomic function	Sub-function	Gene ID	*p* (Site)	*p* (Behavior)	*p* (Int)	*p* (Site)	*p* (Behavior)	*p* (Int)
Cytosol catabolism		*hsl-a*	0.334	0.524	0.639	0.659	0.179	0.298
		hsl-b	0.773	0.721	0.138	0.060	0.222	0.501
		mgl	0.409	0.510	0.728	0.008**	0.869	0.777
		pnpla2a	0.552	0.393	0.045*	0.336	0.511	0.651
		pnpla2b	0.988	0.204	0.256	0.283	0.206	0.723
		pnpla2c	0.636	0.004**	0.209	0.002**	0.478	0.223
		fbxo32	0.119	0.385	0.035*	0.262	0.807	0.029*
		murf1	0.513	0.194	0.178	0.002**	0.205	0.219
Mitochondria-related functions	Mitochondrial biogenesis	*pgc1α1*	0.242	0.836	0.980	0.008**	0.096	0.717
		pgc1α2	0.263	0.263	0.320	0.007**	0.581	0.053
		tfam	0.301	0.350	0.222	0.010*	0.686	0.854
		hsp60	0.674	0.670	0.074	0.348	0.830	0.577
	Mitochondrial fusion	*mfn1*	0.921	0.586	0.708	0.198	0.303	0.466
		mfn2	0.226	0.691	0.905	0.411	0.131	0.625
		opa1	0.318	0.030*	0.246	0.143	0.273	0.118
	Mitochondrial fission	*drp1*	0.442	0.145	0.423	0.040*	0.534	0.125
	Mitochondrial metabolism	*mt-ATP6*	0.414	0.101	0.642	0.417	0.910	0.983
		mt-nd5	0.735	0.242	0.827	0.241	0.498	0.977
		cox1	0.088	0.016*	0.703	0.003**	0.664	0.979
		12s rRNA	0.502	0.404	0.348	0.915	0.784	0.919
		cpt1α1	0.058	0.722	0.871	< 0.001***	0.359	0.566
		cpt1α2a	0.002**	0.041*	0.673	0.145	0.049*	0.245
		cpt1α2b	0.297	0.940	0.145	0.898	0.670	0.767
		cpt1β	0.038*	0.074	0.385	< 0.001***	0.431	0.530
		hadh	0.106	0.844	0.692	0.715	0.511	0.166
		glud1	0.794	0.885	0.878	0.174	0.788	0.992
		got1	0.731	0.443	0.497	0.028*	0.629	0.690
		got2	0.293	0.889	0.903	0.574	0.664	0.502
		gpt2a	0.775	0.944	0.458	0.338	0.604	0.376
		gpt2b	0.992	0.872	0.692	0.875	0.634	0.792
Autophagy	Macroautophagy	*atg5*	0.065	0.574	0.779	0.291	0.139	0.365
		atg7	0.177	0.588	0.266	0.476	0.909	0.799
		atg12	0.166	0.414	0.343	0.538	0.811	0.908
		lc3	0.511	0.598	0.589	0.101	0.844	0.726
		ulk1	0.971	0.917	0.260	0.096	0.683	0.535
	Lysosomal catabolism	*catha*	0.460	0.672	0.910	< 0.001***	0.193	0.802
		cathd	0.783	0.304	0.668	0.510	0.274	0.391
		cathf	0.251	0.331	0.062	0.832	0.261	0.413
		cathl	0.035*	0.363	0.845	0.025*	0.645	0.774
		lipa	0.565	0.999	0.107	0.031*	0.776	0.683
		tfeb	0.974	0.735	0.728	0.014*	0.754	0.424
	CMA	*lamp2a*	0.271	0.818	0.863	0.009**	0.583	0.244
		phlpp1	0.181	0.199	0.342	0.177	0.324	0.485
		hsp90	0.865	0.031*	0.606	0.525	0.126	0.062
		hsc70a	0.256	0.370	0.658	0.042*	0.533	0.040*
		hsc70b	0.374	0.971	0.472	0.001**	0.289	0.971
	Mitophagy	*fundc1*	0.780	0.654	0.045*	0.061	0.296	0.905
		pink1	0.313	0.049*	0.668	0.231	0.095	0.145
		parkin	0.035*	0.405	0.707	0.008**	0.271	0.944
		bnip3a	0.701	0.227	0.296	0.002**	< 0.001***	0.844
		bnip3b	0.694	0.015*	0.237	0.101	0.525	0.268
Antioxidant system		*catalase*	0.497	0.220	0.498	0.574	0.195	0.903
		sod1	0.172	0.390	0.132	0.076	0.879	0.850
		sod2	0.764	0.415	0.589	0.859	0.825	0.451
		mtl	0.012*	0.241	0.058	0.012*	0.723	0.105
		gstp	0.065	0.226	0.174	0.859	0.981	0.235
		gpx1	0.424	0.036*	0.965	0.111	0.128	0.245
		gsr	0.038*	0.300	0.454	0.648	0.929	0.963
		gfap	0.986	0.759	0.659	0.523	0.154	0.169

Table S3. Analysis of deviance table for GLM fitted to (A) Swimming activity data, and (B) Swimming rheotaxis data, with site (Marine and Estuary) as a categorical factor. One GLM was fitted for each season (Autumn and Spring).

A

	Source	d.f.	Deviance	Resid. D.f.	Resid. Dev	*P* (> Chi)
Autumn data	Null model			106	6967.3	
	Site effect	1	85.2	105	6882.1	< 0.001***
Spring data	Null model			105	3375.1	
	Site effect	1	437.2	104	2938.0	< 0.001***

B

	Source	d.f.	Deviance	Resid. D.f.	Resid. Dev	*P* (> Chi)
Autumn data	Null model			57	923.5	
	Site effect	1	14.8	56	908.7	< 0.001***
Spring data	Null model			23	674.8	
	Site effect	1	257.4	22	417.4	< 0.001***

Note: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure S1 Sampling stations of glass eels.

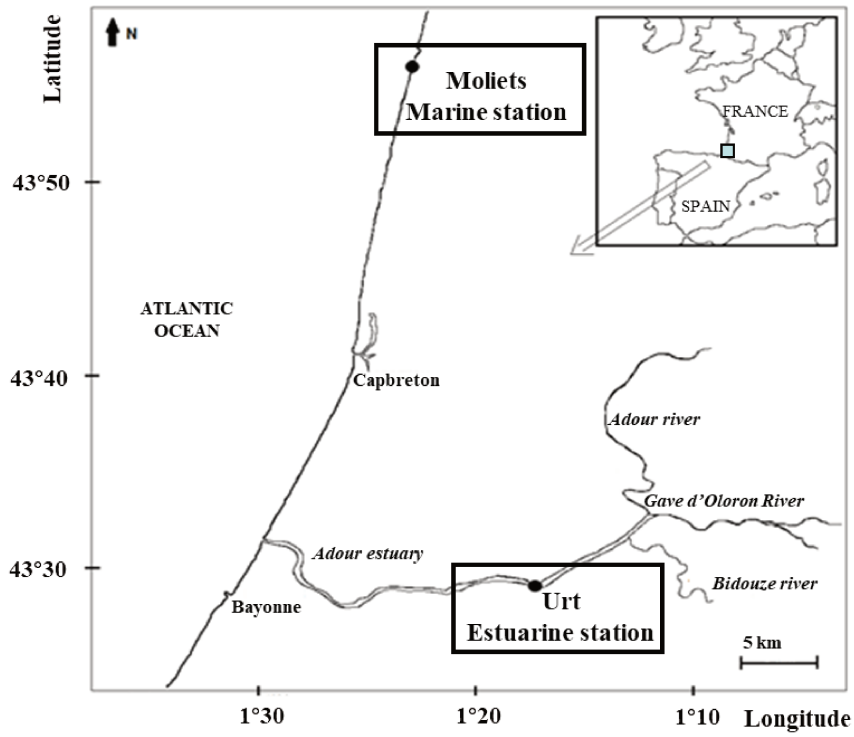
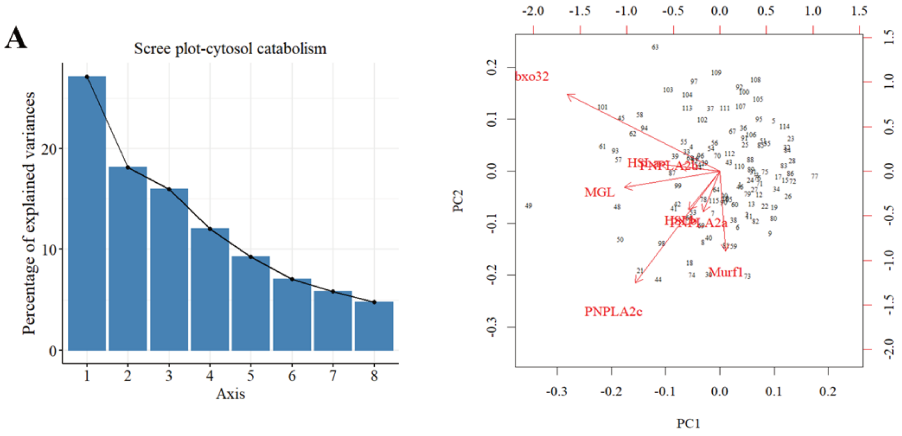
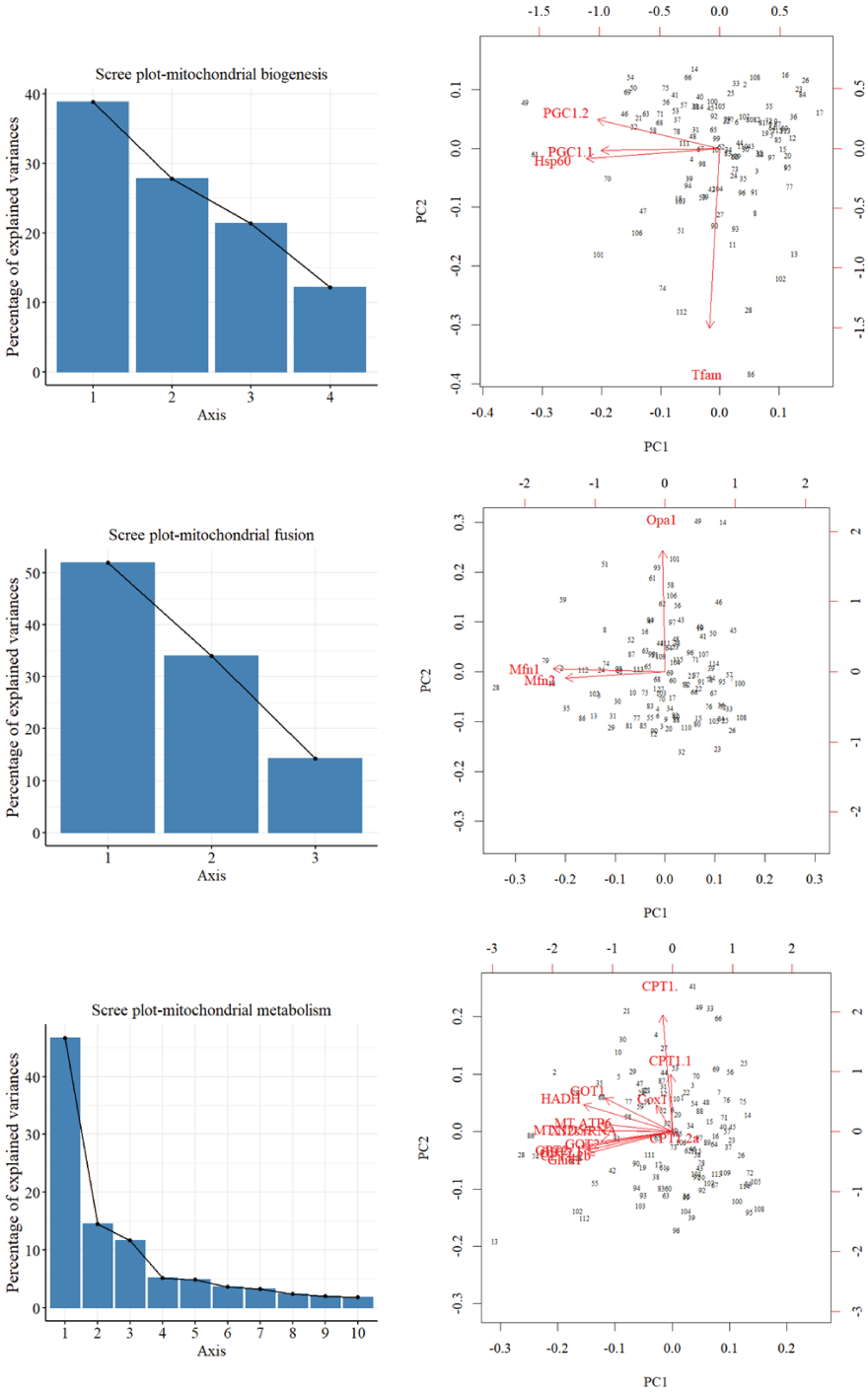


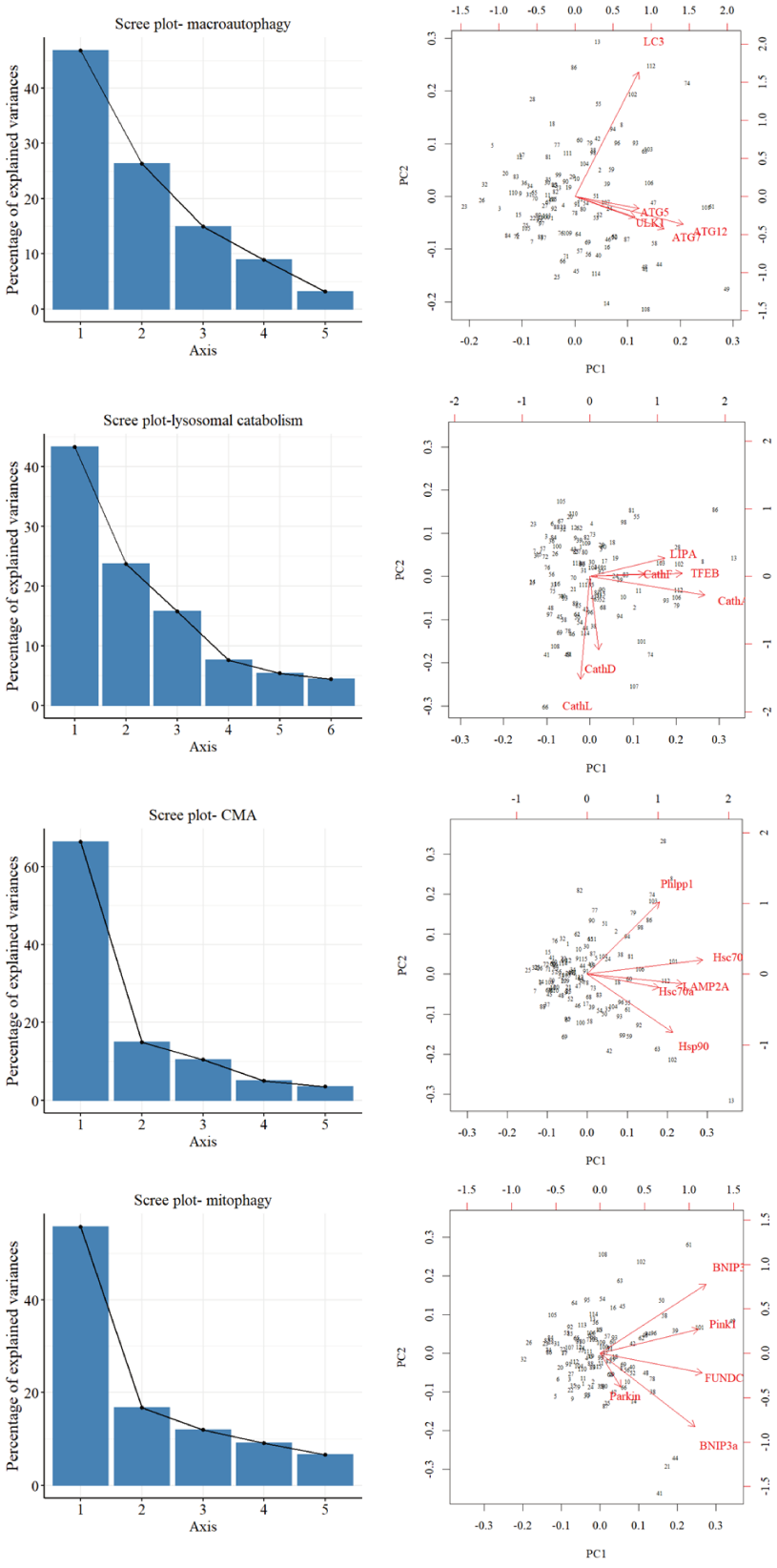
Figure S2. Principal Component Analysis (PCA) on the expression levels of genes involved in each energy-related genomic function: (A) Cytosol catabolism; (B) Mitochondria-related functions; (C) Autophagy; (D) Antioxidant system.



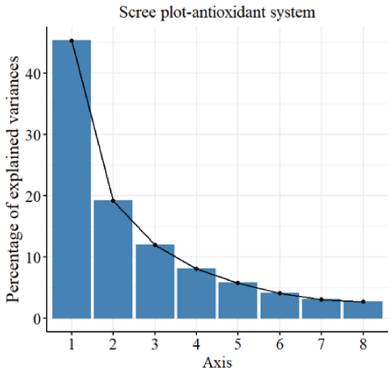
B


Scree plot-mitochondrial biogenesis
Percentage of explained variances
Axis
PGC1.2
PGC1.1
Hsp60
Tfam
PC1
PC2
Scree plot-mitochondrial fusion
Percentage of explained variances
Axis
Opa1
Mfn1
Mfn2
PC1
PC2
Scree plot-mitochondrial metabolism
Percentage of explained variances
Axis
CPT1.
CPT1.1
HADH
GOT1
COX1
MT.ATP6
PC1
PC2

C


Scree plot- macroautophagy
Percentage of explained variances
Axis
LC3
ATG5
ULK1
ATG7
ATG12
PC1
PC2
Scree plot-lysosomal catabolism
Percentage of explained variances
Axis
LIPA
CathF
TFEB
CathD
CathL
PC1
PC2
Scree plot- CMA
Percentage of explained variances
Axis
Pllpp1
Hsc70
Hsc70a
LAMP2A
Hsp90
PC1
PC2
Scree plot- mitophagy
Percentage of explained variances
Axis
BNIP3
Pink1
FUNDC
Parkin
BNIP3a
PC1
PC2

D


Scree plot-antioxidant system
Percentage of explained variances
Axis
0
10
20
30
40
1
2
3
4
5
6
7
8

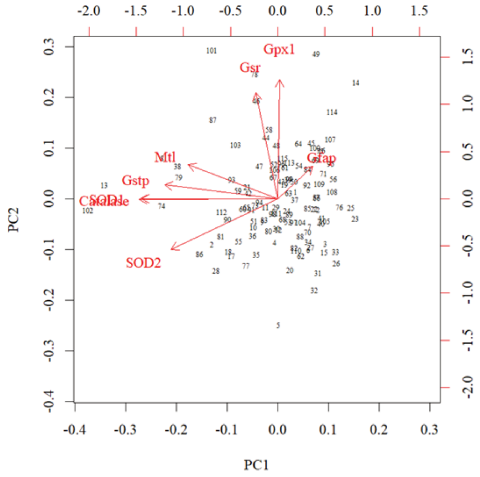
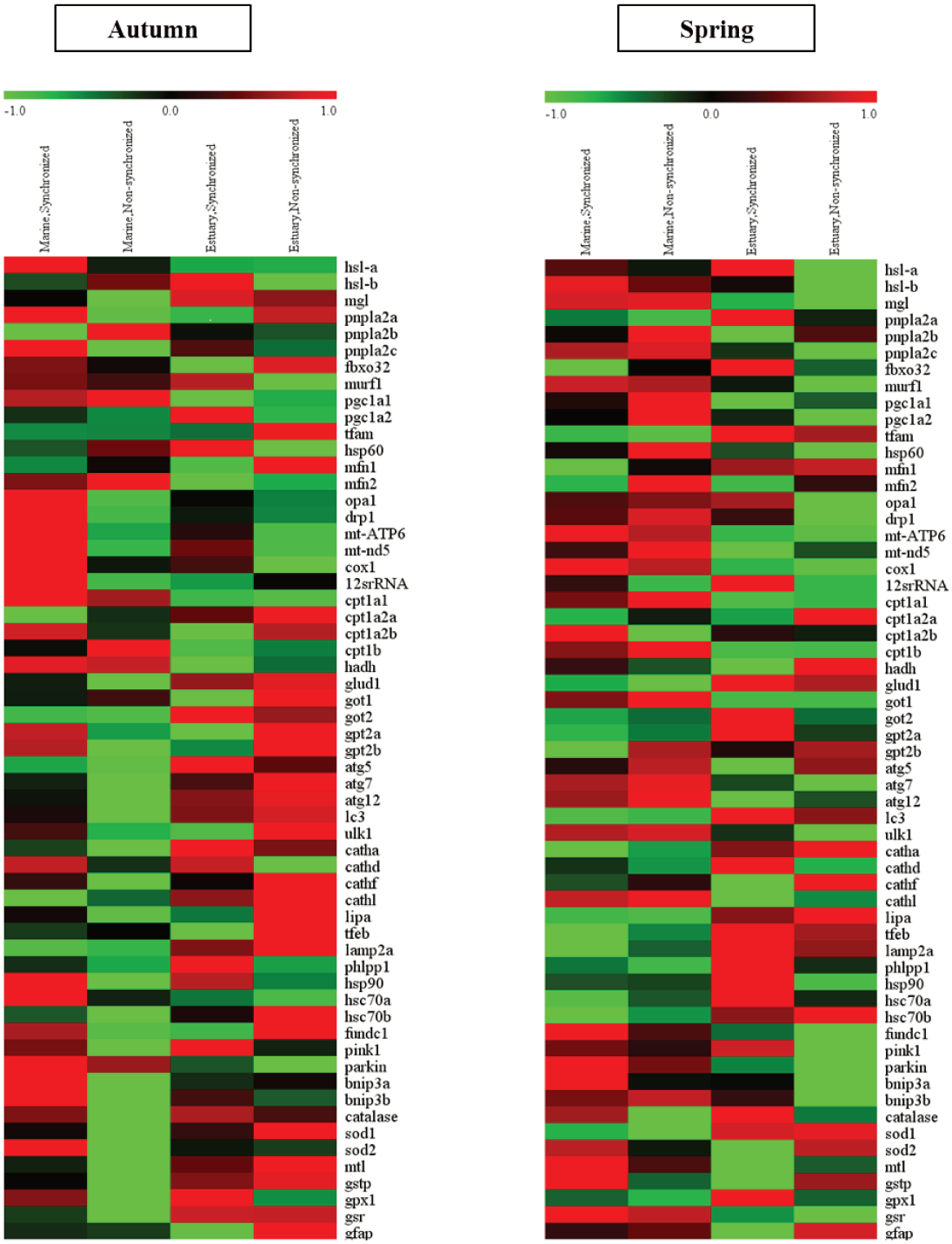

Gpx1
Gsr
Mtl
Gstp
SOD2
PC1
PC2
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

Figure S3. Heatmaps of mean gene expressions, presented in columns of sampling site (Marine and Estuary) and migration behavior (Synchronized and Non-synchronized) in autumn and spring.



Publication 3

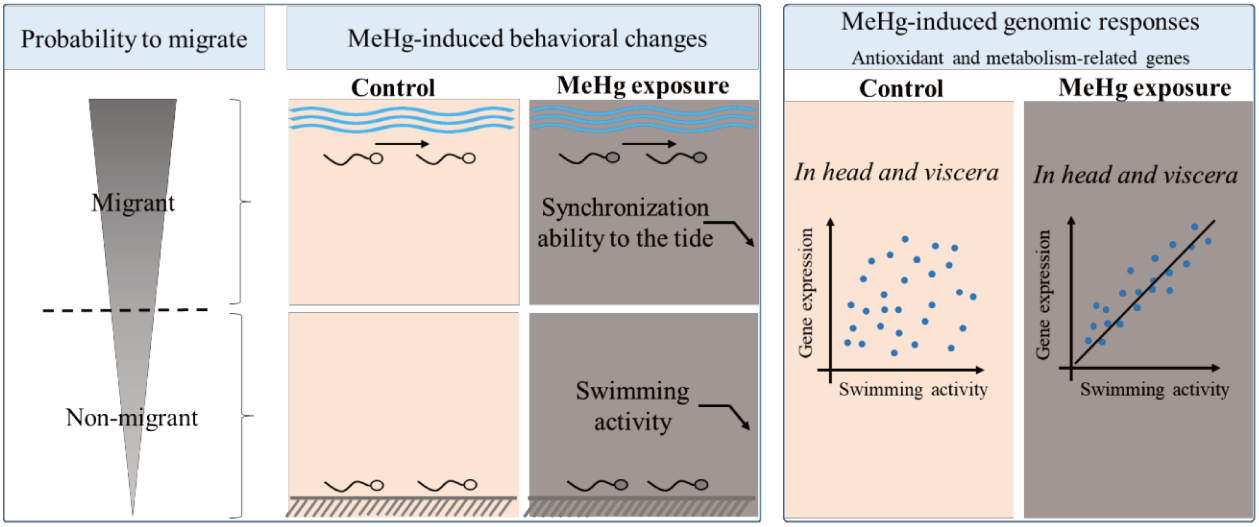


Figure 2-3. Graphical abstract and highlights from Publication 3- Methylmercury induced behavioral and energy related gene transcriptional responses in European glass eel (*Anguilla anguilla*).

Highlights

1. MeHg decreased the swimming activity level in glass eels.

2. The effects of MeHg were stronger in individuals that did not synchronize to the tide.

3. MeHg affected the relation between metabolism-related genes and swimming activity.

4. MeHg mainly targeted the head and viscera in glass eels.

2.3 Effect of Methymercury on marine glass eels migration behavior and their energetic status

Presentation of Publication 3

New insights into methylmercury induced behavioral and energy related gene transcriptional responses in European glass eel (*Anguilla anguilla*)

Objective and methods

In estuarine ecosystem, a remarkable stress factor is chemical pollutants, one of which MeHg is largely emphasized as its severe cytotoxicity. In this chapter, we aimed to assess the effects of MeHg exposure on glass eel's migratory behavior and energy metabolism. To investigate this question, we first carried out a test of the kinetics of MeHg (100 ng L^{-1}) accumulation in glass eel, and results showed an increase of MeHg accumulation in the first 7 days after exposure, followed by a plateau until 30 days. Accordingly, we then exposed another group of glass eels to MeHg (100 ng L^{-1}) for 7 days, followed by a 10-day swimming test as described in the precedent chapters. The propensity to migrate was then related to metabolism and energy-related metabolism. However, because of the recurrent lack of molecular difference between synchronized and non-synchronized fish, gene expression levels were analyzed separately in the head, the viscera and the muscles.

Results and Conclusions

MeHg exposure induced a decrease in the number of synchronized glass eels. The swimming activity level also decreased in exposed glass eels, non-synchronized individuals being more affected than the synchronized ones. This result support the conclusion of the precedent chapters concerning a possible higher sensitivity of non-synchronized individuals to stress. MeHg exposure triggered no change in the expression level of genes related to energy metabolism and antioxidant, regardless of the tissues. However, when swimming activity was individually correlated to transcriptional profiles of metabolism and antioxidant related genes, a significant positive correlation was presented in contaminated glass eels in head and viscera but not in the muscle. Indeed, the expression of genes involved in the antioxidant system, mitochondrial respiratory chains and catabolism and macroautophagy presented in the head a significant positive correlation to swimming activity after MeHg exposure but not in the control group. In the viscera, except for mitochondrial catabolism, all others functions in addition to mitophagy displayed a positive correlation to swimming activity in the contaminated group but not in the control. These results suggest that contaminated glass eels needed to increase energy metabolism more than non-contaminated fish to cope with increasing swimming activity. Regardless of MeHg treatment, higher expression levels of gene involved in antioxidant and energy metabolism in synchronized glass eels than their non-synchronized

conspecifics were observed in head. It is the first time we observed difference between the two behavioral phenotypes on molecular level, which might be masked by whole body analyses carried out previously (Figure 2-3).

In conclusion, our results suggest that MeHg could reduce the migratory propensity in glass eels and particularly affect non-synchronized individuals, possibly more vulnerable to stress factors. Results also support the interest of focusing on head to investigate facultative migration behavior and the effect of environmental stressors on this rhythmic behavior.

Chemosphere 255 (2020) 127020

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

New insights into methylmercury induced behavioral and energy-related gene transcriptional responses in European glass eel (*Anguilla anguilla*)

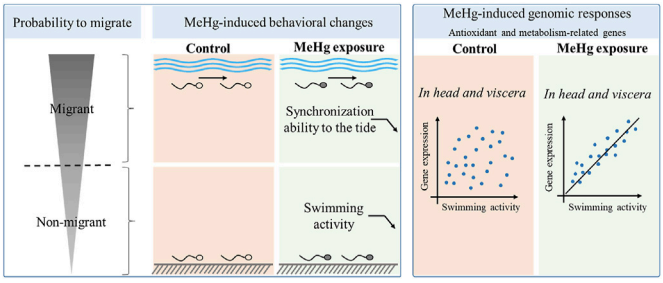
Hengtong Liu [a, b], Amaia Lamarins [a], Jacques Labonne [a], Mathilde Monperrus [c], Pascale Coste [a], Emmanuel Huchet [a], Jacques Rives [a], Iban Seiliez [b, 1], Valérie Bolliet [a, 1, *]

[a] Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, ECOBIOP, Aquapôle INRAE, MIRA, F64310, Saint-Pée-sur-Nivelle, France
[b] INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, NuMéA, F64310, Saint-Pée-sur-Nivelle, France
[c] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux – MIRA, UMR 5254, 64600, Anglet, France

HIGHLIGHTS

- MeHg decreased the swimming activity level in glass eels.
- The effects of MeHg were stronger in individuals that did not synchronize to the tide.
- MeHg affected the relation between metabolism-related genes and swimming activity.
- MeHg mainly targeted the head and viscera in glass eels.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 February 2020
Received in revised form
5 May 2020
Accepted 7 May 2020
Available online 10 May 2020

Handling Editor: Jim Lazorchak

Keywords:
Glass eel
Anguilla anguilla
Migration behavior
Methylmercury
Metabolism

ABSTRACT

The effect of methylmercury (MeHg) was investigated in glass eel migration behavior and metabolism. To migrate up estuary, glass eels synchronize their swimming activity to the flood tide and remain on or in the substratum during ebb tide. Following seven days of exposure to MeHg (100 ng L^{-1}), glass eels migration behavior was expressed by their swimming synchronization to the water current reversal every 6.2 h (mimicking the alternation of flood and ebb tides) and their swimming activity level. In relation to their behavior, we then analyzed the energy-related gene expression levels in individual head, viscera and muscle. Results showed that MeHg decreased the number of glass eels synchronized to the change in water current direction and their swimming activity level. This last effect was more pronounced in non-synchronized fish than in synchronized ones, supporting the idea that non-synchronized glass eels could be more vulnerable to stress. As regard the expression of energy-related genes, no significant difference was observed between control and MeHg-exposed fish. In contrast, when the swimming activity levels were plotted against transcriptional responses, positive correlations were evidenced in viscera and especially in the head of exposed glass eels but not in control. Finally, it is noteworthy that non-synchronized glass eels displayed lower expression level of metabolism genes than their synchronized counterpart, but only in the head. Altogether, these results support the interest of

* Corresponding author. UMR 1224 Ecobiop, Aquapôle, 64310, Saint Pée sur Nivelle, France.
E-mail address: valerie.bolliet@inrae.fr (V. Bolliet).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.chemosphere.2020.127020

focusing on the head to investigate the facultative migration behavior in glass eels and the effect of environmental stressors on this rhythmic behavior.

1. Introduction

The status of European eel (*Anguilla anguilla*) remains critical since the recruitment of glass eels strongly declined from 1980 to about 2010, and have remained at a low level since, which has urged Euro-wide European eel regulation (council regulation (EC) no. 1100/2007) for the protection and recovery of the stock (ICES, 2018). The European eel is a facultative catadromous species that crosses the Atlantic Ocean twice during its life. The present knowledge clearly indicate that the species reproduces in the Sargasso Sea, where the leptocephalus larval stage is first observed (Schmidt, 1923; van Ginneken and Maes, 2005). The larvae move with ocean currents more than 5000 km toward the European continent, after which they transform into a juvenile stage, the glass eel. Then, glass eels migrate up estuary to reach freshwater habitats where they grow and develop into yellow eels. At an age of 5–20 years they commence the silvering process and become silver eels at which point they begin their migration back to the Sargasso Sea to reproduce and probably die (Miller et al., 2015; Righton et al., 2016; Schmidt, 1923; Tesch, 1980, 2003).

Glass eels migrate up estuaries to ascend rivers using selective flood transport: during flood tide, glass eels move up in the water column and migrate with the current while they go down and remain on or in the substratum during ebb tide (Forward and Tankersley, 2001; Gascuel, 1986; Jellyman, 1979). They are also synchronized to the photoperiod, avoiding light and swimming mainly during the night when the water is clear. However, a high degree of geographical dispersion crossing marine and riverine water has been documented regarding to different migratory patterns of settlement and river colonization (Daverat et al., 2006; Secor et al., 1995; Tsukamoto and Arai, 2001; Tsukamoto et al., 1998; Tzeng, 1996). These different patterns of migration could have a strong impact on the fate of the population because of the environmental sex determinism in eels (Geffroy and Bardonnet, 2016; Krueger and Oliveira, 1999). Briefly, in European eels, males are generally observed to dominate in high-density environments, often associated with estuarine or lower river reaches, whereas individuals that migrated upstream to the river tend to become mostly females (Adam et al., 2008; Davey and Jellyman, 2005; Harrison et al., 2014; Laffaille et al., 2006; Parsons et al., 1977).

Although there is no consensus on the reason for the diverse migratory patterns, it has been suggested that early settlement in estuary or coast, if taking place, is partly due to low energy condition (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006; Liu et al., 2019). Indeed, most glass eels do not feed throughout estuarine migration and they depend on energy reserves accumulated by the leptocephalus larvae during oceanic migration to reach the river (Kawakami et al., 1999; Tesch, 2003; Bardonnet and Riera, 2005). Moreover, estuary is a highly stressful ecosystem, where a combination of different stressors including large variations in the temperature, hydraulic conditions, salinities, oxygen availability or contaminants may influence the energy metabolic processes of glass eels during migration. Due to its proximity to urbanized and industrial areas, estuary represents a major sink for various contaminants including various forms of mercury (Hg), both inorganic (Hg(II)) and methylated (MeHg), this latest form being recognized to adversely affect fish physiology, growth, health and behavior (Cambier et al., 2009, 2012; Lee et al., 2011; Murphy et al., 2008; Scheulhammer et al., 2007).

Behavioral effects of MeHg exposure in fish have been largely documented, with a lot of concerns on prey capture ability (Weis and Khan, 1990; Zhou et al., 2001), predator avoidance (Webber and Haines, 2003), reproduction (Sandheinrich and Miller, 2006), habitat selection (Sampaio et al., 2016) and more recently on memory and aggressiveness (Strungaru et al., 2018). The underlying molecular mechanisms of MeHg toxicity are predominantly related with oxidative stress, which in turn may induce lipid peroxidation and DNA damages (Berntssen et al., 2003; Gonzalez et al., 2005). In glass eels, MeHg toxicity could increase energy expenditure through the processes of cellular repairment and detoxification which may thereby reduce glass eels fitness and their migratory success through estuary. Claveau et al. (2015) reported that glass eels exposed to MeHg (50 ng L^{-1}) for 11 days exhibited some perturbations of mitochondrial structures and metabolism associated to an activation of antioxidative defence systems. However, the effect of MeHg differed between behavioral phenotypes of glass eels, those displaying a low propensity to migrate up estuaries being more affected than their 'migrant' counterpart. These findings suggested the existence of differences in sensitivity to MeHg exposure among glass eels that remained to be elucidated.

In the present study we aimed to identify the MeHg-induced repercussions in synchronization of glass eels to the water current reversal, their level of swimming activity and the transcriptomic profiles of energy-related genes. This was achieved by transcriptome analyses related to energy metabolism and direct observations of swimming behavior.

2. Materials and methods

2.1. Ethics

Procedures used in this study have been validated by the ethics committee N°073 (ref: 2017012015086652). The experiment was carried out in strict accordance with the EU legal frameworks, specifically those relating to the protection of animals used for scientific purposes (i.e., Directive, 2010/63/EU), and under the French legislation governing the ethical treatment of animals (Decret no. 2013–118, February 1st, 2013).

2.2. Primary test for MeHg accumulation kinetics over 30 days

2.2.1. Fish collection

The glass eels were collected at the mouth of a small estuary (courant d'Huchet) located 40 km north of the mouth of the Adour estuary, France (43° 55′N, 1° 23′W). They were sampled using a dip-net at night and during flood tide in February 2018. Then, they were transferred to the laboratory and maintained in a tank containing water from the fishing site. During the next 48 h, the water was continuously aerated and progressively diluted with fresh water. Fish were kept under 12 °C and a photoperiod of 12 L/12 D with a very low light intensity during the photophase (0.2–0.3 μW/cm^2).

2.2.2. MeHg exposure and kinetic sampling

After acclimation, glass eels were randomly selected and

allocated by groups of three into a series of seven aquariums (1.5 L of aerated fresh water) with initial compartment concentration of 100 ng L^{-1} of MeHg. Glass eels were exposed to this single spike of MeHg prepared as follow: The stock solution of MeHg at 1000 mg L^{-1} was prepared by dissolving MeHg chloride obtained from Strem Chemicals (Newburyport, MA, USA) in methanol. The spiking solution at 100 μg L^{-1} was then prepared by diluting the stock solution in 1% hydrochloric acid.

Three glass eels were collected in the beginning of this test as controls. All the aquariums were sealed with a transparent cover to prevent water volatilization and to guarantee the photoperiod. The aeration, temperature and photoperiod were maintained as described for the acclimation period. During the exposure period, no mortality neither erratic swimming (abrupt change of direction or swimming speed) were observed.

The MeHg-exposed fish were sampled at 1, 2, 3, 7, 11, 18 and 30 days. One aquarium was recovered on each sampling time point and the three fish in this aquarium were considered to be three replicates. Sampled fish were killed by anesthetic and quickly washed with distilled water. After biometry, all the fish were immediately frozen into liquid nitrogen and stored at −80 °C for future mercury speciation analysis.

2.2.3. Mercury speciation analysis

Each glass eel was lyophilized, mashed using an agate mortar and then submitted to microwave extraction according to the procedure previously published by Navarro et al. (2013). The supernatant was spiked with known amounts of standard solution of MeHg and Hg(II) and submitted to propylation. Mercury speciation analysis was performed by GC-ICPMS using the method with parameters, which are detailed by Navarro et al. (2013). Mercury species concentrations were determined by speciated isotope dilution (Monperrus et al., 2005). Analytical performances were evaluated using the Certified Reference Material DOLT- 4 (Dogfish Liver, NRCC, Ottawa, Canada). Good agreement with certified values was obtained with recoveries of 105 ± 8% and 95 ± 7% for MeHg and Hg(II), respectively. Detection limits of 0.1 and 0.2 ng g^{-1} were found for MeHg and Hg(II), respectively. All concentrations were expressed in ng Hg.g^{-1} dry weight.

2.3. Seven-day MeHg exposure

The kinetics experiment ran over 30 days showed that glass eels exposed to MeHg presented an increase in MeHg concentration measured in whole body for up to seven days which then reached a plateau (Fig. 1). The increase in Hg(II) concentration remained low and stabilized very quickly. According to these results, we chose 7 days as exposure duration to investigate the effect of MeHg on migration behavior and energy metabolism.

2.3.1. Fish collection and tagging

140 glass eels were collected in March 2018 at the same fishing site and using the same capture method described above (see 2.2.1). They were transferred to the laboratory and maintained at 12 °C overnight in an aerated tank containing water from the fishing site. In the morning following their capture, all glass eels were anesthetized (Benzocaine, 0.01 mg L^{-1}) and individually tagged using Visible Implant Elastomer (VIE Tag) (combinations of one or two hypodermic spots of different colors as described by Imbert et al., 2008) in order to trace the swimming behavior individually. Once tagged, glass eels were released to wake up in the water from fishing site. During the next 48 h, the water was continuously aerated and progressively diluted with fresh water.

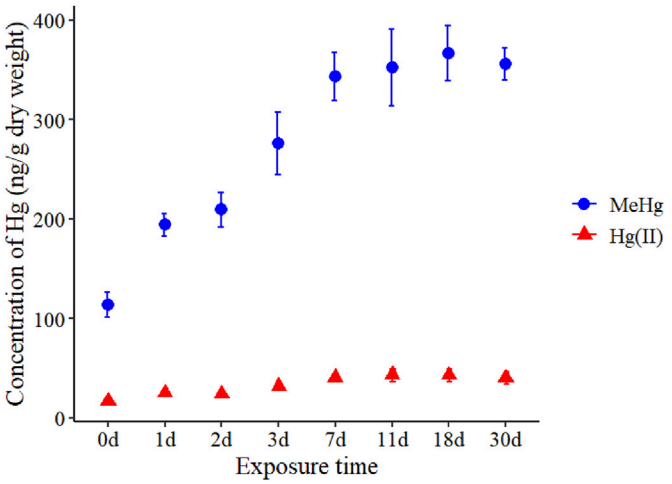


Fig. 1. Kinetics of MeHg (blue circles) and Hg(II) (red triangles) concentrations (mean ± sd) in glass eels along 0–30 days of exposure to MeHg. Data for 0 d are from control fish (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.3.2. MeHg exposure

After acclimation, fish were randomly divided into two groups: control group without Hg addition and MeHg-group exposed to an initial MeHg concentration of 100 ng L^{-1}. Seven glass aquariums (5 L of aerated fresh water) were used for each group, with 10 fish in each aquarium. The exposure lasted for seven days. During the entire experimental period, both control and MeHg-exposed fish were kept under the same conditions as those during the acclimation period. No mortality neither erractic swimming were observed during the experiment.

2.3.3. Observations of the post-exposure swimming behavior

After seven-day exposure, one aquarium of 10 glass eels from the control group and one aquarium of 10 specimens from the MeHg-exposed group were recovered for mercury speciation analysis. Procedures of sample preparation and mercury measurement were the same as primary test (see 2.2.3). All the other control and MeHg-exposed glass eels (n = 6 x 10 glass eels in each group) were transferred into two annular tanks (30 glass eels of both the control and MeHg groups in each tank) installed in two temperature-controlled rooms. The rooms were kept under the same conditions as describes above, except that we added a constant UV light (0.6 μW/cm^2) in order to see the VIE Tag. The water temperature was kept at 12 ± 0.5 °C and continuously recorded by thermistors placed in the tank. The annular tank system was specially designed to mimic tidal rhythm by being equipped with two pumps at its opposite ends (Liu et al., 2019; Supplementary Fig. S1). The two pumps were programmed to alternately work to generate clockwise or counterclockwise water flow every 6.2 h. In each tank, the swimming activity of glass eels was traced continuously during 10 days by a camera programed to record 15 s every 40 min. The UV light allowed the identification of each glass eel during the light and dark phases by its elastomer mark. During the 10 days, a total of 360 sessions of 15 s were obtained.

Glass eels use selective tidal-stream transport to migrate up estuary, wherein individuals synchronize their swimming activity to tidal current, but they also have to sustain the level of swimming activity. Thus, in our experimental conditions, the propensity of glass eels to migrate was firstly evaluated by their capacity to synchronize the swimming activity to the change in water current direction by a period of 12.4 h. Then, their activity levels were

quantitatively analyzed by counting the total number of observations of each elastomer mark in the water column in the 360 sessions of 15 s videos.

2.3.4. Gene expression analysis

2.3.4.1. Sampling procedure. After swimming test, all the glass eels in annular tank were recovered, anaesthetized and then killed by a lethal bath of anaesthesia (Benzocaine, 0.05 mg L^{-1}), flash-frozen in liquid nitrogen, and stored at −80 °C.

2.3.4.2. RNA extraction and quantitative real-time PCR. Frozen glass eels were cut in three sections, containing (i) the head including the gills, (ii) the heart, the liver, the spleen and the stomach and (iii) most of the muscle tissue with the intestine (hereafter referred to as head, viscera and muscle, respectively, Fig. 2). Then, each section was immediately stored in TRIzol reagent for total RNA extraction.

The protocol conditions for quantitative RT-PCR have been previously published (Lansard et al., 2010). The primers specific to 27 genes involved in antioxidant system, mitochondrial function and autophagy activity have been described in previous study by Liu et al. (2019). For the expression analysis, relative quantification of target gene expression was done using the ΔCT method described by Pfaffl (2001). The relative expression of Luciferase was used for data normalization as described previously (Marandel et al., 2016).

2.4. Statistical analyses

2.4.1. Propensity of glass eels to migrate

To characterize the propensity of glass eels to migrate, we first investigated the synchronization of glass eel's swimming activity to the change in water current direction every 6.2h (synchronized/non-synchronized). For this purpose, we used a modeling method to categorize all the recovered glass eels into synchronized ones and non-synchronized ones, which has been previously described by Liu et al. (2019) (See Supplementary Text S1). Since it has been previously shown that glass eels display rhythmic swimming activity in response to current reversal (Bolliet and Labonne, 2008), two parameters, the probability of being swimming of an individual i at time t, $P(t,i)$ and the periodicity of swimming occurrence of an individual i, $per(i)$, were derived from the model. Fish having a P value above the mean of P meanwhile having an activity periodicity close to 12.4 h were considered *synchronized*, others were considered *non-synchronized*. Finally, the number of synchronized fish in the control and MeHg-exposed groups was compared by chi-squared test.

The second aspect to investigate the propensity of glass eels to migrate was the level of *swimming activity*, expressed as the total number of observations of glass eels swimming in the water column during the 360 sessions of 15 s, regardless of synchronization. The comparison between control and MeHg-exposed groups were conducted using chi-squared test.

2.4.2. Principal component analysis (PCA) of gene transcriptional profiles

To determine the transcriptional profile of studied genes, PCA was used as a multivariate statistical approach to reduce the number of the variables considered. The data set of gene expression levels was compressed by PCA procedure without much loss of information. In detail, to evaluate the global transcriptional response in each pathway, we ran a PCA analysis for each cellular function, using a table providing the normalized gene expression levels for all individuals. The first axis of PCA performed on each function in each tissue explained 47%−77% of the total variances of all the genes involved, making it an acceptable synthetic measure of gene expression level of each function (See Supplementary Table S1). Supplementary Fig. S2 ~ Fig. S4 showed the relevance of all the genes involved in each function to the first axis of PCA. We then retrieved the score of individuals on the first axis of the PCA, and used these coordinates as a synthetic indicator of the individual level of expression for the cellular function.

All the statistical analyses relevant to gene transcriptions were examined using the first axis of PCA for each genomic function. Two-way ANOVA was used to analyze the varying gene transcriptional profiles in response to MeHg exposure and synchronization behavior. The interactions in the responses were also evaluated. Differences were considered significant at $p < 0.05$. The relationships of swimming activity to gene transcriptional profiles were estimated by Spearman's Rank Order Correlation test. The correlation was considered significant at $p < 0.05$ level. Then, the slope of the correlations observed in control and MeHg-exposed groups were compared by a Fisher's Z transformation method.

3. Results

3.1. MeHg exposure and accumulation in glass eels

After seven days of exposure, concentrations of MeHg measured in the whole body of glass eels were 3.6 times higher than those measured in the control fish (476 ± 58 and 132 ± 31 ng Hg g^{-1} dry weight, respectively, $p < 0.001$). Concentrations in Hg(II) remained similar with 9 ± 3 and 7 ± 2 ng Hg g^{-1} dry weight in the control and exposed group, respectively.

3.2. MeHg-induced behavioral changes

Two dead glass eels were found in both the control and the exposed groups after swimming test, which left a total of 58 glass eels in each group.

The propensity of glass eels to migrate was first evaluated by their ability to synchronize to the change in water current direction every 6.2 h. Glass eels exhibiting swimming activity with a period of approximately 12.4 h were considered as synchronized and the others as non-synchronized. As shown in Fig. 3a, the number of synchronized fish was lower in MeHg-exposed group relative to the control (30 and 35 individuals, respectively corresponding to 52% and 60% of the total group, respectively), although the difference

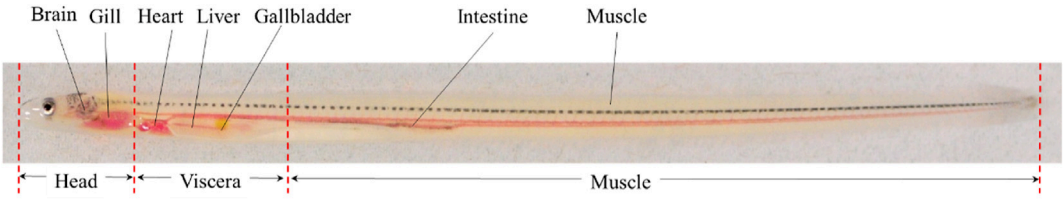


Fig. 2. Sections of glass eel.

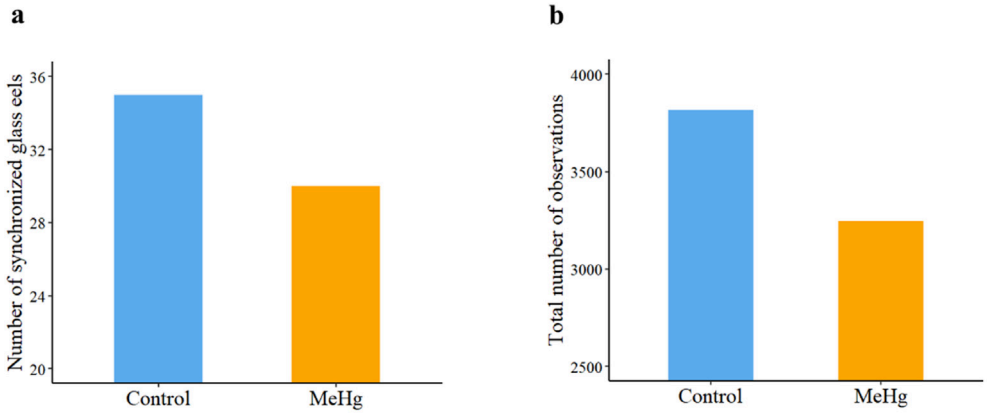


Fig. 3. Swimming behavior of control (blue bar, n = 58) and MeHg-exposed (orange bar, n = 58) glass eels over 10 days. (a) Bar chart showing the total number of glass eels which synchronized their swimming activity to the change in water current direction every 6.2 h, Pearson's chi-squared test, $X^2 = 0.56$, p = 0.454 (b) Bar chart showing the total number of observations of all glass eels, Pearson's chi-squared test, $X^2 = 0.56$, p < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

was not significant using a chi-squared test ($X^2 = 0.56$, $p = 0.454$).

The level of swimming activity was then expressed by the total number of observations of glass eels swimming in the water column during the 360 sessions of 15 s. MeHg treatment significantly decreased the total number of observations when compared to control (chi-squared test, $X^2 = 55.55$, $p < 0.001$, Fig. 3b). When analyzing separately the two behavioral phenotypes, the decrease in the total swimming activity level was significant in non-synchronized glass eels but not in synchronized ones (Table 1). In addition, the maximum number of observations per fish decreased in the exposed group when compared to the control one (222 and 163 observations during the 360 sessions, respectively).

3.3. MeHg- and behavior-induced gene transcriptional responses

The transcriptional profiles of 27 genes involved in five metabolic functions were analyzed. Some of these genes code for proteins involved in the mitochondrial respiratory chain complexes, mitochondrial catabolism and the antioxidant system. The other genes code for proteins involved in macroautophagy (the best-characterized autophagy subclass) and mitophagy (a macroautophagy-dependent specific degradation of mitochondria). As outlined in Materials and Methods, the data set of gene expression levels was compressed by PCA procedure to reduce the number of the variables considered. All the statistical analyses were then examined using the score of individuals on the first axis of the PCA for each function considered.

We first analyzed the transcriptional responses of the five metabolic functions to both MeHg exposure and synchronization behavior. As shown in Table 2, none of the functions considered appear to have been significantly affected by the treatment of glass eels with MeHg, regardless of the tissue examined. In contrast, significant different transcriptional responses were evidenced in head tissues between synchronized and non-synchronized glass eels. Of the five functions considered, only one (mitophagy) did not differ between the two behavioral groups. The expression of genes from the other four functions was significantly higher in synchronized glass eels than the non-synchronized ones (Table 2 and

Table 2
Statistical results of the two-way ANOVA for detecting transcriptional profiles of each genomic function in response to MeHg exposure and synchronization behavior. *P*-values of ANOVA are presented in the table. Significant values are in bold. Factor 'Treatment' is MeHg treatment (control *vs* MeHg exposure), factor 'Behavior' is behavioral phenotype (non-synchronized *vs* synchronized), 'Int' is interaction of both factors. H- head, V- viscera, M – muscle.

Genomic function	Tissue	*p* (Treatment)	*p* (Behavior)	*p* (Int)
Antioxidant system	H	0.338	**0.003**	0.663
	V	0.637	0.344	0.239
	M	0.268	0.636	0.650
Mitochondrial respiratory chain	H	0.585	**0.003**	0.107
	V	0.679	0.135	0.377
	M	0.242	0.489	0.945
Mitochondrial catabolism	H	0.776	**0.006**	0.206
	V	0.762	0.329	0.626
	M	0.753	0.597	0.806
Mitophagy	H	0.307	0.051	0.509
	V	0.784	0.499	0.903
	M	0.375	0.561	0.833
Macroautophagy	H	0.673	**0.004**	0.689
	V	0.958	0.163	0.886
	M	0.513	0.376	0.796

Table 1
Assessment of the MeHg-induced effects on swimming activity within two behavioral phenotypes. Synchronized glass eels: Fish which synchronized their swimming activity to the change in water current direction every 6.2 h. Number of fish: Total number of synchronized or non-synchronized glass eels observed during the behavioral test (for a total of 58 glass eels in both groups). Number of observations: Total number of observations of glass eels swimming in the water column during the 360 sessions recorded. A tagged glass eel can be observed only once during a recorded session. Significant values are in bold.

Behavioral phenotype	Treatment	Number of fish	Number of observations	Pearson's chi-squared test		
				df	X^2	*p*-value
Non-synchronized	Control	23	819	1	40.63	< **0.001**
	MeHg	28	731			
Synchronized	Control	35	2999	1	0.80	0.372
	MeHg	30	2516			

Supplementary Fig. S5).

In order to consider in our analysis the effect of glass eels swimming activity, which could mask (or at least influence) the effect of MeHg, we then performed a Spearman's Rank Order Correlation test between individual swimming activity level and the first axis of PCA for each function in both control and MeHg-exposed fish. As locomotor activity is expected to increase energy expenditure, correlations were tested using glass eels displaying similar range of activity. Although no significant difference could be evidenced between correlations observed in the control and the contaminated groups (using a Fisher's Z transformation), Table 3 shows that all the functions considered, except mitophagy, presented in the head a significant positive correlation to swimming activity after MeHg exposure but not in the control group. In the viscera, except mitochondrial catabolism, all others functions displayed a positive correlation to swimming activity in the contaminated group but not in the control. Finally, in the muscle, no correlation could be observed for the antioxidant system, the mitochondrial chain and the mitochondrial catabolism, while genes involved in mitophagy and macroautophagy responded positively to the activity in both groups.

4. Discussion

To clarify the adverse behavioral and metabolic responses due to MeHg exposure, changes in migration behavior and tissue-level transcriptions related to energetics were assessed with MeHg treatment in glass eels.

After a 7-day exposure, average MeHg concentration in whole body of glass eels was 476 ± 58 ng Hg g^{-1} dry weight, revealing the ability of the whole body to accumulate MeHg in a short-term exposure. In contrast, the concentrations of Hg(II) remained low, supporting a previous study using isotopic tracers and showing a low potential of demethylation in glass eels after 11 days of exposure to MeHg (50 ng L^{-1}) (Claveau et al., 2015).

4.1. Effect of MeHg on glass eels swimming activity and energy-related genes expression

Exposure to MeHg significatively decreased the total number of observations of glass eels swimming in the water column and reduced the maximum number of observations per fish (222 and 163 in the control and the exposed group, respectively). These results are consistent with previous studies conducted in *Salmo salar* and *Diplodus sargus* displaying lower swimming activity after dietary exposure to MeHg at 10 mg kg^{-1} during 4 months and 8.7 μg g^{-1} during seven days, respectively (Berntssen et al., 2003; Puga et al., 2016). Interstingly, when analyzing separately the synchronized and non-synchronized glass eels, the decrease in swimming activity level after MeHg exposure was only significant in the non-synchronized group, which supports the idea that glass eels presenting a low propensity to migrate might be more vulnerable to stress than those displaying a high probability to migrate (Bolliet et al., 2017; Claveau et al., 2015).

A number of studies reported the toxic effects of MeHg, notably on the oxidative status, the mitochondrial function and the Calcium homeostasis in fish (Cambier et al., 2009, 2010; Claveau et al., 2015; Graves et al., 2017; Gonzalez et al., 2005; Nøstbakken et al., 2012; Rasinger et al., 2017; Richter et al., 2011; Yadetie et al., 2016). Surprisingly, we did not observe any effect of MeHg on transcriptional profiles of the studied genes involved in mitochondrial metabolism, antioxidant system or autophagy. Our results contrast with a previous study in glass eels showing an activation of antioxidative defence system at the transcriptomic level after eleven days of exposure to MeHg (50 ng l^{-1}) (Claveau et al., 2015). However, in this last study, gene expression was analyzed just after exposure while in the present one analyses were conducted after the behavioral test, i.e., 10 days after the end of exposure. In addition, both studies focused on gene expression at a single time point that does not give a real picture of the dynamic aspect of the different events at play during complex and integrative processes such as antioxidant system, energy metabolism or autophagy. Furthermore, depending on the genes studied, the levels of transcripts do not always correlate with the amount or the activity of the corresponding proteins (Vogel and Marcotte, 2012; Yadetie et al., 2016). Future functional studies will therefore be necessary to draw definitive conclusions on the effect of MeHg in the functions monitored in glass eels.

Interestingly, we evidenced a significant positive correlation between individual swimming activity levels and the expression of the genes studied. The expression of genes related to autophagy and mitophagy increased with activity in the muscle, both in the control and exposed groups, probably reflecting an increase in energy requirement related to activity. However, in the head and viscera, most of the genomic functions related to antioxidant system and metabolism showed a positive correlation in the contaminated group, but not in the control one. As mentioned above, oxydative stress and mitochondrial impairment are among the most studied effects of MeHg in fish. MeHg targets some specific thiol containing proteins such as gluthatione peroxydase involved in the anti-oxydant system, predisposing cell to oxidative stress and generation of Reactive Oxygen Species (known as ROS) (Farina et al., 2011). In addition, MeHg can also directly target specific thiol-containing enzymes of the respiratory chain complex and both effects may affect cellular energy pathways (Farina et al., 2011; Glaser et al., 2010; Yadetie et al., 2016). Thus, although our results must be taken with caution (because the differences between correlations obtained for the control and contaminated groups were not strong enough to be significant), they strongly suggest that contaminated glass eels were affected by MeHg and needed to increase energy metabolism more than non-contaminated fish to cope with increasing swimming activity.

It is also noteworthy that the head was the most affected section by MeHg, a positive correlation being observed between swimming activity and all genomic functions. Although the head section include not only the brain but also the gills, this result seems consistent with the literature reporting that the brain is a predominant target for MeHg in fish (Gonzalez et al., 2005; Graves

Table 3
Spearman's Rank Order Correlation test between individual swimming activity level and the first axis of PCA for each function in both control and MeHg-exposed fish. R and *p* values of Spearman's correlation test are presented in the table. Significant values are in bold. H- head, V- viscera, M – muscle.

Function	Tissue	Control		MeHg	
		r	*p*-value	r	*p*-value
Antioxidant system	H	0.32	0.169	0.65	**<0.001**
	V	0.05	0.826	0.53	**0.014**
	M	0.33	0.156	0.32	0.135
Mitochondrial respiratory chain	H	0.22	0.346	0.57	**0.005**
	V	0.30	0.193	0.43	**0.049**
	M	0.27	0.251	0.27	0.205
Mitochondrial catabolism	H	0.23	0.326	0.68	**<0.001**
	V	−0.10	0.686	0.28	0.223
	M	−0.07	0.772	0.06	0.774
Mitophagy	H	0.63	**0.003**	0.76	**<0.001**
	V	0.40	0.078	0.51	**0.019**
	M	0.67	**0.001**	0.75	**<0.001**
Macroautophagy	H	0.39	0.091	0.72	**<0.001**
	V	0.38	0.099	0.62	**0.002**
	M	0.75	**<0.001**	0.68	**<0.001**

et al., 2017; Pereira et al., 2014, for review 2019). Indeed, MeHg has been reported to cross the blood-brain-barrier and accumulates in the brain having serious toxic effects including proteome changes related to oxidative stress and mitochondrial dysfunction, morpho-structural changes and dysfunction in neurotransmission processes (Cariccio et al., 2019; for review see Pereira et al., 2019; Pletz et al., 2016). In addition, in both control and exposed glass eels, the expression levels of metabolism-related genes were lower in non-synchronized glass eels than in synchronized ones in the head but not in the other sections. Also in eels sampled below and above successive obstacles along a river, Podgorniak et al. (2015) reported different gene transcription profiles in brain but not in liver or muscle. Altogether, these results suggest that the head may represent the one to focus on for a better understanding of glass eel's migration and the effect of environmental stressors on this migration.

4.2. Effect of MeHg on the rhythmic swimming activity in glass eels

To migrate up estuary, glass eels use selective tidal stream transport and synchronize their swimming activity to the flood thanks to an endogenous clock (Bolliet et al., 2007; Forward and Tankersley, 2001; Hickman, 1981; McCleave and Kleckner, 1982; Wippelhauser and McCleave, 1987). They are also known to avoid light and mainly migrate during the night, likely using a circadian clock (Bolliet et al., 2007). In our experimental conditions, a very low light intensity during photophase was used to avoid synchronization of glass eels activity to photoperiod that could have masked synchronization to the tidal cue. Thus, in the present study, synchronized glass eels corresponded to fish that synchronized their swimming activity to the change in water current direction with a period close to 12.4 h. A lower number of glass eels synchronizing to tidal period were observed in contaminated condition compared to control one, even though it was not statistically significant using a chi-squared test. Xenobiotics have been shown to disturb the circadian system in different fish species (Prokkola and Nikinmaa, 2018) and a couple of studies showed that MeHg disrupted circadian rhythms in rodents, the crayfish *Astacus astacus* and the freshwater crab P*otamon potamios* (Arito et al., 1983; Parmalee and Aschner, 2017; Styrishave and Depledge, 1996). In addition, Depledge (1984) evidenced an effect of mercury exposure on tidal rhythmicity in the heart rate of the shorecrab *Carcinus maenas*. Though the location of the circatidal clock is still unknown in fish, the pacemaker regulating circadian rhythms has been located in the pineal gland. Interestingly, Korbas et al. (2012, 2013) reported an accumulation of inorganic Hg in the pineal gland of zebrafish exposed to MeHg. Thus, the relationship between mercury species and the endogenous clock(s) driving the rhythmic swimming activity in glass eels appears as an interesting avenue to explore.

5. Conclusion

Our results suggest that MeHg may affect the estuarine migration of glass eels by reducing their ability to synchronize to the tide and their level of swimming activity. They also support the idea that non-synchronized fish may be more vulnerable to stress and the first affected by contamination. A decrease in the propensity to migrate *in natura* would lead to an increase in glass eels settlement in estuary and consequently a decrease in population recruitment in upper reaches. Non-migrant glass eels becoming mostly males, such effect would change the fate of the population by influencing the sex ratio in this species. A better understanding of the effect of MeHg on the maximum swimming activity in glass eels and their biological clocks is now required to clearly assess the impact of this contaminant on their synchronization to environmental cues and migration.

The results also support the interest of focusing on the head to investigate facultative migration behavior in glass eels and the effect of environmental stressors on this rhythmic behavior. However, as head samples include the entire brain tissue, eyes but also the gills, they also question the relationships between the ability to migrate and osmoregulatory functions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Hengtong Liu: Methodology, Data curation, Formal analysis, Writing - original draft. **Amaia Lamarins:** Methodology, Formal analysis, Writing - review & editing. **Jacques Labonne:** Formal analysis, Supervision. **Mathilde Monperrus:** Methodology, Supervision. **Pascale Coste:** Methodology. **Emmanuel Huchet:** Methodology. **Jacques Rives:** Methodology. **Iban Seiliez:** Supervision, Validation, Writing - review & editing, Writing - original draft. **Valérie Bolliet:** Methodology, Supervision, Validation, Writing - review & editing, Writing - original draft, Funding acquisition.

Acknowledgements

The research leading to these results has received funding from the ERDF (European Regional Development Fund) and AEAG (Agence de l'Eau Adour-Garonne) under the MICROPOLIT project. We wish to thank the IE ECP for the technical support and the China Scholarship Council for the grant to Hengtong Liu.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.127020.

References

Adam, G., Feunteun, E., Prouzet, P., Rigaud, C., 2008. L'anguille EUROPÉENNE: indicateurs d'abondance et de colonisation, first ed. Coll. Savoir-faire, Versailles.

Arito, H., Hara, N., Torii, S., 1983. Effect of methylmercury chloride on sleep-waking rhythms in rats. Toxicology 28, 335–345. https://doi.org/10.1016/0300-483X(83)90007-0.

Bardonnet, A., Riera, P., 2005. Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: new insights from stable isotope analysis. Estuarine. Coastal Shelf Sci 63, 201–209. https://doi.org/10.1016/j.ecss.2004.11.009.

Berntssen, M.H.G., Aatland, A., Handy, R.D., 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquat. Toxicol. 65, 55–72. https://doi.org/10.1016/S0166-445X(03)00104-8.

Bolliet, V., Claveau, J., Jarry, M., Gonzalez, P., Baudrimont, M., Monperrus, M., 2017. Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European glass eels (*Anguilla anguilla*). Physiol. Behav. 169, 33–40. https://doi.org/10.1016/j.physbeh.2016.11.008.

Bolliet, V., Labonne, J., 2008. Individual patterns of rhythmic swimming activity in *Anguilla anguilla* glass eels synchronised to water current reversal. J. Exp. Mar. Biol. Ecol. 362, 125–130. https://doi.org/10.1016/j.jembe.2008.06.017.

Bolliet, V., Lambert, P., Rives, J., Bardonnet, A., 2007. Rhythmic swimming activity in *Anguilla anguilla* glass eels: synchronisation to water current reversal under laboratory conditions. J. Exp. Mar. Biol. Ecol. 344, 54–66. https://doi.org/10.1016/j.jembe.2006.12.027.

Bureau du Colombier, S.B., Bolliet, V., Lambert, P., Bardonnet, A., 2007. Energy and migratory behavior in glass eels (*Anguilla anguilla*). Physiol. Behav. 92, 684–690. https://doi.org/10.1016/j.physbeh.2007.05.013.

Cambier, S., Bénard, G., Mesmer-Dudons, N., Gonzalez, P., Rossignol, R., Brethes, D., Bourdineaud, J.P., 2009. At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebra fish (*Danio rerio*). Int. J. Biochem. Cell Biol. 41, 791–799. https://doi.org/10.1016/

j.biocel.2008.08.008.
Cambier, S., Gonzalez, P., Durrieu, G., Maury-Brachet, R., Boudou, A., Bourdineaud, J.P., 2010. Serial analysis of gene expression in the skeletal muscles of zebrafish fed with a methylmercury-contaminated diet. Environ. Sci. Technol. 44, 469–475. https://doi.org/10.1021/es901980t.
Cambier, S., Gonzalez, P., Mesmer-Dudons, N., Brethes, D., Fujimura, M., Bourdineaud, J.P., 2012. Effects of dietary methylmercury on the zebrafish brain: histological, mitochondrial, and gene transcription analyses. Biometals 25, 165–180. https://doi.org/10.1007/s10534-011-9494-6.
Cariccio, V.L., Sama, A., Bramanti, P., Mazzon, E., 2019. Mercury involvement in neuronal damage and in neurodegenerative diseases. Biol. Trace Elem. Res. 187, 341–356. https://doi.org/10.1007/s12011-018-1380-4.
Claveau, J., Monperrus, M., Jarry, M., Baudrimont, M., Gonzalez, P., Cavalheiro, J., Mesmer-Dudons, N., Bolliet, V., 2015. Methylmercury effects on migratory behaviour in glass eels (*Anguilla anguilla*): an experimental study using isotopic tracers. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 171, 15–27. https://doi.org/10.1016/j.cbpc.2015.03.003.
Daverat, F., Limburg, K.E., Thibault, I., Shiao, J.C., Dodson, J.J., Caron, F.O., Tzeng, W.N., Iizuka, Y., Wickstrom, H., 2006. Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla, A. japonica* and *A. rostrata*. Mar. Ecol.: Prog. Ser. 308, 231–241. https://doi.org/10.3354/meps308231.
Davey, A.J.H., Jellyman, D.J., 2005. Sex determination in freshwater eels and management options for manipulation of sex. Rev. Fish Biol. Fish. 15, 37–52. https://doi.org/10.1007/s11160-005-7431-x.
Depledge, M.H., 1984. Disruption of circulatory and respiratory activity in shore crabs (*Carcinus maenas* (L.)) exposed to heavy metal pollution. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 78, 445–459. https://doi.org/10.1016/0742-8413(84)90113-0.
Edeline, E., 2007. Adaptive phenotypic plasticity of eel diadromy. Mar. Ecol.: Prog. Ser. 341, 229–232. https://doi.org/10.3354/meps341229.
Edeline, E., Lambert, P., Rigaud, C., Elie, P., 2006. Effects of body condition and water temperature on *Anguilla anguilla* glass eel migratory behavior. J. Exp. Mar. Biol. Ecol. 331, 217–225. https://doi.org/10.1016/j.jembe.2005.10.011.
Farina, M., Aschner, M., Rocha, J.B.T., 2011. Special issue: environmental Chemicals and neurotoxicity oxidative stress in MeHg-induced neurotoxicity. Toxicol. Appl. Pharmacol. 256, 405–417. https://doi.org/10.1016/j.taap.2011.05.001.
Forward, R.B., Tankersley, R.A., 2001. Selective tidal-stream transport of marine animals. Oceanogr. Mar. Biol. 39, 305–353.
Gascuel, D., 1986. Flow-carried and active swimming migration of the glass eel (*Anguilla anguilla*) in the tidal area of a small estuary on the French Atlantic coast. Helgol. Meeresunters. 40, 321–326. https://doi.org/10.1007/BF01983739.
Geffroy, B., Bardonnet, A., 2016. Sex differentiation and sex determination in eels: consequences for management. Fish Fish. 17, 375–398. https://doi.org/10.1111/faf.12113.
Gonzalez, P., Dominique, Y., Massabuau, J.C., Boudou, A., Bourdineaud, J.P., 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (Danio rerio). Environ. Sci. Technol. 39, 3972–3980. https://doi.org/10.1021/es0483490.
Glaser, V., Leipnitz, G., Straliotto, M.R., Oliveira, J., dos Santos, V.V., Wanmacher, C.M., de Bem, A.F., Rocha, J.B., Farina, M., Latini, A., 2010. Oxidative stress-mediated inhibition of brain creatine kinase activity by methylmercury. Neurotoxicology 31, 454–460. https://doi: 10.1016/j.neuro.2010.05.012.
Graves, S.D., Kidd, K.A., Batchelar, K.L., Cowie, A.M., O'Driscoll, N.J., Martyniuk, C.J., 2017. Response of oxidative stress transcripts in the brain of wild yellow perch (*Perca flavescens*) exposed to an environmental gradient of methylmercury. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 192, 50–58. https://doi.org/10.1016/j.cbpc.2016.12.005.
Harrison, A.J., Walker, A.M., Pinder, A.C., Briand, C., Aprahamian, M.W., 2014. A review of glass eel migratory behaviour, sampling techniques and abundance estimates in estuaries: implications for assessing recruitment, local production and exploitation. Rev. Fish Biol. Fish. 24, 967–983. https://doi.org/10.1007/s11160-014-9356-8.
Hickman, R.A., 1981. Densities and swimbladder development of juvenile American eels, *Anguilla rostrata* (Lesueur) as related to energetics of migration. J. Fish. Biol. 18, 507–517. https://doi.org/10.1111/j.1095-8649.1981.tb03792.x.
Imbert, H., Arrowsmith, R., Dufour, S., Elie, P., 2008. Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage dependent mechanisms in European eel upstream migration. Horm. Behav. 53, 69–81. https://doi.org/10.1016/j.yhbeh.2007.06.011.
ICES, 2018. Advice on Fishing Opportunities, Catch and Effort. Ecoregions in the Northeast Atlantic. https://doi.org/10.17895/ices.pub.4601 ele.2737.nea. European eel (*Anguilla anguilla*) throughout its natural range.
Jellyman, D.J., 1979. Upstream migration of glass-eels (*Anguilla* spp.) in the Waikato River. N. Z. J. Mar. Freshw. Res. 13, 13–22. https://doi.org/10.1080/00288330.1979.9515776.
Kawakami, Y., Mochioka, N., Nakazono, A., 1999. Immigration patterns of glass-eels *Anguilla japonica* entering river in northern Kyushu, Japan. Bull. Mar. Sci. 64, 315–327. https://doi.org/10.2331/fishsci.64.235.
Korbas, M., Lai, B., Vogt, S., Gleber, S.C., Karunakaran, C., Pickering, I.J., Krone, P.H., George, G.N., 2013. Methylmercury targets photoreceptor outer segments. ACS Chem. Biol. 8, 2256–2263. https://doi.org/10.1021/cb4004805.
Korbas, M., MacDonald, T.C., Pickering, I.J., George, G.N., Krone, P.H., 2012. Chemical form matters: differential accumulation of mercury following inorganic and organic mercury exposures in zebrafish larvae. ACS Chem. Biol. 7, 410–419. https://doi.org/10.1021/cb200287c.
Krueger, W.H., Oliveira, K., 1999. Evidence for environmental sex determination in the American eel, *Anguilla rostrata*. Environ. Biol. Fish. 55, 381–389. https://doi.org/10.1023/A:1007575600789.
Laffaille, P., Acou, A., Guioullet, J., Mounaix, B., Legault, A., 2006. Patterns of silver eel (*Anguilla anguilla* L.) sex ratio in a catchment. Ecol. Freshw. Fish 15, 583–588. https://doi.org/10.1111/j.1600-0633.2006.00195.x.
Lansard, M., Panserat, S., Plagnes-Juan, E., Seiliez, I., Skiba-Cassy, S., 2010. Integration of insulin and amino acid signals that regulate hepatic metabolism-related gene expression in rainbow trout: role of TOR. Amino Acids 39, 801–810. https://doi.org/10.1007/s00726-010-0533-3.
Lee, J.W., De Riu, N., Lee, S., Bai, S.C., Moniello, G., Hung, S.S.O., 2011. Effects of dietary methylmercury on growth performance and tissue burden in juvenile green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*). Aquat. Toxicol. 105, 227–234. https://doi.org/10.1016/j.aquatox.2011.06.013.
Liu, H.T., Labonne, J., Coste, P., Huchet, E., Plagnes-Juan, E., Rives, J., Veron, V., Seiliez, I., Bolliet, V., 2019. Looking at the complex relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (*Anguilla anguilla*). Sci. Total Environ. 696 https://doi.org/10.1016/j.scitotenv.2019.134039.
Marandel, L., Lepais, O., Arbenoits, E., Veron, V., Dias, K., Zion, M., Panserat, S., 2016. Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (*Oncorhynchus mykiss*) by nutritional status and dietary carbohydrates. Sci. Rep. 6 https://doi.org/10.1038/srep32187.
McCleave, J.D., Kleckner, R.C., 1982. Selective tidal stream transport in the estuarine migration of glass eels of the American eel (*Anguilla rostrata*). J. Cons. Cons. Int. Explor. Mer 40, 262–271. https://doi.org/10.1093/icesjms/40.3.262.
Miller, M.J., Bonhommeau, S., Munk, P., Castonguay, M., Hanel, R., McCleave, J.D., 2015. A century of research on the larval distributions of the Atlantic eels: a re-examination of the data. Biol. Rev. 90, 1035–1064. https://doi.org/10.1111/brv.12144.
Murphy, C.A., Rose, K.A., Alvarez, M.D., Fuiman, L.A., 2008. Modeling larval fish behavior: scaling the sublethal effects of methylmercury to population-relevant endpoints. Aquat. Toxicol. 86, 470–484. https://doi.org/10.1016/j.aquatox.2007.12.009.
Navarro, P., Clemens, S., Perrot, V., Bolliet, V., Tabouret, H., Guérin, T., Monperrus, M., Amouroux, D., 2013. Simultaneous determination of mercury and Butyltin species using a multiple species-specific isotope dilution methodology on the European, *Anguilla anguilla* glass eel and yellow eel. Int. J. Environ. Anal. Chem. 93, 166–182. https://doi.org/10.1080/03067319.2011.629349.
Nøstbakken, O.J., Martin, S.A.M., Cash, P., Torstensen, B.E., Amlund, H., Olsvik, P.A., 2012. Dietary methylmercury alters the proteome in Atlantic salmon (*Salmo salar*) kidney. Aquat. Toxicol. 108, 70–77. https://doi.org/10.1016/j.aquatox.2011.08.017.
Parmalee, N.L., Aschner, M., 2017. Metals and circadian rhythms. Adv. Neurotoxicol. 1, 119–130. https://doi.org/10.1016/bs.ant.2017.07.003.
Parsons, J., Vickers, K.U., Warden, Y., 1977. Relationship between elver recruitment and changes in sex ratio of silver eels *Anguilla anguilla* L. migrating from Lough Neagh, Northern Ireland. J. Fish. Biol. 10, 211–229. https://doi.org/10.1111/j.1095-8649.1977.tb05127.x.
Pereira, P., Korbas, M., Pereira, V., Cappello, T., Maisano, M., Canario, J., Almeida, A., Pacheco, M., 2019. A multidimensional concept for mercury neuronal and sensory toxicity in fish - from toxicokinetics and biochemistry to morphometry and behavior. Biochim. Biophys. Acta Gen. Subj. 1863, 129298. https://doi.org/10.1016/j.bbagen.2019.01.020.
Pereira, P., Raimundo, J., Araujo, O., Canario, J., Almeida, A., Pacheco, M., 2014. Fish eyes and brain as primary targets for mercury accumulation - a new insight on environmental risk assessment. Sci. Total Environ. 494–495, 290–298. https://doi.org/10.1016/j.scitotenv.2014.07.008.
Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45. https://doi.org/10.1093/nar/29.9.e45.
Pletz, J., Sánchez-Bayo, F., Tennekes, H.A., 2016. Dose-response analysis indicating time-dependent neurotoxicity caused by organic and inorganic mercury - implications for toxic effects in the developing brain. Toxicology 347–349, 1–5. https://doi.org/10.1016/j.tox.2016.02.006.
Podgorniak, T., Milan, M., Pujolar, J.M., Maes, G., Bargelloni, L., De Oliveira, E., Pierron, F., Daverat, F., 2015. Differences in brain gene transcription profile advocate for an important role of cognitive function in upstream migration and water obstacles crossing in European eel. BCM Genomics 16, 378. https://doi.org/10.1186/s12864-015-1589.
Prokkola, J.M., Nikinmaa, M., 2018. Circadian rhythms and environmental disturbances - underexplored interactions. J. Exp. Biol. 221 https://doi.org/10.1242/jeb.179267.
Puga, S., Pereira, P., Pinto-Ribeiro, F., O'Driscoll, N.J., Mann, E., Barata, M., Pousao-Ferreira, P., Canario, J., Almeida, A., Pacheco, M., 2016. Unveiling the neurotoxicity of methylmercury in fish (*Diplodus sargus*) through a regional morphometric analysis of brain and swimming behavior assessment. Aquat. Toxicol. 180, 320–333. https://doi.org/10.1016/j.aquatox.2016.10.014.
Rasinger, J.D., Lundebye, A.K., Penglase, S.J., Ellingsen, S., Amlund, H., 2017. Methylmercury induced neurotoxicity and the influence of selenium in the brains of adult zebrafish (*Danio rerio*). Int. J. Mol. Sci. 18, 725. https://doi.org/10.3390/ijms18040725.
Richter, C.A., Garcia-Reyero, N., Martyniuk, C., Knoebl, I., Pope, M., Wright-Osment, M.K., Denslow, N.D., Tillitt, D.E., 2011. Gene expression changes in female zebrafish (*Danio rerio*) brain in response to acute exposure to methylmercury. Environ. Toxicol. Chem. 30, 301–308. https://doi.org/10.1002/etc.409.

Righton, D., Westerberg, H., Feunteun, E., Okland, F., Gargan, P., Amilhat, E., Metcalfe, J., Lobon-Cervia, J., Sjoberg, N., Simon, J., Acou, A., Vedor, M., Walker, A., Trancart, T., Bramick, U., Aarestrup, K., 2016. Empirical observations of the spawning migration of European eels: the long and dangerous road to the Sargasso Sea. Sci. Adv. 2, e1501694 https://doi.org/10.1126/sciadv.1501694.

Sampaio, E., Maulvault, A.L., Lopes, V.M., Paula, J.R., Barbosa, V., Alves, R., Pousao-Ferreira, P., Repolho, T., Marques, A., Rosa, R., 2016. Habitat selection disruption and lateralization impairment of cryptic flatfish in a warm, acid, and contaminated ocean. Mar. Biol. 163, 217. https://doi.org/10.1007/s00227-016-2994-8.

Sandheinrich, M.B., Miller, K.M., 2006. Effects of dietary methylmercury on reproductive behavior of fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. 25, 3053–3057. https://doi.org/10.1897/05-641R.1.

Scheulhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W., 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36, 12–18. https://doi.org/10.1579/0044-7447, 2007)36[12:EOE-MOT]2.0.CO;2.

Schmidt, J., 1923. Breeding places and migrations of the eel. Nature 111, 51–54. https://doi.org/10.1038/111051a0.

Secor, D.H., Hendersonarzapalo, A., Piccoli, P.M., 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. 192, 15–33. https://doi.org/10.1016/0022-0981(95)00054-u.

Strungaru, S.A., Robea, M.A., Plavan, G., Todirascu-Ciornea, E., Ciobica, A., Nicoara, M., 2018. Acute exposure to methylmercury chloride induces fast changes in swimming performance, cognitive processes and oxidative stress of zebrafish (*Danio rerio*) as reference model for fish community. J. Trace Elem. Med. Biol. 47, 115–123. https://doi.org/10.1016/j.jtemb.2018.01.019.

Styrishave, B., Depledge, M.H., 1996. Evaluation of mercury-induced changes in circadian heart rare rhythms in the freshwater crab, *Potamon potamios* and the crayfish, *Astacus astacus* as an early predictor of mortality. Comp. Biochem. Physiol. Mol. Integr. Physiol. 115, 349–356. https://doi.org/10.1016/S0300-9629(96)00105-3.

Tesch, F.W., 2003. The Eel, fifth ed. Wiley, New York.

Tesch, F.W., 1980. Occurrence of eel *Anguilla anguilla* larvae west of the European continental shelf, 1971-1977. Environ. Biol. Fish. 5, 185–190. https://doi.org/10.1007/BF00005354.

Tsukamoto, K., Arai, T., 2001. Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats. Mar. Ecol.: Prog. Ser. 220, 265–276. https://doi.org/10.3354/meps220265.

Tsukamoto, K., Nakai, I., Tesch, W.V., 1998. Do all freshwater eels migrate? Nature 396, 635–636. https://doi.org/10.1038/25264.

Tzeng, W.N., 1996. Effects of salinity and ontogenetic movements on strontium: calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. J. Exp. Mar. Biol. Ecol. 199, 111–122. https://doi.org/10.1016/0022-0981(95)00185-9.

van Ginneken, V., Maes, G.E., 2005. The European eel (*Anguilla Anguilla*, linnaeus), its lifecycle, evolution and reproduction: a literature review. Rev. Fish Biol. Fish. 15, 367–398. https://doi.org/10.1007/s11160-006-0005-8.

Vogel, C., Marcotte, E.M., 2012. Insights into the regulation of protein abundance from proteomic and trascriptomic analyses. Nat. Rev. Genet. 13, 227–232.

Webber, H.M., Haines, T.A., 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environ. Toxicol. Chem. 22, 1556–1561. https://doi.org/10.1002/etc.5620220718.

Weis, J.S., Khan, A.A., 1990. Effects of mercury on the feeding behavior of the mummichog, *Fundulus heteroclitus* from a polluted habitat. Mar. Environ. Res. 30, 243–249. https://doi.org/10.1016/0141-1136(90)90001-5.

Wippelhauser, G.S., McCleave, J.D., 1987. Precision of behavior of migrating juvenile American eels (*Anguilla rostrata*) utilizing selective tidal stream transport. J. Cons. Cons. Int. Explor. Mer 44, 80–89. https://doi.org/10.1093/icesjms/44.1.80.

Yadetie, F., Bjørneklett, S., Garberg, H.K., Oveland, E., Berven, F., Goksoyr, A., Karlsen, O.A., 2016. Quantitative analyses of the hepatic proteome of methylmercury-exposed Atlantic cod (*Gadus morhua*) suggest oxidative stress-mediated effects on cellular energy metabolism. BMC Genom. 17, 554. https://doi.org/10.1186/s12864-016-2864-2.

Zhou, T., Scali, R., Weis, J.S., 2001. Effects of methylmercury on ontogeny of prey capture ability and growth in three populations of larval *Fundulus heteroclitus*. Arch. Environ. Contam. Toxicol. 41, 47–54. https://doi.org/10.1007/s002440010219.

Chapter 3 – GENERAL DISCUSSION

European eels arrive to European coasts all year round with highest recruitment between late autumn and spring in tne south west of France. As known, the estuarine migration in European glass eels is facultative and before returning to the Sargasso Sea to spawn, some glass eels migrate to freshwater, some reside in marine or brackish coastal waters while some others move between marine, estuarine and freshwater habitats (Daverat et al., 2006; Tsukamoto et al., 2002; Tzeng et al., 2000). Energy has been considered as a limiting factor in this facultative migration, given the fasting and endurance swimming period in glass eels. Therefore, this thesis was aiming to investigate the role of energy in eels' migration and settlement processes by monitoring the energetic status of glass eels from distinct seasons (autumn and spring) as well as from different habitats (marine and estuary). Meanwhile, other potential hypotheses in addition to energy aspect have also been discussed.

3.1 Conditional strategy based on individual energy stores

Given the specific life history of eels, which accumulate energy during larval oceanic drift and do not or hardly eat throughout estuarine migration in glass eel stage, endogenous energy stores were previously thought to closely condition glass eel's migratory ability. The energy-based conditional strategy suggests that individuals with higher energetic stores would present a higher migratory propensity to reach rivers (Edeline, 2007). Supporting this hypothesis is the study of Edeline et al. (2006) showing an increase in saltwater-seeking locomotor activity as glass eels' weight and length decrease. Study by Bureau du Colombier et al. (2007) also showed that migrant European glass eels exhibited higher energy content than the inactive ones in February. However, this result was not confirmed in glass eels caught in November, which support only partly a causal role of energy stores in the facultative migration of glass eels. In addition, several other findings in both European and American eels conflicted with the concept of conditional strategy. For example, Bolliet et al. (2017) showed no difference in wet weight and length between migrant and non-migrant European glass eels caught in April and others studies in American glass eels suggested that salinity preference were not influenced by index of body condition (Boivin et al., 2015) or wet mass and total lipid content (Gaillard et al., 2015).

Looking at swimming behavior and weight in autumn and spring glass eels, our results showed that autumn glass eels displayed higher energy stores, a higher proportion of synchronized fish and a higher level of swimming activity than spring individuals. In addition, we also showed that spring estuarine fish displayed similar weight than marine fish despite the 22 km covered from the sea without feeding, suggesting that the smallest marine individuals possibly settled or died during migration. Both results may support the energy-based conditional strategy. However, whatever the season or the habitat, no weight difference was evidenced between synchronized and non-synchronized fish in our behavioral trials. Although we cannot rule out the possibility that the synchronized and non-synchronized swimming behavior discriminated in

our experimental conditions may not totally reflect glass eels' behavioral performance in the wild (see section 3), the low heterogeneity in weight within each experiment could also explain the absence of link between weight and behavior. Indeed, there was high differences and no weight overlap between autumn and spring glass eels, while individuals within autumn or spring groups showed much less variability. Accordingly, we hypothesize that when energy stores are too low, it may become a limiting factor for swimming activity and then trigger settlement. On the other hand, our results also clearly indicate that the observed differences in migration behavior cannot be only linked to differences in energy reserves and that other factors must be at play in the ability of glass eels to migrate or not.

Conclusions and perspectives

Altogether, these findings suggest that a conditional strategy based on energy stores cannot fully explain the facultative migration in glass eels. Energy stores may limit glass eels' migration but only when this factor become limiting, wherein a threshold of energy stores may exist. This hypothesis could be tested, for example, by analyzing the migratory behavior of glass eels caught monthly during the season. This would allow to determine if there is a gradient of migratory propensity or if the propensity to migrate increases markedly in parallel to increased weight below a limit (Figure 3-1).

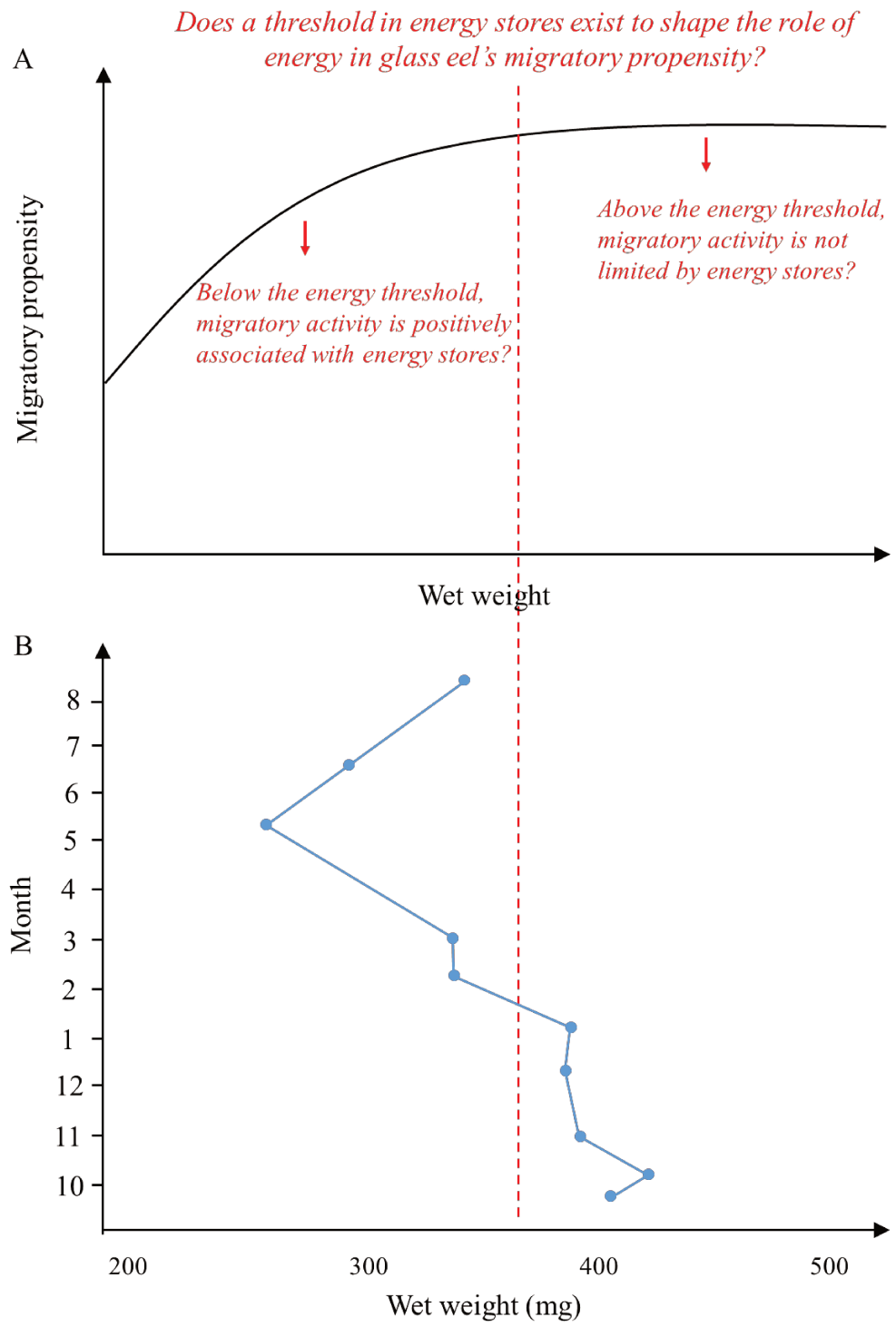


Figure 3-1. A presumed model indicating the fluctuated association between migratory propensity and wet weight of glass eels across season. (A) a positive correlation between glass eel's migratory propensity and wet weight is presumed to appear when the wet weight is below a threshold, while this correlation disappear above the threshold; (B) variation of the wet weight of glass eels captured in marine (at Moliets, modified from Charlon and Blanc, 1982).

3.2 Energy mobilization

In addition to energy stores, we hypothesized that the ability to mobilize energy could also be involved in the propensity of glass eels to migrate (individuals with a more efficient energy mobilization would display a higher migratory capacity).

Surprisingly, in the present thesis, and regardless of the swimming activity, non-synchronized glass eels were found to lose more weight than the synchronized fish after the swimming test suggesting a higher energy expenditure (Publication 2). In fish, the use of energy could be associated to: standard metabolism (SMR), swimming activity and digestive cost, whereby the last two cases are not relevant to sedentary and fasting glass eels. Thus, in order to test whether the higher weight loss in non-synchronized glass eels may result from a higher metabolism than in synchronized fish, we measured the oxygen consumption of glass eels at the individual level as a proxy of SMR. Metabolic results showed that synchronized fish in autumn displayed higher SMR than the non-synchronized ones, while no difference of this factor was observed in spring groups (Publication 2). At first glance, this result seems contradictory, but experimental biases related to our protocol cannot be excluded. Indeed, SMR was measured after the swimming test that may have increased the SMR of synchronized fish in relation to their activity, possibly masking a higher SMR of non-synchronized fish. Thus, index of SMR here may not be informative enough to conclude.

On the other hand, SMR is only one of the parameters reflecting metabolic status, where maximum metabolism (MMR) and aerobic scope (AS, the difference between MMR and SMR) are also included. AS predicts animal capacity to increase its oxygen consumption and aerobic exercise (Norin and Clark, 2016) and may be a better proxy to be used to compare the energy mobilization ability of glass eels during migration.

Altogether, this suggests that future studies would be necessary to measure individual standard metabolism before and after a swimming test, but also to evaluate AS in synchronized and non-synchronized glass eels.

We then measured the expression of several energy-related genes as another proxy for glass eel's ability of energy mobilization (Publications 1 and 2) but no clear difference between synchronized and non-synchronized fish was evidenced. In American glass eels captured from Mersey River and Grande-Rivière-Blanche (Canada), Gaillard et al. (2015, 2016) evidenced a lower expression level of triacylglycerol lipase, and higher energy stores and higher triacylglycerol content in the Mersey River than the other site, indicating an association between transcriptional response and energy storage strategy depending on the site of capture. The absence of differential expression in metabolism-related genes observed in our results may be first related to the homogeneity in body mass and energy contents (i.e. lipid contents) between

synchronized and non-synchronized glass eels. In addition, as for the SMR, the transcriptional analysis was performed after the swimming test, which may have induced a bias in the results obtained. Furthermore, these analyses were also performed at a single time point that does not give a real picture of the dynamic aspect of the different events at play during complex and integrative metabolic processes such as energy expenditure (which, in addition, include not only gene transcription but also protein translation and enzyme activity). Finally, analyses have been carried out on the whole fish and we cannot rule out the existence of a dilution effect that could mask some effects in particular tissues. Evidence supporting this idea was provided in the Publication 3 showing higher levels of transcripts related to antioxidant system, mitochondrial activity and autophagy in the head of synchronized fish than in the head of non-synchronized ones. Obviously, this result cannot explain the higher energy consumption in non-synchronized glass eels than the other behavioral phenotype. However, we could hypothesize that this result may be due to the specific role of energy metabolism in brain. Indeed, energy consumption in brain mainly serves to maintain cellular and systemic function, and has been shown to be critical to the CNS networks and normal behavioral rhythms (Cavey et al., 2016; Harris et al., 2012). In this context, the different gene transcription profiles we detected in head of glass eels prompted us to hypothesize that the enhanced genetic regulation related to energy metabolism and antioxidant functions may contribute to an enhanced synchronization capacity in European glass eels. In this regard, different gene transcription profiles were previously evidenced in in the brain of European glass eels (Podgorniak et al., 2015): genes related to signaling pathways such as calcium-mediated synaptic connections that control neuronal activity expressed differentially among glass eels sampled from different locations on their upstream migration. But, an evidenced metabolism-neuron-synchronization interaction is lacking in glass eels. Our results together with these mentioned studies probably support a future interest to investigate this potential interaction to help understand glass eels' migratory behavior.

As seen above, neither the SMR nor the expression of genes considered, allow understanding the apparent higher weight loss of non-synchronized fish compared to their synchronized counterparts during the swimming test. However, some of our results showing a higher energy consumption in non-sunchronized fish than the synchronized ones tend to support an attractive hypothesis related to a higher vulnerability to stress of non-synchronized glass eels than synchronized ones. This hypothesis was first proposed by Claveau et al. (2015a) who used the photoperiod as a synchronizer of swimming activity instead of the water current reversal used in this thesis. In this study, the authors showed that after exposure to isotopically enriched ^{201}MeHg (50 ng L^{-1}), *cat* and *gstr* expression levels were significantly higher in non-synchronized glass eels than in synchronized ones suggesting that non-synchronized glass eels may be more sensitive to contamination than synchronized individuals. In a more recent study, Bolliet et al. (2017) using the same

synchronizer, also presented evidence that non-synchronized glass eels displayed higher levels of *cat* and *gstr* expression than synchronized individuals caught both in marine and estuarine sites in spring.

Thus the higher weight loss observed in non-synchronized glass eels than in synchronized ones may result from a higher stress/vulnerability to stress in the former. In accordance with this hypothesis, our data also showed that exposure to MeHg (100 ng L^{-1}) during seven days decreased the total swimming activity in non-synchronized glass eels but not in synchronized ones. Estuaries are known to represent a stressful environment as discussed in the Publication 3. In this context, we hypothesize that in addition to the conditional strategy, individual's vulnerability to stress may be involved in facultative migration: the most sensitive would be no longer able to continue the migration and would have to settle down before reaching the river. The underlying mechanisms remain to be elucidated but we already showed that marine non-synchronized glass eels displayed lower expression levels of metabolism-related genes than their synchronized counterpart in the head (but not in viscera nor in muscle).

Conclusions and perspectives

Overall, these findings suggest a link between individual energy mobilization and migratory ability: non-synchronized glass eels tend to mobilize more energy than synchronized fish, possibly due to the higher sensitivity to stress in the former. We hypothesize that during the stressful upstream migration, sensitive individuals could devote more energy to cope with environmental stresses and thus less energy to migrate, which produces progressive settlement, especially in spring where energy stores are low. To test this hypothesis, it might be interesting at different seasons to sample glass eels along a spatial gradient from marine coast to estuary, and then analyze several parameters of energy mobilization (including the levels of transcripts related to lipid/protein catabolism, as well as activities of lipases, phospholipases and proteases) but also stress indicators (levels of stress hormone and stress protein, and oxidative state) on different tissues (brain, liver, muscle). Meanwhile, the swimming behavior (activity, proportion of synchronized fish) of sampled glass eels could be determined in order, finally, to decipher the existing links between energy mobilization systems, stress and behavioral traits along the spatial gradient from marine coast to estuary (Figure 3-2).

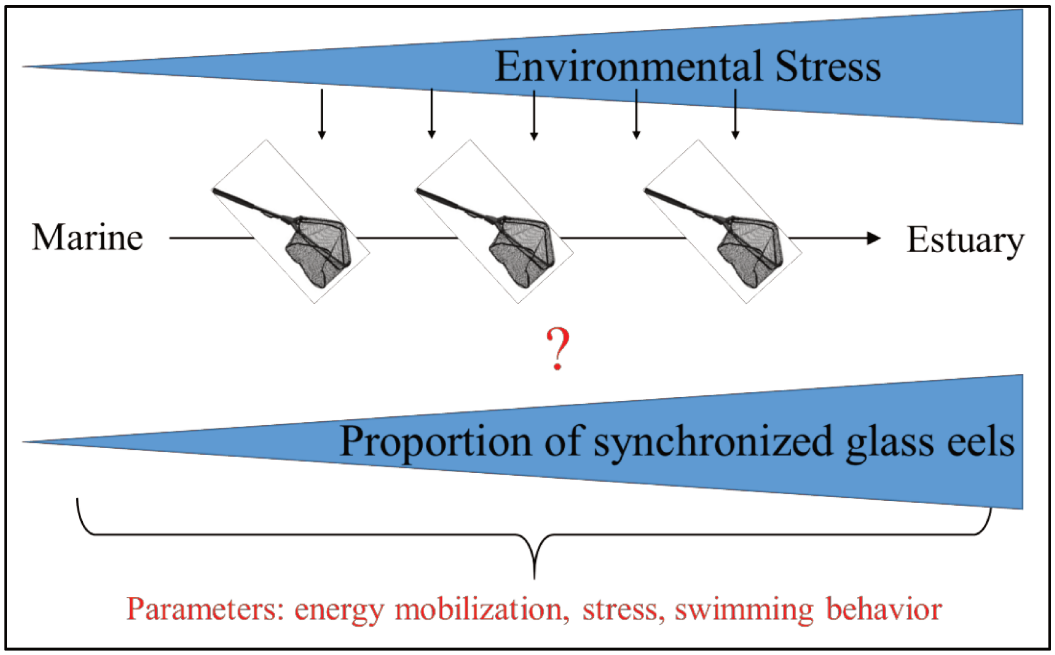


Figure 3-2. Experimental design for future research focusing on environmental stress and estuarine settlement - A protocol showing the processes of gradient sampling from marine to estuarine sites, and the parameters to be analyzed to indicate individual energy mobilization, stress state and swimming behavior.

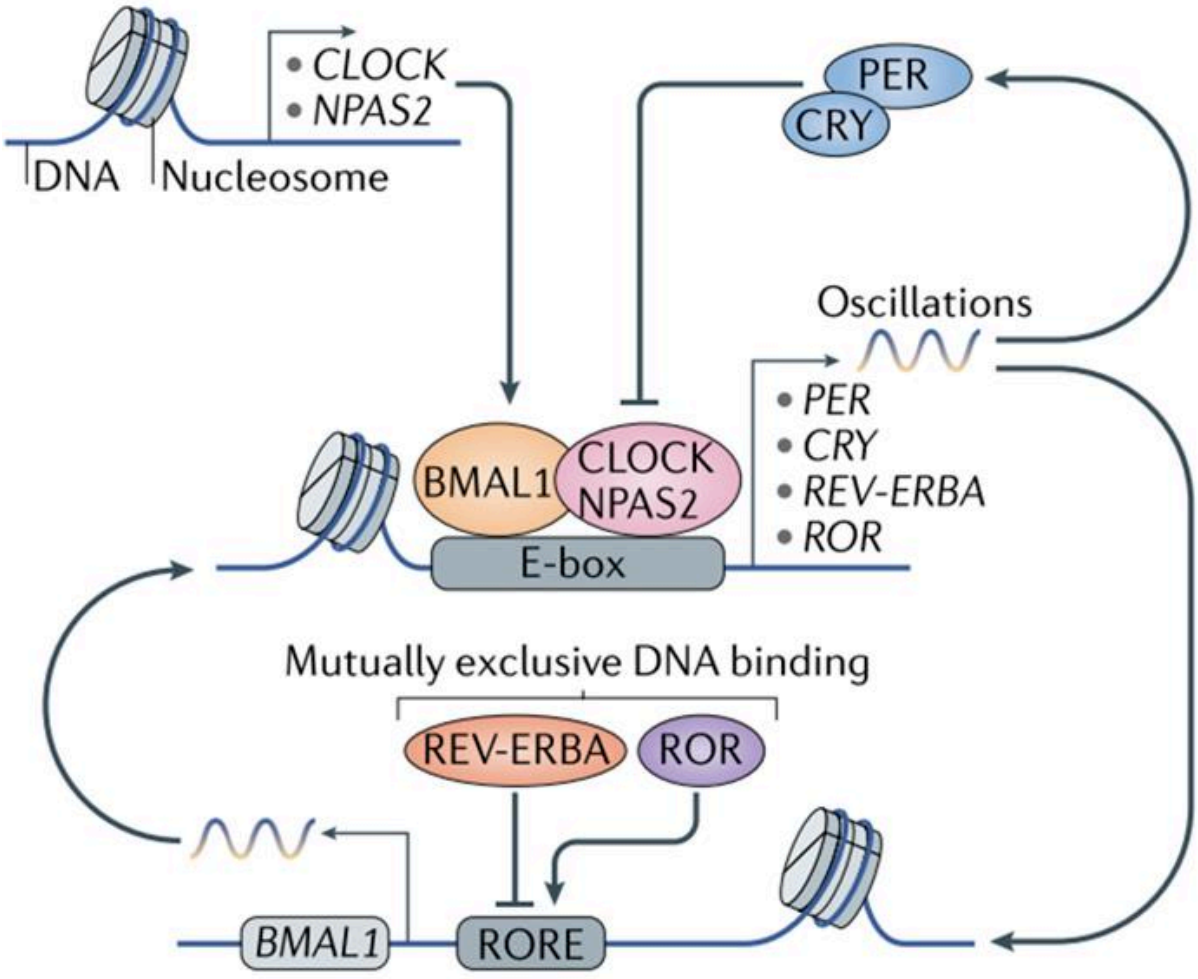


Figure 3-3. Transcriptional/translational feedback loops (TTFL) in mammalian circadian clock. Circadian locomotor output cycles kaput-aryl hydrocarbon receptor nuclear translocator- like protein 1 (CLOCK-BMAL1) and neuronal PAS domain protein 2 (NPAS2)-BMAL1 heterodimers activate the transcription of *PER*, *CRY*, *REV-ERBA* and *ROR* genes. Period (PER) and cryptochrome (CRY) proteins repress CLOCK-BMAL1-dependent and NPAS2–BMAL1-dependent transcription. Nuclear receptor subfamily 1 group D (REV-ERBA) and RAR- related orphan receptor (ROR) proteins drive rhythmic BMAL1 transcription from ROR response elements (ROREs) in the promoter region (Reinke and Asher, 2019).

3.3 Clocks

Once recruited to the coast, European glass eels migrate up estuary using a selective tidal stream transport (STST) as a main mechanism moving towards rivers. They synchronize their swimming activity to the tide with a period of 12.4 h, as well as to a diurnal cycle with a period of 24 h, avoiding light and swimming mainly during the night (Forward and Tankersley, 2001; Tesch, 2003). This circatidal/circadian rhythmic migration has been recognized to be controlled through endogenous clock(s) (Bolliet et al., 2007; Wippelhauser and McCleave, 1987).

The essential role of the circadian clock is to synchronize cellular, physiological, and behavioral processes, such as eating habits and locomotor activity (Isorna et al., 2017). In mammals, the molecular circadian oscillators in both central and peripheral tissues are controlled by the several transcriptional-translational events driven by the so-called "CLOCK" genes. In details, the products of the genes *BMAL1* (also known as *ARNTL*), *CLOCK* and *NPAS2* form CLOCK-BMAL1 and NPAS2-BMAL1 heterodimers, which activate the transcription of the genes *PERIOD* (*PER*) and *CRYPTOCHROME* (*CRY*). Once PER and CRY have reached a critical concentration, they in turn repress CLOCK-BMAL1-dependent and NPAS2-BMAL1-dependent transcription. The alternating activation and suppression of the BMAL1/CLOCK/NPAS2-driven positive loop and PER/CRY-controlled negative loop result in a circadian oscillation in the molecular clock (Figure 3-3; Lande-Diner et al., 2013; Reinke and Asher, 2019; Shearman et al., 2000). Expression of these core clock genes inside cells influence many signaling pathways which allows the cells to identify the time of day and perform appropriate function, thus organize behavior and physiology to adapt to daily environmental cycles (Allada and Chung, 2010). In fish, circadian clocks were initially thought to be located in the central nervous system. However, there is now strong evidences that the circadian system is formed by a network of central and peripheral oscillators that are coordinated to control circadian rhythms (Albrecht, 2012; Schibler et al., 2015).

In contrast to the well-mapped circadian clock, knowledges on the circatidal system, the location of the clock(s) and its molecular orchestration remain scarce. Different to the circadian pacemaker driven mainly by light cues, there are evidences showing that circatidal clock are adjusted to more complex external cues - i.e. odour, turbulence, salinity, electrical fields and water current reversal (Bolliet et al., 2007; Cresci et al., 2019; Creutzberg, 1961; Edeline et al., 2005; McCleave and Kleckner, 1982; Wippelhauser and McCleave, 1987), and different cues may probably act synergistically to synchronize a tidal function or behaviour (Warman and Naylor, 1995). Given the gap in the mechanistic knowledge concerning circatidal clock, circadian clock genes have been studied to investigate their potential interaction with circatidal rhythms. Intertidal fiddler crabs (*Uca pugnax*) and green shore crabs (*Carcinus maenas*) held in constant conditions can continue their locomotor activity bouts at the times of expected low water or high water with

a 12.4 h interval between peaks (Wilcockson and Zhang, 2008). Similar robust circatidal locomotor rhythms in constant conditions have been also monitored in smaller crustacea such as the cirolanid isopod *Eurydice pulchra* (Zhang et al., 2013). This free running behavior suggests the existence of circatidal endogenous oscillator in these animals. In *E. pulchra*, RNAi mediated disruption of the circadian clock had no effect on circatidal swimming rhythms, suggesting that the pacemakers driving circatidal rhythms is distinct from the circadian system of this species (Zhang et al., 2013). However, some interactions between these clocks regarding metabolic control has been evidenced. In O'Neill et al. (2015), the overoxidation of peroxiredoxin (PRX), whose oxidation–reduction cycles constitute a marker for circadian rhythms, and the expression of 10 mitochondrially encoded genes (such as subunits of NADH dehydrogenase, cytochrome b, and cytochrome c oxidase), both follows a circatidal (approximately 12.4 h) pattern in *E. pulchra*. But, to date, insight into the cellular or physiological basis of the tidal oscillators in fish is very limited, and it remains to be determined whether circatidal clocks share some common substrates with circadian clocks, or whether these two types of clocks are totally dissociated.

In the present thesis, we used one cue, the change in water current direction every 6.2 h, to mimic tide and synchronize the clock(s) and glass eels swimming activity. This allowed to distinguish two main swimming behaviors: glass eels which synchronized to the cue and swam in the water column in one direction during one period of 6.2 h and fish that did not synchronized and stayed buried in the substratum. The use of only one synchronizer to mimic tides may have not be strong enough to identify all individuals with a good propensity to migrate, explaining the lack of differences between synchronized and non-synchronized fish in some of our experiments. However, in an experiment that we conducted but did not include in the present thesis, three groups of glass eels originating from a same catch were submitted successively to a change in water current direction during 6.2 h. Results showed that the number of synchronized fish was not significantly different between the groups revealing at least the repeatability of the synchronizer. Thus, we hypothesize that although glass eels may present a heterogeneity in their ability to synchronize to the environmental cues, those which synchronize to only one cue should represent individuals with a high ability to synchronize to the tide. On the other hand, it is possible that our experimental design led to identify a group of non-synchronized fish more heterogeneous, including fish with low to medium ability to synchronize to the tide or, at least, fish which need several synchronizers to strengthen the synchronization of their swimming activity.

Energy-clock interaction

Interestingly, our results showed a higher number of glass eels synchronized to the change in water current direction in autumn than in spring (Publications 1 and 2). Our results also provided evidences that the strongest difference between autumn and spring fish concern their energetic status, autumn individuals

presenting higher energy reserves and likely a higher ability to produce energy. This raises the question of the potential relationships between energy and clocks mechanisms. Previous finding in mammals demonstrated the existence of such a crosstalk between metabolism and the circadian clock. AMP-activated protein kinase (AMPK), a critical sensor of energy status that maintains cellular energy homeostasis, has thus been reported to transmit energy-dependent signals to the mammalian circadian clock (Jordan and Lamia, 2013). AMPK does so, not only by driving the phosphorylation and destabilization of CRY and PER proteins (Lamia et al., 2009; Um et al., 2011), but also by inducing autophagy, which has been shown to affect the circadian clock by selectively degrading CRY (Toledo et al., 2018). Another fuel-sensing molecule positioned at the crossroads of nutritional status and circadian regulation is SIRT1 (silent mating type information regulation 2 homolog 1). Like AMPK, SIRT1 has emerged as a critical cellular energy sensor (Bordone and Guarente, 2005; Haigis and Guarente, 2006). SIRT1 is a class III histone deacetylase (HDAC) that, in addition to histones, deacetylates numerous transcription factors and co-regulators (Imai et al., 2000; Landry et al., 2000). The circadian transcription factor CLOCK has been reported to have histone acetyltransferase (HAT) activity, and SIRT1 was identified as the HDAC that counteracts the HAT activity of CLOCK (Asher et al., 2008; Nakahata et al., 2008). Together, these findings clearly demonstrate the existence of a close link between energy status and clock machinery at least in mammals. It is then tempting to speculate that such interactions may be at play in the observed higher number of synchronized glass eels in autumn than in spring. However, an important research effort still needs (i) to determine the conservation in European eel of the link between energy metabolism and clock regulation, and (ii) to precisely characterize the factors involved in circatidal rhythm.

Stress-clock interaction

Our results also showed that estuarine glass eels presented a higher number of synchronized individuals than marine one suggesting a possible selection of glass eels during migration based on their ability to synchronize to local environmental cues (Publication 2). In addition, glass eels exposed to MeHg presented a lower percentage of synchronized individuals than controls (Publication 3). Both migration in an estuary and exposure to contaminants may be considered as a stress for glass eels, and numerous studies link stress to the arhythmicity and loss of oscillation in clock networks (Hernández-Pérez et al., 2019; Koch et al., 2017). The influence of stress on circadian rhythms has been addressed in rodents and Tahara et al. (2015) reported that an acute stress at the photoperiod onset causes a phase advance shift in mRNA expression rhythms of several core clock genes in peripheral organs. Meanwhile, when the stress was applied at different times during the photophase, it causes a phase delay or even loss of synchrony in the expression of these clock genes (Tahara et al., 2015) as well as loss of activity rhythms (Bartlang et al., 2015), indicating that the influence of stress on circadian clocks depends on the time of day. In fish, the most

frequent stressors are those related to changes in water quality such as temperature, salinity, oxygenation, and contaminants (Gesto et al., 2013; Wendelaar-Bonga, 1997), as well as stocking-density stress brought by intensive fish aquaculture (Hernández-Pérez et al., 2019). Short-term exposure to treated sewage and a sub-lethal PPCP mixture were reported to completely abolish the diurnal activity pattern in male mosquitofish (*Gambusia holbrooki*) reflected by reduced daytime locomotor activity (Melvin et al., 2016). Several studies have shown that exposure of fish to waterborne copper (Kim et al., 2017), bisphenol A (Rhee et al., 2014), ammonia (Jung et al., 2016), and the anti-inflammatory drug diclofenac (Lubiana et al., 2016; Prokkola et al., 2015) induces a strong disruption in the expression of "rhythm-generating" genes.Hypoxia can also reverse circadian rhythms in the spontaneous activity of fish (Svendsen et al., 2014), where the mechanism probably lies in the interplay of HIF-CLOCK-PER as demonstrated in mouse (Chilov et al., 2001).

Stress-induced phase resetting also occurs through the crosstalk between clock system and fish hypothalamic-pituitary-interrenal axis (HPI axis, comparable to hypothalamic-pituitary-adrenal (HPA) axis in mammals), which is a neurohormonal pathway mediating the adaptive response to stressors through the rhythmic activity of its end-effector, the glucocorticoid receptor. Circadian system imposes a daily rhythm on the HPI axis resulting in rhythmic synthesis and release of glucocorticoids (GCs, i.e. cortisol) (Lamia et al., 2011). In a reciprocal fashion, the HPI axis strongly influences the activity/circadian rhythm of the clock system through GCs (Helfrich-Forster, 2017). These hormones affect the peripheral clocks in almost all organs and tissues via the action of glucocorticoid receptor (GR) (Chrousos and Kino, 2005; Nader et al., 2010). As reported by Sánchez-Bretaño et al. (2016), GCs treatment enhances the expression of *per1* and inhibit *clock* and *bmal1* in the liver of goldfish. In addition, GRs signal on various kinases, among which are mitogen-activated protein kinases (MAPKs). Several MAPKs have been shown to affect clock function in various context in mammals (Akashi and Nishida, 2000), in birds (Sanade et al., 2000), and in flies (Dusik et al., 2014). Thus, we could hypothesize in fish that kinases activated by GC signaling may directly affect molecules of the circadian clock and constitute the crosstalk between the stress and circadian systems. Taken together, these reports suggest a possible negative role of stress influencing animal clock rhythm through the regulation of HPI axis produces.

The stress-induced change in melatonin synthesis may be another pathway that prevents fish from integrating environmental rhythmic information (Lopez-Patino et al., 2014). Melatonin is known as ‘time-keeper-hormone’ acting in the circadian system of vertebrates, and mainly produced by the pineal gland and the retina (Besseau et al., 2006; Cahill, 1996; Falcon and Meissl, 1981; Vuilleumier et al., 2007). Photoperiodic information is transduced by the retina/pineal organ into a rhythmic secretion of melatonin, which is released into blood circulation with high concentrations at night and low during day (Sanchez-

Vazquez et al., 2019). The pineal melatoninergic system in vertebrates has been reported to be influenced by stress (Benyassi et al., 2001; Zhao and Touitou, 1993) and in rodents, stress of physical activity every 2 h for the 24 h around the clock resulted in lower melatonin levels at night (Paredes et al., 2005). In fish, rainbow trout transferred from freshwater conditions (6 ‰) to isosmotic (12 ‰) and hyperosmotic conditions (18 ‰) showed an increased melatonin level at night in both short-term (6 h) and long-term (5 days) exposure (Lopez-Patino et al., 2011). Exposure to waterborne copper decreased plasma levels of serotonin and arylalkylamine N-acetyltransferase (AANAT2) proteins, which are two indicators of melatonin synthesis (Kim et al., 2017). Lopez-Patino et al. (2014) also indicated that stress of chasing and high-stocking density in rainbow trout decreased serotonin content in pineal, *aanat2* gene expression level, and AANAT enzyme activity at night.

Taken together, these studies argue in favor of a strong relationship between stress and circadian clocks. To my knowledge, such relationship has never been investigated with the circatidal system but it cannot be excluded that stress-clocks interactions may also exist in circatidal rhythms. Our results showed that both stressful environment and MeHg exposure markedly decreased the number of tidally synchronized individuals suggesting that stress may affect circatidal activity rhythm in glass eels. As proposed in the previous section, glass eels may present different sensitivity to stress. Concerning rhythmic behavior and considering that stress may affect the clock system, the most vulnerable individuals to stress may not be able to adopt selective tidal transport to migrate up estuary and be obliged to stop migration and settle in the estuary. In order to test the putative link of stress and tidal clock in glass eels, it is challenging but crucial to firstly identify the elements at play in modulating behavioral and physiological function in tune to tidal cycle, and then determine whether these elements respond to different stressors.

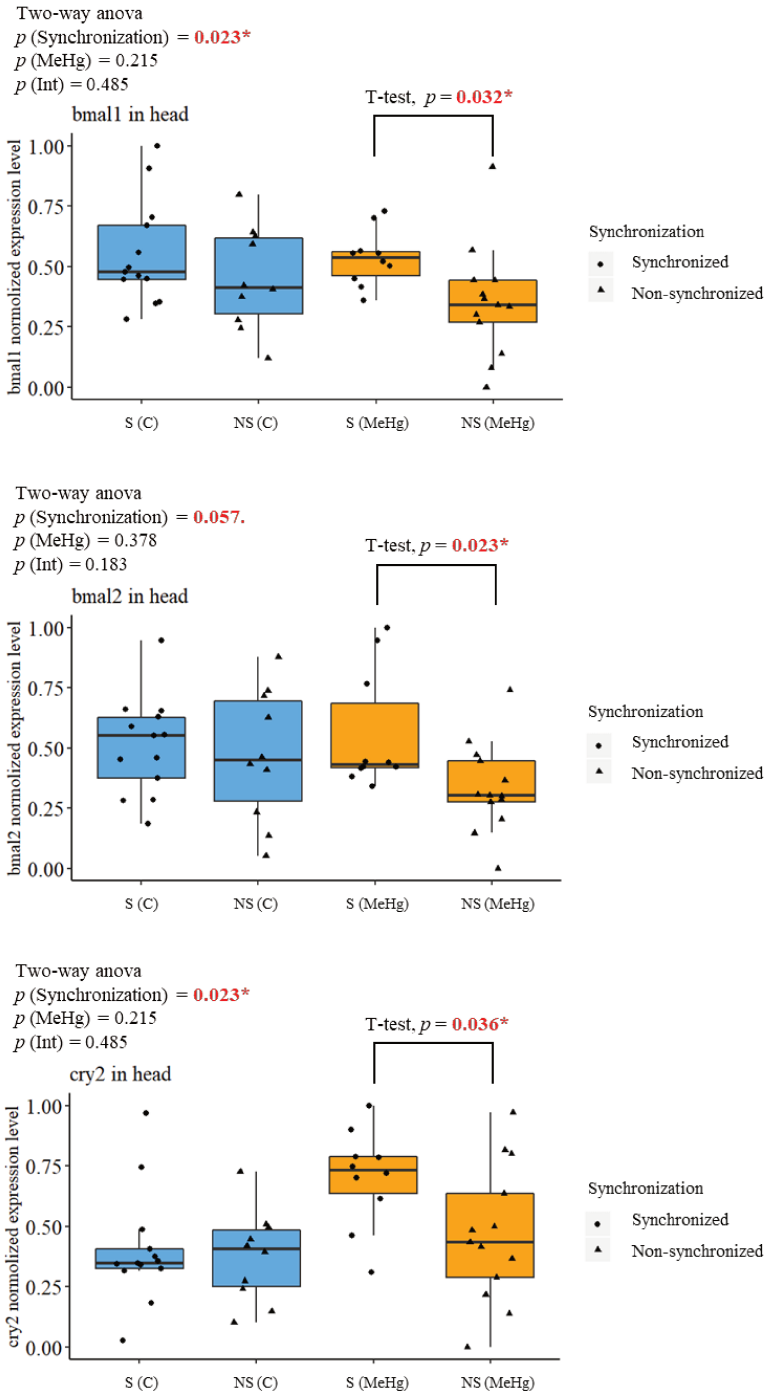


Figure 3-4. Comparison of the expression level of clock-related genes in the head of glass eels, in response to synchronization behavior and MeHg treatment. S- Synchronized behavior, NS- Non-synchronized behavior; Blue box- control group, yellow box- MeHg treatment group. P-values from two-way ANOVA are listed on each plot. Gene expression level in response to synchronization behavior within control and MeHg treatment group were tested, significant t-test p-values are marked on each plot.

Conclusions and perspectives

To sum up, our results suggest that the ability of glass eels to synchronize to the environment cues may be closely linked to both energetic status and stress, which may influence circatidal clocks. We hypothesize that stress derived from either endogenous energy condition or external environments may play a negative role in synchronization ability, wherein the most stressed /sensitive to stress may stop their migration.

To test this hypothesis, we should first test our experimental design and eliminate a potential bias from the synchronizer used. We could test fish swimming behavior using two tidal synchronizers such as water current direction and salinity changes and compare the strength of these combined synchronizers in terms of synchronized fish. Alternatively, we could synchronize glass eels to LD cycle, as what has been done in Bureau du Colombier et al. (2007, 2008), Claveau et al. (2015a) and Bolliet et al. (2017), and then submit the sorted synchronized and non-synchronized glass eels to the change in water current direction to check if the synchronized individuals under the two migratory cues are the same. Then, in order to test the relationship between glass eel's synchronization ability and its energy and stress conditions, we could carry out a gradient sampling from marine to estuary as described in the previous section (Figure 3-2), and the parameters would be focused here on energy metabolism-related gene expression, stress indicators, and clock-related gene expression. Parallel experiments need to be carried out in both autumn and spring.

To investigate the molecular mechanism of clock, we should first identify the potential clock genes in glass eel. We have already monitored the expression profile of several marker genes involved in circadian clock between synchronized and non-synchronized glass eels after 7-day MeHg exposure in a photoperiod controlled condition (unpublished data). It is interesting to note that three genes (bmal1, bmal2 and cry2) were differentially expressed in head, but not in viscera or muscle. These genes expressed more in synchronized fish than the non-synchronized fish, particularly when the fish were contaminated by MeHg (Figure 3-4). This result suggests a probability that the marker genes in circadian system, at least bmal and cry2, may also have a contribution to circatidal rhythm of glass eel's swimming, and environmental stress may influence these pacemakers. Given the large knowledge gap in circatidal pacemakers in aquatic organisms, initiating the investigation from circadian makers to seek out the makers of circatidal clock could be worth trying.

3.4 Potential genetic basis for divergent sensitivities to stress

Above all, we have stated the potential roles of energy stores, energy mobilization and clock system in glass eel's facultative migration, and our results strongly direct at their close associations with stress sensitivity: individuals which are more sensitive to stress factors may subsequently settle, instead of reaching in freshwater habitat. However, the underlying mechanisms behind the interindividual variability in stress sensitivity, and the process of local selection on this phenotypic trait remain unclear.

Because of panmixia, the existence of different eel lines varying in allele frequencies at some loci suggest a natural selection occurring in this species (Koehn and William, 1978). Indeed, large geographic range occupied by eel brings dramatically different environmental conditions corresponding to different local selection, i.e. latitudinal variation in temperature (Gagnaire et al., 2012). Meanwhile, eel also occupies a wide variety of freshwater, brackish and saltwater habitats, which also represent selective forces (Pavey et al., 2015). Thus, it is believed in American eel that a genetic basis exists behind the dispersal and habitat choice in this species (Babin et al., 2017). Gagnaire et al. (2012) have advanced the understanding of the genetic basis of local adaption through identifying several genes that present spatially varying selection associated with habitat heterogeneity, where these genes are related to lipid/saccharide/protein metabolism, defense response and molecular function. Pavey et al. (2015) collected genetic samples from yellow eel at freshwater and brackish/saltwater habitats, two known ecotypes, and through a genome-wide association study demonstrated a polygenic discrimination of habitat ecotypes in American eels. In this study, 331 SNPs out of 42,424 were associated with the divergent ecotypes. These 331 SNPs are associated with 101 genes encoding proteins: the freshwater module subset is characterized by enrichment of transcription factors and calcium ion regulation, while the brackish/saltwater module subset is enriched in growth factor receptor, vascular and morphological development. This study brings support to the hypothesis that genetic variation is at play behind the ecotypic differences in American eels. In a genome scan study based on 50,354 RAD-seq markers in European glass eels collected from eight locations between 34 and 64 ˚N, Pujolar et al. (2014) identified 754 loci probably influenced by local selection. These 754 SNPs include genetic functions of calcium signaling, neuroactive ligand-receptor interaction and circadian rhythm. It is interesting that within the circadian rhythm pathway, a central clock gene PERIOD (*per*) shows the strongest pattern of covariance with two environmental variables, temperature and latitude, may indicate a genetic basis of associated local selection and European glass eel clock system (Pujolar et al., 2014). This finding is consistent with our hypothesis about the selection occurring on clock regulation during glass eel's estuarine migration. Although the link between these genetic functions identified in these genotyping studies and stress response is not clear, in European eel as a sister species of American eel we probably

could also hypothesize the presence of genetic differences underpinning the phenotypic differences in stress sensitivity. For example, some genotypes may be more or less capable to cope with stress encountered.

Conclusions and perspectives

Taken together, these studies and our results lead to the hypothesis of the existence of a genetic basis for stress sensitivity in the European eel, with some individuals having a greater ability to cope with stress than others, making them more able to migrate up the estuary.

Genotyping studies carried out in autumn and spring among glass eels captured along a gradient from marine to estuarine habitats may help to shed light on the actions of genetic variation on glass eels' stress response, for example we could try to determine the spatially-varying enrichment of the stress sensitivity-related, metabolism-related, and clock-related factors along downstream to upstream habitats, thus to seek out the possible stress-metabolism and stress-clock interactions (Figure 3-5).

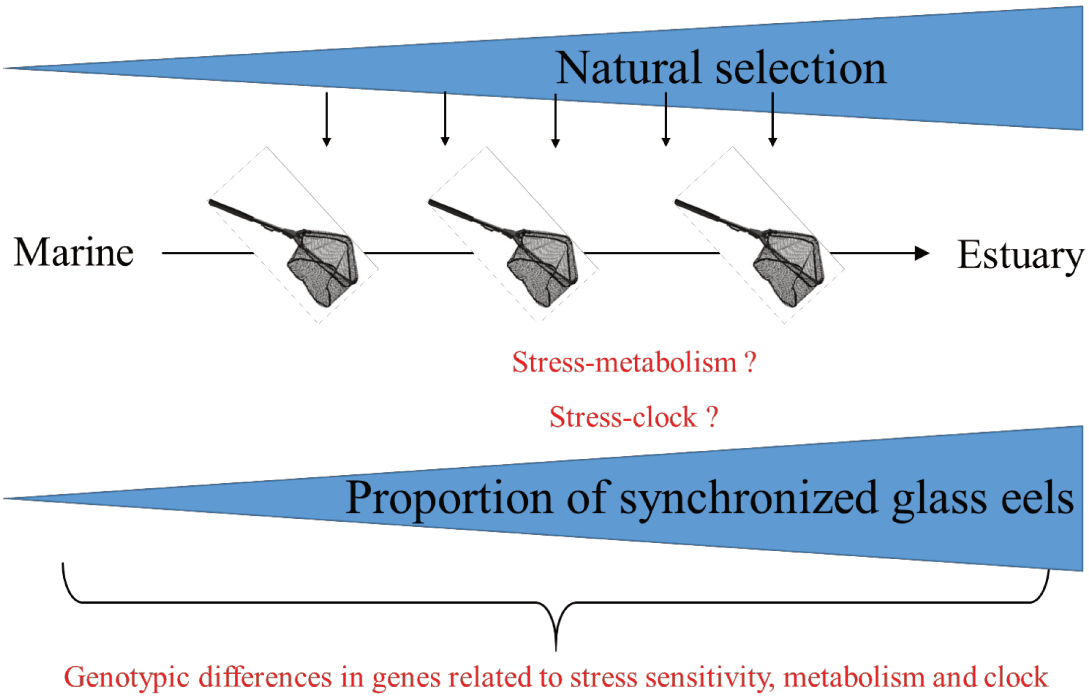


Figure 3-5. Experimental design for future research focusing on genotypes in relation to stress, metabolism and clocks - A protocol showing the processes of gradient sampling from marine to estuarine sites, and the genotyping study aiming to map the spatially-varying enrichment of the stress sensitivity-related, metabolic, and clock-related factors.

Chapter 4 – CONCLUSIONS

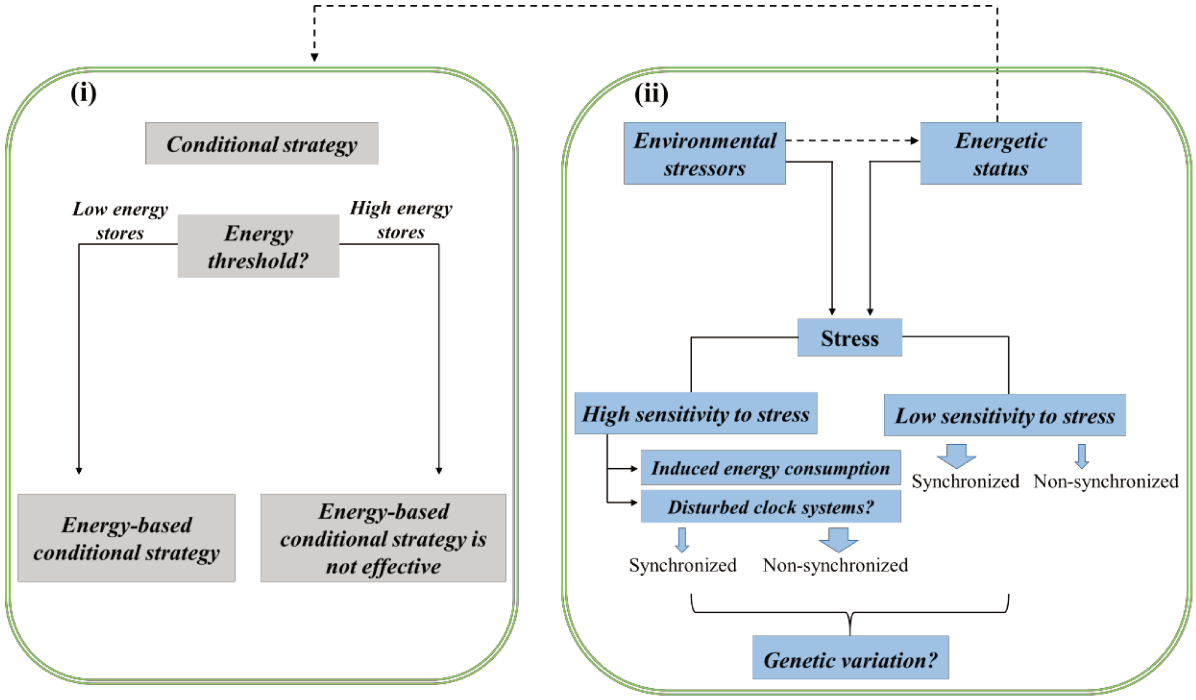


Figure 4-1. Hypothetical mechanisms of facultative migration in European glass eel concerning the putative energy threshold in conditional strategy, and the various sensitivities to stress. (i) Hypothesis regarding conditional strategy (in grey): an energy threshold may exist, below which glass eel's swimming behavior should closely depend on its energy status, while above which fish swimming behavior is not conditioned by energy status; (ii) Hypothesis regarding environmental stressors (in blue): glass eels may display a variation in their sensitivities to stress derived from both environment and endogenous physiology (energetic status). High sensitivity to stress may contribute to an increased proportion of non-synchronized fish which will settle in estuaries. A genetic basis may be underlying the varied sensitivities to stress or abilities to cope with stress. Blue arrows in (ii) represent the observed migratory behavior (thick arrows and narrow arrows respectively represent increased and decreased probability of synchronized/non-synchronized behavior). Links between these two hypotheses may exist that low energetic status could increase individual's stress, meanwhile environmental stressor could in turn increase energy distress thus influence the energy-based conditional strategy (represented by dash arrows).

Facultative migration in European eels is closely associated with diverse habitat use between individuals and has likely a profound impact on the species sex determination. The present thesis investigated the role of a conditional strategy based on energy on the propensity to migrate in glass eels. Energy stores but also the ability of energy mobilization were analyzed in relation to individual's swimming behavior when submitted to water current reversal every 6.2 h to mimic tide.

Our results showed that the theory of conditional strategy based on energy stores cannot fully explain the facultative migration in glass eels. We proposed that energy stores may limit glass eels' migration but only when this factor become limiting, wherein a threshold of energy stores may exist (Figure 4-1).

Results also provided some evidences that glass eels may present different sensitivity to physiological (energetic) and/or environmental stress. Individuals showing a low ability to cope with stress may have a higher propensity to settle before reaching freshwater than fish less sensitive to stress.

Inter-individual variations in the sensitivity to stress, mechanisms involved in the effect of stress on glass eels (metabolism, clocks…) and the potential relationships with the conditional strategy represent interesting and exciting avenues to explore in the future to better understand facultative migration. The head may be particularly interesting to focus on in relation to rhythmic behavior and/or metabolism regulation.

BIBLIOGRAPHY

Adam, G., Feunteun, E., Prouzet, P., and Rigaud, C. (2008). L'*anguille* européenne: Indicateurs d'abondance et de colonisation. Editions QUAE, Collection Savoir-Faire, 393.

Akashi, M., and Nishida, E. (2000). Involvement of the MAP kinase cascade in resetting of the mammalian circadian clock. Genes & Development 14, 645-649.

Albrecht, U. (2012). Timing to Perfection: The Biology of Central and Peripheral Circadian Clocks. Neuron 74, 246-260.

Allada, R., and Chung, B.Y. (2010). Circadian organization of behavior and physiology in Drosophila. Annual review of physiology, 72, 605-624.

Alonso, D., Pineda, P., Olivero, J., Gonzalez, H., and Campos, N. (2000). Mercury levels in muscle of two fish species and sediments from the Cartagena Bay and the Cienaga Grande de Santa Marta, Colombia. Environmental Pollution 109, 157-163.

Alvarez, D., and Nicieza, A.G. (2005). Is metabolic rate a reliable predictor of growth and survival of brown trout (*Salmo trutta*) in the wild? Canadian Journal of Fisheries and Aquatic Sciences 62, 643-649.

Antunes dos Santos, A., Ferrer, B., Marques Gonçalves, F., Tsatsakis, A. M., Renieri, E. A., Skalny, A. V., Farina, M., Rocha, J. B. and Aschner, M. (2018). Oxidative stress in methylmercury-induced cell toxicity. Toxics, 6(3), 47.

Ara, T., Kotake, A., and Ohji, M. (2008). Variation in migratory history of Japanese eels, *Anguilla japonica*, collected in the northernmost part of its distribution. Journal of the Marine Biological Association of the United Kingdom 88, 1075-1080.

Arai, T. (2014). Do we protect freshwater eels or do we drive them to extinction? Springerplus 3.

Arai, T. (Ed.). (2016). Biology and Ecology of Anguillid Eels. Boca Raton: CRC Press.

Arai, T., Chino, N., and Kotake, A. (2009). Occurrence of estuarine and sea eels *Anguilla japonica* and a migrating silver eel *Anguilla anguilla* in the Tokyo Bay area, Japan. Fisheries Science 75, 1197-1203.

Arai, T., Kotake, A., and McCarthy, T.K. (2006). Habitat use by the European eel *Anguilla anguilla* in Irish waters. Estuarine Coastal and Shelf Science 67, 569-578.

Arai, T., Kotake, A., Aoyama, T., Hayano, H., and Miyazaki, N. (2002). Identifying sea-run brown trout, *Salmo trutta*, using Sr : Ca ratios of otolith. Ichthyological Research 49, 380-383.

Arai, T., Kotake, A., Lokman, P.M., Miller, M.J., and Tsukamoto, K. (2004). Evidence of different habitat use by New Zealand freshwater eels *Anguilla australis* and *A-dieffenbachii*, as revealed by otolith microchemistry. Marine Ecology Progress Series 266, 213-225.

Arai, T., Kotake, A., Ohji, M., Miller, M.J., Tsukamoto, K., and Miyazaki, N. (2003a). Occurrence of sea eels of *Anguilla japonica* along the Sanriku Coast of Japan. Ichthyological Research 50, 78-81.

Arai, T., Kotake, A., Ohji, M., Miyazaki, N., and Tsukamoto, K. (2003b). Migratory history and habitat use of Japanese eel *Anguilla japonica* in the Sanriku Coast of Japan. Fisheries Science 69, 813-818.

Arai, T., Otake, T., and Tsukamoto, K. (2000). Timing of metamorphosis and larval segregation of the Atlantic eels *Anguilla rostrata* and *A-anguilla*, as revealed by otolith microstructure and microchemistry. Marine Biology 137, 39-45.

Archer, S., Hope, A., and Partridge, J.C. (1995). The molecular basis for the green-blue sensitivity shift in the rod visual pigments of the European eel. Proceedings of the Royal Society B-Biological Sciences 262, 289-295.

Arito, H., Hara, N., and Torii, S. (1983). Effect of methylmercury chloride on sleep-waking rhythms in rats. Toxicology 28, 335-345.

Armstrong, J.D., Priede, I.G., and Lucas, M.C. (1992). The link between respiratory capacity and changing metabolic demands during growth of northern pike, *Esox-lucius* L. Journal of Fish Biology 41, 65-75.

Arribas, C., Fernandez-Delgado, C., Oliva-Paterna, F.J., and Drake, P. (2012). Oceanic and local environmental conditions as forcing mechanisms of the glass eel recruitment to the southernmost European estuary. Estuarine Coastal and Shelf Science 107, 46-57.

Aschner, M. (1989). Brain, kidney and liver ^{203}Hg-methyl mercury uptake in the rat: relationship to the neutral amino acid carrier. Pharmacology & toxicology, 65(1), 17-20.

Asehoff, J. and Pohl, H. (1970). Rhythmic variations in energy metabolism. Fed. Proc. 29:1541-1552.

Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., Mostoslavsky, R., Alt, F.W., and Schibler, U. (2008). SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 134, 317-328.

Auer, S.K., Bassar, R.D., Salin, K., and Metcalfe, N.B. (2016a). Repeatability of metabolic rate is lower for animals living under field versus laboratory conditions. Journal of Experimental Biology 219, 631-634.

Auer, S.K., Salin, K., Anderson, G.J., and Metcalfe, N.B. (2016b). Flexibility in metabolic rate and activity level determines individual variation in overwinter performance. Oecologia 182, 703-712.

Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J., and Metcalfe, N.B. (2015). Flexibility in metabolic rate confers a growth advantage under changing food availability. Journal of Animal Ecology 84, 1405-1411.

Baar, K., Wende, A.R., Jones, T.E., Marison, M., Nolte, L.A., Chen, M., Kelly, D.P., and Holloszy, J.O. (2002). Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. Faseb Journal 16, 1879-1886.

Baatrup, E., and Doving, K.B. (1990). Histochemical-demonstration of mercury in the olfactory system of salmon (*Salmo-salar* L) following treatments with dietary methylmercuric chloride and dissolved mercuric-chloride. Ecotoxicology and Environmental Safety 20, 277-289.

Babin, C., Gagnaire, P.A., Pavey, S.A., and Bernatchez, L. (2017). RAD-Seq Reveals Patterns of Additive Polygenic Variation Caused by Spatially-Varying Selection in the American Eel (*Anguilla rostrata*). Genome Biology and Evolution 9, 2974-2986.

Badgujar, P.C., Pawar, N.N., Chandratre, G.A., Telang, A.G., and Sharma, A.K. (2015). Fipronil induced oxidative stress in kidney and brain of mice: Protective effect of vitamin E and vitamin C. Pesticide Biochemistry and Physiology 118, 10-18.

Baltazar-Soares, M., Biastoch, A., Harrod, C., Hanel, R., Marohn, L., Prigge, E., Evans, D., Bodles, K., Behrens, E., Boning, C.W., et al. (2014). Recruitment Collapse and Population Structure of the European Eel Shaped by Local Ocean Current Dynamics. Current Biology 24, 104-108.

Bardonnet, A., and Riera, P. (2005). Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: new insights from stable isotope analysis. Estuarine Coastal and Shelf Science 63, 201-209.

Bartlang, M.S., Oster, H., and Helfrich-Forster, C. (2015). Repeated Psychosocial Stress at Night Affects the Circadian Activity Rhythm of Male Mice. Journal of Biological Rhythms 30, 228-241.

Benyassi, A., Schwartz, C., Ducouret, B., and Falcon, J. (2001). Glucocorticoid receptors and serotonin N-acetyltransferase activity in the fish pineal organ. Neuroreport 12, 889-892.

Berg, K., Puntervoll, P., Valdersnes, S., and Goksoyr, A. (2010). Responses in the brain proteome of Atlantic cod (*Gadus morhua*) exposed to methylmercury. Aquatic Toxicology 100, 51-65.

Bermejo-Nogales, A., Calduch-Giner, J.A., and Perez-Sanchez, J. (2015). Unraveling the Molecular Signatures of Oxidative Phosphorylation to Cope with the Nutritionally Changing Metabolic Capabilities of Liver and Muscle Tissues in Farmed Fish. Plos One 10.

Berntssen, M.H.G., Aatland, A., and Handy, R.D. (2003). Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquatic Toxicology 65, 55-72.

Bertin, L. (1951). Les anguilles. Variation, croissance, euryhalinite, toxicite, hermaphrodisme juvenile et sexualite, migrations, me´tamorphoses. Paris: Payot.

Besseau, L., Benyassi, A., Moller, M., Coon, S.L., Weller, J.L., Boeuf, G., Klein, D.C., and Falcon, J. (2006). Melatonin pathway: breaking the 'high-at-night' rule in trout retina. Experimental Eye Research 82, 620-627.

Beullens, K., Eding, E. H., Gilson, P., Ollevier, F., Komen, J. and Richter, C. J. J. (1997). Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L.) maintained in captivity. Aquaculture 153, 135–150.

Biro, P.A., and Stamps, J.A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology & Evolution 25, 653-659.

Bishop, R.E., and Torres, J.J. (1999). Leptocephalus energetics: Metabolism and excretion. Journal of Experimental Biology 202, 2485-2493.

Bishop, R.E., and Torres, J.J. (2001). Leptocephalus energetics: assembly of the energetics equation. Marine Biology 138, 1093-1098.

Bodine, S.C., Latres, E., Baumhueter, S., Lai, V.K.M., Nunez, L., Clarke, B.A., Poueymirou, W.T., Panaro, F.J., Na, E.Q., Dharmarajan, K., et al. (2001). Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294, 1704-1708.

Boivin, B., Castonguay, M., Audet, C., Pavey, S.A., Dionne, M., and Bernatchez, L. (2015). How does salinity influence habitat selection and growth in juvenile American eels Anguilla rostrata? Journal of Fish Biology 86, 765-784.

Boldsen, M.M., Norin, T., and Malte, H. (2013). Temporal repeatability of metabolic rate and the effect of organ mass and enzyme activity on metabolism in European eel (*Anguilla anguilla*). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 165, 22-29.

Bolliet, V., and Labonne, J. (2008). Individual patterns of rhythmic swimming activity in *Anguilla anguilla* glass eels synchronised to water current reversal. Journal of Experimental Marine Biology and Ecology 362, 125-130.

Bolliet, V., Claveau, J., Jarry, M., Gonzalez, P., Baudrimont, M., and Monperrus, M. (2017). Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European glass eels (*Anguilla anguilla*). Physiology & Behavior 169, 33-40.

Bolliet, V., Lambert, P., Rives, J., and Bardonnet, A. (2007). Rhythmic swimming activity in *Anguilla anguilla* glass eels: Synchronisation to water current reversal under laboratory conditions. Journal of Experimental Marine Biology and Ecology 344, 54-66.

Bolsoni-Lopes, A., and Alonso-Vale, M.I.C. (2015). Lipolysis and lipases in white adipose tissue - An update. Archives of Endocrinology Metabolism 59, 335-342.

Bonhommeau, S., Le Pape, O., Gascuel, D., Blanke, B., Treguier, A.M., Grima, N., Vermard, Y., Castonguay, M., and Rivot, E. (2009). Estimates of the mortality and the duration of the trans-Atlantic migration of European eel *Anguilla anguilla* leptocephali using a particle tracking model. Journal of Fish Biology 74, 1891-1914.

Bordone, L. and Guarente, L. (2005). Calorie restriction, SIRT1 and metabolism: understanding longevity. Nature reviews Molecular cell biology, 6(4), 298-305.

Bourdineaud, J.P., Rossignol, R., and Brethes, D. (2013). Zebrafish: A model animal for analyzing the impact of environmental pollutants on muscle and brain mitochondrial bioenergetics. International Journal of Biochemistry & Cell Biology 45, 16-22.

Bower, N.I., Taylor, R.G., and Johnston, I.A. (2009). Phasing of muscle gene expression with fasting-induced recovery growth in Atlantic salmon. Frontiers in Zoology 6.

Bradley, M.A., Barst, B.D., and Basu, N. (2017). A Review of Mercury Bioavailability in Humans and Fish. International Journal of Environmental Research and Public Health 14.

Branco, V., and Carvalho, C. (2019). The thioredoxin system as a target for mercury compounds. Biochimica Et Biophysica Acta-General Subjects 1863.

Branco, V., Canario, J., Holmgren, A., and Carvalho, C. (2011). Inhibition of the thioredoxin system in the brain and liver of zebra-seabreams exposed to waterborne methylmercury. Toxicology and Applied Pharmacology 251, 95-103.

Branco, V., Coppo, L., Sola, S., Lu, J., Rodrigues, C.M.P., Holmgren, A., and Carvalho, C. (2017). Impaired cross-talk between the thioredoxin and glutathione systems is related to ASK-1 mediated apoptosis in neuronal cells exposed to mercury. Redox Biology 13, 278-287.

Brett, J.R. (1962). Some considerations in the study of respiratory metabolism in fish, particularly salmon. Journal of the Fisheries Research Board of Canada 19, 1025-1038.

Bridges, C.C., and Zalups, R.K. (2010). Transport of inorganic mercury and methylmercury in target tissues and organs. Journal of Toxicology and Environmental Health-Part B-Critical Reviews 13, 385-410.

Bruni, F, Polosa, P.L., Gadaleta, M.N., Cantatore, P. and Roberti, M. (2010). Nuclear Respiratory Factor 2 Induces the Expression of Many but Not All Human Proteins Acting in Mitochondrial DNA Transcription and Replication. J Bio Chem, 285: 3939-3948.

Bustamante, H.A., Gonzalez, A.E., Cerda-Troncoso, C., Shaughnessy, R., Otth, C., Soza, A., and Burgos, P.V. (2018). Interplay Between the Autophagy-Lysosomal Pathway and the Ubiquitin-

Proteasome System: A Target for Therapeutic Development in Alzheimer's Disease. Frontiers in Cellular Neuroscience 12.

Cahill, G.M. (1996). Circadian regulation of melatonin production in cultured zebrafish pineal and retina. Brain Research 708, 177-181.

Cai, Q., and Tammineni, P. (2016). Alterations in Mitochondria! Quality Control in Alzheimer's Disease. Frontiers in Cellular Neuroscience 10.

Cambier, S., Benard, G., Mesmer-Dudons, N., Gonzalez, P., Rossignol, R., Brethes, D., and Bourdineaud, J.P. (2009). At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebra fish (*Danio rerio*). International Journal of Biochemistry & Cell Biology 41, 791-799.

Cambier, S., Gonzalez, P., Durrieu, G., Maury-Brachet, R., Boudou, A., and Bourdineaud, J.P. (2010). Serial Analysis of Gene Expression in the Skeletal Muscles of Zebrafish Fed with a Methylmercury-Contaminated Diet. Environmental Science & Technology 44, 469-475.

Cambier, S., Gonzalez, P., Mesmer-Dudons, N., Brethes, D., Fujimura, M., and Bourdineaud, J.P. (2012). Effects of dietary methylmercury on the zebrafish brain: histological, mitochondrial, and gene transcription analyses. Biometals 25, 165-180.

Campana, S.E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188, 263-297.

Campbell, C.T., Kolesar, J.E., and Kaufman, B.A. (2012). Mitochondrial transcription factor A regulates mitochondrial transcription initiation, DNA packaging, and genome copy number. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms 1819, 921-929.

Campello, L., Esteve-Rudd, J., Cuenca, N., and Martin-Nieto, J. (2013). The Ubiquitin-Proteasome System in Retinal Health and Disease. Molecular Neurobiology 47, 790-810.

Cardoso, P.G., Pereira, E., Duarte, A.C., and Azeiteiro, U.M. (2014). Temporal characterization of mercury accumulation at different trophic levels and implications for metal biomagnification along a coastal food web. Marine Pollution Bulletin 87, 39-47.

Careau, V., Thomas, D., Humphries, M.M., and Reale, D. (2008). Energy metabolism and animal personality. Oikos 117, 641-653.

Cariccio, V.L., Sama, A., Bramanti, P., and Mazzon, E. (2019). Mercury Involvement in Neuronal Damage and in Neurodegenerative Diseases. Biological Trace Element Research 187, 341-356.

Carratù, M. R. and Signorile, A. (2015). Methyl mercury injury to CNS: mitochondria at the core of the matter. Open Acc. Toxicol, 1(2), 1-6.

Carty, A.J., Malone, S.F. (1979). The chemistry of mercury in biological systems. In: The Biogeochemistry of Mercury in the Environment (Nriagu J.O., ed). New York: Elsevier/North Holland, 433–459.

Carvalho, C.M.L., Chew, E.H., Hashemy, S.I., Lu, J., and Holmgren, A. (2008). Inhibition of the human thioredoxin system - A molecular mechanism of mercury toxicity. Journal of Biological Chemistry 283, 11913-11923.

Cassan-Wang, H., Soler, M., Yu, H., Camargo, E.L.O., Carocha, V., Ladouce, N., Savelli, B., Paiva, J.A.P., Leple, J.C., and Grima-Pettenati, J. (2012). Reference Genes for High-Throughput Quantitative Reverse Transcription-PCR Analysis of Gene Expression in Organs and Tissues of *Eucalyptus* Grown in Various Environmental Conditions. Plant and Cell Physiology 53, 2101-2116.

Casselman, J.M. and Cairns, D.K. (2003). Worldwide decline of eel resources necessitates immediate action - Quebec declaration of concern. Fisheries 28, 28-30.

Cassidy, A.A., Saulnier, R.J., and Lamarre, S.G. (2016). Adjustments of Protein Metabolism in Fasting Arctic Charr, *Salvelinus alpinus*. Plos One 11.

Castle, P. H. J. and Williamson, G. R. (1974) On the validity of the freshwater eel species *Anguilla ancestralis* Ege from Celebes. Copeia 2:569-570.

Castonguay, M., Hodson, P.V., Moriarty, C., Drinkwater, K.F., Jessop, B.M. (1994). Is there a role of ocean environment in American and European eel decline? Fish. Oceanogr. 3, 197–203.

Castro, D., Mieiro, C.L., Coelho, J.P., Guilherme, S., Marques, A., Santos, M.A., Duarte, A.C., Pereira, E., and Pacheco, M. (2018). Addressing the impact of mercury estuarine contamination in the European eel (*Anguilla anguilla* L., 1758) - An early diagnosis in glass eel stage based on erythrocytic nuclear morphology. Marine Pollution Bulletin 127, 733-742.

Cavey, M., Collins, B., Bertet, C., and Blau, J. (2016). Circadian rhythms in neuronal activity propagate through output circuits. Nature Neuroscience 19, 587-+.

Chabot, D., Steffensen, J.F., and Farrell, A.P. (2016). The determination of standard metabolic rate in fishes. Journal of Fish Biology 88, 81-121.

Chan, N.C., Salazar, A.M., Pham, A.H., Sweredoski, M.J., Kolawa, N.J., Graham, R.L.J., Hess, S., and Chan, D.C. (2011). Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Human Molecular Genetics 20, 1726-1737.

Charlon, N. and Blanc, J. M. (1982). Etude des civelles d'Anguilla anguilla L. dans la région du bassin de l'Adour. 1. Caractéristiques biométriques de longueur et de poids en fonction de la pigmentation. Arch. Hydrobiol. 93 (2), 238–255.

Chen, H.C., Detmer, S.A., Ewald, A.J., Griffin, E.E., Fraser, S.E., and Chan, D.C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. Journal of Cell Biology 160, 189-200.

Chilov, D., Hofer, T., Bauer, C., Wenger, R.H., and Gassmann, M. (2001). Hypoxia affects expression of circadian genes PER1 and CLOCK in mouse brain. Faseb Journal 15, 2613-2622.

Choi, D.W. (1992). Excitotoxic cell death. J. Neurobiol., 23, 1261–1276.

Chrousos, G. P., and Kino, T. (2005). Intracellular glucocorticoid signaling: a formerly simple system turns stochastic. Science's STKE, 2005(304), pe48-pe48.

Ciechanover, A., Finley, D., and Varshavsky, A. (1984). Ubiquitin dependence of selective protein-degradation demonstrated in the mammalian-cell cycle mutant ts85. Cell 37, 57-66.

Cingolani, F., and Czaja, M.J. (2016). Regulation and Functions of Autophagic Lipolysis. Trends in Endocrinology and Metabolism 27, 696-705.

Clarkson, T. (1992). Mercury: major issues in environmental health. Environ. Health Perspect. 100, 31–38.

Clarkson, T.W. (1972). The pharmacology of mercury compounds. Annu.Rev. Pharmacol. 12, 375–406.

Claveau, J., Monperrus, M., Jarry, M., Baudrimont, M., Gonzalez, P., Cavalheiro, J., Mesmer-Dudons, N., and Bolliet, V. (2015b). Methylmercury effects on migratory behaviour in glass eels (*Anguilla*

anguilla): An experimental study using isotopic tracers. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 171, 15-27.

Claveau, J., Monperrus, M., Jarry, M., Pinaly, H., Baudrimont, M., Gonzalez, P., Amouroux, D., Bardonnet, A., and Bolliet, V. (2015a). Spatial and seasonal variations of methylmercury in European glass eels (*Anguilla anguilla*) in the Adour estuary (France) and relation to their migratory behaviour. Environmental Science and Pollution Research 22, 10721-10732.

Cleveland, B.M., and Evenhuis, J.P. (2010). Molecular characterization of atrogin-1/F-box protein-32 (FBXO32) and F-box protein-25 (FBXO25) in rainbow trout (*Oncorhynchus mykiss*): Expression across tissues in response to feed deprivation. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 157, 248-257.

Collins, G.A., and Goldberg, A.L. (2017). The Logic of the 26S Proteasome. Cell 169, 792-806.

Colombo, G., and Grandi, G. (1996). Histological study of the development and sex differentiation of the gonad in the European eel. Journal of Fish Biology 48, 493-512.

Colombo, G., Grandi, G., and Rossi, R. (1984). Gonad differentiation and body growth in *Anguilla anguilla* L. J. Fish Biol. 24, 215–228.

Compeau, G.C., and Bartha, R. (1985). Sulfate-reducing bacteria - principal methylators of mercury in anoxic estuarine sediment. Applied and Environmental Microbiology 50, 498-502.

Cook, J.T., Sutterlin, A.M., and McNiven, M.A. (2000). Effect of food deprivation on oxygen consumption and body composition of growth-enhanced transgenic Atlantic salmon (*Salmo salar*). Aquaculture 188, 47-63.

Cresci, A., Durif, C.M., Paris, C.B., Shema, S.D., Skiftesvik, A.B., and Browman, H.I. (2019a). Glass eels (*Anguilla anguilla*) imprint the magnetic direction of tidal currents from their juvenile estuaries. Communications Biology 2.

Cresci, A., Durif, C.M., Paris, C.B., Thompson, C.R.S., Shema, S., Skiftesvik, A.B., and Browman, H.I. (2019b). The relationship between the moon cycle and the orientation of glass eels (*Anguilla anguilla*) at sea. Royal Society Open Science 6.

Cresci, A., Paris, C.B., Durif, C.M.F., Shema, S., Bjelland, R.M., Skiftesvik, A.B., and Browman, H.I. (2017). Glass eels (Anguilla anguilla) have a magnetic compass linked to the tidal cycle. Science Advances 3.

Creutzberg, F. (1959). Discrimination between ebb and flood tide in migrating elvers (*Anguilla vulgaris* Turt.) by means of olfactory perception. Nature 184, 1961-1962.

Creutzberg, F. (1961). On the orientation of migrating elvers (*Anguilla vulgaris* Turt.) in a tidal area. Netherlands Journal of Sea Research 1, 257-338.

Cutts, C.J., Metcalfe, N.B., and Taylor, A.C. (1998). Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. Journal of Fish Biology 52, 1026-1037.

Dagnino-Subiabre, A., Orellana, J.A., Carmona-Fontaine, C., Montiel, J., Diaz-Veliz, G., Seron-Ferre, M., Wyneken, U., Concha, M.L., and Aboitiz, F. (2006). Chronic stress decreases the expression of sympathetic markers in the pineal gland and increases plasma melatonin concentration in rats. Journal of Neurochemistry 97, 1279-1287.

Dang, F., Li, Z.Z., and Zhong, H. (2019). Methylmercury and selenium interactions: Mechanisms and implications for soil remediation. Critical Reviews in Environmental Science and Technology 49, 1737-1768.

Dash, S., Aydin, Y., and Moroz, K. (2019). Chaperone-Mediated Autophagy in the Liver: Good or Bad? Cells 8.

Daverat, F., Limburg, K.E., Thibault, I., Shiao, J.C., Dodson, J.J., Caron, F.O., Tzeng, W.N., Iizuka, Y., and Wickstrom, H. (2006). Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A-japonica* and *A-rostrata*. Marine Ecology Progress Series 308, 231-241.

Daverat, F., Tomas, J., Lahaye, M., Palmer, M., and Elie, P. (2005). Tracking continental habitat shifts of eels using otolith Sr/Ca ratios: validation and application to the coastal, estuarine and riverine eels of the Gironde-Garonne-Dordogne watershed. Marine and Freshwater Research 56, 619-627.

Davey, A.J.H., and Jellyman, D.J. (2005). Sex determination in freshwater eels and management options for manipulation of sex. Reviews in Fish Biology and Fisheries 15, 37-52.

Dawkins, R. (1980). Good strategy or evolutionary stable strategy? In Sociobiology: beyond nature/ nurture? (ed. Barlow G. W. and Silverberg J.), pp. 331-367. Boulder, Colorado: Westview.

De Duve, C. and Wattiaux, R. (1966). Functions of lysosomes. Annu. Rev. Physiol. 28, 435-492.

De Marco, S.G., Botte, S.E., and Marcovecchio, J.E. (2006). Mercury distribution in abiotic and biological compartments within several estuarine systems from Argentina: 1980-2005 period. Chemosphere 65, 213-223.

De Veen J. F. (1967). On the Phenomenon of Soles (*Solea solea* L.) swimming at the Surface, ICES Journal of Marine Science, Volume 31, Issue 2, August, Pages 207-236.

De Veen, J. F. (1978). On selective tidal transport in the migration of North Sea plaice (*Pleuronectes platessa*) and other flatfish species. Neth. J. Sea Res. 12, 115-147.

Deelder, C. L. (1952). On the migration of the elver (*Anguilla vulgaris* L.) at sea. J. Cons. Perm. Int. Explor. Mer, l8(2): 187-2l8.

Dekker, W. (2003). Status of the European eel stock and fisheries. In: Aida, K., Tsukamoto, K. and Yamauchi, K., eds. Eel biology. Tokyo: Springer Japan, pp. 237-254.

Dekker, W. (2008). Coming to Grips with the Eel Stock Slip-Sliding Away. In International Governance of Fisheries Eco-systems: Learning from the Past, Finding Solutions for the Future, pp. 335-355. Ed. by Schlechter M.G., Leonard N.J. and Taylor W.W. American Fisheries Society, Symposium 58, Bethesda, MD.

Dekker, W., and Beaulaton, L. (2016). Faire mieux que la nature? The History of Eel Restocking in Europe. Environment and History 22, 255-300.

Demishtein, A., Fraiberg, M., Berko, D., Tirosh, B., Elazar, Z., and Navon, A. (2017). SQSTM1/p62-mediated autophagy compensates for loss of proteasome polyubiquitin recruiting capacity. Autophagy 13, 1697-1708.

Depledge, M.H. (1984). Disruption of circulatory and respiratory activity in shore crabs (*Carcinus-maenas* (L)) exposed to heavy-metal pollution. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 78, 445-459.

Desaunay, Y., and Guerault, D. (1997). Seasonal and long-term changes in biometrics of eel larvae: a possible relationship between recruitment variation and North Atlantic ecosystem productivity. Journal of Fish Biology 51, 317-339.

Dhar, S.S., Wong-Riley, M.T. (2009). Coupling of energy metabolism and synaptic transmission at the transcriptional level: role of nuclear respiratory factor 1 in regulating both cytochrome c oxidase and NMDA glutamate receptor subunit genes. J Neurosci 29, 483-492.

Dikic, I. (2017). Proteasomal and autophagy degradation systems. Annu. Rev. Biochem. 86, 193–224.

Do Nascimento, J.L., Oliveira, K.R., Crespo-Lopez, M.E., Macchi, B.M., Maues, L.A., Pinheiro, M., Silveira, L.C., and Herculano, A.M. (2008). Methylmercury neurotoxicity & antioxidant defenses. Indian J Med Res, 128(4), 373-382.

Dorow, M., Beardmore, B., Haider, W., and Arlinghaus, R. (2010). Winners and losers of conservation policies for European eel, Anguilla anguilla: an economic welfare analysis for differently specialised eel anglers. Fisheries Management and Ecology 17, 106-125.

Dou, S.Z., and Tsukamoto, K. (2003). Observations on the nocturnal activity and feeding behavior of *Anguilla japonica* glass eels under laboratory conditions. Environmental Biology of Fishes 67, 389-395.

Dreiem, A., and Seegal, R.F. (2007). Methylmercury-induced changes in mitochondrial function in striatal synaptosomes are calcium-dependent and ROS-independent. Neurotoxicology 28, 720-726.

Drevnick, P.E., Roberts, A.P., Otter, R.R., Hammerschmidt, C.R., Klaper, R., and Oris, J.T. (2008). Mercury toxicity in livers of northern pike (*Esox lucius*) from Isle Royale, USA. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 147, 331-338.

Drouineau, H., Bau, F., Alric, A., Deligne, N., Gomes, P., and Sagnes, P. (2017). Silver eel downstream migration in fragmented rivers: use of a Bayesian model to track movements triggering and duration. Aquatic Living Resources 30.

Du Colombier, S.B., Bolliet, V., Lambert, P., and Bardonnet, A. (2007). Energy and migratory behavior in glass eels (*Anguilla anguilla*). Physiology & Behavior 92, 684-690.

Du Colombier, S.B., Bolliet, V., Lambert, P., and Bardonnet, A. (2011). Metabolic loss of mass in glass eels at different salinities according to their propensity to migrate. Estuarine Coastal and Shelf Science 93, 1-6.

Dubland, J.A. and Francis, G.A. (2015). Lysosomal acid lipase: at the crossroads of normal and atherogenic cholesterol metabolism. Front. Cell Dev. Biol. 3, 3.

Dupont-Prinet, A., Chatain, B., Grima, L., Vandeputte, M., Claireaux, G., and McKenzie, D.J. (2010). Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed deprivation in the European sea bass (*Dicentrarchus labrax*). Journal of Experimental Biology 213, 1143-1152.

Durif, C. (2003). La migration d'avalaison de l'*anguille* europeenne *Anguilla anguilla*: Caracterisation des fractions devalantes, phenomene de migration et franchisse-ment d'obstacles. These de doctorat, Universite Paul Sabatier, Toulouse.

Dusik, V., Senthilan, P.R., Mentzel, B., Hartlieb, H., Wulbeck, C., Yoshii, T., Raabe, T., and Helfrich-Forster, C. (2014). The MAP Kinase p38 Is Part of *Drosophila melanogaster*'s Circadian Clock. Plos Genetics 10.

Eaton, S. (2002). Control of mitochondrial beta-oxidation flux. Prog. Lipid Res. 41, 197-239.

Ebisawa, T., Fukuchi, M., Murakami, G., Chiba, T., Tanaka, K., Imamura, T., and Miyazono, K. (2001). Smurf1 interacts with transforming growth factor-β type I receptor through Smad7 and induces receptor degradation. J. Biol. Chem.276, 12477-12480.

Edeline, E. (2007). Adaptive phenotypic plasticity of eel diadromy. Marine Ecology Progress Series 341, 229-232.

Edeline, E., Dufour, S., and Elie, P. (2005). Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. Marine Ecology Progress Series 304, 191-199.

Edeline, E., Dufour, S., Briand, C., Fatin, D., and Elie, P. (2004). Thyroid status is related to migratory behavior in *Anguilla anguilla* glass eels. Marine Ecology Progress Series 282, 261-270.

Edeline, E., Lambert, P., Rigaud, C., and Elie, P. (2006). Effects of body condition and water temperature on *Anguilla anguilla* glass eel migratory behavior. Journal of Experimental Marine Biology and Ecology 331, 217-225.

Efeyan, A., Comb, W.C., and Sabatini, D.M. (2015). Nutrient-sensing mechanisms and pathways. Nature 517, 302-310.

Ege, V. (1939). A revision of the genus *Anguilla Shaw*, a systematic, phylogenetic and geographical study. Dana Rep. 16: 1–256.

Egusa, S. (1979). Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese-eel farming ponds. Rapp. P-V. Reun. Comm. Int. Explor. Mer 174, 51–58.

Eliason, E.J., and Farrell, A.P. (2016). Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. Journal of Fish Biology 88, 359-388.

Elie, P. (1979). Contribution à l'étude des montées de civelles d'*Anguilla anguilla* L., dans l'estuaire de la Loire: Pêche écophysiologie et élevage. Ph.D. thesis. University of Rennes I (383 p).

Elie, P., Lecomte-Finiger, R., Cantrelle, I. and Charlon, N. (1982). Définition des limites des différents stades pigmentaires durant la phase civelle d'Anguilla anguilla L. (Poisson Téléostéen Anguilliforme). Vie et Milieu 32 (3), 149-157.

Erickson, R.J., Nichols, J.W., Cook, P.M., et al. (2008). Bioavailability of chemical contaminants in aquatic systems, In: Di Giulio RT, Hinton DE, The Toxicology of Fishes, Florida, USA: CRC Press, 9-45.

Evans, D.W., Dodoo, D.K., and Hanson, P.J. (1993). Trace-element concentrations in fish livers - implications of variations with fish size in pollution monitoring. Marine Pollution Bulletin 26, 329-334.

Falcon, J. (1999). Cellular circadian clocks in the pineal. Progress in Neurobiology 58, 121-162.

Falcon, J., and Meissl, H. (1981). The photosensory function of the pineal organ of the pike (*Esox-lucius* L) correlation between structure and function. Journal of Comparative Physiology 144, 127-137.

Falcon, J., Besseau, L., Sauzet, S., and Boeuf, G. (2007). Melatonin effects on the hypothalamo-pituitary axis in fish. Trends in Endocrinology and Metabolism 18, 81-88.

Fan, T., Huang, Z.X., Wang, W., Zhang, B.Y., Xu, Y., Mao, Z.F., Chen, L., Hu, H., and Geng, Q. (2018). Proteasome inhibition promotes autophagy and protects from endoplasmic reticulum stress in rat alveolar macrophages exposed to hypoxia-reoxygenation injury. Journal of Cellular Physiology 233, 6748-6758.

Fang, Y.T., Guo, C.J., Zhang, P.P., Zhao, W.J., Wang, S.H., Xing, G.W., Shi, H.F., Lu, J., Peng, W.X., Feng, Y., et al. (2016). Role of autophagy in methylmercury-induced neurotoxicity in rat primary astrocytes. Archives of Toxicology 90, 333-345.

Farina, M., and Aschner, M. (2019). Glutathione antioxidant system and methylmercury-induced neurotoxicity: An intriguing interplay. Biochimica Et Biophysica Acta-General Subjects 1863.

Farina, M., Aschner, M., and Rocha, J. B. (2011). Oxidative stress in MeHg-induced neurotoxicity. Toxicology and applied pharmacology, 256(3), 405-417.

Farina, M., Brandao, R., Lara, F. S., Soares, F. A. A., Souza, D. O., and Rocha, J. B. T. (2003). Mechanisms of the inhibitory effects of selenium and mercury on the activity of δ-aminolevulinate dehydratase from mouse liver, kidney and brain. Toxicology letters, 139(1), 55-66.

Finaud, J., Lac, G., and Filaire, E. (2006). Oxidative stress - Relationship with exercise and training. Sports Medicine 36, 327-358.

Finn, P.F., and Dice, J.F. (2006). Proteolytic and lipolytic responses to starvation. Nutrition 22, 830-844.

Finstad, A.G., Forseth, T., Ugedal, O., and Naesje, T.F. (2007). Metabolic rate, behaviour and winter performance in juvenile Atlantic salmon. Functional Ecology 21, 905-912.

Forey, P.L., Littlewood, D.T.J., Ritchie, P. and Meyer, A. (1996). Interrelationships of elopomorph fishes. In: Stiassny, M.L.J., Parenti, L.R. and Johnson, G.D. (eds.). Interrelationships of Fishes: 175-191; New York (Academic Press).

Forward, R. B., and Tankersley, R. A. (2001). Selective tidal-stream transport of marine animals. Oceanogr. Mar. Biol. 39 (39), 305–353.

Franco, J.L., Posser, T., Dunkley, P.R., Dickson, P.W., Mattos, J.J., Martins, R., Bainy, A.C.D., Marques, M.R., Dafre, A.L., and Farina, M. (2009). Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. Free Radical Biology and Medicine 47, 449-457.

Franco, J.L., Posser, T., Missau, F., Pizzolatti, M.G., Santos, A.R.S., Souza, D.O., Aschner, M., Rocha, J.B.T., Dafre, A.L., and Farina, M. (2010). Structure-activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction. Environmental Toxicology and Pharmacology 30, 272-278.

Franco, J.L., Teixeira, A., Meotti, F.C., Ribas, C.M., Stringari, J., Pomblum, S.C.G., Moro, A.M., Bohrer, D., Bairros, A.V., Dafre, A.L., et al. (2006). Cerebellar thiol status and motor deficit after lactational exposure to methylmercury. Environmental Research 102, 22-28.

Friedland, K.D., Miller, M.J., and Knights, B. (2007). Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. Ices Journal of Marine Science 64, 519-530.

Fry, F.E.J. and Hart, J.S. (1948). The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. 94, 66-77.

Gagnaire, P.A., Normandeau, E., Cote, C., Hansen, M.M., and Bernatchez, L. (2012). The Genetic Consequences of Spatially Varying Selection in the Panmictic American Eel (*Anguilla rostrata*). Genetics 190, 725-U703.

Gailer, J. (2007). Arsenic-selenium and mercury-selenium bonds in biology. Coordination Chemistry Reviews 251, 234-254.

Gaillard, M., Bernatchez, L., Tremblay, R., and Audet, C. (2015). Regional variation in energy storage strategies in American glass eels from Eastern Canada. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 188, 87-95.

Gaillard, M., Pavey, S.A., Cote, C.L., Tremblay, R., Bernatchez, L., and Audet, C. (2016). Regional variation of gene regulation associated with storage lipid metabolism in American glass eels (*Anguilla rostrata*). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 196, 30-37.

Gascuel, D. (1986). Flow-carried and active swimming migration of the glass eel (*Anguilla-anguilla*) in the tidal area of a small estuary on the French Atlantic coast. Helgolander Meeresuntersuchungen 40, 321-326.

Gascuel, D., Feunteun, E., and Fontenelle, G. (1995). Seasonal dynamics of estuarine migration in glass eels (*Anguilla-anguilla*). Aquatic Living Resources 8, 123-133.

Ge, P.F., Zhang, J.Z., Wang, X.F., Meng, F.K., Li, W.C., Luan, Y.X., Ling, F., and Luo, Y.N. (2009). Inhibition of autophagy induced by proteasome inhibition increases cell death in human SHG-44 glioma cells. Acta Pharmacologica Sinica 30, 1046-1052.

Geffroy, B., and Bardonnet, A. (2016). Sex differentiation and sex determination in eels: consequences for management. Fish and Fisheries 17, 375-398.

Geffroy, B., Guilbaud, F., Amilhat, E., Beaulaton, L., Vignon, M., Huchet, E., Rives, J., Bobe, J., Fostier, A., Guiguen, Y., et al. (2016). Sexually dimorphic gene expressions in eels: useful markers for early sex assessment in a conservation context. Scientific Reports 6.

Geisler, S., Holmstrom, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J., and Springer, W. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nature Cell Biology 12, 119-U170.

Gentes, S., Maury-Brachet, R., Feng, C.Y., Pedrero, Z., Tessier, E., Legeay, A., Mesmer-Dudons, N., Baudrimont, M., Maurice, L., Amouroux, D., et al. (2015). Specific Effects of Dietary Methylmercury and Inorganic Mercury in Zebrafish (*Danio rerio*) Determined by Genetic, Histological, and Metallothionein Responses. Environmental Science & Technology 49, 14560-14569.

Gesto, M., Lopez-Patino, M.A., Hernandez, J., Soengas, J.L., and Miguez, J.M. (2013). The response of brain serotonergic and dopaminergic systems to an acute stressor in rainbow trout: a time course study. Journal of Experimental Biology 216, 4435-4442.

Gibson, R.N. (1992). Tidally-synchronized behavior in marine fishes. Rhythms in Fishes 236, 63-81.

Glaser, V., Leipnitz, G., Straliotto, M.R., Oliveira, J., dos Santos, V.V., Wannmacher, C.M.D., de Bern, A.F., Rocha, J.B.T., Farina, M., and Latini, A. (2010). Oxidative stress-mediated inhibition of brain creatine kinase activity by methylmercury. Neurotoxicology 31, 454-460.

Gomes, L.C., Di Benedetto, G., and Scorrano, L. (2011). During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nature Cell Biology 13, 589-U207.

Gomes, M.D., Lecker, S.H., Jagoe, R.T., Navon, A., and Goldberg, A.L. (2001). Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proceedings of the National Academy of Sciences of the United States of America 98, 14440-14445.

Gonzalez, P., Dominique, Y., Massabuau, J.C., Boudou, A., and Bourdineaud, J.P. (2005). Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). Environmental Science & Technology 39, 3972-3980.

Graves, S.D., Kidd, K.A., Batchelar, K.L., Cowie, A.M., O'Driscoll, N.J., and Martyniuk, C.J. (2017). Response of oxidative stress transcripts in the brain of wild yellow perch (*Perca flavescens*) exposed to an environmental gradient of methylmercury. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 192, 50-58.

Grilo, T.F., Mendes, T., Coelho, J.P., Pereira, E., Pardal, M.A., and Cardoso, P.G. (2015). Kinetics of Mercury Accumulation and Elimination in Edible Glass Eel (*Anguilla anguilla*) and Potential Health Public Risks. Water Air and Soil Pollution 226.

Gross, M.R. (1984). Sunfish, salmon, and the evolution of alternative reproductive strategies and tactics. In: Fish Reproduction: Strategie and Tactics (Ed. by Potts G.W. and Wootton R.J.), pp. 55–75. London: Academic Press.

Gross, M.R. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. Trends in Ecology & Evolution 11, 92-98.

Gross, M.R., and Repka, J. (1998). Stability with inheritance in the conditional strategy. Journal of Theoretical Biology 192, 445-453.

Grumati, P., Coletto, L., Schiavinato, A., Castagnaro, S., Bertaggia, E., Sandri, M., and Bonaldo, P. (2011). Physical exercise stimulates autophagy in normal skeletal muscles but is detrimental for collagen VI-deficient muscles. Autophagy 7, 1415-1423.

Guardiola, F.A., Chaves-Pozo, E., Espinosa, C., Romero, D., Meseguer, J., Cuesta, A., and Esteban, M.A. (2016). Mercury Accumulation, Structural Damages, and Antioxidant and Immune Status Changes in the Gilthead Seabream (*Sparus aurata* L.) Exposed to Methylmercury. Archives of Environmental Contamination and Toxicology 70, 734-746.

Guppy, M., and Withers, P. (1999). Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biological Reviews 74, 1-40.

Haas, A.L., Warms, J.V.B., Hershko, A., and Rose, I.A. (1982). Ubiquitin-activating enzyme - mechanism and role in protein-ubiquitin conjugation. Journal of Biological Chemistry 257, 2543-2548.

Haenen O. L. M. (1995). *Anguillicola crassus* (Nematoda, Dracunculoidea) infections of European eel (*Anguilla anguilla*) in the Netherlands: epidemiology, pathogenesis and pathobiology. Ph.D. thesis, Agricultural University Wageningen, pp 127.

Haigis, M.C., and Guarente, L.P. (2006). Mammalian sirtuins - emerging roles in physiology, aging, and calorie restriction. Genes & Development 20, 2913-2921.

Hall, B.D., Bodaly, R.A., Fudge, R.J.P., Rudd, J.W.M., and Rosenberg, D.M. (1997). Food as the dominant pathway of methylmercury uptake by fish. Water Air and Soil Pollution 100, 13-24.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441, 885-889.

Harris, H.H., Pickering, I.J., and George, G.N. (2003). The chemical form of mercury in fish. Science, 301(5637), 1203-1203.

Harris, J.J., Jolivet, R., and Attwell, D. (2012). Synaptic energy use and supply. Neuron, 75(5), 762-777.

Harrison, A.J., Walker, A.M., Pinder, A.C., Briand, C., and Aprahamian, M.W. (2014). A review of glass eel migratory behaviour, sampling techniques and abundance estimates in estuaries: implications for assessing recruitment, local production and exploitation. Reviews in Fish Biology and Fisheries 24, 967-983.

Hazel, W., Smock, R., and Lively, C.M. (2004). The ecological genetics of conditional strategies. American Naturalist 163, 888-900.

Hazel, W.N., Smock, R., and Johnson, M.D. (1990). A polygenic model for the evolution and maintenance of conditional strategies. Proceedings of the Royal Society B-Biological Sciences 242, 181-187.

Helfrich-Forster, C. (2017). Interactions between psychosocial stress and the circadian endogenous clock. Psych Journal 6, 277-289.

Herhaus, L., and Dikic, I. (2015). Expanding the ubiquitin code through post-translational modification. Embo Reports 16, 1071-1083.

Hernandez-Perez, J., Naderi, F., Chivite, M., Soengas, J.L., Miguez, J.M., and Lopez-Patino, M.A. (2019). Influence of Stress on Liver Circadian Physiology. A Study in Rainbow Trout, Oncorhynchus mykiss, as Fish Model. Frontiers in Physiology 10.

Hershiko, A. and Ciechanover, A. (1998). The ubiquitin-proteasome pathway. Annu Rev Biochem 67: 425-479.

Hershko, A., and Heller, H. (1985). Occurrence of a polyubiquitin structure in ubiquitin-protein conjugates. Biochemical and Biophysical Research Communications 128, 1079-1086.

Hickman, R.A. (1981). Densities and swimbladder development of juvenile American eels, *Anguilla-rostrata* (Lesueur) as related to energetics of migration. Journal of Fish Biology 18, 507-517.

Hoppins, S., and Nunnari, J. (2009). The molecular mechanism of mitochon-drial fusion. Biochim. Biophys. Acta1793, 20-26

Hoppins, S., Lackner, L., and Nunnari, J. (2007). The machines that divide and fuse mitochondria. Annual Review of Biochemistry 76, 751-780.

Horne, J. and Birnie, K. (1978). Catching, Handling and Processing Eels. Ministry of Agriculture, Fisheries and Food, Torry Research Station.

Hrenchuk, L.E., Blanchfield, P.J., Paterson, M.J., and Hintelmann, H.H. (2012). Dietary and Waterborne Mercury Accumulation by Yellow Perch: A Field Experiment. Environmental Science & Technology 46, 509-516.

Huertas, M., and Cerda, J. (2006). Stocking density at early developmental stages affects growth and sex ratio in the European eel (*Anguilla anguilla*). Biological Bulletin 211, 286-296.

Huett, A., Heath, R.J., Begun, J., Sassi, S.O., Baxt, L.A., Vyas, J.M., Goldberg, M.B., and Xavier, R.J. (2012). The LRR and RING Domain Protein LRSAM1 Is an E3 Ligase Crucial for Ubiquitin-Dependent Autophagy of Intracellular Salmonella Typhimurium. Cell Host & Microbe 12, 778-790.

Hughes, W.L. (1957). A physicochemical rationale for the biological activity of mercury and its compounds. Annals of the New York Academy of Sciences 65, 454-460.

Huntingford, F.A., Andrew, G., Mackenzie, S., Morera, D., Coyle, S.M., Pilarczyk, M., and Kadri, S. (2010). Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*. Journal of Fish Biology 76, 1576-1591.

Imai, S., Armstrong, C.M., Kaeberlein, M., and Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403, 795-800.

Imbert, H., Arrowsmith, R., Dufour, S., and Elie, P. (2008). Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage-dependent mechanisms in European eel upstream migration. Hormones and Behavior 53, 69-81.

Isorna, E., de Pedro, N., Valenciano, A.I., Alonso-Gomez, A.L., and Delgado, M.J. (2017). Interplay between the endocrine and circadian systems in fishes. Journal of Endocrinology 232, R141-R159.

Itakura, E., Kishi-Itakura, C., and Mizushima, N. (2012). The Hairpin-type Tail-Anchored SNARE Syntaxin 17 Targets to Autophagosomes for Fusion with Endosomes/Lysosomes. Cell 151, 1256-1269.

IUCN 2020. The IUCN Red List of Threatened Species. Version 2020-2.

Jamart, C., Benoit, N., Raymackers, J.M., Kim, H.J., Kim, C.K., and Francaux, M. (2012). Autophagy-related and autophagy-regulatory genes are induced in human muscle after ultraendurance exercise. European Journal of Applied Physiology 112, 3173-3177.

Jegstrup, I.M., and Rosenkilde, P. (2003). Regulation of post-larval development in the European eel: thyroid hormone level, progress of pigmentation and changes in behaviour. Journal of Fish Biology 63, 168-175.

Jellyman, D.J. (1979). Upstream migration of glass-eels (*Anguilla* spp.) in the Waikato River. New Zealand Journal of Marine and Freshwater Research 13, 13-22.

Jensen, S., and Jernelov, A. (1969). Biological methylation of mercury in aquatic organisms. Nature 223:753-754

Jiang, L.B., Cao, L., Yin, X.F., Yasen, M., Yishake, M., Dong, J., and Li, X.L. (2015). Activation of autophagy via Ca^{2+}-dependent AMPK/mTOR pathway in rat notochordal cells is a cellular adaptation under hyperosmotic stress. Cell Cycle 14, 867-879.

Jones, F.R.H., Arnold, G.P., Walker, M.G., and Scholes, P. (1979). Selective tidal stream transport and the migration of plaice (*Pleuronectes-platessa* L) in the Southern North-Sea. Journal Du Conseil 38, 331-337.

Jordan, S.D., and Lamia, K.A. (2013). AMPK at the crossroads of circadian clocks and metabolism. Molecular and Cellular Endocrinology 366, 163-169.

Jorgensen, E.H., Martinsen, M., Strom, V., Hansen, K.E.R., Ravuri, C.S., Gong, N.P., and Jobling, M. (2013). Long-term fasting in the anadromous Arctic charr is associated with downregulation of metabolic enzyme activity and upregulation of leptin A1 and SOCS expression in the liver. Journal of Experimental Biology 216, 3222-3230.

Jung, M.M., Kim, T.H., Choi, Y.J., Kim, N.N., Choi, J.Y., Oh, S.Y., Lim, S.G., and Choi, C.Y. (2016). Variations in the antioxidant system and circadian rhythms of goldfish, *Carassius auratus*, exposed to ammonia: profile of the effects of green LED spectra. Biological Rhythm Research 47, 583-596.

Kagedal, K., Johansson, U., and Ollinger, K. (2001). The lysosomal protease cathepsin D mediates apoptosis induced by oxidative stress. Faseb Journal 15, 1592-+.

Karlsen, O.A., Sheehan, D., and Goksoyr, A. (2014). Alterations in the Atlantic Cod (*Gadus morhua*) Hepatic Thiol-Proteome After Methylmercury Exposure. Journal of Toxicology and Environmental Health-Part a-Current Issues 77, 650-662.

Kaur, J., and Debnath, J. (2015). Autophagy at the crossroads of catabolism and anabolism. Nature Reviews Molecular Cell Biology 16, 461-472.

Kaur, P., Aschner, M., and Syversen, T. (2006). Glutathione modulation influences methyl mercury induced neurotoxicity in primary cell cultures of neurons and astrocytes. Neurotoxicology 27, 492-500.

Kaur, P., Aschner, M., and Syversen, T. (2007). Role of glutathione in determining the differential sensitivity between the cortical and cerebellar regions towards mercury-induced oxidative stress. Toxicology 230, 164-177.

Kaushik, S., and Cuervo, A.M. (2015). Degradation of lipid droplet-associated proteins by chaperone-mediated autophagy facilitates lipolysis. Nature Cell Biology 17, 759-+.

Kawakami, Y., Mochioka, N., and Nakazono, A. (1999b). Immigration patterns of glass-eels *Anguilla japonica* entering river in northern Kyushu, Japan. Bulletin of Marine Science 64, 315-327.

Kawakami, Y., Mochioka, N., Kimura, R., and Nakazono, A. (1999a). Seasonal changes of the RNA/DNA ratio, size and lipid contents and immigration adaptability of Japanese glass-eels, *Anguilla japonica*, collected in northern Kyushu, Japan. Journal of Experimental Marine Biology and Ecology 238, 1-19.

Kawamura, T., and Muraoka, I. (2018). Exercise-Induced Oxidative Stress and the Effects of Antioxidant Intake from a Physiological Viewpoint. Antioxidants 7.

Kennish, M.J., ed. (1997) Practical Handbook of Estuarine and Marine Pollution. Boca Raton, USA: CRC Press: 524 pp.

Kerner J., Hoppel C. Fatty acid import into mitochondria. Bio-chim Biophys Acta1486: 1-17, 2000.

Kiffin, R., Christian, C., Knecht, E., and Cuervo, A.M. (2004). Activation of chaperone-mediated autophagy during oxidative stress. Molecular Biology of the Cell 15, 4829-4840.

Killen, S.S., Marras, S., and McKenzie, D.J. (2011). Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology 80, 1024-1033.

Kim, T.H., Choi, J.Y., Park, I.S., and Choi, C.Y. (2017). Waterborne copper disrupts circadian rhythm in red seabream (*Pagrus major*). Biological Rhythm Research 48, 837-843.

Kirk, R.S. (2003). The impact of *Anguillicola crassus* on European eels. Fisheries Management and Ecology 10, 385-394.

Klengel, T., and Binder, E.B. (2015). Epigenetics of Stress-Related Psychiatric Disorders and Gene x Environment Interactions. Neuron 86, 1343-1357.

Knights, B. (2003). A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. Science of the Total Environment 310, 237-244.

Kocaturk, N.M., and Gozuacik, D. (2018). Crosstalk Between Mammalian Autophagy and the Ubiquitin-Proteasome System. Frontiers in Cell and Developmental Biology 6.

Koch, C.E., Leinweber, B., Drengberg, B.C., Blaum, C., and Oster, H. (2017). Interaction between circadian rhythms and stress. Neurobiology of stress, 6, 57-67.

Koehn, R.K., and Williams, G.C. (1978). Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. II. Temporal stability of geographic patterns. Evolution, 624-637.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E., and Tanaka, K. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature441, 880–884.

Komatsu, M., Waguri, S., Ueno, T., Iwata, J., Murata, S., Tanida, I., Ezaki, J., Mizushima, N., Ohsumi, Y., Uchiyama, Y., et al. (2005). Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. Journal of Cell Biology 169, 425-434.

Korbas, M., Lai, B., Vogt, S., Gleber, S.C., Karunakaran, C., Pickering, I.J., Krone, P.H., and George, G.N. (2013). Methylmercury Targets Photoreceptor Outer Segments. Acs Chemical Biology 8, 2256-2263.

Korbas, M., MacDonald, T.C., Pickering, I.J., George, G.N., and Krone, P.H. (2012). Chemical Form Matters: Differential Accumulation of Mercury Following Inorganic and Organic Mercury Exposures in Zebrafish Larvae. Acs Chemical Biology 7, 410-419.

Korolchuk, V.I., Mansilla, A., Menzies, F.M., and Rubinsztein, D.C. (2009). Autophagy Inhibition Compromises Degradation of Ubiquitin-Proteasome Pathway Substrates. Molecular Cell 33, 517-527.

Korolchuk, V.I., Menzies, F.M., and Rubinsztein, D.C. (2010). Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems. Febs Letters 584, 1393-1398.

Krueger, W.H., and Oliveira, K. (1999). Evidence for environmental sex determination in the American eel, *Anguilla rostrata*. Environmental Biology of Fishes 55, 381-389.

Kuma, A., and Mizushima, N. (2010). Physiological role of autophagy as an intracellular recycling system: With an emphasis on nutrient metabolism. Seminars in Cell & Developmental Biology 21, 683-690.

Laffaille, P., Acou, A., Guioullet, J., Mounaix, B., and Legault, A. (2006). Patterns of silver eel (*Anguilla anguilla* L.) sex ratio in a catchment. Ecology of Freshwater Fish 15, 583-588.

Lafoncazal, M., Pietri, S., Culcasi, M., and Bockaert, J. (1993). NMDA-dependent superoxide production and neurotoxicity. Nature 364, 535-537.

Lambert, P., Durozoi, B., and Beaulaton, L. (2007). Validation des hypotheses comportementales utilisees dans la quantification du flux d'anguille (*Anguilla anguilla*) dans l'Isle. Contrat Indicang. Rapport Cemagref no. 115.

Lamia, K.A., Papp, S.J., Yu, R.T., Barish, G.D., Uhlenhaut, N.H., Jonker, J.W., Downes, M., and Evans, R.M. (2011). Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. Nature 480, 552-556.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., et al. (2009). AMPK Regulates the Circadian Clock by Cryptochrome Phosphorylation and Degradation. Science 326, 437-440.

Lamson, H.M., Shiao, J.C., Iizuka, Y., Tzeng, W.N., and Cairns, D.K. (2006). Movement patterns of American eels (*Anguilla rostrata*) between salt- and freshwater in a coastal watershed, based on otolith microchemistry. Marine Biology 149, 1567-1576.

Lande-Diner, L., Boyault, C., Kim, J.Y., and Weitz, C.J. (2013). A positive feedback loop links circadian clock factor CLOCK-BMAL1 to the basic transcriptional machinery. Proceedings of the National Academy of Sciences of the United States of America 110, 16021-16026.

Landry, J., Sutton, A., Tafrov, S. T., Heller, R. C., Stebbins, J., Pillus, L., and Sternglanz, R. (2000). The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proceedings of the National Academy of Sciences, 97(11), 5807-5811.

Lansard, M., Panserat, S., Plagnes-Juan, E., Seiliez, I., and Skiba-Cassy, S. (2010). Integration of insulin and amino acid signals that regulate hepatic metabolism-related gene expression in rainbow trout: role of TOR. Amino Acids 39, 801-810.

Lawson, N.M., Mason, R.P., 1998. Accumulation of mercury in estuarine food chains. Biogeochemistry 40 (2–3), 235–247.

Leaner, J.J., and Mason, R.P. (2004). Methylmercury uptake and distribution kinetics in sheepshead minnows, Cyprinodon variegatus, after exposure to Ch_3Hg-spiked food. Environmental Toxicology and Chemistry 23, 2138-2146.

Lecker, S.H., Solomon, V., Mitch, W.E., and Goldberg, A.L. (1999). Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. Journal of Nutrition 129, 227S-237S.

Lecomte-Finiger R., Maunier C., Khafif M. (2004). Les larves leptocephales, ces meconnues. Cybium 28: 83-95.

Lecomte-Finiger, R. (1994). The early life of the European eel. Nature 370, 424.

Lee, J.M., Wagner, M., Xiao, R., Kim, K.H., Feng, D., Lazar, M.A., and Moore, D.D. (2014). Nutrient-sensing nuclear receptors coordinate autophagy. Nature 516, 112-U291.

Lee, J.W., De Riu, N., Lee, S., Bai, S.C., Moniello, G., and Hung, S.S.O. (2011). Effects of dietary methylmercury on growth performance and tissue burden in juvenile green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*). Aquatic Toxicology 105, 227-234.

Lee, J.W., Kim, J.W., De Riu, N., Moniello, G., and Hung, S.S.O. (2012). Histopathological alterations of juvenile green (*Acipenser medirostris*) and white sturgeon (*Acipenser transmontanus*) exposed to graded levels of dietary methylmercury. Aquatic Toxicology 109, 90-99.

Leong, K.H., Tan, L.L.B., and Mustafa, A.M. (2007). Contamination levels of selected organochlorine and organophosphate pesticides in the Selangor River, Malaysia between 2002 and 2003. Chemosphere 66, 1153-1159.

Levine, B. and Kroemer, G. (2008). Autophagy in the pathogenesis of disease. Cell 132, 27-42.

Limke, T.L., Bearss, J.J., and Atchison, W.D. (2004). Acute exposure to methylmercury causes Ca^{2+} dysregulation and neuronal death in rat cerebellar granule cells through an M3 muscarinic receptor-linked pathway. Toxicological Sciences 80, 60-68.

Liu, H.T., Labonne, J., Coste, P., Huchet, E., Plagnes-Juan, E., Rives, J., Veron, V., Seiliez, I., and Bolliet, V. (2019). Looking at the complex relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (Anguilla anguilla). Science of the Total Environment 696.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. Methods 25, 402-408.

Lock, R.A.C., Wendelaar Bonga, S.E., 2008. The osmoregulatory system. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. Taylor and Francis, New York, pp. 401-416.

Lokireddy, S., Mouly, V., Butler-Browne, G., Gluckman, P.D., Sharma, M., Kambadur, R., and McFarlane, C. (2011). Myostatin promotes the wasting of human myoblast cultures through promoting ubiquitin-proteasome pathway-mediated loss of sarcomeric proteins. American Journal of Physiology-Cell Physiology 301, C1316-C1324.

Lopez-Patino, M.A., Gesto, M., Conde-Sieira, M., Soengas, J.L., and Miguez, J.M. (2014). Stress inhibition of melatonin synthesis in the pineal organ of rainbow trout (*Oncorhynchus mykiss*) is mediated by cortisol. Journal of Experimental Biology 217, 1407-1416.

Lopez-Patino, M.A., Rodriguez-Illamola, A., Gesto, M., Soengas, J.L., and Miguez, J.M. (2011). Changes in plasma melatonin levels and pineal organ melatonin synthesis following acclimation of rainbow trout (*Oncorhynchus mykiss*) to different water salinities. Journal of Experimental Biology 214, 928-936.

Loumbourdis, N.S., and Danscher, G. (2004). Autometallographic tracing of mercury in frog liver. Environmental Pollution 129, 299-304.

Lubiana, P., Prokkola, J.M., Nikinmaa, M., Burmester, T., Kanerva, M., and Gotting, M. (2016). The effects of the painkiller diclofenac and hypoxia on gene transcription and antioxidant system in the gills of three-spined stickleback. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 185, 147-154.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., and Volckaert, F.A.M. (2005). The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: Relationship between heavy metal bioaccumulation, condition and genetic variability. Aquatic Toxicology 73, 99-114.

Mammucari, C., Milan, G., Romanello, V., Masiero, E., Rudolf, R., Del Piccolo, P., Burden, S.J., Di Lisi, R., Sandri, C., Zhao, J., et al. (2007). FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metabolism 6, 458-471.

Marandel, L., Lepais, O., Arbenoits, E., Veron, V., Dias, K., Zion, M., and Panserat, S. (2016). Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (*Oncorhynchus mykiss*) by nutritional status and dietary carbohydrates. Scientific Reports 6.

Martinez-Lopez, N., Garcia-Macia, M., Sahu, S., Athonvarangkul, D., Liebling, E., Merlo, P., Cecconi, F., Schwartz, G.J., and Singh, R. (2016). Autophagy in the CNS and Periphery Coordinate Lipophagy and Lipolysis in the Brown Adipose Tissue and Liver. Cell Metabolism 23, 113-127.

Mashek, D.G. (2013). Hepatic Fatty Acid Trafficking: Multiple Forks in the Road. Advances in Nutrition 4, 697-710.

Mathews, T., and Fisher, N.S. (2008). Evaluating the trophic transfer of cadmium, polonium, and methylmercury in an estuarine food chain. Environmental Toxicology and Chemistry 27, 1093-1101.

Maulvault, A.L., Custodio, A., Anacleto, P., Repolho, T., Pousao, P., Nunes, M.L., Diniz, M., Rosa, R., and Marques, A. (2016). Bioaccumulation and elimination of mercury in juvenile seabass (*Dicentrarchus labrax*) in a warmer environment. Environmental Research 149, 77-85.

Maximino, C., Araujo, J., Leao, L.K.R., Grisolia, A.B.A., Oliveira, K.R.M., Lima, M.G., Batista, E.D.O., Crespo-Lopez, M.E., Gouveia, A., and Herculano, A.M. (2011). Possible role of

serotoninergic system in the neurobehavioral impairment induced by acute methylmercury exposure in zebrafish (*Danio rerio*). Neurotoxicology and Teratology 33, 727-734.

McCleave, J.D. (2001). Simulation of the impact of dams and fishing weirs on reproductive potential of silver-phase American eels in the Kennebec River basin, Maine. North American Journal of Fisheries Management 21, 592-605.

McCleave, J.D., and Edeline, E. (2009). Diadromy As a Conditional Strategy: Patterns and Drivers of Eel Movements in Continental Habitats. Challenges for Diadromous Fishes in a Dynamic Global Environment 69, 97-+.

McCleave, J.D., and Kleckner, R.C. (1982). Selective tidal stream transport in the estuarine migration of glass eels of the American eel (*Anguilla-rostrata*). Journal Du Conseil 40, 262-271.

McCue, M.D. (2010). Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 156, 1-18.

McKechnie, A.E. (2008). Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: a review. Journal of Comparative Physiology B-Biochemical Systems and Environmental Physiology 178, 235-247.

McKenzie, D.J., Vergnet, A., Chatain, B., Vandeputte, M., Desmarais, E., Steffensen, J.F., and Guinand, B. (2014). Physiological mechanisms underlying individual variation in tolerance of food deprivation in juvenile European sea bass, Dicentrarchus labrax. Journal of Experimental Biology 217, 3283-3292.

Mela, M., Neto, F.F., Yamamoto, F.Y., Almeida, R., Grotzner, S.R., Ventura, D.F., and Ribeiro, C.A.D. (2014). Mercury distribution in target organs and biochemical responses after subchronic and trophic exposure to Neotropical fish *Hoplias malabaricus*. Fish Physiology and Biochemistry 40, 245-256.

Mela, M., Randi, M.A.F., Ventura, D.F., Carvalho, C.E.V., Pelletier, E., and Ribeiro, C.A.O. (2007). Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicology and Environmental Safety 68, 426-435.

Melser, S., Lavie, J., and Bénard G. (2015). Mitochondrial degradation and energy metabolism, BBA Mol. Cell Res. 1853 2812–2821.

Melvin, S.D., Buck, D.R., and Fabbro, L.D. (2016). Diurnal activity patterns as a sensitive behavioural outcome in fish: effect of short-term exposure to treated sewage and a sub-lethal PPCP mixture. Journal of Applied Toxicology 36, 1173-1182.

Metcalfe, J.D., Arnold, G.P., and Webb, P.W. (1990). The energetics of migration by selective tidal stream transport - an analysis for plaice tracked in the Southern North-Sea. Journal of the Marine Biological Association of the United Kingdom 70, 149-162.

Metcalfe, N.B., Taylor, A.C., and Thorpe, J.E. (1995). Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim. Behav. 49, 431–436.

Metcalfe, N.B., Van Leeuwen, T.E., and Killen, S.S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? Journal of Fish Biology 88, 298-321.

Meybeck, M., Lestel, L., Bonte, P., Moilleron, R., Colin, J.L., Rousselot, O., Herve, D., de Ponteves, C., Grosbois, C., and Thevenot, D.R. (2007). Historical perspective of heavy metals contamination

(Cd, Cr, Cu, Hg, Pb, Zn) in the Seine River basin (France) following a DPSIR approach (1950-2005). Science of the Total Environment 375, 204-231.

Milenkovic, N., Damjanovic, M., and Ristic, M. (2005). Study of heavy metal pollution in sediments from the iron gate (Danube river), Serbia and Montenegro. Polish Journal of Environmental Studies 14, 781-787.

Miller, M. (2009). Ecology of anguilliform leptocephali: remarkable transparent fish larvae of the ocean surface layer. Aqua-BioScience Monographs 2, 1-94.

Miller, M.J., Bonhommeau, S., Munk, P., Castonguay, M., Hanel, R., and McCleave, J.D. (2015). A century of research on the larval distributions of the Atlantic eels: a re-examination of the data. Biological Reviews 90, 1035-1064.

Mitchell, P. (2011). Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biochimica Et Biophysica Acta-Bioenergetics 1807, 1507-1538.

Mizushima, N., and Komatsu, M. (2011). Autophagy: Renovation of Cells and Tissues. Cell 147, 728-741.

Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2004). In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Molecular Biology of the Cell 15, 1101-1111.

Mommsen, T.P. (2004). Salmon spawning migration and muscle protein metabolism: the August Krogh principle at work. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 139, 383-400.

Monperrus, M., Pecheyran, C., and Bolliet, V. (2020). Imaging Differential Mercury Species Bioaccumulation in Glass Eels Using Isotopic Tracers and Laser Ablation Inductively Coupled Plasma Mass Spectrometry. Applied Sciences-Basel 10.

Monperrus, M., Point, D., Grall, J., Chauvaud, L., Amouroux, D., Bareille, G., and Donard, O. (2005). Determination of metal and organometal trophic bioaccumulation in the benthic macrofauna of the Adour estuary coastal zone (SW France, Bay of Biscay). Journal of Environmental Monitoring 7, 693-700.

Morcillo, P., Chaves-Pozo, E., Meseguer, J., Esteban, M.A., and Cuesta, A. (2017). Establishment of a new teleost brain cell line (DLB-1) from the European sea bass and its use to study metal toxicology. Toxicology in Vitro 38, 91-100.

Morcillo, P., Cordero, H., Meseguer, J., Esteban, M.A., and Cuesta, A. (2015). Toxicological in vitro effects of heavy metals on gilthead seabream (*Sparus aurata* L.) head-kidney leucocytes. Toxicology in Vitro 30, 412-420.

Morcillo, P., Esteban, M.A., and Cuesta, A. (2016). Heavy metals produce toxicity, oxidative stress and apoptosis in the marine teleost fish SAF-1 cell line. Chemosphere 144, 225-233.

Morcillo, P., Esteban, M.A., and Cuesta, A. (2017). Mercury and its toxic effects on fish. AIMS Environmental Science, 4(3), 386.

Mori, N., Yasutake, A., and Hirayama, K. (2007). Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. Archives of Toxicology 81, 769-776.

Moriarty, C. and Dekker, W. (Eds.) (1997). Management of European eel fisheries. Irish Fish. Bull., 15, p.108.

Mozhdeganloo, Z., Jafari, A.M., Koohi, M.K., and Heidarpour, M. (2015). Methylmercury-Induced Oxidative Stress in Rainbow Trout (*Oncorhynchus mykiss*) Liver: Ameliorating Effect of Vitamin C. Biological Trace Element Research 165, 103-109.

Muller, A.K., Brinkmann, M., Baumann, L., Stoffel, M.H., Segner, H., Kidd, K.A., and Hollert, H. (2015). Morphological alterations in the liver of yellow perch (*Perca flavescens*) from a biological mercury hotspot. Environmental Science and Pollution Research 22, 17330-17342.

Murphy, C.A., Rose, K.A., Alvarez, M.D., and Fuiman, L.A. (2008). Modeling larval fish behavior: Scaling the sublethal effects of methylmercury to population-relevant endpoints. Aquatic Toxicology 86, 470-484.

Murton, A.J., Constantin, D., and Greenhaff, P.L. (2008). The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy. Biochimica Et Biophysica Acta-Molecular Basis of Disease 1782, 730-743.

Nader, N., Chrousos, G. P., and Kino, T. (2010). Interactions of the circadian CLOCK system and the HPA axis. Trends in Endocrinology & Metabolism, 21(5), 277-286.

Naderi, F., Hernandez-Perez, J., Chivite, M., Soengas, J.L., Miguez, J.M., and Lopez-Patino, M.A. (2018). Involvement of cortisol and sirtuin1 during the response to stress of hypothalamic circadian system and food intake-related peptides in rainbow trout, *Oncorhynchus mykiss*. Chronobiology International 35, 1122-1141.

Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P., and Sassone-Corsi, P. (2008). The NAD^+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134, 329-340.

Narendra, D., Tanaka, A., Suen, D.F., and Youle, R.J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. Journal of Cell Biology 183, 795-803.

Narendra, D.P., Jin, S.M., Tanaka, A., Suen, D.F., Gautier, C.A., Shen, J., Cookson, M.R., and Youle, R.J. (2010). PINK1 Is Selectively Stabilized on Impaired Mitochondria to Activate Parkin. Plos Biology 8.

Navarro, A., Quiros, L., Casado, M., Faria, M., Carrasco, L., Benejam, L., Benito, J., Diez, S., Raldua, D., Barata, C., et al. (2009). Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. Aquatic Toxicology 93, 150-157.

Navarro, P., Clemens, S., Perrot, V., Bolliet, V., Tabouret, H., Guerin, T., Monperrus, M., and Amouroux, D. (2013). Simultaneous determination of mercury and butyltin species using a multiple species-specific isotope dilution methodology on the European, *Anguilla anguilla* glass eel and yellow eel. International Journal of Environmental Analytical Chemistry 93, 166-182.

Neilson, J.D., and Perry, R.I. (1990). Diel vertical migrations of marine fishes - an obligate or facultative process. Advances in Marine Biology 26, 115-168.

Nespolo, R.F., and Franco, M. (2007). Whole-animal metabolic rate is a repeatable trait: a meta-analysis. Journal of Experimental Biology 210, 3877-3878.

Nguetseng, R., Fliedner, A., Knopf, B., Lebreton, B., Quack, M., and Rudel, H. (2015). Retrospective monitoring of mercury in fish from selected European freshwater and estuary sites. Chemosphere 134, 427-434.

Norin, T., and Clark, T.D. (2016). Measurement and relevance of maximum metabolic rate in fishes. Journal of Fish Biology 88, 122-151.

Norin, T., and Malte, H. (2012). Intraspecific Variation in Aerobic Metabolic Rate of Fish: Relations with Organ Size and Enzyme Activity in Brown Trout. Physiological and Biochemical Zoology 85, 645-656.

Nostbakken, O.J., Martin, S.A.M., Cash, P., Torstensen, B.E., Amlund, H., and Olsvik, P.A. (2012). Dietary methylmercury alters the proteome in Atlantic salmon (*Salmo salar*) kidney. Aquatic Toxicology 108, 70-77.

O'Connor, K.I., Taylor, A.C., and Metcalfe, N.B. (2000). The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. Journal of Fish Biology 57, 41-51.

Oliveira, K., and McCleave, J.D. (2000). Variation in population and life history traits of the American eel, *Anguilla rostrata*, in four rivers in Maine. Environmental Biology of Fishes 59, 141-151.

Olsvik, P.A., Amlund, H., Saele, O., Ellingsen, S., and Skjaerven, K.H. (2015). Impact of dietary selenium on methylmercury toxicity in juvenile Atlantic cod: A transcriptional survey. Chemosphere 120, 199-205.

O'Neill, J.S., Lee, K.D., Zhang, L., Feeney, K., Webster, S.G., Blades, M.J., Kyriacou, C.P., Hastings, M.H., and Wilcockson, D.C. (2015). Metabolic molecular markers of the tidal clock in the marine crustacean *Eurydice pulchra*. Current Biology 25, R326-R327.

Ongwijitwat, S., Liang, H.L., Graboyes, E.M., and Wong-Riley, M.T.T. (2006). Nuclear respiratory factor 2 senses changing cellular energy demands and its silencing down-regulates cytochrome oxidase and other target gene mRNAs. Gene 374, 39-49.

Otake T. Metamorphosis. (2003). In: Aida K, Tsukamoto K, Yamauchi K (eds). Eel Biology. Springer, Tokyo. pp. 61–74.

Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., and van den Thillart, G. (2006). Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? Naturwissenschaften 93, 145-148.

Pankhurst, N.W. (1982). Relation of visual changes to the onset of sexual-maturation in the European eel *Anguilla-anguilla* (L). Journal of Fish Biology 21, 127-140.

Pankhurst, N.W., and Lythgoe, J.N. (1982). Structure and color of the integument of the European eel *Anguilla-anguilla* (L). Journal of Fish Biology 21, 279-296.

Pankhurst, N.W., and Lythgoe, J.N. (1983). Changes in vision and olfaction during sexual-maturation in the European eel *Anguilla-anguilla* (L). Journal of Fish Biology 23, 229-240.

Paredes, S.D., Sánchez, S., Rial, R.V., Rodríguez, A.B., and Barriga, C. (2005). Changes in behaviour and in the circadian rhythms of melatonin and corticosterone in rats subjected to a forced-swimming test. J Appl Biomed, 3(1), 47-57.

Parsons, J., Vickers, K., and Warden, Y. (1977). Relationship between elver recruitment and changes in the sex ratio of silver eels *Anguilla anguilla* L. migrating from Lough Neagh, Northern Ireland. Journal of Fish Biology 10, 211-229.

Passmore, L.A., and Barford, D. (2004). Getting into position: the catalytic mechanisms of protein ubiquitylation. Biochemical Journal 379, 513-525.

Pavey, S.A., Gaudin, J., Normandeau, E., Dionne, M., Castonguay, M., Audet, C., and Bernatchez, L. (2015). RAD Sequencing Highlights Polygenic Discrimination of Habitat Ecotypes in the Panmictic American Eel. Current Biology 25, 1666-1671.

Pereira, M.E., Lillebo, A.I., Pato, P., Valega, M., Coelho, J.P., Lopes, C.B., Rodrigues, S., Cachada, A., Otero, M., Pardal, M.A., et al. (2009). Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. Environmental Monitoring and Assessment 155, 39-49.

Pereira, P., Korbas, M., Pereira, V., Cappello, T., Maisano, M., Canario, J., Almeida, A., and Pacheco, M. (2019). A multidimensional concept for mercury neuronal and sensory toxicity in fish - From toxicokinetics and biochemistry to morphometry and behavior. Biochimica Et Biophysica Acta-General Subjects 1863.

Pereira, P., Raimundo, J., Araujo, O., Canario, J., Almeida, A., and Pacheco, M. (2014). Fish eyes and brain as primary targets for mercury accumulation - A new insight on environmental risk assessment. Science of the Total Environment 494, 290-298.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29.

Pfaffl, M.W., Horgan, G.W., and Dempfle, L. (2002). Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research 30.

Picca, A., Lezza, A.M.S., Leeuwenburgh, C., Pesce, V., Calvani, R., Landi, F., Bernabei, R., and Marzetti, E. (2017). Fueling Inflamm-Aging through Mitochondrial Dysfunction: Mechanisms and Molecular Targets. International Journal of Molecular Sciences 18.

Pickhardt, P.C., Stepanova, M., and Fisher, N.S. (2006). Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*). Environmental Toxicology and Chemistry 25, 2132-2142.

Pintard, L., Willems, A., and Peter, M. (2005). Cullin-based ubiquitin ligases: Cul3-BTB complexes join the family (vol 23, pg 1681, 2004). Embo Journal 24, 1092-1092.

Piper, R. (2007). Extraordinary Animals: An Encyclopedia of Curious and Unusual Animals, Greenwood Press.

Pletz, J., Sanchez-Bayo, F., and Tennekes, H.A. (2016). Dose-response analysis indicating time-dependent neurotoxicity caused by organic and inorganic mercury-Implications for toxic effects in the developing brain. Toxicology 347, 1-5.

Podgorniak, T., Angelini, M., De Oliveira, E., Daverat, F., and Pierron, F. (2017). Selective pressure of fishways upon morphological and muscle enzymatic traits of migrating glass eels. Canadian Journal of Fisheries and Aquatic Sciences 74, 445-451.

Podgorniak, T., Milan, M., Pujolar, J.M., Maes, G.E., Bargelloni, L., De Oliveira, E., Pierron, F., and Daverat, F. (2015). Differences in brain gene transcription profiles advocate for an important role of cognitive function in upstream migration and water obstacles crossing in European eel. Bmc Genomics 16.

Powers, S.K., and Jackson, M.J. (2008). Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. Physiological Reviews 88, 1243-1276.

Priede, I.G. (1985). Metabolic scope in fishes. In: Fish Energetics New Perspectives (eds P. Tytler, and P. Calow), Croom-Helm, London, pp.33-64.

Prokkola, J.M., and Nikinmaa, M. (2018). Circadian rhythms and environmental disturbances - underexplored interactions. Journal of Experimental Biology 221.

Prokkola, J.M., Nikinmaa, M., Lubiana, P., Kanerva, M., McCairns, R.J.S., and Gotting, M. (2015). Hypoxia and the pharmaceutical diclofenac influence the circadian responses of three-spined stickleback. Aquatic Toxicology 158, 116-124.

Prouzet P., 2003, Étude sur la civelle (*Anguilla anguilla*) dans l'estuaire de l'Adour: Pêche, biologie, comportement, modélisations hydrodynamique et comportementale, estimations des flux de civelles en estuaire. Rapport Institution Adour-Ifremer-Univ. Pau-Adour.

Puga, S., Pereira, P., Pinto-Ribeiro, F., O'Driscoll, N.J., Mann, E., Barata, M., Pousao-Ferreira, P., Canario, J., Almeida, A., and Pacheco, M. (2016). Unveiling the neurotoxicity of methylmercury in fish (*Diplodus sargus*) through a regional morphometric analysis of brain and swimming behavior assessment. Aquatic Toxicology 180, 320-333.

Pujolar, J.M., Jacobsen, M.W., Als, T.D., Frydenberg, J., Munch, K., Jonsson, B., Jian, J.B., Cheng, L., Maes, G.E., Bernatchez, L., et al. (2014). Genome-wide single-generation signatures of local selection in the panmictic European eel. Molecular Ecology 23, 2514-2528.

Rabinowitz, J. D. and White, E. Autophagy and metabolism. Science 330, 1344-1348 (2010).

Rambold, A.S., Kostelecky, B., and Lippincott-Schwartz, J. (2011b). Together we are stronger Fusion protects mitochondria from autophagosomal degradation. Autophagy 7, 1568-1569.

Rambold, A.S., Kostelecky, B., Elia, N., and Lippincott-Schwartz, J. (2011a). Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. Proceedings of the National Academy of Sciences of the United States of America 108, 10190-10195.

Rasinger, J.D., Lundebye, A.K., Penglase, S.J., Ellingsen, S., and Amlund, H. (2017). Methylmercury Induced Neurotoxicity and the Influence of Selenium in the Brains of Adult Zebrafish (*Danio rerio*). International Journal of Molecular Sciences 18.

Regnier, T., Bolliet, V., Labonne, J., and Gaudin, P. (2010). Assessing maternal effects on metabolic rate dynamics along early development in brown trout (*Salmo trutta*): an individual-based approach. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 180, 25-31.

Reinke, H., and Asher, G. (2019). Crosstalk between metabolism and circadian clocks. Nature Reviews Molecular Cell Biology 20, 227-241.

Rescan, P.Y., Montfort, J., Ralliere, C., Le Cam, A., Esquerre, D., and Hugot, K. (2007). Dynamic gene expression in fish muscle during recovery growth induced by a fasting-refeeding schedule. Bmc Genomics 8.

Rhee, J.S., Kim, B.M., Lee, B.Y., Hwang, U.K., Lee, Y.S., and Lee, J.S. (2014). Cloning of circadian rhythmic pathway genes and perturbation of oscillation patterns in endocrine disrupting chemicals (EDCs)-exposed mangrove killifish kryptolebias rnarrnoratus. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 164, 11-20.

Riahi, H., Brekelmans, C., Foriel, S., Merkling, S.H., Lyons, T.A., Itskov, P.M., Kleefstra, T., Ribeiro, C., van Rij, R.P., Kramer, J.M., et al. (2019). The histone methyltransferase G9a regulates tolerance to oxidative stress-induced energy consumption. Plos Biology 17.

Ribeiro, C.A.D., Belger, L., Pelletier, E., and Rouleau, C. (2002). Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). Environmental Research 90, 217-225.

Ribeiro, C.A.D., Nathalie, M.D., Gonzalez, P., Yannick, D., Jean-Paul, B., Boudou, A., and Massabuau, J.C. (2008). Effects of dietary methylmercury on zebrafish skeletal muscle fibres. Environmental Toxicology and Pharmacology 25, 304-309.

Ribeiro, C.A.O., Neta, F.F., Mela, M., Silva, P.H., Randi, M.A.F., Rabitto, I.S., Costa, J., and Pelletier, E. (2006). Hematological findings in neotropical fish Hoplias malabaricus exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. Environmental Research 101, 74-80.

Ribeyre, F., and Boudou, A. (1984). Bioaccumulation et repartition tissulaire du mercure - $HgCl_2$ et CH_3HgCl - chez Salmo gairdneri apres contamination par voie directe. Water, Air, and Soil Pollution, 23(2), 169-186.

Ricciarelli, R., Zingg, J.M., and Azzi, A. (2001). Vitamin E: protective role of a Janus molecule. Faseb Journal 15, 2314-2325.

Riemann, L., Alfredsson, H., Hansen, M.M., Als, T.D., Nielsen, T.G., Munk, P., Aarestrup, K., Maes, G.E., Sparholt, H., Petersen, M.I., et al. (2010). Qualitative assessment of the diet of European eel larvae in the Sargasso Sea resolved by DNA barcoding. Biology Letters 6, 819-822.

Righton, D., and Walker, A.M. (2013). Anguillids: conserving a global fishery. Journal of Fish Biology 83, 754-765.

Righton, D., Westerberg, H., Feunteun, E., Okland, F., Gargan, P., Amilhat, E., Metcalfe, J., Lobon-Cervia, J., Sjoberg, N., Simon, J., et al. (2016). Empirical observations of the spawning migration of European eels: The long and dangerous road to the Sargasso Sea. Science Advances 2.

Riley, B.E., Kaiser, S.E., Shaler, T.A., Ng, A.C.Y., Hara, T., Hipp, M.S., Lage, K., Xavier, R.J., Ryu, K.Y., Taguchi, K., et al. (2010). Ubiquitin accumulation in autophagy-deficient mice is dependent on the Nrf2-mediated stress response pathway: a potential role for protein aggregation in autophagic substrate selection. Journal of Cell Biology 191, 537-552.

Robinet, T.T., and Feunteun, E.E. (2002). Sublethal effects of exposure to chemical compounds: A cause for the decline in Atlantic eels? Ecotoxicology 11, 265-277.

Rochard, E. and Elie P. (1994). La macrofaune aquatique de l'estuaire de la Gironde. Contribution au livre blanc de l'Agence de l'Eau Adour Garonne. p. 1-56. In J.-L. Mauvais and J.-F. Guillaud (eds.) État des connaissances sur l'estuaire de la Gironde. Agence de l'Eau Adour-Garonne, Éditions Bergeret, Bordeaux, France. 115 p.

Roff, D. A. (1996). The evolution of threshold traits in animals. Quarterly Review of Biology 71:3-35.

Roncarati, A., Melotti, P., Mordenti, O., and Gennari, L. (1997). Influence of stocking density of European eel (*Anguilla anguilla*, L.) elvers on sex differentiation and zootechnical performances. Journal of Applied Ichthyology 13, 131-136.

Roos, D., Seeger, R., Puntel, R., and Barbosa, N.V. (2012). Role of Calcium and Mitochondria in MeHg-Mediated Cytotoxicity. Journal of Biomedicine and Biotechnology.

Roos, D.H., Puntel, R.L., Farina, M., Aschner, M., Bohrer, D., Rocha, J.B.T., and Barbosa, N.B.D. (2011). Modulation of methylmercury uptake by methionine: Prevention of mitochondrial dysfunction in rat liver slices by a mimicry mechanism. Toxicology and Applied Pharmacology 252, 28-35.

Rossi, R., and Villani, P. (1980). A biological analysis of eel catches, Anguilla-anguilla L, from the lagoons of Lesina and Varano, Italy. Journal of Fish Biology 16, 413-423.

Saftig, P., Beertsen, W., and Eskelinen, E.L. (2008). LAMP-2 - A control step for phagosome and autophagosome maturation. Autophagy 4, 510-512.

Salin, K., Auer, S.K., Rudolf, A.M., Anderson, G.J., Cairns, A.G., Mullen, W., Hartley, R.C., Selman, C., and Metcalfe, N.B. (2015). Individuals with higher metabolic rates have lower levels of reactive oxygen species in vivo. Biology Letters 11.

Sampaio, E., Maulvault, A.L., Lopes, V.M., Paula, J.R., Barbosa, V., Alves, R., Pousao-Ferreira, P., Repolho, T., Marques, A., and Rosa, R. (2016). Habitat selection disruption and lateralization impairment of cryptic flatfish in a warm, acid, and contaminated ocean. Marine Biology 163.

Sanada, K., Hayashi, Y., Harada, Y., Okano, T., and Fukada, Y. (2000). Role of circadian activation of mitogen-activated protein kinase in chick pineal clock oscillation. Journal of Neuroscience 20, 986-991.

Sánchez-Bretaño, A., Alonso-Gómez, A. L., Delgado, M. J., & Isorna, E. (2016). Clock genes expression rhythms in liver of goldfish *in vivo* and *in vitro*. Possible role of glucocorticoids as a synchronizer. J. Comp. Physiol. B, 186, 73-82.

Sanchez-Vazquez, F.J., Lopez-Olmeda, J.F., Vera, L.M., Migaud, H., Lopez-Patino, M.A., and Miguez, J.M. (2019). Environmental Cycles, Melatonin, and Circadian Control of Stress Response in Fish. Frontiers in Endocrinology 10.

Sandheinrich, M.B., and Miller, K.M. (2006). Effects of dietary methylmercury on reproductive behavior of fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry 25, 3053-3057.

Sauvaget, B., Fatin, D., and Briand, C. (2003). Contamination by Anguillicola crassus of five coastal eel populations (*Anguilla anguilla*) in Brittany (France). Bulletin Francais De La Peche Et De La Pisciculture, 21-26.

Scheulhammer, A.M., Meyer, M.W., Sandheinrich, M.B., and Murray, M.W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36, 12-18.

Schibler, U., Gotic, I., Saini, C., Gos, P., Curie, T., Emmenegger, Y., Sinturel, F., Gosselin, P., Gerber, A., Fleury-Olela, F., et al. (2015). Clock-Talk: Interactions between Central and Peripheral Circadian Oscillators in Mammals. 21st Century Genetics: Genes at Work, Vol 80, 2015 80, 223-232.

Schmidt, J. (1922) The breeding places of the eel. Philosophical Transactions of the Royal Society 211, 179–208.

Schmidt, J. (1923). The breeding places of the eel. Philosophical Transactions of the Royal Society B: Biological Sciences, 211, 179–208.

Schmidt, J. 1909. On the distribution of the freshwater eels (*Anguilla*) throughout the world. I. Atlantic Ocean and adjacent region. Meddr. Kommn Havunders. Ser. Fisk.3: 1–45.

Scott, G.R., and Sloman, K.A. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology 68, 369-392.

Secor, D.H., Hendersonarzapalo, A., and Piccoli, P.M. (1995). Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes. Journal of Experimental Marine Biology and Ecology 192, 15-33.

Selimovic, D., Porzig, B., El-Khattouti, A., Badura, H.E., Ahmad, M., Ghanjati, F., Santourlidis, S., Haikel, Y., and Hassan, M. (2013). Bortezomib/proteasome inhibitor triggers both apoptosis and autophagy-dependent pathways in melanoma cells. Cellular Signalling 25, 308-318.

Seo, A.Y., Joseph, A.M., Dutta, D., Hwang, J.C.Y., Aris, J.P., and Leeuwenburgh, C. (2010). New insights into the role of mitochondria in aging: mitochondrial dynamics and more. Journal of Cell Science 123, 2533-2542.

Sha, Z., Schnell, H.M., Ruoff, K., and Goldberg, A. (2018). Rapid induction of p62 and GABARAPL1 upon proteasome inhibition promotes survival before autophagy activation. Journal of Cell Biology 217, 1757-1776.

Shaid, S., Brandts, C.H., Serve, H., and Dikic, I. (2013). Ubiquitination and selective autophagy. Cell Death & Differentiation, 20, 21-30.

Shanker, G., Syversen, T., Aschner, J.L., and Aschner, M. (2005). Modulatory effect of glutathione status and antioxidants on methylmercury-induced free radical formation in primary cultures of cerebral astrocytes. Molecular Brain Research 137, 11-22.

Shanks, A.L. (1995). Orientated swimming by megalopae of several eastern North Pacific crab species and its potential role in their onshore migration. Journal of Experimental Marine Biology and Ecology 186, 1-16.

Shearman, L.P., Sriram, S., Weaver, D.R., Maywood, E.S., Chaves, I., Zheng, B., Kume, K., Lee, C.C., Hastings, M.H. and Reppert, S.M., 2000. Interacting molecular loops in the mammalian circadian clock. Science, 288(5468), 1013-1019.

Sheldon, M. R., and McCleave, J. D. (1985). Abundance of glass eels of the American eel, *Anguilla rostrata*, in mid-channel and near shore during estuarine migration. Natural-iste can., 112: 425-430.

Shi, D., and Gu, W. (2012). Dual roles of MDM2 in the regulation of p53: ubiquitination dependent and ubiquitination independent mechanisms of MDM2 repression of p53 activity. Genes Cancer 3, 240–248.

Shin, Y.G., Klucken, J., Patterson, C., Hyman, B.T., and McLean, P.J. (2005). The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. Journal of Biological Chemistry 280, 23727-23734.

Sindayigaya, E., Vancauwenbergh, R., Robberecht, H., and Deelstra, H. (1994). Copper, zinc, manganese, iron, lead, cadmium, mercury and arsenic in fish from lake Tanganyika, Burundi. Science of the Total Environment 144, 103-115.

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M. and Czaja, M.J. (2009). Autophagy regulates lipid metabolism. Nature 458, 1131-1135.

Skak, C., and Baatrup, E. (1993). Quantitative and histochemical-demonstration of mercury deposits in the inner-ear of trout, *Salmo-trutta*, exposed to dietary methylmercury and dissolved mercuric-chloride. Aquatic Toxicology 25, 55-70.

Stacey, J.A., Pratt, T.C., Verreault, G., and Fox, M.G. (2015). A caution for conservation stocking as an approach for recovering Atlantic eels. Aquatic Conservation-Marine and Freshwater Ecosystems 25, 569-580.

Steindal, I.A.F., and Whitmore, D. (2019). Circadian Clocks in Fish-What Have We Learned so far? Biology-Basel 8.

Stringari, J., Nunes, A.K.C., Franco, J.L., Bohrer, D., Garcia, S.C., Dafre, A.L., Milatovic, D., Souza, D.O., Rocha, J.B.T., Aschner, M., et al. (2008). Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. Toxicology and Applied Pharmacology 227, 147-154.

Strungaru, S.A., Robea, M.A., Plavan, G., Todirascu-Ciornea, E., Ciobica, A., and Nicoara, M. (2018). Acute exposure to methylmercury chloride induces fast changes in swimming performance, cognitive processes and oxidative stress of zebrafish (Danio rerio) as reference model for fish community. Journal of Trace Elements in Medicine and Biology 47, 115-123.

Styrishave, B., and Depledge, M.H. (1996). Evaluation of mercury-induced changes in circadian heart rare rhythms in the freshwater crab, *Potamon potamios* and the crayfish, *Astacus astacus* as an early predictor of mortality. Comparative Biochemistry and Physiology a-Physiology 115, 349-356.

Sugeha, H.Y., Arai, T., Miller, M.J., Limbong, D., and Tsukamoto, K. (2001). Inshore migration of the tropical eels *Anguilla* spp. recruiting to the Poigar River estuary on north Sulawesi Island. Marine Ecology Progress Series 221, 233-243.

Sun, J.S., Wang, M.H., and Ho, Y.S. (2012). A historical review and bibliometric analysis of research on estuary pollution. Marine Pollution Bulletin 64, 13-21.

Sunderland, E.M., Gobas, F., Heyes, A., Branfireun, B.A., Bayer, A.K., Cranston, R.E., and Parsons, M.B. (2004). Speciation and bioavailability of mercury in well-mixed estuarine sediments. Marine Chemistry 90, 91-105.

Svendsen, J.C., Genz, J., Anderson, W.G., Stol, J.A., Watkinson, D.A., and Enders, E.C. (2014). Evidence of Circadian Rhythm, Oxygen Regulation Capacity, Metabolic Repeatability and Positive Correlations between Forced and Spontaneous Maximal Metabolic Rates in Lake Sturgeon *Acipenser fulvescens*. Plos One 9.

Sweet, L.I., and Zelikoff, J.T. (2001). Toxicology and immunotoxicology of mercury: A comparative review in fish and humans. Journal of Toxicology and Environmental Health-Part B-Critical Reviews 4, 161-205.

Tahara, Y., Shiraishi, T., Kikuchi, Y., Haraguchi, A., Kuriki, D., Sasaki, H., Motohashi, H., Sakai, T., and Shibata, S. (2015). Entrainment of the mouse circadian clock by sub-acute physical and psychological stress. Scientific Reports 5.

Takanezawa, Y., Nakamura, R., Sone, Y., Uraguchi, S., and Kiyono, M. (2016). Atg5-dependent autophagy plays a protective role against methylmercury-induced cytotoxicity. Toxicology Letters 262, 135-141.

Takanezawa, Y., Nakamura, R., Sone, Y., Uraguchi, S., and Kiyono, M. (2019). An autophagy deficiency promotes methylmercury-induced multinuclear cell formation. Biochemical and Biophysical Research Communications 511, 460-467.

Tasset, I., and Cuervo, A.M. (2016). Role of chaperone-mediated autophagy in metabolism. Febs Journal 283, 2403-2413.

Tatara, C.P., Newman, M.C., and Mulvey, M. (2001). Effect of mercury and Gpi-2 genotype on standard metabolic rate of eastern mosquitofish (*Gambusia holbrooki*). Environmental Toxicology and Chemistry 20, 782-786.

Tesch F. W. (1978). Telemetric observations on the spawning migration of the eel (*Anguilla anguilla*) west of the European continental shelf. Environ Biol Fish 3:203–209.

Tesch F. W. (1995). Vertical movements of migrating silver eels (*Anguilla anguilla*) in the sea. Bull Sea Fish Inst 2: 24–30.

Tesch F. W. (1977). The Eel: Biology and Management of Anguillid Eels. London: Chapman & Hall. 434 pages.

Tesch F. W. (1989). Changes in swimming depth and direction of silver eels (*Anguilla anguilla* L.) from the continental shelf to the deep sea. Aquat Living Resour 2: 9-20

Tesch F. W., Westerberg H., and Karlsson L. (1991). Tracking studieson migrating silver eels in the Central Baltic. Meeres-forschung 33:183-196.

Tesch, F. W. (1980). Occurrence of eel Anguflla anguflla larvae west of the European continental shelf, 1974-1977. Environ. Biol. Fish 5, 185-190.

Tesch, F. W. (2003). The Eel. fifth ed. Wiley, New York.

Thacker, P.D. (2005). The brain is defenseless against mercury. Environmental Science & Technology 39, 234A-234A.

Toledo, M., Batista-Gonzalez, A., Merheb, E., Aoun, M.L., Tarabra, E., Feng, D.R., Sarparanta, J., Merlo, P., Botre, F., Schwartz, G.J., et al. (2018). Autophagy Regulates the Liver Clock and Glucose Metabolism by Degrading CRY1. Cell Metabolism 28, 268-+.

Tomoda, H., and Uematsu, K. (1996). Morphogenesis of the brain in larval and juvenile Japanese eels, *Anguilla japonica*. Brain Behavior and Evolution 47, 33-41.

Tosi, L., Spampanato, A., Sola, C., and Tongiorgi, P. (1990). Relation of water odor, salinity and temperature to ascent of glass-eels, *Anguilla-anguilla* (L) - a laboratory study. Journal of Fish Biology 36, 327-340.

Toyama, T., Shinkai, Y., Yasutake, A., Uchida, K., Yamamoto, M., and Kumagai, Y. (2011). Isothiocyanates Reduce Mercury Accumulation via an Nrf2-Dependent Mechanism during Exposure of Mice to Methylmercury. Environmental Health Perspectives 119, 1117-1122.

Tsukamoto, K. (2009). Oceanic migration and spawning of anguillid eels. Journal of Fish Biology, 74, 1833-1852.

Tsukamoto, K., and Arai, T. (2001). Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats. Marine Ecology Progress Series 220, 265-276.

Tsukamoto, K., Aoyama, J., and Miller, M.J. (2002). Migration, speciation, and the evolution of diadromy in anguillid eels. Canadian Journal of Fisheries and Aquatic Sciences 59, 1989-1998.

Tsukamoto, K., Nakai, I., and Tesch, W.V. (1998). Do all freshwater eels migrate? Nature 396, 635-636.

Tzeng W.N. (1985). Immigration timing and activity rhythms of the eel, *Anguilla japonica*, elvers in the estuary of northern Taiwan, with emphasis on environmental influences. Bull. Jap. SOC. Fish. Oceanogr. 41, 48, 11-28.

Tzeng, W.N. (1996). Effects of salinity and ontogenetic movements on strontium:calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. Journal of Experimental Marine Biology and Ecology 199, 111-122.

Tzeng, W.N., Severin, K.P., and Wickstrom, H. (1997). Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. Marine Ecology Progress Series 149, 73-81.

Tzeng, W.N., Wang, C.H., Wickstrom, H., and Reizenstein, M. (2000). Occurrence of the semi-catadromous European eel *Anguilla anguilla* in the Baltic Sea. Marine Biology 137, 93-98.

Ullrich, S.M., Tanton, T.W., and Abdrashitova, S.A. (2001). Mercury in the aquatic environment: A review of factors affecting methylation. Critical Reviews in Environmental Science and Technology 31, 241-293.

Um, J.H., Pendergast, J.S., Springer, D.A., Foretz, M., Viollet, B., Brown, A., Kim, M.K., Yamazaki, S., and Chung, J.H. (2011). AMPK Regulates Circadian Rhythms in a Tissue- and Isoform-Specific Manner. Plos One 6.

Usuki, F., Fujita, E., and Sasagawa, N. (2008). Methylmercury activates ASK1/JNK signaling pathways, leading to apoptosis due to both mitochondria- and endoplasmic reticulum (ER)-generated processes in myogenic cell lines. Neurotoxicology 29, 22-30.

Usuki, F., Yasutake, A., Umehara, F., Tokunaga, H., Matsumoto, M., Eto, K., Ishiura, S., and Higuchi, I. (2001). In vivo protection of a water-soluble derivative of vitamin E, Trolox, against methylmercury-intoxication in the rat. Neuroscience Letters 304, 199-203.

van Ginneken, V., Dufour, S., Sbaihi, M., Balm, P., Noorlander, K., de Bakker, M., Doornbos, J., Palstra, A., Antonissen, E., Mayer, I., et al. (2007). Does a 5500-km swim trial stimulate early sexual maturation in the European eel (*Anguilla anguilla* L.)? Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 147, 1095-1103.

van Ginneken, V., Palstra, A., Leonards, P., Nieveen, M., van den Berg, H., Flik, G., Spanings, T., Niemantsverdriet, P., van den Thillart, G., and Murk, A. (2009). PCBs and the energy cost of migration in the European eel (*Anguilla anguilla* L.). Aquatic Toxicology 92, 213-220.

Vane, C.H., Harrison, I., and Kim, A.W. (2007). Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in sediments from the Mersey Estuary, UK. Science of the Total Environment 374, 112-126.

Vatine, G., Vallone, D., Gothilf, Y., and Foulkes, N.S. (2011). It's time to swim! Zebrafish and the circadian clock. Febs Letters 585, 1485-1494.

Vaughan, M., Berger, J.E., and Steinberg, D. (1964). Hormone-SensitiveLipase and Monoglyceride Lipase Activities in Adipose Tissue. J. Biol.Chem.239, 401–409.

Verhelst, P., Buysse, D., Reubens, J., Pauwels, I., Aelterman, B., Van Hoey, S., Goethals, P., Coeck, J., Moens, T., and Mouton, A. (2018). Downstream migration of European eel (*Anguilla anguilla* L.) in an anthropogenically regulated freshwater system: Implications for management. Fisheries Research 199, 252-262.

Vieira, L.R., Gravato, C., Soares, A., Morgado, F., and Guilhermino, L. (2009). Acute effects of copper and mercury on the estuarine fish Pomatoschistus microps: Linking biomarkers to behaviour. Chemosphere 76, 1416-1427.

Vuilleumier, R., Boeuf, G., Fuentes, M., Gehring, W.J., and Falcon, J. (2007). Cloning and early expression pattern of two melatonin biosynthesis enzymes in the turbot (*Scophthalmus maximus*). European Journal of Neuroscience 25, 3047-3057.

Wagner, C., Sudati, J.H., Nogueira, C.W., and Rocha, J.B.T. (2010). *In vivo* and *in vitro* inhibition of mice thioredoxin reductase by methylmercury. Biometals 23, 1171-1177.

Walker, M.G., Jones, F.R.H., and Arnold, G.P. (1978). The movements of plaice (*Pleuronectes platessa* L.) tracked in the open sea. Journal du Conseil international pour l'Exploration de la Mer, 38: 58–86.

Walker, M.G., Riley, J.D., and Emerson, L. (1980). On the movements of sole (*Solea-solea*) and dogfish (*Scyliorhinus-canicula*) tracked off the east Anglian coast. Netherlands Journal of Sea Research 14, 66-77.

Wang, S.P., Soni, K.G., Semache, M., Casavant, S., Fortier, M., Pan, L.G., and Mitchell, G.A. (2008). Lipolysis and the integrated physiology of lipid energy metabolism. Molecular Genetics and Metabolism 95, 117-126.

Wang, X.J., Yu, J., Wong, S.H., Cheng, A.S.L., Chan, F.K.L., Ng, S.S.M., Cho, C.H., Sung, J.J.Y., and Wu, W.K.K. (2013). A novel crosstalk between two major protein degradation systems Regulation of proteasomal activity by autophagy. Autophagy 9, 1500-1508.

Ward, C., Martinez-Lopez, N., Otten, E.G., Carroll, B., Maetzel, D., Singh, R., Sarkar, S., and Korolchuk, V.I. (2016). Autophagy, lipophagy and lysosomal lipid storage disorders. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1861, 269-284.

Warman, C.G., and Naylor, E. (1995). Evidence for multiple, cue-specific circatidal clocks in the shore crab *Carcinus maenas*. Journal of Experimental Marine Biology and Ecology 189, 93-101.

Watanabe, S. (2003). Taxonomy of the freshwater eels, genus Anguilla schrank, 1798. Eel Biology, 3-18.

Webber, H.M., and Haines, T.A. (2003). Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environmental Toxicology and Chemistry 22, 1556-1561.

Weber, D.N., Connaughton, V.P., Dellinger, J.A., Klemer, D., Udvadia, A., and Carvan, M.J. (2008). Selenomethionine reduces visual deficits due to developmental methylmercury exposures. Physiology & Behavior 93, 250-260.

Weihs D. (1978) Tidal stream transport as an efficient method formigration. J Conseil 38: 92–99.

Weis, J.S., and Khan, A.A. (1990). Effects of mercury on the feeding-behavior of the mummichog, *Fundulus-heteroclitus* from a polluted habitat. Marine Environmental Research 30, 243-249.

Weis, J.S., and Khan, A.A. (1991). Reduction in prey capture ability and condition of mummichogs from a polluted habitat. Transactions of the American Fisheries Society 120, 127-129.

Wendelaar-Bonga, S.E. (1997). The stress response in fish. Physiol. Rev. 77, 591-625.

West, A.K., Hidalgo, J., Eddins, D., Levin, E.D., and Aschner, M. (2008). Metallothionein in the central nervous system: Roles in protection, regeneration and cognition. Neurotoxicology 29, 489-503.

Westerberg H. (1979). Counter-current orientation in the migration of the European eel. Rapp P-V Reun Cons IntExplor Mer 174:134-143

Westermann, B. 2008. Molecular machinery of mitochondrial fusion and fission. J. Biol. Chem. 283:13501-13505.

Wiberg, U. (1983). Sex determination in the European eel (*Anguilla anguilla* L.). A hypothesis based on cytogenetic results, correlated with the finding of skewed sex ratios in eel culture ponds. Cytogenet. Cell Genet.36, 589-598.

Wilcockson, D., and Zhang, L. (2008). Circatidal clocks. Current biology, 18(17), R753-R755.

Wilson, J.M., Antunes, J.C., Bouca, P.D., and Coimbra, J. (2004). Osmoregulatory plasticity of the glass eel of *Anguilla anguilla*: freshwater entry and changes in branchial ion-transport protein expression. Canadian Journal of Fisheries and Aquatic Sciences 61, 432-442.

Winter, H.V., Jansen, H.M., and Breukelaar, A. (2007). Silver eel mortality during downstream migration in the River Meuse, from a population perspective. Ices Journal of Marine Science 64, 1444-1449.

Wippelhauser, G.S., and McCleave, J.D. (1987). Precision of behavior of migrating juvenile American eels (*Anguilla-rostrata*) utilizing selective tidal stream transport. Journal Du Conseil 44, 80-89.

Wippelhauser, G.S., and McCleave, J.D. (1988). Rhythmic activity of migrating juvenile American eels *Anguilla-rostrata*. Journal of the Marine Biological Association of the United Kingdom 68, 81-91.

Wolfe, M.F., Schwarzbach, S., and Sulaiman, R.A. (1998). Effects of mercury on wildlife: A comprehensive review. Environmental Toxicology and Chemistry 17, 146-160.

Wood, P., and Partridge, J.C. (1993). Opsin substitution induced in retinal rods of the eel (*Anguilla-anguilla* (L)) - a model for g-protein-linked receptors. Proceedings of the Royal Society B-Biological Sciences 254, 227-232.

Wren, C.D., Harris, S., and Harttrup, N. (1995). Ecotoxicology of mercury and cadmium. In: Handbook of Ecotoxicology, ed. D.J. Hoffman, B.A. Rattner, G.A. Burton Jr and J. Cairns Jr, pp. 392-423. Boca Raton, USA: Lewis Publishers.

Wu, W.K.K., Wu, Y.C., Yu, L., Li, Z.J., Sung, J.J.Y., and Cho, C.H. (2008). Induction of autophagy by proteasome inhibitor is associated with proliferative arrest in colon cancer cells. Biochemical and Biophysical Research Communications 374, 258-263.

Wurtz, J., Taraschewski, H., and Pelster, B. (1996). Changes in gas composition in the swimbladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). Parasitology 112, 233-238.

Xu, J., Ji, J., and Yan, X.H. (2012). Cross-Talk between AMPK and mTOR in Regulating Energy Balance. Critical Reviews in Food Science and Nutrition 52, 373-381.

Yadetie, F., Bjorneklett, S., Garberg, H.K., Oveland, E., Berven, F., Goksoyr, A., and Karlsen, O.A. (2016). Quantitative analyses of the hepatic proteome of methylmercury-exposed Atlantic cod (*Gadus morhua*) suggest oxidative stress-mediated effects on cellular energy metabolism. Bmc Genomics 17.

Yadetie, F., Karlsen, O.A., Lanzen, A., Berg, K., Olsvik, P., Hogstrand, C., and Goksoyr, A. (2013). Global transcriptome analysis of Atlantic cod (*Gadus morhua*) liver after in vivo methylmercury exposure suggests effects on energy metabolism pathways. Aquatic Toxicology 126, 314-325.

Yasutake, A., and Hirayama, K. (1990). Selective quantification of inorganic mercury in tissues of methylmercury-treated rats. Bulletin of Environmental Contamination and Toxicology 45, 662-666.

Yin, Z.B., Milatovic, D., Aschner, J.L., Syversen, T., Rocha, J.B.T., Souza, D.O., Sidoryk, M., Albrecht, J., and Aschner, M. (2007). Methylmercury induces oxidative injury, alterations in permeability and glutamine transport in cultured astrocytes. Brain Research 1131, 1-10.

Zannas, A.S., and Binder, E.B. (2014). Gene-environment interactions at the FKBP5 locus: sensitive periods, mechanisms and pleiotropism. Genes Brain and Behavior 13, 25-37.

Zannas, A.S., Wiechmann, T., Gassen, N.C., and Binder, E.B. (2016). Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and Translational Implications. Neuropsychopharmacology 41, 261-274.

Zechner, R., Madeo, F., and Kratky, D. (2017). Cytosolic lipolysis and lipophagy: two sides of the same coin. Nature Reviews Molecular Cell Biology 18, 671-684.

Zechner, R., Zimmermann, R., Eichmann, T.O., Kohlwein, S.D., Haemmerle, G., Lass, A., and Madeo, F. (2012). FAT SIGNALS - Lipases and Lipolysis in Lipid Metabolism and Signaling. Cell Metabolism 15, 279-291.

Zeng, L.Q., Zhang, A.J., Killen, S.S., Cao, Z.D., Wang, Y.X., and Fu, S.J. (2017). Standard metabolic rate predicts growth trajectory of juvenile Chinese crucian carp (*Carassius auratus*) under changing food availability. Biology Open 6, 1305-1309.

Zhang, L., Hastings, M.H., Green, E.W., Tauber, E., Sladek, M., Webster, S.G., Kyriacou, C.P., and Wilcockson, D.C. (2013). Dissociation of Circadian and Circatidal Timekeeping in the Marine Crustacean *Eurydice pulchra*. Current Biology 23, 1863-1873.

Zhang, Y., Claireaux, G., Takle, H., Jorgensen, S.M., and Farrell, A.P. (2018). A three-phase excess post-exercise oxygen consumption in Atlantic salmon *Salmo salar* and its response to exercise training. Journal of Fish Biology 92, 1385-1403.

Zhang, Y.R., Huang, Q.D., Liu, S.T., He, D.C., Wei, G., and Luo, Y.P. (2014). Intraspecific mass scaling of metabolic rates in grass carp (*Ctenopharyngodon idellus*). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 184, 347-354.

Zhao, J.H., and Goldberg, A.L. (2016). Coordinate regulation of autophagy and the ubiquitin proteasome system by mTOR. Autophagy 12, 1967-1970.

Zhao, J.H., Zhai, B., Gygi, S.P., and Goldberg, A.L. (2015). mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. Proceedings of the National Academy of Sciences of the United States of America 112, 15790-15797.

Zhao, Z.Y., and Touitou, Y. (1993). Kinetic changes of melatonin release in rat pineal perifusions at different circadian stages - effects of corticosteroids. Acta Endocrinologica 129, 81-88.

Zhou, T., and Weis, J.S. (1998). Swimming behavior and predator avoidance in three populations of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. Aquatic Toxicology, 43, 131-148.

Zhou, T., Scali, R., and Weis, J.S. (2001). Effects of methylmercury on ontogeny of prey capture ability and growth in three populations of larval *Fundulus heteroclitus*. Archives of Environmental Contamination and Toxicology 41, 47-54.

Zhu, K., Dunner, K., and McConkey, D.J. (2010). Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. Oncogene 29, 451-462.

Zimmermann, R., Strauss, J.G., Haemmerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., Lass, A., Neuberger, G., Eisenhaber, F., Hermetter, A., et al. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306, 1383-1386.

LIST OF PUBLICATIONS

[1] **Liu, H.T.**, Labonne, J., Coste, P., Huchet, E., Plagnes-Juan, E., Rives, J., Veron, V., Seiliez, I., Bolliet, V., 2019. Looking at the complex relationships between migration behavior and conditional strategy based on energy metabolism in the European glass eel (*Anguilla anguilla*). *Science of the Total Environment, 696.* https://doi.org/10.1016/j.scitotenv.2019.134039.

[2] **Liu, H.T.**, Lamarins, A., Labonne, J., Monperrus, M., Coste, P., Huchet, E., Rives, J., Seiliez, I., Bolliet, V., 2020. New insights into methylmercury induced behavioral and energy-related gene transcriptional responses in European glass eel (*Anguilla anguilla*). *Chemosphere, 255.* https://doi.org/10.1016/j.chemosphere.2020.127020.

[3] **Liu, H.T.**, Labonne, J., Coste, P., K. Dias, Huchet, E., Plagnes-Juan, E., Rives, J., Veron, V., Seiliez, I., Bolliet, V., 2020. Rethinking the role of selective pressure upon estuarine migration behavior in European glass eel (*Anguilla anguilla*). *Physiology & Behaviour* (under review).

The relationship between migration behavior and energetic status in the European glass eel (*Anguilla anguilla*)

ABSTRACT: The European eel (*Anguilla anguilla*) present a complex life cycle with a glass eel stage migrating up estuary to reach river for growth. However, this estuarine migration is known to be facultative, with some individuals settling at sea, in estuaries or alternating stays in rivers and estuaries. As glass eels feed little or not at all during their migration, their divergent migration patterns may be closely associated with individual's body condition. To date, one major theory of conditional strategy proposed that the facultative migration in European glass eels is based on energy stores, the individuals with a high migratory capacity presenting high energy stores. However, this theory has been proved controversial and the aim of this thesis was to investigate the conditional strategy in European glass eels based on more comprehensive measures of energetic status, including not only energy stores but also energy mobilization (metabolism and energy-related genes expression). We also focused on both autumn and spring glass eels, which present dramatic difference in energy stores.

We first characterized the individual energetic status of marine glass eels sampled in autumn and spring and related this status to their migration behavior assessed in experimental facilities. Autumn glass eels presented higher energy stores than spring individuals. Molecular analyses suggested that autumn glass eels present a higher ability to produce energy while the spring ones display an energy distress. This confirmed that autumn and spring glass eels present strong differences in their energetic status and that they have to be studied separately. We hypothesized that a potential threshold in energetic status may exist below which migration could be conditioned by energetics.

Then, to unveil the underlying mechanisms of settlement processes in estuaries in relation to energy-based conditional strategy, we investigated the relationship between energetic status and migration behavior in both marine and estuarine glass eels. Estuarine individuals displayed lower weight than marine ones in autumn but not in spring supporting the idea that a conditional strategy based on energy may explain facultative migration when energy reserves become a limiting factor. We also observed a higher percentage of individuals synchronized to the current direction in estuarine fish than in marine ones suggesting that the selection may also target their ability to synchronize swimming activity to the tide. Weight loss, standard metabolism and the expression of metabolism-related genes suggested that estuarine glass eels were more stressed and had a lower capacity of energy production than marine fish. The non-synchronized glass eels also presented a higher energy expenditure than synchronized individuals possibly reflecting a higher stress and/or vulnerability to stress in the former.

In this regard, we further exposed glass eels to a potential stressor in estuaries ie methylmercury (MeHg) in order to investigate the effects of this contaminant on glass eels' migratory behavior and energetic status. Our results first suggest that non-synchronized glass eels were more vulnerable to MeHg contaminant reflected by a decrease in swimming activity. MeHg also affected the relation between individual metabolism-related genes expression level and swimming activity, supporting our hypothesis that stress factors may influence the settlement processes in glass eels. Finally, it is noteworthy that non-synchronized glass eels displayed lower expression level of metabolism genes than their synchronized counterpart in the head but not in muscle nor in the viscera.

Altogether, these results provided evidences that the energetic status and sensitivity to stress may condition estuarine migration in glass eels but the underlying mechanisms and relationships between these factors but also with the endogenous clocks driving migration remain to be elucidated.

Keywords: *Anguilla anguilla*, glass eel, facultative migration, metabolism, autophagy, methylmercury, stress

Relation entre le comportement migratoire et le statut énergétique de la civelle d'anguille européenne (*Anguilla anguilla*)

RÉSUMÉ : L'anguille européenne (*Anguilla anguilla*) présente un cycle de vie complexe avec un stade civelle qui remonte les estuaires pour atteindre les rivières et entamer une phase de croissance. Cependant, cette migration estuarienne est connue pour être facultative, certains individus s'installant en mer, en estuaire ou alternant les séjours en rivière et en estuaire. Les civelles ne se nourrissent pas ou peu pendant leur migration et leurs schémas migratoires pourraient donc être étroitement associés aux réserves énergétiques des individus. La migration facultative des civelles pourrait donc reposer sur une stratégie conditionnelle, les individus présentant d'importantes réserves d'énergie ayant une capacité migratoire élevée. Cependant, certaines études s'avèrent contradictoires et l'objectif de cette thèse était ? d'étudier la stratégie conditionnelle chez les civelles européennes en se basant non seulement sur les réserves d'énergie mais également sur la mobilisation de l'énergie (métabolisme et expression des gènes liés à l'énergie). L'essentiel des travaux a été mené à la fois sur des civelles d'automne et de printemps, car elles présentent d'importantes différences de réserves énergétiques.

Nous avons tout d'abord caractérisé le statut énergétique individuel des civelles marines échantillonnées en automne et au printemps et l'avons relié à leur comportement migratoire évalué dans des installations expérimentales. Les civelles d'automne présentaient des réserves énergétiques plus élevées que celles de printemps. Les analyses moléculaires suggèrent que les civelles d'automne présentent une plus grande capacité à produire de l'énergie alors que les civelles de printemps affichent une importante détresse énergétique. Ces résultats confirment que les civelles d'automne et de printemps présentent de fortes différences dans leur statut énergétique et qu'elles doivent être étudiées séparément. Nous émettons l'hypothèse qu'il pourrait exister un seuil énergétique en dessous duquel la migration pourrait être conditionnée par ce facteur.

Afin de mieux comprendre les mécanismes de sédentarisation dans les estuaires, la relation entre le statut énergétique et le comportement migratoire des civelles marines et estuariennes a ensuite été étudié parallèlement en automne et au printemps. Nos résultats soutiennent l'hypothèse d'une stratégie conditionnelle basée sur l'énergie lorsque les réserves énergétiques deviennent un facteur limitant (civelles de printemps). Nous avons également observé un pourcentage plus élevé d'individus synchronisés avec la direction du courant (considérés comme migrants) chez les civelles estuariennes que chez les marines, suggérant que la sélection pourrait aussi cibler leur capacité à synchroniser leur activité de nage avec la marée. La perte de poids, la mesure du métabolisme et l'expression de gènes liés au métabolisme suggèrent également que les civelles estuariennes étaient plus stressées et avaient une capacité de production d'énergie plus faible que les marines. Les civelles non synchronisées au courant (considérées comme ayant une faible probabilité de migration) présentaient une dépense énergétique plus élevée que les individus synchronisés, ce qui pourrait refléter un stress et/ou une vulnérabilité au stress plus élevés chez les premières.

Afin de tester cette hypothèse, des civelles ont été exposées à un facteur de stress potentiel dans les estuaires, à savoir le méthylmercure (MeHg), et les effets de ce contaminant sur le comportement migratoire et le statut énergétique des individus a été étudié. Nos résultats mettent en évidence une diminution de l'activité de nage chez les civelles non synchronisées mais pas chez les synchronisés, suggérant que les premières pourraient être plus sensibles au MeHg. Le MeHg a également affecté la relation entre le niveau d'expression des gènes liés au métabolisme et l'activité de natation, ce qui soutient l'idée que les facteurs de stress pourraient influencer la migration des civelles. Enfin, les civelles non synchronisées présentaient un niveau d'expression des gènes du métabolisme inférieur à celui de leurs homologues synchronisées dans la tête et pas dans le muscle ni dans les viscères.

Les travaux réalisés dans le cadre de cette thèse suggèrent que le statut énergétique et la sensibilité au stress pourraient conditionner la migration estuarienne des civelles mais les mécanismes sous-jacents et les relations entre ces facteurs ainsi qu'avec les horloges endogènes qui contrôlent la migration restent à élucider.

Keywords: *Anguilla anguilla*, civelle, migration facultative, metabolisme, autophagie, méthylmercure, stress

Ecole doctorale :
211-SCIENCES EXACTES ET LEURS APPLICATIONS

LABORATOIRE :
UMR 1224 Ecobiop & UMR 1419 NuMéA

CONTACT
UMR 1224 Ecobiop & UMR 1419 NuMéA, Aquapôle,
F-64310 Saint Pée sur Nivelle, France
hengtongouc@gmail.com