

# Facteurs du contrôle de la dispersion continentale chez l'anguille

Thèse présentée pour l'obtention du titre de

**DOCTEUR DE L'UNIVERSITE TOULOUSE III**

**Discipline : Ecologie Aquatique**

Par **ERIC EDELIN**



Photo Pierre Elie

**Sous la direction de PIERRE ELIE**

Soutenue le 30 Juin 2005

## JURY

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| Pierre LAVANDIER   | Professeur             | UPS                    | Président          |
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## Remerciements

Je tiens avant tout à remercier les membres du jury de l'honneur qu'ils me font: Pr. Lavandier, Université Paul Sabatier, Toulouse; Dr. Castonguay, chef de section Poisson, Institut Maurice Lamontagne, Québec; Dr. Gaudin, directeur de recherche à l'INRA, St Pée sur Nivelle, Dr. Chemineau, directeur de recherche à l'INRA, Tours; ainsi que Dr. Dufour, directeur de recherche CNRS/MNHN à Paris et Dr. Elie, directeur de recherche au Cemagref de Bordeaux.

Par leurs grandes qualités scientifiques, la confiance qu'ils m'ont accordée, par leur enthousiasme et leur amitié, Sylvie Dufour et Pierre Elie ont fait de cette thèse une expérience très formatrice et enrichissante. Je leur en suis profondément reconnaissant.

Je remercie Paul Gonthier de m'avoir accueilli au sein de son unité de recherche et de son soutien.

Ce travail, réalisé dans le cadre scientifique des GIS Grisam et Ecobag, a été rendu possible par les contributions financières et/ou matérielles du Cemagref, de la Région Aquitaine, du FEDER objectif 2 Aquitaine, du Ministère de l'Ecologie et du Conseil Supérieur de la Pêche.

La collaboration de Cédric Briand à cette thèse a été inestimable. Pour avoir développé les collecteurs, m'avoir donné accès au barrage d'Arzal, pour ses idées toujours pertinentes, et pour son amitié qui m'a été un grand soutien, merci à lui. Merci aussi à Denis Fatin et Brice Sauvaget, qui ont largement contribué à la réalisation des manipulations à Arzal.

La bonne humeur qui règne dans l'équipe de Sylvie Dufour au MNHN, et l'accueil chaleureux que j'y ai reçu, ont toujours rendu mes séjours à Paris très agréables. Merci à Bernadette Vidal, dont l'humour et l'énergie sont très motivants, à Nadine Le Belle, Finn-Arne Weltzien, Miskal Sbaihi, Sylvie Baloche, et à toute l'équipe.

Romaric LeBarh, agent technique au Cemagref de Bordeaux, a toujours accompagné d' humour et de bonne humeur nos sorties de terrain, de jour comme de nuit, hiver comme été. Ces sorties sur la Dordogne resteront toujours pour moi d'excellents souvenirs.

Je ne saurais oublier Claude et Maryse Durand et leurs enfants pour leur amitié, leur gentillesse, leur aide matérielle et leur grande expérience de la rivière et des poissons, qu'ils ont bien voulu partager avec Romaric et moi.

Ma gratitude va également à Michel Vignaud, et à tous les membres de la brigade du CSP de la Gironde. Leur aide sur le terrain, leur expérience, leur gentillesse et leur bonne humeur m'ont été très précieuses. Merci à Jean-Noël, Pierre, Olivier et les autres, qu'ils me pardonnent de ne pas tous les citer.

Merci également à Philippe Gaudin, Agnès Bardonnet et Valérie Bolliet de m'avoir accueilli au sein de leur laboratoire. Les nombreuses connaissances que j'ai acquis à leur contact m'ont beaucoup apporté.

Mes pensées vont également à Jean-Noël Tourenq, qui m'a mis le pied à l'étrier à l'Université de Toulouse. Je suis aussi très reconnaissant envers Sylvain Mastorillo, Docteur de l'UPS, pour m'avoir donné la possibilité d'enseigner. Mes discussions avec lui et Frédéric Santoul ont toujours été agréables et intéressantes.

J'aimerais remercier Maryse Desenlis et Franc Bouin pour leur gentillesse, leur aide et leur patience. Que tous les personnels administratifs du Cemagref, qui ont souvent dû travailler pour moi dans l'urgence, soient sincèrement remerciés.

Merci à Patrick Lambert et Christian Rigaud pour nos fructueuses discussions scientifiques, à Chantal Gardes, Patricia Lamaison et Marie-Pierre Maleyrand pour leur efficacité en documentation. Merci à Thierry Rouault, Daniel Mercier, Louis Jacobs et Marcel Pelard, sans qui j'aurais été incapable d'élever des anguilles durant deux mois. Merci à Philippe Camoin pour le dessin de nombreuses figures, et Jean-Marie Boudigues pour la regraphie des documents.

Enfin, je tiens à remercier tous ceux qui, par leur soutien, leur amitié ou leur aide, ont contribué à l'avancée de mes travaux.

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## RESUME

Les larves leptocéphales de l'anguille européenne *Anguilla anguilla* migrent depuis la mer des Sargasses jusqu'au talus continental européen où elles se métamorphosent en civelles qui envahissent les milieux continentaux côtiers, estuariens, et dulcicoles. L'anguille montre une grande variété de comportements dispersifs différents autorisant la colonisation de tous les milieux continentaux. Or, les facteurs contrôlant ces comportements dispersifs divergents sont très mal connus.

Durant le présent travail de thèse, nous avons étudié les facteurs proximaux, internes (physiologiques) et externes (environnementaux), du contrôle de la dispersion continentale de l'anguille aux stades civelle et anguille. Nous avons donc développé une approche multidisciplinaire couplant des études de terrain et en structures expérimentales à des descripteurs comportementaux et endocriniens.

Au stade civelle, l'utilisation du transport tidal sélectif (TTS) pour migrer induit un phénomène d'accumulation au niveau de la limite de renverse des courants de la zone de limite de marée dynamique (ZLM). Nos résultats montrent que les civelles sont fortement dépendantes du TTS pour la migration, et qu'une mauvaise utilisation des courants de marée peut induire une sédentarisation précoce en estuaire par épuisement des individus. La transformation en anguille des civelles ayant atteint la ZLM a lieu dans la partie amont de la ZLM, et coïncide avec l'acquisition d'un comportement alimentaire intense. Nos données suggèrent que, lors de la transformation en anguille, les processus comportementaux de la dispersion continentale passent de densité-indépendants (migration vers l'amont) à densité-dépendants (sélection de l'habitat). Les ZLM abritent donc un processus clé de la dispersion continentale de l'anguille, et doivent être protégées des pressions anthropiques afin d'améliorer la gestion de l'espèce.

Au stade civelle, le comportement migrateur est influencé par des facteurs internes et des facteurs externes. Nous montrons que les hormones thyroïdiennes (TH), la thyroxine ( $T_4$ ) et la triiodothyronine ( $T_3$ ), jouent un rôle fondamental dans le contrôle de la dispersion. Des taux de TH élevés induisent la colonisation des milieux de rivière, alors qu'une diminution du statut thyroïdien provoque une sédentarisation précoce des civelles dans les habitats marins ou estuariens. De plus, nos résultats suggèrent qu'une baisse de la condition corporelle (statut énergétique) induit (i) un changement de préférence de salinité de l'eau douce vers l'eau salée et (ii) une sédentarisation précoce en estuaire. Enfin, nos résultats suggèrent que la température, modulant fortement l'activité locomotrice et la préférence pour l'eau douce, est un facteur environnemental clé du contrôle de la dispersion continentale. Il est probable que les effets du statut énergétique et de la température sur le comportement migrateur des civelles soient régulés à travers les sécrétions de TH, mais aussi d'hormone de croissance (GH).

En conclusion, nos résultats suggèrent que la stratégie de la dispersion continentale de l'anguille dépend de son statut physiologique et varie selon le stade de développement. C'est donc une stratégie de dispersion conditionnelle, résultant d'une sélection statut-dépendante. Cette stratégie permet à l'individu d'incorporer des informations sur sa capacité à obtenir de la fitness à travers des tactiques dispersives alternatives et d'exprimer la tactique qui maximise sa fitness. Les TH, et aussi probablement GH, sont des médiateurs proximaux des tactiques alternatives de la dispersion continentale chez la civelle. L'anthropisation récente des milieux de rivière, en diminuant la fitness associée à un comportement de colonisation des eaux douces, induit probablement une baisse de l'expression du comportement migrateur chez l'anguille.

Mots clé: *Anguilla anguilla*, dispersion continentale, comportement, tactiques migratoires alternatives, transport tidal sélectif, contrôle endocrinien, facteurs environnementaux, gestion.

## ABSTRACT

The leptocephalus larvae of the European eel *Anguilla anguilla* migrate from the Sargasso Sea until the continental shelf where they metamorphose into glass eel that invade continental marine, estuarine and freshwater habitats. The eel shows a great variety of dispersal behaviors, allowing the colonization of marine, estuarine and freshwater continental habitats. However, the factors controlling these divergent dispersal behaviors remain relatively unknown.

During the present PhD work, we studied the proximate factors, both internal (physiological) and external (environmental) controlling the eel continental dispersal at the glass eel and elver stages. Thus, we developed a multidisciplinary approach, coupling both field and experimental studies to behavioral and endocrine measurements.

At the glass eel stage, the use of selective tidal stream transport (STST) for migration induces an accumulation of migrants at the limit of flow reversals in the tidal limit area (TLA). Our results show that glass eels are strongly dependent of STST for movements and that a misuse of tidal streams, exhausting individuals, may induce an early settlement in the estuary. Glass eels that reach the TLA transform into elver in its upstream section. This transformation matches with the acquisition of an intense feeding activity. Our data suggest that, during the transformation of glass eels into elvers, continental dispersal processes switch from density-independent (upstream migration) to density-dependent (habitat selection). Therefore, key processes of the eel continental occur in the TLAs. This suggests that these zone should be preserved from any anthropogenic pressure to improve species management.

During the glass eel continental dispersal, migratory behavior is influenced by both internal and external factors. We show that thyroid hormones (TH), thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ), play a critical role in the control of dispersal. High TH levels induce river colonization, whereas decreased TH levels promote early settlement in marine and estuarine habitats. Furthermore, our results suggest that a decreased body condition (energetic status) induces (i) a switch from freshwater- towards saltwater-preference and (ii) a precocious settlement in the estuary. In addition, our results suggest that water temperature, modulating both locomotor activity and preference for FW, is a key environmental factor for the control of continental dispersal. The effects of the energetic status and water temperature on glass eel migratory behavior are likely regulated through the endocrine secretions of TH, and also growth hormone (GH).

In conclusion, our results show that the continental dispersal strategy of the eel depends on its physiological status and developmental stage. Thus, it is a conditional strategy resulting from a status-dependent selection. This strategy allows an individual to incorporate information about its ability to gain fitness through alternative dispersal tactics and express the tactic that maximizes its fitness. TH, and probably also GH, are proximate mediators of the alternative dispersal tactics in glass eel. The recent anthropogenic pressure on river habitats, decreasing the fitness gained in colonizing the rivers, probably reduces the expression of the migratory behavior in the eel.

**Key-words:** *Anguilla anguilla*, continental dispersal, behavior, alternative migratory tactics, selective tidal stream transport, endocrine control, environmental factors, management.

# INTRODUCTION

En écologie, le terme de dispersion peut désigner des phénomènes différents, tels que la dispersion natale d'un individu entre ses lieux de naissance et de première reproduction (natal dispersal), ou les migrations d'individus adultes pour des raisons alimentaires et de reproduction (Clobert et al. 2001). Dans cette thèse, le terme de dispersion désignera le processus de colonisation des habitats de croissance continentaux côtiers, estuariens et d'eau douce par les anguilles immatures, aux stades civelle et anguille jaune.

L'introduction commence par une brève description du cycle biologique de l'anguille, puis nous soulignons l'importance de l'étude de la dispersion continentale pour la compréhension de l'écologie des anguilles. Nous exposons ensuite l'état des connaissances sur le processus de dispersion continentale de l'anguille, puis nous passons en revue les facteurs internes (physiologiques) et externes (environnementaux) influençant, ou pouvant potentiellement influencer, le comportement dispersif. Cet état de l'art nous permet de pointer certains points obscurs d'importance majeure, que nous avons choisi d'aborder durant la thèse. L'introduction se termine par une présentation générale des travaux effectués durant la thèse. Nos résultats seront donnés sous forme d'articles. Dans la discussion, nous présenterons une analyse générale de nos résultats et proposerons une théorie générale du contrôle de la dispersion continentale de l'anguille. Nous terminerons en explorant les perspectives les plus intéressantes pour d'éventuels travaux futurs.

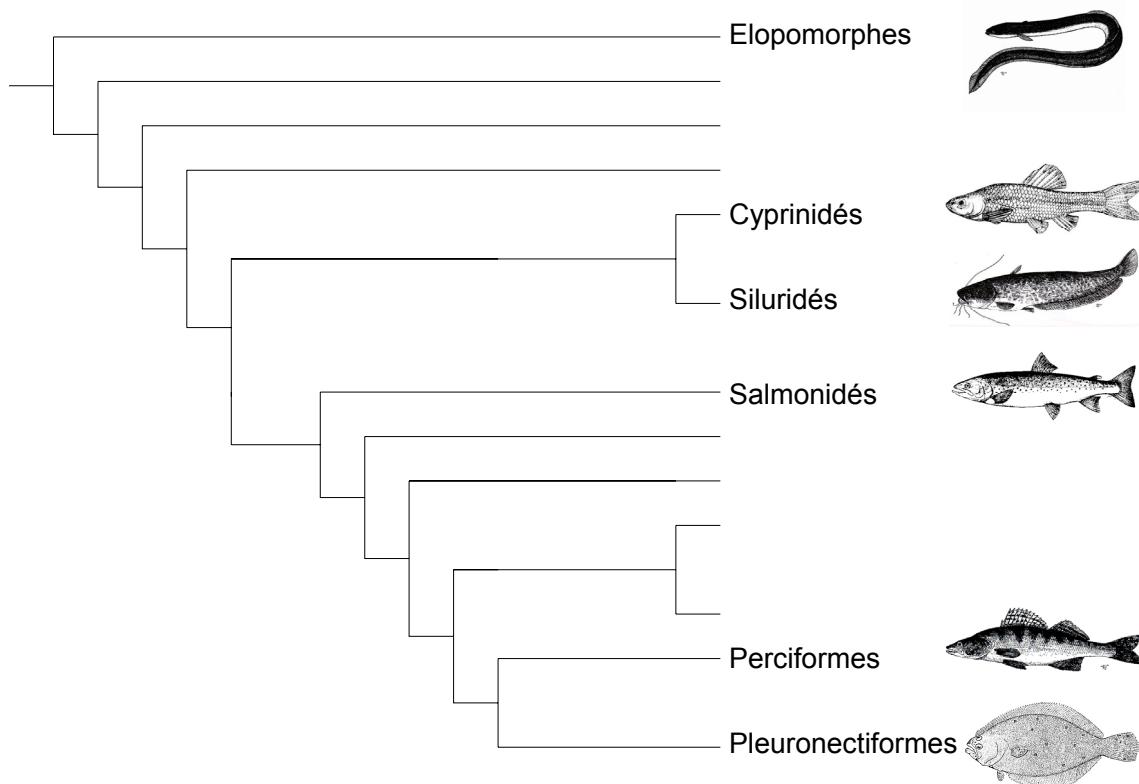


Fig. 1. Phylogénie simplifiée des Téléostéens, montrant la position relative des Elopomorphes vis à vis d'autres groupes importants, d'après Rousseau (2000).

## 1. Cycle biologique et métamorphoses de l'anguille

L'anguille fait partie du superordre des Elopomorphes, un taxon de Téléostéens phylogénétiquement anciens (Fig. 1). Les Elopomorphes sont principalement marins et sont caractérisés par une phase larvaire dite leptocéphale (Hulet & Robins 1989, Pfeiler 1999). Dans l'ordre des Anguilliformes (les anguilles vraies), le genre *Anguilla* compte 15 espèces dans le monde, réparties dans tous les océans, excepté l'atlantique sud (Ege 1939 , Tsukamoto & Aoyama 1998). Toutes les espèces ont un cycle biologique similaire. La reproduction se fait dans l'océan, puis les larves leptocéphales se développent et migrent jusqu'au talus continental où elles se métamorphosent en civelles (Schmidt 1909). La civelle envahit les eaux continentales (incluant les zones marines, estuariennes et de rivière, les marais et les lacs), et se pigmente progressivement jusqu'au stade anguillette (petite anguille jaune). Commence alors une nouvelle phase de croissance, plus longue, (phase anguille jaune) qui se termine avec le début de la maturation sexuelle (ou argenture). L'anguille argentée effectue alors une nouvelle migration vers les sites de reproduction océaniques (Fig. 2). L'anguille subit donc au cours de sa vie deux métamorphoses et réalise deux migrations transocéaniques de longue distance.

La métamorphose de la larve leptocéphale en civelle correspond à de profondes modifications morphologiques, physiologiques et comportementales, qui peuvent être considérées comme une adaptation à la colonisation des milieux continentaux. La larve leptocéphale est translucide et en forme de feuille de saule, une morphologie adaptée à la vie pélagique et à la dérive océanique. Le corps du leptocéphale est très riche en glycoaminoglycanes (GAG), composés hydrophiles constituant une matrice corporelle extracellulaire richement hydratée (Pfeiler 1999). Ces composés ont non seulement un rôle de maintien de la structure et d'hydratation du corps gélatineux de la larve, mais sont aussi supposés fournir des polysaccharides de réserve catabolisés lors de la métamorphose en civelle, phase durant laquelle l'anguille perd ses dents ne s'alimente pas (Bertin 1951). Ce catabolisme des GAG à la métamorphose induit également une baisse très importante (environ 80%) de la teneur corporelle en eau (Bertin 1951, Pfeiler 1999).

Lors de la métamorphose du leptocéphale en civelle, l'anguille acquiert une forme sub-cylindrique (anguilliforme), adaptée à la nage à contre courant, au repos sur le fond ou à l'enfouissement dans le sédiment, des comportements nécessaires à la colonisation des habitats continentaux. La reprise de l'activité alimentaire est conditionnée par l'acquisition d'une nouvelle denture et le développement de l'intestin, qui s'allonge fortement et développe un estomac (Vilter 1945, Elie 1979). De plus, bien que les civelles gardent la capacité de boire de l'eau salée (donc d'osmoréguler en eau de mer), la physiologie osmotique de l'intestin se développe irrémédiablement pour l'osmorégulation en eau douce, quelle que soit la salinité du milieu (Ciccoti et al. 1993). Lors du stade civelle, le corps se pigmente, ce qui permet de définir des stades pigmentaires indiquant la progression de la métamorphose (Elie 1979, 1982). La fin du stade civelle (fin de la métamorphose) correspond à la pigmentation totale du corps et au passage au stade anguillette, ou jeune anguille jaune, au cours de laquelle la croissance reprend.

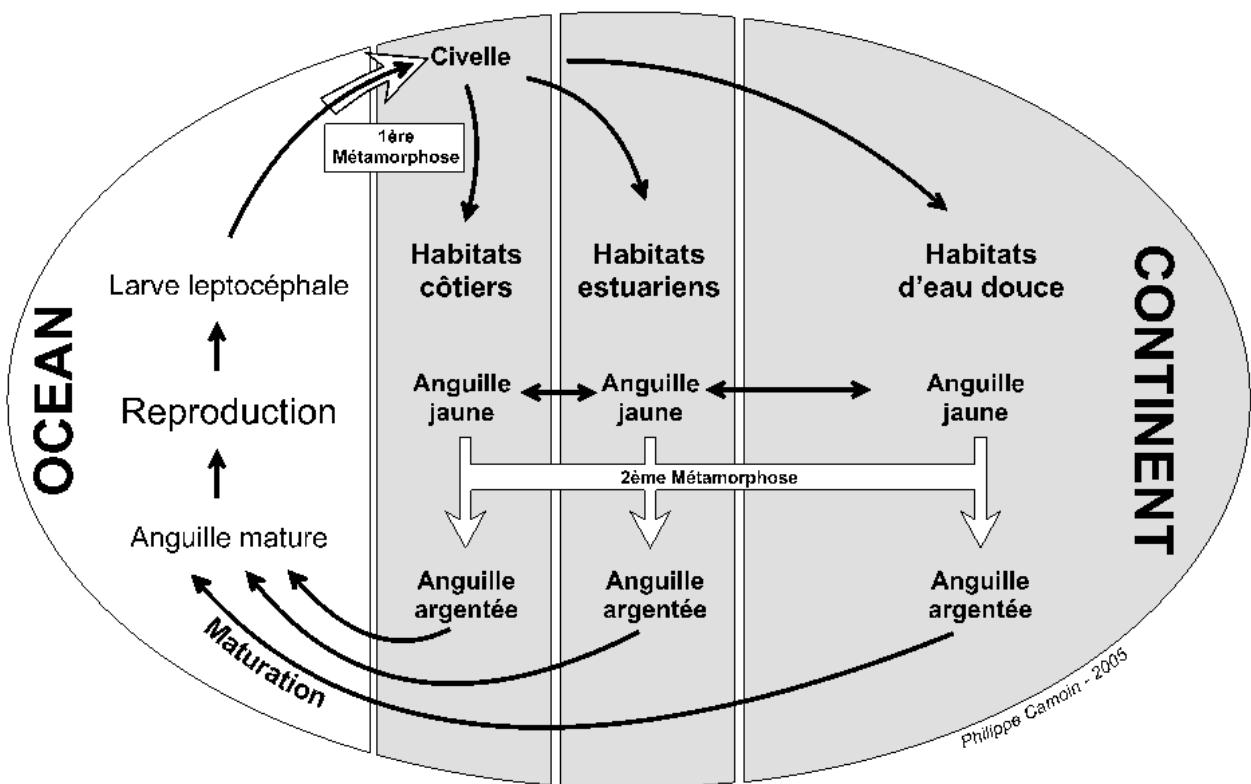


Fig. 2. Cycle biologique schématique de l'anguille. Les habitats continentaux englobent tous les milieux de croissance possibles de l'anguille, i. e. les habitats littoraux, d'estuaire ou d'eau douce.

Le stade anguille jaune est la phase de croissance proprement dite, au cours de laquelle l'anguille va acquérir la taille et les réserves énergétiques suffisantes pour la migration de ponte et la maturation des gonades (Van Ginneken & Van Den Thillart 2000, Van Den Thillart et al. 2004, Van Ginneken et al. 2005). L'anguillette (jeune anguille jaune) est hermaphrodite non fonctionnelle (Bertin 1951). En effet, l'individu n'a pas de gonades différencierées, mais un organe de Syrski possédant des cellules reproductrices germinales qui commencent à se multiplier chez l'anguillette de 10-12 cm. Les cellules germinales se différencient ensuite pour partie en oogonies (à 14 cm de longueur du corps), puis d'autres se différencient en spermatogonies (à 18 cm de longueur du corps). La différenciation sexuelle définitive (sans maturité des gonades) intervient lorsque le poisson atteint une taille d'environ 25-30 cm. Les facteurs précis du déterminisme du sexe sont inconnus, mais sont principalement environnementaux et corrélés à la densité de la population (Krueger & Oliveira 1999).

L'âge et la taille auxquels intervient le début de la maturation sexuelle, ou argenture, sont sexe-dépendants. Les mâles s'argentent plus jeunes et à une taille inférieure à celle des femelles (Bertin 1951, Vøllestad 1992). L'argenture marque le début de la migration de reproduction, mais est réversible, les anguilles pouvant régresser au stade jaune après l'argenture si besoin (Durif 2003). L'argenture constitue une pré-adaptation à la migration océanique à grande profondeur (Fontaine 1994). En effet, en plus de l'acquisition d'une robe argentée caractéristique des poissons marins, l'anguille développe un épaississement de la paroi de la vessie natatoire et ses yeux s'agrandissent (Bertin 1951). Le nombre de cellules à chlorure des branchies augmente, afin de faciliter l'adaptation à l'hypoosmorégulation en eau.

salée. De plus, en raison du début de la maturation des gonades, la fin de la phase continentale de l'argenture (anguilles dévalantes de stades V et VI) est marquée par une nette augmentation du rapport gonadosomatique (Durif et al. 2000 , Durif 2003 , Durif et al. 2005).

## 2. Importance de l'étude de la dispersion continentale

L'anguille, en plus de son intérêt écologique, est un poisson d'une grande importance économique. En effet, les anguilles européenne *A. anguilla*, américaine *A. rostrata* et japonaise *A. japonica*, sont exploitées commercialement à tous les stades du développement continental (Elie 1979 , Tesch 2003). Or, les stocks de ces trois espèces montrent actuellement un déclin dramatique (Stone 2003). Les raisons de ce déclin sont probablement multiples, incluant des facteurs climatiques affectant la circulation océanique (Castonguay et al. 1994b, Knights 2003), et des facteurs anthropiques tels que la pollution, la réduction des habitats et la surpêche (Castonguay et al. 1994a, Elie 1998, Feunteun 2002, Briand et al. 2003, Dekker 2004, Lafaille et al. 2005). Dans ce contexte, une meilleure connaissance de la biologie de l'anguille peut permettre des améliorations significatives des mesures actuelles de gestion.

L'anguille colonise tous les types d'habitats continentaux, des milieux marins côtiers jusqu'aux rivières éloignées de plusieurs centaines de kilomètres de la mer (Bertin 1951, Elie 1979, Elie & Fontenelle 1982, Tesch 2003). De plus, au cours de la phase continentale, les histoires de vie sont très variables. Les anguilles peuvent effectuer l'intégralité de leur croissance en mer, en estuaire, ou en eau douce (Tzeng et al. 1997, Tsukamoto & Arai 2001, Jessop et al. 2004). Enfin, certaines anguilles changent de milieu une ou plusieurs fois au cours de leur vie (Arai et al. 2003, Daverat et al. 2004). Ces nouveaux résultats modifient la vision classique d'une phase continentale exclusivement dulcicole . Nous avons illustré cette diversité des histoires de vie dans la Fig. 2.

Etant donnée l'importance de la pression environnementale sur le déterminisme du sexe, et la variation des ages et longueur à l'argenture en fonction des sexes (Vøllestad 1992, Krueger & Oliveira 1999), la distribution dans les habitats conditionne largement la structure des populations à l'échelle du bassin versant. En effet, les fractions de populations d'estuaire produisent surtout des mâles, s'argentant après environ 4 ans de vie continentale pour une longueur inférieure à 40 cm. En revanche, les anguilles de rivière se différencient préférentiellement en femelles, après 7 à 8 ans pour une longueur supérieure à 45 cm (Bertin 1951, Parsons et al. 1977, Deelder 1985 , Aprahamian 1988, Tesch 2003). De plus, les anguilles d'estuaire ont tendance à grandir plus vite et à s'argenter à des tailles inférieures à celles de rivière, quel que soit le sexe (Gandolfi Hornyold 1929, d'Ancona 1959). Les facteurs du déterminisme du sexe chez l'anguille sont mal connus, mais semblent être corrélés au taux de croissance (Holmgren & Mosegaard 1996, Holmgren et al. 1997) et à la densité de population (Krueger & Oliveira 1999). En conséquence, les mécanismes de dispersion, contrôlant la répartition des individus dans les différents milieux continentaux, jouent un rôle fondamental dans l'écologie de l'anguille.

### **3. Les phases de la dispersion continentale chez l'anguille**

La dispersion, contrôlant les flux de gènes, conditionnant la structure des populations et influençant la fitness, est un des processus les plus importants en écologie des populations (Clobert et al. 2001). Les concepts de la dispersion sont particulièrement utiles pour l'étude de la colonisation des milieux continentaux par les anguilles.

La dispersion animale peut être séparée en trois phases comportementales distinctes: l'émigration, la migration et la sédentarisation (Andreassen et al. 2002). De plus, si la sédentarisation est de longue durée, on parle alors de résidence (Stamps 2001). Dans le cas de la dispersion continentale de l'anguille (stade civelle et anguille jaune), les civelles arrivant de la mer sont déjà en phase de migration, la séquence comportementale sera alors plutôt migration, sédentarisation et émigration si l'habitat est défavorable, ou migration, sédentarisation et résidence si l'habitat est favorable. Les processus de sédentarisation, de résidence et d'émigration sont liés à la sélection de l'habitat par l'individu. Durant ce travail, nous séparerons donc les facteurs affectant la dispersion continentale de l'anguille en deux catégories: les facteurs affectant la migration, et les facteurs affectant la sélection de l'habitat. De plus, ces facteurs seront eux-mêmes divisés en facteurs environnementaux (externes) et physiologiques (internes).

Les habitats continentaux colonisés par l'anguille ont deux niveaux d'organisation. Sur un gradient aval-amont, nous allons distinguer les milieux marins (incluant tous les habitats d'eau de mer), les milieux estuariens (incluant tous les habitats d'eau saumâtre) et les milieux de rivière (incluant en fait tous les habitats d'eau douce). De plus, au sein de chacun de ces grands types d'habitat, les patchs n'auront pas la même valeur en terme de qualité d'habitat. Le comportement de migration déterminera la répartition des individus dans les grands types de milieux (marin, estuaire et de rivière). Le comportement de sélection de l'habitat déterminera la répartition à la fois sur un gradient aval-amont, et surtout dans les patchs au sein des grands types d'habitats.

#### **La dispersion chez les civelles**

Durant la traversée du plateau continental, les civelles utilisent probablement une nage à la fois active et portée par les courants marins (Elie 1979 , Tesch 2003). Près des côtes, et dans les estuaires, les courants de marées deviennent des facteurs hydrodynamiques majeurs pour les civelles qui adoptent alors un comportement de transport tidal sélectif (TTS) (Creutzberg 1958, Elie 1979, McCleave & Kleckner 1982, Gascuel 1986 , McCleave & Wippelhauser 1987). Durant le flot, les poissons sont portés par le courant de marée. A l'étalement de pleine mer, les civelles rejoignent le fond (zones protégées des courants) où elles restent cachées

durant le jusant. Au retour du flot, les poissons remontent dans la colonne d'eau. La migration est donc menée par étapes saltatoires.

Durant le TTS, l'activité de nage est déclenchée par une horloge interne circatidale, synchronisée par les renverses de courant (Wipperhauser & McCleave 1988). Ce mécanisme de synchronisation de l'horloge interne permet aux civelles d'adapter leur rythme d'activité à la déformation de l'onde de marée lors de sa progression vers l'amont des estuaires. Les civelles utilisent également les gradients environnementaux olfactifs et halins pour s'orienter. En effet, les civelles sont fortement attirées par l'odeur des eaux douces continentales (Creutzberg 1961), liée à des composés organiques terrigènes typiques comme par exemple la géosmine (Sorensen 1986, Tosi & Sola 1993, Sola 1995). Ce comportement se rapproche du phénomène de homing du saumon, qui, au cours de la migration de reproduction, s'oriente vers sa rivière de naissance en fonction de signaux olfactifs (Barinaga 1999). Les civelles semblent également être attirées par l'odeur des autres civelles (Sorensen 1986, Briand et al. 2002). De plus, les civelles arrivant de la mer préfèrent majoritairement l'eau douce à l'eau salée (Tongiorgi et al. 1988, Tosi et al. 1988, 1989), un comportement favorisant probablement l'orientation vers les habitats d'eau douce. Enfin, il semble que les gradients thermiques puissent également jouer un rôle dans l'orientation de la migration (Tongiorgi et al. 1986, Tosi et al. 1988). Ces résultats indiquent tous que la migration des civelles est fortement orientée vers la colonisation des habitats d'eau douce.

Lorsque les civelles en migration atteignent la limite de marée dynamique, elles doivent modifier leur comportement en passant du TTS à une nage constante à contre-courant. McCleave & Wipperhauser (1987) supposent que cette transition comportementale, qui permet la colonisation des rivières, induit un retard à la migration. Cependant, le phénomène de migration en limite de marée dynamique est encore très mal connu et cette hypothèse d'un retard de migration en limite des renverses des courants de marée n'a jamais été validée. Ce point a été abordé dans ce présent travail de thèse (voir Chapitre 8).

## **La dispersion chez les anguilles jaunes**

Les suivis des passes montrent que les migrants sont quasi exclusivement des anguillettes (jeunes anguilles jaunes de longueur < 15 cm), ce qui suggère que les anguilles entament la colonisation du bassin versant après au moins 1 an de vie continentale (Haro & Krueger 1988, Michaud et al. 1988, Naismith & Knights 1988, Dutil et al. 1989, McGovern & McCarthy 1992, White & Knights 1997b). Les raisons pour lesquelles l'anguille n'entame pas la colonisation des rivières au stade civelle ne sont pas connues. Notre travail nous a permis d'apporter certaines explications à ce phénomène.

Comparée aux poissons qui remontent les rivières pour se reproduire, l'anguille colonise les rivières à une faible vitesse (Tesch 2003) et donc, plus la distance à la mer est importante, plus les individus interceptés en migration sont grands (Moriarty 1986, Aprahamian 1988, Naismith & Knights 1988, Lobon-Cervia et

al. 1995, White & Knights 1997a, 1997b). Ces résultats indiquent que le comportement migrateur peut s'exprimer jusqu'à un stade de croissance avancé. De plus, ces résultats suggèrent que l'anguille alterne des périodes de migration et d'alimentation lors de la colonisation des rivières. En effet, le coût énergétique de la digestion s'oppose à l'expression de l'activité locomotrice chez l'anguille (Owen 2001), ce qui impose une séparation des phases de croissance et de migration.

D'autre part, plus la taille augmente, plus la tendance à la migration diminue chez l'anguille. En effet, les suivis de migration sur des passes montrent que les migrants sont principalement des anguillettes, et très rarement des anguilles de plus de 30 cm de longueur (Moriarty 1986, Naismith & Knights 1988, White & Knights 1997a, 1997b). La faible vitesse de colonisation des habitats continentaux au stade jaune, soulignée par Tesch (2003), est donc probablement liée à ces phénomènes de croissance et de diminution de la tendance migratrice.

Les moteurs de la dispersion chez l'anguille jaune, hormis le comportement migrateur, ne sont pas précisément connus. Pour plusieurs auteurs, les mouvements des anguilles jaunes semblent être fortement densité-dépendants (Feunteun et al. 2003). En effet, des modèles diffusifs (diffusion aléatoire de particules dépendant de la densité au point source) prévoient bien la distribution des anguilles dans les habitats de rivière (Smogor et al. 1995, Ibboston et al. 2003).

Cependant, d'autres facteurs pourraient intervenir dans le contrôle de la dispersion. Dans les sections suivantes, nous allons passer en revue les facteurs internes (physiologiques) et externes (environnementaux), avérés ou potentiels, pouvant influencer la dispersion continentale de l'anguille.

## 4. Facteurs internes contrôlant le comportement migrateur

### Facteurs endocriniens

Lors des migrations des poissons, les hormones interviennent dans la régulation du développement, du métabolisme énergétique, de l'adaptation physiologique au milieu (osmorégulation) et du comportement (Fontaine 1975, Woodhead 1975). Le contrôle endocrinien des migrations des poissons a surtout été étudié chez les salmonidés.

La transformation du tacon en saumoneau (smoltification) constitue une préadaptation à la migration d'avalaison et à la vie marine (Boeuf 1993). Au cours de la smoltification, la robe du poisson s'argente, ses pigments rétiniens deviennent typiques de ceux des poissons marins, son corps s'allonge, et il développe sa capacité à osmoréguler en eau de mer (hypoosmorégulation) (McCormick et al. 1998). De plus, sa physiologie musculaire, adaptée à la nage de faible intensité en aérobic, devient spécialisée pour les efforts intenses et courts en anaérobic (Katzman & Cech 2001).

La smoltification du tacon est régulée par un pic de production des hormones thyroïdiennes (TH), et par un accroissement des taux d'hormone de croissance (GH), de facteur de croissance insuline-analogue 1 (IGF1) et de cortisol (Hoar 1988, Boeuf 1993, McCormick et al. 1998). Les TH, la thyroxine ( $T_4$ ) et la triiodothyronine ( $T_3$ ), connues pour leur rôle central lors des métamorphoses chez les amphibiens (Denver 1998), sont particulièrement intéressantes. En effet, lors de la smoltification, les TH régulent une grande variété de processus adaptatifs, et sont impliquées dans des transformations morphologiques, physiologiques et comportementales (Boeuf 1993).

Chez les Elopomorphes, le contrôle endocrinien de la migration est beaucoup moins bien connu que chez les salmonidés. Il a été montré que l'activité de la glande thyroïde et les taux de TH s'accroissent fortement lors de la métamorphose du leptocéphale en civelle chez les anguilles des espèces *A. anguilla* (Callamand & Fontaine 1942), *A. japonica* et *A. bicolor pacifica* (Ozaki et al. 2000) et chez le congre *Conger myriaster* (Yamano et al. 1991). De plus, chez *Conger myriaster*, le pic de TH à la métamorphose coïncide avec un accroissement de l'expression des récepteurs  $\alpha$  et  $\beta$  aux TH dans les tissus (Kawakami et al. 2003b, a).

Cela suggère que les TH jouent un rôle fondamental dans la régulation de la métamorphose de la larve en civelle. De plus, il semble que les TH puissent être impliquées dans le développement des capacités hyperosmorégulatrices. En effet, l'intestin des civelles d'*A. anguilla* se développe pour osmoréguler en eau douce (Ciccoti et al. 1993), et la  $T_4$  régule le développement intestinal (Vilter 1946). De plus, comparée à la vie en eau salée, l'acclimatation en eau douce accroît l'activité de la glande thyroïde des civelles d'*A. anguilla* (Monaco et al. 1981).

Un rôle des TH dans la régulation de la migration chez l'anguille peut être supposé. En effet, Castonguay et al. (1990) ont montré que des anguilles jaunes américaines *A. rostrata* prises en train d'escalader des chutes d'eau ont des taux plasmatiques de  $T_4$  plus élevés que des anguilles jaunes prises en aval des chutes et considérées comme sédentaires.

Durant ce présent travail de thèse, nous avons choisi d'explorer le rôle des TH dans la régulation de la dispersion continentale au stade civelle (voir Chapitre 8).

## Facteurs génétiques

Durant les migrations de longue distance comme celle de l'anguille, des instructions génétiques sont nécessaires pour contrôler le déclenchement, la durée et la distance de migration, et les adaptations physiologiques et comportementales de la navigation (Alerstam et al. 2003). En effet, chez les insectes, les traits morphologiques (taille du corps, taille et morphologie des ailes), comportementaux (propension à la migration, durée de la migration), et physiologiques (enzymes associées directement ou indirectement à la locomotion) impliqués dans la capacité dispersive ont une forte héritabilité, indiquant que les variations du comportement migrateur ont une forte base génétique (Roff & Fairbairn 2001). De même, chez les

fauvettes *Sylvia* spp., l'influence génétique est très importante et probablement dominante sur l'influence environnementale dans l'expression des comportements migrateur ou sédentaire (Berthold 1988).

Chez les poissons, les études du déterminisme génétique du comportement migrateur sont rares. Cependant, sur une échelle microévolutive, des études conduites sur le saumon suggèrent une forte composante génétique dans les variations interpopulationnelles du comportement migrateur et d'autres traits d'histoire de vie (Nielsen & Holdensgaard 2001, Quinn et al. 2001). Chez le corégone *Coregonus clupeaformis*, les variations interpopulationnelles du comportement de nage (profondeur et intensité maximale de nage) semblent avoir une base génétique (Rogers et al. 2002).

Chez l'anguille, la panmixie s'oppose à l'établissement de fortes différences génétiques interindividuelles, limitant probablement le contrôle génétique de la dispersion continentale. Chez *A. rostrata*, la panmixie a été récemment confirmée (Wirth & Bernatchez 2003), et seule une sélection naturelle par le milieu a été mise en évidence (Williams et al. 1973, Williams & Koehn 1984). Chez *A. anguilla*, les niveaux d'hétérozygotie des civelles arrivant sur les côtes sont bas, mais parfois significatifs entre des zones géographiques différentes de l'aire de répartition (Daemen et al. 2001, Wirth & Bernatchez 2001, Maes & Volckaert 2002), suggérant que la panmixie n'est pas totale. Cependant, il est pour l'instant inconnu si ces légères différences génétiques sont suffisantes pour induire des comportements migrateurs différents.

## Condition corporelle et taux de croissance

La condition corporelle, traduisant le niveau des réserves énergétiques, est un facteur important influençant la dispersion chez les oiseaux et les mammifères (Dufy & Belthoff 2001). Chez les jeunes salmonidés, les tactiques migratoires alternatives (smoltification ou résidence en rivière) sont dépendantes du statut énergétique (Thorpe et al. 1998). Les poissons qui smoltifient (et donc migrent) sont les plus grands de leur classe d'âge chez la truite fario *Salmo trutta* (Forseth et al. 1999), l'omble de fontaine *Salvelinus fontinalis* (Thériault & Dodson 2003), l'omble chevalier *Salvelinus alpinus* (Rikardsen & Elliott 2000) et le saumon atlantique *Salmo salar* (McCormick et al. 1998). Ces individus ont un métabolisme de base plus élevé, induisant un taux de croissance plus fort, lié à un comportement alimentaire beaucoup plus intense que chez les futurs résidents (Forseth et al. 1999).

Chez les Elopomorphes, un lien entre le taux de croissance larvaire et l'âge à la métamorphose semble également exister. Chez les congres *Conger oceanicus* et *C. conger*, le taux de croissance de l'otolithe du leptocéphale est négativement corrélé à l'âge à la métamorphose et à l'entrée en estuaire au stade civelle (Correia et al. 2003, Correia et al. 2004). Le même phénomène est observé chez *A. japonica* (Tzeng 1990). D'autre part, chez les civelles, la condition corporelle décroît au fur et à mesure de la saison de migration chez *A. anguilla* (Elie 1979, Charlon & Blanc 1982), *A. rostrata* (Jessop 1998), *A. japonica* (Kawakami et al. 1999), *A. reinhardtii* et

*A. australis* (Sloane 1984), et *A. dieffenbachii* (Jellyman & Lambert 2003). Cette décroissance saisonnière du facteur de condition serait liée à des variations saisonnières de la productivité des écosystèmes océaniques (Désaunay 1997).

Jusqu'à maintenant, le lien entre le facteur de condition et le comportement migrateur chez la civelle n'a pas été étudié. Pourtant, cette voie d'exploration semble prometteuse. En effet, il a été montré chez la civelle d'*A. anguilla* que le facteur de condition est inversement corrélé à la teneur du corps en GH (Lambert et al. 2003). Cet accroissement du taux de GH avec la décroissance du facteur de condition pourrait révéler une détresse physiologique, défavorable à l'expression d'un comportement migrateur intense et durable chez les poissons arrivant en fin de saison de migration. Ce point a été abordé dans le présent travail de thèse (voir Chapitre 8).

## 5. Facteurs externes contrôlant le comportement migrateur

### Température

Chez la civelle, il a été montré que la température est le principal facteur environnemental agissant sur la pigmentation, et donc probablement sur le développement (Strubberg 1913, Briand et al. 2004). De plus, les civelles ne s'alimentent pas à des températures inférieures à 10°C (Elie & Daguzan 1976, Charlon & Blanc 1983).

De nombreuses études montrent que la température est un facteur majeur de contrôle du nombre de civelles en migration (Elie 1979, Cantrelle 1981, Gascuel 1986, Vøllestad & Jonsson 1988, McGovern & McCarthy 1992, Elie & Rochard 1994, Martin 1995, Jessop 2003). Ainsi, Vøllestad & Jonsson (1988), étudiant la migration durant 13 ans (entre 1975 et 1987) sur la rivière Imsa (Norvège), ont montré que le niveau des recrutements annuels était positivement corrélé à la moyenne des températures de l'année.

Des seuils de température seraient liés au déclenchement de différents comportements migrateurs chez la civelle. En estuaire, les captures de civelles deviennent négligeables lorsque la température de l'eau descend à 5°C (Elie 1979, Elie & Rochard 1994), ce qui semble indiquer que toute activité locomotrice cesse à cette température. Ce comportement pourrait indiquer l'entrée des civelles en dormance. Gascuel (1986) considère que, entre 5 et 12°C, les civelles montrent une nage exclusivement portée par les courants de marée, et passent à une nage plus active (à contre-courant) à partir d'une température de 12°C. En effet, les montées de civelles sur les passes des barrages estuariens n'interviennent que si la température atteint ou dépasse 12°C (Gascuel 1986, McGovern & McCarthy 1992). Sur les côtes françaises atlantiques, l'essentiel des arrivées de civelles se produit en fin d'automne et en hiver, alors que les températures sont comprises entre 5 et 15°C.

La migration des anguillettes en rivière est également affectée par la température, le pic de migration semblant être déclenché par une hausse des températures printanières (Moriarty 1986, Naismith & Knights 1988). En revanche, plus l'anguille grandit, moins ses déplacements sont température-dépendants (Moriarty 1986, White & Knights 1997b). Cependant, même chez les anguilles jaunes sédentaires, les niveaux d'activité sont influencés par la température (Baras et al. 1998, Baisez 2001).

Les mécanismes physiologiques par lesquels la température peut influencer l'activité locomotrice sont multiples. Une baisse de la température du milieu a des effets instantanés sur l'activité des enzymes, et la fluidité des membranes cellulaires et des liquides internes, induisant une baisse de l'activité métabolique générale (Johnston & Dunn 1987). A plus long terme (jusqu'à un mois), le poisson peut développer des mécanismes compensatoires comme l'accroissement du nombre des mitochondries et des capillaires dans les muscles, le foie, le cerveau et les branchies. Sous un certain seuil thermique, variable selon les espèces, les compensations métaboliques ne sont plus possibles et les poissons entrent en dormance (Johnston & Temple 2002).

Chez le maquereau japonais *Scomber japonicus*, une baisse de la température de 24 à 18°C entraîne une baisse significative de la consommation d'oxygène et de la performance de nage (Dickson et al. 2002). De faibles températures induisent également une baisse de l'activité locomotrice et de la performance de nage chez la morue *Gadus morhua* (Castonguay & Cyr 1998) et le jeune bar *Dicentrarchus labrax* (Koumoundouros et al. 2002). De plus, de faibles températures, en accroissant la viscosité de l'eau, rendent la nage plus difficile, surtout pour les poissons de petite taille (Hunt von Herbing 2002).

La température influence l'activité du cerveau. Chez le poisson chat *Heteropneustes fossilis*, la température influence l'activité de la tyrosine hydroxylase du cerveau et donc la synthèse de la dopamine (Senthilkumaran & Joy 1995, Chaube & Joy 2002), or, les taux de catécholamine du cerveau sont corrélés à l'activité locomotrice chez la truite arc en ciel *Oncorhynchus mykiss* (Jönsson et al. 2003) et l'anguille européenne *A. anguilla* (Le Bras 1978).

Chez les Téléostéens, la température a également des effets endocriniens. Chez le saumon atlantique *Salmo salar*, de faibles températures limitent la smoltification à travers une réduction des sécrétions de GH, IGF1, cortisol et TH (McCormick et al. 2000). Une baisse de la température d'acclimatation induit une diminution des taux plasmatiques de GH chez la truite arc en ciel *Oncorhynchus mykiss* (Gabillard et al. 2003). Chez l'anguille *A. anguilla*, l'élévation de la température induit un accroissement de l'activité de synthèse de la thyroïde (Leloup 1958, Leloup & De Luze 1985). De plus, *in vitro*, la température influence la liaison des TH à leur récepteur chez l'anguille *A. anguilla* et la truite fario *Salmo trutta* (Lebel & Leloup 1989). Cependant, *in vivo* chez la morue *G. morhua*, la température n'a pas d'effet sur la stimulation de l'activité locomotrice par des injections de TH (Castonguay & Cyr 1998).

Enfin, la température influence puissamment les horloges internes des organismes ectothermes (Rensing & Ruoff 2002). Chez des civelles d'anguille

japonaise *A. japonica* maintenues en "free running", on observe des rythmes circatidiaux de consommation de l'oxygène, liés aux rythmes d'activité locomotrice (Kim et al. 2002). Or, en l'absence de stimulation par les renverses de courant, une légère augmentation de la température (de 1°C toutes les 12 ou 24h) suffit à entraîner l'horloge interne et donc à modifier le rythme d'activité. Il est donc possible que des modifications de la température de l'eau puissent perturber l'utilisation des courants de marée (TTS) par les civelles en migration.

Durant le présent travail de thèse, nous avons étudié l'impact de la température sur le comportement migrateur de la civelle à travers son influence sur la préférence de salinité et l'activité locomotrice (voir Chapitre 8).

## Lumière et photopériode

Lors de leur migration transocéanique, les larves leptocéphales effectuent des mouvements verticaux sur un rythme jour/nuit (Kracht 1982 , Schoth & Tesch 1984, Castonguay & McCleave 1987 , Otake et al. 1998 ). Les larves se tiennent en profondeur durant le jour et remontent près de la surface durant la nuit, ce qui suggère un comportement photonégatif, probablement en relation avec l'évitement des prédateurs.

Dans les eaux continentales, les civelles en migration montrent également un fort comportement photonégatif (Bardou et al. 2005). En captivité, l'activité de nage et d'alimentation a lieu la nuit, et les civelles restent cachées le jour (Dou & Tsukamoto 2003). Ce comportement photonégatif des civelles se retrouve lors de la migration en estuaire (Elie 1979, Elie & Rochard 1994), même vis à vis de la lumière lunaire (De Casamajor et al. 2000). Cependant, dans certaines conditions, notamment lorsque les eaux sont suffisamment turbides, ou lorsqu'elles sont contraintes de sortir de l'eau pour migrer, les civelles peuvent migrer de jour (Elie, Comm. Pers.).

Les anguilles jaunes sédentaires montrent également nettement une activité nocturne (Bertin 1951, Baras et al. 1998, Baisez 2001, Tesch 2003), bien qu'une activité diurne puisse être observée à certaines périodes de l'année (Baisez 2001). Ce comportement lucifuge est moins marqué lors des montées d'anguillettes en rivière (Tesch 2003) et chez les anguilles argentées en dévalaison (Durif 2003).

Si il est clair que la lumière a tendance à inhiber le comportement migrateur de l'anguille, l'effet des variations de la photopériode sur le comportement est beaucoup moins bien connu.

Chez les animaux et les plantes, la lumière est un des plus puissants synchroniseurs des rythmes physiologiques (Hastings et al. 1991). L'information photopériodique est transformée en rythme sécrétoire de mélatonine, qui agit sur l'hypothalamus pour réguler l'ensemble des fonctions physiologiques de l'organisme, notamment la maturation sexuelle (Malpaux et al. 2001, Falcon et al. 2003). Des oscillateurs internes permettent de maintenir les rythmes circadiens en l'absence de

stimulation lumineuse (free-running) (Malpaux et al. 2001). Chez les animaux, on enregistre un rythme circadien de sécrétion pour presque toutes les hormones (Brady 1982). Chez les Téléostéens, les variations de la photopériode influencent les taux d'hormones circulantes (Brown & Stetson 1985, Ehrenström & Johansson 1987, Hastings et al. 1991, Björnsson et al. 1994, Leiner et al. 2000, Amano et al. 2001, Leiner & McKenzie 2001, Bayarri et al. 2004, Rodriguez et al. 2004), avec des effets sur le comportement, la croissance et la maturation sexuelle. Par exemple, chez les jeunes salmonidés, la smoltification est déclenchée par un accroissement de la durée du jour (Hoar 1988).

L'anguille présente un rythme circadien d'activité en conditions de free-running, au stade civelle (Wipphäuser & McCleave 1988), et au stade argenté (Edel 1976). Il a été montré chez l'anguille jaune que ces rythmes d'activité étaient liés à des variations circadiennes de la production des catécholamines du cerveau, du cœur et du plasma (Le Bras 1978, 1984). Cependant, l'effet des variations saisonnières de la photopériode sur l'activité migratrice de l'anguille n'a pas été étudié. Le déclenchement des migrations d'anguillettes en rivière est toujours brusque, et intervient lorsque les températures dépassent un seuil de 14-16°C, en Mai pour le Royaume Uni (Moriarty 1986, Naismith & Knights 1988, White & Knights 1997b). Il est possible que le déclenchement de cette migration soit conditionné par l'accroissement printanier de la durée du jour, le facteur déclencheur étant le dépassement d'un seuil de température.

## Odeur de l'eau

Chez les Téléostéens, l'olfaction joue un rôle fondamental dans les processus sociaux (Sorensen 1996, Wisenden 2000) et pour l'orientation des migrations. Chez les salmonidés, l'imprégnation par l'odeur du ruisseau natal lors de la smoltification permet à l'adulte de s'orienter lors de la migration de ponte (Dittman & Quinn 1996, Barinaga 1999). De plus, chez les poissons de récif, l'olfaction jouerait un rôle important dans la sélection de l'habitat (Montgomery et al. 2001). Ces résultats suggèrent que l'olfaction influence à la fois la migration et la sélection de l'habitat.

Chez l'anguille, l'odeur de l'eau joue un rôle à la fois de déclencheur des mouvements et de facteur orienteur de la migration. En effet, il a été montré que l'odeur des eaux douces continentales permettrait de discriminer les courants de flot et de jusant en estuaire, chez la civelle d'*A. anguilla* en migration (Creutzberg 1959), et chez l'anguille américaine *A. rostrata* au stade jaune (Barbin 1998). L'olfaction serait donc un facteur déclencheur dans l'expression du comportement de TTS.

D'autre part, lorsque les poissons sont en mouvement, les signaux olfactifs servent à l'orientation des déplacements. Les civelles d'*A. anguilla* en migration montrent une forte attraction vers l'odeur des eaux douces continentales (Creutzberg 1961). Cette attraction est due, comme discuté plus haut, à divers composés d'origine terrigène (Sorensen 1986, Sola & Tongiorgi 1996), notamment la géosmine (Tosi & Sola 1993), un composé produit par les actinomycètes du sol.

Chez l'anguille argentée, les lobes olfactifs se développent considérablement lors de la préparation à la migration d'avalaison (Bertin 1951). En effet, les signaux olfactifs joueraient un rôle important dans l'orientation des anguilles argentées en migration de reproduction en estuaire (Barbin et al. 1998) et sur le talus continental (Westin 1998). Ces données suggèrent que, comme chez le saumon, une imprégnation olfactive permettant l'orientation de la migration de reproduction pourrait se faire lors des stades juvéniles, au moment de la métamorphose et pendant la migration marine de la civelle.

## Salinité

Les gradients de salinité sont utilisés par les civelles pour s'orienter durant la migration. En effet, des civelles capturées en migration et testées expérimentalement sur leur préférence vis à vis d'un courant d'eau douce et d'un courant d'eau salée préfèrent l'eau douce (Tosi et al. 1988, 1989, 1990). La salinité serait plus importante pour l'orientation que les gradients thermiques et olfactifs (Tosi et al. 1990). De plus, la salinité semble moduler la réponse des civelles vis à vis des composés odorants des eaux douces continentales (Sola & Tongiorgi 1996).

Selon Fontaine (1975), les civelles perdent la spécialisation à osmoréguler en eau de mer lors de la métamorphose, et acquièrent des capacité hyperosmotiques. Ce changement physiologique les pousseraient à préférer les eaux douces, et serait un puissant moteur de la migration.

Durant notre travail de thèse, afin d'explorer plus profondément le rôle de la salinité dans le contrôle de la dispersion continentale, nous avons étudié le lien entre préférence de salinité, croissance, facteur de condition et activité locomotrice chez la civelle (voir Chapitres 6 et 8).

## Marée

Les marées sont fondamentales dans leur action de synchronisation de l'horloge interne des civelles en migration exprimant le TTS (Wipperhauser & McCleave 1988). De plus, l'ampleur des marées est positivement corrélée aux captures de civelles en estuaire (Elie 1979, Jellyman 1979, Elie & Rochard 1994, Jellyman & Lambert 2003), et à l'interface estuaire/rivière (Martin 1995, McKinnon & Gooley 1998, Jessop 2003). Cet effet est probablement mécanique puisque les marées de vives eaux, durant plus longtemps, transportant plus d'eau à des vitesses plus importantes que les marées de mortes eaux, constituent de meilleurs vecteurs de transport pour les civelles.

## Lune

L'effet de la lune sur le comportement migrateur de l'anguille est controversé. Tzeng (1985) rapporte un rythme circalunidal d'activité chez les civelles en migration dans l'estuaire. Cependant, Jellyman et Lambert (2003) et Elie et Rochard (1994) considèrent que la lune agit sur le comportement migrateur des civelles uniquement à travers ses effets tidaux et lumineux. Chez l'anguille jaune sédentaire, l'activité nocturne est la plus forte en lune noire et la plus faible en pleine lune (Adam & Elie 1994), un phénomène probablement dû à l'évitement de la lumière lunaire par les individus. Chez l'anguille argentée, Durif (2003) n'a pas observé d'effet du quartier de lune sur l'intensité des dévalaisons.

## Vents et pression atmosphérique

Les vents et la pression atmosphérique peuvent agir sur la migration des civelles par leur action sur les marées, en renforçant ou en réduisant les courants de flot (Elie & Rochard 1994). De plus, les vents peuvent induire des courants utilisables par les civelles en migration dans des zones où les courants de marée ne sont pas disponibles.

## Facteurs sociaux

Chez les poissons, le comportement d'un individu peut être fortement influencé par le comportement de ses congénères (Magurran 1993). Chez les salmonidés, alors que les tacons sont territoriaux, les smolts acquièrent un comportement de groupe et forment des bancs lors de la migration d'avalaison (Iwata 1995, Hutchison & Iwata 1998, McCormick et al. 1998). Ce comportement de groupe favorise probablement un phénomène d'entraînement (émulation) favorable à la migration et, comme chez beaucoup d'autres poissons, une protection vis à vis des prédateurs.

La formation de "cordons" par les civelles en migration est bien connue (Bertin 1951, Elie 1979, Deelder 1985, Tesch 2003). De même, les anguillettes migrant en rivière ont tendance à former des bancs. Il est probable que la formation de ces groupes par les individus en migration favorise l'expression d'un comportement migrateur par un phénomène d'entraînement (contagion), et limite l'énergie dépensée pour la nage par un phénomène d'aspiration. Cependant, en raison du déclin dramatique des recrutements, ces phénomènes de migration en bancs (cordons) ne sont plus observés, et l'entraînement de la migration par les congénères intervient probablement peu dans le contrôle du comportement migrateur chez l'anguille.

## 6. Facteurs internes contrôlant la sélection de l'habitat

Le pré-requis à l'intervention de processus liés à la sélection de l'habitat est la perte (temporaire ou non) du comportement migrateur. L'animal peut alors explorer l'habitat dans lequel il se trouve et prendre la décision de rester ou de partir.

Les processus physiologiques qui déterminent un individu à rester ou à partir du milieu où il se trouve peuvent être considérés comme liés à sa plasticité phénotypique, c'est à dire sa capacité à adapter son phénotype (physiologie, morphologie et comportement) aux contraintes biotiques et abiotiques du milieu (Murren et al. 2001). En effet, contrairement à un individu migrateur, un individu sédentaire restera suffisamment longtemps dans le milieu pour que son phénotype soit influencé par les conditions environnementales.

La plasticité phénotypique a principalement une base génétique (Pigliucci 1996). Chez l'anguille, la panmixie limite probablement les variations interindividuelles de plasticité phénotypique. Cependant, chez *A. rostrata*, il semble se produire une sélection par le milieu (Williams et al. 1973, Williams & Koehn 1984), ce qui suggère que tous les individus ne sont pas équivalents en terme d'adaptabilité au milieu, i. e. que la plasticité phénotypique pourrait varier selon les individus.

Ces variations interindividuelles de plasticité phénotypique pourraient être liées au taux d'hétérozygotie. Par exemple, chez le guppy *Poecilia reticulata*, la plus forte tolérance à la salinité des souches sauvages comparées aux souches domestiques est liée à une plus forte hétérozygotie des allozymes (Shikano et al. 2000).

La plasticité phénotypique peut également être influencée par les conditions environnementales rencontrées par les individus (disponibilité en nourriture durant les phases de croissance précédant la sédentarisation). Par exemple, chez les poissons de récif, la capacité d'adaptation dans un nouvel habitat dépendrait fortement de la condition corporelle des larves (Booth & Beretta 2004).

On considère classiquement que l'anguille a une grande plasticité phénotypique (Vøllestad 1992), liée entre autre à une grande tolérance physiologique aux variations des facteurs abiotiques du milieu (voir chapitre 7). Cette grande plasticité phénotypique pourrait avoir évolué en réponse à la distribution océanique des larves, qui est très aléatoire et se fait au sein d'aires de répartition très vastes (Europe, Asie, Amérique du nord...), regroupant des types d'habitats très différents (Vøllestad 1992). En effet, les migrations, même modestes, favoriseraient la sélection de traits plastiques (Kingsolver et al. 2002).

## **7. Facteurs externes contrôlant la sélection de l'habitat**

### **Salinité**

La salinité est un facteur environnemental d'importance majeure, qui conditionne la structure des peuplements de poissons dans les zones côtières et estuariennes (Davenport & Sayer 1993, Elie 1998, Jung & Houde 2003).

Il est classiquement admis que la civelle possède une grande tolérance aux variations brusques de la salinité du milieu (Fontaine & Raffy 1932, Bertin 1951, Wilson et al. 2004). Cependant, lors de la migration estuarienne, une stabulation des civelles se produit à la limite de salure des eaux, suggérant que les civelles ont besoin d'un délai physiologique d'adaptation à l'eau douce avant d'entamer la colonisation des milieux d'eau douce (Petit & Vilter 1944, Deelder 1958, Sorensen & Bianchini 1986). De plus, lors d'expériences comportementales de choix binaire entre des courants d'eau douce et salée, une proportion variant entre 30 et 50 % des individus préfèrent l'eau salée (Tosi et al. 1988, 1989). Ces résultats suggèrent que la tolérance aux changements de salinité chez la civelle n'est pas totale, et pourrait varier selon les individus.

D'autre part, la tolérance à la salinité varie au cours du développement de l'anguille. Ainsi, lors d'un transfert brusque de l'eau douce vers l'eau salée, l'osmolalité du sang revient à la normale après 8 jours chez l'anguille argentée, se stabilise sans revenir à la normale après 10 jours chez l'anguille jaune, et augmente sans être stabilisée après 12 jours chez l'anguillette (Boucher-Firly 1935, Bertin 1951).

Ces données suggèrent que, contrairement à ce qui est classiquement admis, la salinité pourrait jouer un rôle dans la sélection de l'habitat par l'anguille. Ce sujet sera abordé dans notre travail de thèse (voir Chapitre 8).

### **Odeur de l'eau**

L'olfaction chez l'anguille semble être impliquée dans le contrôle de la sélection de l'habitat lors de la dispersion continentale. En effet, comme souligné dans le chapitre 5, les civelles sont fortement attirées par les odeurs des eaux douces continentales. De plus, chez la civelle d'*A. anguilla*, les acides aminés (Crnjar et al. 1992, Sola et al. 1993, Sola & Tongiorgi 1998) et les sels biliaires (Sola & Tosi 1993) sont également attracteurs.

Les sels biliaires pourraient être responsables de l'attrait exercé par les odeurs liées aux congénères. En effet, les civelles et les anguillettes d'*A. anguilla* en migration sont 1,4 fois plus nombreuses sur la passe du barrage estuaire d'Arzal

(France) lorsque l'eau d'évacuation du piège à civelles est dirigé sur la rampe de la passe (Briand et al. 2002). L'attrait exercé par l'odeur des congénères sur les civelles a également été montré expérimentalement chez *A. rostrata* (Sorensen 1986). De plus, chez l'anguille jaune *A. anguilla*, les extraits épidermiques sont hautement attractifs en milieu naturel (Saglio 1982).

La réponse comportementale aux odeurs des congénères chez les animaux est généralement considérée comme adaptative (Sorensen 1996). En effet, chez l'anguille, l'attrait représenté par les odeurs terrigènes et par les congénères pourrait permettre la sélection des habitats les plus productifs (Sorensen 1986).

## Autres facteurs abiotiques du milieu

Chez les Téléostéens, tous les facteurs abiotiques du milieu peuvent potentiellement influencer la sélection de l'habitat (Davenport & Sayer 1993). Nous parlerons de la température, du pH, et des teneurs en ammoniacal, nitrites et oxygène dissous.

Chez *A. dieffenbachii* et *A. australis* la température maximale létale varie selon les stades (Richardson et al. 1994). En effet, cette dernière varie entre 25 et 28°C au stade civelle, 33 et 35°C au stade anguillette et 37 et 40°C au stade jaune. La température de préférence varie entre 24 et 27°C au stade anguillette. Des civelles d'*A. japonica* testées expérimentalement sur 24h montrent des préférences thermiques variables mais toujours supérieures à la température d'acclimatation utilisée avant le test (Chen & Chen 1991). Ces préférences thermiques correspondent aux températures pour lesquelles les plus fortes croissances sont obtenues en élevage (Elie & Daguzan 1976, De Silva et al. 2001), et sont donc probablement liées au fonctionnement optimal de la machinerie enzymatique.

En milieu naturel, la proximité d'un effluent d'eau chaude (circuit de refroidissement d'une centrale thermique) accroît la sédentarité, la croissance et la survie chez *A. anguilla* (Andersson et al. 1990). Ce résultat suggère que la température du milieu peut influencer la sélection de l'habitat par l'anguille.

Les variations de pH entraînent des préférences d'habitat chez les civelles et les anguillettes d'*A. australis* et *A. dieffenbachii* (West et al. 1997), le pH préféré se situant aux alentours de 10.

Enfin, l'anguille est un des poissons les plus tolérants aux fortes teneurs en ammoniacal (Richardson 1997) et nitrites (Tomasso & Grossel 2005), ainsi qu'à l'hypoxie (Dean & Richardson 1998). Ces résultats suggèrent que ces facteurs (teneur en ammoniacal, nitrites et oxygène), dans leurs limites habituelles rencontrées en milieu naturel, ne jouent pas un rôle central dans le contrôle de la distribution des anguilles dans les habitats.

## **Facteurs sociaux**

D'une façon générale, les interactions sociales jouent un grand rôle dans le contrôle de la dispersion animale (Clobert et al. 2001). Chez l'anguille, l'hypothèse de densité-dépendance de la dispersion continentale (voir Chapitre 3) suppose que les facteurs sociaux jouent un rôle important dans le phénomène de sélection de l'habitat.

D'une façon générale, les facteurs sociaux de la dispersion densité-dépendante sont la territorialité, la prédation intraspécifique (cannibalisme), l'interférence pour la prise de nourriture et la déplétion des ressources alimentaires par les concurrents (Sutherland et al. 2002). L'occurrence de tous ces phénomènes a bien été montrée chez l'anguille (voir ci-dessous), confortant l'hypothèse de densité-dépendance de la dispersion continentale.

Au stade jaune, la forte activité alimentaire est couplée à un comportement territorial et cannibale marqué (Peters et al. 1980, Degani & Levanon 1983, Chisnall 1996, Glova 1999, 2001, Tesch 2003). De plus, des phénomènes d'interférence pour la prise de nourriture ont été observés en captivité (Wickins 1987). En milieu naturel, les taux de croissance peuvent être inversement corrélés à la densité, suggérant des phénomènes d'interférence et de déplétion (Bertin 1951, Baisez 2001, Beentjes & Jellyman 2003, Graynoth & Taylor 2004). Cependant, ce lien négatif entre densité et croissance n'est pas toujours observé (Aprahamian 2000). D'autre part, il est important de noter qu'en milieu saturé, la territorialité peut s'opposer à la dispersion, comme observé par Baras et al. (1998) chez la grande anguille jaune.

Nous avons abordé le sujet de la densité-dépendance de la dispersion continentale dans l'article 1, où nous avons étudié la distribution des jeunes anguilles jaunes en limite de marée dynamique sur la Dordogne.

## **Qualité de l'habitat**

Nous entendons par qualité de l'habitat l'ensemble des variables biotiques et abiotiques qui définissent la capacité d'accueil du milieu et la connectivité des habitats.

D'une façon générale, la capacité d'accueil du milieu sera liée à la disponibilité en ressources. Ce terme de ressource inclut un grand nombre de variables différentes (nourriture, espace, température, oxygène...). Ainsi, les variables liées à la densité et/ou la taille des anguilles dans le milieu sont multiples (et corrélées): la profondeur (Chisnall 1996, Baisez 2001, Broad et al. 2001a, 2001b, Lafaille et al. 2003), l'altitude (Broad et al. 2001a, 2001b), la végétation rivulaire (Chisnall 1996,

Broad et al. 2001a, 2001b), la disponibilité en abris, la taille du sédiment et la vitesse du courant (Glova et al. 1998, Glova 1999, Lafaille et al. 2003).

D'autre part, dans les milieux anthropisés, les barrages constituent un facteur très important de diminution de l'accessibilité des habitats (Elie & Rigaud 1984, 1987, White & Knights 1997a, Elie 1998, Lafaille et al. 2005).

## 8. Présentation de la thèse

Au terme de cette analyse des données bibliographiques existant actuellement, nous pouvons dégager un schéma conceptuel général du contrôle de la dispersion continentale de l'anguille (Fig. 3). Certains liens de ce schéma restent obscurs, faute de données. Quatre aspects ont plus particulièrement retenu notre attention en raison de leur importance:

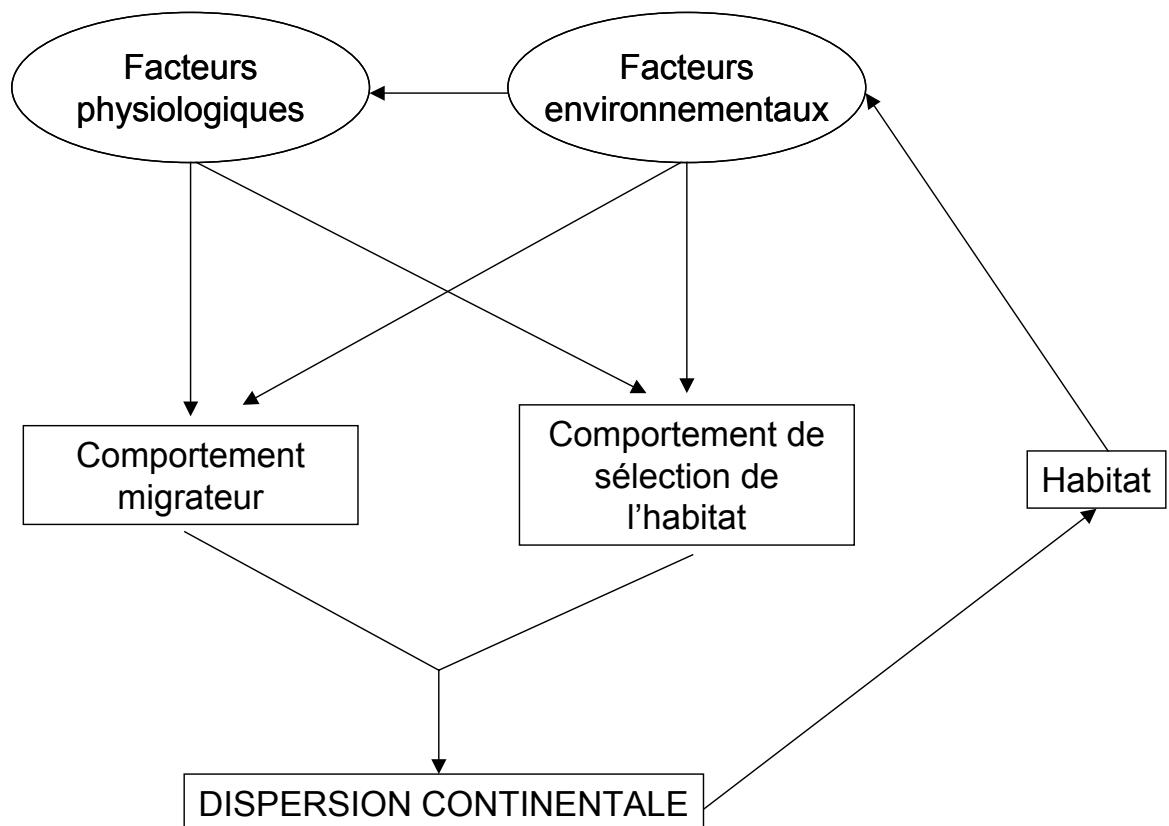


Fig. 3. Schéma général du fonctionnement de la dispersion continentale de l'anguille.

1. Bien que plusieurs auteurs supposent que des processus clé de la dispersion continentale aient lieu en limite de marée dynamique, le phénomène de migration à ce point des bassins versants est encore très imparfaitement connu.

2. D'autre part, aucune donnée n'existe concernant le contrôle endocrinien de la dispersion chez l'anguille, alors que les hormones sont d'une importance capitale dans la régulation des migrations chez les salmonidés.

3. Bien que de très nombreuses études de microchimie des otolithes montrent l'importance du lien entre la salinité du milieu et les tactiques dispersives divergentes, le comportement de l'anguille vis à vis de la salinité reste obscur, et le rôle de la salinité dans la sélection de l'habitat est inconnu.

4. Le rôle de la condition corporelle dans le contrôle du comportement dispersif n'a toujours pas été étudié chez l'anguille.

Pour aborder la question de la dispersion continentale de l'anguille à travers l'étude de ces quatre points, nous avons donc développé une approche multidisciplinaire, incluant des études de terrain, utilisant différents outils d'échantillonnage adaptés aux variations du comportement dispersif des anguilles, des études comportementales et de croissance en structures expérimentales, et des dosages hormonaux (Fig. 4)

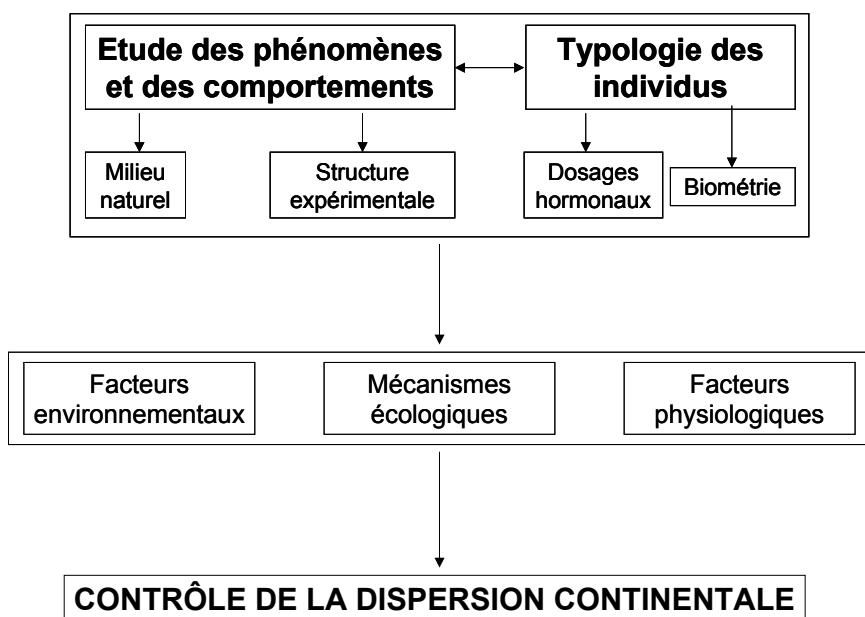


Fig. 4. Schéma conceptuel méthodologique de l'étude des facteurs internes (physiologiques) et externes (environnementaux) contrôlant la dispersion continentale de l'anguille.

Les résultats des travaux suivants sont présentés:

- Article 1. Etude du phénomène de migration en limite de marée dynamique sur la Dordogne.
- Article 2. Etude du lien entre le comportement migrateur des civelles et l'activité de la glande thyroïde en milieu naturel.
- Article 3. Effet des hormones thyroïdiennes sur l'activité locomotrice et la rhéotaxie des civelles en milieu expérimental.

Article 4. Etude des liens entre la préférence de salinité, l'activité locomotrice et la croissance des civelles en milieu expérimental.

Article 5. Etude de l'effet de la température et de la condition corporelle sur l'activité locomotrice et la préférence de salinité de la civelle en milieu expérimental.

## RESULTATS

### ARTICLE 1: Hydrodynamic effects on *Anguilla anguilla* juvenile migration at the tidal limit: a key-process of the eel continental dispersal

### Effets des facteurs hydrodynamiques sur la migration des juvéniles d'*Anguilla anguilla* en limite de marée dynamique: un processus clé de la dispersion continentale de l'anguille

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Article soumis à *Marine Biology*

#### Résumé

Les civelles d'*Anguilla anguilla* envahissent les eaux continentales côtières et estuariennes en utilisant le transport tidal sélectif (TTS), un mécanisme de transport utilisé par de nombreux autres organismes marins. Nous avons ici examiné comment la migration des juvéniles d'anguille était influencée par les facteurs hydrodynamiques dans la zone de limite de marée (ZLM) de la Dordogne (France). Nos résultats montrent que, en raison de la réduction de l'advection tidale disponible pour le TTS, les civelles s'accumulaient à la limite de renverse des courants, ce qui indique que la limite de marée dynamique représentait une barrière physique pour les individus en migration. De plus, nos données suggèrent que peu de civelles étaient capables de franchir cet obstacle hydraulique. En amont de la limite de renverse des courants, la vitesse de migration chutait et les civelles terminaient leur métamorphose en anguillette (i. e. en jeune anguille jaune) avant d'atteindre les habitats non tidaux. Cela suggère que les civelles sont fortement dépendantes du TTS pour la migration et mal adaptées à la nage constante à contre-courant. Le schéma de distribution des anguillettes n'était pas lié à la position de la limite de renverse des courants et les abondances étaient constantes dans la ZLM, suggérant un phénomène d'exclusion mutuelle due à la recherche de nourriture après l'accumulation initiale au stade civelle. En conséquence, nos données suggèrent que, durant la transformation des civelles en anguillettes, la dispersion bascule d'une migration anadrome densité-indépendante vers des mouvements densités-dépendants, motivés par la recherche de nourriture. La protection de l'anguille durant ces processus clé de la dispersion continentale pourrait contribuer à freiner l'effondrement des stocks d'anguille d'eau douce.

## **Abstract**

Glass eels of *Anguilla anguilla* invade coastal and estuarine waters using selective tidal stream transport (STST), a transport mechanism used by many other marine animals. We here investigated how the migration of juvenile eels was affected by hydrodynamics in the tidal limit area (TLA) of the Dordogne River (France). Our results show that, due to the reduction of tidal advection available for STST, glass eels accumulated at the limit of flow reversals, indicating that the tidal limit represented a physical barrier for migrating individuals. Our data further suggest that few glass eels were able to overcome this hydraulic obstacle. Upstream the limit of flow reversals, migration speed dropped and glass eels completed their metamorphosis into elvers (i. e. young yellow eels) before reaching the non tidal habitats. This suggests that glass eels are strongly dependent on STST for migration and maladapted to constant counter-current swimming. The distribution pattern of elvers was not related to the position of the limit of flow reversals and abundances were constant in the TLA, suggesting mutual exclusion due to food-search motivated movements after the initial accumulation at the glass eel stage. Therefore, our data suggest that, during the transformation of glass eels into elvers, dispersal switches from a density-independent, upstream migration to density-dependent, food-search motivated movements. Protection of the eel during these key-processes of the continental dispersal could contribute to brake the collapse of freshwater eel stocks.

## Introduction

Animal dispersal is an issue of crucial importance for population ecology, that shapes population structure, controls gene flow, and influences fitness (Clobert et al. 2001). Among the variety of factors that may affect fish dispersal, hydrodynamics are often of great importance in explaining the variability observed in population patterns (Werner et al. 1997). Here, we investigated how hydrodynamics affected the distribution patterns of *Anguilla anguilla* juveniles.

The leptocephalus larvae of *Anguilla anguilla* migrate from the Sargasso Sea breeding area to the European continental shelf, where they metamorphose into unpigmented glass eels (Schmidt 1909). As their pigmentation develops, glass eels recruit into coastal waters at an age ranging between 7 and 9 months (Lecomte-Finiger 1992; Arai et al. 2000). At that time, some individuals engage in the colonization of river systems, whereas others settle early into marine or estuarine habitats (Tzeng et al. 2000; Kotake et al. 2003; Edeline et al. 2004). This phenomenon, which constitutes the first step of the eel continental dispersal, is still poorly understood.

Glass eels invade continental marine and estuarine waters using selective tidal stream transport (STST) (McCleave and Kleckner 1982; Gascuel 1986; McCleave and Wipperhauser 1987). Fishes ascend from the bottom and are carried by the flood tide currents. During slack water, they return to the bottom where they remain during the ebb tide. Hence, movements are achieved through saltatory steps. Compared to constant counter-current swimming, STST allows important energy savings (Weihs 1978; Metcalfe et al. 1990). This low energy cost of STST is of central importance for glass eels since feeding activity stops at the onset of the metamorphosis, feeding resumption being conditioned by the development of a new teeth set and completion of changes in gut morphology (Elie 1979). Until feeding resumption, energy for migration is therefore provided by catabolism of energy stores (Tesch 2003; Bardonnet and Riera 2005).

For navigation towards inland habitats, migrating glass eels rely on environmental gradients such as odorous clues from river waters (Creutzberg 1961; Sorensen 1986; Tosi and Sola 1993; Sola 1995). Orientation towards salinity gradients may also play a critical role in driving the distribution of glass eels into continental habitats (Edeline et al. 2005b). When they reach the limit of flow reversals, migrating glass eels lose the tidal advection and have to switch from STST to constant counter-current swimming (McCleave and Wipperhauser 1987). This behavioral transition is related to a high thyroid gland activity, and thyroid hormones are deeply involved in the control of the glass eel continental dispersal (Edeline et al. 2004, 2005a). However, the migration processes related to this behavioral shift remain quite obscure and this gap in our knowledge prevents from a good understanding of how eels colonize continental habitats.

Studies of river migration, all conducted on obstacles where eels have to crawl out of the water and climb moist surfaces (artificial eel ladders or natural falls), show that the great majority of migrants are fully pigmented elvers (i. e. young yellow eels) of body length < 15 cm (Haro and Krueger 1988; Michaud et al. 1988; Naismith and Knights 1988; Dutil et al. 1989; McGovern and McCarthy 1992; White and Knights 1997). This suggests that glass eels exhibit a period of estuarine residency during which they complete their metamorphosis into elvers before carrying out the river colonization (Michaud et al. 1988; Naismith and Knights 1988). The reasons for this

period of estuarine residency are far from being clear and the precise location of the watershed where it takes place is unknown. In addition, it is also unknown whether the waiting period in estuary occurs when no obstacle impedes the migration into the river.

During the present study, we studied the migration of juvenile eels (glass eels and elvers) at the tidal limit. To this aim, we examined the distribution, pigment stages and feeding activity of glass eels and fully pigmented elvers (young yellow eels of body length < 15 cm) at the tidal limit of the Dordogne river (France), where no obstacle impedes tidal streams or eel migration. In addition, we analyzed the hydrodynamic effects of tides and river discharge on the distribution patterns, pigment stages and feeding activity of glass eels and elvers.

## Materials and methods

On the French Atlantic coast (Bay of Biscay), glass eels are found all year round, but the migration mainly occurs between November and April (Elie 1979; Elie and Rochard 1994). Sampling was carried out at the tidal limit of the Dordogne River (France) during two sampling periods, corresponding to about two migration runs, from 21 February 2002 to 25 June 2002 and from 5 November 2002 to 10 July 2003.

### Study area

The Dordogne River, which is 482 km long and drains a catchment of 23700 km<sup>2</sup>, meets the Garonne River to form the Gironde estuary (Fig. 1). The tidal limit of the Dordogne River is located about 50 km upstream of the freshwater interface (Fig. 1), providing a rare opportunity to separate the effects of hydrology and salinity on the migration dynamics of juvenile eels.

The Gironde-Dordogne River basin is free of physical barriers (falls or dam) in its lower reaches; the hydrological regime of the tidal limit is therefore undisturbed. Consequently, the tidal limit varies between a most downstream and a most upstream positions, depending on tidal height and river discharge, and constituting a tidal limit area (TLA). The TLA can be divided in two sections. As presented in the Fig. 2, the downstream section is submitted to tidal variations in both water level and flow reversals, whereas the upstream section is only submitted to tidal variations in water level, without flow reversals. The Dordogne River, flowing in lowland plains with low variations in height, has a TLA laying on about 40 km of river line, with well separated downstream and upstream sections.

### Sampling sites

The sampling sites were chosen to fit with, and to include the different sections of the TLA (Fig. 2). The most downstream sampling site, St Sulpice de Faleyrens (site A), was located downstream the TLA, so that flow reversals occurred at this site at each tide, even during strong flood events. The most upstream sampling site, Pessac sur Dordogne (site E), was right upstream the TLA, so that no tidal variations in water level occurred at this site. Vignonet (site B) and Lamothe Montravel (site D), were respectively located in the downstream and upstream sections of the TLA. Castillon la Bataille (site C), was located at the interface between the two sections of

the TLA, and was therefore the most frequent limit of flow reversals. Sampling sites characteristics are detailed in the table 1.

### Sampling protocol

Fishing was carried out on 69 days over both sampling periods. Glass eels were sampled using collectors derived from those used by Silberschneider et al. (2001). Collectors consist of polyethylene thread tufts inserted into a heavy 50 x 60 x 10 cm PVC base and represent an artificial vegetation where eels can shelter. Both yellow and glass eels are negatively phototactic and therefore do not migrate during the day time (Tesch 2003). Sampling was made at day time during the ebb tide, as both migrating and non migrating eels were sheltering in the collectors.

Care was taken to avoid sites where aquatic vegetation eventually competed with collectors as shelters. Moreover, collectors were distributed on at least 100 m on a river side or on both river sides in order to avoid patchy effects on abundances. Sampling was made by boat. Each time collectors were cleared, we measured water depth with a ballasted rope and current speed with a Doppler current meter (Flo-Mate). All sampling sites were sampled during a single day, except the site E which was isolated by a ford impassable with our main boat. The site E was therefore sampled on separated days using another boat. 5 to 6 collectors per site were attached with ropes to the riparian vegetation and cleared at intervals ranging between 2 to 15 days.

### Morphological analysis

Eels were measured to the nearest cm aboard. Individuals of body length inferior or equal to 15 cm were kept, transported to the laboratory and frozen after anesthesia in clove oil (10% diluted in ethanol, 4 ml l<sup>-1</sup>). Subsequently, after thawing, fishes were examined under microscope for determination of pigment stages. Then, we dissected glass eels for determination of presence/absence of food in the digestive tract as an indicator of the feeding resumption and activity. Elvers were considered to have restarted feeding and were therefore not dissected.

The glass eel stage is the late metamorphic stage between the translucent leaf-shaped leptocephalus larvae and the fully pigmented elver (young yellow eel). Hence, the extent of skin pigmentation is related to the progress of the metamorphosis, and is used as an indicator of the relative time spent into the estuary. We determined pigment stages according to the extent of skin pigmentation over the head, tail and body regions, through stages VA, VB, VIA<sub>0</sub>, VIA<sub>1</sub>, VIA<sub>2</sub>, VIA<sub>3</sub> and VIA<sub>4</sub> to VIB, following Elie (1979) and Elie et al. (1982). The distinction between glass eel and elver stages is unclear (Tabeta and Mochioka 2003). In this paper, we use the term "glass eel" for all pigment stages from VA to VIB. Hence, we consider that the "elver" stage corresponds to the young yellow stage eels (stage VII) of body length < 15 cm.

### Data analysis

We analyzed our data with generalized linear models (GLMs), using SAS 8.0 (PROC GENMOD) (SAS 2000). A GLM links a linear combination (sum) of independent variables (effects) to one dependent variable (McCullagh and Nelder 1989). The dependent variables analyzed in the present study were the abundances

of glass eels and elvers, and the feeding activity and pigment stages of glass eels (see Table 2). The effects tested in these models were as follows:

- The "Number of collectors" used at each sampling site (either 5 or 6).
- The "Pigment stage".
- The "River discharge" at sampling site E, classified into four classes according to the quartiles for the period 1997-2003.
- The "Sampling period", from 21 February 2002 to 25 June 2003, and from 5 November 2002 to 10 July 2003.
- The "Sampling sites", corresponding to different distances from the river mouth and related to the position of the TLA (Fig. 2).
- The "Season", corresponding to the three steps of the glass eel recruitments. In French Atlantic rivers, migration peaks in February (Elie 1979; Elie and Rochard 1994; Beaulaton and Castelnau In Press). We therefore separated the migration run into three seasons corresponding to increasing, decreasing and late recruitments. The first season corresponded to the period from the 1<sup>st</sup> November to the neap tide of February, the second season from the neap tide of February to the neap tide of May, and the third season from the neap tide of May onwards.
- *Tides*, classified in two classes (neap or spring tides).

To examine the contribution of the covariates to model fit, we compared the parsimony of each of an exhaustive set of models comprising combinations of independent variables using the Akaike's Information Criterion (AIC) (Akaike 1973). Details of the models are presented in the Table 2.

- Modeling abundances

Due to the high number of zero values for abundances, we modeled separately the probability for a zero value ( $p$ ), i. e. presence/absence of glass eels at a sampling site, and the distribution of the non-zero values (positive catches) (Pennington 1983; Stefánsson 1996). The data was first recorded so that for each sampling site and day, the value 0 was recorded if no fish was caught, and the value 1 was recorded for non-zero catches to obtain Bernoulli-type 0/1 measurements (binomial distribution). The usual model for probabilities is via the logit function. The GLM produced for modeling the probability  $p$  for a 1 value was therefore of the general form:

$$\ln [p/(1-p)] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + \varepsilon$$

where  $\beta_0$  is a constant,  $\beta_1 \dots \beta_n$  are coefficients of independent variables  $X_1 \dots X_n$ , and  $\varepsilon$  is random error.

In a second time, we modeled non-zero catches using a GLM where the number of fish was related to other measured variables using a log link. Hence non-zero catches  $\mu$  were modeled as follows:

$$\ln \mu = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + \varepsilon$$

A log-log plot of the variance versus means for positive catches (effectives per site per day) gave a regression slope close to 1 for glass eels (= 1.43) and elvers (= 1.58), indicating that a Poisson distribution had to be used for modeling positive catches (McCullagh and Nelder 1989).

Having obtained fitted values for the probability  $p$  of a non-zero catch, and for the positive catches  $\mu$ , conditional on it being positive, the predicted unconditional catches are given by  $p\mu$ . The resulting overall model for the distribution will be referred to as the Delta-Poisson model.

- Modeling pigment stages.

Pigment stages, increasing as glass eels develop into fully pigmented elver, were used as an indicator of the developmental stage of fishes. As for presence/absence of fishes, we modeled the probability  $p'$  for a given pigment stage to be encountered with a logit function. However, the probability  $p'$  for a given pigment stage to be encountered includes also the probability to encounter all less developed pigment stages (multinomial distribution). Therefore, instead of using a binomial logit,  $p'$  was modeled using a cumulative logit function (see Table 2).

- Modeling feeding activity of glass eels. As for presence/absence of fishes, probability for presence  $p''$  of food in the gut of glass eels was coded as Bernoulli-type 0/1 measurements (Binomial distribution) and was therefore modeled using a binomial logit function (see Table 2).

## Results

Total catches represented 2063 eels ranging from stage VB glass eels to 45 cm yellow eels. In a single collector, catches ranged from 0 to 59 eels.

### Modeling the dependent variables

The model retained for each dependent variable was that having the lowest AIC among the set of tested models including exhaustive combinations of all independent variables (see Table 2). Therefore, the effects not included in the models were considered to not contribute significantly to the variations of the dependent variables.

### Abundances of glass eels

The distance to the river mouth, related to the "*Sampling site*" variable, affected significantly the probability of presence (GLM 1, df = 4,  $p < 0.0001$ ), positive catches (GLM 2, df = 4,  $p < 0.0001$ ), and unconditional catches of glass eels (Delta-Poisson model coupling the GLMs 1 and 2) (Fig. 3).

Abundances markedly increased with the distance to the river mouth in the downstream section of the TLA (sampling sites B and C), peaking at the limit of flow reversals (sampling site C). This result suggests that an accumulation of glass eels occurred in the downstream section of the TLA, as tidal advection available for STST was reduced. Abundances of glass eels sharply decreased at the sampling site D, with values equivalent to that encountered downstream the TLA (sampling site A), suggesting that no accumulation occurred at these two sampling sites. At the sampling site E (upstream the TLA), abundances of glass eels were almost null (Fig. 3).

Positive catches of glass eels significantly decreased with increasing river discharge (GLM 2, df = 3,  $p < 0.0001$ ), indicating that high river discharge, hindering the tidal flow, reduced glass eels recruitments in the TLA. Increasing tidal height also decreased significantly positive catches (GLM 2, df = 1,  $p < 0.0001$ ), probably

through increased pelagic behavior (and then reduced sheltering behavior in collectors) and transport in habitats located between the sampling sites C and D.

As expected, the "Season" variable, reflecting the seasonality of recruitments, significantly affected both presence (GLM 1, df = 2, p < 0.0001) and positive catches of glass eels (GLM 2, df = 2, p < 0.0001). The highest abundances were logically found in the middle season, during peaking recruitments. Lastly, positive catches of glass eels significantly declined over the two sampling periods (GLM 2, df = 1, p < 0.0001), confirming the trend for decreased recruitments of glass eels on European coasts.

#### Abundances of elvers

The distance to the river mouth ("Sampling site" variable) affected significantly both probability of presence (GLM 3, df = 4, p = 0.0004) and positive catches of elvers (GLM 4, df = 4, p = 0.0040) (Fig. 4). Abundances of elvers were the lowest downstream the TLA (sampling site A) and tended to be the highest upstream the TLA (sampling site E) (Fig. 4). In contrast, abundances were constant throughout the TLA (sampling sites B, C and D).

Whereas the response of elvers to tides was similar to that of glass eels, the response to river discharge was the opposite. Indeed, both presence (GLM 3, df = 1, p = 0.0435) and positive catches (GLM 4, df = 1, p = 0.0377) of elvers decreased significantly with increasing tidal height, suggesting that, as glass eels, elvers adopted a less benthic behavior during periods of increased tidal advection. In contrast, positive catches of elvers increased significantly with increasing river discharge (GLM 4, df = 1, p = 0.0009), suggesting that increased flow velocity promoted sheltering near to the banks, where collectors were located.

In addition, as for glass eels, the "Season" variable significantly affected positive catches of elvers (GLM 4, df = 2, p = 0.0154), and, logically, the peak of elver abundance followed that of glass eels (during the third "Season"). Finally, both presence (GLM 3, df = 1, p < 0.0001) and positive catches of elvers (GLM 4, df = 1, p = 0.0260) declined over the two sampling periods, following the same trend than glass eel recruitments.

#### Pigment stages of glass eels in the TLA

The "Sampling site" variable significantly affected glass eels pigment stages (GLM 5, df = 4, p < 0.0001). As expected, pigmentation increased with increasing distance to the river mouth, traducing the kinetic of watershed colonization (Table 3). However, despite a higher distance to the river mouth, glass eels were less pigmented at the sampling site C than at site B, indicating frequent arrivals of young migrants at the limit of flow reversals. This confirms the abundance results of an accumulation of migrating glass eels in the downstream section of the TLA, peaking at the limit of flow reversals.

Pigmentation abruptly increased in the upstream section of the TLA (sampling site D, see Table 3), indicating a drop of migration speed as tidal advection was no longer available to facilitate movements. Upstream the TLA (sampling site E), 97.4 % of the catches were elvers (stage VII), confirming this trend.

The "Season" variable significantly affected pigment stages (GLM 5, df = 2, p < 0.0001), glass eels being less pigmented as recruitments were at maximum during the middle of the migration run (migration peak). This indicates that increased

recruitments, providing young animals, decreased the relative age of the glass eel population.

### Feeding activity of glass eels in the TLA

As expected, the probability of presence of food in the digestive tract of glass eels significantly increased with increasing pigmentation (GLM 6, df = 6, p < 0.0001). Indeed, food was found in the gut of about 50% of stage VB, and 90% of stage VIB glass eels, indicating that the majority of glass eels had restart feeding before reaching the TLA. However, whereas scarce food particles were found until the stage VA<sub>4</sub>, guts of stage VIB glass eels were often full of food, indicating a sharp increase in feeding activity at this latter developmental stage.

The hydrological regime, likely constraining fish movements and conditioning food supply from the drift, was significantly linked to the feeding activity of glass eels for both river discharge (GLM 6, df = 3, p < 0.0001) and tides (GLM 6, df = 2, p = 0.0014).

The "Sampling site" variable was significantly linked to the feeding activity (GLM 6, df = 3, p = 0.0140). Indeed, all other things being equal, feeding activity of glass eels decreased significantly at sampling site D.

## Discussion

Glass eels use STST to colonize continental habitats. Numerous coastal and estuarine animals, including arthropods and fishes, at either the larval or adult stages, also use STST for movements (Arnold and Metcalfe 1996; Gibson 1997; Forward and Tankersely 2001). To our knowledge, the effects of a loss of tidal advection on the distribution of animals dispersing by mean of STST was still not documented. In the present study, through the links between hydrodynamics, feeding resumption and distribution patterns, we have investigated how the loss of tidal advection affected the migration of juvenile eels at the tidal limit of the Dordogne River.

### Glass eels migration at the tidal limit

Both abundance and pigment stage data show that, due to the loss of tidal advection available for STST, migrating glass eels accumulated in the downstream section of the TLA. This result indicates that the tidal limit represents a physical barrier for migrating glass eels, and suggests that many individuals are unable to pass through this hydraulic obstacle. Indeed, glass eels were very few upstream the limit of flow reversals. Our previous results show that switch from STST to constant counter-current swimming and river colonization necessitates a high thyroid gland activity (Edeline et al. 2004, 2005a). Therefore, the tidal limit could act as a filter on migrating glass eels, selecting for river colonization only the individuals with the highest thyroid gland activity.

Pigment stage data show that, upstream the limit of flow reversals, migrating glass eels transformed into elvers between the sampling sites C and E, over only 13 km of river line. Such a transformation takes at least one month (P. Elie, pers. comm.), giving a maximum migration speed of about  $13 / 30 = 0.4 \text{ km day}^{-1}$  after the

loss of tidal advection. In contrast, during STST, glass eel average migration speed is about 3-4 km day<sup>-1</sup> (L. Beaulaton, unpub. data). Therefore, migrating glass eels were unable to maintain their initial migration speed as tidal advection was no longer available for transport. This suggests that glass eels are strongly dependent on tidal streams for migration, supporting our previous results showing that estuarine glass eels misusing tidal streams present a physiological stress, likely related to a too high energy cost of migration (Edeline et al. 2004).

The metamorphosis from the translucent, leaf-shaped and pelagic leptocephalus larvae to the fully pigmented, eel-shaped and benthic yellow eel may be seen as an adaptive shift from oceanic drift to river colonization and continental residency (Tesch 2003). Our results therefore suggest that, during the metamorphosis, the morphological, physiological and behavioral adaptations of glass eels restrict them mainly to the colonization of coastal and estuarine areas by mean of STST.

Accumulation downstream the limit of flow reversals probably increases sensitivity of glass eels to the fishing pressure. Indeed, the glass eel fishery of the Vilaine River, located downstream the Arzal dam (France), dramatically depletes the stock (Briand et al. 2003). Numerous glass eel fisheries are located in the upstream sections of estuaries (Gascuel et al. 1995; Dekker 2003), likely targeting natural accumulation zones and impairing the colonization of freshwater habitats by the eel. In the context of the worldwide collapse of freshwater eel stocks (Stone 2003) and dramatic decline of glass eel recruitments (Dekker 1998; Briand et al. 2003; Beaulaton and Castelnau In Press), we suggest that TLAs should be intensively protected from anthropogenic pressures.

### Yellow eel movements

Our results show that the distribution pattern of elvers was quite different to that of glass eels, suggesting a change in dispersal behavior at the end of the larval metamorphosis. In estuaries, sedentary yellow eels use tidal streams for homing movements (Parker 1995; Parker and McCleave 1997). Accordingly, during the present study, increasing tidal height significantly decreased elver abundances in collectors, suggesting enhanced displacements as more tidal streams were available for motion. However, elver distribution was unrelated to the position of the limit of flow reversals, indicating that, in contrast to glass eels, tidal streams did not drive the distribution of yellow eels in the TLA. Accordingly, elver and glass eel abundances responded in an opposite way to increasing river discharge. Because glass eels used the TLA as a migration route, increased river discharge, resulting in a reduced tidal advection available for STST, decreased the recruitments. This effect of river discharge on glass eels recruitments has been previously reported (Elie 1979; De Casamajor et al. 2000). In contrast, in elvers, increasing river discharge increased the number of individuals sheltering near to the banks in collectors, indicating a resident population rather than individuals migrating upstream by mean of STST. Therefore, our data suggest that a pronounced change in the eel dispersal behavior occurred as glass eels transformed into elvers. This result is in accordance with the classical view that eels switch from a "pelagic" to a "benthic" behavior at the end of the larval metamorphosis (Bertin 1951; Tesch 2003).

The physiological control of this developmental change in dispersal behavior likely involves thyroid hormones. Indeed, thyroid hormones have been shown to promote the migratory behavior in both glass eel (Edeline et al. 2004, 2005a) and

yellow eel (Castonguay et al. 1990), and the termination of the larval metamorphosis is related to a decreased thyroid gland activity (Callamand and Fontaine 1942). Furthermore, the completion of the larval metamorphosis is marked by both the end of the progression of pigmentation and a fully developed gut (Elie 1979; Jegstrup and Rosenkilde 2003). Finishing of the gut development, allowing starting of an intense feeding activity, likely promote a drop in the migratory propensity and switch to the "benthic" behavior.

Indeed, it has been shown that the energy requirements of digestion and locomotor activity are conflicting in the eel (Owen 2001). Accordingly, our data obtained on glass eels show that, all other things being equal, feeding activity decreased with increasing locomotor activity as migrating individuals switched from STST to constant counter-current swimming at sampling site D. In contrast, the majority of glass eels had restarted feeding before reaching the TLA, indicating that first feeding resumption did not hinder STST behavior.

In fishes, food-search motivated movements promote density-dependent dispersal through intraspecific competition (Huntingford 1993; Sutherland et al. 2002). In yellow eels, movements are thought to be mainly driven by density-dependence (Smogor et al. 1995; Feunteun et al. 2003; Ibboston et al. 2003; Briand et al. 2005), supporting the view that food-search is a major driver of yellow eel dispersal. During the present study, elver abundances remained nearly constant in the TLA (sampling sites B, C and D), supporting the hypothesis of a mutual exclusion after an initial accumulation at the glass eel stage. These data suggest that, during the transformation of glass eels into elvers, dispersal behavior switches from density-independent to density-dependent.

In a constant environment (homogenous distribution of resources), food-search motivated movements would lead to an homogenous distribution within the habitat. Yet, our present results show that abundances of elvers were the lowest downstream the TLA (sampling site A) and tended to be the highest upstream the TLA (sampling site E), suggesting that movements of elvers preferentially occurred towards upstream. Hence, elver movements were likely controlled by interacting processes of habitat selection and upstream swimming behavior (positive rheotaxis).

In conclusion, our results indicate that tidal streams are fundamental in structuring the estuarine distribution of migrating glass eels. The limit of flow reversals represented a physical barrier that all individuals were probably not able to overcome. Upstream this point, the migration speed dropped and glass eels completed their metamorphosis into yellow eels before reaching the non tidal river area. A profound change in dispersal behavior, likely related to the drop of the thyroid gland activity and acquisition of an intense feeding activity, occurred during this transformation. This change may be interpreted as a switch from a density-independent, upstream migratory behavior to a food-search motivated, density-dependent dispersal. Protection of the eel during these key-processes of the continental dispersal may be crucial for enhancement of recruitments in freshwater habitats.

## Acknowledgements:

We are indebted to Claude Durand, professional fisherman on the Dordogne River, for his kindness, his help in learning us his experience of the river, and providing us a permanent boat anchorage. We are deeply grateful to Michel Vignaud and all the Gironde team of the French Conseil Supérieur de la Pêche (CSP) for their invaluable help throughout this experiment. We thank Cédric Briand (IAV) for providing collectors and for valuable comments on the manuscript. We also want to thank all personal of the Cemagref who participated to sampling, Aymeric Guibert, Charles Roqueplo, Franc Bouin, Mario Lepage, Gérard Castelnau, Laurent Brosse, Aude Lochet and Christine Gazeau. Philippe Camoin (Cemagref), drew Fig. 1. This work was partly supported by research grants from the Région Aquitaine and from the French Ministère de l'Ecologie.

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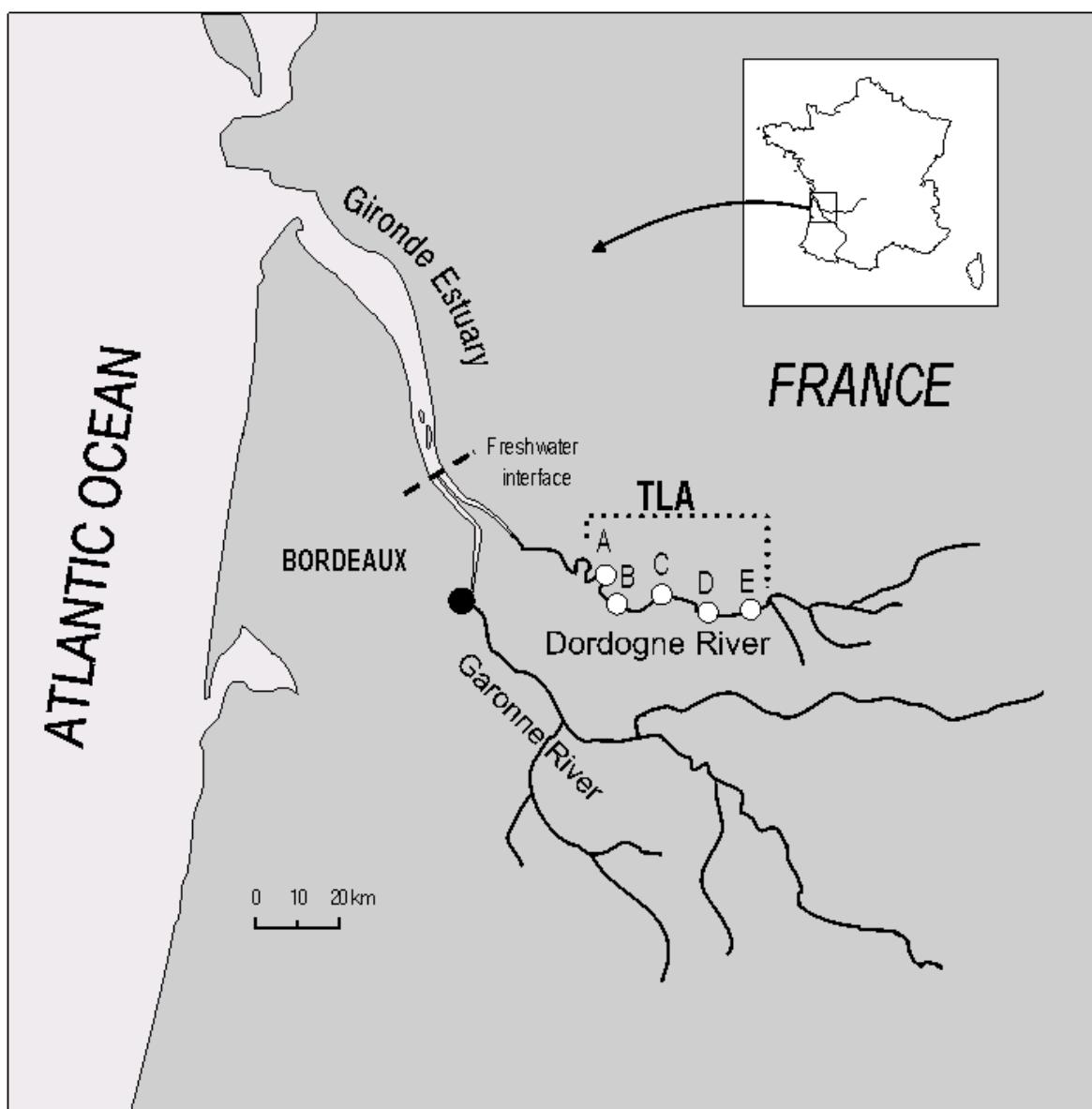


Fig. 1

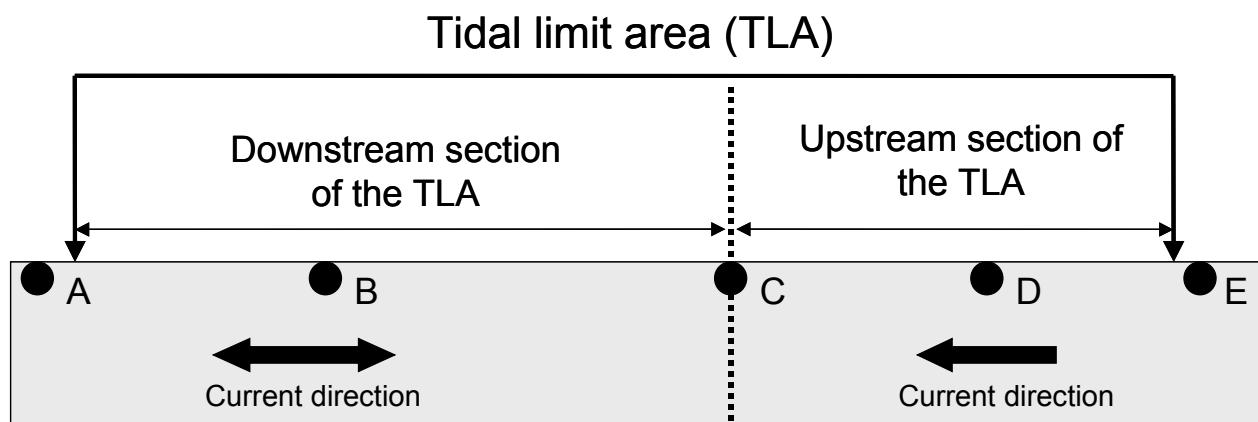


Fig. 2

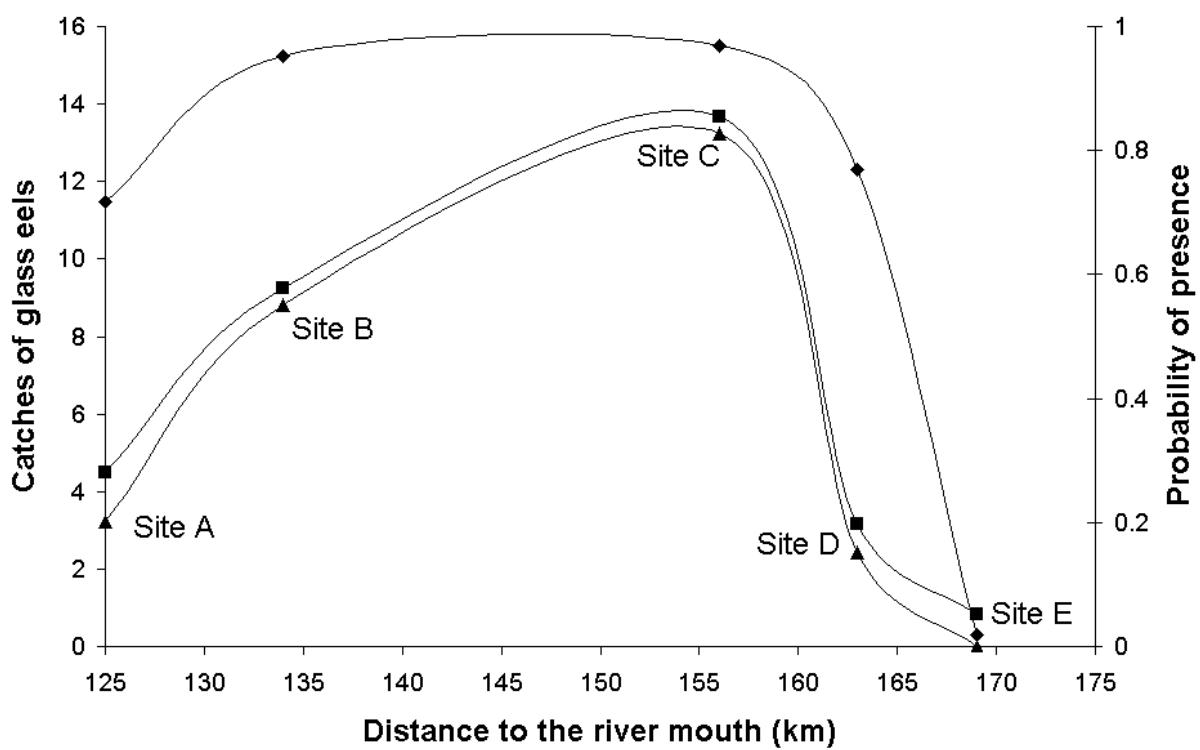


Fig. 3

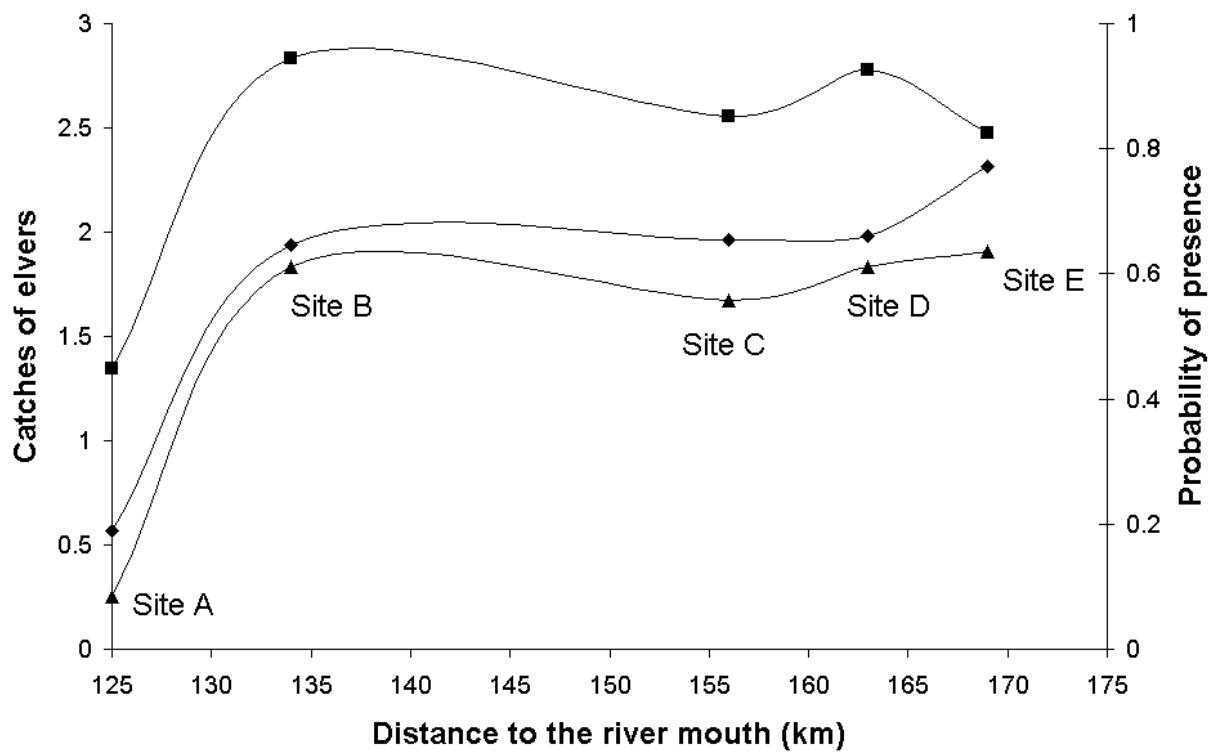


Fig. 4

Table 1. Characteristics of each sampling site chosen to study juvenile eel migration in the tidal limit area (TLA) of the Dordogne River (France). The position of each sampling site relative to the different sections of the TLA are presented in the Fig. 2.

| Sampling site           | Code | Distance to the river mouth (km) | Substrate            | Water depth of sampling (m) |                  | Water velocity ( $\text{m s}^{-1}$ ) |                 |
|-------------------------|------|----------------------------------|----------------------|-----------------------------|------------------|--------------------------------------|-----------------|
|                         |      |                                  |                      | range                       | mean $\pm$ SD    | range                                | mean $\pm$ SD   |
| St Sulpice de Faleyrens | A    | 125                              | Mud                  | 0.1 - 5                     | 1.67 $\pm$ 0.91  | 0 - 0.70                             | 0.30 $\pm$ 0.18 |
| Vignonet                | B    | 134                              | Mud + vegetal wastes | 0.1 - 4.7                   | 1.69 $\pm$ 0.83  | 0 - 0.70                             | 0.22 $\pm$ 0.16 |
| Castillon la Bataille   | C    | 156                              | Sand + gravels       | 0.1 - 4                     | 1.30 $\pm$ 0.842 | 0 - 0.90                             | 0.15 $\pm$ 0.19 |
| Lamothe-Montravel       | D    | 163                              | Sand + gravels       | 0.3 - 5                     | 1.44 $\pm$ 0.75  | 0 - 0.72                             | 0.26 $\pm$ 0.20 |
| Pessac sur Dordogne     | E    | 169                              | Sand + mud           | 0.2 - 3.7                   | 1.64 $\pm$ 0.91  | 0.01 - 0.8                           | 0.19 $\pm$ 0.15 |

Table 2. Details of the different GLMs used in the present study. The combination of independent variables (effects) retained for each model in this table is that having the lowest AIC. The GLMs (1) and (2), and (3) and (4) were coupled to produce Delta-Poisson models for unconditional catches of glass eels and elvers, respectively.

| GLM number | Dependent variable | Developmental stage | Link function    | Distribution | Independent variables  | Effect probability   |
|------------|--------------------|---------------------|------------------|--------------|--|--|
| 1          | Presence           | Glass eel           | Logit            | Binomial     | Sampling site<br>Season<br>Number of collectors  | < 0.0001<br>< 0.0001<br>0.0003                                     |
| 2          | Positive catches   | Glass eel           | Log              | Poisson      | Sampling site<br>Season<br>Sampling period<br>River discharge<br>Tides<br>Number of collectors | < 0.0001<br>< 0.0001<br>< 0.0001<br>< 0.0001<br>< 0.0001<br>0.0008 |
| 3          | Presence           | Elver               | Logit            | Binomial     | Sampling period<br>Sampling site<br>Number of collectors<br>Tides                              | < 0.0001<br>0.0004<br>0.0042<br>0.0435                             |
| 4          | Positive catches   | Elver               | Log              | Poisson      | Number of collectors<br>River discharge<br>Sampling site<br>Season<br>Sampling period<br>Tides | < 0.0001<br>0.0009<br>0.0040<br>0.0154<br>0.0260<br>0.0377         |
| 5          | Pigment stages     | Glass eel           | Cumulative logit | Binomial     | Sampling site<br>Season  | < 0.0001<br>< 0.0001   |
| 6          | Feeding activity   | Glass eel           | Logit            | Binomial     | Pigment stage<br>River discharge<br>Tides<br>Sampling site                                     | < 0.0001<br>< 0.0001<br>0.0014<br>0.0114                           |

Table 3. Percentage of each pigment stage among total catches of juvenile eels at each sampling site in the tidal limit area of the Dordogne River. For details about the sampling sites, see Fig. 2 and Table 1.

| Pigment<br>stage (%) | Sampling site |      |      |      |      |
|----------------------|---------------|------|------|------|------|
|                      | A             | B    | C    | D    | E    |
| VB                   | 2.7           | 0.3  | 0    | 0.0  | 0    |
| VIA <sub>0</sub>     | 3.4           | 0.7  | 1.1  | 0.0  | 0    |
| VIA <sub>1</sub>     | 6.0           | 2.3  | 6.9  | 0.3  | 0    |
| VIA <sub>2</sub>     | 12.8          | 4.6  | 21.3 | 0.3  | 0    |
| VIA <sub>3</sub>     | 13.4          | 7.0  | 25.1 | 4.1  | 0    |
| VIA <sub>4</sub>     | 20.1          | 28.8 | 23.1 | 25.1 | 0    |
| VIB                  | 12.8          | 29.8 | 8.0  | 13.4 | 2.6  |
| VII                  | 28.9          | 26.5 | 14.4 | 56.8 | 97.4 |

## FIGURE LEGENDS

Fig. 1. Map of France showing the Dordogne River and the location of the sampling sites (A, B, C, D and E) used to study the migration of juvenile eels in the tidal limit area (TLA). For detailed description of the sampling sites, see the Fig. 2 and Table 1.

Fig. 2. Scheme of the tidal limit area (TLA) of the Dordogne River showing the locations of the five sites sampled during the present study. The positions of the upstream and downstream sections of the TLA are presented. The tidal influence in the downstream section induced flow reversals coupled with variations in water level. In the upstream section, tidal influence was limited to variations in the water level. The positions of the sampling sites (A, B; C, D, and E) relative to the different sections of the TLA are presented.

Fig. 3. Effect of the distance to the river mouth (and related sampling sites) on probability of presence of glass eels (diamonds, GLM 1), positive catches (squares, GLM 2), and unconditional catches (triangle, delta-Poisson model coupling the GLMs 1 and 2) in the tidal limit area (TLA) of the Dordogne River. For details about the GLMs, see Table 2.

Fig. 4: Effect of the distance to the river mouth (and related sampling sites) on probability of presence of elvers (young yellow eels of body length < 15 cm) (diamonds, GLM 3), positive catches (squares, GLM 4), and unconditional catches (triangle, Delta-Poisson model coupling GLMs 3 and 4) in the tidal limit area (TLA) of the Dordogne River. For details about the GLMs, see Table 2.

## **ARTICLE 2: Thyroid status is related to migratory behavior in *Anguilla anguilla* glass eels**

### **Le statut thyroïdien est lié au comportement migrateur chez les civelles d'*Anguilla anguilla***

Article publié dans *Marine Ecology Progress Series* (2004) 282 : 261-270

#### Résumé:

Afin de déterminer si le statut thyroïdien est lié au comportements de migration et de sédentarisation chez les civelles d'*Anguilla anguilla*, nous avons échantillonné des civelles montrant différents comportements migrateurs au niveau du barrage d'Arzal, qui constitue la limite de marée dynamique de la rivière Vilaine (France). Nous avons collecté quatre groupe de civelles : les civelles de flot et de jusant ont été capturées au filet dans l'estuaire, respectivement durant le flot et le jusant, les civelles de piège ont été échantillonnées dans le piège de la passe du barrage, et les civelles de fond étaient cachées sur le fond durant le flot. Nous avons mesuré les taux corporels individuels de triiodothyronine ( $T_3$ ) et de thyroxine ( $T_4$ ) pour ces groupes, et calculé les taux d'hormone thyroïdienne (TH) équivalents à la somme  $T_3+T_4$  et reflétant l'activité sécrétoire de la glande thyroïde, et les rapports  $T_3:T_4$  reflétant l'activité de la désiodase de l'anneau externe de la  $T_4$  ( $T_4$ ORD). Les civelles de piège avaient les plus hauts taux de TH, indiquant une activation de la glande thyroïde via l'axe thyréotrope. Cela pourrait être responsable de la transition comportementale (perte du rythme circatidal et passage à une nage à contre-courant) en limite de marée dynamique et de la colonisation active des habitats de rivière par les civelles. Les civelles de jusant et de flot avaient des taux similaires de TH, plus bas que chez les civelles de piège, indiquant une diminution de l'activité sécrétoire de la thyroïde. Les civelles de jusant avaient des rapports  $T_3:T_4$  plus élevés que ceux des civelles de flot et de jusant, indiquant un stress physiologique lié à une mauvaise utilisation des courants de marée. Les civelles de fond avaient les taux de TH les plus bas, et des rapports  $T_3:T_4$  élevés et similaires à ceux des civelles de jusant ; cela suggère que le stress physiologique induit par une nage fréquente à contre-courant mène à une sédentarisation précoce des civelles dans les habitats estuariens. Nos données soulignent le rôle central du statut thyroïdien dans la régulation des comportements de migration et de sédentarisation des civelles dans les habitats marins et estuariens.

# Thyroid status is related to migratory behavior in *Anguilla anguilla* glass eels

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**ABSTRACT:** To determine whether thyroidal status is related to migratory and settling behavior in *Anguilla anguilla* glass eels, we sampled glass eels showing different migratory behaviors in the Arzal dam, which constitutes the tidal limit of the Vilaine River (France). We collected 4 groups of glass eels: flood and ebb tide glass eels were netted in the estuary during the flood and ebb tides, respectively; trap glass eels were sampled from the eel ladder trap, and bottom glass eels sheltering on the bottom of the estuary during flood tide. We measured individual whole-body triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) levels for these groups, and calculated total thyroid hormone (TH) levels as  $T_3 + T_4$  contents reflecting the thyroid gland secretory activity, and  $T_3:T_4$  ratios reflecting  $T_4$  outer-ring desiodase ( $T_4$  ORD) activity. Trap glass eels had the highest TH levels, indicating an activation of the thyroid gland via the thyrotrop axis. This could be responsible for the behavioral transition (loss of circatidal rhythm and switch to counter-current swimming) at the tidal limit and active colonization of river habitats by glass eels. Ebb and flood tide glass eels had similar TH levels that were lower than those of trap glass eels, indicating a lower thyroid gland secretory activity in the former. Ebb tide glass eels had higher  $T_3:T_4$  ratios than flood tide and trap glass eels, indicating physiological stress related to inefficient use of tidal streams. Bottom glass eels had the lowest TH levels, and high  $T_3:T_4$  ratios similar to those of ebb tide glass eels; this suggests that physiological stress induced by frequent counter-current swimming leads to precocious settlement of glass eels in estuarine habitats. Our data supports the critical role of the thyroid status in the migratory and settling behavior of glass eels in estuarine and marine habitats.

**KEY WORDS:** Glass eel · Migration · Settlement · Habitat colonization · Thyroid gland activity · Selective tidal stream transport

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## INTRODUCTION

At the end of their transoceanic migration, *Anguilla anguilla* leptocephalus larvae metamorphose into glass eels off the continental shelf of Europe (Schmidt 1909), and subsequently colonize continental waters. Within estuaries, they use selective tidal stream transport (STST) for upstream movement (Creutzberg 1961, Elie 1979, McCleave & Kleckner 1982, Gascuel 1986, McCleave & Wippelhauser 1987). To do so, glass eels remain on or near to the bottom during ebb tide and

move up into the water column on flood tide, timing their sojourn by an internal clock (Wippelhauser & McCleave 1987, 1988). This mechanism enables rapid landward transport of the glass eels at low energetic cost (Weihs 1978, Metcalfe et al. 1990). At the tidal limit of the river, glass eels show a pronounced change in migratory behavior (Gascuel 1986, McCleave & Wippelhauser 1987) involving loss of the circatidal rhythm and acquisition of a strictly counter-current swimming behavior. This may involve a migration delay at the tidal limit (McCleave & Wippelhauser

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1987). The mechanisms that regulate this behavioral transition are unknown. Moreover, some individuals never colonize freshwater; they settle in estuaries or along the coastline (Tsukamoto & Arai 2001, Tzeng et al. 2003) and do not even reach the tidal limit. The internal factors leading to this divergent migratory behavior are also unknown.

In teleosts, thyroid hormones (THs), L-thyroxine ( $T_4$ ) and 3,5,3'-triiodo-L-thyronine ( $T_3$ ), are involved in a wide range of biological processes such as development, growth, reproduction, osmoregulation, ionic regulation (Eales 1979, Eales & Brown 1993) and probably also metabolism (Woodhead 1975). Further, THs have been linked to migratory and homing behavior in many fish species (Fontaine 1975, Woodhead 1975, Comeau et al. 2001). In teleosts, production of  $T_4$ , the main hormone secreted by the thyroid gland, is regulated by the hypothalamo-pituitary axis via the thyroid stimulating hormone (TSH) (Fig. 1). TSH is released into the blood and deiodinated by  $T_4$  outer-ring deiodases ( $T_4$  ORD) into  $T_3$  in the peripheral tissues. Subsequently,  $T_3$  is deiodinated to the biologically inactive 3,3' diiodo-L-thyronine ( $T_2$ ) by  $T_3$  inner-ring deiodases ( $T_3$  IRD) or excreted through urine or bile (Fig. 1). The binding affinity of  $T_3$  to TH receptors is approximately 10-fold higher than  $T_4$ , as shown in the hepatocytes of *Salmo gairdneri* and *Oncorhynchus kisutch* (Darling et al. 1982, Bres & Eales 1986).

In the American eel *Anguilla rostrata* at the yellow stage, migrating individuals caught climbing waterfalls exhibited higher plasma  $T_4$  levels than individuals caught below the waterfalls (Castonguay et al. 1990). In young salmon, the  $T_4$  surge associated with smoltifi-

cation induces a change in their rheotactic behavior (Iwata 1995, Specker et al. 2000, Iwata et al. 2003). During development of the red sea bream *Pagrus major* and the metamorphosis of the summer flounder *Paralichthys dentatus*, a  $T_4$  surge may promote the transition from pelagic toward settling behavior (Hirata et al. 1989, Schreiber & Specker 1999). In glass eels, a role of THs in rheotropism and in the capacity to migrate away from the sea has been hypothesized (Fontaine & Callamand 1941, Fontaine 1975). More recently, a correlation between decreasing  $T_4$  levels and the transition from pelagic toward benthic behavior was shown in glass eels *A. anguilla* held in an aquarium (Jegstrup & Rosenkilde 2003).

As a first step to understanding the potential internal mechanisms controlling migration and habitat colonization, we measured TH ( $T_3$  and  $T_4$ ) levels in *Anguilla anguilla* glass eels exhibiting different migratory behavior under natural conditions. The ecological implications of our findings are discussed.

## MATERIALS AND METHODS

**Sampling site.** Glass eels were sampled during April 2003 in the Vilaine River estuary in South Brittany, France ( $1^{\circ}5'$ W,  $45^{\circ}5'$ N, Fig. 2). The Arzal estuarine dam, equipped with an eel ladder, is located 12 km from the mouth of the estuary and constitutes the tidal limit. The eel ladder consists of 2 ramps lined with tufts of synthetic brushes (described by Legault 1992). These ramps lead to a central canal and onto a second ramp, from which glass eels fall into a holding tank constituting a trap that is cleared daily (Briand et al. 2002). Water flowing over the eel ladder is freshwater from the pond created by the dam. Climbing takes place at night, and glass eels are able to climb the entire ladder within 1 or 2 h. Water depth below the dam ranges from 5 to 11 m and salinity from 0 to 25 psu depending on tide and river discharge.

**Sampling.** We collected 4 groups of glass eels, classified according to place of capture and tidal conditions: 'trap glass eels' were sampled from the eel ladder trap after they had climbed the 2 ramps; 'flood-tide glass eels' and 'ebb tide glass eels' were netted from the estuary in a fishing boat, 100 m below the dam during flood and ebb tides respectively; 'bottom glass eels' were sampled from collectors on the estuary bottom during flood tide. Collectors consist of polyethylene-thread tufts inserted into a heavy  $50 \times 60 \times 10$  cm PVC base to represent artificial vegetation (Silberschneider et al. 2001). Although as many as 9 collectors were used, only 5 bottom glass eels were caught and analyzed for TH content. A subsample from each of the other 3 groups was kept for analysis of pigment stages

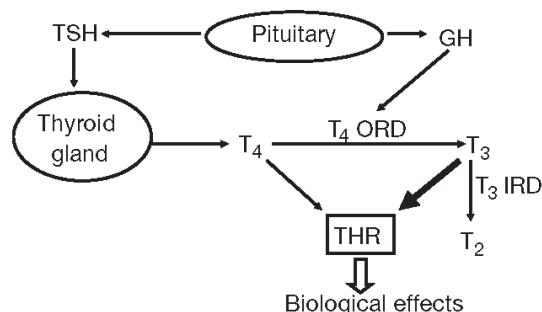


Fig. 1. General thyroid model. Thyroid stimulating hormone (TSH) produced by pituitary gland regulates thyroid synthesis and secretion of L-thyroxine ( $T_4$ ).  $T_4$  is released into blood and deiodinated in 3,5,3'-triiodo-L-thyronine ( $T_3$ ) in peripheral tissues by  $T_4$  outer-ring deiodases ( $T_4$  ORD). Pituitary growth hormone (GH) stimulates activity of  $T_4$  ORD.  $T_3$  binds thyroid hormone receptors (THR) with higher affinity than  $T_4$ .  $T_3$  can be deiodinated by  $T_3$  inner-ring deiodinases ( $T_3$  IRD) in 3,3' diiodo-L-thyronine ( $T_2$ ), which is ineffective in stimulating THR.

or for TH level assessment. Details are presented in Table 1. Glass eels were collected during either full moon or during its last quarter. Lunar phase may influence the migration of glass eels by means of both tides and moonlight (Jellyman & Lambert 2003); however, it does not affect glass eel migration in the Vilaine River estuary (C. Briand unpubl. data). This may be due to the high turbidity of the estuary waters hindering penetration of moonlight through the water column, and/or to the proximity of the dam to the sea, which has a strong tidal influence below the dam even during neap tides.

**Sample storage.** Glass eels were dried on blotting paper, individually stored in cryotubes that had been previously weighted to the nearest  $10^{-4}$  g, and then frozen in liquid nitrogen vats. The fresh weight of the glass eels could thus be obtained without thawing, by weighing the cryotube containing the glass eel and subtracting the weight of the tube. Samples were stored at  $-20^{\circ}\text{C}$  until extraction. Glass eels used for pigment-stage analysis were put into bags and frozen at  $-20^{\circ}\text{C}$ .

**Determination of pigment stages.** After thawing, the glass eels were examined microscopically to determine pigment stage according to Elie et al. (1982). Briefly, pigment stages describe the extent of skin pigmentation over the head, tail and body regions through Stages VA, VB, VIA<sub>0</sub>, VIA<sub>1</sub>, VIA<sub>2</sub>, VIA<sub>3</sub> and VIA<sub>4</sub> to VIB. The distinction between glass eel and elver stages is unclear (Tabeta & Mochioka 2003). In this paper, we use the term 'glass eel' for all pigment stages between Stages VA and VIB.

**Radio immunoassay (RIA).** The method followed was that of Cis Bio International for total T<sub>4</sub> and total T<sub>3</sub> (RIA) kits (Gif s/Yvette), using tubes coated with anti-T<sub>3</sub> or T<sub>4</sub> antibodies and <sup>125</sup>I radiolabelled T<sub>3</sub> or T<sub>4</sub>. Sensitivity was 0.1 ng ml<sup>-1</sup> for T<sub>3</sub> and 2.5 ng ml<sup>-1</sup> for T<sub>4</sub>. Briefly, after addition of the extract or standard and radiolabelled hormone to antiserum-coated tubes, the tubes were incubated at  $37^{\circ}\text{C}$  for 2 h and then decanted. The radioactive fraction bounded to the tube

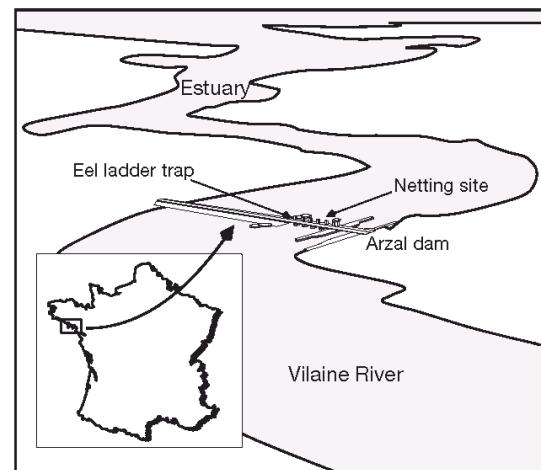


Fig. 2. Map of France (inset) showing location of Vilaine River estuary. Details of Arzal dam with locations of eel ladder trap and netting site in estuary where glass eels *Anguilla anguilla* were sampled are shown

was counted in a gamma counter (MDA 312, Kontron Analytical). The bounded radioactive fraction (*B*) is expressed as a percentage of the maximal bounded radioactive fraction (*B*<sub>0</sub>) which was measured without the addition of cold TH to the antiserum-coated tube. Percentage *B:B*<sub>0</sub> was inversely proportional to the amount of cold TH in samples, which was calculated from the standard dilution-curve parameters. Individual whole-body T<sub>3</sub> and T<sub>4</sub> levels were measured in duplicate in both assays and expressed as ng hormone g<sup>-1</sup> wet body mass. Total thyroid hormone (TH) content was calculated as T<sub>3</sub> content + T<sub>4</sub> content for each individual. Similarly, the ratio T<sub>3</sub>:T<sub>4</sub> was also calculated for each individual.

**Pilot experiments.** Pilot experiments were conducted to determine the best extraction medium and the volume of glass eel extract necessary to work

Table 1. *Anguilla anguilla*. Characteristics of glass eel groups used for measurement of whole-body thyroid hormones levels and pigment stages. Groups: Ebb, sampled by netting at ebb tide; Flood, sampled by netting at flood tide; Trap, sampled from eel ladder trap; Bottom, sampled from collectors on bottom of estuary at flood tide. All samples collected during night in April 2003 (mid-column = mid-water column; bottom = bottom of estuary water column); T: water temperature; N TH: no. analyzed for thyroid hormone; N stage: no. analyzed for pigment stage

| Group  | Sampling    |        |                     | Tide, moon phase    | T<br>(°C) | Salinity<br>(psu) | N<br>TH | N<br>stage |
|--------|-------------|--------|---------------------|---------------------|-----------|-------------------|---------|------------|
|        | Gear        | Date   | Location            |                     |           |                   |         |            |
| Ebb    | Net         | 23 Apr | Estuary, mid-column | Ebb, last quarter   | 14        | 21                | 46      | 74         |
| Flood  | Net         | 16 Apr | Estuary, mid-column | Flood, full moon    | 12        | 20                | 114     | 62         |
| Trap   | Ladder trap | 16 Apr | Eel ladder          | Flood, full moon    | 12        | 0                 | 69      | 18         |
| Bottom | Collector   | 23 Apr | Bottom              | Flood, last quarter | 14        | 21                | 5       | 0          |

within the sensitivity range of the RIA kits. We used pools of extracts of 5 to 8 glass eels from the Dordogne River (France) caught in 2002. The extraction method is described below. We tested 4 different extraction media: 0.01 M phosphate-buffer saline, pH 7.4 (PBS); phosphate-buffer saline, pH 7.4, containing 1 mM 5-propyl-2-thiouracil (Sigma) (PBS-PTU); absolute ethanol (EtOH) and absolute ethanol containing 1 mM PTU (EtOH-PTU). PTU was used to block endogenous desiodase activity (Denver 1993). This pilot experiment showed that the most efficient extraction medium was EtOH-PTU. Details are presented in 'Results'.

**Extraction.** The extraction procedure chosen was similar to that of Tagawa & Hirano (1987), with minor modifications. Extraction was made in cryotubes in ice-cold EtOH-PTU. Homogenization was carried out using an Ultra-Turrax homogenizer (Labo Moderne, Paris) and followed by sonication for 20 s with a Vibra

Cell 72434 sonicator (Bioblock Scientific). The blades of the homogenizer were rinsed with 0.3 ml ice-cold EtOH-PTU and the rinse was added to the homogenate. After centrifugation at  $2950 \times g$  for 20 min at 4°C, the supernatant was kept and the pellet was re-extracted in 0.3 ml ice-cold EtOH-PTU by 20 s sonication. After a second centrifugation, both supernatants were pooled and centrifuged at  $64 \times g$  for 5 min at 4°C. The supernatant was vacuum-dried at 37°C for 24 h in a SVC 100H SpeedVac (Savant). Samples were reconstituted by sonication in 800 µl ice-cold PBS-PTU, and were analysed for  $T_3$  and  $T_4$  content by RIA.

**Statistics.** For analysis of pigment-stage differences between groups with a  $\chi^2$ -test (SYSTAT), we pooled pigment stages into 3 pools (VB, VIA<sub>0</sub> and VIA<sub>1</sub> and more) to avoid pools of less than 5 individuals. For validation of extraction procedures, after log-linearization, parallelism of dilution curves of extract with the standard dilution curve in the RIA were tested with an ANCOVA (SYSTAT).  $T_3$ ,  $T_4$ , TH,  $T_3:T_4$  ratio levels and mass distribution differences between groups were tested with a Kolmogorov-Smirnov test (2-sample KS, SYSTAT). Results are given as means  $\pm$  SD.

## RESULTS

### Pilot extraction experiments

Serial dilutions of tissue extracts inhibited the binding of TH radiotracer, to produce a dose-response curve parallel to that of the TH standard ( $p = 0.483$  for  $T_3$ ,  $p = 0.959$  for  $T_4$ , ANCOVA). This was obtained with the 4 extraction media (Fig. 3). The extraction efficiency was EtOH-PTU > EtOH > PBS-PTU > PBS for  $T_3$  and EtOH-PTU  $\geq$  PBS-PTU > EtOH > PBS for  $T_4$  (Fig. 3). For the subsequent study of individual whole-body TH, we therefore chose the extraction medium providing the best efficiency, i.e. EtOH-PTU. Extract volumes for individual samples which corresponded to the linear parts of the standard dilution curves were 75 µl for  $T_3$  and 200 µl for  $T_4$ .

### Body mass and pigment stages

Ebb tide glass eels ( $0.286 \pm 0.057$  g) had significantly smaller masses than trap

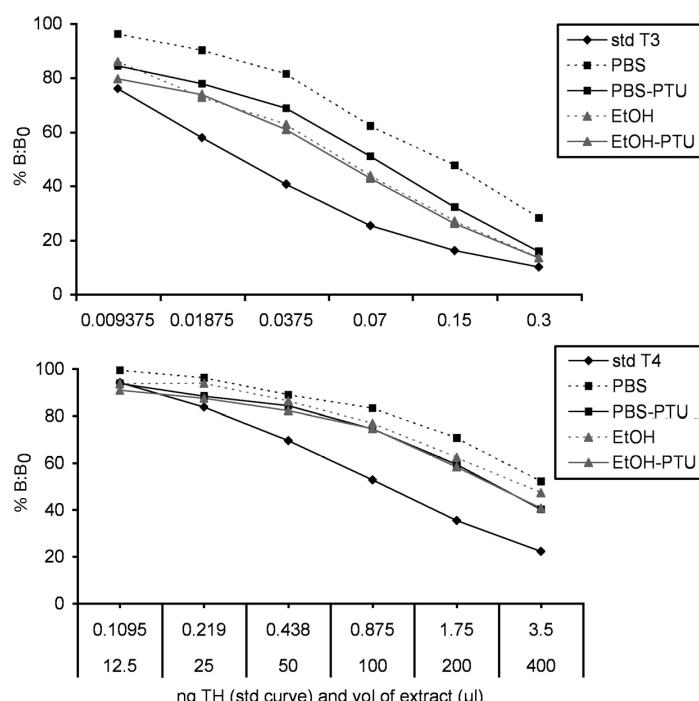


Fig. 3. *Anguilla anguilla*. Dose-response curves of glass eel extracts in radio immunoassays (RIAs) for thyroid hormones (THs)  $T_3$  and  $T_4$ . RIA competitive binding curves are expressed as percentage of maximal binding of radiotracer (%  $B:B_0$ ) for serial dilutions of  $T_3$  and  $T_4$  standards (std) and glass eel extracts. Each point represents average of duplicates. Note that for both hormones, curves for serial dilutions of extracts are parallel to curve for serial dilutions of standard. PBS: phosphate buffer saline; PBS-PTU: phosphate buffer saline containing 1 mM 5-propyl-2-thiouracil; EtOH: absolute ethanol; EtOH-PTU: absolute ethanol containing 1 mM 5-propyl-2-thiouracil

Table 2. *Anguilla anguilla*. Mean body mass and pigment stages (after Elie et al. 1982) for each glass eel group. -: no data. Collection details in Table 1

| Glass eel groups | Body mass (g)     |             | Pigment stage (%) |                  |                  |                  |                  |                  |     |
|------------------|-------------------|-------------|-------------------|------------------|------------------|------------------|------------------|------------------|-----|
|                  | Mean $\pm$ SD     | Range       | VB                | VIA <sub>0</sub> | VIA <sub>1</sub> | VIA <sub>2</sub> | VIA <sub>3</sub> | VIA <sub>4</sub> | VIB |
| Ebb              | 0.286 $\pm$ 0.057 | 0.175–0.441 | 77                | 14               | 2                | 5                | 2                | 0                | 0   |
| Flood            | 0.302 $\pm$ 0.056 | 0.182–0.522 | 73                | 18               | 8                | 1                | 0                | 0                | 0   |
| Trap             | 0.304 $\pm$ 0.048 | 0.213–0.425 | 0                 | 11               | 17               | 33               | 17               | 22               | 0   |
| Bottom           | 0.291 $\pm$ 0.062 | 0.221–0.356 | –                 | –                | –                | –                | –                | –                | –   |

glass eels ( $0.304 \pm 0.048$  g) ( $p < 0.05$ , KS), but masses were not significantly different between any other combination of groups ( $p > 0.227$ , KS) (Table 2). Pigment stages of trap glass eels were more advanced than those of ebb and flood tide glass eels ( $p < 0.001$ ,  $\chi^2$ ) but were not significantly different between ebb tide and flood tide glass eels ( $p > 0.8$ ,  $\chi^2$ ) (Table 2).

### T<sub>3</sub> content

Whole-body T<sub>3</sub> levels were  $1.70 \pm 0.26$  ng g<sup>-1</sup> in bottom glass eels,  $1.79 \pm 0.38$  ng g<sup>-1</sup> in ebb tide glass eels,  $1.50 \pm 0.43$  ng g<sup>-1</sup> in flood tide glass eels and  $1.79 \pm 0.44$  ng g<sup>-1</sup> in trap glass eels (Fig. 4). Whole-body T<sub>3</sub> levels were not significantly different between trap, ebb tide and bottom glass eels, but were significantly lower in flood tide glass eels than in ebb tide ( $p < 0.001$ , KS) and trap ( $p < 0.003$ , KS) glass eels. Moreover, whole-body T<sub>3</sub> levels were not significantly different between bottom and flood tide glass eels ( $p > 0.2$ , KS).

### T<sub>4</sub> content

Whole-body T<sub>4</sub> levels were  $10.41 \pm 0.9$  ng g<sup>-1</sup> in bottom glass eels,  $13.02 \pm 4.01$  ng g<sup>-1</sup> in ebb tide glass eels,  $14.23 \pm 4.18$  ng g<sup>-1</sup> in flood tide glass eels and  $16.24 \pm 3.85$  ng g<sup>-1</sup> in trap glass eels (Fig. 4). Whole-body T<sub>4</sub> levels were not significantly different between bottom and ebb tide glass eels ( $p > 0.05$ ; KS). When compared to flood tide glass eels, bottom and ebb tide glass eels had significantly lower whole-body T<sub>4</sub> levels ( $p < 0.008$  and  $p < 0.05$ , KS, respectively). Moreover, whole-body T<sub>4</sub> levels of flood tide glass eels were significantly lower than those of trap glass eels ( $p < 0.005$ , KS).

### TH content (T<sub>3</sub> + T<sub>4</sub>)

Whole-body TH (T<sub>3</sub> + T<sub>4</sub>) content was  $12.12 \pm 1.12$  ng g<sup>-1</sup> in bottom glass eels,  $14.81 \pm 4.19$  in ebb tide glass eels,  $15.73 \pm 4.44$  ng g<sup>-1</sup> in flood tide glass eels and  $18.04 \pm 4.02$  ng g<sup>-1</sup> in trap glass eels (Fig. 5). Whole-body TH levels were not significantly different between bottom and ebb tide glass eels ( $p > 0.07$ , KS) and between flood and ebb tide glass eels ( $p > 0.05$ , KS), but were significantly higher in flood tide than in bottom glass eels ( $p < 0.05$ , KS) and significantly higher in trap glass eels than in flood tide glass eels ( $p < 0.002$ , KS).

### T<sub>3</sub>:T<sub>4</sub> ratio

T<sub>3</sub>:T<sub>4</sub> ratios were  $0.16 \pm 0.02$  for bottom glass eels,  $0.14 \pm 0.04$  for ebb tide glass eels,  $0.11 \pm 0.03$  for flood tide glass eels and  $0.11 \pm 0.03$  for trap glass eels (Fig. 5). T<sub>3</sub>:T<sub>4</sub> ratios were not significantly different between bottom and ebb tide glass eels (KS,  $p > 0.1$ ) and between trap and flood tide glass eels (KS,  $p > 0.4$ ) but were significantly higher in bottom and ebb tide glass eels than in trap and flood tide and trap glass eels (KS,  $p < 0.002$ ).

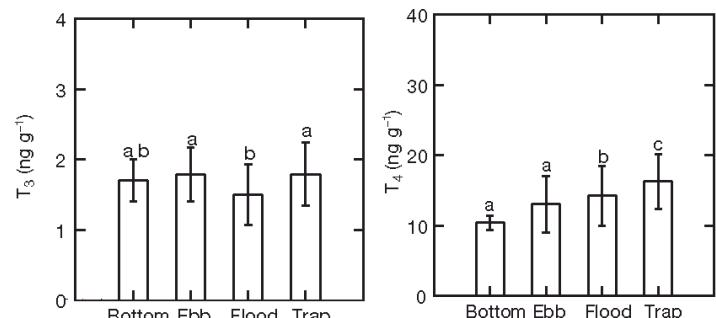


Fig. 4. *Anguilla anguilla*. Mean ( $\pm$  SD) whole-body T<sub>3</sub> and T<sub>4</sub> levels (ng hormone g<sup>-1</sup> wet body mass) measured in glass eels by RIA. Bottom: bottom glass eels (N = 5), ebb: ebb tide glass eels (N = 46), flood: flood tide glass eels (N = 114), trap: trap glass eels (N = 69). Different letters above bars indicate significant differences between distributions ( $p < 0.05$ , Kolmogorov-Smirnov test)

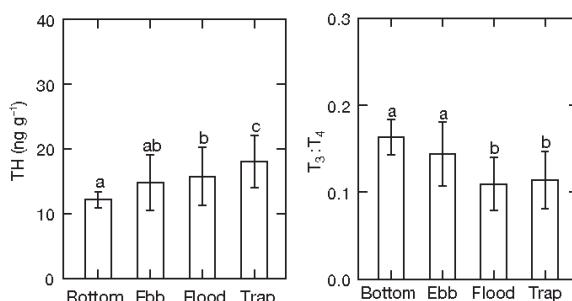


Fig. 5. *Anguilla anguilla*. Mean ( $\pm$  SD) whole-body TH ( $T_3 + T_4$ ) levels (ng hormone  $g^{-1}$  wet body mass) and  $T_3:T_4$  level ratios for glass eels. Further details as in Fig. 4

## DISCUSSION

In this study, we compared differences in whole-body TH levels between glass eels showing different migratory behaviors at the tidal limit with the aim of determining whether and how these hormones could be implicated in migration and settlement phenomena.

### Extraction

Comparison of different extraction media showed that the best extraction efficiency was obtained with EtOH-PTU, with PTU blocking the action of deiodases (Denver 1993). Our results show that in *Anguilla anguilla* glass eels,  $T_4$  content is approximately 10-fold that of  $T_3$ . Since the binding affinity of  $T_3$  to TH receptors is approximately 10-fold higher than that of  $T_4$  (Darling et al. 1982, Bres & Eales 1986), this suggests that in *A. anguilla* glass eels,  $T_3$  and  $T_4$  may contribute to TH receptor stimulation in the same order of magnitude. Whole-body  $T_3$  and  $T_4$  levels (1.5 to 1.79 and 10 to 16 ng  $g^{-1}$ , respectively) were similar to those in metamorphosing *Conger myriaster* leptocephalus larvae (0.15 to 2 and 5 to 30 ng  $g^{-1}$ , respectively) (Yamano et al. 1991) and of the same magnitude as those in salmonid fry (Tagawa & Hirano 1987, Greenblatt et al. 1989).

### Body mass and pigment stages

The body mass of trap glass eels was slightly but significantly larger than that of ebb tide glass eels, but similar to that of the other groups. Trap glass eels were significantly more pigmented than glass eels of the other groups, indicating that they were more advanced in metamorphosis. On the one hand, this may suggest that a migration delay takes place between arrival below the dam and the climbing of the eel ladder as

suggested by McCleave & Wippelhauser (1987). On the other hand, since  $T_4$  treatment accelerates pigmentation in glass eels (Vilter 1946, Jegstrup & Rosenkilde 2003), the increased metamorphosis rate in trap glass eels was also possibly induced by increased thyroid gland activity (Fig. 5). Otolith ageing of the individuals would allow discrimination between these 2 hypotheses (Cieri & McCleave 2001).

### Thyroid status parameters

Because glass eels are too small to sample their blood, we measured (by RIA) whole-body  $T_3$  and  $T_4$  contents, and calculated TH content ( $T_3 + T_4$ ) as well as the  $T_3:T_4$  ratio.  $T_4$  levels depend on thyroid gland production and  $T_4$  ORD activity rates. The plasma  $T_4$  level is generally considered as the most common proxy for evaluating thyroïdal status (Eales & Brown 1993). Whole-body  $T_4$  content includes the thyroid gland, blood and tissue stocks. Consequently, whole-body  $T_4$ , and *a fortiori* TH, may be considered reliable parameters for thyroid gland synthesis-activity.

Plasma  $T_3$  levels depend on  $T_4$  ORD activity and degradation rates. A complex system of deiodase pathways tend to maintain intracellular  $T_3$  tone at some set point determined by the physiologic state (Eales & Brown 1993). In fishes, as in mammals, thyroïdal status, through  $T_3$  production, may regulate energy-demanding processes (Eales & McLatchy 1989). Thus, whole-body  $T_3$  levels in glass eels was assumed to reflect the energy demand of tissues.

The plasma  $T_3:T_4$  ratio reflects the  $T_4$  ORD activity rate (Eales & Brown 1993). In the eel,  $T_4$  ORD activity increases with increasing temperatures and salinity (Leloup & De Luze 1985). Temperatures were similar in all samples (12 to 14°C), and salinity was only different for trap glass eels, which were sampled in freshwater. The  $T_3:T_4$  ratio was not significantly different between trap and flood tide glass eels, suggesting that entry into freshwater was too recent to modify  $T_4$  ORD activity rate. Thus, we assumed that temperature and salinity did not influence  $T_3:T_4$  ratio in our study, and whole-body  $T_3:T_4$  was assumed to be a proxy for  $T_4$  ORD activity.

### Thyroid status and its ecological interpretation

#### Trap glass eels

Whole-body  $T_4$  and TH levels were highest in trap glass eels, indicating increased thyroid gland synthesis-activity (Figs. 4 & 5). Trap glass eels had climbed the eel ladder and entered freshwater, a phenomenon

corresponding to the colonization of river habitats. Hence, our results strongly suggest that the activation of the thyroid gland is related to the colonization of river habitats by *Anguilla anguilla* glass eels. The effects of salinity on thyroid gland synthesis-activity reported in the literature are unclear. TH, interacting with other hormones, may be involved in acclimation to salinity (Leloup & De Luze 1985). In the stickleback *Gasterosteus aculeatus*,  $T_4$  treatment induces a change in salinity preference from saltwater to freshwater (Baggerman 1962), suggesting that, in the trap glass eels, activation of the thyroid gland also induced a freshwater-seeking behavior. Young yellow eels *A. rostrata* caught climbing waterfalls with no salinity change exhibit higher plasma  $T_4$  levels than eels caught below waterfalls, but no increase in plasma  $T_3$  levels, indicating an increased thyroid gland synthesis-activity without increased  $T_4$  ORD activity (Castonguay et al. 1990). In trap glass eels, we also monitored a sharp activation of thyroid gland coupled with no change in  $T_3:T_4$  ratios. This physiological pattern was probably related to the climbing of the eel ladder, but also to the transition of behavior from the STST to the active colonization of the river, as first hypothesized by McCleave & Wipfelhauser (1987). The activation of thyroid gland synthesis-activity in trap glass eels is the result of the stimulation of the thyrotrop axis through TSH and would represent deep physiological changes in the glass eels. Further experiments are needed to investigate the temporal relationships between the activation of the thyroid gland and the triggering of transition behavior and (or) climbing behavior.

Whole-body  $T_3$  levels were significantly higher in trap than in flood tide glass eels, suggesting an increased energy demand in tissues of the former. Flood tide glass eels were caught in the water column on flood tide, probably exhibiting an STST behavior which is a flow-supported swimming behavior (Gas-cuel 1986). In contrast, trap glass eels had climbed the eel ladder. This behavior requires strong locomotory activity, and consequently an increased requirement for  $T_3$  by the tissues to meet the increased energy demand. A stimulating role of TH on energetic metabolism during fish migration was previously reported by Woodhead (1975). Our results support this.

#### Bottom glass eels

Whole-body  $T_4$  and TH levels in bottom glass eels were the lowest of all groups and significantly lower than those of flood tide and trap glass eels, indicating decreased thyroid gland activity (Figs. 4 & 5). The bottom glass eels collected had remained on the bottom of the estuary despite the presence of a flood tide. This

behavior does not correspond to that of migrating glass eels in the estuary (STST); these swim up into the water column at flood tide and are carried upstream by the current. This suggests that the bottom glass eels had switched from a migrating to a settling behavior. Our data, showing a correlation between settling behavior and decreasing thyroid gland synthesis activity, are in accordance with the experimental data of Jegstrup & Rosenkilde (2003), who showed that glass eels *Anguilla anguilla* have lower whole-body  $T_4$  contents when they switch from swimming to resting on the bottom of aquaria. Consequently, our results suggest that the loss of migratory behavior in glass eels is linked to decreased thyroid gland activity.

#### Flood tide versus ebb tide glass eels

Whole-body TH content did not differ significantly between flood and ebb tide glass eels, and was intermediate in these 2 groups compared to that of bottom and trap glass eels (Fig. 5). Ebb tide and flood tide glass eels were both collected while swimming in the water column of the estuary, suggesting that they were exhibiting estuarine migratory behavior (STST). This may indicate that STST corresponds to a thyroid gland activity intermediate between that of river-colonizing and that of settling glass eels. Nevertheless, despite similar TH contents, ebb tide glass eels had significantly lower  $T_4$  levels than flood tide glass eels (Fig. 4), indicating a decrease in thyroid gland secretory activity. This suggests that ebb tide glass eels were close to the switch from migratory to settling behavior.

In contrast, whole-body  $T_3$  levels were significantly higher in ebb-tide than in flood tide glass eels, suggesting an increased energy demand in the former. Ebb tide glass eels had to swim actively against the current to maintain their position below the dam against the downstream ebb, whereas flood tide glass eels were swimming with the flow. Therefore, in contrast to flood tide individuals ebb tide glass eels did not take advantage of tidal transport, nor did they seek shelter on the substrate. Their counter-current swimming behavior induced increased locomotory activity and a consequent increased energy demand of the tissues. The increased  $T_3$  content of ebb tide individuals was sustained despite decreased thyroid gland activity, and was therefore probably related to increased  $T_4$  ORD activity.

As indicated by the  $T_3:T_4$  ratios,  $T_4$  ORD activity was significantly higher in ebb tide and bottom glass eels than in trap and flood tide glass eels. This indicates that both bottom and ebb tide glass eels have increased capacities for producing  $T_3$  from  $T_4$  com-

pared to trap and flood tide glass eels. Through the higher affinity of  $T_3$  rather than  $T_4$  to TH receptors, this mechanism increases the biological activity of thyroid hormones despite decreased thyroid gland synthesis-activity. It may be the consequence of physiological stress. In the eel, the growth hormone (GH) promotes  $T_4$  ORD activity (De Luze & Leloup 1984), and it has been shown that stress, fasting, or a low condition factor induces an increase in GH production by the pituitary gland in the yellow eel (Marchelidon et al. 1996, Rousseau et al. 1999, Dufour et al. 2001) and glass eel (Lambert et al. 2003) (present Fig. 1). Ebb tide glass eels had the lowest body masses of all groups, indicating low condition and physiological stress. Environmental conditions were similar throughout the sampling period and, as indicated by pigment stages, ebb tide glass eels spent the same amount of time in the estuary as flood tide glass eels. Hence, the physiological stress observed in ebb tide glass eels could not be the consequence of different environmental factors but was rather the result of divergent migratory behavior.

We therefore propose an ecological and physiological mechanism for the divergent migratory behavior of ebb tide glass eels. These individuals were caught in the mid water column at ebb tide, whereas glass eels using STST sought shelter on the bottom, waiting for the next flood tide. This suggests that the rhythm of the biological clock of ebb tide individuals was no longer in phase with the flood tide, inducing a non-optimal use of currents requiring frequent counter-current swimming. For juvenile fishes, STST enables an energy conservation (cost-per-unit-distance) of >90% compared to constant swimming (Weihs 1978). Therefore, the physiological stress detected in ebb tide glass eels was presumably induced by a too high energetic expenditure during migration. The similar physiological patterns (decreased thyroid gland and increased  $T_4$  ORD activities) of bottom and ebb tide glass eels suggests that physiological stress induced by a misphased rhythm of the biological clock may lead to precocious settling behavior. This settling behavior may represent the beginning of estuarine residency, although our present data cannot exclude the possibility that the settling behavior of bottom glass eels may be only transient. If settlement leads to residency, this mechanism could explain the colonization of estuarine or marine habitats by *Anguilla anguilla*. We do not yet know why the rhythm of the biological clock of ebb tide glass eels becomes misphased, but suggest that the thyroid gland may play a role in the regulation of activity rhythms in glass eels.

In conclusion, our data has revealed that thyroidal status is related to the migratory behavior of glass eels.

Increased thyroid gland activity is linked to the active colonization of river habitats, and may also induce freshwater-seeking behavior. Behavioral experiments, including TH treatment, are necessary to elucidate the role of TH in triggering migratory behavior. If some individuals constantly exhibit a higher thyroid gland activity and migratory behavior than the rest of the population, this may indicate the existence of pioneer individuals (Fontaine 1975, Feunteun et al. 2003). Precocious settlement in estuarine habitats at the glass eel stage seems to involve decreased activity of the thyroid gland but increased  $T_4$  ORD activity, probably reflecting physiological stress. Therefore, as a mechanism for the loss of migratory behavior in estuaries at the glass eel stage we propose that the rhythm of the biological clock becomes misphased, preventing the glass eels from efficient use of tidal streams as transport means and inducing a physiological stress through the resultant high energetic cost of migration. This leads to the loss of the migratory behavior and consequently to settlement. Our data further reveals the role of TH in glass eel migration and settlement. One facet of our findings is the awareness that endocrine disruptors such as PCBs, by blocking the action of TH on receptors (Iwasaki et al. 2002), may also reduce the colonization of river habitats by glass eels.

**Acknowledgements.** We are grateful to Dr. R. Denver, University of Michigan, for fruitful methodological discussions. We sincerely want to thank B. Sauvaget (IAV) for his help during night sampling. P. Lambert (Cemagref) critically read the manuscript and helped us with the statistical analysis. We thank Dr. I. Mayer (University of Bergen) for English corrections. P. Camoin (Cemagref) drew Fig. 2. This work was partly supported by a research grant from GRISAM.

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*Editorial responsibility:* Otto Kinne (Editor),  
Oldendorf/Luhe, Germany

*Submitted:* March 4, 2004; *Accepted:* July 4, 2004  
*Proofs received from author(s):* October 29, 2004

**ARTICLE 3: Endocrine control of *Anguilla anguilla* glass eels dispersal: effect of thyroid hormones on locomotor activity and rheotactic behavior**

**Le contrôle endocrinien de la dispersion des civelles d'*Anguilla Anguilla* : effet des hormones thyroïdiennes sur l'activité locomotrice et la rhéotaxie**

Article publié dans *Hormones and behavior* (2005) 48: 53-63

Résumé :

La dispersion, un des processus les plus importants en écologie des populations, est un thème reliant la physiologie au comportement. Cependant, le contrôle endocrinien de la dispersion animale reste mal compris. Ici, nous testons si et comment les hormones thyroïdiennes peuvent influencer la dispersion des civelles *d'Anguilla anguilla*, en testant leur influence sur l'activité locomotrice et la rhéotaxie. Les civelles ont été capturées durant leur migration estuarienne et traitées par immersion dans une solution de L-thyroxine ( $T_4$ ) ou de thiourée (TU). Comme mesuré par essai radioimmunologique, les traitements par  $T_4$  et TU ont induit, respectivement, un accroissement et une diminution les taux d'hormones thyroïdiennes corporels comparé aux témoins non traités. Nous avons testé un total de 960 civelles distribué en groupes de traitement témoin,  $T_4$  et TU, sur leur comportement de nage dans des canaux expérimentaux équipés avec des pièges amont et aval permettant de mesurer simultanément l'activité locomotrice et la rhéotaxie. Comparé aux témoins, l'activité locomotrice s'est accrue significativement chez les civelles hyperthyroïdiennes traitées à la  $T_4$ , et a déclenché significativement chez les civelles hypothyroïdiennes traitées à la TU. Les résultats obtenus sur la rhéotaxie suggèrent des mécanismes de régulation plus complexes, car seul le traitement à la TU a modifié significativement la rhéotaxie. L'influence des hormones thyroïdiennes sur l'activité locomotrice suggère un rôle central de ces hormones dans la régulation des mécanismes menant à la colonisation des habitats continentaux par les civelles. Les hormones thyroïdiennes sont également impliquées dans le contrôle de l'activité locomotrice chez les mammifères et les oiseaux migrateurs, ce qui suggère que ces hormones représentent des médiateurs proximaux conservés de la dispersion chez les vertébrés.



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Hormones and Behavior 48 (2005) 53–63

Hormones  
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## Endocrine control of *Anguilla anguilla* glass eel dispersal: Effect of thyroid hormones on locomotor activity and rheotactic behavior

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Received 27 October 2004; revised 1 February 2005; accepted 1 February 2005

Available online 17 March 2005

### Abstract

Dispersal, one of the most important processes in population ecology, is an issue linking physiological and behavioral features. However, the endocrine control of animal dispersal remains poorly understood. Here, we tested whether and how thyroid hormones may influence dispersal in glass eels of *Anguilla anguilla*, by testing their influence on locomotor activity and rheotactic behavior. Glass eels were caught during their estuarine migration and treated by immersion in either a L-thyroxine ( $T_4$ ) or a thiourea (TU) solution. As measured by radioimmunoassay,  $T_4$  and TU treatments induced, respectively, increased and decreased whole-body thyroid hormone levels relative to untreated controls. We tested a total of 960 glass eels distributed into control, and  $T_4$  and TU treatment groups, on their swimming behavior in experimental flume tanks equipped with upstream and downstream traps that allowed us to concurrently measure both the locomotor activity and the rheotactic behavior. Compared to controls, locomotor activity significantly increased among the hyperthyroid,  $T_4$ -treated eels, but significantly decreased among the hypothyroid, TU-treated eels. The results on rheotactic behavior suggested a more complex regulatory mechanism, since TU but not  $T_4$  treatment significantly affected rheotactic behavior. The influence of thyroid hormones on locomotor activity suggests a central role for these hormones in the regulation of mechanisms leading to the colonization of continental habitats by glass eels. Thyroid hormones are also implicated in the control of locomotor activity in mammals and migratory behavior in birds, suggesting that these hormones represent conserved, proximate mediators of dispersal in vertebrates.

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**Keywords:** Thyroid hormones; Dispersal; Migration; *Anguilla anguilla*; Locomotor activity; Rheotactic behavior

### Introduction

Animal dispersal is of great ecological importance, shaping population structure, conditioning gene flow and influencing fitness (Clobert et al., 2001). Dispersal is affected by a range of factors including the organization of social systems, growth forms, seasonality, habitat and a host of other ecological and behavioral parameters. Hormones that may influence dispersal and migration include glucocorticoids and androgens (Dufay and Belthoff, 2001; Holekamp and Smale, 1998). However, the endo-

crine control of dispersal remains poorly understood in ectothermic vertebrates, most studies having focused on mammals and birds (Andreassen et al., 2002; Clobert et al., 2001).

The life cycle of the elopomorph fish, *Anguilla anguilla* (the European eel), includes two long migrations between continental growth and marine breeding areas. In *A. anguilla*, the hatched leptocephalus larva migrates from the Sargasso sea and metamorphoses into a glass eel off the continental shelf of Europe (Schmidt, 1909). Subsequently, glass eels colonize continental marine waters and migrate up-estuary using selective tidal stream transport (STST) (Creutzberg, 1958; McCleave and Wipfelhauser, 1987), a transport mechanism used by numerous marine and

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estuarine animals (Forward and Tankersely, 2001). Fishes ascend from the bottom and are carried by tidal currents during the flood tide. During slack water, they return to the bottom where they remain during the ebb tide. Movements are therefore achieved through saltatory steps. In fishes, the STST allows important energy savings and provides rapid transport compared to constant counter-current swimming (Metcalfe et al., 1990; Weihl, 1978). When they reach the tidal limit, migrating glass eels have to face a constant downstream water current. At this point, they lose the circatidal rhythm and adopt a strict counter-current swimming (McCleave and Wipperhauser, 1987; Wipperhauser and McCleave, 1988). This behavioral shift, affecting both locomotor activity and rheotactic behavior, allows the colonization of river systems. Therefore, due to their life cycle, eels experience marine, estuarine and freshwater habitats. Throughout their migration, they have to cope with oceanic, tidal and river currents, using either flow-carried or active swimming. Hence, the eel is an especially good model to study fish dispersal.

During migrations, amphihaline fishes show a suite of physiological, morphological and behavioral modifications linked to endocrine alterations (Fontaine, 1975; Woodhead, 1975). However, the endocrine control of migratory behavior is still relatively unknown, especially in eels. The thyroid hormones (THs) L-thyroxine ( $T_4$ ) and 3,5,3'-triiodo-L-thyronine ( $T_3$ ) are phylogenetically conserved molecules that affect many aspects of development, growth and metabolism of vertebrates. In juvenile salmonids, THs play a central role in the regulation of smoltification that transforms river-dwelling parrs into migratory smolts that are adapted to the marine environment (McCormick et al., 1998). Moreover, during smolting, a surge of THs is proposed to trigger the downstream migratory behavior (Iwata, 1995; Katzman and Cech, 2001; Specker et al., 2000). In adult salmon caught during their spawning migration, THs are related to the river discharge rate, suggesting a further role in counter-current swimming ability (Youngson and Webb, 1992). Correlations between migratory behavior and plasma TH levels were observed in field studies of the Arctic charr *Salvelinus alpinus* (Hogasen and Prunet, 1997) and the cod *Gadus morhua* (Comeau et al., 2000, 2001). In eels, THs also play a fundamental role in regulating the metamorphosis from the leptocephalus larval to the elver stage (Jegstrup and Rosenkilde, 2003; Ozaki et al., 2000; Vilter, 1946; Yamano et al., 1991). In subadult American eels *Anguilla rostrata*, elevated  $T_4$ , but not  $T_3$ , plasma levels are correlated with increased locomotor activity under natural conditions (Castonguay et al., 1990). In European glass eels *A. anguilla*, river-colonizers caught on a fish pass exhibit an increased thyroid status compared to estuarine migrants (Edeline et al., 2004). Moreover, compared to estuarine migrants, glass eels caught on the bottom of the estuary have decreased whole-body THs. Consistent with these observations, laboratory experiments show a stimulating effect of THs on locomotor activity in *G.*

*morhua*, juvenile salmon *Oncorhynchus* spp. and goldfish *Carassius auratus* (Castonguay and Cyr, 1998; Hoar et al., 1952, 1955; Woodhead, 1970). However, there remain no studies of eels or any other elopomorph fish, one of the major groups of teleosts (see Nelson, 1994), of the role of THs in the behavioral changes occurring during the metamorphosis from the leptocephalus larval to the elver life history stages.

Eel stocks are currently collapsing (Briand et al., 2003; Dekker, 1998; Stone, 2003). Therefore, a greater knowledge of the behavioral biology of eels is essential to the improvement of conservation policies. Moreover, the study of the rather specialized life history stages of elopomorph fish may provide important insight into the endocrine mechanisms of vertebrate migration and dispersal. Here, we investigated the role of THs in controlling dispersal in glass eels of *A. anguilla* by assessing their influence on the intensity of locomotor activity and rheotactic behavior. To this end, glass eels caught during their estuarine migration were treated by immersion in either a  $T_4$  or thiourea (TU, an antithyroid drug) solution. Swimming activity and its orientation were concurrently monitored in flume tanks equipped with upstream and downstream traps. In addition, subsamples of control, TU-treated and  $T_4$ -treated fishes were analyzed for whole-body TH content to assess the effects of the hormonal treatment on thyroid status.

## Materials and methods

Animal manipulations were performed in compliance with the recommendations of the French ethical committee and under the supervision of authorized investigators.

### Fish collection and maintenance

Glass eels were sampled with a pushed surface net at night and during flood tide on 22 March 2004 in the tidal freshwater zone of the Isle River, at Libourne, in southwestern France. The sampling site was located 100 km upstream of the mouth of the Gironde estuary. Water temperature was 11°C. Glass eels were transferred to the INRA station of St. Pée sur Nivelle in aerated tap water from which chlorine had been eliminated by aeration. Fishes were maintained in 5 l containers of aerated tap water. Ten percent of the water volume was renewed daily. Pilot experiments showed that  $T_4$  treatment sharply increased the aggressive behavior of glass eels (identified by skin injuries) when stocked at a high density of 35 individuals  $l^{-1}$ . Therefore, during the present experiment, we acclimatized glass eels at a low density of 9.6 individuals  $l^{-1}$ . Moreover, the animal's welfare was improved by providing shelters (flat stones placed on the bottom of the holding containers). Under these conditions, we did not observe any skin injuries. Water temperature was regulated with an air conditioner at  $11 \pm 0.5^\circ\text{C}$ . The room was maintained from

8:00 am to 18:00 pm under low light intensity ( $1.8 \pm 0.11$  lux) that corresponded to the natural photoperiod (10 L/14 D). This light intensity did not constitute a stress for glass eels and has been shown to not affect their levels of activity (Wipperhauser and McCleave, 1988).

#### Hormonal treatments

In teleost fishes, production of  $T_4$ , the main hormone secreted by the thyroid gland, is regulated by the hypothalamo-pituitary axis via thyroid stimulating hormone (Eales and Brown, 1993).  $T_4$  is released into the circulation and deiodinated by  $T_4$  outer ring deiodases ( $T_4$ ORD) into  $T_3$  in peripheral tissues. Subsequently,  $T_3$  is either deiodinated into 3,3' diiodo-L-thyronine ( $T_2$ ) by  $T_3$  inner ring deiodases ( $T_3$ IRD) or excreted through urine or bile. The binding affinity of  $T_3$  to TH receptors is approximately ten fold higher than  $T_4$ , as shown in hepatocytes of the rainbow trout *Oncorhynchus mykiss* and coho salmon *Oncorhynchus kisutch* (Bres and Eales, 1986; Darling et al., 1982).

Administration of exogenous  $T_4$  and TU by immersion is noninvasive and stress free (Higgs et al., 1982). This method is therefore especially well suited to examine the effects of hormonal manipulation on behavior. Therefore, we immersed glass eels in water into which we had previously added either  $T_4$  to increase circulating TH levels or thiourea (TU) to decrease TH production. We used  $T_4$  rather than  $T_3$  in order to mimic, as close as possible, the natural secretion of the thyroid gland. TU prevents the iodination of the thyroglobulin in the thyroid gland (Davidson et al., 1979; Raby et al., 1990). Therefore, due to thyroglobulin stocks, TU treatments have to be applied for long periods of time to strongly deplete TH levels. Such a treatment would have involved a long period of captivity that may induce other behavioral alterations. Thus, we used a short term 7-day TU treatment. Thiourea also exerts an inhibitory action on  $T_4$ ORD activity over short periods (Frith and Eales, 1996) and was likely the primary mechanism by which TU influenced TH levels in the present experiment. Following standard concentrations used in prior studies of eels, we applied doses of  $0.5 \text{ mg T}_4 \text{ l}^{-1}$  and  $500 \text{ mg TU l}^{-1}$  (Jegstrup and Rosenkilde, 2003; Pradet-Balade et al., 1997).

$T_4$ -sodium salt (Sigma) was dissolved in 0.1 N sodium hydroxide ( $\text{NaOH}, 1.25 \text{ mg ml}^{-1}$ ), and TU (Sigma) was dissolved in distilled water ( $1.25 \text{ g ml}^{-1}$ ). Solutions were kept at  $4^\circ\text{C}$ , and 2 ml was added to water (5 l containers) to reach  $T_4$  and TU concentrations of  $0.5$  and  $500 \text{ mg l}^{-1}$ , respectively. Both TU-treated and control groups received 2 ml  $5 \text{ l}^{-1}$  water of 0.1 N NaOH ( $T_4$  solvent). Concentrations of TU,  $T_4$  and NaOH were maintained constant throughout the experiment by adding drugs daily to the 500 ml renewed water.

A total of 960 glass eels coming from a single catch were randomly distributed into 4 batches of 240 individuals. Each batch was randomly distributed in 3 treatment groups ( $T_4$ ,

TU, control; 80 glass eels per treatment) and treated for 7 days before testing their swimming behavior. The behavioral test required 24 h for a given trial (see below). Therefore, in order to keep hormonal treatment duration constant between batches, treatment started with a 24 h lag between each batch. For example, hormonal treatment began 36 h after capture for batch 1 and 108 h after capture for batch 4.

#### Experimental flume tanks and behavioral tests

Behavioral tests were performed using six experimental flume tanks (180 cm long, 30 cm wide, 17 cm in water depth); one of which is represented in Fig. 1. Upstream and downstream walls were pierced by a hole (4 cm in diameter) shut by a perforated plug (1 mm mesh), allowing water to circulate but preventing the escape of fish. Plugs were easily replaced by traps during the behavioral tests. Dechlorinated tap water, flowing through a pipe, was delivered to each flume tank with a constant discharge providing a flow velocity (mean  $\pm$  SD) of  $20 \pm 2 \text{ cm s}^{-1}$  at the mouth of the upstream trap,  $2 \pm 2 \text{ cm s}^{-1}$  in mid channel and  $2 \pm 1 \text{ cm s}^{-1}$  at the mouth of the downstream trap. Therefore, flow velocity at the mouth of the upstream trap did not limit the swimming capacity of the eels (McCleave, 1980) but was still strong enough to select glass eels showing a pronounced upstream swimming behavior. In contrast, flow velocity at the mouth of the downstream trap was low, avoiding the accidental trapping of glass eels that did not clearly show a downstream swimming behavior. Moreover, in mid-channel, glass eels were able to either rest on the bottom by hiding under the stones or swim in the water column. Hence, the experimental device was designed to test the effect of hormonal treatment on the voluntary movements of glass eels. Trapped eels (upstream and downstream swimming) were considered to have exhibited locomotor activity consistent with dispersal behavior. In contrast, glass eels remaining in the channel, often hidden under the stones, were considered to have exhibited no locomotor activity. Following preliminary trials, eels were kept in the flume tanks without any water current for 22 h before being tested. This procedure avoided accidental catches due to exploratory behavior. Behavioral tests started at 14:00, when water flow was open. Traps were not immediately set in order to avoid the effect of the sudden flow (stress) on swimming behavior. Traps were set at 15:00 and trapping ran for 1 h. At 16:00, eels were removed from the traps and the flume tanks. After a 10 min rinse with clean water, new batches of glass eels were put into the flume tanks (40 glass eels per flume). To avoid any possible "flume tank effect", we randomly assigned the tanks to a treatment for each trial, ensuring that a rotation between tank/treatment had been made. Moreover, behavioral tests were done by observers unaware of the treatments. For behavioral tests, two trials were performed per treatment group (40 glass eels in one flume tank per trial). All

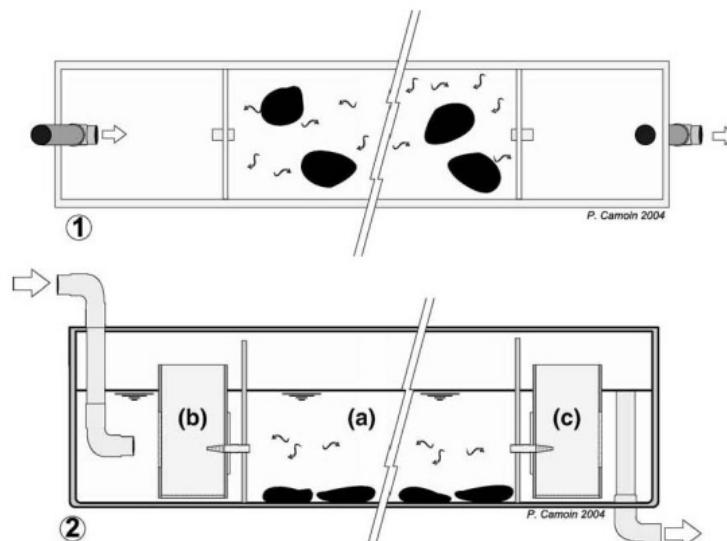


Fig. 1. Top (1) and side (2) views of an experimental flume tank (180 cm long, 30 cm wide, 17 cm in water depth) used to test the effect of an altered thyroid status on both the locomotor activity and the rheotactic behavior in *A. anguilla* glass eels. Water circulation is indicated by arrows. Top view: glass eels ( $n = 40$ ) were put in label (a) for 22 h before behavioral trials. Flat stones provided shelters and perforated plugs (mesh 1 mm) prevented escape. Side view: after 1 h, upstream (b) and downstream (c) traps were set and trapping ran for 1 h. Lateral tarpaulins that hid the movements of experimenters are not represented here. The whole experimental apparatus included 6 parallel flume tanks at each trial.

together, a total of 8 behavioral trials were carried out for each hormonal treatment ( $T_4$ , TU, control).

As emphasized above, we aimed to avoid an effect of stress on the swimming behavior of glass eels. Therefore, four flat stones were regularly arranged on the bottom of the flume tanks to provide shelters, and lateral tarpaulins hid to the fishes the movements of experimenters. Due to the low acclimatization density (9.6 glass eels  $l^{-1}$  during hormonal treatments and 0.4 glass eel  $l^{-1}$  during behavioral tests), no aggressive behavior was observed in any group (including  $T_4$ -treated glass eels). The temperature during trials was identical to that of the stocking water and the light intensity was homogeneous among and inside the flume tanks. Photoperiod and light intensity were identical during the acclimatization period and the trials.

#### Morphological analysis

The glass eel stage is a late metamorphic stage between the translucent leaf-shaped leptocephalus larva and the fully pigmented elver. The extent of skin pigmentation is therefore indicative of the progress of metamorphosis.  $T_4$  and TU immersions are known to, respectively, enhance and reduce the development of pigment in glass eels over a 20 day treatment period (Jegstrup and Rosenkilde, 2003; Vilter, 1946). Furthermore, glass eels that stop feeding at metamorphosis are known to shrink and lose weight until feeding resumes (Elie et al., 1982). In order to detect potential developmental alterations due to the hormonal treatment and late glass eel metamorphosis, we monitored

changes in body length, weight and pigmentation during the experiment. After being anesthetized with clove oil (10% diluted in ethanol, 4 ml  $l^{-1}$ ), glass eels were analyzed for total length to the nearest mm and body mass to the nearest  $10^{-3}$  g. Pigment stages were determined under a microscope and classified according to the extent of skin pigmentation over the head, tail and body regions through stages VA, VB, VIA<sub>0</sub>, VIA<sub>1</sub>, VIA<sub>2</sub>, VIA<sub>3</sub> and VIA<sub>4</sub> to VIB following Elie et al. (1982). Stage VA is the earliest pigment stage, and stage VIB is the latest development pigment stage. A brief description of these pigment stages is given in the Table 1. For this morphological analysis, subsamples were made at capture ( $N = 69$ ) from the whole batch and at the end of the experiment ( $N = 103$ ) from batch 4 (see Table 2).

#### Whole-body TH extractions

After 7 days of treatment and before the behavioral test, subsamples of control,  $T_4$ -treated and TU-treated glass eels from batches 1, 2 and 3 ( $N = 5$  batch $^{-1}$  treatment $^{-1} = 45$ ) were sampled from the containers for hormonal assays. After anesthesia with clove oil, glass eels were individually frozen in cryotubes which had been previously weighed to the nearest  $10^{-3}$  g. The fresh mass of glass eels was then obtained without thawing by weighing the cryotube containing the glass eel and subtracting the mass of the tube.

The extraction procedure was previously described in Edeline et al. (2004). Briefly, extraction was performed in

Table 1

Brief description of the main features of each pigment stage of glass eels according to Elie et al. (1982) that was used for the morphological analysis in the present study

| Pigment stage    | Main features   |
|------------------|---|
| VA               | The overall pigmentation is limited to the end of the caudal fin.   |
| VB               | Pigmentation appears on the head, and caudal pigmentation begins to extend on the back.                                     |
| VIA <sub>0</sub> | Few pigments appear behind the head and dorsal pigmentation extends on the back.  |
| VIA <sub>1</sub> | Head and tail dorsal pigments overlap, and lateral pigmentation begins to extend towards the head from the end of the tail. |
| VIA <sub>2</sub> | Lateral pigmentation do not exceeds the dorsal fin. Moreover, ventral pigmentation develops from the tail towards the head. |
| VIA <sub>3</sub> | Lateral pigmentation exceeds the dorsal fin, but there is no ventral pigmentation between the anus and pectoral fins.       |
| VIA <sub>4</sub> | Ventral pigmentation develops between pectoral fins and the anus.   |
| VIB              | Ventral pigmentation is compact, visibility of internal organs becomes reduced.   |

Skin pigmentation is related to the progress of metamorphosis from the translucent leptocephalus to the fully pigmented elver stages. For further details on eel metamorphosis, see Tabeta and Mochioka (2003).

cryotubes in 0.9 ml ice cold absolute ethanol containing 1 mM 5-Propyl-2-Thiouracil (Sigma) (EtOH-PTU). PTU was used to block endogenous desiodinase activity (Denver, 1993). Homogenization was carried out using an Ultra-Turrax homogenizer (Labo Moderne, Paris) and followed by sonication for 20 s with a Vibra Cell 72434 sonicator (Bioblock). The blades of the homogenizer were rinsed with 0.3 ml ice cold EtOH-PTU and the rinse was added to the homogenate. After centrifugation at 2950 × g for 20 min at 4°C, the supernatant was kept and the pellet was re-extracted in 0.3 ml ice cold EtOH-PTU by 20 s sonication. After a second centrifugation, both supernatants were pooled and centrifuged at 64 × g for 5 min at 4°C. The supernatant was vacuum dried at 37°C for 18 h in a Savant SVC 100 H Speed Vac. Samples were reconstituted by sonication in 800 µl of ice cold phosphate-buffered saline, pH 7.4, containing 1 mM PTU, and were analyzed for T<sub>3</sub> and T<sub>4</sub> content by radioimmunoassays (RIAs).

Table 2

Morphological characters of glass eels from batch 4 just after capture (day 0 of acclimatization) and at the end of the experiment (day 13 of acclimatization), including 7 days of hormonal treatment

| Acclimatization period (days) | Hormonal treatment     | Number of fish analyzed | Total length (mm)<br>(mean ± SD) | Body mass (g)<br>(mean ± SD) | Pigment stages (%) |                  |                  |                  |                  |                  |     |
|-------------------------------|------------------------|-------------------------|----------------------------------|------------------------------|--------------------|------------------|------------------|------------------|------------------|------------------|-----|
|                               |                        |                         |                                  |                              | VB                 | VIA <sub>0</sub> | VIA <sub>1</sub> | VIA <sub>2</sub> | VIA <sub>3</sub> | VIA <sub>4</sub> | VIB |
| 0 (at capture)                | None                   | 69                      | 69.4 ± 4.8                       | 0.294 ± 0.066                | 4                  | 10.1             | 52.2             | 24.6             | 8.7              | 0                | 0   |
| 13                            | Control                | 31                      | 69.1 ± 4.7                       | 0.266 ± 0.063                | 0                  | 0                | 22.6             | 45.2             | 32.3             | 0                | 0   |
| 13                            | T <sub>4</sub> 0.5 ppm | 28                      | 66.9 ± 4.5                       | 0.235 ± 0.054                | 0                  | 3.6              | 14.3             | 39.3             | 42.9             | 0                | 0   |
| 13                            | TU 500 ppm             | 44                      | 68.2 ± 4.0                       | 0.259 ± 0.054                | 0                  | 0                | 36.4             | 34.1             | 27.3             | 2.3              | 0   |

Total length is to the nearest mm, body mass is to the nearest 10<sup>-3</sup> g, and pigment stage is according to Elie et al. (1982).

### Radioimmunoassay (RIA)

The RIA method followed that of Edeline et al. (2004), using RIA kits for total T<sub>4</sub> and total T<sub>3</sub> (Cis Bio International, Gif sur Yvette) with tubes coated with anti-T<sub>3</sub> or -T<sub>4</sub> antibodies and <sup>125</sup>I radio-labeled T<sub>3</sub> or T<sub>4</sub>. Sensitivity was 2.5 ng ml<sup>-1</sup> for T<sub>4</sub> and 0.1 ng ml<sup>-1</sup> for T<sub>3</sub>. Intra-assay variation ranged between 4.1 and 6.6% for T<sub>4</sub> and 3.7 and 6.5% for T<sub>3</sub>. Inter-assay variation, given by the manufacturer, was estimated to range between 6.5 and 10.1% for T<sub>3</sub> and 4.6 and 14.3% for T<sub>4</sub>.

Briefly, after addition of extract or standard and radio-labeled hormone to antiserum coated tubes, tubes were incubated at 37°C for 2 h and decanted. The radioactive fraction bounded to the tube was counted in a gamma counter (Kontron Analytical MDA 312). The bounded radioactive fraction (B) was expressed as a percentage of the maximal bounded radioactive fraction (B<sub>0</sub>) which was obtained with no addition of cold TH in the antiserum coated tube. % B:B<sub>0</sub> was inversely proportional to the amount of cold TH in samples, which was calculated from the standard dilution curve parameters. Individual whole-body T<sub>3</sub> and T<sub>4</sub> levels were measured in duplicate in both assays and expressed as ng hormone per g wet body mass.

### Data analysis

All statistics were conducted with Systat 10. We tested how hormonal treatment affected locomotor activity and rheotaxis in glass eels using a logistic regression model (logit, dummy coding procedure), linking the probability for a behavior to be exhibited to one or more independent variable(s) with a logistic function. First, the effect of treatment on locomotor activity was modeled with a binomial logit, grouping the upstream and downstream swimming behaviors under a common swimming activity and comparing the probability to swim to that to be sedentary. Second, the effect of treatment on the rheotactic behavior was modeled with a multinomial logit, equivalent to two binomial logits processed simultaneously, comparing concurrently the probability to swim upstream to that to be sedentary and the probability to swim downstream to that to be sedentary.

The differences of individual whole-body T<sub>3</sub> and T<sub>4</sub> levels, body lengths and weights between groups were

tested with a Wilcoxon non-parametric test. For the analysis of pigment stages, differences between groups with a Pearson's  $\chi^2$  test, we pooled pigment stages VB and VIA<sub>0</sub>, and stages VIA<sub>3</sub> and VIA<sub>4</sub>, to avoid pools of less than 5 individuals. A Pearson's  $\chi^2$  test was also used to compare the proportions of upstream and downstream swimmers for each treatment. Results are given as mean  $\pm$  SD.

## Results

### Morphology

Detailed results are presented in Table 2. Over the acclimatization period, control glass eels did not shrink (Wilcoxon,  $df = 1$ ,  $\chi^2 = 0.497$ ,  $P = 0.481$ ). However, there was a significant loss of weight due to the end of metamorphosis (Wilcoxon,  $df = 1$ ,  $\chi^2 = 4.481$ ,  $P = 0.034$ ). The pigmentation of control fish significantly increased during the experiment (Pearson's  $\chi^2$  tests,  $df = 3$ ,  $\chi^2 = 19.178$ ,  $P < 0.001$ ), indicating that the development of glass eels was normal. Compared to controls, neither T<sub>4</sub>-nor TU treatments had a significant effect on pigmentation (Pearson's  $\chi^2$  test, T<sub>4</sub>:  $df = 3$ ,  $\chi^2 = 2.213$ ,  $P = 0.529$  and TU:  $df = 2$ ,  $\chi^2 = 1.747$ ,  $P = 0.418$ ). Moreover, neither body length (Wilcoxon, T<sub>4</sub>:  $df = 1$ ,  $\chi^2 = 1.814$ ,  $P = 0.178$ ; TU:  $df = 1$ ,  $\chi^2 = 0.036$ ,  $P = 0.849$ ) nor wet mass (Wilcoxon, T<sub>4</sub>:  $df = 1$ ,  $\chi^2 = 2.779$ ,  $P = 0.095$ ; TU:  $df = 1$ ,  $\chi^2 = 0.075$ ,  $P = 0.784$ ) were significantly affected by the hormone treatments. Together, these results suggested that hormonal treatments did not induce any significant degree of stress.

### Alteration of the thyroid status

Whole-body T<sub>4</sub> and T<sub>3</sub> contents of control fish were similar to those found in a previous study in glass eels of *A. anguilla* (Edeline et al., 2004). Whole-body T<sub>4</sub> levels after 7 days of treatment were  $11.5 \pm 4.9$  ng g<sup>-1</sup> in controls,

$104.7 \pm 17.9$  ng g<sup>-1</sup> in T<sub>4</sub>-treated fish and  $11.1 \pm 1.8$  ng g<sup>-1</sup> in TU-treated fish (Fig. 2). T<sub>4</sub> immersion induced, as predicted, a significant increase in whole-body T<sub>4</sub> levels (Wilcoxon,  $df = 1$ ,  $\chi^2 = 21.774$ ,  $P < 0.0005$ ). In contrast, due to the short treatment period, the effect of TU treatment on whole-body T<sub>4</sub> levels was not significant (Wilcoxon,  $df = 1$ ,  $\chi^2 = 0.097$ ,  $P = 0.756$ ).

Whole-body T<sub>3</sub> concentrations after 7 days of treatment were  $2.1 \pm 0.4$  ng g<sup>-1</sup> in controls,  $4.9 \pm 1.4$  ng g<sup>-1</sup> in T<sub>4</sub>-treated fish and  $1.5 \pm 0.3$  ng g<sup>-1</sup> in TU-treated fish (Fig. 2). T<sub>4</sub>-treated glass eels had significantly higher whole-body T<sub>3</sub> levels than controls (Wilcoxon,  $df = 1$ ,  $\chi^2 = 21.389$ ,  $P < 0.0005$ ), indicating that exogenous T<sub>4</sub> was physiologically deiodinated into T<sub>3</sub>. Moreover, whole-body T<sub>3</sub> levels were significantly lower in TU-treated fish than in controls (Wilcoxon,  $df = 1$ ,  $\chi^2 = 11.995$ ,  $P = 0.001$ ), indicative of the inhibitory action of TU on T<sub>4</sub>ORD activity.

### Effect of thyroid status alteration on the locomotor activity

Average percentages of locomotor activity (upstream and downstream swimming/total number of glass eels ( $n = 8$  trials per treatment; 40 glass eels per trial)) were  $19.2 \pm 7.3\%$  in controls,  $31.9 \pm 13.2\%$  in T<sub>4</sub>-treated fish and  $12.8 \pm 11.7\%$  in TU-treated fish (Fig. 3). When tested with the binomial logistic regression model ( $n = 8$  trials per treatment; 40 glass eels per trial), locomotor activity was significantly higher in T<sub>4</sub>-treated fish ( $\times 1.7$ ) (logit,  $df = 2$ ,  $t$  ratio = 3.494,  $P < 0.0005$ ) and significantly lower in TU-treated fish ( $\times 0.7$ ) (logit,  $df = 2$ ,  $t$  ratio = -2.094,  $P = 0.036$ ) in comparison to controls.

### Effect of thyroid status alteration on the rheotactic behavior

For all the treatments, the proportion of downstream swimmers was significantly higher than that of upstream swimmers (Pearson's  $\chi^2$  test,  $df = 1$ , control:  $\chi^2 = 14.254$ ,  $P < 0.0005$ , TU:  $\chi^2 = 4.122$ ,  $P = 0.042$ , T<sub>4</sub>:  $\chi^2 = 12.629$ ,  $P < 0.0005$ ), likely reflecting that glass eels were exhibiting

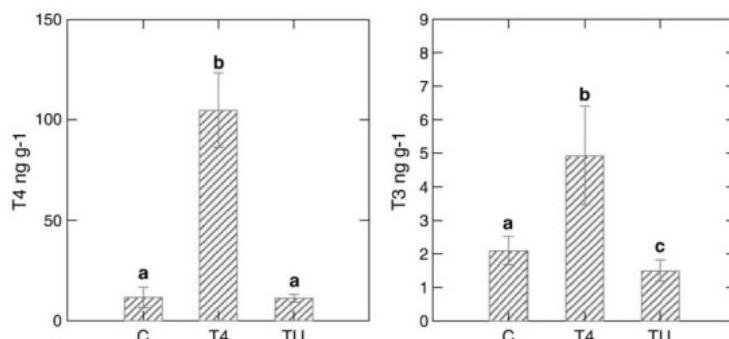


Fig. 2. Whole-body T<sub>4</sub> and T<sub>3</sub> levels (mean  $\pm$  SD) as ng hormone per g of wet body mass (ng g<sup>-1</sup>) measured in *A. anguilla* glass eels by RIA. C: control glass eels ( $N = 15$ ), T<sub>4</sub>: T<sub>4</sub>-treated glass eels ( $N = 15$ ), TU: TU-treated glass eels ( $N = 15$ ). Different letters indicate significant differences between distributions. For detailed statistics, see the Results.

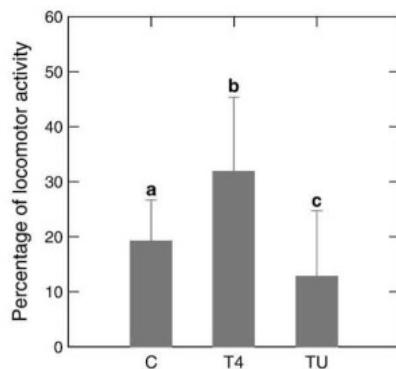


Fig. 3. Effect of THs on the locomotor activity of *A. anguilla* glass eels tested in experimental flume tanks. The average percentages of locomotor activity (upstream and downstream swimming/total number of glass eels ( $n = 8$  trials per treatment; 40 glass eels per trial)) are given as mean  $\pm$  SD for control (C), T<sub>4</sub>-treated (T<sub>4</sub>) and TU-treated (TU) fishes. Different letters indicate a significant effect of the treatment on the locomotor activity, tested on 8 trials with a binomial logistic regression model. For detailed statistics, see the Results.

STST when caught. This suggested that the natural behavior of glass eels was not strongly affected by the acclimatization procedure. Average percentages of upstream and downstream swimmers for each treatment are presented in Fig. 4.

When tested with a multinomial logistic regression model ( $n = 8$  trials per treatment; 40 glass eels per trial), T<sub>4</sub>-treated fishes showed both significantly more upstream ( $\times 2.1$ ) (logit,  $df = 4$ ,  $t$  ratio = 2.719,  $P = 0.007$ ) and downstream ( $\times 1.5$ ) (logit,  $df = 4$ ,  $t$  ratio = 2.647,  $P = 0.008$ ) swimming activity than controls, suggesting that thyroid status did not affect rheotactic behavior.

In contrast, TU-treated glass eels showed a significantly lower downstream ( $\times 0.6$ ) (logit,  $df = 4$ ,  $t$  ratio = -2.264,  $P = 0.024$ ), but not upstream ( $\times 0.9$ ) (logit,  $df = 4$ ,  $t$  ratio = -0.442,  $P = 0.659$ ) swimming activity than control glass eels, indicating an effect of thyroid status on rheotactic behavior.

## Discussion

The present study demonstrates that THs are involved in the regulation of eel locomotor activity. Indeed, T<sub>4</sub> and TU treatments, respectively, increased and decreased glass eel locomotor activity. The results of this study support our previous field data showing that thyroid status is related to the migratory behavior of glass eels (Edeline et al., 2004). In our previous field studies of *A. anguilla* glass eels, individual whole body T<sub>4</sub> and T<sub>3</sub> levels ranged between 6.9 and 50 ngT<sub>4</sub> g<sup>-1</sup> and 0.57 and 3.2 ngT<sub>3</sub> g<sup>-1</sup>, respectively (Edeline et al., 2004). Therefore, the TH levels experienced by glass eels after T<sub>4</sub> or TU treatments in the present experiment were similar in magnitude to those found in a wild population of glass eels.

Our findings are consistent with previous laboratory experiments conducted in a wide range of teleosts including salmonids, cods and cyprinids (see Introduction) and show for the first time in an elopomorph fish, one of the major groups of teleosts (see Nelson, 1994), that thyroid status is involved in the control of locomotor activity. Other studies show that the onset of the migration is related to increased circulating TH levels in birds (Pathak and Chandola, 1982, 1984), while THs have been shown to be involved in the control of locomotor activity in mammals (Rastogi and Singhal, 1976, 1979). Together, these support the hypothesis that thyroid control of locomotor activity may be a conserved vertebrate trait that reflects a more general role for THs in the mediation of dispersal and migration among vertebrates.

The physiological mechanisms by which THs alter locomotion are not clear. In teleosts, THs activate the production of metabolic enzymes in brain, liver and skeletal muscle that lead to increased energy production and aerobic capacity (Tripathi and Verma, 2003; Varghese et al., 2001). THs are known to have general effects on cell metabolism through mitochondrial activation (Goglia et al., 2002; Lanni et al., 2001; Leary et al., 1996), independently of  $\beta$ -adrenergic stimulation (Bachman et al., 2004). Therefore,

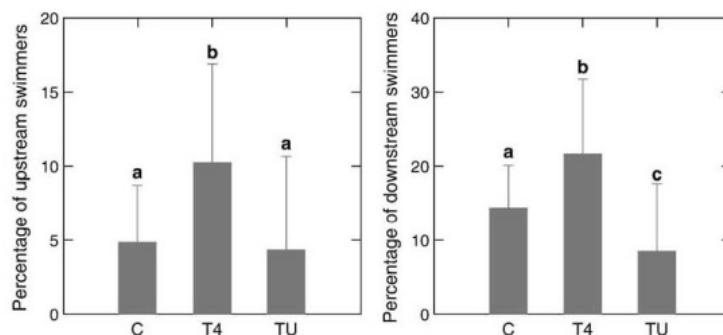


Fig. 4. Effect of THs on the rheotactic behavior of *A. anguilla* glass eels tested in experimental flume tanks. The average percentages of upstream (left panel) and downstream (right panel) swimmers (upstream or downstream swimming/total number of glass eels ( $n = 8$  trials per treatment; 40 glass eels per trial)) are given as mean  $\pm$  SD for controls (C), T<sub>4</sub>-treated (T<sub>4</sub>) and TU-treated (TU) fishes. Different letters indicate a significant effect of the treatment on the rheotactic behavior, tested on 8 trials with a multinomial logistic regression model. For detailed statistics, see the Results.

THs probably affect fish activity and locomotion through an activation of cellular metabolic pathways.

THs have also been shown to affect a variety of target genes in brain (Anderson, 2001; Viguerie and Langin, 2003). In elopomorphs and other teleosts, TH receptors are present in the brain (Bres and Eales, 1988; Dasmahapatra et al., 1991; Kawakami et al., 2003a,b; Van Der Kraak and Eales, 1980) and THs have been shown to alter catecholaminergic activity (Chabe and Joy, 2003). Importantly, catecholamines have previously been implicated in the control of locomotion in several vertebrate groups, including fish (Johansson et al., 2004; Jönsson et al., 2003; Le Bras, 1978) and mammals (Rastogi and Singhal, 1976, 1979). Although we did not specifically address cathecholamine effects in the present study, this line of research offers opportunities for future work.

We report that  $T_4$  treatment significantly increased both upstream and downstream movements, suggesting that an increased thyroid status may promote migration, irrespective of the current direction. In contrast, TU treatment resulted in a significant decrease in downstream, but not upstream, swimming behavior. We predict that a stronger depletion of TH levels would have also decreased upstream swimming. This result suggests an influence of THs on rheotactic behavior and that in the wild, decreased TH levels could reduce STST. This trend is supported by our previous field data suggesting that glass eels settling in an estuary have significantly decreased whole-body TH levels compared to eels using STST (Edeline et al., 2004).

In juvenile salmonids, the TH surge during the parr-smolt transformation is associated with a suite of changes that occur during the downstream migration, including the acquisition of negative rheotaxis (Specker et al., 2000). During the present experiment, the hyperthyroidism and slight hypothyroidism induced, respectively, by the  $T_4$  and TU treatments affected glass eel rheotactic behavior in different ways, suggesting a complex regulatory mechanism. In the Singi fish *Heteropneustes fossilis*, protein synthesis in different brain regions was stimulated by different threshold doses of  $T_4$ , suggesting region-specific TH sensitivity (Ghosh and Medda, 1982); similar results were obtained in mammals (Rastogi and Singhal, 1979). In the rainbow trout *O. mykiss* and sockeye salmon *Oncorhynchus nerka*, it has been shown that THs may differentially regulate brain desiodase activity (Plate et al., 2002). Various sensitivity thresholds to THs could explain our results, but further experiments are needed to more completely investigate the neuroendocrine control of rheotaxis. External orienting signals or “clues” (Harden-Jones, 1984), such as salinity and olfactory gradients, may also influence the rheotactic behavior of migrating glass eels (Tosi et al., 1988, 1989, 1990). For example, THs were shown to influence salinity preference in the stickleback *Gasterosteus aculeatus* and juvenile Pacific salmon *Oncorhynchus* spp. (Baggerman, 1960, 1962; Iwata, 1995).

THs are also implicated in olfactory processes. In mammals, THs affect the maturation and the turnover of olfactory receptor neurons (Paterno and Meisami, 1996a,b), while recent data in salmonids suggest that THs may be involved in the control of olfactory sensitivity during diadromous migrations (Lema and Nevitt, 2004; Plate et al., 2002). THs induce olfactory cell proliferation (Lema and Nevitt, 2004) and are likely involved in odor imprinting during the parr-smolt transformation (Dukes et al., 2004). In glass eels, olfaction also apparently plays a central role in orientation behavior, as indicated by their strong attraction towards green and earthy odors such as geosmin that are typical of inland waters (Sola, 1995; Sorensen, 1986; Tosi and Sola, 1993). Thus, by promoting the proliferation of olfactory neurons, THs could increase the sensitivity of migrating glass eels towards odorous clues. These mechanisms may constitute additional pathways by which THs influence rheotaxis, further supporting the view that the regulation of the rheotactic behavior involves complex interactions between external and internal stimuli. During the present experiment, we deliberately chose not to investigate the effects of salinity and olfactory clues on rheotaxis. This could explain why  $T_4$  treatment had a significant effect on locomotor activity but not on rheotactic behavior. Further studies should address the role of THs on responses to combined migratory clues (water current direction and salinity/terrestrial olfactory clues).

During the parr-smolt transformation, THs mediate physiological, morphological and behavioral changes that represent an adaptative specialization for downstream migration, seawater entry and marine residency (Boeuf, 1993; McCormick et al., 1998). Similarly, in glass eels, an adaptative role of THs in the colonization of continental habitats may be hypothesized. The metamorphosis from the leaf-shaped translucent leptocephalus to the eel-shaped fully pigmented elver may be considered an adaptive, morphological switch from oceanic drift to STST and river colonization. THs mediate this transformation (Jegstrup and Rosenkilde, 2003; Ozaki et al., 2000; Yamano et al., 1991), as well as the adaptation of the gut to osmoregulation in freshwater (Ciccoti et al., 1993; Monaco et al., 1981; Vilter, 1946). Accordingly, Specker (1988) suggested that THs may play a fundamental role in preparing animals to exploit a new environment, especially through developmental changes in the gut. THs could also be involved in other physiological adaptations to continental waters colonization. In juvenile salmonids,  $T_3$  treatment induces increased muscle twitch rates, relaxation and maximum force, decreasing aerobic capacity and probably making smolts unable to maintain their position against the current (Katzman and Cech, 2001). This suggests that during smoltification, THs promote a transformation of muscle physiology from low intensity aerobic swimming towards the burst-like anaerobic swimming required for downstream migration. In glass eels, adapta-

tions of muscle physiology for the shift from oceanic drift to STST could also be regulated by THs. In addition, during the parr-smolt transformation, THs promote schooling behavior (Hutchison and Iwata, 1998; Iwata, 1995; McCormick et al., 1998). In the present experiments, we did not observe any schooling behavior in any treatment group at the very low density in our flume tanks. However, this does not exclude the possibility that THs may participate in the schooling behavior of migrating glass eels in the wild. Indeed, before the collapse of glass eel recruitments, huge shoals of migrants ascend rivers (Tesch, 2003).

In conclusion, our data show that THs are proximate mediators of transformations in glass eel swimming ability. THs stimulate locomotor activity, possibly through changes in energy metabolism, brain catecholamine synthesis and muscle physiology. From a broader perspective, THs could mediate a suite of morphological, physiological and behavioral adaptations allowing the colonization of continental habitats by glass eels. This includes a role for THs in the control of theotactic behavior possibly by modulating sensitivity to a variety of environmental clues used for orientation. Hence, THs may play a central role in the expression of the glass eel migratory behavior during both the STST and the behavioral shift that occurs at the tidal limit. Together with other studies, the results also suggest that THs are conserved proximate mediators of dispersal among vertebrates.

### Acknowledgments

We are indebted to Pr. Ian Mayer (University of Bergen) for precious critical comments and to Andrew Bass (Cornell university) for help with the manuscript. The authors thank Marc Jarry (INRA) and Laurent Beaulaton (Cemagref) for their help during data analysis. We are grateful to Philippe Camoin (Cemagref) for drawing Fig. 1. This study was partly supported by research grants from the GRISAM and Région Aquitaine.

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## **ARTICLE 4: The role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla***

### **Le rôle de la préférence de salinité des civelles dans le contrôle de la sélection de l'habitat et la plasticité de croissance chez *Anguilla anguilla***

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Article sous presse dans *Marine Ecology Progress Series*

**RESUME:** Les anguilles colonisent les habitats marins, estuariens et de rivière, et les anguilles marines et estuariennes tendent à grandir plus vite que les anguilles de rivière. Les raisons de ces tactiques migratoires et de ces trajectoires de croissance divergentes restent très mal connues. Dans le but d'explorer le rôle de la préférence de salinité des civelles dans le contrôle de la sélection de l'habitat et de la plasticité de croissance chez *Anguilla anguilla*, nous avons trié différents contingents de civelles à travers deux tests de préférence de salinité consécutifs. Cela a permis d'étudier le lien entre préférence de salinité et activité locomotrice, et de distinguer les contingents de civelles qui étaient soit plastiques, soit fixes dans leur préférence pour l'eau douce (ED) ou l'eau salée (SW). Ensuite, nous avons enregistré la cinétique de croissance des différents contingents en conditions d'élevage contrôlées en ED et ES. La préférence pour l'ED était liée à une forte activité locomotrice, un schéma comportemental favorisant probablement la colonisation des habitats de rivière en milieu naturel. En effet, le CED (contingent d'ED, i. e. les civelles ayant préféré deux fois l'ED) a montré de faibles performances de croissance, similaires à ceux observés dans les populations naturelles de rivière. En revanche, la préférence pour l'ES était liée à une faible activité locomotrice, et le CES (contingent d'ES, i. e. les civelles ayant préféré deux fois l'ES) a montré de fortes performances de croissance en ES, comme observé dans les populations naturelles de mer et d'estuaire. Le CPQ (contingent plastique, i. e. les civelles ayant changé de salinité de préférence) a montré des performances de croissance intermédiaires qui pourraient être reliées au style de vie "nomade" des anguilles migrant entre différents types d'habitats au cours de leur vie. La croissance a été significativement plus forte en ES qu'en ED chez tous les contingents, indépendamment de la disponibilité en nourriture. Nos résultats, montrant que la préférence de salinité des civelles était liée à leur activité locomotrice et à leur performance de croissance, apportent des mécanismes écologiques pour le contrôle de la distribution dans les habitats et les schémas de croissance observés dans les populations sauvages d'anguille.

**MOTS CLE :** civelle, *Anguilla anguilla*, Tactiques migratoires divergentes, Sélection de l'habitat, Plasticité d'histoire de vie, Préférence de salinité, Activité locomotrice, Croissance.

**ABSTRACT:** Eels colonize either marine, estuarine or freshwater habitats, with marine and estuarine eels tending to grow faster than freshwater eels. The reasons for these divergent migratory tactics and growth trajectories still remain poorly understood. In order to investigate the role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*, we sorted different contingents of glass eels through two consecutive salinity preference tests. This allowed to study the link between salinity preference and locomotor activity (i. e. positive rheotaxis), and to distinguish contingents of glass eels that were either plastic or fixed in their preference for freshwater (FW) or saltwater (SW). Subsequently, we monitored somatic growth of the different contingents in controlled SW and FW rearing conditions. Preference for FW was linked to high locomotor activity, a behavioral pattern likely promoting colonization of FW habitats in the wild. Accordingly, FWC (FW contingent, i. e. glass eels that preferred FW twice) exhibited poor growth performance, similar to those observed in wild FW eel populations. In contrast, preference for SW was linked to low locomotor activity, and the SWC (SW contingent, i. e. glass eels that preferred SW twice) had high growth rates in SW, as observed in wild marine and estuarine populations. The PCC (plastic contingent, i. e. glass eels that shifted their salinity preference) had an intermediary growth status that could be related to the "nomad" life style of eels migrating between different habitat types during their life. Growth was significantly higher in SW compared to FW in all contingents, indicating that habitat salinity may directly affect growth, irrespective of food availability. Our results, showing that glass eels salinity preference was linked to their locomotor activity and growth performance, provide a comprehensive ecological mechanism for the control of habitat distribution and growth patterns observed in wild eel populations.

**KEY WORDS:** Glass eel, *Anguilla anguilla*, Divergent migratory tactics, Habitat selection, Life history plasticity, Salinity preference, Locomotor activity, Growth.

## INTRODUCTION

Worldwide, freshwater eel stocks are currently collapsing (Stone 2003). In this context, a better understanding of the proximate drivers of the eel distribution in marine, estuarine and freshwater habitats would be invaluable for improvement of the management policies.

At the end of their transoceanic migration, leptocephalus larvae metamorphose into glass eels that subsequently invade coastal and inland habitats using selective tidal stream transport (STST) (McCleave & Kleckner 1982, Gascuel 1986, McCleave & Wippelhauser 1987). They ascend from the bottom and are carried by tidal currents during the flood tide. During slack water, they return to the bottom where they remain during the ebb tide. The migration is therefore achieved through saltatory steps. Vertical movements in the water column are cued by an internal circatidal clock, which rhythm is controlled by flow reversals (Wippelhauser & McCleave 1988). During STST, glass eels also orientate towards the mouth of rivers and up-estuary following olfactory cues from river waters (Sorensen 1986, Tosi & Sola 1993, Sola 1995). The larval feeding activity stops at the onset of metamorphosis (Schmidt 1909), and is resumed and conditioned at the glass eel stage by the acquisition of a new set of teeth and physiological development of the gut (Elie 1979, Tesch 2003).

Microchemical otolith studies have shown that glass eels may either colonize FW habitats or stop their migration and settle in SW as early as they reach coastal and estuarine habitats (Tzeng et al. 1997, Tsukamoto & Arai 2001, Tzeng et al. 2002, Jessop et al. 2004). Moreover, some "nomad" individuals migrate between SW and FW habitats during their life (Feunteun et al. 2003, Daverat et al. 2004). The reasons for these divergent migratory tactics are still only partially understood. Furthermore, marine and estuarine eels grow faster than river eels (Fernandez-Delgado et al. 1989, Mounaix & Fontenelle 1994, Tzeng et al. 2003, Arai et al. 2004, Jessop et al. 2004). The reasons for these growth discrepancies remain obscure.

Salinity is a major environmental factor, that may shape fish community structures (Davenport & Sayer 1993, Jung & Houde 2003) and affect growth in numerous teleost fish species (Boeuf & Payan 2001). In the flathead mullet *Mugil cephalus*, field and laboratory studies showed that variations of salinity tolerance during the development could control habitat selection (Cardona 2000). The behavior of glass eels towards salinity is far from being clearly known. Glass eels always survive to acute salinity challenges during abrupt transfer between fresh and seawater (Fontaine & Raffy 1932, Wilson et al. 2004), but glass eels arriving from the sea seem to need a delay before showing voluntary entry in FW (Petit & Vilter 1944, Deelder 1958, Sorensen & Bianchini 1986). This suggests that glass eels may exhibit salinity tolerance variations in terms of osmoregulatory ability. Accordingly, experimental behavioral studies indicate that, although the majority of *Anguilla anguilla* glass eels is FW-seeking, the proportion of SW-seeking fish may still reach from 30 to 50% (Tosi et al. 1988, 1989, 1990). Moreover, some individuals may reverse their salinity preference from FW towards SW over an acclimatization period in brackish water (E. Edeline et al. unpubl. data). These salinity preference patterns fit with the different salinity histories (marine, estuarine, riverine and nomad) observed in yellow and silver eels during microchemical otolith studies, suggesting that interindividual variations in glass eel salinity preference could be involved in habitat selection and utilization.

In order to investigate how glass eel salinity preference may be involved in the control of the eel life history plasticity, we sorted different contingents of glass eels

through two consecutive salinity preference tests. This allowed us to study the link between salinity preference and locomotor activity, and to separate contingents of glass eels that were either plastic or fixed in their preference for FW or SW. Subsequently, we monitored growth of the different glass eel contingents in controlled SW and FW rearing conditions. The links between salinity preference, locomotor activity and growth provided new insights on the ecological and physiological mechanisms involved in the control of the eel continental distribution and growth patterns.

## MATERIALS AND METHODS

**Fish collection.** Glass eels were netted at the mouth of the Gironde estuary during the night of the 26<sup>th</sup> March 2002 (flood tide, 18 ‰ and 10°C water) at Le Verdon, France. As the eels were caught just after their arrival from the sea, they were considered to be homogenous in terms of salinity history. At this particular geographic location, glass eels are at an early stage of metamorphosis (pigment stage VB, see later for details about pigment stages), i. e. they have not yet restarted feeding and growth (Cantrelle 1981, Elie et al. 1982). The glass eels were immediately transferred to the Cemagref's biological station of Saint Seurin sur Isle in aerated natural estuarine water, and then kept unfed for 1 to 3 days in 18 ‰ salted (synthetic sea salt, Instant Ocean, France) and 18°C drill hole water, under natural photoperiod.

**Behavioral test apparatus.** The behavioral test apparatus was similar to that of Tosi et al. (1988), as modified to include three tanks providing a triplicate for each experiment (Fig. 1). This experimental apparatus allowed us to assess concurrently both salinity preference and locomotor activity (i. e. positive rheotaxis) of glass eels, a behavioral trait strongly related to the propensity to colonize inland habitats in the eel (Castonguay et al. 1990, Edeline et al. 2004, 2005).

Each tank was divided into two compartments by a wall into which opened the mouths of two traps delivering flows of drill hole water, either fresh (0 ‰) or salted (33 ‰, synthetic sea salt), which constituted a binary choice for glass eels. The choice compartment (A), receiving glass eels, was filled with 15 l of 17°C, 18 ‰ salted drill hole water (synthetic sea salt). Each trap (B) consisted of a plastic funnel inserted through a cork into the neck of a flask, through which water flowed. Water was gravity-delivered from 10 l water reservoirs (C) to each tank, flowing through taps limiting the flow to 0.14 l min<sup>-1</sup>. In order to avoid any light effect on choices, tanks were covered with lids. Glass eels were therefore kept in the dark throughout the trials.

In order to decrease stress, glass eels were left for ten minutes in the dark before starting the trials. Temperatures were maintained identical (17°C) between flowing and acclimatization waters. Glass eels were counted volumetrically, and about 100 glass eels per tank were tested during 20 min trials (about 3 x 100 glass eels per trial, 11 trials). Glass eels were subsequently counted individually at the end of each trial. Glass eels caught in traps (B) had actively swum towards a water flow, and were considered as active, i. e. to have exhibited a locomotor activity. Glass eels caught in the traps connected to the FW or SW reservoir were considered to have preferred FW or SW, respectively. In contrast, glass eels that remained in the choice compartment (A) had not responded to the water flow and were considered as

inactive (i. e. sedentary). In this protocol, sedentary behavior may reflect both low locomotor activity and preference for brackish water.

**Behavioral testing of glass eels.** We developed two consecutive behavioral tests (over a 10 day acclimatization period) in order to (1) assess the sustainability of the behavioral responses over two consecutive tests, (2) study the behavioral link between locomotor activity and salinity preference, and (3) to discriminate salinity preference in contingents of glass eels for the growth experiment.

During the first trial series, 3193 glass eels were tested for salinity preference on the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> days after capture. Eight hundred and sixty-four of them were active (i. e. actively swam towards either a FW or a SW flow). All the active glass eels, as well as a batch ( $n = 543$ ) of inactive fishes, were kept at 18°C, 18 ‰ to perform the second behavioral test. The other fishes (the rest of the inactive individuals) were released into the environment.

During second trial series, we tested these two batches (543 inactive and 864 active glass eels) for their salinity preference, on the 9<sup>th</sup> and 10<sup>th</sup> day after capture. After the second trial, the 543 glass eels that had been inactive during the first trial were released into the environment. Among the 864 glass eels that had been active during the first trial series, 526 were again active during the second trial series. These 526 fishes were used to carry out the growth experiment, the others were released into the environment.

We classified the 526 glass eels that were active during the two trials into contingents according to their salinity preference: FWC ( $n = 240$ ) for FW contingent (double preference for FW), SWC ( $n = 47$ ) for SW contingent (double preference for SW), and PCC ( $n = 239$ ) for plastic contingent (shift in salinity preference over the acclimatization period: from SW to FW, or from FW to SW). Glass eels from these three contingents were then reacclimatized for 4 to 6 days in 18 ‰ salted, 18°C, drill hole water under natural light-dark conditions before starting the growth experiment.

**Growth experiment.** Each contingent was divided in two, half the eels for rearing in FW (0 ‰ drill hole water) and half the eels for rearing in SW (34 ‰ salted drill hole water, synthetic sea salt), using a natural photoperiod and a water temperature of 18°C. Glass eels were fed in excess with eel fry food (Biomar, France) at identical rations in FW and SW.

Eight glass aquaria (35 l) were used, each divided in 3 compartments by 3 circular plastic fences with a density of 20 to 24 individuals per fence. In order to avoid a possible "aquarium effect" on growth, the compartments used for rearing of each contingent were distributed among different aquaria. Each aquarium was equipped with a recirculation system. Water treatment was provided by a 25 W UV-lamp and a sponge filter supplied by a small pump fixed on the bottom of the aquarium. Three tubes were inserted at the base of the sponge filter bowl and allowed to reinject water into the aquarium through the top of each fence. Moreover, a small sponge and charcoal filter completed the water treatment and provided a slight water current. Food wastes and faeces were regularly siphoned out of the aquaria.

**Measurements.** The beginning of the growth experiment was termed day 1. Individual measurements were made at days 1, 15, 32, 47 and 66. Glass eels were anesthetized with clove oil (10% diluted in ethanol, 4 ml l<sup>-1</sup>) and individually measured for body length (BL) to the nearest mm. After they had recovered, eels were put back into their aquarium compartment. This procedure allowed reducing handling mortality

to zero. In order to limit additional handling stress that may impair growth (Wickins 1987), glass eels were not weighed during the growth period. However, in order to evaluate productivity, after drying on blotting paper, eels were individually weighed to the nearest  $10^{-2}$  g at the end of the growth experiment (day 66).

**Data analysis.** Statistical analysis was carried out using Systat 11 (SPSS Inc., USA). Results are given as mean  $\pm$  SD.

*Behavioral experiment.* We used a one way Pearson's  $\chi^2$  test for analysis of differences in salinity preferences and locomotor activity during the first trial series. Moreover, we tested how locomotor activity during the first trial (*Locomotor activity1*, i. e. the probability to swim upstream irrespective of salinity preference) affected locomotor activity and salinity preference during the second trial with a binomial logistic regression model (logit) of the following form:

$$\ln [p/(1 - p)] = \alpha + \beta_1(\text{Locomotor activity1}) + \varepsilon$$

where  $p$  is either the probability for being active or the probability for preferring FW to SW when active,  $\alpha$  is a constant,  $\beta_1$  is the coefficient of the independent variable "*Locomotor activity1*", and  $\varepsilon$  is random error. The effect of the tank was not statistically significant and "*Tank*" effect was therefore not included in the model.

*Growth experiment.* For analysis of growth, BL were log-linearized to fit distributions to normality (Kolmogorov-Smirnov test,  $p < 0.0005$ ). Then, after checking for homogeneity of variances (Bartlett's test,  $p < 0.0005$ ), the effects of the "*Rearing salinity*" and "*Contingent*" variables on BL were tested using an ANCOVA with the "*Time*" as a covariate. Pairwise comparisons were made using Bonferroni multiple comparison test.

*Survival rates.* We calculated survival rates from the counts of eels at each measurement at days 1, 15, 32, 47 and 66. Due to the low number of reference points, we analyzed the effects of the "*Contingents*" and "*Rearing salinity*" variables on cumulated mortality percentages using a non parametric test of Kruskal-Wallis (KW) and a Mann-Whitney U test, respectively.

## RESULTS

### Behavioral experiment

First behavioral trial series. During the first trial series ( $n = 3193$  glass eels, 11 trials),  $18 \pm 12\%$  of the glass eels actively swam towards FW,  $9 \pm 7\%$  towards SW, and  $73 \pm 14\%$  were inactive. Inactive glass eels were significantly more numerous than active eels (one way Pearson's  $\chi^2$ ,  $df = 1$ ,  $p < 0.0005$ ). Among active glass eels  $65 \pm 21\%$  preferred FW to SW, a highly significant difference (one way Pearson's  $\chi^2$ ,  $df = 1$ ,  $p < 0.0005$ ).

Second behavioral trial series. Glass eels that had been active during the first trial were significantly more active than glass eels that had been inactive during the first trial (Logit,  $df = 1$ ,  $p < 0.0005$ ). This result indicates that glass eels tended to maintain their locomotor activity level over the acclimatization period.

Glass eels that preferred FW during the first trial significantly preferred FW to SW when active during the second trial ( $n = 6$  tanks, 583 glass eels; FW:  $45 \pm 14\%$ ; SW:

$22 \pm 16\%$  and Inactive:  $33 \pm 10\%$ ). Interestingly, glass eels that had preferred SW during the first trial also significantly preferred FW to SW when active during the second trial ( $n = 3$  tanks, 281 glass eels; FW:  $46 \pm 15\%$ ; SW:  $21 \pm 15\%$  and Inactive:  $33 \pm 10\%$ ). This result indicates that a sustained high locomotor activity over the two behavioral tests was linked to a higher preference for FW than for SW. In contrast, glass eels that had been inactive during the first trial significantly preferred SW to FW when active during the second trial ( $n = 6$  tanks, 542 glass eels; FW:  $10 \pm 5\%$ ; SW:  $16 \pm 7\%$  and Inactive:  $74 \pm 6\%$ ), indicating that a low locomotor activity was linked to a higher preference for SW than for FW. When tested with the logistic regression model, this link between locomotor activity and salinity preference was highly significant (Logit,  $df = 1$ ,  $p < 0.0005$ ).

### Growth experiment

Effects of the "*Time*", "*Rearing salinity*" and "*Contingent*" variables on growth. The average BL of glass eels on day 1 was  $75.2 \pm 4.3$  mm ( $n = 526$ ). At the end of the experiment, this value was  $75.5 \pm 9.8$  in FW ( $n = 197$ ), and  $86.2 \pm 14.7$  in SW ( $n = 108$ ). The "*Time*" significantly affected BL (ANCOVA,  $df = 1$ ,  $p < 0.0005$ ), indicating that, irrespective of rearing salinity and contingents, BL significantly changed during the experiment (Fig. 2). Irrespective of contingents, growth of juvenile eels was significantly higher in SW than in FW (ANCOVA,  $df = 1$ ,  $p < 0.0005$ ). This result was confirmed by the productivity data showing that the final total biomass was 75.39 g in SW and 57.30 g in FW. Accordingly, we observed a more intense feeding activity and much less food left over in SW compared to FW.

Regardless of the rearing salinity, growth was significantly influenced by the "*Contingent*" variable (ANCOVA,  $df = 2$ ,  $p = 0.018$ ). Indeed, irrespective of the rearing salinity, growth of FWC eels was significantly lower than that of PCC (Bonferroni,  $p = 0.031$ ) and SWC eels (Bonferroni,  $p = 0.017$ ). No other combination of contingent was significantly different for growth.

Effects of the "*Time*" and "*Contingent*" variables on growth in each salinity. In FW, BL were not significantly influenced by the "*Time*" variable, indicating that growth was not significant over the experimental period (ANCOVA,  $df = 1$ ,  $p = 0.606$ ). Nevertheless, in FW, the "*Contingent*" variable significantly affected BL (ANCOVA,  $df = 2$ ,  $p = 0.019$ ) (Fig. 2A). Indeed, PCC eels grew significantly more than FWC eels (Bonferroni,  $p = 0.006$ ). No other combination of contingents was significantly different for growth in FW.

In SW, BL was significantly affected by the "*Time*" variable (ANCOVA,  $df = 1$ ,  $p < 0.0005$ ), indicating that body growth was significant during the experimental period. Moreover, the "*Contingent*" variable also significantly affected BL (ANCOVA,  $df = 2$ ,  $p = 0.015$ ) (Fig. 2B). Growth of the SWC eels was significantly higher than that of PCC (Bonferroni,  $p = 0.012$ ) and FWC eels (Bonferroni,  $p = 0.004$ ). No other combination of contingents was significantly different for growth in SW.

Survival rates. An increase in mortality was monitored between day 47 (6 % mortality) and day 66 (35 % mortality). We observed that this effect was mainly due to cannibalism that started after day 47, and seemed to occur more frequently in SW than in FW. However, survival rates were not significantly different between contingents (KW,  $df = 2$ ,  $p = 0.715$ ) and salinities (U-test,  $df = 1$ ,  $p = 0.848$ ).

## DISCUSSION

Microchemical otolith studies have recently demonstrated that eels exhibit a flexible pattern of continental habitats colonization, either migrating up-river, or settling in marine and estuarine habitats (Tzeng et al. 1997, Tsukamoto & Arai 2001, Tzeng et al. 2002, Daverat et al. 2004, Jessop et al. 2004). Furthermore, marine and estuarine eels tend to grow faster than river eels (Fernandez-Delgado et al. 1989, Mounaix & Fontenelle 1994, Tzeng et al. 2003, Arai et al. 2004, Jessop et al. 2004). The drivers of these divergent life histories are still poorly understood. In the present study, we investigated the role of glass eel salinity preference in the control of the eel life history plasticity, by evaluating the link between salinity preference, locomotor activity and body growth.

### Link between salinity preference and locomotor activity

Our first salinity preference test shows that, at arrival from the sea, most of active glass eels preferred FW to SW, in accordance with the previous results of Tosi et al. (1988, 1989, 1990). However, through the second salinity preference test, we further show a highly significant link between salinity preference and locomotor activity. Glass eels showing a high, sustained locomotor activity (swimming upstream during the two consecutive tests) preferred significantly FW to SW, whereas glass eels showing a low locomotor activity (inactive during one of the two tests) preferred significantly SW to FW when active. In the wild, locomotor activity is a behavioral trait strongly related to the migratory propensity, in both glass eels (Edeline et al. 2004, 2005) and yellow eels (Castonguay et al. 1990). Preference for FW in highly active glass eels is a behavioral pattern likely promoting the colonization of FW habitats by glass eels, including low-density and lake habitats, that promote production of large female eels (Krueger & Oliveira 1999, Oliveira et al. 2001). In contrast, low locomotor activity coupled to preference for SW likely promotes precocious settlement in marine or estuarine habitats. Hence, our results support the view that salinity preference is connected to locomotor activity during the behavioral control of the distribution in habitats.

At the watershed scale, estuarine eels seem to be the most prevalent, ranging between 64 and 81% of the population (Tsukamoto & Arai 2001, Tzeng et al. 2002, Jessop et al. 2004). This proportion is comparable to that of inactive glass eels during our first behavioral trial series (73 %), which exhibited no locomotor activity and remained in the brackish water of choice compartments. This result is a further support to the view that both locomotor activity and salinity preference may interact in driving the distribution in continental habitats. These sedentary brackish water glass eels, together with those recurrently choosing SW, likely represent the marine/estuarine ecotype.

The endocrine control of locomotor activity and salinity preference is likely regulated by a complex action of several mediators. In glass eels, increased thyroid hormones (TH) levels enhance locomotor activity and migratory behavior (Edeline et al. 2004, 2005) and, in other Teleosts, TH have been shown to also affect salinity preference according to species and/or life stage (Baggerman 1960, 1962, Iwata 1995). Hence, TH could be involved in the regulation of the observed link between preference for FW and locomotor activity in glass eels. However, PCC glass eels changed their salinity preference while maintaining their locomotor activity, and a minority of active eels kept exhibiting a preference for SW. This indicates that the triggering

mechanisms of locomotor activity and salinity preference may also be separated. This differential regulation could involve in addition to TH, growth hormone and cortisol, that both affect locomotor activity in Teleosts (Overli et al. 2002, Johansson et al. 2004) and have a synergistic action for acclimation to SW (McCormick 2001).

### Link between habitat salinity and growth

During the present experiment performed under controlled conditions, glass eel growth was significantly higher in SW compared to FW conditions, irrespective of previous salinity preference. This indicates that environmental salinity may directly affect juvenile eel growth. This result is in accordance with preliminary data from extensive eel pond culture, showing that glass eels and elvers grow faster in SW than in FW (P. Elie, unpubl. data). Growth rate is a life history trait of major importance, which was suggested to influence sexual differentiation, and also age and size at silverying in both sexes (Vøllestad 1992, Holmgren & Mosegaard 1996). Our data suggest that habitat salinity, through its effects on growth rates, may influence both population structure and dynamics.

In FW, growth curves showed that lengths restarted to increase after 47 days. Similar observations of arrested or negative growth rates at the start of glass eel rearing experiments have been reported previously (Bertin 1951, Elie & Daguzan 1976, Elie 1979, De Silva et al. 2001). Indeed, the stop of feeding and intense catabolism of energy stores associated to the metamorphosis induces length and weight reduction (Schmidt 1909, Tesch 2003). When reared at temperatures above 20°C in FW with various diets, the delay before glass eels restart growing ranged from 28 days in *Anguilla australis* (De Silva et al. 2001) to 50 days in *A. anguilla* and *A. rostrata* (Appelbaum et al. 1998). Consequently, growth rates observed in FW at 18°C in the present study are within the range of standard growth rates for glass eels in captivity. At the end of the growth experiment (66 days), glass eels reared in FW had recovered their initial body length. In contrast, glass eels reared in SW recommenced growth earlier (after 15 days) and reached significantly longer body length at the end of the experiment.

In teleosts, salinity may affect growth through various mechanisms such as the standard metabolic rate, food intake and conversion, and hormonal stimulation (Boeuf & Payan 2001). In glass eels, the early work of Fontaine & Raffy (1932) showed that oxygen consumption is lower in SW than in FW, suggesting that a decreased standard metabolic rate may contribute to the higher growth rates monitored here in SW than in FW. Our observations suggest that appetite and feeding activity was higher in SW compared to FW. We also noticed that the intense feeding activity in SW promoted cannibalism, a well known phenomenon in eel culture (Degani & Levanon 1983). In teleosts, hypo-osmoregulation has been shown to enhance secretion of growth hormone (GH), which promotes growth, appetite and aggressive behavior (Le Bail & Boeuf 1997, Jönsson et al. 1998, Boeuf & Payan 2001). Therefore, we suggest that the stimulatory effects of salinity on glass eel growth may result from various effects on metabolism, appetite, feeding and aggressive behaviors, some of them being possibly mediated by increased GH secretion.

The habitats productivity, higher in SW than in FW at high latitudes, was suggested to induce the higher growth rates of marine and estuarine eels compared to river eels in *A. rostrata* (Jessop et al. 2004). Our results indicate that water salinity may also directly promote this phenomenon, irrespective of food availability. Furthermore, our

data suggest that intra-specific predation (cannibalism) may be higher in SW than in FW. This may eliminate slow growers and contribute to the apparent fast growth rates of estuarine and marine eel populations.

### **Link between salinity preference and growth**

In the present experiment, growth was significantly affected by the "Contingent" variable, as defined by the behavioral sorting. This indicates that glass eel growth may be linked to salinity preference. Indeed, FWC eels were the poorer growers, whatever the rearing salinity condition during the growth experiment. In contrast, SWC eels had significantly higher growth rates in SW than FWC and PCC eels. If we consider that, as discussed above, the SWC and FWC glass eels are representative of marine and estuarine- versus river-colonizers, respectively, our results are in agreement with growth patterns observed in wild populations where estuarine and marine eels grow faster than river eels. Some individuals, called "nomads", may migrate between marine, estuarine and FW habitats on both seasonal and annual time scales (Arai et al. 2003, Feunteun et al. 2003, Daverat et al. 2004). Growth of PCC eels was not significantly different to that of SWC eels in FW, and to that of FWC eels in SW. Such a growth pattern suggests an intermediary status between SWC and FWC eels, that could lead to a nomad life style.

The link between glass eel salinity preference and growth rates likely relies on endocrine controls as discussed previously, but also on genetic factors. Indeed, on the one hand, salinity tolerance in the guppy *Poecilia reticulata* (survival after transfer to 35 ‰ SW) is positively correlated to allozyme heterozygosity (Shikano et al. 2000). On the other hand, data from eel farms show the occurrence of strong interindividual growth variations (Elie 1979, Usui 1991). Recent data in *Anguilla anguilla* revealed that heterozygous individuals for allozyme loci have a significantly higher growth in comparison with more homozygous individuals (Pujolar et al. 2005). These results suggest that, in SWC glass eels, high allozyme heterozygosity could have promoted preference for high salinity and high growth rates.

In conclusion, our results show that glass eel salinity preference was linked to their locomotor activity and growth performance. This provides a comprehensive ecological mechanism for the control of both habitat distribution and growth patterns observed in wild populations. Furthermore, it suggests that glass eel salinity preference may represent a behavioral trait of major importance for the control of the eel life history plasticity in continental habitats. Studies on the genetic and endocrine control of glass eel migration would allow to further investigate the regulatory mechanisms of the eel continental dispersal.

## Acknowledgements:

We are indebted to D. Mercier, L. Jacobs, M. Pelard and T. Rouault (Cemagref's biological station of Saint Seurin sur Isle) for their invaluable technical support. We thank Dr. F. A. Weltzien (University of Oslo) for critical reading of the manuscript and English editing. We also want to thank P. Camoin (Cemagref) for drawing the figures 1 and 2. This study was partly supported by research grants from GRISAM and Région Aquitaine.

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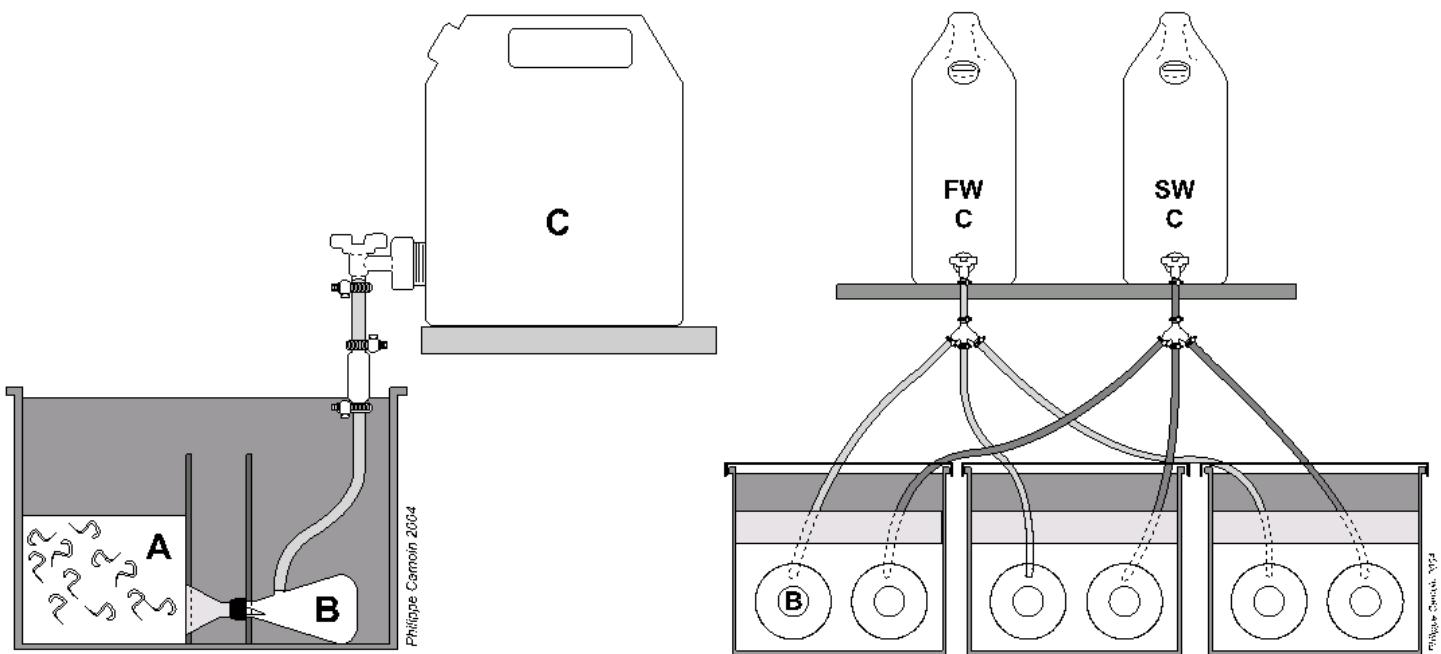


Fig. 1

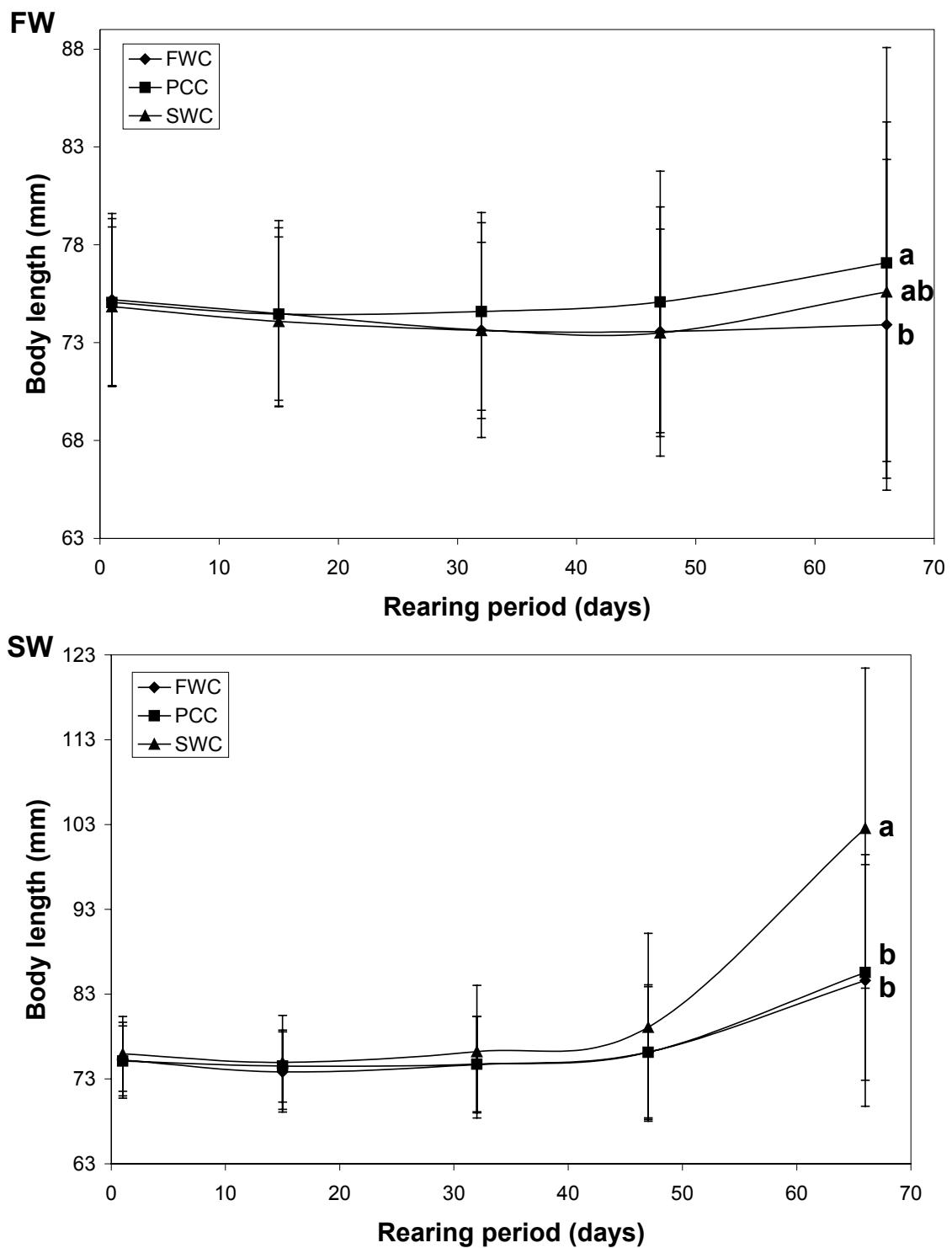


Fig. 2

## FIGURE LEGENDS

Fig. 1. Experimental device used to test salinity preference of glass eels. A: choice compartment, B: trap compartment, C: water header reservoir, FW: freshwater (0 ‰), SW: saltwater (33 ‰). Glass eels caught in traps were considered to show locomotor activity (i. e. positive rheotaxis) while glass eels remaining in the choice compartment were considered as inactive (i. e. sedentary). Glass eels caught in traps connected with FW and SW were considered to prefer FW and SW, respectively.

Fig. 2: *Anguilla anguilla*. Body length (mean  $\pm$  SD) of three contingents of juvenile eels reared in freshwater (FW, top panel) and saltwater (SW, down panel). FWC: FW contingent, i. e. glass eels that showed a double preference for FW during two consecutive salinity preference tests; SWC: SW contingent i. e. glass eels that showed a double preference for SW during two consecutive salinity preference tests, and PCC: plastic contingent, i. e. glass eels that showed a shift in salinity preference over the two tests. Different letters (lower cases) indicate significant differences between contingents over the whole rearing period.

## **ARTICLE 5: Physiological and environmental drivers of *Anguilla anguilla* glass eel migratory behavior: body condition and water temperature effects on locomotor activity and salinity preference**

### **Facteurs physiologiques et environnementaux contrôlant le comportement migrateur de la civelle d'*Anguilla anguilla*: effets de la condition corporelle et de la température sur l'activité locomotrice et la préférence de salinité**

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Article soumis à *Journal of Experimental Marine Biology and Ecology*.

#### **Résumé**

Les civelles arrivant de la mer présentent des tactiques migratoires alternatives menant soit à la colonisation des rivières, soit à une sédentarisation précoce dans les habitats marins ou estuariens. Les facteurs contrôlant ces tactiques migratoires divergentes restent mal connus. Dans la nature, le comportement migrateur des civelles peut être influencé par la température de l'eau, et nos résultats précédents suggèrent qu'il peut également dépendre de la condition corporelle (statut énergétique). Dans le but d'explorer expérimentalement comment ces effets environnementaux et physiologique régulent le comportement migrateur, nous avons testé les effets de la température de l'eau et de la condition corporelle sur l'activité locomotrice (nage vers l'amont) et la préférence de salinité des civelles d'*Anguilla anguilla*. La baisse de la température d'acclimatation a réduit significativement l'activité locomotrice et la préférence pour l'eau douce, en accord avec les données de terrain montrant que des températures basses entravent à la fois la migration estuarienne et les recrutements en rivière. La condition corporelle a affecté significativement la préférence de salinité, les civelles passant d'une préférence pour l'eau douce à une préférence pour l'eau salée à mesure que la condition corporelle décroissait. Ce résultat est en accord avec nos précédentes données de terrain qui suggéraient que les civelles se sédentarisant en estuaire avaient une condition corporelle diminuée par rapport à celle des migrants. En conséquence, comme observé chez les jeunes salmonidés, le statut énergétique peut être décisif pour le contrôle des tactiques migratoires alternatives des civelles. Nos résultats montrent que le comportement migrateur des civelles peut être contrôlé par des facteurs physiologiques et environnementaux à travers l'activité locomotrice et la préférence de salinité.

Mots clé : Civelle, Dispersion continentale, tactiques migratoires alternatives, Température, Physiologie, Activité locomotrice, Préférence de salinité.

## **Abstract**

Glass eels arriving from the sea present alternative migratory tactics, leading either to the colonization of rivers or to an early settlement in marine or estuarine habitats. The drivers of these divergent migratory tactics remain poorly known. In the wild, the glass eel migratory behavior may be affected by water temperature, and our previous results suggest that it could also be physiologically dependent on the body condition (energetic status). In order to experimentally investigate how these environmental and physiological effects on the migration are behaviorally mediated, we tested the effects of water temperature and body condition on locomotor activity (upstream swimming) and salinity preference in *Anguilla anguilla* glass eels. Decreasing acclimatization temperature reduced significantly both locomotor activity and preference for freshwater, in accordance with field data showing that low water temperatures hinder both the estuarine migration and river recruitments. Body condition significantly affected salinity preference since glass eels switched from a freshwater- towards a saltwater-preference as their body condition decreased. This result is in accordance with our previous field data suggesting that glass eels settling precociously in estuary have a decreased body condition compared to migrants. Hence, as observed in juvenile salmonids, the energetic status may be critical to the control of the alternative migratory tactics in glass eels. Our results show that the glass eels migratory behavior, through locomotor activity and salinity preference, may be controlled by both physiological and environmental factors.

Key-words: Glass eel, continental dispersal, alternative migratory tactics, temperature, physiology, locomotor activity, salinity preference.

## 1. Introduction

The eels *Anguilla* spp. are ubiquitous fish of commercial importance, that colonize coastal, estuarine and freshwaters (Tesch, 2003). However, freshwater eel stocks are currently collapsing at a worldwide scale (Stone, 2003), shifting the fishing pressure on coastal and estuarine stocks. In this context, a better understanding of the factors controlling eel recruitments into its different growth habitats would be invaluable for improvement of the management policies.

The willow leaf-shaped, translucent, leptocephalus larvae migrate from the marine spawning grounds to the continental shelf where they metamorphose into unpigmented glass eels. Subsequently, glass eels colonize coastal, estuarine and river habitats using selective tidal stream transport (McCleave and Kleckner, 1982; McCleave and Wippelhauser, 1987), a transport mechanism used by many other coastal and estuarine organisms (Forward et al., 1998; Forward and Tankersely, 2001). To do so, glass eels swim with the current during flood tide, and avoid to be moved back during the ebb tide by sheltering near to or on the bottom of the estuary, or even buried into the sediment. The migration is therefore achieved by saltatory steps. This transport mechanism allows landward and up-estuary movements at a low energy cost (Weihs, 1978; Metcalfe et al., 1990). Feeding activity stops at the beginning of the metamorphosis, and consumption of energy stores for migratory activity therefore induces a reduction in length and weight until glass eels restart feeding (Elie, 1979; Tabeta and Mochioka, 2003).

To guide their movements, migrating glass eels also refer to a variety of environmental gradients, or clues according to the definition of Harden-Jones (1984). Indeed, glass eels orientate towards freshwater habitats using inland waters odor (Creutzberg, 1961; Sorensen, 1986; Tosi and Sola, 1993; Sola, 1995), temperature (Tongiorgi et al., 1986) and salinity gradients (Tosi et al., 1988, 1989, 1990). Tosi et al. (1990), studying the relative importance of water odor, salinity and temperature on the orientation of glass eels, concluded that salinity gradients were the most important environmental clues guiding glass eels towards river habitats. Accordingly, Fontaine (1975) considered that, because of the metamorphosis, glass eels had a change in their osmotic capacities, inducing a physiological preference for freshwater.

However, microchemical otolith studies, using the Sr/Ca ratio as a tracer for saline history (Secor et al., 1995; Tzeng, 1996), have shown that diadromy is facultative in the eel (Tsukamoto and Arai, 2001). Indeed, some glass eels stop their migration and settle as soon as they reach the coastline or the estuary, and individuals may carry out their entire growth phase in marine or estuarine habitats (Tzeng et al., 1997; Tsukamoto and Arai, 2001; Daverat et al., 2004). The drivers for these divergent migratory tactics have long remained unknown. We recently demonstrated that thyroid hormones (TH), controlling locomotor activity and affecting rheotaxis, are proximate physiological mediators of these alternative migratory tactics (Edeline et al., 2004, 2005). Nevertheless, other internal factors could be involved in the control of the glass eel migratory behavior.

For instance, body condition (a proxy for the energetic status) is a life-history trait of major importance, that influences dispersal in birds and mammals (Duffy and Belthoff, 2001), and affects the age at smoltification in salmonids (McCormick et al., 1998). In glass eels, early settling individuals have significantly lower body masses and decreased thyroid status compared to estuarine migrants, probably indicating a depressed body condition (Edeline et al., 2004). This suggests that the energetic

status is a physiological trait involved in the control of glass eels migration. However, it remains unknown how the influence of body condition on the migration is behaviorally mediated.

In addition, numerous field studies indicate that migration and river recruitments of glass eels are negatively correlated with water temperatures (Elie, 1979; Cantrelle, 1981; Gascuel, 1986; Vøllestad and Jonsson, 1988; McGovern and McCarthy, 1992; Elie and Rochard, 1994; Martin, 1995; Jessop, 2003), suggesting an environmental control on the migratory behavior. However, it is not known how water temperature affects the glass eels migratory behavior.

In the present study, we investigated whether water temperatures and body condition may affect glass eels migration and river recruitments through a modulation of locomotor activity and salinity preference. To this aim, glass eels were collected at arrival from the sea and acclimatized at two temperatures. Then, we concurrently tested both locomotor activity (upstream swimming behavior) and salinity preference of glass eels from each temperature group in experimental behavioral tanks. The links between acclimatization temperature, body condition, salinity preference and locomotor activity were analyzed. The ecological and physiological implications of our finding are discussed.

## 2. Materials and methods

### 2.1. Fish collection and maintenance

Glass eels freshly arrived from the sea were netted at the mouth of the Gironde estuary, France, during the night March the 7<sup>th</sup> 2001 (Fig. 1). Netting took place near to the harbor of Royan, at a locality where freshwater from a small river is released through a pipe, resulting in a low salinity of 10 ‰, and a water temperature of 10°C. These conditions resulted in a localized concentration of newly arrived glass eels.

Fishes were immediately transported to the laboratory and, in order to limit disturbance, kept unfed in the dark in de-chlorinated domestic tap water maintained at  $10 \pm 1^\circ\text{C}$ , and with the same salinity as that of the netting site (i. e. salinity 10 ‰). This acclimatization water was freshly made up using synthetic aquarium marine salt (Instant Ocean). On day 6 of acclimatization, the batch of glass eels was separated into two groups ( $n =$  about 1200 / group), one group kept at  $10 \pm 1^\circ\text{C}$ , the other group kept at  $18^\circ\text{C} \pm 1^\circ\text{C}$ , after gradually increasing the temperature from 10 °C over a 7-hour period.

### 2.2. Experimental device and behavioral tests

The behavioral test apparatus was similar to that of Tosi et al. (1988), as modified to include three tanks providing a triplicate for each experiment (Fig. 2). This experimental apparatus allowed to assess concurrently both salinity preference and locomotor activity (i. e. upstream swimming behavior) of glass eels, a behavioral trait strongly related to the propensity to colonize inland habitats in the eel (Castonguay et al., 1990; Edeline et al., 2004, 2005).

Each tank was divided into two compartments by a wall into which opened the mouths of two traps delivering a flow of either odorless freshwater (FW) or saltwater (SW), which constituted a binary choice for the glass eels. In order to

avoid any odorous effect, and because tap water quality may be inconsistent, we used odorless commercial spring water as the flowing water, either fresh (0 ‰) or SW (27 ‰) freshly made up using synthetic aquarium marine salt. The choice compartment (A), receiving glass eels, was filled with 15 l of 10‰ SW, at either 10 or 18°C (acclimatization water). Each trap (B) consisted of a plastic funnel inserted through a cork into the neck of a flask, through which water flowed. Water was gravity delivered from 10 l water reservoirs (C) to each tank, flowing through taps limiting the flow to 0.14 l min<sup>-1</sup>. Temperatures were maintained identical between flowing and acclimatization waters. In order to avoid possible light effects that may affect glass eel behavior (Bardonnet et al., 2005), the tanks were covered with lids during all the trials. Glass eels were therefore kept in the dark throughout the trials.

Glass eels were counted volumetrically and about 90 glass eels per tank were tested in 20 min trials (about 3 x 90 glass eels per trial). Glass eels were subsequently counted individually at the end of each trial. Each individual was tested once. From days 6 to 10 of acclimatization, 2524 glass eels from the cold (10°C) and warm (18°C) acclimatized groups were tested alternately in the morning or in the afternoon during 10 trials.

### 2.3. Morphological analysis

At the end of the behavioral trials of days 6 and 9 of the acclimatization period, subsamples of glass eels (inactive, FW- and SW-seeking individuals) from both the cold and warm acclimatized groups were frozen for subsequent measurements of body length (L, to the nearest mm) and body mass (W, to the nearest 10<sup>2</sup>g), and determination of pigment stages (n total = 150).

The glass eel stage is a late metamorphic stage between the translucent leaf-shaped leptocephalus larva and the fully pigmented elver (Elie, 1979; Tabeta and Mochioka, 2003). Hence, the extent of skin pigmentation is indicative of the progress of the metamorphosis (developmental stage). Pigment stages were determined under a microscope and classified according to the extent of skin pigmentation over the head, tail and body regions, through stages VA, VB, VIA<sub>0</sub>, VIA<sub>1</sub>, VIA<sub>2</sub>, VIA<sub>3</sub> and VIA<sub>4</sub> to VIB following Elie et al. (1982). Stage VA is the earliest pigment stage, and stage VIB is the latest development pigment stage. A brief description of these pigment stages is given in the Table I.

### 2.4. Data analysis

Statistics were conducted with SYSTAT 10 (SPSS Inc.). Results are given as mean ± SE.

**Behavior.** Individuals that chose either FW or SW were considered as active, i. e. exhibiting an upstream locomotor activity, the others were considered as inactive. The effects of the "Day" and "Temperature" on the upstream locomotor activity and salinity preference of active glass eels were simultaneously tested with a logistic regression (Logit) using the following model:

$$\ln [p/(1-p)] = a + bT + cD$$

where p is either the probability for an individual to be active or the probability for an active individual to choose FW, T is the "Temperature" variable (coded as categorical variable), D is the "Day" variable (coded as a continuous variable) and a, b and c are coefficients.

Morphology. For analysis of glass eel developmental stages variations with a Pearson's  $\chi^2$  test, we pooled stages VB and VIA<sub>0</sub> (low pigmented individuals), on the one hand, and stages VIA<sub>1</sub> to VIA<sub>3</sub> (highly pigmented individuals) on the other hand, to avoid pools of less than 5 individuals.

We calculated the body condition of glass eels as the relative mass  $W_r = 100 W / W_{std}$ , where  $W$  is the mass of an individual fish and  $W_{std}$  the predicted mass of a fish of the same  $L$ , as calculated according the  $\log_{10}W$ - $\log_{10}L$  regression equation (least squares means fit) for the whole sample ( $n = 180$ ) (Le Cren, 1951). Then, after checking for equality of variances (Levene's and Bartlett's tests), we analysed  $W_r$  differences among the variables "Day", "Temperature", "Activity" and "Salinity preference" using an ANOVA. Pairwise comparisons were made with Bonferroni post hoc test.

### 3. Results

#### 3.1. Temperature effect on locomotor activity

Decreasing acclimatization temperature decreased significantly glass eel locomotor activity, which was on average 65.9 % in warm acclimatized glass eels and 29.6 % in cold acclimatized glass eels (Logit, d.f. = 2,  $p < 0.0005$ ) (Fig. 3). Locomotor activity was not significantly influenced by the "Day" variable, indicating that glass eel locomotor activity remained constant during the testing period (Logit, d.f. = 2,  $p = 0.434$ ).

#### 3.2. Temperature effect on salinity preference

Decreasing acclimatization temperature decreased significantly the preference for FW in active glass eels (Logit, d.f. = 2,  $p = 0.002$ ) (Fig. 4). Moreover, preference for FW decreased over the testing period (Fig. 4). Salinity preference of active glass eels was significantly affected by the "Day" variable, indicating that this decreased FW-preference was significant (Logit, df = 2,  $p < 0.0005$ ).

#### 3.3. Links between behavior, body condition and developmental stage

The glass eels pigment stages significantly increased over the testing period (Pearson's  $\chi^2$ ,  $df = 2$ ,  $p < 0.0005$ ), indicating that the developmental processes linked to the end of metamorphosis occurred normally. In addition, the acclimatization temperature affected significantly neither  $W_r$  (ANOVA,  $df = 1$ ,  $p = 0.293$ ) nor pigment stages of glass eels (Pearson's  $\chi^2$ ,  $df = 1$ ,  $p = 0.893$ ). This result suggests that the testing period was short enough to avoid body condition and developmental alterations due to temperature variations.

Pigment stages were not significantly different between activities (Pearson's  $\chi^2$ ,  $df = 1$ ,  $p = 0.614$ ) and between salinity preferences (Pearson's  $\chi^2$ ,  $df = 1$ ,  $p = 0.110$ ), suggesting no link between the developmental stage and glass eels behavior within the limits of the observed stages (from VB to VIA3).

The  $W_r$  was not significantly different between active and inactive fishes (ANOVA,  $df = 1$ ,  $p = 0.054$ ). Rather,  $W_r$  was significantly higher in FW-seeking than in SW-seeking glass eels (GLM,  $df = 1$ ,  $p < 0.0005$ ) and inactive glass eels (ANOVA,  $df = 1$ ,  $p = 0.001$ ) (Fig. 5), suggesting that high body condition promoted preference for

FW. In contrast,  $W_r$  was not significantly different between SW-seeking and inactive glass eels (ANOVA,  $df = 1$ ,  $p = 0.691$ ), suggesting that these two behaviors were related to the same energetic status (Fig. 5). In addition, because glass eels were kept unfed, body condition significantly decreased over the experiment (ANOVA,  $df = 2$ ,  $p = 0.010$ ) (Fig. 6).

#### 4. Discussion

In this study, we tested whether and how body condition and acclimatization temperature may affect glass eels migration through locomotor activity and salinity preference.

##### 4.1. Temperature effects on behavior

During the present experiment, decreased acclimatization water temperature significantly decreased both locomotor activity and preference for FW in glass eels. Field studies have shown that the glass eel migration and river recruitments are strongly dependent on water temperatures (Elie, 1979; Cantrelle, 1981; Gascuel, 1986; Vøllestad and Jonsson, 1988; McGovern and McCarthy, 1992; Elie and Rochard, 1994; Martin, 1995; Jessop, 2003). Our results suggest that this temperature control of the migration may act through a regulation of both locomotor activity and FW-preference.

There are several physiological mechanisms by which temperature may affect both locomotor activity and salinity preference. In ectotherms, reduced temperature primarily acts in decreasing enzymes activity and the fluidity of membranes and internal liquids (Johnston and Dunn, 1987). These immediate effects entrain a fall in the power output available from the muscle, affecting swimming performance and locomotor activity (Johnston and Temple, 2002). Decreased locomotor activity and swimming performance at reduced temperature have been previously reported for cod *Gadus morhua* (Castonguay and Cyr, 1998), chub mackerel *Scomber japonicus* (Dickson et al., 2002) and sea bass juveniles *Dicentrarchus labrax* (Koumoundouros et al., 2002). In addition, in Teleost fish, temperature may regulate the endocrine secretions (Grau, 1988). In glass eels, the endocrine control of locomotor activity involves thyroid hormones (TH) (Edeline et al., 2004; Edeline et al., 2005). Yet, cold water temperature decreases the thyroid gland activity in the eel (Leloup, 1958; Leloup and De Luze, 1985) and, *in vitro*, reduces the binding affinity of TH to their receptors in both trout *Salmo trutta* and eel *Anguilla anguilla* (Lebel and Leloup, 1989). These results suggest that, during the present experiment, cold acclimatized glass eels could had reduced TH secretion and biological activity (through binding on receptors), inducing a reduced locomotor activity.

A decreased thyroid status in cold acclimatized glass eels could also explain their reduced preference for FW. Indeed, TH have been shown to influence salinity preference in the stickleback *Gasterosteus aculeatus* (Baggerman, 1962) and in juvenile Pacific salmons (Baggerman, 1960; Iwata, 1995). In glass eels, a thyroid surge triggers the migration from the estuary to an eel ladder supplied by freshwater (Edeline et al., 2004). These data suggest that, in glass eels, TH could also be involved in the control of the preference for FW. Accordingly, in glass eels, TH secretion increases significantly 10 days after transfer from SW to FW (Monaco et al., 1981).

Numerous authors report that, in estuary, glass eels show a sharp reduction in the upstream migratory propensity below a threshold temperature of about 11-12°C (Gascuel, 1986; McGovern and McCarthy, 1992; Jessop, 2003). This threshold temperature could correspond to a limit for activation of important physiological processes such as TH secretion.

#### 4.2. Link between body condition and behavior

During the present experiment, body condition was significantly linked to salinity preference, and the decrease in body condition monitored during the testing period probably explains the shift of glass eels from FW- towards SW-preference. This result suggests that, in the wild, low condition glass eels may preferentially colonize SW habitats, whereas high condition glass eels may rather orientate towards FW habitats. Accordingly, we have previously showed that, compared to estuarine migrants, early settling glass eels undergo a physiological stress and have a decreased body weight, suggesting a low body condition (Edeline et al., 2004).

*Anguilla* spp. glass eels are recruited into estuaries during migration peaks, lasting for several months (Tesch, 2003). Yet, the body condition of estuarine recruits decreases over this migration period in *A. anguilla* (Elie, 1979; Charlon and Blanc, 1982), *A. rostrata* (Jessop, 1998), *A. japonica* (Kawakami et al., 1999), *A. reinhardtii* and *A. australis* (Sloane, 1984) and *A. dieffenbachii* (Jellyman and Lambert, 2003). This may be due to seasonal variations in oceanic ecosystems productivity, affecting growth of leptocephalus larvae (Désaunay, 1997). Our results, showing increased preference for SW as body condition decreases, suggest that the tendency to colonize marine and estuarine rather than river habitats may be higher in late than in early recruits.

Such a condition-mediated early settlement in SW habitats could be of adaptive significance. Indeed, low condition individuals (showing a physiological stress) probably do not have the capacity to migrate far from the sea. Hence, in low body condition glass eels, an early settlement in marine or estuarine habitats likely increases survival (i. e. fitness) by stopping the energy expenditure related to the migration. Accordingly, minimizing the probability of death due to exhaustion may be the major factor constraining the migratory behavior in anadromous fishes (Bernatchez and Dodson, 1987).

Our results suggest that the alternative migratory tactics in glass eels may be controlled by the energetic status. Similarly, migratory behavior depends on body condition parameters in juvenile salmonids (McCormick et al., 1998; Metcalfe, 1998; Thorpe et al., 1998; Forseth et al., 1999; Thériault and Dodson, 2003) and the ayu *Plecoglossus altivelis* (Tsukamoto et al., 1987). Hence, we propose that the energetic status may be a key physiological factor for the control of the alternative migratory tactics in fish populations.

We propose that the endocrine control of the adaptive condition-dependent migratory behavior in glass eels may involve both TH and growth hormone (GH). Indeed, a depressed caloric state induces a decreased thyroid status in Teleost fishes (Eales, 1988). Accordingly, we have previously shown that a decreased body mass may be correlated to a depressed thyroid status in glass eels (Edeline et al., 2004). In addition, a depressed caloric state also induces increased GH and cortisol secretions in the eel (Marchelidon et al., 1996; Rousseau et al., 1999; Dufour et al., 2001; Lambert et al., 2003). Yet, in Teleosts, cortisol and GH have synergistic actions for SW adaptation (McCormick, 2001) and therefore probably promote preference for

SW. In Teleosts, GH and cortisol also stimulate locomotor activity (Overli et al., 2002; Jönsson et al., 2003; Johansson et al., 2004), suggesting that, in our study, increased GH and cortisol levels could have compensated the effects of decreased TH levels on locomotor activity as observed in low condition glass eels.

## **5. Conclusion**

In conclusion, our results show that, in the wild, low water temperatures may hinder the glass eel migration and river recruitments through decreased locomotor activity and preference for FW. We suggest that this temperature effect on locomotor activity and salinity preference could be mediated by TH. Moreover, a lowered body condition decreased preference for FW and promoted preference for SW. We suggest that this condition-dependent salinity preference is of adaptive significance and may be mediated by both TH and GH. We further propose that, as observed for salmonid smoltification, the energetic status is a key physiological factor for the control of the facultative diadromy in the eel. Therefore, our results show that both environmental (external) and physiological (internal) factors may act and interact in controlling the glass eel migratory behavior.

## Acknowledgements

We particularly wish to thank Dr. Sylvie Dufour (CNRS, MNHN, Paris) and Dr. Agnès Bardonnet (INRA, St Péé s/Nivelle) for their helpful comments on the manuscript. We also wish to thank Philippe Camoin (Cemagref) for drawing Figs. 1 and 2, and Jean-Louis Keller (Cemagref) for technical assistance. This work was partly supported by a research grant from the GRISAM and Région Aquitaine.

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Table I. Brief summary of the main features of the pigment stage classification used in the present study. For further details, see Elie et al. (1982).

| Pigment stage    | Main features   |
|------------------|---|
| VA               | The overall pigmentation is limited to the end of the caudal fin.   |
| VB               | Pigmentation appears on the head, and caudal pigmentation begins to extend on the back.                                     |
| VIA <sub>0</sub> | Few pigments appear behind the head and dorsal pigmentation extends on the back.  |
| VIA <sub>1</sub> | Head and tail dorsal pigments overlap, and lateral pigmentation begins to extend towards the head from the end of the tail. |
| VIA <sub>2</sub> | Lateral pigmentation do not exceeds the dorsal fin. Moreover, ventral pigmentation develops from the tail towards the head. |
| VIA <sub>3</sub> | Lateral pigmentation exceeds the dorsal fin, but there is no ventral pigmentation between the anus and pectoral fins.       |
| VIA <sub>4</sub> | Ventral pigmentation develops between pectoral fins and the anus.   |
| VIB              | Ventral pigmentation is compact, visibility of internal organs becomes reduced.   |

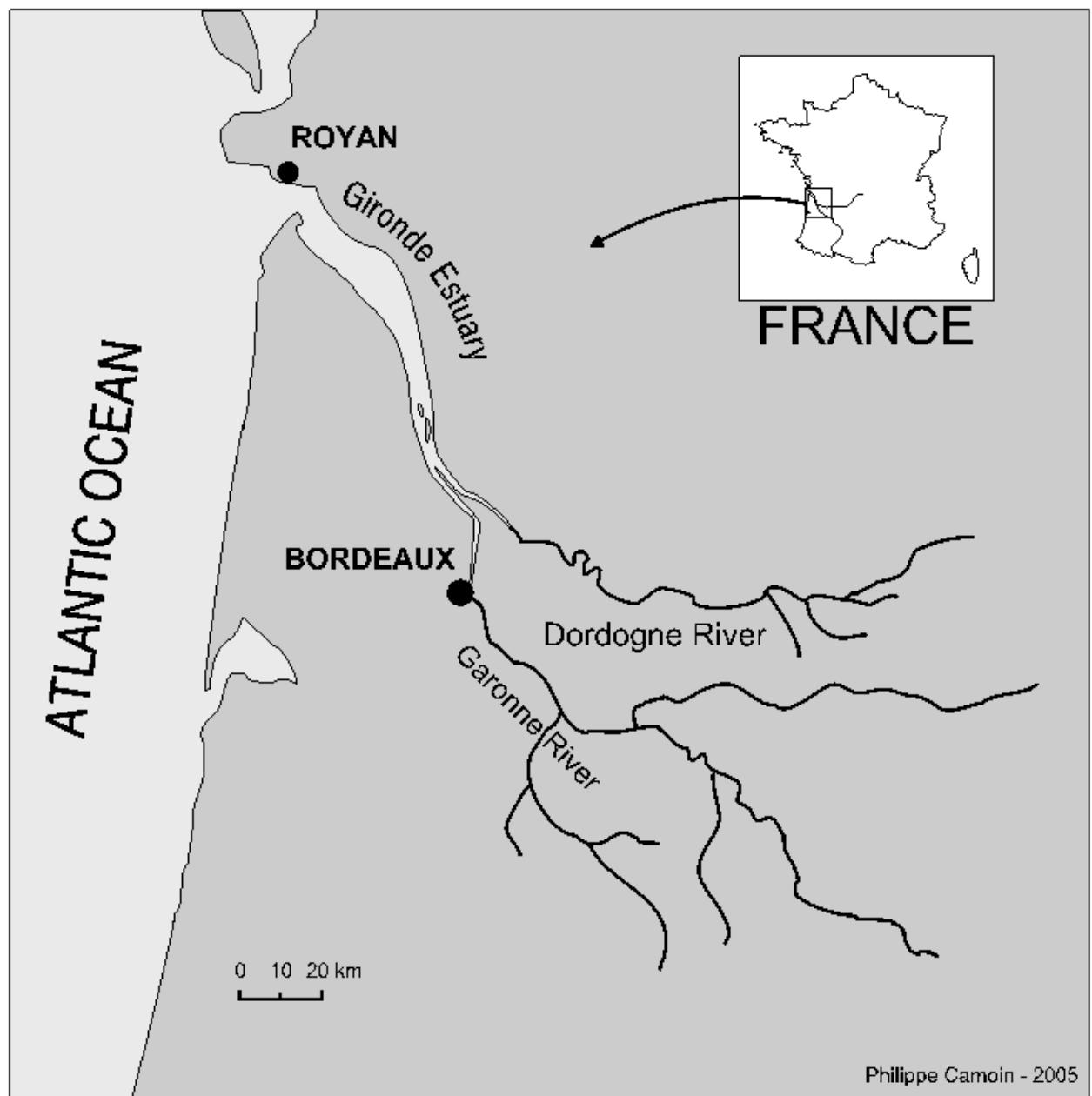


Fig. 1.

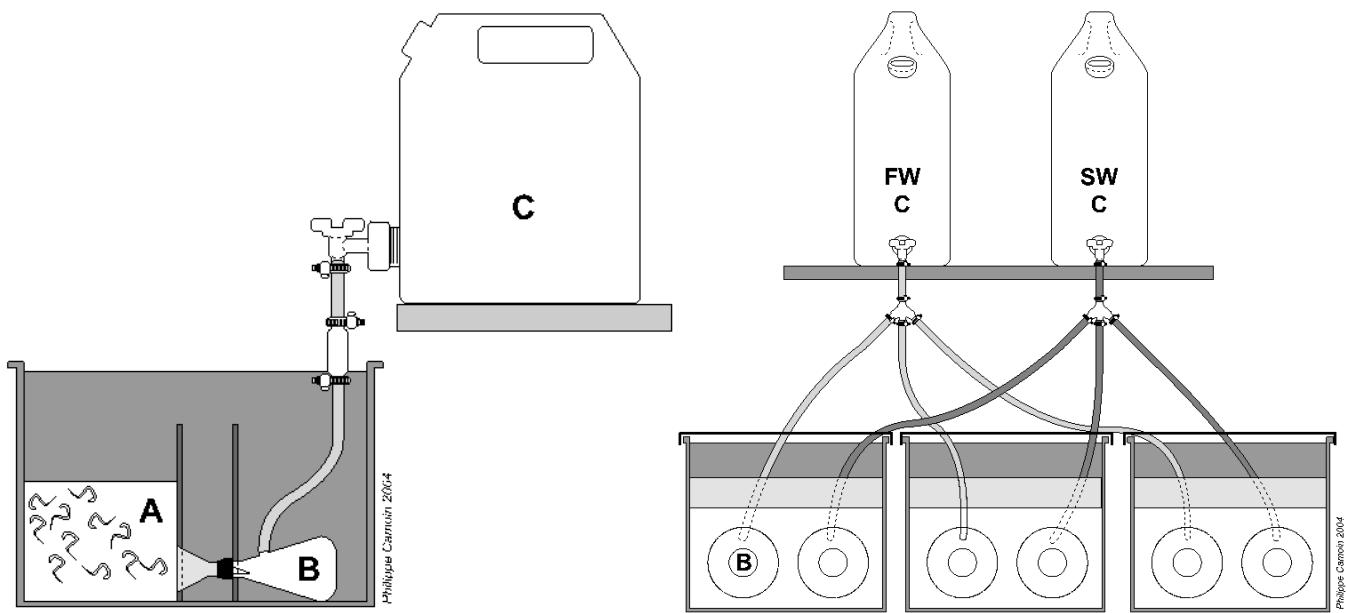


Fig. 2.

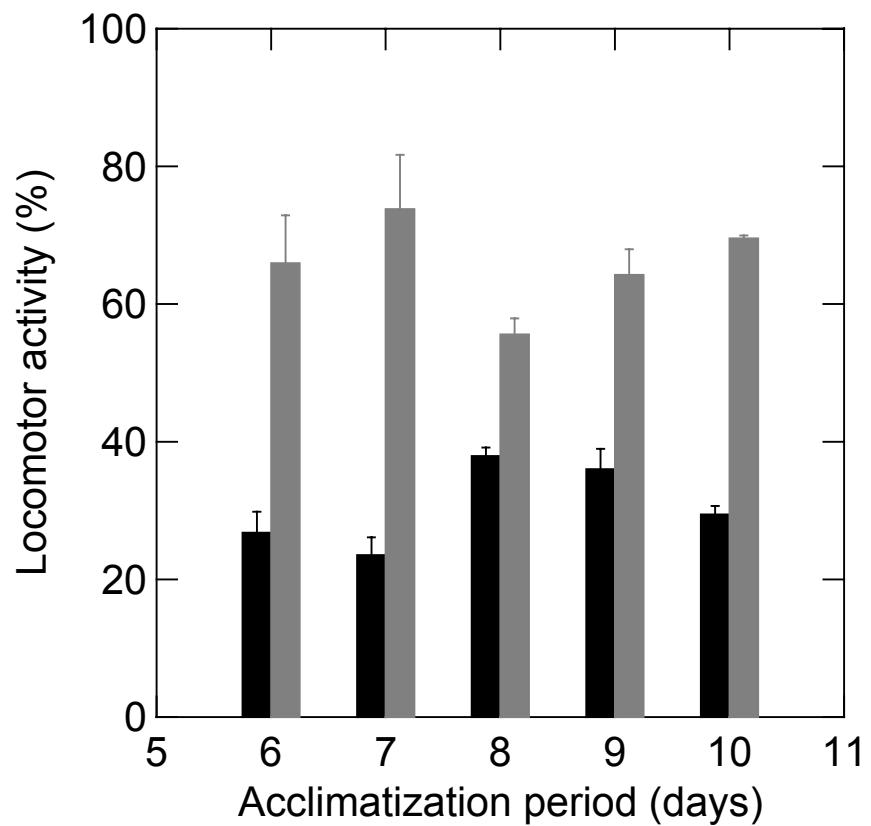


Fig. 3.

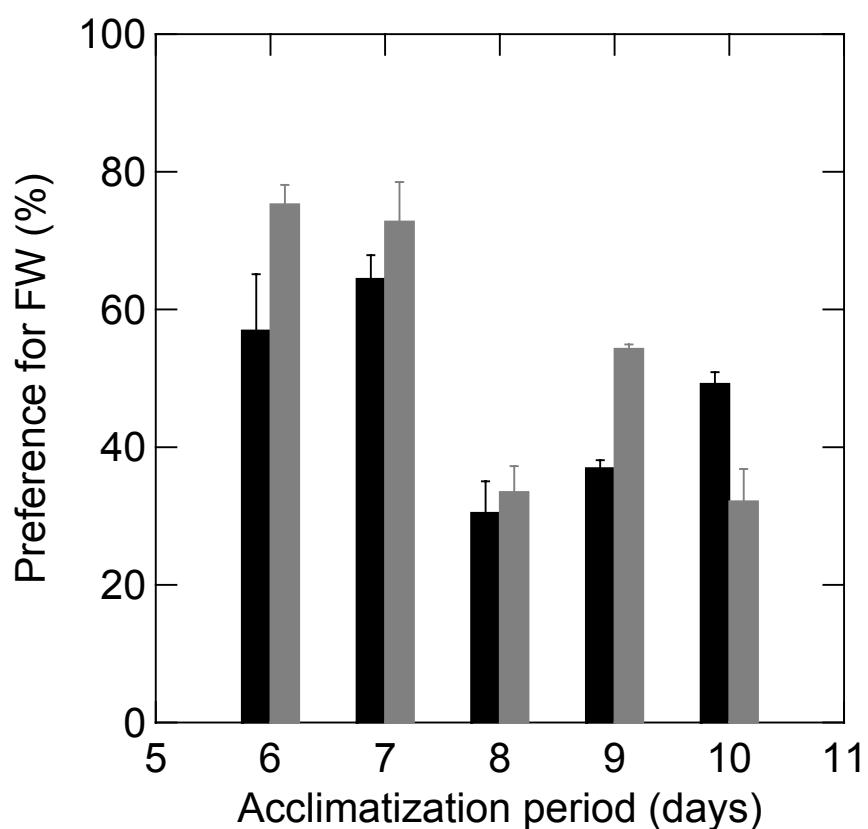


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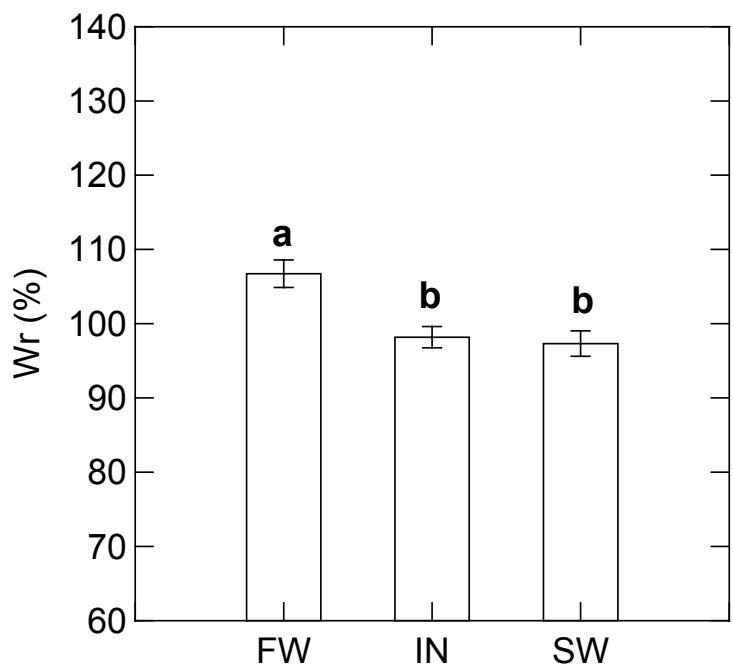


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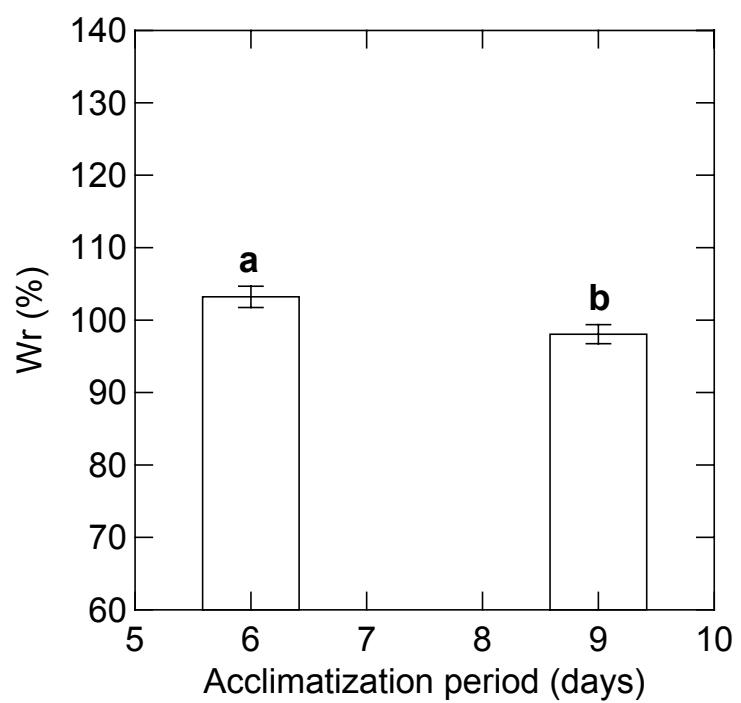


Fig. 6.

## FIGURE LEGENDS

Fig. 1: Map of France showing the location of the sampling site of glass eels (Royan, at the mouth of the Gironde estuary).

Fig. 2: Experimental device used to test locomotor activity and salinity preference of glass eels. A: choice compartment, B: trap compartment, C: water header reservoir, FW: freshwater, SW: saltwater. Glass eels trapped in B were considered as active. Among them, those caught in traps connected with FW or SW were considered to prefer FW or SW, respectively.

Fig. 3: Upstream locomotor activity (mean  $\pm$  SE) of glass eels acclimatized at either 10°C (black columns) or 18°C (grey columns). N = approximately 90 glass eels per tank, 3 tanks per trial. Glass eels were significantly more active at 18°C than at 10°C (Logit, d.f. = 2, p < 0.0005). Locomotor activity did not change significantly over the testing period (Logit, d.f. = 2, p = 0.434).

Fig. 4: Preference for FW (mean  $\pm$  SE) of active glass eels acclimatized at either 10°C (black columns) or 18°C (grey columns). N = approximately 90 glass eels per tank, 3 tanks per trial. Active eels were significantly more FW-seeking at 18°C than at 10°C (Logit, d.f. = 2, p = 0.002). Preference of glass eels for FW decreased significantly during the testing period (Logit, d.f. = 2, p < 0.0005).

Fig. 5. Body condition ( $W_r$ ) of glass eels as a function of their behavior: FW: preference for freshwater, SW: preference for SW, and IN: inactive. Different letters indicate significant differences.

Fig. 6. Body condition ( $W_r$ ) of glass eels at days 6 and 9 of the acclimatization period. Different letters indicate significant differences.

## DISCUSSION

Les parties précédentes de cette thèse ont permis de constater à quel point l'anguille constitue un bon modèle biologique pour l'étude de la dispersion animale. La discussion va nous permettre, dans une première partie, de synthétiser nos découvertes sur certains mécanismes écologiques du contrôle de la dispersion continentale de l'anguille. Ces mécanismes font intervenir des facteurs que nous avons étudié, mais aussi d'autres facteurs, qui sont spéculatifs mais qui, à notre avis, méritent d'être mentionnés.

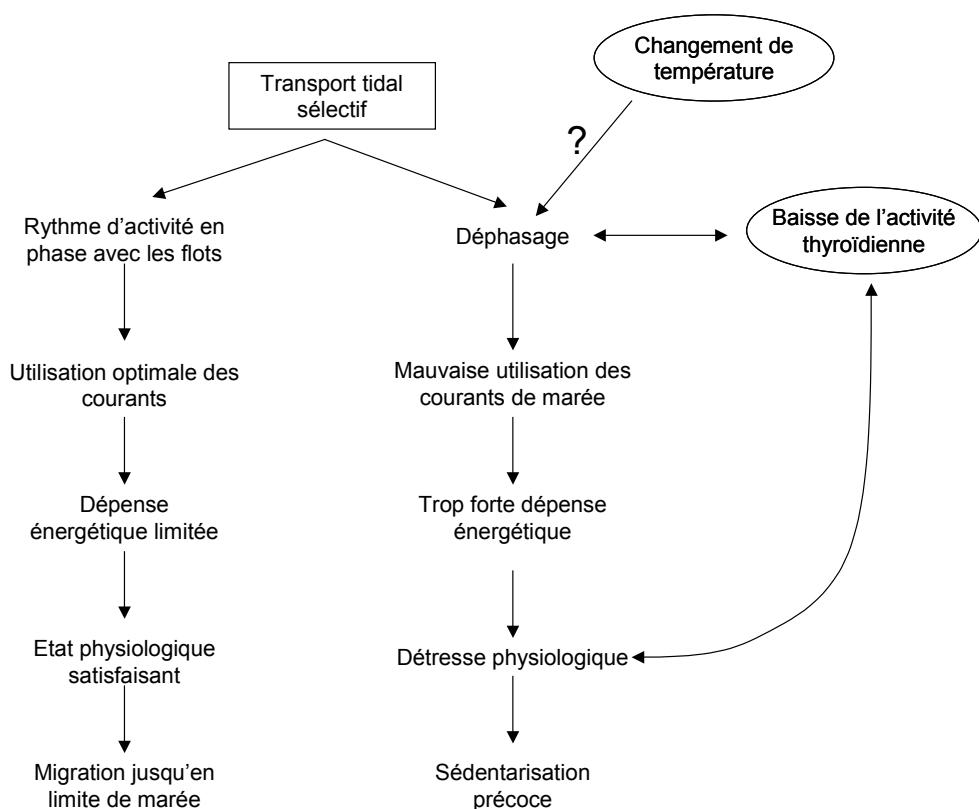


Fig. 8. Proposition de mécanisme écologique pour un contrôle de la dispersion continentale lié à l'utilisation des courants de marée par les civelles (Article 2). Une simple flèche représente un effet, une double flèche un lien.

D'autre part, nos résultats nous ont permis d'échafauder un modèle stratégique (théorique) de dispersion continentale pour l'anguille, inscrit dans le cadre conceptuel de la théorie des jeux. Ce modèle est présenté dans la deuxième partie de la discussion.

## 1. Mécanismes du contrôle de la dispersion continentale

### Courants de marée

Les civelles en migration tirent avantage des courants de marée en utilisant le TTS (Transport Tidal Sélectif, voir Introduction). Les courants de marée sont donc des vecteurs de transport pour les individus en migration.

Nos résultats suggèrent que les courants de marées sont d'une importance capitale pour la dispersion continentale. En effet, nous avons montré qu'un ralentissement de l'advection tidale induit une accumulation des civelles en aval de la limite de renverse des courants et que, en amont de ce point, la vitesse de migration chute (Article 1). Ce résultat suggère que les civelles sont mal adaptées à la nage constante à contre courant et dépendent fortement des courants de marée pour coloniser les eaux continentales.

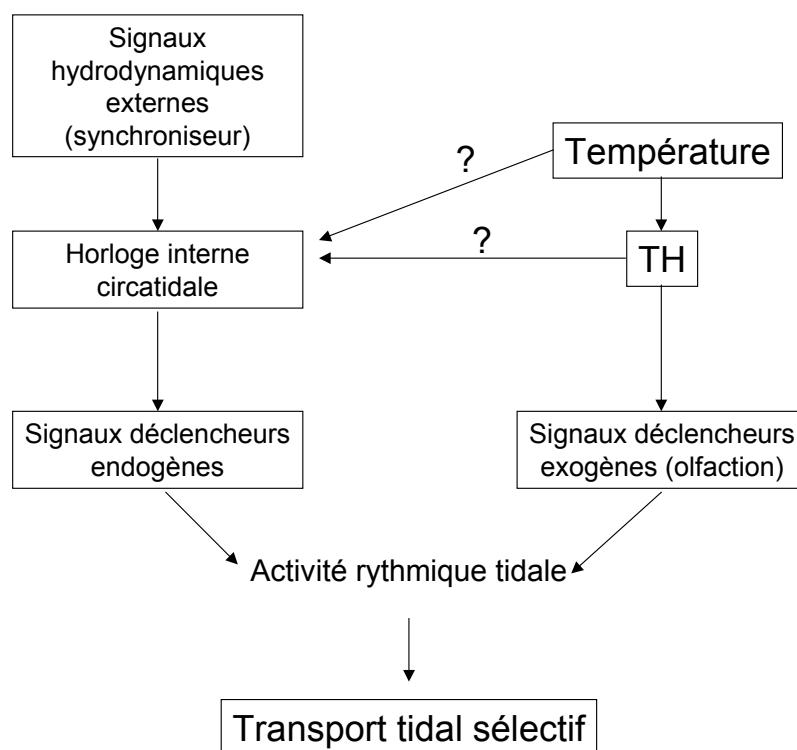


Fig. 9. Régulation du transport tidal sélectif (TTS) chez la civelle. L'activité rythmique tidale est contrôlée par des signaux déclencheurs endogènes (produits par une horloge interne circatidale) et par des signaux déclencheurs exogènes de nature olfactive liés aux marées. L'horloge interne circatidale est synchronisée par des signaux hydrodynamiques (renverses de courants). Nous proposons l'hypothèse d'un effet de la température et des hormones thyroïdiennes (TH) sur la perception des signaux olfactifs et le calage de l'horloge interne.

De plus, dans l'article 2, nous avons montré un lien entre une mauvaise utilisation des courants de marée par les civelles (présence dans la colonne d'eau durant le jusant) et une détresse physiologique, entraînant probablement une sédentarisation précoce en estuaire. Ce résultat suggère que des variations interindividuelles d'efficacité dans l'utilisation des courants de marée pourraient induire une distribution différente des jeunes individus dans le bassin versant. A partir de ces données, il nous est possible de proposer un mécanisme écologique liant l'utilisation des courants de marée à la dispersion (Fig. 8).

L'activité rythmique circatidale des civelles durant le TTS est contrôlée par une horloge interne, synchronisée par les renverses de courant (Wippelhauser & McCleave 1988), et par des signaux olfactifs exogènes (Creutzberg 1959, Barbin et al. 1998) (Fig. 9). Un décalage du rythme d'activité chez les civelles pourrait donc être lié à une modification des rythmes endogènes et/ou à la mauvaise perception des signaux olfactifs exogènes.

La perturbation du rythme endogène circatidal pourrait être provoquée par l'intervention de synchroniseurs plus puissants que les renverses de marée. Pour les ectothermes, la température est un puissant synchroniseur (Rensing & Ruoff 2002). En effet, chez des civelles en free-running, des variations de température, même faibles, peuvent entraîner un décalage du rythme de l'horloge circatidale interne (Kim et al. 2002). Hélas, l'expérience n'a pas été conduite sur des civelles toujours soumises à des renverses de courant. L'effet des changements de température sur le rythme circatidal endogène des civelles en milieu naturel est donc toujours inconnu (Fig. 9).

Nos résultats montrent une baisse de l'activité thyroïdienne chez les civelles de jusant (Article 2), suggérant que les TH pourraient être impliquées dans la régulation du rythme circatidal (Fig. 8), peut-être par une action sur la rhéotaxie (Article 3). Chez les poissons, la perception des courants (et donc des renverses liés aux marées) se fait par plusieurs modalités sensorielles, impliquant la ligne latérale, l'oreille interne, la vision et le toucher (Montgomery et al. 2000, Montgomery et al. 2003). Des études complémentaires seraient nécessaires afin d'explorer l'action des TH sur ces modalités sensorielles. D'autre part, les TH semblent être impliquées dans la sensibilité aux signaux olfactifs (Plate et al. 2002, Lema & Nevitt 2004). Il est donc possible que les TH affectent la sensibilité des civelles vis à vis des signaux olfactifs exogènes.

## Hormones thyroïdiennes

Nous avons montré que les TH jouent un rôle très important dans le contrôle de la dispersion continentale (Articles 2 et 3). En effet, à travers une régulation de l'activité locomotrice et de la rhéotaxie négative lors du TTS, les TH jouent un rôle central dans l'expression du comportement migrateur (Articles 2 et 3). De plus, nos travaux suggèrent que les TH sont également impliquées dans l'orientation de la migration et la sélection de l'habitat. En effet, les TH pourraient contrôler la préférence pour l'eau douce (Articles 2, 4 et 5). De plus, comme discuté plus haut, il

semble que les TH soient impliquées dans la sensibilité olfactive durant les migrations diadromes (Plate et al. 2002, Lema & Nevitt 2004). Ces données suggèrent que les TH jouent un rôle central dans la régulation de la colonisation des habitats de rivière, et qu'une baisse du statut thyroïdien peut induire une sédentarisation précoce en mer ou en estuaire (Fig. 10).

L'activité thyroïdienne peut être influencée par le statut énergétique (Eales 1988) et des facteurs environnementaux divers (Grau 1988), incluant la température (Leloup 1958, Leloup & De Luze 1985). En effet, la température, agissant sur l'activité locomotrice et la préférence pour l'eau douce, semble pouvoir moduler l'activité thyroïdienne des civelles (Article 5) (Fig. 10).

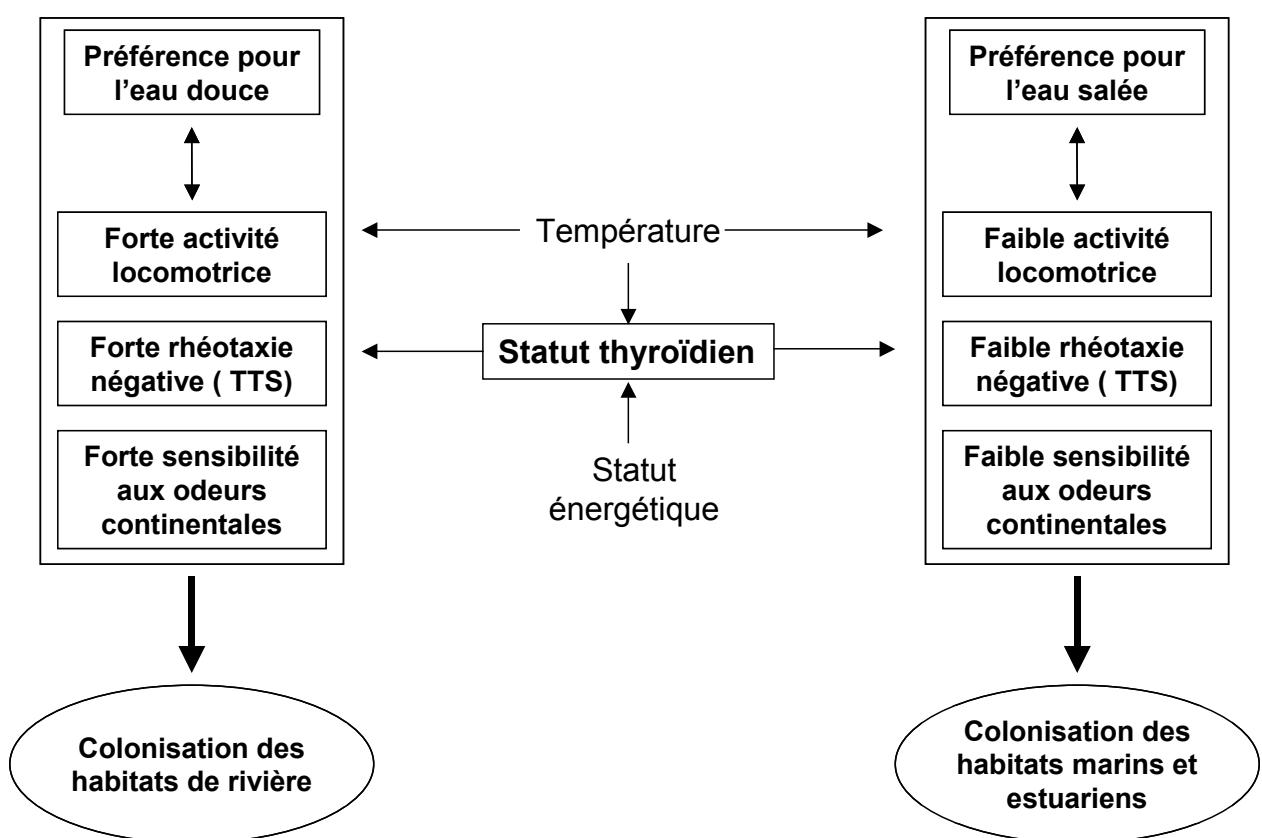


Fig. 10. Proposition de mécanisme écologique pour le rôle du statut thyroïdien dans le contrôle de la dispersion continentale de l'anguille (Articles 2, 3, 4 et 5). Le statut thyroïdien régule l'activité locomotrice et semble jouer un rôle dans le contrôle de la rhéotaxie (Article 3). De plus, les hormones thyroïdiennes pourraient réguler la préférence de salinité et la sensibilité olfactive. La température influence l'activité locomotrice et la préférence pour l'eau douce, probablement à travers une modulation de l'activité thyroïdienne (Article 5). D'autre part, il semble que le statut énergétique puisse également influencer l'activité thyroïdienne (Articles 2 et 5).

La métamorphose de la larve leptocéphale en civelle est marquée par un pic de production de TH (Callamand & Fontaine 1942, Yamano et al. 1991, Ozaki et al. 2000). Ensuite, l'activité de la thyroïde est forte au stade civelle, puis diminue au stade jaune pour ensuite remonter lors de l'argenture (Callamand & Fontaine 1942). Les TH interviennent donc principalement au cours des métamorphoses et des changements de milieu. En effet, Specker (1988) considère que les TH ont un rôle de préadaptation des poissons à l'exploitation de nouveaux milieux, notamment à travers des changements de la physiologie de l'intestin. Chez la civelle, les TH

induisent la croissance de l'intestin (Vilter 1946), et régulent probablement son adaptation pour l'osmorégulation en eau douce (Ciccoti et al. 1993).

En conséquence, les TH, régulant les changements morphologiques, physiologiques et comportementaux nécessaires à la colonisation des habitats de rivière, peuvent être considérées comme des médiateurs fondamentaux de la dispersion continentale chez l'anguille (Article 3).

### **Condition corporelle, TH, GH et cortisol**

La condition corporelle est un facteur primordial dans le contrôle de la dispersion chez les mammifères, les oiseaux et les Salmonidés (voir Introduction). Chez la civelle, nos résultats suggèrent qu'une sédentarisation précoce en estuaire peut être induite par une faible condition corporelle, liée à une baisse de l'activité thyroïdienne et une détresse physiologique (Article 2). En effet, une diminution de la condition corporelle induit une baisse de la préférence pour l'eau douce et une hausse de la préférence pour l'eau salée (Article 5). Il est donc probable que la condition corporelle joue un rôle central dans le contrôle de la dispersion continentale de l'anguille.

En effet, une baisse du statut calorique induit une baisse de l'activité thyroïdienne chez les Téléostéens (Eales 1988) et une hausse de la sécrétion de GH chez la civelle (Lambert et al. 2003) et l'anguille jaune (Marchelidon et al. 1996, Rousseau et al. 1999, Dufour et al. 2001). Or, chez les salmonidés, la GH, en synergie avec le cortisol (une autre hormone de stress), régule l'acclimatation à l'eau salée (McCormick 2001). Il est donc probable que, chez les civelles en mauvaise condition, la hausse des taux de GH soit responsable d'une préférence accrue pour l'eau salée. De plus, chez l'anguille, les TH exercent un rétrocontrôle négatif sur la production de GH par l'hypophyse (Rousseau et al. 2002). Il est donc probable que la baisse des taux de TH, initiée par un statut énergétique déficient (faible condition corporelle), favorise une hausse des taux de GH (Fig. 11).

La baisse du statut thyroïdien, corrélée à une hausse des taux de GH (stress physiologique), est donc probablement le mécanisme physiologique contrôlant le changement comportemental d'une migration orientée vers la colonisation des milieux de rivière à une sédentarisation précoce en mer ou en estuaire.

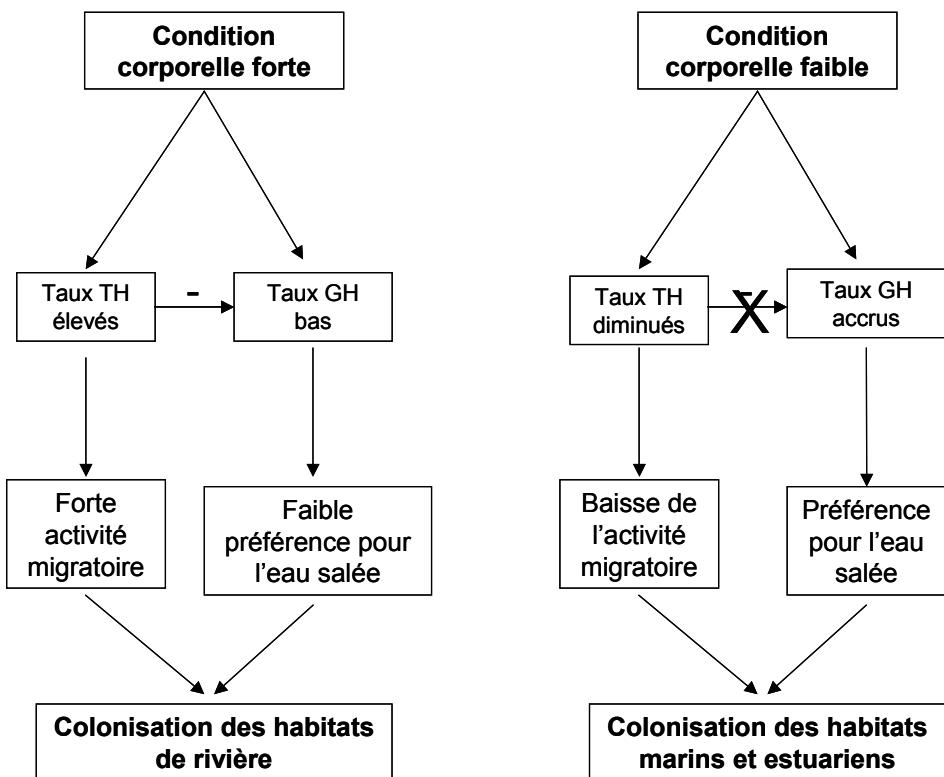


Fig. 11. Proposition de mécanisme physiologique pour le contrôle de la dispersion continentale de l'anguille au stade civelle. Un statut énergétique satisfaisant (condition corporelle forte) permet l'expression d'une forte activité thyroïdienne, qui limite la production d'hormone de croissance (GH) (rétrocontrôle négatif) et favorise un comportement de colonisation des habitats de rivière. En revanche, une faible condition corporelle induit des taux d'hormones thyroïdiennes (TH) bas, permettant la hausse des taux de GH. Les taux de TH bas réduisent la propension à coloniser les rivières, et la hausse de GH augmente la préférence pour l'eau salée, favorisant la sédentarisation précoce en mer ou en estuaire.

## Dispersion et croissance

A l'échelle des bassins versants, les sous populations d'anguilles présentent des variations de leurs taux de croissance. Les anguilles estuariennes ont tendance à grandir plus vite que les anguilles de rivière et à s'argenter à des tailles inférieures (Fernandez-Delgado et al. 1989, Mounaix & Fontenelle 1994, Tzeng et al. 2003, Arai et al. 2004, Jessop et al. 2004). En effet, chez l'anguille, le taux de croissance est lié à la différenciation sexuelle, à l'âge et à la taille à l'argenture (Vøllestad 1992, Holmgren & Mosegaard 1996, Holmgren et al. 1997). Jusqu'à présent, les raisons des différences de croissance entre populations de rivière et d'eau salée étaient inconnues.

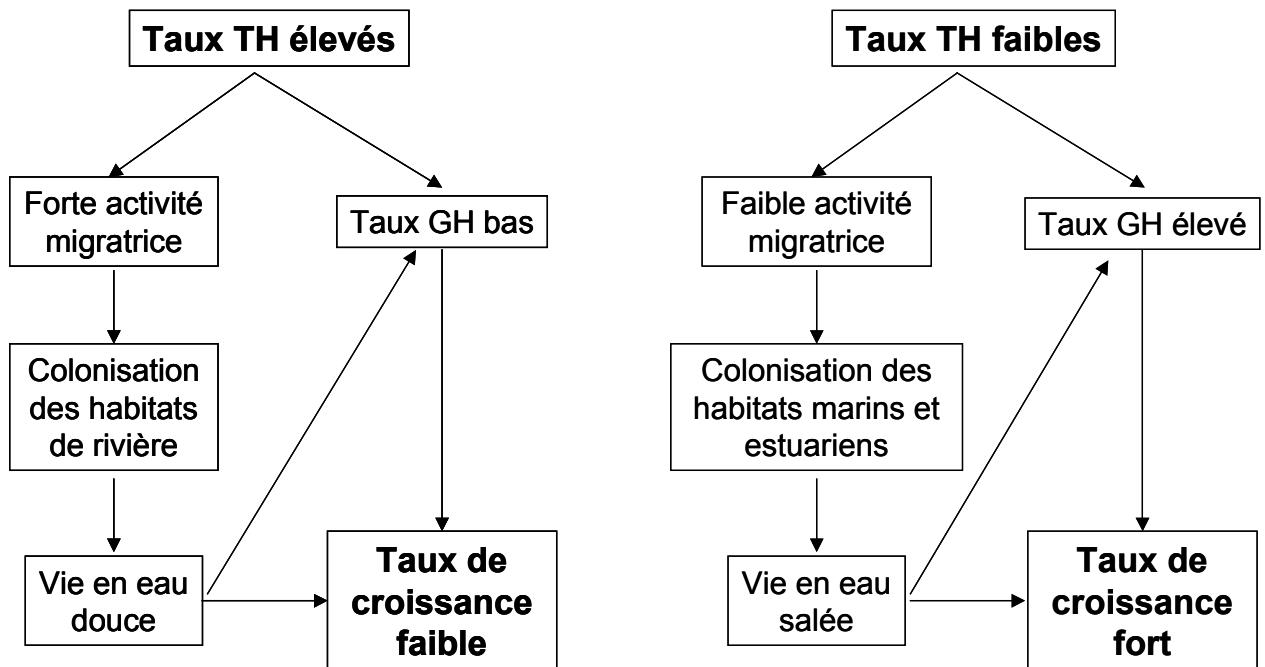


Fig. 12. Proposition de mécanisme physiologique liant la dispersion à la croissance chez l'anguille. Des taux d'hormones thyroïdiennes (TH) élevés induisent une forte activité migratrice et la colonisation des habitats de rivière, tout en limitant la sécrétion d'hormone de croissance (GH). D'autre part, l'osmorégulation en eau douce ne nécessite pas des taux élevés de GH. Par contre, une activité thyroïdienne faible favorise la sédentarisation en eau salée et l'expression de forts taux de GH. L'osmorégulation en eau salée nécessite une sécrétion forte de GH.

Nous avons montré que, chez les civelles, des comportements liés à la dispersion (activité locomotrice et préférence de salinité) sont corrélés à la croissance (Article 4). En effet, les civelles présentant le schéma activité locomotrice forte / préférence pour l'eau douce (type colonisateur des rivières) montrent une croissance inférieure à celle des civelles présentant le schéma activité locomotrice faible / préférence pour l'eau salée (type colonisateur des côtes et estuaires).

Ces schémas de comportement et de croissance sont en accord avec les données de terrain, montrant que les anguilles marines et d'estuaire grandissent plus vite que les anguilles de rivière. Ces résultats suggèrent que les contrôles physiologiques de la dispersion et de la croissance ont des points communs, et impliqueraient les TH et la GH.

En effet, la GH, en plus de ses effets anaboliques directs, accroît l'appétit (Le Bail & Boeuf 1997) et le comportement agressif chez les Téléostéens (Jönsson et al. 1998). Il est donc probable que de forts taux de GH favorisent une croissance rapide par une prise accrue de nourriture et une dominance sociale, comme observé dans l'Article 4. Au contraire, de forts taux de TH, tout en favorisant la colonisation des habitats de rivière, inhibent la synthèse de GH et réduisent donc la croissance (Fig. 12).

Nous avons également montré que la salinité d'acclimatation joue un rôle très important dans le contrôle de la croissance (Article 4). Le comportement dispersif est donc également lié à la croissance par son contrôle sur les facteurs environnementaux que subira l'animal dans son habitat de croissance (Fig. 12).

Nos données de croissance ne concernent que les premiers mois suivant la reprise d'alimentation des civelles (Article 4). Il n'est donc pas possible de savoir si le lien entre comportement et croissance se poursuit tout au long de la vie de l'animal, i.e. si ce lien a une base génétique. Des données récentes montrent que des facteurs génétiques sont impliqués dans le déterminisme des différences interindividuelles de croissance chez l'anguille (Pujolar et al. 2005). D'autre part, des études menées chez les insectes (Roff & Fairbairn 2001) et chez les oiseaux (Berthold 1988) montrent un fort déterminisme génétique du comportement migrateur. Il est donc probable que des facteurs génétiques soient impliqués dans le lien entre dispersion et croissance chez l'anguille.

## 2. Stratégie de la dispersion continentale de l'anguille

### Tactiques dispersives divergentes

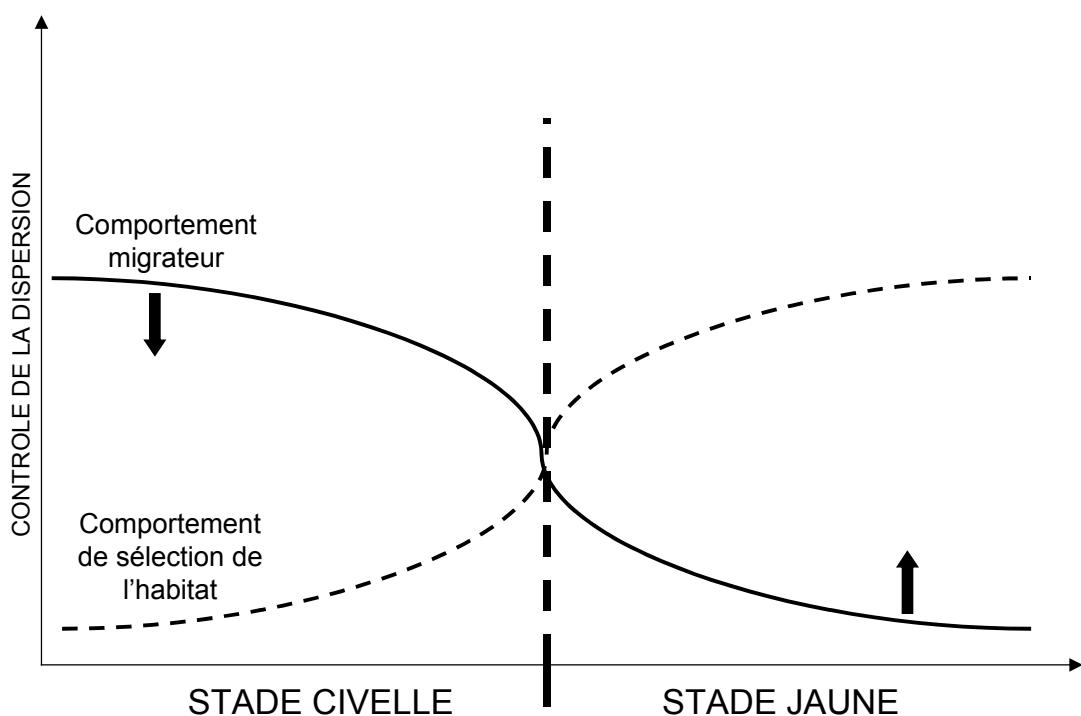


Fig. 13. Stratégie de la dispersion continentale de l'anguille. La dispersion résulte de l'action de deux traits comportementaux: le comportement migrateur (densité-indépendant) et le comportement de sélection de l'habitat (densité-dépendant). Au stade civelle, le comportement migrateur domine, alors qu'au stade anguille jaune, le comportement de sélection de l'habitat est prépondérant. Le changement de dominance est lié à la fin de la métamorphose et à l'acquisition d'une activité alimentaire intense. Ce phénomène a lieu en limite de marée dynamique pour beaucoup d'individus, mais peut avoir lieu en estuaire, voire en mer. La grande flexibilité du comportement migrateur (représenté ici par les flèches) permet l'émergence de tactiques alternatives (ou divergentes) adaptées au statut de l'individus et la colonisation d'une grande variété d'habitats différents.

Une stratégie est définie comme un programme génétique (règle de décision) induisant l'allocation d'énergie parmi différents phénotypes alternatifs (tactiques) (Gross 1996). Une tactique est donc un phénotype (physiologie, morphologie, comportement...) résultant d'une stratégie. A partir de notre travail et de données de la littérature, nous allons proposer un modèle pour la stratégie de la dispersion continentale de l'anguille.

Lors de leur migration, les civelles sont clairement orientées vers la colonisation des habitats d'eau douce. En effet, au cours du développement, l'intestin se spécialise pour osmoréguler en eau douce, quelle que soit la salinité d'acclimatation (Ciccoti et al. 1993). Ce résultat montre que l'anguille est ontogénétiquement programmée pour effectuer sa phase de croissance continentale en eau douce.

En effet, à leur arrivée de l'océan, la majorité des civelles actives recherche l'eau douce (Articles 4 et 5). De plus, le TTS, ainsi que l'attraction vers l'odeur des eaux de rivière, induisent l'orientation vers l'amont de l'estuaire. Ainsi, nos résultats montrent qu'en raison du ralentissement de l'advection tidale, les civelles en migration ont tendance à s'accumuler à l'aval immédiat de la limite de renverse des courants (Article 1). Au stade civelle, la tactique de dispersion typique est donc un comportement migrateur, orienté vers les habitats d'eau douce, utilisant le TTS jusqu'en limite de renverse des courants de marée.

En amont de ce point, en raison de la perte d'advection tidale, la vitesse de migration chute et les civelles se transforment en anguillettes (fin de la métamorphose). La tactique dispersive semble alors passer d'un comportement migrateur (densité-indépendant) à un comportement de sélection de l'habitat (densité-dépendant) en raison de l'acquisition d'une activité alimentaire intense (Article 1). Cette séquence comportementale peut être considérée comme la tactique de base de la dispersion continentale de l'anguille (Fig. 13).

Cependant, à partir de cette tactique de base, une modification du compromis entre comportements de migration et de sélection de l'habitat peut se produire (répondant à un impératif de maximisation de la fitness), grâce à la flexibilité du comportement migrateur et à la grande plasticité phénotypique de l'anguille (Fig. 13). Par exemple, certaines civelles perdent le comportement migrateur et se sédentarisent en mer, alors que certaines anguilles jaunes continuent à migrer à plusieurs centaines de kilomètres de la mer.

Cette flexibilité du comportement induit une grande variété de tactiques alternatives et peut être considérée comme adaptative (maximisant la fitness).

En revanche, le comportement de sélection de l'habitat, qui repose sur l'acquisition du comportement alimentaire, est probablement beaucoup moins flexible que le comportement migrateur. En effet, la phase continentale de l'anguille est une phase de croissance, au cours de laquelle l'individu doit acquérir suffisamment de réserves énergétiques pour la migration de ponte, la maturation des gonades et la reproduction (Van Ginneken & Van Den Thillart 2000, Van Den Thillart et al. 2004 , Van Ginneken et al. 2005). Une forte pression selective agit donc probablement pour

l'expression d'un comportement alimentaire intense, limitant fortement la flexibilité du comportement de sélection de l'habitat.

## Stratégie dispersive conditionnelle

La théorie de la stratégie conditionnelle a été développée par Gross (1996) pour expliquer les variations du comportement reproducteur chez les animaux en termes de coûts et bénéfices de fitness. Cependant, elle peut être appliquée à tous les comportements, y compris le comportement dispersif (migration et sélection de l'habitat).

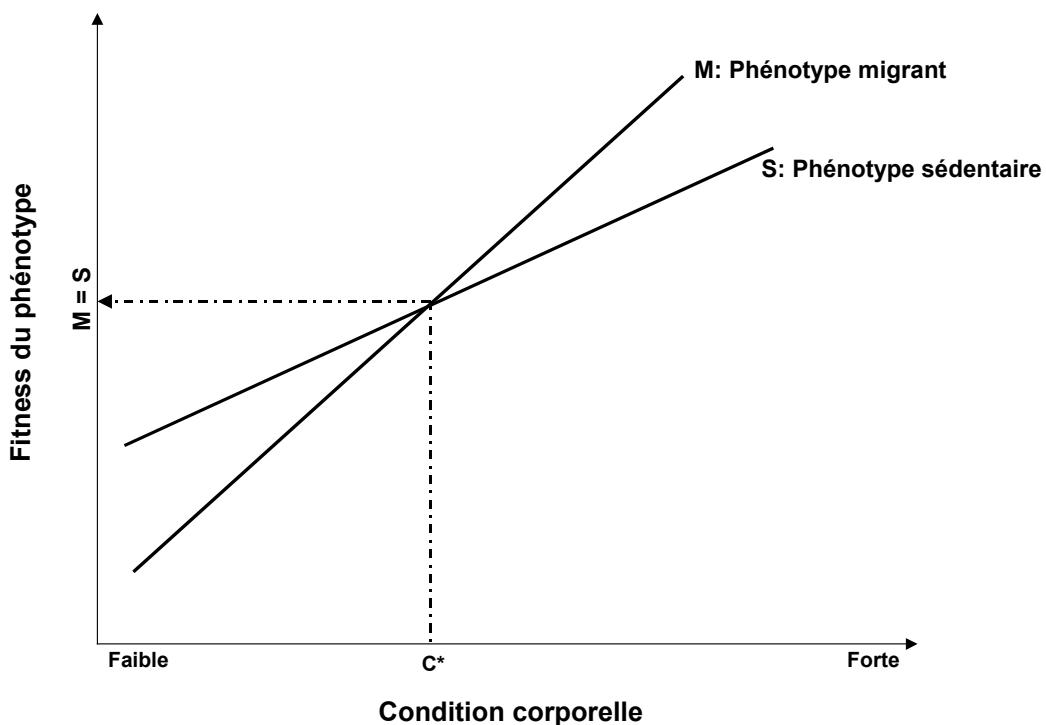


Fig. 14. Modèle de sélection statut-dépendante pour les tactiques alternatives de dispersion chez la civelle. Les phénotypes sédentaire et migrant ont une fitness dépendante de la condition corporelle : leur fitness dépend du statut de l'individu. Il existe une condition corporelle intermédiaire  $c^*$  où les fitness des deux phénotypes sont égales. La tactique choisie en fonction du statut permet donc à l'individu d'augmenter sa fitness. La stratégie (programme génétique) définit la position du point  $c^*$ .

La théorie de la stratégie conditionnelle s'applique lorsque se produit dans la population une sélection statut-dépendante. La sélection statut-dépendante apparaît lorsque les fitness des phénotypes alternatifs (migrant ou sédentaire), dépendent de l'état de l'individu (énergétique, hormonal,...). L'état diffère toujours entre les individus en fonction d'influences environnementales (température, disponibilité en nourriture,...), de la variance génétique (recombinaisons, mutations) et du stade de développement. La stratégie dispersive conditionnelle permet à l'individu d'incorporer l'information concernant sa capacité à acquérir de la fitness par l'expression de phénotypes (comportements dispersifs) alternatifs, et d'exprimer le phénotype qui maximise la fitness.

Ce modèle s'applique particulièrement bien au cas des civelles qui adaptent leur comportement migrateur à leur condition corporelle (Articles 2 et 5) (Fig. 14). En effet, en cas de faible condition corporelle (réserves énergétiques basses), les civelles adoptent un comportement de sédentarisation précoce en eau salée. Ce comportement permet probablement d'accroître la survie en stoppant la dépense énergétique liée à la migration. En effet, chez les poissons migrants, minimiser la probabilité de mourir d'épuisement est probablement le facteur majeur contrôlant le comportement migrateur (Bernatchez & Dodson 1987).

En revanche, les civelles possédant une condition corporelle satisfaisante ont probablement la capacité d'atteindre les habitats de rivière, où les faibles densités améliorent la survie (réduction de la compétition intraspécifique), et où les conditions de croissance permettent de maximiser la taille à maturité. Des civelles en bonne condition ont donc intérêt en terme de fitness à exprimer un fort comportement migrateur.

D'autre part, chez l'anguille, les tactiques dispersives alternatives sont également dépendantes du stade de développement. En effet, la transformation des civelles en anguillettes induit bien une modification du comportement dispersif, qui peut être reliée à un impératif de maximisation de la fitness (voir discussion plus haut).

Le modèle de la stratégie dispersive conditionnelle peut être étendu à toute la phase continentale de l'anguille, et expliquer l'existence des grands types de disperseurs décrits par Feunteun et al. (2003). Les différentes tactiques dispersives observées chez l'anguille en milieu continental sont donc le résultat d'une stratégie conditionnelle, induisant des décisions à certains moments clé de la vie de l'individu, en fonction de son statut (physiologique, social, développement...). Dans ce modèle, les facteurs environnementaux influencent le statut de l'individu. Chez la civelle, les médiateurs proximaux de ces tactiques alternatives sont les TH et, dans une moindre mesure, GH (Fig. 11).

Un des postulats de la théorie de la stratégie conditionnelle est la monomorphie génétique des individus pour la décision, c'est à dire que les tactiques alternatives ne sont pas déterminées par des allèles alternatifs (Gross 1996). Chez la civelle, il est probable que les variations de condition corporelle soient effectivement principalement d'origine environnementale. En effet, la condition corporelle est liée à la productivité des écosystèmes océaniques durant la phase larvaire (Désaunay 1997) et à l'efficacité de l'utilisation des courants de marée (Article 2).

Cependant, on ne peut exclure un effet génétique sur le statut des individus, notamment au niveau de l'hétérozygotie. Certaines données suggèrent même que les effets génétiques sont quasi systématiques en dispersion (Berthold 1988, Roff & Fairbairn 2001). Chez l'anguille, le niveau d'hétérozygotie, influençant la croissance (Pujolar et al. 2005) pourrait avoir également des effets sur la dispersion à travers la sélection de l'habitat (plasticité phénotypique) et le comportement migrateur (variété des allozymes métaboliques par exemple...). Il est donc probable que la théorie de la stratégie conditionnelle soit violée sur ce point. Cependant, comme le reconnaît

Gross (1996), jusqu'à présent aucune étude n'a démontré le jeu complet de postulats de la théorie de la stratégie conditionnelle.

## **Evolution de la stratégie conditionnelle de dispersion continentale chez l'anguille**

Comme discuté plus haut, la stratégie dispersive de l'anguille est principalement orientée vers la colonisation des habitats d'eau douce, tout en maintenant une plasticité phénotypique importante, permettant l'expression d'une grande diversité de tactiques alternatives menant à la colonisation de tous les habitats continentaux. Dans ce chapitre, nous allons tenter de proposer des explications évolutives pour l'apparition de cette stratégie chez un poisson d'origine marine.

L'anguille est un Téléostéen très ancien (Fig. 1), qui a probablement été un des premiers membres de ce taxon à coloniser les habitats d'eau douce. Les habitats de rivière ont donc probablement représenté pour l'anguille des milieux de croissance où la compétition interspécifique était fortement amoindrie. En effet, les interactions sociales induisent une pression évolutive forte, et une réduction de la compétition est un facteur majeur sélectionnant pour la dispersion (Gandon & Michalakis 2001). Dans le cas de l'anguille, il a été proposé que la compétition avec le congre, un autre anguilliforme mais de taille plus importante, a pu jouer un rôle important dans l'acquisition de la diadromie (Moriarty 1987, Tsukamoto & Arai 2001).

D'autre part, la productivité relative des habitats (différences entre mer et rivière) semble également exercer une forte contrainte évolutive pour l'acquisition de la diadromie (Gross et al. 1988). Les anguilles seraient apparues sous des latitudes tropicales (Tsukamoto & Aoyama 1998), où la productivité des habitats marins est plus faible que celle des habitats d'eau douce. Cette différence de productivité aurait également favorisé l'acquisition de la diadromie chez l'anguille (Tsukamoto & Arai 2001).

Cependant, sous les latitudes tempérées, la productivité des habitats marins dépasse celle des habitats d'eau douce, diminuant la fitness associée à la diadromie et favorisant les tactiques de sédentarisation précoce en milieux salés (Tsukamoto & Arai 2001). De plus, nous proposons qu'une stratégie de diadromie conditionnelle (flexibilité du comportement migrateur) apporte un avantage évolutif en permettant (1) d'adapter la dépense énergétique (coût de la dispersion) au statut de l'individu, (2) de s'adapter à des destructions d'habitat catastrophiques.

Un bon exemple de changement de type catastrophique des écosystèmes provient de l'impact récent de l'espèce humaine sur les habitats d'eau douce. En effet, en raison des barrages, de la pollution, de la réduction des habitats et de la pêche, la colonisation des eaux douces devient très difficile, voire impossible. L'existence d'une tactique de colonisation des habitats marins et estuariens a probablement, et pour l'instant, évité l'extinction des anguilles américaine, japonaise et européenne.

Dans les bassins versants fortement anthropisés, l'expression d'un fort comportement migrateur pourrait entraîner une baisse de la fitness. En effet, les individus migrants risquent plus d'être pêchés que les sédentaires et vont s'accumuler au pied des obstacles où l'accroissement de la densité favorise probablement les mortalités. L'anthropisation des bassins versants induit donc probablement un bouleversement des fitness associées aux différentes tactiques dispersives (chute de l'avantage à coloniser les rivières), qui pourrait se traduire par une chute de l'expression d'un comportement migrateur fort chez l'anguille.

Un changement évolutif rapide de la stratégie de dispersion des anguilles américaine, japonaise et européenne est donc à prévoir en réponse à l'anthropisation des milieux d'eau douce, avec une baisse de l'expression du comportement de colonisation des milieux de rivière. En effet, dans de nombreux bassins versants, la proportion d'anguilles estuariennes et marines dépasse à présent largement celle d'anguilles de rivière (Tsukamoto & Arai 2001, Tzeng et al. 2002, Jessop et al. 2004). Cependant, nous ne savons pas si ce phénomène est adaptatif, ou s'il résulte simplement de la plasticité phénotypique de l'anguille (sédentarisation sous contrainte). La survie des anguilles dépendra sans doute de leur adaptabilité (vitesse d'évolution) à ces nouvelles conditions environnementales.

## Conclusion et perspectives

Notre travail de thèse a permis de découvrir des facteurs et des mécanismes proximaux du contrôle de la dispersion continentale de l'anguille. La dispersion continentale est un processus se déroulant en deux phases, liées aux stades de développement. Au stade civelle, les poissons montrent un comportement migrateur (densité-indépendant) utilisant les courants de marée pour se disperser dans les habitats continentaux. Ce comportement induit une accumulation d'individus en limite de renverse des courants. Lors de la transformation des civelles en anguillettes, l'acquisition d'une forte activité alimentaire induit un comportement dispersif de sélection de l'habitat (densité-dépendant).

Les TH, qui contrôlent les mécanismes liés à la colonisation des milieux de rivière, jouent un rôle fondamental dans la régulation de la dispersion continentale. Il est probable que la GH et le cortisol jouent un rôle important pour réguler la colonisation des habitats d'eau salée. La condition corporelle (statut énergétique) influence les sécrétions de ces hormones et est fortement reliée aux tactiques alternatives de sédentarisation précoce en zone salée et de colonisation des rivières chez la civelle. De plus, les sécrétions endocriniennes, influencées également par la température, semblent pouvoir jouer un rôle dans le contrôle de la croissance.

Notre approche nous a permis de montrer que la stratégie de la dispersion continentale de l'anguille, qui permet à l'individu de choisir en fonction de son statut (endocrinien, développement, énergétique, social...) la tactique dispersive la plus avantageuse en terme de fitness. C'est donc une stratégie de type conditionnelle.

Ce travail permet d'envisager de nombreuses voies pour des recherches futures sur le contrôle des comportements migrateur et de sélection de l'habitat. En ce qui concerne la régulation du comportement migrateur, il serait important de tester les effets des TH sur la préférence pour l'eau douce, l'olfaction et la synchronisation des rythmes internes (notamment circatidiaux). De plus, GH et cortisol semblent être des bons candidats pour la régulation de la préférence pour l'eau salée. L'étude de l'effet du statut énergétique sur les sécrétions de ces hormones serait également très intéressante.

L'exploration des processus de sélection de l'habitat et des variations de la plasticité phénotypique pourrait apporter des résultats importants pour la compréhension du phénomène de dispersion au stade jaune. Des tests expérimentaux de densité-dépendance des déplacements sont en cours au Cemagref. La définition de normes de réaction (en termes de dispersion) des anguilles en fonction des facteurs biotiques et abiotiques du milieu est particulièrement prometteuse. A terme, la mise en évidence de différences interindividuelles de plasticité phénotypique, liées éventuellement à des variations du niveau d'hétérozygotie, permettrait de progresser substantiellement dans notre connaissance des processus contrôlant la dispersion continentale.

En terme de gestion, nos travaux montrent que les zones de limite de marée dynamique doivent être préservées en priorité. En effet, l'accumulation des civelles et l'étape clé de transformation en anguille se font dans ces secteurs, y rendant

l'anguille particulièrement sensible à la pression humaine. Nos résultats suggèrent également que l'anthropisation généralisée des bassins versants pourrait se traduire par une diminution forte de la fitness associée à la tactique de migration en rivière. A terme, cet effet pourrait induire une modification profonde de la stratégie de dispersion continentale de l'anguille, avec une perte de la tactique de migration en rivière.

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