**UNIVERSITE DE LA ROCHELLE**

**ÉCOLE DOCTORALE**
*Sciences pour l'Environnement – Gay Lussac (GL)*

**Laboratoire IFREMER**, L'Houmeau

**THÈSE**

présentée par :

David BENHAÏM

Soutenue le 17 novembre 2011
pour l'obtention du grade de Docteur de l'Université de La Rochelle

Discipline : Biologie de l'environnement, des populations, Ecologie

# Caractérisation de l'adaptation comportementale des téléostéens en élevage : plasticité et effets de la domestication

**JURY :**

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| **DICKEL Ludovic, Professeur, Université de Caen** | **Rapporteur** |
| **FERON Christophe, MCF (HDR), Université de Paris 13** | **Rapporteur** |
| **BEGOUT Marie-Laure, Cadre de Recherche, IFREMER L'Houmeau** | **Co-Directrice de thèse** |
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| **HUNTINGFORD Felicity, Professeur, Univ. De Glasgow** | **Examinatrice** |
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| Christel LEFRANCOIS, Maître de Conférence, Univ. De La Rochelle | Examinatrice |

*Celui qui connaît vraiment les animaux est par là même capable de comprendre pleinement le caractère unique de l'homme.*

*Konrad Lorenz, L'agression.*

# Avant-propos

Cette thèse s'intègre dans des programmes de recherche nationaux menés dans le **GDR Ifremer-INRA** « Amélioration génétique pour une pisciculture durable » 2009-2012 (**AG**π), axé sur la génétique quantitative et dont les grandes lignes sont les suivantes : la forte augmentation de la production mondiale de téléostéens repose désormais sur le développement de l'aquaculture alors que les captures de la pêche mondiale stagnent depuis 15 ans. Ce développement doit donc se faire de façon raisonnée et prendre en compte, dès à présent, les grandes questions sociétales, comme (i) la consommation de ressources non renouvelables ou limitées (énergie, épuisement de la biodiversité marine à travers l'utilisation d'huiles et farines de poissons) et (ii) l'impact environnemental des élevages, qu'il s'agisse de pollution chimique ou de 'pollution génétique' à travers les interactions entre populations d'élevage et populations sauvages.

Deux démarches complémentaires ont été proposées dans le GDR pour réaliser une amélioration génétique susceptible d'accompagner le développement de l'aquaculture dans une perspective de durabilité :

1. Engager une réflexion sur le choix des futurs objectifs de sélection, incluant notamment l'estimation des valeurs marchande et non-marchande du progrès génétique acquis ou potentiel sur différents caractères et selon différents scénarios de production et d'évolution des systèmes (Axe 1)

2. Identifier les bases génétiques de caractères zootechniques *a priori* déterminants pour la diversification et la durabilité des productions piscicoles et affiner la description des phénotypes associés. Les caractères retenus sont l'adaptation à la captivité (Axe 2) et l'aptitude à utiliser les aliments d'origine végétale (Axes 3).

Ces deux démarches reposent sur une approche pluridisciplinaire faisant intervenir outre la génétique, les approches physiologiques et comportementales.

Ce travail de thèse a contribué à la caractérisation comportementale de différentes populations produites pour répondre aux questions posées dans les axes 2 et 3 du GDR.

Il a été soutenu par les laboratoires Ifremer LRH-LR de L'Houmeau et la station d'aquaculture de Palavas, et les activités financées sur divers fonds provenant du projet Ifremer « Domestication et Sélection », notamment ceux du GDR et du projet FUI[1] « Génétique de l'adaptation aux aliments végétaux en aquaculture (Vegeaqua) ».

Les travaux réalisés en Islande ont été financés par le 'Hólar University College' ainsi que par des fonds provenant du centre pour la recherche en Islande (Rannis) et de l'Université Eimskip (Université d'Islande).

Plusieurs expériences ont également été réalisées à l'Institut National des Sciences et Techniques de la Mer (Le CNAM, Cherbourg) me permettant ainsi de bénéficier des installations existantes et de combiner mon activité de recherche à une formation personnelle diplômante.

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[1] Fonds Uniques Interministériels

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C'est un plaisir et un privilège de vous connaître toutes les deux !

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# Sommaire

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# Introduction générale

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Ce travail est une étude visant la **compréhension de l'adaptation aux conditions d'élevage par une approche comportementale** basée sur la comparaison de diverses populations de téléostéens (domestiquées et sauvages) pour trois espèces, l'une marine, le bar (*Dicentrarchus labrax)*, les deux autres d'eau douce, l'omble chevalier (*Salvelinus alpinus*) et la truite commune (*Salmo trutta*).

La notion d'adaptation revêt des sens différents selon les disciplines scientifiques. Il nous a donc semblé important de commencer par définir, avant d'entrer dans le cœur de nos travaux, notre vision de l'adaptation, en regard et par rapport à cette discipline, de tenter de mettre en relation les principaux concepts de l'éthologie avec le concept d'adaptation et de cerner le potentiel de l'approche comportementale dans le contexte de notre travail.

Ces définitions nous ont semblé d'autant plus importantes, que comme nous l'avons précisé en avant propos, ce travail de thèse s'intègre dans les programmes de recherche du GDR Ifremer-INRA-AGπ faisant intervenir des généticiens, des physiologistes et des comportementalistes pour appréhender l'adaptation dans sa globalité et développer une démarche et des outils efficaces pour répondre aux objectifs d'une amélioration génétique. Nous exposerons donc ici quelques notions essentielles propres à la génétique et à la physiologie et nous verrons par la suite leurs niveaux de recouvrement ou de divergence avec l'approche comportementale qui sera développée dans les sections suivantes.

## *1. La notion de réponse adaptative à un changement environnemental*

La nature des réponses d'un organisme face à des changements environnementaux dépend du temps que dure le changement et du temps de génération de l'organisme. A courts termes, la réponse adaptative se fera à l'échelle de l'individu et peut être contournée, ou ses effets réduits, en faisant intervenir une réponse comportementale. Si le changement persiste, des mécanismes biochimiques et physiologiques seront mis en jeu, permettant alors à l'organisme de s'acclimater. A une échelle de temps plus longue, des changements au niveau des traits du cycle biologique apparaîtront (taux de croissance, âge à la maturité, etc.). Enfin, à une échelle de temps encore plus longue, les réponses effectuées par les individus seront détectées dans les caractéristiques démographiques de la population avec des modifications au niveau des taux de natalité et de mortalité. Finalement, la population peut s'adapter à ces changements à long terme, les modifications concernent alors les gènes. Dans ce cas, le changement environnemental agit comme un facteur évolutif causant des modifications dans le pool génique de la population par le biais de la sélection naturelle (Slobodkin & Rapoport, 1974).

Arms & Camp (1989) distinguent trois types d'adaptation : anatomiques, physiologiques et comportementales. Les adaptations **anatomiques** concernent la structure physique de l'organisme, *e.g.* les nageoires d'un manchot sont des adaptations anatomiques qui lui permettent de nager. Les adaptations **physiologiques** concernent l'ensemble des mécanismes internes de l'échelle cellulaire à l'échelle macroscopique. Elles comprennent la biochimie cellulaire et les processus qui permettent à l'organisme d'assurer les grandes fonctions (nutrition, reproduction, locomotion), *e.g.* l'adaptation à la température chez les bactéries des sources hydrothermales. Un exemple d'adaptation **comportementale**, enfin, est la capacité du kangourou-rat de manger les feuilles de l'arroche du désert qu'aucun autre animal ne peut manger parce que ses feuilles contiennent des cristaux de sel. Le kangourou-rat utilise ses incisives pour peler la couche externe de la feuille, remplie de sel et il mange ensuite la partie interne qui ne contient pas de sel. Cette capacité d'apprêter sa nourriture est un des nombreux exemples d'adaptation comportementale permettant à un animal d'avoir accès à une nourriture que les autres animaux ne peuvent se procurer.

En reprenant la définition donnée au début de ce chapitre, l'adaptation est donc la modification d'un caractère anatomique, d'un processus physiologique ou d'un trait comportemental dans une population d'individus, sous l'effet d'une sélection naturelle ou dirigée. Elle peut désigner le processus ou le résultat du processus.

### *2. La définition de l'adaptation vue par les généticiens*

Pour appréhender le caractère « adaptation », les généticiens spécialisés dans l'étude des processus de sélection tentent d'intégrer, en dehors des objectifs zootechniques classiques (croissance, survie, qualité des rendements), des objectifs non marchands devant satisfaire à des contraintes ou besoins environnementaux, sociétaux et de bien-être animal (Bodin et al., 2010). Parmi ces caractères, plusieurs concernent la relation de l'animal à son milieu et les notions de robustesse, flexibilité, plasticité, rusticité ou d'adaptation à des milieux difficiles. Ces termes possèdent des définitions très controversées (Debat & David, 2001). La robustesse est définie par Knap (2005) comme la capacité de survie de l'animal et le maintien d'un potentiel global de production dans des environnements stressants. La robustesse peut être également définie comme le maintien d'un potentiel de production spécifique où l'animal est dit « robuste » pour un caractère donné, si les performances pour ce caractère se maintiennent dans des environnements variés ce qui rejoint la notion d'interaction génotype-milieu expliquée par différents mécanismes génétiques : modèle de surdominance, modèle pléiotropique, modèle épistatique (Scheiner & Lyman, 1991). La « canalisation » d'un caractère correspond alors à l'action de sélectionner pour diminuer la sensibilité aux variations environnementales (San Cristobal-Gaudy et al., 1998).

Cette sensibilité à l'environnement fait également intervenir la notion de plasticité phénotypique, concept qui vu par le généticien, s'inscrit dans l'équation: $V_p=V_G+V_E+V_{GxE}+V_{erreur}$ (Scheiner & Goodnight, 1984; Via & Lande, 1985) où $V_P$ est la variance phénotypique, $V_G$ la variance génétique, $V_E$ la variance environnementale et $V_{GE}$ (la covariance de la génétique et de l'environnement). Cette équation inclut donc explicitement l'influence d'un effet environnemental et la plasticité phénotypique se définit comme étant la capacité d'un génotype à produire plus d'un phénotype lorsqu'il est exposé à différents environnements. Ceci peut se produire par le biais de la modification d'évènements au cours du développement ou par la capacité de l'organisme à ajuster son phénotype en réponse à des changements de conditions environnementales (Gordon, 1992; Scheiner, 1993; Via et al., 1995; Freeman & Herron, 2004; Pigliucci, 2005; Stearns & Hoekstra, 2005). Il faut noter que la mesure du déterminisme génétique de la sensibilité à l'environnement peut s'effectuer de manière très précise en utilisant des clones, l'équation précédente étant dans ce cas, réduite à la simple expression : $V_p = V_E$ (Dupont-Nivet et al., 2007).

Les évolutionnistes, quant à eux, définissent l'adaptation comme étant un trait génétiquement déterminé pour lequel il y a eu sélection et qui se retrouve dans une grande partie de la population parce qu'il augmente les chances de l'individu qui le possède de se reproduire avec succès (Arms & Camp, 1989). Ce point de vue inclut la capacité de changer selon les conditions environnementales aussi bien que celle d'apprendre. Nous y reviendrons plus loin, mais d’ores et déjà, cette définition intègre clairement une composante comportementale (*i.e.* l’apprentissage).

### 3. La définition de l’adaptation vue par les physiologistes

En physiologie, l’adaptation prend tout son sens dans la notion d’**homéostasie**. Ce concept a été évoqué pour la première fois par Bernard (1859) puis défini précisément par Cannon (1932) comme étant un état stable entretenu par tous les processus actifs qui s’opposent aux changements des propriétés de l’organisme. Cette résistance au changement est une caractéristique de tous les organismes et l’essentiel de la physiologie peut être décrit en termes d’homéostasie et des mécanismes régulateurs qui la sous-tendent (Rieutort, 1998). L’homéostasie existe du niveau cellulaire à l’organisme entier. Les modifications de l’environnement déclenchent des réactions de l’organisme aboutissant à des perturbations internes qui sont normalement maintenues dans des limites étroites par des ajustements automatiques à l’intérieur du système, destinés à éviter les oscillations amples et à maintenir les conditions internes à peu près constantes (Cannon, 1932). Ces réactions ou réponses de l’organisme, définissent l’adaptation physiologique. Le même auteur associait déjà les processus homéostatiques de maintien de la vie, au phénomène de stress. Selon lui, l’homéostasie est menacée si les substances essentielles manquent ou sont en excès (origines endogènes) ou encore, si un facteur externe est délétère (facteurs exogènes) pour l'organisme. Au niveau adaptatif à court terme, le stress va conduire aux réactions de fuite ou de lutte qui sont la conséquence d'un hyperfonctionnement sympathique. Il a donc un rôle adaptatif essentiel car, « tout comme un matériau ne peut résister qu'à des contraintes modérées, l'homéostasie ne peut être maintenue que si les écarts à la normale restent relativement faibles. Au-delà, des processus correctifs permettant de faire face sont nécessaires : c'est le « stress » (Dantzer, 2002). Le stress est donc le complément à l'homéostasie qui permet de réduire au maximum des dégâts subits par l'organisme. C'est une réaction d'urgence à court terme qui conduit à l'évitement de la situation pathogène. Et en ce sens, le stress est fondamental à l'adaptation d'un organisme.

Plus récemment, le concept d’**allostasie** a été introduit pour expliquer le maintien d’un équilibre face à des changements (McEwen & Wingfield, 2003). L’allostasie est un processus fondamental par lequel les organismes vont activement s’ajuster, par le biais de réponses morphologiques, physiologiques et comportementales, à des évènements prévisibles ou non. Un **état allostatique** fait référence à des niveaux d’activités de médiateurs primaires (*e.g.* glucocorticoïdes) modifiés de façon durable en réponse à un facteur de stress. Le résultat cumulatif d’un état allostatique est la **charge allostatique**. Dans certaines limites, l’organisme peut « faire avec » et s’adapter ou tolérer les facteurs de stress pour maintenir son homéostasie. Cependant, lorsque le système ne parvient pas à s’accorder avec le facteur perturbateur, l’augmentation de la charge allostatique résulte en une **surcharge allostatique**. La surcharge allostatique est soit un état dans lequel la demande énergétique excède l’énergie disponible (type I), soit un état dans lequel des challenges délétères (*e.g.* sociaux) sont chroniques et conduisent à un état allostatique prolongé et indépendant des changements saisonniers (type II). De sérieuses pathologies peuvent en résulter si la surcharge n’est pas réduite. L’allostasie forme en fait un continuum entre les évènements du cycle de vie normal et les perturbations imprévisibles avec des transitions et des conséquences bien définies.

Nous reviendrons plus loin sur les réponses neuroendocriniennes au stress chez les téléostéens qui ont fait l’objet de nombreux travaux (Prunet & Auperin, 2007) car certaines d’entre elles seront utilisées comme indicateurs dans ce travail en complément de l’approche comportementale. Notons toutefois que l’utilisation de ce terme est ambiguë car il désigne souvent indifféremment l'évènement (le facteur de stress) et la réponse (réponse de stress). A la lumière du concept d'allostasie, le terme de stress est utilisé pour décrire les évènements qui menacent un individu et qui déclenchent des réponses physiologiques et comportementales faisant partie de l'allostasie et qui viennent s'ajouter à celles imposées par le cycle biologique dans les conditions normales.

## 4. L'approche comportementale.

Avant de développer l'approche comportementale de l'adaptation, il convient de définir le concept de comportement ainsi que l'éthologie, discipline à laquelle il est rattaché. Encore une fois, il n'existe pas de définition unique pour le terme « comportement ».

### 4.1. La notion de comportement et d'éthologie

Dans une approche écologique, les comportements vont être appréhendés de manière relativement globale : les séquences de comportement sont analysées en termes d'unités, appelées « actions », et définies par rapport à la fonction ou au but de la séquence observée (Feyereisen & De Lannoy, 2001). De telles actions chez un organisme, ont pour fonction globale de lui assurer une certaine probabilité de survie dans certaines conditions de milieu. D'autres auteurs définissent le comportement comme l'ensemble des processus de décisions par lesquels les individus ajustent leur état et leur situation par rapport aux variations abiotiques et biotiques du milieu (Danchin et al., 2005). Watson (1917) définit quant à lui, le comportement comme l'ensemble des réactions objectivement observables qu'un organisme, généralement pourvu d'un système nerveux, exécute en réponse aux stimulations du milieu, elles-mêmes objectivement observables. Cette définition souligne l'imbrication de l'éthologie et de la physiologie. L'ensemble de ces définitions place toujours le comportement en interaction avec les facteurs environnementaux.

Tinbergen (1963), définit l'éthologie comme « l'étude biologique du comportement » et, caractérise cette science par un phénomène observable (comportement ou mouvement) et par un type d'approche, une méthode d'étude (la méthode biologique). Ce même auteur posera dans l'ouvrage précité, les 4 questions fondamentales de l'éthologie : (1) Quelles sont les causes immédiates du comportement ? (2) Quelle est sa valeur de survie ? (3) Comment s'est-il mis en place au cours de l'ontogenèse ? (4) Comment s'est-il mis en place au cours de la phylogenèse ? Les définitions se sont en fait diversifiées depuis les travaux fondateurs de Konrad Lorenz, Nikolaas Tinbergen et Karl von Frisch et l'apport des disciplines voisines qui ont suivi, notamment celle de la physiologie, des neurosciences comportementales, de la zoologie, des sciences de l'évolution et de la psychologie expérimentale. Ainsi, la définition de Campan & Scapini (2002) tente-t-elle d'intégrer les apports de ces différents courants d'influence : « l'éthologie est l'étude du comportement dans toutes ses manifestations et à tous les niveaux, de la cellule à l'organisme entier et aux sociétés animales, des causes qui le déterminent et de ses fonctions ». Parmi les sciences du comportement enfin, **l'éthologie animale appliquée** est une discipline moderne qui se définit comme l'étude du comportement des animaux dans leur milieu habituel et en interaction plus ou moins constante avec l'homme, ses productions ou son environnement. L'animal est alors décrit en fonction de son utilité sociale pour l'homme : animal de rente, de compagnie, de laboratoire ou encore de sport, ou animal sauvage maintenu en captivité, entomofaune utile ou nuisible (Boissy et al., 2009). L'approche comportementale développée dans ce travail de thèse se rattache à cette dernière définition.

### 4.2. La plasticité phénotypique comportementale

La définition de la plasticité phénotypique a été développée précédemment en abordant la définition de l'adaptation par les généticiens (cf. 2). Ce concept peut s'étendre au comportement. Dans ce cas, l'organisme va présenter des modifications comportementales en réponse aux changements de l'environnement. Il s'agit, comme nous l'avons déjà évoqué d'un mécanisme essentiel de l'adaptation. Classiquement, on distingue deux types de plasticité phénotypique qui diffèrent par la vitesse et la réversibilité de la réponse (Sih et al., 2004). La première, appelée plasticité phénotypique développementale, est lente et irréversible et elle devrait être favorisée si l'environnement varie de façon lente et prévisible alors que la plasticité phénotypique comportementale ou flexibilité, rapide et réversible, s'exprimerait dans un environnement qui varie rapidement et de façon imprévisible (Piersma & Drent, 2003). La flexibilité du comportement permettrait donc une adaptation rapide aux changements de l'environnement. A l'aide d'un modèle mathématique basé sur la théorie des jeux, Fauvergue & Tenielier (2004) illustrent l'avantage adaptatif de la flexibilité comportementale lors de changements brusques de l'environnement. Ces auteurs définissent la décision comportementale comme un phénotype déterminé par un génotype, et la flexibilité phénotypique comme la possibilité pour un génotype donné de déterminer plusieurs phénotypes de façon réversible. Pour une population d'individus au comportement canalisé génétiquement, la convergence vers le comportement optimal nécessite plusieurs générations de sélection fréquence-dépendante. En revanche, des individus capables d'apprendre la qualité de l'environnement convergent vers l'optimum au cours d'une seule génération (Figure 1).

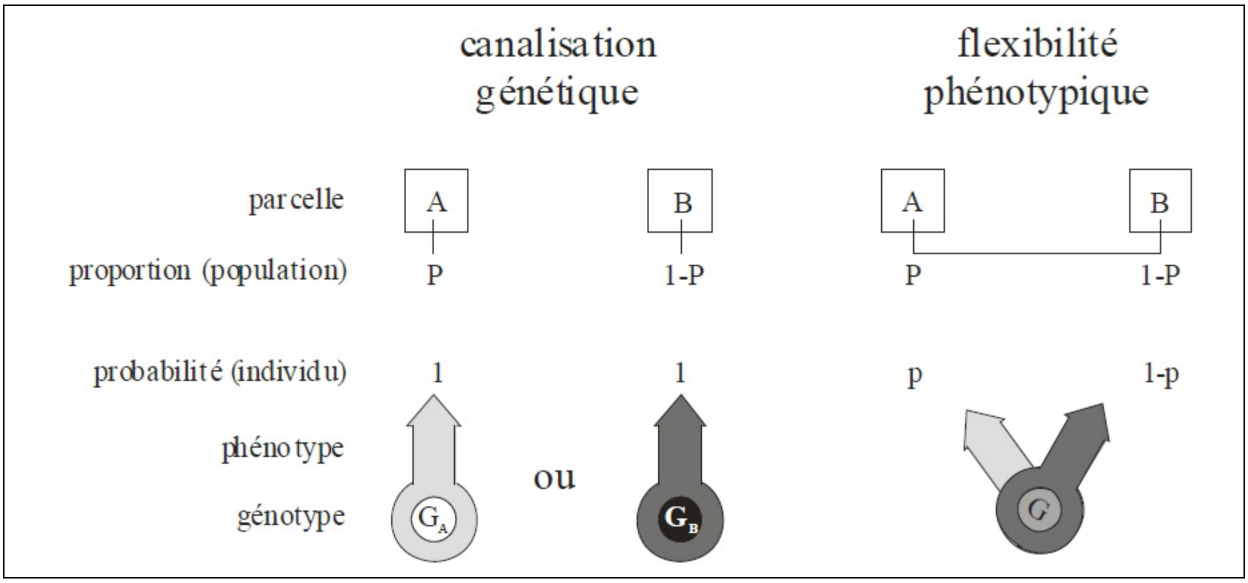

*Figure 1. Relation entre génotype et phénotype dans le cas d'un phénotype (une décision comportementale) à deux alternatives (Fauvergue & Tenielier, 2004)*

Dans le cadre de cette thèse, nous nous intéresserons à la **flexibilité comportementale**, notion différente de la « plasticité développementale » au sens qui lui est accordé en physique : la propriété d'un solide de se déformer sous l'action d'une force, et de conserver la forme acquise lorsque la force cesse d'agir. Dans ce sens, il existe peu de décisions comportementales plastiques (Fauvergue & Tenielier, 2004).

Cette flexibilité comportementale peut s'exprimer notamment *via* l'apprentissage qui est un processus d'optimisation plus rapide que la sélection naturelle. Il est important de préciser que cette flexibilité n'est pas un phénomène totalement épigénétique. Les structures et les mécanismes de traitement de l'information (perception, mémoire, intégration) qui permettent la flexibilité sont eux-mêmes des phénotypes [enzymes, régions cérébrales (Silva et al., 1997) sur lesquels la sélection naturelle agit (Mery & Kawecki, 2002)].

### 4.3. L'acclimatation

La notion d'acclimatation est un autre terme lié à l'adaptation et à la plasticité phénotypique. Wilson & Franklin (2002) la définissent comme toute modification facultative d'un trait physiologique en réponse aux changements d'une ou de plusieurs variables en conditions naturelles ou de laboratoire. Ces changements peuvent survenir au cours du développement ou à des stades ultérieurs du cycle biologique de l'organisme. Les réponses peuvent être bénéfiques, neutres ou négatives. L'acclimatation ainsi que l'apprentissage et la mémoire, sont compris dans la définition la plus complète de la plasticité phénotypique.

### 4.4. La personnalité et les « coping styles »

Autres concepts relativement récents en éthologie, les traits de personnalités et les « coping styles » peuvent également être reliés à la plasticité phénotypique et à l'adaptation.

Les traits de personnalité sont des tendances qui s'expriment dans différents contextes (deux ou plus), varient entre les individus d'une population donnée et qui sont stables dans le temps (Sih et al., 2004; Réale et al., 2007). En écologie comportementale, la plasticité comportementale est habituellement considérée comme étant illimitée (les individus peuvent toujours exprimer l'optimum), immédiate (peu ou pas de décalage) et infiniment réversible (Sih et al., 2004). Les traits de personnalité se situent entre les deux extrêmes constitués par la plasticité développementale et la flexibilité. La personnalité traduit l'existence de différences individuelles dans le comportement des membres d'une même espèce. Des études de plus en plus nombreuses suggèrent en effet, que les individus d'une même espèce diffèrent dans leur niveau moyen d'expression comportementale dans divers contextes et dans leur niveau de réponse aux variations environnementales. Plasticité et personnalité ne s'opposent pas, ce sont deux aspects complémentaires du phénotype de l'individu (Dingemanse et al., 2009).

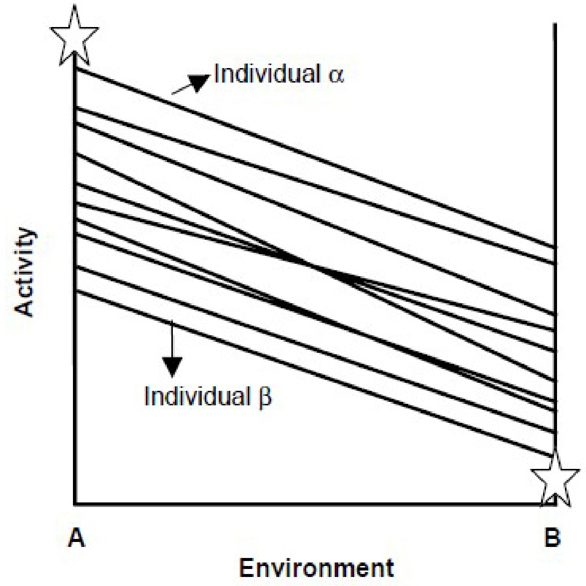

*Figure 2. Représentation graphique d'un syndrome comportemental (D'après Sih et al., 2004). Chaque ligne sur cette figure représente un individu différent et comment son activité change dans deux environnements distincts (A : sans prédateur ; B : avec prédateur). Les étoiles indiquent le niveau optimal d'activité pour chaque environnement (niveau haut en A, bas en B). Le syndrome comportemental apparaît quand les lignes de plasticité tendent à être parallèles. Les individus les plus actifs en A sont également les plus actifs en B, maintenant leur rang d'un environnement à l'autre.*

Récemment, Réale et al. (2007) ont suggéré que personnalité, tempérament et individualité étaient des termes équivalents qui pouvaient être classés en 5 catégories : activité, timidité ou témérité (en réponse à des situations de risque), exploration (en réponse à des situations nouvelles), agressivité et sociabilité. Lorsque des corrélations existent entre traits de personnalité, ils forment un syndrome comportemental (Sih et al., 2004). C'est le cas par exemple, de l'axe proactif-réactif où les individus proactifs sont à la fois agressifs et téméraires (Koolhaas et al., 1999; Réale & Festa-Bianchet, 2003). Ces traits de personnalité ont été décrits pour une variété de mammifères (Nettle, 2006), d'oiseaux, de reptiles, d'amphibiens, d'insectes, d'araignées, de céphalopodes et de téléostéens (Gosling, 2001) et sont souvent caractérisés par des traits physiologiques sous-jacents (Koolhaas et al., 1999; Van Riel et al., 2002; Carere et al., 2003; Feldker et al., 2003; Sluyter et al., 2003; Veenema et al., 2003; Øverli et al., 2006). Ces traits de personnalité peuvent également être héritables (van Oers et al., 2005). D'un point de vue adaptatif, DeWitt et al. (1998) soulignent que dans un monde idéal, les individus seraient infiniment plastiques et adapteraient leur comportement en toute circonstance, mais l'existence de syndromes comportementaux implique comme nous l'avons déjà dit, une plasticité limitée, peut-être en raison du coût élevé engendré par la plasticité.

Les «coping styles», quant à eux, ne se distinguent pas des concepts de personnalité, de syndrome ou de tempérament pour un grand nombre d'auteurs (Sih et al., 2004; Bell, 2007). Pourtant, ce terme est souvent associé au stress. Selon Koolhass et al. (1999), les « coping styles » sont en effet une série cohérente de réponses comportementales et physiologiques au stress qui persistent au cours du temps et qui caractérisent un certain nombre d'individus. L'impact de stimuli aversifs ou de facteurs de stress est souvent mesuré par la capacité de l'organisme à « faire face » (cope) à la situation (Ursin & Olff, 1995; Ursin, 1998) et les « coping styles » sont les stratégies comportementales et physiologiques mises alors en place par l'organisme (Wechsler, 1995). Henry & Stephens (1977) ont suggéré que l'on pouvait distinguer deux types de réponses au stress. La première ou réponse active (réponse de fuite ou de combat) est caractérisée par un comportement territorial et de l'agressivité. La seconde est caractérisée à l'inverse par de l'immobilité et de faibles niveaux d'agression.

### 4.5. L'apprentissage et la cognition

L'apprentissage et la cognition jouent également un rôle majeur dans l'adaptation. La cognition inclut la perception, l'attention, la mémoire et les fonctions exécutives relatives au traitement de l'information dont l'apprentissage et la résolution de problèmes (Brown et al., 2007). L'étude de la cognition a été largement centrée sur les oiseaux et les mammifères, mais depuis quelques décennies, il a été également montré que les téléostéens à l'image des autres vertébrés, étaient capables d'exprimer une grande diversité de comportements sophistiqués et que l'apprentissage jouait un rôle majeur dans leur développement comportemental. Des capacités de mémoire à long terme ont été mises en évidence (Brown & Laland, 2001; Warburton, 2007) ainsi que des capacités cognitives comparables à celles des primates (Odling-Smee & Braithwaite, 2003). Ces différentes aptitudes vont clairement favoriser l'adaptation des téléostéens en élevage. Il existe également un lien entre traits de personnalités ou « coping styles » et cognition. En effet, les téléostéens possédant comme les mammifères, des traits de personnalité tels que la timidité ou la témérité, ont également des capacités d'apprentissage et des motivations différentes (Koolhaas et al., 1999; Kristiansen & Fernö, 2006). Certains aspects comportementaux des « coping styles » pourraient même être modifiés par l'expérience (Ruiz-Gomez et al., 2008).

### 4.6. Le comportement, la physiologie, la morphologie et l'adaptation

Le comportement, la physiologie, mais aussi la morphologie relèvent de mécanismes imbriqués. Les traits comportementaux sont complexes car ils sont composés de nombreux niveaux sous-jacents, eux-mêmes imbriqués de façon hiérarchisée (Figure 3). En général, les sélections naturelle et sexuelle vont avoir tendance à agir plus fortement aux niveaux d'organisations biologiques les plus élevés. La sélection agit plutôt sur la variation phénotypique qui reflète la variation de l'expression du gène.

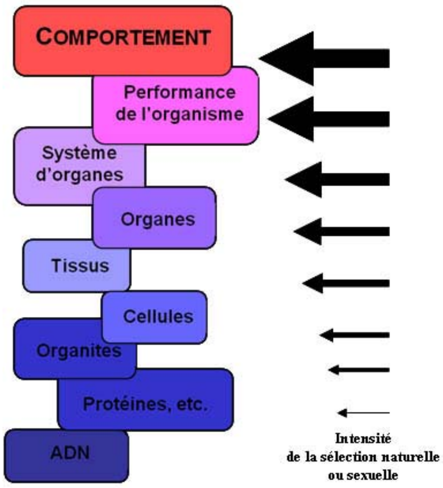

*Figure 3. Le comportement et ses niveaux sous-jacents en relation avec l'intensité de la sélection naturelle ou sexuelle (Garland Jr & Kelly, 2006)*

Le comportement est donc souvent le mécanisme par lequel s'exerce la spécialisation chez de nombreuses espèces animales. Une modification comportementale dans l'évolution d'une espèce conduit fréquemment à un changement de niche écologique et une sélection dirigée sur d'autres traits tels que la morphologie et la physiologie (Futuyma & Moreno, 1988). En effet, le comportement est une composante plus flexible que la morphologie et la physiologie (Schluter, 1996; Skúlason & Jónsson, 1999) et de nombreuses études, en particulier chez les téléostéens (Klemetsen et al., 2006; Sacotte and Magnan, 2006; Svanbäck and Eklöv, 2006), ont montré que la différentiation comportementale précède la différentiation morphologique. Ainsi, variabilités comportementale et morphologique sont intimement liées. De très nombreuses études, en particulier chez les salmonidés, montrent le lien entre la taille et les comportements d'agressivité (Abbot et al., 1985; Huntingford et al., 1990). D'autres auteurs (Benhaïm et al., 2003) ont montré qu'au moment de la première alimentation exogène, il existait des différences comportementales entre petits et gros juvéniles d'omble chevalier (*Salvelinus alpinus*) de mêmes âges. Les résultats de ces observations montrent que, dès ces stades précoces, les individus les plus gros sont plus actifs, ont un temps de réponse plus court à la distribution de l'aliment et se nourrissent principalement à la surface de l'eau. Les individus les plus petits sont moins mobiles, passent plus de temps au fond et font moins de tentatives que les gros pour happer la nourriture et le font indifféremment au fond ou en surface.

## *5. Les facteurs environnementaux intervenant sur la plasticité comportementale*

Dans presque toutes les sections précédentes, l'influence de l'environnement a été évoquée au travers de son impact sur la plasticité phénotypique : l'interaction génotype-milieu (§2), les réactions physiologiques de l'organisme en réponse à des modifications environnementales (§3), l'éthologie qui étudie l'interaction des individus avec leur environnement (§4.1). Il convient maintenant de préciser la nature de ces facteurs environnementaux et en particulier, ceux rencontrés en conditions d'élevage. D'une manière générale, nous les classerons en deux grandes catégories : les facteurs abiotiques et les facteurs biotiques.

### 5.1. Les facteurs abiotiques

En écologie, les facteurs abiotiques représentent l'ensemble des facteurs physico-chimiques d'un écosystème influençant une biocénose donnée (Ramade, 1984). Parmi ces facteurs, l'auteur distingue des facteurs climatiques (température, éclairement, etc.) et des facteurs physico-chimiques non-climatiques (pression, teneur en sels minéraux, teneur en oxygène dissous etc.). Ces définitions s'appliquent aussi au milieu d'élevage aquatique où le maintien de la plupart de ces facteurs dans une certaine gamme de valeurs est primordial pour la survie des espèces et la productivité des entreprises.

Ainsi, l'eau est à la fois un simple support physique pour l'animal aquatique (apportant l'oxygène et diluant les produits du métabolisme) et un milieu biologique. Par rapport au milieu aérien, l'eau présente plusieurs caractéristiques originales comme une densité élevée et la capacité de dilution d'éléments toxiques (ammoniaque). Les organismes aquatiques sont amenés à supporter des contraintes de milieu et des variations d'environnement dont les amplitudes sont souvent beaucoup plus importantes que celles habituellement observées pour les organismes vivant en milieu aérien. Les principaux facteurs impliqués sont la pression partielle en oxygène, la composition chimique et les propriétés physiques du milieu comme la température, la pression hydrostatique et l'intensité lumineuse (Billard, 2005). Nous ne reviendrons pas sur ces paramètres qui ont été largement étudiés du fait de leur caractère essentiel dans la maîtrise zootechnique, mais en revanche, nous étendrons la liste des facteurs abiotiques à l'aliment artificiel, aux pratiques et aux structures d'élevage pour les diverses raisons expliquées ci-après :

- En élevage intensif, les animaux sont nourris exclusivement avec un aliment composé dans lequel est incorporé l'ensemble des nutriments indispensables. Nous y reviendrons dans le §8.1.4, mais soulignons déjà ici la grande complexité de ce facteur et le fait que l'essor de l'aquaculture mondiale conduit à revoir la formulation complète des aliments pour téléostéens, notamment leurs teneurs en lipides et protéines d'origine marine.

- Le mode de distribution de l'aliment varie également d'une espèce à l'autre et d'un stade de développement à l'autre. Selon la méthode utilisée (distribution manuelle à satiété, distribution automatique, systèmes d'auto-alimentation), les performances d'élevage, le comportement des téléostéens, la structure sociale des populations seront impactés. Une ressource spatialement ou temporellement limitée peut favoriser l'émergence de comportements agressifs (Grant & Guha, 1992; Grant & Kramer, 1992).

- Des tris sont souvent réalisés en routine pour réduire l'hétérogénéité en taille. Cette dernière, outre les difficultés liées à l'utilisation de la nourriture et à la gestion de la vente du cheptel, peut favoriser le cannibalisme et le stress et affecter ainsi de manière très significative les taux de survie (Baras, 1998; Kestemont et al., 2003). Cette pratique engendre néanmoins du stress et pourrait déstabiliser la structure sociale d'un groupe avec des conséquences sur la quantité d'aliment ingérée, le rythme alimentaire et le taux de croissance (Millot, 2008).

- Les enceintes d'élevage, enfin, peuvent jouer un rôle important sur les performances de production comme c'est le cas par exemple de certains dispositifs d'élevage en particulier chez les salmonidés où la présence d'un refuge artificiel ou d'un substrat permettent de meilleures performances de croissance et de survie (Eriksson & Westlund, 1983).

### 5.2. Les facteurs biotiques

En écologie, les facteurs biotiques intègrent les interactions entre les êtres vivants et celles des êtres vivants sur le non-vivant. Parmi les premiers, on peut citer les interactions intraspécifiques (relations entre les individus d'une même espèce) et interspécifiques (relations entre individus de deux ou plusieurs espèces) (Ramade, 1984). Cette définition s'applique entièrement au milieu d'élevage aquatique, mais dans le cadre de cette thèse, nous ne nous intéresserons qu'aux seules interactions intraspécifiques.

Le comportement social signifie que les individus d'une même espèce tendent à former des relations coopératives et interdépendantes et qu'ils vivent et se reproduisent dans des communautés plus ou moins organisées (Webster, 1980). Banks & Heisey (1977) précisent que le comportement social recouvre tous les modèles comportementaux qui impliquent au moins deux membres d'une même espèce et qui incluent l'agressivité, la reproduction, les soins parentaux ou le comportement d'entraide. Une autre nuance apportée par McFarland

(1982) et décrivant une interaction sociale comme une interaction où chaque individu influence le comportement de l'autre, sera aussi utile dans le cadre de ce travail.

Parmi les interactions sociales, la relation entre la taille et la dominance, est considérée comme l'un des plus importants facteurs conduisant à une hiérarchie alimentaire (Koebele, 1985) en favorisant éventuellement l'agressivité (Abbot & Dill, 1989) et dont la conséquence directe est la diminution de croissance chez les individus de faible rang. Chez les salmonidés par exemple, il existe une grande variabilité de taille des œufs qui résulte en une large distribution en taille des juvéniles au moment de la première nutrition exogène (Wallace & Aasjord, 1984). Outre le développement de hiérarchies sociales, des différences comportementales entre petits et gros juvéniles d'omble chevalier de même âge ont aussi été démontrées (Benhaïm et al., 2003, cf. §4.6). Cette différence de taille entre juvéniles peut donc être reliée à une stratégie alimentaire. Il est intéressant de noter que la corrélation entre la taille des œufs et des juvéniles de cette espèce persiste au-delà d'une année (Wallace & Aasjord, 1984), ce qui n'est pas le cas chez les autres espèces de salmonidés.

Il est cependant important de noter que l'agressivité n'est pas toujours le levier par lequel s'exprime la compétition entre les individus. Ward et al. (2006) définissent en effet trois types de compétition : « la compétition d'exploitation » où il n'existe pas d'interactions directes entre compétiteurs (un individu exploite et épuise la nourriture disponible avant qu'un second individu n'arrive, la « scramble competition » où chaque individu peut détecter ses compétiteurs et tente d'être le premier à obtenir la particule alimentaire et « le combat » qui implique une agression directe entre les compétiteurs.

Enfin la densité ou charge en téléostéens que l'éleveur est tenté de maximiser pour des questions de rentabilité, est également un facteur source d'interactions sociales. Elle peut s'avérer bénéfique ou négative pour les phénomènes de dominance selon les espèces. Chez la truite arc-en-ciel (*Oncorhynchus mykiss)*, les fortes densités ont un effet négatif sur l'activité alimentaire et les taux de croissance (Alanärä, 1996). C'est également le cas chez le saumon coho (*Oncorhynchus kisutch*) (Schreck et al., 1985) et chez l'omble de fontaine (*Salvelinus fontinalis*) (Vijayan & Leatherland, 1988). En revanche, les taux de croissance chez l'omble chevalier sont corrélés positivement à la densité du groupe (Wallace & Kolbeinshavn, 1988; Jørgensen et al., 1993). Cette variabilité des réponses à la densité est probablement liée à l'écologie et au type d'habitat naturel du téléostéen (Ruzzante, 1994). La plupart des salmonidés précités sont des espèces territoriales et c'est probablement la raison pour laquelle ils répondent négativement à l'augmentation de la densité qui correspond à une diminution de l'espace disponible dans les bassins d'élevage. Des espèces comme le bar (*Dicentrarchus labrax*) ou la daurade (*Sparus aurata*), espèces relativement grégaires en milieu naturel, montrent une meilleure tolérance aux fortes densités. Chez le bar, il a même été clairement démontré que le facteur limitant n'était pas la densité, mais la dégradation de la qualité de l'eau qu'une mauvaise gestion de l'élevage entraîne rapidement en cas de fortes charges (Roque d'Orbcastel et al., 2010). Paspatis et al. (2003) montrent aussi chez le bar juvénile que le taux de croissance augmente avec l'accroissement de la densité quel que soit le mode de nourrissage (manuel ou distribution à la demande).

## *6. La domestication*

Selon Price (1999), la domestication est le processus par lequel une population d'animaux devient adaptée à l'homme et à un environnement de captivité par le biais de changements génétiques survenant générations après générations ainsi que par des effets environnementaux sur les étapes de développement renouvelées à chaque génération. Ce processus conduit à un certain nombre de changements phénotypiques comme l'apparition de caractères morphologiques et comportementaux modifiés par rapport à la forme sauvage ancestrale (Bilio, 2007a). Certains de ces caractères ont été stabilisés en raison de leur intérêt pour l'homme. Par exemple, certains poulets ont été sélectionnés pour être plus gros, des bovins pour rester plus petits et les moutons pour perdre leur couche externe de poils drus (crin), mais pour conserver la couche interne de poils doux (la laine) (Diamond, 2002).

Il est important de noter que la plupart des animaux sauvages qui ont contribué à fournir des animaux domestiqués d'intérêt sont de grands mammifères terrestres herbivores et omnivores dont le processus de domestication a commencé il y a 10 500 ans (Diamond, 2002).

Le processus de domestication peut être divisé en trois phases (Bilio, 2007b) :

1) Phase de pré-domestication : adaptation à l'environnement d'élevage et succès persistant de la reproduction contrôlée ;
2) Domestication non contrôlée et sélection dirigée (sélection de caractères désirés et élimination éventuelle de caractères non désirés) ;
3) Etablissement de populations aux pédigrées connus (« stabilisation » génétique des lignées les plus adaptées aux futurs besoins en termes de production).

Ce processus ne peut donc pas être dissocié de celui de la sélection car il intègre trois types de sélection (Figure 4) : (i) la sélection artificielle, (ii) la sélection induite par la captivité et iii) le relâchement de la sélection naturelle (Price & King, 1968; Price, 1998).

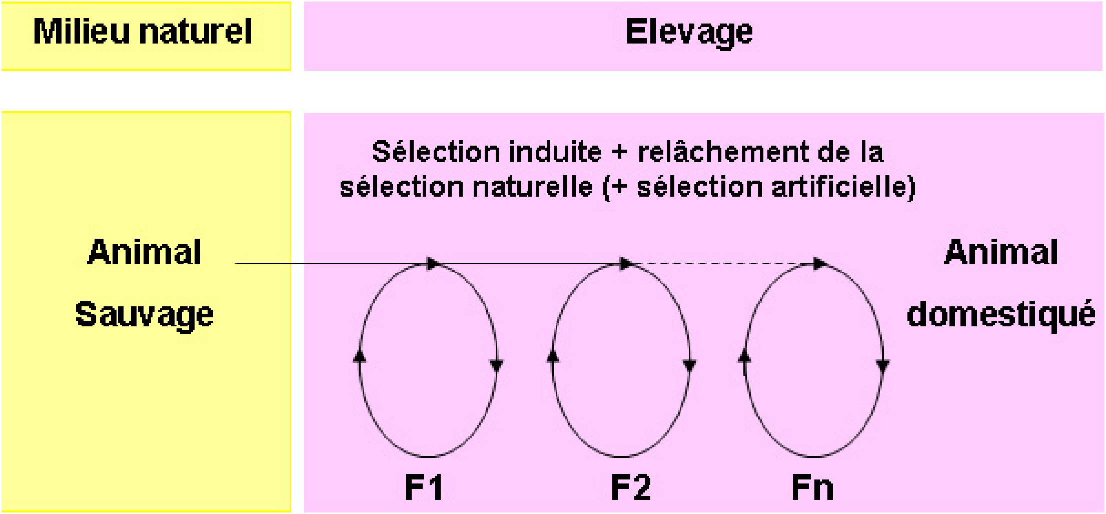

*Figure 4: Le concept de domestication et de sélection (schéma d'après Millot, 2008 et concept selon Price & King, 1968). F : génération ; 1, 2, ….n : nombre de générations.*

Fraser et al. (1997) montrent trois circonstances qui permettent d'illustrer l'importance de la pré-adaptation pour les animaux en cours de domestication (Figure 5). Selon ces auteurs, un animal peut posséder : (i) des adaptations basées sur son histoire évolutive qui n'ont plus d'utilité fonctionnelle en captivité, (ii) des adaptations qui peuvent occasionner des expériences négatives en captivité ou (iii) ne pas posséder les adaptations nécessaires à la vie en captivité.

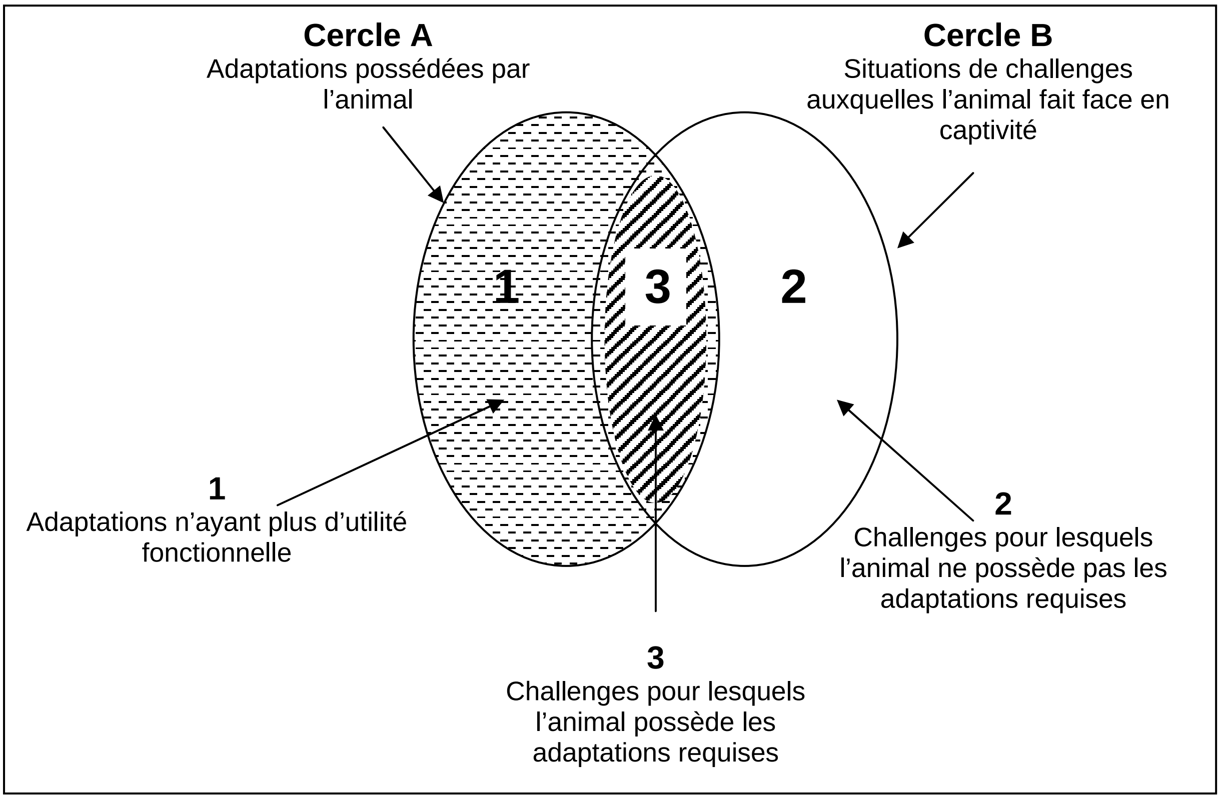

*Figure 5 : modèle conceptuel illustrant 3 types de problèmes rencontrés par l'animal lorsque les adaptations qu'il possède (cercle A) ne répondent pas parfaitement aux situations de challenge rencontrés en élevage (cercle B). Dans les aires 1 et 2, les adaptations de l'animal ne correspondent pas aux situations de challenge auxquelles il fait face (d'après Fraser et al., 1997).*

### 6.1. Les téléostéens : espèces sauvages captives ou espèces domestiquées ?

Balon (2004) défend l'idée que seules quelques espèces de téléostéens peuvent être considérées comme réellement domestiquées. Parmi ces dernières, on peut citer la carpe commune (*Cyprinus carpio* L.) introduite en France par les Romains lors de l'occupation de la Gaule à des fins de consommation et le poisson rouge (*Carassius auratus* L.) dont la domestication à des fins ornementales est mentionnée en Chine en 970 av. J.C. A part quelques exceptions donc, l'aquaculture reste une filière très récente par rapport aux autres élevages d'animaux terrestres. En effet, 97% des espèces cultivées ont été domestiquées depuis le début du 20$^{ème}$ siècle (Duarte et al., 2007) et la production aquacole mondiale repose sur quelques espèces seulement qui ont été choisies sur des critères économiques et techniques (Fontaine et al., 2003). La plupart des téléostéens d'élevage devrait donc davantage être considérée comme des espèces captives exploitées plutôt que domestiquées et seules quelques unes d'entre elles seraient en voie de franchir le seuil de domestication (Balon, 2004). Cet auteur argumente son point de vue en avançant les faibles différences existant entre les espèces captives exploitées et leur forme sauvage ancestrale, ce qui leur permettrait de retourner dans le milieu naturel. De son côté Denis (2004) souligne l'existence d'un continuum de domestication, sachant qu'aucune espèce animale ne peut être considérée comme définitivement domestiquée.

Il est important de préciser aussi que l'aquaculture est le domaine de production animale qui connaît la plus forte croissance au niveau mondial (9% par an, FAOstat, 2008) et que le nombre d'espèces élevées a fortement augmenté ces dernières décennies, certaines d'entre elles à des fins alimentaires, d'autres à des fins de repeuplement. Les statistiques de la FAO (2007) couvrant la période 1950-2005, révèlent une croissance constante de 43 à 219, du nombre d'espèces de téléostéens utilisées en aquaculture (Figure 6), ce qui suggère que le développement aquacole de ces dernières décennies repose sur l'élevage de nouvelles espèces (Fontaine et al., 2009).

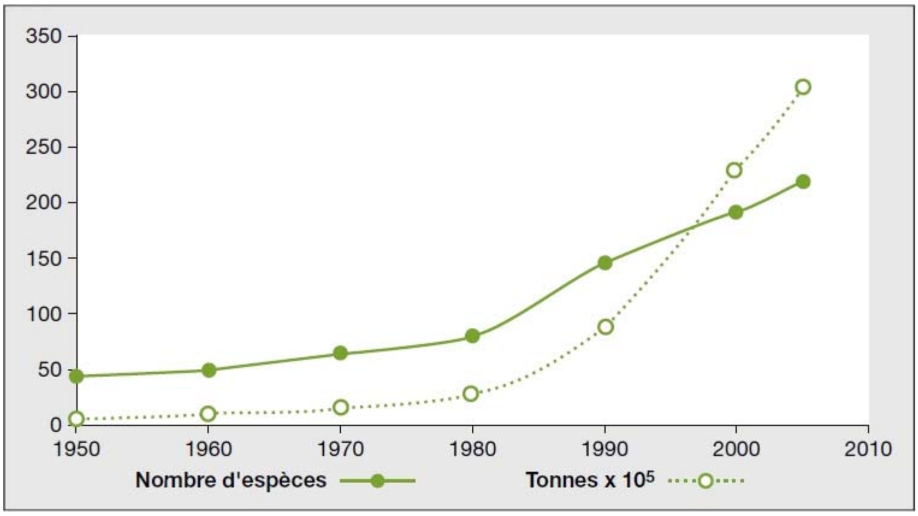

*Figure 6 : Evolutions, de 1950 à 2005, de la production aquacole (en tonnes) et du nombre d'espèces en élevage (d'après Fontaine et al., 2009).*

Cette augmentation du nombre de nouvelles espèces cultivées place la domestication au cœur des débats scientifiques actuels et notamment ceux ayant trait à la notion de développement durable piscicole. Comme nous allons le voir dans la partie suivante, les populations mises en élevage montrent une différentiation rapide de diverses caractéristiques essentielles (à la survie en milieu naturel) par rapport à leurs congénères sauvages (Araki et al., 2007; Pearsons et al., 2007) et le relâcher accidentel ou volontaire à des fins de repeuplements pourrait donc mettre en danger les populations sauvages en affectant leur valeur adaptative (McGinnity et al., 2003).

## 6.2. Les effets de la domestication chez les téléostéens

Beaucoup d'espèces de téléostéens n'en sont qu'aux premières générations de domestication et se situent donc au cœur de la phase de transition entre l'état sauvage et domestiqué. Une autre caractéristique est, qu'à l'inverse de la majorité des animaux terrestres domestiqués, les populations sauvages sont encore accessibles dans le milieu naturel. La comparaison des populations sauvages et domestiquées de la même espèce permet donc de mettre en évidence les principales différences qui apparaissent avec le processus de domestication. C'est la stratégie que nous avons choisie dans ce travail où l'accent sera mis sur l'étude des effets de la domestication au niveau des réponses comportementales.
Pour cerner l'étendue des effets potentiels de la domestication, nous présentons ici, ceux qui figurent déjà dans la littérature pour différents espèces de téléostéens.

### 6.2.1. Les effets sur la génétique, la morphologie, l'anatomie et la croissance

Beaucoup d'études, en grande partie faites sur les salmonidés, ont montré des différences morphologiques et génétiques entre populations domestiquées et sauvages de la même espèce.

Les téléostéens d'élevage, souvent dérivés de populations non indigènes, diffèrent génétiquement des populations naturelles (Philipp, 1991; Youngson et al., 1991). De plus, ils proviennent souvent de populations fondatrices de faible effectif ce qui peut entraîner des changements génétiques aléatoires (Allendorf & Phelps, 1980). Enfin, les sélections induites et artificielles évoquées précédemment contribuent également à leur différenciation génétique.

Côté morphologie, chez le saumon atlantique d'élevage (*Salmo salar*), une divergence nette a été notée par rapport à celle des congénères sauvages : corps plus robuste et nageoires rayonnées plus petites (Fleming & Einum, 1997). Au niveau anatomique, ce sont des modifications importantes telles que le ralentissement du développement des organes sensoriels (yeux, ligne latérale et odorat) qui ont été observées chez diverses larves en élevage (Kawamura et al., 1989) ou chez le turbot (*Psetta maxima*) tel que le développement surnuméraire de branches de la ligne latérale (Ellis et al., 1997a).

Les stocks de salmonidés domestiqués présentent une vitesse de croissance généralement plus rapide que les sauvages et ont un poids supérieur pour une même taille (Beamish, 1978). Les compositions chimiques d'ombles de fontaine sauvages et domestiqués sont similaires à l'exception des lipides qui sont présents en plus grande quantité chez les domestiqués dans des conditions d'élevage similaires depuis le stade œuf (Vincent, 1960). Chez le bar rayé (*Morone saxatilis*), la composition en EPA et DHA (acides gras polyinsaturés à longues chaînes de la série ω3) est considérablement plus faible que chez leurs congénères sauvages (Harrell & Woods, 1995).

### 6.2.2. Les effets sur les performances de nage

Des performances de nage plus faibles chez les lignées domestiquées par rapport aux lignées sauvages (vitesse de nage critique) ont été montrées chez la truite arc-en-ciel (Winz, 1986) et chez l'omble de fontaine (Vincent, 1960), mais pas chez le bar européen après une génération de sélection (Luna-Acosta et al., 2011).

### 6.2.3. Les effets sur la réponse aux prédateurs

Les traits comportementaux sont probablement les premiers à être affectés par la domestication (Swain & Riddell, 1990; Ruzzante & Doyle, 1991; Ruzzante & Doyle, 1993; Price, 1999). En général, les répertoires comportementaux sont similaires chez les téléostéens sauvages et domestiqués et c'est davantage la fréquence ou l'intensité avec laquelle un comportement particulier s'exprime qui est affectée par la domestication (Ruzzante, 1994).

Parmi eux, le comportement anti-prédateur est particulièrement sensible à l'environnement d'élevage (Johnsson & Abrahams, 1991; Berejikian, 1995; Dellefors & Johnsson, 1995; Johnsson et al., 1996; Einum & Fleming, 1997; Fernö & Järvi, 1998; Johnsson et al., 2001). Il a été montré par exemple que des guppys sauvages (*Poecilia reticulata*) échappaient avec succès à un prédateur plus fréquemment que des guppys domestiqués (Walker et al., 2005). Il en est de même chez le saumon d'élevage qui montre une réponse de fuite réduite face à une menace de prédation aérienne par rapport au congénère sauvage (Johnsson et al., 2001) ou encore chez la morue (*Gadus morhua*) (Nordeide & Svasand, 1990). Ces différences entre congénères sauvages et domestiqués pourraient être en partie expliquées par des expériences de vie différentes (Huntingford, 2004) car en effet le milieu aquacole est incontestablement différent de l'habitat naturel des téléostéens. Les téléostéens d'élevage font face à des conditions *a priori* moins contraignantes : plus simples, avec de la nourriture facile d'accès, une absence de prédateurs. Néanmoins, ils doivent également s'adapter à des densités élevés, un espace réduit, une nourriture artificielle et peu diversifiée, et des manipulations fréquentes (Fernö et al., 2007).

### 6.2.4. Les effets sur le comportement alimentaire

Les espèces domestiquées tendent à perdre la reconnaissance des proies vivantes. C'est ce qu'on observe chez le bar en élevage semi-intensif en marais maritime (Reymond, 1989; Bégout-Anras et al., 2001) où les plus gros individus utilisent surtout l'aliment tandis que les petits recherchent davantage les proies naturelles. Les différences au niveau du comportement alimentaire entre téléostéens sauvages et domestiqués pourraient davantage tenir à des différences de traits de personnalité qu'à des différences d'appétit (Cf. §4.4) : les téléostéens domestiqués pourraient être moins perturbés par la présence d'un aliment nouveau (Truite commune, Sundström et al., 2004a) ou par la présence d'un danger potentiel (Saumon atlantique, Einum & Fleming, 1997).

### 6.2.5. Les effets sur l'agressivité

En règle générale, l'environnement artificiel du milieu d'élevage tend à réduire l'agressivité chez bon nombre de salmonidés (Petersson & Jarvi 2000), mais en fait, cela dépend fortement des pratiques d'élevage (Bégout & Lagardère, 2004) (cf. §5.1). En réalité, les patrons comportementaux utilisés lors des interactions agonistiques sont souvent similaires chez les téléostéens d'élevage et les téléostéens sauvages (Mork et al., 1999), mais la domestication opère une sélection au niveau de l'intensité de l'agressivité qui peut être plus basse ou plus élevée chez les stocks domestiqués en fonction des régimes alimentaires (Ruzzante, 1994). Chez le médaka (*Oryzias latipes*), par exemple, la sélection pour la croissance favorise des individus moins agressifs lorsque l'accès à la nourriture est très localisé, mais pas lorsqu'elle est dispersée (Ruzzante & Doyle, 1991). Ces auteurs avancent l'hypothèse d'un coût sans doute trop élevé de l'agressivité dans les élevages caractérisés par de fortes charges et une nourriture localisée. Chez le saumon atlantique, lorsque des dyades composées d'un individu sauvage et d'un individu domestiqué sont testées, l'individu sauvage tend à devenir dominant (Metcalfe et al., 2003). Dans ce cas, l'hypothèse avancée est qu'aux charges élevées rencontrées en élevage, l'apprentissage de la défense territoriale n'est plus possible.

Il est également possible d'aborder le sujet de l'agressivité par le biais des traits de personnalité (Cf. §4.4). Des différences marquées ont ainsi été montrées dans la prise de risque et le niveau d'agressivité chez des populations naturelles de nombreuses espèces de salmonidés (Adams et al., 1998; McLaughlin et al., 1999). Des truites communes élevées en « sea ranching » sont plus téméraires lorsqu'elles sont confrontées à un nouvel objet que des truites d'origine sauvage, et en conditions de test dyadique, le plus téméraire des deux individus est aussi le plus agressif (Sundström et al., 2003).

### 6.2.6. Les effets sur la cognition

Il a été montré que la domestication avait une influence sur la taille du cerveau. C'est le cas, chez la truite arc-en-ciel pour laquelle les individus domestiqués ont de plus petits cerveaux que des individus sauvages de même taille (Marchetti & Nevitt, 2003). Ceci pourrait avoir un impact sur les capacités cognitives. Comme déjà évoqué précédemment (Cf. §6.2.3), le milieu d'élevage conduit à une succession de générations d'individus non exposés aux stimuli habituellement rencontrés dans leur habitat naturel (prédateurs par exemple) (Brokordt et al., 2006). Ces auteurs émettent l'hypothèse qu'en considérant la plasticité de certains traits comportementaux et morphologiques basés sur la présence ou l'absence de prédateurs, certaines réponses à des stimuli naturels pourraient être atténuées ou même absentes chez les téléostéens domestiqués. D'autres auteurs parlent même de déficits comportementaux exacerbés par l'appauvrissement engendré par le milieu d'élevage (Brown & Laland, 2001; Braithwaite & Salvanes, 2005).

### 6.2.7. Les conséquences au niveau du repeuplement

De nombreuses populations de téléostéens d'origine marine et continentale, ont décliné durant les dernières décennies en raison de la forte pression de pêche (Brown & Laland, 2001; Brown & Day, 2002). Ceci a conduit au développement d'écloseries à des fins de repeuplement au début du siècle (Smith, 1999). Cependant, la majorité de ces programmes donnent des résultats décevants avec des taux de mortalité très importants chez les téléostéens relâchés par rapport à ceux de leurs congénères sauvages de même âge (Godin, 1978; Nordeide et al., 1994; Olla et al., 1994; Phillipart, 1995; Hilborn, 1998; Brown & Laland, 2001). Afin d'améliorer les résultats de ces programmes, les biologistes ont focalisé davantage leur attention sur le comportement des téléostéens relâchés (Olla et al., 1994). Si comme nous l'avons vu précédemment (Cf. §6.2.6), la domestication engendre des déficits comportementaux, il a été néanmoins démontré qu'il était possible d'y remédier en utilisant des bassins d'élevage dotés « d'enrichissements ». Les téléostéens élevés dans ces conditions ont des comportements plus flexibles, possèdent des taux de survie plus importants par le biais de comportements plus adaptés au milieu naturel (comportement alimentaire, réponse anti-prédateur, comportement social, Salvanes & Braithwaite, 2006).

## 7. L'adaptation et le bien-être animal

En reprenant le modèle conceptuel de Frazer et al. (1997) (Cf. §6 et figure 5), lorsque les adaptations de l'animal ne permettent pas de répondre aux situations de challenge rencontrées en élevage, ceci peut résulter en une altération du bien-être. Pour étudier la complexité de ce concept, une communauté s'est constituée récemment (l'Animal Welfare) qui rassemble des éthologistes, des spécialistes de l'univers émotionnel et des aptitudes cognitives, des physiologistes de l'adaptation, des philosophes, des théologiens et des spécialistes d'éthique animale (Bourdon, 2003; Larrère, 2003). Leurs conclusions principales peuvent se résumer comme suit :

Il n'existe pas de définition du bien-être unanimement reconnue. Néanmoins, la plupart des auteurs sont en accord avec la définition de Hughes (1976) selon laquelle le bien-être est un état de complète santé mentale et physique, où l'individu est en harmonie avec son environnement. La notion d'harmonie signifie que les besoins et les désirs d'un individu sont satisfaits (Veissier et al., 2000). Classiquement, trois approches du bien-être animal sont définies : l'approche naturaliste, l'approche adaptative et l'approche mentale (Appleby, 1999).

*L'approche naturaliste*, développée avec l'éthologie, est basée sur l'idée que l'animal doit pouvoir vivre sa vie « naturelle » (Rollin, 1993), c'est-à-dire que l'animal doit posséder une motivation pour réaliser des comportements du répertoire de son espèce. Veissier & Boissy (2009) estiment toutefois que la notion de comportement naturel ne suffit pas à justifier celle de bien-être animal. Il suffit en effet, de prendre l'exemple des comportements anti-prédateurs qui ne sont pas sous-tendus par une motivation positive : c'est l'évitement du prédateur qui compte et non pas la réalisation du comportement.

*L'approche adaptative*, comme nous l'avons vu précédemment, repose sur le principe que tout individu possède des mécanismes d'adaptation à son environnement permettant de maintenir l'homéostasie ou l'allostasie. Lorsque les ajustements de l'individu permettent de réduire aisément l'écart entre l'environnement actuel et les conditions optimales, on juge l'individu adapté et son bien-être préservé (Broom, 2001). En revanche, lorsque l'homéostasie ne peut être maintenue, une réponse de stress est observée, résultant de l'activation de systèmes biologiques particuliers : la branche orthosympathique du système nerveux autonome et l'axe corticotrope (Mormède, 1995). Si l'écart perdure, l'animal s'épuise en cherchant à s'adapter. Le bien-être est alors sur un *continuum* allant d'un niveau très faible à un niveau excellent (Broom, 1993). Cette approche peut permettre d'améliorer les conditions de vie des animaux en réduisant l'écart entre l'environnement réel et l'optimum ou de sélectionner les animaux ayant des capacités d'adaptation élevées (Faure, 1979) qui sont également les plus productifs dans l'environnement qui leur est imposé (Sandoe & Christensen, 1998). Il est néanmoins difficile de déterminer la limite entre le normal (l'adaptation) et l'anormal (le stress) (Veissier & Boissy, 2009).

*L'approche mentale*, enfin, spécifie que le bien-être est un état qui résulte de l'absence d'émotions négatives prolongées (peur, douleur, frustration), voire de la présence d'émotions positives (joie, plaisir, Dawkins, 1983; Boissy et al., 2007).

### 7.1. Les indicateurs de bien-être et de mal-être

En l'état actuel des connaissances, le niveau de bien-être d'un animal s'apprécie en déterminant où il se situe entre les deux extrêmes que constituent l'harmonie et le mal-être. Elle procède donc de deux démarches complémentaires : la recherche des éléments qui concourent à atteindre l'harmonie entre les besoins de l'individu et les conditions environnementales (motivations de l'animal) et l'évaluation des efforts d'adaptation de l'animal placé dans un environnement qui s'éloigne des conditions idéales (Veissier & Boissy, 2009).

Les critères permettant d'atteindre l'harmonie sont évalués par l'analyse des besoins des animaux destinés à définir l'environnement idéal. Ces besoins peuvent être connus à travers l'étude de la physiologie, de l'ergonomie (niveau de confort de l'animal) par le biais de tests de préférence (Chaplin et al., 2000). La référence au comportement spontané de l'animal et l'observation sur de longues périodes au lieu de tests de courte durée permettent d'éviter des conclusions hâtives erronées (Veissier & Boissy, 2009).

Pour les critères permettant d'évaluer le mal-être, plusieurs indicateurs sont utilisés pour apprécier les difficultés rencontrées par un animal dans une situation non optimale. Ce sont des critères comportementaux, physiologiques (réaction de stress), la capacité à produire et à se reproduire, et enfin l'état sanitaire.

#### *7.1.1. Les critères comportementaux*

La première réponse d'un animal face à un évènement extérieur est généralement de nature comportementale. La forme de la réponse dépend de l'espèce et de critères individuels tels que l'âge de l'animal. Elle semble conditionnée par le rapport de force entre l'individu et l'objet du danger et à l'intérieur d'une même catégorie d'individus, certains peuvent systématiquement présenter des réponses passives alors que d'autres présentent des réponses actives (Cf. « coping styles » §4.4).

A plus long terme, lorsque l'animal ne dispose pas de substrats adéquats pour satisfaire un besoin comportemental, des anomalies peuvent apparaître dans l'expression des comportements : reports d'activités vers un autre objet (activités de substitution) ou activités à caractère stéréotypé (Figure 7). Par conséquent, dans un élevage donné, si un fort taux d'activités de substitution, d'activités à vide ou de stéréotypies est relevé, on pourra effectivement conclure à une certaine médiocrité des conditions de vie des animaux. Néanmoins les animaux qui présentent une forte fréquence de comportements anormaux ne sont pas forcément ceux qui souffrent le plus puisque ces activités constituent pour l'animal un moyen d'adaptation (Mason, 1991).

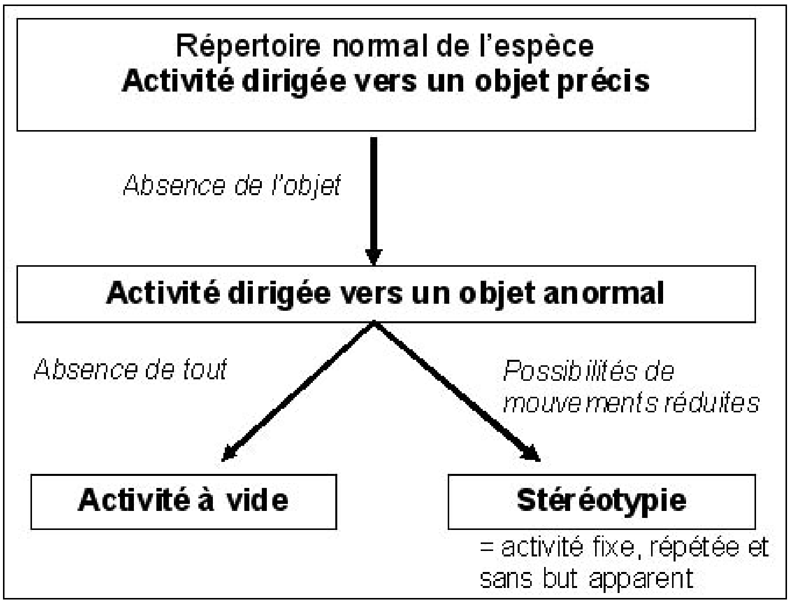

*Figure 7. Apparition d'activités anormales selon les contraintes perçues par l'animal.*
*Le répertoire comportemental d'un animal d'une espèce donnée comprend des activités généralement dirigées vers des objets précis (interactions sociales vers un congénère, activités alimentaires vers un aliment etc.). En l'absence d'objets adéquats pour effectuer une activité particulière pour laquelle l'animal est motivé, celui-ci peut rediriger son activité vers un autre objet. En l'absence d'objet de substitution, des activités à vide peuvent apparaître. Enfin, lorsque les possibilités d'activités sont très limitées, des stéréotypies peuvent apparaître (Veissier & Boissy, 2009).*

### 7.1.2. Les critères physiologiques

Deux grands types d'activation neuroendocrinienne découlent de situations de stress : l'activation de la branche sympathique du système nerveux autonome et l'activation de l'axe corticotrope (Mormède, 1995). La première aboutit à la libération de catécholamines au niveau des terminaisons nerveuses et dans le sang. La seconde se traduit par la libération de corticolibérine (CRF : Cortico-Releasing Factor) par l'hypothalamus, laquelle stimule la production de corticotropine (ACTH : Adreno-Cortico-Tropin Hormone) par l'hypophyse qui elle-même stimule la libération de glucocorticoïdes par les surrénales. Le cortisol qui est le glucocorticoïde majeur des vertébrés est directement mesurable dans le plasma, mais les conditions expérimentales de mesure sont déterminantes. Lorsque l'animal est soumis à un stress chronique, le cortisol qui est un indicateur de stress aigu, ne varie pas forcément.

### 7.1.3. Les critères de production

Lorsque les contraintes persistent, une baisse de l'état général de l'animal peut être observée au travers de critères de production (baisse de croissance, d'appétit, difficultés de reproduction). Ces altérations peuvent être dues à l'activation des systèmes neuroendocriniens impliqués dans le stress (activation consommatrice d'énergie). Toutefois, si on peut supposer un état de souffrance en observant une production amoindrie, l'inverse n'est pas valable. Ainsi certaines pathologies peuvent être associées à de fortes performances de production (Sanotra et al., 2003). La présence de bons résultats zootechniques ne permet pas systématiquement de conclure à l'absence de problèmes liés au bien-être dans la mesure où certains animaux, dominés par les autres par exemple, peuvent avoir une production très affaiblie sans que cela affecte de façon notable la moyenne du groupe (Veissier & Boissy, 2009).

#### 7.1.4. Les critères sanitaires

Dans un élevage, l'apparition de pathologies liées à des agents pathogènes opportunistes peut révéler un stress qui a conduit à une diminution des défenses immunitaires. Toutefois, il existe des cas inverses où le stress va induire une stimulation des défenses immunitaires (Siegel & Latimer, 1975).

### 7.2. Les particularités des téléostéens

Les téléostéens possèdent des structures nerveuses très similaires au plan fonctionnel à celles des vertébrés supérieurs qui sont impliquées dans le vécu émotionnel (Sneddon, 2002). De plus, les téléostéens sont capables de mémoriser des informations sur le caractère aversif d'un évènement en vue d'une utilisation ultérieure (Topal & Csanyi, 1999). Si certains auteurs considèrent que tous les vertébrés sont capables d'éprouver de la souffrance (Lazarus, 1993; Scherer, 2001), la question n'est toujours pas tranchée de façon unanime pour les téléostéens. A l'heure actuelle cependant, de nombreux auteurs considèrent qu'ils éprouvent certains des états physiques et émotionnels qui sont associés chez l'homme à la douleur, sans pour autant avoir la même conscience de la souffrance que les humains (Adron et al., 1973; Braithwaite & Huntingford, 2004; Chandroo et al., 2004; Huntingford et al., 2006). C'est la raison pour laquelle, une première recommandation pour la protection des téléostéens utilisés en élevage a été adoptée par le Conseil de l'Europe le 5 décembre 2005 et est entrée en vigueur le 5 juin 2006. La plupart des critères d'évaluation du bien-être évoqués précédemment (Cf. §7.1) s'appliquent au cas des téléostéens (Barton, 1997; Wendelaar Bonga, 1997; Huntingford & Adams, 2005). Dans la partie qui suit, nous évoquerons uniquement quelques points spécifiques.

#### 7.2.1. Les critères comportementaux

Face à une situation de stress, un téléostéen peut exprimer une large gamme de réponses comportementales. Comme le signale Conte (2004) dans sa revue consacrée au stress et au bien-être des téléostéens d'élevage, puisque le stress peut provoquer des modifications comportementales et qu'inversement, des modifications comportementales provoquées peuvent induire du stress, la connaissance des comportements normaux de chaque espèce est indispensable pour assurer leur bien-être en conditions d'élevage. La cause initiale du stress est souvent liée à un facteur environnemental (Cf. §5) et un changement de comportement reflète la façon dont le téléostéen ressent ce changement (Schreck et al., 1997).

Les principales réponses comportementales sont les caractéristiques et performances de nage, les postures, les réactions d'évitement, la chémoréception, le comportement alimentaire, la réponse anti-prédateur et l'apprentissage (Schreck et al., 1997). Une alimentation inadaptée, en particulier au stade larvaire, conduit souvent à des malformations qui altèrent le développement de réponses comportementales adaptées à des situations de challenges environnementaux (Fletcher, 1997). Inversement un stress aigu répété peut modifier le rythme et la prise alimentaires du bar (Millot et al., 2010). Des valeurs excessives de dioxyde de carbone provoquent des comportements de nage erratique chez le bar rayé (Conte, 2004), des températures inadaptées altèrent les performances de nage du tilapia (*Tilapia mossambica*) (Kutty & Sukumaran, 1975), des pathologies induisent une modification du niveau d'activité : forte activité au départ accompagnée de mouvements en surface, puis diminution de l'activité et léthargie (Warren, 1991). La réponse de fuite peut aussi être altérée par la pollution (Faucher et al., 2006), la température de l’eau (Johnson et al., 1996) ou l’hypoxie (Lefrançois & Domenici, 2006). Le stress peut également altérer les performances d’apprentissage chez le saumon coho (Schreck et al., 1997).

Nous ne reviendrons pas sur les différentes pratiques d’élevage évoquées précédemment (Cf. §5.1), ni sur les facteurs biotiques qui peuvent également constituer des sources de mal-être et de stress (Cf. §5.2).

### *7.2.2. Les critères physiologiques*

Tout ce qui a été décrit chez les vertébrés supérieurs concernant les indicateurs physiologiques du stress (Cf. §7.1.2) est valable pour les téléostéens. Ici, nous donnerons quelques précisions sur les réponses primaires, secondaires et tertiaires observées chez les téléostéens (Figure 8).

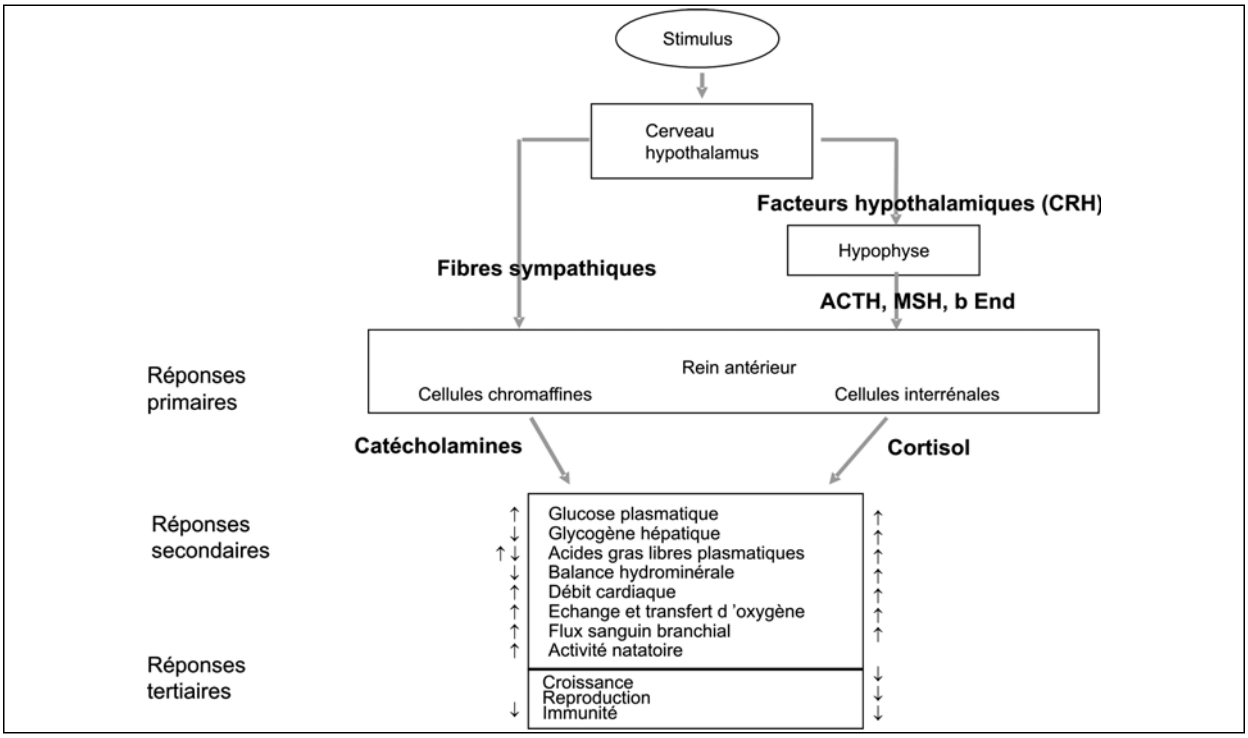

*Figure 8. Principales voies neuroendocriniennes d’intégration de la réponse au stress chez les téléostéens. ACTH : adrénocorticotropine, MSH : hormone stimulant les mélanophores, b End : β-endorphine (d’après Wendelaar Bonga, 1997 ; Prunet & Auperin, 2007).*

Faisant suite aux réponses primaires aboutissant à la sécrétion de cortisol par l’interrénale et de catécholamines par le tissu chromafin du rein antérieur, des réponses secondaires vont être provoquées telles que la respiration, le métabolisme énergétique, l’osmorégulation et la fonction cardiovasculaire (Prunet & Auperin, 2007) puis lorsque la situation de stress se prolonge et que l’animal ne peut plus s’adapter, les modifications de ces fonctions physiologiques peuvent entraîner des réponses tertiaires comme la perte d’appétit, la réduction de croissance, le blocage de la reproduction, la chute de la réponse immunitaire (système essentiellement non spécifique chez les téléostéens).

En pratique, un certain nombre de paramètres sensibles au stress tels que l’hématocrite et d’autres paramètres hématologiques vont pouvoir être mesurés pour évaluer le niveau de stress des téléostéens (Soivio & Oikari, 1976). Le cortisol est probablement le paramètre le plus mesuré pour évaluer le stress, mais il existe de grandes différences interspécifiques avec les niveaux d'élévation les plus importants chez les salmonidés qui sont des espèces métaboliquement actives (Davis & Parker, 1983). Une augmentation de la concentration sanguine en glucose constitue un autre indicateur de stress très sensible chez les téléostéens (Wedermeyer & McLeay, 1981), mais avec des différences intra et interspécifiques elles-mêmes liées à des différences de sécrétion de catécholamines (Mazeaud & Mazeaud, 1981). Il en est de même pour le lactate, produit final du métabolisme anaérobie, qui peut être mesuré chez les téléostéens pour étudier la réponse secondaire au stress (Barton, 1997).

### 7.2.3. L'amélioration du bien-être en élevage

Le respect et le maintien des paramètres physico-chimiques propres à une espèce, une densité d'élevage adaptée, une limitation des manipulations et une attention portée aux changements comportementaux des animaux et à l'apparition de maladies permettent de gérer en partie le stress et d'améliorer le bien-être (Conte, 2004).

Concernant la manipulation des téléostéens, Fernö et al. (2007) précisent que des conditionnements à la peur non planifiés apparaissent fréquemment dans les élevages (par exemple, l'association entre la présence de l'épuisette et le stress de confinement conduit à une réponse de fuite intense dès que l'épuisette est placée à l'intérieur du bassin). L'incertitude quant à la récompense ou à la « punition » associée avec les routines d'élevage pourrait bien augmenter les niveaux de stress. En conséquence, il pourrait s'avérer judicieux d'éviter toute routine d'élevage conduisant à un conditionnement à la peur non planifié et de rendre ces routines prévisibles et de les associer à des évènements positifs. Pour faciliter les manipulations et réduire le stress, divers anesthésiants sont couramment utilisés en aquaculture dont certains tels que le métomidate qui bloquent en plus la synthèse du cortisol et préviennent l'augmentation de glucose sanguin (Thomas & Robertson, 1991).

Le respect du bien-être concerne également les phases d'abattage et de transport des téléostéens. Le transport est une opération comportant de multiples phases qui devraient être organisées de façon à minimiser le stress (Piper et al., 1982). Certaines espèces ne demandent qu'un minimum de précautions (maintien de la qualité de l'eau et de la température) alors que d'autres nécessitent des précautions spécifiques afin d'éviter des problèmes de déséquilibre ionique pouvant causer de fortes mortalités (McDonald & Milligan, 1997). Une période de récupération lors de laquelle les téléostéens ne sont pas manipulés, est généralement observée. Les téléostéens récupèrent de leur réponse physiologique primaire liée à la manipulation et au transport au bout de 6 à 24h, mais la récupération physiologique complète peut prendre de 10 à 14 jours (Schreck et al., 1997). L'abattage quant lui, doit se faire rapidement et de façon à minimiser la peur et la douleur avec des méthodes qui varient localement et d'un pays à l'autre : choc électrique ou mécanique suivis d'une décapitation (méthodes jugées les plus acceptables en terme de bien-être), immersion dans la glace jugée moins acceptable (Conte, 2004).

Une autre possibilité pour **améliorer le bien-être est la sélection génétique de caractères physiologiques et/ou comportementaux favorisant une meilleure adaptation des téléostéens à l'environnement d'élevage** et une tolérance ou une récupération accrues au stress. A ce jour, peu de caractères de bien-être ont cependant été sélectionnés à l'échelle industrielle à part la résistance aux maladies (Chevassus & Dorson, 1990; Gjedrem et al., 1991). La faible réponse au stress basée sur la concentration sanguine en cortisol (Fevolden et al., 1991; Fevolden et al., 1993; Pottinger & Carrick, 1999; Tanck et al., 2001; Fevolden et al., 2002) et du lyzozyme (Fevolden et al., 2002) est également une perspective prometteuse. La sélection de ces critères passe par la mise au point de challenges standardisés et réalisables à grande échelle. Une « meilleure » adaptation peut aussi correspondre à sélectionner des téléostéens possédant une plus grande plasticité phénotypique, mais le problème dans ce cas, est la difficulté de conduire une domestication universelle pour tout système d'élevage (Vandeputte & Launey, 2004). L'interaction génotype-milieu conduira probablement à des divergences importantes entre les différents sites d'élevage.

## *8. La structure de la thèse*

Les deux objectifs principaux de ce travail (Figure 9) sont :

1) De caractériser des facteurs favorisant l'expression de la **plasticité comportementale**, d'analyser la **variabilité des réponses**, de mieux comprendre les liens entre variabilité morphologique et comportementale chez des espèces de téléostéens d'importance écologique et aquacole. Cette approche exploratoire vise l'identification **d'indicateurs précoces** de compétences comportementales ou de traits de personnalité utilisables dans des processus de domestication et de sélection (incluant des critères et objectifs basés sur l'adaptation, le bien-être et la santé des téléostéens). Certains de ces travaux ont pour objectif de mieux comprendre des mécanismes évolutifs généraux tels que les variabilités comportementale et morphologique chez de jeunes téléostéens qui pourraient favoriser dans la nature, l'évolution du polymorphisme.

2) De caractériser les **effets de la domestication sur le comportement**. Cette démarche revient à identifier des indicateurs comportementaux sensibles à une ou plusieurs générations de domestication. Elle servira également à déterminer les comportements adaptés au milieu d'élevage et au milieu sauvage dans la perspective de l'amélioration des programmes de repeuplement. Ces analyses sont essentiellement réalisées à travers une approche comportementale, mais des indicateurs physiologiques, des indicateurs de croissance et d'utilisation de l'aliment viendront parfois compléter les descriptions.

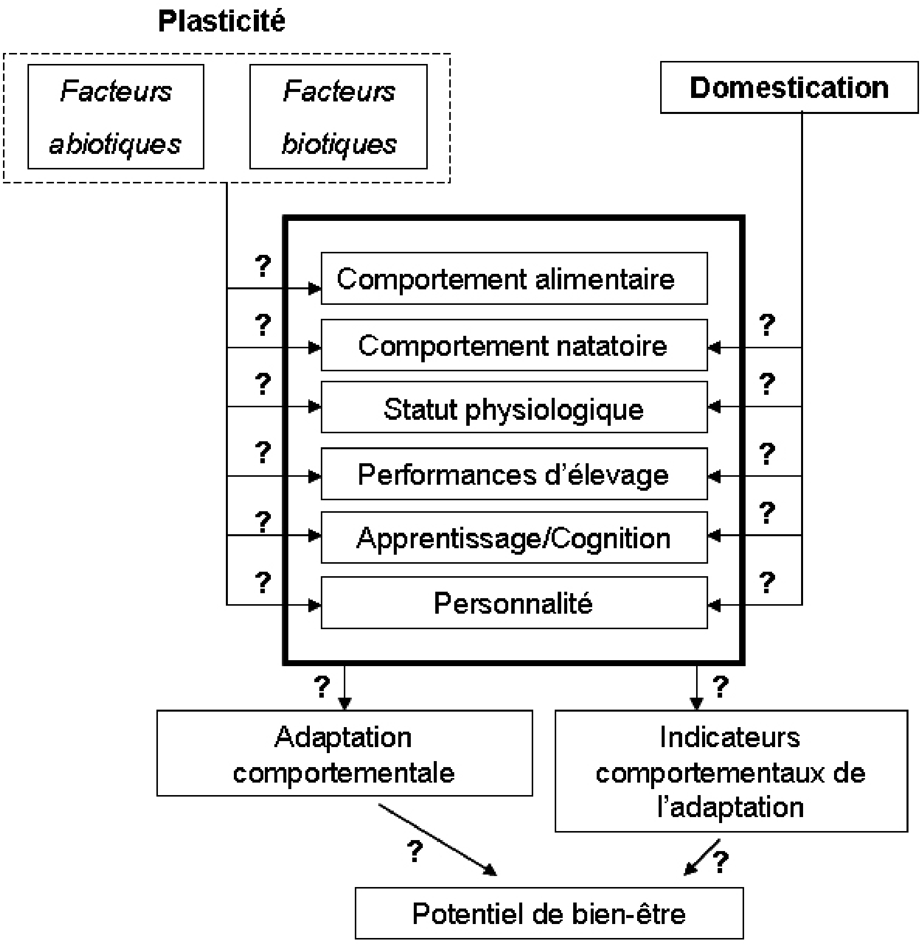

*Figure 9 : Objectifs de la thèse visant à caractériser la plasticité comportementale en réponse à des facteurs biotiques et abiotiques et de caractériser les effets de la domestication sur le comportement, dans le but d'identifier des critères adaptatifs potentiellement utiles pour l'amélioration du bien-être des téléostéens en élevage.*

### 8.1. Les modèles biologiques

Trois modèles biologiques d'intérêt économique ont été retenus dans le cadre de cette thèse (l'omble chevalier *Salvelinus alpinus*, la truite commune *Salmo trutta* et le bar européen *Dicentrarchus labrax*) sur la base de plusieurs critères nous permettant de répondre aux questions posées dans cette thèse. Il s'agissait en effet de choisir des espèces (i) très différentes en termes de cycle biologique, de physiologie et de comportement afin d'obtenir des réponses exploitables à une plus grande échelle, (ii) possédant divers niveaux de domestication/sélection mais aussi des populations sauvages de référence (bar et truite commune) et (iii) des caractéristiques particulières pour approcher les mécanismes de l'écologie de l'évolution (l'omble chevalier). Selon les expériences, les individus ont été étudiés à des âges différents : du stade larvaire au stade adulte. Le stade larvaire désigne la période qui suit le stade embryonnaire et précède le stade juvénile. Précisons que le terme de larve est plutôt réservé aux téléostéens marins qui possèdent en général des œufs pauvres en vitellus (cas du bar). La différence entre le stade larvaire et le stade juvénile ne se traduit pas toujours par une métamorphose très marquée. Chez le bar, le début de la phase juvénile n'est pas strictement défini par un évènement unique. Certains auteurs considèrent que c'est la différentiation de l'estomac qui indique la fin de la période larvaire. Chez les salmonidés, chez qui les œufs sont relativement riches en vitellus, les individus possèdent déjà un estomac fonctionnel au moment de la première nutrition exogène, l'usage courant les désignant sous le terme d'alevins plutôt que celui de larves (Gatesoupe et al., 1999).

### 8.1.1. L'omble chevalier

L'omble chevalier est un téléostéen arctique de l'ordre des salmoniformes et de la famille des salmonidés ayant une distribution boréale circumpolaire. Il est commun dans les régions arctiques et subarctiques de l'Amérique du nord et de l'Europe (Guillard et al., 1992). L'espèce peut se rencontrer dans des milieux très différents (lacs, rivières, mers) sous des formes migratrices anadromes ou sédentaires. Elle est caractérisée par une forte plasticité phénotypique qui s'est exprimée au cours de l'évolution à travers les adaptations locales postglaciaires (Skúlason et al., 1992). La variabilité des propriétés physiques et biologiques des systèmes d'eau douce en Islande est liée en grande partie à l'âge et à la nature du substrat volcanique qui forme diverses parties de l'île au même titre que la végétation, le climat et l'activité humaine (Gardarsson, 1979; Gudjónsson, 1990). Cette variabilité phénotypique touche de nombreux caractères dont la taille des œufs (entre 4,5 et 5 mm), divers aspects morphologiques liés à la nage et à l'alimentation, diverses caractéristiques comportementales en relation avec l'habitat, l'alimentation et la reproduction (Skúlason et al., 1992; Skúlason & Jónsson, 1999).

Outre son intérêt considérable pour une meilleure compréhension de mécanismes évolutifs, cette espèce est également élevée en aquaculture. Depuis les années 80, la production de l'Omble chevalier est en augmentation constante, principalement en Islande, au Canada et dans les pays du nord de l'Europe. L'Islande est à ce jour le plus important producteur d'Omble chevalier. Il s'agit d'une espèce à forte valeur ajoutée qui a atteint en 2007 sa production maximale (3515 tonnes) dont près de 60% pour l'Islande (Statistiques FAO, 2009). La population du lac Ölvesvatn (65°58' N; 20°04' O), fait l'objet d'un programme de sélection au Hólar University College (Islande), désignée en 1993 comme le centre de recherche de référence sur l'Omble chevalier en Islande. Cette population sera utilisée dans le cadre des expérimentations réalisées en Islande.

### 8.1.2. La truite commune

La truite commune est un téléostéen de la famille des salmonidés répartie originellement en Europe et dans le Nord de l'Afrique (Baglinière, 1999). Des études génétiques (Ferguson & Fleming, 1983; Krieg & Guyomard, 1985; Guiffra, 1993; Presa-Martinez et al., 1994) ont révélé une très grande diversité de cette espèce en Europe occidentale avec l'identification de trois rameaux évolutifs pouvant s'apparenter à des sous-espèces géographiques. Il existe trois formes, en rivière, mer et lac, pouvant cohabiter sur un même réseau hydrographique. Cette espèce possède une grande capacité d'adaptation à différents milieux et une forte tolérance vis-à-vis des changements d'habitat (Baglinière, 1999), mais apprécie les eaux riches en oxygène ne dépassant pas 20°C et en rivière, des fonds de pierres ou de graviers. De toutes les espèces de salmonidés, il s'agit de celle qui s'est le mieux établie et d'une manière très large en dehors de son aire de répartition (Baglinière, 1999). La ponte a lieu en automne dans les eaux courantes lorsque la température baisse jusqu'à 6-7°C et la taille des œufs est d'environ 4 mm (Lobon-Cervia et al., 1997) pour un poids frais compris entre 60 et 80 mg (Einum & Fleming, 1999). Passé le stade alevin, la truite abandonne la vie en groupe, mais reste la plupart du temps cachée sur le fond. Cette espèce, plus fragile que la truite arc-en-ciel a une faible croissance en eau douce et n'est donc quasiment pas élevée pour sa chair en pisciculture continentale. En revanche, son intérêt économique réside dans le fait que les truites produites sont pour la plupart destinées au repeuplement et commercialisées à des âges divers en fonction des besoins des sociétés de pêche.

La truite n'a fait l'objet que d'une seule expérience dans le cadre de cette thèse, mais son intérêt est grand dans le cadre de la caractérisation des effets de la domestication, dans la mesure où elle fait partie des espèces les mieux connues en Europe (Baglinière & Maisse, 2002) et que l'INRA possède plusieurs lignées sélectionnées et une grande connaissance des populations sauvages. Deux lots ont été comparés dans cette expérience (Tableau 1) :

1) Un lot sauvage provenant du rameau méditerranéen constitué à partir de géniteurs prélevés dans une zone indemne de repeuplement (Bassin du Fier, Haute Savoie). Le taux d'introgression pour cette souche était de 4.4%. Au total, 16 mâles et 11 femelles ont été utilisés pour réaliser le plan de croisement. Une première génération a été produite en captivité pour réaliser une F2 résultant du croisement entre 13 femelles et 9 mâles sauvages (lot SS, 1000 œufs fécondés) et elle a constitué notre modèle expérimental.

2) Un lot provenant du croisement entre 13 femelles sauvages et de 10 mâles provenant d'une souche synthétique de l'INRA (30 à 40 générations de domestication ; lot SD, 600 œufs décondés).

*Tableau 1. Plan de croisement utilisé pour l'obtention des juvéniles testés dans le cadre de la thèse. S : Sauvage ; D : Domestiqué.*

| | 9 mâles sauvages (Bassin du Fiers) | 10 mâles domestiqués (souche INRA) |
|---|---|---|
| 13 femelles sauvages (Bassin du Fiers) | Lot SS (1 000 œufs fécondés) | Lot SD (600 œufs fécondés) |

### 8.1.3. Le bar européen

Le bar européen est un téléostéen de l'ordre des perciformes et de la famille des Moronidés. Il s'agit d'une espèce marine eurytherme et euryhaline dont l'aire de répartition naturelle comprend les côtes Atlantiques (de la Norvège au Maroc), la Méditerranée et la mer noire. Cette espèce fraie dans les eaux dont la salinité est inférieure à 35‰, près des embouchures des rivières et des estuaires ou dans les zones littorales où la salinité est supérieure à 30‰. Les œufs sont pélagiques et de petite taille (1,02-1,39 mm) et contrairement aux salmonidés, l'éclosion donne suite à un stade larvaire. Dès l'ouverture de la bouche, les larves de bar qui occupent le méroplancton littoral, vont être caractérisées par des comportements de prédation mettant en jeu un stimulus visuel (Barnabé, 1989). Au stade juvénile, cette espèce va occuper la zone littorale, les étangs ou les estuaires au printemps avant de gagner la pleine mer à l'automne. A l'âge adulte, le bar jusque-là pélagique, devient démersal vivant le plus souvent au voisinage du fond. C'est à partir de ce moment que le caractère ubiquiste de cette espèce est acquis. Des phénomènes de grégarisme ont été mis en évidence en captivité et observés en milieu naturel chez des spécimens de 30-40 mm (Barnabé, 1978).

Il s'agit d'une espèce de grande importance commerciale qui fait partie des quelques espèces pionnières de l'aquaculture marine. C'est en France, dans les années 80 qu'a démarrée la production de cette espèce avant de se développer en Europe, essentiellement dans le bassin Méditerranéen pour atteindre en 2000, le seuil des 50 000 tonnes (Source FAO, 2006). Plusieurs groupes de bars ont été utilisés dans le cadre de cette thèse :

1) Un lot de larves issu de l’Ecloserie Marine de Gravelines (France) dont l’origine génétique n’est pas réellement connue (probablement issu d’une cinquième génération de domestication et de sélection sur la longueur) et qui a été utilisé comme standard industriel.

2) Un lot de juvéniles issu de l’entreprise de grossissement Aquanord (France), précédemment produit à l’Ecloserie Marine de Gravelines et également utilisé comme standard industriel.

3) Un lot de bars sauvages capturés dans le milieu naturel (sur la côte Méditerranéenne, au cap d’Agde: 43° 58’ N; 03° 30’ 19’’E) à l’état de larves.

4) Deux lots sélectionnés à la station Ifremer de Palavas sur les performances de croissance en relation avec deux types d’aliments expérimentaux isoénergétiques (Figure 10) : un aliment dont les lipides et les protéines proviennent exclusivement de matières premières d’origine végétale (Vg) et un aliment à base de matières premières d’origine marine (Mar) (projet FUI *Vegeaqua*). Les deux lots sont le résultat d’un croisement factoriel complet entre 9 mères et 25 pères, les 225 familles ayant été élevées en commun depuis le stade de l’œuf. Ils sont nourris avec les aliments expérimentaux à partir d’un poids initial de 2,5 g. A 20 g, les individus sont marqués à l’aide d’un PIT tag et un échantillon d’ADN est prélevé pour assignation *a posteriori* des parentés à l’aide de marqueurs microsatellites.

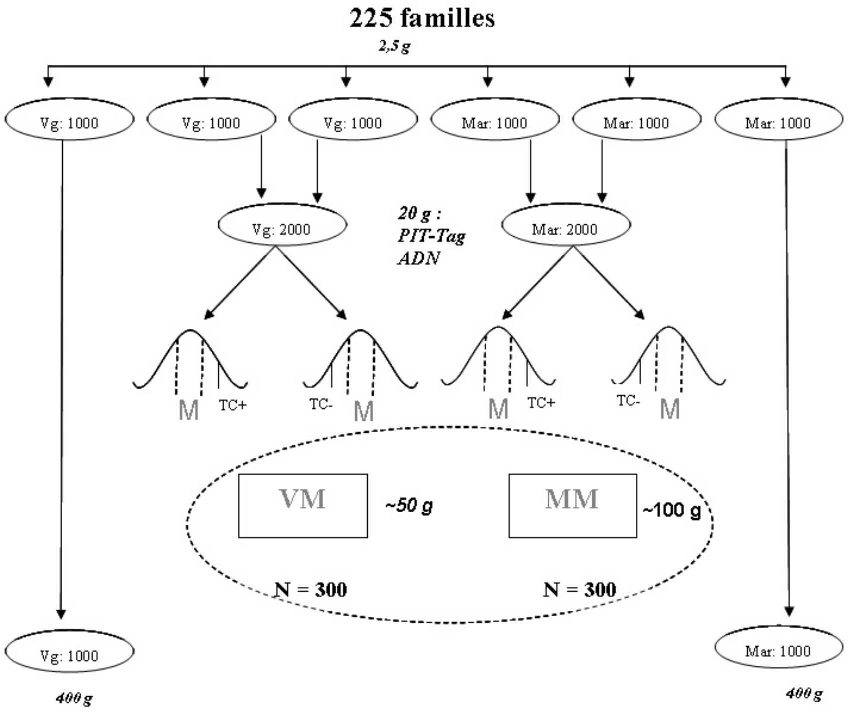

*Figure 10 : Lots de bars sélectionnés sur des critères de croissance à la station Ifremer de Palavas-les-Flots. Vg : lot nourri avec de l'aliment expérimental constitué de 100% de matières premières d'origine végétale ; Mar : lot nourri avec de l'aliment expérimental constitué de 100% de matières premières d'origine marine ; TC+ : taux de croissance les plus élevés ; TC- : taux de croissance les plus faibles ; M : lots possédant un taux de croissance moyen. Le cercle en pointillés désigne les deux lots de 300 individus utilisés dans le cadre de la caractérisation comportementale.*

### 8.1.4. Les études réalisées dans le cadre de cette thèse

Les deux objectifs de la thèse structurent ce manuscrit en deux parties. Les divers lots, variables étudiées et techniques utilisées sont résumés dans le tableau 2.

Dans la partie 1 (Plasticité comportementale) certains **facteurs abiotiques et biotiques pouvant agir sur la plasticité comportementale**, sont étudiés :

-Chapitre 1 : l'influence d'un premier facteur abiotique (un **refuge artificiel**) sur le comportement (mobilité), les performances d'élevage (poids, taille), la survie et la date de première alimentation exogène de l'**omble chevalier,** est étudiée durant la phase d'alimentation endogène.

-Chapitre 2 : le second facteur abiotique testé est l'influence d'un **aliment** constitué de 100% de lipides et de protéines d'origine végétale sur le comportement du **bar**. Dans un premier temps, le comportement alimentaire (quantité d'aliment demandée, ingérée et gaspillée, rythme alimentaire) sous self-feeder (distributeur d'aliment à la demande) est comparé à celui d'un lot témoin nourri avec de l'aliment classique. L'étude est focalisée sur la phase d'apprentissage sur self-feeder (30 premiers jours). Tous les individus sont marqués par un PIT-Tag. Dans un second temps, nous comparons les capacités cognitives et l'exploration (variables cinématiques, temps passé dans chaque zone) de 10 individus de chaque lot, testés un par un dans un labyrinthe en T doté de symboles associés à une récompense (présence d'un congénère derrière une paroi transparente totalement étanche). Le statut physiologique de ces individus est également testé (variables sanguines dont le glucose et le cortisol).

-Chapitre 3 : l'interaction de deux facteurs biotiques (la **taille de l'œuf et l'environnement social**) sur le comportement alimentaire, la mobilité et l'agressivité est analysée dans une expérience réalisée sur des juvéniles d'**omble chevalier** observés à différents âges à partir de la période de première alimentation exogène. L'influence relative de ces deux facteurs est estimée à partir de la comparaison de conditions d'isolement (isolement depuis l'éclosion *versus* isolement à court terme) et de groupe (groupes mixtes en taille *versus* groupes homogènes).

-Chapitre 4 : une expérience réalisée sur le **bar** (lot standard industriel) sous self-feeder, permet de tester l'influence d'un facteur abiotique (le **tri**, pratique d'élevage courante en aquaculture, cf.4.1.2) puis d'étudier, l'influence du facteur biotique qui en découle, à savoir, l'influence de la structure en taille de deux lots (trié ou non) sur diverses variables : le comportement alimentaire (quantité d'aliment demandée, ingérée et gaspillée, rythme alimentaire), la structure sociale (basée sur le niveau d'activation du self-feeder), les performances de croissance et le statut physiologique des téléostéens (paramètres sanguins dont le glucose).

-Chapitre 5 : une expérience menée sur les mêmes lots, a permis de comparer les mêmes variables avant et après l'application d'une période de jeûne afin de déterminer le rôle de la motivation alimentaire sur l'acquisition du statut de manipulateur principal. La comparaison des structures sociales établies dans ces deux conditions permet de tester l'hypothèse de traits de **personnalité** gouvernant l'activité d'alimentation à la demande chez le **bar** et d'estimer son niveau de plasticité.

Dans la partie 2 (domestication *versus* sauvage) les effets de la domestication sur le comportement des individus sont étudiés en s'appuyant sur la comparaison de lots sauvages et domestiqués.

-Chapitre 6 : l'activité de **nage** (variables cinématiques) est comparée entre deux lots SS et SD (Cf. §8.1.2) de **truites** juvéniles avant et après l'application d'un stress sous forme d'une courte phase de 5 minutes d'obscurité suivi d'un rétablissement brutal de la lumière. L'appartenance au groupe d'origine (Fier x domestique atlantique ou Fier x Fier) est vérifiée par génotypage. Cette comparaison permet d'évaluer l'influence d'une hybridation **sauvage** x **domestiqué** sur le comportement de la truite.

-Chapitre 7 : l'effet de la **domestication** sur le comportement de la larve de **bar** est abordé par la comparaison d'un lot de larves **sauvages** capturées dans le milieu naturel et d'un lot de larves **domestiquées**. Diverses variables cinématiques ainsi que le taux de **réponse de fuite**, sont été analysés sur des larves placées individuellement dans des enceintes et soumises à un stimulus (tige lâchée dans l'eau). L'expérience est répétée à différents âges afin d'évaluer l'impact de la **captivité** sur le comportement des larves sauvages.

-Chapitre 8 : le comportement d'exploration (variables cinématiques et temps passé dans chaque zone) de juvéniles de **bars sauvages** et **domestiqués** est testé individuellement dans un labyrinthe en T avec un congénère non familier placé derrière une paroi transparente à l'extrémité d'un des bras. Cette expérience permet d'évaluer l'impact de la domestication sur le comportement **social**, sur le comportement **natatoire**, l'**exploration** en situation de challenge (découverte d'un environnement non familier) et de tester la possibilité d'utiliser un congénère non familier en tant que récompense associée à un apprentissage.

-Chapitre 9 : dans un autre type de labyrinthe en T dont chacune des deux extrémités est dotée d'une paroi transparente derrière laquelle est placé un individu familier ou non (sauvage ou domestiqué), deux lots de 20 bars **sauvages** et **domestiqués** sont testés individuellement en se référant aux mêmes variables que celles décrites pour le chapitre 8. Cette expérience permet de tester la capacité de **reconnaissance visuelle** et d'évaluer l'influence de la domestication sur la reconnaissance visuelle.

-Chapitre 10 : les **capacités cognitives** et l'**exploration** (variables cinématiques, temps passé dans chaque zone) de 10 **bars** juvéniles **sauvages** et **domestiqués** sont testées individuellement dans un labyrinthe en T doté de symboles associés à une récompense (présence d'un congénère derrière une paroi transparente totalement étanche).

*Tableau 2 : Récapitulatif des différentes parties et chapitres de cette thèse, du matériel biologique, des facteurs testés, des variables étudiées et des techniques ou méthodes utilisées. Vg : lot nourri avec de l'aliment constitué de 100% de matières premières d'origine végétale ; Mar : lot nourri avec de l'aliment constitué de 100% de matières premières d'origine marine ; S : lot sauvage ; D : lot domestiqué ; SS : lot sauvage ; SD : lot hybride (sauvage X domestiqué).*

| **Partie/Chapitre** | **Matériel biologique** | **Facteurs testés** | **Variables étudiées** | **Techniques ou méthodes utilisées** |
|---|---|---|---|---|
| 1/1 | Omble chevalier | Abiotique<br>(refuge artificiel) | Mobilité<br>Croissance<br>Survie<br>Première nutrition exogène | Enregistrements et analyses vidéo |
| 1/2 | Bar<br>Lots VG et MAR | Abiotique<br>(aliment végétal) | Comportement alimentaire<br>Croissance<br>Statut physiologique<br>Apprentissage/Cognition<br>Comportement natatoire<br>Exploration | PIT tag<br>Self-feeder<br>Analyses de sang<br>Labyrinthe<br>Enregistrements et analyses vidéo |
| 1/3 | Omble chevalier | Biotiques<br>(taille de l'œuf x environnement social) | Comportement alimentaire<br>Mobilité<br>Agressivité | Enregistrement vocal |
| 1/4 | Bar<br>Standard industriel | Abiotique/Biotique<br>(tri) | Comportement alimentaire<br>Croissance<br>Structure sociale<br>Statut physiologique | PIT tag<br>Self-feeder<br>Analyses de sang |
| 1/5 | Bar<br>Standard industriel | Abiotique<br>(jeûne) | Comportement alimentaire<br>Croissance<br>Structure sociale<br>Statut physiologique | PIT tag<br>Self-feeder<br>Analyses de sang |
| 2/6 | Truite<br>Lots SS et SD | Réponse à un stimulus lumineux | Comportement natatoire | Enregistrements et analyses vidéo |
| 2/7 | Bar<br>Lots D et S | Réponse à un stimulus mécanique et visuel | Taux de réponse de fuite<br>Comportement natatoire<br>Exploration | Stimulus standardisé<br>Enregistrements et analyses vidéo |
| 2/8 | Bar<br>Lots D et S | Congénère non familier | Comportement natatoire<br>Exploration | Enregistrements et analyses vidéo |
| 2/9 | Bar<br>Lots D et S | Congénère non familier *vs.* familier | Comportement natatoire<br>Exploration | Enregistrements et analyses vidéo |
| 2/10 | Bar<br>Lots D et S | Cognition | Apprentissage/Cognition<br>Comportement natatoire<br>Exploration | Enregistrements et analyses vidéo |

# Partie 1 : Plasticité en réponse à des facteurs biotiques et abiotiques

# Chapitre 1

# Impact of a new artificial shelter on Arctic charr (*Salvelinus alpinus*, L.) behaviour and culture performance during the endogenous feeding period

D. Benhaïm, C.A. Leblanc, G. Lucas

*Aquaculture, 2009, 295, 38-43*

# Résumé

Nous abordons dans ce premier chapitre l'influence d'un facteur abiotique, la présence d'un refuge artificiel, sur le comportement de l'omble chevalier à un stade de développement très précoce puis les conséquences sur les performances d'élevage qui en découlent sont analysées.

Il est bien connu que les refuges sont d'une importance majeure chez de nombreuses espèces de téléostéens que ce soit dans un contexte naturel ou d'élevage. Le comportement de recherche de refuge de l'omble chevalier a été cependant peu étudié dans un contexte aquacole. Ici, nous testons l'utilisation d'un nouveau type de refuge constitué d'un drain agricole coupé en deux et analysons les effets sur les performances d'élevage et le comportement de l'omble chevalier durant la période de nutrition endogène. Ce dispositif présente des sillons dans lesquels les alevins viennent spontanément se placer en position verticale. Une première expérience a comparé l'activité des ombles en présence ou non d'un refuge disposé dans les compartiments d'un incubateur. Tous les individus ont été mesurés à 122, 158 et 190 jpf (jours post fertilisation) et observés à 126, 139 et 157 jpf. Les ombles disposant d'un refuge ont montré de meilleures performances de croissance, une mortalité plus faible et ont entamé le stade de première nutrition exogène environ six jours plus tôt que les individus qui n'en disposaient pas. Ces performances sont clairement associées à une mobilité beaucoup plus faible chez les individus disposant d'un refuge. Ainsi à 126 jpf, tous les ombles disposant d'un refuge sont immobiles tandis que les autres sont caractérisés par des mouvements stationnaires horizontaux (86%) ou nagent contre le courant (7%). A 157 jpf, 85% des individus sans refuge sont immobiles contre 95% des individus avec refuge. Le plus souvent, un seul individu par sillon est observé et une valeur moyenne de 2.4 sillons est maintenue entre deux individus successifs durant toute l'expérience. La seconde expérience est un test de préférence où les individus ont été placés dans des compartiments similaires, mais occupés par la moitié d'un refuge. Dans cette configuration, 61% des individus sont localisés sous le refuge à 122 jpf alors qu'à 157 jpf, 58% sont situés sur le refuge et 42 % sont en dehors du refuge.

Ces résultats révèlent une plasticité des caractéristiques comportementales chez l'omble chevalier en présence d'un nouveau type de refuge artificiel à un stade de développement très précoce. Ce refuge permet aux individus de se positionner verticalement sans produire de mouvement latéral et de choisir par eux-mêmes leur localisation tout au long de leur développement. Ce dispositif pourrait donc être utilisé pour améliorer les performances d'élevage et le bien-être de l'omble chevalier durant la période de nutrition endogène.

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# Impact of a new artificial shelter on Arctic charr (*Salvelinus alpinus*, L.) behaviour and culture performance during the endogenous feeding period

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ABSTRACT

Shelter is of major importance in many species of fish both in the wild and in aquaculture. Sheltering behaviour of Arctic charr has been poorly studied in aquaculture. A new type of shelter made of PVC agricultural drain cut in half was tested on culture performance and behaviour during the endogenous feeding period. This device offered grooves where alevins could position themselves and lie in a vertical position. A first experiment compared fish with and without shelter in incubator compartments. All fish were measured at 122, 158, and 190 days post fertilization (dpf) and observed at 126, 139 and 157 dpf. Fish provided with shelter showed better growth performances, lower mortality and started first exogenous feeding about six days later compared to fish without shelter. These effects from shelter provision were also associated with much less mobility in fish provided with shelter. At 126 dpf, all fish provided with shelter were immobile while other fish displayed horizontal stationary body movement (86%) or swam against the water current (7%). At 157 dpf, 85% of fish without shelter were immobile compared to 95% in fish provided with shelter. Both categories displayed stationary body movements. In most cases, there was one single fish per groove and a stable average value of 2.4 grooves was maintained between two successive fish throughout the study. The second experiment was a preference test where the fish were placed in similar compartments but occupied by a half shelter. We found that 61% of the fish were located under the shelter at 122 dpf while at 157 dpf, 58% were on the shelter and 42% out of the shelter. The present results revealed Arctic charr behavioural characteristics in the presence of a new type of artificial shelter at very early stages of development. This shelter enables the fish to stabilize in a vertical position without producing any movement and to choose by itself its favorite location throughout development. This device could be used to improve Arctic charr growth, performance and fish welfare during the endogenous feeding period.

## 1. Introduction

Cryptic or sheltering behaviour has been extensively documented in fishes, including the European minnow (*Phoxinus phoxinus* (L.) (Greenwood and Metcalfe, 1998), coho salmon *Oncorhynchus kisutch* (Walbaum), steelhead trout *Oncorhynchus mykiss* (Walbaum) (Bustard and Narver, 1975), Atlantic salmon *Salmo salar* (L.) (Cunjak, 1988) and the coelacanth *Latimeria chalumnae* (smith) (Fricke et al., 1991). It has been reported that among these species, salmonids show some kind of sheltering behaviour during the day in winter (Cunjak, 1988; Heggenes et al., 1993; Greenwood and Metcalfe, 1998). The occurrence of such behaviour has been linked to risk of predation, light intensity and/or developmental stages (e.g. Valdimarsson and Metcalfe, 1998; Klemetsen et al., 2003).

Field and laboratory studies (Smith and Griffith, 1994, Suttle et al., 2004) have compared salmonid performance in very different substrates (e.g. fine sand *versus* coarse gravel). Sheltering behaviour was beneficial as it led to higher survival even when predators were excluded (Smith and Griffith, 1994). Indeed, the presence of rocks allowed trout to shelter in spaces between rocks which provided them with a reduction in day-time energy expenditure. A number of other advantages of sheltering behaviour have been reported in the literature including thermal regulation and avoidance of high water flows (Valdimarsson and Metcalfe, 1998), avoidance of anchor ice (Heggenes et al., 1993; Whalen et al., 1999), and protection from light at cold temperatures (Cunjak, 1988; Contor and Griffith, 1995).

Sheltering behaviour is of great importance at early life stages in fishes. For example, it is known that juvenile salmonids shelter in streambed interstitial spaces to avoid predators (Griffith and Smith, 1993; Heggenes et al., 1993; Valdimarsson and Metcalfe, 1998). This behaviour has also been reported in Arctic charr (*Salvelinus alpinus*) juveniles in very shallow water and close to the shore of the lake where they showed a clear preference for boulder and bedrock substrates (Klemetsen et al., 2003). Overall, sheltering behaviour and availability of refuges seem to benefit alevins and juveniles of

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salmonids in terms of growth and avoidance of predator. However little is known about the sheltering behaviour of yolk-sac alevins and especially the benefits of such behaviour.

The recognized importance of sheltering behaviour in natural conditions has led to experiments in salmonid aquaculture in order to promote growth. This interest is driven mostly by the fact that yolk-sac alevins and later alevins incubated in hatcheries are often smaller than wild ones, even if they come from the same sized eggs (Quinn, 2004). In the hatchery, yolk-sac alevins and alevins are commonly reared on flat-screened hatching trays without any substrate, which may lead to increased swimming activity and decreased growth due to the increasing energy expenditure (Bailey and Taylor, 1974; Leon, 1975; Dill, 1981). Several attempts have been made to achieve a more natural environment in the hatchery by using gravel or artificial substrates in the trays (Leon and Bonney, 1979; Leon, 1975; Dill, 1981). Artificial substrates included polyethylene astro-turf (Hansen and Torrissen, 1984; Nortvedt, 1986) and other inert synthetic shade materials (Coulibaly et al., 2007). Arctic charr embryos reared in gravel during the endogenous feeding period showed higher yolk conversion efficiency, and converted more yolk to body tissue when compared to alevins reared on a flat bottom (Alanärä, 1993). However, gravel is not a suitable substrate in aquaculture because of the risk of fungal infections and the difficulty of removing embryos mechanically without injuring them (Alanärä, 1993). Instead, Alanärä (1993) suggested the use of some temporal, easily removable artificial substrate. When incubators with artificial substrates are compared to the traditional flat-screened system, artificial substrates have been shown to improve fish growth and survival during feeding (Leon, 1975; Leon and Bonney, 1979; Eriksson and Westlund, 1983; Hansen and Moller, 1984; Hansen, 1985).

In Icelandic Arctic charr farming, a specific type of astro-turf mat is placed underneath the eggs some time before hatching. The mat is supposed to simulate the natural environment and be a kind of shelter or supportive element to the yolk-sac alevins. In the mats there are narrow furrows which individuals can enter when they hatch. It has been hypothesized that in this system the yolk-sac alevins do not move much and use yolk sac nourishment preferentially for growth. Here we test the potential benefits of a new type of artificial shelter for early developmental stages of Arctic charr in culture. The system tested in this study has similar properties to the mat described above and provides the yolk-sac alevins with both substrate and shelter, but the device also allows for behavioural observations throughout the development process. Other advantages of this device include ease of cleaning and facilitation of the transfer of embryos at the onset of feeding.

The objective of this study was to examine the importance of this new device for the behaviour (mobility) and culture performance of free swimming Arctic charr embryos, from hatching and until the

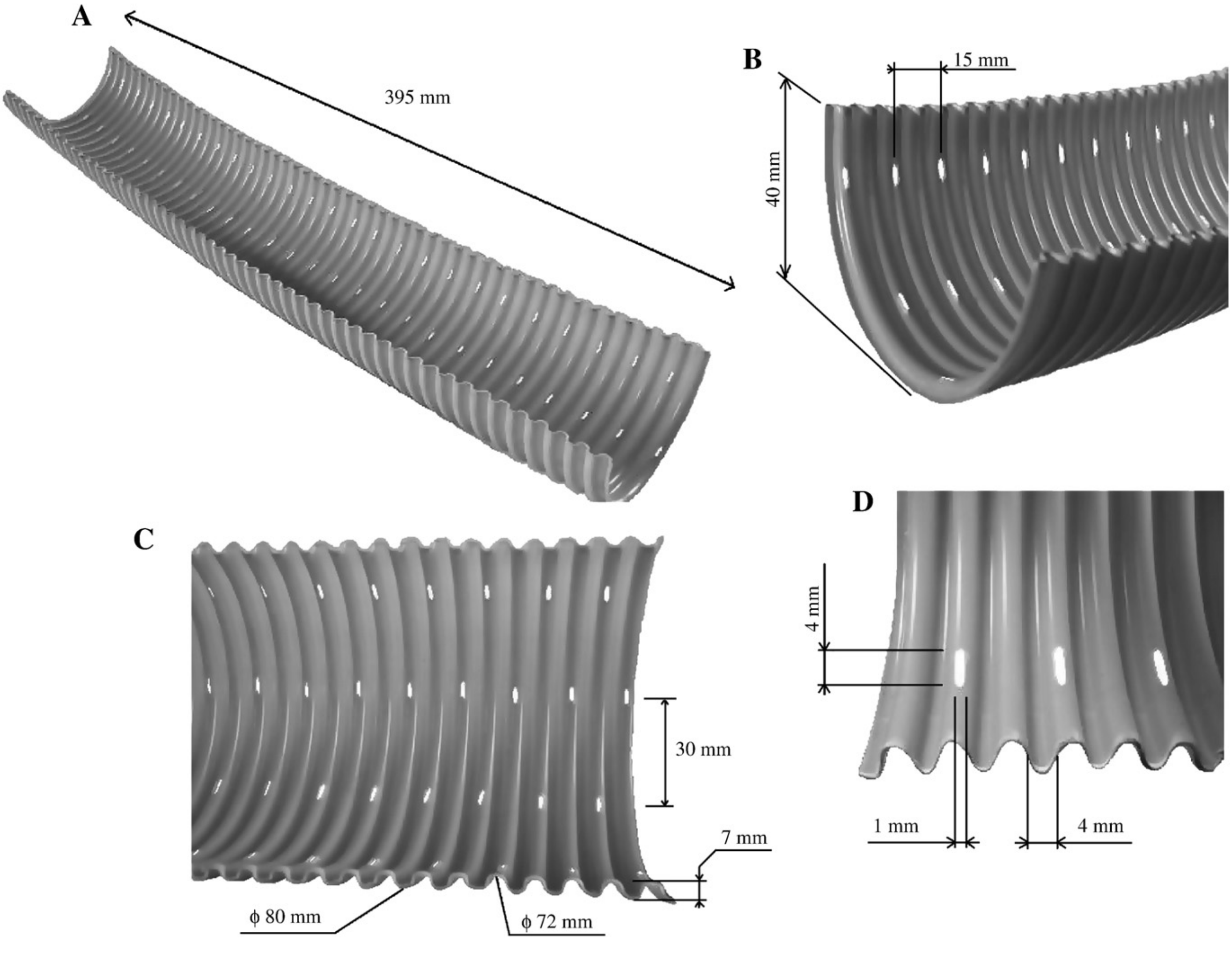

**Fig. 1.** Shelter made of agricultural drain. (A) Overall view of the shelter. (B) Lateral view showing shelter height and the distance between two slots. (C) Overhead view showing external and internal diameters, distance between two central slots and height of one groove. (D) Magnified view of internal part of the shelter showing slot dimensions (lenght and width) and the groove width.

onset of first feeding. We approached this objective by addressing three main questions:

1) What is the influence of the device on fish behaviour, survival, growth, and the date of first feeding?
2) How do fish use the device throughout the yolk-sac alevin period?
3) Do yolk-sac alevins prefer to use the device or a flat bottom?

## 2. Materials and methods

### 2.1. Fish and experimental set-up

In the study we used Arctic charr from Hólar University College breeding program. The experimental fish came from the fertilization of eggs from 13 females with the sperm from one male on 19th of November 2007. After hatching, juveniles were placed in three EWOS hatching trays (39.5 × 42.5 × 17.2 cm). In each EWOS tray, we installed six compartments (39.5 × 7 × 17.2 cm) as the arena for observations. The three trays were placed randomly in one tank (250 L) and moved each week to reduce the impact of small differences in environmental variables such as temperature, light, or oxygen availability.

The shelter was easy to build and made of yellow PVC agricultural drain (Raudren G, DN 80, produced by Rehau company) cut in half (80 mm external diameter). The pipe had both internal and external grooves. We created a shelter by cutting a 39.5 cm length of this pipe which had 53 grooves on both sides (Fig. 1). The shelter had holes in it that allowed water to flow through the internal part of the device.

Water temperature was maintained at 4.9 ± 0.5 ºC throughout the observation period and water level was held at 12 cm in each compartment. The flow rate was 53 ml $s^{-1}$ in each compartment. Opaque black plastic covered the trays to reduce any disturbance to the fish. The plastic was removed during observations, but light was kept below 50 lux at the water surface.

### 2.2. Experiments

We conducted two experiments to estimate the importance of the shelter for the fish. The first experiment focused on growth, survival and behaviour. The second experiment focused on examining if the yolk-sac alevins selected the shelter over no shelter. Both experiments started on the 17th of March 2008, when the fish had hatched.

In the first experiment 12 compartments were set up, six assigned with a shelter treatment and six with a no shelter treatment i.e. plain flat bottom. Shelters were created by covering the inner surface on the floor of the compartment with PVC agricultural drain in the longitudinal direction (flow direction). We randomly assigned 100 fish from a pool of the 13 families to each compartment. To measure growth throughout the experiment, a sample of 20 fish were collected from each compartment at 122, 158 and 190 days post fertilization (dpf). Age of fish referred to day post fertilization (dpf) since hatching is influenced by many epigenetic and environmental stimuli and thus is a poor indicator of developmental state (Balon, 1985).

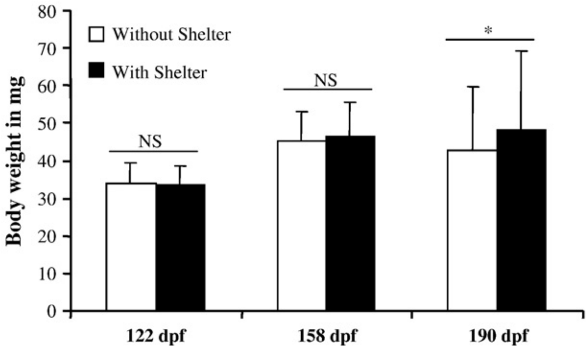

**Fig. 2.** Mean ± S.D. Weight difference in fish. Fish were weighted at three different dates expressed in days post fertilization (dpf). Star over bar indicates significant difference (Mann–Whitney test, $p < 0.05$).

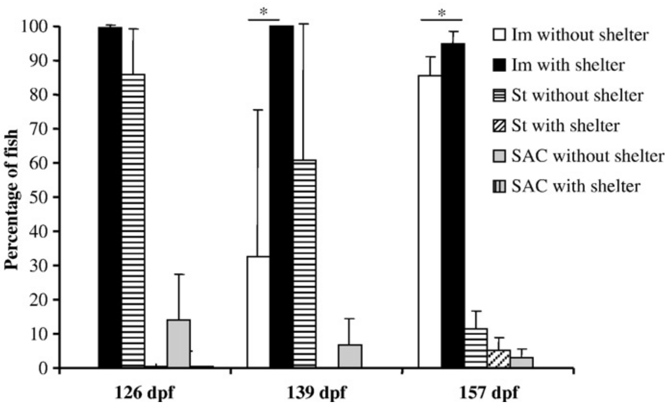

**Fig. 3.** Mean ± S.D. Behavioural activity in fish provided or not with shelter during endogenous feeding period at three different dates expressed in days post fertilization (dpf). Im: Immobility, St: Horizontal stationary body movement, SAC: Swimming against current. Star over bar indicates significant difference (Mann–Whitney test, $p < 0.05$).

The fish were anesthetized using 2-phenoxyethanol (0.3 ml $l^{-1}$) and weighed (to the nearest 0.0001 mg). The fish were then allowed to recover and released back into their compartments. At 190 dpf length (to the nearest mm) was measured as well in order to calculate condition factor $K$ for each fish using the formula: $K = W/L^3$ where $W$ is weight (g) and $L$ (mm) the total length. Specific Growth Rate (SGR) was also calculated using the following formula:

SGR (% body weight per day) = 100 (Ln BWf–Ln BWi)/$t$ where BWf and BWi are the final and the initial mean body weight (g) respectively and $t$ the number of days.

Mortality was recorded and dead fish removed every day during the two months the experiment was conducted. For data analysis, the total number of fish that died each week was used.

When more than 50% of fish in a compartment displayed foraging activity we designated that as the date of first feeding (in dpf). We could estimate approximately when this was going to happen and for 10 days around the date of first feeding, the fish were observed for 5 min after food delivery to detect foraging.

At 126, 139 and 157 dpf, fish behaviour was measured. A camera (Canon XL2) was placed 70 cm above the tray, thus allowing for recording of four compartments at the same time. The compartments were videotaped for 2 min that started when the opaque plastic was slowly removed. To ensure the mobility was not overestimated, the first minute of the video sequence was not used for the data analysis.

One minute into the video, a unique scan sample (Altmann, 1974) of all fish was performed. Five behavioural variables were recorded: Immobility, Horizontal stationary body movement, Speed swimming as defined by Benhaïm et al. (2003), and Swimming against current which corresponds to a fish movement clearly oriented against the current. The position of fish in compartments provided with shelter was also recorded as either: on the shelter, or under the shelter. An index was created to examine the space between fish. The scores were 0 when two fish were in the same groove, 1 when one fish was in one groove and one fish was in the next one, 2 when two fish were separated by one empty groove etc. The number of fish in each groove expressed in percentage of groove occupancy was also examined.

The second experiment took place in six compartments where a shelter device, similar plastic pipe as described in the first experiment, was placed. Here, the device covered only half the length of each compartment, dividing the compartment in two equal parts: bottom covered with the device (shelter) and plain bottom (no shelter).

Thirty fish were introduced in each compartment at 5:00 pm. The next day at 10:00 am, the numbers of fish on, under and out of the device were counted. This preference test was run at 122, 137 and 157 dpf.

### 2.3. Data analysis

The results of the two experiments were compared using *t*-tests and one way ANOVA, with Scheffe post-hoc tests. When the data did not fulfill requirements of homoscedasticity and normality of the residuals we used Mann–Whitney and Kruskal Wallis tests. *Z*, the normal approximation to the Mann–Whitney test, was reported when sampling size was greater than 20 (Zar, 1996). To test for the effects of shelter on body weight, condition factor, and behavioural items at each date, a *t*-test or a Mann–Whitney test were used. Effects on mortality and day of first feeding were tested using a Mann–Whitney test. The averaged space between two fish in compartments provided with shelter, and the number of fish under or on shelter were compared between dates (126, 139, 157 dpf) using a Kruskal Wallis test. The percentages of groove occupancy were tested using ANOVA, where the type of groove occupancy (0, 1, 2 or 3 fish per groove) and date (126, 139, 157 dpf) were defined as fixed factors. Post-hoc Scheffe tests were conducted to compare the differences between the three different groove occupancy types.

The preference test was conducted using ANOVA, where fish location (under, on or out of the shelter) was defined as fixed factor. Post-hoc Scheffe tests were conducted to compare the differences between the three locations.

All statistical analyses were conducted in SPSS 14 (SPSS, Inc., Chicago, Illinois, U.S.A.).

## 3. Results

### 3.1. Culture performances

Body weight at the end of the yolk-sac alevin period was higher in fish provided with shelter ($Z = -2.06$, $P = 0.04$; Fig. 2). SGR between the first and the last date was higher for fish provided with shelter (0.39 ± 0.47%) compared to fish without shelter (0.22 ± 0.39%). Fish in shelter had higher condition factor (4.26 ± 1.21) compared to fish without shelter (4.02 ± 0.97) but the difference was not significant ($Z = -1.84$, $P = 0.06$). The mortality was always lower in fish provided with shelter but only the cumulative mortality difference was significantly higher in fish without shelter ($U = 1875$, $P < 0.0001$). Over the study period, the mortality rate was 3.2% among fish provided with shelter and 7.2% among fish without shelter. Mortality increased shortly before the swim-up stage. First feeding occurred earlier in fish without shelter (182.66 ± 4.13 dpf) than in those with shelter (188.00 ± 1.79 dpf) ($U = 4$, $P = 0.025$).

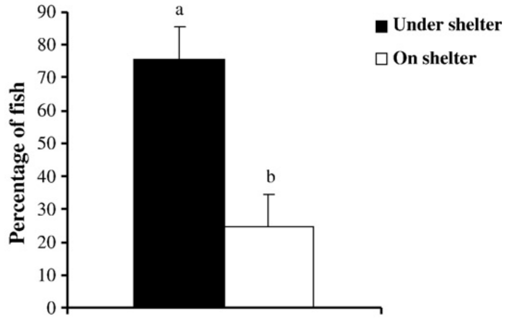

**Fig. 4.** Mean ± S.D. Percentage of fish located on or under shelter. Significant difference ($P < 0.05$) between fish located under or on shelter are represented by a different letter above the bar (Kruskal Wallis test).

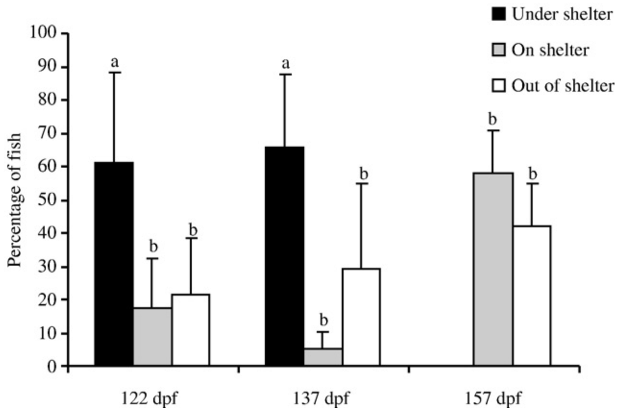

**Fig. 5.** Mean ± S.D. Fish preference test in the fish location : under, on or out of the shelter. At each date expressed in days post fertilization (dpf), significantly different fish locations ($P < 0.05$) are represented by a different letter above the bar (Post hoc sheffé tests).

### 3.2. Fish behaviour

At 126 dpf, almost all of the fish provided with shelter were immobile while 85% of fish without shelter displayed horizontal stationary body movement and 15% swam against current (Fig. 3). At 139 dpf, all the fish provided with shelter were immobile compared to only 32.7% of fish without shelter ($U = 0$, $P = 0.004$). The fish without shelter displayed horizontal stationary body movement (60.6%) or swam against current (6.8%). At 157 dpf, 94.9% of fish provided with shelter were immobile compared to 85.4% of fish without shelter ($U = 3$, $P = 0.016$). In fish without shelter, 11.5% displayed horizontal stationary body movement whereas 5.1% of fish provided with shelter showed the same, although the difference was not significant ($U = 6$, $P = 0.054$).

The average space between two fish in compartments provided with shelter remained stable throughout the experiment ($H(2,18) = 3.02$, $P = 0.22$): the distance between two fish was on average 2.42 ± 0.96 grooves. The percentages of fish were significantly different when considering the type of groove occupancy (0, 1, 2 or 3 fish per groove) ($F(3,68) = 206.09$, $P < 0.001$). The date effect was not significant: 56 to 70% of grooves remained empty during the period. At 126, 139 and 157 dpf, the percentage of empty grooves were significantly higher than other situations ($P < 0.001$). Twenty-six to 36% of grooves were occupied by a single fish. At 126, 139 and 157 dpf, the percentages of grooves occupied by a single fish were significantly higher compared to those occupied by 2 or 3 fish ($P < 0.001$). Other situations (2 and 3 fish per grooves) were few and there was no significant difference between them.

The percentage of fish located under the shelter was higher than the percentage of fish located on the shelter ($H(1,36) = 26.27$, $P < 0.0001$) (Fig. 4). No difference was found between dates for either fish located under or on the shelter ($H(2,36) = 2.56$, $P = 0.28$).

### 3.2. Preference test

At 122 dpf, 61.1% of the fish were located under the shelter (Fig. 5). The rest of the fish were equally distributed on and out of the shelter. The same pattern was observed at 137 dpf except that the number of fish located out of the shelter increased from 21.7% to 29.4%. However this difference was not significant. At 157 dpf, no fish was located under the shelter. They were distributed either on shelter (57.8%) or out of the shelter (42.2%) but the difference was not significant.

## 4. Discussion

This study showed a significant impact of a new type of shelter on culture performance and behaviour of yolk-sac alevins of Arctic charr. The final weight and the specific growth rate, as well as the survival rate, were higher in fish provided with shelter. This was associated with much higher mobility in fish without shelter, whereas most of the fish provided with shelter were immobile in the grooves either on or under the shelter. This mobility was characterized by horizontal stationary body movements or swimming against current. As shown by other authors on Atlantic salmon juveniles (Finstad et al., 2007), improved performance in shelter environments is thus most likely caused by reduced metabolic costs. The inactivity in this study probably favoured a more efficient conversion of the yolk into somatic growth, as occurs for yolk-sac alevins deposited in gravel redds (Alanärä, 1993). The grooves of the shelter allowed the yolk-sac alevins to stabilize in the vertical orientation more easily than fish reared on a flat bottom, which spent a lot of effort, especially in the first tested days to do the same. For this specific reason, yolk-sac alevins without shelter increased their swimming activity to display a righting response (Marr, 1963; Bams, 1969) and therefore had less energy to convert into body tissue (Hansen, 1985). The mortality recorded in fish without shelter corresponded to the usual rate when yolk reserves are depleted in the absence of food (Ivlev, 1961, Viljanen and Koho, 1991; Wilkonska et al., 1994; Keckeis et al., 2000). Provision of shelter is an important means of increasing survival and growth as already observed in farmed fish (Curran and Able, 1998: Winter flounder: *Pseudopleuronectes americanus*; Hossain et al., 1998: African Catfish: *Clarias gariepinus*). In these studies, fish without shelter had greater energy loss associated with much higher mobility. They could have been therefore more susceptible to mortality than those with lower energy loss rates especially during periods when feeding is energetically less profitable (Millidine et al., 2006). Several authors have demonstrated that individual survival probability is closely linked to the ability to retain stored energy above critical levels (Biro et al., 2004; Finstad et al., 2004).

The onset of exogenous feeding occurred six days earlier in fish without shelter, and those fish were also lighter than fish provided with shelter. It is well known that the active search for food commences when most of the yolk reserves have been depleted. Exogenous feeding coincides with the “swim-up” stage, when approximately 30% of the yolk sac remains (Hansen and Torrissen, 1984; Wallace and Aasjord, 1984). In the present work, fish without shelter probably used their yolk more rapidly and less efficiently and had to emerge at a smaller size and earlier time. These results are similar to those on sockeye salmon *(Oncorhynchus nerka)* reared under hatchery conditions in flat throughs (Mead and Woodall, 1968).

The study of the average space between two adjacent fish gave interesting results to optimize the use of the shelter. It appeared that a stable average value of 2.4 grooves was maintained between yolk-sac alevins located on the shelter throughout the study. Also, there was very often a single fish per groove throughout the period. These results are agree with previous experiment showing that both juvenile Atlantic salmon and brown trout *(Salmo trutta)* prefer to shelter singly when tested at densities ranging from one to potentially five fish per shelter (Armstrong and Griffiths, 2001).

Here we have demonstrated for the first time that when a choice was given to yolk-sac alevins they preferred to use shelter rather than a flat bottom. At the first date, most of the fish preferred to go under the shelter. These results have striking similarities with the initial alevin behavioural response described in the wild. After they hatch, salmonids such as Pacific salmon or trout, are not able to swim in streams and would find harsh conditions if they emerged (Quinn, 2004). Their initial responses are to wriggle downward through the interstitial spaces in the gravel of the stream or lake to orient away from light (Quinn, 2004). On the last day of our study, most of the fish were located either on or out of the shelter. This behaviour also corresponds to what has been described in the wild when the yolk-sac alevin with largely depleted yolk finally move upward and emerge, i.e. swim-up stage (Quinn, 2004).

Our results have implications for hatchery rearing techniques of Arctic charr since their behaviour is associated with clear culture performance improvements, including welfare. An increasing concern for fish welfare, especially in aquaculture, has developed in recent years (Ellis et al., 2002). There is a need to identify welfare indicators, both species-specific and operational (Millot et al., 2008). Taking into account the behavioural choice of the yolk-sac alevins and alevins could minimize stress and its negative consequences. As shown recently in young rainbow trout, the responsiveness of the corticotropin axis in 5–6 month old rainbow trout is influenced both by early stress exposure and by initial egg cortisol levels (Auperin and Geslin, 2008).

Our shelter device is available in several external diameters ranging from 50 to 200 mm and can therefore be easily adapted to specific cultural needs i.e. the number of fish, size of incubator, or species of fish. Finally, it opens a wide range of further research focusing on the link between shelter, behaviour and morphology, and the importance of welfare and early growth of fishes.

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# Chapitre 2

# Impact of plant-based diet on behavioural and physiological traits in sea bass (*Dicentrarchus labrax*)

D. Benhaïm, M-L. Bégout, S. Péan, M. Manca, P. Prunet & B. Chatain

*Soumis*

# Résumé

Dans ce chapitre, nous abordons l'influence sur le comportement et la physiologie du bar d'un autre facteur abiotique, un aliment dont les protéines et les lipides proviennent exclusivement de matières premières d'origine végétale.

Des études récentes menées sur le bar permettent d'envisager la possibilité de remplacer totalement les matières premières d'origine marine par des matières premières d'origine végétale, avec des conséquences toutefois variables sur les performances de croissance et la composition en lipides de la chair selon l'âge où démarre ce type de régime alimentaire. En dehors de la croissance, il est également important d'étudier d'autres critères afin de vérifier que de tels régimes alimentaires n'altèrent pas le bien-être, la santé et la qualité des téléostéens. Le but de cette étude est d'évaluer l'impact d'un régime alimentaire constitué de 100% de matières premières d'origine végétale (PB), donné dès un stade de développement précoce, sur le comportement du bar. Une première expérience a comparé en triplicats, le processus d'apprentissage en conditions d'auto-alimentation entre des individus naïfs nourris avec de l'aliment PB (PBF) et des individus nourris avec de l'aliment marin classique (MBF). La seconde expérience a comparé l'activité de nage, l'exploration et la capacité de discrimination entre deux objets en 2 dimensions associés à une récompense (contact visuel avec un congénère non familier) de bars issus des deux traitements (PBF *vs.* MBF) testés individuellement en labyrinthe. Des paramètres sanguins comprenant notamment des indicateurs de stress (cortisol et glucose) ont été également mesurés. La première expérience montre que le comportement en condition d'auto-alimentation ne varie pas entre les PBF et les MBF durant les 30 premiers jours. Les premières activations de la tige tactile démarrent 2-3 jours après le début de l'expérience, mais la demande alimentaire reste faible chez les deux catégories d'individus ne commençant à augmenter qu'à partir de la deuxième période de 15 jours (~6 g $kg^{-1}$). Cette période d'apprentissage est donc caractérisée par de faibles performances de croissance avec de nombreux individus présentant des taux de croissance négatifs même si la totalité de l'aliment distribué a été consommée. La seconde expérience montre que la distance parcourue et la vitesse moyenne sont supérieures chez les MBF tandis que les vitesses angulaires sont similaires entre les deux catégories d'individus. Les MBF et les PBF passent une forte proportion de leur temps dans la zone de démarrage et lorsqu'ils la quittent, ils occupent préférentiellement la zone située près du congénère ou la zone située à l'opposé de cette dernière. Le pourcentage de « non-choix » est élevé pour chaque catégorie (~ 60%), mais lorsqu'ils quittent la zone de démarrage, les individus s'orientent préférentiellement vers la zone située près du congénère, effectuant leur tout premier virage dans la direction de cette dernière, ce qui montre leur capacité de discrimination entre les deux objets proposés. La significativité des tests est néanmoins altérée par la proportion importante de « non-choix ». Le principal résultat physiologique est le haut niveau de cortisol induit par le test de cognition en labyrinthe, indiquant un fort niveau de stress. Ce résultat pourrait permettre d'expliquer la forte proportion de temps passée dans la zone de démarrage et le nombre élevé de « non-choix » chez les deux catégories d'individus. Ce niveau de cortisol est en outre significativement plus élevé chez les MBF que chez les PBF, ce qui suggère un impact du régime alimentaire sur la libération à court terme du cortisol.

Cette étude fournit les premiers éléments de réponse concernant l'influence d'un régime alimentaire à base de matières premières d'origine végétale sur le comportement et la physiologie du bar. Ces éléments pourraient être utiles dans la perspective d'établir les critères de bien-être et de santé des téléostéens soumis à ce type de régime alimentaire.

# Impact of plant-based diet on behavioural and physiological traits in sea bass (*Dicentrarchus labrax*)

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## Abstract

Recent study on sea bass allowed expecting that total and early replacement of marine products by plant products could be performed with moderate effect on late fish growth and body lipid content. Apart from growth it is however important to investigate for other traits to ensure such diet will not impair fish welfare, health and quality. The aim of this study was to assess for potential late behavioural impact induced by a total plant-based diet (PB) started at an early stage of sea bass development. Moreover, acute stress response as indicated by plasma cortisol measurement was assessed in this study. A first experiment compared the learning process in self-feeding conditions between naive fish fed PB diet (PBF) to fish fed a classical marine diet (MBF). The second experiment compared swimming activity, exploration and ability to learn discriminating between two 2-D objects associated to a reward of fish (PB *vs*. MB) individually tested in a maze. Blood physiological variables including stress indicators (cortisol and glucose) were measured to complete the characterisation. The first experiment showed that self-feeding behaviour was not different between PBF and MBF during the 30 first days. This learning period was characterized by low growth performances with numerous individuals presenting a negative specific growth rate but all the food distributed was consumed. The second experiment showed that the distance travelled and the velocity mean were higher in MBF than PBF while angular velocities were similar. The percentage of "no-choice" was high in both fish categories (~ 60%) but when they left the start box, they went preferentially toward the congener zone, performing their very first turnings in the adequate direction indicating that they were able to discriminate between two 2-D objects to achieve a simple task. The significance of tests was however lowered by the high level of "no-choice" responses in both fish categories. The main physiological result was a high level of cortisol induced by the maze test procedure indicating acute stress in both fish categories. This could explain the high "no-choice" responses level in both fish categories. Interestingly the plasma cortisol concentration was higher in MBF suggesting an impact of PB diet on cortisol short-term release. This study provides a first insight into the impact of PB diet on sea bass behavioural traits and confirms impact on cortisol in response to stress, both traits which can be helpful in determining relevant indicators of health and welfare.

*Keywords:* vegetable; self-feeder; feeding behaviour; maze; learning; cortisol.

## 1. Introduction

Aquaculture is currently the fastest growing animal food-producing sector (FAO 2008, State of World Aquaculture) but it is confronted with important challenges related to a responsible development of farming activities. The sustainability of aquaculture largely depends upon the reduction of the reliance on feed based ingredients derived from wild fishery resources (Tacon and Metian, 2008). Fish meal (FM) and fish oil (FO) prices increases could undermine the profitability of many aquaculture enterprises (Tacon, 1998). This opened recently new research priorities to develop substitutes for these feed ingredients. Several studies have shown that at least in salmonids, it is possible to totally replace FO by plant oil (PO) sources without affecting growth rate or feed conversion (Reinitz and Yu, 1981; Hardy et al., 1987; Thomassen and Rosjo, 1989; Greene and Selivonchick, 1990; Guillou et al., 1995). Similar results were obtained with total replacement of FM by plant proteins (PP) (Watanabe et al., 1998; Kaushik et al., 2004). In marine fish such as European Sea bass (*Dicentrarchus labrax*), 50 to 98% of FM can be replaced using PP sources with a resulting growth similar to what is obtained when fish oil is used as the lipid source (Kaushik et al., 2004; Dias et al., 2009) although in some cases, total replacement of FM by single PP sources has been shown to lead to depressed growth (Dias et al., 2009). Replacement of 60% of FO by PO in the same species has been shown to have no negative impact on fish survival, growth and health (Izquierdo et al., 2003b; Figueiredo-Silva et al., 2005; Mourente et al., 2005) whereas growth performances were reduced at higher levels of substitution (80%) (Montero et al., 2005).

Apart from growth performances in such PB diets, it is also important to investigate for other traits. For example, this kind of substitution is known to modify muscle fatty acid composition (Thomassen and Rosjo, 1989; Greene and Selivonchick, 1990; Arzel et al., 1994). The inclusion of PO in fish feeds can lead to alterations of the fatty acid profile *i.e.* mainly the reduction in the n-3 HUFA, particularly Eicosapentaenoic acid (EPA) (Izquierdo et al., 2003a; Montero et al., 2005) and in some cases it may significantly affect fish fillet quality and sensory characteristics (Guillou et al., 1995; Martínez-Llorens et al., 2007), while some effects on the odour active compounds is also possible (Serot et al., 2001; Serot et al., 2002). Finally, inclusion of linseed oil in diet at either 60% or 100% replacement of anchory oil increased plasma cortisol levels in sea bream exposed to an acute confinement stress (Montero et al., 2003; Ganga et al., 2011).

In marine fish culture, the use of PO as the sole lipid source in feeds seems to be limited since these species have low capacity to convert linoleic and linolenic acids, into arachidonic, EPA and Docosahexaenoic acid (DHA) which are essential for marine fish (Sargent et al., 2002). However, a recent study showed genotype-diet interaction in late growth of European sea bass fed with either a FM or a FO diet or an all-plant-based suggesting that genetic improvement can be impacted by extreme changes in diet and the use of plant-based products (Le Boucher et al., 2011).

To the best of our knowledge, there are however no studies showing potential effects of PB diets on fish behavioural traits except a few ones showing reduced swimming activity in gilthead seabream (*Sparus aurata*) larvae (Benítez-Santana et al., 2007) or affecting ontogeny of schooling behaviour as well as brain development in yellowtail (*Seriola quinqueradiata*) (Ishizaki et al., 2001). Specific attention should be paid to this aspect since reduced DHA levels in brain have been shown to be associated with poor water-maze learning memory performance in rat (Moriguchi et al., 2000; Lim et al., 2005), and lead to increased fear and anxiety in mice (*Mus musculus*) (Owada et al., 2006).

The aim of this study was therefore to assess for potential late behavioural impact induced by a PB diet started at an early stage of sea bass development. It was encompassed in a larger research program which investigated heritability of growth performances in sea bass fed a total PB diet and other complementary aspects such as tissues biochemical compositions, physiology (osmoregulatory capacity) etc.

This was approached by two different experiments. The first one compared the learning process in self-feeding conditions between fish fed a total PB diet to fish fed a classical marine diet (MB). Indeed, this species has demonstrated a great ability and high plasticity to learn to press a lever to receive a food reward (Anthouard et al., 1993; Covès et al., 1998; Covès et al., 2006; Millot et al., 2008). This experiment was focused on the 30 first days, the self-feeding activity reaching a suitable level within 22 days in this species (Sánchez-Vázquez et al., 1994).

The second experiment compared behaviour and cognition of fish (PB *vs.* MB) individually tested in a maze. It is now recognised that fish exhibit a rich array of sophisticated behaviours (Brown et al., 2007), and several studies showed that they have long term memories (Brown and Laland, 2001; Warburton, 2007) and cognitive capacity comparable with that of non-human primates (Odling-Smee and Braithwaite, 2003). Here we assessed in particular, swimming activity, exploration and ability to learn discriminating between two 2-D objects associated to a reward (visual contact with an unfamiliar congener). Finally, in addition to these behavioural parameters, we also analyse various plasma physiological parameters which include stress biomarkers.

## 2. Material and methods

### *2.1. Fish origin*

The fish used in this experiment were hatched and grown at the Ifremer aquaculture station (Palavas-les-flots, France). They were produced from wild-caught West Mediterranean European sea bass broodstock. A full factorial mating design was used in order to obtain 225 families from 9 dams and 25 sires. They were all reared in the same tank from egg stage to almost 3 months-old (2.5g). During this period, fish were fed Artemia for 40 days, and then weaned on classical marine-based artificial feed until they reached 2.5 g. From this stage, 3 000 individuals were fed a total plant-based diet (PB) and 3 000 a classical marine diet (MB) (Table 1). When fish reached a mean body weight of 20 g, they were tagged using a Passive Integrated Transponder (AEG-Id, Ulm, Germany) then split into 3 classes according to their growth performances (low, medium and high growth rates). At the end, 300 individuals from the medium class in each diet category were transferred to the Ifremer experimental station located in L'Houmeau (France) for the behavioural and physiological characterization.

| Diets | MD | PB |
|---|---|---|
| *Ingredients (g kg$^{-1}$)* | | |
| Fishmeal | 380.0 | |
| Corn gluten meal | 180.0 | 200.0 |
| Soybean meal | 0.0 | 182.0 |
| Wheat gluten | 72.0 | 20.0 |
| Extruded wheat | 253.0 | 72.0 |
| White lupin | | 140.0 |
| Fish oil | 85.0 | |
| Linseed oil | | 94.0 |
| Soya lecithin | | 10.0 |
| L-Lysine | | 27.0 |
| $CaHPO_4 . 2H_2O$ | | 30.0 |
| Binder (sodium alginate) | 10.0 | 10.0 |
| Attractant mix[a] | | 15.0 |
| Min. premix[b] | 10.0 | 10.0 |
| Vit. premix[b] | 10.0 | 10.0 |
| *Chemical composition* | | |
| Dry matter (DM in %) | 88.9 | 94.5 |
| Crude protein (% DM) | 49.4 | 49.6 |
| Crude fat (% DM) | 14.4 | 14.1 |
| Ash (% DM) | 7.0 | 6.1 |
| Nitrogen-free extract (NFE) (% DM)[c] | 18.1 | 24.7 |

*Table 1. Ingredients and proximal composition of the experimental diets.*

*(a): Attractant mix contained (g kg$^{-1}$ feed) taurine (3), betaine (3), glycine (2), alanine (2) and glucosamine (5), glycine (2), alanine (2) and glucosamine (5).*
*(b): As in Guillaume et al. (2001).*
*(c): NFE = dry matter-crude protein-crude fat-ash.*
*M: fish meal and fish oil diet. PB: all fish meal and fish oil replaced by plant products.*

### 2.2. Experiment 1: self-feeding behaviour in fish fed PB vs. MB diets

Immediately after arrival, fish fed a MB diet (MBF) were randomly dispatched into 3 tanks (50 fish per tank, tanks 1, 2, 3), fish fed a PB diet (PBF) into 3 other tanks (60 fish per tank, tanks 4, 5, 6) held in one single experimental room described hereafter. This fish number difference was chosen to take into account the weight difference between MBF and PBF at the beginning of the experiment (Mean ± SD, 146.6 ± 31.2 and 93.2 ± 28.5 g respectively). Rest of the fish were stocked in other tanks and kept for experiment 2.

The experiment was thus carried out in six 400 l tanks supplied with filtered seawater in a recirculated system (flow rate = 4 $m^3$ $h^{-1}$ in each tank, and water renewal = 10% per day). The mean water temperature, oxygen concentration, and salinity were the whole experiment along, 21.2 ± 0.8 °C, 7.6 ± 1.4 mg $L^{-1}$, and 23.2 ± 1.4 g $L^{-1}$ respectively. Tanks were surrounded by black curtains to reduce any visual disturbance to the fish, and lighted by individual 120W lamp located about 90 cm above the water surface. Light regime was 16:8 LD (light onset at 06:00 U.T. + 1) with twilight transition periods of 30 min. Fish were fed pellets which composition is given in table 1. Both diets were isoproteic (digestible protein: 43 % of dry matter), isolipidic (fat: 13 % of dry matter) and isoenergetic (17 MJ kg$^{-1}$). Because the MBF were larger than PBF, pellets were 6 and 2.5 mm respectively. For further details see Le Boucher et al. (2011). Each tank was provided with a self-feeder. The device to operate the feeders comprised a screened type sensor (a metal rod protected in a PVC cylinder surrounded by the PIT tag detection antenna; Covès et al., 2006), and a control box. After each actuation, fish were rewarded with pellets, feed dispensers being regulated to distribute 0.7 g $kg^{-1}$ for MBF and 0.4 g $kg^{-1}$ for PBF. The reward level was a compromise between minimizing wastage, and optimizing feed allocation to the group. Such a set up allowed us to monitor the group (*i.e.* tank) apparent feed consumption (food quantity dispensed minus waste counted on the bottom of the tank, and in the sediment trap). Triggering activity recordings were done continuously for 30 days, and only stopped 48 h before (no recordings and fasting of fish), and during fish handling (2 days off in total).

Experiment started after a 10-day acclimation period, fish being fed by hand one daily meal until apparent satiation during this period. Fish were then placed under self-feeding conditions (day 1, D1) of the experiment, and food access was possible all day (24 h) even during tank cleaning, and waste counting from 10:00 to 11:00 (U.T. + 1). The experiment lasted 30 days in total, and fish were weighed, and measured (anesthetized with clove oil at 40 ppm) at the beginning (D1) and at the end of the experiment (D32).

The variables chosen to evaluate rearing performances, and feeding behaviour within periods were the following (most are normalized against fish biomass to account for inter-tank differences):

- Body weight (BW in grams)
- Total body length (BL in cm)
- Specific growth rate : SGR (% body weight per day) = 100 (Ln BWf - Ln BWi) / t, with BWf and BWi being the final and initial body weight (g) respectively, and t the total number of days
- Feed demand (FD in g $kg^{-1}$ of fish biomass)
- Food wastage (FW in g)
- Feeding rhythms calculated by taking into account the feeding demand per hour

At the end of experiment (D32), all fish were sacrificed and sexed.

### 2.3. Experiment 2: behaviour of MBF and PBF in a maze

Two days before the beginning of observations, MBF and PBF from the residual stock were anesthetized with clove oil at 40 ppm and 10 individuals from each category were selected. Total length was 21.7 ± 0.9 cm in MBF and 22.0 ± 1.4 cm in PBF; weight was 91 ± 4.2 g in MBF and 73.5 ± 0.7 g in PBF. All PIT tag numbers were noted for each individual before being placed in two 400 l tanks. Additionally, a stock of sea bass of similar size was used from another tank (fish not familiar with tested fish) as the reward in the experiment.

Fish were individually tested in a maze constructed from opaque white expanded PVC plastic and transparent Plexiglas® (Fig. 1A). The start box was a 30x40 cm rectangle separated from the rest of the maze by a removable transparent Plexiglas® wall. At the end of each arm of the maze, one aquarium (60x26cm, 60 l) continuously provided with air pump was placed against a not removable transparent Plexiglas® wall. This Plexiglas wall was located 2.6 cm behind the lengthening of the line of the start box wall to ensure the tested fish was not able to see the reward before turning on left or right side of the maze. Four white plastic supports were used to show two different laminated printouts 2-D objects either on left or right side of the fish (Fig. 1B). Both objects (equal black and white area) were already successfully tested in previous experiment (Snekser et al., 2009). Shortly before observations, the maze was filled with water which level was maintained at 20 cm representing a total volume of 267 l. Temperature, salinity and oxygen level were verified before and after the end of observations performed on each fish and were respectively 21.0 ± 0.5 °C, 7.7 ± 1.5 mg L-1, and 23.0 ± 1.1 g $L^{-1}$ before, 21.0 ± 0.4 °C, 7.6 ± 1.4 mg $L^{-1}$, and 23.0 ± 1.2 g $L^{-1}$ after. Digital camera (Imaging Source DMK 21AUO4) with a frame rate of 25 Hz and a resolution of 640 x 480 pixels was positioned at 220 cm above the water surface. Three 80 W light spots were placed around the maze providing an indirect and homogenous lighting on the maze.

Before the beginning of observations, all individuals were randomly associated to one of the two 2-D objects. The positions of the supports showing the 2-D objects were also randomly placed on left or right side of the maze arms for each individual and each trial. The reward (non-familiar fish) was placed according to the 2-D object position corresponding to the tested individual. The choice for an unfamiliar fish rather than a familiar one was driven by several reasons. First, two unfamiliar sea bass separated by a transparent barrier allowing only for visual contact have already been shown to spend most of their time in the zone nearest to the transparent barrier (Di-Poï, 2008). Secondly, in this case, compare to chemical stimuli or multimodal combination of both visual and chemical stimuli, the visual stimulus only, seemed to modulate differently the behaviour of fish which showed an increased interest in the presence of the congener on the other side (Di-Poï, 2008). The only constraint on the randomisation process was that the stimulus never appeared in the same position more than twice in a row. These objects were the most salient and detectable visual cues in the maze environment. The first tested fish was gently collected from the tank using a net and immediately placed inside a bucket closed by a cover then placed in the maze start box. After a 5min acclimatization period, the transparent wall was removed and the video capture started. The maze was filmed during 10 min. At the end of video recording, the individual was placed again in the start box and tested a second time after a 5 min acclimatization period. In total, each individual was tested 3 consecutive times in a day, this procedure being repeated for 5 consecutive days. At the end of the 3 consecutive trials, individuals were returned to their tank. In order to test all individuals, 14 days were required. The water was entirely renewed at the end of each day and the non-familiar fish used as the reward was changed every hour to minimize stress due to confinement and handling.

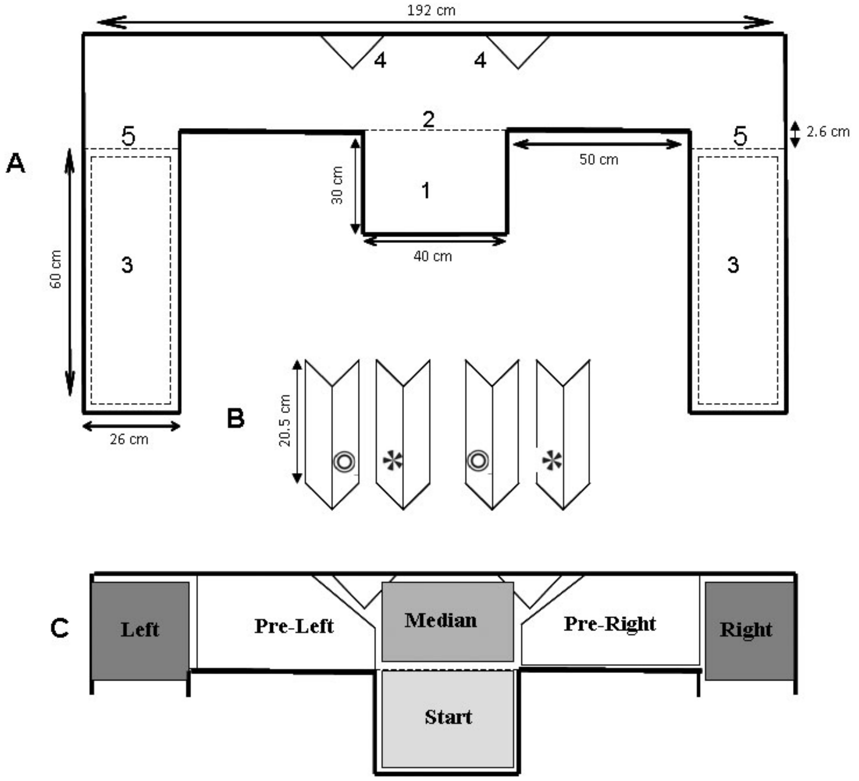

***Fig. 1.*** *A. Schematic representation of the T-maze apparatus. Dotted lines are transparent Plexiglas walls, continous lines are white opaque plastic.*

*1.Start box; 2. removable transparent plexiglass wall; 3. Aquarium where congener was placed. 4. white plastic support for laminated printouts 2-D objects. 5. Not removable transparent plexiglass wall.*

*B. Detail of the 4 supports and the 2-D objects.*

*C. Virtual zones delimitation on the bottom of the maze defined for the video recordings analysis.*

The video recordings were analysed using the software EthoVision XT (Noldus, The Netherlands), which allowed to define six virtual zones to be defined in the maze (Fig. 1C) and to track the fish swimming behaviour.

Each video was also viewed to report several learning criteria: first turning of the fish (left or right-turn) leading to the reward zone (success) or the opposite zone (failure), latency time to reach the reward zone and/or the opposite zone, the last place of the fish just before the end of video recording, the fish orientation from the 2-D object it was associated to (Fish head purely oriented toward the 2-D object).

Different variables of interest were chosen to analyse fish exploration and swimming:

-The time spent in each zone expressed in seconds (s): Start Box (Start), Median area (Median), Reward zone near the congener (ZCong), zone opposite to the reward zone (OpCong), zone located between Median and ZCong (PreCong), zone located between Median and OpCong (PreOpcong).
-The fish absolute angular velocity expressed in degree per second (Vang in ° $s^{-1}$) was calculated by the software as followed:
$Vang_n = RTA_n / t_n - t_{n-1}$ where $RTA_n$ is the relative turn angle for sample n and $t_n - t_{n-1}$ , the time difference between the current and previous sample. Here the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for head direction. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity.
-The distance travelled by each fish in the maze (Dtot in mm)
-The velocity mean expressed in body length per second (Vel in BL $s^{-1}$)
The last three variables quantified the fish swimming activity level in the maze.

Different variables were chosen to assess the fish learning process and to check for possible bias such as spatial memory (fish turning according to its previous left or right choice) or side preference:
-Number of successful and failed turnings. Only the very first turning was accounted to meet the successful criteria. The maximum score is 14 successful turnings when the fish goes toward the reward zone at the first trial but not necessarily after its first turning choice. If the fish goes to the reward zone at the second trial, it can not exceed 13 and so on.
-The latency time to reach ZCong or/and OpCong (in s)
-The last place occupied by the fish at the end of the video recording: 0 or 1 (absence or presence) and the score is summed per zone over all trials (*e.g.* if the last place for one fish is always ZCong, the number is 15 for ZCong and 0 for all other zones).
-The number of times the fish was oriented toward the 2-D object associated with the reward (StCong), oriented toward the opposite 2-D object (StOpCong), just before the wall removal. All other situations were classified in the category “Other” (StOther).

In order to evaluate physiological status *i.e.* to compare the physiological blood responses between MBF and PBF, all individuals were submitted to one more trial performed under similar conditions as described above (only this trial was not filmed). At the end of the trial, each fish was immediately anesthetized with clove oil at 40 ppm and a blood sample (c. 1 ml) was collected from caudal vessels with 1-ml preheparinized syringes (in less than 2 min). A small part of the blood sample was immediately analysed using an i-Stat® Portable Clinical Analyzer (Abbott; cartridges CG8+; Heska corporation, Fort Collins, CO, USA; (Harrenstien et al., 2005)). The following parameters were analyzed:

- Potential of hydrogen (pH)

- Carbon dioxide partial pressure ($PCO_2$ in mm Hg)
- Oxygen partial pressure ($PO_2$ in mm Hg)
- Base excess (BE in mmol $L^{-1}$),
- Bicarbonate ($HCO_3$ in mmol $L^{-1}$)
- Total carbon dioxide ($TCO_2$ in mmol $L^{-1}$)
- Oxygen saturation ($sO_2$ in %)
- Potassium ion ($K^+$ in mmol $L^{-1}$)
- Sodium ion ($Na^+$ in mmol $L^{-1}$)
- Ionized calcium (iCA in mmol $L^{-1}$)
- Plasma glucose (Glu in mg $dL^{-1}$)
- Hematocrit (Hct in % PCV), and haemoglobin (Hb in mmol $L^{-1}$)

Most of the blood sample was centrifuged at 3000 ×g for 20 min at 4 °C, and plasma samples were frozen and stored at −80 °C until cortisol analyses were performed (in duplicate for each individual). Cortisol was assayed by a 3H cortisol radioimmunoassay according to the method described by Auperin et al. (1997).

Fish were finally killed by cervical section and then sexed.

### *2.4. Statistical analysis*

All variables were compared using parametric analysis of variance (ANOVA) after verification of distribution normality and homoscedasticity (Dagnélie, 1975). When data did not fulfil these requirements, non parametric Kruskall-Wallis tests were used. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls), and Kruskall-Wallis test by a rank-based multiple comparisons (Zar, 1984). All statistical analyses were conducted using Statistica 8 (Statsoft, USA), and for all tests, the significant threshold was $p < 0.05$.

#### *Experiment 1*

SGR means were compared using a 2-way ANOVA with the treatment (PB *vs.* MB diets) as fixed factor and the tank as a random factor nested to treatment.

FD and FW means were compared using a 3-way ANOVA with the treatment (PB *vs.* MB diets), the period (2 periods of 15 days) as fixed factors, and the tank as a random factor nested to treatment.

Feeding rhythms were compared using a 3 factors ANOVA taking Treatment (PB *vs.* MB diets), Hours as fixed factors, Tank as random factor nested to Treatment.

A Chi-square test was employed to compare the sex ratio in both treatments.

#### *Experiment 2*

All variables related to the swimming activity were compared using a repeated measures analysis of variance with Treatment (PB *vs.* MB diets) as between-subjects factor and Trial (15 trials) as within-subjects factor.

The time spent in each zone were compared using a repeated measures analysis of variance with Treatment (PB *vs.* MB diets) and Zone (6 zones) as between-subjects factor, Trial as within-subjects factor (15 trials).

Criteria for successful/failed turnings and side-turning preference within each treatment were determined using a binomial test at a 5% level of significance.

The number of turnings performed according or not to the previous one and the number of times in each zone accounting for the last place were compared within each treatment using a Wilcoxon matched pairs test.

The latency times to reach the reward zone was compared using a Kruskal-Wallis test taking Treatment (PB *vs.* MB diets) and trial (15 trials) as independent variables.

The percentages of successful turnings when fish orientation from the 2-D object just before wall removal was adequate or not were compared using a Kruskall-Wallis for each fish category taking into account in both cases fish orientation (StCong, StOpCong and StOther) as independent variable.

The percentages of successful turnings in relation to the fish last place were compared using a Kruskall-Wallis test for each fish category taking into account in both cases fish last place (LastCong, LastOpcong and LastOther) as independent variable.

For physiological blood parameters, a Kruskall-Wallis test was used taking Treatment (PB *vs*. MB diets) as independent variable. A test of Mann-Whitney was used to compare cortisol levels between MBF and PBF.

## 3. Results

### *3.1. Experiment 1*

During the experiment, mortality rate was 3.3 ± 4.2% in MBF and 8.3 ± 5.0% in PBF but the difference was not significant. In average MBF biomass per tank was 7328 ± 18 g at the beginning of the experiment and 7058 ± 462 g at the end; in PBF, it was 5591 ± 96 g and 4943 ± 181 g.

SGR was not significantly different between treatments but there was a significant Tank(Treatment) effect ($F_{(4,304)} = 13.9$, $P < 0.001$) with one tank of PBF (Tank 6) being lower than all other tanks and one MBF tank (Tank 2) being higher than all other tanks ($P < 0.001$ in all pairwise comparisons). Most of the fish in each tank had negative SGR at the end of the period except for one MBF tank (Tank 2, 29%). In all other cases, there were 61 and 74% of fish with negative SGR in Tanks 1 and 3 (MBF) and 71, 73 and 95% in PBF tanks.

FD was similar in PBF and MBF (Fig. 2). There were neither Tank(Treatment) nor Treatment effects but a highly significant Period effect ($F_{(1,160)} = 27.0$, $P < 0.001$), FD being higher in the second period than in the first one (mean ± SE: 6.3 ± 0.4 and 2.3 ± 0.2 g $kg^{-1}$ respectively). No FW was recorded in any tanks the whole period along.

No hour effect was recorded for FD but a highly significant Tank(Treatment) effect was measured ($F_{(4,787)} = 5.5$, $P < 0.001$).

There was 87±2% of males in MBF and 82±3% in PBF, the difference between treatments being not significant.

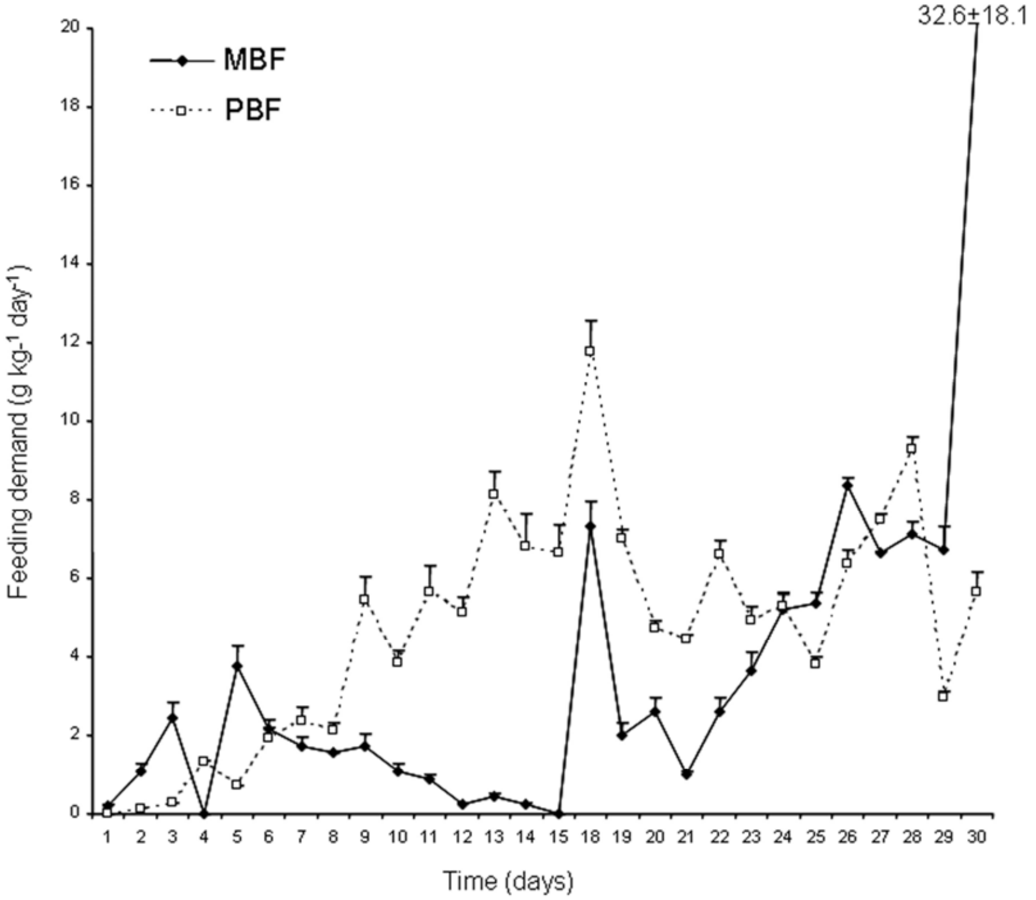

***Fig. 2**. Mean±SE. Daily feeding demand during the experiment in fish fed a marine-based diet (MBF) vs. fish fed a plant-based diet (PBF).*

### *3.2. Experiment 2*

### *Exploration and kinematics in the maze*

MBF and PBF spent most of the time in the Start Box (71 ± 39% and 76 ± 38% respectively, Fig. 3). The rest of the time, they were preferentially located in ZCong (8 ± 20% and 11 ± 20% respectively) and OpCong (8 ± 18% and 7 ± 16% respectively). There was a Zone*Treatment significant effect ($F_{(75,373)}$=1.49, $P<0.01$) with no significant differences at trial 1, with MBF*Start, PBF*Start, MBF*ZCong and PBF*ZCong showing higher values than all other categories at trial 4 ($P<0.05$ for each pairwise category), with MBF*Start and PBF*Start being higher than all other categories at all other trials.

In average, MBF showed higher Dtot and Vel than PBF (1038 ± 1217 mm, 0.08 ± 0.10 BL $s^{-1}$ and 577 ± 533 mm, 0.05 ± 0.06 BL $s^{-1}$ respectively). There was no Treatment effect for Dtot but Newman-Keuls post hoc tests showed that MBF had higher Dtot than PBF at trials 14 and 15. A similar result was shown for Vel. Vang was not significantly different between MBF (793 ± 380° $s^{-1}$) and PBF (887 ± 360° $s^{-1}$).

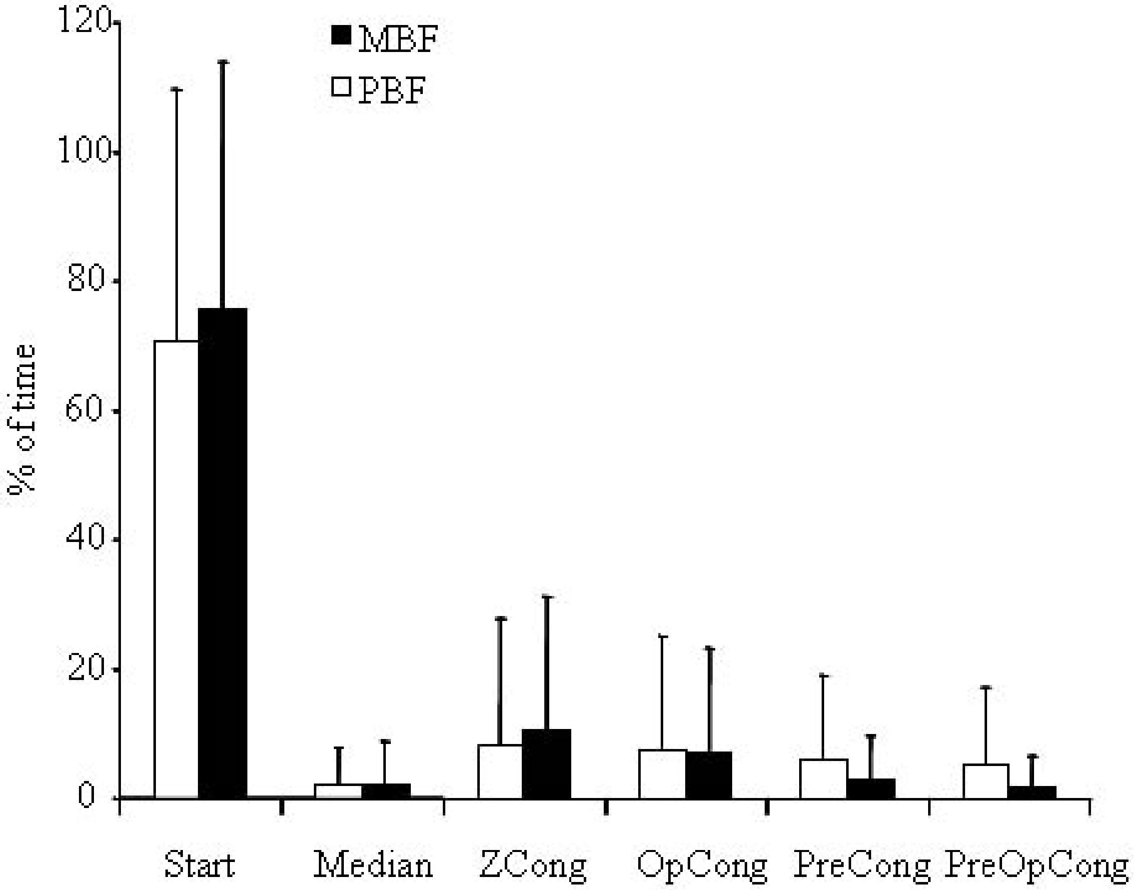

***Fig. 3***. *Proportion of time spent (mean±SD in %) by a fish in each zone of the maze.*

*Start: Start Box; Median: Median area; ZCong: reward zone near the congeneric; OpCong: zone opposite to the reward zone; PreCong: zone located between Median and ZCong; PreOpcong: zone located between Median and OpCong.*

### *Learning performances*

Both MBF and PBF performed more successful turnings than failed ones (Fig. 4) but only two of ten MBF (100% of successful turnings, $P = 0.01$; 80% of successful turnings, $P = 0.04$ respectively) and one of ten PBF (85% of successful turnings, $P < 0.01$) showed a significant association between the 2-D object and the reward. The proportion of “no-choice” responses was higher than 60% in both fish categories (Fig. 4).

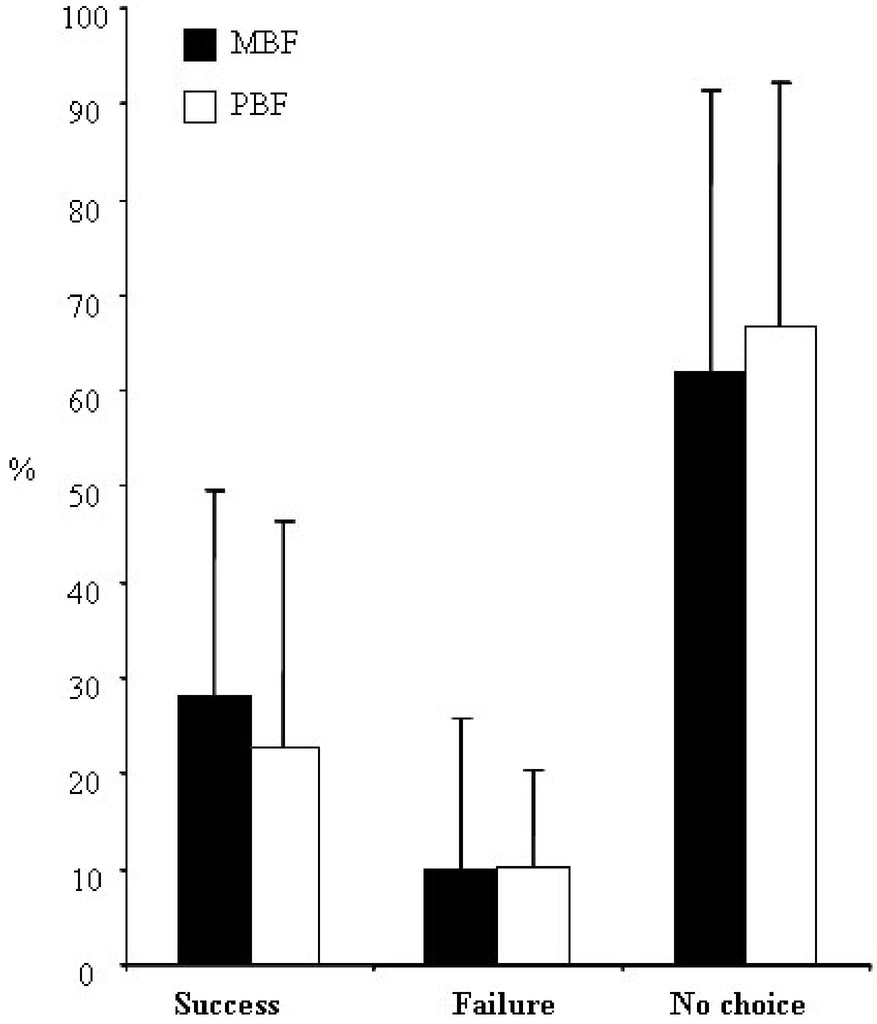

***Fig. 4***. *Learning process assessment based on the comparison between the percentages of successful and failed turnings (mean±SD in %) in fish fed a marine-based diet (MBF) vs. fish fed a plant-based diet (PBF).*

MBF and PBF went to the right side of the maze 23 ± 20% and 11 ± 10% of the time, 26 ± 21% and 29 ± 23% on the left side, and did not turn 51±30% and 60 ± 26%. There was no significant side-turning preference for any fish in both treatments.

The latency time to reach the reward zone was (Min, Max) comprised between 4.5 and 335 $s^{-1}$ in MBF and 3.8 and 372 $s^{-1}$ in PBF. The difference was not significant but there were latency time differences between trials ($H_{(14,\ 89)}=39.3$, $P<0.001$) with latency being lower at trial 1 than at trials 9, 10, 13, 15 ($P<0.05$ for each pairwise comparison).
The turnings did not significantly depend on the fish previous choice in MBF and PBF and there was no significant difference between fish categories (Mean ± SD: 34 ± 21% of turnings according to the previous ones in MBF, 32 ± 19% in PBF).

MBF and PBF performed successful turnings 69.9 ± 25.0% and 68.5 ± 40.2 of the time when they were oriented toward the adequate 2-D object just before the wall removal (Fig. 5A). In MBF this percentage was significantly higher than those recorded in StOpcong ($Z = 2.5$, $P = 0.01$) and in StOther ($Z = 2.4$, $P = 0.01$). In PBF, this percentage was significantly higher than the one recorded for StOpcong ($Z = 2.4$, $P = 0.02$). In MBF, the percentage of StOpcong was significantly higher than the one for PBF ($H_{(1,\ 17)} = 5.9$, $P = 0.01$). MBF and PBF performed successful turnings when their last position was in ZCong (LastCong, 38.9 ± 36.4% and 66.5 ± 35.5% respectively) or LastOther (55.3 ± 40.7% and 27.3 ± 36.7% respectively; Fig. 5B). These percentages were significantly higher than those recorded for LastOpCong in MBF (Z = 2.2, P = 0.03 and Z = 2.2, P = 0.02). In PBF, only the difference between LastCong and LastOpCong was significant (Z = 2.2, P = 0.03). No significant difference between MBF and PBF were recorded for any of these variables (Fig. 5B).

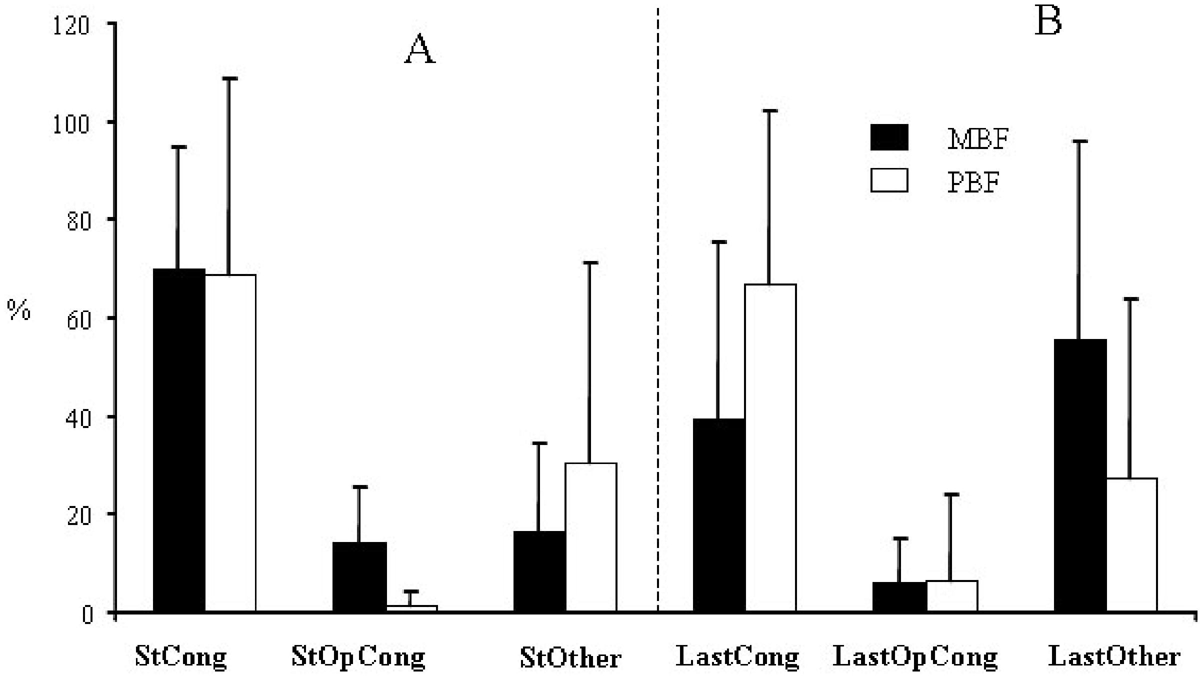

***Fig. 5***. *Mean±SD. Fish fed a marine-based diet (MBF) vs. fish fed a plant-based diet (PBF) position at the beginning and the end of observation period in relation to success.*

*A. The percentages of successful turnings in relation to fish orientation from the 2-D object just before wall removal.*

*StCong: % of times the fish was oriented to the 2-D object it was associated to; StOpcong: fish oriented to the opposite 2-D object; StOther : all other situations.*

*B. The percentages of successful turnings in relation to the fish last place before the end of observation.*

*LastCong: last place in ZCong; LastOpcong: last place in Opcong; LastOther: last place in all other situations.*

### *Physiological variables*

No difference in physiological variables were recorded between treatments except for BE that was higher in MBF ($H_{(1, 17)} = 5.5$, P = 0.02) and for $HCO_3$ and $TCO_2$ that were lower in MBF than in PBF ($H_{(1, 17)} = 6.3$, P = 0.02 and $H_{(1, 17)} = 6.3$, P = 0.01 respectively) (Table 2).

Cortisol concentration was significantly lower in PBF than MBF (Z = -2.2, P = 0.02; Fig. 6). All tested individuals were males except one individual in PBF.

|  | MBF | PBF |  |
|---|---|---|---|
| pH | 7.1 ± 0.1 | 7.2 ± 0.1 |  |
| $PCO_2$ (mmhg) | 17.6 ± 4.1 | 19.3 ± 3.2 |  |
| $PO_2$ (mmhg) | 29.0 ±17.1 | 28.0 ± 9.0 |  |
| BE (mmol $l^{-1}$) | -23.4 ± 2.8 | -20.5 ± 1.2 | * |
| $HCO_3$ (mmol $l^{-1}$) | 5.7 ± 0.9 | 7.3 ± 1.1 | * |
| $TCO_2$ (mmol $l^{-1}$) | 6.2 ± 1.2 | 8.0 ± 1.3 | * |
| $sO_2$ (%) | 34.7 ±24.4 | 41.5 ±19.2 |  |
| Na (mmol $l^{-1}$) | 161.2 ± 6.1 | 157.9 ±11.9 |  |
| K (mmol $l^{-1}$) | 5.9 ± 1.8 | 5.3 ± 1.6 |  |
| iCA (mmol $l^{-1}$) | 1.1 ± 0.3 | 1.0 ± 0.5 |  |
| Glu (mg $dl^{-1}$) | 92.3 ±25.0 | 83.8 ±39.0 |  |
| Hct (% PCV) | 22.0 ± 5.4 | 24.0 ± 7.6 |  |
| Hb (mmol $l^{-1}$) | 7.5 ± 1.8 | 8.2 ± 2.6 |  |

***Table 2**. Blood plasma variables in fish fed a marine-based diet (MBF) vs. fish fed a plant-based diet (PBF).* Results are mean ± SD of ten fish from each treatment.
* $P<0.05$.

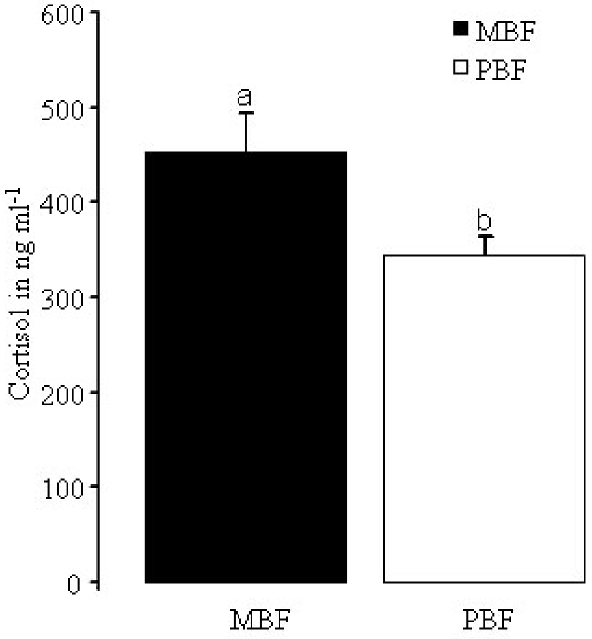

***Fig. 6**. Mean±SE. Plasma cortisol concentrations in fish fed a marine-based diet (MBF) vs. fish fed a plant-based diet (PBF). When two treatments have different letters they significantly differ from each other with P<0.05.*

## 4. Discussion

The aim of this study was to assess for potential late behavioural impact induced by a PB diet started at an early stage of sea bass development. This was approached by a first experiment which compared the learning process in self-feeding conditions between both fish categories (MBF and PBF) and a second one which compared behaviour, cognition and physiology of fish individually tested in a maze. The first experiment showed that PB diet did not impact self-feeding behaviour. The second one showed that PB diet induced a few behavioural, cognitive and physiological differences in fish submitted to a challenge such as a maze.

### *4.1. Self-feeding behaviour*

Self-feeding behaviour was not different between PBF and MBF during the 30 first days which suggests that PB diet did not impact feed-demand behaviour and growth performances over the studied period. Sea bass used in this experiment were naive to the self-feeding apparatus and triggering activity started 2-3 days after the beginning of the experiment which is even earlier than previous studies (Rubio et al., 2003; Rubio et al., 2004; Covès et al., 2006; Millot et al., 2008). Such a delay is known to vary greatly according to the apparatus design (Rubio et al., 2004) and species. However, feed-demand was lower compared to the cited studies reaching about 6 g kg$^{-1}$ during the second 15-day period. As Kohbara et al. (2003) mentioned, it takes time for the food demand to reach a suitable level. The learning period was consequently characterized by low growth performances and similarly to Millot et al. (2008), numerous individuals presented a negative SGR. There was no food wastage during this period in any tanks. Feeding rhythm was not well established in both treatments but the food demand was mostly recorded in the morning and late afternoon indicating diurnal feeding pattern which is in accordance with previous studies on the same species (Azzaydi et al., 1999). Sex ratios were similar in both treatments with more than 80% of males which is in accordance with previous studies on sea bass (Saillant et al., 2003). It suggests that plant-based diet does not influence the sex determination contrary to other environmental factors such as temperature (Baroiller et al., 1999) or social interactions (Shapiro, 1980).

### *4.2. Swimming activity and spatial distribution*

Behavioural differences and similarities were found between MBF and PBF. The distance travelled and the velocity mean were higher in MBF than PBF even though significant differences were not found at each trial. This demonstrated an impact of plan-diet on swimming activity. In particular, plant-diet does not contain any DHA that has been demonstrated to play an essential role in brain development in human and rodents by affecting gene expression associated to major component of membrane lipids, as for example the synaptic membranes (Wainwright, 2002). In our study, plant-diet could have impacted swimming activity because DHA is involved in synaptic plasticity, neurotransmission, neurogenesis and has protective properties against oxidative damage to brain lipids and membranes which could cause loss of cognitive or motor skills (Wainwright, 2002; Cohen et al., 2005; Innis, 2007). This is in accordance with the reduced swimming activity of sea bream larvae fed rotifers enriched with vegetable oils (Benítez-Santana et al., 2007). On the contrary, angular velocity was similar in MBF and PBF and similar to the one found in previous study performed in a maze (Benhaïm et al., unpublished data) which indicated that plant-diet did not impact the swimming complexity path. In this study fish from both categories spent a large proportion of time in the start box whereas this was not the case in similar study performed on wild *vs.* domesticated sea bass (Benhaïm et al., unpublished data). This could be due to higher stress in the present work where fish were much bigger than in the cited study and were therefore more difficult to handle. Fish remained motionless in the start box for a large proportion of the time similarly to fish characterized by a freezing behaviour after a stressful event (Malavasi et al., 2004; Millot et al., 2009). Apart from this zone, both fish categories spent most of the time in the zone located near the congener but also at the opposite side. It is in accordance with previous study already cited above (Benhaïm et al., unpublished data). It suggests that plant-based diet did not impact exploration behaviour.

### 4.3. Learning abilities

When fish left the start box, they went preferentially toward the congener zone, performing their very first turnings in the adequate direction. This indicates that they were able to discriminate between two 2-D objects. However, only a very few individuals of both treatments showed significant association between the 2-D object and the reward. The significance of the tests was actually lowered by the high level of "no-choice" responses in both categories.

The best learning criteria was the first turning performed by the fish. Similarly to previous study (Alves et al., 2007) latency did not appear to be a pertinent indicator in our study because of inter-individual differences. Successful individuals also showed anticipatory behaviour since most of them were oriented toward the proper stimulus before the wall was removed. This type of behaviour has recently also been found in the cod *Gadus morhua* (Nilsson et al., 2008) and referred to as goal tracking (Siebeck et al., 2009). The first turning of the fish did not depend on its previous choice and there were no evidence for preferences to turn right or left that could have biased the test. Side preference reflecting behavioural lateralization has long been shown in lower vertebrate (Bisazza et al., 1998) *e.g.* the preference for the right side of a T-maze in tilapia (*Oreochromis niloticus*) (Gonçalves and Hoshino, 1990a). It has been shown that an initial side preference for turning right or left in a T-maze can influence subsequent performance in a trained task in rats, (Andrade et al., 2001) and cephalopods (Boal, 1996; Karson et al., 2003). In our study, successful fish used obviously place strategy rather than response strategy. The place strategy refers to animals that can learn an association between a given place and a reward (Dudchenko, 2001; Gibson and Shettleworth, 2005). Previous studies have shown that fish employ multiple spatial strategies that closely parallel those described in mammals and birds (Lopez et al., 1999) but one of the two strategies can be favoured by the conditions of the experiment (Restle, 1957).
Both fish categories actually showed very similar responses to the test they were submitted to. This indicated that plant-based diet would not have a major effect on spatial orientation such as place learning in sea bass.

### 4.4. Physiological traits

Blood parameters measured after one test in a maze were similar in both fish categories and within the usual values for sea bass (Coeurdacier et al., 1997; Dosdat et al., 2003; Millot et al., 2008). Only BE, $HCO_3$ and $TCO_2$ were significantly different between categories but the values did not indicate any major physiological disturbance. Glucose and ion ($Na^+$, $Cl^-$, $Ca^{2+}$) levels were similar in both fish categories and did not indicate any secondary stress induced by the test procedure since they were within the resting values of sea bass (Cerdá-Reverter et al., 1998; Peruzzi et al., 2005; Di Marco et al., 2008). As already noticed by Marino et al. (2001), briefly handling of sea bass do not induce significant changes in these plasma parameters and one could assume that the present protocol used in experiment 2 was mild enough to leave these plasma parameters unchanged. On the converse, exposure of the sea bass to the test procedure induced high cortisol concentration levels in both fish categories (~ 450 ng $ml^{-1}$ in MBF and ~ 340 ng $ml^{-1}$ in PBF). In the literature, cortisol levels for unstressed sea bass range from 15 to 133 ng $ml^{-1}$ (Roche and Boge, 1996; Cerdá-Reverter et al., 1998; Marino et al., 2001) which lead us to suggest that the present test procedure induced rapid significant increases in the hormone concentration in the blood. The test procedure included acute handling of the fish which typically elicits a cortisol response in numerous species (Schreck, 1982; Barton, 2000). Moreover, tested fish are submitted to isolation stress, a situation which is also known to induce plasma cortisol increase (Allen et al., 2009). Altogether, these plasma cortisol values probably indicate that sea bass exposed to protocol of experiment 2 developed acute stress responses. Consequently, one could question whether such protocol may have modified behavioural responses when comparing PBF and MBF treatments. Thus, results of learning could have also been impaired by this acute stress responses as suggested by previous studies on rat in water maze (Hölscher, 1999) and in Zebrafish in maze (Gaikwad et al., 2011).

An unexpected result obtained in the present study is the significantly lower levels of cortisol observed in stressed PBF fish when compared to stressed MBF sea bass. This results suggest that an important consequence of PB is to reduce cortisol release by interrenal after acute stress exposure. Previous studies carried out in sea bream have shown that replacement in the diet of fish oil by vegetable oil (such as linseed oil but not soyabeen oil) lead to a significant increase of plasma cortisol level after acute stress (Montero et al., 2003) and to a significant increase of cortisol release by head kidney after ACTH stimulation (Ganga et al., 2011). Replacement of fish oil by these vegetable oils which are devoid of n-3 HUFA resulted in reduced tissue levels of acid arachidonic, essential fatty acid and DHA (Ganga et al., 2011). Several studies have shown that these fatty acids can modulate cortisol release in fish (Van Anholt et al., 2004) although their mechanisms of action are still debated (Ganga et al., 2006). In the present study, we observed that manipulation of sea bass (catching by net and transfer during 15 minutes in the maze) led to an acute stress as indicated by the high levels of plasma cortisol and this cortisol response was reduced in fish receiving plant-based diet. One can notice that such discrepancy have already been observed in sea bream between animal fed with linseed oil or with soyabean oil, the latter having no effect on cortisol release (Ganga et al. 2011). In that study, the authors suggested that differences in the ratios between n-3 and n-6 fatty acids in the head kidney may explain such differences. In absence of information on the PUFA (of n-3 or n-6 series) content in the head kidney of our fish, it is difficult to explain the differences between our data in sea bass and those reported in sea bream and further studies should be developed to clarify the undelaying mechanisms. In conclusion, our data clearly confirm that plant-based diet has significant effect on responsiveness of HPI axis to acute stress as already suggested in other fish species (see review by Montero and Izquierdo, 2010).

### *4.5. Conclusion*

This study demonstrated for the first time an impact of a total and early plant-based diet use on sea bass behavioural and physiological traits evaluated at a later stage. Indicators such as swimming activity are relevant in characterizing plant-based diet impact. The results on learning need to be confirmed by further research in which the number of learning sessions and/or individuals would be increased and by designing experiments minimizing the stress and allowing specifying the modalities of spatial learning *i.e.* testing response *vs.* place learning. Results on physiological traits and particularly the cortisol levels also indicate an impact of plant-based diet that need to be further studied. Further research at earlier stage especially during the feed transition *i.e.,* marine to plant-diet could also be useful to better understand the mechanisms underlying the culture performances differences observed between fish fed a marine diet and fish fed a plant-based diet. At last, behavioural, physiological and other traits such as growth performances and survival, need to be linked together to confirm that sea bass fed a plant-based diet can really adapt to the challenges it will face in its environment and that it will be compatible with welfare, quality and health of this species.

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# Chapitre 3

# The importance of egg size and social effects for behaviour of Arctic charr juveniles

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# Résumé

Dans ce chapitre, nous abordons l'influence combinée de deux facteurs biotiques (la taille de l'œuf et l'environnement social) sur le comportement alimentaire et la mobilité de l'omble chevalier.

Les comportements observés à un stade précoce de développement peuvent s'avérer déterminants pour la prise de nourriture, la croissance et par conséquent, ils peuvent jouer un rôle important dans le cycle biologique et la survie individuelle. La taille de l'œuf est corrélée à la croissance des juvéniles d'omble chevalier, mais son influence sur les comportements précités a été peu étudiée. Cette influence de la taille de l'œuf sur la croissance et le comportement des juvéniles pourrait diminuer au cours du temps à cause de l'émergence des interactions sociales. Ce travail examine chez des juvéniles d'omble chevalier, juste après la période de première nutrition exogène, les différences de mobilité et de comportement alimentaire en relation avec la taille initiale de l'œuf et l'environnement social. Les réponses comportementales de juvéniles provenant de petits et de gros œufs ont été comparées à 5 âges différents au cours du développement et dans 3 contextes sociaux : isolement à long terme (avant éclosion), isolement à court terme *vs.* groupe, groupe hétérogène *vs.* homogène en taille. La taille de l'œuf a une influence sur la mobilité et le comportement alimentaire : les individus provenant de gros œufs s'alimentent davantage et sont plus mobiles que ceux provenant de petits œufs. L'environnement social influence le comportement alimentaire, la mobilité et l'utilisation de l'espace : les individus en groupe sont plus mobiles, se nourrissent davantage et répondent plus rapidement après distribution de l'aliment que les individus isolés. L'interaction entre la taille de l'œuf et les effets sociaux a été identifiée pour l'activité alimentaire, mais pas pour la mobilité ni pour l'utilisation de l'espace. Les gros individus en groupe se nourrissent davantage que dans les 3 autres catégories observées : gros individus en isolement, petits individus en groupe et petits individus en isolement. Les comportements agonistiques ont été rarement observés dans cette expérience et aucune différence significative n'existe entre les différents groupes.

Cette étude a donc montré l'importance de la taille de l'œuf et des effets sociaux pour les stades de développement précoces et ces résultats sont discutés sous l'angle de l'écologie évolutive de l'omble chevalier.

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# The Importance of Egg Size and Social Effects for Behaviour of Arctic Charr Juveniles

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## Abstract

Early behaviour can determine food intake and growth rate with important consequences for life history and survival in fishes. Egg size is known to affect growth rate of young Arctic charr but its influence on the development of behaviour is poorly documented. It is believed that egg size influence on growth and potentially on the behaviour of young fish decreases over time, minimized by the effects of social factors. Shortly after first feeding, we examined differences in mobility and foraging of Arctic charr in relation to egg size and social environment. The behaviour of juveniles from small and large eggs was compared five times over the course of development and in three different experimental settings: long-term isolation (isolation before hatching), short-term isolation vs. group rearing and mixed size group vs. homogeneous size groups. Egg size affected foraging behaviour and mobility of fish: fish coming from large eggs were more mobile and foraged more than fish coming from small eggs. Social environment affected foraging behaviour, mobility and space use: fish in a group were more mobile, foraged more and responded faster to food delivery than isolated fish. The interaction of egg size and social effects was seen primarily in foraging activities but did not affect mobility or space use. Large fish in groups foraged more than the three other groups: large fish in isolation, small fish in groups and small fish in isolation. Agonistic behaviour was rarely observed and there was no significant effect of group composition on agonistic behaviour. We discuss the importance of egg size and social effects at early stages of development with a focus on the evolutionary ecology of Arctic charr.

## Introduction

Egg size varies considerably in fishes from microscopic eggs (e.g. 0.75 mm for the greasy grouper *Epinephelus tauvina* up to some very large eggs in sharks and coelacanths (8-cm egg diameter). Usually, a trade-off exists between egg number and egg size because ovarian space and available energy for egg development are limited. Several models have attempted to explain this trade-off between quality and quantity. Lack's (1947) hypothesis and Smith & Fretwell (1974)'s model predicted that each population should have a single optimal egg size to produce the highest number of surviving offspring. Two assumptions were made in this model: (1) there is a trade-off between size and number of offspring and (2) larger offspring have a better chance of surviving i.e. 'bigger is better'. Empirical data support this model in reptiles and fishes (Einum & Fleming 2002; Heath et al. 2003) but not in birds where optimal egg size was consistently smaller than the optimal egg size predicted by the model (Roff 1992). Additionally, between-female variations within population are in disagreement with the model predictions.

Such variation is commonly associated with female phenotype (e.g. body size/age, see Roff 1992) and parental care (Sargent et al. 1987).

Extensions of the single-optimum egg size model (Smith & Fretwell 1974) were developed to explain intra-population variation in egg size (e.g. Sargent et al. 1987; Hendry et al. 2001). These authors made the basic assumption that the egg size–offspring fitness function varies with the phenotype of the mother. For instance, larger female coho salmon *Oncorhynchus kisutch* produce larger eggs and provide better maternal care by guarding the redd (Quinn 2005) resulting in higher survival of young from large eggs. Such maternal effects may explain the discrepancies with the Smith and Fretwell's model. Recent work has also explored the idea that within-clutch variation in egg size may be a bet-hedging tactic as an adaptation to fluctuating environments or that it may result from other constraints (Marshall et al. 2008).

In fishes, the correlation between egg size and female body size has been of interest for decades (Thorpe et al. 1984; Chambers & Leggett 1996). Much of this work has been conducted on salmonids because of their fairly large eggs (3–8 mm in diameter) and their important commercial value (Hendry & Stearns 2004). They have, as well, been the focus of theoretical and empirical studies on evolutionary and ecological significance of egg size (Hendry et al. 2001; Einum & Fleming 2002; Hendry & Stearns 2004), but the importance of egg size for population divergence has seldom been studied.

Arctic charr *Salvelinus alpinus* females show considerable variability in egg size and yolk quality resulting in a wide size distribution of juvenile at first feeding (Balon 1980; Wallace & Aasjord 1984; Beacham et al. 1985; Kamler 1992; Seppä 1999; Jonsson & Svavarsson 2000). The correlation between egg size and Arctic charr juvenile size persists for up to 1 yr after first feeding (Wallace & Aasjord 1984). Embryos from smaller eggs develop faster than those from larger eggs, suggesting that different timing of development is connected to egg size (Valdimarsson et al. 2002). Because Arctic charr is a species lacking parental care, egg size and thus embryo size can be considered as a direct measure of maternal investment in individual offspring.

Previous studies have emphasized the high degree of polymorphism in Arctic charr (Skúlason et al. 1993, 1999; Snorrason & Skúlason 2004; Klemetsen 2010). Sympatric forms have been found to use different resources (habitat and food) and to differ in phenotypes: growth, age and size at maturity, body coloration, behaviour and body shape (summarized in Skúlason et al. 1999; Klemetsen 2010). It has been suggested that differences in early behaviour may be important for the observed diversification (Skúlason et al. 1999; McLaughlin & Grant 2001), where early behaviour is likely to influence individual behaviour later in life (Metcalfe 1993; Salvanes & Braithwaite 2006). Thus, small size differences at first feeding stemming from differences in egg size may promote differences in mobility patterns with important consequences for subsequent differences in habitat and food selection. Such differences in resource use could lead to variable life histories and promote the evolution of resource polymorphism (McLaughlin & Grant 2001).

A common pattern proposed in the literature is that the effect of egg size on body size of progeny declines rapidly throughout development, especially when the fish starts feeding (reviewed by Mousseau & Fox 1998; e.g. Heath et al. 1999). A reduction in maternal effects, e.g. egg size, through ontogeny could arise because the relative importance of the environment and genetic factors increases later in development (Lindholm et al. 2006). Little is known about the interplay between the role of egg size and the environment during early developmental stages. Any effect of egg size on progeny fitness might even disappear faster in salmonid juveniles because they develop territoriality soon after exogenous feeding (Quinn 2005). For example, the effects of egg size on growth disappear at emergence in chinook salmon *Oncorhynchus tshawytscha* (Heath et al. 1999). A few days after first feeding, juveniles develop a feeding territory involving agonistic interactions with conspecifics (Quinn 2005). Such social interactions may erase the effect of egg size on growth and behaviour of salmonids early in life.

The aim of this study was to investigate the mechanisms of the behavioural differences between small and large Arctic charr juveniles at the onset of first feeding as described by Benhaïm et al. (2003). These authors showed that large and small Arctic charr coming from large and small eggs differed in terms of mobility and foraging tactics. Those observations were conducted on fish raised in homogenous size groups but they did not account for potential agonistic behaviours that could occur in heterogeneous ones. Based on the literature, we predicted that social effects will explain most of the differences between large and small fish in fish groups. We assessed both egg size and social effects in experiments based on isolation of fish vs. raising them in groups. We addressed several questions: (1) How can egg size affect early behaviour of individual offspring? (2) How do egg size, the social environment and their interaction affect behaviour of Arctic charr? We predicted that social interactions will affect the behaviour of both size classes of fish, minimizing egg size effects on foraging and mobility: feeding behaviour and mobility will be higher in fish held in groups because of interactions with conspecifics. (3) Finally, we assessed the importance of agonistic behaviours in behavioural differences between small and large fish maintained in a group, i.e. can agonistic interactions between different size fish explain the behavioural differences previously observed by Benhaïm et al. (2003)?

## Methods

### Eggs, Fish and Experimental Set-up

We used Arctic charr from the breeding programme of Hólar University College. Hatchery broodstock originated mainly from the lake Ölvesvatn in north-west Iceland. Intra-clutch variation in egg size has been previously reported in Icelandic Arctic charr (Benhaïm et al. 2003) although not studied in detail. All eggs and juveniles used in our study came from the fertilization of one female (age: 4+) with the sperm from one male (4+). We decided to use only one family as our study is the first step towards understanding how egg size and social environment affect the behaviour of salmonid juveniles. Fertilized eggs were incubated in EWOS hatching trays with flowing water ($\bar{x} \pm \text{SD} = 5.2 \pm 0.3°\text{C}$) and maintained in darkness using an opaque black plastic cover. At the eyed stage, 50 embryos were sampled to estimate size variation. Eggs were visually sorted creating two size classes, with as much size difference as possible (paired *t*-test, $t_{(48)} = 15.8$, $p < 0.0001$), small eggs ($\bar{x} \pm \text{SD} = 36.6 \pm 3.1$ mg, n = 25) and large eggs ($\bar{x} \pm \text{SD} = 51.2 \pm 3.5$ mg, n = 25). We placed 100 from each size class in net cages (10.5 × 10.5 × 6 cm, mesh size 0.5 mm), and six from each size class were individually isolated in net cages. After the eggs had reached eyed stage, dead embryos or unfertilized eggs were removed daily. Incubation took on average 465 degree days, and hatching date was 21 February 2005 (98 dpf), i.e. 50% of the embryos had hatched. In our experiment, small fish came from small eggs and large fish came from large eggs (e.g. at 159 dpf: $\bar{x} \pm \text{SD}$ small fish = 65.2 ± 6.6 mg vs. large fish 98.2 ± 8.3 mg). From hatching, one group of small fish and one group of large fish were raised separately in incubating trays until being assigned to the treatment. Long-term isolated embryos were isolated at the eyed stage and reared in the compartment for observation.

Water temperature was maintained at 4.9 ± 0.5°C throughout the observation period, and water level was held at 12 cm in each compartment. A flow velocity of 0.2 cm/s was maintained in every tray. The 12 trays were placed randomly in two tanks (250 L) and moved each week to reduce the impact of small differences on environmental variables such as temperature, light or oxygen availability. Light intensity was approximately 50 lux, and a 12:12 LD photoperiod was applied. The entire system was isolated from any disturbance by black opaque plastic curtains.

Juveniles were fed commercial food (EWOS micro 013C, 0.1–0.2 mm). Food rations were established during pre-observation periods. Fish in groups were fed a ration of 30 mg, while fish kept isolated were fed 10 mg regardless of body size. Such rations were selected to allow observations of foraging by fish according to the unit volume of the trays. The food was hand-delivered once during each observation, and both amounts of food were sufficient to sustain regular growth. Daily food leftover and faeces were removed after each observation. Between observations, fish were fed three times a day according to aquaculture ration for Arctic charr juveniles.

### Experiments and Behavioural Observations

Three social environments were tested: no isolation, i.e. group of six fish, short isolation and long isolation. Short isolation refers to fish that were maintained in a group and were then isolated 24 h before observation. Long isolation refers to embryos that were isolated since eyed stage. We had six small and six large fish in long isolation. Mobility, foraging behaviours and space use were estimated by comparing behaviour of large and small fish maintained in the three different social contexts. Behaviour was observed using focal animal sampling (Altmann 1974) before and after food delivery. Behaviour before food delivery was recorded as a baseline of activity before feeding.

The first experiment aimed to compare during development the behaviour between small and large fish coming, respectively, from small and large eggs, isolated since eyed embryo stage (cf. first question in the Introduction). The same 12 fish (six large and six small) were individually observed five times during development (Table 1). In a second experiment, we compared small and large fish in different social

**Table 1:** Experimental design table

| | Age | Group composition | Replicates | Nature of fish | Environment | Statistics |
|---|---|---|---|---|---|---|
| Experiment 1 | 5 | 1 small fish vs. 1 large fish | 6 | Same individuals followed over time | Long term isolation | Repeated measures ANOVA |
| Experiment 2 | 5 | Group of 6 small or 6 large fish<br>vs. 1 small or 1 large fish | 3<br>6 | Different individuals/groups at each age | Homogenous group vs. short term isolation | ANOVA |
| Experiment 3 | 5 | Group of 6 small or 6 large fish<br>vs. group of 3 small and 3 large fish | 3<br>3 | Different individuals/groups at each age | Homogenous vs. heterogeneous group | ANOVA |

This table shows for each environment the group composition, the number of replicates and the nature of the fish used in the three experiments. Experiment 1 compared small and large fish isolated at eyed stage. Experiment 2 compared small and large fish maintained in groups of 6 fish vs. small and large fish placed in short isolation. Experiment 3 compared three groups of small or large fish vs. three groups of mixed small and large fish. Observations were made at 5 ages: 159, 173, 180, 187 and 194 d post-fertilization. The column replicates refers to the number of replicates for each treatment at each age.

environments, i.e. group of six fish vs. six shortly isolated fish (cf. question 2 in the Introduction). We used three replicates of six small fish in groups and six large fish in homogenous group and six replicates of individual small and large fish in short isolation (Table 1). In a third experiment, we compared agonistic behaviour of juveniles from different size classes maintained in a group (cf. question 3 in the Introduction). We compared three replicates of small and large fish maintained in homogenous groups and three replicates of mixed groups, i.e. three small and three large fish in a group (Table 1). The behavioural sampling method (Altmann 1974), i.e. counts of behaviour occurrences before and after food presentation, was used to compare agonistic behaviour between homogenous and mixed groups.

In these three experiments, every trial lasted for 3 min, i.e. 1 min before food delivery and 2 min after (Benhaïm et al. 2003). The behaviour of fish was voice-recorded to collect both the occurrence and the duration of behavioural items. The target fish was selected randomly as the first individual crossing a randomly selected area. Food pellets were supplied by hand above the left side of the unit where the food tended to drift out of the compartment. Therefore, the mobility of fish was maximized towards the feeding area. Observations were carried out daily between 09:00 and 13:00 h. At each time, fish were observed 4 d in a row in each treatment.

Experiments started 6 d after the onset of first feeding, and observations were repeated five times at 159, 173, 180, 187 and 194 days post-fertilization (dpf). Different fish were observed at each observation date except for the fish in long isolation. Fish were not fed for 2 d before observation, providing a similar level of appetite without causing discomfort from food deprivation. A 2-d fasting period has been used in Arctic charr (Lahti & Lower 2000) and other fish species without causing starvation of juveniles (e.g. Enders et al. 2005). One day before observation, juveniles were anaesthetized and measured for length and weight (to the nearest 0.1 mm and 0.001 g). Then, the juveniles were assigned to one of the two social environments: group or short isolation.

### Behavioural Variables

After hatching, juveniles were kept in 12 EWOS hatching trays (39.5 × 42.5 × 17.2 cm). Each tray was longitudinally divided into six compartments, each compartment being a unit of observation for a single fish or a group of fish. To collect data on fish movement, we visually divided each compartment into five equal viewing areas (8 × 7 cm) in length and three areas in depth: areas were marked with a waterproof marker. Using these visual landmarks, we were able to describe the position of the fish horizontally and vertically and to record mobility. The depth of the compartment was divided into three equal parts: the surface, the water column and the bottom. Each snap by a fish at a particle in these locations was, respectively, called surface foraging, foraging in the water column and bottom foraging. Reaction time was also recorded and defined as the latency (in seconds) before the first bite at a food particle.

Immobility and mobility were recorded in a similar way to that described by Benhaïm et al. (2003): horizontal and vertical stationary movements, slow and regular swimming, jerky swimming and speed swimming (see Benhaïm et al. 2003 for ethogram). We recorded both the occurrence and the duration of each activity. The total number of items corresponded to the sum of all behavioural occurrences in one observation. Additionally, space use was assessed for each fish recording the number of zones (horizontal dimension) and levels (vertical dimension) visited. We also calculated the total number of crossed areas, i.e. the sum of all visited zones and levels.

Aggression level was characterized by two relevant agonistic behavioural items previously described in juvenile fish. Chase was defined as pursuit of one individual by another for at least one body length (Kim et al. 2004). Escape behaviour referred to a burst and fast swimming by one individual to move away from a conspecific (Noakes 1980).

### Data Analysis

We used SPSS 14.0 Windows Student Version (SPSS, Inc., Chicago, IL, USA) for statistical analyses. Difference of weight between fish coming from large and small eggs was analysed with a *t*-test. Data before food delivery provided a baseline of behaviour/activity shown by the fish before feeding, while those after food delivery were analysed to assess mobility, foraging behaviour and space use. Data were obtained by averaging the behaviour from 4 d of observation for each treatment, each replicate and each time. Data from the focal animal sampling method were behaviour durations in seconds, while those from behaviour sampling method were behaviour occurrences. Data were analysed for normality with a Shapiro–Wilk test and for homoscedasticity with a Bartlett's test.

In the first experiment, differences in behaviour, mobility and space use between small and large fish, reared in isolation, were assessed using a repeated measures analysis of variance (ANOVA) because the same fish were followed over time (Table 1). Egg size (small and large) was between-subjects factor, and time was within-subjects factor. In the second experiment, we used an ANOVA where egg size, social environments and time were defined as fixed factors (Table 1). The model included three fixed factors, 2- and 3-way interactions. To analyse the origin of the significant differences, we conducted *post hoc* Newman–Keuls tests in both ANOVAs.

## Results

Over the course of the experiment, fish coming from large eggs were on average 32.5 ± 8.5% larger than fish coming from small eggs. At the end of the experiment, the large fish weighted 194.9 ± 24.3 mg and small fish 131.1 ± 13.3 mg ($t = 14.89$ df = 82 $p < 0.001$).

Before food presentation, all fish kept alone, independently of their previous social context, were immobile at least 90% of the time.

### Experiment 1: Egg Size Effect on Behavioural Development (Long-Term Isolation)

Differences between small and large fish were detected in foraging activity, mobility and space use. Large fish foraged significantly more (i.e. total foraging) and faster (i.e. reaction time) than smaller ones (Table 2). On average, larger fish foraged 4.6 ± 3.3 times more than smaller ones (Fig. 1a) and reacted to food delivery 1.4 ± 0.6 times faster than smaller ones. They were also more mobile (Fig. 1b) and had more active behaviour than smaller ones (Table 2). For instance, larger fish spent 72.8 ± 21.3% of the time immobile, whereas smaller fish spent 88.0 ± 24.0% (Table 1). Additionally, larger fish crossed in averaged 2.4 ± 1.9 more areas than smaller ones.

Interestingly, such differences became significant through development with the exception of the last observation (e.g. Fig. 1). Reaction time to food delivery illustrates this trend where differences between large and small fish increased over time (with the exception of 194 dpf): large fish foraged on average

**Table 2:** Summary of repeated measures ANOVA results for fish placed in long-term isolation

| | Egg size df = 1 | |
|---|---|---|
| | *F* | *P* |
| Foraging | | |
| Reaction time to food | 3.1 | 0.098 |
| Foraging in water column and surface | 2.3 | 0.160 |
| Bottom foraging | 2.5 | 0.140 |
| Total foraging | 4.7 | 0.036 |
| Mobility | | |
| Immobility | 3.6 | 0.074 |
| Stationary | 0.6 | 0.680 |
| Vertical stationary | 0.7 | 0.630 |
| Slow and regular swimming | 0.4 | 0.802 |
| Jerky swimming | 1.2 | 0.394 |
| Rapid swimming | 1.5 | 0.311 |
| Number of items | 8.6 | 0.010 |
| Space use | | |
| Total number of crossed areas | 2.0 | 0.212 |
| Visited zones | 4.6 | 0.046 |
| Visited level | 3.3 | 0.095 |

We compared the effect of egg size on each behavioural variable for six small fish and six large fish placed in isolation since eyed stage. Fish were observed at five different times.

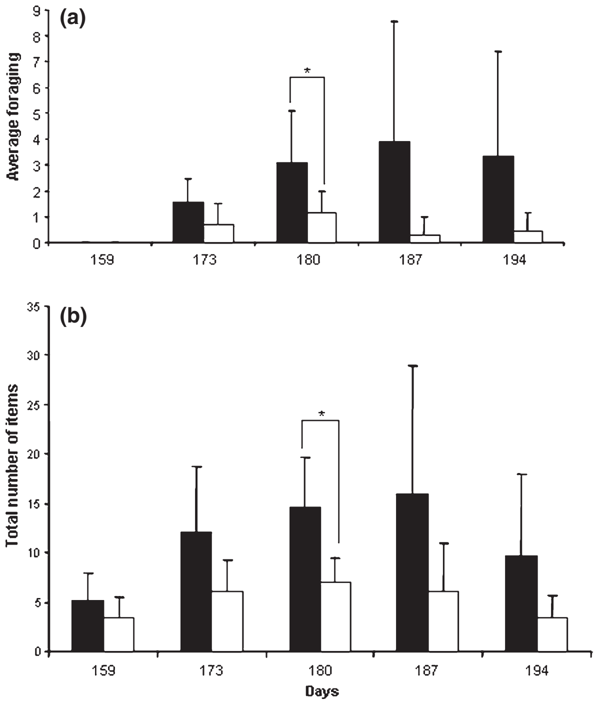

**Fig. 1:** Foraging and mobility of large and small juveniles of Arctic charr isolated since hatching. Foraging (a) and the total number of behavioural items performed (b) during the two minutes of observation after food presentation are shown. Mean + SD values are given. Differences between small and large fish (Newman–Keuls *post hoc* tests) are shown: *$p < 0.05$.

27.5 s earlier than small fish. This relationship became significant at 180 dpf ($p = 0.024$), 187 dpf ($p = 0.007$) and 194 dpf ($p = 0.043$). Same trend was observed in the number of visited zones (173 dpf, $p = 0.035$; 180 dpf, $p = 0.020$; 187 dpf, $p = 0.050$) and the total foraging activity (180 dpf, $p = 0.055$; 187 dpf, $p = 0.093$; and 194 dpf, $p = 0.110$).

### Experiment 2: Interaction Between Egg Size and Social Effects on Behavioural Development (Short-Term Isolation vs. Group)

Egg size, social environment and time affected foraging, mobility and space use of young Arctic charr. Egg size significantly affected bottom and total foraging but not mobility or space use (Table 3). However, most variables characterizing foraging and mobility and all variables characterizing space use showed a social effect (Table 3). For instance, fish in groups reacted faster (36.4 ± 17.5 s) to food delivery than fish in short isolation (93.9 ± 15.5 s). Fish became more mobile over the course of the experiment: stationary, rapid swimming, total number of visited areas and number of displayed items increased (time factor in Table 3). Additionally, the reaction time to food delivery significantly decreased and foraging activities increased (significantly for bottom foraging and marginally significant for foraging in water column and surface; factor time in Table 3) resulting in weight gain: small fish gained in average 66.1 ± 8.9 mg and large fish gained in average 100.7 ± 14.0 mg over the experimental period of 45 d.

Bottom and total foraging activities were affected by a two-way interaction between egg size and social effect (Table 3). Large fish in groups foraged more than large fish in isolation, small fish in group and small fish in isolation (*post hoc* tests: all $p < 0.001$; Fig. 2a). Only rapid swimming activity, a rather rare and brief behaviour, showed the same interaction with large fish in groups displaying more rapid swimming than other groups (*post hoc* tests: all $p < 0.001$). The interaction between egg size and time was not found in any variables, but the interaction between social effect and time was found mainly in foraging activities and rapid swimming (Table 3). Fish in group at 180 and 187 dpf displayed more bottom foraging and total foraging than fish shortly isolated at all times (*post hoc* tests: all $p < 0.001$; Fig. 2a). Similarly at 187 dpf, fish in groups displayed more rapid swimming than other groups at all times (*post hoc* tests: all $p < 0.001$).

Additionally, foraging, mobility activities and the total number of crossed areas were affected by a three-way interaction of factors (egg size, social factor and time; Table 3 and Fig. 2). Overall, this interaction illustrates a gradient of activity (foraging, mobility and space use) with large fish in group being more active than small fish in group being more active than large fish in isolation being more active than small fish in isolation. A 3-way interaction may indicate that the interaction between egg size and social effect changed over time. For example, the average total number of foraging (Fig. 2a) was as follows: large fish in group at 180 and 187 dpf foraged more than small fish in group and small and large isolated fish at all time (*post hoc* tests: all $p < 0.001$). Another type of 3-way interaction was observed in the number of items (Fig. 2) and in the total number of crossed area (Table 3) where similar results were observed: both variables were higher in large fish in groups at 187 dpf compared with all other categories (*post hoc* tests: all $p < 0.001$) except for small fish in groups at 173 and 180 dpf, large fish in short isolation at 159 and 194 dpf and large fish in groups at 194 dpf.

**Table 3:** Summary of ANOVA results for fish in groups vs. fish in short isolation

| | Size df = 1 | | Social effect df = 1 | | Time df = 4 | | Size*Social df = 1 | | Size*Time df = 4 | | Social*Time df = 4 | | Size*Social* Time df = 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Foraging | | | | | | | | | | | | | | |
| Reaction time to food | 1.4 | 0.250 | 90.9 | 0.000 | 3.4 | 0.010 | 0.9 | 0.340 | 1.2 | 0.310 | 0.4 | 0.830 | 1.2 | 0.300 |
| Foraging in water column and surface | 0.1 | 0.780 | 0.6 | 0.450 | 2.1 | 0.091 | 0.0 | 0.940 | 0.0 | 0.890 | 1.1 | 0.350 | 0.2 | 0.910 |
| Bottom foraging | 6.1 | 0.016 | 15.5 | 0.000 | 3.1 | 0.020 | 7.8 | 0.007 | 1.1 | 0.370 | 5.7 | 0.001 | 3.4 | 0.010 |
| Total foraging | 5.4 | 0.020 | 14.8 | 0.000 | 2.0 | 0.100 | 6.3 | 0.014 | 1.0 | 0.440 | 3.9 | 0.007 | 3.5 | 0.010 |
| Mobility | | | | | | | | | | | | | | |
| Immobility | 0.6 | 0.430 | 10.2 | 0.002 | 1.9 | 0.130 | 0.0 | 0.950 | 0.6 | 0.650 | 0.4 | 0.840 | 3.0 | 0.025 |
| Stationary | 2.8 | 0.097 | 4.4 | 0.040 | 2.4 | 0.060 | 0.0 | 0.910 | 1.6 | 0.180 | 2.3 | 0.070 | 2.6 | 0.045 |
| Vertical stationary | 3.5 | 0.067 | 0.1 | 0.730 | 1.6 | 0.186 | 4.7 | 0.540 | 1.3 | 0.280 | 0.4 | 0.780 | 0.7 | 0.680 |
| Slow and regular swimming | 0.6 | 0.450 | 1.2 | 0.270 | 1.4 | 0.240 | 0.3 | 0.610 | 0.2 | 0.950 | 0.2 | 0.910 | 1.4 | 0.250 |
| Jerky swimming | 0.1 | 0.820 | 2.3 | 0.130 | 2.9 | 0.003 | 0.0 | 0.870 | 0.7 | 0.560 | 1.6 | 0.180 | 0.6 | 0.610 |
| Rapid swimming | 2.5 | 0.110 | 8.0 | 0.006 | 2.7 | 0.039 | 7.5 | 0.008 | 1.4 | 0.260 | 2.6 | 0.046 | 1.1 | 0.360 |
| Number of items | 2.1 | 0.150 | 14.7 | 0.000 | 5.4 | 0.001 | 0.0 | 0.930 | 0.8 | 0.510 | 1.1 | 0.340 | 3.8 | 0.018 |
| Space use | | | | | | | | | | | | | | |
| Total number of crossed areas | 1.1 | 0.290 | 7.5 | 0.008 | 7.2 | 0.001 | 0.0 | 0.860 | 0.8 | 0.560 | 0.6 | 0.650 | 2.5 | 0.053 |
| Visited zones | 0.2 | 0.660 | 10.6 | 0.002 | 3.3 | 0.015 | 0.0 | 0.890 | 1.2 | 0.300 | 1.5 | 0.230 | 2.1 | 0.090 |
| Visited level | 2.5 | 0.120 | 2.7 | 0.100 | 3.2 | 0.018 | 0.8 | 0.360 | 1.1 | 0.370 | 0.6 | 0.670 | 1.1 | 0.390 |

*F* value, degrees of freedom and the probability p are displayed for each dependent variable. The factor 'size' refers to the effect of egg size (large vs. small). 'social' effects refer to the two different social treatments tested: group of six fish vs. short isolation. Short isolation refers to fish that were maintained in group and were isolated 24 h before observation. The symbol '*' is used to characterize the interaction between factors. The factor time refers to the five different ages at which fish were observed.

### Experiment 3: Agonistic Behaviour (Mixed vs. Homogeneous Size Groups)

In groups, agonistic behaviour (chase or escape) was rarely observed, and no significant differences were detected between heterogeneous and homogenous size groups.

## Discussion

Our results show how social environment and body size may affect behaviour at early stages of development and indicate as well how behavioural patterns may change over time. They highlight the relative importance of both egg size and social effects for small and large fish in foraging, mobility and the use of space. In long isolation, egg size affects both mobility and foraging activities. We also demonstrated that social interactions, other than agonistic behaviour, play an important role in mobility and foraging of first feeding fish. Overall, a social effect was observed in almost all behavioural items we looked at. Fish in groups were more mobile over time and space and foraged more than fish placed in short isolation. Egg size clearly affected foraging activities (larger fish foraging more than smaller fish) but did not affect mobility or space use. However, we observed 2-way interactions (egg size × social environment and social environment × time) and 3-way interactions (egg size × social environment × time) in foraging and mobility indicating that social effects alone did not explain the observed behavioural differences. The interaction egg size × social environment affected foraging behaviours and one mobility variable (i.e. rapid swimming) revealing that the combination of factors egg size and social environment does not influence much mobility or space use of the fish (Table 3). The influence of time was difficult to interpret: overall mobility and foraging activities increased over time up to 187 dpf. However, activities were overall lower at 194 dpf. This could reflect plasticity or an artefact of measures.

Our study supports the hypothesis that variation in feeding behaviour may not be primarily the result of social hierarchies but rather the result of a strong genetic component and/or parental effects (Ferguson & Noakes 1982, 1983; Kamler 2005; Martins et al. 2005a,b). There is substantial genetic basis for many observed differences in early history and behaviour (Noakes 1989; Boake 1994). When behavioural

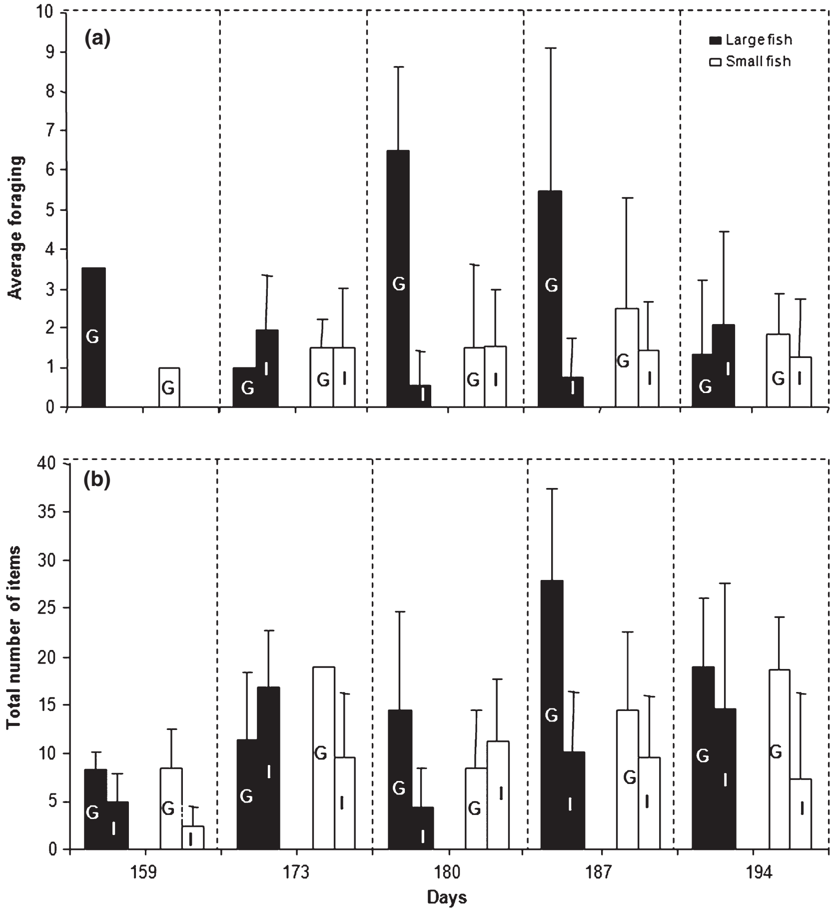

**Fig. 2:** Foraging and mobility of large and small juveniles of Arctic charr in different social environments. Foraging (a) and the total number of behavioural items performed (b) during the two minutes of observation after food presentation are shown. Means + SD values are given. G: fish observed in group condition; I: fish shortly isolated. Large fish are in black and small fish in white.

differences are observed between two populations, the assumption is often made that those differences stem from inherited, i.e. genetic differences rather than maternal effects (Huntingford 2004). However, our study is one of few showing the importance of egg size on the behaviour of juvenile salmonids. These behavioural differences may have their roots in differences in egg chemical composition provided by the mother. Differences in egg size may reflect differences in egg content with potential consequences for later development of embryos. Preliminary results on total energy content of individual eggs of Arctic charr indicate that larger eggs have more energy content than smaller eggs (Leblanc, C. unpublished data). In charr, non-genetic maternal effects, i.e. all materials transferred from mother to egg beyond genes, may play an important role in early stages of fish development including the development of behaviour. Behavioural differences may also be the result of interaction between genetic and maternal effects, but our experiments were not designed to measure such effects.

Our study showed that early behaviour of fish can be influenced by egg size with direct consequences for growth. Such results may be important in terms of evolution of fishes and dynamics of populations (Green 2008). In fact, egg size may be a tool used by the mother to adapt to fluctuating environments to increase her fitness. Our results and those of Benhaïm et al. (2003) indicate that each egg size may correspond to a different behavioural tactic, especially in terms of mobility and foraging behaviour. Different phenotypes may arise from different egg size as seen in spadefoot tadpoles *Spea multiplicata*. Martin & Pfennig (2010) showed that larger females invested in larger eggs, which in turn produce larger tadpoles better able to capture shrimp that induce carnivore morphology. Egg size may indeed be a source of novel resource use phenotype. More work is needed regarding the considerable scope for egg size and egg quality for fish behaviour and morphology. Experimental designs including several females will help to better understand the importance of egg size and maternal investment on the behaviour of fishes and its potential role in the evolution of fish phenotypes.

From our study, it is possible to conclude that social environment plays an important role in mobility and foraging of first feeding fish, where fish in groups were more active than fish maintained in isolation. These results are consistent with previous studies examining isolated fish (Koebele 1985; Jobling & Baardvik 1994; Martins et al. 2005a,b) where isolation generally induces fewer foraging attempts, longer food biting latency (Gómez-Laplaza & Morgan 1991), decreases in mobility (Gómez-Laplaza & Morgan 2003) and less flexibility in behaviour (Salvanes et al. 2007) by reducing competition pressure, predation risk and the absence of social facilitation. The greater feeding latency that we observed in isolated fish is most likely due to the absence of social interactions (Gómez-Laplaza & Morgan 1991) and the lack of visual contact with conspecific providing increased feed intake and growth rate in a group of fish(Sundstrom & Johnsson 2001; Martins et al. 2006). Additionally, it has been hypothesized that aggressive interactions are higher in heterogeneous size groups, especially in salmonids (Abbott et al. 1985). Unlike other studies, we observed almost no differences in aggressive interaction between mixed and homogenous groups. These results are similar to low levels of aggression previously found in similar-sized Arctic charr (Benhaïm et al. 2003).

We have demonstrated that differences in behaviour between small and large Arctic charr juveniles were triggered by egg size, social environment, time and the interaction of those factors. Additionally, our results show that egg size effects were not cancelled out by the effect of social environment but rather interact with the social environment to affect early behaviour. This is surprising for salmonid juveniles where the importance of social interactions has been widely reported in both laboratory and field studies (e.g. Glova 1986). Heath et al. (1999) reported that the effect of maternal size on offspring size disappeared shortly after emergence in chinook salmon *Oncorhynchus tshawytscha*, with offspring tending to resemble their fathers more than their mothers. We showed that egg size affects behaviour early in development and may still affect mobility and foraging of fish later in life.

In a polymorphic system like Arctic charr, differences in feeding tactics between small and large fish could be linked to evolutionary processes. Indeed variation in behaviour, stemming from small size differences at first feeding, may influence habitat and food selection that can lead to divergence of fish populations, especially if there are clear interactions between maternal and genetic effects (Leblanc et al. unpublished observations). In wild populations, Sturlaugsdóttir (2008) showed important genetically fixed differences in mobility among Icelandic Arctic charr morphs (pelagic/benthic). Evidence for genetic differences in the behaviour of offspring of a 'profundal' and a 'littoral' morph has previously been suggested by Klemetsen et al. (2002) in a Norwegian population of Arctic charr. Those differences may be related to habitat and diet specialization of the morphs. Considering the importance of egg size may greatly improve our understanding in many areas of evolutionary biology (Räsänen & Kruuk 2007), especially our understanding of maintenance of diversity within a species. Such a maternal effect may for example be an important contribution to the large intra-specific diversity seen in Icelandic populations of Arctic charr (Skúlason et al. 1999). The importance of egg size and more generally the importance of maternal effects for resource polymorphism and evolution of diversity of fishes is a new field that needs to be further studied.

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# Chapitre 4

# Effect of size grading on Sea bass (*Dicentrarchus labrax*) juvenile self-feeding behaviour, social structure and culture performances

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# Résumé

Dans ce chapitre, nous abordons l'influence d'un facteur abiotique de grande importance en aquaculture : la pratique du tri. Ce facteur abiotique conduit à la formation de deux structures en taille (homogène *vs*. hétérogène). Ce nouvel environnement social constitue donc un facteur biotique. Cette expérience combine en conséquence un facteur abiotique et un facteur biotique.

L'hétérogénéité de croissance bien qu'ayant été largement étudiée en pisciculture, demeure un problème central. La méthode usuelle pour la réduire consiste à trier les cheptels, ce qui permet de faciliter la gestion de l'alimentation et les opérations de vente. Cette procédure permettrait de rompre le développement des hiérarchies sociales, offrant ainsi la possibilité aux plus petits individus de connaître une meilleure croissance en l'absence des compétiteurs de plus grande taille. Les systèmes d'auto-alimentation répondent aux nouvelles préoccupations concernant le bien-être animal et constituent des dispositifs adaptés à l'étude du comportement alimentaire. Cette étude est la première visant à tester l'influence du tri sur le comportement alimentaire, la structure sociale et les performances de croissance de juvéniles de bars marqués individuellement à l'aide d'un PIT-tag et suivis grâce à un système informatisé d'alimentation à la demande couplé à une antenne de détection. Une expérience en deux phases a été réalisée : une première phase de 27 jours (6 bassins de 100 bars, 40 g de poids moyen initial), une deuxième phase où les individus ont été triés de façon à créer deux lots de poids similaires, mais de coefficients de variation (CV en %) différents (3 lots hétérogènes de 60 individus : 20% ; 3 lots homogènes de 60 individus : 10%). La première phase a été comparée à une période de 27 jours suivant le tri ; puis les performances des lots homogènes et hétérogènes ont été comparées sur une période de 56 jours. La première phase inclut une période d'apprentissage rapide (entre 2 et 12 jours). La demande alimentaire augmente après le tri avant de se stabiliser à une valeur journalière de18 g kg$^{-1}$ de biomasse par la suite. La structure sociale a été modifiée par le tri et/ou en raison du temps d'apprentissage initial. Les manipulateurs principaux ne sont apparus qu'après le tri, les non-manipulateurs ont diminué (90 à 80%) et les manipulateurs occasionnels ont augmenté (10 à 18%). En revanche, les performances de croissance et les demandes alimentaires ne se sont pas révélées différentes entre les lots homogènes et hétérogènes durant la seconde phase de l'expérience. Les rythmes alimentaires sont similaires dans les deux types de lots et caractérisés par deux pics à 6:00 et 11:00. La structure sociale ne diffère pas entre les lots et consiste en 1 ou 2 manipulateurs principaux, environ 80% de non-manipulateurs et 18% de manipulateurs occasionnels. Aucune différence n'est relevée au niveau des paramètres sanguins.

En conclusion de cette étude, il semble que la pratique du tri, commune en aquaculture, ne modifie que légèrement le comportement de demande alimentaire et la structure sociale qui se construit autour du dispositif d'auto-alimentation et qu'elle n'améliore pas les performances de croissance chez le bar.

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# Effect of size grading on sea bass (*Dicentrarchus labrax*) juvenile self-feeding behaviour, social structure and culture performance

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**Abstract –** This study aims to test the influence of size grading on self-feeding behaviour, social structure (measured by the percentage of triggering acts per individual), growth performances, and blood physiological variables of individually passive integrated transponder (PIT)-tagged sea bass juveniles, using a computerized on-demand feeding system coupled with a PIT tag monitoring device. Three consecutive periods of 27 days each were compared: a first period ($P1$) before grading (6 tanks of 100 fish; 40.2 ± 8.9 g) followed by a second period ($P2$) after grading. The protocol applied aimed to create two groups of fish of similar mean weight but with either a low or a high coefficient of variation of weight ($CV_w$) corresponding to an imposed difference in social disruption ($T_{low}$: $CV_w \sim 10\%$, 3 tanks of 60 fish each with social disruption; $T_{high:}$ $CV_w \sim 20\%$, 3 tanks of 60 fish each, without social disruption). $T_{low}$ and $T_{high}$ groups were studied over $P2$, and an additional 27-day period under identical conditions ($P3$). The grading protocol used and/or time modified the social structure when comparing $P1$ and $P2$. Thereafter, during $P2$ and $P3$, no difference could be observed in growth performances, feed demand, or physiological variables between $T_{low}$ and $T_{high}$ groups. Feeding rhythms and social structures were similar in both groups. In conclusion, such grading practice only transiently modifies feed demand behaviour and social structure built around the self-feeder, without further improvement in individual growth performances in sea bass.

**Key words:** Feeding behaviour / Self feeder / Triggering activity / Heterogeneity / Growth / Grading

## 1 Introduction

Variation in individual growth is a common feature in many cultured fish stocks (Huntingford et al. 1990; Stefánsson et al. 2000; Smith and Fuiman 2003). Magnuson (1962) defined growth depensation as the increase in the variance of size distribution over time, due to differences in growth rates. Such variation is generally considered as a drawback in commercial fish culture (Barki et al. 2000). Indeed good aquaculture practices usually aim to minimize growth depensation to reduce food wastage, and water quality degradation. Even though growth heterogeneity has been extensively studied in both natural and cultured fish population, it remains a central problem in aquaculture.

Kestemont et al. (2003) stated that growth heterogeneity is induced by a wide range of intrinsic and environmental factors, of which the relative influence is largely unknown but could be qualified as either inherent (i.e. having a strong genetic component that is expressed to a varying degree according to the environmental conditions) or imposed (i.e. requiring specific biotic or abiotic conditions to be manifested). Among the biotic factors, social interaction has been identified as a major cause of individual variation in growth (Jobling et al. 1993; Cutts et al. 1998) if it leads to feeding hierarchies that decrease growth of low ranked fish (Koebele 1985). It is also well known that size-related dominance determines aggressive behaviour, feeding and growth performances in fish (Abbot and Dill 1989). Some mechanisms have been proposed to explain how such hierarchies could influence growth, e.g., physiological stress (Jobling 1985; Abbot and Dill 1989; Huntingford et al. 1993; Griffiths and Armstrong, 2002), disproportional food acquisition (Koebele 1985 ; Grant 1997), activity differences (Adams et al. 1998; Sloman and Armstrong 2002), and cost of dominance (Yamagishi et al. 1974; Rubenstein 1981).

In commercial fish farming, size grading is routinely carried out to ease feeding and harvesting operations (Lee 1988; Baardvik and Jobling 1990), and to avoid reduced survival via cannibalism in piscivorous species (Goldan et al. 1997).

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This procedure is based on the assumption that grading disrupts the development of social hierarchies, and allows the smaller fish to grow better in the absence of large dominant competitors (Strand and Øiestad 1997; Liao and Chang 2002). Some experiments supported the usefulness of grading procedures based on asymmetric competition (abalone, *Haliotis tuberculata*, Mgaya and Mercer 1995), while others reported either lower growth rates in graded fish (cod, *Gadus morhua* (Lambert and Dutil 2001) or no biomass gain: Arctic charr, *Salvelinus alpinus* (Wallace and Kolbeinshavn 1988; Baardvik and Jobling 1990); Atlantic salmon, *Salmo salar* (Gunnes 1976); eel, *Anguilla anguilla* (Kamstra 1993); Dover sole, *Solea solea* L. (Overton et al. 2010). In some species, destruction of size hierarchies does improve the growth of the small fish but the growth of the large fish could be adversely affected by the stronger agonistic interaction and intraspecific aggression occurring among the graded large individuals (Baardvik and Jobling 1990; Sunde et al. 1998; Stefánsson et al. 2000). Furthermore, size grading is in itself a stressful procedure for fish (Pickering 1981) and is labour-intensive, with risks of handling damage, disease outbreak, and growth reduction (Sunde et al. 1998). This further suggests that a better understanding of size variation mechanisms would be of high interest, and that efficient feeding modes and age-for-size grading methods could be better designed (Jørgensen and Jobling 1990; Benhaïm et al. 2003).

Study of feeding behaviour may contribute to a better understanding of size variation mechanisms. Feeding is a complex behaviour encompassing several behavioural responses associated with eating, including feeding modes and habits, mechanisms of food detection, feeding frequency, food preferences (Volkoff and Peter 2006), and foraging strategies (Benhaïm et al. 2003). Self-feeders are particularly useful for the study of feeding behaviour in fish (Boujard et al. 1992) and, when coupled with a PIT tag detection antenna, have contributed to a better understanding of individual behaviour within fish groups (Alanärä and Brännäs 1993, 1996; Brännäs and Alanärä 1993; Covès et al. 1998; Rubio et al. 2004; Covès et al. 2006). This combination of techniques was successfully used in European sea bass, a commercially important species in the Mediterranean (Covès et al. 1998; Covès et al. 2006; Di-Poï et al. 2007; Millot et al. 2008, 2009). Several authors have shown that, within a group of 50 juvenile sea bass, only a few individuals defined as high-triggering fish were responsible for the majority of food demands in the group, whereas the rest of the population exhibited the defined low- or zero-triggering activity (Covès et al. 2006; Di-Poï et al. 2007; Millot et al. 2008, 2009; Millot and Bégout 2009). Lastly, self-feeding systems are also very promising for fish farming because they can improve growth and feed conversion ratios (Azzaydi et al. 1998), they are an attractive alternative to either hand-feeding or automated feeding systems due to their low labour costs (Aloisi 1994), and offer a response to the new concerns about animal welfare, even though their commercial development has been rather limited.

Surprisingly, size-grading effects have never been studied under self-feeding conditions. The aim of the present work was, therefore, to analyse the influence of a size- grading protocol on feed demand behaviour, and social structure (in terms of individual triggering activity) in sea bass. In order to mimic grading procedures used in common fish husbandry, the approach consisted of a comparison of two groups of juveniles characterized by similar mean weight but contrasting coefficient of variation on weight ($CV_{\mathrm{w}}$ = 10 vs. 20%) and initial social structure (disrupted vs. non disrupted) while held under self-feeding conditions. Growth performance and blood physiological variables were measured to complete the characterisation.

## 2 Materials and methods

### 2.1 Fish

Sea bass used in this experiment were hatched and grown in a private farm, Aquanord (France), and were never size-graded from 2 g until the beginning of the present experiment. At the mean weight of 40 g, 600 fish were transferred to the Ifremer experimental station located in L'Houmeau (France). After a two-week acclimation period, each fish was PIT-tagged by inserting a tag horizontally just behind the head to prevent any change of position after implantation. Fish were then weighed (to the nearest mg), and measured for total length (to the nearest mm). At the beginning of the experiment, fish of the experimental population had an average initial body weight ($BW_{\mathrm{i}}$) of 40.3 ± 8.9 g and a $CV_{\mathrm{w}}$ = 22.2% ($N$ = 600). They were randomly distributed between 6 tanks (100 fish per tank) held in a single experimental room, as described below.

### 2.2 Experimental set-up

The experiment was carried out in six 400 L tanks supplied with filtered seawater in a recirculating system (flow rate: 4 $\mathrm{m}^3\ \mathrm{h}^{-1}$ in each tank, and water renewal: 10% per day). The mean water temperature, oxygen concentration, and salinity were 21.0 ± 0.9 °C, 7.5 ± 1.5 mg $\mathrm{L}^{-1}$, and 23.4 ± 1.3 g $\mathrm{L}^{-1}$, respectively, throughout the experiment. Tanks were surrounded by black curtains to reduce any visual disturbance to the fish, and lit by individual 120 W lamps located about 90 cm above the water surface. Light regime was 16:8 LD (light onset at 06:00 h, UT + 1 h) with twilight transition periods of 30 min (06:00 to 06:30 and 22:00 to 22:30 h). Fish were fed a commercial sea bass diet (Neo Start 3 Le Gouessant, France; 47% crude protein, 18% lipid according to the manufacturer; 3.2 mm). Each tank was provided with a self-feeder. The device that operates these feeders is made up of a screened-type sensor (a metal rod protected in a PVC cylinder surrounded by the PIT tag detection antenna; Covès et al. 2006), and a control box. After each actuation, fish were rewarded with pellets. The feed dispensers were regulated to distribute between 0.8 g $\mathrm{kg}^{-1}$ and 0.6 g $\mathrm{kg}^{-1}$ of fish at the beginning and end of the experiment, respectively. The reward level was a compromise between minimizing wastage, and optimizing feed allocation to the group. Such a set up allowed us to monitor two variables of interest on a daily basis: the individual feed demand behaviour, and the group (i.e. tank) apparent feed consumption (food quantity dispensed minus waste counted on the bottom of the tank and in the sediment trap). Triggering activity was recorded continuously for 82 days, and only stopped 48 h before and during fish handling (when no recordings were made and the fish were fasted; 10 days off in total). Fish were placed under self-feeding conditions from the very first day (D1) of the experiment, food access was possible all day (24 h) even during tank cleaning, and waste counting occurred from 10:00 to 11:00 h (UT + 1 h).

The experiment lasted 82 days in total, during which the fish were weighed, and measured (anesthetized with clove oil at 40 ppm) at fortnightly intervals: days 13 (D13), 27 (D27), 41 (D41), 54 (D54), 69 (D69), and 82 (D82).

## 2.3 Experimental periods

The experiment was run from 7 May to 28 July 2009, structured into three periods where the same set of variables (self-feeding behaviour, social structure, and growth performances) were measured on graded, and non-graded fish:

- The first 27-day period (*P*1) allowed variable estimation in non-graded fish. It included the self-feeder learning process, i.e., the time taken by fish in each tank to learn self-feeder triggering (6 tanks of 100 fish; $BW = 40.2 \pm 8.9$ g).
- The second 27-day period (*P*2) allowed variable estimation in graded fish. The grading was carried out at D27 by creating two groups of similar mean weight but either a high or low $CV_w$ ($T_{high}$: $CV_w \sim 20\%$, 3 tanks of 60 fish; $T_{low}$: $CV_w \sim 10\%$, 3 tanks of 60 fish).
- The third 27-day period (*P*3) was added to *P*2, allowing the variable estimation in graded vs. non graded fish over a 54-day period (*P*2 + *P*3).

$CV_w$ was calculated as: $100\ SD \times BW^{-1}$, where *SD* and *BW* are the standard deviation and mean body weight respectively. To make the $T_{high}$ groups, fish from both extremes of the weight distribution were eliminated in each of three randomly-selected tanks; grading was done within tanks in order to maintain the social structure previously established during *P*1. All other weight classes were retained, i.e., from 21.1 to 65.1 g. To make the $T_{low}$ groups, fish from all three remaining tanks were pooled in a 2 $m^3$ tank supplied with pure oxygen, and hand-graded to select fish belonging to a weight class ranging between 33.7 and 49.7 g, thereby composing groups with a disrupted social structure compared with *P*1. Handling stress was identical for each fish since they were all weighed, and measured before being placed in their new tank. These procedures created the following group characteristics:

- $T_{low}$ groups: $BW = 41.9$ g, $CV_w = 9.5\%$ in Tank 1; $BW = 41.7$ g, $CV_w = 9.4\%$ in Tank 2; $BW = 41.7$ g, $CV_w = 9.4\%$ in Tank 3
- $T_{high}$ groups: $BW = 38.1$ g, $CV_w = 20.4\%$ in Tank 4; $BW = 41.1$ g, $CV_w = 22.5\%$ in Tank 5; $BW = 38.6$ g, $CV_w = 22.6\%$ in Tank 6.

## 2.4 Measured and calculated variables

The variables chosen to evaluate rearing performances, and feeding behaviour within periods were the following (most were normalized against fish biomass to account for inter-tank differences): body weight, *BW* (g); total body length, *BL* (cm); specific growth rate (% body weight per day), $SGR = 100\ (\ln BW_f - \ln BW_i)/t$, whith $BW_f$ and $BW_i$ being the final and initial body weight (g) respectively, and *t* the total number of days; Fulton condition factor ($K = 100\ BW \times BL^{-3}$); Feed demand, *FD* (g $kg^{-1}$ of fish biomass); Food wastage, *FW* (g); Feed conversion ratio ($FCR = FD$ / biomass gain); Feeding rhythms calculated by taking into account the feeding demand per hour;

Social structure was established according to fish individual triggering level, classifying them into 3 categories according to their proportional contribution to total number of trigger actuations within a group: high-triggering (HT) (>15% actuations), low-triggering (LT) (<15%), and zero-triggering (ZT) (<2%) individuals (based on Covès et al. 2006).

Physiological status of graded and non-graded fish was evaluated from blood samples (0.1 ml taken from the caudal vessel with a 1-ml heparinized syringe) of 4 anesthetized fish randomly taken from each tank at D82. Blood was immediately analysed using an I-Stat® Portable Clinical Analyzer (Abbott; cartridges CG8+; Heska Corporation, Fort Collins CO, USA; Harrenstien et al. 2005) to evaluate the 13 following variables:

pH; carbon dioxide partial pressure, $pCO_2$ (mm Hg); oxygen partial pressure, $pO_2$ (mm Hg); base excess, BE (mmol $L^{-1}$); bicarbonate, $HCO_3^-$ (mmol $L^{-1}$); total carbon dioxide, total $CO_2$ (mmol $L^{-1}$); oxygen saturation, $O_2$ saturation (%); potassium ion, $K^+$ (mmol $L^{-1}$); sodium ion, $Na^+$ (mmol $L^{-1}$); ionized calcium, iCa (mmol $L^{-1}$); plasma glucose, Glu (mg $dl^{-1}$); hematocrit, Hct (% packed cell volume PCV), and haemoglobin, Hb (mmol $L^{-1}$).

## 2.5 Statistical analyses

All variables were compared using parametric analysis of variances (ANOVA) after verification of distribution normality, and homoscedasticity (Dagnélie 1975). When data did not fulfil these requirements, non parametric Kruskall-Wallis tests were used. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls) and significant Kruskall-Wallis tests by rank-based multiple comparisons (Zar 1984). All statistical analyses were conducted using Statistica 8 (Statsoft, USA) with the significant threshold $p < 0.05$ for all tests.

To assess for differences between tanks during *P*1, all rearing performances were compared using a two-way ANOVA with Date (three dates corresponding to 5 biometry measurement dates) and Tank as fixed factors; *FD*, *FW*, and *FCR* during *P*1 were compared using a two-way analysis of the variance with Date (two dates corresponding to two biometry dates) and Tank as fixed factors.

To assess the influence of size grading, mean *FD*, *FW*, and *FCR* during *P*1 and *P*2 were compared using a two-way ANOVA with Period as a fixed factor, and Tank as a random factor.

To assess for differences between $T_{low}$ and $T_{high}$ groups during *P*2 and *P*3, all rearing performance variables were compared using a 3-way ANOVA with Treatment ($T_{low}$ vs. $T_{high}$), and Date (5 dates corresponding to 5 biometry dates) as fixed factors, and the Tank as a random factor nested to Treatment.

Data on feeding demand per hour from each treatment ($T_{\text{low}}$ and $T_{\text{high}}$) were submitted to Cosinor analysis to test for the existence of statistically significant daily rhythms. Cosinor analysis is based on least squares approximation of time series data with a cosine function of known period of the type $Y = \text{Mesor} + \text{Amplitude}\cos((2\pi\,(t\text{-Acrophase})/\text{Period})$, where *Mesor* is the time series mean; amplitude is a measure of the amount of temporal variability explained by the rhythm; period ($t$) is the cycle length of the rhythm, i.e., 24 h for circadian rhythms; and acrophase is the time of the peak value relative to the designated time scale. Cosinor analysis also provided a statistical value for a null hypothesis of zero amplitude. Therefore, if this null hypothesis was rejected for a statistical significance of $p < 0.05$, the amplitude could be considered as differing from 0, thereby constituting evidence for the existence of a statistically significant rhythm of the period considered. In order to statistically compare $T_{\text{low}}$ and $T_{\text{high}}$ feeding rhythms, ANOVA was used, with Treatment and Hour as fixed factors and Tank as a random factor nested to Treatment.

For physiological blood parameters, a 2-way ANOVA was used, with Treatment as a fixed factor and Tank as a random factor nested to Treatment. Pearson coefficients (*Rs*) were used to test for associations between initial and final weight between biometry dates (D27-D41, D41-D54, D54-D69, D69-D82) in each tank.

To assess for differences between triggering fish categories (HT, LT and ZT), *BW*, *BL*, and *SGR* were compared between biometry dates and tanks using Kruskal–Wallis tests, while percentages of triggering fish were compared using Mann–Whitney tests.

## 3 Results

During the experiment, mortality rate was 2.2 ± 3.4%. On average (all tanks), biomass was 4025 ± 108 g (density 10.1 ± 0.3 kg $\text{m}^{-3}$) at the beginning of $P1$ and 4380 ± 517 g (10.9 ± 1.3 kg $\text{m}^{-3}$) at the end; 2822 ± 119 g (7.0 ± 0.3 kg $\text{m}^{-3}$) at the beginning of $P2$ and 3886 ± 136 g (9.7 ± 0.3 kg $\text{m}^{-3}$) at the end; and 3886 ± 136 g (9.7 ± 0.3 kg $\text{m}^{-3}$) at the beginning of $P3$ and 5144 ± 254 g (12.9 ± 0.6 kg $\text{m}^{-3}$) at the end.

### 3.1 Differences between tanks during *P*1

*BW*, *BL*, and K increased more from D13 (41.1 ± 8.9 g, 15.8 ± 1.1 cm, 1.0 ± 0.1%) to D26 (47.7 ± 9.9 g, 16.4 ± 1.1 cm, 1.1 ± 0.1%) than they had from D1 (40.2 ± 8.9 g, 15.5 ± 1.1 cm, 1.0 ± 0.1%) to D13. There were significant interactions between Date and Tank in all cases ($F_{(10,1674)} = 111.4$, $p < 0.001$; $F_{(10,1674)} = 2.8$, $p < 0.001$ and $F_{(10,1674)} = 29.6$, $p < 0.001$ respectively) with tanks 1 and 6 being significantly higher at D26 than all other tanks at any date ($p < 0.05$ in all cases). *SGR* strongly increased from D1-D13 (0.13 ± 0.35) to D13-D26 (1.00 ± 0.26) with a significant interaction between Date and Tank ($F_{(5,1088)} = 14.2$, $p < 0.001$). Newman–Keuls tests showed significant differences for each pairwise comparison ($p < 0.05$).

*FD* increased from D13 (10.9 ± 11.1 g $\text{kg}^{-1}$) to D26 (16.7 ± 9.7 g $\text{kg}^{-1}$) and there were significant Date and Tank effects ($F_{(1,107)} = 13.5$, $p < 0.001$ and $F_{(5,107)} = 3.2$, $p < 0.001$) respectively) values being higher in tank 4 than in tank 5 ($p < 0.05$). *FCR* decreased from D13 (1.0 ± 4.1) to D26 (0.8 ± 2.8) and there was a significant interaction between Tank and Date ($F_{(5,107)} = 2.9$, $p < 0.05$) with Tank 3 at D13 being higher than all other tanks at any date ($p < 0.05$ for each pairwise comparison). *FW* was negligible during $P1$ (lower than 1 g per day on average).

### 3.2 Influence of size grading protocol

FD increased by 24% from $P1$ (14.3 ± 6.1 g $\text{kg}^{-1}$ $\text{day}^{-1}$) to $P2$ (18.7 ± 10.7 g $\text{kg}^{-1}$ $\text{day}^{-1}$) ($F_{(1,180)} = 10.7$, $p = 0.02$), and neither a Tank effect nor an interaction between Tank and Period were observed. FW was 0.2 ± 0.6 g during $P1$ and 4.5 ± 4.9 g during $P2$. Differences could not be tested because an interaction existed between Period and Tank ($F_{(5,180)} = 22.8$, $p < 0.001$) due to one tank where the amount of uneaten food pellets collected daily increased after size grading (from 0.5 ± 1.1 to 23.6 ± 21.0 g). In all other tanks, this amount remained very low (between 0–2 g per day) before and after size grading. Mean food conversion rate were 1.5 ± 2.7 and 1.5 ± 0.5 for $P1$ and $P2$ respectively but differences could not be tested for the same reason as above: an interaction between Tank and Period was observed ($F_{(5,180)} = 2.7$, $p = 0.02$).

During $P1$, percentages of fish observed in the ZT category were between 87 and 93% (Fig. 1 $P1$). These percentages decreased during $P2$ in each tank except tank 2 (Fig. 1 $P2$) ($Z = -2.9$, $p < 0.05$). At the same time, percentages of HT fish increased ($Z = -1.89$, $p = 0.057$), except in tank 6: 9–11 vs. 15–17% in tank 1, 12–14 vs. 33–35% in tank 2, 6–8 vs. 9–11% in tank 4, 6–8 vs. 19–20% in tank 5, for $P1$ and $P2$ respectively (Fig. 1), and percentages of LT fish increased ($Z = -2.9$, $p < 0.05$) in all tanks (14.2 ± 2.5 vs. 31.5 ± 8.0%).

### 3.3 Comparison of $T_{\text{low}}$ and $T_{\text{high}}$ treatments during *P*2 and *P*3

#### 3.3.1 Rearing performances

The evolution of mean *BW*, *BL* and K of all groups during $P2 + P3$ is presented in Figure 2. There was no difference in growth performances between the $T_{\text{low}}$ and $T_{\text{high}}$ groups i.e., no Treatment effect was recorded for any dependant variable (Table 1). This was confirmed by the stability of the $CV_{\text{w}}$ (Fig. 2D). In $T_{\text{low}}$ groups, $CV_{\text{w}}$ even tended to slightly decrease from the beginning to the end of the experiment (from 20.9 ± 1.3% to 18.8 ± 1.0%) whereas it slightly increased in $T_{\text{high}}$ groups (from 9.9 ± 0.2% to 10.8 ± 1.0%). The *SGR* decreased irregularly during the course of the experiment (Fig. 2E), being similar at D26 and D41, lower at D54, higher again at D69, and reaching the lowest value at D82 (Table 1). *SGR* was not exactly the same among tanks, as tanks 1 and 6 showed a different evolution over the experiment. Tank 1 *SGR* increased from D41 to D54 (but was at the lowest level compared with the other tanks at D41), decreased between D54 and D69 and

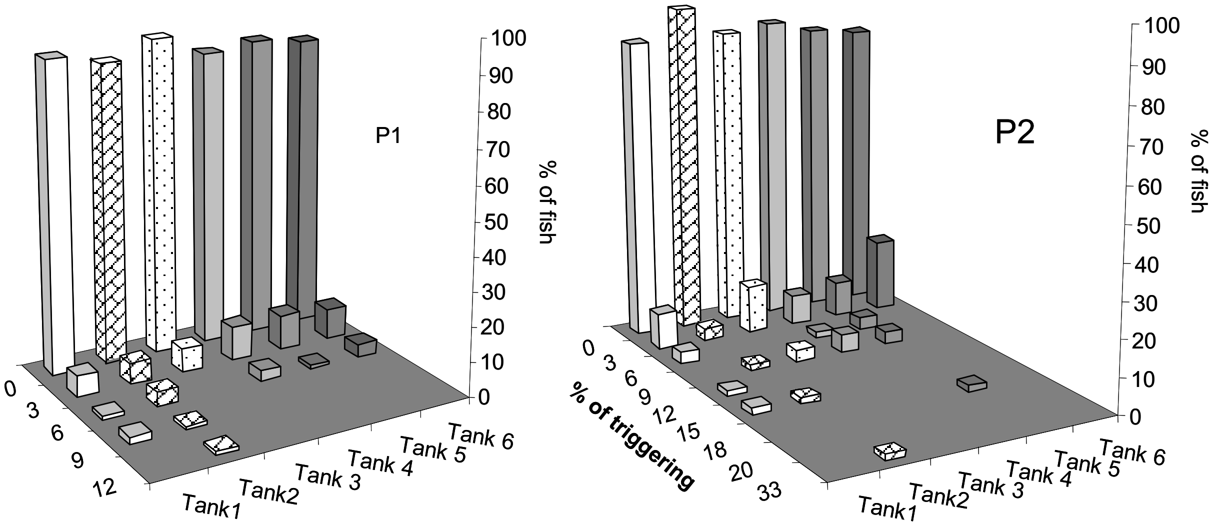

**Fig. 1.** Social structure measured by the percentage of triggering acts per individual before and after size grading. Percentages of fish are given in each triggering activity class: 0 refers to 0–2, 3 to 3–5 etc. *P*1: before size grading (Day 1-Day 27). *P*2: after size grading (Day 28-Day 55). In *P*2, $T_{low}$: tanks 1, 2 and 3; $T_{high}$: tanks 4, 5 and 6.

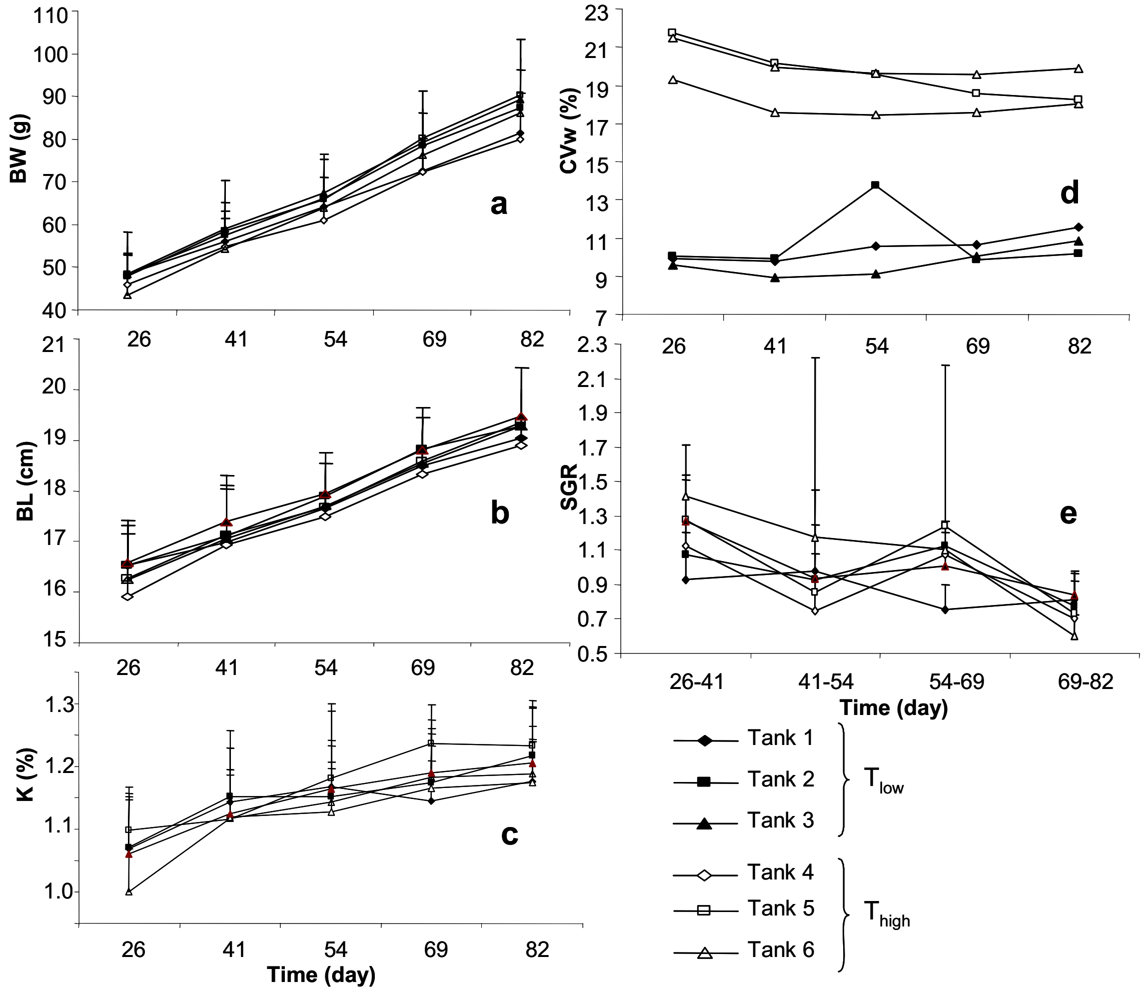

**Fig. 2.** Growth performances (mean ± *SD*) of two groups of sea bass graded for high ($T_{high}$) and low ($T_{low}$) coefficient of variation of weight. $T_{low}$: $CV_w$ ~ 10%, and $T_{high}$: $CV_w$ ~ 20%. *BW*: body weight; *BL*: total body length; K: Fulton index; $CV_w$: coefficient of variation of body weight. *SGR*: specific growth rate; *SD*: standard deviation.

**Table 1.** Results of ANOVA and Newman–Keuls tests used to analyse the mean differences between treatments ($T_{\text{low}}$: *CV* ~ 10%: low *CV* of weight; $T_{\text{high}}$: *CV* ~ 20%: high *CV* of weight) (fixed factor), Date (fixed factor) and Tanks (random factor nested to Treatment). *BW*: Body weight; *BL*: Total body length; *SGR*: specific growth rate. D1: Day 26; D2: Day 41; D3: Day 54; D4: Day 69; D5: Day 82. Significance threshold was $p < 0.05$.

| | Source | *df* | *F* | *p* | Newman–Keuls |
|---|---|---|---|---|---|
| *BW* (g) | Treatment | 1&1771 | 0.5 | 0.519 | |
| | Tank (treatment) | 4&1771 | 31.3 | <0.001 | |
| | Date | 4&1771 | 782.6 | <0.001 | D5 > D4 > D3 > D2 > D1 |
| *BL* (cm) | Treatment | 1&1771 | 1.7 | 0.268 | |
| | Tank (treatment) | 4&1771 | 0.7 | 0.562 | |
| | Date | 4&1771 | 115.9 | <0.001 | D5 > D4 > D3 > D2 > D1 |
| *SGR* | Treatment | 1&1771 | 0.1 | 0.805 | |
| | Tank (treatment) | 4&1771 | 5.1 | <0.001 | |
| | Date | 4&1771 | 65.3 | <0.001 | D5 < D1=D2 < D4 < D3 |

**Table 2.** Spearman coefficients (*r*) between initial and final body weight in $T_{\text{low}}$ (*CV* ~ 10% : low *CV* of weight) and $T_{\text{high}}$ (*CV* ~ 20%: high *CV* of weight). $T_{\text{low}}$ (Tanks 1, 2 and 3) and $T_{\text{high}}$ (Tanks 4, 5 and 6). ** $p < 0.01$, *** $p < 0.001$. Results of rank-based multiple comparisons between tanks are shown with the letters a and b. When two tanks have different letters they significantly differ at $p < 0.05$.

| | D41 | | D54 | | D68 | | D82 | |
|---|---|---|---|---|---|---|---|---|
| Tank | *r* | *p* | *r* | *p* | *r* | *p* | *r* | *p* |
| 1 | 0.92 | *** | 0.94 | *** | 0.96 | ** | 0.95 | ** |
| $2^a$ | 0.85 | *** | 0.90 | *** | 0.92 | *** | 0.97 | ** |
| 3 | 0.88 | *** | 0.96 | ** | 0.95 | ** | 0.99 | ** |
| 4 | 0.96 | *** | 0.99 | ** | 0.98 | ** | 0.98 | ** |
| $5^b$ | 0.98 | ** | 0.99 | ** | 0.99 | ** | 0.99 | ** |
| 6 | 0.97 | ** | 0.98 | ** | 0.98 | ** | 0.99 | ** |

**Table 3.** Mean ± SE. Cosinor values for feeding demand per hour (*FD*, g kg$^{-1}$) in groups with high ($T_{\text{high}}$) and low ($T_{\text{low}}$) coefficient of variation in weight. $T_{\text{low}}$: $CV_W$ ~ 10%, and $T_{\text{high}}$: $CV_W$ ~ 20%. Mesor and amplitude are expressed in g kg$^{-1}$. The reference phase for the acrophase refers to the time of day and is expressed in hours (h:min).

| Feeding demand (*FD*) | $T_{\text{low}}$ | $T_{\text{high}}$ |
|---|---|---|
| Acrophase (h:min) | 8:17 ± 0:27 | 11:34 ± 0:34 |
| Amplitude (g kg$^{-1}$) | 2.05 ± 0.11 | 1.40 ± 0.10 |
| Mesor (g kg$^{-1}$) | 2.67 ± 0.08 | 2.01 ± 0.07 |
| *p* | *** | *** |

increased again till the end of the experiment. Tank 6 was the only one to show a regular decrease in *SGR* from the beginning to the end of these periods. At D82, all tanks had reached similar *SGR* values.

Spearman rank correlation coefficients for *BW* calculated between two successive dates were close to 1 in both treatment groups and significant in all cases (Table 2). Nevertheless, observed tendencies were different between the $T_{\text{low}}$ and $T_{\text{high}}$ groups. In $T_{\text{high}}$ groups, coefficients remained very stable during the experiment, demonstrating a linear relationship at a value of 0.98 between initial and final body weight. In $T_{\text{low}}$ groups, Spearman rank correlation coefficient increased (0.85 to 0.98 in average) from the beginning to the end of the experimental period. There was a significant Tank effect ($H_{(5,23)} = 13.7$, $p = 0.017$), with tank 2 having a higher coefficient than tank 5 ($p = 0.04$).

### 3.3.2 Feeding behaviour

FD and FW were similar in $T_{\text{low}}$ (18.3 ± 0.7 g kg$^{-1}$day$^{-1}$ and 14.0 ± 24.0 g) and $T_{\text{high}}$ groups (17.6 ± 4.5 g kg$^{-1}$day$^{-1}$ and 0.9 ± 1.3 g) throughout the whole period but Tank (Treatment) effect was significant for both variables ($F_{(4,121)} = 7.8$, $p < 0.001$ and $F_{(4,121)} = 33.6$, $p < 0.001$). Indeed, *FW* was very low in all tanks (0.6 ± 1.6 g) except tank 3 (41.7 ± 33.7 g). Date effect was not significant for *FD* or for *FW*. No difference in mean *FCR* was evident between the two groups: 1.3 ± 0.1 to 2.4 ± 0.3 from the beginning to the end of the experiment for $T_{\text{low}}$, and 1.3 ± 0.3 to 1.9 ± 0.4 for $T_{\text{high}}$, but there were significant Date and Tank (Treatment) effects ($F_{(3,121)} = 13.3$; $p <$ 0.001 and $F_{(4,121)} = 3.7$, $p < 0.01$). The Tank (Treatment) effect came from tank 4, where *FCR* was lower than in the other tanks (1.09 ± 0.27 vs. 1.50 ± 0.52). Newman–Keuls post- hoc tests showed that *FCR* increased with time ($p < 0.01$ for each pairwise comparison).

### 3.3.3 Feeding rhythm

A highly significant daily rhythm in *FD* was observed in both treatments, with the acrophase in the morning (Table 3). Mesor and Amplitude were higher in $T_{\text{low}}$ than $T_{\text{high}}$ groups.

Feeding demand varied between a minimum of 1.2 and a maximum of 3.8 g kg$^{-1}$ during *P*2 + *P*3. Two main feeding activity peaks were observed at 06:00 and 11:00 h (Fig. 3). No difference could be observed in the mean overall demand of $T_{\text{low}}$ and $T_{\text{high}}$ groups but Hour effect was highly significant ($F_{(23,2100)} = 9.1$, $p < 0.001$), with higher demands at 6:00 and 11:00 h compared to all other time slots ($p < 0.01$ for each pairwise comparison). The number of demands observed during these two peaks did not differ, but Tank (Treatment) effect was significant $F_{(4,2100)} = 23.5$, $p < 0.001$).

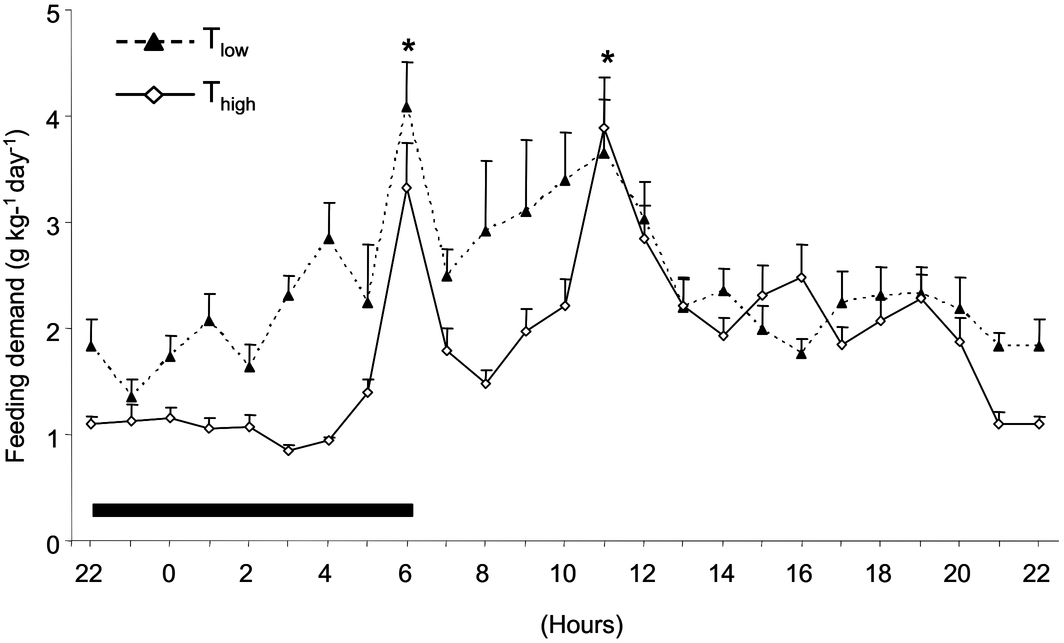

**Fig. 3.** Feeding rhythm difference (mean ± SE) between $T_{\text{low}}$ ($CV$ ~ 10%: low $CV$ of weight) and $T_{\text{high}}$ ($CV$ ~ 20%: high $CV$ of weight). The black mark on the $X$-axis represents the night period. * indicates a significant feeding peak ($p < 0.05$).

**Table 4.** Percentage of fish in each tank for each feed-demand behaviour category; *SGR*: specific growth rate (mean ± *SD*). Data are given for *P*1 (Day 1-Day 27), *P*2 (Day 28-Day 55) and *P*3 (Day 56-Day 82),. $T_{\text{low}}$ (group with low coefficient of variation in weight, $CV$ ~ 10%) are in tanks 1, 2 and 3; $T_{\text{high}}$ (group with high coefficient of variation in weight, $CV$ ~ 20%) are in tanks 4, 5 and 6. High triggering: >15% actuations, low-triggering: <15% and zero-triggering: <2%.

| Tank | High-triggering | | | | | | Low triggering | | | | | | Zero-triggering | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | *SGR* | | | % | | | *SGR* | | | % | | | *SGR* | | |
| | *P*1 | *P*2 | *P*3 | *P*1 | *P*2 | *P*3 | *P*1 | *P*2 | *P*3 | *P*1 | *P*2 | *P*3 | *P*1 | *P*2 | *P*3 | *P*1 | *P*2 | *P*3 |
| 1 | 0.0 | 1.3 | 1.7 | | 1.1 ± 0.0 | 0.9 ± 0.0 | 11.5 | 23.7 | 21.7 | 1.8 ± 0.6 | 1.1 ± 0.4 | 0.9 ± 0.1 | 88.5 | 75.0 | 76.7 | 1.5 ± 0.9 | 1.0 ± 0.2 | 0.8 ± 0.2 |
| 2 | 0.0 | 3.0 | 1.7 | | 0.9 ± 0.0 | 0.8 ± 0.0 | 15.6 | 25.3 | 13.6 | 1.5 ± 1.2 | 1.2 ± 0.2 | 0.8 ± 0.1 | 87.5 | 71.7 | 84.7 | 1.7 ± 0.8 | 1.0 ± 1.5 | 0.8 ± 0.1 |
| 3 | 0.0 | 0.0 | 1.7 | | | 0.8 ± 0.0 | 11.5 | 30.0 | 18.3 | 1.4 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1 | 91.7 | 70.0 | 80.0 | 1.4 ± 1.0 | 1.0 ± 0.0 | 0.8 ± 0.1 |
| 4 | 0.0 | 0.0 | 0.0 | | | | 17.7 | 36.7 | 27.1 | 1.5 ± 0.3 | 0.9 ± 0.1 | 0.7 ± 0.1 | 86.5 | 63.3 | 72.9 | 1.2 ± 0.3 | 0.7 ± 0.2 | 0.7 ± 0.2 |
| 5 | 0.0 | 1.7 | 1.9 | | 0.8 ± 0.0 | 0.7 ± 0.0 | 13.5 | 28.3 | 18.5 | 1.4 ± 0.4 | 0.9 ± 0.1 | 0.7 ± 0.1 | 88.5 | 70.0 | 79.6 | 1.4 ± 0.4 | 0.9 ± 0.2 | 0.7 ± 0.1 |
| 6 | 0.0 | 0.0 | 0.0 | | | | 15.6 | 45.0 | 22.0 | 0.5 ± 1.1 | 1.2 ± 0.2 | 0.8 ± 0.1 | 88.5 | 55.0 | 78.0 | 0.3 ± 0.4 | 1.3 ± 0.3 | 0.9 ± 0.1 |

### 3.3.4 Social structure

The percentage of high, low or zero triggering fish is presented in Table 4. No HT fish were observed during *P*1. This category appeared during *P*2 in two of the three $T_{\text{low}}$ groups (1 and 3%) and in one $T_{\text{high}}$ group (2%). At the end of *P*3, there were 2% of HT fish in all $T_{\text{low}}$ groups, and 2% in one tank out of the $T_{\text{high}}$ groups. In $T_{\text{low}}$, 2 fish were responsible for 28% of the triggering-activity in tank 1, 1 fish for 32% in tank 2, and 1 fish for 18% in tank 3. In $T_{\text{high}}$ groups, none of the fish showed a triggering activity higher than 15% except in tank 5 (1 fish responsible for 19% of the triggering activity). In tank 4, five fish were responsible for 15% of the triggering activity; and in tank 6, 10 fish were responsible for 50% of the triggering activity. At the same time, 18 and 81% of LT and ZT fish were observed respectively in $T_{\text{low}}$ groups, vs. 23 and 77% in $T_{\text{high}}$ groups. None of these percentages were significantly different between $T_{\text{low}}$ and $T_{\text{high}}$ groups.

The growth performances of HT, LT and ZT fish (Table 4) were not significantly different. For all variables, Date effect was significant ($p < 0.001$ in all cases): *BW* ($H_{(4,1780)} = 1203.0$); *BL* ($H_{(4,1780)} = 1064.0$); *SGR* ($H_{(4,1758)} = 546.0$); *K* ($H_{(4,1780)} = 430.4$). The variability was higher between tanks than between social categories i.e. Tank effect was significant in all cases ($p < 0.01$): *BW* ($H_{(5,1780)} = 25.9$); *BL* ($H_{(5,1780)} = 21.1$); *SGR* ($H_{(5,1758)} = 39.1$); *K* ($H_{(5,1780)} = 64.5$). *SGR* variability came from the HT category at *P*1 (Table 4), *SGR* was indeed lower in tanks 1 and 2 compared to LT and ZT categories, but higher in tanks 5 and 3. Conversely, *SGR* was similar in low and zero-triggering individuals.

### 3.3.5 Fish physiology

Blood plasma characteristics were similar in both $T_{\text{low}}$ and $T_{\text{high}}$ groups (Table 5). No Treatment effect was recorded for any variables and there was only a Tank effect for blood $pO_2$.

**Table 5.** Blood plasma parameters (mean ± *SD*) in in groups with high ($T_{\text{high}}$) and low ($T_{\text{low}}$) coefficient of variation in weight. $T_{\text{low}}$: $CV_{\text{w}} \sim 10\%$, and $T_{\text{high}}$: $CV_{\text{w}} \sim$ 20% at the end of the experiment (Day 82). *df*: degree of freedom; * indicates a significant difference, threshold: $p < 0.05$.

| | $T_{\text{low}}$ | $T_{\text{high}}$ | Treatment | | Tank (treatment) | |
|---|---|---|---|---|---|---|
| | | | *df* 1 & 18 | | *df* 4 & 18 | |
| | | | *F* | *p* | *F* | *p* |
| pH | 7.2 ± 0.1 | 7.2 ± 0.1 | 0.0 | 0.9 | 1.0 | 0.4 |
| $pCO_2$ (mm Hg) | 32.2 ± 7.1 | 30.4 ± 4.8 | 0.0 | 0.8 | 0.4 | 0.8 |
| $pO_2$ (mm Hg) | 36.1 ± 13.5 | 37.8 ± 10.9 | 0.0 | 0.8 | 3.6 | 0.02* |
| BE (mmol $L^{-1}$) | –14.6 ± 2.6 | –16.2 ± 2.0 | 0.0 | 0.9 | 0.9 | 0.5 |
| $HCO_3^-$(mmol $L^{-1}$) | 13.2 ± 2.3 | 11.8 ± 1.4 | 0.0 | 0.9 | 0.8 | 0.5 |
| Total $CO_2$ (mmol $L^{-1}$) | 14.2 ± 2.5 | 12.6 ± 1.6 | 0.0 | 0.9 | 0.8 | 0.5 |
| $O_2$ saturation (%) | 57.4 ± 21.1 | 57.8 ± 16.9 | 0.0 | 0.9 | 0.7 | 0.6 |
| $Na^+$ (mmol $L^{-1}$) | 156.8 ± 8.5 | 159.6 ± 10.2 | 0.5 | 0.5 | 1.1 | 0.4 |
| $K^+$ (mmol $L^{-1}$) | 4.4 ± 1.1 | 4.3 ± 0.7 | 0.1 | 0.8 | 0.8 | 0.5 |
| iCa (mmol $L^{-1}$) | 1.1 ± 0.2 | 1.2 ± 0.4 | 0.5 | 0.5 | 0.7 | 0.6 |
| Glucose (mg $dl^{-1}$) | 137.5 ± 58.4 | 129.5 ± 32.8 | 0.9 | 0.4 | 0.1 | 0.9 |
| Hct (% PCV) | 25.6 ± 3.3 | 26.6 ± 6.4 | 0.6 | 0.5 | 1.1 | 0.4 |
| Hb (mmol $L^{-1}$) | 8.7 ± 1.0 | 9.0 ± 2.2 | 0.9 | 0.4 | 1.0 | 0.4 |

This was due to tank 3, where blood $pO_2$ was lower compared with the other tanks, but only the difference with tank 1 was significant ($p < 0.05$).

# 4 Discussion

## 4.1 Effects of the size grading protocol

The main modification observed after applying the size grading protocol in this experiment was a 24% increase in feed demand. This difference cannot be totally explained by the grading protocol because the period preceding its application included a self feeder learning phase that was characterized by a low food demand but short, in accordance with the classic behaviour of this species (Rubio et al. 2003, 2004; Covès et al. 2006; Millot et al. 2008). This feed-demand increase was neither accompanied by food wastage nor feed conversion rate improvement, but showed similar and high day to day variations before and after size grading. This would confirm the need for a period of 22 days for the feed-demand to reach a suitable and stable level of feed reward for a group of European sea bass, as pointed out by Sánchez-Vázquez et al. (1994). The data also showed that the high variability of the food conversion rate between tanks at the beginning of the experiment was probably linked to the learning process, as this disappeared after size grading.

Triggering activity was also modified after size grading, with no fish displaying more than 12–14% of the total activity before size grading and conversely, almost none displaying less than 15% after size grading. According to the feed-demand behaviour classification previously described (Covès et al. 2006; Di-Poï et al. 2007, 2008; Millot et al. 2008), the present study therefore confirmed that within a group of 60–100 sea bass juveniles, only a few individuals were responsible for the majority of food demands, whereas the rest of the population exhibited low- or zero-triggering activity. It also showed, however, that high-triggering fish appeared only after size grading.

## 4.2 The effects of a reduction in the body weight coefficient of variation

The main result is the absence of impact on growth performances over the 54-days of the experiment, a phenomenon that has already been described in many species such as eel, turbot, *Scophthalmus maximus*, or Arctic charr (Kamstra 1993; Strand and Øiestad 1997; Sunde et al. 1998; Wallace and Kolbeinshavn 1988), although this is the first time this has been observed under self-feeding conditions. The growth was almost linear and mean *SGR* values were slightly higher than values already published for sea bass of the same size reared under similar conditions (Muller-Feuga 1998; Millot et al. 2008).

Another interesting result was in the evolution of the body weight coefficients of variation, which increased in the $T_{\text{low}}$ groups while slightly decreasing in $T_{\text{high}}$ groups. This finding agrees with previous work done by Carmichael (1994) in channel catfish *(Ictalurus punctatus)*, and by Sunde et al. (1998) in turbot. In the latter species, growth heterogeneity of graded groups was enhanced until it reached the value of the un-graded group. This has been called heterogeneity stabilisation or convergence. According to these authors, this phenomenon is always due to altered feeding behaviour or social hierarchical order. They hypothesized that fish competition was very high just after size grading, and decreased with time. In some species, size grading might lead to the destruction of size hierarchy, the growth of the large fish being adversely affected by the stronger agonistic interaction, and intraspecific aggression occur among the graded large individuals (Baardvik and Jobling 1990; Stefánsson et al. 2000; Sunde et al. 1998). In sea bass, no studies have reported a high level of aggression under self-feeding conditions, as the reward level after each trigger actuation usually optimizes feed allocation to the group. This was verified in the present study, where the regular growth evaluations and very low mortality rate showed no evidence that any injuries occurred over the course of the experiment. However, low aggression level does not mean absence of social interactions between fish. Indeed, in exploitation competition, there are no direct interactions between competitors; instead, one individual exploits and depletes a food patch before a second individual arrives (Ward et al. 2006). Recent work based on growth heterogeneity modelling in fish rearing systems rejected the hypothesis of competition decrease within time (Campéas 2009). According to this author, growth heterogeneity stability is not correlated with the stabilisation of social interactions (i.e., size had little or no effect on fight determinism), and $CV_w$ stability is the result of the dominance pressure of large fish on small fish. Indeed, according to Drews (1993), dominance can be defined as the priority to food access, and small subordinates may have a reduced feeding rate in the presence of dominant fish (Abbot et al. 1985) despite surplus feed availability (Jobling and Wandsvik 1983; Øverli et al. 1998; Sloman and Armstrong 2002). In our study, this idea is supported by the fact that correlations between initial and final weights tended to be lower in $T_{low}$ groups, but at the end of experiment, when $CV_w$ increased, correlations reached the same values as those of the $T_{high}$ groups. Indeed, Campéas (2009) showed that this tendency was related to a modification of fish rank (weight rank between the beginning and the end of the studied period) in low $CV_w$ groups.

Growth heterogeneity was frequently shown to be accompanied by food wastage (McCarthy et al. 1992; Jobling and Baardvick 1994; McDonald et al. 1996), but in our trial neither feed-demand nor feed wastage were different between the high and low $CV_w$ groups. In both cases, all the food delivered was ingested and, since all fish grew positively, it is very likely that they all had access to food.

Feeding rhythms were identical in both treatments, with two main peaks at 06:00 and 11:00 h confirming that sea bass given free access to the self-feeder have a predominantly diurnal feeding pattern. Nevertheless, the greatest activity was not concentrated at the end of the day, as has been described in previous work (Azzaydi et al. 1999). Such morning activity has already been observed for this species under similar conditions (Millot 2008; Millot and Bégout 2009), but varying patterns of feeding rhythms have also been reported by several authors (Anthouard et al. 1993; Sánchez-Vázquez et al. 1994, 1995, 1998; Bégout-Anras 1995; Boujard et al. 1996).

Over $P2$ and $P3$, the social structure was similar in low and high $CV_w$ groups, and in line with that described by several authors for the species (Covès et al. 2006; Di-Poï et al. 2007; Millot et al. 2008). In both treatments, only two or three fish were responsible for about 35% of the triggering activity and in most cases, there was only one high-triggering fish. However, at the end of $P3$, there were HT fish in all of the $T_{low}$ groups, but in only one tank of the $T_{high}$ groups. The remainder of the population was divided into two groups: about 20% of LT fish and 78% of ZT fish. Size structure is well known to play an important role in social interactions (Abbot and Dill 1989; Dou et al. 2004), and relative size is the most determinant factor in fighting ability during aggressive behaviour, as well as for attacking opponents and obtaining preferential access to food (Dou et al. 2004). However, our study shows that the juvenile sea bass group structure (according to the triggering activity) was not influenced by the size structure under self-feeding conditions. This group structure seems to be very stable and self-organised even after the removal of the regular feed-demand leader (Di-Poï et al. 2007; Millot and Bégout 2009) or chronic and acute stress experiments (Millot et al. 2010, 2011). In all cases, these changes were followed by the reconstruction of the group with the same structure and function as the initial group (Di-Poï et al. 2008).

In low and high $CV_w$ groups, HT fish showed similar culture performances as the other fish, which confirmed results obtained by previous authors (Di-Poï et al. 2007, 2008; Millot et al. 2008). Covès et al. (2006) also showed that HT sea bass did not monopolize the trigger or the distributed pellets, therefore indicating that they were not displaying hierarchy. This could explain why the group structure was not influenced by the size structure. It should be remembered that the self-feeder was regulated to provide tanks with a reward level optimizing feed allocation to the group, and that direct competition for food only occurs when food is limited and defensible (Karplus et al. 2000). There is a need for further behavioural research in order to better understand the link between size-based social structure, and group structure according to triggering activity. For instance, it would be interesting to induce competition by restricting food delivery under self-feeding conditions, and to look at the influence of these two overlapping constraints on feed demand and social interactions.

Finally, all measured physiological variables were very similar in both low and high $CV_w$ groups, and were within the usual values for sea bass (Coeurdacier et al. 1997; Dosdat et al. 2003; Millot et al. 2008) indicating that no important physiological changes were promoted by the population heterogeneity.

## 5 Conclusion

In the light of these results, it appears that grading practices, which are common in fish farming, induce only a transient modification of feed demand behaviour under self-feeding conditions. Juvenile sea bass seemed to adapt very quickly to new situations occurring in self-feeding conditions, including modifications in the size distribution of the population. The stability of the social structure built up around the self-feeder devices already known to be better in terms of feed conversion (Azzaydi et al. 1998), constitutes a new argument for their use (instead of automatic-feeders) Furthermore, grading practice does not improve the growth of graded fish in self-feeding conditions and the remarkable stability of $CV_W$ over time indicates that size-grading frequency could be largely reduced, not only to avoid social structure disturbances but also to limit handling which is a labour-intensive and costly procedure.

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# Chapitre 5

## Effect of fasting on self-feeding activity in juvenile Sea bass (*Dicentrarchus labrax*)

D. Benhaïm, M-L. Bégout, S. Péan, B. Brisset, D. Leguay & B. Chatain

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# Résumé

Ici, nous abordons l'influence d'un facteur abiotique particulier : le jeûne. Ce facteur est utilisé pour analyser les causes initiales de l'activité de manipulation d'un système d'auto-alimentation.

Les systèmes d'auto-alimentation couplés à des antennes de détection de PIT-tag ouvrent de nouvelles perspectives depuis quelques années. Ils permettent de mieux comprendre les comportements individuels des animaux vivant en groupe. Plusieurs études ont révélé la mise en place d'une structure sociale singulière qui se construit autour du dispositif d'auto-alimentation, basée sur l'activité de manipulation des individus. Dans des conditions expérimentales variables, la population peut-être divisée en trois catégories : manipulateurs principaux, occasionnels et non-manipulateurs. Dans tous les cas, un faible nombre de manipulateurs principaux est responsable d'un fort pourcentage d'activité. Une question pertinente concerne le rôle joué par la motivation alimentaire dans l'acquisition du statut de manipulateur principal. Cette question a été abordée en appliquant une période de 3 semaines de jeûne de manière à induire un taux de croissance spécifique négatif chez tous les individus appartenant à des lots homogènes et hétérogènes (2 contextes, cf. Chapitre 4) en poids étudiés en triplicats. Au niveau du groupe, le comportement de demande alimentaire n'est pas fortement modifié par la période de jeûne et aucune différence n'est observée entre les lots homogènes et hétérogènes. Une compensation complète de croissance est relevée en fin d'expérience. Au niveau individuel, les manipulateurs principaux sont exactement les mêmes avant et après jeûne. Ils sont représentés par un, deux ou quatre individus selon les bassins. Quand plusieurs individus montrent une activité importante avant jeûne, leur classement est parfois modifié après le jeûne. Les manipulateurs principaux augmentent leur niveau d'activité après le jeûne, démontrant ainsi une forme de plasticité comportementale en réponse à une variation environnementale. Cette réponse est liée aux besoins nutritionnels du groupe dont la biomasse augmente fortement dans les semaines qui suivent la période de jeûne. L'acquisition du statut de manipulateur principal ne peut donc pas être expliquée par un taux de croissance spécifique négatif, ni par un effet sexe, ni par l'un des paramètres physiologiques mesurés dans cette expérience.

Cette étude permet de conclure que l'activité de manipulation qui se développe dans chaque bassin pourrait être expliquée par l'existence de traits de personnalité (les tendances comportementales observées s'expriment en effet dans deux contextes dans cette étude, varient entre les individus d'une population donnée et sont stables dans le temps) et/ou des traits métaboliques (les manipulateurs principaux augmentent leur activité après le jeûne). Le jeûne pourrait donc renforcer un trait métabolique caractérisant ces individus, mais des recherches complémentaires sont nécessaires pour confirmer ces hypothèses.

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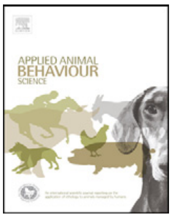

# Effect of fasting on self-feeding activity in juvenile sea bass (*Dicentrarchus labrax*)

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## ABSTRACT

In various experiments under self-feeding conditions, sea bass groups could be divided into three categories regarding feeder actuation: high, low and zero-triggering fish. In all cases few high-triggering fish were responsible for a high percentage of the feed delivery. A question was raised about the role played by feeding motivation in such high-triggering status acquisition. It was approached by applying a 3-week fasting period in order to induce similar negative specific growth rate (SGR) in two groups of fish of similar mean weight but with either a low or a high coefficient of variation for weight ($CV_w$) ($T_{low}$: $CV_w \sim 11\%$, 3 tanks of 60 fish each; $T_{high}$: $CV_w \sim 20\%$, 3 tanks of 60 fish each). These groups were created to test the consistency of behavioural responses in two different contexts (*i.e.* two population size-distributions). During the follow-up period of 40 days, the group level feed-demand behaviour was not strongly modified by the fasting period and there were no differences between $T_{low}$ and $T_{high}$ groups. Complete growth compensation was the same in all tanks as observed at the end of the experiment. At the individual level, high-triggering fish were exactly the same individuals before and after the fasting period. Up to four high-triggering fish could be observed according to the tank and when several fish were performing high-triggering activity, their rankings were sometimes reversed after the fasting period. High-triggering fish increased their activity levels after the fasting period showing behavioural plasticity. High-triggering status could neither be explained by an initial lower SGR nor a sex effect, nor by any of the measured physiological blood parameters. Thus, individual's triggering activity levels could be related to personality and/or metabolic traits but further research is required to confirm this assumption.

## 1. Introduction

Self-feeding systems have been primarily developed to allow fish to obtain food according to their energy and nutrition requirements (Brännäs and Alanärä, 1993; Paspatis and Boujard, 1996; Sánchez-Vázquez et al., 1998, 1999; Yamamoto et al., 2000a, 2000b) at preferred feeding times (Boujard and Leatherland, 1992; Sánchez-Vázquez et al., 1995; Boujard et al., 1996; Heilman and Spieler, 1999). In the recent years, when coupled with a PIT tag detection antenna, they opened up new research perspectives. They contributed to better understand the individual behaviours of fish living in groups (Alanärä and Brännäs, 1993; Covès et al., 2006). For example, dominance hierarchies have been described in rainbow trout, *Oncorhynchus mykiss* (Alanärä and Brännäs, 1993, 1996; Alanärä et al., 1998) and arctic charr, *Salvelinus alpinus*

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(Brännäs and Alanärä, 1993) reared under self-feeding conditions.

Numerous studies using this device concerned European sea bass, *Dicentrarchus labrax*, a commercially important species in the Mediterranean that has demonstrated a great ability and high plasticity to use such systems (Covès et al., 1998; Rubio et al., 2004). In this species, an intriguing individual specialization exists when using self-feeders with three triggering categories coexisting: high-triggering (HT), low-triggering (LT) and zero-triggering (ZT) fish (Covès et al., 2006; Di-Poï et al., 2007; Millot et al., 2008; Millot and Bégout, 2009). In small populations composed of 50–100 fish, whatever the experimental conditions, the same pattern is always observed: HT fish are very few with only one or two animals being responsible for 80% of the triggering activity under a reward regime of 1 or 2 pellets per individual given after each actuation (Covès et al., 2006) or 2–3 fish responsible of about 45% of the triggering activity under a reward regime equivalent to 1 pellet per individual (Millot et al., 2008). On the other hand, the ZT status would be attributed to fish that never actuate the device (Covès et al., 2006; Millot et al., 2008) or less than 4% of the time or if they perform a mean triggering activity lower than once a day (Di-Poï et al., 2007). This ZT category represented about 10% of the population (Covès et al., 2006), the rest being composed of individuals that seldom actuated the trigger (LT) (Covès et al., 2006), ≤25% of actuations (Millot et al., 2008), 4–15% (Di-Poï et al., 2007), or 0–30% (Covès et al., 2006). This individual specialization in three categories has been shown to be homogenous and stable in time over 200 days (Millot et al., 2008) with HT fish keeping their status during 60 days on average (Millot and Bégout, 2009). These authors showed that an HT fish that looses its status rarely recovers it, and becomes LT. They also hypothesized that the frequent change of HT fish in a tank could be regarded as the consequence of an imbalance group social structure (Millot, 2008), pointing out that changes occurred at the time of stressful events or spontaneously but without changing the overall population composition (Millot, 2008; Millot and Bégout, 2009). The characteristics of HT, LT or ZT fish could be summarised as followed:

- None of these fish showed differential access to delivered food whatever the category they belong to (Covès et al., 2006) and there was no clear evidence of a link between sex and food demand, *i.e.* HT fish are either females or males (Covès et al., 2006).
- In most cases, the three categories exhibited no difference in mean initial or final weights or in mean specific growth rate (Covès et al., 2006; Di-Poï et al., 2007, 2008).
- The serotonergic turnover of LT and ZT individuals is higher than the HTs one indicating that they could be under social stress due to the high activity of HT individuals (Di-Poï et al., 2007) or that they are subordinate fish (Winberg and Nilsson, 1993).

Finally, Millot et al. (2008) pointed out an interesting result from a long term experiment where the future HT individuals had negative specific growth rate at the beginning of their active period. These authors hypothesized that fish with a negative growth might be searching more for pellets and/or spent more time in the feeding zone, *i.e.* had a higher feeding motivation, which in turn, may enhance the self-feeder learning process and hence its actuation. Another hypothesis would be that HT specialization could be underpinned by personality traits.

Animal personality traits can be defined as behavioural tendencies that affect behaviour in different contexts, vary across the individuals in a given population, and are consistent within individuals across time (Sih et al., 2004; Réale et al., 2007). Among personality traits, shyness or boldness has been described in several fish species: sunfish, *Lepomis gobbosus* (Coleman and Wilson, 1998); guppies, *Poecilia reticulata* (Godin and Dugatkin, 1996); rainbow trout (Werner and Anholt, 1993; Lima et al., 1998); sea bass (Millot et al., 2009). In rainbow trout, boldness could be associated to learning abilities (Sneddon, 2003). As mentioned by several authors, personality traits often underlie physiological or neuroendocrine correlates (Koolhaas et al., 1999; Van Riel et al., 2002; Carere et al., 2003; Feldker et al., 2003; Sluyter et al., 2003; Veenema et al., 2003; Øverli et al., 2006). This intrinsic characteristic of the individual should not be confused with non-repeatable variation in behaviours that may be determined by recent experience or environmental conditions (Dingemanse and de Goede, 2004).

Fasting has been extensively studied in several fish species since many species live through natural fasting periods and can survive for months without food (Love, 1980). This lack of food is a frequent occurrence for wild fish as a consequence of temporal and spatial food availability in the aquatic environment (Aranda et al., 2001). Numerous studies aimed to investigate metabolic consequences of fasting in fish (Black and Love, 1986; Gutiérrez et al., 1991; Blasco et al., 1992), while others focused on compensatory growth after fasting (Weatherley and Gill, 1987; Kim and Lovell, 1995; Paul et al., 1995; Hayward et al., 1997; Nicieza and Metcalfe, 1997; Jobling et al., 1999). Behaviour under fasting conditions has also been reported for larvae, juveniles, and adults of several species (Beukema, 1968; Laurence, 1972; Rice et al., 1987; Croy and Hughes, 1991) but to the best of our knowledge, there is no study testing the influence of fasting on individual specialization and the resulting group structure in self-feeding conditions for any species.

Thus, the aim of this study was to determine if HT behaviour is only the consequence of temporary and contextual feeding motivation. This question was approached by applying a period of fasting in order to induce similar negative specific growth among fish. If feeding motivation is directing HT behaviour acquisition and status, we hypothesize that rank-order (in relation with triggering-activity) of individuals should be modified after the fasting period. Further, the experiment was performed with two different size distribution groups, *i.e.* large and narrow coefficient of variation for weight, in order to test for results consistency in two usual situations met in aquaculture (graded *vs.* non-graded groups). Indeed, it has already been shown that social environment may be determined by factors such as size heterogeneity. In particular, it could affect feeding behaviour and swimming activity (Martins et al.,

2005) or promote social hierarchy resulting in feed intake and specific growth rate that are proportional to fish size and weight (Alanärä and Brännäs, 1996).

## 2. Materials and methods

### 2.1. Fish

Sea bass juveniles used in this experiment were hatched and grown at the farm Aquanord SA (France). They were transferred to the experimental station of Ifremer L'Houmeau when they were 284 days old. After a two-week acclimation period, each fish was individually tagged by inserting a PIT-tag® horizontally just behind the head to prevent any change of position subsequent to implantation. Fish were weighed (to the nearest mg) and measured for total length (to the nearest mm).

### 2.2. Experimental set-up

The experiment was carried out in six 400 L tanks supplied with sand filtered seawater in a recirculated system (flow rate of $4\,m^3\,h^{-1}$ in each tank, and 10% water renewal per day). Water temperature was maintained at $21.0 \pm 0.9\,^{\circ}C$, oxygenation at $7.5 \pm 1.5\,mg\,L^{-1}$, and salinity at $23.4 \pm 1.3$‰. Tanks were surrounded by black curtains to reduce any disturbance to the fish, and individually lighted with a 120 W lamp located at 90 cm above the water surface. Light regime was 16:8 LD (light onset at 06:00 U.T. + 1) with twilight transition periods of 30 min. Fish were fed a commercial sea bass diet (Neo Start 3, France: 47% crude protein, 18% lipid according to the manufacturer, 3.2 mm) provided with a self-feeder. The device to operate the feeder comprised a screened type sensor (a metal rod protected in a PVC cylinder surrounded by the tag detection antenna; Covès et al., 2006), and a control box. After each actuation, fish were rewarded with pellets, feed dispensers being regulated to distribute always the same quantity of food which corresponded, according to the fish biomass in tanks, to $0.8\,g\,kg^{-1}$ of fish at the beginning of the experiment and $0.6\,g\,kg^{-1}$ at the end. The reward level was a compromise between minimizing wastage, and optimizing feed allocation to the group. Such a set up allowed to monitor two variables of interest on a daily basis: the individual feed demand behaviour and the group (*i.e.* one group per tank) apparent feed consumption (uneaten pellets were counted). Apparent group feed consumption (food quantity dispensed minus waste counted on the bottom of the tank and in the sediment trap) was monitored daily. Triggering activity recordings were done continuously except before and during fish biometry sessions (no recordings during 48 h at each biometry session, triggers were taken out of the water). Fish were placed under self-feeding conditions at D1 (first day of the experiment), and food access was possible the whole day along (24 h) even during the cleaning of rearing units and the waste counting from 10:00 to 11:00 (U.T. + 1). The experiment lasted 145 days, and fish were weighed and measured at fortnight interval (9 measures in total). Fish were anesthetized with clove oil ($40\,mg\,L^{-1}$) during handling.

### 2.3. Experiment steps

The first step consisted in a 25-day learning phase, *i.e.* the time taken by the fish to learn actuation of the self-feeding trigger in each tank. At this stage, each tank hosted 100 fish of similar weight and size.

At D26, fish were size graded to create two different treatments: groups of fish of similar mean weight but with either a low or a high CV of weight ($CV_w$) ($T_{low}$: $CV_w \sim 10\%$, 3 tanks of 60 fish each; $T_{high}$: $CV_w \sim 20\%$, 3 tanks of 60 fish each). Their feed demand was recorded for 54 days (D27–80). At D81, self-feeder systems were removed, and a 3-week fasting period applied (D81–102). At D103, self-feeder systems were set-up again, and a second feed demand period was recorded for 40 days (D103–142).

### 2.4. Measured and calculated variables

The variables chosen to evaluate biological performances and feeding behaviour within periods were the following:

- Body weight (BW in g), Total body length (BL in cm), Specific growth rate: SGR (% body weight per day) = 100(Ln BWf − Ln BWi)/$t$, with BWf and BWi being the final and initial body weight (g), respectively, and $t$ the total number of days, Fulton index ($K = 100 \times BW \times (BL^3)^{-1}$) were measured on each fish.
- Feed demand (FD in $g\,kg^{-1}$ of fish biomass), Food wastage (FW in g), and Feed efficiency ratio (FER = biomass gain (g)/total feed eaten (g)) were measured at the tank level.
- Daily feeding rhythms (measured at the tank level also) were calculated by taking into account the feeding demand per hour.

Fish individuals were characterized according to their triggering activity classifying them into 3 categories according to their proportional contribution to total number of trigger actuations within a group: high-triggering HT (>15% actuations), low-triggering LT (<15%), and zero-triggering ZT (<2%) individuals (based on Covès et al., 2006).

In order to evaluate physiological status, *i.e.* to compare the physiological blood compositions before and after fasting and between $T_{high}$ and $T_{low}$ groups, 4 fish were randomly taken in each tank at D83 and D103 taking care however not to sample HT fish. Four more fish from each tank were similarly analysed at the end of the experiment (D145), including HT fish, ZT fish and random fish samplings. They were anesthetized, and a blood sample (ca. 0.1 mL) was collected from caudal vessels with 1-mL preheparinized syringes. These samples were immediately analysed using an i-Stat® Portable Clinical Analyzer (Abbott; cartridges CG8+; Heska Corporation, Fort Collins, CO, USA; Harrenstien et al., 2005). This apparatus analysed the 13 following variables:

Potential of hydrogen (pH)
Carbon dioxide partial pressure ($pCO_2$ in mm Hg)
Oxygen partial pressure ($pO_2$ in mm Hg)
Base excess (BE in $mmol\,L^{-1}$)

Bicarbonate ($HCO_3$ in $mmol\,L^{-1}$)
Total carbon dioxide ($TCO_2$ in $mmol\,L^{-1}$)
Oxygen saturation ($sO_2$ in %)
Potassium ion ($K^+$ in $mmol\,L^{-1}$)
Sodium ion ($Na^+$ in $mmol\,L^{-1}$)
Ionized calcium (iCA in $mmol\,L^{-1}$)
Plasma glucose (Glu in $mg\,dL^{-1}$)
Hematocrit (Hct in % PCV), and
Hemoglobin (Hb in $mmol\,L^{-1}$).

At D145, all fish were sacrificed and sexed. The gonads, liver and viscera of 4 fish randomly sampled in each tank were dissected, weighted and hepatosomatic (HS), viscerosomatic (VS) and gonadosomatic (GS) indexes were calculated as described by Storebakken et al. (1991).

### 2.5. Data analysis

All variables were compared using parametric analysis of variances (ANOVA) after verification of distribution normality and homoscedasticity (Dagnélie, 1975). When data did not fulfil these requirements, non-parametric Kruskall–Wallis tests were used. Significant ANOVA were followed by a post hoc multiple comparison test (Newman–Keuls), and Kruskall–Wallis test by a rank-based multiple comparisons (Zar, 1984).

Statistical analyses were conducted using Statistica 8 (Statsoft, USA), and for all tests, the significant threshold was $P < 0.05$.

Individual SGR values were compared using repeated measures analysis of variance with Treatment ($T_{low}$ *vs.* $T_{high}$ groups) and Tank (random factor) nested to Treatment as between-subjects factors and Date (9 dates corresponding to 9 fish measurement dates) as within-subjects factor.

FD, FW, FER means were compared before (D27–80) and after fasting (D102–142) in each tank using a Student's *t*-test for independent samples. To test for treatment effect ($T_{low}$ *vs.* $T_{high}$ groups) on the same variables, means calculated from each triplicate were compared before fasting then after fasting using a Student's paired *t*-test.

Mann–Whitney tests were used to compare: (i) the percentages of fish falling in each triggering category before and after fasting at D81 *vs.* D103, and (ii) the latency time, *i.e.* the time taken by fish to perform the first self-feeder actuation between HT and first triggering fish at D103.

At last, some of the characteristics of the 3 fish triggering categories were compared: (i) their sex-ratio using a chi-square test, (ii) their SGR using a two-way ANOVA with treatment ($T_{low}$ *vs.* $T_{high}$), fish triggering category (HT, LT and ZT), Sex as fixed factors and Tank as a random factor nested to treatment. Analyses were performed separately at D82, D102, D117, D130 and D144.

Physiological blood parameters were compared using a Kruskal–Wallis test with Treatment ($T_{low}$ *vs.* $T_{high}$ groups) and Date (day 54, 82 and 144) as fixed factors.

GS, VS and HS recorded at the end of the experiment were compared using Kruskal–Wallis test with Tank, Categories (triggering) and Sex as independent variables.

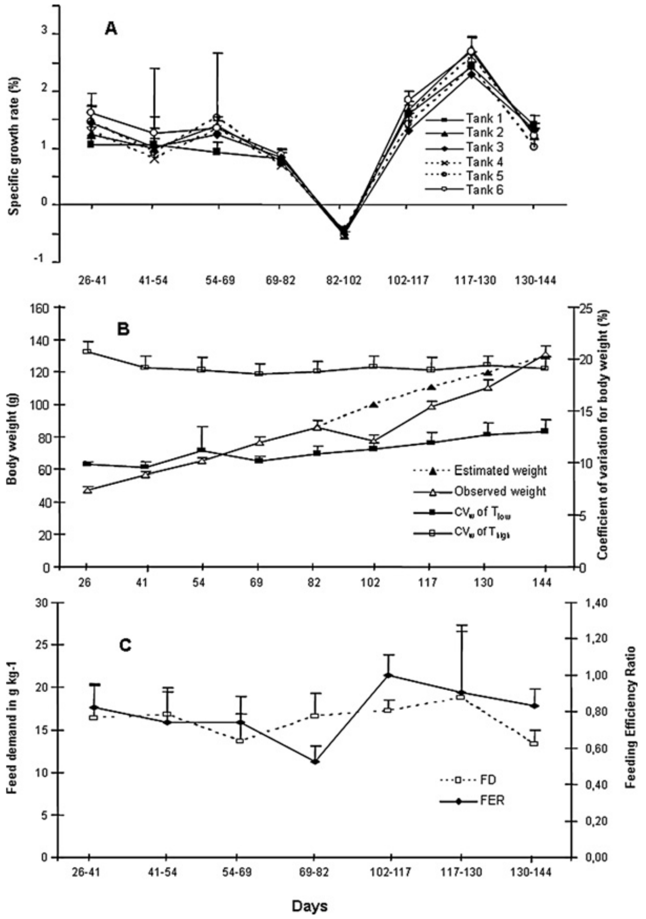

**Fig. 1.** Growth performances before and after fasting period. Fish were fasted between day 81 and 102. (A) Mean ± standard deviation of specific growth rates in $T_{low}$ (CV ∼ 10%: low CV of weight) and $T_{high}$ (CV ∼ 20%: high CV of weight). Tanks 1–3 are $T_{low}$ groups; tanks 4–6 are $T_{high}$ groups. (B) Mean ± standard deviation of observed (white dots) and theoretical (black dots) body weight evolution within age (all tanks data pooled). The theoretical growth was an extrapolation of the growth fish would have stand without fasting (between day 82 and 102). This curve is based on the regression ($y = 0.6941x - 27{,}693$) calculated from day 26 to day 82 with a coefficient of determination $r^2 = 0.998$. Mean ± standard deviation of coefficients of variation for weight ($CV_w$) in $T_{low}$ and $T_{high}$ groups. (C) Mean ± standard deviation of feeding efficiency ratio (FCR) and feeding demand (FD).

## 3. Results

### 3.1. Growth and feed-demand behaviour at the group level

Before fasting, all SGR were around 1.1% of body weight gain per day with low variation (1.11 ± 0.15%) between treatment or tank, while during fasting, they all reached negative values with a lower variation between treatment or tanks (−0.50 ± 0.08%) (Fig. 1A). After fasting, SGR strongly increased reaching almost twice (1.7× after a month) the values observed before fasting. At the end of the experiment, weight gain per day returned to the levels recorded before fasting (1.23 ± 0.13%). There was no SGR difference between $T_{low}$ and $T_{high}$ groups but significant tank (treatment) effect ($F_{(4,278)} = 30.2$, $P < 0.001$). Newman–Keuls tests showed that this significant effect was due to tank 6 where SGR values were significantly

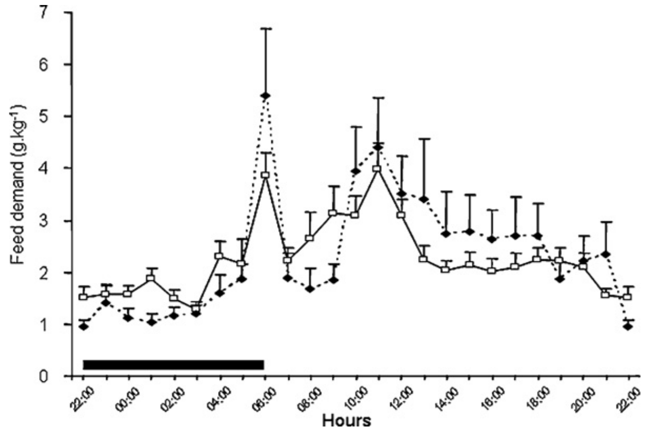

**Fig. 2.** Mean ± standard error of feeding rhythm before (white dots) and after (black dots) fasting period. The black mark on the X-axis represents the night period.

lower to all other tanks at date 1 ($P < 0.001$ for each pairwise comparison), significantly higher to tanks 4 and 5 at date 3 and 5 ($P < 0.05$ for each pairwise comparison), significantly higher to all other tanks at date 8 ($P < 0.001$ for each pairwise comparison). It was also due to tank 1 where SGR values were significantly lower to all other tanks at date 2 and 4 ($P < 0.05$ for each pairwise comparison), higher to all other tanks at Date 6 except for tank 4 ($P < 0.001$ for each pairwise comparison).

The theoretical growth curve calculated without fasting showed that the estimated final weight is almost identical to the observed data at the end of the experiment, *i.e.* 62 days after the end of the fasting period (Fig. 1B). $CV_W$ (Fig. 1B) remain stable in $T_{low}$ and $T_{high}$ groups. They tended to slightly decrease from the beginning to the end of the experiment in $T_{high}$ groups (from 20.70 ± 1.03% to 19.10 ± 1.12%) whereas they slightly increased in $T_{low}$ groups (from 9.86 ± 0.23% to 12.97 ± 1.18%).

The whole experiment along, FD varied between 13.28 and 18.69 g $kg^{-1}$ (min–max values), FER between 0.52 and 0.99 (Fig. 1C) and FW remained very low varying between 0.0 and 5.1 g per day. There were no FD and FW significant differences but significant FER difference before and after fasting in tanks 1, 4, and 5 ($df = 73$, $t = 2.9$, $P < 0.05$; $df = 76$, $t = 2.37$, $P = 0.02$; $df = 76$, $t = 2.07$, $P = 0.04$, respectively); no FD, FW and FER significant differences in tanks 2 and 6; FD and FER significant differences in tank 3 ($df = 78$, $t = -4.3$, $P < 0.001$; $df = 76$, $t = -4.1$, $P < 0.001$, respectively).

No differences could be observed either between $T_{low}$ and $T_{high}$ groups or before and after fasting for FD or FW (15.85 ± 1.46 g $kg^{-1}$, 0.60 ± 0.50 g, before fasting and 16.40 ± 2.80 g $kg^{-1}$, 0.29 ± 0.26 g, after fasting,), tank 3 data being removed from the analysis because of the very high level of food wasted after the fasting period. The sole difference was recorded for FER that was significantly higher in $T_{low}$ groups before fasting ($df = 41$, $t = 2.25$, $P = 0.03$).

Feeding rhythms were similar before and after fasting with peaks of higher activity observed at 6:00, 10:00, 11:00 and 12:00 (Fig. 2).

### 3.2. Individual feed demand behaviour

Triggering activity categories were very similar before and after fasting (Fig. 3). Most of the fish almost never (*i.e.* 0–1% of triggering activity) actuated the trigger (65.6 ± 7.4% before fasting and 67.8 ± 6.1% after fasting). Another important category was fish having a triggering activity of 2–5%: 29.1 ± 8.8% of the fish before fasting and 27.3 ± 5.9 after fasting for this class. The most important triggering activity was therefore performed by 1–10 individuals per tank before fasting and 1–6 individuals per tank after fasting (Fig. 4). The only difference recorded before and after fasting was the increase of the higher triggering activity performed by a single fish (Fig. 3).

HT fish remained the same before and after fasting (Fig. 4). In tanks 1 and 4, this was verified for a single fish per tank that performed a very high level of activity that doubled after the fasting period (Fig. 4): 28.4 ± 19.5% and 33.2 ± 20.4% of the triggering activity of their respective tank. In other tanks, similar results were observed but for a group of 2 or 4 fish. In tank 6, there was no real HT but the higher ranked fish before fasting slightly increased its activity after fasting while the fish that was in second position strongly increased its activity.

In tank 5, 2 fish performed similar activity before and after fasting. In tank 2, 2 fish performed the highest triggering activity but their ranks were reversed before and after fasting. In tank 3, 4 fish performed similar triggering activity before fasting, but this activity decreased for two of them after fasting while it increased for the other two.

The latency time to the first triggering activity after fasting (22.2 ± 13.6 min) was not different from the HT fish latency time ($Z = -1.28$, $P = 0.20$). First trigger actuations were performed by HT fish in tanks 2 and 4 and by LT fish in tanks 1, 3, 5, and 6 (Fig. 5).

### 3.3. Individual characteristics

Sex ratio was similar in all tanks (1.49 ± 0.21% of females) and not correlated with triggering activity. For SGR, no effect of Treatment, Triggering category or Sex could be observed at any dates but Tank (Treatment) effect was significant ($F_{(4, 334)} = 13.2$, $P < 0.01$; $F_{(4, 334)} = 8.3$, $P < 0.01$; $F_{(4, 334)} = 9.3$, $P < 0.01$; $F_{(4, 334)} = 5.3$, $P < 0.01$; $F_{(4, 334)} = 30.0$, $P < 0.01$, respectively from D82 to D144).

Neither Tank or Treatment ($T_{low}$ *vs.* $T_{high}$ groups) effects were observed for any physiological variables but they differed according to Date (Table 1). Numerous variables significantly decreased after the fasting period, *i.e.* $pCO_2$, $HCO_3$, $TCO_2$, Glucose, Hct and Hb. These values increased again at the end of the experiment reaching similar values as the ones recorded before fasting or even higher values, *i.e.* $pCO_2$, Na, K and Glucose. Only $sO_2$ was significantly lower after fasting, and at the end of the experiment.

No Tank, Category or Sex differences were recorded for any physiological variables at the end of the experiment. The only difference concerned GS that was higher in females (0.20 ± 0.05%) than in males (0.05 ± 0.01, $H_{(1, 23)} = 16.5$, $P < 0.0001$).

**Table 1**
Mean ± standard deviation of blood plasma variables before fasting (day 82), after fasting (day 102) and at the end of the experiment (day 144) ($N = 24$).

| | Day 82 | Day 102 | Day 144 | |
|---|---|---|---|---|
| Ph | $7.2 \pm 0.0^a$ | $7.2 \pm 0.1^a$ | $6.9 \pm 0.2^b$ | *** |
| $pCO_2$ (mm Hg) | $31.3 \pm 5.9^a$ | $21.9 \pm 2.9^b$ | $57.8 \pm 12.1^c$ | *** |
| $pO_2$ (mm Hg) | $36.9 \pm 11.9^a$ | $32.4 \pm 13.3^a$ | $14.1 \pm 11.4^b$ | *** |
| BE (mmol $L^{-1}$) | $-15.4 \pm 2.4$ | $-18.9 \pm 1.9$ | $-19.5 \pm 5.2$ | |
| $HCO_3$ (mmol $L^{-1}$) | $12.5 \pm 1.9^a$ | $8.9 \pm 1.2^b$ | $12.3 \pm 2.9^a$ | *** |
| $TCO_2$ (mmol $L^{-1}$) | $13.4 \pm 2.2^a$ | $9.6 \pm 1.2^b$ | $14.1 \pm 2.7^a$ | *** |
| $sO_2$ (%) | $57.6 \pm 18.6^a$ | $47.7 \pm 22.2^a$ | $11.4 \pm 18.3^b$ | *** |
| Na (mmol $L^{-1}$) | $158.2 \pm 9.3^a$ | $150.0 \pm 29.6^a$ | $168.0 \pm 8.7^b$ | *** |
| K (mmol $L^{-1}$) | $4.3 \pm 0.9^a$ | $4.1 \pm 0.6^a$ | $5.9 \pm 1.4^b$ | *** |
| iCA (mmol $L^{-1}$) | $1.1 \pm 0.3$ | $0.9 \pm 0.4$ | $1.4 \pm 0.3$ | |
| Glucose (mg $dL^{-1}$) | $133.5 \pm 46.5^a$ | $72.5 \pm 16.4b^b$ | $354.8 \pm 74.1^c$ | *** |
| Hct (% PCV) | $26.1 \pm 5.0^a$ | $21.2 \pm 3.4^b$ | $29.9 \pm 4.7^a$ | *** |
| Hb (mmol $L^{-1}$) | $8.8 \pm 1.7^a$ | $7.2 \pm 1.2^b$ | $10.2 \pm 1.6^a$ | *** |

Letters following means indicate statistical differences between sampling dates, means not sharing a common letter are significantly different and no letter means no significant difference. Significant differences are represented by an asterisk: $^{***}P < 0.001$.

## 4. Discussion

This work aimed to determine if HT behaviour is only the consequence of temporary and contextual feeding motivation. Therefore, a period of fasting was applied in order to induce similar negative SGR values among two groups of fish (differing by their size-distribution). Consistency in HT behaviour was evaluated between periods and groups in order to test the hypothesis of feeding motivation governing the observed triggering responses.

This study showed that feed demand behaviour was not strongly modified by a three-week fasting period. At the individual level, it was confirmed that only a few individuals were responsible for most of the triggering activity. The HT status could neither be explained by an initial lower SGR nor by a sex effect, nor by any of the measured physiological blood parameters. Individuals triggering activity that took place in each tank was consistent within individuals across time and in two contexts (two different size distribution). This could be related to personality and/or metabolic traits but further research is needed to confirm this assumption.

### 4.1. Growth, feed-demand behaviour and physiology at the group level

Growth performances were similar in low and high $CV_W$ groups. The fasting period induced similar negative SGR values in all individuals that were associated to plasma glucose decrease in accordance with levels given by previous study on sea bass (Echevarria et al., 1997), and to hematocrit decrease, which has been already described in other fish species (Muller et al., 1983; Boismenu et al., 1992; Gillis and Ballantyne, 1996; Neubert et al., 1999).

A parallel decrease of $pCO_2$, $HCO_3$ and $TCO_2$ was also observed that could be linked to the fact that fish deprived of food reduce their oxygen consumption (and therefore their $CO_2$ production) by decreasing their energy expenditure in an attempt to conserve body energy reserves (Cook et al., 2000; O'Connor et al., 2000; Rios et al., 2002).

During the four weeks following the fasting period, fish exhibited accelerated growth. They grew indeed very fast, and at the end of the experiment they achieved the same

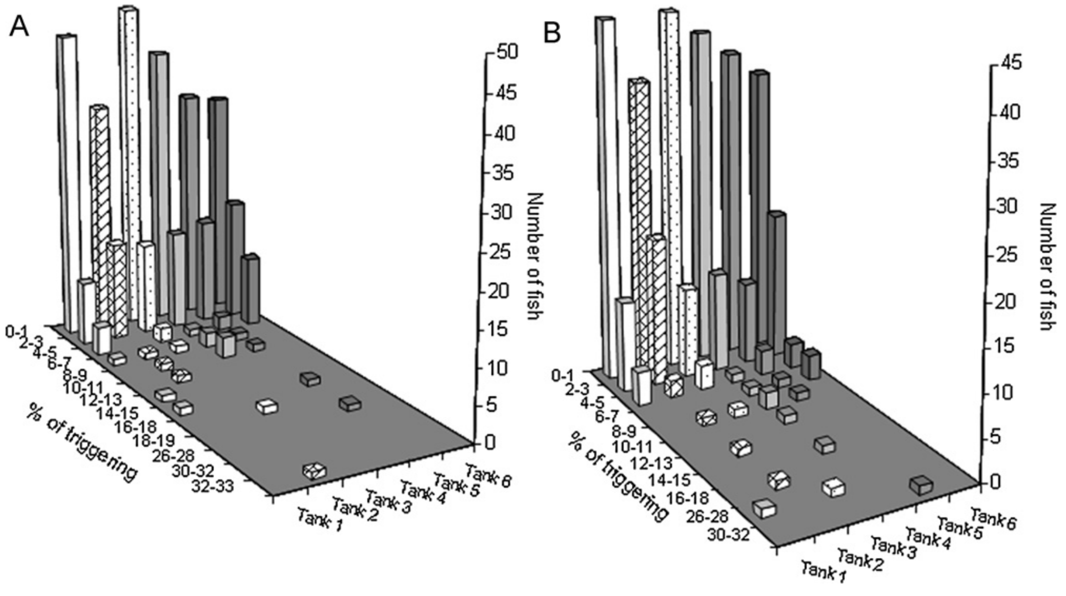

**Fig. 3.** Number of fish in different triggering classes (expressed in %) in $T_{low}$ ($CV \sim 10\%$: low CV of weight) (tanks 1–3) and $T_{high}$ ($CV \sim 20\%$: high CV of weight) (tanks 4–6) groups. (A) Before fasting (day 27–80); (B) after fasting (day 103–142). Fish were fasted during the 81–102-day period.

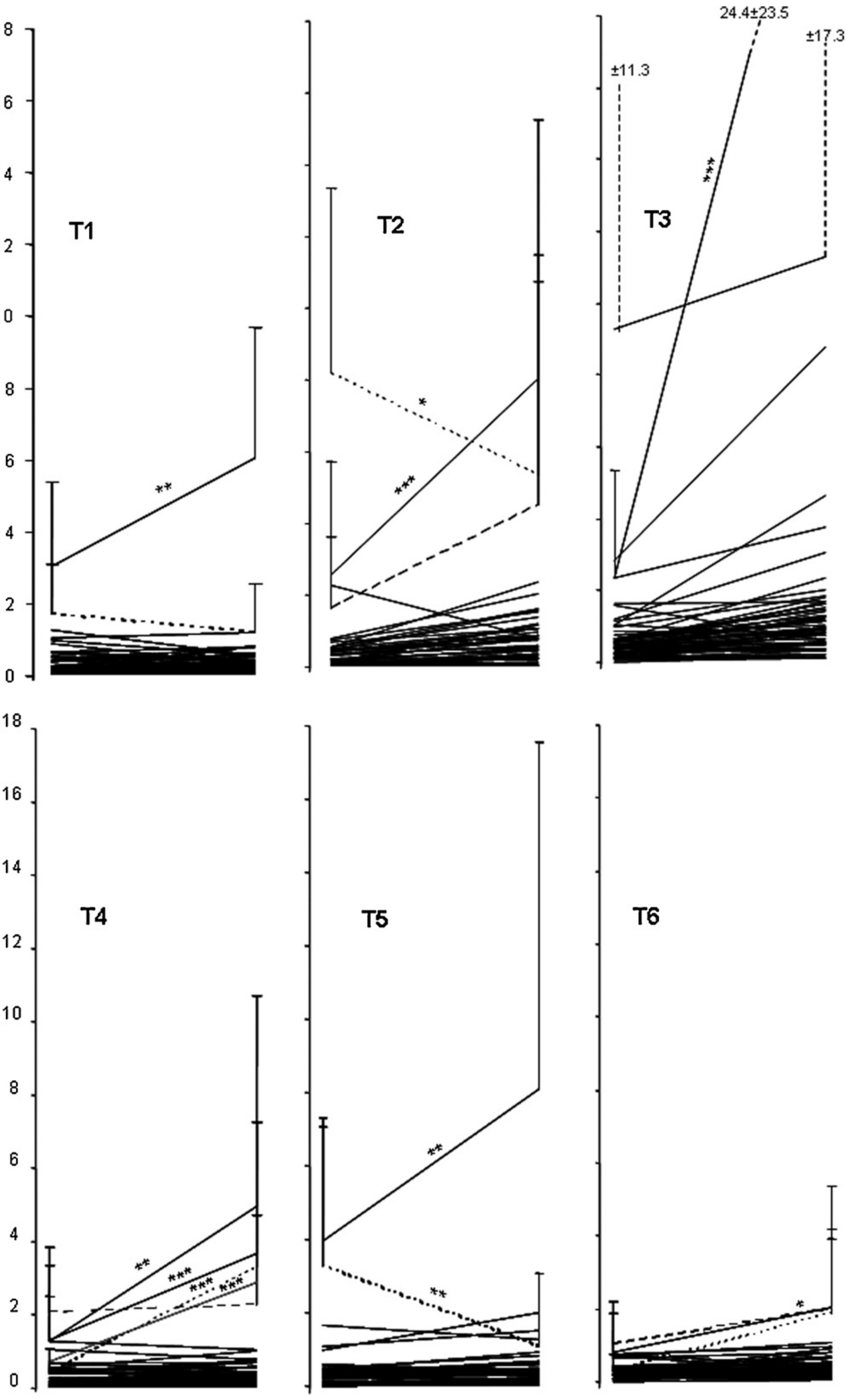

**Fig. 4.** Mean ± standard deviation of number of trigger actuations performed by each individual before and after fasting period (day 27–80 and day 103–142 periods) in $T_{low}$ (CV ~ 10%: low CV of weight) (tanks 1–3) and $T_{high}$ (CV ~ 20%: high CV of weight) (tanks 4–6) groups. Significant differences before and after fasting are represented by an asterisk (Mann–Whitney test): *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

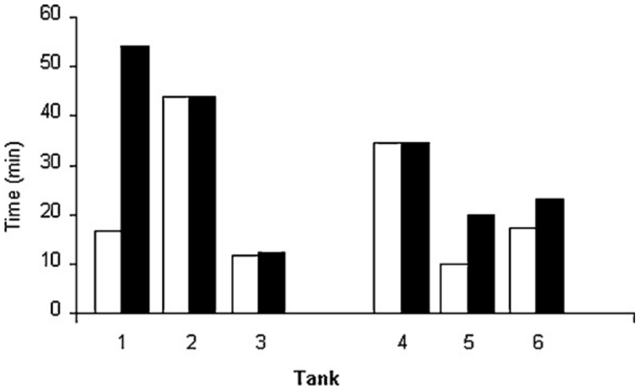

**Fig. 5.** Latency time taken by any fish (black bars) to perform the first actuations after the fasting period (at day 103) in $T_{low}$ (CV ~ 10%: low CV of weight) (tanks 1–3) and $T_{high}$ (CV ~ 20%: high CV of weight) (tanks 4–6) groups. In white bars: latency time taken by fish that were high-triggers before fasting.

body mass than fish that would have been continuously fed, indicating a complete compensation (Kim and Lovell, 1995; Nicieza and Metcalfe, 1997; Jobling et al., 1999). Previous studies already showed complete compensatory growth in Atlantic cod *Gadus morhua* after deprivation for 3 weeks (Jobling and Baardvick, 1994). In the present study, this growth acceleration was not associated with hyperphagia (*i.e.* feed demand did not strongly increase after the fasting period), but with food conversion efficiency improvement, which is one of the major mechanisms causing compensatory growth during the period of re-feeding (Dobson and Holmes, 1984; Russell and Wootton, 1992; Qian et al., 2000).

Feeding rhythm was neither influenced by size distribution (low and high $CV_W$ groups) nor by fasting and 4 main peaks were recorded at 6:00, 10:00, 11:00 and 12:00, which are close to rhythms described previously under similar conditions (Millot et al., 2009).

### 4.2. Individual feed-demand behaviour

The self-feeder triggering activity was very similar before and after fasting. This study confirms that, in a group of 50–140 g sea bass (60 individuals), a few individuals only are responsible of more than a third of the triggering activity, the rest of the population being composed here, of around 66% of ZT fish and 28% of fish performing 2–5% of triggering. An interesting result is that fasting induced higher activity only in fish that had already acquired the HT status before fasting.

The most important result of this study came up when comparing the triggering activity of each tagged fish before and after fasting: feeding motivation was not revealed to be the major factor explaining the different levels of activity. Indeed, because all fish were similarly fasted, it was expected according to the feeding motivation hypothesis (Millot et al., 2008), that several fish should have acquired HT status after the fasting period, but this was not the case because HT fish maintained their status before and after fasting. In some cases, a single fish could even perform a third of the total triggering activity that represented 3–4 times more than the most active LT fish. In other cases, the same group of 2 or 4 fish was found before and after fasting but with different rankings (according to the triggering activity level). This social structure seemed therefore to remain very stable, and here, a small number of fish (1–4) kept a high level of triggering activity during 96 days including a period of 55 days before fasting and a period of 41 days after fasting. Millot et al. (2008) showed that a HT fish could keep its status during approximately 63 ± 16 days on average (12–186, min–max) and when loosing it, a new fish previously identified as a LT fish, was replacing it. The present work showed that fish rankings according to their triggering activity levels in each tank should also be considered. Indeed, we hypothesize that not all LT fish have the same potential to become HT fish, and that only those that are able to maintain themselves at a high ranking level can do it. In the present work, fish that lost their first position in term of activity were still among the more active individuals. Rankings reversal could indicate a competition situation among the more active fish that would explain the HT status changes described previously (Millot et al., 2008), and the fact that HT fish removed from tanks are replaced by the same number of new HT fish (Di-Poï et al., 2008). Millot et al. (2008) already mentioned the hypothesis of HT fish dominance on other fish categories. Dominance does not necessarily imply aggression among fish, *e.g.* exploitation or scramble competitions (Ward et al., 2006). The observed stability of the social structure could be explained by social interactions that are known to influence animal activities resulting in efficient adaptative behaviour (Galef, 1995; Galef Jr. et al., 1996).

The latency time to actuate the trigger after the fasting period was very short in all tanks (11–43 min) while it was 17.7 ± 2.7 h during the learning phase. This confirms that trigger actuation relies upon cognition, which includes memory formation and executive functions related to information processing such as learning and problem solving functions (Brown et al., 2006). Even though the very first actuations were performed in 4 of the 6 tanks by LT fish, their latency time was not significantly different from HT ones. It is also notable that the feeding rhythm was established again immediately without food wastage increase in 5 tanks out of 6.

This suggested that trigger actuation among sea bass populations cannot be fully explained by feeding motivation. One hypothesis is that trigger actuation could be classified in “activity”, which is one of the 5 personality traits category described by Réale et al. (2007) because, in all cases some individuals consistently differed in activity levels (HT *vs.* LT *vs.* ZT fish). As already mentioned above, further research would be useful to determine the frontier between LT and HT fish, *i.e.* to check if all fish belonging to the LT fish category have really the same potential to become HT fish. Some authors already pointed out that more studies were also needed to discern generalities on the breadth of behavioural syndromes (*i.e.* on the range of behavioural correlations, Sih et al., 2004). The personality traits need reflecting consistency in behaviour between individual across two or more situations (Sih et al., 2004), which was the case here, across two situations (before and after fasting; low and high $CV_W$ groups), and for the 96 days that lasted the experiment.

There were neither blood parameters responses nor growth performances (except a higher body weight in LT fish compared to ZT fish) nor sex differences between HT, LT and ZT fish. However, we did not investigate metabolic traits that could explain the HT status acquisition. Indeed, in our study the HT increased their activity after fasting and their status lasted 96 days while Millot et al. (2008), when performing a long-term study on sea bass under self-feeding conditions, showed that the HT were keeping their status during 60 days on average. This longer period could be explained by a metabolic trait that could be reinforced by the fasting period. It would have been interesting to compare HT physiological parameters between the beginning and the end of the experiment to investigate more accurately the metabolic trait explanation but the blood sampling itself can induce stress which could have changed the triggering status (Millot, 2008; Millot and Bégout, 2009).

Nevertheless, the plasticity of the behavioural response in relation to environment variation is not a sticking point with the personality traits definition since some authors stated that personality and plasticity could be considered as complementary aspects of the individual phenotype (Dingemanse et al., 2009). Furthermore, personality can underlie physiological or neuroendocrine correlates (Koolhaas et al., 1999; Van Riel et al., 2002; Carere et al., 2003; Feldker et al., 2003; Sluyter et al., 2003; Veenema et al., 2003; Øverli et al., 2006). Specifically in sea bass, Di-Poï et al. (2007) already showed that HT fish had lower serotonergic turnover than LT and ZT fish and hypothesized it could be linked to a bold personality trait.

In conclusion, this study brought forward evidence to demonstrate that in sea bass: (i) high-triggering status is not regulated by a negative growth, (ii) feeding motivation seems not to be the sole factor that determines individual activity level, and (iii) triggering activity could be associated to personality traits but our protocol did not allow to reject the hypothesis stating that there were no difference in feeding motivation before and after fasting and therefore to directly assess personality. Further research is needed to better understand the HT status acquisition. In particular, new questions arose about the low-triggering category, which could be probably better described, *i.e.* subcategories should be identified. We suggest investigating HT personality traits in dedicated experiments since the triggering activity could be correlated to other personality traits as described in behavioural syndromes (Koolhaas et al., 1999; Réale et al., 2000). At last, experiments in calorimetric chambers could be useful to determine the potential importance of metabolic traits associated with HT behaviour status.

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# Partie 2 : Impact de la domestication sur le comportement

# Chapitre 6

# Effect of domestication history on behavioural traits in juvenile brown trout (*Salmo trutta* L.)

D. Benhaïm, R. Guyomard, B. Chatain, E. Quillet & M-L. Bégout

*Soumis*

# Résumé

Dans ce chapitre, nous abordons le processus de domestication en utilisant un matériel biologique caractérisé à l'aide de l'outil génétique et en prenant les caractéristiques de l'activité de nage et la réponse à un challenge standardisé comme indicateurs pour comparer deux populations distinctes.

En effet, le processus de domestication peut être étudié en comparant les traits comportementaux de téléostéens domestiqués et de téléostéens sauvages car ces traits sont généralement les premiers à être impactés. Cependant, de nombreuses études s'intéressant aux différences entre ces populations ne contrôlent pas les interactions entre génétique et environnement. Le but de cette étude est de tester la variabilité génétique pour certains traits comportementaux chez des juvéniles de truite commune provenant du croisement de femelles issues d'une population Méditerranéenne pratiquement pure avec des mâles de même origine (WW) ou des mâles issus d'une lignée Atlantique domestiquée (WD). Les activités de nage des WW et des WD sont comparées individuellement avant et après l'application d'un stress lumineux (30 min à la lumière, suivi de 5 min à l'obscurité puis à nouveau 30 min à la lumière). Un génotypage est réalisé sur chaque individu observé pour vérifier son origine. Des différences comportementales sont observées entre WW et WD. En particulier, la vitesse angulaire et le temps passé immobile sont supérieurs chez les WW avant et après l'extinction brutale de la lumière. La vitesse moyenne et la distance totale parcourue sont plus importantes chez les WD notamment durant les 30 mn qui suivent la période au cours de laquelle la lumière a été à nouveau rétablie. Les WD semblent alors récupérer une plus grande activité de nage tandis que les WW demeurent à un niveau inférieur. Chaque individu observé a pu être réassigné à son origine supposée.

Cette étude démontre donc un impact de la domestication sur certains traits comportementaux natatoires et plusieurs discriminants ont pu être identifiés (vitesse angulaire, temps passé immobile, vitesse moyenne et distance parcourue). Les résultats sont discutés avec une perspective biologie de la conservation et repeuplement.

# Effect of domestication history on behavioural traits in juvenile brown trout (*Salmo trutta* L.)

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## Abstract

Behavioural traits are likely to be affected early in domestication. A comparison of these traits between wild and domesticated fish can therefore be used to investigate this process. The aim of this study was to assess genetic variation by comparing behavioural traits in juvenile brown trout generated by cross-fertilising females from an almost pure native wild Mediterranean population with males of the same origin (WW) or with males from an Atlantic domesticated strain (WD). This experimental design made it possible to distinguish between environmental and genetic effects. The swimming activity characteristics of individual WW and WD juveniles were compared before and after the application of a stress (light switched off suddenly, followed by a 5-minute period of darkness). For each of the fish observed, mating type origin (WW or WD) was unambiguously reassigned by genotyping. Behavioural responses differed between WD and WW fish. Angular velocity and the time spent immobile were greater for WW fish both before and after the short period of darkness, indicating higher reactivity. Once the light had been turned on again, mean velocity and total distance travelled were higher in WD than in WW fish. WD fish tended to recover levels of swimming activity higher than those before the dark period. This study therefore demonstrates an impact of genetic origin and domestication on swimming activity repertoire (higher reactivity in WW fish), a behavioural trait of particular importance for individual ecological performance.

*Keywords:* domestication, genotyping, microsatellite, swimming activity, restocking.

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## Introduction

Domestication is the process by which a population of individuals becomes adapted to humans and to the captive environment, through the recurrence of environmentally induced developmental events in each generation and genetic changes occurring over generations (Price 1999). These changes involve both deliberate and inadvertent selection, together with random genetic changes known as genetic drift, in which genetic variation is lost due to stochastic changes in allelic frequencies over several generations (Crow and Kimura 1970). For most farmed animals, essentially large terrestrial herbivorous and omnivorous mammals, domestication began 10 500 years ago (Diamond 2002). By contrast, the domestication of 97 % of cultured fish species did not begin until the start of the 20th century (Duarte *et al.* 2007). Farmed fish are therefore unlikely to differ markedly from the corresponding wild ancestral forms and only a few would be expected to be on the threshold of becoming domesticated (Balon 2004). Nevertheless, domestication effects can be observed in some fish, in some cases within as little as one or two generations of their removal from the natural environment (Dunham 1996a). These modifications concern morphological and behavioural characters (Bilio 2007). According to Denis (2004), domestication can best be described as a continuum of genetic transformations over generations constituting a general progression from the wild state to a state of genetic adaptation to captivity.

In fish, the coexistence of wild and domesticated stocks of the same species provides us with an opportunity to investigate the process and dynamics of the domestication process. A number of studies have been conducted to investigate differences between wild and domesticated fish.

Investigations of behavioural traits in wild and domesticated fish constitute an effective approach to studies of the domestication process, because these traits are likely to be among the first affected (Price 1999; Ruzzante and Doyle 1991; Ruzzante and Doyle 1993; Swain and Riddell 1990), in some cases within a generation of initial domestication (Bégout Anras and Lagardère 2004; Huntingford 2004; Vandeputte and Prunet 2002). Antipredator behaviour, one of the most studied of these traits, has been shown to be very sensitive to artificial rearing (Berejikian 1995; Dellefors and Johnsson 1995; Einum and Fleming 1997; Fernö and Järvi 1998; Johnsson and Abrahams 1991; Johnsson *et al.* 2001; Johnsson *et al.* 1996), and swimming performances have been shown to be poorer in domestic stocks (Beamish 1978). These differences between wild and cultured fish may be accounted for partly by differences in experience in the life histories of individual fish (Huntingford 2004). Farmed fish are faced with conditions that appear to be less challenging than natural habitats, with structurally simpler environments, a ready supply of food and an absence of predators. However, they also have to deal with high animal densities, space restrictions, artificial and uniform food and frequent handling (Fernö et al. 2007). Another way of studying the impact of domestication on fish is to investigate their behavioural responses to novel environments and/or challenging situations. Indeed, the most important effect of domestication on behavior is a decrease in emotional reactivity or responsiveness to fear-evoking stimuli (*i.e.* environmental change, Price 2002). Measurements of behavioural reactivity are thus sensitive indicators of the complex of biochemical and physiological changes occurring in response to stress (Schreck *et al.* 1997). For example, environments such as the light/dark plus maze, based on the tendency of fish to seek dark backgrounds (or to avoid light backgrounds) in unfamiliar environments, can be used to study such behaviour (Champagne *et al.* 2010; Gould 2011; Serra *et al.* 1999; Steenbergen *et al.* 2011a). Another method involves the sudden exposure of fish to darkness, which has been used as an acute ecologically relevant challenge producing robust changes in locomotor activity in larval zebrafish (Steenbergen *et al.* 2011b).

Brown trout, *Salmo trutta* L., is the most common salmonid in Europe and is of considerable socio-economic importance and heritage value because of its intraspecies diversity (Caudron *et al*. 2009). Two evolutionary lineages have been identified in France on the basis of allozymes, mtDNA and nuclear DNA markers: the Atlantic lineage (AL), which occurs in the rivers of the Atlantic catchment area, and the Mediterranean lineage, which occurs in the rivers of the Mediterranean basin (Bernatchez *et al*. 1992; Cortey *et al*. 2004; Guyomard 1989; Launey *et al*. 2003).

An important issue in studies of the domestication of brown trout (and of many other fish) is the replacement of many natural populations by domesticated stocks through intensive stocking or escapees. For example, most of the native Mediterranean lineage in France has been replaced by domesticated stocks belonging to the Atlantic lineage (Guyomard 1989; Launey *et al*. 2003; Krieg and Guyomard 1985; Barbat-Leterrier *et al*. 1989; Beaudou *et al*. 1994; Berrebi *et al*. 2000; Largiadèr *et al*. 1996; Poteaux *et al*. 1998) and this is also probably true for most of the native Atlantic populations. Thus, in most comparisons of domestic and wild stocks, it is not possible to exclude the possibility that the two genotypes investigated originate from domesticated stocks and differ only in one of them having being released into the wild for a short period of time. This may bias the experiments carried out. Fortunately, remnant unstocked or almost unstocked native Mediterranean populations of brown trout have been identified (Caudron *et al*. 2011) and provide a suitable genetic source for studying the domestication process in this species. Furthermore, many studies of phenotypic differences between wild and domesticated stocks of fish species do not include the necessary fertilisation designs for separation of the effects of genetic and environmental factors. It is also important to take into account maternal effects, which contribute to phenotypic complexity for many traits, and may complicate attempts at phenotypic analysis (Bernardo 1996). Maternal effects in fish are due to the energy reserves within the yolk (reviewed by Love 1980), which depend on the reserves the female is able to commit to oogenesis and oocyte maturation (Kerrigan 1997). Many studies comparing wild fish with those from hatcheries fail to recognise the influence of maternal effects on egg quality, with repercussions for the survival and behaviour of the offspring (Huntingford 2004). Gene-environment interactions can also be limited by placing all the fish in the same environment (Dupont-Nivet *et al*. 2008) or by using replicates to assess tank effects.

There have been few studies of the domestication process based on differences in behaviour between wild and domesticated fish and an experimental design allowing the identification of lineages and the control of environmental sources of variation. However, a recent study on zebrafish, *Danio rerio*, confirmed the potential of such studies for the QTL mapping of behavioural traits and for dissecting the consequences of selection during domestication (Wright *et al*. 2006).

In this study, we assessed the genetic variation underlying differences in behavioural traits between genetically differentiated populations of juvenile brown trout. This study is innovative in its use of genotypes corresponding to well characterized stages of domestication. In particular, we compared juveniles produced by mating females from a near-pure Mediterranean population with males from the same population (WW) or with males from an Atlantic domesticated strain (WD). The swimming activity characteristics of individual WW and WD fish were compared before and after a short period of darkness, and each of the fish studied was genotyped to check its origin.

## Materials and methods

### *Experimental animals and housing conditions*

This study was conducted with the approval of the French Animal Care Committee under the terms of the official licence of M.L. Bégout (17-010).

In December 2006, an experimental captive population was founded with 11 females and 16 males (effective population size = 22.8) caught in the Fier, a tributary of the Rhône inhabited by an almost pure Mediterranean population (Guyomard and Caudron, unpublished data). We checked that the parents had a Mediterranean genetic profile by genotyping with Str541 and Str591, two microsatellites that have proved useful for differentiating between Atlantic stock strains and Mediterranean populations (see Estoup *et al*. 2000 and Caudron *et al*. 2006, for details on the method). Fish were fertilised, hatched and reared to sexual maturity at La Puya fish farm (Annecy, Haute-Savoie, France).

In 2009, an experiment with a semi-factorial design was carried out with mature males and females of the Fier captive population and males from the INRA synthetic strain (INRA experimental fish- arm, Le Drennec, Finistère, France). We pooled the eggs from all females and divided them up into the same number of groups as individual males used. Each group of eggs was fertilized by an individual male. Fifteen minutes after fertilisation, all the eggs fertilised by males of the same origin were pooled and each pool was divided into two replicates (R1 and R2, Table 1). Eggs were incubated at temperatures of 6 to 10 °C, in small stainless steel incubators, until the first feed, after which they were transferred to small concrete tanks until the end of the experiment. The fry were initially fed with zooplankton, which was gradually replaced with dry pellets. The fish were reared according to standard trout farming practice. The behavioural experiment was started 280 days after fertilisation of the eggs.

**Table I** *Half-factorial design features: numbers of male and female brown trout used.*

| | 13 Captive Fier ♀ x 9 captive Fier ♂ | | 13 captive Fier ♀ x 10 INRA ♂ | |
|---|---|---|---|---|
| | $[WxW]_1$ | $[WxW]_2$ | $[WxD]_1$ | $[WxD]_2$ |
| Fertilisation | 500 | 500 | 350 | 350 |
| Day 180 | 358 (0.062) | 336 (0.155) | 302 (0.199) | 204 (0.091) |
| Day 220 | 150 | 150 | 150 | 150 |

*Replicate sizes at fertilisation; from day 180 to day 220, replicate sizes were adjusted to 150. In brackets: mean individual weight (in grammes) on day 180. W: wild captive Fier (Mediterranean lineage); D: the domesticated INRA synthetic strain.*

*Experimental set-up*

Observations were made in a specially modified dark room dedicated to this experiment at La Puya farm. The apparatus consisted of 12 transparent rectangular arenas (24.5 x 15 x 13.5 cm, Aquabox® 3, AQUA SCHWARZ GmbH, Göttingen, Germany), each filled with 1.5 l of water (the water used had characteristics identical to those of the water in the original tanks) giving a water depth of 7 cm. The temperature and oxygen level were checked before and after the observations. Temperature was 11.6 ± 0.6°C before and 12.8 ± 0.3°C after the observations, whereas oxygen concentration was 7.4 ± 0.2 mg $l^{-1}$ before and 7.2 ± 0.2 mg $l^{-1}$ after the observations. Arenas were numbered from 1 to 12 and placed on a waterproof infrared casing (1 x 1 m, Noldus, The Netherlands) for the recording of videos in total darkness. A frame made of opaque white cardboard was placed against each arena, preventing visual interaction between fish. A camera (Imaging Source DMK 21AUO4) with a frame rate of 30 Hz and a resolution of 640 x 480 pixels was positioned 87 cm above the infrared casing. Three 120 W spot lights placed around the infrared casing were used to light the arenas indirectly. The light intensity measured at the surface of the water in each arena was 150 Lux.

The spot lights were connected to a programmable timer, making it possible to turn the light on or off automatically at specific times.

*Experimental protocol*

Six WW (3 R1 and 3 R2) and six WD (3 R1 and 3 R2) fish were gently collected from the tanks and individually transferred into arenas in random order. Care was taken to select juveniles of the two origins that were visibly similar in size. The fish were allowed to acclimatise to the arenas for 5 minutes before the start of video recording. The arenas were filmed for 65 min: 30 min in the light, 5 min of darkness and then another 30 minutes in the light. The light was turned off abruptly, resulting in sudden darkness, and then switched back on, with the programmable timer. At the end of the video recording, individuals were placed in 12 numbered 1-litre beakers. They were anaesthetised with 2-phenoxyethanol (0.3 ml $l^{-1}$) for measurement (total body length, BL, to the nearest mm) and weighing (BW, to the nearest mg). Thereafter, fish were sacrificed by severance of the spinal cord.

Fin clips were taken from each of the juveniles observed and stored in 95% ethanol for genotyping. DNA extraction and genotyping with Str541 and Str591 microsatellite markers was performed as described by Caudron *et al*. (2006). We also took fin clips from each of the males and females used to generate the juveniles studied.

This procedure was carried out eight times in all (*i.e.* 48 WW and 48 WD fish were observed over two days).

The water in each arena was replaced, in its entirety, after each observation session.

*Video analyses and behavioural variables*

The video recordings were analyzed with EthoVision XT software (Noldus, The Netherlands), which was used to track the swimming fish in each arena.
Each video recording was analysed in three sequences:

- L1: 30 minutes in the light.
- D: 5 minutes in the dark.
- L2: 30 minutes in the light.

For each sequence, the following variables of interest were used to characterise the swimming behaviour of the fish:
-The cumulative distance travelled by the fish in each arena (Dtot in mm),
-The mean velocity, expressed in body lengths per second (Vel in BL $s^{-1}$),
-The time spent immobile (NotMov in second), with a threshold of 1.7 cm $s^{-1}$,
-The absolute angular velocity (Vang) of the fish, expressed in degrees per second (° $s^{-1}$) was calculated by the software as follows:
$Vang_n = RTA_n / t_n - t_{n-1}$ where $RTA_n$ is the relative turn angle for sample n, and $t_n - t_{n-1}$ is the time difference between the current and previous samples. Here, the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for heading direction. This variable was used as an indicator of the extent to which the fish turned per unit time and to quantify the complexity of the swimming path.

*Statistical analysis*

All variables were compared by parametric analysis of variance (ANOVA), after checking that the normality and homoscedasticity requirements were met (Dagnélie 1975). All statistical analyses were conducted with Statistica 8 (Statsoft, USA) and, for all tests, $p < 0.05$ was considered significant.

BL and BW were compared in a one-way analysis of variance, with Replicate (R1 and R2) as the fixed factor, and then in a one-way analysis of variance with Origin (WW and WD) as the fixed factor.

The regression between each swimming variable and either BL or BW was analysed, to check for allometric relationships fish of the two origins during each period (L1, D and L2).

The effects of fish size were resolved, by comparing all variables relating to swimming activity in a repeated measures analysis of covariance after checking for parallelism (Dagnélie, 1975). Fish origin (WW and WD) was taken as a between-subject factor, Sequence (L1, D and L2) as a within-subject factor and body size or body weight as a covariate. Significant ANCOVA results were followed by a post-hoc multiple comparison test (Newman–Keuls).

## Results

### *Biometry*

No significant differences in weight or size were found between replicates of the same origin and data were therefore pooled for further analyses. However, WD fish were significantly heavier and larger than WW fish (1.54 ± 0.52 and 1.19 ± 0.55 g, $F_{(1,94)} = 9.8$, $P = 0.002$; 5.49 ± 0.63 and 5.08 ± 0.70 cm, $F_{(1,94)} = 8.8$, $P = 0.004$, respectively).

### *Genotyping*

The INRA males had only Atlantic alleles (Str541*132, Str591*150 and *152), whereas the Fier males and females had only Mediterranean alleles (Str541*136, Str591*164, *166 and *170), with the exception of a single female with one Atlantic allele (Str541*132). The juveniles were therefore unambiguously reassigned to the WW or WD group by genotyping for these two markers. All the genotyping results were consistent with the expected genotypes of the fish analyzed.

### *Swimming activity*

In most cases, correlations between swimming variables and BL or BW were weak and not significant. However, two significant correlations were identified in WW fish during L1: a correlation between Dtot and BL or BW ($r^2 = 0.22$, $P = 0.001$; $r^2 = 0.18$, $P = 0.003$ respectively) and a correlation between NotMov and BL or BW ($r^2 = 0.22$, $P = 0.001$ in both cases). Dtot tended to decrease with increasing BL or BW and NotMov tended to increase with increasing BL. There was also one significant correlation in WD fish, during L2, between Vang and BL ($r^2 = 0.1$, $P = 0.03$). Vang tended to increase with increasing BL.

The hypothesis of parallelism was verified for each of the variables studied. This involved that the slope of the regression line for swimming traits against fish size or fish weight did not differ significantly between fish of different origins. Similar results were obtained whether BL or BW was used as a covariate, so we present only the results obtained with BL below.

The difference in Vang between WW and WD was significant ($F_{(3,85)} = 10.9$, $P < 0.001$). Further Newman-Keuls tests showed that Vang was higher in WW fish than in WD fish during L1 (marginally significant: $P = 0.06$, Figure 1) and L2 ($P < 0.001$) but not during D ($P = 0.55$), when Vang decreased in fish of both origins. The significant difference observed during L2 results from the combination of an increase in Vang during this period in WW fish and a tendency for Vang to decrease in WD over the same period (Figure 1). If we consider measurements taken every minute (Figure 2), Vang was always higher in WW fish than in

WD fish during L1 and L2. During the dark phase (D), the two groups of fish reacted similarly, with an immediate decrease in Vang, which lasted from the beginning to the end of D; Vang continued to decrease during the first minute of L2 and then increased thereafter.

Dtot differed significantly between WW and WD fish ($F_{(3,85)} = 11.4$, $P < 0.001$). Further Newman-Keuls tests showed that the difference was significant only during L2 ($P < 0.001$). In WD fish, Dtot increased more strongly in L2 than in L1, whereas similar values were obtained for these two periods for WW fish (Figure 1).

Similarly, Vel differed significantly between WW and WD fish ($F_{(3,85)} = 11.9$, $P < 0.001$, Figure 1). As for Dtot, the difference was significant only during L2, when Vel increased in WD fish ($P < 0.001$, Figure 1). If we consider measurements taken every minute (Figure 2), the two groups of fish reacted similarly, with an immediate decrease in Vel at the beginning of D, followed by an increase until the end of D; Vel then decreased again at the beginning of L2. However, during L2, Vel fell to values lower than those for L1 in WW fish, whereas it remained higher than during L1 in WD fish.

Finally, NotMov differed significantly between WW and WD fish ($F_{(3,85)} = 13.9$, $P < 0.001$). This difference was significant during L1 and L2 ($P = 0.04$ and $P < 0.001$, respectively, Figure 1), but not during D ($P = 0.48$). WD fish were immobile for 50.4 ± 3.8% of the time during L1 and for 43.1 ± 3.1 of the time during L2, whereas WW fish were immobile for 60.5 ± 3.3% of the time during L1 and 61.6 ± 2.7 of the time during L2.

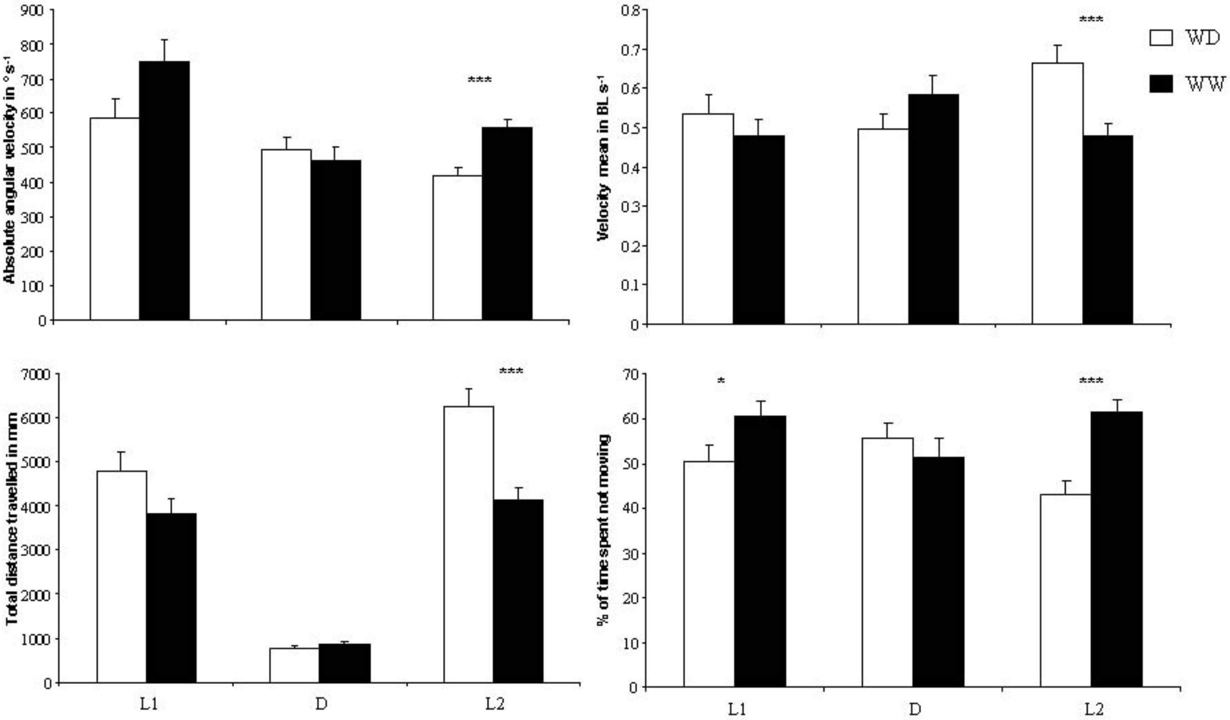

***Fig. 1*** *Mean ± SEM. Swimming variables in WD and WW fish during three sequences:*

*L1: 30 min in normal light conditions; D: 5 min in dark conditions; L2: 30 min in normal light conditions.*

*P<0.05, ***: P<0.001.*

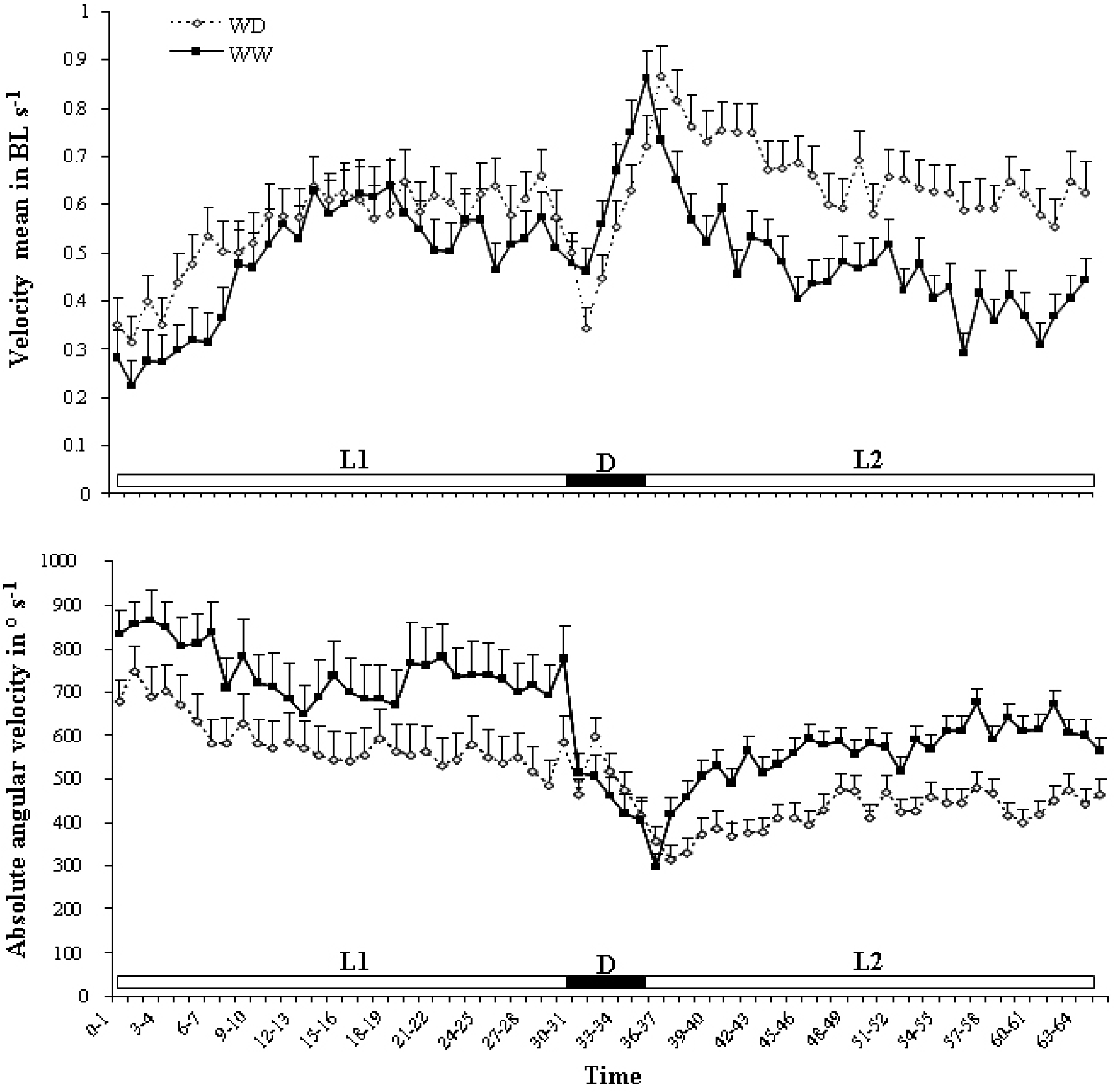

***Fig. 2*** *Mean ± SEM. Swimming variables in WD and WW fish during three sequences:*

*L1: 30 min in normal light conditions; D: 5 min in dark conditions; L2: 30 min in normal light conditions. Data are given for each minute.*

## Discussion

In this study, we compared the swimming behaviour (in a challenging situation) of two groups of brown trout juveniles, the origin of which was clearly identified by genotyping. These two groups differed in terms of the domestication history of the male parents. The Atlantic INRA strain has a long history of domestication that largely predates the foundation of the strain (more than 10 generations), which itself originates from a mixture of domesticated stocks, whereas the Mediterranean La Puya strain has been in captivity for only one generation. Since the two groups differed only in terms of the domestication history of the male parents, the observed significant differences should provide an estimate of strictly paternal genetic effects (both additive and dominance effects) for the behavioural traits investigated. These genetic differences result from two sources of variation: 1) the two populations used initially originated from two different well differentiated lineages and 2) they also differ in terms of their domestication history. With the experimental design used, it was not possible to dissect the relative contributions of the two factors, but the nature of the differences between the two genotypes strongly support the hypothesis, discussed below, that these differences partly reflect an impact of the domestication process on behavioural traits.

Growth performance is known to increase rapidly with the stage of domestication in fish (Gjedrem 1979). The results obtained for the WW and WD groups were consistent with this observation. A substantial difference (~ 20 %) was already evident 180 days after fertilisation (see table 1) and was maintained until sampling for the behavioural assay. The visual selection of juveniles was not sufficient to ensure that the WW and WD groups were homogeneous in terms of size and weight. However, we found that swimming activity was only weakly correlated with fish size or weight.

Significant differences were recorded between WD and WW fish for several behavioural traits. Angular velocity and time spent immobile were greater in WW than in WD fish, both before and after the light was turned off. Mean velocity and total distance travelled were higher in WD fish, particularly during the 30-minute period after the light was switched back on. These results reflect a lower swimming complexity in WD fish and a higher vigilance threshold (Bégout and Lagardère 2004) or stronger fear response (Arai *et al.* 2007) in WW fish, regardless of the challenge period (initial introduction into the test tank, or after the period of darkness). These findings are consistent with previous studies comparing wild-caught and domesticated sea bass juveniles under similar conditions (Benhaïm *et al.*, submitted) and probably reflect an antipredator response that has already been shown to be eroded in several farmed species, including Atlantic salmon, *Salmo salar* (Einum and Fleming 1997), rainbow trout, *Oncorhynchus mykiss* (Johnsson and Abrahams 1991), brown trout, (Fernö and Järvi 1998), and Atlantic cod, *Gadus morhua* (Nordeide and Svasand 1990).

The observed differences are mostly accounted for by the inheritance of differentially selected behavioural characters over several generations (Huntingford 2004). Indeed, during the domestication process, the frequency distributions of various behavioural traits change, partly because the fish selected from the source populations generally thrive in the predator-free and food-rich hatcheries (Salvanes and Braithwaite 2006).

The behavioural differences between WW and WD fish, which are likely to reflect only half the genetic differences between the two strains, were quantitative rather than qualitative in nature (see Price 2002), because both groups of fish reacted similarly to the two stimuli (light off, light on). Indeed, the sudden onset of darkness triggered a decrease in mean velocity rapidly followed by an increase, indicating avoidance of a potentially dangerous area and risk assessment (Millot *et al.* 2009). A similar decrease in locomotor activity following a sudden onset of darkness has been described in zebrafish, in which sudden changes in illumination can temporarily override the activity levels set by the circadian clock (Steenbergen *et al.* 2011b). However, recovery differed between the two groups of fish: 30 minutes after the second stimulation, WD fish had recovered higher levels of swimming activity, whereas the level of swimming activity remained low in WW fish. Thus, fish remained fearful, as reported for sea bass by Millot *et al.* (2009). Similarly, sudden exposure to darkness triggered a decrease in angular velocity in both groups of fish. Thirty minutes after exposure to darkness, only the WW fish displayed an increase in angular velocity, although values remained lower than those before the first stimulation in both groups of fish.

Clearly, these differences have implications for the management of aquaculture systems and for the success of restocking programmes (Huntingford 2004). Indeed, it is increasingly recognized that restocking programmes often fail because of behavioural deficits in the domesticated fish released (Huntingford 2004; Brown and Day 2002; Brown and Laland 2001; Olla *et al.* 1994), resulting in lower fitness in natural environments (Einum and Fleming 1997; Fleming and Einum 1997; Garant *et al.* 2003; McGinnity *et al.* 2003; Metcalfe *et al.* 2003). These findings also support the notion that fish farm escapees present a potential risk to wild fish stocks (see Huntingford 2004). This is of particular importance in salmonids, given the long history of restocking and farming of this taxonomic group (Salvanes and Braithwaite 2006).

This study constitutes one of the first attempts to demonstrate the existence of genetic variation for behavioural traits between populations with different domestication profiles. The behavioural traits identified here as relevant indicators of the domestication process in brown trout are probably also applicable to other fish species. The experimental set-up described here will be improved in several ways in future studies. One major improvement will involve the use of full 2x2 factorial designs comparing F2-F3 captive Mediterranean broodstocks with their wild source populations. This will make it possible to avoid differences due to geographic variation and to assess domestication effects only. Moreover, we will estimate the total differences between the two strains rather than the paternal effect only (corresponding to half the expected additive genetic variability between strains). Finally, we suggest that particular attention should be paid to determining how gene x environment interactions affect behavioural and physiological adaptation in fish (Johnsson *et al.* 2001).

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# Chapitre 7

# Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*)

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*Soumis*

# Résumé

Dans ce chapitre, le protocole expérimental et le matériel biologique utilisés ne permettent pas de contrôler précisément l'origine des individus, mais la comparaison porte sur des larves de bar domestiquées (standard industriel) et des larves sauvages capturées dans le milieu naturel et ayant subi par conséquent l'influence de la sélection naturelle. Ce protocole permet d'aborder l'impact de la captivité et du développement sur divers traits comportementaux. Des techniques d'otolithométrie ont permis de contrôler l'âge des larves sauvages afin d'assurer une comparaison pertinente avec les larves domestiquées.

Les études comportementales comparant des téléostéens sauvages capturés dans le milieu naturels et des téléostéens d'élevage sont rares alors qu'elles pourraient permettre d'améliorer à la fois les programmes de sélection en aquaculture et les programmes de repeuplement. Ici, nous examinons l'activité de nage chez des juvéniles de bars capturés dans le milieu naturel et des juvéniles domestiqués, avant et après application d'un stimulus standardisé permettant de déclencher une réponse de fuite. L'expérience est répétée à 8 âges différents sur des individus naïfs. Deux questions sont abordées : (i) La domestication a-t-elle un impact sur le comportement des juvéniles de bar ? (ii) Les premiers mois de captivité induisent-ils des modifications comportementales chez les juvéniles d'origine sauvage ? Un dispositif a été conçu pour déclencher la chute d'un stimulus visuel et mécanique (tige en plastique noir) simultanément dans 8 arènes où les animaux ont été placés individuellement et filmés. La réponse de fuite est évaluée (0 ou 1) ainsi que différentes variables liées à l'activité de nage : vitesse angulaire (Vang), distance totale parcourue (Dtot), vitesse moyenne (Vel), temps d'immobilité (Im) et distance par rapport au stimulus (Dstim) analysées 5 min avant, 5 et 15 min après déclenchement du stimulus. La lecture des otolithes sur un échantillon de juvéniles a montré que les bars domestiqués et sauvages étaient d'âges similaires (~ 55 jours au début de l'expérience, ~ 125 jours à la fin). Des différences comportementales ont été mises en évidence entre les deux catégories d'individus (Vang et Dstim plus élevées chez les individus sauvages) démontrant un impact de la domestication (réduction de la réponse de fuite et de la complexité de nage). Des similarités entre les deux catégories d'individus ont également été démontrées (réponse similaire après déclenchement du stimulus : diminution de Dtot, Vel et augmentation de Vang et de Im). Une diminution au cours du temps de la réponse de fuite et de la variabilité de la réponse (calculée à partir des variables liées à l'activité de nage) chez les deux catégories d'individus, montre que la captivité n'explique pas complètement les changements comportementaux chez les téléostéens sauvages, mais que des modifications d'ordre ontogéniques sont également mises en jeu.

En conclusion, cette étude souligne des différences comportementales entre bars sauvages et domestiqués et elle décrit une variabilité individuelle et des modifications survenant à un stade de développement précoce. Les résultats sont discutés avec une perspective de repeuplement et d'amélioration des programmes de sélection en aquaculture.

# Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*)

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## Abstract

Behavioural studies comparing hatchery and wild-caught fish are useful to improve selection for aquaculture and restocking programmes. We examined swimming behaviour characteristics in wild captured and domesticated sea bass juveniles before and after eliciting a startle response at 8 different ages and always on naive individuals. We specifically investigated whether domestication impacts juvenile sea bass behaviour and whether the first months of captivity induce behavioural modifications in wild juveniles. An apparatus was designed to mimic a predator attack by presenting sudden visual and mechanical stimuli simultaneously in 8 arenas where single individuals were placed and video recorded. The reactivity response was evaluated and different swimming variables including angular velocity, total distance travelled, mean velocity, immobility and distance from stimulus point were analysed from videos taken 5 min before stimulus actuation, 5 and 15 min after. Otolith readings showed that wild and domesticated juveniles were of similar age (~ 55 days at the start of the experiment and ~ 125 at the end of experiment). There were consistent behavioural differences (e.g. higher angular velocity and distance from stimulus point in wild fish) demonstrating that domestication reduces flight response behaviour. There were also similarities between both fish origins (similar response to stimulus actuation: decrease of total distance travelled and mean velocity, increase of angular velocity and immobility). A decrease over time in reactivity and variability in swimming responses among fish of both origins showed that captivity only does not fully explain wild fish behaviour changes and ontogenic modifications are likely interplaying.

Keywords: domestication; swimming activity; restocking, selection; coping styles.

## 1. Introduction

According to Price (1999) domestication is the process in which a population of animals becomes adapted to man and to the captive environment through genetic changes occurring over generation and environmentally-induced developmental events recurring in each generation. This can lead to phenotypical changes *e.g.* appearance of modified morphological and behavioural characters compared with the ancestral wild forms (Bilio, 2007). Some of these variations have been stabilised because of beneficial interests to humans. For example**,** chickens were selected to be larger, wild cattle (aurochs) to be smaller, and sheep to lose their bristly outer hairs (the kemp) and not to shed their soft inner hairs (the wool) (Diamond, 2002)**.** Most wild animals that yielded valuable domesticates were large terrestrial mammalian herbivores and omnivores and their domestication started 10 500 years ago (Diamond, 2002)**.**

When compared to terrestrial agriculture, aquaculture is still a new industry. Fish domestication is so recent that most fish in culture are still exploited captives but a few are on the threshold of becoming domesticated (Balon, 2004). However it is also the fastest growing animal food-production sector and the number of farmed fish species has increased rapidly during the last few decades, some as food fish, others for stocking in the wild (Balon, 2004). Furthermore, the domestication process includes inadvertent and artificial selections (Price, 1999). Artificial selection is the process of changing the characteristics of animals by artificial means such as directional selection, genomic selection (Hamblin et al., 2011), or familial selection (Theodorou and Couvet, 2003). Artificial selection has substantially contributed to modern agriculture and animal husbandry, though aquaculture has yet to gain much from efficient breeding and selection programmes (Jobling, 2007). Furthermore, domestication may play a role in the distribution of individual characteristics such as behavioral and physiological responses which, if they are consistent over time and characteristic of a certain group of individuals, define a coping style (Koolhaas et al., 1999). For example, genetic selection of pigs that are more adapted to farming conditions may indirectly result in the selection for one type of coping style and a consequent reduction in individual variation (Ruis et al., 1999). Indeed, domestication and selection could rapidly impact fish behaviour, sometimes as soon as at the first generation of domestication (Vandeputte and Prunet, 2002; Bégout Anras and Lagardère, 2004; Huntingford, 2004) and it is therefore important to check for the distribution of behavioural traits among populations, expecting bimodal distributions when coping styles are defined (Verbeek et al., 1994).

Among behavioural characteristics, several studies have implied that antipredator behaviour is highly sensitive to artificial rearing (Johnsson and Abrahams, 1991; Berejikian, 1995; Dellefors and Johnsson, 1995; Johnsson et al., 1996; Einum and Fleming, 1997; Fernö and Järvi, 1998; Johnsson et al., 2001). The single most important effect of domestication on behavior is reduced emotional reactivity or responsiveness to fear-evoking stimuli (i.e. environmental change, Price, 2002). Behavioral measures of reactivity are sensitive indicators of the complex of biochemical and physiological changes occurring in response to stress (Schreck et al., 1997). In particular, swimming performances in brook trout, Salvelinus fontinalis and in Guppy, Poecilia reticulate (Beamish, 1978; Walker et al., 2005) were reported to be better in wild stocks of fish versus domestic stocks. Changes in this behaviour were good indicators of the effects of the domestication process on the stress response (Millot et al., 2009a; Millot et al., 2009b). Standardized stimulation has been used to study the escape response in fish which is an important aspect of the swimming performances for escaping a predator (Wardle, 1993) and particularly the "C-start" response in relation to different environmental constraints: group versus solitary response (Domenici and Batty, 1997); pollution (Faucher et al., 2006); water temperature (Johnson et al., 1996) and hypoxia (Lefrançois and Domenici, 2006).

Little is known about the antipredator behaviour of hatchery-reared and wild-caught juveniles of other non-salmon fish species (Malavasi *et al.*, 2004) or on the behavioral response to fear evoking stimuli in the early life stages of fish**.** The european sea bass, *Dicentrarchus labrax* is a major species in Mediterranean aquaculture although little is known about the effects of the early phases of domestication or selection on growth apart from classical traits of commercial interest (Dupont-Nivet *et al.*, 2008; Vandeputte *et al.*, 2009). Attempts have been made to analyse behavioural responses to challenges in fish aged 12 to 24 months (Millot *et al.*, 2010; Millot *et al.*, 2011). Increased understanding of early behavioral swimming responses in sea bass should help determine early indicators that could be used for further domestication and selection programs or for restocking.
The present study aimed at comparing the swimming behavior characteristics of juvenile wild-caught sea bass with domesticated counterparts using an apparatus specifically designed to elicit a standardized and synchronized anti-predator escape response in several arenas. The comparison between origins was done using always naive individuals over time to address the following questions:

(i) Does domestication have an impact on juvenile sea bass behavior, especially regarding swimming activity before and after applying a visual and mechanical stimulus?
(ii) Do the first months of captivity induce behavioral modifications in wild juveniles?

## 2. Material and methods

### *2.1. Experimental animals and housing conditions*

Domestic sea bass larvae were hatched at a farm in Aquanord SA (France). They were transferred on February 23rd, 2009 to the experimental station of INTECHMER (Cherbourg) when they were 3 days old (D3) and grown in a recirculated system. In total, 150 000 individuals were placed into a 1 $m^3$ cylindrical tank with conical bottom. All parameters were set according to the protocol used by the Aquanord hatchery. The tank was supplied with water treated by both sand and biological filters (flow rate between 150 and 500 L $h^{-1}$ and 10% water renewal per hour). Light regime was 12:12 LD (light onset at 08:00 U.T. + 1) and intensity was between 0 and 500 Lux. Salinity was maintained at 35 g $L^{-1}$ except during the twenty first days where it was gradually decreased to 25 g $L^{-1}$ and increased again to 35 g $L^{-1}$ to facilitate the swimbladder formation. The oxygenation level was 7.8 ± 0.2 mg $L^{-1}$, temperature was 15.2 ± 0.53°C. The temperature usually reaches 21°C in a sea bass hatchery but here it was intentionally maintained lower to avoid creating large size differences with the wild stock that was thought to be captured later according to the natural conditions. Larvae were fed Artemia nauplii from D9 to D21 (5 nauplii per ml), a mixture of Artemia nauplii and enriched meta-nauplii (SUPER SELCO®) from D22 to D27 (2.5 nauplii and 2.5 meta-nauplii per ml) and enriched meta-nauplii from D28 to D54 (5 meta-nauplii per ml). Twenty four hours before the arrival of wild fish (D53), 560 individuals were placed in a 20 L container supplied with pure oxygen and transported by car several times in the day to place them under similar conditions to those of their wild counterparts during transport.

Wild sea bass juveniles were captured off the Mediterranean coast of France (Harbour of Cap d'Agde, Southern France, 43° 58' N; 03° 30' 19'' E) by Aquarid, a society specialized in catching fry for restocking and aquariology purposes. A whole school of 560 wild fish observed from the boat was collected at low depth (280 cm). Immediately after capture they were transported in a 20 L container supplied with pure oxygen to the experimental station (INTECHMER, Cherbourg) where they arrived 24 h later, on April 15th (D54).
On D54, both fish groups (wild *vs.* domesticated) were transferred in two separate hatching trays (42.5 x 39.5 x 17.2 cm) placed in a 200 L tank (215 x 42 x 17 cm). Two more trays were placed into this tank to separate tested individuals from naive ones. This tank was connected to the recirculated system described above. Water flowed through the left side and bottom of

the trays and exited through the bottom of a 500 µm diameter stitched grid replaced at D90 with a 1mm diameter grid. At this stage, all parameters were maintained stable for the total experiment duration. Temperature was 16.7 ± 0.5°C, salinity, 33.9 ± 1.0 g $L^{-1}$, Oxygen level, 7.24 ±0.43 mg $L^{-1}$ and flow rate was 200 L $h^{-1}$. Fish were fed enriched meta-nauplii from D54 to D76 (5 meta-nauplii per ml), enriched meta-nauplii and Marine Start (150-300 µm, Le Gouessant) from D77 to D80, Marine Start (150-300 µm) from D81 to D85, Marine Start (150-300 and 300-500 µm) from D86 to D91, Marine Start (300-500 µm) from D92 to D94, Marine Start (300-500 and 500-800 µm) from D95 to D98, Marine Start (500-800 µm) from D99 to D102 and Marine Start (500-800 and 800-1200 µm) from D103 to D125. The amount of food to be automatically distributed over 12 h was calculated according to feeding tables provided by Le Gouessant.

### *2.2. Experimental setup*

Observations were made in a dedicated room. The apparatus (Figure 1) was composed of 8 circular arenas (diameter 11 cm, height 9 cm) with opaque white walls and a transparent floor filled with 300 ml of water (from the recirculated system) which represented a water level of 5 cm. Arenas were numbered from 1 to 8 and placed on an infrared waterproof casing (1 x 1 m, Noldus, The Netherlands) that enabled recording of videos at low light intensity. The upper and internal part of each arena was composed of a piece of transparent plastic pipe (diameter 1.5 cm, length 5.5 cm) that guided a falling stimulus (a black plastic tube, diameter 0.5 cm, length 15 cm). One extremity of a fishing wire was attached to the end of the tube while the other was fixed to a plastic tablet located 50 cm above the infrared casing. The upper extremity of the wire was composed of a screw nut hanging on an electromagnet. The fall of the tube was then triggered by interrupting the electromagnet. The length of each wire was adjusted for eliciting a standardized and synchronized stimulus in each arena, with the tube coming into contact with the arena bottom. The color of the stimulus as well as the acoustic and shock waves therefore provided a mechanical and visual stimulus to the fish. A camera (Imaging Source DMK 21AUO4) with a frame rate of 60 Hz and a resolution of 640 x 480 pixels was positioned at 42.5 cm above the infrared casing. Two 60 W light bulbs were horizontally placed on walls located on the left and right sides of the infrared casing. They were located 100 cm above the infrared casing and provided an indirect lighting on the arenas. The light intensity measured at the water surface of each arena was 25 Lux.

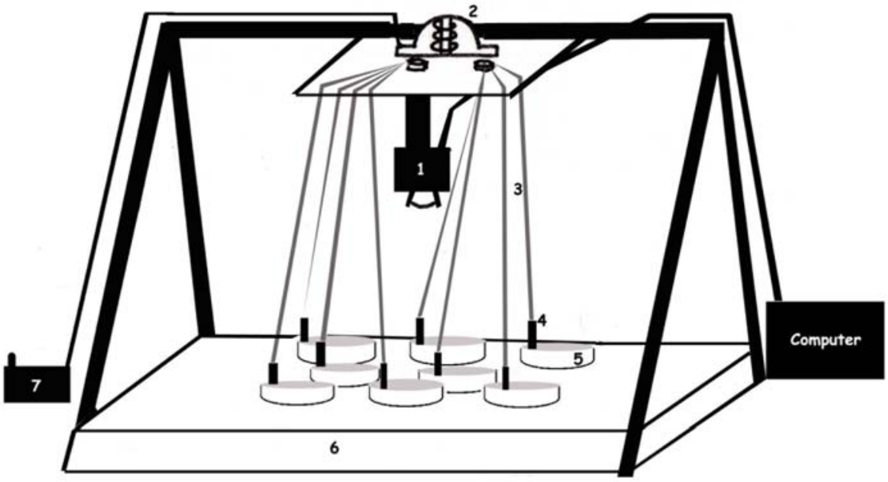


***Fig. 1.*** *Representative scheme of the experimental setup.*
*1: Digital camera (60 hz); 2: Electromagnet; 3: Fishing wire attached to the end of the stimulus; 4: Black plastic tube used as a stimulus; 5: Circular arena; 6: Infrared casing; 7: Electrical switch button allowing to interrupt the electromagnet and then to drive the fall of the stimulus.*

### *2.3. Age determination of wild fish*

At D138 i.e., 13 days after completing all observations, 30 wild and 30 domesticated individuals were randomly sampled from the tested fish. They were weighed and measured before dissection. The age of the domesticated fish was known but they were used as a control in the age-determination method. The fish were sacrificed using a lethal dose of 2-phenoxyethanol. The left and right sagitta otoliths were removed from the cranium, cleaned, encased in resin and mounted with CrystalBondTM glue on microscope slides. They were polished in the sagittal plane to the central primordial. All increment counts were made using the TNPC®5 image analysis software for calcified structure (Ifremer, Noesis) (Fablet and Ogor 2005). Age was estimated as the mean of the left and right otolith values.

### *2.4. Experimental protocol*

At each observation session, fish were collected after a two-hour feeding period. Then, four wild and four domesticated fish were gently collected from the trays and placed inside 8 opaque 1 l beakers which were covered and numbered. Care was taken to visually select juveniles of similar size. They were individually transferred into arenas where order was randomly predetermined. Video capture started after a 15 min acclimatization period. Arenas were filmed for 20 min, the stimuli being actuated at the fifth minute. At the end of the video recording, individuals were anesthetized with 2-phenoxyethanol (0.3 ml $L^{-1}$) for measuring (total body length, BL to the nearest mm: D55 to D125) and weighing (BW, to the nearest mg, D91 to D125). They were then observed under stereo microscope to check for stomach fullness (0: no food inside stomach; 1: food inside stomach; D59 to D69). Swimbladder presence was also verified on D59 to D91. Finally, each individual was returned to its beaker and when they had recovered, they were placed into separate trays dedicated to tested individuals (one for wild and one for domesticated).

This procedure was repeated 8 times in a day *i.e.* 32 wild and 32 domesticated individuals were observed during a session. In total, 8 sessions were performed at D55, 59, 63, 69, 91, 98, 111, and 125 accounting for a total of 256 observations per group.

### *2.5. Video analyses*

The video recordings were analyzed using the software EthoVision XT (Noldus, The Netherlands), which allowed a virtual point to be defined in each arena (position of the stimulus on the bottom of arena) and to track the fish swimming behavior. Six dates were analyzed from D63 to D125. A technical problem due to the infrared casing did not allow the swimming activity to be analyzed (*i.e.* no tracks extraction but only qualitative observations made) on the two first dates (D55 and D59).

Each video recording was analyzed in three sequences of 5 min:
- sequence 1 (S1): before the stimulation,
- sequence 2 (S2): just after the stimulation, and
- sequence 3 (S3): 10 min after the stimulation.

Each video (including the two first dates) was also viewed to see whether the reactivity (React) was modified after stimulus actuation: 0: the fish does not display neither escape response nor any swimming change, 1: the fish displays a clear escape response.

The position of the fish just before the end of the fall stimulus was noted (F: fish head oriented toward the stimulus and axis of the fish making an angle between -45 and +45° to the stimulus; S: axis of the fish making an angle between 180 and 225° or between -90 and -135°; B: fish head oriented facing away from the stimulus and axis of the fish making an angle between 225 and 315° to the stimulus; Figure 2a).

### *2.6. Behavioural variables*

For each 5 min sequence, different variables of interest were chosen to characterize fish behavior:

- Distance moved: the distance travelled by the centre point of the subject between two consecutive X-Y coordinates acquired (Dtot in mm);
- Mean Velocity: the distance moved by the centre point of the individual fish per unit time between two consecutive X-Y coordinates acquired expressed in body lengths per second (Vel in BL $s^{-1}$);
- Time immobile: the total duration the fish displayed no movement (Im in s);
- The fish absolute angular velocity expressed in degrees per second (Vang in ° $s^{-1}$) was calculated by the software as followed:
  $Vang_n = RTA_n / t_n - t_{n-1}$ where $RTA_n$ is the relative turn angle for sample n, and $t_n - t_{n-1}$ is the time difference between the current and previous sample. Here, the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for heading direction. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity.
- The mean distance of the fish from the stimulus point (Dstim in mm),

For each challenge, the fish reaction to the stimulus (React) was reported as a binary occurrence (0: no response, 1: response),

-To assess for individual variability between wild and domesticated fish and between the first and the last date of the experiment (D63 and D125), two indexes based on previous behavioral variables were calculated for each individual as follows:

(1) Reactivity index (RI):

$$RI = \frac{Xi(S1) - Xi(S2)}{Xi(S1)} - \frac{1}{32}\sum_{1}^{32} Xi(S1) - Xi(S2)$$

(2) Recovery index (RcI)

$$RcI = \frac{Xi(S3) - Xi(S2)}{Xi(S3)} - \frac{1}{32}\sum_{1}^{32} Xi(S3) - Xi(S2)$$

RI and RcI were calculated for each behavioural variable expressed as Xi. Xi(S1), Xi(S2), and Xi(S3) representing Xi values during sequences 1, 2, or 3; N = 32 referred to the number of individuals observed during a session per origin.

### *2.7. Statistical analysis*

All variables were compared using parametric analysis of variance (ANOVA) after verification of distribution normality and homoscedasticity (Dagnélie, 1975). When data did not fulfill these requirements, non parametric Kruskall-Wallis tests were used. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls), and Kruskall-Wallis test by a rank-based multiple comparisons (Zar, 1984). All statistical analyses were conducted using Statistica 8 (Statsoft, USA), and for all tests, the significant threshold was $p < 0.05$.

Logarithmic regressions of wild fish size *vs.* age, and body weight *vs.* age were calculated to estimate wild fish age at each observation session. The correlation between estimated domesticated fish age *vs.* weight or size was also examined. Wild and domesticated fish ages were compared using a Mann-Whitney test.

A Kruskal-wallis test was used to compare the reaction to the stimulus (React) with Origin (Wild *vs.* Domesticated fish), Date (8 ages), and Fish position just before the end of the fall stimulus (F, S and B) as independent variables.

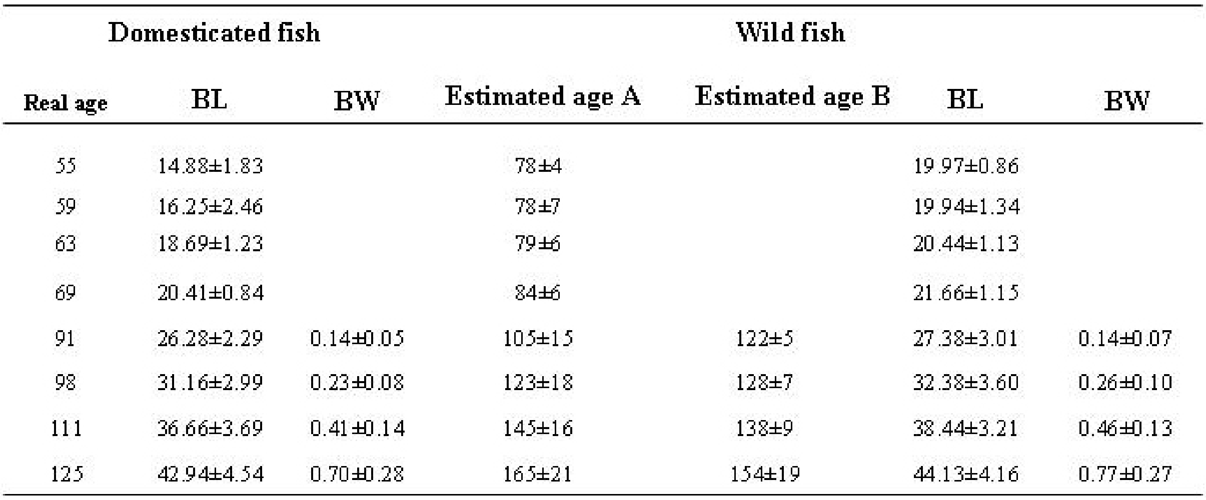
All variables related to swimming activity were compared using ANOVA with Sequence (S1, S2 and S3) as the within-subjects factor, Origin (Wild *vs.* Domesticated fish) and Date (6 ages) as between-subjects factors. For the variable Dstim, the Sequence factor was reduced to 2 levels (S2 and S3).

RI and RcI were compared inside each Origin (wild or domesticated fish) using a Kruskal-wallis test with Date (Day 63 and Day 125) as an independent variable. They were also compared between fish origins at each date (Day 63 or Day 125) using the same test with Origin (wild or domesticated) as an independent variable.

## 3. Results

### *3.1. Wild fish age*

The estimated age of wild fish at D139 (age of domesticated fish when sacrificed) was (Mean ± SD) 167.3 ± 22.9 days. Estimation of fish age based on domesticated fish otoliths was 133.2 ± 7.5 days. Both relationships between wild fish age and body length ($Y = 155.5 \, \mathrm{Ln}(x) - 422.3$, $R^2 = 0.76$), age and body weight ($Y = 49.9 \, \mathrm{Ln}(x) + 169.8$, $R^2 = 0.75$) were highly significant ($P < 0.001$). These data allowed estimation of the age of wild fish at each observation session (Table 1). There were no correlations between domesticated fish age and size ($R^2 = 0.01$, $P = 0.89$) or between domesticated fish age and weight ($R^2 = 0.001$, $P=0.97$). In conclusion, there was no significant difference between the ages of wild and domesticated fish used in the experiments ($U=16.5$, $P=0.81$).



| Domesticated fish | | | Wild fish | | | |
|---|---|---|---|---|---|---|
| **Real age** | **BL** | **BW** | **Estimated age A** | **Estimated age B** | **BL** | **BW** |
| 55 | 14.88±1.83 | | 78±4 | | 19.97±0.86 | |
| 59 | 16.25±2.46 | | 78±7 | | 19.94±1.34 | |
| 63 | 18.69±1.23 | | 79±6 | | 20.44±1.13 | |
| 69 | 20.41±0.84 | | 84±6 | | 21.66±1.15 | |
| 91 | 26.28±2.29 | 0.14±0.05 | 105±15 | 122±5 | 27.38±3.01 | 0.14±0.07 |
| 98 | 31.16±2.99 | 0.23±0.08 | 123±18 | 128±7 | 32.38±3.60 | 0.26±0.10 |
| 111 | 36.66±3.69 | 0.41±0.14 | 145±16 | 138±9 | 38.44±3.21 | 0.46±0.13 |
| 125 | 42.94±4.54 | 0.70±0.28 | 165±21 | 154±19 | 44.13±4.16 | 0.77±0.27 |

***Table 1****. Mean ± SD. Comparison between real domesticated fish age at each observation session and estimated wild fish age.*

*Estimation is either calculated on body length vs. age logarithmic regression equation (Estimated age A[1]) or on body weight vs. Age logarithmic regression equation (Estimated age B[2]).*

*1: $Y = 155.5 \, \mathrm{Ln}(x) - 422.3$.*

*2: $Y = 49.9 \, \mathrm{Ln}(x) + 169.8$.*

*BL : Total body length measured on fish at each observation session.*

*BW: Body weight measured on fish at each observation session.*

*Wild fish age was determined upon otolith readings.*

### *3.2. Stomach fullness and swimbladder rates*

Swimbladders were observed in all fish observed from D55 to D91. At D55, artemias were identified in 87.5% of domesticated fish stomachs, but were only present in 50% of wild fish stomachs at this time. At D59, 63, and 69, these percentages had increased (Mean ± SD) to 96.9 ± 5.4% in domesticated fish, and 93.8 ± 0.0% in wild fish.

### *3.3. Reactivity to the stimulus*

During the five first assessment dates, the percentages of reactivity were between 75 and 90% in domesticated fish and between 62 and 94% in wild fish (Figure 2b). During the last three assessment dates, the percentages decreased in both fish groups: they were between 44 and 59% in domesticated fish and between 44 and 65% in wild fish. There was no significant difference between domesticated and wild fish ($H_{1,\ 480}$ = 1.9, P=0.17), but there was significant effect of date ($H_{7,480}$ = 36.4, P<0.001) with reactivity being significantly higher at D55 and D59 than at D111 (P < 0.01).

Fish positions before the end of the fall stimulus were similar in both groups regardless of whether there was a subsequent reaction. Fish were oriented in front of the stimulus 30-35% of the time, behind it 40-45% of the time, and on the side 15-20% of the time (Figure 2c and d). The fish position before the end of the fall stimulus was not linked to the subsequent escape response *i.e.,* the initial orientation of the fish did not significantly differ ($H_{2,469}$ = 1.3, P=0.53).

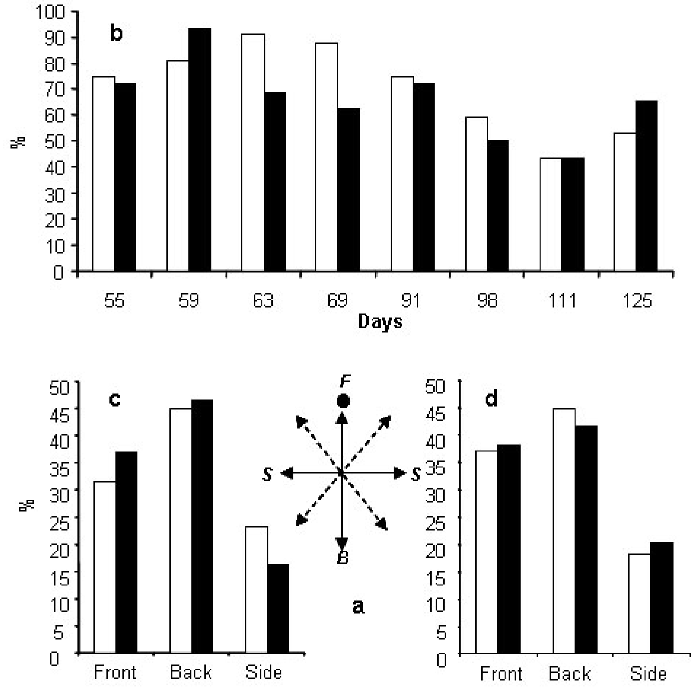


***Figure 2.*** *Escape response after stimulus actuation in wild-caught vs. domesticated fish. Black bars are wild-caught fish, white bars are domesticated fish.*

*(a): different positions of the fish just before the end of the fall stimulus : F: fish head oriented toward the stimulus and axis of the fish making an angle between -45 and +45° to the stimulus; S: axis of the fish making an angle between 180 and 225° or -90 and -135°; B: fish head oriented facing away from the stimulus and axis of the fish making an angle between 225 and 315° to the stimulus.*

*(b): Percentage of escape responses at different dates corresponding to observation sessions.*

*(c): Position of the fish just before the end of the fall stimulus when no escape response was observed.*

*(d): Position of the fish just before the stimulus fall end when escape response was observed.*

### *3.4. Swimming activity*

There was a significant interaction between Date and Origin for the angular velocity (Vang) (Table 2). On the four first assessment dates, wild fish performed higher Vang than domesticated fish; the values (Mean ± SE) during S1, S2, and S3 were respectively 1118 ± 117, 1539 ± 134, 1454 ± 135° $s^{-1}$ for domesticated fish and 1308 ± 133, 1629 ± 139, 1751 ± 143° $s^{-1}$ for wild fish (Figure 3). This tendency was reversed on the last two assessment dates where domesticated fish had higher Vang than wild fish (1163 ± 110, 1423 ± 105, 1278 ± 114° $s^{-1}$ and 996±89, 1191 ± 98, 1025 ± 101° $s^{-1}$ for S1, S2 and S3 respectively). Further Newman-Keuls tests showed that Vang was significantly higher in wild fish at D63 during S1 compared with wild and domesticated fish at D125 (Table 2, Figure 3). During S2, Vang was significantly lower in wild fish at D125 compared with all other categories at any date except for domesticated fish at D69, D98 and D125; significantly lower in domesticated fish at D125 compared with wild fish at D63, D91 and domesticated fish at D111; significantly higher in wild fish at D63 compared with domesticated fish at D69. During S3, Vang was significantly higher in wild fish at D63 compared with all other categories at any date except for wild fish at D91; significantly lower in wild and domesticated fish at D125 compared with all other categories at any date; higher in wild fish at D91 compared with domesticated fish at D98 and wild fish at D111.

| | Source | df | F | P | Newman-Keuls S1 | Newman-Keuls S2 | Newman-Keuls S3 |
|---|---|---|---|---|---|---|---|
| Vang | Origin | 3 & 392 | 1.2 | 0.320 | | | |
| | Date | 15 & 1082 | 4.6 | < 0.001 | | | |
| | Date*Origin | 15 & 1082 | 1.8 | 0.003 | W*D1 > W*D6=D*D6 | W*D6 < all but D*D2=D*D4=D*D6;<br>D*D6 < W*D1=W*D3=D*D5;<br>W*D1 > D*D2 | W*D1 > all but W*D3;<br>W*D6=D*D6 < all;<br>W*D3 > D*D4=W*D5 |
| Dtot | Origin | 3 & 392 | 0.4 | 0.730 | | | |
| | Date | 15 & 1082 | 3.1 | < 0.001 | D6 > all | | D6 > all but D3<br>D3 > D1 |
| | Date*Origin | 15 & 1082 | 1.4 | 0.140 | | | |
| Vel | Origin | 3 & 392 | 1.4 | 0.230 | | | |
| | Date | 15 & 1082 | 3.7 | < 0.001 | | | |
| | Date*Origin | 15 & 1082 | 2.0 | 0.01 | D*D1 > W*D4, W*D5, D*D5 ;<br>D*D5 < D*D1, D*D2 | D*D1 > all but D*D2 ;<br>D*D2 > all but D*D1, W*D2, W*D3, D*D3 | D*D3 > all but D*D1, W*D2, D*D2 |
| Im | Origin | 3 & 392 | 0.3 | 0.790 | | | |
| | Date | 15 & 1082 | 4.6 | < 0.001 | D6 < all | D5 > all | D6 < all |
| | Date*Origin | 15 & 1082 | 1.6 | 0.076 | | | |
| Dstim | Origin | 2 & 394 | 2.8 | 0.059 | | W > D | W > D (0.06) |
| | Date | 10 & 788 | 11.8 | < 0.001 | | D1=D2>all ;<br>D 3 < D4=D6 ;<br>D5 < D6 | D1=D2 > all |
| | Date*Origin | 10 & 788 | 1.0 | 0.430 | | | |

***Table 2.*** *Results of repeated measures ANOVA and Newman-Keuls post-hoc tests used to analyse the mean differences between Origins (Wild vs. Domesticated fish) and Dates (6 ages). Origin and Date are between-subjects factors and Sequence (S1, S2, S3) is the within-subjects factor.*

*W: wild fish; D: domesticated fish; S1 : 5 min before stimulus actuation, S2: 5 min after stimulus actuation, S3: 10 min after stimulus actuation.*

*D1 (Day 63); D2 (Day 69); D3 (Day 91); D4 (Day 98); D5 (Day 111); D6 (Day 125). For example, D1*W means domesticated fish at day 63.*

*Vang: absolute angular velocity (° $s^{-1}$); Dtot: total distance travelled in the arena (mm); Vel: mean velocity (BL $s^{-1}$); Im: time spent in immobility (seconds); Dstim: mean distance of the fish from the stimulus point (mm).*

*Significant threshold was $P<0.05$.*

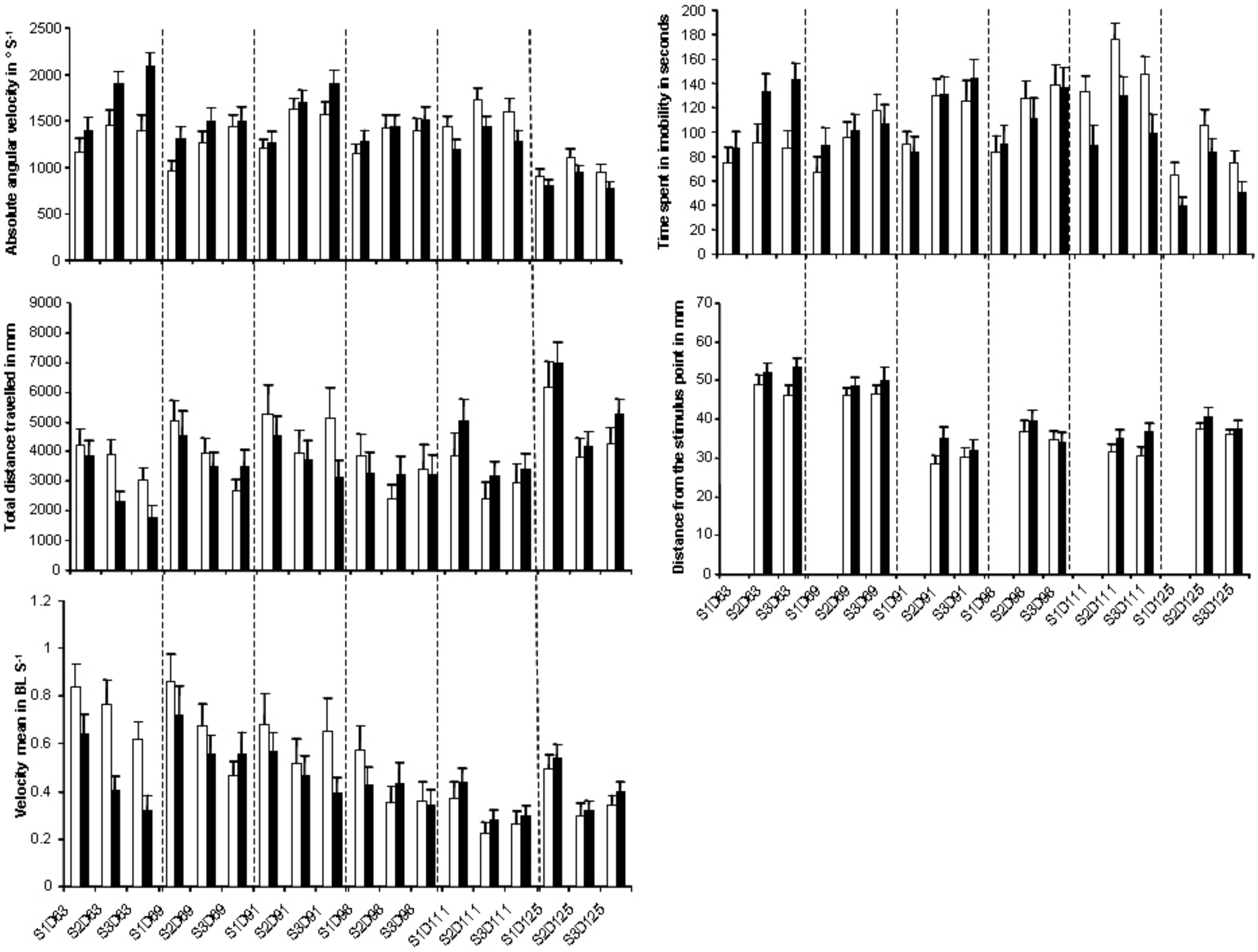


***Figure 3.*** *Mean ± SE. Behavioural variables in domesticated vs. wild fish at different ages (D) during three sequences (S) of 5 minutes (Black bars are wild-caught fish, white bars are domesticated fish):*
*S1 : 5 mn before stimulus actuation. S2: 5 mn after stimulus actuation; S3: 10 mn after stimulus actuation.*
*Day 63 (D63); Day 69 (D69); Day 91 (D91); Day 98 (D98); Day 111 (D111); Day 125 (D125).*

The total distance travelled (Dtot) was not significantly different between wild and domesticated fish but there was a significant Date effect (Table 2). On average, values were 4732 ± 766, 3390 ± 596, 3567 ± 645 mm from S1 to S3 in domesticated fish and 4713 ± 677, 3341 ± 529, 3389 ± 539 mm in wild fish (Figure 3). Newman-Keuls tests on Date showed that Dtot was significantly higher at D125 compared with all other dates during S1. During S3, Dtot was significantly higher at D125 compared with all other dates except for D91 and significantly higher at D91 than at D63 (Table 2).

There were significant effects of Date and Date*Origin on Velocity (Vel) (Table 2). During S1, domesticated fish at D63 performed significantly higher Vel than wild fish at D98, D111 and domesticated fish at D111; domesticated fish at D111 performed significantly lower Vel than domesticated fish at D69. During S2, domesticated fish at D63 performed significantly higher Vel compared with all other categories at any date except for domesticated fish at D69 performing itself significantly higher Vel than all other categories at any date except for wild fish at D69, wild and domesticated fish at D91 (Table 2). During S3, domesticated fish at D91 performed higher Vel compared with all other categories at any date except for domesticated fish at D63, D69 and wild fish at D69.

Immobility (Im) was not significantly different between wild and domesticated fish but there was a significant Date effect (Table 2). Newman-Keuls tests on Date during S1 and S3 showed that Im was significantly lower at D125 compared with all other dates. During S2, Im was significantly higher at D111 compared with all other dates.

The mean distance of the fish from the stimulus point (DStim) was significantly higher in wild fish than in domesticated fish, with a significant effect of date. During S2, DStim was higher at D63 and 69 (Figure 3, Table 2) than at all other dates but lower at D91 and 111 compared with D125. During S3, DStim was higher at D63 and 69 than at all other dates.

### *3.5. Effects of fish age: comparing responses at D63 and D165*

In domesticated fish, Reactivity index (RI) and Recovery index (RcI) calculated for Vang did not significantly differ between D63 and D125. RI and RcI calculated for Im both significantly increased at D125 ($H_{1,64}$ = 36.2, P<0.001 and $H_{1,64}$ = 47.3, P<0.001 respectively). RI calculated for Dtot and Dstim did not differ between dates but RcI for Dtot (ranging from -4.7 to 1.6 at D63 and from -1.9 to 0.4 at D125) and Dstim (ranging from -0.9 to 1.1 at D63 and from -0.5 to +0.5 at D125) were significantly higher at D63 ($H_{1,64}$ = 6.1, P<0.05 and $H_{1,64}$ = 18.2, P<0.001, respectively) (Figure 4a1 and b1). RI calculated for Vel did not differ between dates and RcI decreased at D125 although the difference was not significant ($H_{1,64}$ = 2.9, P=0.09).

In wild fish, RI calculated for Vang increased at D165 but the difference was not significant ($H_{1,64}$ = 3.3, P=0.07) and RcI did not differ between dates. RI calculated for Im did not differ between dates but RcI significantly increased at D125 ($H_{1,64}$ = 20.7, P<0.001). RI calculated for Dtot and Dstim did not differ between dates but RcI for Dtot (ranging from -2.7 to 3.4 at D63 and from -0.9 to 0.5 at D125) and Dstim (ranging from -1.1 to 0.5 at D63 and from -1.3 to 0.3 at D125) were significantly higher at D63 ($H_{1,64}$ = 26.4, P<0.001 and $H_{1,64}$ = 4.6, P<0.05 respectively) (Figure 4a2 and b2).

At D63, RI calculated for Dtot did not differ between fish origins but RcI calculated for Dtot was significantly higher in wild fish than in domesticated fish ($H_{1,64}$ = 22.3, P<0.001). RI calculated for Dstim did not differ between fish origins but RcI was significantly higher in domesticated fish than wild fish ($H_{1,64}$ = 13.1, P<0.001). At D125, no differences were recorded between wild and domesticated fish in any of the variables.

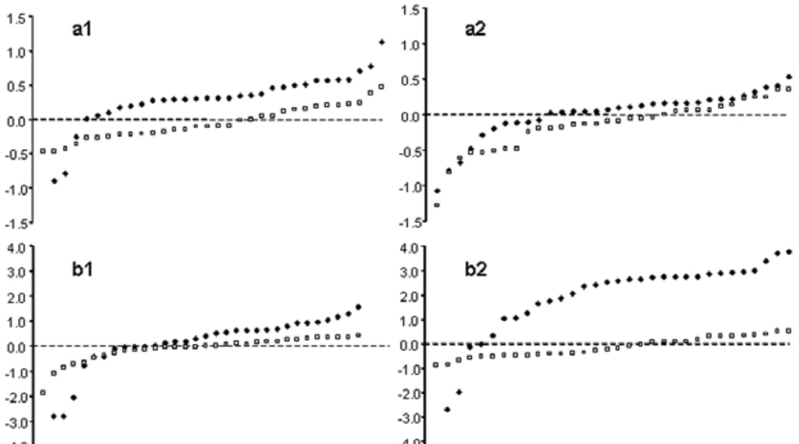


***Figure 4.*** *Distribution of recovery indexes (RcI[1]) based on different behavioural variables (Xi) and ranged in ascending order (N=32 individuals). Black rhombus are data at day 63, white squares are data at day 125. (a1): Distance from the stimulus point in domesticated fish; (a2): Distance from the stimulus point in wild fish; (b1): Cumulative distance travelled in domesticated fish; (b2): Cumulative distance travelled in wild fish.*

$$RcI = \frac{Xi(S3) - Xi(S2)}{Xi(S3)} - \frac{1}{32}\sum_{1}^{32} Xi(S3) - Xi(S2)$$

*With S3: sequence of 5 min, 10 min after stimulation, S2: sequence of 5 min just after stimulation. Xi: value of the individual for the variable X.*

## 4. Discussion

This study compared the swimming behavior of wild and domesticated juvenile sea bass before and after applying a visual and mechanical stimulus. This allowed two questions to be addressed. First was the assessment of the effect of domestication. The results showed consistent behavioral differences as well as similarities between both groups of fish developed hereafter. Second was the assessment of behavioural modifications of wild fish during the first month of captivity. Some changes were indeed recorded but most were also recorded in domesticated fish showing that the captive environment was not the only factor involved in such behavioral modifications. Furthermore, individual variability was strongly reduced among fish from both origins from the first to the last day of the experiment.

### *4.1. Experimental conditions and wild fish age*

The apparatus designed in this experiment could elicit a standardized and synchronized response whatever the initial position of the fish in the arena. It allowed collection of behavioral data on a large sample of fish (a total of 480 individuals observed over 8 sessions). The age of captured wild sea bass was verified whereas most published studies comparing wild and domesticated juveniles have only selected individuals of similar average size (Malavasi *et al.*, 2004). The age estimation method used in this study was precise and accurate *i.e.,* the estimated age of domesticated fish was close to the actual age. Otolith readings showed that wild caught sea bass were in the same age range as domesticated fish and both were also of similar size at each experimental date. All of the observed fish did not present any deformities: 100 % had a swimbladder and most of them fed during periods preceding the observation sessions. However, the very first session showed a lower percentage of stomach fullness in wild fish (only 50%). This could be explained by the effect of transport that may have been greater in wild fish than in domesticated fish or by a short-term accommodation to artemia (7 days later, stomach fullness percentage doubled).

### *4.2. Impact of domestication: comparing wild and domesticated fish responses*

Differences were recorded between wild and domesticated fish demonstrating an impact of domestication on behavior. During the four first sessions, angular velocity was higher in wild fish and mean velocity was lower than in domesticated fish even before stimulus actuation. The most consistent variable was the distance from the stimulus point, which was always higher in wild fish. This can be linked to anti-predator response which has already been shown to be eroded in several farmed species: Atlantic salmon, *Salmo salar* (Einum and Fleming, 1997), Steelhead trout, *Oncorhynchus mykiss* (Johnsson and Abrahams, 1991), Brown trout, *Salmo trutta* (Fernö and Järvi, 1998), and Atlantic cod, *Gadus morhua* (Nordeide and Svasand, 1990). A similar result was already recorded on Japanese flounder *Paralichthys olivaceus* juveniles where predator-experienced fish showed a longer response distance to the predator, reflecting a fear response or increased caution (Arai *et al.*, 2007). The difference in mean velocity and angular velocity reflected a lower swimming complexity in domesticated fish that could be linked to a decrease in the vigilance threshold (Bégout and Lagardère, 2004). Indeed, the environment experienced by cultured fish is strikingly different from that experienced by their wild counterparts *e.g.* the physical environment is much simpler, space is restricted and migration is not possible, it is less challenging in that good quality food is readily available and fish are protected against predators (Gross, 1998; Price, 1999; Waples, 1999). Furthermore, the hatchery environment is known to favour 'high-risk high-gain' phenotypes (Swain and Riddell, 1990). In our study, wild fish were captured when they were approximately 43±7 days old meaning that they had survived to natural predation which is very high at this stage. Indeed, this natural selection also leads to some behavioral phenotypes that increase rates of survival (Huntingford, 2004).

Some similarities were also recorded between both fish origins. They reacted similarly to the stimulus presentation: the distance travelled decreased as did the mean velocity and mobility while the angular velocity increased. These are typical indicators of fish avoidance of a dangerous area and of risk assessment (Millot et al, 2009). Ten minutes after the stimulation, the fish tended to recover a higher swimming activity though it remained at higher level than before stimulation. As already indicated by Millot et al. (2009), this means that fish remained fearful toward the stimulus. These results confirm that in nearly all cases, behavioral differences between wild and domestic populations are quantitative rather than qualitative in character and are best explained by differences in response threshold (Price, 2002).

### *4.3. Impact of captivity on wild fish*

Some behavioural modifications occurred in wild fish over time although they also occurred in domesticated fish in most cases. The reactivity decreased from the first to the last day of the experiment (75% to 53% in domesticated fish, 72 to 66% in wild fish after 70 days).

Swimming differences were mainly recorded during the last two stimuli exposures where mean velocity tended to decrease. Distance from the stimulus was especially high during the two first exposures and it decreased during the four following ones. Angular velocity was higher in wild fish during the first four assessments but became lower than domesticated fish on the last two assessments. However, angular velocity was lower for both fish origins at the end of the study. It seems that the vigilance threshold decreased in both domesticated and wild fish over time. Therefore, it is not possible to conclude that the captive environment was the only factor involved in the behavioral modifications of wild fish. The ability to evade predators may be particularly important during the early stages of life history (Houde, 1997). Gibb et al. (2005) proposed a model explaining performance changes across life-history stages in teleost fishes that could be related to our results. In this model, performance increases during early development, peaks at the larva-juvenile transition, and declines in juveniles and adults. At the juvenile stage, the performance decreases because the axial muscle cross-sectional area cannot increase rapidly enough to match the concomitant increase in body mass. We hypothesize that these performances modifications due to the biomechanical consequence of interrelated developmental changes in the size and shape of fish as they metamorphose from larvae to juvenile could also be associated with behavioral modifications *e.g.* decrease of vigilance threshold. Several studies showed that behavior and morphology were intimately related *e.g.* size and aggressiveness in salmonids (Abbot *et al.*, 1985).

Nevertheless, the captive environment was also likely to play a role in wild fish behavioural modifications. Indeed, as with other behaviors, anti-predator responses have both inherent and learned components shaped by rearing conditions and most behavior patterns should be viewed as lying somewhere on the continuum between these two extremes (Kieffer and Colgan, 1992).

At last, a decrease of intra-group variability was shown in both wild and domesticated fish between the first observation session and the last session performed 62 days later. This was mostly seen in the recovery indexes calculated for the distance travelled and the distance from the stimulus in both fish origins. At D63, the recovery index for the distance travelled was higher in wild fish whereas the recovery index for the distance from the stimulus was higher in domesticated fish. This variability decreased in both fish origins and could be related to the rearing environment which provided a constant plain environment influencing a range of behavioral traits (Salvanes and Braithwaite, 2006). Deficits have been already shown in virtually all aspects of hatchery-reared fish behavior owing to the impoverished conditions in which they are raised (Brown and Laland, 2001). Millot et al. (2009a) showed that selected 2-years old sea bass were characterized by a higher swimming activity and a lower path complexity than wild and domesticated strains before and after stimulus actuation but no significant differences were recorded between domesticated and wild ones. In this experiment, wild fish were not caught in the sea but were issued from wild caught parents. Therefore, the authors hypothesized that a 20-month period of rearing (first generation fish) could be sufficient to obtain fish presenting the same behavioural characteristics than second generation fish. In the present study, we hypothesize that an even shorter period could lead to behavioral modifications in wild-caught fish. However, after 70 days of captivity, wild fish were still characterized by a higher distance from stimulus than domesticated counterparts. At the same time it is interesting to note that domesticated fish response to aging was similar: at the beginning of the experiment *i.e.* at an early stage of development, reactivity was higher than 70 days later.

### *4.4. Conclusions: implications for selection and restocking programs*

Our study showed consistent behavioural differences between wild-caught and domesticated sea bass juveniles which demonstrated an impact of domestication on behavior. However, it also demonstrated a wider repertoire of responses at an early stage (~ 60 days old) and selection and restocking programs could be developed on this basis. Indeed, some domesticated individuals presented similar behavioral responses to wild fish. These individuals could be selected for restocking programs that often fail because released hatchery-reared fishes show remarkable deficits in many aspects of their behavioral performance, *e.g.* antipredator response, resulting in high levels of mortality in the post-release phase (Berejikian, 1995; Brown and Day, 2002). Conversely, other domesticated individuals showing lower reactivity to stimulus and lower path complexity could represent an opportunity for primary or directed selection in aquaculture. Indeed, selected sea bass have already been shown to be characterized by behavioral traits demonstrating a better adaptation than wild and domesticated strains (Millot et al., 2009) and this could be reinforced with such primary selection. Behavioural convergence between wild and domesticated sea bass juveniles showed the strong impact of the rearing environment. Numerous behaviors are partly innate- and partly environment-dependant *e.g.* predator avoidance (Magurran, 1990; Berejikian *et al.*, 2003). In our study, the presence of a clear reactivity in the domesticated fish suggested that some innate antipredator response remained in hatchery-reared sea bass juveniles (Malavasi *et al.*, 2004). However, the fact that behavioral responses modifications occurred in both wild and domesticated fish also shows that behavior can be rapidly modified by the environment. The behavioural patterns of fish result from innate patterns of maturation (developmental changes) and learning processes (Kieffer and Colgan, 1992). In our study, a sensitive period could also be involved at an early stage that could have long-term effects on the individual’s development (Bateson and Martin, 1999). Indeed deficiencies originating in early life are likely to affect later success (Salvanes and Braithwaite, 2006). However, behaviors can also arise through experience (Kelley *et al.*, 2003). Salvanes & Braithwaite (2006) showed that early experience with both variable spatial and food cues consistently produces cod that were faster in their attraction to, and their consumption of, live prey; in their speed of exploration of a new environment, and in their recovery from a stressful experience. This demonstrates behavioral plasticity in fish. Our study highlights the behavioral differences between wild-caught and domesticated fish and describes modification and repertoire variability at an early stage in sea bass life. However, further research is required to assess the developmental origin of behavioural modification and how it can be combined with restocking and selection programs.

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# Chapitre 8

# Unfamiliar congener used as a visual attractor in wild caught and domesticated sea bass *(Dicentrarchus labrax)* placed in a T-maze

D. Benhaïm, M-L. Bégout, G. Lucas & B. Chatain

*Soumis*

# Résumé

Les téléostéens domestiqués et ceux capturés dans le milieu naturel décrits dans le chapitre précédent sont à nouveau utilisés dans ce chapitre, mais à un stade de développement ultérieur et dans des conditions expérimentales très différentes. En plus de l'activité de nage, le protocole permet ici d'étudier l'exploration des individus et leur tendance grégaire.

A l'instar de la plupart des espèces de téléostéens, le bar européen a été domestiqué très récemment par rapport aux autres animaux terrestres et aux plantes. Les réponses comportementales constituent des indicateurs sensibles du processus de domestication car une seule génération suffit généralement à les modifier. Ce travail a comparé l'activité de nage, l'exploration et l'attraction visuelle exercée par un congénère non familier placé derrière une paroi transparente à l'extrémité de l'un des deux bras d'un labyrinthe, entre des juvéniles de bars sauvages capturés dans le milieu naturel et des juvéniles domestiqués. L'hypothèse testée est que lorsqu'un individu est placé dans un environnement nouveau (et donc stressant), il adopte un comportement grégaire même si le congénère proposé ne lui est pas familier. Vingt individus de taille similaire (~ 11.7 cm) de chaque origine ont été testés individuellement. Après une période d'acclimatation de 5 min, l'individu est libéré de la zone de démarrage et le labyrinthe est filmé durant 20 min. Différentes variables liées à l'activité de nage ont été mesurées telles que la vitesse angulaire (Vang), la distance totale parcourue (Dtot), la vitesse moyenne (Vel), le temps d'immobilité (Im) ainsi que le temps passé dans chacune des 6 zones virtuelles définies : Zone de démarrage (Start), la zone localisée juste après Start (PostStart), la zone située près du congénère non familier (ZCong), la zone située à l'opposé de ZCong (OpCong), la zone située près de ZCong (PreCong) et la zone localisée près de Opcong (PreOpcong). Vang et Vel sont plus élevées chez les individus domestiqués, mais les deux catégories d'individus ont passé la plus forte proportion de temps dans ZCong. Ceci démontre une attraction visuelle comparable entre les deux catégories induite par un congénère non familier de taille similaire. Cependant, il existe une variabilité avec des individus se réfugiant dans Start, d'autres qui sont visuellement attirés par le congénère mais qui restent localisés dans OpCong.

Ces résultats montrent un impact de la domestication sur l'activité de nage mais pas sur le comportement grégaire. Les résultats sont interprétés dans les contextes écologique et aquacole. L'intérêt potentiel pour de futures expériences sur la cognition de cette espèce est également abordé.

# Unfamiliar congener used as a visual attractor in *wild caught and domesticated sea bass* *(Dicentrarchus labrax) placed in a T-maze*

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## Abstract

Behavioural responses are sensitive indicators of the domestication process since they are generally impacted as soon as the first generation. The present work compared wild-caught and domesticated sea bass juveniles swimming activity, exploration and visual attraction induced by an unfamiliar congener located behind a transparent wall at the end of one arm of a T-maze. This cognitive challenge was based on the hypothesis that placed into a novel and therefore stressful environment, fish would adopt a gregarious behaviour even though they were not familiar with the present congener. Twenty individuals of similar size (11.7 ± 0.6 cm) from both origins were individually tested. After a 5min acclimatization period, the wall of the start-box was removed and the maze was filmed during 20 min. Different swimming variables including angular velocity (Vang), total distance travelled (Dtot), velocity mean (Vel), time spent in Immobility (Im) were analysed from videos as well as the time spent in each of 6 virtual zones including the start-box zone (Start), the zone located just after Start (PostStart), the zone near the congener (ZCong), the zone opposite to ZCong (OpCong), the zone located near ZCong (PreCong), and the zone located near OpCong (PreOpCong). Vang and Vel were higher in domesticated fish but fish from both origins spent most of the time in ZCong showing a similar visual attraction induced by an unfamiliar congener of similar size. Nevertheless, individual variability was shown, including fish choosing to shelter in Start zone and fish visually attracted to the congener but located in OpCong. These results demonstrated an impact of domestication on swimming activity characteristics but not on gregarious behaviour. The findings are discussed with focus on ecological and aquaculture concerns and their potential interest for future cognition-based experiments on this species.

Keywords: domestication, maze, visual cue, personality, swimming activity.

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## 1. Introduction

The aquaculture sector is so recent that most cultured fishes might be rather considered as exploited captives and only a few of them would be on the threshold of becoming domesticated (Balon, 2004). Indeed, farmed fishes are little changed from their wild ancestral form and could usually be returned to the wild. However, consistent differences between wild and domesticated fish have been reviewed by several authors (Ruzzante, 1994; Gross, 1998; Vandeputte and Prunet, 2002). Likely to be the first to be affected, behavioural traits are good indicators of the domestication process (Swain and Riddell, 1990; Ruzzante and Doyle, 1991; Ruzzante and Doyle, 1993; Price, 1999). Among the most studied of all, antipredator behaviour has been shown to be very sensitive to artificial rearing (Johnsson and Abrahams, 1991; Berejikian, 1995; Dellefors and Johnsson, 1995; Johnsson et al., 1996; Einum and Fleming, 1997; Fernö and Järvi, 1998; Johnsson et al., 2001), and swimming performances to be poorer in domestic stocks (Beamish, 1978). These differences between wild and cultured fishes may be partly explained by different previous experiences (Huntingford, 2004). Farmed fishes are facing conditions that seem to be less challenging than natural habitats e.g. structurally simpler environments, food easy to catch and absence of predators but they also have to adapt to high densities, restricted space, artificial and uniform food, and quite frequent handling (Fernö et al., 2007).

A way to identify differences between wild and domesticated fish *i.e.*, to study the impact of domestication, is to examine their behavioural responses to a novel environment. Initial introduction into novel environments has been shown to produce in Zebrafish and other small teleost fish, behaviors consistent with predator evasion, fear, and/or anxiety (Gould, 2011). For example, environment such as a light/dark plus maze, based on the tendency of the fish to seek dark backgrounds (or avoid light backgrounds) in unfamiliar environments, can be used to study these behaviors (Serra et al., 1999; Gould, 2011). This device enabled to show behavioural differences in Zebrafish lines including ZIRC, AB and WIK, the latter of which descends more recently from wild populations in India (Sackerman et al., 2010).

European sea bass (*Dicentrarchus labrax*) is a leading species in Mediterranean aquaculture that was recently domesticated. This explains why very little is known on effects of the early step of domestication or selection for growth apart from classical traits of commercial interest (Dupont-Nivet et al., 2008; Vandeputte et al., 2009) and first attempts made to analyse behavioural responses to challenges in 12-24 months old fish (Millot et al., 2010; Millot et al., 2011). The present work compared wild-caught and domesticated sea bass juveniles swimming activity, exploration in T-maze in which one unfamiliar congener of similar size was placed at the extremity of one of the two arms, behind a transparent Plexiglass wall precluding olfactory cues. Because natural selection has ceased in the domesticated line, we postulated that innate predator vigilance in an unfamiliar environment could wane from this latter, leading to behavioural responses different from those of wild ones. We also intended to test the hypothesis of visual attraction on both fish origins induced by the unfamiliar fish after being placed into this novel and therefore stressful environment. Indeed, when stressed, a fish seeks either to shelter (Bustard and Narver, 1975; Cunjak, 1988; Greenwood and Metcalfe, 1998) or cover behind other group members to reduce the chance of being caught by a predator (Hamilton, 1971). It has been shown that fish reduces neighbour distance to obtain easier information about whether other group members have detected a predator (Pöysä, 1994) and that social interaction plays an important and beneficial role in regulating the stress response in cohesive social species such as sturgeon *Acipenser fulvescens* (Allen et al., 2009). Since sea bass in the wild, at the juvenile stage, is a demersal and gregarious species (Barnabé, 1989), it could respond similarly. Binary choice tests between familiar and unfamiliar congener were already performed on several fish species showing that they preferentially school with familiar individuals (*e.g.* Van Havre and Fitzgerald, 1988; Miklósi

et al., 1992; Brown and Smith, 1994; Griffiths and Magurran, 1997; Brown, 2002; Farmer et al., 2004). Here we intended to examine the attraction induced by an unfamiliar congener. On one hand; there is actually little direct evidence for shoal fidelity among wild fish (Helfman, 1984; Hoare et al., 2000; Svensson et al., 2000). But on the other hand, in aquaculture, fish are somehow forced to shoal with unfamiliar fish because of the stock management that implies frequent size grading procedures accompanied with tanks population reorganization.
Thus the following questions were addressed:
(i) Are there differences in exploration and swimming activities between wild-caught and domesticated fish in a maze?
(ii) Are wild-caught and/or domesticated sea bass juveniles visually attracted by an unfamiliar congener?

## 2. Material and methods

### 2.1. Experimental animals and housing conditions

Domestic sea bass were hatched at the farm Aquanord SA (France), transferred on February 23rd, 2009 to the experimental station of INTECHMER (Cherbourg) when they were 3 days old (D3) and grown in a recirculated system. All parameters were set according to the protocol used by Aquanord hatchery except for the temperature that was 15.2±0.53°C. The temperature usually reaches 21°C in a sea bass hatchery but here it was intentionally maintained at a lower level to avoid creating large size differences with the wild stock that was thought to be captured later according to the natural hatching conditions.

Wild sea bass juveniles were captured off the Mediterranean coast of France (Harbour of Cap d'Agde, Southern France, 43° 58' N; 03° 30' 19'' E). A whole school of 560 wild fish observed from the boat was collected at low depth (280 cm). Immediately after capture they were transported to the experimental station (INTECHMER, Cherbourg) where they arrived 24 hours later, on April 15th. For further details, see Benhaïm et al. (submitted).
Both fish origins (about 400 individuals each) were later grown in open water system in two separate 2m$^3$ tanks until the beginning of this experiment which started on March 28th 2010. At this date, domesticated fish were 390 days old. Wild-caught fish ages were determined on a 30-individual sample (see Benhaïm et al., submitted) and were of similar age as domesticated fish. During the experimental period, light regime was 16:8 LD (light onset at 06:00 U.T. + 1). In both tanks, temperature, salinity and oxygen level were (Mean ± SD), 15.4 ± 0.3°C, 35.0 ± 0.0 g L$^{-1}$, 5.6 ± 0.3 mg L$^{-1}$.
Two days before the beginning of observations, wild and domesticated fish were anesthetized with 2-phenoxyethanol (0.3 ml l$^{-1}$) and based on previous biometry data, 20 individuals from each origin were selected. Total length (mean ± SD) was 11.7 ± 0.6 cm in domesticated fish and 11.7 ± 0.5 cm in wild ones (t-test: t=0.02, P=0.98); weight was 16.7 ± 3.7 g in domesticated fish and 15.3 ± 2.7 g in wild ones (t-test: t=-1.33, P=0.19). These individuals were placed in two 200 l tanks. Additionally, a stock of 400 domesticated fish of same age but from a different tank was used to select 30 individuals of similar size and weight (11.8 ± 0.9 cm, 16.4 ± 2.3 g). These fish were placed in a third 200 l tank. They were used as unfamiliar attractors in the experiment. The three tanks were supplied with water of identical characteristics as original tanks.

### 2.2. Experimental setup

Individuals were tested one by one in a maze constructed from opaque white plastic and transparent Plexiglas® (Figure 1). The start box (20x10 cm) was separated from the rest of the maze by a removable opaque wall. At the end of each arm of the maze, two strictly waterproof compartments (20x15 cm) were also separated by a not removable transparent wall. The maze which floor was made of transparent Plexiglas® was placed on an infrared waterproof casing (1x1 m, Noldus, The Netherland) that enables to record videos at low light intensity and to improve video analysis. Shortly before observations, the maze was filled with water which level was maintained at 12 cm. Temperature, salinity and oxygen level were verified before and after the end of observations performed on each fish and were respectively 16.0 ± 0.5°C, 35.0 ± 0.0 g $L^{-1}$, 7.8 ± 1.5 mg $L^{-1}$ before, 16.0 ± 0.5°C, 35.0 ± 0.0 g $L^{-1}$, 7.3 ± 1.2 mg $L^{-1}$ after. Digital camera (Imaging Source DMK 21AUO4) with a frame rate of 30 Hz and a resolution of 640 x 480 pixels was positioned at 77 cm above the water surface. Two 60 W light bulbs were horizontally placed on walls located on the left and right sides of the infrared casing. They were located 100 cm above the infrared casing and provided an indirect and homogenous lighting on the maze. The light intensity measured at the water surface of the maze was 100 Lux.

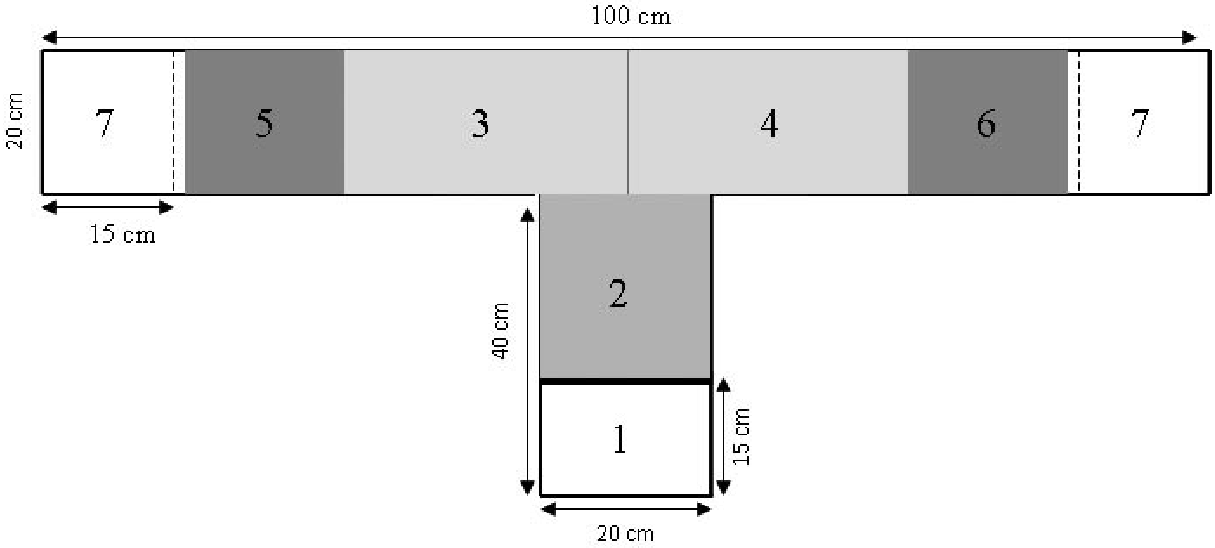

*Figure 1. Schematic representation of the T-maze apparatus and virtual zones delimitation on the bottom of the maze defined for the video recordings analysis.*

*Dotted lines are transparent Plexiglas walls, continous lines are white opaque plastic. The bottom of the maze is made of transparent Plexiglas.*

*1.Start-box closed by a removable opaque wall; 2. Post-Start; 3. Pre-left: area located on the right side of the left area; 4. Pre-right: area located on the left side of the right area; 5. left area located near the compartment where congener was placed; 6. right area located near the compartment where congener was placed; 7. left and right compartments closed by a waterproof transparent Plexiglas wall where congener was placed*

## 2.3. Experimental protocol

Before the beginning of observations, the position of the unfamiliar congener was randomly determined for each individual on the left or right arm of the maze. The first tested fish was gently collected from the tank using a net and immediately placed inside a bucket closed by a cover then placed in the maze start-box. After a 5min acclimatization period, the wall was removed and the video capture started. The maze was filmed during 20 min. In order to test all individuals, 4 days were required. On the first day, five domesticated fish were consecutively tested in the morning and five wild fish were tested in the afternoon. On the second day, this protocol was reversed and so on for the third and fourth days. The water was entirely renewed between wild and domesticated individual batches. The unfamiliar fish used as the attractor was changed every hour to minimize stress due to confinement and handling.

## 2.4. Video analyses

The video recordings were analysed using the software EthoVision XT 5 (Noldus, The Netherland), which allowed six virtual zones to be defined in the maze (Figure 1) and to track the fish swimming behaviour.

Each video was also viewed to measure the time spent oriented toward the congener *vs.* toward the empty compartment when the fish was located in OpCong.

### 2.5. Behavioural variables

Different variables of interest were chosen to analyse the fish behaviour:

-The time spent in each zone expressed in seconds (s): Start-Box (Start), Post-Start (PostStart), zone near the congener (ZCong), zone opposite to ZCong (OpCong), zone located near ZCong (PreCong), zone located near OpCong (PreOpCong).
-The orientation toward the congener or toward the empty compartment when the fish was located in OpCong.
-The fish absolute angular velocity expressed in degree per second (Vang in ° $s^{-1}$) was calculated by the software as followed:
$Vang_n = RTA_n / t_n - t_{n-1}$ where $RTA_n$ is the relative turn angle for sample n and $t_n - t_{n-1}$ , the time difference between the current and previous sample. Here the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for head direction. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity.
-The distance travelled by each fish in the maze (Dtot in mm)
-The velocity mean expressed in body length per second (Vel in BL $s^{-1}$)
-The time spent in immobility (Im in s)
The last three variables quantified the fish swimming activity level in the maze for each sequence.

### 2.6. Statistical analysis

All variables were compared using parametric analysis of variances (ANOVA) after verification of distribution normality and homoscedasticity (Dagnélie, 1975). When data did not fulfil these requirements, non parametric tests were used. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls), and Kruskall-Wallis test by a rank-based multiple comparisons (Zar, 1984). All statistical analyses were conducted using Statistica 8 (Statsoft, USA), and for all tests, the significant threshold was $p < 0.05$.
All variables were compared using ANOVA with Origin (wild and domesticated fish) and Zone (6 virtual zones) as fixed factors.
The time spent oriented toward the congener zone was compared to the time spent oriented toward the empty compartment when the fish was located in OpCong using a Wilcoxon matched pair test inside each fish origin (wild and domesticated). The same comparison between wild and domesticated fish was done using a Mann-Withney test.
Principal components analysis was used to search for individual variability in zone exploration among wild and domesticated fish.

## 3. Results

### 3.1. Spatial distribution

All the fish tested left the start box after the wall was removed *i.e.,* none of the fish spent 100% of the time in Start or in PostStart zones. Wild-caught and domesticated fish spent most of the time in ZCong (mean ± SE, 41 ± 7% and 38 ± 8% respectively, Figure 2). There was only a Zone significant effect ($F_{(5,\ 216)}$= 13.2, P<0.001) with the time spent in ZCong being higher than in all other zones (P<0.05 for each pairwise comparisons) and time spent in

OpCong (20 ± 5% in wild fish and 24 ± 7% in domesticated ones) being lower than in ZCong but higher than in all other Zones (P<0.05 for each pairwise comparison).

When wild and domesticated fish were located in OpCong, they were oriented significantly more toward ZCong than OpCong (84±2% and 63±44% for wild and domesticated fish respectively, Z=2.3, P=0.02 in both cases) and there was no Origin effect for both orientations

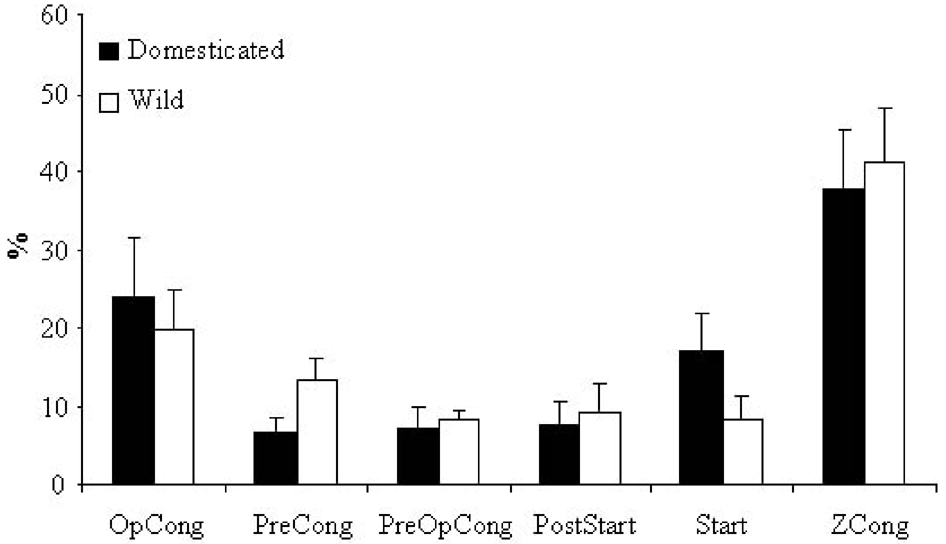

***Figure 2**. Proportion of time spent (mean S.E., in %) by a fish in each zone of the maze.*

*Start : Start Box, PostStart: area located after Start, ZCong: reward zone near the congene, OpCong: zone opposite to the reward zone, PreCong: zone located just before ZCong, PreOpcong: zone located just before OpCong*

### 3.2. Individual variability

PCA showed that the first two components accounted for 94% of the variability of the data (Figure 3). The first component was related to two variables: the negative pole to high values of OpCong (contribution to the first component was 0.40 and the correlation was -0.81), the positive one to high values of ZCong (contribution to the first component was 0.55 and the correlation was 0.94). As already shown in the previous section, PCA confirmed that most of the individuals were characterized by high values of ZCong (Figure 3). It represented 70% of wild fish and 50% of domesticated ones. There were also 20% of domesticated fish and 15% of wild ones associated to high value of OpCong (Figure 3); 20% of domesticated fish and 5% of wild ones associated to high values of Start (Figure 3). At last, 10% of wild fish associated to both Start and ZCong (W5 and W16, Figure 3) while 5% of both origins were associated to ZCong and OpCong (D11 and W8, Figure 3).

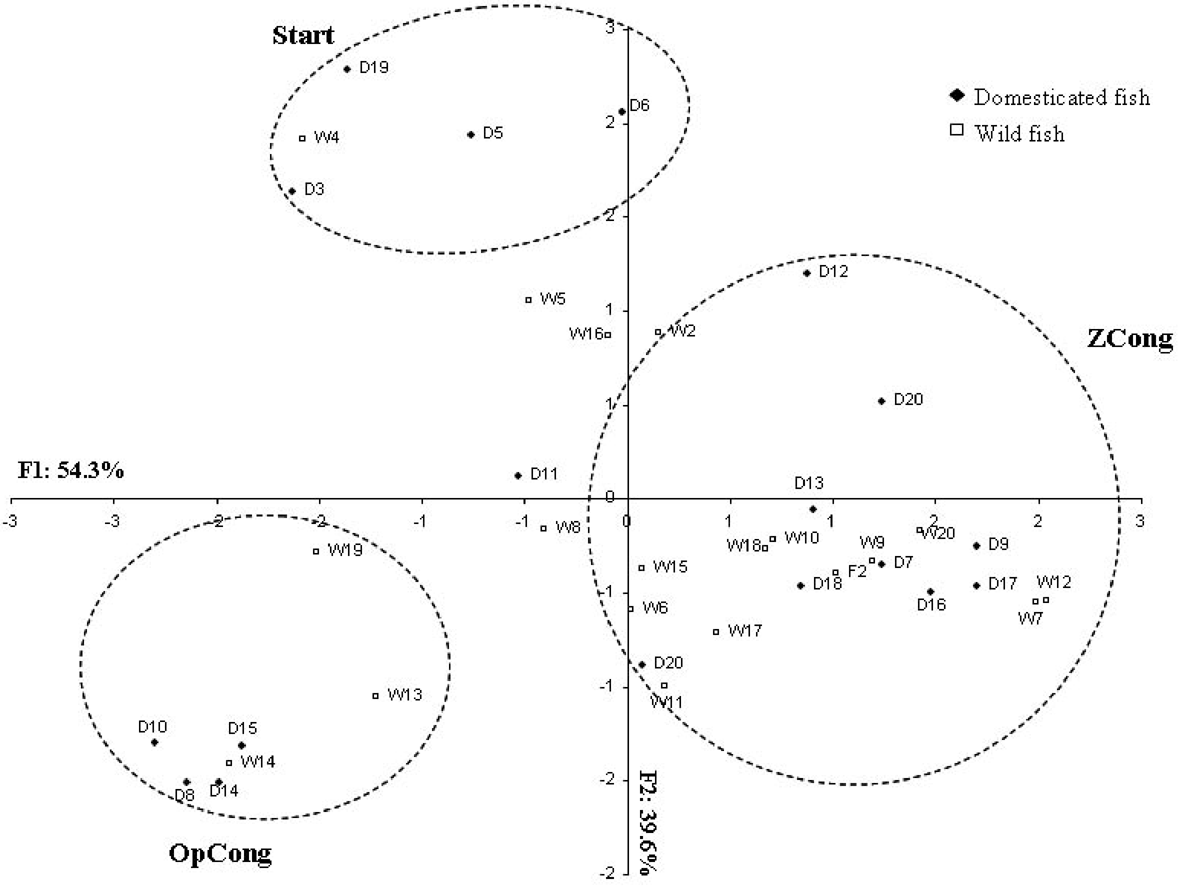

*Figure 3. Principal component analysis (PCA) applied to Zones explored in the maze.*

*W: wild fish; D: domesticated fish; Start: Start Box ; ZCong: zone near the congener, OpCong: zone opposite to ZCong.*

### 3.3. Swimming activity

Vang was higher in domesticated fish than in wild ones (Mean ± SE, 338 ± 41 and 223 ± 30 ° $s^{-1}$). There was a significant Origin effect ($F_{(1,198)}$=28.6, P<0.001) and a significant Zone effect ($F_{(5,198)}$=11.5, P<0.001) with Vang being higher in Start than in all other zones (P<0.05 for each pairwise comparison, Figure 4), being higher in ZCong and in OpCong than in all other zones but lower than in Start (P<0.05 for each pairwise comparison).

Wild and domesticated fish travelled similar distance (1935 ± 416 and 1938 ± 487 mm respectively). There was a significant Zone effect ($F_{(5,198)}$=6.7, P<0.001) with Dtot being higher in ZCong than in all other zones (P<0.05 for each pairwise comparison, Figure 4).

Vel was significantly higher in domesticated fish than in wild ones (Mean ± SE, 0.77 ± 0.18 and 0.52 ± 0.12 BL $s^{-1}$, $F_{(1,198)}$=5.6, P=0.018) and there was a significant Zone effect ($F_{(5,198)}$=5.2, P<0.001) with Vel being higher in PostStart and in PreOpcong than in ZCong and OpCong (P<0.05 for each pairwise comparison, Figure 4).

At last, wild-caught and domesticated fish spent similar time immobile (Mean ± SE, 73 ± 13% and 68 ± 16% respectively). There was a significant Zone effect with Im being higher in ZCong than in all other zones and Im being lower in OpCong than in ZCong but higher than in all other zones (P<0.05 for each pairwise comparison, Figure 4).

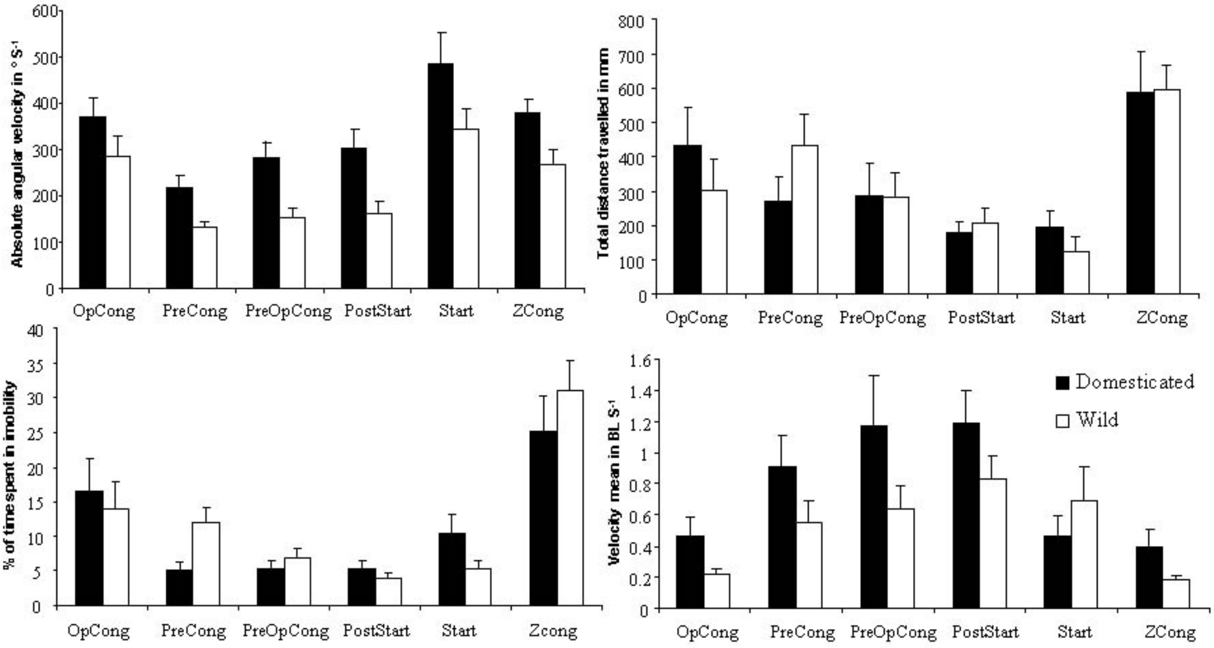

***Figure 4**. Mean ± SE. Behavioural variables in domesticated vs. wild fish in each virtual zone defined for the video recordings analysis.*

*Start: Start Box, PostStart: zone located just after Start, ZCong: zone near the congener, OpCong: zone opposite to the congener zone, PreCong: zone located just before ZCong, PreOpCong: zone located just before OpCong.*

## 4. Discussion

The aim of this study was to assess for domestication effect on juvenile sea bass swimming activity, exploration and visual attraction to an unfamiliar congener. This was approached by comparing wild-caught and domesticated fish individually tested in a maze. The results showed consistent behavioural differences between fish origins but a similar attraction to an unfamiliar congener.

Behavioural differences were found between wild-caught and domesticated fish of similar size and age. Angular velocity and mean velocity were higher in domesticated fish. These differences between wild and domesticated fish demonstrated an impact of domestication on swimming activity. Difference in velocity mean could be linked to a decrease of the vigilance threshold (Bégout and Lagardère, 2004) induced by the environment experienced by cultured fish that is strikingly different from that experienced by their wild counterparts *e.g.* the physical environment is much simpler, space is restricted and migration is not possible, it is less challenging in that good quality food is readily available and fishes are protected against predators (Gross, 1998; Price, 1999; Waples, 1999). On the contrary wild fish behaviour could indicate higher vigilance and then uncertainty in a novel environment such as a maze. Other studies on the same species have shown similar mean velocity differences between wild and domesticated fish at an early stage (Benhaïm et al, submitted) and at a later stage (Millot et al., 2009). In the present study, angular velocity was lower in wild fish than domesticated ones while the opposite tendency was observed in the previous cited studies. However, the values recorded in the present work were much lower than those recorded previously. Angular velocity difference between wild and domesticated sea bass has also been shown to be reversed in relation to aging (Benhaïm et al, submitted). Here the swimming activity varied according to the zones occupied by the fish in the maze. The highest angular velocities were recorded in the start-box. This can be explained by the wall removal that induced a stress reaction. Lowest mean velocities were recorded in the zone located either near the congener or at the opposite side. In both cases, the fish remained motionless with its head oriented to the unfamiliar congener located behind the transparent wall.

Fish from both origins spent most of the time in the zone near the congener. This study demonstrated therefore a visual attraction in both wild-caught and domesticated fish induced by an unfamiliar congener of similar size. Placed into a novel and therefore stressful environment, fish from both origins seem to benefit from the presence of an unfamiliar congener which was associated to a decrease of the swimming activity. It could be related to the fact that social or gregarious species may greatly benefit from social interaction. Group behaviour has already been shown to increase growth as a result of social facilitation (Stirling, 1977; Peuhkuri et al., 1995) and to reduce predation risk (Roberts, 1996). The vigilance decreases when neighbour distance decreases because information about whether other group members have detected a predator is easier to obtain from nearer individuals (Pöysä, 1994). Such gregarious behaviour is widespread among fishes, with many species forming non-random shoals according to species, size, parasite load and familiarity (Pitcher and Parrish, 1993; Krause et al., 1996; Hoare et al., 2000; Krause and Ruxton, 2002; Griffiths, 2003; Griffiths and Ward, 2006). The present work showed that gregarious behaviour could also occur very fast even when fish are not with familiar conspecifics. In natural habitats shoals may encounter one another frequently, and field observations suggest that transfer of individuals between shoals may be substantial (Helfman, 1984; Hilborn, 1991; Hoare et al., 2000; Svensson et al., 2000). This reinforces the idea that the decision to shoal or not strongly depends on the context encountered by the fish which is in accordance with Hamilton's theory of the selfish herd (Hamilton, 1971). For example, European minnows *Phoxinus phoxinus* shoal to obtain shelter, but only when there is insufficient physical structure available (Orpwood et al., 2008). Placed in a T-maze without shelter, most of the tested individuals pitched on gregarious behaviour even though the congener was unfamiliar. This could be also linked to the fact that social interaction can play an important and beneficial role in regulating the stress response in cohesive social species (Allen et al., 2009). Domestication did not impact this behaviour. However, some individuals from both origins spent more time on the opposite side of the congener but mostly oriented toward it while a few others spent most of the time in the start-box. Previous studies have shown that in accordance with the observed behaviour, individuals can be divided into coping style categories (Koolhaas et al., 1999; Øverli et al., 2004) or been arranged along a behaviour gradient, such as the bold–shy continuum (Wilson et al., 1993). In the present work, a few individuals went out the start-box and decided thereafter to return to it where they remained till the end of video recording. It could be related to a shy personality trait and/or a subordinate status. Indeed, it is well known that staying alone could be a better strategy for subordinates (Ranta et al., 1993) allowing them to have a lower probability of suffering injury in an escalated contest (Abbot et al., 1985). Fish that spent most of the time on the opposite side of the congener were visually attracted to the unfamiliar conspecific but made the choice to maintain maximum distance to it. Finally, individuals that spent the larger proportion of time near the congener could be considered as bold fish. However, this hypothesis needs to be confirmed by further research designed to test these behavioural tendencies in several contexts to match the definition of personality traits (Sih et al., 2004).

This preliminary experiment provided evidence for a gregarious behaviour in juvenile sea bass *i.e.* attraction to an unfamiliar congener, on the basis of visual cues alone. It also demonstrated individual variability including fish choosing to shelter in the start-box and fish visually attracted to the congener but maintaining maximum distance to it. These findings are in accordance with the life history of sea bass that develop a schooling life style at juvenile stage (Barnabé, 1978). Domestication does not seem to impact this behaviour which is not surprising because farmed fish are often raised under high rearing density all along the production process and submitted to frequent population reorganization *i.e.* pooled with unfamiliar congeners. At the same time, swimming behaviour was impacted by domestication.

These findings may have implications for restocking programs that often fail because of the dramatic level of mortality of newly released individuals (Olla et al., 1998) which are immediately placed in a novel and variable environment and exposed to predation risk (Brown and Laland, 2001). The gregarious behaviour of domesticated fish in our study, similar to those of their wild-caught congeners may facilitate the fish shoaling (with unfamiliar congeners) when released in the wild which is crucial for predator avoidance. At last, these findings could also be useful to design cognition experiments on this species. Indeed, in these experiments, fish were trained to solve different tasks that were rewarded (Huntingford and Wright, 1989; Odling-Smee and Braithwaite, 2003; Warburton, 2007). The use of an unfamiliar fish (on the basis of visual cues only) as a reward in such experiments seems to be particularly well adapted and more relevant than food.

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# Chapitre 9

# Does familiarity matter in wild caught and domesticated sea bass (*Dicentrarchus labrax*) placed in a T-maze?

D. Benhaïm, M-L. Bégout, G. Lucas & B. Chatain

*Soumis*

# Résumé

Les bars domestiqués et ceux capturés dans le milieu naturel décrits dans les chapitres précédents sont à nouveau utilisés dans ce chapitre, mais à un stade de développement ultérieur. En plus de l'activité de nage, le protocole permet ici d'étudier l'exploration des individus et leur capacité à discriminer un congénère familier.

Les caractéristiques comportementales constituent des indicateurs sensibles du processus de domestication car une seule génération suffit généralement à les modifier. Ce travail a comparé l'activité de nage, l'exploration et la capacité à discriminer un congénère familier dans un labyrinthe en T. Dix-huit individus (~ 19 cm) de chaque origine ont été testés individuellement. L'extrémité de chaque bras du labyrinthe est fermée par une paroi transparente contre laquelle est positionnée une enceinte indépendante contenant un congénère familier ou non familier. Après une période d'acclimatation de 5 min, l'individu est libéré de la zone de démarrage et le labyrinthe est filmé durant 15 min. A la fin de l'enregistrement, l'individu est à nouveau placé dans la zone de démarrage pour un second essai après avoir inversé la position des congénères familier et non familier. Différentes variables liées à l'activité de nage ont été mesurées telles que la vitesse angulaire (Vang), la distance totale parcourue (Dtot), la vitesse moyenne (Vel), le temps d'immobilité (Im) ainsi que le temps passé dans chacune des 6 zones virtuelles définies : Zone de démarrage (Start), la zone localisée juste après Start (PostStart), la zone située près du congénère familier (Fam), la zone située près du congénère non familier (UnF), la zone située près de Fam (PreFam) et la zone localisée près de UnF (PreUnF). Aucune différence n'a pu être mise en évidence au niveau de l'activité de nage entre les bars sauvages et domestiqués. Les Vang et Dtot les plus élevées ont été enregistrées dans les zones Start, Fam et UnF tandis que les Vel les plus basses ont été relevées dans les zones Fam et UnF. La préférence pour la Zone Fam n'a pu être montrée que chez les bars domestiqués et en particulier lors du second essai. Ceci démontre la capacité de ces animaux à discriminer la familiarité sur la base de signaux visuels. Chez les animaux des deux origines, il existe cependant une forte variabilité entre les individus et entre les deux essais. Certains individus préfèrent s'associer avec le congénère familier, d'autres avec le congénère non familier et d'autres encore, passent autant de temps dans les zones situées près du congénère familier et du congénère non familier.

En conclusion, plusieurs hypothèses reposant sur les influences combinées du niveau de stress et du statut hiérarchique sont mises en avant pour expliquer les différences observées entre bars sauvages et domestiqués ainsi que la variabilité intra individuelle en ce qui concerne la préférence pour le congénère familier ou non familier.

# Does familiarity matter in wild caught and domesticated sea bass *(Dicentrarchus labrax)* *placed in a T-maze?*

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## Abstract

Behavioural responses are sensitive indicators of the domestication process since they are generally impacted as soon as the first generation. The present work compared wild-caught and domesticated sea bass juveniles swimming activity, exploration and ability to discriminate a familiar congener in a T-maze. Eighteen individuals of similar size (~ 19 cm) from both origins were individually tested. The end of each arm of the maze was closed by a transparent and not removable wall in front of which were placed separate compartments containing either familiar or unfamiliar congeners. After a 5 min acclimatization period, the wall of the start-box was removed and the maze was filmed during 15 min. At the end of the video recording, the individual was placed again in the start box and tested a second time after a 5 min acclimatization period, the position of familiar and unfamiliar congeners being reversed. Different swimming variables including angular velocity (Vang), total distance travelled (Dtot), velocity mean (Vel), time spent in Immobility (Im) were analysed from videos as well as the time spent in each of 6 virtual zones including the start-box zone (Start), the zone located just after Start (PostStart), the zone near the familiar congener (Fam), the zone near the unfamiliar congener (UnF), the zone located near Fam (PreFam), and the zone located near UnF (PreUnF). No swimming behaviour differences were found between wild-caught and domesticated fish. The highest Vang and Dtot were recorded in Start, Fam and UnF. Lowest Vel were also recorded in Fam and UnF. The preference for associating with the familiar was shown only in domesticated fish and especially at trial 2 demonstrating that they were able to discriminate familiarity on the basis of visual cues alone. In fish from both origins there was however a high variability between individuals and trials. Some individuals preferred associating with the familiar congener, others with the unfamiliar congener and finally some individuals spent equal time in Fam and UnF. Various hypotheses relying on the interplay of stress level and hierarchical status are discussed to explain differences between wild and domesticated fish and the variability among individuals for the fish association preference with unfamiliar or familiar congener.

Keywords: domestication, maze, visual cue, swimming activity, shoaling behaviour.

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## 1. Introduction

Studying the differences between wild and cultured-stocks of fish is useful to better understand the domestication process. Indeed, domestication of most cultured fish is so recent that they are little changed from their wild ancestral form (Balon, 2004). Behavioural traits are good indicators of this process because they are likely the first to be affected, sometimes as soon as the first generation (Swain and Riddell, 1990; Ruzzante and Doyle, 1991; Ruzzante and Doyle, 1993; Price, 1999). Among the most studied of all, antipredator behaviour has been shown to be very sensitive to artificial rearing (Johnsson and Abrahams, 1991; Berejikian, 1995; Dellefors and Johnsson, 1995; Johnsson et al., 1996; Einum and Fleming, 1997; Fernö and Järvi, 1998), and swimming performances to be poorer in domestic stocks (Beamish, 1978). These differences between wild and cultured fishes may be partly explained by different previous experiences (Huntingford, 2004) as well as by the rearing conditions themselves.

In the wild, gregarious species form shoals that represent non-random assemblages according to species, size, parasite load and familiarity *i.e.* individuals prefer to join groups with which they had a previous experience (Pitcher and Parrish, 1993; Krause et al., 1996; Hoare et al., 2000; Krause and Ruxton, 2002; Griffiths, 2003; Griffiths and Ward, 2006). Shoaling behaviour in fishes often serves as an important antipredator strategy (Magurran, 1990; Pitcher and Parrish, 1993). The effects of familiarity have been shown to enhance the benefits of grouping and mediates association decisions in shoaling species (Griffiths and Ward, 2007). However, due to the difficulty to monitor the social dynamic and fidelity of fish shoals in the wild, its importance remain unclear (Helfman, 1984; Hilborn, 1991; Hoare et al., 2000; Svensson et al., 2000). Furthermore, the decision to shoal or not strongly depends on the context encountered by the fish *e.g.* European minnows *Phoxinus phoxinus* shoal to obtain shelter, but only when there is insufficient physical structure available (Orpwood et al., 2008). Given this, one could expect that the preference for familiars should increase under a predation threat, but several authors found no significant increase in the preference of fish for familiar individuals on the appearance of a model predator (Griffiths, 1997a; Brown, 2002) suggesting that in habitats where fishes often experience a predation risk, it is adaptive to maintain a consistent preference for familiar congeners.

Cultured fishes are kept in an environment very different from the natural habitats (Fernö et al., 2007). This is primarily a predator-free and foodrich environment where morphological, physiological and behavioural responses to predation seem to be attenuated (Huntingford, 2004). Nevertheless, cultured fish need to adapt to frequent disturbances such as grading procedures which involves unfamiliar fish to be often pooled together to create similar sized groups. Furthermore, because of high fish densities (hundred of fish per $m^3$), individuals are somehow forced to shoal together whatever their familiarity levels and anyway, they cannot recognize all of them because there is an upper limit of around 40 different individuals that a single fish can identify as familiar (Griffiths and Magurran, 1997). These points raise the question of whether the domestication could impact the ability and/or the motivation to recognize one individual from another.

European sea bass (*Dicentrarchus labrax*) is a leading species in Mediterranean aquaculture that was recently domesticated. This explains why very little is known on effects of the early step of domestication or selection for growth apart from classical traits of commercial interest (Dupont-Nivet et al., 2008; Vandeputte et al., 2009) and first attempts made to analyse behavioural responses to challenges in 12-24 months old fish (Millot et al., 2010; Millot et al., 2011). In the wild, at the juvenile stage, sea bass is a demersal and gregarious species (Barnabé, 1989) but little is known about shoal fidelity, dispersal or even site fidelity.

The present work compared wild-caught and domesticated sea bass juveniles swimming activity, exploration in a T-maze designed to determine if individuals prefer associating with familiar congeners.

## 2. Material and methods
### 2.1. Experimental animals and housing conditions

Domestic sea bass were hatched at the farm Aquanord SA (France), transferred on February 23rd, 2009 to the experimental station of INTECHMER (Cherbourg) when they were 3 days old (D3) and grown in a recirculated system. All parameters were set according to the protocol used by Aquanord hatchery except for the temperature that was 15.2±0.5°C. The temperature usually reaches 21°C in a sea bass hatchery but here it was intentionally maintained at a lower level to avoid creating large size differences with the wild stock that was thought to be captured later according to the natural hatching conditions.

Wild sea bass juveniles were captured off the Mediterranean coast of France (Harbour of Cap d'Agde, Southern France, 43° 58' N; 03° 30' 19'' E). A whole school of 560 wild fish observed from the boat was collected at low depth (280 cm). Immediately after capture they were transported to the experimental station (INTECHMER, Cherbourg) where they arrived 24 hours later, on April 15th. For further details, see Benhaïm et al. (submitted a).
Both fish origins (about 400 individuals each) were later grown in open water system in two separate 2m$^3$ tanks until the beginning of this experiment which started on May 5th 2011. At this date, domesticated fish were 793 days old. Wild-caught fish ages were determined on a 30-individual sample at earlier stage (Benhaïm et al., submitted a) and were of similar age as domesticated fish.
Wild and domesticated fish were anesthetized with 2-phenoxyethanol (0.3 ml l$^{-1}$) and based on previous biometry data, 30 individuals from each origin were selected. Total length (mean ± SD) was 19.2 ± 1.1 cm in domesticated fish and 18.8 ± 0.8 cm in wild ones (t-test: t=1.3, P=0.21); weight was 77.7 ± 15.3 g in domesticated fish and 70.8 ± 9.3 g in wild ones (t-test: t=-1.33, P=0.19). Some of the fish (in each tank from both origins) were likely to be already familiar since they were maintained since the beginning in the same tank but the 30 selected individuals were placed in two 200 l tanks during 32 days to optimize the familiarization process between all individuals. The tanks were provided with water from a recirculated system. During this period, light regime was 16:8 LD (light onset at 06:00 U.T. + 1). In both tanks, temperature, salinity and oxygen level were (Mean ± SD), 16.5 ± 0.3°C, 35.0 ± 0.0 g L$^{-1}$, 6.5 ± 0.2 mg L$^{-1}$. Fish were fed manually until satiation three times daily with a commercial diet (Neo Grower Extra Marin, 4 mm, Le Gouessant, France).

### 2.2. Experimental setup

Individuals were tested one by one in a maze constructed from opaque white plastic and transparent Plexiglas® (Fig. 1). The start box (20 x 15 cm) was separated from the rest of the maze by a removable opaque wall. The end of each arm of the maze was closed by a transparent and not removable wall. A separate compartment with one transparent wall was placed in front of the transparent wall of the maze precluding olfactory cues. These compartments, continuously supplied with air (air pump), were used to place either familiar or unfamiliar congeners. The familiarity test was therefore based on optical cues that have been shown to underpin individual recognition in a variety of species (Fricke, 1973; Hert, 1985; Balshine-Earn et al., 1998).
The maze which floor was made of transparent Plexiglas® was placed on an infrared waterproof casing (1 x1 m, Noldus, The Netherland) that enables to record videos at low light

intensity and to improve video analysis. Shortly before observations, the maze was filled with water which level was maintained at 14.5 cm. Temperature, salinity and oxygen level were verified before and after the end of observations performed on each fish and were respectively 17.4 ± 0.5°C, 35.0 ± 0.0 g $l^{-1}$, 6.6 ± 1.3 mg $l^{-1}$ before, 17.4 ± 0.3°C, 35.0 ± 0.0 g $l^{-1}$, 6.6 ± 1.2 mg $l^{-1}$ after. Digital camera (Imaging Source DMK 21AUO4) with a frame rate of 30 Hz and a resolution of 640 x 480 pixels was positioned at 99 cm above the water surface. Two 60 W light bulbs were horizontally placed on walls located on the left and right sides of the infrared casing. They were located 100 cm above the infrared casing and provided an indirect and homogenous lighting on the maze. The light intensity measured at the water surface of the maze was 100 Lux.

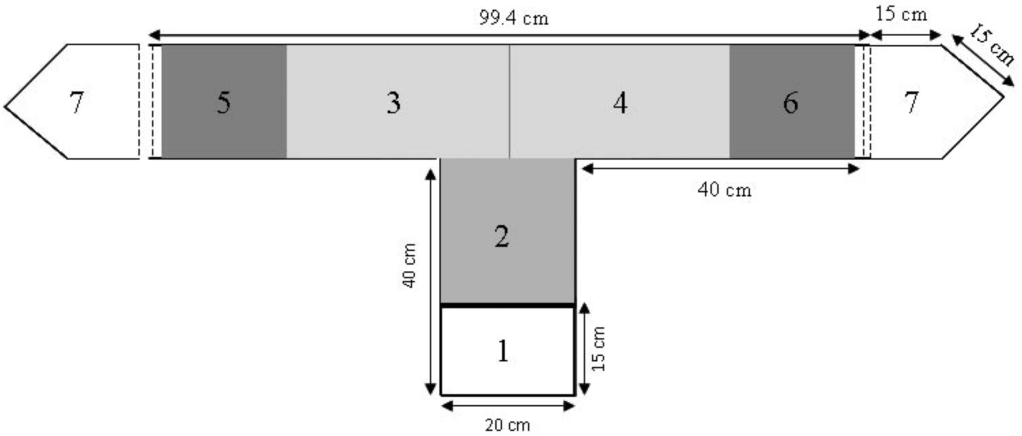

***Fig.1.*** *Schematic representation of the T-maze apparatus and virtual zones delimitation on the bottom of the maze defined for the video recordings analysis.*

*Dotted lines are transparent Plexiglas® walls, continous lines are white opaque plastic. The bottom of the maze is made of transparent Plexiglas®.*

*1.Start-box closed by a removable opaque wall; 2. Post-Start; 3. Pre-left: area located on the right side of the left area; 4. Pre-right: area located on the left side of the right area; 5. left area located near the compartment where congener (familiar or unfamiliar) was placed; 6. right area located near the compartment where congener was placed (familiar or unfamiliar); 7. left and right separate compartments closed by a transparent Plexiglas® wall where congeners (familiar or unfamiliar) were placed.*

## 2.3. Experimental protocol

Before the beginning of observations, the position of both unfamiliar and familiar congeners was randomly determined for each individual on the left or right arm of the maze. The first tested fish was gently collected from the tank using a net and immediately placed inside a bucket closed by a cover and then placed in the maze start-box. After a 5 min acclimatization period, the wall was removed and the video capture started. The maze was filmed during 15 min. At the end of the video recording, the individual was placed again in the start box and tested a second time after a 5 min acclimatization period, the position of familiar and unfamiliar congeners being reversed between trials. In total, 18 fish were tested in each category, other fish being used as familiar or unfamiliar congeners. In order to test all individuals, 2 days were required. On the first day, 9 domesticated fish were consecutively tested in the morning and 9 wild fish were tested in the afternoon. On the second day, this protocol was reversed. The water was entirely renewed between wild and domesticated individual batches. The unfamiliar and familiar fish used as the attractors were changed every hour to minimize stress due to confinement and handling. Before being returned to their initial tank (2 $m^3$), fish were anesthetized to be measured and weighted.

## 2.4. Video analyses and behavioural variables

The video recordings were analysed using the software EthoVision XT 8 (Noldus, The Netherland), which allowed six virtual zones to be defined in the maze (Fig. 1) and to track the fish swimming behaviour.

Different variables of interest were chosen to analyse the fish behaviour:

-The time spent in each zone expressed in seconds (s): Start-Box (Start), Post-Start (PostStart), zone near the familiar congener (Fam), zone near the unfamiliar congener (UnF), zone located near Fam (PreFam), zone located near UnF (PreUnF).
-The distance travelled by each fish in the maze (Dtot in mm)
-The fish absolute angular velocity expressed in degree per second (Vang in ° $s^{-1}$) was calculated by the software as followed:
$Vang_n = RTA_n / t_n - t_{n-1}$ where $RTA_n$ is the relative turn angle for sample n and $t_n - t_{n-1}$ , the time difference between the current and previous sample. Here the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for head direction. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity.
-The velocity mean expressed in body length per second (Vel in BL $s^{-1}$)
-The time spent in immobility (Im in s)
The last four variables quantified the fish swimming activity level in the maze for each sequence.

## 2.6. Statistical analysis

All variables were compared using parametric analysis of variances (ANOVA) after verification of distribution normality and homoscedasticity (Dagnélie, 1975). Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls). All statistical analyses were conducted using Statistica 8 (Statsoft, USA), and for all tests, the significant threshold was $p < 0.05$.
All variables were compared using a repeated measures analysis of variance with Origin (Wild and domesticated fish) and Zone (6 zones) as between-subjects factor and Trial (2 trials) as within-subjects factor.
Principal components analysis was used to investigate for individual variability in zone exploration among wild and domesticated fish. Two separate analyses were performed at trials 1 and 2.

## 3. Results

### 3.1. Spatial distribution

All the fish tested left the start box after the wall was removed *i.e.,* none of the fish spent 100% of the time in Start or in PostStart zones. Wild-caught and domesticated fish spent most of the time in UnF and Fam (mean ± SE, 42.9 ± 10.6% and 35.5 ± 9.7%; 26.2 ± 8.8% and 40.7 ± 9.1 % respectively, Fig. 2). There was only a Zone significant effect ($F_{(10, 406)}$= 11.2, P<0.001) with the time spent in Fam and UnF at trial 1 being higher than in all other zones (P<0.05 for each pairwise comparisons); the time spent in Fam at trial 2 being higher than in all other zones and time spent in UnF being lower than in Fam but higher than in all other zones (P<0.05 for each pairwise comparisons). The result recorded at trial 2 was due to the strong increase of the time spent by domesticated fish in Fam and the correlated decrease in UnF (33.3 ± 8.1 to 48.1 ± 10.2% and 32.6 ± 8.8% to 19.8 ± 11.0% respectively, Fig. 2). The

Zone * Origin effect was not significant but when looking at the Newman-Keuls post-hoc test at trial 2, the time spent by domesticated fish in Fam was significantly higher than in UnF ($P<0.05$) and time spent by wild fish in Fam is significantly lower than in UnF ($P<0.05$).

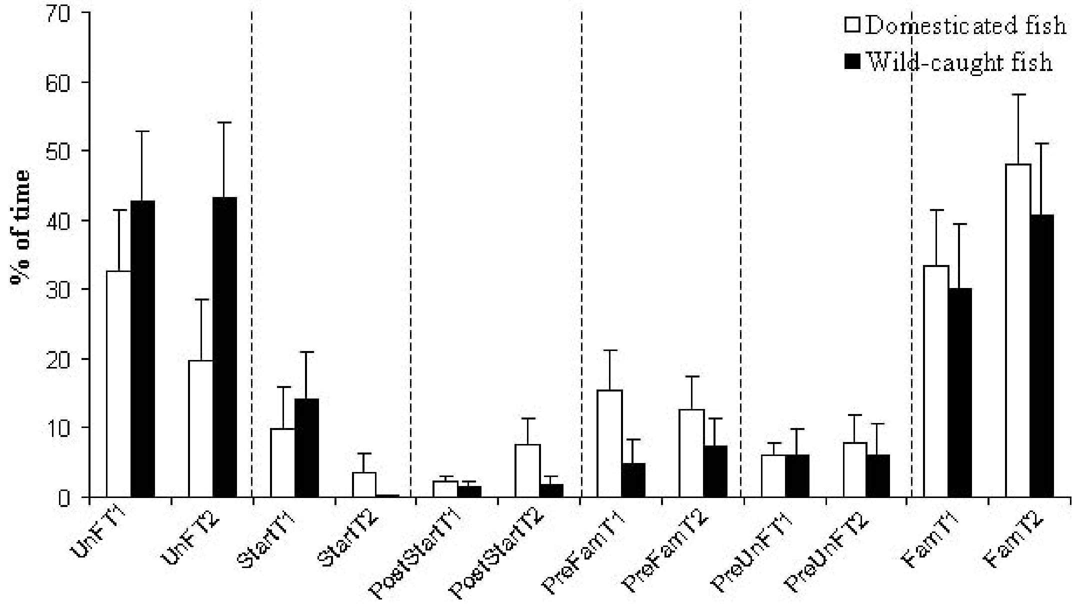

***Fig. 2.*** *Proportion of time spent (mean  S.E., in %) by a fish in each zone of the maze.*
*T1: trial 1, T2: trial 2.*
*Start: Start-Box, PostStart: zone located just after Start, Fam: zone near the familiar congener, UnF: zone near the unfamiliar congener, PreFam: zone located just before Fam, PreUnF: zone located just before UnF.*

### 3.2. Individual variability

At trial 1, PCA showed that the first two components accounted for 59% of the variability of the data (Fig. 3a). The first component was related to two variables: the negative pole to high values of Fam (contribution to the first component was 0.39 and the correlation was -0.82), the positive one to high values of UnF (contribution to the first component was 0.49 and the correlation was 0.91). Domesticated and wild-caught fish were distributed as follows: 44 and 28% of individuals characterized by high values of Fam, 33 and 50% by high values of UnF, 11 and 0% by equal values for Fam and UnF, 11 and 22% by high values of Start (Fig. 3a).

At trial 2, PCA showed that the first two components accounted for 61% of the variability of the data (Fig. 3b). The first component was related to two variables: the negative pole to high values of UnF (contribution to the first component was 0.46 and the correlation was -0.90), the positive one to high values of Fam (contribution to the first component was 0.47 and the correlation was 0.91). Domesticated and wild-caught fish were distributed as follows: 56 and 39% of individuals characterized by high values of Fam, 17 and 33% by high values of UnF, 11 and 17% by equal values for Fam and UnF, 11 and 6% by high values of PreUnF, 6 and 6% by high values of PreFam (Fig. 3b).

When comparing individuals from trial 1 to trial 2, 28% of domesticated fish were characterized by high values of Fam at trials 1 and 2 but only 11% of wild fish; 11% of fish from both origins showed high values of UnF at trials 1 and 2; 17% of wild fish showed high values of Fam at trial 1 followed by high values of UnF at trial 2 while it was only 6% of domesticated fish; 22% of wild fish showed high values of UnF at trial 1 followed by high values of Fam at trial 2 while it was only 11% of domesticated fish.

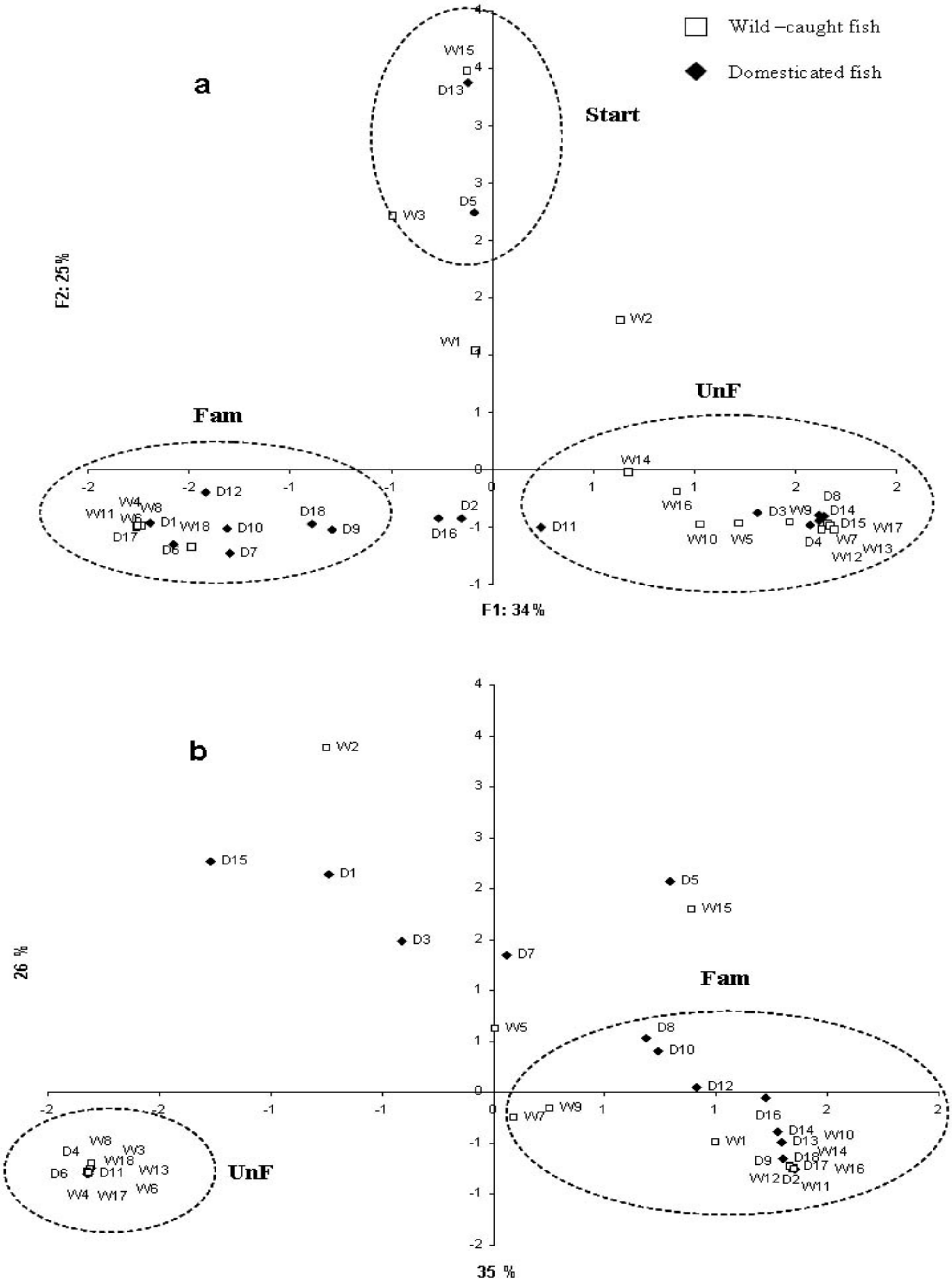

*Fig. 3*. *Principal component analysis (PCA) applied to Zones explored in the maze.*
*(a): at trial 1, (b): at trial 2.*
*W: wild fish; D: domesticated fish; Start: Start Box ; Fam: zone near the familiar, Unfam: zone near the unfamiliar congener.*

### 3.3. Swimming activity

There were no significant differences between wild-caught and domesticated fish for any swimming variables but a significant zone effect for all of them (Dtot: $F_{(10,238)}$= 6.5, P<0.001; Im: $F_{(10,234)}$= 14.1, P<0.001; Vang: $F_{(10,238)}$= 5.1, P<0.001; Vel: $F_{(10,238)}$= 5.7, P<0.001).
Dtot was higher in Fam and UnF than in all other zones at trials 1 and 2 (mean ± SE, 449 ± 88 and 370 ± 59 cm respectively, P<0.05 for each pairwise comparison, Fig. 4a).
Vang was higher in Fam, UnF and Start than in all other zones at trials 1 and 2 (Mean ± SE, 446 ± 46, 417 ± 41, 361 ± 48 ° $s^{-1}$ respectively, P<0.05 for each pairwise comparison, Fig. 4 b).

At trials 1 and 2, Vel was lower in Fam and UnF than in all other zones (P<0.05 for each pairwise comparison, Fig. 4c).

At trial 1, Im was higher in UnF than in all other zones (mean ± SE, 456 ± 96 s, P<0.05 for each pairwise comparison, Fig. 4d), lower in Fam (mean ± SE, 381 ± 84 s than in UnF, P<0.05) but higher than in all other zones (P<0.05 for each pairwise comparison). At trial 2, Im was higher in Fam than in all other zones (mean ± SE, 622± 70 s, P<0.05 for each pairwise comparison), lower in UnF than in Fam (mean ± SE, 475 ± 89 s, P<0.05) but higher than in all other zones (P<0.05 for each pairwise comparison).

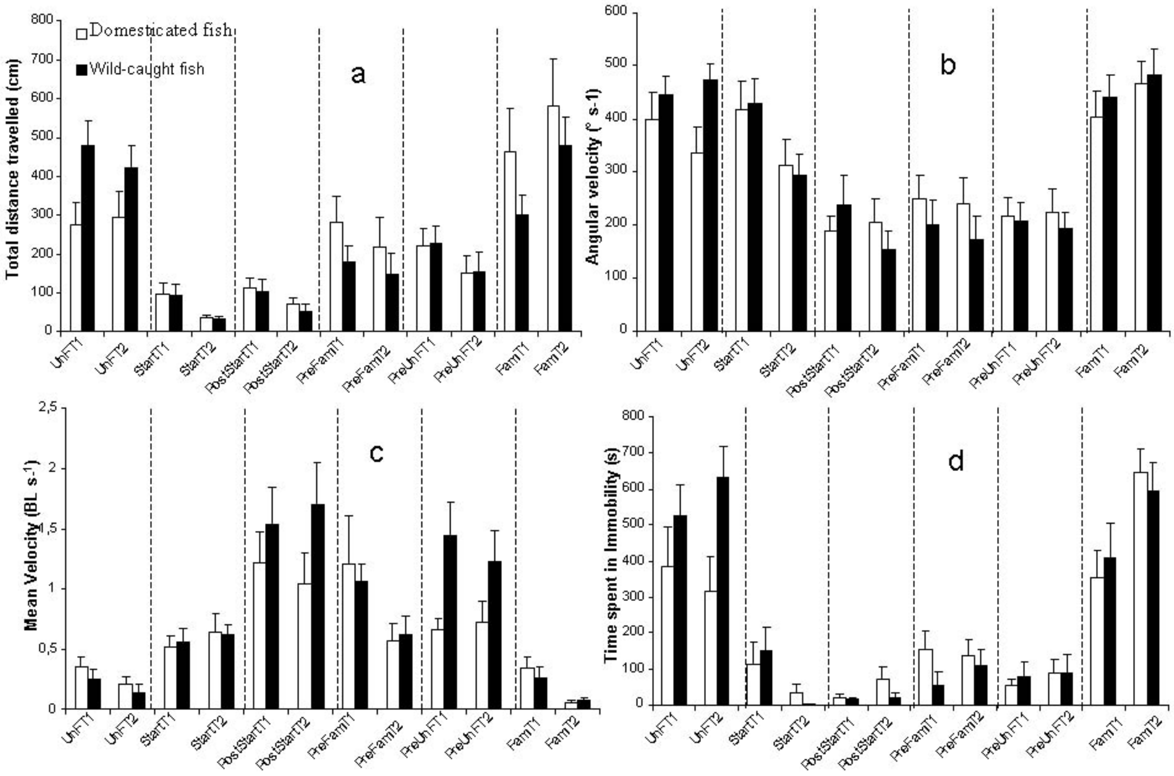

***Fig. 4**. Mean ± SE. Behavioural variables in domesticated vs. wild-caught fish in each virtual zone defined for the video recordings analysis.*

*T1: trial 1, T2: Trial 2.*

*Start: Start-Box, PostStart: zone located just after Start, Fam: zone near the familiar congener, UnF: zone near the unfamiliar congener, PreFam: zone located just before Fam, PreUnF: zone located just before UnF.*

## 4. Discussion

The aim of this study was to assess for domestication effect on juvenile sea bass swimming activity, exploration and visual attraction to unfamiliar *vs.* familiar congeners in a T-maze. The results showed a similar swimming activity in both fish origins. Domesticated fish showed preference for associating with familiar congeners while wild-caught fish spent similar time in the zones near the familiar and the unfamiliar congeners. There was however a high variability among both fish origins and between trials.

No swimming characteristics differences were found between wild-caught and domesticated fish of similar size and age *i.e.* angular velocity, mean velocity, distance total travelled and immobility were similar in both fish origins which is not in accordance with previous studies performed on sea bass in same conditions (Benhaïm et al., submitted b). However, in the present study, fish were 793 days old which was 330 days older than previous study. This could indicate that a 738-day period of rearing is sufficient to obtain wild-caught fish presenting the same swimming characteristics than domesticated fish. Such tendency has already been observed in previous study comparing exploration and swimming activity in wild and domesticated sea bass strains of similar age submitted to an acute stress (600 days, Millot et al., 2009).

Here the swimming activity varied according to the zones occupied by the fish in the maze. The highest angular velocities were recorded in the start-box and in the zones located near unfamiliar and familiar congeners. The value recorded in the start-box can be explained by the wall removal that induced a stress reaction. The values recorded in the zones near both unfamiliar and familiar congeners were in relation with the visual interaction between the fish separated by the transparent wall. It has been already shown that visual information alone can change behaviour (Chen and Fernald, 2011). Lowest mean velocities were also recorded in the zones near both unfamiliar and familiar congeners. In both cases, the fish remained motionless with its head oriented to the unfamiliar congener located behind the transparent wall but in the same time since it spent most of the time in these zones, it is also where the fish travelled the greatest distance.

Fish from both origins spent most of the time in the zones near the familiar and unfamiliar congeners. The preference for associating with the familiar was shown only in domesticated fish and especially at trial 2. This result is in accordance with previous studies performed on European Minnow *Phoxinus phoxinus* (Griffiths, 1997b), juveniles bluegill sunfish *Lepomis macrochirus* (Brown and Colgan, 1986), rainbowfish *Melanotaenia* spp. (Brown, 2002), guppy *Poecilia reticulata* (Magurran *et al.*, 1994; Griffiths and Magurran, 1997), paradise fish *Macropodus opercularis* (L.) (Miklósi *et al.*, 1992), fathead minnow *Pimephales promelas* (Chivers *et al.*, 1995) and on domesticated sea bass juveniles (Di-Poï, 2008). Our study bring forward evidence that domesticated sea bass are able to discriminate familiarity on the basis of visual cues alone as already shown in the three-spined sticklebacks *Gasterosteus aculeatus* (Waas and Colgan, 1994). There was however a high variability between individuals and trials. In both fish origins, there were fish that prefer associating with the familiar congener, others with the unfamiliar congener and also individuals that spent equal time in familiar and unfamiliar zones. A higher proportion of domesticated fish preferred associating with the familiar congener at trials 1 and 2 while a higher proportion of wild fish preferred associating with the unfamiliar congener at trials 1 and 2. At last, a higher proportion of wild fish changed from unfamiliar at trial 1 to familiar congener at trial 2. Several hypotheses can be put forward to explain the results observed on wild-caught sea bass which are not in accordance with previous study performed on wild-caught and captive reared crimson spotted rainbowfish *Melanotaenia duboulayi* (Kydd and Brown, 2009). Indeed, these authors found that captive-reared rainbowfish showed no preference for familiar individuals while wild fish spent significantly more time shoaling with familiar individuals which could be the result of relaxed selection and inbreeding in the captive environment. First, even though little is known about dispersal, shoal site fidelity and shoal fidelity in the wild about sea bass and rainbowfish, it seems that environmental and biological conditions could rather favour the development of familiarity in the latter species. It is actually known that eggs hatched by rainbowfish females have two sticky web-like threads that prevent them from being displaced by current and that fry typically remain in this freshwater microhabitat. It is therefore likely that emerging shoals of adults could show some degree of relatedness (Arnold, 2000; Pusey *et al.*, 2001). On the contrary, sea bass pelagic eggs are largely dispersed by estuarine currents and individuals are gregarious especially at the juvenile stage (Barnabé, 1978). Another hypothesis is that, in the wild, shoals are generally composed of a large number of individuals while lab studies have shown that there is an upper limit of around 40 different individuals that a single fish can identify as familiar (Griffiths and Magurran, 1997). Other mechanisms apart from familiarity are indeed important in determining shoaling preferences *e.g.* ecological conditions such as physical structure availability (Orpwood et al., 2008) and social dynamics of the species such as the social organization difference between female and male guppies (Croft et al., 2003). Our study could indicate that familiarity does not play a major role in the wild sea bass shoal formation. Another explanation could be a greater fear response and/or caution in wild fish as already described in numerous studies on different species (Nordeide and Svasand, 1990; Johnsson and Abrahams, 1991; Einum and Fleming, 1997; Fernö and Järvi, 1998; Arai et al., 2007) but also on sea bass juveniles coming from the same population tested in our study at earlier stage (Benhaïm et al., submitted a). Indeed, the maze is a novel and therefore stressful environment in which wild fish could associate with the first encountered congener whatever its familiarity level with it. This hypothesis is reinforced by the results of a previous study showing that sea bass juveniles from both origins made the choice to shoal with an unfamiliar congener when placed in a T-maze presenting an unfamiliar congener and an empty compartment (Benhaïm et al., submitted b). In the wild, the priority for many fish species is to reduce predation risk using a shoaling strategy (Roberts, 1996) and field observations suggest that transfer of individuals between shoals (*i.e.* unfamiliar congeners) may be substantial (Helfman, 1984; Hilborn, 1991; Hoare et al., 2000; Svensson et al., 2000).

At last, the strong individual variability in fish from both origins could refer to dominance-subordinance relationships established during the 32-day familiarization period. The formation of dominance hierarchies is known to especially occur in small groups of fish (Adams et al., 1995) which was the case in our study during the familiarization period (groups were composed with 30 individuals). The proportion of individuals that preferred associating with unfamiliar congeners at trials 1 and 2 could be subordinates that were actually able to discriminate the familiar congener but preferred avoiding it. Indeed, familiarity is also known to stabilize dominance hierarchies (Brick, 1998).

This first experiment provided evidence for the ability of domesticated sea bass to discriminate familiar congeners on the basis of visual cues alone. It also showed an unexpected difference between wild-caught and domesticated fish. The hypothesis of higher stress that could underpin the results recorded in wild fish needs to be verified using physiological indicators since there were no obvious swimming behaviour differences between both fish origins. This study also showed that in a context such as a maze *i.e.* novel and therefore stressful environment especially in isolated conditions, familiarity could not be always the main factor driving the choice of the fish. Further research is needed to better understand the interplay of stress level and hierarchical status in the fish association preference with unfamiliar or familiar congeners.

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# Chapitre 10

# Exploration and cognition in wild caught and domesticated sea bass (*Dicentrarchus labrax*) in a maze

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*Soumis*

# Résumé

Les bars domestiqués et ceux capturés dans le milieu naturel décrits dans les chapitres précédents sont à nouveau utilisés dans ce chapitre. En plus de l'activité de nage, le protocole permet ici d'étudier l'exploration des individus et leurs capacités cognitives.

Ce travail a comparé l'activité de nage, l'exploration et la capacité de discrimination entre deux objets en 2 dimensions associés à une récompense (contact visuel avec un congénère non familier) de bars sauvages capturés dans le milieu naturel et de bars domestiqués testés individuellement en labyrinthe. Dix individus de chaque catégorie ont été testés trois fois de suite et durant 3 jours (9 essais au total) avec une période d'acclimatation de 5 min entre chaque essai. Les individus ont été placés dans la zone de démarrage fermée par une paroi transparente positionnée en face de deux objets en deux dimensions et dont la position a été fixée de façon aléatoire à droite ou à gauche selon l'essai considéré. Les individus ont été filmés durant 10 min après le retrait de la paroi fermant la zone de démarrage. Différentes variables associées à l'activité de nage dont la vitesse angulaire (Vang), la distance totale parcourue (Dtot) et la vitesse moyenne, ont été mesurées ainsi que le temps passé dans chacune des 6 zones virtuelles définies : Zone de démarrage (Start), la zone localisée juste après Start (PostStart), la zone située près du congénère non familier (ZCong), la zone située à l'opposé de ZCong (OpCong), la zone située près de ZCong (PreCong) et la zone localisée près de Opcong (PreOpcong). Plusieurs critères d'apprentissage ont été choisis : le nombre de virages réussis (premier virage effectué en direction de ZCong), le temps de latence pour rejoindre ZCong, le nombre de fois où l'individu testé est orienté vers l'objet auquel il est associé avant le retrait de la paroi transparente fermant la zone de démarrage. Des différences comportementales ont été mises en évidence entre les bars domestiqués et sauvages. Vang est plus élevée chez les bars sauvages alors que Dtot et Vel sont plus élevées chez les bars domestiqués. Les individus sauvages et domestiqués passent la plus forte proportion de leur temps dans ZCong et OpCong. Aucune différence entre les deux catégories de bars n'existe au niveau des capacités d'apprentissage, mais il existe cependant des différences individuelles marquées.

Cette étude comparative entre deux populations démontre donc un impact de la domestication sur l'activité de nage, mais pas sur les capacités d'apprentissage spatial chez le bar.

# Exploration and cognition in wild caught and domesticated sea bass (*Dicentrarchus labrax*) in a maze

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## Abstract

European sea bass aquaculture is so recent that very little is known on the effects of the early step of its domestication. Behavioural parameters are sensitive indicators of the domestication process since they are generally impacted as soon as the first generation. The present work compared wild-caught and domesticated sea bass juveniles swimming activity, exploration and ability to learn discriminating between two 2-D objects associated to a simple spatial task that enabled the tested individual to visually interact with an unfamiliar congener (the reward) located behind a transparent wall at the end of one of the two arms of a maze. Ten fish from each origin were individually tested 3 times in a row during 3 days (9 trials in total) with a 5min acclimatization period between each trial. Fish were placed in a start box closed by a transparent wall located in front of two 2-D objects which position was randomly assigned on left or right side. Each fish was also randomly associated to one of the two 2-D objects. Fish were filmed during 10 min after the removal of the start box wall. Different swimming variables including angular velocity (Vang), total distance travelled (Dtot), velocity mean (Vel), were analysed from videos as well as the time spent in each of 6 virtual zones including the reward zone near the congener (Cong) and the zone opposite to the reward zone (OpCong). Several learning criteria were chosen: the number of successful turnings, the latency time to reach Cong, the number of times the fish was oriented toward the 2-D object before the wall removal. Behavioural differences were found between domesticated and wild fish. Angular velocity was higher in wild fish while the distance travelled and the velocity mean were higher in domesticated ones. Wild and domesticated fish spent most of the time in Cong and in OpCong. No differences were reported in learning abilities between wild and domesticated fish. However, intra-individual differences existed. This study demonstrated therefore an impact of domestication on swimming behaviour but not on spatial learning.

Keywords: domestication, learning, maze, spatial orientation, visual cue.

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# 1.Introduction

Most cultured fish have been domesticated since the beginning of the twentieth century (Duarte et al. 2007) and the world aquaculture production of fish relies only on a few species that have been mostly selected upon economic and technical criteria (Fontaine et al. 2003). This process is actually so recent that most cultured fishes might be rather considered as exploited captives and only a few of them would be on the threshold of becoming domesticated (Balon 2004). This statement relies on the fact that farmed fishes are little changed from their wild ancestral form and could usually be returned to the wild. However, consistent differences between wild and domesticated fish have been reviewed, the main effects being an increase in growth, perturbations of reproduction (Gross 1998; Ruzzante 1994; Vandeputte and Prunet 2002) and behavioural traits alteration (Price 1999; Ruzzante and Doyle 1991; Ruzzante and Doyle 1993; Swain and Riddell 1990). Among the latter, antipredator behaviour has been shown to be very sensitive to artificial rearing (Berejikian 1995; Dellefors and Johnsson 1995; Einum and Fleming 1997; Fernö and Järvi 1998; Johnsson and Abrahams 1991; Johnsson et al. 2001; Johnsson et al. 1996) and swimming performances to be poorer in domestic stocks (Beamish 1978). These differences between wild and cultured fishes may be partly explained by different previous experiences (Huntingford 2004). Aquaculture and natural habitats are obviously very different. Farmed fishes face conditions that seem to be less challenging than natural habitats e.g. structurally simple environments, food easy to catch and absence of predators but they also have to adapt to high densities, restricted space, artificial and uniform food, quite frequent handling (Fernö et al. 2007). This raises the question of whether domestication could have an impact on fish cognition. Indeed, it is known that domestication influences brain size, since hatchery-reared, domesticated rainbow trout *Oncorhynchus mykiss* have smaller brains than do wild-caught fish of the same size (Marchetti and Nevitt 2003) and even first generation lab-reared guppies *Poecilia reticulata* can have smaller brains than wild counterparts (Burns et al. 2009).

Cognition includes perception, attention, memory formation and executive functions related to information processing such as learning and problem solving (Brown et al. 2007). Study of animal cognition has been largely centred on birds and mammals but over the last decades, it has been shown that fishes like the rest of vertebrates exhibit a rich array of sophisticated behaviours and that learning plays a pivotal role in the behavioural development of fishes (Brown et al. 2007). Several studies have shown that they have long term memories (Brown and Laland 2001; Warburton 2007) and that their cognitive capacity in many domains is comparable with that of non-human primates (Odling-Smee and Braithwaite 2003). In particular, studies report that fishes use systematic exploration to extract spatial information in unfamiliar environments e.g. they use organized pattern of exploration when introduced into a novel environment, avoiding previously visited locations (Kleerekoper et al. 1974) and increase exploratory activity to environmental modifications (Russell 1967).

Several fish species have been successfully trained to use landmark informations to solve a range of spatial tasks (Huntingford and Wright 1989; Odling-Smee and Braithwaite 2003; Warburton 2007). Tasks are generally food rewarded to reinforce the learning process but shelter use and shoaling behaviour that are particularly important (*i.e.* correlated to survival time, Mathis and Smith 1993) can also be used. Visual orientation is known to be strongly involved in the development of cognitive skills (spatial learning and problem solving) in well –structured habitats (Brown and Braithwaite 2005; Kotrschal et al. 1998). More recently, it has been demonstrated that reef fish such as Ambon damselfish *Pomacentrus amboinensis*, can discriminate between a range of visual stimuli including simple shapes drawn on a flat surface and that the choice of reward stimulus is unimportant as it can be learned (Siebeck et al. 2009).

European sea bass (*Dicentrarchus labrax*) is a leading species of Mediterranean aquaculture that was recently domesticated. This explains why very little is known on effects of the early step of domestication or selection for growth apart from classical traits of commercial interest (Dupont-Nivet et al. 2008; Vandeputte et al. 2009) and personality traits differences between wild and selected fish (Millot et al. 2009b).This species has indeed demonstrated a great ability and high plasticity to learn to press a lever to receive a food reward (Covès et al. 2006; Covès et al. 1998; Di-Poï et al. 2007; Millot et al. 2009a; Millot et al. 2008; Anthouard et al. 1993). Under other experimental conditions *i.e.* response to acute stress, exploration and swimming activities have been compared in wild, domesticated and selected strains (Millot et al. 2009a). To the best of our knowledge, there are however no studies on sea bass focusing on cognition, and the impact of domestication on cognition.

The present work compared wild-caught and domesticated sea bass juveniles swimming activity, exploration and ability to learn discriminating between two 2-D objects associated to a reward (visual contact with an unfamiliar congener) in a maze. This reward allowed precluding olfactory cues associated to food reward. Further, in the wild, at the juvenile stage, sea bass are demersal and gregarious species able to orientate in relation to the benthic substrate preferentially composed of rocks (Barnabé 1989) and have a very well developed visual sense that they specially use for very active predation (Langridge 2009). Finally, demersal fishes often stay within a certain home range (Matthews 1990) and may therefore be assumed to rely on learned spatial cues (Reese 1989). The choice for an unfamiliar fish rather than a familiar one was driven by several reasons. First, two unfamiliar sea bass separated by a transparent barrier allowing only for visual contact have already been shown to spend most of their time in the zone nearest to this latter (Di-Poï 2008). Secondly, in this case, compare to chemical stimuli or multimodal combination of both visual and chemical stimuli, the visual stimulus only, seemed to modulate differently the behaviour of fish which showed an increased interest in the presence of the congener on the other side (Di-Poï 2008). Finally, it allowed using a similar stimulus *i.e.* unfamiliar fish for both wild and domesticated fish.

Several questions were addressed by the present study:

(i) Are there differences in exploration and swimming activities between wild-caught and domesticated fish in a maze?

(ii) Are sea bass juveniles able to associate 2-D objects to a reward?

(iii) Does domestication have an impact on cognition?

## 2. Material and methods

### 2.1. Experimental animals and housing conditions

Domesticated sea bass were hatched at a farm Aquanord SA (France), transferred on February 23rd, 2009 to the experimental station of INTECHMER (Cherbourg) when they were 3 days old (D3) and grown in a recirculated system. All parameters were set according to the protocol used by Aquanord hatchery except for the temperature that was 15.2±0.5°C. The temperature usually reaches 21°C in a sea bass hatchery but here it was intentionally maintained lower to avoid creating large size differences with the wild stock that was thought to be captured later according to the natural hatching conditions.

Wild sea bass juveniles were captured off the Mediterranean coast of France (Harbour of Cap d'Agde, Southern France, 43° 58' N; 03° 30' 19'' E). A whole school of 560 wild fish observed from the boat was collected at low depth (280 cm). Immediately after capture they were transported to the experimental station (INTECHMER, Cherbourg) where they arrived 24 hours later, on April 15th. For further details, see Benhaïm et al. (submitted).

Both fish origins (about 400 individuals each) were later grown in open water system in two separate 2 $m^3$ tanks until the beginning of this experiment which started on July 28th 2010. At this date, domesticated fish were 463 days old. Wild-caught fish ages were determined on a 30-individual sample (see Benhaïm et al., submitted) and were of similar age as domesticated fish. During the experimental period, light regime was 16:8 LD (light onset at 06:00 U.T. + 1). In both tanks, temperature, salinity and oxygen level were (Mean±SD), 18.1±0.2°C, 35.0±0.0 g $L^{-1}$, 5.5±0.2 mg $L^{-1}$ respectively.

Two days before the beginning of observations, wild and domesticated fish were anesthetized with 2-phenoxyethanol (0.3 ml $l^{-1}$) and based on previous biometry data, 10 individuals from each origin were selected. Total length was 14.2±0.1 cm in domesticated fish and 14.2±0.4 cm in wild-caught ones (t-test: $t = 0$, $P = 1$); weight was 33.5±1.9 g in domesticated fish and 33.9±1.0 g in wild ones (t-test: $t = -0.53$, $P = 0.6$). Wild-caught and domesticated fish were individually placed in two 200 l tanks divided into 10 numbered compartments. Additionally, a stock of 10 domesticated fish of similar size were selected from another tank *i.e.* fish not familiar with tested domesticated fish, and placed in a third 200 l tank. These fish were used as congener reward in the experiment. The three tanks were supplied with water of identical characteristics as original tanks.

### 2.2. Experimental setup

Observations were made in a dedicated room. Fish were individually tested in a maze constructed from opaque white plastic and transparent Plexiglas® (Fig. 1A).

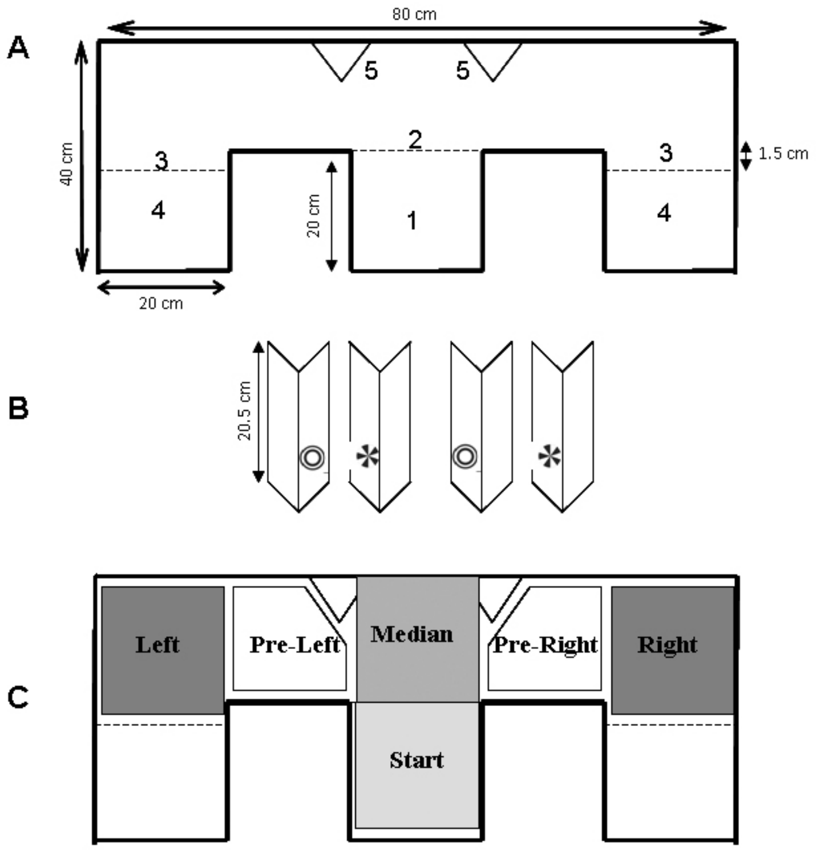

***Fig. 1.*** *A. Schematic representation of the T-maze apparatus. Dotted lines are transparent Plexiglas walls, continous lines are white opaque plastic. The bottom of the maze is made of transparent Plexiglas.*

*1.Start box; 2. removable transparent plexiglass wall; 3. not removable transparent Plexiglas walls; 4. compartment strictly waterproof where congeners were placed. 5. white plastic support for laminated printouts 2-D objects.*

*B. Detail of the 4 supports and the 2-D objects.*

*C. Virtual zones delimitation on the bottom of the maze defined for the video recordings analysis.*

The start box was a 20 x 20 cm square separated from the rest of the maze by a removable transparent wall. At the end of each arm of the maze, two strictly waterproof compartments (20 x 18.5 cm) were also separated by a no removable transparent wall. These compartments were designed 1.5 cm shorter than the start box to ensure the tested fish was not able to see the reward before turning on left or right side of the maze. The maze which floor was made of transparent Plexiglas was placed on an infrared waterproof casing (1 x 1 m, Noldus, The Netherland) that enables to record videos at low light intensity and to improve video analysis. Four white plastic supports were used to show two different laminated printouts 2-D objects either on left or right side of the fish (Fig. 1B). Both objects (equal black and white area) were already successfully tested in previous experiment (Snekser et al. 2009). Shortly before observations, the maze was filled with water which level was maintained at 12 cm. Temperature, salinity and oxygen level were verified before and after the end of observations performed on each fish and were respectively 17.5±0.5°C, 35.0±0.0 g $L^{-1}$, 7.8± 1.5 mg $L^{-1}$ before, 17.5±0.5°C, 35.0±0.0 g $L^{-1}$, 7.5± 1.4 mg $L^{-1}$ after. Digital camera (Imaging Source DMK 21AUO4) with a frame rate of 30 Hz and a resolution of 640 x 480 pixels was positioned at 42.5 cm above the water surface. Two 60 W light bulbs were horizontally placed on walls located on the left and right sides of the infrared casing. They were located 100 cm above the infrared casing and provided an indirect and homogenous lighting on the maze. The light intensity measured at the water surface of the maze was 100 Lux.

## 2.3. Experimental protocol

Before the beginning of observations, all individuals were randomly associated to one of the two 2-D objects. The positions of the supports showing the 2-D objects were also randomly placed on left or right side of the maze arms for each individual and each trial. The reward (non-familiar fish) was placed according to the 2-D object position corresponding to the tested individual. The only constraint on the randomisation process was that the stimulus never appeared in the same position more than twice in a row. These objects were the most salient and detectable visual cues in the maze environment. The first tested fish was gently collected from the tank using a net and immediately placed inside a bucket closed by a cover then placed in the maze start box. After a 5min acclimatization period, the transparent wall was removed and the video capture started. The maze was filmed during 10 min. At the end of the video recording, the individual was placed again in the start box and tested a second and a third time after a 5 min acclimatization period. In total, each individual was tested 3 consecutive times in a day, this procedure being repeated for 3 consecutive days. At the end of the 3 consecutive trials, individuals were returned to their holding tank compartments. In order to test all individuals, six days were required (two pools of 5 wild *vs.* 5 domesticated fish). The water was entirely renewed after each individual was tested and the non-familiar fish used as the reward was changed every hour to minimize stress due to confinement and handling.

## 2.4. Video analyses

The video recordings were analysed using the software EthoVision XT 5 (Noldus, The Netherlands), which allowed six virtual zones to be defined in the maze (Fig. 1C) and to track the fish swimming behaviour.

Each video was also viewed to report several learning criteria: first turning of the fish (left or right-turn) leading to the reward zone (success) or the opposite zone (failure) (Fig. 1C), latency time to reach the reward zone and/or the opposite zone, the last place of the fish just before the end of the video recording, the fish orientation from the 2-D object it was associated to (Fish head purely oriented toward the 2-D object).

## 2.5. Behavioural variables

Different variables of interest were chosen to analyse fish exploration and swimming:

-The time spent in each zone expressed in seconds (s): Start Box (Start), Median area (Median), Reward zone near the congener (Cong), zone opposite to the reward zone (OpCong), zone located between Median and Cong (PreCong), zone located between Median and OpCong (PreOpCong).
-The fish absolute angular velocity expressed in degree per second (Vang in $° s^{-1}$) was calculated by the software as followed:
$Vang_n = RTA_n / t_n - t_{n-1}$ where $RTA_n$ is the relative turn angle for sample n and $t_n - t_{n-1}$ , the time difference between the current and previous sample. Here the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for heading direction. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity.
-The distance travelled by each fish in the maze (Dtot in mm)
-The mean velocity expressed in body length per second (Vel in BL $s^{-1}$)
The last three variables quantified the fish swimming activity level in the maze.
Different variables were chosen to assess the fish learning process and to check for possible bias such as spatial memory (fish turning according to its previous left or right choice) or side preference:
-Number of successful and failed turnings. Only the very first turning was accounted to meet the successful criteria. The maximum score is 8 successful turnings when the fish goes toward the reward zone at the first trial but not necessarily after its first turning choice. If the fish goes to the reward zone at the second trial, it can not exceed 7 and so on.
-The latency time to reach Cong or/and OpCong (in s)
-The last place occupied by the fish at the end of video: 0 or 1 (absence or presence) and the score is summed per zone over all trials (e.g. if the last place for one fish is always Cong, the number is 9 for Cong and 0 for all other zones).
-The number of times the fish was oriented toward the 2-D object associated with the reward (StCong), oriented toward the opposite 2-D object (StOpCong), just before the wall removal. All other situations were classified in the category “Other” (StOther).

## 2.6. Statistical analysis

All variables were compared using parametric analysis of variances (ANOVA) after verification of distribution normality and homoscedasticity (Dagnélie 1975). When data did not fulfil these requirements, non parametric tests were used. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls), and Kruskall-Wallis test by a rank-based multiple comparisons (Zar 1984). All statistical analyses were conducted using Statistica 8 (Statsoft, USA), and for all tests, the significant threshold was $p < 0.05$.

All variables related to the swimming activity were compared using a repeated measures analysis of variance with Origin (Wild and domesticated fish) as between-subjects factor and Trial as within-subjects factor.

The times spent in each zone were compared using a repeated measures analysis of variance with Origin (Wild and domesticated fish) and Zone (6 zones) as between-subjects factor and Trial as within-subjects factor.

Criteria for successful/failed turnings and side-turning preference within each treatment were determined using a binomial test at a 5% level of significance. The number of turnings performed according or not to the previous one and the number of times in each zone accounting for the last place were compared within each treatment using a Wilcoxon matched pairs test.

The latency time to reach the reward zone was compared using a Kruskall-Wallis test taking Origin (wild vs. domesticated) and trial (9 trials) as independent variables.

The percentages of successful turnings when fish orientation to the 2-D object just before wall removal was adequate or not were compared using a Kruskall-Wallis for each fish origin considering in both cases fish orientation (StCong, StOpCong and StOther) as independent variable.

The percentages of successful turnings in relation to the fish last place were compared using a Kruskall-Wallis test for each fish origin considering in both cases fish last place (LastCong, LastOpCong and LastOther) as independent variable.

## 3. Results

### 3.1. Spatial distribution

Wild-caught and domesticated fish spent most of the time in Cong (37 and 32% respectively) and in OpCong (33 and 29%) (Fig. 2). There were Zone and Zone*Origin significant effects ($F_{(45,\,450)}$= 4.0, $P<0.001$ and $F_{(45,\,450)}$= 1.8, $P<0.001$ respectively). Newman-Keuls test including all trials showed that wild fish time in Cong was higher than all other categories (Fig. 2). When looking at each Trial, Wild fish * Cong was significantly higher than all other categories at Trial 1 ($P<0.05$), no significant differences were reported at Trials 2, 3 and 8, Wild fish * Cong and Wild fish * OpCong were significantly higher than all other categories at Trials 4 and 9 ($P<0.05$), Wild fish * OpCong and Domesticated fish *OpCong were significantly higher than all other categories at Trial 5 ($P<0.05$), Wild fish * Cong and Domesticated fish * Cong were significantly higher than all other categories at Trial 6 ($P<0.05$), Wild fish * OpCong, Wild fish * Cong and Domesticated fish * Cong were significantly higher than all other categories at Trial 7 ($P<0.05$).

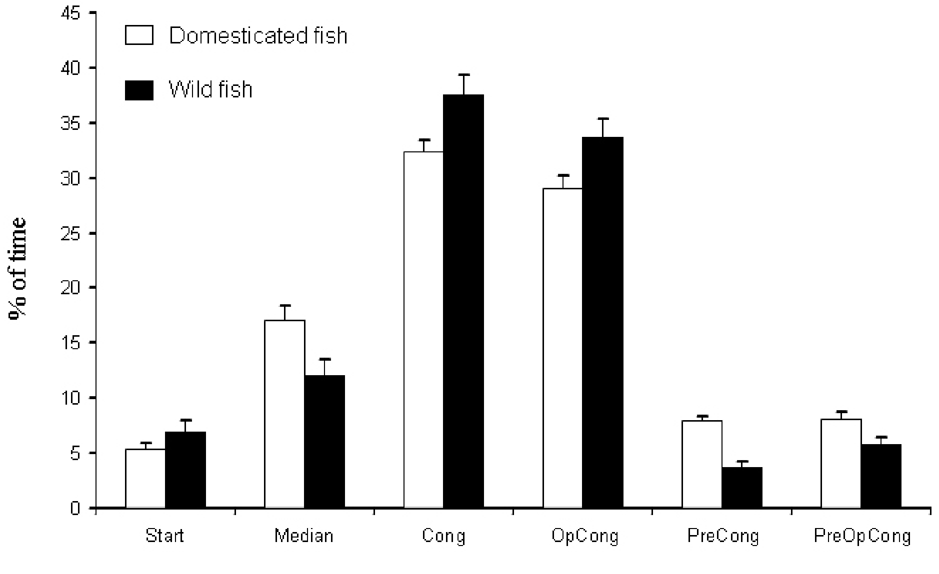

***Fig. 2.*** *Proportion of time spent (mean S.E., in %) by a fish in each zone of the maze.*
*Start : Start Box, Median: Median area, Cong: reward zone near the congener, OpCong: zone opposite to the reward zone, PreCong: zone located between Median and ZCong, PreOpCong: zone located between Median and OpCong.*

### 3.2. Swimming activity

Vang was, though not significantly, higher in wild fish than domesticated ones (Mean±SE: 616.8±13.7 and 466.5±16.7° $s^{-1}$, $F_{(9,10)}$=2.8, P=0.064). Differences were significant at each trial ($P<0.05$ for each pairwise comparisons) except for the three first trials.

Domesticated fish travelled significantly more distance and performed higher mean velocities than wild ones (Mean±SE: 629.9±42.3 mm and 0.07±0.00 BL $s^{-1}$, 285.4±17.1 mm and 0.03±0.00 respectively; $F_{(9,10)}=3.6$, P=0.027 and $F_{(9,10)}=3.8$, P=0.025). Differences were significant at each trial ($P<0.05$ for each pairwise comparisons) except for the two first ones.

### 3.3. Learning

Both wild and domesticated fish performed more successful turnings than failed ones but none individual from both fish origins showed a significant association between the 2-D object and the reward. However three domesticated and three wild individuals showed probabilities close to the significance level (D3 and D5: 75% of successful turnings, P = 0.11; D7: 83% of successful turnings, P = 0.09; W1, W2, W5: 75% of successful turnings, P = 0.11; Table 1). When looking at individual variability (Table 1), one fish from each origin showed very low percentages of successful turnings (D8: 12.5%, P = 0.03, W6: 25.0%, P = 0.11). In both cases, these fish performed their first turnings most of the time toward the opposite stimulus (D8: 87.5%, W6: 75.0%). Last position was 85.7% of the time in Cong for D8, 100% of the time in OpCong for W6. If these fish are considered as successful when they go toward the opposite zone, this leads to 64.6±15.1% of success in domesticated fish and 67.5±6.4% in wild ones.

Domesticated and wild fish went to the right side of the maze 63.7±23.3% and 51.9±19.4% (Mean±SD) of the time respectively. There was significant side-turning preference for three domesticated fish (D4 and D5: 89% of right turnings, P = 0.02; D7: 100% of right turnings, P = 0.004; Table 1) and one wild individual (89% of right turnings, P = 0.02).

| | Right-turnings (%) | Success (%) | P | Trials |
|---|---|---|---|---|
| D1 | 63 | 63 | 0.22 | 8 |
| D2 | 44 | 63 | 0.22 | 8 |
| D3 | 33 | 75 | 0.11 | 8 |
| D4 | 89* | 43 | 0.27 | 7 |
| D5 | 89* | 75 | 0.11 | 8 |
| D6 | 44 | 50 | 0.27 | 8 |
| D7 | 100* | 83 | 0.09 | 6 |
| D8 | 33 | 13 | 0.03* | 8 |
| D9 | 67 | 50 | 0.27 | 8 |
| D10 | 75 | 57 | 0.27 | 7 |
| W1 | 44 | 75 | 0.11 | 8 |
| W2 | 78 | 75 | 0.11 | 8 |
| W3 | 33 | 63 | 0.22 | 8 |
| W4 | 44 | 38 | 0.22 | 8 |
| W5 | 71 | 75 | 0.11 | 8 |
| W6 | 44 | 25 | 0.11 | 8 |
| W7 | 44 | 63 | 0.22 | 8 |
| W8 | 89* | 63 | 0.22 | 8 |
| W9 | 44 | 63 | 0.22 | 8 |
| W10 | 25 | 63 | 0.22 | 8 |

***Table 1.*** *Learning performances and side-turning preference in domesticated and wild individuals. Side-turning preference (based on 9 trials per individual) and successful turnings are assessed by a binomial test at the 5% level of significance. Significant successful turnings are signified with a single asterisk beside the probability value (P). Trials: number of accounted trials performed by each individual for the calculation of the learning performance, the maximum being 8. D: domesticated individuals; W: Wild individuals.*

The latency time to reach the reward was (Mean±SE) 56.8±11.1 s in domesticated fish and 47.0±10.5 s in wild ones but the difference was not significant ($H_{(1, 133)}$=0.00, P=0.99). There was no latency time differences between trials ($H_{(8, 133)}$=7.9, P=0.45).

The turnings did not significantly depend on the fish previous choice and there was no significant difference between fish origins (Mean±SD: 52±14.5% of turnings according to the previous ones in domesticated fish, 40±18.1% in wild ones).

Both wild and domesticated fish were significantly located more often in Cong than OpCong and Other at the end of the observation period ($H_{(2, 60)}$=23.5, P<0.001) (Fig. 3). Difference between fish origins was not significant ($H_{(1, 60)}$=0.0, P=0.83).

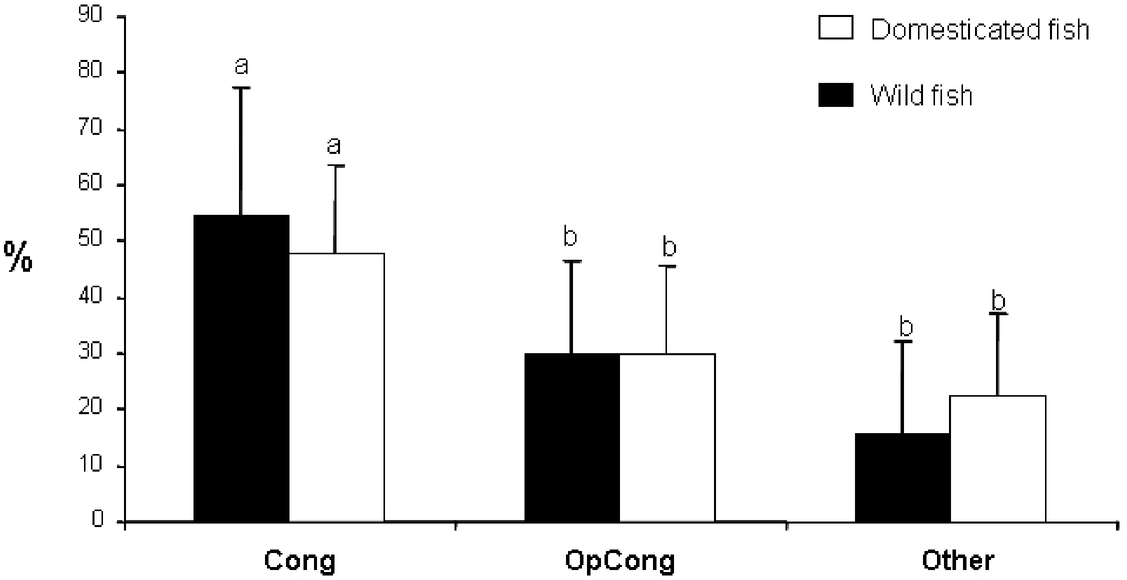

***Fig. 3.*** *Mean±SD. Last place occupied by wild vs domesticated fish before the end of the 10-minute observation period.*

*Cong: reward zone near the congener, OpCong: zone opposite to the reward zone, Other: all other situations. Results of rank-based multiple comparisons are shown with the letters a and b. When two treatments have different letters they significantly differ from each other with P< 0.05.*

Domesticated and wild fish performed successful turnings 63.0±30.4% and 74.0±25.0 of the time when they were oriented toward the adequate 2-D object just before the wall removal (Fig. 4A). These percentages were significantly higher than those recorded in other zones ($H_{(2, 30)}$=12.5, P<0.05 and $H_{(2, 30)}$=19.7, P<0.001 respectively). Similarly, domesticated and wild fish performed successful turnings when their last position was in Cong 61.5±32.4% and 54.33±22.9% of the time respectively (Fig. 4B). These percentages were significantly higher than those recorded in other zones ($H_{(2, 30)}$=9.8, P<0.007 and $H_{(2, 30)}$=10.0, P<0.007 respectively).

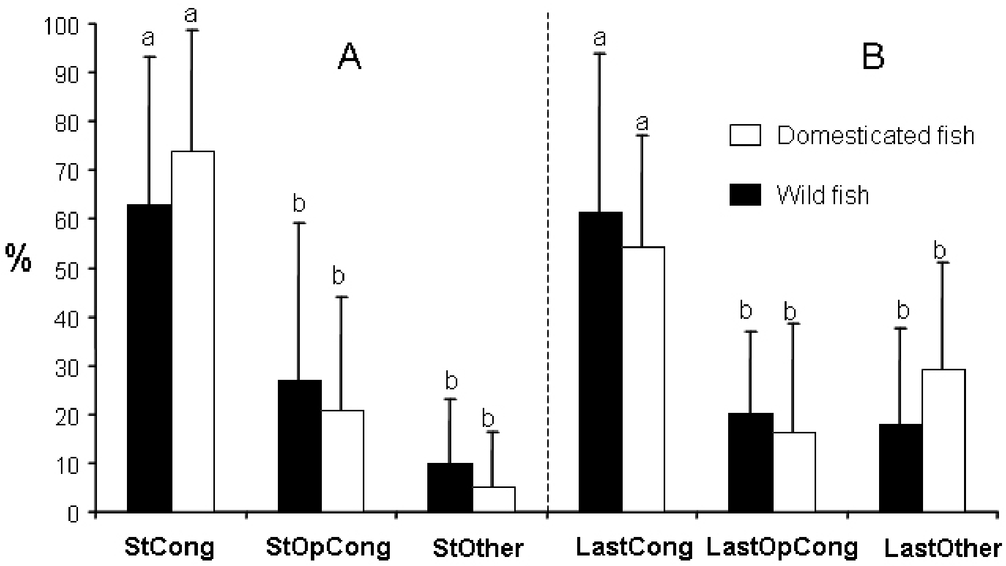

***Fig. 4.*** *Mean±SD. Wild and domesticated fish position at the beginning and the end of observation period in relation to success.*
*A. The percentages of successful turnings in relation to fish orientation from the 2-D object just before wall removal.*
*StCong: % of times the fish was oriented to the 2-D object it was associated to, StOpCong: fish oriented to the opposite 2-D object, StOther : all other situations.*
*B. The percentages of successful turnings in relation to the fish last place before the end of observation.*
*LastCong: last place in Cong , LastOpCong: last place in OpCong, LastOther: last place in all other situations.*
*Results of rank-based multiple comparisons are shown with the letters a and b. When two treatments have different letters they significantly differ from each other with P< 0.05.*

## 4. Discussion

The aim of this study was to assess for domestication effect on swimming behaviour, exploration and learning abilities of juvenile sea bass in a maze. This was approached by comparing wild-caught and domesticated fish. The results showed consistent behavioural differences between fish origins. It also provided a first insight for this species into learning abilities such as abstract 2-D discrimination and interpretation. No differences were reported in learning abilities between wild and domesticated fish. However, intra-individual differences existed that can reduce the power of statistical tests especially when using small samples in cognition-based experiments.

### 4.1. Spatial distribution and exploratory swimming activity

Behavioural differences were found between domesticated and wild fish. Angular velocity was higher in wild fish while the distance travelled and the velocity mean were higher in domesticated ones. These differences between wild and domesticated fish demonstrate an impact of domestication on swimming behaviour. Difference in velocity mean and angular velocity reflected lower swimming complexity in domesticated fish that could be linked to a decrease in the vigilance threshold (Bégout and Lagardère 2004) induced by the environment experienced by cultured fish that is strikingly different from that experienced by their wild counterparts e.g. the physical environment is much simpler, space is restricted and migration is not possible, it is less challenging in that good quality food is readily available and fishes are protected against predators (Gross 1998; Price 1999; Waples 1999). On the contrary wild fish behaviour could indicate higher vigilance and then uncertainty in a novel environment such as a maze. Indeed, previous studies have already shown a high frequency of turnings and a slower travelling speed in solitary fish placed under similar conditions (Ward et al. 2010). These results are also in accordance with previous studies on sea bass at an early stage (Benhaïm et al, submitted) or later stage (Millot et al. 2009a).

In general fish from both origins spent most of the time in the zone closest to the congener, the stay duration being higher in wild fish. However, differences sometimes occurred between trials. In these cases, wild and/or domesticated fish spent similar time in Cong and OpCong. This work was actually based on the hypothesis that fish would consider Cong as a reward zone so it is not surprising to see that fish spent generally most of the time in that zone. It confirms that social or gregarious species may greatly benefit search for social interaction. Group behaviour has already been shown to increase growth as a result of social facilitation (Peuhkuri et al. 1995; Stirling 1977) and to reduce predation risk (Roberts 1996). The vigilance decreases when neighbour distance decreases because information about whether other group members have detected a predator is easier to obtain from nearer individuals (Pöysä 1994). More recently, it has also been shown that social interaction plays an important and beneficial role in regulating the stress response in cohesive social species such as sturgeon *Acipenser fulvescens,* Allen et al. 2009). The fact that wild fish spent more time in the congener zone could reinforce the hypothesis of higher vigilance in wild fish described above leading them to reduce distance from a congener. Fish from both origins spent also time in OpCong. It can be explained by the proportion of fish that failed to find the reward zone. More likely, some fish could voluntarily avoid Cong and prefer to spend time in the opposite zone. In an experiment with two unfamiliar sea bass individuals separated by a transparent barrier, Di-Poï (2008) hypothesized that contacts with the barrier can be considered as an agonistic attempt (intimidation) of the initiator towards the congener on the other side. The fish that spent most of the time in the opposite zone could then be subordinates. It is well known that staying alone could be a better strategy for subordinates (Ranta et al. 1993) allowing them to have a lower probability of suffering injury in an escalated contest (Abbot et al. 1985). Congener avoidance could also refer to a particular coping style because fish differed within origin in the nature of their response to the challenge (Koolhaas et al. 1999).

### 4.2. Learning abilities

Most of individuals from both origins went preferentially toward the congener zone indicating that they were able to discriminate between two 2-D objects. However, the power of the binomial test was clearly weakened by the low number of trials. Experiments on learning process usually require numerous training sessions (Alves et al. 2007; Siebeck et al. 2009; Sovrano and Bisazza 2008). This enables to compare for example the learning performances between the first and the last session. In our study, preliminary observations showed that individuals could not be tested more than 3 trials in a row. Indeed, most of individuals remained immobile in the startbox after 3 trials indicating high level of stress. However, the required task was quite simple compare to previous cited studies and tested fish were placed in an environment where visuals cues were mostly restricted to the 2-D object. Indeed, the latency time to reach the reward was lower than 1 min. Some studies have already shown that associative learning occurs just one simultaneous presentation of the cue and the stimulus (Magurran 1989) and the response can be retained for up to 2 months (Chivers and Smith 1994). Here the association between the visual cue and the reward could have been learned very fast. Even though it needs to be confirmed by further research e.g., increasing the number of day sessions, we assume that fish from both origins would be able to discriminate between two 2-D objects with equal areas of dark and light removing therefore any remaining spurious differences in luminance between the stimuli (Siebeck et al. 2009) to achieve a simple task. The best learning criteria was the first turning performed by the fish. Similarly to previous study (Alves et al. 2007) latency did not appear to be a pertinent indicator in our study because of inter-individual differences. Successful individuals also showed anticipatory behaviour since most of them were oriented toward the proper stimulus before the transparent wall was removed. This type of behaviour has recently also been found in cod *Gadus morhua* (Nilsson et al. 2008) and referred to as goal tracking (Siebeck et al. 2009). The first turning of the fish did not depend on its previous choice but a few individuals. A few individuals (three domesticated and one wild) showed preferences to turn right reflecting behavioural lateralization as already shown in lower vertebrate (Bisazza et al. 1998) e.g. the preference for the right side of a T-maze in tilapia *Oreochromis niloticus* (Gonçalves and Hoshino 1990a). In our study, successful fish used obviously place strategy rather than response strategy. The place strategy refers to animals that can learn an association between a given place and a reward (Dudchenko 2001; Gibson and Shettleworth 2005). Previous studies have shown that fish employ multiple spatial strategies that closely parallel those described in mammals and birds (Lopez et al. 1999) but one of the two strategies can be favoured by the conditions of the experiment (Restle 1957).

This study also demonstrated inter-individual differences, with some fish showing a preference for the side opposite to the reward. As stated in previous section, these fish were more likely to prefer the opposite side of the reward than to be “bad learners”.

### 4.3. Impact of cognition on learning abilities

The main focus of this work was to investigate for learning abilities differences between wild and domesticated fish. Both origins actually showed very similar responses to the test they were submitted to. In particular, the same proportion of “good and bad learners” was recorded. This indicates that domestication would not have a major effect on spatial orientation such as place learning in sea bass. Fish reared in tanks and cages are kept in an environment very different from the natural habitats but in both cases, they benefit from spatial learning. Wild fish need to relocate various biologically important locations such as shelter or profitable food patch (Odling-Smee et al. 2007). To achieve this, the fish needs to monitor its location with reference to an external point of reference as it moves through its environment (Odling-Smee et al.; 2007). This is the case for sea bass juvenile that need to orientate in relation with visuals cues (benthic substrate composed of rocks) when becoming dermersal (Barnabé 1989). In the same time, cultured fish must also cognitively process the sensory information presented by the farming systems such as food location when automatically distributed (Fernö et al. 2007).

### 4.4. Conclusion

This study demonstrated behavioural differences in wild and domesticated fish facing to a new environment but no difference in spatial learning. Further research is however needed on this species to confirm these results. It would be useful to increase the number of learning sessions and/or individuals in experiments designed to minimize the stress and allowing specifying the modalities of spatial learning *i.e.* testing response and place learning.

### Acknowledgments

We are grateful to Aquanord company for providing domesticated animals and to AQUARID (Sète) for providing us with wild sea bass juveniles.

### Ethical standards

This study was conducted under the approval of the Animal Care Committee of France under the official licence of M.L. Bégout (17-010).

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# Discussion générale

Les objectifs de cette thèse étaient :

1) D'identifier des indicateurs précoces de compétences comportementales ou de traits de personnalité utilisables dans des processus de domestication et de sélection (incluant des critères et objectifs basés sur l'adaptation, le bien-être et la santé des téléostéens). Pour ce faire, nous avons (i) testé l'influence d'un certain nombre de facteurs abiotiques (effet refuge, aliment végétal, pratique du tri, effet du jeûne) et biotiques (taille de l'œuf et environnement social) sur les réponses comportementales (plasticité comportementale) de deux espèces modèles très différentes (l'omble chevalier, le bar européen), (ii) analysé la variabilité des réponses (traits de personnalité), (iii) complété les observations par l'analyse de variables physiologiques (paramètres sanguins) et des performances d'élevage. Les réponses comportementales utilisées ont été le comportement alimentaire, l'activité de nage, l'exploration et la cognition. Certains de ces travaux avaient pour objectif de mieux comprendre des mécanismes évolutifs généraux tels que la variabilité comportementale et morphologique chez de jeunes téléostéens qui pourrait favoriser dans la nature, l'évolution du polymorphisme (*i.e.* influence de la taille de l'œuf et de l'environnement social sur le comportement de l'omble chevalier).

2) De déterminer les réponses comportementales adaptées au milieu d'élevage et au milieu sauvage dans la perspective de l'amélioration des programmes de repeuplement et/ou de sélection. Pour atteindre ce second objectif, nous avons comparé des populations sauvages et domestiquées de deux espèces (truite commune et bar européen) à des âges différents (larves et juvéniles), dans des contextes variables (challenges, labyrinthes). Selon les cas, le protocole expérimental contrôle l'origine des téléostéens par le biais de croisements appropriés et à l'aide de « l'outil génétique » (truite) ou la comparaison porte sur des populations sauvages capturées dans le milieu naturel et des standards industriels domestiqués (bar européen). Ces comparaisons ont permis de tester l'influence de la domestication, de la captivité et du développement sur le comportement des téléostéens. Les réponses comportementales utilisées ont été l'activité de nage, le taux de réponse de fuite, l'exploration et la cognition.

Les apports majeurs de notre travail ayant permis d'atteindre ces deux objectifs sont synthétisés et discutés ici.

## Des indicateurs précoces de compétences comportementales ou des traits de personnalité utilisables en domestication et/ou sélection ?

### *Chez l'omble chevalier*

Parmi ceux étudiés, les indicateurs pertinents pour mettre en évidence la plasticité comportementale sont le **comportement alimentaire** et la **mobilité** (Figure 11). Il s'agit d'indicateurs très précoces (pendant la nutrition endogène, et la première nutrition exogène) directement liés à des performances d'élevage (croissance, survie et âge de la première nutrition exogène). Les leviers utilisés pour la mise en évidence de cette plasticité comportementale sont la présence d'un refuge, la taille de l'œuf et l'environnement social.

#### *Les limites*

Il est difficile de trancher avec certitude quant à la réversibilité plus ou moins totale des réponses comportementales. La réversibilité totale renvoie à la définition de la flexibilité comportementale (Piersma & Lindstrom, 1997) mais il n'est pas exclu que certains des facteurs testés induisent des changements irréversibles caractérisant une plasticité développementale.

La situation d'interaction entre les deux facteurs biotiques (taille de l'œuf et environnement social) est plus complexe à interpréter que celle du facteur abiotique (refuge) puisqu'il existe une interaction significative entre ces derniers pour certains des comportements observés (*e.g.* le comportement alimentaire). Il est également difficile de trancher avec certitude sur la plasticité liée à la taille de l'œuf (ou effet maternel) dans la mesure où elle peut être régulée par des facteurs génétiques et environnementaux (Mousseau & Fox, 1998) et que le protocole utilisé dans ce travail, ne permet pas de faire la distinction.

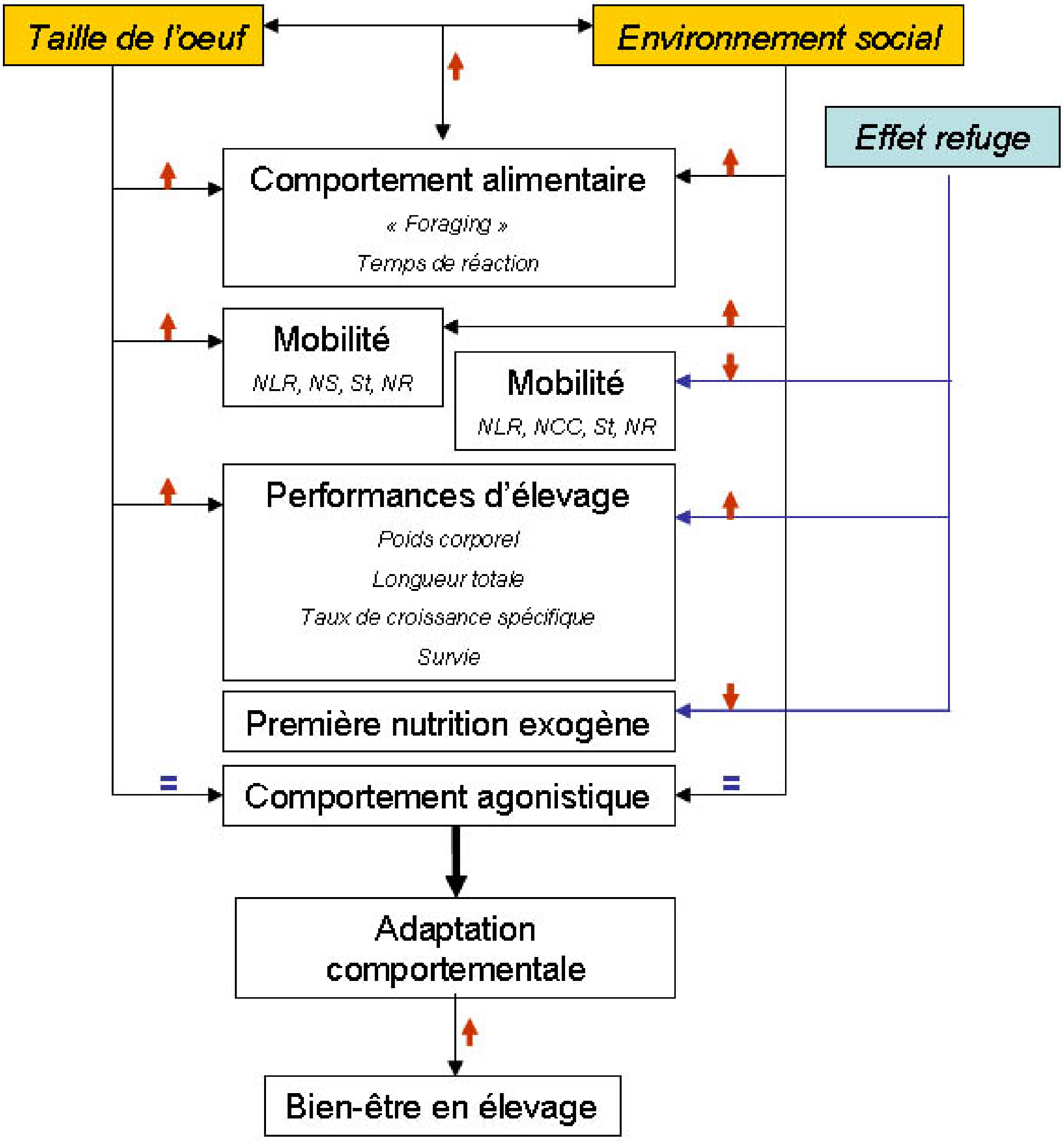

*Figure 11 : Synthèse des résultats concernant l'influence d'un facteur abiotique (effet refuge) et de deux facteurs biotiques (taille de l'œuf et environnement social) sur le comportement et les performances d'élevage de l'omble chevalier dans le but d'évaluer les capacités d'adaptation et le potentiel de bien-être en élevage. Les effets sont représentés par :*
*↑ : augmentation ; ↓ : diminution ; = : pas d'influence. NLR : nage lente régulière ; NS : nage saccadée ; St : mouvements stationnaires ; NR : nage rapide ; NCC : nage à contre courant.*

### *Les applications*

Les potentielles améliorations zootechniques suggérées par nos résultats sont qu'un tri au stade œuf pourrait s'avérer davantage bénéfique que le tri réalisé en routine à des stades de développement plus avancés. En effet, chez l'omble chevalier, le gain de croissance obtenu par des tris successifs ne semble pas évident et ceci indépendamment de l'âge des individus (Wallace & Kolbeinshavn, 1988; Baardvik & Jobling, 1990). Plusieurs auteurs ont testé l'influence du tri pour différentes classes de taille et dans tous les cas, le gain de biomasse est plus élevé pour les lots non triés (Baardvik & Jobling, 1990; Guillard et al., 1992).

Par ailleurs, les améliorations envisageables au niveau du bien-être résideraient dans l'utilisation de refuges artificiels, pratique qui donne des résultats manifestement en accord avec la notion d'harmonie (Veissier et al., 2000) et entre donc dans la définition du bien-être en élevage (Cf. §4).

### *Les apports conceptuels*

Les résultats de ces travaux permettent également d'émettre des hypothèses quant à l'influence de ces facteurs abiotiques et biotiques (interagissant avec de nombreux autres facteurs non testés dans le cadre de cette thèse) sur l'écologie et l'évolution de cette espèce. L'impact des facteurs testés sur le comportement et la croissance de téléostéens à un stade de développement très précoce pourraient favoriser dans la nature, l'émergence de stratégies de vie différentes : sélection de l'aliment et de l'habitat. De précédentes études ont démontré la forte plasticité de l'omble chevalier (Skúlason et al., 1992; Skúlason et al., 1993; Skúlason & Jónsson, 1999). Il existe par exemple quatre morphes dans le Thingvallavatn, le plus grand lac d'Islande : un grand morphe benthique, un petit morphe benthique, un morphe piscivore et un morphe planctonivore. Ces morphes sont donc séparés par leur régime alimentaire et leur position dans la colonne d'eau. Les différences comportementales décrites dans cette thèse à un stade très précoce de développement, en réponse à divers facteurs environnementaux (biotiques et abiotiques) pourraient favoriser dans la nature, la sélection de l'aliment et de l'habitat en conduisant à une variabilité au niveau de l'histoire de vie et à l'évolution du polymorphisme. La figure 12 résume cette hypothèse en s'inspirant du modèle de Skúlason & Smith (1995) qui décrit comment le polymorphisme peut conduire à une spéciation.

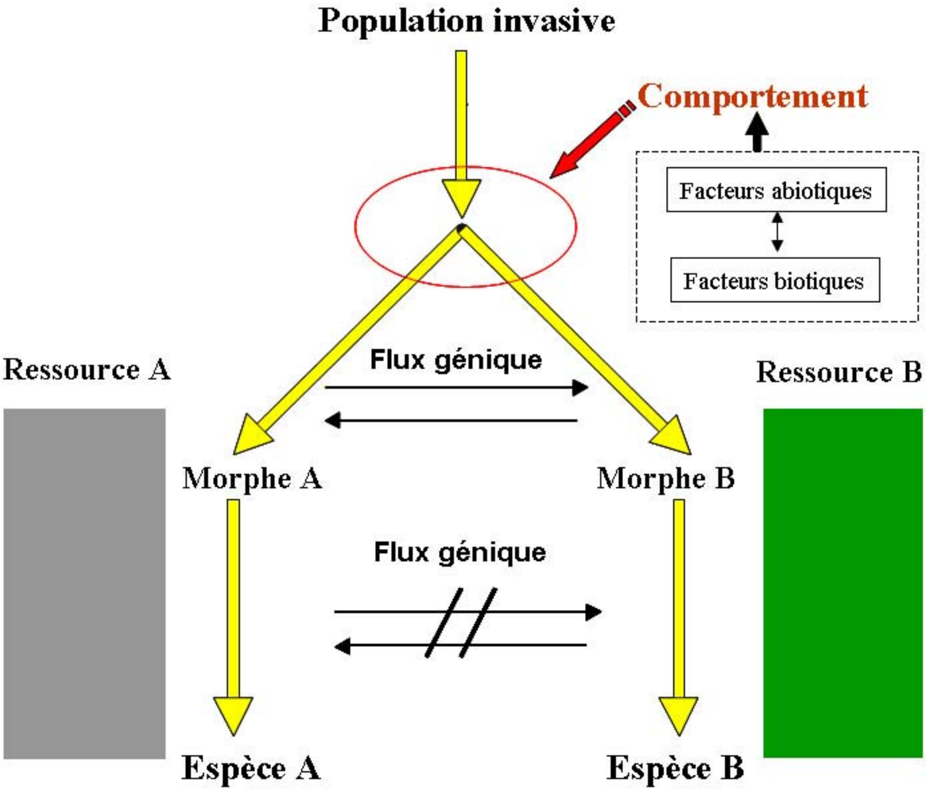

*Figure 12 : Schéma général des possibles étapes et mécanismes impliqués dans l'apparition du polymorphisme et de nouvelles espèces (inspiré de la figure 1 de Skúlason & Smith, 1995).*

***Chez le bar***

Les indicateurs comportementaux pertinents pour caractériser la plasticité comportementale sont des variables cinématiques caractérisant la locomotion (**vitesse moyenne** et **distance totale parcourue**) et une variable physiologique (le taux de cortisol). Parmi les trois facteurs utilisés comme « leviers expérimentaux » chez cette espèce (Figure 13), *i.e.* l'usage d'un aliment à base de matières premières d'origine végétale (aliment végétal), le tri (sur le poids) et le jeûne, seul le premier semble induire une plasticité comportementale à des stades juvéniles (poids de 90g environ). En effet, l'usage d'un aliment végétal impacte l'activité de nage (réduction de la vitesse moyenne et de la distance parcourue en labyrinthe) et la libération à court terme du cortisol cependant qu'il n'affecte ni les capacités d'apprentissage en condition d'auto-alimentation ou en situation de test cognitif en labyrinthe, ni le comportement alimentaire, ni le taux de croissance spécifique durant la période d'apprentissage. Le tri lui, n'a qu'une faible influence sur les variables mesurées en condition d'auto-alimentation : des lots homogènes et hétérogènes en poids ne divergent pas davantage au niveau de leur réponse comportementale, de leurs performances de croissance et de leur structure sociale. Enfin, le jeûne (3 semaines) ne modifie pas la structure sociale qui se construit autour du dispositif d'auto-alimentation (manipulateurs principaux du distributeur d'aliment identiques avant et après jeûne).

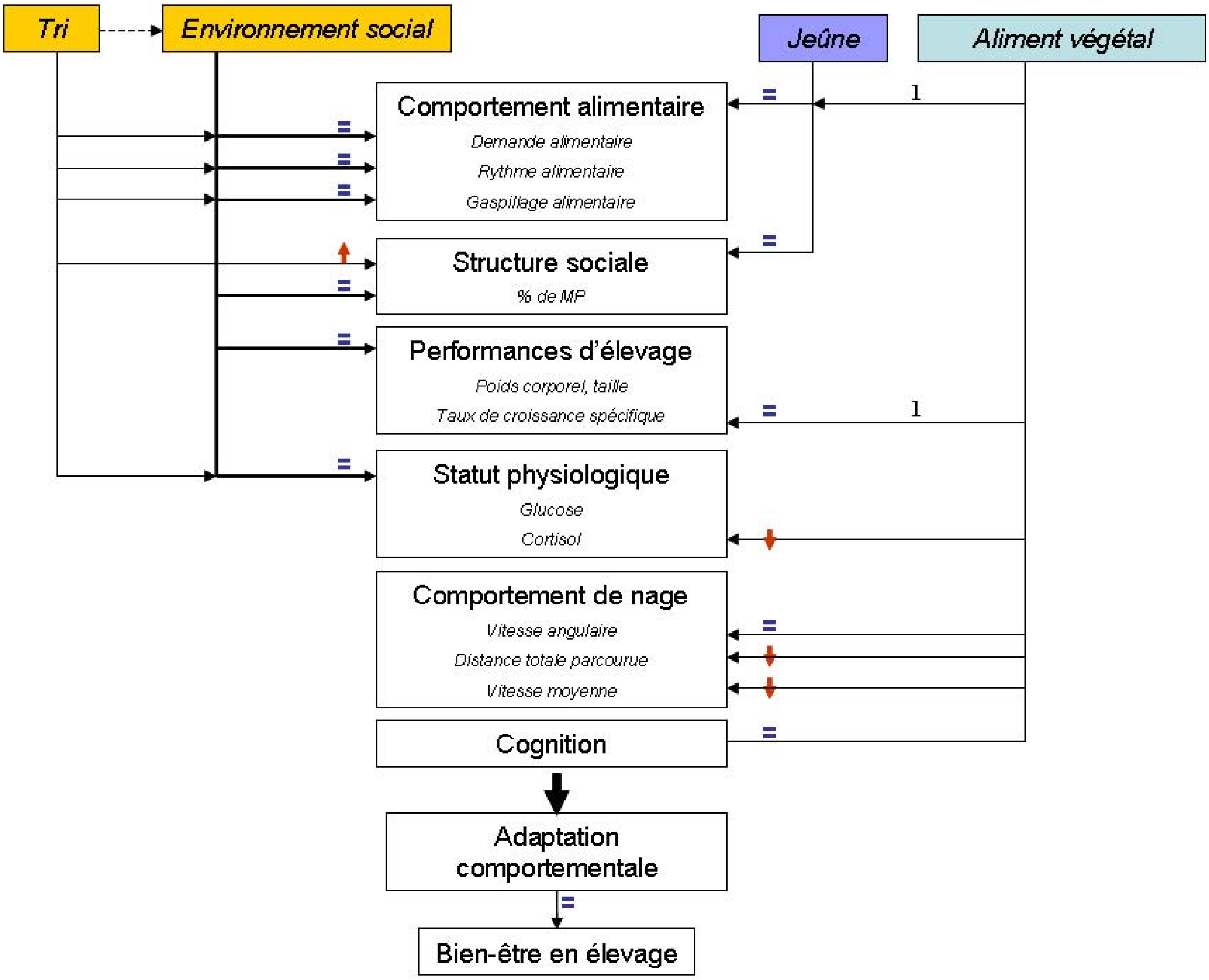

*Figure 13 : Synthèse des résultats concernant l'influence d'un facteur abiotique (pratique du tri) et du facteur biotique qui en résulte (environnement social : lot homogène vs. hétérogène en poids), d'un facteur abiotique (aliment végétal), et du jeûne sur le comportement, la physiologie et les performances d'élevage du bar dans le but d'évaluer les capacités d'adaptation et le potentiel de bien-être en élevage. Les effets sont représentés par : ↑ : augmentation ; ↓ : diminution ; = : pas d'influence. MP : manipulateurs principaux. 1 : pendant la phase d'apprentissage en condition d'auto-alimentation.*

### *Les limites*

Pour ce qui concerne l'aliment, les résultats devront être confirmés sur une plus longue période car (i), le taux de croissance spécifique était très faible pour les deux régimes pendant la période étudiée et (ii), le lot végétal accusait déjà un déficit de 36% en poids corporel par rapport au lot témoin en début d'expérience, même si cela n'a rien de surprenant, d'autres études menées avec des taux de substitution d'huiles de poisson de 80% ayant déjà montré une telle réduction des performances de croissance (Montero et al., 2005). Il est également difficile d'affirmer qu'une carence à plus long terme n'affecterait pas les fonctions d'apprentissage. Les conclusions concernant le cortisol quant à elles (niveau des lots nourris d'aliment végétal plus bas en situation de test en labyrinthe), doivent être modulées par le fait que les taux mesurés pour les deux régimes traduisent un niveau de stress aigu qui pourrait avoir altéré les capacités de réponse lors du test cognitif en labyrinthe.

### *Les applications*

Les résultats sur le tri suggèrent qu'il serait possible de supprimer cette procédure stressante (Pickering, 1981), coûteuse en terme de main-d'œuvre (Person-Le Ruyet et al., 1991) et pas toujours efficace en terme de gain de croissance (Doyle & Talbot, 1986; Baardvik & Jobling, 1990), même si elle reste le moyen le plus communément utilisé pour gérer les rationnements alimentaires (taille des particules) et la commercialisation du cheptel dans les fermes (Wallace & Kolbeinshavn, 1988). En condition d'auto-alimentation, la procédure n'améliore pas la croissance, sans pour autant avoir d'effets négatifs, les paramètres sanguins mesurés n'indiquant pas, par exemple, de perturbation physiologique ce qui est en accord avec de précédentes études menées dans des conditions comparables (Coeurdacier et al., 1997; Dosdat et al., 2003; Millot et al., 2008). La faible évolution du coefficient de variation des lots étudiés est un argument de plus en faveur de l'utilisation des dispositifs d'auto-alimentation. Ces derniers avaient en effet déjà été annoncés comme très prometteurs en raison des performances de croissance et des indices de conversion alimentaire obtenus sur cette espèce (Azzaydi et al., 1998), et du fait qu'ils permettent aussi de réduire les coûts de main-d'œuvre liés à la distribution de l'aliment (Aloisi, 1994; Azzaydi et al., 1998). De plus, le concept même d'alimentation à la demande, où les animaux se nourrissent en fonction de leurs besoins, répond aux prérogatives actuelles en matière de bien-être animal. A ce jour pourtant, le développement commercial de ces dispositifs reste limité.

### *Les apports conceptuels*

L'absence de perturbation des fonctions cognitives constaté dans le cas d'une utilisation d'aliment végétal dépourvu d'acides gras polyinsaturés (EPA, DHA principalement), laisse penser que, dans les conditions imposées dans notre expérience, une telle carence n'a pas altéré les membranes lipidiques (en particulier celles situées au niveau du tissu cérébral) comme cela a pu être démontré chez les rongeurs (Su, 2010).

L'absence d'effet de 3 semaines de jeûne sur la structure sociale qui se construit autour du dispositif d'auto-alimentation (pas de modification de l'identité des manipulateurs principaux), nous conduit à émettre l'hypothèse de l'existence de traits de personnalité basés sur le pourcentage d'activation de la tige tactile du dispositif d'auto-alimentation en nous conformant à la définition de Sih et al. (2004) et de Réale et al. (2007). Millot (2008) suggère que le manipulateur principal joue un rôle directeur dans la répartition spatiale du groupe et qu'il pourrait être considéré comme un individu caractérisé par un trait de personnalité de type « téméraire » par rapport aux individus non-manipulateurs qui eux, seraient caractérisés par un trait de personnalité de type « timide ». La corrélation entre les traits de personnalité décrits dans cette thèse et celle de Millot (2008) reste à démontrer, mais elle évoque l'existence d'un syndrome comportemental (Sih et al, 2004), un peu à l'image de l'axe proactif-réactif (Koolhaas et al., 1999; Réale & Festa-Bianchet, 2003, cf. §4.4).

De manière plus globale, dans toutes ces expériences, les poissons ont montré qu'ils possédaient les capacités d'adaptation nécessaires pour répondre aux situations de challenge testées, sans que l'on puisse constater de « dépassement » de leurs capacités d'adaptation. Ceci plaide en faveur d'un important potentiel de bien-être chez le bar (cf. Frazer et al., 1997). Si le facteur « aliment végétal » est le seul à s'être révéler efficace pour mettre en évidence des indicateurs comportementaux et physiologiques de plasticité précoce chez cette l'espèce, il reste néanmoins évident que cette conclusion n'exclut pas l'existence d'une plasticité phénotypique vis à vis des autres facteurs. Il est en effet très vraisemblable que les téléostéens procèdent à des ajustements physiologiques et comportementaux qui n'ont pas pu être détectés avec les méthodes utilisées. Ces ajustements relèvent de l'homéostasie (cf. §3) qui indique qu'un état stable est entretenu par tous les processus actifs qui s'opposent aux changements des propriétés de l'organisme (Rieutort, 1998). La relative constance des réponses comportementales et autres variables mesurées (structure sociale, demande alimentaire, performance de croissance, paramètres sanguins etc.) pourraient être simplement le résultat de ces ajustements, traduisant ainsi la bonne capacité d'adaptation du bar aux changements subits.

### *Conclusions*

Les leviers utilisés pour caractériser la plasticité comportementale ont été choisis différemment chez le bar et l'omble chevalier pour tenir compte des différences existant entre ces deux espèces, en particulier au stade de développement précoce. Une comparaison interspécifique ne serait donc pas pertinente, mais certaines hypothèses peuvent être néanmoins avancées sur l'importance de la plasticité comportementale pour ces deux modèles biologiques aux stratégies de vie bien distinctes. L'omble chevalier serait caractérisé par une plus forte plasticité comportementale que le bar, hypothèse supportée par les observations réalisées dans le milieu naturel où, l'omble est connu pour sa forte plasticité phénotypique qui se traduit par divers aspects morphologiques et comportementaux (cf. §8.1.1). Notons toutefois que l'hypothèse d'une plasticité plus faible chez le bar reste valable dans la limite des facteurs et des stades de développement testés dans ce travail.

## Des réponses comportementales adaptées au milieu d'élevage ou au milieu sauvage ?

Pour discriminer le comportement des individus sauvages et domestiqués, nous avons utilisé des stimulations lumineuse (truite), mécanique et visuelle (larve de bar), diverses situations de challenge en labyrinthe (juvéniles de bar) et mesuré une dizaine de variables. Les résultats sont synthétisés sur la figure 14.

### *Chez la truite*

Trois variables liées à l'activité de nage permettent de différencier les deux types de populations : l'individu sauvage se caractérise par une vitesse angulaire plus élevée, mais une vitesse moyenne et une distance totale parcourue moins importantes que l'individu domestiqué. Son niveau de récupération est également plus élevé (retour à une activité de nage comparable à celle observée avant l'application du stimulus). On notera que le stimulus lumineux a tendance à réduire la vitesse angulaire.

#### *Les limites*

Les résultats sont valables pour des truites de 280 jpf et d'une taille d'environ 5 cm.

Si l'expérience a permis de mettre en évidence un effet de la domestication sur le comportement en réduisant au maximum les sources de variation liées à l'environnement, le protocole utilisé ne permet cependant pas de distinguer complètement les sources de variation (seuls les effets génétiques paternels ont été estimés). Un plan factoriel complet comparant des individus captifs d'origine méditerranéenne (F2-F3) à leurs congénères sauvages permettrait de faire la distinction entre les effets de la domestication et la variation liée à l'origine géographique.

### *Les applications*

Les différences de réponses comportementales observées ont des implications pour les programmes de repeuplement et de domestication/sélection. Les programmes de repeuplement sont en effet souvent l'objet de nombreux échecs en raison des réponses comportementales inadaptées des individus relâchés (Olla et al., 1994; Brown and Laland, 2001; Brown and Day, 2002; Huntingford, 2004). Ce travail souligne que l'aptitude des individus à être relâchés peut être évaluée en situation de challenge en laboratoire, leur potentiel étant mesuré avec les indicateurs décrits ici.

En l'état de nos connaissances, on pourra considérer qu'un animal testé est apte au repeuplement s'il présente les 3 caractéristiques suivantes : vitesses angulaires élevées, distances totales parcourues et vitesses moyennes faibles lorsqu'ils sont placés en situation de challenge en laboratoire. A l'inverse, l'application de notre test sur une nouvelle espèce candidate à l'élevage, pourrait nous révéler son aptitude à la domestication. Cependant, jusqu'à que ces chiffres absolus soient confirmés, voire vérifiés chez d'autres espèces, il serait plus prudent de n'utiliser notre test qu'en situation comparative (animaux testés *versus* animaux domestiqués ou animaux sauvages). Cela nécessite que les institutions responsables du repeuplement entretiennent des souches domestiquées dans leurs installations ou que les populations sauvages soient accessibles pour évaluer l'aptitude à la domestication d'une nouvelle espèce candidate à l'élevage.

Nos tests demeurent cependant longs de mise en œuvre pour caractériser des milliers d'individus. Il serait donc indispensable d'avancer dans leur automatisation avant d'envisager qu'ils puissent servir à des programmes de sélection pour les aptitudes ou contre aptitudes, à la domestication.

### *Les apports conceptuels*

Les résultats indiquent, que des truites ayant subi 30 à 40 générations de domestication sont effectivement caractérisées par des réponses comportementales distinctes de celles de leurs congénères sauvages et semblent notamment mieux adaptées à l'environnement d'élevage.

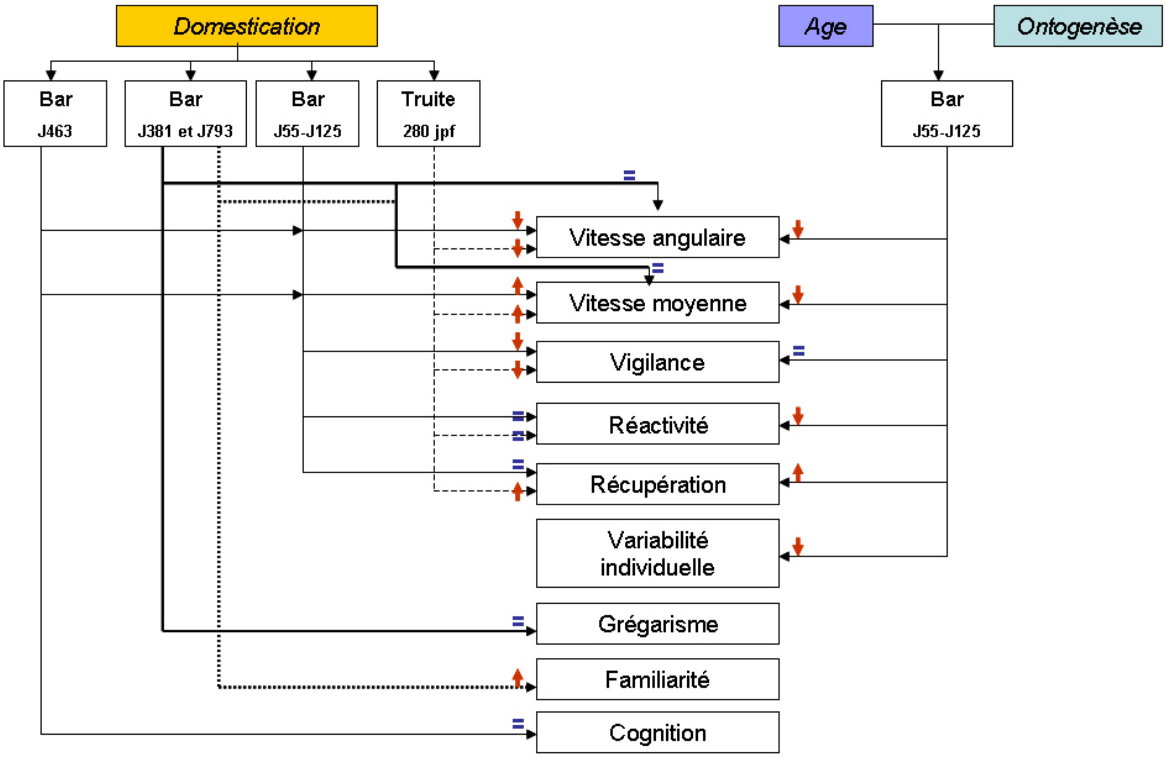

*Figure 14 : Synthèse des résultats concernant l'influence de la domestication et de la combinaison de l'âge et de l'ontogenèse sur le comportement du bar et de la truite. Les effets sont représentés par : ↑ : augmentation ; ↓ : diminution ; = : pas d'influence.*

### *Chez le bar*

Trois variables liées à l'activité de nage et une tendance à la familiarité (préférence d'un congénère familier) permettent de différencier les deux types de populations : l'individu sauvage se caractérise (i) par une vitesse angulaire plus élevée, (ii) par une distance totale parcourue et une vitesse moyenne moins élevées et (iii) par un comportement de familiarité restreint. On notera que la vitesse angulaire des individus sauvages a tendance à diminuer lorsque la durée de captivité augmente (valeurs les plus élevées chez les larves et notamment après un stimulus) et en test de labyrinthe. La vitesse moyenne quant à elle, a tendance à diminuer après stimulus et aussi en labyrinthe.

#### *Les limites*

Nos conclusions sont celles qui s'imposent dans le cas de la comparaison d'une population sauvage capturée à l'état larvaire dans le milieu naturel et d'un lot standard industriel (5 ou 6 générations de domestication/sélection) élevés dans les mêmes conditions entre l'âge de 2 mois et 2 ans.

Les limites concernant l'utilisation des variables cinématiques caractérisant la locomotion résident principalement dans l'interférence entre des mécanismes d'ordre ontogénique et les réponses comportementales. En effet, les différences de réponses entre animaux sauvages et domestiqués se réduisent avec l'âge. En comparant les deux types de larves au cours du temps, nous souhaitions évaluer l'influence du temps de captivité sur la réponse de fuite et l'activité de nage, les larves domestiquées ayant été suivies en tant que témoins. Or, les résultats ont montré une diminution des taux de réponse de fuite au cours du temps chez les larves des deux origines. De la même façon, les différences entre les vitesses angulaires, les vitesses moyennes et les indices de récupération s'amenuisent entre les individus des deux origines entre le début et la fin de l'étude. Le temps de captivité et des modifications d'ordre ontogénique pourraient donc intervenir simultanément pour moduler l'expression des réponses comportementales. En constatant l'absence de différences significatives au niveau de l'activité de nage entre des lignées domestiqués et sauvages, Millot et al. (2009a) avait déjà émis l'hypothèse qu'une période de 20 mois d'élevage pourrait être suffisante pour obtenir des bars possédant les mêmes caractéristiques que ceux issus d'une seconde génération de sélection.

Il faut également souligner les limites expérimentales de nos tests cognitifs en labyrinthe puisqu'ils n'ont pas permis de trancher avec certitude sur les capacités d'apprentissage. Nous pensons que cela incombe (i) au nombre relativement faible de sessions réalisées par rapport à ce qui a pu être effectué sur d'autres espèces moins sensibles aux manipulations et au stress induit (Alves et al., 2007; Sovrano & Bisazza, 2008; Siebeck et al., 2009), mais également (ii) à l'existence de différences individuelles au niveau des capacités d'apprentissage. Certains individus semblent en effet, associer correctement le symbole adéquat à la récompense alors que d'autres semblent choisir volontairement le côté opposé.

### *Les applications*

Les exploitations envisagées pour la truite précédemment peuvent être listées également pour le bar, *i.e.* possibilité de tester l'aptitude au milieu naturel ou aux conditions d'élevage à l'aide de challenges réalisés en laboratoire, et utilisation potentielle des résultats pour des programmes de repeuplement et/ou de domestication moyennant l'automatisation des tests. En l'occurrence, nos résultats suggèrent qu'on pourrait considérer qu'un animal est apte à l'élevage s'il présente une faible réactivité au stimulus mécanique et visuel, une plus faible complexité de nage, *i.e.* une vitesse angulaire basse, une vitesse moyenne et une distance totale parcourue élevée (par comparaison avec des congénères sauvages).

Chez le bar cependant, l'existence d'une forte variabilité individuelle pour la plupart des variables mesurées (sans doute liée à la domestication récente de cette espèce par rapport à la truite) permet d'envisager d'autres possibilités. En effet, les populations domestiquées depuis 5-6 générations, abritent encore des individus présentant des caractéristiques sauvages (à l'âge de deux mois) qui pourraient donc être réservés pour le repeuplement.

### *Les apports conceptuels*

Nos résultats complètent ceux de Millot et al. (2009a et 2009b) qui fournissaient des indicateurs du processus de domestication et de sélection liés, eux aussi, à la réponse de fuite et la prise de risque chez le bar à des stades de développement plus tardifs et en situation isolée ou de groupe. Le niveau de récupération incomplet, que ces auteurs mesurent après l'application du stress, est une tendance que nous retrouvons dans le présent travail.

Nous confirmons également une complexité de nage plus faible chez les populations domestiquées en liaison avec une diminution du seuil de vigilance (Bégout & Lagardère, 2004). L'éloignement important au point de chute du stimulus qui caractérise les animaux sauvages, même après deux mois de captivité, vient renforcer cette hypothèse et semble aller dans le sens d'une érosion de la réponse anti-prédateur au cours de la domestication déjà démontrée chez plusieurs espèces en élevage (Nordeide & Svasand, 1990; Johnsson & Abrahams, 1991; Einum & Fleming, 1997; Fernö & Järvi, 1998; Arai et al., 2007).

Si les analyses portant sur le grégarisme ou les capacités cognitives en labyrinthe ne se sont pas avérées discriminantes, elles révèlent néanmoins un certain nombre de points particuliers du comportement de cette espèce. Par exemple, une attraction positive vers un congénère non-familier tout à fait similaire chez les individus domestiqués et les individus sauvages, traduit un comportement grégaire qui a été décrit en milieu naturel au stade juvénile (cf. Barnabé, 1978) et qui ne semble pas affecté par la domestication. Cette expérience montre également qu'une stimulation de nature strictement visuelle est suffisante pour obtenir ce résultat. Autre fait intéressant : l'importante variabilité individuelle que nous décrivons (individus passant la majorité du temps près du congénère, individus se réfugiant dans la zone de démarrage, individus situés à l'opposé du congénère, mais orientés vers lui), suggère l'existence de traits de personnalité qui reste cependant à vérifier à l'aide de protocoles expérimentaux plus adaptés. Enfin, l'expérience réalisée sur la cognition semble montrer que certains individus sont capables d'associer un symbole à une récompense, le symbole en question étant un objet en 2 dimensions qui ne fait référence à aucun repère environnemental familier.

### *Conclusions*

**Chez la truite comme chez le bar**, il est possible de discriminer les individus sauvages, des individus domestiqués par leur comportement dès le plus jeune âge et avec des **indicateurs identiques liés à l'activité de nage**. Ce sont des variables cinématiques comme la vitesse angulaire, la distance totale parcourue et la vitesse moyenne qui évoluent dans le même sens. Les leviers pertinents pour mettre en évidence ces comportements sont la stimulation lumineuse pour la truite, et les stimulations mécanique et visuelle ou les challenges en labyrinthe pour le bar.

# Conclusions et perspectives

La pisciculture reste une filière très récente par rapport aux élevages d'animaux terrestres offrant ainsi de multiples perspectives de recherche sur le processus de domestication car chez les téléostéens, la différenciation entre populations sauvages et domestiqués est encore dans une phase de transition (Dobney & Larson, 2006).

Dans ce vaste champ d'étude, cette thèse n'a qu'effleuré le problème de **l'adaptation à l'élevage** de quelques téléostéens **par le biais de la plasticité comportementale** en réponse à quelques facteurs biotiques et abiotiques. Il reste évident que la compréhension approfondie des mécanismes qui sous-tendent la plasticité comportementale, véritable levier adaptatif des poissons en élevage, passera par une poursuite de l'étude d'autres facteurs abiotiques et biotiques et de leurs interactions. Il n'en demeure pas moins que **l'approche comportementale s'est avérée pertinente** car elle a permis, à l'aide de méthodes non-invasives, de caractériser l'adaptation et de la relier à d'autres indicateurs (morphologie, performances de croissance, physiologie). Les résultats qui ont été obtenus ici peuvent également déjà permettre de contribuer à l'amélioration zootechnique et au bien-être des espèces étudiées (effet refuge, taille de l'œuf et environnement social, influence du tri et du jeûne, influence de l'aliment végétal). **En termes de perspective, il serait cependant important d'étudier l'impact des différences précoces observées dans des expériences sur les stades plus tardifs**. Par exemple, les conséquences tardives des gains de croissance et de survie lorsqu'on utilise un refuge artificiel chez les juvéniles d'omble chevalier ou les conséquences de l'utilisation d'un aliment végétal sur le comportement du bar adulte ou entrant en reproduction. Dans ce dernier cas, la connaissance des conséquences sur la physiologie de l'animal, juste effleurées dans nos expériences, comme la cinétique du cortisol plasmatique en réponse à un stress standardisé et le niveau basal des animaux nourris avec l'aliment végétal, serait sans nul doute d'une grande utilité.

Notre travail tente également de donner quelques clés pour discriminer, par leur comportement, les populations sauvages et domestiquées et **les différentes démarches développées ont permis de cerner une partie du processus de domestication, d'ouvrir de nouvelles perspectives de recherche, et de soulever certaines difficultés méthodologiques.** Nous en résumons ici les points forts.

### *La compréhension du processus de domestication*

Toutes les expériences ont montré des différences, mais aussi de grandes similitudes au niveau des réponses comportementales entre les populations sauvages et domestiquées des espèces testées, mais aussi entre les espèces testées. C'est pourquoi, à l'instar de Balon (2004) nous pensons qu'il serait préférable de parler pour la plupart des téléostéens d'élevage, d'**espèces captives exploitées** plutôt que domestiquées.

Notre travail semble confirmer l'existence de traits de personnalité associés à l'activité de manipulation du dispositif d'auto-alimentation. En termes de perspective, il serait intéressant de voir s'il existe des corrélations entre ce type de trait de personnalité et d'autres traits comportementaux en situation de challenge. D'une manière générale, nous recommandons de combiner des tests en situation d'isolement et de groupe car nous avons confirmé l'importance de l'environnement social sur plusieurs types de réponses comportementales (mobilité et comportement alimentaire). L'étude du comportement d'auto-alimentation en groupe nous semble d'autant plus pertinente que les outils actuels d'analyse cinématique des variables caractérisant la locomotion (logiciel Ethovision) sont essentiellement adaptés à l'approche individuelle et restent largement perfectibles en situation de groupe (en particulier pour des individus de petite taille).

### *Les approches méthodologiques*

Comme cela avait été réalisé chez le bar précédemment en comparant des réponses comportementales (Millot et al., 2009 a&b) et physiologiques (Luna Acosta et al., 2011) entre populations de composition génétique connue, ici, une nouvelle approche méthodologique, nous a permis de mettre en évidence une variabilité d'ordre génétique pour les différences comportementales chez la truite commune. Cette expérience constitue une première tentative de différentiation de populations sauvages et domestiquées rendue possible grâce à un matériel biologique original. Ainsi, des zones indemnes de repeuplement identifiées par l'étude des polymorphismes moléculaires de populations domestiques de repeuplement et de populations naturelles de Haute Savoie, ont permis à l'INRA de reconstituer des souches sauvages et de les comparer à des souches domestiquées dans un plan de croisement maximisant les effectifs génétiques. La compréhension du processus de domestication passe clairement par la poursuite de ce genre d'études, **l'approche comportementale en situation de challenge s'étant révélée pertinente pour différentier les populations sauvages et domestiquées** et le génotypage, essentiel pour contrôler l'origine des téléostéens observés.

Cette thèse souligne aussi l'importance d'utiliser des indicateurs physiologiques en complément des indicateurs comportementaux. Cette approche n'a pas été menée de façon systématique dans cette thèse, mais elle a contribué, par exemple, à montrer que les manipulateurs principaux ne semblent pas différer des autres individus du groupe au niveau de leurs paramètres sanguins. Il nous semble également important de compléter les mesures ponctuelles d'indicateurs tels que le cortisol par des approches cinétiques (*e.g.* Di Marco et al., 2008; Saera-Vila et al., 2009) qui pourraient révéler des différences significatives entre les individus en terme de temps de retour à un niveau basal. Cela nécessite par conséquent de mesurer systématiquement le niveau basal des individus testés.

### *Les dispositifs expérimentaux*

Le dispositif réalisé pour déclencher une réponse de fuite chez les larves de bar a permis d'obtenir des résultats très encourageants dans la perspective de développer des outils de sélection. Cette procédure de challenge, ainsi que toutes les expériences réalisées en labyrinthe, permettent d'identifier rapidement une variabilité au niveau des réponses comportementales qui laisse en effet envisager la possibilité de développer des méthodes de sélection précoces pour l'élevage ou le repeuplement moyennant une future automatisation. Ces expériences contribueront aussi au développement d'une **procédure multicritère, nécessaire pour identifier sans ambigüité les traits de personnalité** des téléostéens.

Les tests en labyrinthe réalisés pour l'étude de la cognition ne constituent qu'une approche préliminaire. Il serait utile de poursuivre ces travaux en développant de nouveaux dispositifs expérimentaux permettant de multiplier les sessions d'apprentissage tout en en réduisant les effets du stress sur les individus testés. Dans la perspective de futurs challenges à tester, ils fournissent cependant des données utiles sur l'activité de nage et l'exploration dans un environnement nouveau, et à des âges différents. Les variables à retenir sont, de toute évidence, la vitesse moyenne, la vitesse angulaire absolue, la distance totale parcourue et la mobilité qui se sont avérées pertinentes pour discriminer des populations sauvages et domestiquées chez la truite et le bar. Les tests en labyrinthe réalisés pour l'étude de la familiarité et du grégarisme fournissent, quant à eux, des indications importantes pour élaborer des challenges ou des expériences cognitives faisant intervenir une récompense basée sur des signaux strictement visuels et ils pourraient être complétés par des expériences permettant de déterminer les rôles respectifs et combinés des signaux visuels et olfactifs.

### *L’avenir de ces recherches*

L’objectif fondamental de tous ces travaux sera de contribuer à une meilleure compréhension des bases mécanistiques (comportementales, neurophysiologiques, physiologiques, et génomiques) des réponses adaptatives des téléostéens. L’objectif appliqué lui, sera d’utiliser cette connaissance pour développer des programmes de sélection axés sur une meilleure adaptation à l’élevage. Cela nécessitera évidemment (i) l’évaluation de la variabilité familiale des traits de personnalité en s’appuyant sur le test précoce le plus discriminant et le plus apte à être mesuré sur un grand nombre d’individus, et (ii) l’estimation de leur héritabilité et corrélations génétiques. L’intervalle de génération de la truite ou du bar étant long (3 ans environ), il sera sans doute judicieux pour réaliser ces travaux, d’utiliser des modèles biologiques complémentaires à cycle de vie court tel que le poisson zèbre (*Danio rerio*), espèce couramment utilisée comme modèle en développement, génétique et en analyse comportementale (Miklósi & Andrew, 2006; Norton & Bally-Cuif, 2010). Une partie de ces travaux est déjà engagée depuis 2011 dans les laboratoires Ifremer de l’Houmeau et de Palavas dans le cadre d’un projet européen (COPEWELL ‘A new integrative framework for the study of fish welfare based on the concepts of allostasis, appraisal and coping styles’). Ils consisteront à caractériser les traits de personnalité en soumettant les individus à plusieurs challenges en situation isolée et à différents âges. Une situation de groupe associée au challenge d’apprentissage sur dispositif d’auto-alimentation, est également envisagée de même que l’analyse des corrélations entre traits comportementaux et physiologiques (indicateurs de stress et divers paramètres sanguins).

En conclusion, si tout le monde s’accorde sur le fait que le succès de la domestication et de la sélection des téléostéens passe par une bonne adaptation des animaux aux conditions d’élevage, la notion d’adaptation, elle, est particulièrement difficile à appréhender de façon globale. Elle peut en effet revêtir de nombreux aspects différents selon les espèces et les conditions d’élevage. A titre d’illustration, si la sélection de certains caractères comportementaux et physiologiques peut favoriser une tolérance ou une récupération accrue à certains types de stress, cette forte plasticité phénotypique ne concernera que les types de stress ciblés et ne permettra pas de conduire à une domestication universelle pour tous les systèmes d’élevage existants en raison de l’importante diversité des sites (Vandeputte & Launey, 2004). Le graal ultime pour parvenir à sélectionner les capacités d’adaptation à l’élevage passera donc par la mise en évidence d’un caractère intégrateur global d’adaptation à tout type de conditions d’élevage et nous sommes persuadés que les indicateurs comportementaux (en particulier les traits de personnalité) pourraient répondre à ce défi. Nous plaidons donc en faveur de la poursuite de l’effort de caractérisation des traits comportementaux, de leur variabilité et de leur héritabilité.

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# Abstract

The farming of aquatic animals has started recently in comparison with that of terrestrial species which domestication started 10 500 years ago. Aquaculture is however the fastest growing animal food-production sector. This sector has now to face several challenges threatening its sustainability. Indeed, aquaculture future largely depends upon the reduction of the reliance on feeds based ingredients derived from wild fishery resources, upon a better knowledge of fish domestication process and of the adaptation mechanisms and related welfare potential.

The present work aims at a better understanding of the adaptation to farming conditions through a behavioural approach based on the comparison between wild *vs.* domesticated populations of three species: a marine species, sea bass (*Dicentrarchus labrax*), two freshwater species, arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*). The thesis is divided into two parts which objectives were : 1) to test the impact of biotic and abiotic factors selected based on their importance in aquaculture (shelter, plant-based diet, egg size and social environment, size grading, and fasting effects) on sea bass and artic charr behavioural plasticity (behavioural modifications in response to environmental changes). Behavioural indicators (feeding behaviour, mobility, swimming activity, exploration, personality traits) were investigated and completed by the analysis of physiological variables (blood parameters) and growth performances. 2) to characterize the domestication effect and the interplay of aging and ontogeny on behaviour (swimming activity, escape response rate, exploration and cognition) through the comparison between wild *vs.* domesticated sea bass and brown trout at different ages and under different experimental conditions (challenges, maze).

In the first part, experiments performed on arctic charr showed that an artificial shelter (abiotic factor) induced a lower mobility, better growth performances and a higher level of survival during the endogenous feeding period, that egg size and social environment play an important role on feeding behaviour and mobility during the weeks following the first exogenous feeding period. These results illustrated behavioural flexibility in response to abiotic and biotic factors. The findings could be used to improve culture performances and welfare of arctic charr. The experiments performed on sea bass showed that a plant-based diet do neither modify the learning abilities under self-feeding conditions nor the cognition under a test situation in a maze, but has an impact on swimming activity (the mean velocity decreased and the total distance travelled decreased) and on the short-term release of cortisol (plasmatic concentration lower than the control group). Another experiment showed that, under self-feeding conditions, size grading and the resulting social environment have no major influence on feed-demand, feeding rhythm, food wastage, social structure, growth performances and physiological status. At last, a three-week fasting period does not modify the social structure and high-triggering fish are exactly the same individuals before and after the fasting period. Individual's triggering activity levels could therefore be considered as personality traits but further research is needed to verify this hypothesis.

In the second part of the thesis, the experiment performed on brown trout showed an impact of domestication on swimming activity before and after a light-elicited stress, using a biological material controlled by genotyping. Angular velocity was higher in wild fish while mean velocity and total distance travelled were higher in domesticated fish. All the experiments carried out on sea bass compared wild-caught fish (from the larval stage) to domesticated fish (industrial standard). The first one showed the interplay of age and ontogeny on swimming activity and escape response rate. These variables decreased in fish from both origins (from D63 to D125). The impact of domestication was shown through differences between wild-caught and domesticated larvae (angular velocity and vigilance level higher in wild fish). These differences were often observed at later stages in the three next experiments performed in a maze. They showed that domestication has no influence on gregarism, and cognition in sea bass but there was a high individual variability. The ability to discriminate familiarity was shown only in domesticated fish.

The behavioural approach developed in this thesis was demonstrated to be relevant to reveal plasticity in response to a few abiotic and biotic factors among the numerous ones that remain to be investigated. Plasticity plays an important role in the adaptation of fish to culture conditions. Domestication impact on behaviour was also demonstrated as well as the interplay of age and ontogeny. This thesis followed the path and brings new insights for research programs targeting fish selection based upon personality traits in order to improve adaptation for aquaculture or restocking purposes.

**Mots clés :** adaptation, plasticity, learning, domestication, welfare, feeding behaviour, swimming activity, personality, plant-based diet, maze, physiological status, growth, aquaculture, restocking.

# Résumé

L'élevage des espèces aquatiques est une activité très récente par rapport à celle des espèces terrestres qui ont été domestiquées il y a 10 500 ans. Depuis quelques années, l'aquaculture est cependant le secteur agroalimentaire qui connaît la plus forte expansion. Ce développement rapide se heurte à de nombreux problèmes qui menacent la durabilité de ce secteur. Entre autres, il s'agit de la dépendance vis-à-vis des ressources marines sauvages pour la formulation des aliments, du faible niveau de domestication des espèces marines, d'une connaissance partielle des capacités d'adaptation et du potentiel de bien-être des téléostéens en élevage. Ce travail vise une meilleure compréhension de l'adaptation aux conditions d'élevage par une approche comportementale basée sur la comparaison de diverses populations de téléostéens (domestiquées et sauvages) pour trois espèces, l'une marine, le bar (*Dicentrarchus labrax),* les deux autre d'eau douce, l'omble chevalier (*Salvelinus alpinus*) et la truite commune (*Salmo trutta*). La thèse s'articule en deux parties qui ont permis : 1) de tester des facteurs biotiques et abiotiques sélectionnés en fonction de leur importance en élevage (effet refuge, aliment végétal, taille de l'œuf et environnement social, pratique du tri, effet du jeûne) sur la plasticité comportementale (modifications comportementales en réponse aux changements de l'environnement) du bar et de l'omble chevalier. Des indicateurs comportementaux (comportement alimentaire, activité de nage, exploration, cognition, traits de personnalité) ont été recherchés et complétés par l'analyse de variables physiologiques (paramètres sanguins) et des performances d'élevage. 2) de caractériser les effets de la domestication, de la captivité et du développement sur le comportement (activité de nage, taux de réponse de fuite, exploration, cognition), par le biais d'une comparaison entre des bars ou des truites sauvages et domestiqués à des âges différents et dans des contextes variables (challenges, labyrinthe).

Dans la première partie, les travaux réalisés sur l'omble chevalier ont permis de montrer que la présence d'un refuge artificiel (facteur abiotique) durant la phase de nutrition endogène induisait une forte diminution de la mobilité accompagnée de meilleures performances de croissance et d'une survie plus importante, que la taille de l'œuf et l'environnement social jouaient un rôle important sur le comportement alimentaire et la mobilité des juvéniles dans les semaines qui suivent la première nutrition exogène. Ces résultats, illustrant des cas de flexibilité comportementale en réponse à des facteurs biotiques et abiotiques, pourraient permettre des améliorations zootechniques et potentiellement d'améliorer le bien-être de cette espèce. Les travaux réalisés sur le bar ont montré qu'un aliment végétal ne modifiait pas les capacités d'apprentissage en condition d'auto-alimentation ni leurs capacités cognitives en situation de test en labyrinthe, mais qu'il semble avoir un impact sur l'activité de nage (réduction de la vitesse moyenne et de la distance parcourue) et sur la libération à court terme du cortisol (concentration plasmatique plus faible que le lot témoin). Une autre expérience a montré que la pratique du tri et l'environnement social qui en résulte, en conditions d'auto-alimentation, a peu d'influence sur la demande, le rythme, le gaspillage alimentaires, la structure sociale, les performances de croissance et le statut physiologique. Une période de trois semaines de jeûne, enfin, ne modifie pas la structure sociale et les manipulateurs principaux sont les mêmes avant et après cette période, ce qui pourrait être lié à l'existence de traits de personnalité basés sur le pourcentage d'activation de la tige tactile du dispositif d'auto-alimentation.

Dans la seconde partie de la thèse, l'expérience sur la truite a permis de mettre en évidence un effet de la domestication sur l'activité de nage avant et après application d'un stress lumineux, en utilisant un matériel biologique contrôlé par génotypage. La vitesse angulaire est plus élevée chez les individus sauvages alors que la vitesse moyenne et la distance parcourue sont plus importantes chez les individus domestiqués. Les expériences réalisées sur le bar ont comparé des populations sauvages capturées à l'état larvaire dans le milieu naturel à des populations standard industrielles. La première a montré l'influence combinée de l'âge et de mécanismes d'ordre ontogénique sur l'activité de nage et le taux de réponse de fuite. Ces paramètres ont en effet tendance à diminuer chez les deux catégories de larve (J63 à J125). L'impact de la domestication se traduit par des différences entre individus sauvages et domestiquées (vitesse angulaire et vigilance plus élevés chez les individus sauvages). Ces différences au niveau de l'activité de nage seront souvent observées dans les expériences suivantes réalisées à un stade de développement ultérieur en situation de test en labyrinthe. Les trois expériences concernées montrent aussi que la domestication ne semble pas avoir d'influence sur le grégarisme et la cognition chez le bar, mais elles révèlent une forte variabilité individuelle alors que la discrimination de la familiarité n'a pu être mise en évidence que chez les individus domestiqués.

L'approche comportementale développée dans cette thèse s'est avérée pertinente pour révéler une plasticité en réponse à quelques facteurs abiotiques et abiotiques parmi les nombreux autres qui restent à déterminer. Cette plasticité constitue un véritable levier adaptatif pour les téléostéens en élevage. L'effet de la domestication sur le comportement a également été démontré ainsi que l'influence de l'âge et des mécanismes ontogéniques dont la nature reste à déterminer. Cette thèse s'inscrit dans la lignée des travaux qui vise à sélectionner les téléostéens sur leurs traits de personnalité dans l'optique d'une adaptation optimale en élevage ou dans la perspective de programmes de repeuplement.

**Mots clés :** adaptation, plasticité, domestication, bien-être, comportement alimentaire, activité de nage, personnalité, aliment végétal, labyrinthe, statut physiologique, croissance, aquaculture, repeuplement.