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Frédéric Bertucci

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THESE

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par Frédéric BERTUCCI

**Communication acoustique chez un poisson Cichlidé**  
**Analyse expérimentale du rôle et de la structure des signaux**



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**COMMUNICATION ACOUSTIQUE CHEZ UN  
POISSON CICHLIDE**

ANALYSE EXPERIMENTALE DU ROLE ET DE LA STRUCTURE DES SIGNAUX

**ACOUSTIC COMMUNICATION IN A CICHLID FISH**

EXPERIMENTAL ANALYSIS OF THE ROLE AND THE STRUCTURE OF SIGNALS

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# **Communication acoustique chez un poisson Cichlidé**

**Analyse expérimentale du rôle et de la structure des signaux**



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# **INTRODUCTION**

# I. INTRODUCTION

## A. Interactions sociales et communication chez les poissons

### 1. Structures sociales

Chez les poissons, les structures sociales vont du banc, défini comme un regroupement social plus ou moins temporaire de nombreux individus à nage polarisée et synchronisée (Hoare & Krause, 2003), à des regroupements plus réduits et plus stables, voire à des individus solitaires comme chez le brochet (*Esox lucius*) (Eklöv, 1997). Si un banc ne présente généralement pas de système de dominance visible, les groupes sociaux de nombreuses espèces s'organisent selon une hiérarchie sociale (Jameson et al., 1999). On peut trouver des hiérarchies linéaires avec des individus "alpha" dominant tous les autres membres du groupe, suivis par des "beta" dominant les autres membres à l'exception des "alpha", et ainsi de suite jusqu'aux rangs les plus inférieurs composés d'individus complètement dominés. C'est le cas chez le tilapia du Mozambique (*Oreochromis mossambicus*) chez lequel les individus "alpha" sont habituellement des mâles et des individus de grande taille alors que les femelles, plus petites, occupent les rangs les plus inférieurs (Oliveira & Almada, 1996).

Les hiérarchies sont généralement le reflet des compétences individuelles, basées sur l'évaluation d'indices comme la taille ou l'expérience passée. Bien que faisant appel à des interactions agressives, la dominance ne correspond pas forcément au niveau d'agressivité d'un individu. Le genre, l'âge et le statut social sont autant de facteurs influençant l'organisation sociale. Chez le cichlidé jonquille (*Neolamprologus pulcher*) par exemple, le groupe se compose d'un couple reproducteur dominant, et d'une quinzaine d'individus subordonnés, de taille et de sexe différents. Ces derniers aident le couple reproducteur en assurant diverses tâches comme la défense du territoire ou l'élevage des jeunes (Bruitjes & Taborsky, 2008), leur spécialisation pour accomplir telle ou telle tâche étant fonction de leur taille et leur statut (Desjardins et al., 2008a ; Bruitjes & Taborsky, 2011).

L'acquisition et la sauvegarde d'un statut social (ou de dominance) assurent un accès privilégié aux ressources telles que la nourriture ou les partenaires sexuels. Les individus dominants sont également matures plus tôt, ce qui augmente leur succès reproducteur (Gagliardi-Seeley et al., 2009 ; Oliveira et al., 2000).

### 2. La territorialité

Chez certaines espèces, les interactions sociales conduisent à l'espacement des individus, créant des territoires, définis comme des zones plus ou moins exclusives défendues par un individu ou un groupe (Davies & Houston, 1984). Ces zones ont des limites dynamiques qui

peuvent se modifier suivant les saisons, la densité de la population ou les interactions entre les individus. Chez le poisson combattant du Siam (*Betta splendens*), chaque mâle possède un territoire centré autour d'un nid distant d'environ 1 mètre du nid voisin (Jaroensutasinee & Jaroensutasinee, 2001). Les disputes agressives entre voisins le long des frontières de territoires sont donc fréquentes (Jaroensutasinee & Jaroensutasinee 2003).

Défendre son territoire peut être coûteux en termes de temps et d'énergie. Une fois les limites territoriales établies, un individu résident devient généralement moins agressif envers ses voisins familiers (Leiser & Itzkowitz, 1999). C'est le cas par exemple chez le cichlidé jonquille qui ajuste son comportement en patrouillant plus souvent autour des frontières de territoires et en se montrant plus agressif en présence d'individus inconnus que face à des individus familiers (Frostman & Sherman, 2004). L'effort nécessaire à la défense d'un territoire augmente également avec la taille de ce dernier. Il est donc parfois plus avantageux de partager ce territoire avec d'autres individus afin de diviser l'effort entre les membres du groupe. Les co-occupants ont un accès aux ressources mais devront être tolérés par le propriétaire, le dominant s'ils participent au maintien du territoire, à la reproduction ou aux soins des jeunes (Bruitjes & Taborsky, 2008).

### **3. Cognition et reconnaissance individuelle chez les poissons**

Afin qu'un groupe social soit stable, les individus qui le composent doivent être capables de juger de la position hiérarchique de leurs congénères et de les reconnaître. La cognition est l'ensemble des processus allant de la perception d'un stimulus, la reconnaissance, l'apprentissage et la mémorisation d'une information permettant la mise en place de comportements sociaux tels que la discrimination entre des individus familiers et étrangers, l'attribution d'aptitudes au combat ou la recherche des points de nourriture (Adolphs, 2001 ; Shettleworth, 2001, Brown & Braithwaite, 2005).

Chez les espèces grégaires, la reconnaissance de ses voisins ou partenaires est essentiel à la structuration d'un banc ou d'un territoire et intervient dans de nombreux comportements sociaux comme le choix du partenaire, le partage de nourriture ou la prédation (Griffiths, 2003). Ce processus se fait à deux niveaux : une reconnaissance sociale et une reconnaissance individuelle. La reconnaissance sociale permet la distinction de congénères à partir de traits généraux alors que la reconnaissance individuelle fait appel à une mémoire des caractéristiques individuelles comme la taille ou la couleur (Gheusi et al., 1994 ; Dale et al., 2001).

Les associations d'individus et la structuration d'un groupe se font généralement sur la base d'interactions sociales répétées entre des individus qui deviennent ainsi "familiers" et

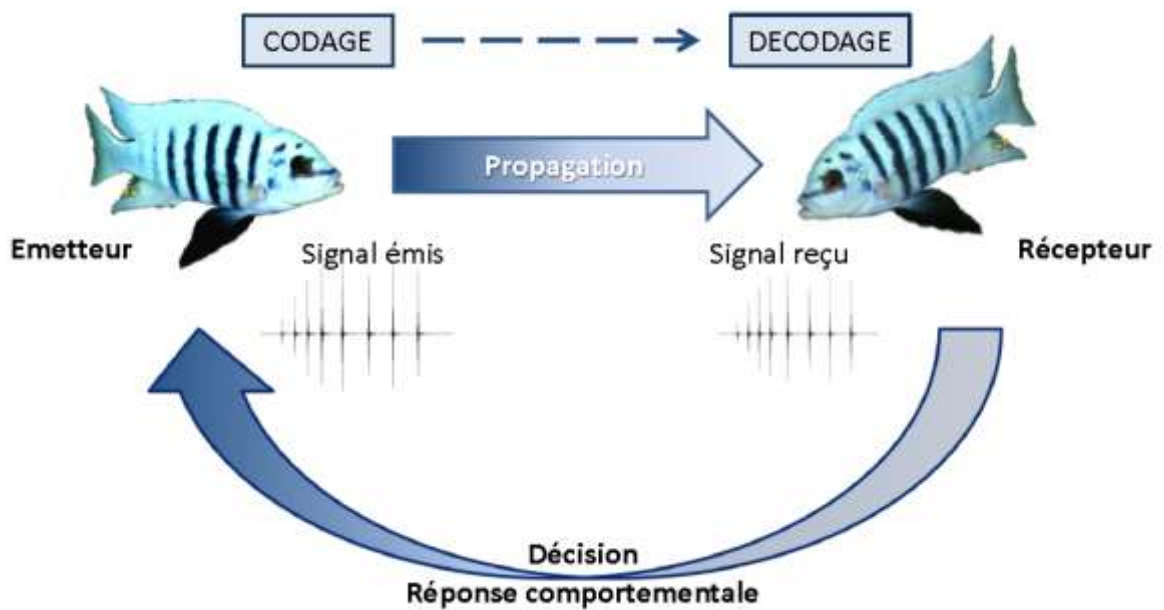
acquièrent des expériences mutuelles (Griffiths, 2003 ; Lee-Jenkins & Godin, 2010). Ces associations confèrent de nombreux avantages, en particulier une cohésion du groupe plus forte qui améliore la réponse anti-prédateur (Griffiths et al., 2004), une coopération accrue dans la recherche de nourriture (Ward & Hart, 2005) ou une stabilisation des hiérarchies en réduisant l'agressivité entre les membres d'un groupe (Höjesjö et al., 1998).

Les interactions au sein d'un groupe favorisent également le transfert d'informations entre les individus et donc l'apprentissage de nouveaux comportements (par imitation par exemple). Cette capacité d'apprentissage social sera un avantage pour s'adapter à une situation nouvelle et réduira également les coûts associés à l'apprentissage d'une tâche (Giraldeau et al., 1994). Cet apprentissage se retrouve pour de nombreux comportements comme le choix du partenaire ou la recherche de nourriture (Brown & Laland, 2003). Ainsi, chez le guppy (*Poecilia reticulata*), des individus observateurs trouvent et apprennent leur chemin vers une source de nourriture plus rapidement en suivant des individus familiers (Swaney et al., 2001).

Les comportements sociaux impliqués dans le regroupement d'individus et/ou la séparation de l'espace en territoires ainsi que la reconnaissance des individus entre eux impliquent des échanges d'information -une communication- entre les différents protagonistes (e.g. Amorim & Neves, 2008).

#### **4. Les canaux de communication**

La communication correspond à un transfert d'information via un signal. Ce processus implique des étapes successives de production, transmission et réception du signal entre au moins deux individus, l'émetteur et le récepteur (figure 1). Les poissons utilisent divers canaux de communication. L'utilisation de tel ou tel canal est déterminée par différents facteurs comme les capacités de production de l'individu émetteur, l'information à transmettre, le destinataire du message ou encore l'environnement à travers lequel le signal sera transmis. Chaque canal de communication a des caractéristiques propres, susceptibles de lui conférer divers avantages et inconvénients (tableau 1). De plus, un émetteur peut ajuster la transmission d'une information afin de s'adapter à des conditions environnementales changeantes en augmentant l'intensité d'un signal acoustique dans un milieu bruyant (Egnor & Hauser, 2006) ou encore en changeant la durée de signalisation (Brumm & Slater, 2006).



**Figure 1 – Chaîne de transmission de l'information.**

*Un signal codant de l'information est échangé entre un individu émetteur et un récepteur. Durant sa transmission, le signal subira des modifications diminuant la qualité de l'information portée. Le récepteur décodera le signal reçu afin d'extraire l'information et répondre à l'aide d'un comportement approprié.*

**Information transmission chain.**

*A signal encoding an information is exchanged between an emitting individual and a receiver. During transmission, the signal will undergo modification which will decrease the quality of the carried information. The receiver will decode the received signal in order to extract and respond with an appropriate behaviour.*

De nombreux animaux, dont les poissons, utilisent souvent plusieurs canaux de communication de manière concomitante. L'information est alors portée par des signaux de différentes modalités sensorielles. On parle alors de communication multimodale (Partan & Marler, 1999, Gordon & Uetz, 2011). On observe ainsi des interactions durant lesquelles interviennent signaux acoustiques et visuels comme chez les mâles *Tramitichromis intermedius* qui produisent de plus en plus de sons en association avec des tremblements de leur corps au moment de la ponte (Ripley & Lobel, 2004), ou signaux visuels et chimiques lors de la parade sexuel chez le blennie paon (*Salaria pavo*) (Gonçalves et al., 2002).

Caractéristiques du canal	Type de canal			
	Chimique	Acoustique	Visuel	Tactile
Portée	Longue	Long	Moyenne	Courte
Vitesse de changement	Lente	Rapide	Rapide	Rapide
Passage d'obstacles	Bon	Bon	Mauvais	Mauvais
Localisation	Variable	Moyenne	Forte	Forte
Coût énergétique	Bas	Elevé	Bas	Bas

**Tableau 1 – Caractéristiques de différents canaux de communication.**

(Extrait de Krebs & Davies, 1993; d'après Alcock 1984).

**Features of different communication channels.**

(Extracted from Krebs & Davies, 1983, after Alcock, 1984).

- **Le canal chimique** : les molécules (*phéromones*) utilisées par les poissons comme signal de communication sont solubles dans l'eau et diffusent donc facilement dans ce milieu. Le système olfactif des poissons, très sensible, détecte des doses de l'ordre du nano ou pico molaire, permettant une communication sur de longues distances (Hamdani & Døving, 2006). Au moment de l'ovulation, des phéromones femelles déclenchent le comportement reproducteur des mâles (Stacey et al., 2003). Des substances d'alarme peuvent également être secrétées par la peau suite à une blessure et informer les membres d'un groupe de la présence d'un prédateur (Wisenden & Sargent, 1997 ; Brown & Brennan, 2000). D'autres informations, ayant par exemple trait au statut social, peuvent aussi être échangées par voie chimique. Sur la base de ces signaux, un individu pourra donc repérer un partenaire (Serrano et al., 2008), mais aussi potentiellement discriminer entre différents statuts sociaux, des membres de groupes différents (McLennan & Ryan, 1997 ; McLennan & Ryan, 1999),

voire les individus de son groupe social (Giaquinto & Volpato, 1997 ; Wisenden & Dye, 2009).

Chez les cichlidés, les signaux chimiques sont surtout employés comme indicateurs du statut social (Almeida et al., 2005) et la reconnaissance des congénères (Giaquinto & Volpato, 1997).

- **Le canal électrique** : si seulement 1.5% des poissons produisent des signaux électriques, la plupart des espèces sont capables de les percevoir. Alors que des poissons tels que les anguilles produisent de fortes décharges électriques dans le but de se défendre ou d'attaquer, quelques groupes utilisent de faibles décharges servant à la communication intra-spécifique (Lorenzo et al., 2006 ; Moller, 2006 ; Scheffel & Kramer, 2006). Des études récentes montrent que ces signaux électriques peuvent renseigner sur le statut social des individus (Fugère et al., 2011) et être impliqués dans la sélection d'un partenaire (Curtis & Stoddard, 2003).
  
- **Le canal visuel** : chez les poissons, la vision est l'un des canaux de communication les plus utilisés. Les patterns de couleur observés sont produits à partir de cellules à pigments spécialisées : les chromatophores (tableau 2). A l'intérieur de ces cellules, les organelles contenant les pigments ont une forte motilité permettant d' "activer" ou de "désactiver" certaines couleurs. Certains chromatophores sont sous contrôle hormonal et permettent des modifications de couleurs à long terme (plusieurs semaines). D'autres sont sous contrôle nerveux permettant des modifications beaucoup plus rapides (inférieurs à la seconde). Ces changements de robes peuvent exprimer le statut social, l'état d'agressivité, la motivation ou l'état physiologique des individus (e.g. Kodric-Brown, 1998 ; O'Connor et al., 2000 ; Siebeck, 2004).

Type de chromatophores	Melanophores	Erythrophores	Xanthophores	Iridophores
Pigment	Mélanine	Ptéridines et Caroténoïdes		Guanine
Couleur	Noir / Brun	Rouge	Orange / Jaune	Bleu / Vert

**Tableau 2 – Principaux types de chromatophores rencontrés chez les poissons.**

(d'après Carleton et al., 2006).

**Main types of chromatophores of fishes.**

(after Carleton et al., 2006).



La plupart des poissons possèdent différents types de cônes pouvant détecter différentes longueurs d'ondes en fonction des propriétés d'absorption des pigments qu'ils contiennent (Hunt & Bowmaker, 2006). Les longueurs d'ondes disponibles pour la communication seront de plus dépendantes de nombreux facteurs environnementaux comme la turbidité, la profondeur ou le moment de la journée.

Les signaux colorés peuvent être associés à des comportements posturaux comme par exemple l'érection des nageoires (Jordan, 2008).

- **Le canal mécanique** : les poissons ont à leur disposition un système sensoriel recevant des stimuli hydromécaniques. Le moindre mouvement d'un poisson, d'un prédateur ou d'un objet dans le milieu produira un signal qui sera capté par des groupes de cellules ciliées localisés sur la peau ou à l'intérieur de canaux, sur la totalité du corps de l'animal et plus particulièrement le long de leur ligne latérale (Janssen & Strickler, 2006). Ces stimuli seront utiles dans la traque d'une proie par un prédateur (Hanke et al., 2000), ou pour coordonner les individus au sein d'un banc de poisson par exemple (Partridge, 1982).

Lors de combats ou durant les comportements de parade nuptiale, de nombreux poissons produisent des mouvements à courte distance l'un de l'autre, tels que des tremblements ou des "battements de queue". Ces comportements sont également détectables via la ligne latérale et peuvent permettre de juger en particulier de la taille d'un congénère. Notons que ces signaux peuvent également être détectés par l'oreille du poisson.

Un dernier canal de communication est l'acoustique. Les communications sonores des poissons demeurent mal connues et beaucoup reste à faire pour comprendre leur(s) rôle(s) biologique(s). Cette thèse leur est consacrée.

## **B. La communication acoustique chez les poissons**

Depuis le XIXe siècle, on connaît des centaines d'espèces de poissons produisant des sons lors d'interactions inter et intra-spécifiques (Müller, 1857). Cependant ce n'est que récemment que les mécanismes de production et de réception ainsi que le rôle biologique de ces sons ont commencé d'être étudiés.

### **1. Mécanismes de production et diversité des sons**

Les poissons présentent la plus grande diversité de mécanismes de production sonore rencontrée chez les vertébrés (Crawford & Huang, 1999 ; Parmentier et al., 2003 ; Connaughton, 2004, Longrie et al., 2009 ; Rice et al., 2011). Les muscles entourant la vessie

natatoire et les structures pectorales sont très souvent impliquées (Fine et al., 1997, Parmentier & Diogo, 2006). D'autres mécanismes plus rares sont basés sur les frottements des mâchoires pharyngiennes (Rice & Lobel, 2002), ou encore l'expulsion de bulles de gaz par l'anus (chez le hareng *Clupea harengus*, Wahlberg & Westerberg, 2003). Cette diversité des modalités de production se retrouve dans les types de sons émis par les poissons. (Amorim, 2006, Rice & Bass, 2009). Les sons produits par des muscles associés à la vessie natatoire sont généralement de basse fréquence (inférieures à 1kHz) et sous forme d'impulsions ("pulses"). Ces caractéristiques sont principalement dues à des contraintes morphologiques telles que la contractilité des muscles, déterminant la répétition des impulsions, et la structure de la vessie natatoire, déterminant la fréquence du son. Les sons produits à partir de structures pectorales ont aussi une forme pulsatile mais ont une fréquence plus haute (supérieure à 1kHz) (Ladich & Fine, 2006). Ces modes de production sonores permettent en particulier des variations du nombre de pulses, de leur intensité ainsi que de la fréquence des sons.

De nombreuses études rapportent des différences significatives dans les vocalisations au niveau interspécifique (Crawford et al., 1997a), parfois entre espèces très proches (Amorim et al., 2004a). Plus récemment, des variations au niveau intraspécifique ont été mises en évidence (Crawford et al., 1997a ; Amorim & Vasconcelos, 2008 ; Parmentier et al., 2010). Cette variabilité de vocalisations pourrait être la base d'une communication acoustique qui pourrait jouer un rôle très important dans la vie sociale des poissons.

## **2. Les contextes d'émission**

Bien que des sons puissent être produits par certains poissons dans des situations de détresse comme par exemple lors de captures (Ladich, 1997a ; Ladich & Myrberg, 2006), les signaux acoustiques sont essentiellement émis lors d'interactions intraspécifiques, principalement lors d'agressions entre mâles et durant la période de reproduction (Lugli & Torricelli, 1999 ; Myrberg & Lugli, 2006 ; Kierl & Johnston, 2010).

Un des contextes sociaux principaux donnant lieu à des interactions agressives entre individus conspécifiques, généralement mâles, est l'établissement d'un territoire et la défense de ressources telles que la nourriture ou des partenaires. Dans ce cas, les productions sonores interviennent dans les premières phases d'une dispute (Henglmüller & Ladich, 1999 ; Simoes et al., 2008) et permettent par exemple de chasser les intrus présents autour du territoire (Vasconcelos et al., 2010) avant de s'engager dans des combats physiques.

Les mâles peuvent également produire des sons afin d'attirer des femelles dans leur territoire au moment de la période de reproduction (Lugli et al., 1996a). Alors que certaines

espèces ne possèdent qu'un seul type de vocalisation de parade nuptiale, certains mormyridés possèdent un répertoire plus fourni (Crawford et al., 1997a, b). Ces productions vocales apparaissent durant les stades précoces des interactions, s'intensifient au fur et à mesure de l'approche d'une femelle et cessent après la ponte (Lugli et al., 1996a, b ; Crawford et al., 1997b). Ces productions sonores pourraient ainsi être utilisées par les femelles pour reconnaître leurs conspécifiques et ensuite choisir un partenaire avec qui s'accoupler (Verzijden et al., 2010).

### **3. Les informations transmises**

Les sons produits par les poissons renseignent d'abord sur l'identité spécifique. Pouvoir identifier ses congénères est important dans les cas où, lors de la période de reproduction, les individus d'une espèce sont entourés par d'autres poissons appartenant à des espèces sympatriques et génétiquement proches (Amorim et al., 2004a ; Amorim et al., 2008b ; Phillips & Johnston, 2009 ; Verzijden et al., 2010). Au moment de la reproduction, les sons produits ont également pour fonction de localiser l'émetteur, attirer et préparer le partenaire à la reproduction (Lugli & Torricelli, 1999 ; Ladich, 2007).

Durant un affrontement, les signaux produits peuvent renseigner les protagonistes sur leurs compétences au combat respectives, ce qui permet d'éviter des combats violents et coûteux (Krebs & Davies, 1993 ; Arnott & Elwood, 2009). La production de signaux acoustiques permet par exemple de juger de la motivation ou l'état physiologique d'un individu (Ladich, 1998 ; Amorim & Almada, 2005).

Chez de nombreuses espèces, il existe une corrélation négative entre la taille d'un individu et des paramètres spectraux du son. Des études récentes suggèrent que ces différences intraspécifiques permettent une reconnaissance acoustique individuelle (Myrberg et al., 1993 ; Amorim & Vasconcelos, 2008 ; Colley et al., 2009) et reflètent les qualités de l'individu émetteur (Amorim et al., 2010).

Ainsi, les productions sonores des poissons apparaissent susceptibles de porter des informations allant au-delà de la simple identité spécifique. Cependant, le nombre encore réduit d'études expérimentales ne permet pas d'en avoir une idée précise. En se focalisant sur une espèce de cichlidés, mes travaux cherchent à apporter une contribution à nos connaissances.

### **4. L'audition chez les poissons**

Les poissons possèdent des capacités auditives diverses dues aux nombreuses structures morphologiques impliquées dans l'audition. On peut diviser les poissons téléostéens en deux grands groupes : les spécialistes et les généralistes de l'audition. Les poissons généralistes,

dont font partie les cichlidés (e.g. Yan & Popper, 1993, Ripley et al., 2002), ne détectent les sons uniquement que par leur oreille interne. Leur sensibilité auditive se restreint depuis les basses fréquences jusqu'à quelques centaines de Hz. Les poissons spécialistes, comme les gouramis, possèdent des structures morphologiques spécialisées connectant l'oreille interne à des cavités remplies d'air à l'intérieur de leur corps qui amplifient les différences de pression améliorant leur sensibilité auditive jusqu'à plusieurs kHz (e.g. Ladich & Yan, 1998 ; Ladich, 1999 ; Wysocki, 2006).

Lors de communications acoustiques, l'information contenue dans les sons peut être codée par leur pattern temporel, leur fréquence ou leur intensité et ainsi permettre la reconnaissance spécifique ou individuelle (Marvit & Crawford, 2000a, b ; Bass & McKibben, 2003). Dans le milieu naturel, ces signaux se propagent sur des distances relativement courtes, i.e. quelques mètres chez le poisson électrique *Pollimyrus* (Crawford et al., 1997). La perception des variables temporelles semble être un facteur clé pour le décodage de l'information par les systèmes auditifs des poissons (Wysocki & Ladich, 2003). En effet, l'aspect temporel des sons est moins sujet aux altérations lors de leur transmission que des variables spectrales qui peuvent subir des modifications dues par exemple à des phénomènes d'absorption ou de réverbération. Cependant, la possibilité de combiner la perception de nombreuses caractéristiques acoustiques permet d'optimiser la détection des signaux et de juger de leur pertinence lors du processus de communication, en créant une image complexe du son.

## C. Les Cichlidés africains, modèles de radiation évolutive

### 1. Intérêt évolutif

Les cichlidés des grands lacs africains ont connu l'une des plus rapides et plus vastes radiations adaptatives au sein des vertébrés. On estime à plus de 1000 le nombre d'espèces et la moitié serait endémique du lac Malawi (Konings, 2007). Différents processus peuvent être à l'origine de cette spéciation. Une diversité des niches écologiques disponibles (éboulis rocheux, substrat sableux, algues, pleine eau, ...) accompagnée d'une forte compétition pour l'accès à la nourriture explique une importante spécialisation trophique. Les cichlidés exploitent la quasi-totalité des sources de nourriture disponibles, des espèces sympatriques ayant des régimes alimentaires différents. Une sélection sexuelle est également invoquée pour expliquer l'étendue des variations de colorations observées chez des espèces morphologiquement très proches (Danley & Kocher, 2001 ; Genner et al., 2007). Une étude phylogénétique menée sur le groupe des Mbunas du Malawi démontre que cette diversité serait le résultat de radiations répétées au sein des mêmes types de couleurs, expliquant

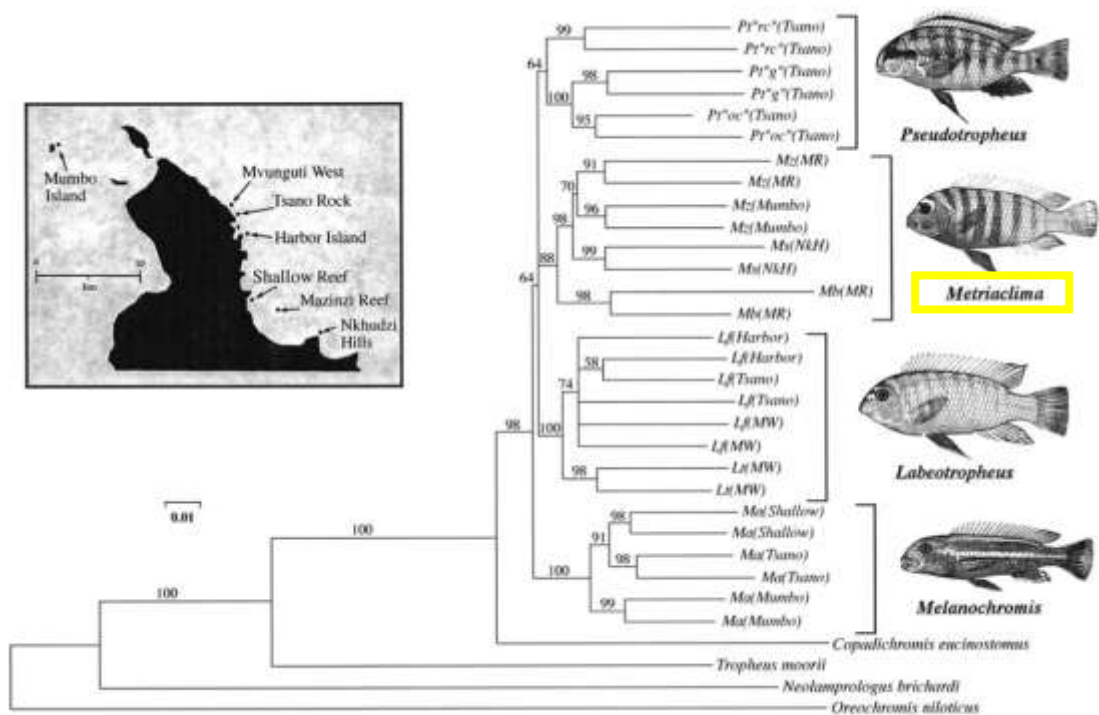
l'existence d'espèces phénotypiquement différentes mais très proches du point de vue génétique au sein des différentes régions du lac alors que des espèces phénotypiquement similaires sont parfois éloignées génétiquement (figure 2) (Albertson et al., 1999 ; Allender et al., 2003).

Lors de l'ensemble de ces processus, les signaux de communication jouent des rôles déterminants (e.g. chimiques, Plenderleith et al., 2005 ; acoustiques, Amorim et al., 2004a).

## **2. Structure sociale**

Alors que certaines espèces vivent en groupes hautement hiérarchisés, comme par exemple le cichlidé jonquille, d'autres espèces, comme *Lethrinops c.f. parvidens* ou *Copadichromis eucinostomus*, ne se regroupent qu'au moment de la reproduction pour former des leks où les mâles construisent des monticules de sables ("bowers") servant de sites de parade puis de pontes (Taylor et al., 1998 ; Kellogg et al., 2000). La grande majorité des espèces de cichlidés est territoriale et les individus résidents excluent tous les intrus s'approchant de leurs frontières (Genner et al., 1999). Généralement, ce sont les mâles qui sont en compétition pour un territoire dans lequel ils essayent d'attirer les femelles grâce à leurs comportements lors de la parade nuptiale. Chez *Neolamprologus pulcher*, certains mâles n'auront accès qu'à une femelle s'ils ne possèdent qu'un territoire mais pourront s'accoupler avec plusieurs d'entre elles dans le cas où ils possèdent de multiples territoires (Desjardins et al., 2008b).

Les poissons cichlidés présentent une grande diversité de comportements reproducteurs, monogames ou polygames, ainsi que de types de soins parentaux. Les soins parentaux incluent le bi-parentalisme, le maternalisme et le paternalisme dans quelques cas (Konings, 2007). Les œufs peuvent être déposés sur le substrat (typique des couples monogames avec soins bi-parentaux) ou incubés dans la bouche du parent (typique des systèmes polygames avec choix des femelles et maternalisme). Ce dernier système est le plus commun dans le lac Malawi (Konings, 2007).



**Figure 2 – Phylogénie de 4 espèces de Cichlidés du Malawi.**

(extrait de Albertson et al., 1999).

Abréviations : Pt, *P. tropheops*; "rc," red cheek; "g," gracilior; "oc," orange chest; Mz, *M. zebra*; Ms, *M. sandaracinos*; Mb, *M. benetos*; Lf, *L. fuelleborni*; Lt, *L. trewavasae*; Ma, *M. auratus*.

**Phylogeny of 4 species of Malawi Cichlids.**

(extracted from Albertson et al., 1999)

### 3. La communication visuelle

Les signaux visuels sont très importants dans la reconnaissance interspécifique et de nombreux auteurs ont démontré l'importance des indices visuels dans le choix du partenaire (Knight & Turner, 1999 ; Jordan et al., 2003). La sélection sexuelle sur la coloration nuptiale des mâles a ainsi pu grandement influencer la spéciation rapide des Cichlidés (Genner & Turner, 2005 ; Genner et al., 2007).

Les eaux du lac Malawi sont claires, la transmission de la lumière y est bonne et les teintes bleues à jaunes sont les mieux transmises (grâce à un pic de transmission proche des 500nm). Cependant, en fonction de leurs habitats, de leurs régimes alimentaires et de leur organisation sociale, différentes espèces auront des capacités visuelles différentes (Dobberfuhl et al., 2005, cichlidés du Tanganyika). Les espèces se nourrissant de larges proies n'ont en effet pas besoin d'une grande acuité visuelle, contrairement aux espèces planctivores, comme *Metriaclima zebra*, qui ont une bonne vision (Carleton et al., 2006). Associées aux préférences des femelles, les contraintes écologiques influençant la sensibilité visuelle des différentes espèces ont conduit à l'évolution des nombreux patterns de couleurs observés en particulier chez les mâles. Les cichlidés du Malawi présentent des patterns de couleurs allant donc des teintes bleues à jaunes, ce qui maximiserait leur détectabilité par les femelles (figure 3).

Durant les combats pour les territoires ou lors de parades destinées aux femelles (Seehausen & Schluter, 2004), les mâles utiliseront des démonstrations (displays) visuelles, mettant en particulier leur couleur en avant, en modifiant rapidement leur coloration. De plus, la présence d'ocelles, i.e. points de couleur jaune, sur la nageoire annale des poissons attirent les femelles et les individus territoriaux/dominants possèdent des ocelles plus larges et/ou en plus grand nombre, ce qui augmente leur attractivité (e.g. Couldridge, 2002).

Cette sensibilité aux signaux visuels des cichlidés est renforcée par une sensibilité aux UV en particulier pour les espèces vivant dans les eaux peu profondes (Jordan et al., 2004 ; 2006).



**Figure 3 – Cichlidés du Malawi dans un éboulis rocheux en bord du lac.**  
(extrait de Konings, 2007).

**Malawi Cichlids along the rocky coast of the lake.**  
(extracted from Konings, 2007).

#### 4. La communication acoustique

Les poissons Cichlidés, mâles comme femelles, émettent des sons tant en contexte agressif que lors de la parade nuptiale (Lobel, 2001). Les vocalisations produites peuvent être classés en trois catégories correspondant vraisemblablement à leur mode de production (Amorim, 2006). Le premier type de sons correspond à une série d'impulsions (pulses) de faible fréquence et souvent assimilés à des grognements dans la littérature (Amorim et al., 2004a). Un second type est constitué de sons dits de "masticage", de nature stridulatoire et de plus haute fréquence. Le dernier type est plus rare et correspond à des bruits plus sourds, semblables à des coups et associés à des mouvements brusques du corps du poisson.

Le premier type reste le plus intéressant pour l'étude de la communication car ces vocalisations sont émises en situation agressive et également lors de la reproduction (e.g. Simões et al., 2008). Des variations de leur caractéristiques, telles que leur durée moyenne, le nombre moyen de pulses dans un grognement ou encore leur rythme d'émission, ont principalement été mises en avant par des études démontrant des différences interspécifiques, même chez des espèces très proches (Amorim, 2004a ; revues par Amorim, 2006). Cela souligne encore une fois le rôle important qu'aurait pu jouer la communication acoustique dans la spéciation des Cichlidés africains.

Les mbunas du Malawi ont été un groupe modèle lors de ces études avec en particulier le cichlidé zèbre bleu, *Metriaclima zebra* (Amorim et al., 2004a ; Amorim et al., 2008b ; Simões et al., 2008).

#### 5. *Metriaclima zebra*, une espèce territoriale

*Metriaclima zebra* (anciennement *Pseudotropheus*) appartient au groupe le plus diversifié des Cichlidés du lac Malawi, les *mbunas*. Ces poissons occupent les parties rocheuses du lac, où la nourriture est abondante et la compétition se fait donc principalement pour l'accès à un territoire. Le régime alimentaire est composé d'algues couvrant les roches et contenant des microorganismes tels que des diatomées et des invertébrés, source de protéines (Konings, 2007). *M. zebra* se nourrit en position perpendiculaire en pressant ses dents contre le substrat pour racler les algues à la surfaces des pierres, d'où son nom commun de "frappeurs de pierres". Ils ont un système d'accouplement polygynandre. Les femelles incubent leurs œufs dans leur cavité buccale durant près de trois semaines à l'abri des mâles qui n'apportent aucunes ressources ou soins parentaux aux femelles et aux jeunes. Les juvéniles se dispersent très peu et les adultes sont fortement philopatriques.



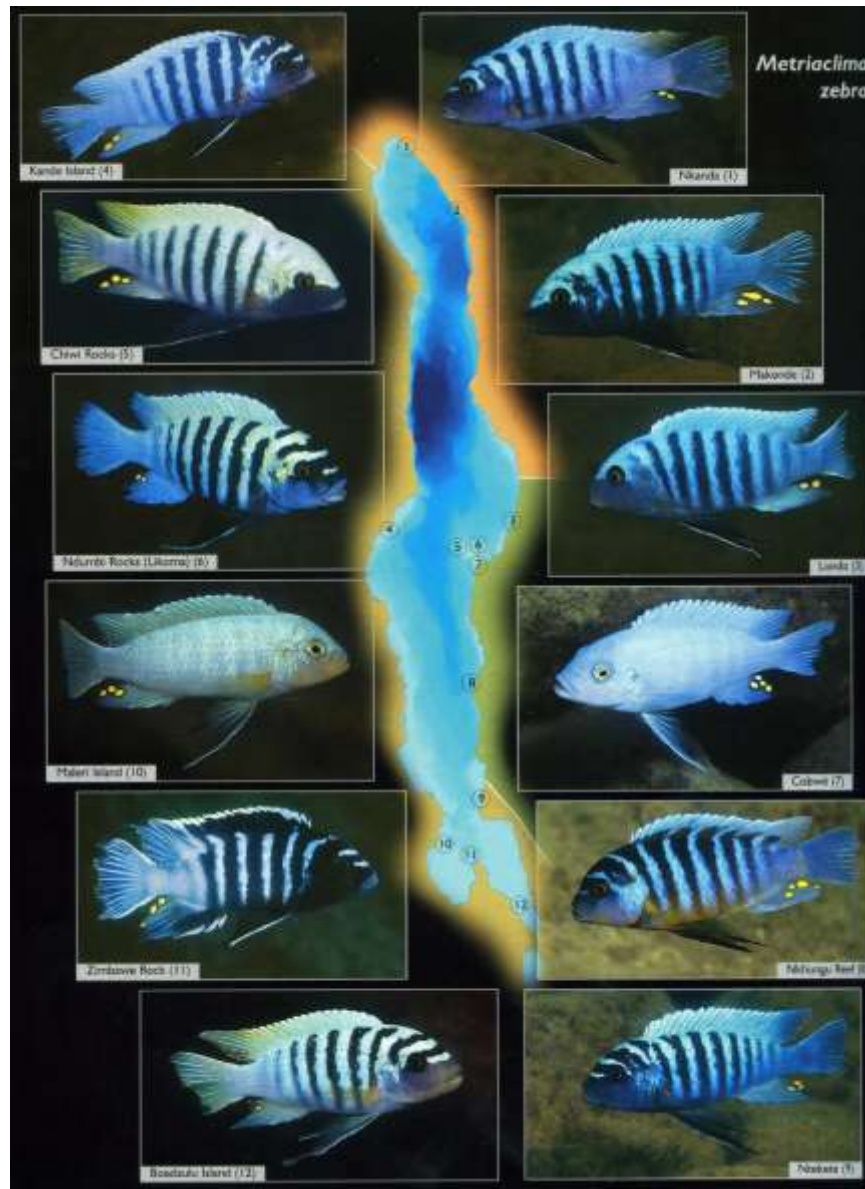
Les mâles de cette espèce sont territoriaux et présentent des barres verticales noires sur un corps bleu (figure 4). Les femelles sont généralement moins colorées. Lors d'interactions agressives ou lors de la parade sexuelle, le corps pâlit et les bandes noires apparaissent plus marquées.

Les agressions consistent en une série de comportements dont l'ordre d'apparition peut varier. Généralement, un male dominant défendra d'abord son territoire en chassant un rival ou en le poursuivant, en adoptant certaines postures comme l'érection des nageoires ou des tremblements (Amorim et al., 2004a). C'est lors de ces comportements que des sons peuvent être produits. Les combats peuvent aller jusqu'à un contact physique entre les adversaires et des morsures.

Lors de la parade nuptiale, les mêmes comportements peuvent être observés chez les mâles auxquels s'ajoutent des mouvements destinés à attirer la femelle vers le site de ponte. A nouveau, les sons seront produits durant les phases précoces des interactions.

Les mâles peuvent cependant vocaliser sans effectuer de comportements spécifiques, en restant immobiles par exemple.

Outre le rôle de la communication acoustique dans les interactions interspécifiques et la spéciation des cichlidés, les productions sonores observées dans ces différents contextes chez *M. zebra* soutiennent le rôle important des sons dans les interactions sociales intraspécifiques de ce poisson.



**Figure 4 – Les différentes variétés de *M. zebra* et leur localisation dans le lac Malawi.**

*Les Metriaclima zebra* ou “cichlidés zèbres classiques” sont rayés de noir sur un corps bleu (ils sont aussi appelés zèbres-BB pour Bleus et Barrés de noir). Ces poissons sont localisés sur les côtes rocheuses du lac avec une distribution en patches (extrait de Konings, 2007).

***Different types of M. zebra and their location in Lake Malawi.***

*Metriaclima zebras* or “classic blue zebras” are black striped on a blue body (also called BB-zebras for Blue and Black striped). These fishes are located on the rocky coast of the lake with a patchy distribution (from Konings, 2007).

## D. Objectif de la thèse et axes de recherche

Avec pour modèle *Metriaclima zebra*, ma thèse a pour objectif d'explorer certains aspects encore peu envisagés de la bioacoustique des poissons. L'aspect majeur de mon travail concerne le rôle des signaux acoustiques et les informations qu'ils sont susceptibles de transmettre. En utilisant une double démarche –analyse de la structure acoustique et expériences de playback-, j'ai cherché à tester l'importance biologique des sons émis par *Metriaclima zebra*. Dans un second temps, j'ai étudié l'ontogenèse de ces signaux au cours du développement des jeunes, leurs comportements au sein d'un groupe et la production de signaux acoustiques.

### 1. Quel est le rôle des sons produits lors des combats entre mâles *Metriaclima zebra* ?

Le fait que de nombreuses espèces de poissons produisent des sons dans différents contextes sociaux souligne l'importance de ces signaux de communication (Ladich & Myrberg, 2006 ; Myrberg & Lugli, 2006). De nombreux auteurs ont ainsi étudié le rôle biologique de ces sons en utilisant diverses approches comme des expériences de playback (Stout, 1963 ; Schwarz, 1974) ou en corrélant des caractéristiques acoustiques avec des traits de l'individu (Ladich, 1998 ; Amorim & Almada, 2005). Les expériences de playback menées jusqu'à présent montrent des résultats contrastés. Chez *Cyprinella analostana*, la repasse de sons enregistrés lors de combats augmente le nombre et la durée des comportements agressifs (Stout, 1963). Chez d'autres espèces comme *Archocentrus centrarchus* (Schwarz, 1974) et *Ictulurus nebulosus* (Rigley & Muir, 1979), l'agressivité est au contraire inhibée par les playbacks.

Ces sons sont généralement produits lors d'interactions rapprochées quand l'émetteur et le receveur sont également en interactions visuelles. Les expériences de playback menées jusqu'à ce jour ont souvent négligé cette association entre stimuli visuels et acoustiques (Lugli, 1997 ; Raffinger & Ladich, 2009 par exemple). Si ce point ne remet pas en cause le fait que les signaux acoustiques puissent avoir différents rôles chez différentes espèces (Ladich & Myrberg, 2006), une compréhension plus claire de la fonction relative des sons lors d'interactions agressives nécessite de tenir compte de cette association.

En couplant ou en dissociant ces deux canaux de communication, l'**article 1** montre que les stimuli acoustiques de *Metriaclima zebra* n'ont aucun effet sur le comportement agressif de l'individu récepteur lorsqu'ils ne sont pas associés aux stimuli visuels. En présence du canal visuel, les signaux acoustiques induisent une diminution du niveau d'agressivité.

## 2. Les sons d'agression codent-ils pour l'identité individuelle de l'émetteur ?

Si les sons émis par les poissons présentent des caractéristiques propres à chaque espèce, y compris lorsqu'elles sont proches, la diversité intraspécifique des signaux acoustiques (répertoire) semble bien inférieure à celle observée chez les oiseaux ou les mammifères mais reste encore assez méconnue (Crawford et al., 1997a ; Amorim et al., 2004a). On peut faire l'hypothèse que les signaux acoustiques puissent être le support d'information permettant une reconnaissance intraspécifique au niveau individuel. Afin d'établir des relations sociales au sein d'un groupe ou entre individus partageant des frontières territoriales, les individus doivent être capables d'identifier le statut hiérarchique de leurs congénères voire leur identité. Si des relations entre les caractéristiques d'un signal acoustique et les caractéristiques individuelles de son émetteur ont été décrites chez la plupart des vertébrés, depuis les amphibiens jusqu'aux mammifères, la relation la plus communément rencontrée chez les poissons est une corrélation négative entre la fréquence dominante du son et la taille de l'émetteur (longueur et poids) (Crawford et al., 1997a ; Colley et al., 2009). Cette taille servirait ensuite d'indicateur du statut social (un gros individu est généralement dominant et plus susceptible de sortir vainqueur d'un combat). Cependant, une étude menée chez *Halobatrachus didactylus* par Amorim & Vasconcelos (2008) suggère qu'une signature individuelle plus élaborée, basée sur différents paramètres acoustiques puisse être présente dans les cris d'accouplement de cette espèce. De même, une étude de Simões et al. (2008) a démontré que certaines caractéristiques temporelles des sons émis par *M. zebra* lors d'agressions différaient en fonction du sexe de l'émetteur et du contexte social, illustrant le degré d'information contenu dans ces sons. Il est donc possible que les sons produits par les poissons codent pour des informations liées à l'identité de l'individu émetteur. Cette hypothèse demande cependant des investigations nouvelles. L'expérience présentée dans l'**article 2** teste ainsi la possibilité d'une signature acoustique individuelle chez *Metriaclima zebra* grâce à une analyse de la structure acoustique de sons émis lors de combats entre mâles. Si des individus de deux classes de taille distinctes sont facilement identifiés sur une base acoustique, l'identification individuelle des poissons au sein des classes demeure difficile : le codage d'une signature individuelle reste donc imparfait.

## 3. Les paramètres temporels, un codage du niveau d'agressivité ?

L'utilisation des sons dans différents contextes sociaux pourrait suggérer que les poissons utilisent des vocalisations spécifiques à ces contextes, définies par des caractéristiques particulières. En plus d'une différence entre mâles et femelles (les sons produits par les mâles sont plus longs et comptent plus de pulses que ceux émis par les femelles), une étude

menée par Simões et al. (2008) montre que les sons produits par *M. zebra* lors d'agressions ou lors de parades sexuelles diffèrent dans leur durée et le nombre de pulses. Les paramètres temporels pourraient donc contenir des informations relatives au sexe et à la motivation de l'émetteur.

Sur la base d'expériences de playback, l'**article 3** étudie dans un premier temps la réponse comportementale des individus suite à la diffusion de playbacks de sons agressifs. Si aucune réponse vocale ou agressive n'a été observée, les mâles ayant acquis un statut de résident répondent plus fortement en augmentant leur activité territoriale. Les signaux acoustiques pourraient donc renseigner un individu sur la présence et/ou la motivation d'un compétiteur potentiel et déclencher une réponse comportementale, i.e. une augmentation de la maintenance du territoire et approche du haut-parleur.

A partir des résultats précédents, l'effet de modifications des paramètres temporels de ces sons a été testé sur des mâles territoriaux afin d'étudier comment est codée l'information portée par ces signaux. Si la réponse observée précédemment reste présente, les signaux dont la période est augmentée accroissent l'activité territoriale. Les autres modifications n'altèrent pas significativement cette réponse, suggérant une grande tolérance aux variations de ces paramètres chez ce poisson.

Si cette étude confirme le rôle social du son dans les comportements territoriaux de *M. zebra* et représente l'une des rares expériences de playback acoustique chez le poisson, la question "comment l'information est-elle codée ?" reste ouverte et d'autres investigations, portant sur d'autres paramètres acoustiques, en particulier fréquentiels, sont nécessaires.

#### **4. Ontogenèse des signaux acoustiques**

Alors que la mise en place des vocalisations au cours du développement chez les oiseaux et les mammifères a fait l'objet de nombreux travaux (Hauser, 1998), une partie beaucoup plus exploratoire de ma thèse a consisté à étudier l'ontogenèse des productions acoustiques chez *M. zebra*. Ce processus a été très peu étudié chez les poissons. Pourtant, chez des poissons tels que *M. zebra*, les comportements agressifs apparaissent dès les premiers jours de vie d'un individu, probablement suite à la compétition pour la nourriture et/ou l'espace. Il est possible que les sons aient un rôle lors de ces interactions entre très jeunes individus.

Le développement des vocalisations produites lors d'interactions agonistiques a été étudié chez le gourami grogneur (*Trichopsis vittata*). Les premières agressions apparaissent trois semaines après l'éclosion et sont accompagnées de sons dès la huitième semaine (Henglmüller & Ladich, 1999). Si les premiers sons apparaissent sous forme de pulses isolés, les productions suivantes voient apparaître des doubles pulses puis finalement un

nombre croissant de doubles pulses par sons avec une diminution de la fréquence dominante des sons corrélée à l'augmentation de la taille de l'animal. Des études de Wysocki & Ladich (2001) et Vasconcelos & Ladich (2008) s'intéressant au développement des systèmes auditifs chez respectivement *T. vittata* et le poisson crapaud lusitanien *Halobatrachus didactylus* ont montré que la détection de sons de basse fréquence se développe avant les premières productions, ce qui rendrait inefficace une communication acoustique lors des premiers stades du développement mais permettrait la détection d'individus de plus grande taille ou de prédateurs par les juvéniles. Dans l'état actuel des connaissances, il est donc difficile de conclure quant à l'importance biologique des sons émis par les juvéniles. Sous la forme de résultats préliminaires, l'**article 4** montre que l'apparition de la production de sons se fait très tôt, avant que la gamme complète des comportements soit observée. Si seuls des pulses isolés ont été enregistrés et que leurs caractéristiques évoluent avec l'âge du poisson, le rôle de ces signaux lors de la structuration d'un groupe social de juvéniles, pendant plusieurs mois, reste à définir de manière précise.

# **MATERIEL ET METHODES**

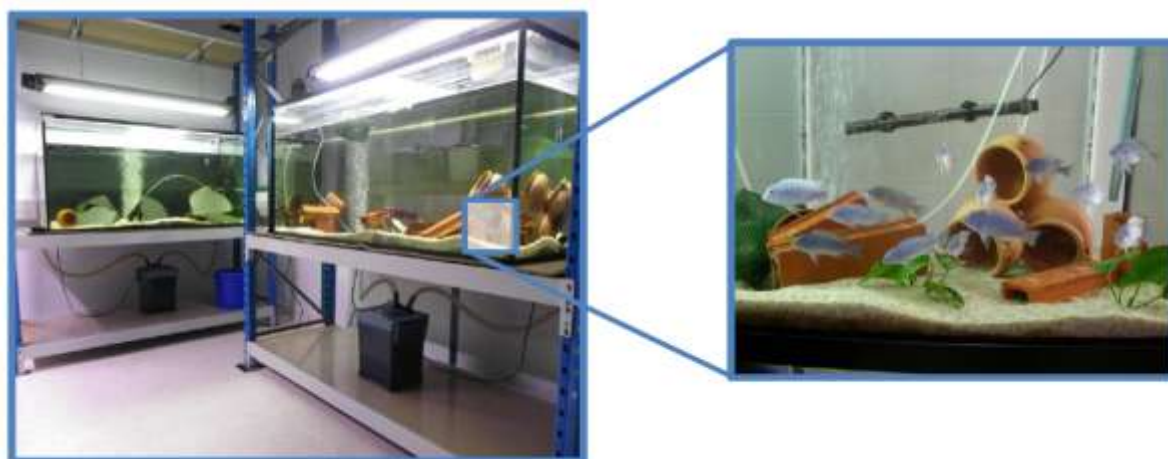
## II. MATERIEL ET METHODES

Cette partie présente les principales méthodes utilisées tout au long de mon travail de thèse depuis le maintien des poissons au laboratoire jusqu'aux techniques utilisées lors des différentes expériences. Des détails relatifs à chaque expérience sont donnés dans les articles correspondants.

### A. Conditions de maintien au laboratoire

#### 1. Elevage au laboratoire

Les poissons utilisés lors des expériences ont été achetés à la société N'Guyen International (Kingersheim, France) et maintenus en groupes hétérosexuels dans des aquariums de 120cm x 60cm x 50cm. Ces aquariums accueillait entre 10 et 20 individus avec un sex-ratio mâles:femelles d'environ 1:2. Chaque aquarium était équipé d'une pompe filtrante externe (Rena Filstar xP3), d'un bulleur, d'abris confectionnés par des pots et des briques de terre-cuite ou des plaques de lauze amoncelées (figure 5). Le fond des aquariums était recouvert de sable. La température de l'eau était maintenue à  $25 \pm 2^\circ\text{C}$  et le pH à 8.0, sous un cycle jour:nuit de 12 L:12 D. Les poissons étaient nourris quotidiennement avec de la nourriture pour Cichlidés sous forme de granulés (JBL NovoRift), complétés une fois par semaine avec un mélange de moules, crevettes et épinards congelés.



**Figure 5 – Aquariums communautaires d'élevage au laboratoire.**

*A gauche : 2 aquariums d'élevage ; à droite : Groupe hétérosexuel de poissons devant des abris de pots et briques en terre-cuite.*

#### **Communautory tanks in the lab.**

*Left: 2 storage tanks ; right : heterosexual group o fish in front of shelters made of pots and bricks.*



## 2. Conditions d'expérience

Pour les besoins des expériences, les poissons étaient introduits dans des aquariums de tailles variables équipés de façon similaire aux aquariums d'élevage. Les poissons utilisés en contexte expérimental ne changeaient pas de cycle jour:nuit et étaient nourris aux mêmes horaires que le reste de l'élevage.

Des aquariums de 60 x 30 x 30 cm placés côte à côte ont été utilisés afin de tester le rôle des productions sonores lors d'interactions agressives (expériences rapportées dans l'[article 1](#)). Une paroi amovible opaque était située entre ces deux aquariums au moment d'isoler les individus (Figure 6).

Les mêmes aquariums ont été utilisés lors des expériences de playbacks rapportées dans l'[article 3](#). Cette fois, chaque individu était isolé et n'avait accès qu'aux stimuli acoustiques diffusés par des haut-parleurs (voir Figure 15). Les parois intérieures étaient recouvertes de papier bulle afin d'éviter les phénomènes de réflexion des sons et réduire le bruit de fond.

Lors de l'enregistrement des sons agressifs constituant la banque de sons ([article 2](#)), des aquariums de 80 x 35 x 35 cm ont été utilisés. Une paroi transparente ainsi qu'une paroi opaque partageaient l'aquarium en deux compartiments pouvant recevoir un poisson (Figure 7).

Afin d'étudier la production de sons chez des juvéniles ([article 4](#)), lorsqu'une femelle gestante était repérée, i.e. présence d'œufs dans sa cavité buccale, elle était isolée de son groupe en étant placée dans une cage à l'intérieur de son bac communautaire. Après environ 21 jours de gestation, les alevins sont retirés de la bouche de la mère et isolés dans des aquariums de 40 x 20 x 25 cm par lot d'une quinzaine d'individus.

Les expériences ont ensuite été menées dans un aquarium de 25 x 25 x 25 cm placé au centre d'un aquarium plus large de 60 x 50 x 50 cm. L'intérieur de ces deux aquariums était recouvert d'une mousse absorbante de forte densité (5 cm d'épaisseur). Ce dispositif permettait de réduire au maximum les effets de résonance et de réflexion du son (Figure 8). Une seconde partie de cette étude porte sur des interactions en dyades. Des aquariums de 20 x 16 x 25 cm ont été utilisés pour les juvéniles de 40 et 200 jours, alors que des aquariums de 40 x 20 x 25 cm ont été utilisés pour les individus adultes. Chacun de ces aquariums était divisé en deux compartiments par des parois amovibles placées côte à côte, l'une opaque et l'autre transparente (Figure 9).



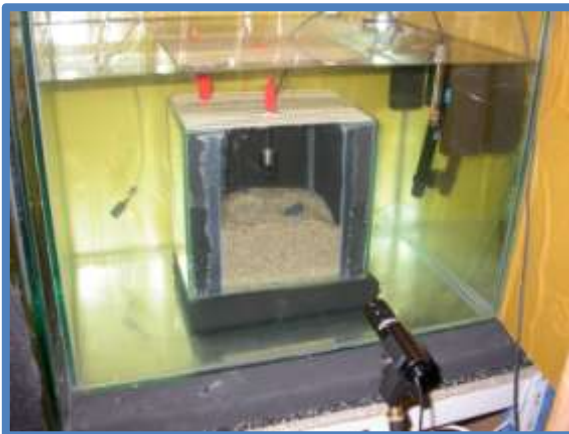
**Figure 6 – Dispositif expérimental utilisé lors de l'expérience rapportée dans l'article 1.**

*Experimental setup used in the experiment presented in article 1.*



**Figure 7 – Dispositif expérimental utilisé lors de l'expérience rapportée dans l'article 2.**

*Experimental setup used in the experiment presented in article 2.*



**Figure 8 – Dispositif expérimental utilisé lors de l'expérience rapportée dans l'article 4 pour la suivie du groupes de juvéniles.**

*Experimental setup used in the experiment presented in article 4 for the recordings of the group of juveniles.*



**Figure 9 – Dispositif expérimental utilisé lors de l'expérience rapportée dans l'article 4 pour l'enregistrement des interactions en dyades des juvéniles.**

*Experimental setup used in the experiment presented in article 4 for the recordings of dyadic interactions in juveniles.*

Pour chacune des expériences, chaque aquarium et/ou chaque compartiment disposait d'un hydrophone.

Toutes les expériences ont été menées dans une pièce insonorisée dans laquelle les aquariums expérimentaux étaient placés sur des étagères et reposaient sur des plaques de caoutchouc aggloméré surmontés de panneaux de fibres de bois. Ce dispositif permettait de réduire le bruit de fond, provenant en particulier des vibrations se transmettant depuis le sol vers les aquariums.

## B. Ethogramme de *M. zebra*

Cette présentation des comportements observés chez *M. zebra* est issue des descriptions faites par Jordan (2008) et Simões et al. (2008), complétées par mes observations personnelles.

### 1. Comportements agressifs

Lors des différentes expériences, les poissons étaient la plupart du temps placés dans des aquariums ou des compartiments séparés afin de réduire les risques de blessures. Il était donc impossible aux individus d'établir des contacts physiques et ainsi d'exprimer la totalité de leur répertoire comportemental. Malgré cette contrainte, la plupart des comportements agressifs ont pu être observés.

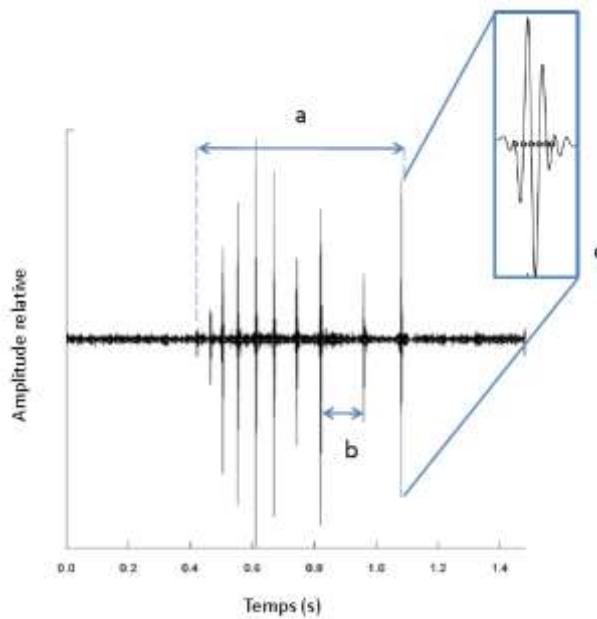
**Les displays latéraux** : le poisson se place de façon perpendiculaire à son adversaire, présentant son flanc. Le corps est légèrement arqué et les nageoires impaires (dorsale, caudale, anale) sont déployées.

**Les quivers (tremblements)** : Le poisson s'immobilise un bref instant avant de faire vibrer son corps. Ce comportement est souvent associé à une production de son.

**Le sink** : ce comportement suit souvent un display latéral. Le poisson, perpendiculaire à son adversaire, se laisse tomber dans la colonne d'eau avant de reprendre sa nage et l'interaction en cours.

A ces comportements visuels est parfois associée la production de signaux sonores.

**Les sons** : ils sont produits lorsque deux individus sont proches l'un de l'autre. Ils consistent en une série de pulses successifs de basse fréquence. La durée des sons, le nombre de pulses et leur période sont variables (figure 10).



**Figure 10 – Oscillogramme d'un son de mâle *M. zebra*.**

Quelques variables mesurables sont présentées : a) nombre d'impulsions (pulses) et durée du son, depuis le début du premier pulse jusqu'à la fin de la dernière ; b) période des pulses ; c) détail d'un pulse.

**Oscillogram of a sound of a male *M. zebra*.**

Some measured variables are shown: a) number of pulses and sound duration ; b) pulse period ; c) detail of a pulse.

Si un conflit n'est pas résolu à ce stade, le niveau d'agressivité peut alors augmenter et des interactions physiques peuvent alors apparaître si le dispositif expérimental le permet.

**Le circling :** deux individus engagés dans une interaction agressive se placent de façon antiparallèle l'un à l'autre et débutent une nage en cercle. Cette nage est parfois interrompue par l'un des comportements précédents ou par la morsure ou la charge d'un des individus. Ce comportement est rarement observable lors des expériences.

**Les charges/poursuites :** afin de repousser un congénère qui s'approche de son territoire ou durant un combat, un poisson nage rapidement en direction de son adversaire. Il y a parfois contact entre la tête de l'attaquant et le flanc de sa cible. Souvent, le poisson cible rebrousse chemin, poursuivi sur une courte distance par le mâle territoriale avant que ce dernier ne retourne dans son abri. Lors de l'établissement de la hiérarchie, les poursuites peuvent être beaucoup plus longues, et l'un des adversaires harcèle l'autre. Lors des expériences où une paroi transparente séparait les deux compartiments, les poissons heurtaient cette dernière en tentant de charger.

**Les morsures :** suite à une charge, lors du contact physique, l'un des combattants peut tenter de mordre les flancs de son adversaire. Les deux poissons peuvent également se mordre mutuellement au niveau de leur bouche et l'un des protagonistes bloque la bouche de son adversaire dans la sienne (mouth locking). Durant les expériences, on assistait à des tentatives de morsures avec des poissons frottant leurs dents contre la paroi transparente.

## 2. Autres comportements

**La maintenance** : en devenant territoriaux, les mâles “aménagent” leur territoire autour d’un abri. En particulier, ils déplacent le substrat depuis cet abri vers la périphérie (figure 11) en collectant du sable dans leur cavité buccale pour le rejeter aux frontières de leur territoire.

**Les visites de l’abri** : dans les aquariums communautaires ou expérimentaux, les individus disposaient de pots, briques en terre-cuite ou d’amas de pierres créant des abris. Les poissons, en particulier dominants, visitent ces abris régulièrement, les entretiennent en déplaçant du substrat et peuvent même rester à l’intérieur et n’en sortir que pour repousser un congénère qui s’approche.



**Figure 11 – Mâle *M. zebra* à l’entrée de son abri.**

*L’abri, composé d’un pot en terre cuite recouvert d’une plaque de lauze (relevée ici), est situé sous un hydrophone.*

**Male *M. zebra* in front a a shelter.**

*The shelter, a pot and a flat stone (lift up), is located under an hydrophone.*

**La nage Haut/Bas** : ce comportement est typiquement observé dans les aquariums expérimentaux lorsque deux individus sont séparés par une paroi transparente. Les poissons vont alors essayer d’atteindre leur adversaire et se retrouvent face à la paroi.

**Les comportements de reproduction** : durant le comportement de parade nuptiale (court), les mâles utilisent des comportements également observés lors des interactions agressives tels que les charges, les quivers ou le circling. Un comportement typique est le “lead swim” durant lequel le mâle dirige la femelle vers son territoire et le site de ponte. Le mâle peut également effectuer des boucles ou “loops” sur lui-même.

## 3. Observation

La collecte des données comportementales a été réalisée par observation focale des individus, durant le visionnage de vidéos enregistrées lors des sessions expérimentales. Afin de quantifier les comportements (agressifs et d’occupation de l’espace), j’ai utilisé le logiciel

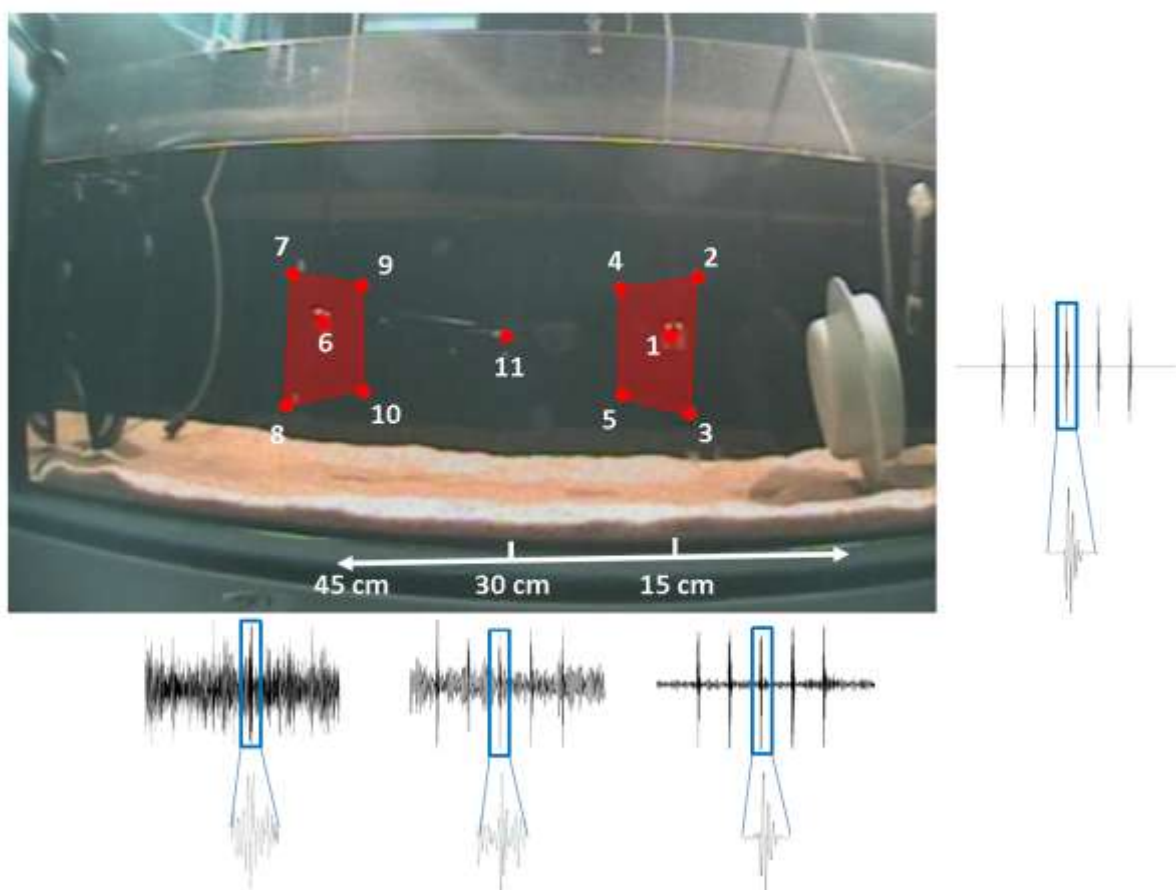
de transcription Etholog 2.2.5 (Ottoni, 1995-1999). Chaque session d'observation durait entre 10 et 15 minutes et résultait en une feuille de données comprenant le nombre de chacun des comportements réalisés, leur moment d'occurrence et leurs durées.

### C. Propagation du son et positionnement de l'hydrophone

Afin d'étudier le rôle biologique des sons, une observation du comportement des individus ainsi qu'une analyse précise des caractéristiques acoustiques sont nécessaires. Les expériences en aquarium permettent une observation aisée des comportements dans un milieu contrôlé. Cependant, pour l'enregistrement de sons, une des contraintes majeures reste la taille de l'aquarium qui peut induire des phénomènes physiques tels que la résonance ou la réverbération. Si la fréquence de résonance d'un aquarium est proche de la fréquence du son produit par un poisson, les caractéristiques spectrales enregistrées peuvent être altérées. La production d'un son dans un espace clos peut également conduire à une persistance du son après sa production causée par de multiples réflexions. Il devient alors impossible de séparer le son original du son réfléchi.

Afin de réduire la réverbération, trois des faces internes des aquariums utilisés lors de mes expériences étaient recouvertes d'une double couche de papier bulle (papier d'emballage). La dernière face étant laissée libre afin de permettre les enregistrements vidéo.

Les sons de faible intensité comme ceux de *Metriaclima zebra* se dégradent très rapidement. J'ai donc réalisé des expériences témoins visant à juger de la distance à laquelle l'enregistrement n'est plus fidèle aux caractéristiques originales du son. En utilisant un haut-parleur sous-marin Electrovoice, UW-30 connecté à un amplificateur Denon PMA-100M, différents sons étaient diffusés dans un aquarium de 80 x 35 x 35 cm à l'aide d'un enregistreur numérique Marantz PMD670. Les enregistrements se sont effectués à des distances de 15 cm, 30 cm et 45 cm de la source. Les résultats ont montré qu'à 30 cm, l'intensité des sons était très diminuée et qu'aucun son n'était enregistrable à 45 cm (Figure 12).



**Figure 12 – Dispositif expérimental utilisé pour étudier la propagation des sons dans nos aquariums.**

Les sons ont été diffusés à l'aide d'un haut parleur et enregistrés à 15 cm (5 points différents), 30 cm (un point au centre de l'aquarium) et à 45 cm (5 points différents). L'oscillogramme du son avant diffusion et le détail d'un pulse sont fournis (à droite). Pour chaque distance, l'oscillogramme du même son et le détail d'un pulse sont également figurés.

**Experimental setup used to test sound propagation in our aquaria.**

Sounds were played back using a loudspeaker and recorder distances of 15 cm (5 different recordings), 30 cm (one recording in the middle of the aquarium) and at 45 cm (5 recordings). The oscillogram of a sound before the playback and the detail of a pulse are shown (right). For each distance, the oscillogram of the same sound and the detail of a pulse are given.

De plus, d'après Akamatsu et al. (2002), un enregistrement minimisant les effets de résonance sur les caractéristiques spectrales d'un son doit se faire entre la source d'émission et la distance d'atténuation du son. Pour les sons de mâles adultes *M. zebra*, la distance d'atténuation se situe entre 13 cm et 16 cm (d'après l'équation d'Akamatsu et al., 2002). Ainsi :

- les hydrophones étaient placés au centre des aquariums,
- seuls les sons produits et enregistrés à une distance maximale de 2 longueurs de poissons (environ 15 cm) de l'hydrophone ont été retenus lors des analyses,
- les hydrophones étaient situés à 15 cm des haut-parleurs lors des expériences de playback.

Les hydrophones (Aquarian Audio Products H2a-XLR) étaient donc placés au centre de chaque aquarium ou partie de l'aquarium où un individu passerait le plus de temps, c'est-à-dire entre un abri et l'aquarium ou compartiment adjacent contenant un adversaire. Les hydrophones étaient connectés à un préampli (Yamaha MLA8) relié à une carte d'acquisition vidéo (Osprey-450e) permettant de synchroniser les enregistrements acoustiques et vidéos.

## D. Analyse des sons

### 1. Préparation des fichiers sons

A partir des vidéos enregistrées lors des sessions expérimentales, j'ai utilisé le logiciel GoldWave (version 5.25 ; [www.goldwave.com](http://www.goldwave.com)) afin d'isoler et d'extraire les signaux acoustiques en vue de leur analyse. Après avoir repéré l'instant de production d'un son sur la piste audio correspondante, ce dernier était isolé dans un nouveau fichier. Un filtre passe-bande entre 100 Hz et 2 kHz était appliqué à chaque son afin de retirer le bruit de fond présent sur les enregistrements.

Ces fichiers étaient ensuite classés selon l'identité de leur émetteur dans une banque de sons.

### 2. Extraction des différents paramètres acoustiques

L'un des objectifs de ce travail de thèse étant d'étudier le type d'information transmise via les signaux acoustiques, il fallait analyser précisément la structure acoustique des sons produits par les poissons. En particulier, alors que la plupart des analyses acoustiques préalablement menées chez le poisson utilise la transformée rapide de Fourier (FFT) pour construire des spectrogrammes, la méthode choisie ici est celle dite du zero-crossing. Sur des signaux brefs – tels que les pulses émis par les poissons -, les fenêtres temporelles du spectrogramme sont inadaptées et ne permettent pas d'accéder à la dynamique du signal. En particulier, le



spectrogramme d'un pulse donne une idée peu précise de la composition fréquentielle du signal et surtout de sa modulation. La technique du zero-crossing permet de calculer la fréquence instantanée directement à partir des demi-périodes de chaque sinusoïde constituant un son (Staddon et al., 1978 ; Mbu Nyamsi et al., 1994). L'analyse s'affranchit ainsi du compromis entre précisions temporelle et fréquentielle lié à la transformée de Fourier.

L'autre nouveauté de ce travail réside dans le fait que cette analyse par zero-crossing s'est faite au niveau du pulse lui-même et non seulement du son dans son ensemble. Au lieu d'une moyenne plus ou moins approximative de la composition fréquentielle du son, on a donc obtenu une mesure exacte de la fréquence instantanée et de son décours temporel pour chacun des pulses constituant le son.

Les paramètres acoustiques ont été extraits grâce à une routine d'analyse semi-automatique écrite dans R (version 2.8.1) avec le package Seewave (version 1.5.4) (Sueur et al., 2008).

Parmi les différents paramètres pouvant être extraits, sept ont principalement été utilisés pour mon travail (Figure 13).

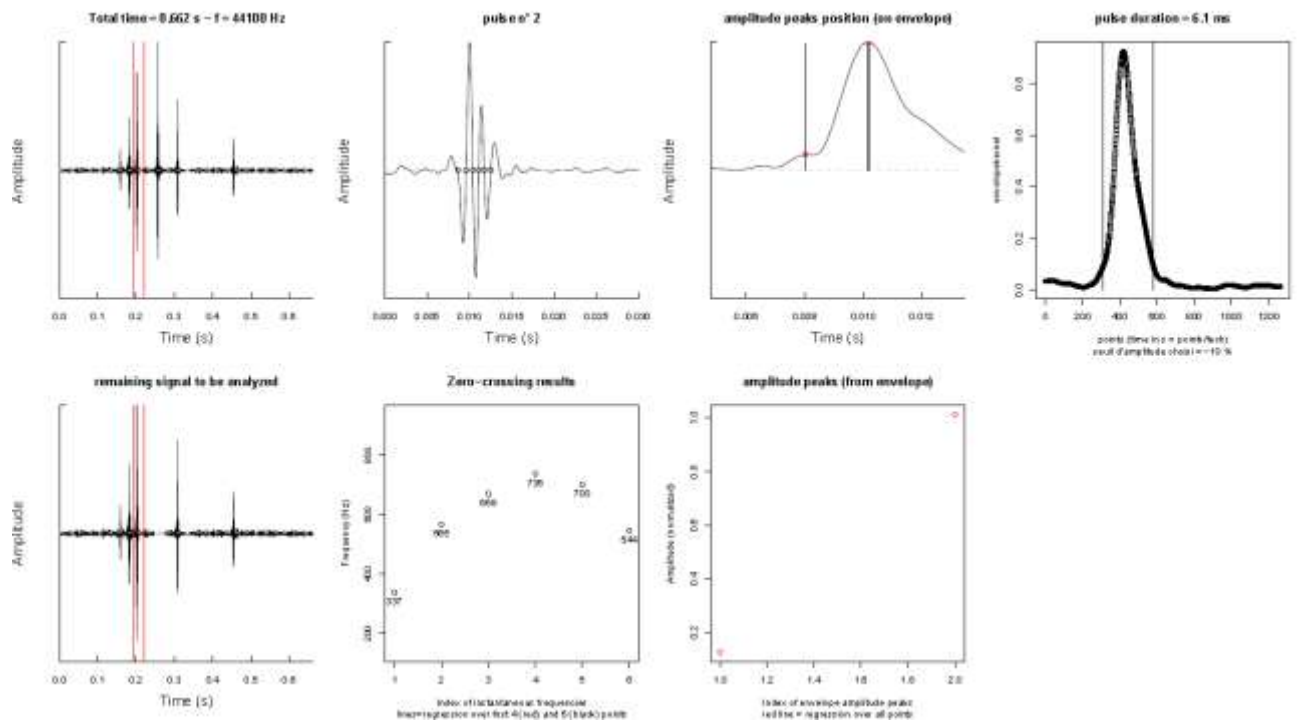
- **la nombre de pulse composant un son** : la détection des pulses commençait par le pulse de plus grande amplitude (pulse max) puis se poursuivait de façon décroissante en amont et en aval jusqu'à ce que les pulses ne dépassent pas 10% de l'amplitude du pulse de plus grande amplitude. Chaque pulse dont l'amplitude ne dépassait 10% de l'amplitude du pulse max était exclu de l'analyse. Ce seuil a été fixé de façon à permettre la détection d'un maximum de pulses ayant une structure assurant une analyse convenable.

- **la durée totale du son** : mesurée depuis le début du premier pulse à la fin du dernier pulse.

- **la modulation d'amplitude des pulses** : correspond à la pente de la régression linéaire calculée sur les valeurs maximales d'amplitudes de chacun des pulses.

- **la période des pulses** : correspond à la durée moyenne de l'intervalle de temps entre les pics d'amplitude de deux pulses consécutifs.

- **la durée moyenne des pulses** : mesurée à partir de l'enveloppe d'amplitude de chaque pulse. La détection du début et de la fin du pulse était fixée au seuil de 20% de l'amplitude maximale du pulse étudié. Ce seuil a été fixé car il assurait la mesure la plus fiable de la durée d'un pulse.



**Figure 13 – Résultats graphiques de l'analyse semi-automatique d'un son de mâle *M. zebra* avec Seewave (Sueur et al., 2008).**

**Graphical results of the semi-automatic analysis of a male *M. zebra* sound using Seewave (Sueur et al., 2008).**

- **la fréquence instantanée moyenne des pulses** : calculée comme la moyenne des six mesures de la fréquence instantanée du pulse obtenues par la méthode du zero-crossing.

- **la modulation de fréquence dans les pulses** : correspond à la pente de la régression linéaire calculée sur les 6 valeurs de fréquence instantanée du pulse mesurées par zero-crossing.

### 3. Analyse de l'individualité des sons

J'ai mené une analyse multivariée de la signature individuelle, basée sur une analyse discriminante à validation croisée et permutée. Cette analyse se fait en deux étapes. La première (figure 14a) consiste à :

- 1- calculer des fonctions discriminantes à partir d'une fraction des sons préalablement enregistrés auprès des individus considérés.
- 2- La fraction restante des sons est ensuite utilisée pour tester la précision de classification de la fonction (cross-validation).
- 3- Cette étape est répétée 100 fois, avec un tirage aléatoire des deux fractions afin d'obtenir une moyenne de classification correcte.

La deuxième étape consiste à créer des jeux de données où les sons sont assignés aléatoirement à un individu (permutations) (figure 14b).

Comme précédemment, des fonctions discriminantes sont construites à partir d'une fraction des sons (1) puis testées avec les sons restants (2). Cette deuxième étape permet d'obtenir le niveau de significativité de la cross-validation menée sur les données non-permutées (pour plus de précisions concernant la méthode, voir Mathevon et al., 2010).

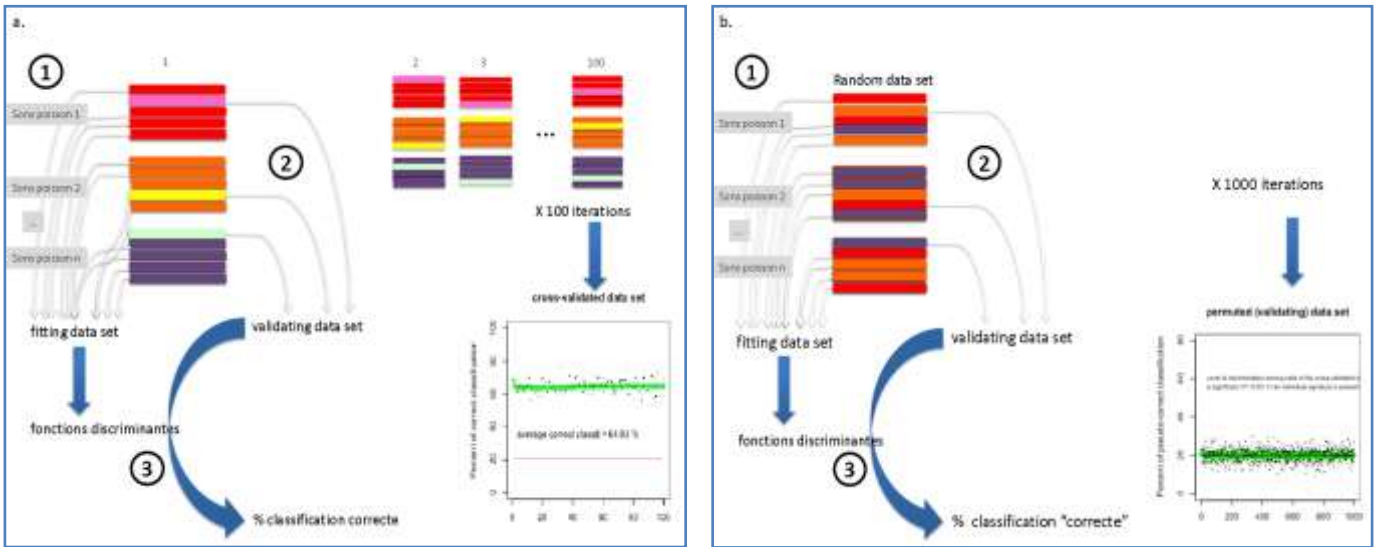
## E. Préparation des playbacks

La banque de sons utilisée pour l'analyse a également été employée afin de réaliser les expériences de repasse (playbacks). Les sons émis provenaient toujours de poissons étrangers qui n'avaient jamais été vus ou entendus par les poissons testés lors des expériences. La méthode de playback constitue un outil efficace pour tester la fonction biologique de signaux acoustiques, et est couramment utilisées avec de nombreux animaux. Si les sons agressifs produits par les mâles portent une information, l'émission de sons agressifs, la modification de leurs paramètres et la réponse comportementale observée renseignent sur leur importance dans la communication de *M. zebra*.

Des haut-parleurs sous-marin Electrovoice, UW-30 connectés à un amplificateur Denon PMA-100M étaient placés dans une zone de l'aquarium, contre une paroi (Figure 15). Un enregistreur numérique Marantz PMD670 connecté à l'amplificateur servait à diffuser les sons.

### 1. Rôle des signaux acoustiques

Afin de tester le rôle relatif de l'acoustique par rapport au visuel (**article 1**), deux types de playbacks ont été utilisés. Une séquence de sons produits durant une interaction agressive entre deux mâles était diffusée durant 20 minutes à deux poissons isolés visuellement. Dans un deuxième traitement, des sons agressifs isolés étaient diffusés dans le compartiment d'un poisson dès qu'un opposant, situé dans un compartiment adjacent, produisait un son. Dans ce deuxième cas, les individus étaient en contact visuel durant 20 minutes.



**Figure 14 – Les différentes étapes d’une analyse de fonctions discriminantes à validation croisée (a.) et permutée (b.).**

La première étape fournit un pourcentage moyen de classification correcte (ligne verte) et le pourcentage de classification correcte attendue par chance (ligne rouge). La seconde étape fournit le niveau de significativité de la validation croisée (première étape).

**The different steps of a cross-validated (a.) and permuted (b.) discriminant functions analysis.**

The first step gives an average percentage of correct classification (green line) and the correct classification expected by chance (red line). The second step give the significance level of the cross-validation (first step).

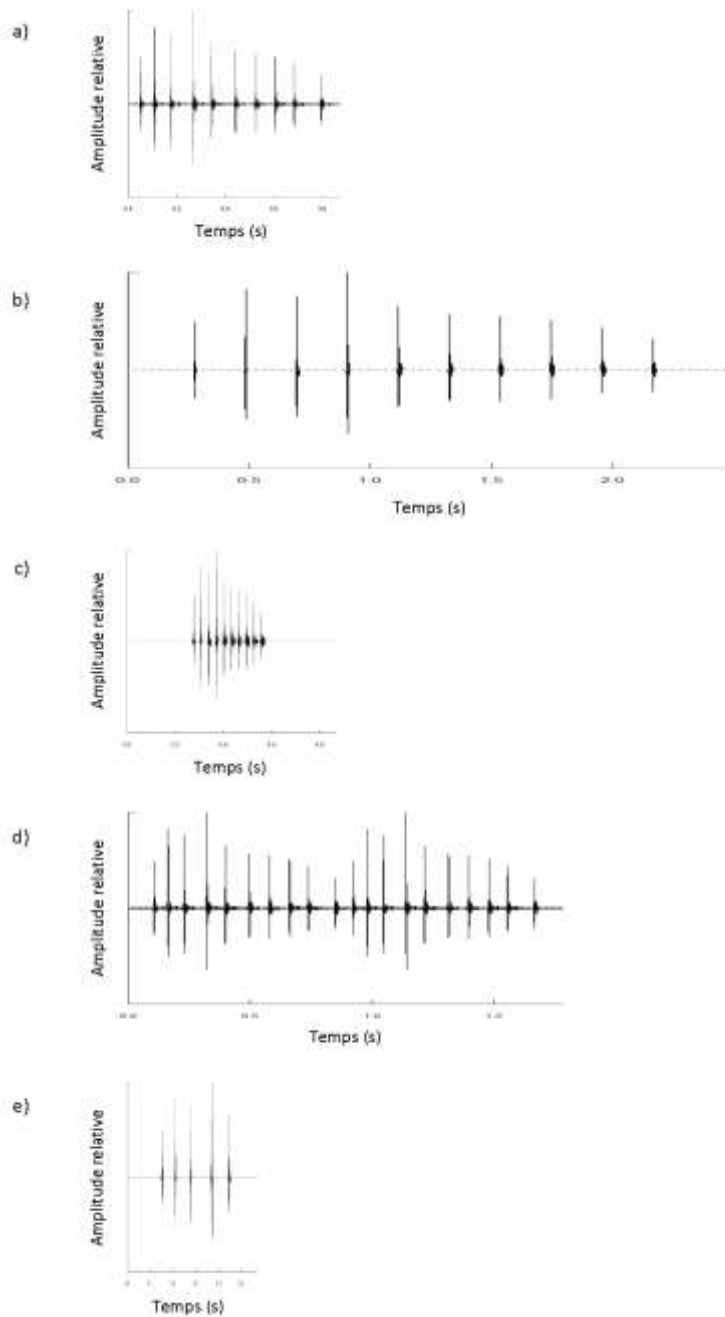


**Figure 15 – Dispositif expérimental utilisé lors des expériences de playback rapportées dans l’article 3.**

**Experimental setup used in the playback experiments presented in article 3.**

## ***2. Modification des signaux acoustiques***

Les paramètres temporels des sons (Figure 16a) ont été modifiés en utilisant le logiciel Praat (version 5.0.35). En particulier, les modifications ont porté sur la période des pulses (les pulses d'un son étaient tous espacés d'une durée égale à deux fois la valeur de l'intervalle pic à pic maximal (Figure 16b) ou à la moitié de la valeur de l'intervalle minimal (Figure 16c) ; et le nombre des pulses, doublé en diffusant un même son deux fois de suite (Figure 16d) ou divisé par 2 en ne jouant que la première moitié d'un son (Figure 16e). Les sons de playbacks étaient émis dès que le poisson testé s'approchait du haut-parleur, dans le but de mimer la présence d'un congénère dans cette zone de l'aquarium (**article 3**).



**Figure 16 – Oscillogrames des différents signaux modifiés utilisés lors des playbacks.**

*Le son original (a) a subit deux types de modifications. Une modification de la période des pulses – qui a été augmentée (b) ou diminuée (c) – et une modification du nombre de pulses – doublé (d) ou divisé par deux (e).*

**Oscillograms of the different modified signals used during playback experiments.**

*The original sound (a) underwent two types of modifications. A modification of the pulse period – increased (b) or decreased (c) – and a modification of the number of pulses – doubled (d) or halved (e).*

# RESULTATS

## Sounds modulate males' aggressiveness in a cichlid fish

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## Résumé de l'article 1

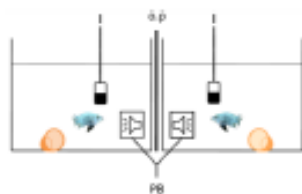
### Introduction

Lors d'interactions agressives, de nombreux poissons produisent des sons, souvent associés à des comportements visuels. L'expérience présentée dans cet article utilise la méthode du playback acoustique afin d'étudier le rôle de ces signaux et de leur association avec le canal visuel.

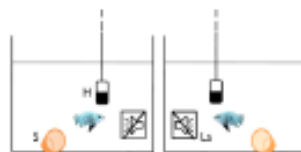
### Matériel et méthodes

Deux aquariums contenant chacun un hydrophone, un haut-parleur et un abri étaient disposés face à face. Un poisson mâle était introduit dans chaque aquarium 24h avant le début de l'expérience. Durant cette période, les deux individus étaient isolés acoustiquement et visuellement à l'aide d'une paroi opaque.

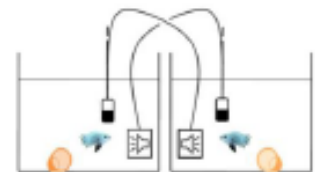
Suite à cette période d'isolement, les individus recevaient l'un des traitements suivants:



**Traitement acoustique:** la paroi opaque est en place et un playback de sons émis lors d'une interaction aggressive est diffusé dans chaque aquarium.



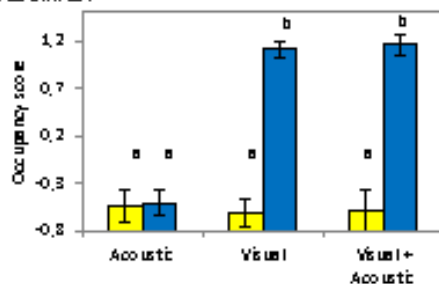
**Traitement visuel:** la paroi opaque est retirée et les haut-parleurs sont inactifs.



**Traitement visuel et acoustique:** la paroi opaque est retirée et les sons produits par un individu sont diffusés en direct dans l'aquarium voisin.

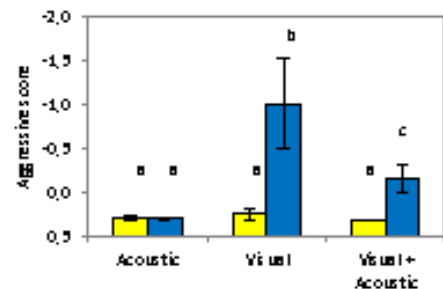
### Résultats

Des variables d'occupation de l'espace (temps passé près de la paroi, nage contre la paroi, maintenance du territoire, visite de l'abri, temps passé proche du haut parleur et nombre de pauses) ainsi que des variables d'agressivité (nombre de sons produits, displays latéraux, tremblements, charges et morsures) ont été recueillies.



L'occupation de l'espace n'est pas affectée par les stimuli acoustiques.

Dès que des stimuli visuels sont disponibles, un score élevé montre une focalisation sur l'adversaire.



Les stimuli acoustiques seuls n'ont pas d'effet sur le niveau d'agressivité. L'agressivité élevée rencontrée lorsque seuls les stimuli visuels sont disponibles est diminuée par l'association de l'acoustique.

### Conclusion

L'agression est principalement déclenchée en réponse à une stimulation visuelle. Les signaux acoustiques réduisent l'agressivité lorsqu'ils sont associés aux stimuli visuels mais ne suffisent pas à provoquer cette agression. Les signaux acoustiques complètent ainsi les signaux visuels produits lors d'interactions agressives chez *Methylinis zebra*.

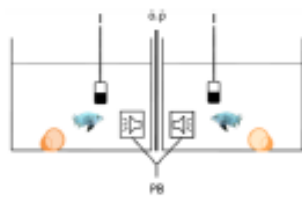
## Summary of article 1

### Introduction

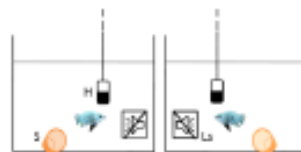
During aggressive interactions, many fishes produce sounds, often associated to visual displays. The experiment presented in this article uses acoustic playback method in order to study the role of these signals and their association with the visual channel.

### Material and methods

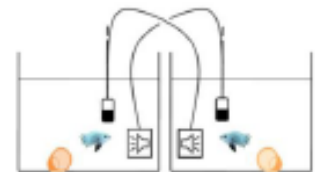
Two aquaria each containing an hydrophone, a loudspeaker and a shelter were placed side by side. A male individual was introduced in each aquarium 24h before the start of the experiment. During this period, the two individuals were acoustically and visually isolated by means of an opaque partition. After this isolation period, individuals received one of the following treatments:



**Acoustic treatment:** the opaque partition is in place and a playback of sounds produced during a fight is broadcasted in each aquarium.



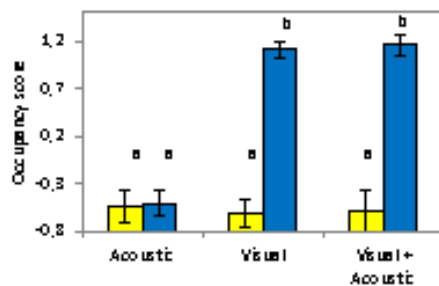
**Visual treatment:** the opaque partition is removed and loudspeakers are off.



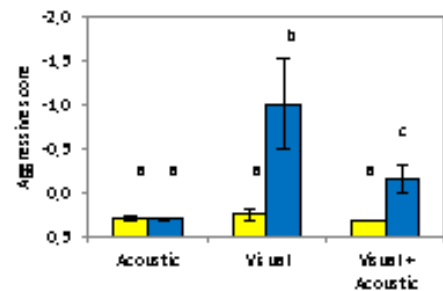
**Visual and acoustic treatment:** the opaque partition is removed and sounds produced by an individual are played "live" in the other aquarium.

### Results

Variables defining space occupancy (time spent near the partition, swimming against the partition, territory maintenance, visit of the shelter, time spent near the loudspeaker and number of breaks) and aggressive variables (number of sounds produced, lateral displays, quivers, charges and bites) were collected.



Space occupancy is not affected by acoustic stimuli. As soon as visual stimuli are available, a high score shows that the subjects focus on their opponent.



Acoustic stimuli alone have no effect on the aggressiveness. The high level of aggressiveness observed when only visual stimuli are available is lowered by the association of acoustics.

### Conclusion

Aggression is mainly triggered by visual stimuli. Acoustic signals reduce aggressiveness when they are associated to visual stimuli but are not sufficient to trigger aggression. Thus, acoustic signals complement visual signals produced during aggressive interactions in *Metrioclima zebra*.



## Sounds Modulate Males' Aggressiveness in a Cichlid Fish

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### Abstract

Acoustic signals are produced in many fish species during agonistic or courtship interactions. A way to test the biological role of these sounds is the use of acoustic playback experiments. However, sounds are usually associated with visual displays and playback experiments performed in fish so far, often failed to match acoustic and visual stimuli. To avoid this mismatch issue, we experimentally separated or coupled visual and acoustic channels to test the role of sounds produced during male–male aggressive interactions in a cichlid fish, *Metriaclima zebra*. Results show that aggressive behaviour is based on visual stimuli and that acoustic signals alone never trigger aggression. Furthermore, the association between visual and acoustic channels lowers the level of aggressiveness found when fish can only interact visually. This suggests that acoustic signals used during a dispute may complement visual displays to modulate males' behaviour by reducing their aggressiveness and the risk of escalated fights.

### Introduction

Fishes of various species are well known to produce sounds in different social contexts, mainly aggressive interactions and courtship, which suggests that sounds may be important in communication. For instance, males of the European river bullhead (*Cottus gobio*) and the croaking gourami (*Trichopsis vittata*) both emit sounds accompanying behavioural displays during contests against an intruder (for a review, see Ladich & Myrberg 2006). Courtship sounds may also occur when males try to attract females into their territory (for a review, see Myrberg & Lugli 2006). Besides deciphering the mechanisms of sound production (Ladich & Fine 2006), the literature on fish bioacoustics underlines the diversity of emitted sounds and repertoire size among species (e.g. Mann & Lobel 1998; Ladich 2007; Amorim et al. 2008a; Phillips & Johnston 2008). Some authors especially focused on the functional effect of the acoustic channel (e.g. Ladich 1997; De Jong et al. 2007; Vasconcelos et al. 2010). This can be achieved by means of

different experimental approaches like playback experiments, muting experiments and correlative experiments (Ladich & Myrberg 2006). In most cases, the hypothesis that sound production could be a by-product, or at least a far less important communication signal than other cues, has not been experimentally tested. To investigate the biological role of fish sounds during aggressive contests for resources like territories, mates or food, playback experiments represent a valuable tool that has been classically used for decades in other animal taxa like amphibians, birds or mammals (Hauser 1998). Previous studies using acoustic playback in fish have yet been attempted and found contrasting results. In the satinfish shiner (*Cyprinella analostana*), Stout (1963) found that the playback of fighting sounds increases the number and duration of aggressive behaviour between males. Conversely, playback of agonistic sounds seems to inhibit aggressiveness in, e.g. *Archocentrus centrarchus* (Schwarz 1974) and *Itulurus nebulosus* (Rigley & Muir 1979) (see Ladich & Myrberg 2006 for other examples). Fish sounds are typical

close-range signals, implying that they are usually coupled with other stimuli, particularly visual (Amorim 2006). While some playback studies lacked additional visual stimuli (e.g. Lugli 1997), others used mirror images (e.g. Raffinger & Ladich 2009). In this case, a real challenger was missing and the mismatch between visual and acoustic stimuli could have affected the results. Other experiments played back aggressive sounds during fights between two fish. For instance, Rigley & Muir (1979), working on the brown bullhead (*I. nebulosus*), showed that playing back aggressive 'ratchet' sounds produced by an intruder fish reduced the number of attacks performed by a resident. The playback was composed of the intruder's previously recorded own sounds, and one 10-s sound was played every 3 min over the 15 min of the test. Nevertheless, it was played in addition to the natural sounds produced by both contestants during the dispute and was not associated with any visual behaviour the intruder would have been performing. It is thus difficult to understand the biological role of sounds because of the existing interactions between acoustic and visual channels. The use of playback techniques in fish is thus challenging and requires that aggressive acoustic playbacks match the visual stimuli to which aggressive sounds are usually associated.

In this study, we focus on a cichlid fish known for its sound-producing habit and aim to test the significance of sounds produced during male-male aggressive interactions by experimentally separating or coupling visual and acoustic channels. *Metriaclima* (formerly *Pseudotropheus*) *zebra* is a sound-producing rock-dwelling cichlid fish living in the sediment-free rocky coasts of Lake Malawi. Members of the genus *Metriaclima* are characterized by their jaw morphology, a moderately sloped vomer leading fishes to feed perpendicular to the substrate on algae attached to the rock surface (Konings 2007). Males of *M. zebra* are blue with 6–8 vertical black bars on their body and defend territories in which they try to attract females. Sounds are produced during both aggressive and courtship interactions, in association with visual displays (Amorim et al. 2004, 2008b; Simões et al. 2008).

Trials consisted in exposing pairs of males to three different situations where the two tested fish were allowed to interact either (1) *via* acoustic channel only (playback experiment), (2) *via* visual channel only or (3) *via* both channels. As territorial interactions usually occur at close range with both animals seeing each other and given the colour phenotype of the species, we predicted that (1) vision would be

the major communication channel triggering agonistic behaviour and (2) acoustic stimuli would modulate individuals' aggressiveness.

## Methods

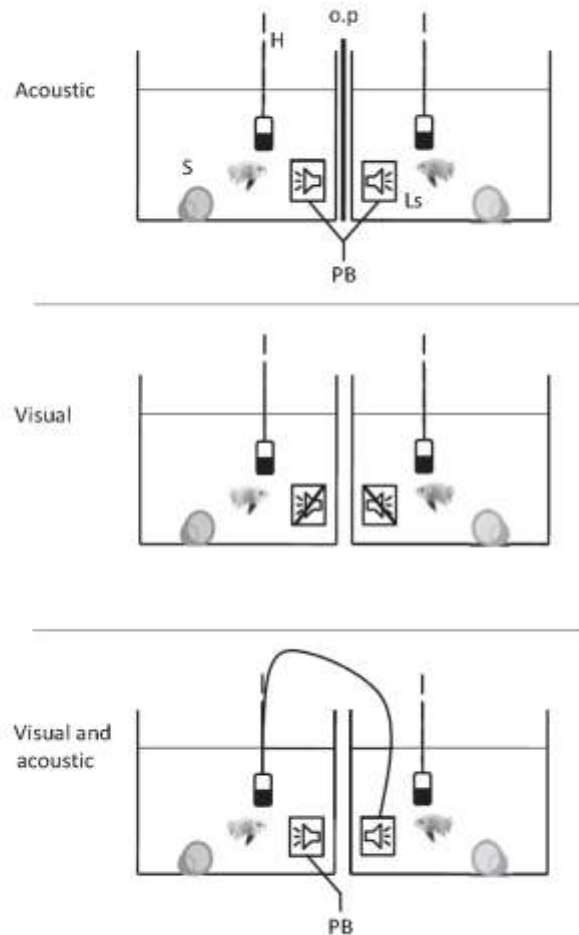
### Fish

*Metriaclima zebra* were purchased from N'Guyen International (Kingersheim, France) and stored in heterosexual groups in two holding tanks (120 cm long, 60 cm wide and 50 cm height) containing 25 individuals each, with a sex ratio of 1:2. Each tank was equipped with an external filter (Rena Filstar xP3, Rena France, Annecy, France), aeration, sand substrate, terracotta pots and bricks as shelters. The temperature was maintained at  $25 \pm 2^\circ\text{C}$  and the pH at 8.0 on a 12 L:12 D d cycle. The fish were fed daily with commercial cichlid food (JBL NovoRift, JBL GmbH & Co. KG, Neuhofen, Germany) complemented with cubes of a mixture of mussels, shrimps and spinach once a week. The fish acclimatized in the holding tanks for 3 mo before the start of the experiment. They were approximately 2 yr old and mature at the time of the experiment.

We used 16 experimental males (eight from each holding tank). A few days prior to experimentation, all individuals were measured for their standard length (from the tip of the head to the caudal peduncle, mm), total length (from the tip of the head to the tip of the caudal fin, mm) and weight (g). Subjects had a  $\bar{x} \pm \text{SE}$  standard length of  $71.93 \pm 0.84$  mm, total length of  $86.56 \pm 0.77$  mm and weight of  $10.48 \pm 0.36$  g. To identify the individuals, besides natural features such as colour, number and position of egg-spots and marks on the body, all fish were individually tagged by means of two-colour (red and/or green) spots combinations of Visible Implant Elastomer (VIE Tags) in three flank positions. Observation of the holding tanks revealed that neither the number nor the colour of the VIE tags affected the behaviour of fish or the status an individual would acquire in the group.

### Experimental Set-up

The set-up consisted of two aquaria (60 cm long, 30 cm wide and 30 cm height) separated by a removable opaque partition (Fig. 1) and placed on a vibration-insulated shelf, i.e. covered with a layer of agglomerated rubber panel and a layer of wood-fibre footfall sound insulation board. The whole set-up was located in an acoustically insulated room to



**Fig. 1:** Experimental setup (not to scale) for the three different treatments. H: hydrophone, Ls: loudspeaker, o.p: opaque partition, PB: playback, S: shelter. Dashed lines represent connections to the computer.

minimize background noise. Each aquarium of the set-up contained a filter, aeration, an internal heater, a sand substrate and a terracotta pot in the middle. We placed an underwater loudspeaker (University sound, Electrovoice, UW-30, EVI Audio France S.A., Lognes, France) in each aquarium, in the zone next to the partition (Fig. 1). Loudspeakers were connected to an amplifier (Denon PMA-100M, Denon France, D&M France SAS, Suresnes, France) and a Marantz PMD670 (Marantz Europe B.V., Eindhoven, The Netherlands) solid-state recorder. To record sounds and behaviour in response to stimuli, we placed a hydrophone (Aquarian Audio Products H2a-XLR, AFAB Enterprises, Anacortes, WA, USA, sensitivity:  $-180$  dB re  $1$  V/ $\mu$ Pa, flat frequency response:  $\pm 4$  dB 20–4.5 kHz) between the shelter and the loudspeaker, i.e. where the individuals would spend

most of their time displaying. A video camera (BUL520, brand, Active Media Concept, Vallauris, France) was positioned in front of the set-up. Hydrophones were connected both to a preamplifier (Yamaha MLA8, Yamaha Music France, Marne-la-Vallée, France) linked to the video capture card of a PC (Osprey-450e) that synchronized audio and video signals, and to the amplifier to play sounds from one aquarium into the other through the loudspeakers (Fig. 1).

During the experiment, we tested pairs of same-sized unfamiliar individuals (coefficient of variation between two individuals' total length  $< 5\%$ ). To avoid familiarization, we never paired the same individuals twice together. We used a repeated measures design with three treatments: (1) visual alone, (2) acoustic alone, (3) visual and acoustic stimuli. To avoid order effects, the treatments' sequences were balanced among subjects.

#### Acoustic Stimuli

Acoustic stimuli were created from recordings made in our laboratory from fish different from those used in the present experiment. The recorded fish were of same age and in the same size range as those tested. Sounds were recorded at a distance of 1–2 body lengths from the focal fish (approximately 15 cm), which is close to the attenuation distance of the fish sound (13.4 cm) calculated with the equation from Akamatsu et al. (2002).

To test the impact of the acoustic channel alone, we played back a sequence of 20 min, recorded from resident males involved in agonistic interactions. The sequence was made of 60 sounds with a  $\bar{x} \pm$  SE duration of  $733.70 \pm 96.37$  ms. Each sound was composed of  $7.79 \pm 0.93$  pulses, with a pulse period of  $98.07 \pm 8.25$  ms. Pulses had a dominant frequency of  $638.18 \pm 16.28$  Hz. The interval between the sounds was  $8.62 \pm 1.69$  s. The playback corresponded to a standard sequence of agonistic sounds recorded from a male that was unknown by the individuals involved in the present experiment. For the visual and acoustic treatment where fish had access to both stimuli, we also used sounds previously recorded from resident males engaged in agonistic interactions. Sounds were different for each experiment ( $n = 10$ , with a  $\bar{x} \pm$  SE duration of  $650.02 \pm 10.02$  ms). They were composed of  $6.33 \pm 0.79$  pulses, with a pulse period of  $103.23 \pm 10.59$  ms. Pulses had a dominant frequency of  $591.37 \pm 13.17$  Hz. These recorded males have also never been heard or seen by the males of the experiment. There

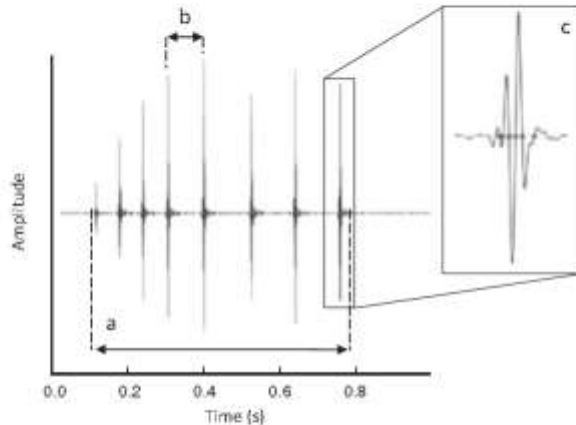
was no difference between the characteristics of the sounds used in the two treatments (unpaired *t*-test:  $t = 0.25$ – $1.16$ ,  $n_1 = 60$ ,  $n_2 = 10$ ,  $p > 0.05$ ). To adjust the level of playback, we made preliminary experiments where we first recorded fish while assessing precisely their distance to the hydrophone. We subsequently replaced the fish by a loudspeaker positioned at the same distance and adjusted the level of playback of the fish sound to get exactly the same level of recording. All acoustic stimuli were digitized at 44.1 kHz (16 bit resolution) with PRAAT software version 5.0.35 (Boersma & Weenink 1992–2008). To make sure that the playback sounds were not altered, two variables of 10 randomly selected sounds have been compared, i.e. mean frequency and pulse period before and after a playback. Sounds had a  $\bar{x} \pm \text{SE}$  frequency of  $590.62 \pm 14.86$  Hz before the playback and  $607.50 \pm 19.93$  Hz after the playback. A paired *t*-test did not reveal any significant difference ( $t = 1.91$ ,  $n = 10$ ,  $p = 0.09$ ). Sounds had a  $\bar{x} \pm \text{SE}$  pulse period of  $80.61 \pm 6.69$  ms before the playback and  $80.66 \pm 6.68$  ms after the playback. Again, a paired *t*-test did not reveal any significant difference ( $t = 1.25$ ,  $n = 10$ ,  $p = 0.24$ ).

#### Progress of the Experiment

A pair of fish was introduced in the set-up (one fish per experimental aquarium) and kept visually and acoustically isolated from each other for 24 h before the beginning of the experiment. This procedure allowed each of the two fish to acclimatize and become resident in its own aquarium. At the beginning of the trials, filters, aeration and heaters were switched off to reduce background noise. Audio and video recordings started with a 10-min control period during which both fish were still isolated. At the end of this control period, the two experimental subjects received one of the three possible treatments. In the visual treatment, the opaque partition separating the two aquaria was removed, the loudspeakers were off, and the two subjects were allowed to interact visually for 20 min. In the acoustic treatment, the opaque partition remained between the aquaria and the 20-min sequence of sounds produced by a resident male was played back to both fish simultaneously. For the visual and acoustic treatment, the opaque partition was removed and the treatment period was divided into two subperiods of 10 min during which one fish could see and simultaneously hear its opponent (live condition), while the second fish could only see its opponent but was challenged with playback of recorded

aggressive sounds whenever aggressive sounds were produced by the first fish (simulated condition). The hydrophone recording the second fish was placed at a sufficient distance from the loudspeaker (20 cm, i.e. above the attenuation distance of the fish sound) so that it was unable to record again the played-back sound. The hydrophone thus only recorded the sounds emitted by the live fish. That way, the fish in the live condition was only challenged with the sounds emitted by its opponent. We carefully checked that this fish never heard the replacement signals. After 10 min, we switched the plugging so that both fish got the same conditions. This peculiar design was set up to avoid a Larsen feedback loop, i.e. audio feedback that occurred when the live recordings of both subjects were played back simultaneously. Although there was a delay (around 500 ms) between the sound emitted by the fish and the playback of the replacement sound, we assume that the replacement sound well mimicked the sound production behaviour of the fish. In practice, the experimenter never made any false positives or negatives in detected signals. Anyway, only the results obtained in the live condition were considered for the statistical analysis of the behavioural response. At the end of a treatment period, the playback, if any, was stopped and the opaque partition was put back in place.

Behavioural data were collected from the videos recorded during the trials. Using the behavioural transcription software EthoLog 2.2.5 (Ottoni 1995–1999) and for each trial, we quantified the number of aggressive behaviours performed by each of the two fish, i.e. lateral display, quiver, charges and bite attempts. We also quantified behaviours that define space occupancy, i.e. time spent swimming near, i.e. less than two fish lengths from the partition between aquaria (s), number of up-and-down swims (when the fish was swimming up and down against the wall of the aquarium close to the wall of the second aquarium), number of maintenance behaviour (when the fish was moving sand within its territory), number of times that the tested fish entered into the shelter, number of times it went under the loudspeaker (the fish could consider the space between the loudspeaker and the substrate as a shelter) and the number of breaks (when the fish was staying still in the water column). Besides these motion behaviours, we counted the total number of sounds produced by each interactant. Sounds were digitized at 44.1 kHz (16 bit resolution). To take into account the peculiarity of sound transmission within the closed environment of aquaria, we calculated the minimum



**Fig. 2:** Oscillogram of a sound produced by a male *M. zebra* in an agonistic context. Some of the measured parameters are represented: sound duration (a), pulse period (b) and number of pulses (c) with the 6 estimates of the instantaneous pulse frequency (c).

resonance frequency of the experimental tanks using the equation from Akamatsu et al. (2002) and consequently applied a low-pass filter of 4.1 kHz to all recordings. The acoustic structure of recorded sounds was analysed with PRAAT software (version 5.0.35; Boersma & Weenink 1992–2008). A sound consisted in a bout of several successive pulses (Amorim et al. 2004), and the following acoustic features were thus measured (Fig. 2): sound duration (ms), number of pulses in a sound, pulse period (average peak to peak interval between two consecutive pulses of a sound, ms) and mean frequency (Hz) (measured as the mean of six zero-crossing-based instantaneous frequency estimations of pulses; see Fig. 2c) (Staddon et al. 1978; Mbu Nyamsi et al. 1994). By simply measuring the instantaneous period (and thus frequency) of each sine wave constituting a pulse, the zero-crossing approach avoids the compromise between precision in time and frequency that is linked to the fast Fourier transform calculation used to build spectrograms.

#### Statistical Analysis

The behavioural responses of each tested individual in the three different treatments (visual, acoustic, visual and acoustic) were compared with a repeated measures ANOVA if the data were normally distributed, followed by the Tukey Honestly Significant Difference (HSD) *post hoc* test if a significant result was found. Otherwise, a Friedman ANOVA was performed, followed by a Tukey Honestly Significant Difference (HSD) *post hoc* test for non-parametric

repeated measures ANOVA in case of significant difference. We performed a principal component analysis (PCA) to summarize the information contained in each set of motion behavioural variables, i.e. space occupancy (time spent near the partition between aquaria (s), number of up-and-down swims, number of maintenance behaviours, number of times in the shelter, number of times under the loudspeakers and number of breaks) and aggressiveness (number of sounds, number of lateral displays, number of quivers, number of bites and number of charges). All tests were two-tailed. Statistical analysis was carried with STATISTICA 6.0 (Statsoft Inc 2004).

## Results

### Sounds Produced During Aggressive Interactions

Ten of the 16 tested males produced sounds during the experimental trials (min–max number of sounds: 1–34). Sounds have been produced during six of eight interactions in the visual treatment and during three of eight interactions in the visual and acoustic treatment. Sounds were produced when individuals were engaged in aggressive interactions, and were mainly associated with visual behavioural displays like lateral displays (58%) and quivers (23%), while sometimes occurring during swimming and breaks. Fish neither showed aggressive behaviour nor produced sounds in the acoustic treatment.

Sounds had a  $\bar{x} \pm \text{SE}$  duration of  $525.71 \pm 79.95$  ms, which were composed of  $8.62 \pm 0.68$  pulses, with a pulse period of  $67.38 \pm 3.84$  ms. Pulses had a dominant frequency of  $400.42 \pm 7.40$  Hz. The short attenuation distance makes the measurement of the absolute sound pressure level (SPL) extremely difficult (Akamatsu et al. 2002).

### Differential Effects of Visual Channel Alone, Acoustic Channel Alone, or Visual and Acoustic Channels Together

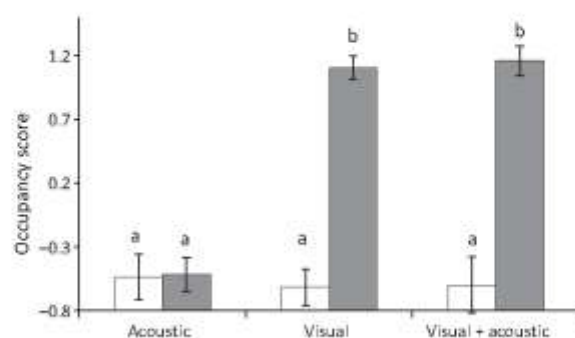
We found no effect of the treatment during the control periods for any variable (repeated measures ANOVA/Friedman ANOVA:  $F/F_r = 1.70\text{--}20.81$ ,  $n = 16$ ,  $p = 0.17\text{--}1$ ). Conversely, we found a significant difference between treatments for the time spent near the partition between aquaria (repeated measures ANOVA:  $F = 11.94$ ,  $n = 16$ ,  $p < 10^{-3}$ ). With a Tukey HSD *post hoc* test, we showed a significant increase in the time spent near the partition when the visual channel was present but not when only acoustic was present [the visual and the visual and

**Table 1:** P values of all pairwise comparisons made using Tukey HSD post-hoc tests which compared aggressiveness and space occupancy in the three different treatments

	Acoustic	Visual	Acoustic	Visual	
Aggressive variables		Motion variables			
<b>Visual</b>					
Number of sounds	<10 <sup>-3</sup>		Time (s) near the partition	<10 <sup>-3</sup>	
Lateral displays	<10 <sup>-3</sup>		Up-and-down swims	<10 <sup>-3</sup>	
Quivers	<0,005		Maintenance	<10 <sup>-3</sup>	
Charges	<0,02		Visit the shelter	NS	
Bite attempts	NS		Breaks	<10 <sup>-3</sup>	
			Under loudspeaker	NS	
Aggressive score	<10 <sup>-3</sup>		Occupancy score	<10 <sup>-3</sup>	
<b>Visual and acoustic</b>					
Number of sounds	NS	<0,02	Time (s) near the partition	<10 <sup>-3</sup>	NS
Lateral displays	NS (<0,10)	<0,005	Up-and-down swims	<10 <sup>-3</sup>	NS
Quivers	NS	<0,05	Maintenance	<0,002	NS
Charges	<0,05	NS	Visit the shelter	NS	NS
Bite attempts	NS	NS	Breaks	<10 <sup>-3</sup>	NS
			Under loudspeaker	NS	NS
Aggressive score	<10 <sup>-3</sup>	<10 <sup>-3</sup>	Occupancy score	<10 <sup>-3</sup>	NS

acoustic treatments were significantly different from the acoustic treatment (at  $p < 0.001$ ) but not from each other] (Table 1). We found the same result for the number of up-and-down swims (repeated measures ANOVA:  $F = 20.81$ ,  $n = 16$ ,  $p < 10^{-3}$ ). A difference between treatments was also found for the number of maintenance behaviours (repeated measures ANOVA:  $F = 8.14$ ,  $n = 16$ ,  $p < 10^{-3}$ ), the number of times fishes visited the shelters (Friedman ANOVA:  $F_r = 8.86$ ,  $n = 16$ ,  $p = 0.012$ ) and the number of breaks (repeated measures ANOVA:  $F = 8.51$ ,  $n = 16$ ,  $p < 10^{-3}$ ). Tukey HSD *post hoc* tests showed a significant decrease in these behaviours when the visual channel was present compared to when only the acoustic channel was present (Table 1). Treatments did not affect the number of times subjects came under the loudspeakers (repeated measures ANOVA:  $F = 1.70$ ,  $n = 16$ ,  $p = 0.15$ ).

The first principal component of the PCA performed on the space occupancy variables was derived as follows:  $0.55 \times$  time spent near the partition between aquaria,  $0.86 \times$  number of up-and-down swims,  $-0.46 \times$  number of maintenance behaviours,  $-0.57 \times$  number of times in the shelter,  $-0.41 \times$  number of times under the loudspeakers,  $-0.71 \times$  number of breaks. This component explained 37.74% of the variance in the data, and a high score indicates a lower occupancy (more active pattern) than a low score (routine-like pattern). We found a significant effect of the treatment for this variable (Friedman

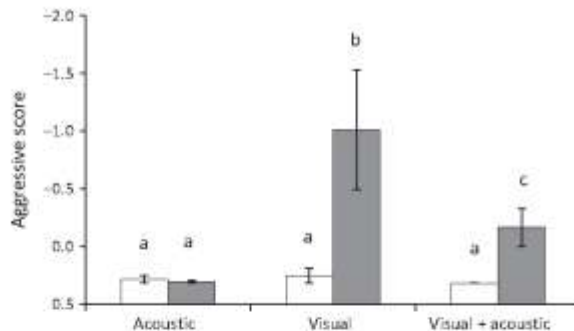


**Fig. 3:** Space occupancy scores for the three different treatments. A high score indicates a lower occupancy (active pattern) than a low score (routine-like pattern) (□: control period, ■: test). Values are mean  $\pm$  SE. Different letters indicate statistically significant differences (HSD post hoc test for non parametric repeated measures ANOVA).

ANOVA:  $F_r = 24.00$ ,  $n = 16$ ,  $p < 10^{-3}$ ) with a lower score in the acoustic treatment than in the visual and in the visual and acoustic treatments (Table 1; Fig. 3).

We found a significant effect of the treatment on the number of sounds produced (Friedman ANOVA:  $F_r = 14.31$ ,  $n = 16$ ,  $p < 10^{-3}$ ), the number of lateral displays (Friedman ANOVA:  $F_r = 20.39$ ,  $n = 16$ ,  $p < 10^{-3}$ ), the number of quivers (Friedman ANOVA:  $F_r = 13.86$ ,  $n = 16$ ,  $p < 10^{-3}$ ) and the number of charges (Friedman ANOVA:  $F_r = 9.39$ ,  $n = 16$ ,  $p < 0.01$ ). No effect on the number of bite attempts was found (Friedman ANOVA:  $F_r = 0.89$ ,  $n = 16$ ,  $p = 0.64$ ). The first principal component of the PCA





**Fig. 4:** Aggressive scores for the three different treatments. A high negative score indicates a stronger aggressive response than a low negative score (□: control period, ■: test). Values are mean  $\pm$  SE. Different letters indicate statistically significant differences (HSD post hoc test for non parametric repeated measures ANOVA).

performed on the aggressive variables was derived as follows:  $-0.94 \times$  number of sounds,  $-0.88 \times$  number of lateral displays,  $-0.90 \times$  number of quivers,  $-0.49 \times$  number of bites,  $-0.76 \times$  number of charges. This component explained 65.7% of the variance in the data, and a high negative score indicates a higher aggressiveness than a low negative score. We found a significant effect of the treatment for this variable (Friedman ANOVA:  $F_r = 15.31$ ,  $n = 16$ ,  $p < 10^{-3}$ ) with a high aggressiveness in the visual treatment, a low aggressiveness in the acoustic treatment and an intermediate level for the treatment with visual and acoustic stimuli (Table 1; Fig. 4). We also used this aggressive score to define potential future winners (the fish with the highest negative score) and potential future losers (the fish with the lowest negative score) within the different dyads. During the test periods of the visual and the visual and acoustic treatments, we could discriminate winners and losers based on their PCA score, i.e. winners were significantly more aggressive than losers (Friedman ANOVA:  $F_r = 22.07$ ,  $n = 8$ ,  $p < 10^{-3}$  and  $F_r = 18.28$ ,  $n = 8$ ,  $p < 10^{-3}$  for, respectively, the visual and the visual and acoustic treatments). No difference was found for the acoustic treatment and the three control periods. Moreover, when considering the interactions during which sounds have been produced, future winners had a tendency to produce more sounds than future losers (Wilcoxon signed-rank test:  $T = 7$ ,  $n = 9$ ,  $p = 0.066$ ).

## Discussion

As reviewed by Ladich & Myrberg (2006), sounds produced during aggressive encounters in fish may

have different roles like repel an intruder, increase or decrease an opponent's aggressiveness, and could also be used in fighting ability assessment. As in other animal taxa, playback experiments have been performed to investigate the biological role of sounds in fishes but often failed to match visual and acoustic stimuli.

Our study examined the role of sounds in agonistic interactions between males *M. zebra* by coupling or separating visual and acoustic stimuli. As soon as a tested fish was visually stimulated by another male, it changed its space occupancy pattern from a routine-like pattern (low occupancy score) consisting in swimming around the aquarium, visiting the shelter and moving the substrate, to a more active pattern (high occupancy score) where the latter behaviours decreased and the motivation to access the opponent and to engage in fight increased, as indicated by up-and-down swims. Acoustic stimuli had no effect on space occupancy. Sound production, however, affected the level of aggressiveness. The highest level of aggressiveness was found when fishes were acoustically isolated and could only interact visually. When sounds were associated with visual stimuli, i.e. as in normal condition, the level of aggression demonstrated by interactants lowered. Besides, sound production always happens during fighting-like behaviours, sounds being produced at a close distance from the opponent. This may explain why acoustic stimuli alone did not produce any effect on motivation nor triggered agonistic behaviour. In agreement with the results of Schwarz (1974) who found a reduction in the number of highly aggressive encounters in response to playbacks of fish sounds in *Cichlasoma centrarchus*, our results strengthen the hypothesis that aggressiveness seems to be firstly based on visual signals and sound production would be a complementary display used during contests. However, in this latter study, there was no acoustic-visual matching either temporal or spatial, i.e. the loudspeaker was positioned in the centre of the tank rather than close to the opponent, and was playing a loop-recording of fish sounds.

The association of sound with other behavioural features suggests that the number of sounds produced and their acoustic characteristics may be used during a dispute to assess an individual's status and/or motivation and may consequently modulate the opponent's behaviour, resulting, in the present case, in a reduction in its aggressiveness. This might avoid escalating interactions and one would expect the future winner of a fight to produce more sounds

than the future loser. In interactions where sounds have been produced (6/8 in the visual treatment, 3/8 in the visual and acoustic treatment), the winner indeed tended to produce more sounds than the loser. Ladich (1998) found that certain sound characteristics of male croaking gouramis (*T. vittata*), i.e. dominant frequency and SPL, were correlated with weight and could predict the outcome of contests. However, winners and losers did not differ in the number of sounds they produced. This latter point would imply that acoustic signals may have different significance and may be used in different ways in different species. Likewise, Colleye et al. (2009) reported a strong correlation between the dominant frequency of a pulse and its duration with the size of an individual in the skunk clownfish (*Amphiprion akallopisos*) which implies that different sound characteristics might be of interest in different species to assess conspecifics. In this study, we observed a lower dominant frequency of sounds in fish used for the experiment than in fish used to obtain the different playbacks although they were of similar size and age, which would mean that this parameter could also be, for example, status dependent (most of the fish used for playbacks recordings were dominant). Nevertheless, because a real control for sound effect using white noise is missing, we cannot rule out the possibility that any other sound would have given the same results. However, the reduction in aggressiveness observed when visual and acoustic stimuli were associated compared to the null response when acoustic stimuli were the only source of information makes us confident in the appeasing role of sounds during agonistic interactions in this species. A fine analysis of the signal may thus help investigating how much information is encoded and which are the important parameters of the sounds in *M. zebra*.

Besides, as natural interactions may occur within communication networks involving several potential signallers and receivers within signalling range of each other, bystanders may eavesdrop upon observed interactions and use this information during future encounters (McGregor & Dabelsteen 1996). However, the active space of our fish's sounds – the volume in which communication signals remain biologically significant to receivers – is certainly limited by their short transmission range, making, together with the hearing abilities of the species and the ambient noise, eavesdropping by distant individuals unlikely.

In accordance with previous studies (Amorim et al. 2004; Phillips & Johnston 2008; Simões et al. 2008), not all individuals of our experiments pro-

duced sounds. Amorim & Almada (2005) showed that recent social experience affected male sound production during courtship in *Oreochromis mossambicus*. Social experience and social status may then also influence sound production during aggressive encounters, and a 24-h period of isolation might not be sufficient to completely erase individuals' social status.

In conclusion, the kick-off of agonistic encounters in *M. zebra* is mostly based on visual signals; acoustic signals alone never trigger aggression. However, sound signals emitted in an agonistic context may influence the behavioural response of an opponent. This result underlines the modulatory role played by sounds in aggressive social interactions. Apart from this, *M. zebra* produces acoustic signals in other contexts like during courtship (Simões et al. 2008). Although no experimental procedure has ever tested the biological significance of those sounds, they might appease females and influence mate choice (Verzijden et al. 2010). In this fish species, sounds are likely to have complex and unforecast roles, bearing information, besides fighting abilities, motivation or status, that remains to be investigated.

#### Acknowledgements

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Oscillograms of a sound produced by a male *M. zebra* in an agonistic context before playing it back in a tank (a) and once played back (b). For each oscillogram, the detail of a pulse is shown.

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# **Sounds produced by the cichlid fish *Metriaclima zebra* allow reliable estimation of size and provide information on individual identity**

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## Résumé de l'article 2

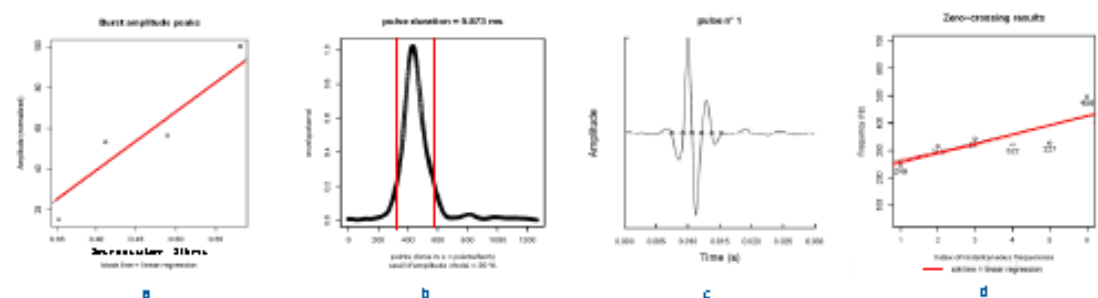
### Introduction

Afin d'établir et maintenir la cohésion d'un groupe, différents individus doivent pouvoir se reconnaître grâce aux informations portées par différentes modalités sensorielles. Les signaux acoustiques jouent un rôle social important chez *M. zebra*. Permettent-ils de coder l'identité individuelle de l'émetteur ?

### Matériel et méthodes

Les sons produits par 11 mâles lors d'interactions agressives ont été analysés de manière fine jus qu'au niveau du pulse. Deux groupes ont été utilisés (7 individus de 2 ans et 4 individus de 4 ans).

Des paramètres temporels (nombre de pulses par son, durée du son (a), durée (b) et période des pulses) et spectraux (fréquence instantanée des pulses (c), modulation de la fréquence instantanée des pulses (d) et modulation d'amplitude des pulses (a)) ont ainsi été mesurés.



Pour chacune de ces variables, nous avons calculé un potentiel pour le codage de l'individualité (PIC) et testé les différences inter-individuelles.

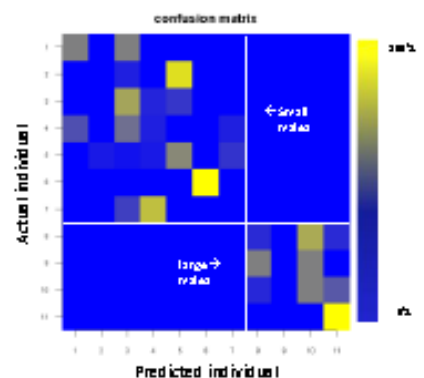
Les variables présentant une différence inter-individuelle significative ont ensuite été utilisées afin de conduire une analyse de fonctions discriminantes permutée (pDFA).

### Résultats

Comme chez de nombreux autres poissons, il existe des corrélations entre des caractéristiques acoustiques et la morphologie (taille et poids) des individus.

De plus, des variables temporelles et spectrales présentent des différences inter-individuelles significatives et pourraient donc permettre le codage d'une information liée à l'identité de l'émetteur.

Cependant, une classification parfaite n'est pas atteinte (50% des sons analysés ont été correctement attribués à leur émetteur). Seuls les deux groupes testés sont clairement identifiés grâce aux sons produits.



### Conclusion

Les signaux acoustiques ne semblent pouvoir porter qu'une partie d'une information complexe comme l'identité individuelle. Les caractéristiques acoustiques restent cependant de bons indicateurs de la taille de l'émetteur mais sont donc susceptibles d'évoluer lors du développement.

Des expériences de playback et de neurophysiologie pourraient permettre d'étudier la perception et l'utilisation de cette information.

Bertucci, F., Attis, J., Beauchaud, M. & Methevon, N. (In revision). Sounds produced by the cichlid fish *Methriaclima zebra* allow reliable estimation of size and provide information on individual identity.

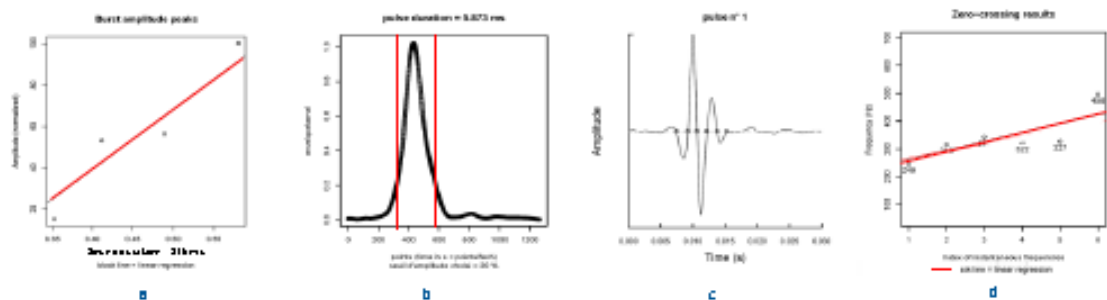
## Summary of article 2

### Introduction

In order to establish and maintain the cohesion of a group, different individuals may be able to recognize each other by means of the information carried by different sensory modalities. Acoustic signals play an important social role in *M. zebra*. Do acoustic signals encode the individual identity of the sender?

### Material and methods

A fine analysis of sounds produced by 11 males during aggressive interactions has been performed down to the pulse level. Two groups were used (7 individuals of 2 years of age and 4 individuals of 4 years of age). Temporal parameters (number of pulses per sound, sound duration (a), pulse duration (b) and period) and spectral (instantaneous frequency of pulses (c), instantaneous pulses frequency modulation (d) and pulses amplitude modulation (a)) were measured.



For each variable, we calculated a potential for individuality coding (PIC) and tested inter-individual differences.

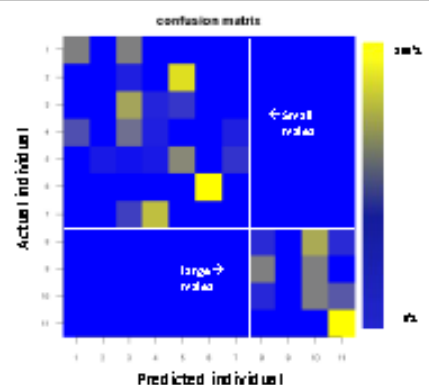
Variables with a significant inter-individual difference were then used in order to conduct a permuted discriminant functions analysis (pDFA).

### Results

Like in many other fish species, we find correlations between some acoustic features and the morphology (size and weight) of the individuals.

Moreover, temporal and spectral variables show significant inter-individual differences and may therefore encode some information related to the identity of the emitter.

However, a perfect classification was not achieved (50% of analysed sounds were correctly attributed to the right fish). The two tested groups are clearly identified by the sounds produced.



### Conclusion

Acoustic signals seem to carry only a part of a complex information like individual identity. Acoustic features are good indicators of the size of an emitter though, but are likely to change during the development of individuals.

Playback and neurophysiology experiments could allow us to study how this information is perceived and used.

Bertucci, F., Attie, J., Beauchaud, M. & Mathévon, N. (In revision). Sounds produced by the cichlid fish *Mtetracoma zebra* allow reliable estimation of size and provide information on individual identity.

## **Abstract**

Sounds produced by male cichlids *Metriaclima zebra* during aggressive interactions were recorded for the purpose of conducting a detailed analysis and searching for the potential of an individual acoustic signature. Eleven fish, divided in two different groups of size (small and large individuals made of respectively 7 and 4 individuals) were analysed. The two groups were significantly different for all acoustical variables considered; six out of seven features demonstrated a significant inter-individual variability and most of them were correlated with the size of the emitter. A cross-validated and permuted discriminant function analysis (pDFA) further clearly separated the two groups and correctly classified around 50% of the sounds to the correct individuals. Acoustic features allowing to best distinguish between males were the instantaneous frequency of sounds and the modulation of pulse amplitude. These results suggest that acoustic signals could bear some information about individual identity.

## **Key Words**

Acoustic communication; aggressive sounds; fish; individual signature.



## INTRODUCTION

Many fish species produce low-frequency pulsed sounds which may play an important role in their social life, e.g. during mate choice or aggressive interactions (Amorim *et al.*, 2011; Ladich & Myrberg, 2006; Myrberg & Lugli, 2006). Even though the diversity of fish acoustic signals is tremendously smaller than that found within birds or mammals, inter-specific differences have been observed between closely related species. For instance, among the five sound types that have been described in mormyrids (four species studied), Crawford *et al.* (1997) demonstrated that the acoustic courtship displays of two closely related species of this group exhibit differences in the pulse repetition rate of one sound type (grunts) and in the fundamental frequency of another sound type (moans). Likewise, studies on cichlids from Lake Malawi highlighted differences in the sound characteristics of sympatric species. For example, Amorim *et al.* (2004) found significant differences in pulse number, pulse duration and peak frequency of three *Pseudotropheus* species (for other examples, see Amorim, 2006).

In addition to inter-specific differences, fish acoustic signals can potentially yield information that allows to discriminate between individuals from the same species. These signals may convey information related to social rank or individual identity and serve in the establishment of social relationships. Correlations between the acoustic structure of acoustic signals and an individual's body size, sex, motivation or physiological state are well-known in vertebrates, from amphibians to mammals (e.g. Reby & McComb, 2003; Pfefferle & Fisher, 2006; Koren *et al.*, 2008; Mathevon *et al.*, 2010). In fish, the most common source of intra-specific variation reported is an inverse relationship between the dominant frequency of the produced sound and the size of the emitter (Crawford *et al.*, 1997; Amorim *et al.*, 2003; Colleye *et al.*, 2009). Previous studies also showed that larger males are more likely to win contests and become dominant, and acoustic features related to body size were good predictors of the outcome of a fight between male croaking gouramis *Trichopsis vittata* (Cuvier 1831) (Ladich, 1998). Furthermore, Amorim & Almada (2005) found that male *Oreochromis mossambicus* (Peters 1852) courtship sounds were affected by recent social experience, i.e. winning or losing a fight. Recent social status could thus be encoded in courtship sounds and play a role in mate choice or even in the assessment of opponents by an eavesdropping male. These results support the idea that some fish sounds may carry more information than simply the size of an emitter. In mormyrids and batrachoidids, intraspecific differences between individuals of comparable body size have thus been found (Amorim, 2006). Although the study of individual vocal characteristics in vociferous fishes, e.g. in cichlids (Amorim & Almada, 2005), gouramis (Ladich *et al.*, 1992), damselfish (Myrberg *et al.*, 1993) or

mormyrids (Crawford et al., 1997), received little attention (Amorim, 2006), individuality in male courtship sounds has already been shown to play a role in neighbour recognition and in female mate choice (Myrberg & Lugli, 2006; De Jong *et al.*, 2007). How could information about individual identity be encoded in fish acoustic signals? Amorim & Vasconcelos (2008) studied the mating call (boatwhistle) of the Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider 1801) and found that the most important variables that would permit discrimination among males were the dominant frequency of the middle part of the boatwhistle, the frequency modulation between the beginning and the middle part, the pulse period of the middle part, the amplitude modulation and the duration of the boatwhistle. Diversity of fish sounds, mostly based on their temporal pattern and their frequency, may thus encode information ranging from the specific to the individual level of recognition. To our best knowledge, the study by Amorim & Vasconcelos (2008) appears to be the only one that tested the existence of an elaborate individual signature in fish. Research effort is thus clearly needed in order to fully understand the role of sound production within the social life of fish.

Sound production in cichlids is well documented (Lobel, 2001). Both sexes commonly produce acoustic signals during courtship or aggression. Within a species, the repertoire is usually made of one or two sound types (Amorim, 2006). The great variability of sounds observed at the interspecific level suggests that acoustic channel must have played a role in the speciation rate observed in the great African lakes (Amorim *et al.*, 2004; Verzijden *et al.*, 2010). In spite of the potential interest to understand this phenomenon, and besides the common correlation with size (Amorim *et al.*, 2003, 2004), little is known about information encoded in acoustic signals at the intraspecific level.

*Metriaclima* (formerly *Pseudotropheus*) *zebra* (Boulenger 1899) is a rock-dwelling cichlid from Lake Malawi known to produce sounds in different social contexts. Males will defend territories against other males and try to attract females (Konings, 2007). During agonistic or courtship interactions, males adopt postures like quivering or lateral displays and produce low frequency sounds consisting in a train of short pulses (burst). Simões *et al.* (2008) reported that larger males produced quiver sounds of lower frequencies during courtship. Moreover, temporal features of agonistic sounds contain information related to sex and to social context: males' sounds include more pulses than females, and male-male contest sounds are longer than female-female contest sounds. In a previous study, it has been shown that when two dominant males interact, sounds contributed to a decreased level of aggressiveness (Bertucci *et al.*, 2010). The acoustic signals of *Metriaclima zebra* may thus be informative to receivers.

In the present study, an experiment in which two dominant males were engaged in agonistic interactions was performed and the sounds that they produced were recorded. The objective of this study was to conduct a detailed analysis of these sounds with the aim of searching for their potential to encode individual characteristics.

## **MATERIAL AND METHODS**

### **FISH**

*Metriaclima zebra* were purchased from N'Guyen International (Kingersheim, France) and stored in heterosexual groups in holding tanks (60 x 120 x 50 cm) containing 10-12 individuals each. Each tank was equipped with an external filter (Rena Filstar xP3, Rena France, Annecy, France), an aeration device, sand substrate, terracotta pots and bricks as shelters. The temperature was maintained at  $25 \pm 2^\circ\text{C}$  by an internal heater (RenaCal 200, Rena France, Annecy, France) and the pH was maintained at 8.0 on a 12L:12D cycle. Fish were fed daily with commercial cichlid food (JBL NovoRift, JBL GmbH & Co. KG, Neuhofen, Germany). Once a week this diet was complemented with a frozen mixture of mussels, shrimps and spinach.

A total of 37 adult males were divided into two groups. The first group comprised 17 individuals approximately two years of age, with a mean  $\pm$  SE standard length (from the tip of the head to the caudal peduncle) of  $61.80 \pm 1.11$  mm, a total length (from the tip of the head to the end of the caudal fin) of  $75.32 \pm 1.34$  mm and a weight of  $6.53 \pm 0.27$  g. The second group was composed by 20 larger individuals, aged three to four years, with a standard length of  $99.35 \pm 2.69$  mm, a total length of  $110.95 \pm 8.15$  mm and a weight of  $29.44 \pm 1.74$  g. Within each group, individuals were identified by means of VIE tags (Visible Implant Elastomer, Northwest Marine Technology, Shaw Island, WA) implanted beneath the skin in three flank positions. The number and size of egg-spots located on the anal fin were also used as a second identifier.

### **EXPERIMENTAL PROCEDURE**

The experimental apparatus consisted in an aquarium (35 x 80 x 35 cm), divided into two equal parts by a removable opaque partition and a removable transparent partition placed side by side in the centre of the aquarium. Each part of the set-up contained a filter, an

aeration device, an internal heater, a sand substrate and a terracotta pot in the middle in order to provide a shelter to the fish.

A video camera (BUL520, Active Media Concept, Vallauris, France) was positioned in front of the aquarium in order to record behaviour during trials. To record acoustic signals, a hydrophone (Aquarian Audio Products H2a-XLR, sensitivity: -180 dB re 1V/ $\mu$ Pa, flat frequency response:  $\pm$  4dB in the range 20 Hz – 4.5 kHz) was placed in each compartment, between the shelter and the partitions separating the two parts of the aquarium, i.e. where individuals were expected to spend most of their time displaying. The hydrophones were connected to a preamplifier (Yamaha MLA8, Yamaha Music France, Marne-la-Vallée, France) and a video capture card (Osprey-450e) of a PC which synchronized audio and video signals.

The experiment was performed in an acoustically insulated room to minimize external background noise. The experimental apparatus was positioned on a shelf covered with a layer of agglomerated rubber panel and a layer of wood-fiber footfall sound insulation board to reduce vibrations transmitted from the floor. Three walls of the aquarium were covered with bubble wrap to reduce sound reverberation within the aquarium. During the experimental sessions, the filter and the aeration device were switched off to allow recordings of fish sound.

Acoustic signals had to be recorded during interactions between males with established territories. The experiment had thus two sessions: 1) a first session of interaction between two males naïve towards the experimental apparatus, 2) a second session after the establishment of territories. The first session started with the introduction of two unfamiliar, size-matched males (coefficient of variation of their total length <5%) in each part of the aquarium. Individuals were given 10-15 minutes to acclimatize prior to interaction. The opaque partition was then removed, allowing the two subjects to interact visually during 20 minutes. If no sounds had been produced during this period, the transparent partition was also removed, allowing the subjects to interact physically. The interaction was terminated and fish were separated by replacing the transparent partition as soon as highly aggressive behaviours, especially biting, were observed. The longest interaction permitted did not exceed 5 minutes.

Between the first and the second sessions, the fish remained in the experimental apparatus, but separated for 24 hours under a 12L:12D day cycle. This period allowed males to establish a territory, i.e. by moving sand substrate (digging) around the shelter. After 24 hours, the interaction procedure was repeated and the fish were returned to their storage tank at the end of the trial. Fish were carefully inspected for injuries although no signs of

external physical damage were observed. For the analysis procedure, only the sounds produced during the second sessions were considered.

In order to collect a sufficient number of sounds for the analysis, the whole experiment was repeated a maximum of three times for each male. Two individuals were never paired more than once to avoid familiarization. Only sounds composed by more than two pulses and recorded within 1-2 body lengths from the hydrophone were considered. Analysis was restricted to males for which we obtained at least 5 sounds (mean: 9.54; min-max: 5-22). By doing so, we were able to analyse sounds of 7 individuals from the first group and 4 individuals from the second group.

## SOUND ANALYSIS

Sounds were digitized at 44.1 kHz (16 bit resolution) and analysed using a custom routine written in R (version 2.8.1) with the Seewave package (version 1.5.4) (Sueur *et al.*, 2008).

The seven parameters extracted from sounds were: 1) the number of pulses within a sound (the custom routine detected the maximum amplitude (peak) within the sound as the pulse max and subsequently sought lower peaks until they no longer exceeded 10% of the pulse max amplitude, peaks that did not exceed 10% of the max were considered as background noise) (Fig. 1a), 2) the sound duration (ms) (measured from the beginning of the first pulse to the end of the last pulse), 3) the pulse amplitude modulation (measured as the slope of the regression line of the relative amplitude peaks in a sound. The maximum amplitude peak was first detected, and normalized to 100. The amplitude peaks preceding this maximum were then measured, relative to this 100 reference. The maximum peak was usually the last one in the series. If not, the amplitude peaks following the maximum was ignored for the regression calculation, Fig. 1b), 4) the pulse period (measured as the average peak to peak time interval between two consecutive pulses, ms), 5) the mean pulse duration (by first calculating the amplitude envelope of the pulse, then smoothing the envelope -with a 30 points moving average, and finally applying a threshold -fixed at 20% of the maximum amplitude of the pulse, see Fig. 1d, ms), 6) the mean instantaneous pulse frequency (measured as the mean of six zero-crossing-based instantaneous frequency estimations of pulses, Hz) (Fig. 1c), 7) the pulse frequency modulation (measured as the slope of the regression line of the six zero-crossing estimations) (Fig. 1e). The use of zero-crossings allows the display of instantaneous frequency, the frequency being calculated as the inverse of a complete cycle duration. Zero-crossing was used to avoid the compromise between

precision in time and frequency that is linked to the fast Fourier transform (FFT) calculation used to build spectrograms (Staddon *et al.*, 1978; Mbu Nyamsi *et al.*, 1994).

Potential differences between the two groups of fish (2 and 3-4 years old) were examined by means of an analysis of variance with group as a fixed factor and fish identity as a random factor. The individual variability among acoustic features was then compared by calculating within-males and between-males coefficients of variation ( $CV = \text{Standard Deviation} / \text{mean}$ ) (e.g. Charrier *et al.*, 2003). The within-male CV ( $CV_w$ ) was calculated from means and standard deviations of each variable of the sounds produced by each individual male. The between-male CV ( $CV_b$ ) was calculated for each variable by dividing the overall standard deviation by its respective overall mean. The Potential for Individual Coding (PIC), calculated as the  $CV_b/CV_w$  ratio, provided a measure of the relative between-males variability for each sound variable. If the PIC value of a variable is greater than one, this variable could potentially be used as a cue for individual recognition. The significance of individual differences among males was tested for each sound features by using Kruskal-Wallis tests.

To further test if fish sounds could bear an individual signature, a multivariate approach was performed by using a cross validated and permuted discriminant function analysis (pDFA) (Mundry & Sommer, 2007, Mathevon *et al.*, 2010). The DFA is usually composed of two steps: a set of discriminant functions is first obtained from a training data set; these functions are then used to test classification on a validation set. In the first step of our analysis, a fitting data set formed by the three fifth of the sounds recorded for each individual was used to generate linear discriminant functions. For the second step, a validation data set formed by the remaining two fifth of the sounds was employed to assay the number of correctly classified sounds using the discriminant functions obtained in the first step of the DFA. This cross-validation step gives a measure of the effect size (the percent of correctly assigned sounds). Those two steps were performed 100 times (i.e. 100 random selections of both training and validation data sets). The mean effect size was calculated from these 100 iterations. By performing the cross-validation step, not only does one obtain a desirable measure of effect size (the percent correct) but also the assumption of normality is relaxed. From the cross-validation results, a confusion matrix was extracted, informing about the conditional probability of guessing that a sound emitted by the individual  $i$  was in fact emitted by  $j$ :  $confusion(i,j)=p(ilj)$ . To obtain the statistical significance of the effect size calculated by the cross-validated step, data sets where the identity of sounds is randomly permuted between individuals were created. The procedure is called permuted DFA. As with the non-randomized sets, the same steps –training and validation– were followed for each of these randomized sets. After 1000 iterations, the proportion of randomized validation data sets

revealing a number of correctly classified sounds being at least as large as the effect size obtained with the non-randomized validation data set was calculated. This proportion gives the significance of the level of discrimination and is equivalent to a p-value (Mundry & Sommers, 2007). The pDFA analysis was made with a customized R routine (Mundry, pers. com., Mathevon *et al.*, 2010).

## RESULTS

### SOUND STRUCTURE

In the first group (animals 2 years of age), in which sounds from 7 out of 17 individuals were analyzed (these individuals produced at least five sounds that could be analyzed; min-max number of sounds: 5-22, Table I), fish had a mean  $\pm$  SE standard length of  $64.57 \pm 1.36$  mm, a total length of  $77.86 \pm 1.91$  mm and a weight of  $7.36 \pm 0.45$  g. They produced sounds with a mean  $\pm$  SE duration of  $654.20 \pm 42.78$  ms which were composed of  $8.09 \pm 0.49$  pulses, with a pulse period of  $85.94 \pm 4.19$  ms and a pulse duration of  $7.45 \pm 0.19$  ms. The mean slope of the regression line of the peaks amplitude of a sound (amplitude modulation) was  $0.52 \pm 0.01$  % per second. Pulses had a mean instantaneous frequency of  $592.49 \pm 9.55$  Hz with a mean slope of the regression line of the six frequency estimations (frequency modulation) of  $94.09 \pm 7.38$  Hz/period.

In the second group (animals 3-4 years of age), in which 4 out of 20 individuals produced enough suitable sounds for analysis (min-max number of sounds: 5-18, Table I), fish had a mean  $\pm$  SE standard length of  $104.50 \pm 1.26$  mm, a total length of  $121.25 \pm 2.09$  mm and a weight of  $30.95 \pm 0.82$  g. Produced sounds had a mean  $\pm$  SE duration of  $571.20 \pm 51.69$  ms, were composed of  $6.77 \pm 0.46$  pulses, with a pulse period of  $87.16 \pm 7.53$  ms and a pulse duration of  $9.44 \pm 0.52$  ms. The mean slope of the regression line of the amplitude peaks of a sound (amplitude modulation) was  $0.80 \pm 0.02$  % per second. Pulses produced by these fish had a mean instantaneous frequency of  $301.10 \pm 8.31$  Hz with a mean slope of the regression line of the six frequency estimations (frequency modulation) of  $19.44 \pm 4.16$  Hz/period.

No significant differences were found between the two groups for the sound duration (ANOVA:  $F_{1,9} = 1.41$ ,  $p = 0.26$ ), the number of pulses within the sounds (ANOVA:  $F_{1,9} = 2.51$ ,  $p = 0.15$ ) and the period of pulses (ANOVA:  $F_{1,9} = 1.01$ ,  $p = 0.34$ ). Larger individuals tended to show a longer pulse duration than smaller individuals (ANOVA:  $F_{1,9} = 3.99$ ,  $p = 0.07$ ).

Larger individuals showed a significantly lower pulse instantaneous frequency (ANOVA:  $F_{1,9} = 98.14$ ,  $p < 10^{-5}$ ) and a lower frequency modulation rate (ANOVA:  $F_{1,9} = 9.34$ ,  $p = 0.01$ ). The amplitude modulation rate was significantly greater in larger individuals than in smaller ones (ANOVA:  $F_{1,9} = 35.91$ ,  $p < 10^{-3}$ ).

Several significant correlations were found among sound features and between sound features and the size (standard length and weight) of the individuals (Table II). Instantaneous frequency and pulse frequency modulation were both negatively correlated with size ( $n = 11$ ,  $r = -0.84$ ,  $p = 0.001$  and  $r = -0.80$ ,  $p = 0.003$  respectively) (Fig. 2a, b) and weight ( $n=11$ ,  $r = -0.95$ ,  $p < 10^{-3}$  and  $r = -0.72$ ,  $p = 0.012$  respectively). The pulse amplitude modulation was positively correlated with size ( $n = 11$ ,  $r = 0.78$ ,  $p = 0.004$ ) (Fig. 2c) and weight ( $n = 11$ ,  $r = 0.92$ ,  $p < 10^{-3}$ ).

## POTENTIAL CODING OF INDIVIDUALITY

All the analyzed acoustic features had a PIC value greater than one. There was thus more variability between than within individuals. The subsequent Kruskal-Wallis tests showed significant differences for all variables but sound duration (Table III). Taken together, these results suggest that the remaining six variables (number of pulses, pulse period, pulse duration, instantaneous pulse frequency, pulse frequency modulation and pulse amplitude modulation) could serve as individual recognition cues.

The DFA based on the six sound features generated six discriminant functions (Table IV). The first function explained 48% of the variance and separated individuals mainly upon the mean instantaneous pulse frequency. The pulse amplitude modulation showed also a high loading. The pulse amplitude modulation and the number of pulses were the main factors that separated individuals on the second function which explained 20% of the total variance. The third function relied on the pulse amplitude modulation and the pulse period. These first three functions explained 80.5% of the total variance between individuals. Thus and given the function loadings, the main factors explaining the difference between individuals were the instantaneous pulse frequency and the amplitude modulation rate. The first function represents the spectral feature of the sound, while the second and third functions reflect the temporal structure of the sound. After cross-validation, the correct average classification between individuals was 48.6% (versus 8.99% of correct classification for random permutations,  $p < 0.001$ ). However, there was extensive variation in the classification success rate among individuals (average = 48.6%, individual range 6.66% - 100%). Among



the fish we considered, some individuals thus produced similar aggressive sounds and were poorly classified (e.g. fish 5 and fish 10 in Fig. 3). On the other hand, some individuals produced relatively unique sounds (e.g. fish 6 and fish 11 in Fig. 3).

As illustrated by the classification matrix (Fig. 3), a striking result is that fish from an age group are never classified in the other age group. Thus, when the measured acoustic parameters are considered together, it is easy to distinguish individuals of the two age classes on the basis of their sounds.

## DISCUSSION

The present study shows that agonistic sounds of male *M. zebra* carry information about the emitter. First, individuals belonging to two different classes of sizes are reliably separated by acoustic analysis. Second, and although perfect individual identification was far from achieved, agonistic sounds of male *M. zebra* have the potential to carry some information regarding individual identity.

Most of the variables, including the instantaneous frequency of pulses and the modulation of pulse amplitude, were correlated with standard length and weight. In accordance with previous studies, acoustic signals may thus allow individuals to be classified according to their size by a receiver. In fish, the size of an opponent allows a good assessment of its social status (Ladich, 1998). Larger males are usually dominant and hold bigger territories and females prefer larger males as mates (Myrberg & Lugli, 2006). Yet, as shown by correlations with size and weight, sound characteristics depend on the anatomy of the sender. The mechanism of sound production in *M. zebra* is probably similar to the mechanism identified in *Oreochromis niloticus* (Linnaeus 1758) (Longrie *et al.*, 2009; Parmentier, pers. com.). Sounds would result from movements of the swimbladder due to the contraction of a set of muscles located close to it. Consequently, if sound production is mediated by the swimbladder, larger individuals with a larger swimbladder will produce sounds with lower frequencies. Likewise, if sounds are produced by the contraction of sonic muscles, larger muscles will generate more and longer sounds with a lower frequency. The correlation between sound features and the morphology of the sender may thus help identifying large and small individuals. Acoustic cues are thus good estimates of size classes.

Besides information about size, this study showed that sounds could carry a piece of information that allows discrimination between morphologically equivalent males of *M. zebra*,

based upon the fine characteristics of their agonistic sounds. So far, it appears that an acoustic 'individual signature' has only been found in fish with a more complex sound structure, such as toadfish, in which the mating sound in particular is divided in three different parts and presents harmonics (Amorim & Vasconcelos, 2008). The present study is the first one to suggest that this information would be possibly coded by a more simple fish sound. Although the 'individual signature' of *Metriaclima zebra* remains poorly reliable in comparison with the signatures that have been observed in several bird and mammal vocalizations (e.g. Sayigh *et al.*, 1998; Vignal *et al.*, 2008), the analysis still correctly classified around 50% of the sounds versus 9% by chance. According to the DFA, the instantaneous frequency seems to be the main factor in separating males on an individual basis. Sounds were further individualized by means of the modulation of pulse amplitude (first function) and then by the temporal pattern of the sounds (second and third functions). These 'individual signatures' are likely to change as the fish will grow. The variability in male aggressive sounds might then provide individual information during a relatively short period of time during which the morphology of an individual does not vary notably. From what could be observed in the storage tanks, agonistic interactions mostly occurred when fish were introduced in the tank and when hierarchy had to be established. This period could last up to a week before one or two dominant males obtained a shelter to defend by chasing away other members of the group. Unless one individual died or the disposition of the shelters (e.g. after a cleaning session) was changed, aggressions remained rare. Sound production is then limited to key periods of aggression or courtship during which individual identification would be needed while changes in morphology are limited.

The analysis highlighted both temporal and spectral features as important in encoding information about individuality. One can wonder whether these characteristics are actually perceived and analyzed. Fish are usually classified in two groups according to their hearing sensitivities (Wysocki, 2006; Popper & Fay, 2011): the hearing generalists, which detect sounds of low frequency only via their inner ears, and the hearing specialists, which developed morphological structures connecting air-filled cavities in the body and the inner ears, allowing them to detect sound pressure level and increasing their hearing range to several kHz. One way to test whether a fish is able to hear a conspecific sound is to correlate the sound spectrum with an audiogram, i.e. investigating the matching between dominant frequencies of sound and the region of the best hearing capacities of a fish (Wysocki, 2006). The larger the overlap between spectrum and audiogram, the more the sound is likely to be perceived. Vasconcelos & Ladich (2008) showed for instance that adult *H. didactylus* could detect agonistic sounds of same-sized individuals while juveniles could barely do so, suggesting that sound production occurs prior to sound detection and therefore

communication. As a result, besides the fact that sounds characteristics may depend on the size of the sender and the social context, the significance of sound signals and their detection by conspecifics may also depend on the developmental stage of the receiver. Previous research showed that small differences in the dominant frequency could be detected by fish, e.g. 6% in specialist croaking gourami (Ladich, 1998) and 8% in generalists (Enger, 1981). In the present case, even though the discrimination threshold is unknown, the instantaneous frequency of pulses varied as much as 123 Hz in the small individuals group and 68 Hz in the larger individuals group (i.e. 21% and 23% respectively). These ranges of variation might thus allow *M. zebra* to use this cue. In order to subsequently determine how the spectral components of acoustic signals are represented in the auditory system, one approach would be to measure auditory evoked potentials in response to conspecific sound playbacks (e.g. Wysocki & Ladich, 2003). To assess the temporal pattern of sounds (number of pulses and pulse period in our case), fish must be able to detect pulses within a sound and not only sound as a single acoustic event. Wisocky & Ladich (2002) found that, in five species of hearing specialists, the minimum detectable pulse periods was below 1.5 ms. The same authors further showed that each pulse within a sound of a conspecific elicited a separated stimulus in the auditory centres of four species of hearing specialist whereas generalists yielded unclear results (Wisocky & Ladich, 2003). Neurophysiological studies in a mormyrid showed how the interval between successive pulses could then be encoded (Suzuki *et al.*, 2002). Peripheral neurones faithfully encode the temporal pattern of pulses into trains of spikes and, in the midbrain, interval-selective neurones are able to respond to different optimal interpulse intervals. Moreover, Vasconcelos *et al.* (2011) recently showed that *Halobatrachus didactylus* could resolve fine temporal characteristics of their complex vocalizations. Each pulse, the different parts of the boatwhistle and its duration were accurately represented in the auditory system. It thus appears that fish auditory system is well suited for temporal processing as it provides a reliable representation of sounds. Moreover, temporal pattern is usually well conserved during propagation through the environment (Wysocki, 2006). From the results and except if its auditory abilities differ greatly from other fish, *Metriaclima zebra* should be able to assess the temporal characteristics of the pulses emitted by a conspecific.

In fish, and especially some cichlid species, visual displays are commonly observed during interactions between individuals. In *M. zebra*, males present some variations in the blue intensity of their body and in the contrast of their black bars on the flanks. During fights or courtship, individuals become brighter and dominant males usually display darker vertical bars. As shown by the low number of sounds that have been recorded, visual signals may be the primary source of information used by individuals while acoustic signals would

complement visual information and provide additional individual-related cues like the quality and /or the motivation of the emitter and participate to conspecific recognition. A previous study on the same species showed that the association between visual and acoustic channels lowers the level of aggressiveness found when fish can only interact visually, suggesting that acoustic signals modulate males' behaviour by reducing their aggressiveness (Bertucci *et al.*, 2010). It would now be interesting to test if variations in the characteristics of these sounds result in a differential behavioural modulation.

In conclusion, this experiment showed that sounds produced by male *M. zebra* during agonistic interactions bear information about the emitter. These results have to be considered as a starting point for future research on recognition abilities in this species. Thus, whether male *M. zebra* do discriminate between conspecifics on the basis of acoustic stimuli remains to be experimentally tested. Approaches combining behavioural and playback experiments with possibly neurophysiological investigations focusing on auditory abilities are now needed to decipher the biological relevance of sound signals in this species.

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**Table I**

Summary of the sound features analysed from n = 11 male individuals. Values are mean  $\pm$  S.E.

Fish	Standard length(mm)	Total length (mm)	Weight (g)	Number of sounds	Sound duration (ms)	Number of pulses	Pulse period (ms)	Pulse duration (ms)	Instantaneous frequency (Hz)	Pulse frequency modulation	Pulse amplitude modulation
1	62	74	6.56	5	850.18 $\pm$ 276.53	8.40 $\pm$ 3.07	104.92 $\pm$ 7.65	7.40 $\pm$ 0.52	635.18 $\pm$ 21.65	60.87 $\pm$ 21.22	0.51 $\pm$ 0.03
2	64	78	7.1	8	438.17 $\pm$ 93.89	8.00 $\pm$ 1.41	53.95 $\pm$ 5.88	8.62 $\pm$ 0.55	631.42 $\pm$ 23.19	141.61 $\pm$ 14.75	0.42 $\pm$ 0.01
3	66	78	8.54	11	666.60 $\pm$ 60.12	8.27 $\pm$ 0.60	80.52 $\pm$ 3.28	7.08 $\pm$ 0.41	539.77 $\pm$ 19.10	95.74 $\pm$ 10.46	0.58 $\pm$ 0.01
4	70	85	8.94	12	650.02 $\pm$ 100.19	6.39 $\pm$ 0.79	103.23 $\pm$ 10.59	7.11 $\pm$ 0.54	598.70 $\pm$ 16.30	41.74 $\pm$ 18.71	0.60 $\pm$ 0.03
5	62	74	6.14	22	733.70 $\pm$ 96.37	7.79 $\pm$ 0.93	98.07 $\pm$ 8.25	6.82 $\pm$ 0.18	636.18 $\pm$ 10.89	137.57 $\pm$ 9.09	0.50 $\pm$ 0.01
6	60	72	6.01	5	582.47 $\pm$ 79.59	14.00 $\pm$ 1.67	41.57 $\pm$ 2.25	7.56 $\pm$ 0.38	514.62 $\pm$ 49.66	52.78 $\pm$ 20.74	0.51 $\pm$ 0.02
7	68	84	8.24	6	572.88 $\pm$ 41.15	7.17 $\pm$ 0.83	86.64 $\pm$ 14.65	9.21 $\pm$ 0.72	526.14 $\pm$ 38.45	56.83 $\pm$ 15.53	0.50 $\pm$ 0.03
8	104	120	30.6	7	666.86 $\pm$ 189.08	6.00 $\pm$ 1.02	102.04 $\pm$ 21.11	10.30 $\pm$ 1.33	256.93 $\pm$ 13.65	2.83 $\pm$ 8.11	0.75 $\pm$ 0.03
9	102	117	29.16	5	700.13 $\pm$ 85.88	6.40 $\pm$ 0.68	113.36 $\pm$ 16.09	14.01 $\pm$ 1.83	270.48 $\pm$ 26.40	5.67 $\pm$ 7.42	0.67 $\pm$ 0.04
10	108	127	33.15	18	477.68 $\pm$ 56.91	7.82 $\pm$ 0.72	59.17 $\pm$ 2.99	8.57 $\pm$ 0.39	325.37 $\pm$ 9.67	27.14 $\pm$ 4.33	0.82 $\pm$ 0.02
11	105	121	30.97	6	617.22 $\pm$ 106.52	5.00 $\pm$ 0.73	127.24 $\pm$ 21.06	7.10 $\pm$ 0.23	309.38 $\pm$ 14.31	28.45 $\pm$ 15.56	0.88 $\pm$ 0.01

**Table II**

Coefficient of correlation (r) between mean values of each sound feature and between sound features and total length and weigh (n = 11).

Sound features	Sound duration (s)	Number of pulses	Pulse period (ms)	Pulse duration (ms)	Instantaneous frequency (Hz)	Pulse frequency modulation	Pulse amplitude modulation
Number of pulses	-0.11						
Pulse period (ms)	0.64 *	-0.76 *					
Pulse duration (ms)	-0.02	-0.25	0.17				
Instantaneous frequency (Hz)	0.11	0.37	-0.30	-0.61 *			
Pulse frequency modulation	-0.12	0.25	-0.36	-0.50	0.81 *		
Pulse amplitude modulation	-0.07	-0.50	0.42	0.18	-0.85 *	-0.72 *	
Standard length (mm)	-0.26	-0.37	0.18	0.48	-0.84 *	-0.80 *	0.78 *
Weight (g)	-0.16	-0.51	0.34	0.51	-0.95 *	-0.72 *	0.92 *

\* significant correlations at  $p < 0.05$



**Table III**

For each of the seven variables extracted: mean  $\pm$  SD, range, mean and range of the within-male CV ( $CV_w$ ), between-male CV ( $CV_b$ ) and PIC values ( $CV_b/CV_w$  ratio). H values are the results of Kruskal-Wallis tests comparing  $CV_b$  and  $CV_w$ .

\* represent significant differences at  $p < 0.05$ .

Sound features	Mean $\pm$ SD	min–max	Mean $CV_w$	$CV_w$ min–max	$CV_b$	PIC	H
Sound duration (s)	0.62 $\pm$ 0.33	0.13–1.89	0.47	0.17–0.75	0.53	1.14	11.94
Number of pulses	7.63 $\pm$ 3.62	3–20	0.41	0.23–0.82	0.47	1.16	18.84 *
Pulse period (ms)	86.36 $\pm$ 37.82	33.56–192.97	0.30	0.12–0.55	0.44	1.44	48.76 *
Pulse duration (ms)	8.14 $\pm$ 2.38	4.93–20.00	0.19	0.08–0.34	0.29	1.54	43.22 *
Instantaneous frequency (Hz)	491.52 $\pm$ 155.41	219.98–709.88	0.13	0.07–0.22	0.31	2.38	78.65 *
Pulse frequency modulation	36.01 $\pm$ 40.57	-63.56–168.89	0.44	-18.42–14.26	1.12	2.53	64.84 *
Pulse amplitude modulation	0.62 $\pm$ 0.16	0.37–0.99	0.11	0.04–0.18	0.26	2.27	75.84 *

**Table IV**

Function loadings of the six significant variables for the linear discriminant functions that can be used to classify aggressive sounds.

Sound features	Function Loadings					
	1	2	3	4	5	6
Number of pulses	- 0.53	0.11	- 0.06	0.01	- 0,08	- 0.07
Pulse period (ms)	0.34	- 0.02	0.12	- 0.06	- 0.08	- 0.02
Pulse duration (ms)	0.44	- 0.03	0.08	0.00	- 0.01	- 0.13
Instantaneous frequency (Hz)	- 2.40	0.05	- 0,08	- 0.04	- 0.01	- 0,15
Pulse frequency modulation	0.23	0.00	0.08	0.06	0.04	- 0.03
Pulse amplitude modulation	1.22	0.12	- 0.15	0.05	0.00	- 0.13
Eigen value	6.90	2.80	1.86	1.40	0.88	0.50
Variance explained (%)	47.94	19.55	12.99	9.82	6.18	3.52
Cumulative variance explained (%)	47.94	67.49	80.48	90.3	96.48	100

### **Figure 1**

(a) Oscillogram of a sound produced by a male *M. zebra* during agonistic interactions. The amplitude peak of the different pulses is extracted in order to measure the modulation of amplitude within the sound and the sound duration is calculated (b). Each pulse is then extracted (c) to be analysed. We measured the duration of pulses (d) and used zero-crossings to calculate the mean instantaneous frequency and the modulation of instantaneous frequency by means of six estimates of the instantaneous frequency of pulses (e).

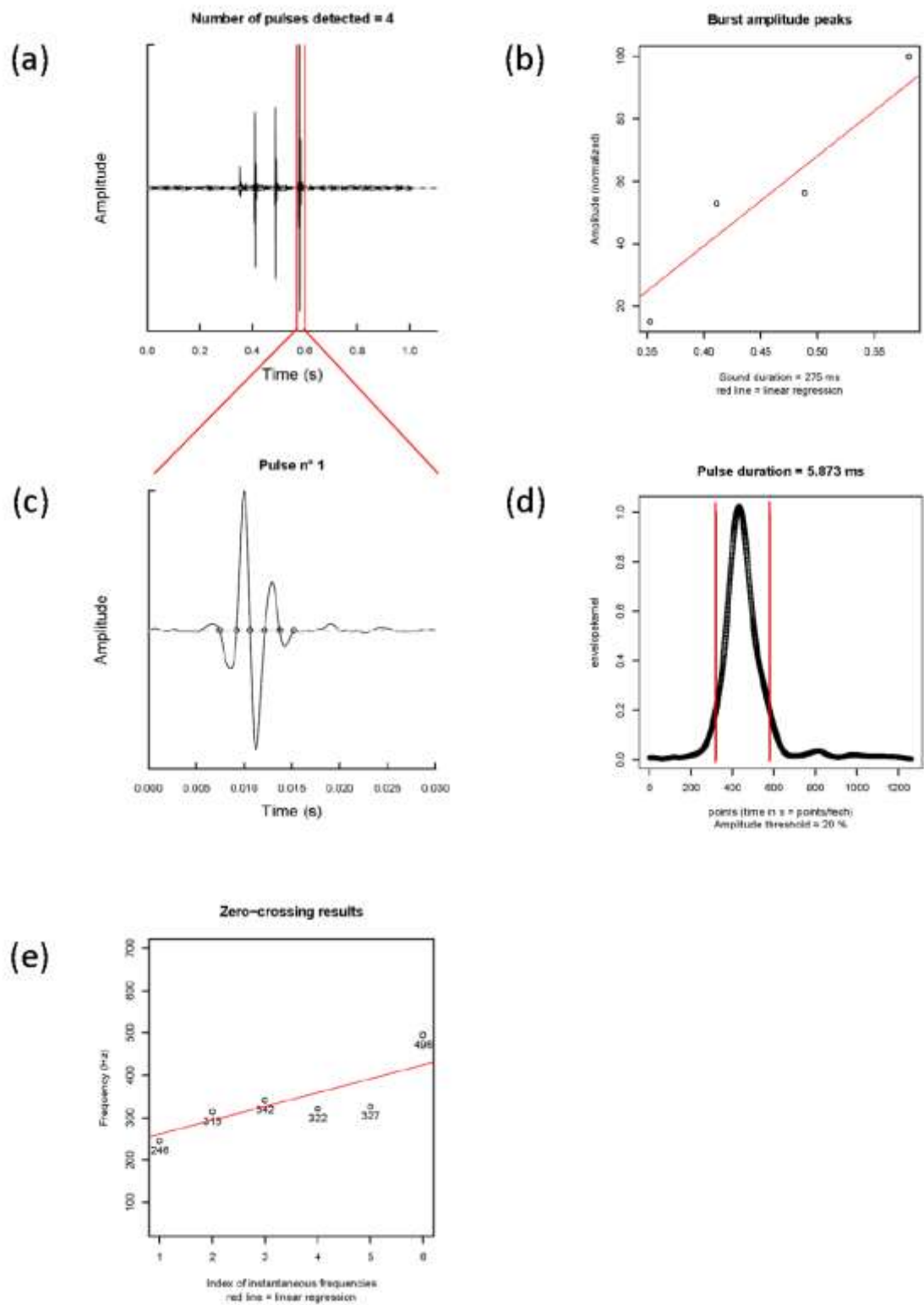
### **Figure 2**

Correlations of (a) instantaneous frequency of pulses ( $n = 11$ ,  $r = -0.84$ ,  $p = 0.001$ ), (b) instantaneous pulse frequency modulation ( $n = 11$ ,  $r = -0.80$ ,  $p = 0.003$ ) and (c) pulse amplitude modulation ( $n = 11$ ,  $r = 0.78$ ,  $p = 0.004$ ) with standard length. Values are individual means  $\pm$  S.D.

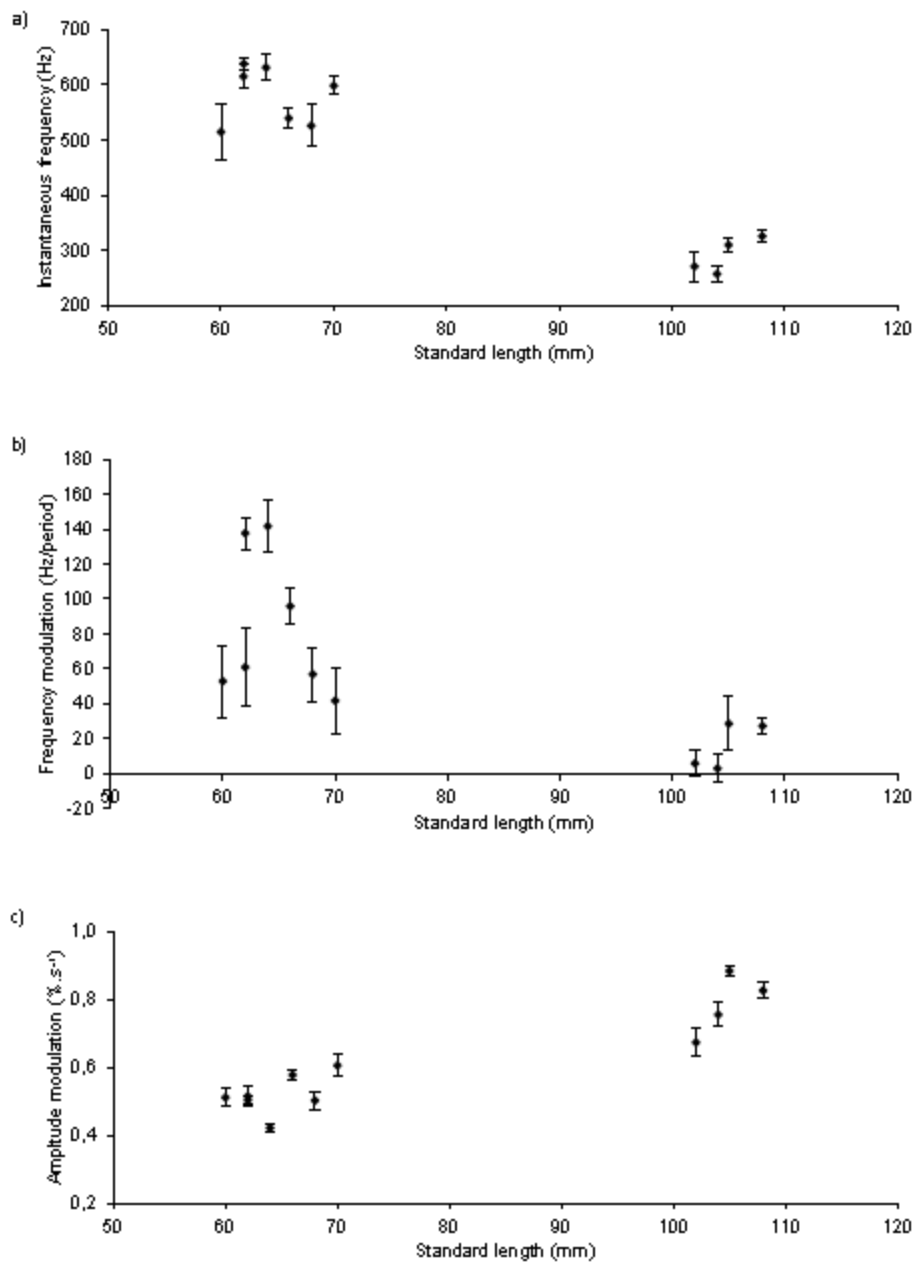
### **Figure 3**

Confusion matrix obtained from the DFA on the cross-validation data set. The colour of the cell (i, j) represents the probability of determining that the sounds came from fish j when it was actually produced by fish i.

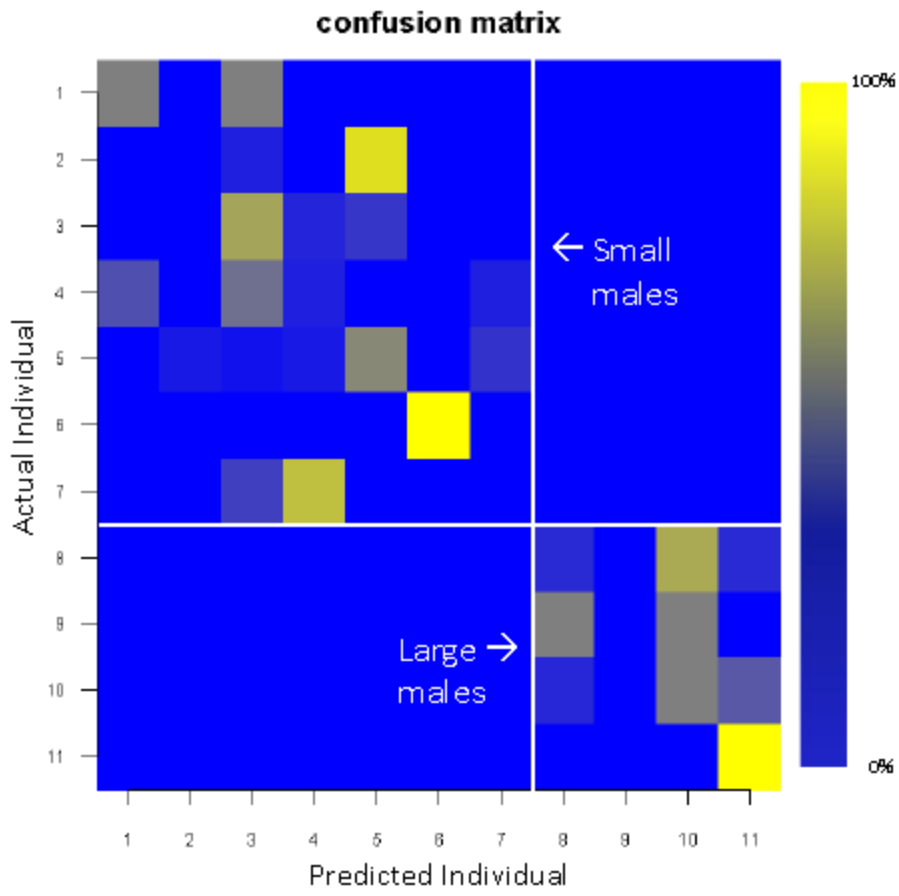
Figure 1



**Figure 2**



**Figure 3**



## Extra material

### Table extra

Percentage values of correct classification (probability of determining that the sounds came from fish j when it was actually produced by fish j) presented in figure 3.

Actual Individual	Predicted Individual											
	1	2	3	4	5	6	7	8	9	10	11	
1	50.0		50.0									
2			12.5		87.5							
3			64.3	14.3	21.4							
4	31.3	43.7	12.5				12.5					
5		10.0	6.7	10.0	53.3		20.0					
6						100.0						
7			25.0	75.0								
8								16.7	66.7	16.6		
9								50.0	50.0			
10								15.4	50.0	34.6		
11											100.0	

# The relevance of temporal cues in a fish sound: a first experimental investigation using modified signals

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## Résumé de l'article 3

### Introduction

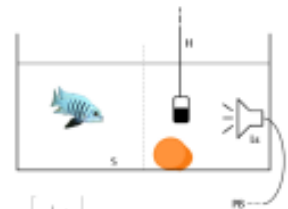
L'association étroite entre signaux acoustiques et visuels chez les poissons restreint l'utilisation d'expériences de playbacks acoustiques pour étudier le rôle des sons.

L'expérience présentée dans cet article tente d'utiliser la méthode du playback afin de connaître la fonction des signaux acoustiques chez *M. zebra* ainsi que la façon dont l'information est codée en diffusant des signaux modifiés.

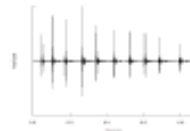
### Matériel et méthodes

#### Expérience contrôlée:

Des sons enregistrés lors d'interactions agonistiques étaient diffusés à des poissons mâles lors de l'approche d'un haut-parleur situé dans leur aquarium. Les individus ont été testés dans deux situations: lors de leur introduction dans l'aquarium (intrus) et 24h plus tard, une fois devenus territoriaux (résidents).

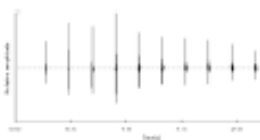


Son original (contrôle)

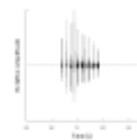


#### Modification des paramètres temporels:

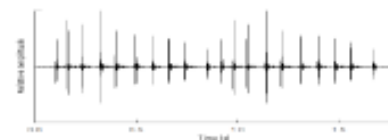
Dans un second temps, des sons dont la période des pulses ou le nombre de pulses ont été modifiés sont diffusés à des mâles résidents.



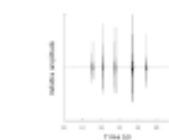
Période augmentée



Période diminuée



Nombre de pulses augmenté



Nombre de pulses diminué

### Résultats

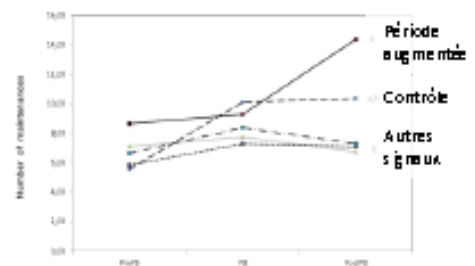
#### Expérience contrôlée:

Aucune réponse aux playbacks n'est observée chez les individus intrus. Chez les individus résidents, si aucune réponse agressive n'est observée, la diffusion de playbacks a pour effet d'augmenter l'activité territoriale des mâles (i.e. augmentation de la maintenance et du temps à proximité du haut parleur).

#### Modification des paramètres temporels:

Seule l'activité de maintenance semble être affectée par la modification de paramètres temporels, avec une augmentation de ce comportement en réponse à l'augmentation de la période des pulses. Les autres modifications conduisent à une diminution de la maintenance.

Aucun des autres comportements des mâles résidents n'est altéré en réponse aux modifications temporelles.



### Conclusion

Cette expérience représente l'une des rares réussites de playback chez un poisson et montre l'importance de considérer le statut social lors de ces expériences. Si les résultats suggèrent une grande tolérance aux modifications des paramètres temporels, des études complémentaires portant sur d'autres paramètres acoustiques permettant d'y voir plus clair dans le processus de codage de l'information chez *M. zebra*.

Bertucci, F., Beuchaud, M., Attie, J. & Mathewson, N. (Submitted). The relevance of temporal cues in a fish sound: experimental investigation using modified signals.

## Summary of article 3

### Introduction

The close association between acoustic and visual signals in fish restrains the use of acoustic playback experiments in order to study the role of sounds.

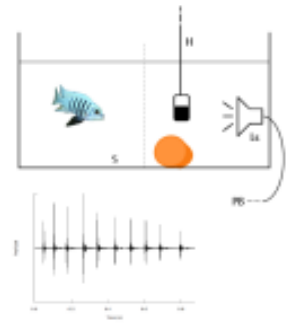
The experiment presented in this article attempts to use this method of playback in order to know the function of acoustic signals in *M. zebra* and the way information is encoded by broadcasting modified signals.

### Material and methods

#### Control experiment:

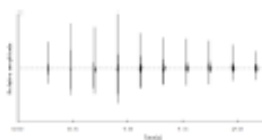
Sounds recorded during agonistic interactions were broadcasted to males when they approached a loudspeaker located in their aquarium.

Individuals have been tested in two situations: after their introduction in the aquarium (as intruder) and 24h later, when they became territorial (as residents).

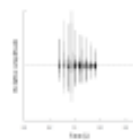


#### Modification of temporal parameters:

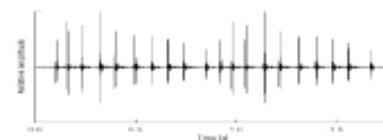
In a second time, sounds with a modified pulse period or with a modified number of pulses were played back resident males.



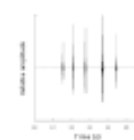
Increased period



Decreased period



Increased number of pulses



Decreased number of pulses

### Results

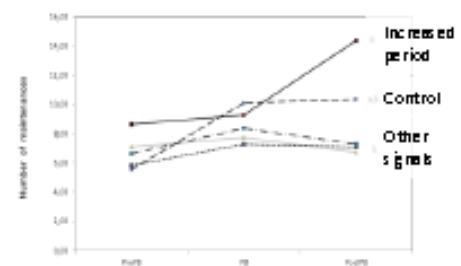
#### Control experiment:

No response to playbacks was observed in intruders. In residents, if no aggressive response was observed, playbacks increased the territorial activity of males (i.e. increase of the maintenance and time spent near the loudspeaker).

#### Modification of temporal parameters:

Only the maintenance activity seems to be affected by the modification of temporal parameters, with an increase of this behaviour in response to the increase of pulse period. Other modifications lead to a diminution of the maintenance.

No other resident males' behaviour were altered in response to temporal modifications.



### Conclusion

This experiment represents one of the rare successful playback experiment in fish and show the importance of considering social status during this kind of experiments. While the results suggest a wide range of tolerance to modifications of temporal parameters, complementary studies focusing on other acoustic parameters will allow us to better understand the encoding process of information in *M. zebra*.

Bertucci, F., Beauchaud, M., Attis, J. & Methevon, N. (Submitted). The relevance of temporal cues in a fish sound: experimental investigation using modified signals.

## **Abstract**

Playback experiments have been a useful tool to study the function of sounds and the relevance of different parameters in signal recognition in many different species of vertebrates. However, only a small number of experiments have been performed in vocal fish, highlighting that the responsiveness to conspecific sounds was higher than to heterospecific sounds and that temporal pattern of sounds was an important feature. Playback experiments in fish remain rare though, due to a tight link between different signal modalities and very few studies have investigated the role of particular features in the encoding of information. In this study, we setup a paradigm in order to test the relevance of acoustic signals in males of the cichlid *Metriaclima zebra* and investigated the role of temporal structure of sounds recorded during aggressive interactions by modifying two parameters, i.e. the pulse period and the number of pulses. We found that territorial males responded more to playbacks by increasing their territorial activity and approaching the loudspeaker during and after playbacks. Modified sounds yielded little if no effects on the behavioural response of territorial males, suggesting a high tolerance for variations in pulse period and number of pulses. The biological function of sounds in *M. zebra* and the lack of responsiveness to our temporal modifications are discussed.

## **Key Words**

Acoustic communication; aggressive sounds; fish; playback experiments, temporal features.

## INTRODUCTION

Several fish species are known to emit sounds in different social contexts, in particular during agonistic interactions and courtship (Ladich & Myrberg, 2006 ; Myrberg & Lugli, 2006). These sounds demonstrate a large inter- and intra-specific diversity and one particular interest has been to study the potential function of these acoustic signals in communication (Amorim, 2006). Cichlids have been one of the most studied groups of sound-emitting fish (Lobel, 2001). In particular, inter-specific differences found among closely related species emphasize the important role acoustic signals may have played in the spectacular rate of speciation witnessed in this group. In Lake Malawi, many species live in sympatry and females could be able to discriminate among male sounds of various species to find a mate (Verzijden et al., 2010). Amorim et al. (2004, 2008) showed that courtship calls of five related species of *Pseudotropheus* (now *Metriaclima*) *zebra* differed in some temporal features, i.e. number of pulses, pulse period and duration; and in peak frequency. Thus, acoustic signals could participate in species recognition.

At the intra-specific level, one of the most widespread differences is an inverse relationship between the frequency of sound and the size of the emitter (e.g. Ladich et al., 1992; Crawford et al., 1997 ; De Jong et al., 2007 ; Amorim et al., 2008 ; Colleye et al., 2009 ; Bertucci et al. *in revision*). Since bigger individuals are usually dominant and territorial, sound characteristics may be useful for a receiver to assess the size of a potential rival. For example, beside the fact that sounds increased the chance of winning a fight in croaking gouramis (*Trichopsis vittata*) (Ladich et al., 1992), some size-related acoustic features could predict the outcome of a fight, i.e. winners produce sounds with a lower dominant frequency and a higher sound pressure level (Ladich, 1998). Acoustic signals could therefore encode social status and/or motivation. Moreover, in some species, sounds also show individual characteristics. Most examples come from Batrachoidids in which several studies reported individuality, both in the temporal and frequency domains of the male courtship calls (e.g. Edds-Walton et al., 2002 ; Amorim & Vasconcelos 2008) and in male aggressive sounds (e.g. Thorson & Fine, 2002). Altogether, these results suggest that sounds of fish hold individual characteristics available for sender recognition and assessment (Amorim et al., 2010).

If sounds seem to bear a wide range of information from the specific to the individual level, one can then wonder whether sounds have context-specific characteristics. In birds and mammals, sounds of aggressive interactions are usually low in frequency and short whereas courtship or sociable sounds are more pure and of higher frequency (Morton, 1977). The same differences seem to exist in fish. In midshipman (*Porichthys notatus*), male advertisement calls directed to females are long and tonal while aggressive sounds are short

and broadband (Bass & McKibben, 2003). More recently, Parmentier et al. (2010) described the acoustic repertoire of a damselfish (*Dacyllus flavicaudus*) and reported six behaviours associated to sound production. Sounds could be classified in three categories, i.e. fighting sounds, mating sounds and chasing sounds. Differences between signals were found in the number of pulses, the pulse period and their relative intensities. For example, sounds associated to aggressive behaviours had a smaller number of pulses than courtship sounds. Moreover, there was a daily cycle of sound production in this species with mating sounds mainly produced at sunrise while chasing sounds were recorded at sunset. In association with three different colour patterns, these results illustrate the potential complexity of context-specific sounds. Nevertheless, fishes usually have smaller acoustic repertoires. Cichlids generally produce only one type of sounds (Lobel, 2001), yet several studies reported context-related differences. In *Oreochromis mossambicus*, pulse period and number of pulses of the courtship sounds produced by males are significantly different depending on the behaviours they are associated to (Amorim et al., 2003). In a subsequent study, Amorim & Almada (2005) found that recent social experience of males affected the structure of the sounds they emitted, as winners produced more sounds with longer pulses and lower peak frequencies than losers.

One way to study the function of sounds and the relevance of different parameters in signal recognition is to use playback experiments. Whereas playbacks have been largely used in many vertebrates (Hauser, 1998), only a small number of experiments have been performed in vocal fish. One possible limitation to the use of this technique would be the ability of current loudspeakers to accurately broadcast sounds with low frequencies (Ladich & Myrberg, 2006). Though, founding experiments especially highlighted that the responsiveness to conspecific sounds was higher than to heterospecific sounds (e.g. Delco, 1960; Myrberg & Spires, 1972). In damselfishes, *Stegastes sp.*, “chirps” sounds are made in response of a female approach and provoke courtship displays in neighbours (for other examples see e.g. Gerald, 1971; Rollo & Higgs, 2008). A series of experiments showed that the higher response to conspecific sounds depended on the number of pulses and pulse rate of chirps (Myrberg et al., 1978; Spanier, 1979). Myrberg et al. (1986) further showed that playbacks of large male bicolor damselfish were more attractive than playbacks of small males. More investigations were conducted in one of the most-studied family of vocal fish, i.e. the toadfish. In the oyster toadfish, playback experiments revealed that besides attracting gravid females (Winn, 1972), male calls affected neighbouring males’ calling activity (Winn 1967). The mate call function of “hums” has been demonstrated in the related plainfin midshipman (*Porichthys notatus*) (Ibara et al., 1983) as well as the fact that these calls may play a role in male-male interaction as a “nesting male present” signal (McKibben & Bass, 1998). Even though this latter analysis did not tested the influence of a particular parameter,

it showed that female midshipman preferred for example longer sounds, with longer pulses, with fundamental frequencies ranging from 80 to 140 Hz. In a subsequent study, McKibben & Bass (2001) were interested in the temporal pattern of sounds emitted by male *P. notatus* and used playbacks in choice test experiments in which the structure of the sounds was altered in terms of pulse duration, the gap between pulses and beat of sounds. Playbacks to gravid females revealed that longer pulses and greater pulse to gap ratios resulted in more attracting signals and that pure tones were more attractive than beats. Altogether, these playback experiments show that temporal pattern of sounds seems to be important in fish acoustic communication.

Beside the toadfish family, playback experiments focusing on the role of sounds at the intra-specific level remain rare (Stout, 1963; Schwarz, 1974; Rigley & Muir, 1979). Lugli et al. (1996) showed that male vocalizations were attractive to females *Padogobius martensii*. In cichlids, Verzijden et al. (2010) showed that female *Pundamilia nyererei* prefer conspecific males associated to playbacks of courtship sounds and sounds emitted during aggressive interactions reduce the level of aggressiveness witnessed during a fight between males of the Malawian cichlid *Metriaclima zebra* (Bertucci et al., 2010). However, Bertucci et al. (2010) found no behavioural response to playbacks of sounds when they were not associated to other (visual) stimuli. The same result was found in male goby exposed to aggressive sounds (Lugli, 1997). Given this tight interaction of acoustic cues with other modalities in fish (Lugli, 1997; Lugli et al., 2004; Ladich & Myrberg, 2006), the results of previous experiments might be confusing and hence, no study comparable to McKibben & Bass (2001) has been performed to investigate the role of, for example, temporal structure of sounds in cichlids.

*Metriaclima* (formerly *Pseudotropheus*) *zebra* is a rock-dwelling cichlid from Lake Malawi. Males produce sounds while defending territories against other males and while courting females (Amorim et al., 2004; Simões et al., 2008). During agonistic postures like quivering or lateral displays, males produce low frequency sounds consisting in a train of short pulses. Simões et al. (2008) highlighted differences between sounds produced by males and females and differences in characteristics of males' sounds emitted during agonistic interactions and courtship, as agonistic sounds were longer in duration and pulse period than courtship sounds.

In this study, we attempt to setup a paradigm allowing us to elicit a behavioural response to acoustic playback, with no other associated stimuli, in order to test the behavioural response of males and the relevance of acoustic cues in the cichlid *Metriaclima zebra*. By modifying two temporal parameters which could be good candidates to encode information, i.e. the number of pulses and the sound duration, and broadcasting these modified stimuli to territorial males, we then aim to determine the possible function of temporal variability of sounds in coding aggressiveness in this species.

## MATERIAL AND METHODS

### FISH

*Metriaclima zebra* were purchased from N'Guyen International (Kingersheim, France) and stored in heterosexual groups in holding tanks (60 x 120 x 50 cm), containing 8–10 individuals with a male:female sex ratio of 1:2. They were 4 years old and sexually mature at the time of the experiment. Each tank was equipped with an external filter (Rena Filstar xP3, Rena France, Annecy, France), an aeration device, sand substrate, terracotta pots and bricks as shelters. The temperature was maintained at  $25 \pm 2^\circ\text{C}$  by an internal heater (RenaCal 200, Rena France, Annecy, France) and the pH remained at 8.0 on a 12L:12D day cycle. Fish were fed daily with commercial cichlid food (JBL NovoRift, JBL GmbH & Co. KG, Neuhofen, Germany). Once a week this diet was complemented with a frozen mixture of mussels, shrimps and spinach.

12 male individuals were involved in the experiments (mean  $\pm$  SE total length –from the tip of the head to the tip of the caudal fin- of  $137.08 \pm 4.47$  mm, standard length -from the tip of the head to the caudal peduncle- of  $112.17 \pm 5.48$  mm, and a weight of  $34.30 \pm 1.66$  g. The fish were identified by the number and the position of egg-spots on their anal fin, any marks on their body, in combination with VIE tags (Visible Implant Elastomer, Northwest Marine Technology, Shaw Island, WA) implanted under the skin.

### EXPERIMENTAL SETUP

The setup was made of one aquarium (60 x 30 x 30 cm) placed on a vibration-insulated shelf and located in an acoustically insulated room to reduce background noise. Additionally, three walls of the aquarium were covered with bubble wrap in order to reduce sound reverberation within the aquarium. The set-up contained a filter, an aeration device, an internal heater, a sand substrate and a terracotta pot in the middle in order to provide a shelter to the fish. During the experimental sessions, the filter and the aeration device were switched off to allow recordings of fish sound. On one side of the aquarium, we placed an underwater loudspeaker (University sound, Electrovoice, UW-30) in (Fig. 1) connected to an amplifier (Denon PMA-100M) and a Marantz PMD670 solid-state recorder.

A video camera (BUL520, Active Media Concept, Vallauris, France) was placed in front of the setup in order to record behaviour during trials. To record acoustic signals, a hydrophone (Aquarian Audio Products H2a-XLR, sensitivity:  $-180$  dB re  $1\text{V}/\mu\text{Pa}$ , flat frequency response:  $\pm 4$  dB in the range 20 Hz – 4.5 kHz) was placed in the centre of the aquarium, i.e. above the shelter. The hydrophone was connected to a preamplifier (Yamaha MLA8, Yamaha Music

France, Marne-la-Vallée, France) and a video capture card (Osprey-450e) of a PC which synchronized audio and video signals.

In order to broadcast sounds at a “natural” level, we placed the loudspeaker at a distance from the hydrophone corresponding to the distance at which sounds were originally recorded. We then adjusted the intensity of playbacks to get the same level of recording. We validated that this paradigm did not alter played back sounds in a previous experiment (Bertucci et al., 2010) by comparing two features of the sound, i.e. mean frequency and pulse period: these sound features did not differ before and after a playback (see supplementary material for results of the comparison tests and a figure showing oscillograms of a recorded sound and of the same sound after transmission through the broadcasting apparatus).

## CONTROL EXPERIMENT: HOW DO MALE *M. ZEBRA* RESPOND TO PLAYBACK OF CONSPECIFIC SOUNDS?

### Experimental procedure

The first step of our study was to set up an efficient playback paradigm which would allow us to study the behavioural response of *M. zebra* to played back signals. As we were also interested by the effect of social status on the behavioural response to playback, each fish was challenged in the experimental tank during two successive playback sessions, separated by 24h of rest. This paradigm allows testing fish in two different social conditions. During the first session, the fish was considered as intruder whereas it was considered as resident during the second session.

The tested fish was introduced in the experimental setup and was given 10-15 minutes to acclimatize. The first session was then divided in three periods of 10 minutes. During the first 10 minutes, no sounds were played back (pre-playback control). At the end of this first period, we started to play back aggressive sounds to the subject every time it was located in the half part of the aquarium containing the loudspeaker (playback period, see Figure 1). The fish then received no playback for 10 minutes, until the end of the first session (post-playback period). We then switched back on the electric devices - filter and aeration - and left the fish in the setup for 24h so that they could become resident. During the second session, we repeated the procedure with the 10 minutes pre-playback, 10 minutes playback and 10 minutes post-playback periods playing back the same sounds as the day before. At the end of the trial, the fish was returned to its storage tank and 2/3 of the water in the test aquarium were renewed and sat for 24h before the introduction of a new fish.



Sounds to be used as acoustic stimuli were obtained from recordings made in our laboratory. Two unfamiliar size-matched males were introduced in adjacent tanks, separated by opaque partitions and were given 24h to acclimatize and become territorial. A hydrophone (Aquarian Audio Products H2a-XLR) was placed in each tank, between the shelter and the walls separating the aquaria, i.e. where individuals would spend most of their time displaying. The hydrophones were connected to a preamplifier (Yamaha MLA8, Yamaha Music France, Marne-la-Vallée, France) and a video capture card (Osprey-450e) of a PC. At the start of a recording session, the opaque partition was removed, allowing the two fish to interact visually during 20 minutes before replacing the partition. We performed a maximum of three recording sessions per male to collect a sufficient number of sounds. We only considered sounds made of more than two pulses and recorded within a distance of 1-2 body lengths from hydrophones. Sounds recorded from four individuals with a mean  $\pm$  S.E. total length of  $115.00 \pm 7.99$  mm, a standard length of  $98.00 \pm 6.38$  mm and a weight of  $25.20 \pm 4.25$  g were used and digitized at 44.1 kHz (16 bit resolution). None of the recorded fish were involved in the present experiment and none of the fish tested had experience with any of the individuals from which the sounds were recorded.

#### *Analysis of behavioural responses*

Behavioural data were collected from the videos recorded during the trials. For each period in the two sessions (intruder and resident), we quantified the number of aggressive behaviours performed, i.e. lateral display, quiver, sound production, bite attempts, using the behavioural transcription software EthoLog 2.2.5 (E.B. Ottoni 1995—1999). We also quantified behaviours defining space occupancy, i.e. time spent swimming (s) in the zone of the loudspeaker, i.e. one half of the aquarium, number of maintenance acts (when the fish was moving – digging - sand within the aquarium when becoming territorial (e.g. Oliveira & Almada, 1996; Simões et al., 2008)), number of times that the tested fish enters into the shelter and number of times it went close to the loudspeaker.

We analyzed the number of lateral displays, quivers, sounds produced, bite attempts, the number of maintenances, times into the shelter, number of times close to the loudspeaker and time spent in the zone of the loudspeaker by means of a repeated measures analysis of variance. The analysis was performed using STATISTICA 6.0 (Statsoft Inc, 2004).

## EXPERIMENT WITH MODIFIED SIGNALS: DO MODIFICATIONS OF SOUND TEMPORAL PATTERN ALTER THE BEHAVIOURAL RESPONSE TO PLAYBACKS?

### *Experimental procedure*

In a second step, we wanted to test if modifications of sound's temporal pattern could elicit differential behavioural responses in the tested fish. Sounds of *Metriaclima zebra* consist in trains of a variable number of short and low frequency pulses. Male sounds show a range of variation in the period of pulses from 40 ms to 180 ms, sound duration varies from 250 ms to 1 s with 5 to 15 pulses per sound (Simões et al. 2008, Bertucci et al., *in revision*). We altered temporal features of these sounds using the PRAAT software version 5.0.35 (Boersma & Weenink 1992—2008) (fig. 2). To test the significance of the pulse period, we built sounds for which we kept the number of pulses of the original sounds (fig. 2a), but with a modified pulse period corresponding either to twice the maximum peak-to-peak distance observed for each individual (PP Max, fig. 2b) or to half the minimum peak-to-peak distance (PP Min, fig. 2c). These two modifications respectively resulted in a series of slow sounds and fast sounds. To test the significance of the number of pulses, we built sounds with no alteration of the pulse period but we either deleted the second half of the original sounds (half pulses, fig. 2d) or pasted a copy of the original sound at its end (double pulses, fig. 2e). For the latter modification, the two parts were separated by the mean peak-to-peak interval of the original sounds. These two modifications respectively resulted in a series of short sounds and long sounds.

Prior to the experiment, fish were put during 24h in the aquarium to let them becoming resident. Resident fish were then challenged with playbacks of modified sounds following the same procedure as previously described for the control experiment. We used a repeated measures design with five different treatments corresponding to the five categories of sounds that we created, i.e. normal sounds, PP Max, PP Min, Double pulses and Half pulses. We tested two fish a day during six consecutive days over a period of five weeks. Each individual thus received a new treatment every seven days in a balanced order to avoid order effects. Since we broadcasted sounds when a fish was located in the half part of the aquarium containing the loudspeaker, the number of playbacks received by each individual depended on its motivation to approach the loudspeaker, which explains why the number of stimulations varied between individuals and treatments (Table 1).

## *Analysis of behavioural responses*

We quantified space occupancy, i.e. time spent swimming (s) in the zone of the loudspeaker, number of maintenances, number of times into the shelter and number of times close to the loudspeaker using EthoLog 2.2.5 (E.B. Ottoni 1995—1999) and we used a repeated measures analysis of variance under STATISTICA 6.0 (Statsoft Inc, 2004) to look for possible effects of temporal modifications on these behaviours.

## RESULTS

### *How do male *M. zebra* respond to playback of conspecific sounds?*

Numbers of aggressions (lateral displays, quivers and sounds) were too low to be analysed. We found significant differences in the number of maintenances (Friedman ANOVA,  $n = 12$ ,  $Fr = 39.59$ ,  $p < 10^{-3}$ ) with no difference in intruders and a significant increase of this number in residents, during the playback of sounds recorded during aggressive interactions and after the playback as revealed by a Fisher post-hoc test (Fig. 3a). Residents also spent significantly less time in the shelter than intruder, whatever the period (prePB, PB or postPB) (Friedman ANOVA,  $n = 12$ ,  $Fr = 16.31$ ,  $p = 0.006$ ) (Fig. 3b). Residents came significantly more often close to the loudspeaker after the playback while no differences were found in intruders (Friedman ANOVA,  $n = 12$ ,  $Fr = 16.26$ ,  $p = 0.006$ , followed by a Fisher post-hoc test) (Fig. 3c). We also found that residents tended to spend more time in the zone of the loudspeaker than intruders (Friedman ANOVA,  $n = 12$ ,  $Fr = 10.58$ ,  $p = 0.06$ ).

In summary, playback of conspecific sounds does not alter the behaviour of fish that have just entered the experimental set up (intruders), whereas it elicits a territorial response by fish that have been present for 24h (residents). This territorial response is well characterized by maintenance behaviour.

### *Do modifications of the temporal pattern of sounds alter the response?*

For the maintenance activity, we found a significant effect of temporal modifications in the post playback period (Repeated measures ANOVA,  $n = 11$ ,  $F = 3.15$ ,  $p = 0.02$ ), with a higher number of maintenance acts in response to sounds with a high pulse period (PP Max) compared to other modifications as revealed by a Fisher post-hoc test. However, none of the treatments differed from the control playback (Table 2, Fig 4).

We found no differences between modifications for the number of time in the shelter (Repeated measures ANOVA,  $n = 11$ ,  $F = 0.96$ ,  $p = 0.44$ ), the number of time close to the loudspeaker (Repeated measures ANOVA,  $n = 11$ ,  $F = 0.79$ ,  $p = 0.54$ ) and the time spent in the zone of the loudspeaker (s) (Repeated measures ANOVA,  $n = 11$ ,  $F = 1.12$ ,  $p = 0.36$ ) (Table 2).

## DISCUSSION

This study used playbacks of sounds produced by males during agonistic interactions in order to investigate the role of these signals in *M. zebra*. Acoustic playbacks have been useful in investigating the biological function of vocalizations in insects, frogs, birds or mammals (e.g. Hauser, 1998; McGregor, 1992). In comparison however, most of the studies using playback experiments in fishes have been unsuccessful, leading to confusing results and thus remain rare. One explanation comes from the close-range feature of fish sounds and the requirement of additional stimuli to elicit a behavioural response (Stout, 1963; Schwarz, 1974; Rigley & Muir, 1979; Ladich, 1997; Bertucci et al, 2010).

*Control experiment: Only resident males respond to acoustic playbacks.*

Even though our paradigm did not allow eliciting any aggressive behaviour like quivers or sound production, we showed in the first part of this experiment that acoustic playbacks of sounds could trigger a behavioural response in residents, with an increased number of maintenance acts (or digging) during and after the playback periods. Substrate plays an important role in cichlids especially during the breeding season when males build nests, i.e. a depression in the substrate, representing their territory in which females would spawn (Oliveira & Almada., 1996). Male Mozambique tilapia (*Oreochromis mossambicus*) have been shown to try to build nests even if substrate was absent (Galhardo et al., 2008), further emphasizing the social need and function of this behaviour in territorial individuals in cichlids (Galhardo et al., 2009). Hence, our results suggest that aggressive sounds are relevant for a resident (territorial) male who would defend or at least advertise its territory against a potential competitor. Aggressive sounds might then play the role of a “territorial male present” signal, similar to the “nesting male present” signal proposed by McKibben & Bass (1998) in midshipman. Such aggressive sounds might therefore not be meaningful for non resident individuals, explaining their lack of reaction in the first session of the trials. Another

explanation could be due to the stress associated to a new environment which could have inhibited locomotion or agonistic behaviours (Øverli et al., 1999). In the same time, males reduced the time they spent in their shelter and came more often close to the loudspeaker. Such phonotactic response is comparable to the one found in former experiments conducted in fishes (e.g. Myrberg & Spies, 1972; Winn, 1972; Ibara et al., 1983). Thus, besides attracting mates or being used during fights (Simões et al., 2008), acoustic signals of cichlids might be used by territorial males to detect potential rivals or interacting with conspecifics in their vicinity.

*This behavioural response seems to resist to modifications of the temporal pattern of sounds.*

In opposition to the previous findings that the number of pulses and pulse rate were important parameters in attracting mate or neighbour's attention in fish (Myrberg et al., 1978; Ibara et al., 1983; Amorim, 2006), most of the modifications of temporal features had here little if no effect on the behaviour of resident males *M. zebra*. Only a tremendous increase of the pulse period (PP Max signal) resulted in a stronger behavioural response than other modifications. Under the "territorial male present" signal hypothesis, a higher reaction to this modification would imply that these sounds evoked a bigger threat in the receiver. A longer pulse period is generally associated to agonistic interactions in *M. zebra* (Simões et al., 2008) and to larger males (Bertucci et al., *in revision*) and might thus promote the observed reaction.

A longer pulse period also results in a longer sound. If the total sound duration was responsible of the observed response to playbacks, we would have expected this latter modification (Double pulses) to show the same, or a higher, effect as PPmax sounds on maintenances number. This was not the case: lengthening the sound while keeping the original pulse period did not result in a stronger behavioural response. This suggests that the increased pulse period, not the sound total duration, may be the cause of the increased territorial activity induced by the PP Max signal.

When increasing the sound duration by playing the same sound twice in a row, not only do we create a twofold longer sound but we alter the sound envelope as well. Indeed, most cichlids sounds start with pulses of low amplitude, pulse amplitude then increases and declines at the end. Even though we found no difference for this modification compared to the control, the repetition of twice the same sound may in fact affect the dynamic pattern of pulses. One way to deal with this issue and preserve this inflection in pulse amplitude would be to double each pulses next to it, pulse period being constant. Pulse amplitude would therefore be a good candidate parameter to be modified in further playback experiments.

Apart from the PPMax modification and in relation to the previous point, playbacks of only the first half of sounds provided the same results as fast sounds (PPMin) and long sounds (Double pulses) with a decreased number of maintenances. Even if this decrease was not significantly different from the control, this would suggest that the first half of sounds (which usually corresponds to an increase in pulse amplitude), a fast sound or a long sound do not provide the same amount or quality of information regarding territorial behaviour as unmodified sounds. The question whether information would be carried by the entire sound or in other (decreasing) parts of the sound thus deserves more investigations focusing on pulse amplitude modulation.

None of the other variables considered (time in the shelter and time around the loudspeaker) were affected by modified playbacks and yielded the same results as control sounds. Most of the previous experiments on attractiveness of modified signal in fish have been performed with male calls broadcasted to females. Rollo & Higgs (2008) however, played back male round goby spawning sounds to both males and females. While responding to a wide range of stimuli round gobies showed a higher response to conspecific sounds. Tonal sounds however were as attractive as heterospecifics calls. Likewise, in many insects, playbacks of a signalling neighbour involve a phonotaxic response and an increase in call duration and rate of signalling of males as the competitor approaches. Temporal pattern has been shown to be one of the most important features in pulsed signals of insects and anurans (reviewed by Gerhard & Huber, 2002). As an example, female field crickets are very selective for pulse rate. In a two-choice experiments, females of *Gryllus bimaculatus* preferred songs with the species-specific mean pulse rate of 22 pulses/s over lower (< 10 pulses/s) and higher (up to 50 pulses/s) pulse rates (Doherty, 1985). In other species, females would be more selective for pulse duration however. Similarly, in the South African frog (*Xenopus laevis*), different pulse frequency and pulse rate can make calls more “female-like” or “male-like” and consequently affect males’ calling and behavioural response (Vignal & Kelley, 2007).

Male aggressive sounds played back to resident males might not be fully appropriate and may explain that sounds were equally evaluated. Moreover, only two temporal acoustic features were considered here and we cannot exclude that the key characteristics of our stimuli were preserved despite our drastic modifications and that other cues might also be involved in the encoding process affecting, in particular, the phonotaxic response. At the moment, the system seems to be able to sustain and tolerate a wide range of variations in term of pulse period and sound duration before the behavioural response of receivers is altered. In other words, neural structures used in signal detection and processing were still able to extract the information carried by the sound and, by assessing its temporal pattern, receivers have considered each sound as equally attractive.

The experimental design of this study constrained the number of treatments we could use. We therefore chose to remove a real negative control treatment. Even though we expected to find little differences in response to our modifications, the weak differences with control sounds may however indicate that tested fish react solely to sounds, whatever their characteristics. Controlling for sound effect using white noise would be needed here to exclude the possibility that any other sound would have given the same results.

The association of the territorial and the phonotaxic response found in the control experiment validates the efficiency and reliability of our results and represents one of the first convincing playback experiments conducted in a cichlid fish. We also provide a good evidence of the important role of acoustic communication at the intraspecific level in *M. zebra*.

There is a major gap in fish literature compared to insects and anurans in particular. The results of the second part of our study have to be considered as a first try to decipher the encoding mechanisms of this fish. Numerous studies showed that fish auditory system was well suited for temporal processing (e.g. Suzuki et al., 2002; Wisocky & Ladich, 2003; Vasconcelos et al., 2011). However, to our knowledge, no neurophysiological studies have been performed to know how pulses interval and other temporal features were represented in the auditory system of this fish. Further playback experiments focusing on other acoustic cues like frequency, amplitudes of pulses or spectral properties of sounds, in association with neurophysiological experiments will be necessary to understand more thoroughly information processing in fish acoustic communication.

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**Table 1**

Number and mean values of playbacks received by each individual in the five different treatments.

	Intrus						Resident						MEAN
	Normal	PP Max	PP Min	Double pulses	Half pulses	Mean	Normal	PP Max	PP Min	Double pulses	Half pulses	Mean	
1	40	20	32	30	24	<b>29,20</b>	32	24	23	21	27	<b>25,40</b>	<b>27,30</b>
2	12	14	28	7	25	<b>17,20</b>	29	24	27	17	29	<b>25,20</b>	<b>21,20</b>
4	14	27	14	18	26	<b>19,80</b>	13	21	21	25	25	<b>21,00</b>	<b>20,40</b>
5	30	28	18	17	3	<b>19,20</b>	23	23	11	13	44	<b>22,80</b>	<b>21,00</b>
6	6	6	10	21	11	<b>10,80</b>	28	19	19	16	31	<b>22,60</b>	<b>16,70</b>
7	9	4	13	16	16	<b>11,60</b>	22	5	10	7	13	<b>11,40</b>	<b>11,50</b>
8	24	21	15	21	24	<b>21,00</b>	5	21	17	13	5	<b>12,20</b>	<b>16,60</b>
9	25	19	23	21	20	<b>21,60</b>	21	19	16	22	14	<b>18,40</b>	<b>20,00</b>
10	22	20	18	19	25	<b>20,80</b>	17	16	17	22	19	<b>18,20</b>	<b>19,50</b>
11	22	23	18	20	22	<b>21,00</b>	14	22	12	22	21	<b>18,20</b>	<b>19,60</b>
12	16	14	19	21	22	<b>18,40</b>	19	14	13	13	16	<b>15,00</b>	<b>16,70</b>
<b>MEAN</b>	<b>20,00</b>	<b>17,82</b>	<b>18,91</b>	<b>19,18</b>	<b>19,82</b>		<b>20,27</b>	<b>18,91</b>	<b>16,91</b>	<b>17,36</b>	<b>22,18</b>		<b>19,14</b>

**Table 2**

Mean  $\pm$  S.E. values of the different variables analysed for each period (PrePB, PB, postPB) and for each sound modification.

	Number of maintenances			Number of time in the shelter			Number of time close to loudspeaker			Time spent in the zone of loudspeaker (s)		
	Pre PB	PB	PostPB	Pre PB	PB	PostPB	Pre PB	PB	PostPB	Pre PB	PB	PostPB
Normal	5.55 $\pm$ 1.60	10.09 $\pm$ 3.17	10.36 $\pm$ 2.16	1.91 $\pm$ 0.96	2.09 $\pm$ 1.50	3.27 $\pm$ 1.21	4.91 $\pm$ 1.15	10.09 $\pm$ 3.74	8.36 $\pm$ 2.59	307,07 $\pm$ 40,25	305,48 $\pm$ 52,55	300,48 $\pm$ 56,95
PP Max	8.64 $\pm$ 4.35	9.27 $\pm$ 3.16	14.36 $\pm$ 3.31	5.73 $\pm$ 3.00	3.09 $\pm$ 1.24	3.09 $\pm$ 0.86	5.82 $\pm$ 1.41	8.45 $\pm$ 2.44	6.27 $\pm$ 1.33	288,69 $\pm$ 47,43	288,28 $\pm$ 50,64	259,04 $\pm$ 46,98
PP Min	7.09 $\pm$ 1.91	7.73 $\pm$ 2.35	6.73 $\pm$ 2.28	0.91 $\pm$ 0.37	1.18 $\pm$ 0.57	1.36 $\pm$ 0.54	9.82 $\pm$ 2.08	10.82 $\pm$ 2.71	9.64 $\pm$ 2.55	340,99 $\pm$ 49,74	370,36 $\pm$ 46,69	375,93 $\pm$ 52,52
Double Pulse	5.82 $\pm$ 2.09	7.27 $\pm$ 1.80	7.09 $\pm$ 2.09	2.18 $\pm$ 1.19	2.00 $\pm$ 0.91	3.18 $\pm$ 1.33	6.00 $\pm$ 2.17	6.91 $\pm$ 2.27	6.36 $\pm$ 2.10	245,85 $\pm$ 59,47	265,10 $\pm$ 58,71	242,93 $\pm$ 51,58
Half Pulse	6.64 $\pm$ 1.97	8.36 $\pm$ 1.96	7.27 $\pm$ 1.64	2.55 $\pm$ 1.13	1.09 $\pm$ 0.39	1.73 $\pm$ 0.81	7.45 $\pm$ 1.59	8.91 $\pm$ 2.64	7.82 $\pm$ 1.52	372,48 $\pm$ 29,35	327,74 $\pm$ 50,75	348,64 $\pm$ 42,90

## FIGURES

### Figure 1

Schematic representation of the experimental setup (not to scale). H: hydrophone, Ls: loudspeaker, PB: playback, S: shelter. Grey dashed line represents the middle of the aquarium.

### Figure 2

Temporal modifications of male *Metriaclima zebra* sounds recorded during aggressive interactions. a.) Oscillogram of an unmodified -control- sound. b.) Oscillogram of the same sound with an increased pulse period (PP), resulting slow sound (PP Max). c.) oscillogram of a sound with a decreased pulse period, resulting in a fast sound (PP Min). d.) Oscillogram of a sound with only the first half of pulses resulting in a short sound (half pulses). e.) Oscillogram of a sound with a double number of pulses, resulting in a long sound (double pulses). Oscillograms are shown to scale.

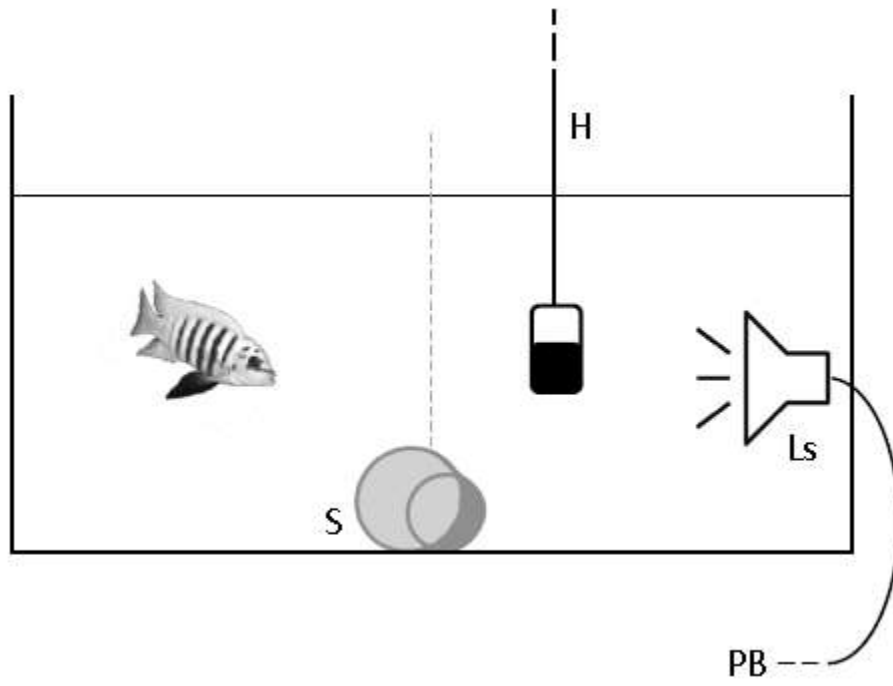
### Figure 3

Behavioural responses to playback of intruders and residents, during the three successive observation periods. PrePB:10 minutes before playback, PB: playback period (duration: 10 minutes ; sound played back each time , PostPB: 10 minutes after playback. a) number of maintenances, b) number of time in the shelter, c) number of time close to the loudspeaker. The boxes represent the first and third quartiles, points (●) are the median (second quartile) and whiskers correspond to the range (min-max) of the distributions. Different letters indicate significant differences ( $p < 0.05$ , Fisher post-hoc test).

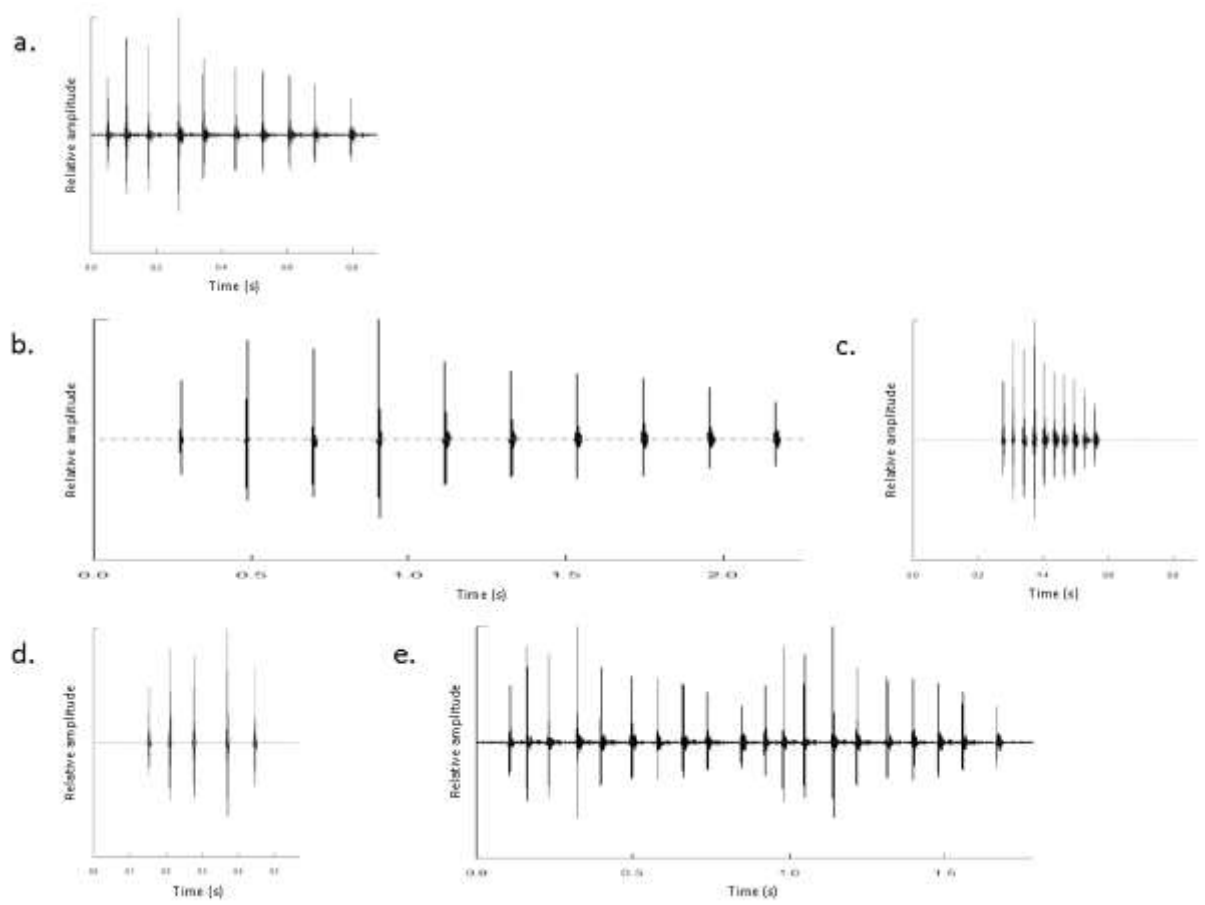
### Figure 4

Behavioural response (number of maintenance behaviours) elicited by sounds with modified time pattern. Fish are tested when resident (i.e., after 24h in the aquarium, see text for details). PrePB:10 minutes before playback, PB: playback period (duration: 10 minutes ; sound played back each time , PostPB: 10 minutes after playback.. Different letters indicate significant differences ( $p < 0.05$ , Fisher post-hoc test).

Figure 1



**Figure 2**



**Figure 3**

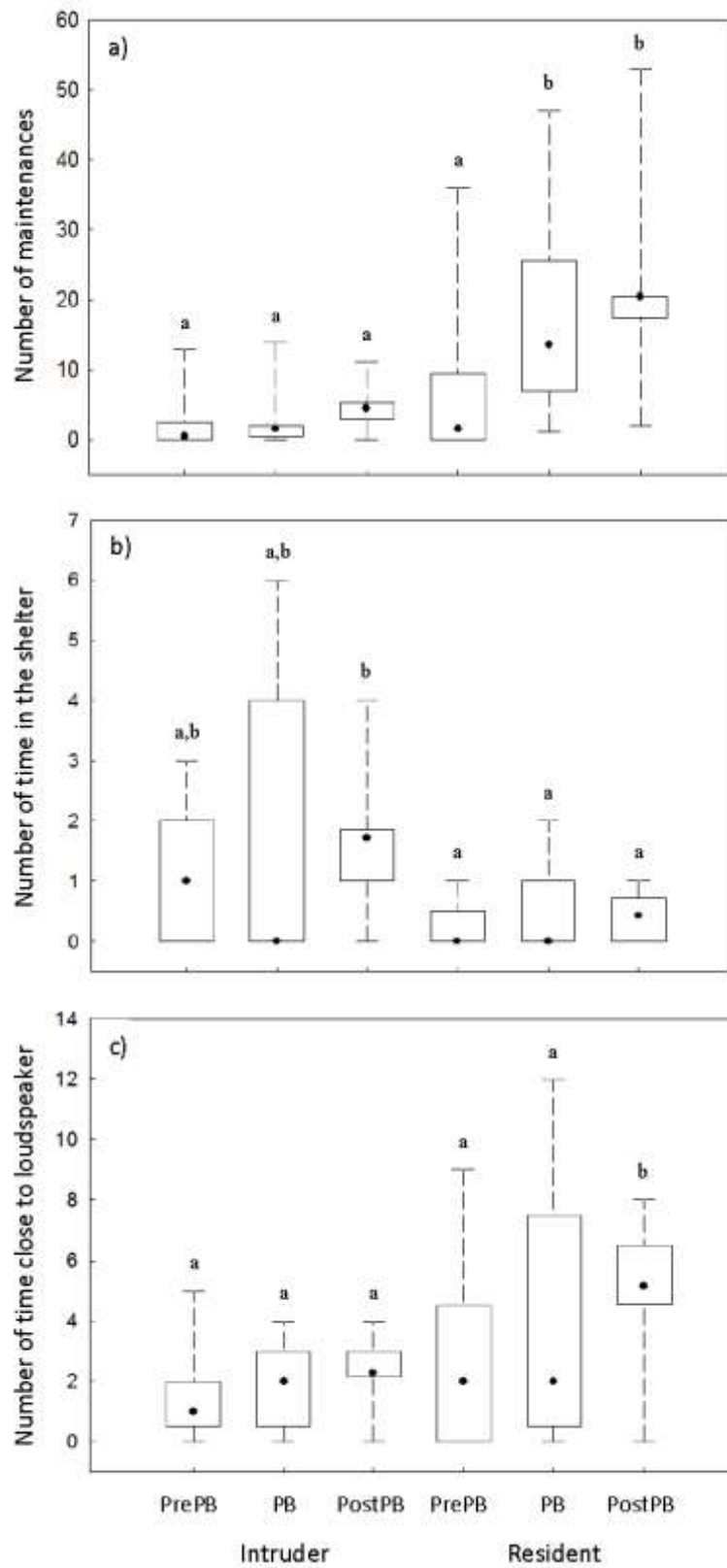
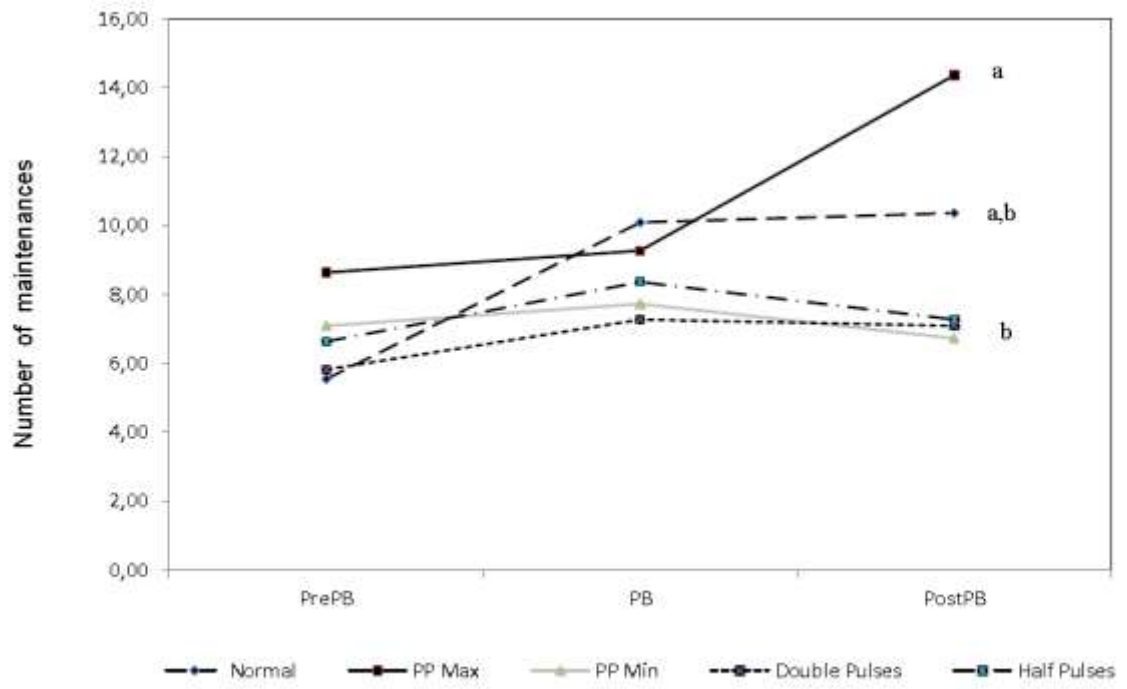




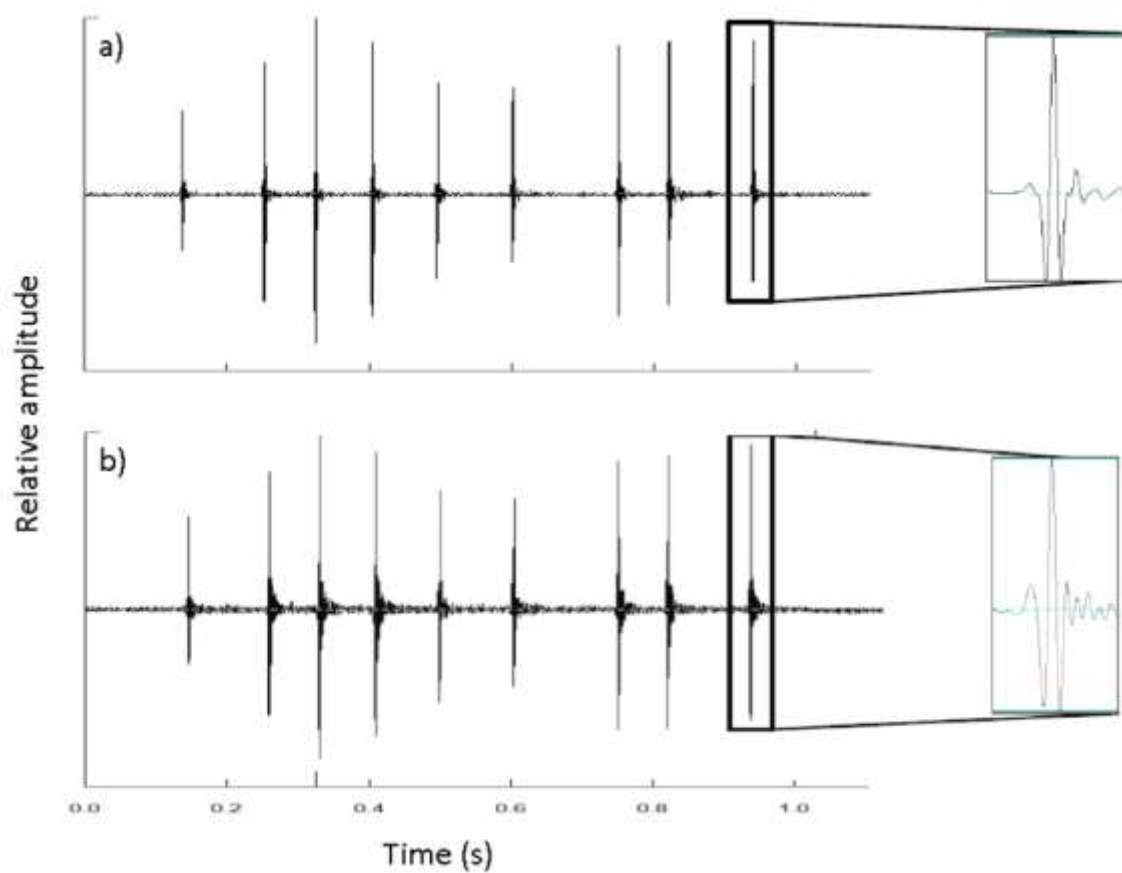
Figure 4



### Supplementary Figure 1

Oscillograms of a sound produced by a male *M. zebra* during an agonistic interaction (a) before playing it back, and (b) once played back through our broadcasting apparatus (recorded at 15 cm from the loudspeaker, within the aquarium filled with water). For each oscillogram, the detail of a pulse is shown.

Two variables (mean frequency and pulse period) of 10 randomly selected sounds were compared before and after playback. A paired t-test revealed no significant differences in the mean frequency ( $n = 10$ ,  $t = 1.91$ ,  $p = 0.09$ ) nor in the pulse period ( $n = 10$ ,  $t = 1.25$ ,  $p = 0.24$ ).



# Ontogenesis of agonistic vocalisations in the cichlid *Metriaclima zebra* (Preliminary results)

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## **Abstract**

While acoustic communication has been described in adults of various fish species, our knowledge about the ontogeny of fish sound production is scarce. Adult fish may use sounds in different social contexts, sending information to potential competitors or sexual partners. Depending on the species, aggressive behaviours might appear in the first days of life due to the competition for food and space. As sound signals are known to be involved during aggressive interactions between adults, it is thus possible that acoustic signals play a role during interactions between juvenile fish. By monitoring a group of juveniles of the cichlid *Metriaclima zebra*, here we describe the evolution of sounds production from hatching to 4 month. We also investigate the possible link between vocalizations and agonistic behaviour by setting dyadic interactions at 3 different ages. We find that sounds production appear early in the development of this fish and increases along with aggressive behaviours. Over the course of this experiment, we only observed sounds consisting in isolated pulses and find changes in two acoustic features, i.e. a decreased pulse frequency and decreased pulse duration, as the fish grow. We discuss these results by comparing them to the existing literature.

**Keywords:** aggressive sounds, dyadic interactions, ontogenesis, group of juveniles, sound characteristics.

## INTRODUCTION

In many fish species, aggressive interactions lead to dominance hierarchies where individuals usually fight for priority access to food, mate or space (Huntingford & Turner 1987). In adult fishes, sound production has been extensively described in these social contexts (Amorim, 2006; Ladich & Myrberg, 2006, Myrberg & Lugli, 2006), which suggests that sounds may be useful to send information to competitors. Aggressive behaviours usually appear in the first days of life due to the competition for food and space. It is possible that acoustic signals play a role during agonistic interactions between juvenile fish. However, and while numerous studies have examined developmental changes in vocalizations in mammals and birds (Hauser, 1998), few studies have focused on the ontogenesis of acoustic communication in fish and little has been done in parallel as it concerns the development of aggression. Ontogenetic development of vocalization and the occurrence of agonistic behaviour have been described in the croaking gourami (*Trichopsis vittata*) (Henglmüller & Ladich, 1999; Wysocki & Ladich, 2001) and in the toadfish (*Halobatrachus didactylus*) (Vasconcelos & Ladich, 2008) during dyadic interactions. In *T. vittata*, first interactions between individuals relate to feeding context and consist in a series of approaches and flight. More aggressive displays, like lateral displays, occur after 3 weeks and first sounds, consisting in single pulses, are recorded after 8 weeks. The most aggressive behaviours, e.g. biting, occur at the age of 10 weeks. During this period of time, sounds characteristics evolve. Besides the transition from single to double pulses, the pulse period, the number of pulses and their intensity increase, while their dominant frequency decreases (Henglmüller & Ladich, 1999). Little is known about the occurrence and role of vocalization in a group of juvenile fish.

In the present study, we focus on *Metriaclima zebra*, a sound-producing rock-dwelling cichlid fish living in the sediment-free rocky coasts of Lake Malawi. Males of *M. zebra* are blue with 6-8 vertical black bars on their body and defend territories in which they try to attract females. In adults, sounds are produced during both aggressive and courtship interactions, in association with visual displays (Amorim et al. 2004; Amorim et al. 2008b; Simões et al. 2008). The aim to this work is (1) to describe the evolution of sounds production in a group of juvenile *M. zebra* from hatching to 4 month old; (2) to test the link between vocalizations and agonistic behaviour by setting dyadic interactions at 3 different stages: 40 days old, 200 days old and adult (two year old, > 600 days, sexually mature).

## MATERIAL AND METHODS

### *FISH*

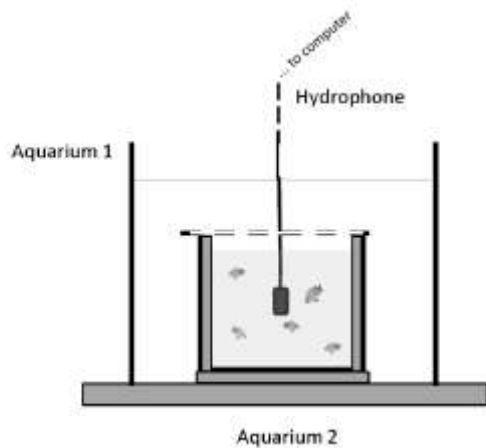
*M. zebra* were purchased from N'Guyen International (Kingersheim, France) and stored in heterosexual groups in two holding tanks (120 cm long, 60 cm wide and 50 cm height) each containing 10 – 12 mature individuals (from 1 to 3 years old), with a sex ratio of 1:2. Each tank was equipped with an external filter (Rena Filstar xP3), aeration, sand substrate, and terracotta pots and bricks as shelters. The temperature was maintained at  $25 \pm 2$  °C and the pH at 8.0 on a LD 12:12 cycle. The fish were daily fed with commercial cichlid food (JBL NovoRift) complemented with cubes of a mixture of mussels, shrimps and spinach once a week.

Every week during 2 months, about one third of the water was changed with colder water (20°C instead of 25°C). These changes of temperature would promote reproduction, and make females begin to spawn. In *M. zebra*, females keep eggs into their mouth once a male has fertilized them. When a mouthbreeding female was detected, we isolated her from the group and let her incubate eggs. After 21 days, tens of juveniles are released in the water, each measuring about 1 cm.

### *EXPERIMENTAL SETUP AND PROCEDURE*

#### Recording of juveniles within a social group

Five days after hatching, 15 juvenile fish coming from a single brood were put in a small aquarium (25 x 25x 25 cm). To improve the quality of sound recording, the small aquarium was placed in a larger one (60 x 50 x 50 cm) (fig 1), and three internal walls of the aquaria were covered with acoustically absorbent material (high density foam) in order to reduce resonance and reflexion. All the experiments were performed in a sound proof chamber to minimize external background noise. During the experimental sessions, the filter and the aeration devices were switched off to allow recordings of fish sound. We simultaneously recorded acoustic signals and videotaped interactions between individuals during 4 months at a rate of 3 times per week during 15 min before feeding.



**Fig. 1** - Experimental setup (not to scale) for the study of a group of juveniles (dashed line represents connection to the computer). The inner aquarium is covered with a net in order to constrain the fish.

### Sound production and aggressive interactions

We used three groups of fish of different ages: two groups of juveniles (group 1: 40 days old individuals, 40D ; group 2: 200 days old individuals, 200D) and a group of adults (> 600 days old).

The 40D group (20 individuals) was obtained from a single hatching. To increase the aggressiveness during dyadic interactions, the group was divided into 2 sub-groups of 10 individuals during 20 days, in two 20-L aquaria equipped with filter, aeration, sand substrate, and bricks as shelters. The two sub-groups were maintained separately with no possible visual, chemical, acoustic or tactile interaction. These fish were 40 days old at the beginning of the experiment: 20 days in the mother mouth plus 20 days in free water.

The 200D group (30 individuals) was obtained from a single hatching of another female from a different tank. We also separated individuals into 2 sub-groups of 15 fish just after the female released them to water. The two sub-groups were maintained separately during 180 days in two 20-L aquaria. These fish were 200 days old at the beginning of the dyadic experiments (20 days in mother mouth plus 180 days in free water). Dissection of the genital tracts showed that 40D and 200D animals were not mature. Sexual maturity in our rearing conditions occurs when fish are at least 9 month old.

The adult group was obtained from our storage tanks. We used 6 males from three different tanks. They were approximately 2 years old at the time of the experiment and we set dyadic interactions between unfamiliar individuals.

For the dyadic interactions, the experimental setup consisted of aquaria (8-L for juveniles and 20L for adults) separated into two compartments by two contiguous removable partitions, one opaque and one transparent. Each aquarium contained a filter, aeration, an

internal heater, a shelter and a sand substrate. A video camera (BUL520) was positioned in front of each aquarium and two hydrophones (H2a-XLR, Aquarian Audio Products) were placed in the aquarium, one per compartment. Hydrophones were connected to a preamplifier (MLA8, Yamaha) and then to the video capture card (Osprey-450e) that was able to synchronize the audio signals to the video signal sent by the video camera. The aquaria were placed on a vibration-insulated shelf and located in an acoustically insulated room in order to minimize background noise.

Two fish of the same age (40D, 200D or adult) were introduced in the experimental aquarium, one fish per compartment and kept isolated from each other for 24 h. This procedure allowed the two fish to acclimatize and become resident in their own compartment. Before the beginning of the trials, filters, aeration and heaters were switched off in order to reduce background noise. Audio and video recordings started with a 10 min control period during which both fish were still isolated. At the end of the control period, the opaque partition was removed, and the two individuals were allowed to interact visually for 10 min.

We performed 5 dyadic interactions between 40D individuals ( $n = 10$ , with a mean  $\pm$  SE total length of  $1.51 \pm 0.03$  mm and weight of  $0.45 \pm 0.03$  g), 5 dyadic interactions between 200D individuals ( $n = 10$ , with a mean  $\pm$  SE total length of  $4.96 \pm 0.41$  mm and weight of  $2.46 \pm 0.54$  g), and 3 dyadic interactions between adult individuals ( $n = 6$ , with a mean  $\pm$  SE total length of  $7.27 \pm 0.29$  mm and weight of  $5.89 \pm 0.11$  g). For each dyadic interaction and at each age, the two fish were taken from the two different stocking aquaria and were chosen to form homogeneous pairs, *i.e.* with similar total length and weight.

#### *Analysis of observed behaviours*

Behavioural data were collected from the videos recorded during the trials. We quantified the number of aggressive behaviours – *i.e.* lateral displays, quivers, charges and bite attempts (the transparent partition prevents real bite) – performed either at the group level for the first experiment or by each of the two fish in the dyadic interactions using the behavioural transcription software EthoLog 2.2.5 (Ottoni, 2000). We calculated the total aggressive score of each individual of a pair by summing the number of aggressive behaviours they displayed.

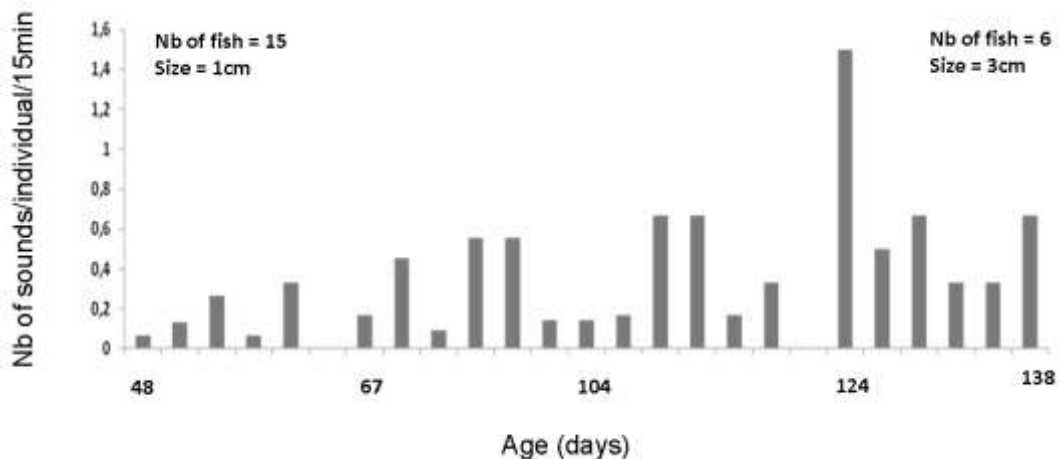
Recorded sounds were digitized at 44.1 kHz (16 bit resolution). We counted the total number of sounds (with clear starts and ends and a good signal-to-noise ratio) produced by each individual. We then measured the duration of pulses and their instantaneous frequency (measured as the inverse of the period of maximum amplitude).



## RESULTS

### *Aggression and Sound production*

Within the group, small specimen of 48 days old produced sound mainly during chases and the number of sounds tended to increase until 138 days (Figure 2).

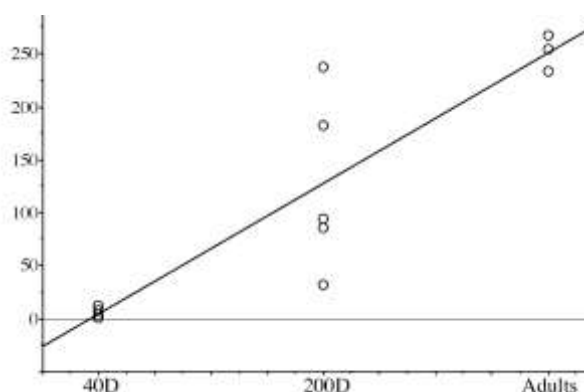


**Fig. 2** – Number of sounds produced within a group of juveniles per individual over a period of 15 minutes from day 48 until day 138.

40D individuals placed in dyadic interaction exhibited very few aggressive behaviours (Table 1), but all the possible aggressive behaviours generally encountered in adults were observed at least one time when considering the whole data (not shown). Due to this low number of behaviours, it has not been possible to distinguish a winner and a loser at the end of the 10 min of interaction. The two individuals mainly swam within their compartment and ignored themselves. Likewise and in accordance with the group observation, very few sounds were emitted. It was even difficult to estimate precisely the number of sounds emitted by individuals during the 10' interactions because of their weak intensity and a low signal-to-noise ratio.

Conversely, in 200D and adults, a larger number of aggressive behaviours was observed during dyadic interactions. The total aggressive score was significantly different between 40D, 200D and adults (one-way ANOVA,  $n = 30$ ,  $F = 12.71$ ,  $p = 0.0002$ ). All the *post-hoc*

Fisher tests were significant ( $p < 0.05$ ). Likewise, the total aggressive score per pair (aggressive score of individual #1 + aggressive score of individual #2) increased as the age increased (simple linear regression analysis,  $n = 15$ ,  $r^2 = 0.81$ ,  $p = 0.0001$ ; figure 3). In 200D and adults, it was possible to define a winner and a loser at the end of the interaction (Table 1). However, there were significant differences between the winners and the losers only for adults regarding the total aggressive score (Mann-Whitney,  $n = 10$ ,  $Z = -1.99$ ,  $p = 0.0463$ ). In adults, winners emitted significantly more sounds than losers (Mann-Whitney,  $n = 10$ ,  $Z = -1.96$ ,  $p = 0.0495$ ), mainly during lateral displays and quivers.



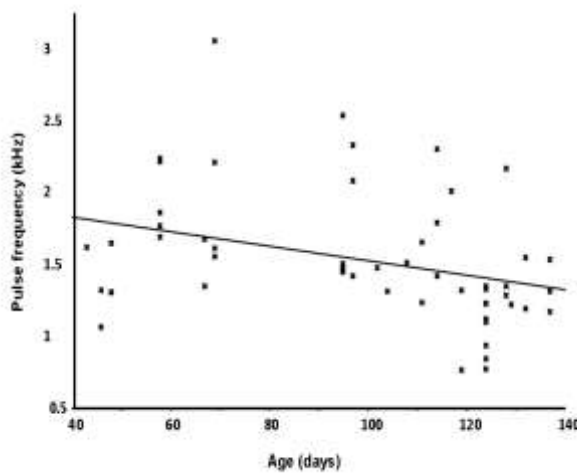
**Fig. 3** – Relationship (linear regression) between the age ( $x$ ) and the total aggressive score per pair of interacting fish ( $y$ ). The line represents the linear regression equation ( $y = 122.87x - 117.76$ )

**Table 1** – Total number of sounds, total number of aggressive behaviours, and status (winner/loser) recorded during dyadic interactions in 40D, 200D, and adults *M. zebra*. Each pair of lines (gray or white) corresponds to a pair of interacting fish. For 40D and 200D, the winner status was obtained when the difference between the total aggressive scores of the two individuals exceeded respectively 2 and 5.

Dyadic interactions in 40D				Dyadic interactions in 200D				Dyadic interactions in adults			
Total number of sounds	Total aggressive score	Status defined from the total aggressive score	Total number of sounds	Total aggressive score	Status defined from the total aggressive score	Total number of sounds	Total aggressive score	Status defined from the total aggressive score	Total number of sounds	Total aggressive score	Status defined from the total aggressive score
1	7	equality	0	64	loser	22	234	winner			
0	5	equality	0	119	winner	2	34	loser			
0	1	loser	2	52	winner	14	187	winner			
0	7	winner	0	43	loser	3	47	loser			
0	3	equality	1	13	loser	16	187	winner			
1	1	equality	0	19	winner	5	67	loser			
0	1	equality	2	44	equality						
0	3	equality	0	42	equality						
2	1	equality	0	110	loser						
0	0	equality	2	127	winner						

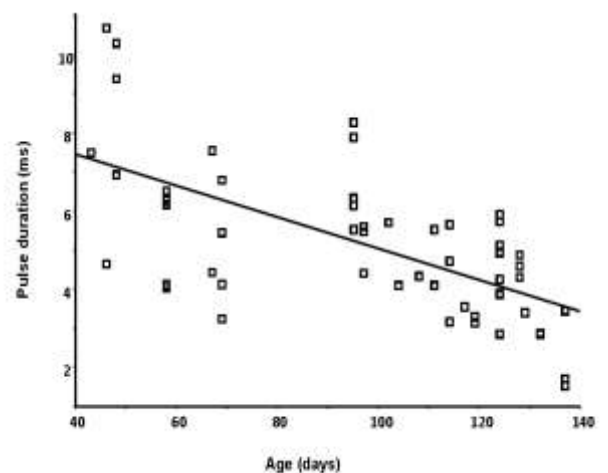
### Sound structure

In both experiments, sounds of juveniles were made of single pulses, while adult sounds were constituted of burst of pulses (5 to 10 pulses typically, see for details Simões et al., 2008; Bertucci et al., 2010). The analysis of sounds characteristics performed for the group showed that pulses had a mean  $\pm$  SE duration of  $5.08 \pm 0.25$  ms, with a instantaneous frequency of  $1610.53 \pm 77.54$  Hz. We found a decrease in the instantaneous frequency ( $r^2 = 0.11$ ,  $p = 0.013$ ) and the duration ( $r^2 = 0.40$ ,  $p < 10^{-3}$ ) of recorded isolated pulses from day 48 to day 138 (figures 4 and 5).



**Figure 4** – Relationship (linear regression) between the age ( $x$ ) and the instantaneous pulse frequency ( $y$ ). The line represents the linear regression equation ( $y = -5.07x + 2036.87$ ).

**Figure 5** – Relationship (linear regression) between the age ( $x$ ) and the pulse duration ( $y$ ). The line represents the linear regression equation ( $y = -0.04x + 9.05$ ).



## DISCUSSION

Sound production has been studied during reproductive context, the defence of a territory or when competing for food in representatives of approximately 30 families (see for reviews Ladich 1997; Ladich and Myrberg, 2006; Myrberg and Lugli, 2006), notably in cichlid fish (Lobel, 2001; see for review Amorin, 2006). The present study shows that the number of agonistic interactions increases with age in juvenile *M. zebra* placed in dyadic interaction conditions. Sounds follow the same way. It is noticeable that juveniles start to vocalize very early, prior to the ability to develop complex agonistic behaviour, producing very few mono-pulse sounds at 40 days of age, a little bit more sounds but still mono-pulse at 200 days of age, and numerous multi-pulses sounds in adults.

The emergence of the aggressive behaviour during development has been studied in a few species of teleost fish (see for review Henglmuller and Ladich, 1999). In these species, the repertoire of agonistic behaviours was considered as fully developed at the age of 3-4 months. However, the first occurrence of the different aggressive acts depends on the type of experiments. Thus, in dyadic conditions, Henglmuller and Ladich (1999) noticed that most of the aggressive patterns observed in adult croaking gouramis (*Trichopsis vittata*) were also observed in fish of less than 3 months of age. In particular, lateral displays, generally regarded as late-occurring, ritualized aggressive acts, first occurred in the third week of life. In dyadic conditions, we observed in 40D *M. zebra* all the behaviours encountered in adults, but in very small numbers. Most of the aggressive acts consisted in charges. The behaviours observed in adult *M. zebra* were similar to those already mentioned by Simões et al. (2008) or Bertucci et al. (2010), *i.e.* lateral displays, quivers, charges, and bites (here bite attempts). In parallel to aggressive behaviours, sound production of *M. zebra* was analysed in dyadic context. Although scarce, some sounds were recorded in association to aggressive behaviours (lateral displays or quivers). According to previous experiments performed in adults *M. zebra* placed in dyadic conditions (Simões et al., 2008; Bertucci et al., 2010), sounds usually occurred during lateral displays and quivers, also in juveniles. The close association between visual and acoustic stimuli thus seems to be present early in the life of this cichlid. Sounds consisted in one-pulse signals, while adult sounds -more numerous- were multi-pulses. Henglmuller and Ladich (1999) obtained similar results in *Trichopsis vittata*: one-pulse sounds accompanied first the lateral displays since the 8<sup>th</sup> week. In this species, the number of sounds increased with age, becoming bi- then multi-pulses at 12 weeks. We did not observe the same evolution in *M. zebra* as the -few- sounds we recorded were still mono-pulse structured in 200D individuals. The change from single pulse to multi-pulse sounds must occur between 200 days and one year of age, as the youngest adults studied here were aged of two years. Changes of the sound structure during ontogenesis are

likely to be due to the development of individual's morphology (size), and sound generating structures. In particular, the decreased sound frequency with the increased body size is a general phenomenon in adult fishes (Ladich et al., 1992; Myrberg et al., 1993; Crawford et al., 1997; Simões et al., 2008, Bertucci et al., *in revision*).

Moreover, the presence, even low, of aggressive behaviours accompanied with sounds in 40D individuals unsurprisingly confirmed the probably innate origin of these behaviours (Longrie et al., 2008).

In conclusion, this study participates to the understanding of ontogenetic development of aggression and the evolution of sound characteristics in a juvenile fish and paves the way for future studies focusing on the role of acoustic signals in the social life of the cichlid *Metriaclima zebra*.

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# **DISCUSSION & PERSPECTIVES**



## IV. DISCUSSION ET PERSPECTIVES

L'objectif de ce travail de thèse est de contribuer à la compréhension du rôle biologique des signaux acoustiques émis en contexte agonistique par un poisson cichlidé du Malawi, *Metriaclima zebra*. J'ai en particulier cherché à cerner l'information pouvant être transmis par ces sons.

La production de sons durant les interactions agonistiques souligne l'importance sociale de ces signaux chez ce poisson. Les signaux sonores participent à une diminution du niveau d'agressivité observée lors d'une dispute territoriale entre deux mâles et portent de l'information quant à l'émetteur. Si la seule perception de signaux sonores ne suffit pas à déclencher des comportements agressifs, des expériences de repasse de signaux (playbacks) montre que les sons augmentent l'activité de maintenance (qui se traduit par le creusage du substrat) de mâles territoriaux. Les effets de la modification des paramètres temporels de ces sons restent peu tranchés ; ils suggèrent que les individus puissent être relativement tolérants aux variations des caractéristiques acoustiques de ces signaux.

### A. Place de l'acoustique dans les interactions mâle-mâle chez *Metriaclima zebra*

#### 1. Emission de signaux sonores et mise en place des comportements agonistiques durant l'ontogenèse

Bien qu'encore préliminaires au regard du nombre d'individus observés, nos résultats concernant le développement des productions acoustiques chez les juvéniles confirment les rares études menées chez d'autres espèces. A 40 jours de vie, tous les comportements agressifs sont rarement observés. La production de sons, bien qu'également peu abondante, montre que l'aptitude à émettre des signaux sonores apparaît très tôt chez *M. zebra*, avant que le répertoire comportemental tel qu'observé chez les adultes ne soit complètement observable. Chez *T. vittata*, la production de sons apparaît lors de la huitième semaine de vie, après les poursuites ou les displays latéraux qui sont présents dès la troisième semaine alors que les comportements les plus agressifs comme les morsures sont observés lors de la dixième semaine (Henglmüller & Ladich, 1999) ou *E. gurnardus* (Amorim & Hawkins, 2005). D'après les expériences d'interactions dyadiques, l'individu dominant produit plus de sons que le dominé. Comportement agonistique et production acoustique semblent donc liés, dès l'âge de 200 jours et possiblement antérieurement. La mise en place bien avant l'âge adulte de comportements agressifs associés à une production sonore est susceptible d'entraîner

des différences de statuts entre les individus. Le rôle des signaux acoustiques dans ce processus reste cependant à préciser.

En ce qui concerne l'ontogenèse de la structure acoustique des signaux émis, les enregistrements réalisés montrent que les premières productions consistent en des pulses isolés, dont la fréquence instantanée diminue avec la croissance en taille des poissons. Cette modification est probablement associée aux changements morphologiques et au développement des muscles soniques (Modesto & Canario, 2003). Sur la période d'âges 48-200 jours, la transition vers des pulses successifs tels qu'on les observe chez l'adulte n'a pas été enregistrée. La période charnière est donc plus tardive et pourrait correspondre à la mise en place de la maturité sexuelle. L'ontogenèse de la structure des sons émis par *M. zebra* demande à être explorée plus en avant si l'on veut suivre finement l'évolution du rôle biologique des signaux acoustiques au cours du développement et de la structuration des interactions sociales.

## **2. Le canal acoustique chez l'adulte : une importance modulée par le statut social**

Nos expériences montrent que la production de signaux acoustiques module –en le diminuant- le niveau d'agressivité lors d'une dispute entre mâles territoriaux. L'intérêt est probablement d'éviter une escalade dans les agressions et un possible combat physique coûteux en temps et en énergie. L'analyse de la structure acoustique des signaux montre qu'ils portent de l'information quant à la taille de l'individu émetteur. Chez de nombreux poissons, la taille est directement liée au statut social d'un individu. On peut faire l'hypothèse –et nous l'avons observé maintes fois dans nos aquariums- qu'il en est ainsi chez *M. zebra*. Les sons pourraient donc permettre au récepteur d'évaluer la taille et donc le statut social relatif de l'individu émetteur, lui permettant ainsi de jauger de l'opportunité d'un affrontement. Reste à savoir si cette information portée par l'acoustique présente un quelconque intérêt en contexte naturel : si l'individu récepteur voit l'émetteur, il est possible que le canal visuel devienne prépondérant pour l'évaluation du risque. Une hypothèse alternative serait que le canal acoustique ne serve qu'à attirer l'attention du récepteur. D'après mes expériences, la diffusion de sons enregistrés en contexte agressif attire les mâles résidents qui en réponse augmentent leur comportement territorial. Une telle réponse n'est pas observée chez des individus non territoriaux. Les réactions comportementales induites par les signaux acoustiques dépendent donc du statut social de l'individu récepteur. De nombreuses études montrent également que le statut social influe sur les caractéristiques acoustiques et que ces dernières varient en fonction de leur contexte d'émission (e.g. Hawkins & Amorim, 2000 ; Amorim et al., 2004b) permettant de renseigner sur le sexe ou la motivation de l'émetteur. Chez l'aiglefin (*Melanogrammus aeglefinus*) par exemple, l'introduction d'un mâle dans un

aquarium occupé par un couple provoque l'augmentation des vocalisations de parade du mâle résident (Bremmer et al., 2002).

### 3. Acoustique et autres canaux de communication

D'après la littérature, les sons produits par les poissons durant des interactions agressives peuvent remplir différentes fonctions : repousser un intrus d'un territoire, modifier l'agressivité d'un opposant, ou encore permettre d'évaluer la qualité d'un congénère (Ladich & Myrberg 2006). Cependant, lors de ces interactions agressives, le principal canal de communication est généralement le visuel. Les individus sont en principe proches, et donc en contact visuel : se voir suffit à initier une approche. Chez les poissons, le canal visuel est donc très employé pour signaler des informations tels le sexe ou le statut social (contraste de couleur par exemple). Le canal acoustique viendrait renforcer ou moduler les informations portées par le visuel. Tout comme chez d'autres cichlidés (Lobel, 2001 ; Ladich & Myrberg 2006), les sons produits lors d'interactions agonistiques chez *M. zebra* sont ainsi souvent associés aux comportements de tremblements et de displays latéraux (Jordan, 2008 ; Simões et al. 2008).

L'association entre stimuli visuels et acoustiques lors des interactions agressives entre mâles de *M. zebra* souligne la complémentarité de différentes modalités sensorielles dans la communication de ce poisson. Mon travail de thèse permet d'identifier plusieurs fonctions possibles de ces signaux. Alors que les différentes postures telles que les tremblements ou displays latéraux peuvent être produites sans production sonore associée, l'ajout de l'acoustique pourrait permettre de particulariser ces comportements, voire de modifier leur qualité en les renforçant du point de vue d'un récepteur. Comme le montrent mes expériences, le canal visuel suffit à initier une interaction aggressive et a provoqué un haut niveau d'agressivité entre deux protagonistes. Le couplage entre les canaux visuel et acoustique diminue cette agressivité. Un signal visuel tel le tremblement pourrait apparaître plus impressionnant accompagné d'un son et ainsi moduler à la baisse la réponse aggressive du récepteur.

De précédentes expériences s'intéressant aux conséquences des signaux sonores lors d'interactions agonistiques ont obtenu des résultats contrastés. Rigley & Muir (1979) chez le poisson chat *Ameiurus nebulosus* ou Schwarz (1974) chez *Cichlasoma centrarchus* montrent que les sons diminuent l'agressivité. Au contraire, chez d'autres espèces de poissons comme *Cyprinella analostana* ou *Botia horae*, on observe une augmentation de l'agressivité en présence de sons (Stout, 1963 ; Valinsky & Rigley, 1981, respectivement). Bien que l'hypothèse d'un rôle différent des sons chez différentes espèces ne soit pas à exclure, ces études ne prenaient pas en compte la possibilité d'une complémentarité avec

d'autres canaux de communication. Mes expériences montrent que l'association des modalités acoustique et visuelle semble particulièrement importante. Il est possible qu'en fonction des espèces, les signaux acoustiques œuvrent différemment en fonction des informations portées par le canal visuel (Van Staaden & Smith, 2011).

Par ailleurs, en assurant une certaine redondance de l'information, une communication multimodale peut participer à sécuriser la transmission du message. La transmission à travers l'environnement suppose en effet un risque de perte d'une partie des informations lors de la transmission et la réception d'un message.

Enfin, des études menées chez les cichlidés montrent que durant la parade sexuelle, ces poissons peuvent utiliser une communication unimodale ou multimodale (Smith & VanStaaden, 2009). S'il apparaît que les signaux sonores complètent les signaux visuels, les stimuli acoustiques ne sont pas indispensables lors des parades nuptiales. Cependant les modifications comportementales observées lors de mes expériences de playbacks soulignent le fait que la compréhension de la communication chez ce poisson nécessite de considérer différents canaux de communication (Partan & Marler, 1999).

Par exemple, en plus des canaux visuel et acoustique, les poissons utilisent également des signaux chimiques. Outre les signaux d'alarme permettant de détecter la présence de prédateurs (Ferrari et al., 2010), les indices chimiques interviennent dans la signalisation de l'état reproducteur ou du rang social chez le tilapia du Mozambique (Miranda et al., 2005 ; Almeida et al., 2005). Giaquinto & Volpato (1997) ont également montré que si les signaux chimiques étaient supprimés, les combats entre mâles duraient plus longtemps et qu'aucune hiérarchie n'était établie. Chez *Metriaclima emmiltos*, la présence d'indices olfactifs en non uniquement visuels influence le choix des femelles pour un mâle conspécifique (Plenderleith et al., 2005) ; au contraire, le rôle du chimique pourrait être plus restreint chez *Metriaclima lombardoi* dans le comportement de parade des mâles (Venesky et al., 2005). Il a également été montré que les jeunes cichlidés bagnards associent les indices visuels et olfactifs afin de s'orienter vers leurs parents (Wisenden & Dye, 2009). Les indices olfactifs pourraient donc porter une partie de l'information échangée lors d'interactions sociales et l'association avec des signaux visuels et acoustiques ainsi que leur interaction permettraient une communication plus complexe chez ce cichlidé.

## B. Le codage d'information dans les signaux acoustiques de *Metriaclima zebra*

### 1. La question de la diversité et de la fiabilité des informations codées

De précédentes études avaient mis en évidence des différences interspécifiques (e.g. Amorim et al., 2004 ; 2008b) et inter-populationnelles (e.g. Mann & Lobel, 1998) des signaux acoustiques émis par les Cichlidés. Cependant, on sait encore très peu de choses sur les informations codées par les signaux acoustiques au niveau intraspécifique.

Une des caractéristiques communément codée par les sons émis par les poissons est la taille de l'émetteur. Les poissons les plus gros produisent des sons de fréquence plus basse (e.g. De Jong *et al.*, 2007). Le codage de l'identité individuelle semble également possible. Chez le poisson crapaud lusitanien, de nombreux paramètres acoustiques ont une variabilité intra-individuelle faible, caractérisant ainsi l'individu émetteur (Amorim et Vasconcelos, 2008). L'étude de la signature individuelle de *M. zebra* menée dans cette thèse a montré que les individus de taille différente étaient clairement identifiés grâce en particulier à l'amplitude des pulses produits et leur pattern temporel. La distinction des individus au sein d'un groupe de taille homogène s'avère plus délicate. Le lien étroit entre les paramètres acoustiques et la morphologie du poisson requiert une analyse à plus long terme afin d'étudier la stabilité ou l'évolution du codage d'une information individuelle (taille et poids). En particulier, les caractéristiques acoustiques peuvent varier au cours de l'année, à la saison de reproduction par exemple durant laquelle des facteurs internes (hormonaux) et externes (température) affecteront la masse musculaire des individus, la contraction musculaire, ou la génération des pulses au niveau du système nerveux central (Bass & Andersen, 1991 ; Bass & Baker, 1991 ; Connaughton et al., 1997 ; Connaughton et al., 2000). Il est ainsi probable que les caractéristiques idiosyncrasiques des signaux acoustiques varient avec ces paramètres, affaiblissant ainsi la fiabilité d'une signature individuelle.

Si la signature acoustique individuelle de *M. zebra* semble être peu développée, peut-être faut-il alors s'intéresser à une information différente comme par exemple une signature de statut social. Des études ou chez le tilapia du Mozambique montrent que les sons produits peuvent renseigner sur l'expérience sociale des individus : les sons de vainqueurs ont une durée de pulses plus longue et également une fréquence dominante plus basse (Amorim & Almada, 2005). Chez le gourami grogneur, les sons des vainqueurs de combats ont une intensité plus élevée et une fréquence dominante plus faible que les sons de perdants (Ladich, 1998).

Les expériences de playback avec signaux modifiés menées durant ce travail de thèse montrent que les *M. zebra* font preuve d'une grande tolérance aux variations des paramètres temporels : la multiplication du nombre de pulses d'un son aussi bien que des sons

largement ralentis produisent des réponses comportementales équivalentes à celles obtenues avec un son témoin. S'il se confirme que ces sons pourraient avoir une valeur équivalente pour un récepteur, alors l'information doit être supportée par d'autres paramètres. Cette tolérance pourrait également être le reflet d'une grande variabilité au niveau inter- et intra-individuel des paramètres acoustiques. L'une des contraintes majeures rencontrées chez les cichlidés du lac Malawi étant la densité élevée d'individus (Konings, 2007), la forte compétition pour acquérir un territoire ou pour se reproduire pourrait alors favoriser l'apparition d'une grande variété de comportements de communication. Une étude récente menée chez deux genres de cichlidés Africains, i.e. *Metriaclima* et *Melanochromis*, propose d'ailleurs que cette variabilité puisse aussi être un signal sur la qualité d'un individu (Van Staaden & Smith, 2011). Une telle variabilité des signaux acoustiques suggère que ces derniers pourraient alors porter une information beaucoup plus complexe et être dépendants de multiples facteurs.

## **2. Perception sensorielle et codage d'information**

Les poissons vivant dans des eaux peu profondes produisent généralement des sons de basse fréquence (e.g. Crawford et al., 1997 ; Ladich & Bass, 2003). Ces vocalisations s'atténuent ainsi rapidement avec la distance et la communication est donc restreinte à des distances de quelques dizaines de centimètres de la source (Lugli et al., 2003). De plus, lors de sa propagation du signal dans le milieu, une partie de l'information contenue dans le signal sera perdue. Peu d'études se sont intéressées aux modifications des paramètres acoustiques lors de la propagation des sons chez le poisson (e.g. Crawford et al., 1997). Chez le poisson demoiselle *Dascyllus albisella*, alors que la période des pulse est préservée, des modifications du pic de fréquence, de la durée des pulses et du nombre de pulses sont observées à 2 mètres de la source (Mann & Lobel, 1997).

Une communication à plus longue distance est surtout limitée par le bruit de fond ambiant créé par différentes sources telles que les vagues, les turbulences, les autres animaux et les bruits anthropogéniques. Les effets du bruit de fond ambiant en eaux douces ont été très peu étudiés (Lugli & Fine, 2003 ; Wysocki, 2006) bien qu'ils puissent dégrader les propriétés et la détectabilité des signaux acoustiques. L'étude des stratégies utilisées pour augmenter le ratio signal-bruit rapporte des solutions similaires à celles observées chez d'autres modèles animaux. Chez la demoiselle sombre (*Stegastes adustus*), les individus produisent des sons à une fréquence correspondant à la région la plus sensible de leur système récepteur (Myrberg & Spires, 1980), d'autres espèces produisent des sons dans des gammes de fréquence moins touchées par le bruit ambiant (Crawford et al., 1997 ; Lugli & Fine, 2003). Se pose alors la question de l'audition chez les poissons. L'une des approches

est de comparer les fréquences dominantes des sons produits avec les régions de plus grandes capacités auditives. Comme chez *H. didactylus*, plus ces deux paramètres sont proches et plus un son sera susceptible d'être perçu (Wysocki, 2006 ; Vasconcelos & Ladich, 2008). Wysocki & Ladich (2003) ont également utilisé des expériences de playback afin de mesurer des potentiels évoqués et étudier le codage de l'information au niveau du système auditif. Ainsi, chaque pulse est codé individuellement dans les centres auditifs des spécialistes de l'audition. Plus récemment, Vasconcelos et al. (2011) ont montré que la structure temporelle des vocalisations du poisson crapaud lusitanien (pulses, différentes parties du son et durée totale du son) était précisément représentée au niveau central. Les caractéristiques temporelles sont donc des éléments clé du codage de l'information dans les signaux acoustiques.

Ce codage temporel est rencontré chez d'autres animaux utilisant des signaux pulsés comme les insectes et les amphibiens (Gerhard & Huber, 2002). Si certaines espèces sont très conservatrices en ce qui concerne la période des pulses ou leur durée (e.g. Doherty, 1985), d'autres seront plus sensibles aux variations et montreront des préférences pour des signaux présentant des caractéristiques jugées plus attractives (Vignal & Kelley, 2007). Chez les poissons cette préférence est surtout dirigée vers les sons de conspécifiques qui sont rendus plus attractifs par l'augmentation du nombre de pulses, de leur durée et/ou de leur taux d'émission (Myrberg & Spires, 1972 ; Myrberg et al., 1978; Spanier, 1979 ; McKibben & Bass, 2001 ; Lugli et al., 2004 ; Rollo & Higgs, 2008). Bien que les mâles *M. zebra* territoriaux semblent être attirés par des sons agressifs, la modification de deux de leur paramètres temporels (nombre de pulses et période de pulses) n'a pas permis d'établir la façon dont l'information est codée. L'analyse de la structure des sons avait mis en évidence le potentiel de ces deux variables à coder une information relative à la taille voire à l'identité de l'individu. Si la perception des patterns temporels semble possible chez les poissons (Suzuki et al., 2002 ; Wysocki & Ladich, 2002 ; 2003 ; Vasconcelos et al., 2011) d'autres études concernant les capacités auditives de *M. zebra* permettront de mieux appréhender le codage de l'information chez cette espèce. De plus, la pertinence des modifications drastiques apportées lors de ces expériences reste à établir. Par exemple, la diminution de la période de pulses permet-elle la détection des pulses constituant le signal et, à l'inverse, les pulses auraient-ils pu être considérés comme isolés dans le cas de l'augmentation de leur période ?

### C. Perspectives de recherche

Les signaux acoustiques de *M. zebra* modulent le comportement agressif des mâles en association avec des stimuli visuels. Leur émission semble liée au statut territorial. Qu'en

est-il du rôle des sons produits lors de la parade nuptiale ? Permettent-ils par exemple d'attirer/apaiser les femelles ? Les femelles ayant également la capacité de vocaliser durant des combats, les sons ont-ils le même rôle ? Le lien entre niveau d'agressivité et productions sonores semble exister très tôt. Quel rôle le son joue-t-il dans la structuration d'un groupe et l'acquisition d'un statut social dès les premiers stades de la vie de ces poissons ?

De plus, si les paramètres morphologiques comme la taille et le poids sont bien codés par les signaux acoustiques et qu'une information plus complexe comme l'identité reste imparfaite, d'autres informations comme le statut social ou l'état physiologique du poisson peuvent être portées par les sons. Quelle serait alors leur pertinence et quels paramètres acoustiques seraient alors impliqués ?

De par les nombreuses variations de patterns de couleurs de ces poissons, les signaux visuels ont très vite été considérés dans l'étude de la communication chez les cichlidés. De nombreuses études, dont cette thèse, ont par la suite montré que les signaux acoustiques ont également un rôle social important. Dans une étude portant sur le choix du partenaire Plenderleith et al. (2005) ont montré que les indices chimiques peuvent également être impliqués. Alors que dans le groupe *Metriaclima*, le comportement de parade peut être initié uniquement par des signaux visuels – comme l'agression chez les mâles –, les indices olfactifs peuvent toutefois intervenir chez *M. lombardoi* (Venesky et al., 2005) alors que chez d'autres espèces proches, le chimique semble n'avoir aucun rôle lors de la parade (Jordan et al., 2003). Les signaux chimiques pourraient aussi intervenir lors des interactions agonistiques et interagir avec d'autres modalités, en particulier l'acoustique.

Au sein d'un groupe ou dans le milieu naturel, les interactions sont susceptibles de se réaliser au sein d'un réseau de communication. Différents émetteurs et récepteurs étant présents dans l'espace de signalisation de leurs voisins. Bien que la distance de transmission des sons de *M. zebra* soit faible, la forte densité d'individus observée dans le lac Malawi (Konings, 2007) rend possible l'interception des signaux produits lors d'interactions par des individus tiers (eavesdropping) ainsi qu'une modification des comportements de signalisation en présence de tiers (effets d'audience). Comment les informations sont-elles alors échangées ? De plus, la reconnaissance des voisins est-elle possible ?

Les expériences de playback menées lors de cette thèse prouvent que l'acoustique joue un rôle social important. Afin de préciser les capacités auditives de ce poisson, une étude couplant playbacks et neurophysiologie permettrait de préciser quels aspects du signal sont importants et comment les informations transmises sont intégrées au niveau cérébral.



L'impressionnante radiation évolutive des cichlidés a conduit à une grande diversité de systèmes sociaux et une colonisation de l'ensemble du lac Malawi. Les différentes contraintes environnementales s'exerçant sur les signaux acoustiques (depuis leur codage, leur propagation, jusqu'à leur décodage) ainsi que les différentes contraintes sociales sont ainsi susceptibles d'influencer l'utilisation des sons chez les différentes espèces ayant la capacité de vocaliser. Des études comparatives des systèmes de communication de différentes espèces permettraient donc de mieux comprendre l'évolution et le rôle joué par la communication acoustique.

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**Frédéric BERTUCCI**

**Titre : COMMUNICATION ACOUSTIQUE CHEZ UN POISSON CICHLIDE :**

*Analyse expérimentale du rôle et de la structure des signaux.*

**Résumé :** De nombreuses espèces de poissons sont connues pour produire des sons dans différents contextes sociaux suggérant un rôle important des signaux acoustiques dans la communication. L'objectif de cette thèse est d'étudier la structure et la fonction des sons produits lors d'interactions agressives entre mâles chez le poisson cichlidé *Metriaclima zebra*. Grâce à des expériences de playback, le rôle relatif des indices acoustiques et visuels lors des interactions agonistiques est évalué. Les résultats montrent que les comportements agressifs reposent essentiellement sur des stimuli visuels. Les stimuli acoustiques seuls ne déclenchent jamais d'agression mais modulent le comportement des mâles en diminuant le niveau élevé d'agressivité observé lorsque le canal visuel est seul présent. Une analyse fine de la structure acoustique des sons produits pendant des conflits montre que les signaux émis par *M. zebra* codent des informations relatives à la taille de l'individu émetteur. La signature individuelle reste assez mal définie. Pour comprendre le décodage de l'information au niveau du poisson récepteur, j'ai mis au point un protocole de test permettant de constater que des mâles territoriaux augmentent leur activité territoriale et approchent le haut-parleur en réponse aux playbacks. Des expériences de playback utilisant des signaux aux paramètres acoustiques artificiellement modifiés suggèrent une grande tolérance des individus envers les variations temporelles.

Cette thèse participe ainsi à la compréhension de la fonction biologique de la communication acoustique chez un poisson. Elle appelle de nouvelles études concernant les informations transmises et leur codage.

**Abstract :** Various species of fish are known to produce sounds in different social contexts suggesting an important communicative role of acoustic signals. The aim of this thesis is to study the structure and the function of sounds produced during aggressive interactions between males of the cichlid fish *Metriaclima zebra*. By means of playback experiments, the relative role of acoustic and visual cues during agonistic interactions is evaluated. The results show that aggressive behaviour is essentially based on visual stimuli. Acoustic stimuli alone never trigger aggression but modulate males' behaviour by decreasing the high level of aggressiveness found when only the visual channel is present. A fine analysis of the structure of sounds produced during disputes shows that signals emitted by *M. zebra* encode information related to the size of the emitter. The individual signature remains poorly defined. In order to understand the decoding process of information by receivers, I set up a paradigm allowing to show that territorial males increase their territorial activity and approach loudspeakers in response to playbacks. Playback experiments using signals with artificially modified acoustic parameters suggest a large tolerance for temporal variations.

This thesis thus participates to the comprehension of the biological function of acoustic communication in a fish. It calls for further studies concerning the transmitted information and its encoding process.

**Discipline :** Ethologie, Neuro-éthologie, Bioacoustique, Biologie

**Mots clés :** communication acoustique, comportements agressifs, cichlidés, expériences de playback, poissons, signature individuelle, information

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