



Etude de la connectivité entre les communautés de poissons de différents habitats du lagon de Nouvelle-Calédonie par microchimie des otolithes et de l'environnement

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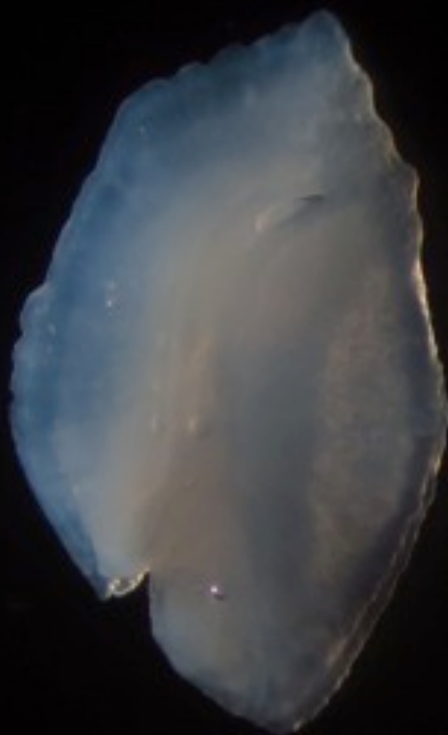
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Étude de la connectivité entre les communautés de poissons de différents habitats du lagon de Nouvelle-Calédonie par microchimie des otolithes et de l'environnement

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Spécialité Ecologie marine
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Soutenue le 22 avril 2014



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Université de la Nouvelle-Calédonie
Ecole doctorale du Pacifique (ED 469)

Thèse de Doctorat
Spécialité Ecologie marine

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Pour obtenir le grade de
Docteur de l'Université de la Nouvelle-Calédonie

**Étude de la connectivité entre les communautés de poissons de
différents habitats du lagon de Nouvelle-Calédonie par
microchimie des otolithes et de l'environnement.**

Soutenue le 22 avril 2014 à l'Université de la Nouvelle-Calédonie, devant le jury composé de :

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« *Necesito del mar porque me enseña...* »

Extrait de *El Mar*

Poème de Pablo Neruda

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Chapitre 1 : Introduction générale



Chapitre 1 : Introduction générale

Les paysages récifaux-lagonaires sont des mosaïques d'habitats discontinus distribués sous forme de patches et représentés par les récifs coralliens (barrière récifale, récifs intermédiaires et frangeants), les herbiers, les fonds meubles, les mangroves, etc. (Jones *et al.* 2009). Ces paysages sont considérés comme des oasis de diversité et de productivité dans les déserts océaniques. Ils font partis des écosystèmes les plus diversifiés de la planète, et sont ainsi souvent comparés aux forêts tropicales humides en terme de diversité spécifique (Bellwood & Hughes 2001). Ils possèdent une valeur économique inestimable et des populations entières vivent grâce aux biens et services qu'ils fournissent (*e.g.* pêche, tourisme, etc.). Cependant, ils sont considérés comme un des écosystèmes marins les plus impactés car à l'échelle mondiale, 30% des écosystèmes récifaux sont d'ores et déjà considérés gravement perturbés tandis que 60% seront dramatiquement menacés à l'horizon 2030 (Hughes *et al.* 2003, Burke *et al.* 2011). Les origines de ces maux sont diverses, les récifs coralliens sont affectés par de nombreuses perturbations d'origine naturelle et anthropique qui peuvent être de nature épisodique (*e.g.* tempêtes, exposition à des polluants toxiques, etc.) ou chronique (*e.g.* surpêche, eutrophisation, aménagement de l'environnement côtier, exposition aux activités minières, etc.) (Nystrom *et al.* 2000, Pandolfi *et al.* 2003, Wantiez *et al.* 2006, Sandin *et al.* 2008). Alors que les perturbations naturelles constituent des opportunités de renouvellement et de développement des récifs coralliens, l'augmentation de leur fréquence ou de leur intensité associée aux changements globaux et l'intensification des perturbations anthropiques sur ces écosystèmes remet en question leur capacité de résilience.

Parmi les écosystèmes associés aux récifs coralliens, la mangrove est un écosystème clé à l'interface entre le milieu terrestre et le milieu marin. Elle fait partie des écosystèmes les plus productifs au monde et possède une forte valeur, aussi bien au niveau écologique qu'économique. La mangrove possède un rôle biologique important comme habitat, zone de nurserie, d'alimentation ou de refuge pour de nombreuses espèces et notamment des poissons et invertébrés comestibles (Nagelkerken *et al.* 2008). Ce rôle biologique est donc à l'origine d'une ressource alimentaire importante pour les populations humaines (Mumby *et al.* 2004). De plus, la mangrove possède un rôle physique majeur en protégeant le littoral de l'érosion ainsi que des événements climatiques violents (*e.g.* tempêtes, raz de marées). Elle agit comme une zone tampon en piégeant les particules issues des apports terrigènes et permet ainsi le

développement des récifs coralliens adjacents dans une eau de faible turbidité. Au cours des cinquante dernières années, 35% de la surface mondiale des mangroves a été perdue (Valiela *et al.* 2001). Cette surface, actuellement comprise entre 160 000 et 200 000 km², disparaît à un taux moyen d'1 à 2 % par an sous l'effet de multiples menaces anthropiques pesant sur cet écosystème (Spalding *et al.* 1997, Nations 2007). Le rôle physique de la mangrove est menacé par l'urbanisation accrue et l'expansion des activités industrielles entraînant une déforestation massive et une conversion des zones de mangrove en terrains agricoles, aquacoles ou en zones constructibles. De même, l'augmentation des rejets d'effluents d'origine anthropique surpasse leur capacité de tampon et entraîne une eutrophisation du milieu. Enfin, l'exploitation à outrance des ressources naturelles menace le rôle écologique de la mangrove.

Associée au régime de perturbations naturelles, la pression anthropique exercée sur les mangroves et les récifs coralliens entraîne de nombreux effets néfastes dont une diminution de la biodiversité de ces écosystèmes, une modification de la structure des communautés, un changement dans la structure de l'habitat voire une perte d'habitats (Nystrom *et al.* 2000, Valiela *et al.* 2001, Alongi 2002, Pandolfi *et al.* 2003, Bellwood *et al.* 2004).

La structure en patchs des paysages récifaux-lagonaires induit une fragmentation des écosystèmes et des espèces associées. Ces dernières s'organisent en métapopulations comprenant de nombreuses sous-populations ou populations locales de différentes tailles. La fragmentation des populations, l'érosion de l'habitat et l'augmentation des perturbations anthropiques que subissent les récifs coralliens et les mangroves en font des écosystèmes côtiers extrêmement fragiles où les connections entre patchs par dispersion ou migration des individus sont indispensables à leur fonctionnement (Mumby & Hastings 2008). Déterminer l'étendue des liens entre les populations locales reste un grand challenge et demeure d'une importance capitale pour l'étude et la préservation des récifs coralliens et des mangroves. En effet, la connectivité entre populations locales a d'importantes implications dans la persistance et la résilience des populations face aux perturbations naturelles et anthropiques. De plus, l'estimation de la connectivité entre populations locales est un objectif clé pour la préservation et la gestion des espèces car le design des réseaux d'aires marines protégées (taille, lieu et espacement) ne peut être optimal sans une estimation préalable de la connectivité existante (Jones *et al.* 2007, Almany *et al.* 2009, Lowe & Allendorf 2010, Mora & Sale 2011).

1. La connectivité

1.1. Définition générale

La connectivité est liée au degré de mouvement des organismes, d'échange de matière ou d'énergie au sein d'un paysage (Crooks & Sanjayan 2006). Il existe deux composantes principales dans le concept de connectivité : 1) la composante structurelle (physique) qui réfère à la propriété de la structure du paysage à faciliter/entraver les mouvements des organismes et 2) la composante fonctionnelle qui correspond à la réponse comportementale des organismes face à cette structure physique (Fig. 1.1). Cette composante fonctionnelle est elle-même subdivisée en deux catégories : la connectivité potentielle englobant les connaissances de base acquises sur les capacités de dispersion des organismes et la connectivité réelle qui va traduire une quantification directe du mouvement réel des individus au sein d'un paysage donné (Calabrese & Fagan 2004, Moilanen & Hanski 2006).

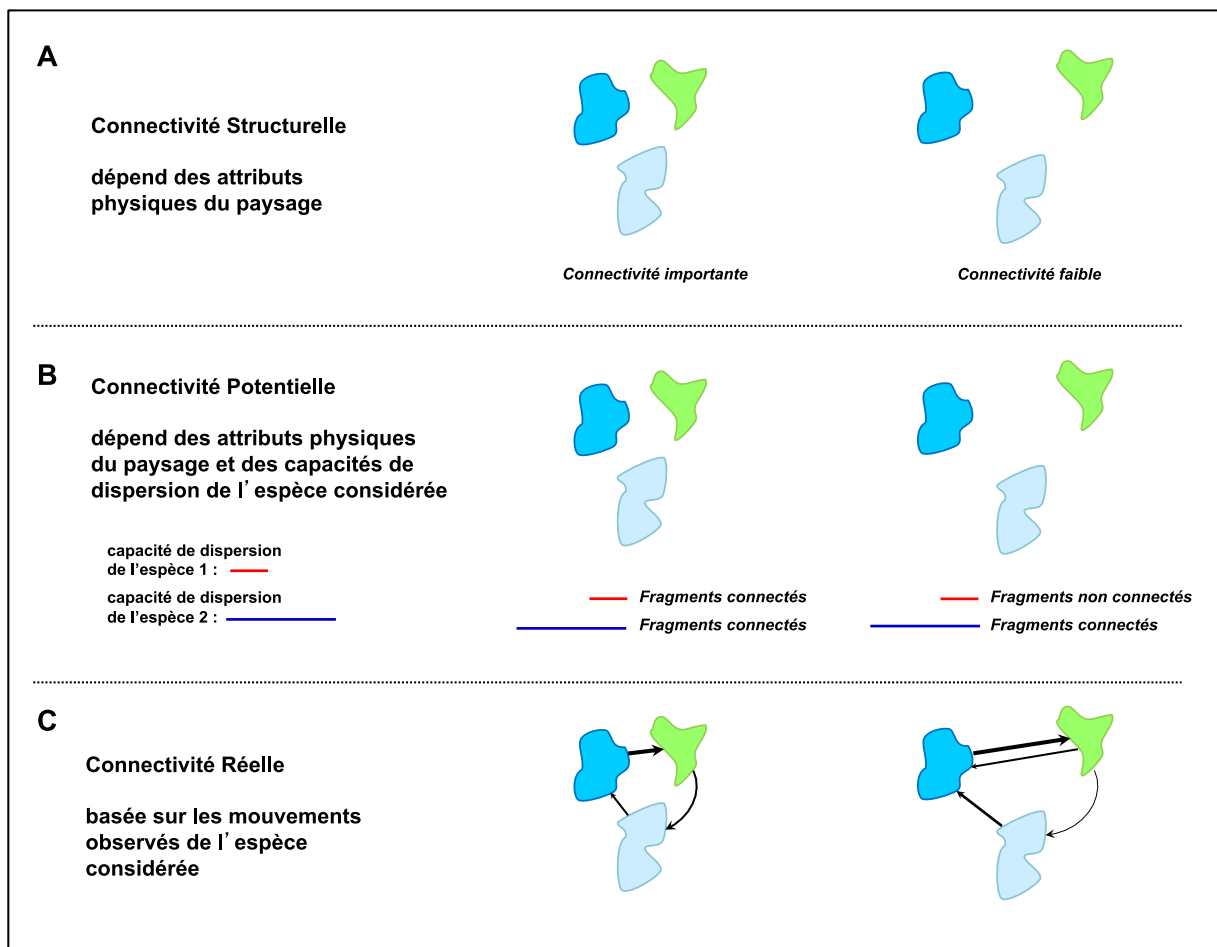


Figure 1.1 : Représentation schématique des trois composantes de la connectivité (d'après Calabrese & Fagan, 2004).

La connectivité réelle se réfère donc aux échanges d'individus entre populations locales spatialement séparées. Ces populations locales, évoluant sur des patchs d'habitats différents, sont liées par des échanges de larves, juvéniles ou adultes résidant de façon permanente ou à long terme dans la nouvelle population (Palumbi 2003). Un temps de résidence minimal est requis afin de 1) distinguer les phénomènes de dispersion/migration des mouvements temporaires associés aux activités de recherche de nourriture ou autres activités quotidiennes et 2) contribuer au pool de gènes des nouvelles populations par transferts de gènes (Lowe & Allendorf 2010). La connectivité réelle peut se mesurer à différentes échelles spatio-temporelles, notamment à l'échelle écologique (connectivité démographique) et à l'échelle évolutive (connectivité génétique) (Moilanen & Hanski 2001, Cowen *et al.* 2007). La connectivité écologique (ou connectivité démographique) se traduit par des échanges d'individus suffisants pour entraîner des impacts mesurables sur la dynamique des populations locales (Sale *et al.* 2010). Les mouvements d'individus se traduisent également par des transferts de gènes permettant le maintien de l'homogénéité génétique entre populations locales, ce qui définit le concept de connectivité génétique (ou connectivité évolutive) (Cowen *et al.* 2007, Cowen & Sponaugle 2009). Des échanges rares et faibles (un individu par génération en moyenne) suffisent à maintenir l'homogénéité génétique entre deux populations locales (Hedgecock *et al.* 2007). Les échelles spatio-temporelles à considérer dans le cas de la connectivité génétique sont bien supérieures par rapport aux échelles à considérer dans le cas de la connectivité écologique. Ces différences d'échelles spatio-temporelles entre les deux types de connectivité sont expliquées plus en détails dans l'exposé des méthodes de mesures de la connectivité (cf. 2. Méthodes de mesure de la connectivité) (Taylor *et al.* 1993, Tischendorf & Fahring 2000, Moilanen & Hanski 2001).

1.2. La connectivité chez les poissons récifaux-lagonaires

Dans les écosystèmes marins, la connectivité réfère aux liens démographiques des populations locales à travers la dispersion des individus aux stades larvaire, juvénile ou adulte (Sale *et al.* 2010). Une des formes de connectivité considérée comme la plus importante pour les organismes marins est représentée à travers le processus de dispersion des larves planctoniques (Almany *et al.* 2007, Almany *et al.* 2009). Chez les poissons récifaux, la phase larvaire pélagique est la première opportunité de dispersion des individus et ce phénomène de connectivité a été particulièrement étudié (Thorrold *et al.* 2007, Cowen & Sponaugle 2009, Jones *et al.* 2009). En effet, il existe de nombreuses études sur la durée de vie larvaire et le

comportement de celles-ci lors du retour vers les récifs (capacités natatoires et sensorielles) (Fisher *et al.* 2000, Kingsford *et al.* 2002, Leis *et al.* 2007), l'estimation du phénomène d'autorecrutement (retour aux sites sources) ou de dispersion à grande échelle (Thorrold *et al.* 2007, Jones *et al.* 2009). D'après Jones *et al.* (2009), les populations marines sont qualifiées d'ouvertes, c'est-à-dire avec un fort potentiel de connectivité, lorsque la majorité du recrutement est issu d'échanges entre populations locales. Au contraire, une connectivité limitée se traduit par des populations fermées c'est-à-dire présentant un fort taux d'autorecrutement (Cowen *et al.* 2000).

De nombreuses espèces de poissons récifaux-lagonaires présentent des populations de juvéniles et d'adultes généralement considérées comme sédentaires et territoriaux, connectées par une large phase de dispersion larvaire (Sale 1991). Cette idée reçue peut entraîner une sous estimation des mouvements des adultes et juvéniles chez de nombreuses espèces. En effet, la phase larvaire ne doit pas être considérée comme l'unique stade de vie impliqué dans la connectivité des communautés marines. Des études acoustiques révèlent qu'en réalité les mouvements des poissons sont bien plus importants que ce que l'on pensait (Chateau & Wantiez 2009, Meyer *et al.* 2010, Claydon *et al.* 2012) et que les processus de dispersion sont toujours possibles chez les populations adultes et juvéniles. D'ailleurs, de récentes études indiquent que le mouvement des adultes et juvéniles permet à de nombreuses espèces de traverser les barrières de connectivité larvaire (Luiz *et al.* 2012) et que l'aire de distribution géographique des poissons de récifs coralliens est plus fortement expliquée par les traits de vie des adultes que par la durée de vie larvaire planctonique (Luiz *et al.* 2013).

1.2.1. La notion de « home range »

Le home range est défini comme la zone utilisée par un animal pour satisfaire les activités quotidiennes telles que l'alimentation ou le repos, durant une partie ou la totalité de son existence (Grüss *et al.* 2011). L'étendue du home range doit pouvoir satisfaire les mouvements entre la zone de refuge et la zone d'alimentation. Suivant l'écologie de l'espèce considérée, l'étendue de la zone de vie peut être de l'ordre de quelques mètres jusqu'à des dizaines de kilomètres et peut couvrir un à plusieurs habitats. Elle est plus importante pour les espèces non territoriales que territoriales. Au sein de la même population, les patrons de mouvements quotidiens peuvent être différents : certains individus présentent des mouvements entre les zones distinctes de refuge et d'alimentation, d'autres individus restent relativement proches de leur refuge en permanence.

1.2.2. Les mouvements d'histoire de vie

Les migrations sont définies comme des mouvements généralement prévisibles dans le temps, se produisant saisonnièrement ou à des instants particuliers du cycle de vie des individus. L'étendue des migrations peut être de l'ordre de quelques centaines de mètres à des milliers de kilomètres. Les migrations d'individus juvéniles et/ou adultes peuvent se produire si les individus ont besoin d'atteindre des zones particulières de frai, de nurseries ou d'alimentation et/ou en réponse à des changements environnementaux (Grüss *et al.* 2011).

Les **migrations ontogéniques** se définissent par des changements d'habitat liés aux différents stades ontogéniques des individus (Fig. 1.2). La plupart des espèces de poissons récifaux-lagonaires possèdent un cycle de vie complexe avec un stade larvaire pélagique puis une phase benthique pour les juvéniles et adultes (Sale 1991). Chez certaines espèces de poissons récifaux, les stades juvéniles et adultes occupent des habitats différents. Lorsque les larves sont compétentes, elles rejoignent le milieu récifal et s'installent au niveau d'habitats côtiers peu profonds tels que les mangroves, les récifs ou les herbiers. A l'installation, elles se métamorphosent en juvéniles qui évoluent au sein de ces habitats qualifiés de zone de nurseries car ils fournissent de nombreux avantages pour leur développement (refuges contre les prédateurs, disponibilité accrue en ressources alimentaires adaptées, etc.) (Parrish 1989, Beck *et al.* 2003). A mesure qu'ils grandissent, les individus vont rejoindre les populations adultes, ce processus correspond au recrutement et se traduit par l'intégration de nouveaux individus parmi les populations d'individus sexuellement matures (Sale 1991).

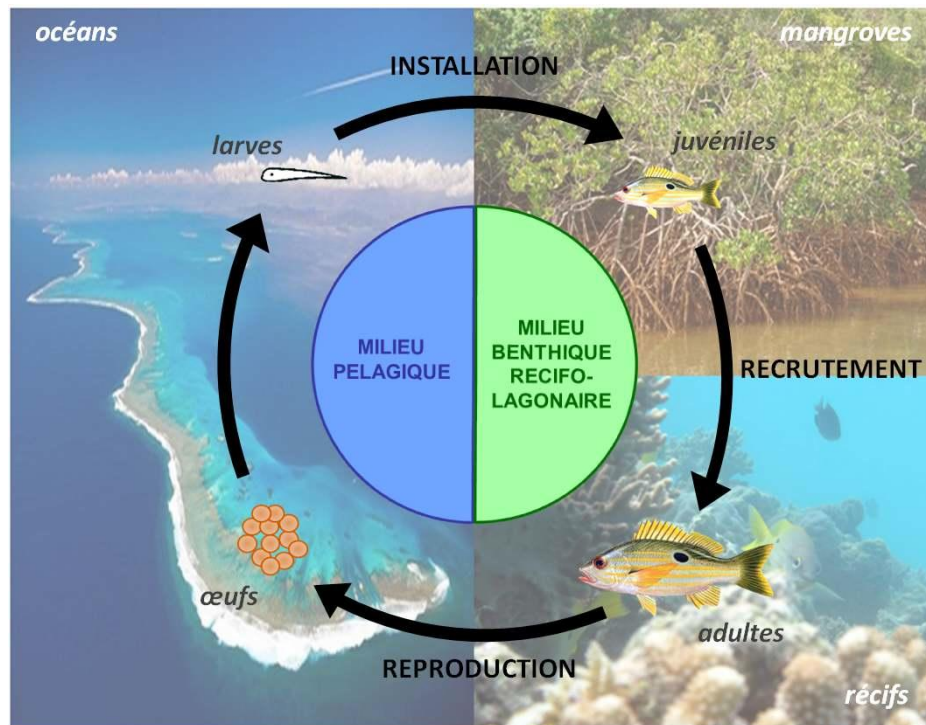


Figure 1.2 : Cycle biologique simplifié de *Lutjanus fulvivflamma*, espèce à œufs pélagiques.

Les **migrations liées à la reproduction** sont associées à la recherche de partenaire ou de lieux de reproduction favorables. Elles sont souvent décrites comme un phénomène de mouvement des animaux de leur lieu de vie à un lieu de reproduction où ils se rassemblent en grand nombre. Cependant, les échelles spatiales et temporelles des migrations de reproduction peuvent s'étendre de distances quotidiennes inférieures à un kilomètre à des mouvements annuels de plusieurs centaines de kilomètres selon l'espèce considérée (Sale 2002). Les migrations courtes consistent généralement en des agrégations formées quotidiennement sur des saisons de reproduction prolongées. Les migrations plus importantes sont généralement caractérisées par des agrégations mensuelles formées sur des saisons de reproduction courtes (Domeier & Colin 1997).

Ces liaisons fonctionnelles entre les habitats et leurs intensités sont essentielles pour le bon fonctionnement des écosystèmes fragmentés du paysage récifo-lagonaire. Identifier et quantifier les patrons de mouvements des juvéniles et des adultes est d'une importance cruciale pour la mise en place et l'efficacité des réseaux d'aires marines protégées (Grüss *et al.* 2011).

1.3. Implications de la connectivité

Le déclin des récifs coralliens à travers le monde appelle au développement de stratégies de protection de la résilience de ces écosystèmes (Pandolfi *et al.* 2003). Le mouvement est un phénomène critique pour la persistance des populations animales et la résilience des écosystèmes (Taylor *et al.* 1993) ; il est donc logique que la connectivité écologique soit considérée comme un objectif clé dans la conservation des écosystèmes et la gestion des ressources (Almany *et al.* 2009, Salas *et al.* 2010, Sale *et al.* 2010). L'idéal serait de pouvoir protéger l'ensemble des écosystèmes constitutifs du paysage récifo-lagonaire au sein des aires marines protégées (AMP), afin de maximiser la probabilité de capturer l'ensemble des connections existantes. Cette solution n'est généralement pas envisageable et l'élaboration de réseaux d'AMP nécessite, au préalable, une quantification de la connectivité écologique entre populations locales. En effet, les paramètres primordiaux intervenants dans le design des réseaux (taille, nombre, surface totale, espacement et emplacement des réserves) ne peuvent être décidés de façon adéquate sans une compréhension de la connectivité écologique existante. Bien qu'il soit impossible d'estimer la connectivité pour l'ensemble des espèces marines présentes, il est important de pouvoir l'estimer pour une gamme d'espèces aux traits d'histoires de vie, degrés de menace et d'exploitation différents ceci dans le but d'étendre les estimations obtenues à des espèces similaires à celles étudiées (Jones *et al.* 2007, McCook *et al.* 2009).

Le maintien de la résilience ne se fait pas uniquement en préservant la connectivité écologique, d'autres processus sont impliqués. L'identification et la protection des sites « sources » c'est-à-dire des sites producteurs nets d'émigrants (Jones *et al.* 2007, McCook *et al.* 2009) est essentielle, ainsi que l'identification et la protection des habitats essentiels pour les espèces présentant un cycle de vie complexe (migrations ontogéniques, de reproduction ou trophiques) (Sale *et al.* 2010). Beck (2001) indique qu'il est crucial d'inclure les habitats qualifiés de nurseries dans les réseaux d'AMP dans le but de protéger à la fois les juvéniles présents dans ces nurseries ainsi que les populations adultes renouvelées à travers le processus de recrutement de ces juvéniles.

Pour l'étude de la connectivité des populations, il est nécessaire d'identifier des échelles de temps pertinentes. Dans un objectif de conservation et de gestion des populations, l'étude de la connectivité à l'échelle écologique est plus pertinente qu'à l'échelle évolutive (Cowen *et al.* 2007).

2. Méthodes de mesure de la connectivité

L'estimation de la connectivité des populations peut être réalisée grâce à différentes méthodes : la génétique, les techniques de marquage et la microchimie des otolithes constituent des approches permettant l'étude de la dispersion et des mouvements des individus à différentes échelles spatiales et temporelles (Fig. 1.3). En effet, les méthodes de mesure de la connectivité changent selon la cible étudiée et surtout les échelles spatiales et temporelles choisies (Moilanen & Hanski 2006). Ainsi, l'estimation de la connectivité écologique ne peut se réaliser à l'aide des mêmes méthodes utilisées pour quantifier la connectivité évolutive (Fig. 1.3).

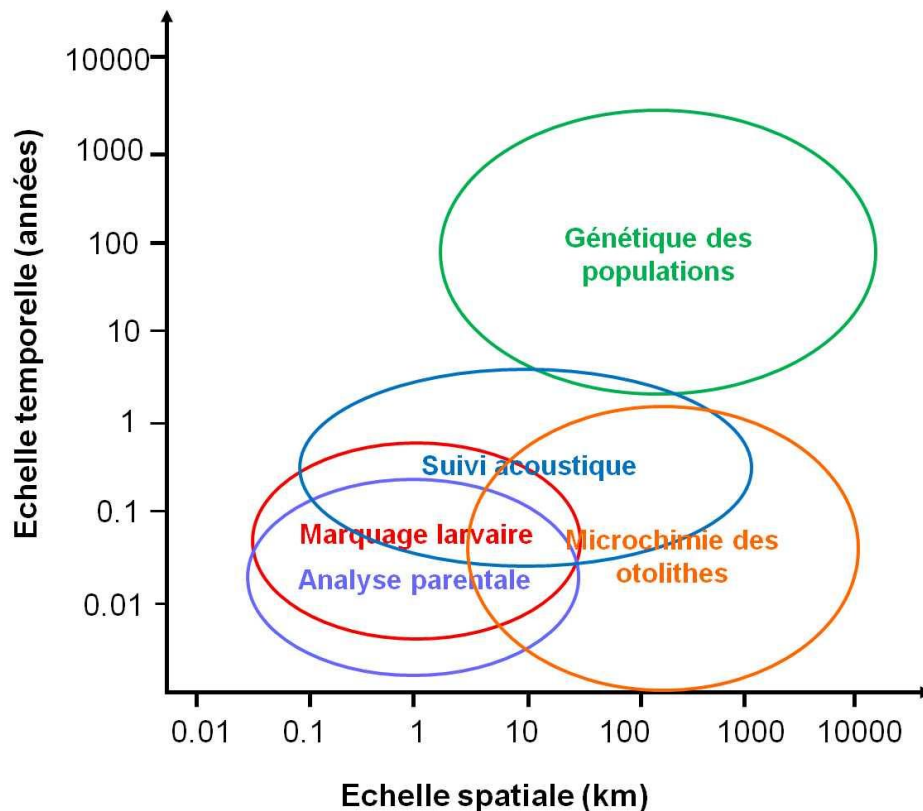


Figure 1.3 : Echelles temporelles et spatiales correspondant aux différentes approches de mesures de la dispersion des organismes récifaux-coralliens (modifiée de Jones *et al.* (2009)).

Les différentes approches permettant d'obtenir des informations sur la connectivité des populations nécessitent toutes l'emploi d'un marqueur. Ce dernier peut être d'origine naturelle ou artificielle (Levin 2006).

2.1. Marqueurs artificiels

Les techniques de marquage artificiel font appel à une large gamme de marqueurs différents, des marqueurs issus d'une technologie avancée (*e.g.* émetteurs satellites) à des techniques basiques (*e.g.* marqueurs externes colorés) (Hastein *et al.* 2001). Les études avec marqueurs externes simples nécessitent de suivre un protocole « capture-marquage-recapture » ne permettant pas de reconstruire en détails les mouvements effectués durant le laps de temps entre la capture et la recapture. De plus, ces expériences deviennent rapidement coûteuses car les taux de recapture sont en général très faibles (Hansen & Jacobsen 2003). Parmi les techniques de marquage artificiel, le marquage acoustique permet d'obtenir des données remarquables sur le détail des déplacements des individus et ce, même pour les espèces mobiles présentant des populations importantes (Kaunda-Arara & Rose 2004a, Chateau & Wantiez 2009, Claydon *et al.* 2012). Cependant le suivi acoustique actif quantifie le comportement de quelques individus sur des périodes de temps relativement courtes (plusieurs semaines). Le suivi acoustique passif est possible à long terme (jusqu'à 2 ans) mais ne permet pas d'étudier les déplacements des individus tout au long de leur vie (Meyer *et al.* 2010).

Les otolithes des poissons sont des structures calcifiées idéales pour les techniques de marquage chimique artificiel. Les composés fluorescents (*e.g.* tétracycline et calcéine) et l'enrichissement en isotopes stables (*e.g.* baryum) sont des techniques utilisées pour marquer les embryons ou les larves élevées en laboratoire avant la dispersion des sites natals (Jones *et al.* 1999, Thorrold *et al.* 2002, Thorrold *et al.* 2007). Le marquage transgénérationnel par enrichissement en isotopes stables est une méthode permettant un marquage en masse des larves de poissons. Le procédé consiste à exposer des femelles gravides à une solution enrichie en isotope stable d'un élément présent naturellement au sein des otolithes. Le marquage des otolithes des embryons se fait donc par transmission maternelle de l'élément dont le rapport isotopique a été modifié artificiellement. Les éléments utilisés sont le strontium ou le baryum via des solutions de chlorure de strontium ou de baryum (Thorrold *et al.* 2002, Thorrold *et al.* 2006, Almany *et al.* 2007). Cette méthode, en plein essor, s'est principalement focalisée sur la connectivité des populations à travers la dispersion larvaire.

2.2. Marqueurs naturels

Les difficultés associées aux techniques de marquage artificiel nécessitant une recapture des individus, ont conduit les chercheurs à s'intéresser aux marqueurs présents naturellement au sein des organismes.

Les méthodes génétiques indirectes (F_{is} , F_{st}) sont généralement inappropriées à l'estimation de la connectivité démographique car elles fournissent des informations sur la connectivité génétique, définie comme étant le degré auquel les flux de gènes affectent les processus évolutifs au sein des populations. Les méthodes génétiques seules apportent peu d'informations sur la connectivité démographique, définie comme le degré auquel la croissance de la population et les taux vitaux sont affectés par la dispersion (Hedgcock *et al.* 2007, Lowe & Allendorf 2010). Les méthodes de mesure basées sur des approches génétiques indirectes sont donc intégrées sur des échelles de temps multi-générationnelles, bien plus longues que pour les autres méthodes de mesure comme les empreintes élémentaires (Levin 2006).

Les méthodes génétiques directes ont émergées bien plus récemment. Ce sont des techniques puissantes pour l'étude de la connectivité à des échelles de temps inférieures aux méthodes génétiques indirectes. Ces méthodes directes se focalisent sur l'assignement des individus à leur population d'origine (Manel *et al.* 2005) ou à leur parent (Jones *et al.* 2005). Elles nécessitent l'établissement de marqueurs génétiques (*e.g.* microsatellites) pour l'espèce étudiée et l'identification de toutes les potentielles sources parentales. Cela représente un travail considérable qui n'est pas toujours logistiquement possible.

Aucune des méthodes précédentes ne permet de mesurer l'utilisation des habitats et la connectivité au cours d'une vie entière, de la naissance à la mort d'un poisson. En revanche, l'analyse de la microchimie des otolithes de poissons est une technique apportant un regard neuf sur les patrons continus de mouvements des poissons et permettant la mesure de la connectivité à des échelles de temps écologiques (Patterson & Kingsford 2005, Levin 2006, Cowen *et al.* 2007, Thorrold *et al.* 2007, Cowen & Sponaugle 2009).

3. Microchimie des otolithes

3.1. Généralités sur les otolithes

Les poissons Téléostéens possèdent, au sein de leurs oreilles internes, des structures extracellulaires minéralisées nommées otolithes. Les otolithes font partie du système sensoriel et sont impliqués dans les fonctions d'équilibration et de mécano-réception du poisson. L'oreille interne des poissons téléostéens est composée d'un ensemble de trois canaux semi-circulaires s'ouvrant à leur base sur une série de trois sacs otiques (Fig. 1.4). Ces trois sacs otiques sont remplis d'endolymphe dans lequel baigne un otolithe. Les sacs otiques sont le *sacculus* (sacculle), l'*utriculus* (utricule) et la *lagena* qui contiennent respectivement la *sagitta*, le *lapillus* et l'*asteriscus*. Les trois otolithes diffèrent par leur taille et leur forme, les *sagittae* étant préférentiellement étudiées car ce sont les plus volumineuses (Fig. 1.4).

Malgré des parties communes entre les espèces (Fig. 1.5), la forme des otolithes présente une importante variation interspécifique. Cette variation morphologique permet l'identification des espèces grâce à l'étude de la forme de leurs otolithes, ce qui se révèle être utile pour la détermination des régimes alimentaires des oiseaux marins ou des hommes de civilisations anciennes puisque les otolithes sont retrouvés dans les fouilles archéologiques (Disspain *et al.* 2011, Mercier *et al.* 2012).

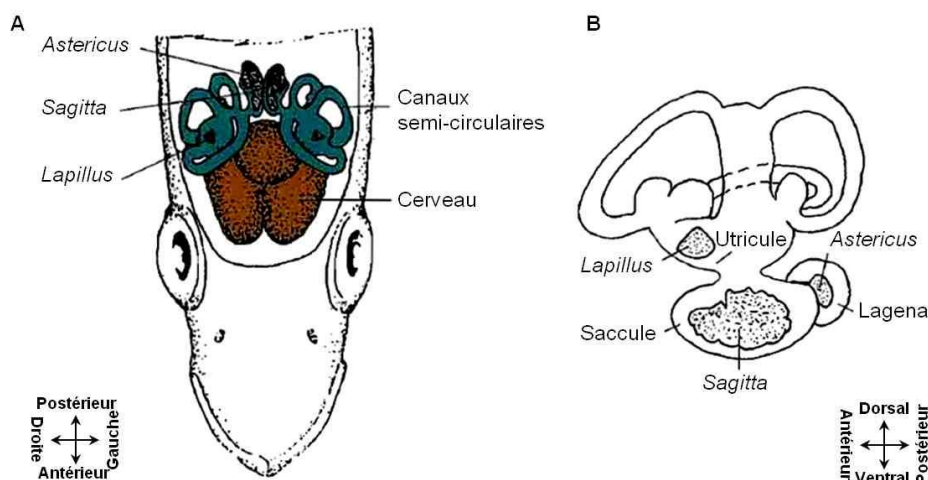


Figure 1.4 : Positionnement des otolithes au sein de l'oreille interne d'un téléostéen typique. (A) Vue dorsale de l'appareil vestibulaire ; (B) Position des otolithes à l'intérieur du système du labyrinthe (issue de Panfili *et al.* (2002)).

Les otolithes sont des formations composées principalement de carbonate de calcium (CaCO_3) sous la forme de cristaux d'aragonite précipités dans une matrice protéique (Campana 1999, Panfili *et al.* 2002). Ils s'accroissent par additions journalières de couches concentriques de CaCO_3 et de protéines (en majorité de l'otoline) à partir du noyau central (*primordium*) (Fig. 1.5). Ces dépôts successifs sont à l'origine de la formation de micro et macrostructures utilisées dans les études d'âge et de croissance des individus (Panfili *et al.* 2002). Les microstructures correspondent aux dépôts journaliers et forment une succession de bandes concentriques sombres et claires permettant d'estimer l'âge en jours des jeunes poissons (larves et juvéniles). Les macrostructures sont constituées d'une succession de zones claires et sombres correspondant aux différentes saisons vécues par les individus. Elles peuvent être interprétées en marques annuelles et permettent d'évaluer l'âge des poissons en années. De même, certains événements marquant de l'histoire de vie des poissons tels que la métamorphose ou la reproduction forment des structures particulières observables sur les otolithes (Campana & Neilson 1985).

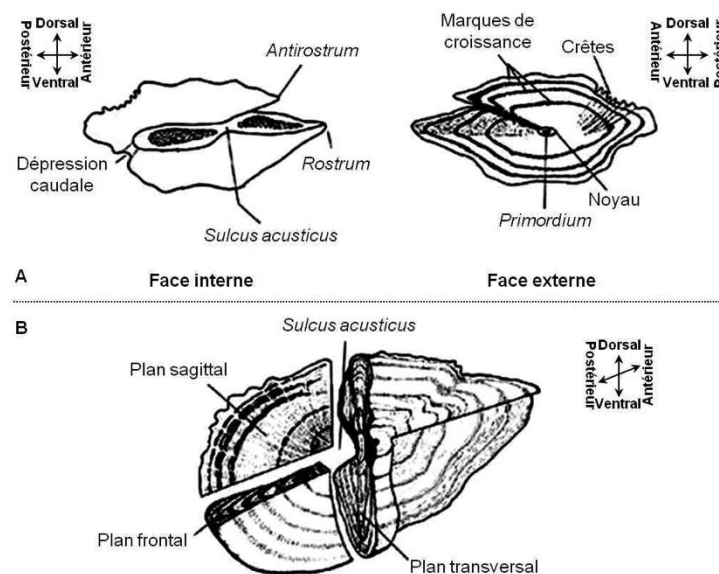


Figure 1.5 : Morphologie d'une *sagitta* typique, face interne et externe (A) et détails des trois plans d'orientations d'une *sagitta* typique (B) (issue de Panfili *et al.* (2002)).

Ces multiples caractéristiques des otolithes sont utilisés dans différents domaines : étude de l'âge et de la croissance (Choat & Robertson 2001, Choat *et al.* 2003, Labonne *et al.* 2008, Vigliola & Meekan 2009), études halieutiques (traits démographiques, dynamique et évaluation des stocks, aide à la gestion des pêches) (Clausen *et al.* 2007), étude des stades marquants de l'histoire de vie des individus (durée de phase larvaire, métamorphose,

recrutement, reproduction) (Lecomte-Finiger 1992, Searcy & Sponaugle 2000, Grandcourt *et al.* 2006) et études taxonomiques (morphométrie des otolithes) (Campana & Casselman 1993, Pothin *et al.* 2006, Barnes & Gillanders 2013).

Depuis le début des années quatre-vingt, la composition chimique des otolithes est de plus en plus étudiée notamment grâce aux progrès technologiques des appareils de mesures employés en chimie analytique et géochimie (*e.g.* ICP-MS solution, laser, etc.) (Campana & Neilson 1985) et pour toutes les perspectives qu'elle ouvre en terme d'évaluation de la connectivité entre populations.

3.2. Microchimie des otolithes

La microchimie des otolithes repose sur une hypothèse simple : à travers les dépôts journaliers, des éléments chimiques provenant de l'environnement dans lequel évolue le poisson sont incorporés dans les otolithes. L'eau environnante est considérée comme la source dominante des éléments. Jusqu'ici 50 éléments ont été détectés à différentes concentrations au sein des otolithes. Des éléments majeurs présents en fortes concentrations (Ca, C, O et N), des éléments mineurs (> 100 ppm : Cl, S, Mg, Na, P, Sr et K) et des éléments traces (< 100 ppm : Ag, Al, As, B, Bi, Ba, Br, Cd, Ce, Co, Cr, Cu, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Lu, Mn, Nd, Ni, Pb, Pr, Rb, Sc, Se, Si, Sm, Tb, Tm, U, V, Y, Yb et Zn) (Sturrock *et al.* 2012). L'incorporation des éléments se fait en plusieurs étapes car les otolithes ne sont pas en contact direct avec l'environnement extérieur. En effet, les éléments doivent franchir différentes barrières biologiques avant d'être incorporés : ils passent de l'eau environnante au plasma sanguin *via* les branchies ou les intestins, traversent les membranes de l'oreille interne vers l'endolymphe, pour finalement atteindre la surface de l'otolithe (Fig. 1.6). Les éléments sujets à de fortes régulations physiologiques (*e.g.* Na, K, S, P, Cl) sont d'un intérêt limité en tant que traceurs de l'environnement (Campana 1999, Sturrock *et al.* 2012). A l'inverse, les éléments qui ne sont pas soumis à de fortes régulations physiologiques présentent potentiellement une concentration dans les otolithes à l'image de leur concentration dans l'eau environnante.

La méthode de microchimie repose sur les deux propriétés clés des otolithes 1) une croissance continue au cours de la vie des poissons et 2) une nature métaboliquement inerte assurant l'inaltération de la composition chimique à travers le temps. Ces propriétés ont

permis de qualifier les otolithes d'enregistreurs permanent de l'environnement, archives de l'histoire de vie environnementale des poissons (Campana 1999).

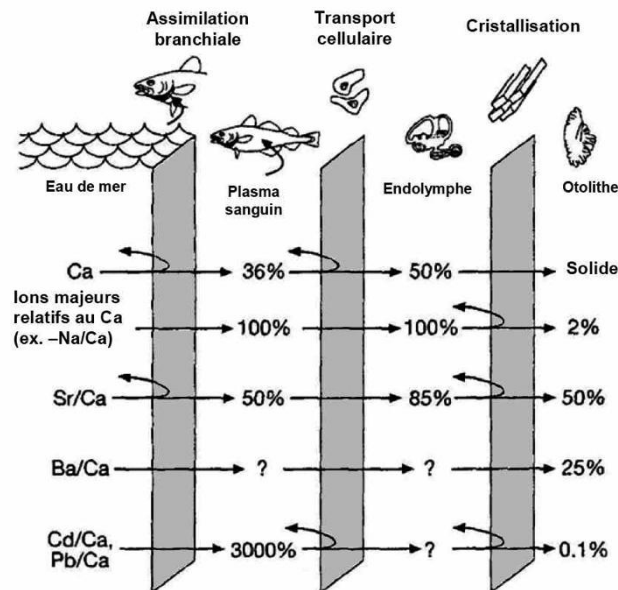


Figure 1.6 : Voies d'incorporation des éléments, barrières physiologiques entre l'eau et les otolithes et estimations des taux de transfert pour certains éléments à chaque barrière physiologique (issue de Campana (1999)).

La composition chimique des otolithes constitue un traceur puissant des masses d'eaux traversées par les poissons. Elle a permis au cours de plusieurs études de retracer l'histoire environnementale des individus (McCulloch *et al.* 2005) et de discriminer spatialement, et à différentes échelles, des populations ou groupes de poissons (Kingsford & Gillanders 2000, Gillanders *et al.* 2001, Rooker *et al.* 2003, Chittaro *et al.* 2004, Patterson *et al.* 2004). Cependant, certaines études n'ont pas pu mettre en évidence une variabilité spatiale suffisante des signatures élémentaires pour discriminer différentes zones géographiques (Chittaro *et al.* 2005, Patterson & Kingsford 2005). Cette absence de discrimination serait expliquée par un manque de variabilité spatiale au niveau de la chimie de l'environnement étudié. En effet, ces études ont été conduites dans des systèmes côtiers où les apports terrigènes limités entraînent une chimie de l'environnement homogène. L'hétérogénéité spatiale des signatures microchimiques et les concentrations élevées de certains éléments dans les otolithes seraient favorisées par des apports naturels d'éléments traces (*e.g.* géologie locale, apports fluviaux, etc.) ou par des apports liés aux activités anthropiques (pollution, rejet des eaux usées, exploitation minière, etc.) (Dove & Kingsford 1998, Morales-Nin *et al.* 2007).

4. Contexte en Nouvelle-Calédonie

Les lagons de Nouvelle-Calédonie se présentent comme un terrain particulièrement intéressant pour l'étude de la connectivité par microchimie. Inscrits au patrimoine mondial de l'UNESCO depuis 2008, ils constituent un des trois systèmes récifaux les plus importants au monde (Andréfouët *et al.* 2009). Ce paysage récifaux-lagonaire rassemble une grande diversité d'écosystèmes contrastés : mangroves, herbiers et algueraies, fonds meubles, récifs coralliens (Kulbicki & Rivaton 1997, Andréfouët & Torres-Pulliza 2004). Leur distribution, plus ou moins fragmentée, forme une mosaïque d'habitats abritant de multiples assemblages de poissons interconnectés (Parrish 1989, Wantiez 2008). De part la superficie et la morphologie de la Nouvelle-Calédonie et de ses lagons, les écosystèmes lagunaires sont sous influences terrigène et océanique contrastées à l'origine d'une microchimie de l'environnement hétérogène. Cette hétérogénéité est soupçonnée de se traduire par des gradients environnementaux marqués, notamment un gradient côte-large.

La Nouvelle-Calédonie présente également une géologie spécifique, extrêmement riche en différents minerais. Elle constitue la troisième réserve mondiale de nickel dont l'exploitation occupe une place majeure dans l'économie locale. D'autres minerais tels que le chrome, cobalt, fer, cuivre, plomb sont présents dans le sol calédonien (Ambatsian *et al.* 1997). Certains de ces gisements (nickel, chrome, cobalt et fer) sont associés aux massifs ultrabasiques (ou ultramafiques) couvrant environ le tiers de la superficie de la Grande Terre, principalement dans le sud et sur la côte ouest (Paris 1981, L'Huillier *et al.* 2010). Sur le territoire, les activités de prospection et d'exploitation de minerais ont commencé à la fin du 19^{ème} siècle et les aménagements du territoire associés (dévégétalisation, dragages, mines à ciel ouvert, etc.) constituent les principales sources de perturbations environnementales en Nouvelle-Calédonie (Labrosse *et al.* 2000, Fichez *et al.* 2008). Elles seraient susceptibles de venir enrichir les signatures microchimiques en éléments tels que le nickel, le chrome ou le cobalt. De ce fait, la microchimie de l'environnement lagonaire pourrait dévoiler des signatures multi-élémentaires spécifiques aux sites et habitats étudiés (Fernandez *et al.* 2006).

La morphologie du lagon, le contexte géologique singulier additionnés à des études antérieures prometteuses (Labonne *et al.* 2008, Sigura 2009), indiquent que l'outil microchimique aurait un potentiel informatif et discriminant élevé en Nouvelle-Calédonie.

5. Objectifs de la thèse

La thèse étant construite sous forme d'articles scientifiques, les matériel et méthodes sont détaillés au sein de chaque article/chapitre. Cependant, il est apparu indispensable de décrire la méthodologie générale en français avant d'aborder les différentes thématiques. Une fois la méthodologie générale exposée dans le **chapitre 2**, ce travail de recherche se divise en deux axes principaux que sont 1) le développement méthodologique de l'outil microchimique en Nouvelle-Calédonie et 2) son utilisation dans l'étude de la connectivité.

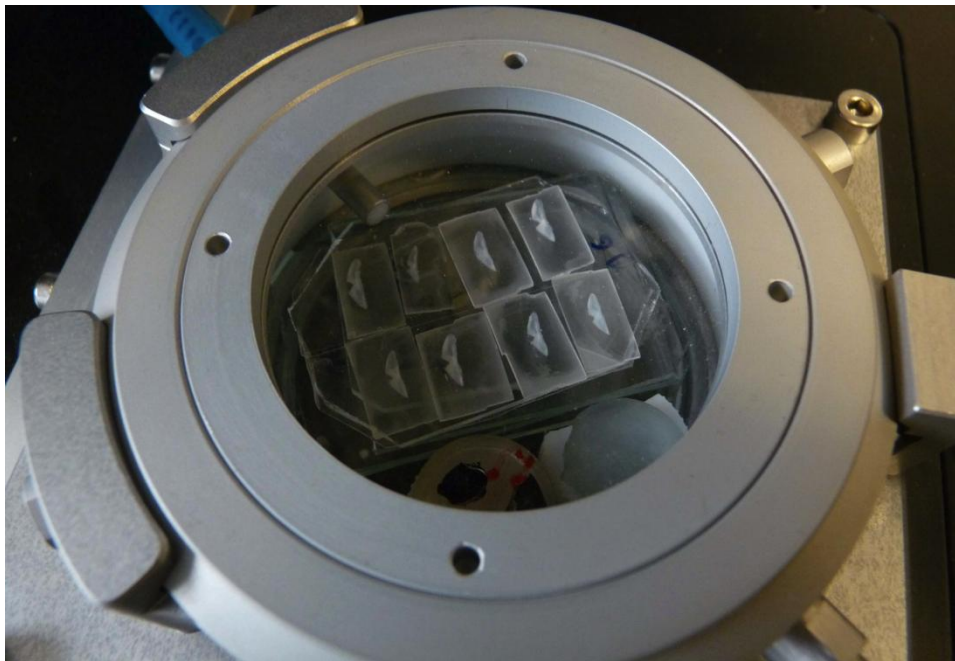
Le premier axe, constitué des chapitres 3 et 4, est composé d'un travail de détermination et de précision des signatures microchimiques des otolithes et de l'environnement. **Le chapitre 3** s'intéressera uniquement à la caractérisation de la microchimie des otolithes en répondant aux problématiques suivantes : **Quels éléments caractérisent les variations spatiales à l'échelle des sites et habitats en Nouvelle-Calédonie ? Quelle est la précision des signatures microchimiques mono-spécifiques et comment varie-t-elle en fonction de différentes espèces à traits de vie contrastés ? Quelle est la perte de précision lors de l'utilisation de signatures microchimiques multi-spécifiques comme proxy des signatures mono-spécifiques ?** Ce chapitre utilisera les données de microchimie issues de l'analyse du bord des otolithes traitées indépendamment pour plusieurs espèces cibles (signatures mono-spécifiques) et traitées en regroupant toutes les espèces indépendamment de leur identité ou en groupes taxonomiquement ou écologiquement proches (signatures multi-spécifiques).

Le chapitre 4, se penchera sur l'étude des relations entre la microchimie de l'environnement et des otolithes, en répondant aux questions suivantes : **Quels éléments issus de l'environnement caractérisent les variations spatiales à l'échelle des sites et habitats en Nouvelle-Calédonie ? Quelle est la précision des signatures chimiques issues de l'environnement et comment varie-t-elle en fonction du compartiment environnemental ? Quelles sont les relations entre la microchimie des otolithes de plusieurs espèces, celles de l'environnement et celles des paramètres physico-chimiques de la masse d'eau ?** Ce chapitre utilisera les données de température et de salinité de l'eau ainsi que les données de microchimie issues des analyses de l'eau de mer, des sédiments de surface et du bord des otolithes pour différentes espèces traitées indépendamment (signatures mono-spécifiques) et pour l'ensemble des espèces (signature multi-spécifique).

Le volet méthodologique permettra de poser les bases microchimiques pour le second axe : l'observation et la quantification de la connectivité entre différents habitats du lagon à travers l'étude de la daurade à tâche noire, *Lutjanus fulviflamma* (Forsskål, 1775). Cet axe est composé de deux chapitres. **Le chapitre 5** focalisera sur l'estimation du rôle des mangroves de Nouvelle-Calédonie comme sites juvéniles pour *L. fulviflamma* à travers les questions suivantes : **Quelle proportion d'individus adultes ont évolué au sein d'une mangrove en tant que juvénile ? La présence de mangrove est-elle un déterminant primordial à la distribution de l'espèce à l'échelle de l'archipel de la Nouvelle-Calédonie ?** Ce chapitre utilisera les données microchimiques du bord des otolithes de *L. fulviflamma* pour les mangroves et les récifs de l'ensemble de l'île en vue de construire les fonctions discriminantes de ces deux habitats. Une fois construites, les fonctions discriminantes seront appliquées à la partie des données microchimiques des sections transversales d'otolithes d'individus adultes correspondant à leur phase juvénile. Elles permettront de prédire les habitats utilisés au cours de la période juvénile et de définir le statut des mangroves calédoniennes comme des habitats juvéniles accessoires, essentiels ou obligatoires pour *L. fulviflamma*. A cela s'ajouteront des données de comptages sous-marins (UVC – Underwater Visual Census) et une cartographie des mangroves en Nouvelle-Calédonie qui permettra de mettre en relation la surface des mangroves avec la présence et l'abondance de *L. fulviflamma* dans les récifs adjacents.

Le chapitre 6 se focalisera sur la reconstruction de l'histoire environnementale des *L. fulviflamma* via les questions suivantes : **Quel est le laps de temps d'utilisation des mangroves par les juvéniles et varie-t-il selon les individus ou les sites ? Quelle transition est la plus fréquente entre les habitats juvéniles et adultes ? Quels sont les patterns de mouvement des adultes ?** Ce chapitre sera constitué des données microchimiques du bord des otolithes de *L. fulviflamma* pour les mangroves et les récifs frangeants, intermédiaires et barrières de quatre sites de la côte Ouest de l'île en vue de construire les fonctions discriminantes de ces habitats. Une fois les fonctions construites, elles seront utilisées pour prédire les habitats fréquentés au cours de la vie des individus via la classification des données des transects microchimiques réalisés sur les sections transversales d'otolithes de *L. fulviflamma* adultes.

Chapitre 2 : Méthodologie générale



Chapitre 2 : Méthodologie générale

1. Sites d'étude

1.1. La Nouvelle-Calédonie

La Nouvelle-Calédonie est une collectivité française d'outre mer située dans le Pacifique Sud-Ouest à 1500 km à l'est de l'Australie, entre les latitudes 19°S et 23°S et les longitudes 163°E et 168°E. L'archipel d'une superficie de 19 954 km² est constitué d'une île principale (Grande-Terre), de trois groupes d'îles (Loyautés, Ile des Pins et Bélep) et de quelques îles inhabitées (Entrecasteaux, Chesterfield, etc.). La Grande Terre présente une superficie de 17 000 km², allongée sur environ 400 km selon une direction nord-ouest/sud-est et d'une largeur de 60 km au maximum (Paris 1981, Andréfouët *et al.* 2009) (Fig. 2.1).

Le lagon de la Grande Terre est constitué d'un récif barrière de 1744 km de long entrecoupé de passes (Andréfouët & Torres-Pulliza 2004). La distance entre la Grande Terre et le récif barrière varie de 1 à 65 km. Cette barrière délimite un lagon dont la superficie s'élève à 31 336 km² et dont la profondeur atteint 40 m en certains endroits (Andréfouët *et al.* 2009). Depuis 2008, quatre zones du lagon de la Grande Terre sont inscrites sur la Liste du Patrimoine Mondial de l'Unesco (le Grand Lagon Sud, la Zone Côtière Ouest, la Zone Côtière Nord et Est et le Grand Lagon Nord).

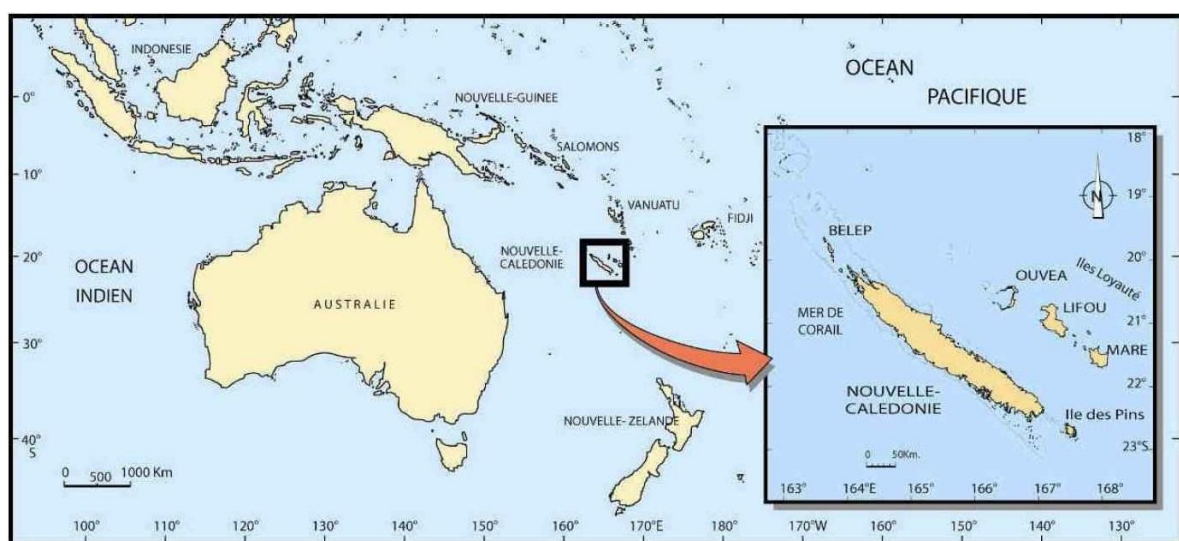


Figure 2.1 : Situation géographique de la Nouvelle-Calédonie dans le Pacifique Sud-Ouest.

1.2. Sites d'échantillonnages

Onze sites répartis autour de la Grande Terre ont été choisis en fonction de différents critères : le type et la présence de certains habitats, les activités minières voisines, l'existence d'études scientifiques antérieures, le statut et les projets de gestions (*e.g.* inscription au Patrimoine Mondial de l'Unesco ou classement en AMP) et l'accessibilité (Tab. 2.1 et Fig. 2.2). Pour les onze sites, l'échantillonnage est effectué dans deux habitats : la mangrove et le récif barrière interne.

Parmi ces onze sites, quatre sites ateliers situés sur la Côte Ouest de l'île ont été identifiés : deux sites potentiellement « impactés » par des projets miniers majeurs : les régions de Voh (Gatope) (projet Koniambo Nickel-SAS) et Prony (projet Vale Inco Nouvelle Calédonie) et deux sites « non impactés » : Ouano (Patrimoine mondial) et St Vincent. Dans ces quatre sites, quatre habitats sont échantillonnés selon un gradient côte-large: la mangrove, les récifs coralliens frangeants, intermédiaires et barrières internes.

Tableau 2.1: Les onze sites d'échantillonnages (* Sites ateliers; M : mangrove ; RF : récif frangeant ; RI : récif intermédiaire ; RB : récif barrière interne).

N°	Sites	Caractéristiques	n stations	Habitats
1	Nouméa	Usine pyrometallurgique de Doniambo (SLN : Société Le Nickel) Pollution urbaine	2	M / RB
2*	St Vincent	Site non-minier dans sa partie échantillonnée Etudes antérieures	4	M / RF / RI / RB
3*	Ouano	Site non-minier Inscription au Patrimoine Mondial de l'Unesco	4	M / RF / RI / RB
4	Népoui	Exploitation minière de Népoui-Kopéto (SLN)	2	M / RB
5*	Gatope	Projet minier Koniambo Nickel SAS (KNS) Développement de la zone urbaine Voh-Koné-Pouembout	4	M / RF / RI / RB
6	Golonne	Site non-minier	2	M / RB
7	Amos	Site non-minier	2	M / RB
8	Tchambouenne	Site non-minier Inscription au Patrimoine Mondial de l'Unesco	2	M / RB
9	Paama	Site non-minier Inscription au Patrimoine Mondial de l'Unesco	2	M / RB
10	Port Bouquet	Exploitation minière de Thio (SLN)	2	M / RB
11*	Prony	Projet minier Vale Inco Nouvelle-Calédonie Présences de cheminées hydrothermales	4	M / RF / RI / RB
Nombre total de stations			30	

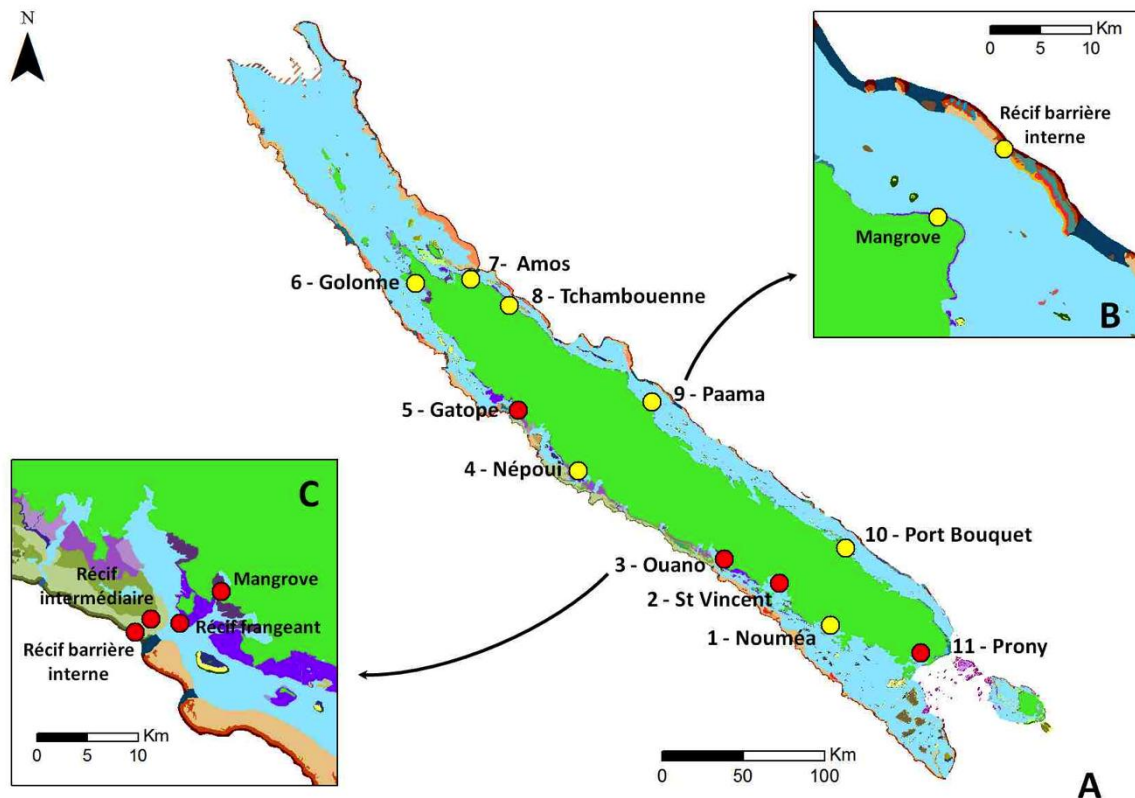


Figure 2.2 : (A) Sites d'étude : les cercles jaunes et rouges désignent respectivement les sites à 2 et 4 habitats échantillonnés. (B et C) Détails des habitats échantillonnés au sein des sites à 2 et 4 habitats échantillonnés. (Base de la carte : Millennium Coral Reef Mapping (Andréfouët & Torres-Pulliza 2004) ; réalisation : M. A. Hamel).

1.3. Description des sites ateliers

La baie de **Saint-Vincent** est située à 40 km au nord de Nouméa. D'une superficie de 172 km², c'est la baie la plus vaste de la côte ouest (Testau & Conand 1983) qui englobe de nombreux îlots (Fig. 2.3). Les arrivées d'eau douce proviennent de trois rivières : la Tontouta, la Tamoa et la Ouenghi. La zone de mangrove St Vincent/Ouenghi, définie comme un sous ensemble géographique par Thollot (1992b), couvre une surface égale à 2.6 km². Il existe deux exploitations minières en activité au niveau du bassin versant de la Tontouta : Tomo (SMGM) et Opoué (SLN/SMGM). Le point de chargement du minerai issu des deux sites miniers se situe au sein de la baie de Saint-Vincent, à l'embouchure de la Tontouta. Cependant, les sites échantillonnés étant éloignés du point de chargement, Saint-Vincent est considéré comme non impacté.

Le site de **Ouano** se situe au niveau de la baie de Chambeyron, à plus de 100 km au nord de Nouméa (Fig. 2.3). Ce site est inscrit au patrimoine mondial de l'Unesco, au sein de la Zone Côtière Ouest et présente des zones protégées juxtaposées à des aires non protégées. La surface de mangrove de la zone Chambeyron/Ouano est égale à 6.5 km².

La ville de Voh, située à 300 km de Nouméa au nord-ouest de la Grande Terre, est proche du site de **Gatope** (Fig. 2.3). Ce site se situe au niveau sud de la baie de Chasseloup, d'une superficie de 75 km² et au sein de laquelle il existe deux arrivées principales d'eau douce issues de deux bras d'une même rivière, la Témala (Testau & Conand 1983). Les premières extractions de minerai se situent sur le massif du Koniambo et ont commencé à la fin du 19^{ème} siècle. L'exploitation minière de Koniambo Nickel SAS, dont les travaux ont commencé en 2008, se situe au niveau de la baie de Vavouto, voisine de Chasseloup (Grenon & Simard 2012). Elle est constituée, entre autres, d'une mine à ciel ouvert, d'une centrale électrique et d'une zone portuaire (chenal d'accès, quai de déchargement, etc.). La production de l'usine a commencé en 2013 et devrait atteindre sa pleine activité en 2015.

La baie de **Prony**, située au sud de la Grande Terre, est une baie fermée d'une superficie totale de 49 km² (Testau & Conand 1983) (Fig. 2.3). Quatre rivières principales constituent les apports d'eaux douces : Rivière du Kaoris, Rivière Bleue, Rivière du Carénage et Rivière Kadji. C'est une baie qui présente une transition entre un environnement sous forte influence terrigène et un environnement océanique sur une courte distance, soupçonnée de présenter une microchimie de l'environnement singulière due à la présence de cheminées hydrothermales et à la présence du projet minier Vale Inco Nouvelle-Calédonie (anciennement Goro Nickel) (Bonvallot & Lardy 2012). Les premiers travaux sur le site ont commencé en 1998 et incluent, entre autres, la construction de l'usine, de la centrale thermique et du port. La mine est une exploitation à ciel ouvert en gradins. La production a progressivement démarré depuis 2010 mais, à l'heure actuelle, n'a pas atteint sa pleine capacité.

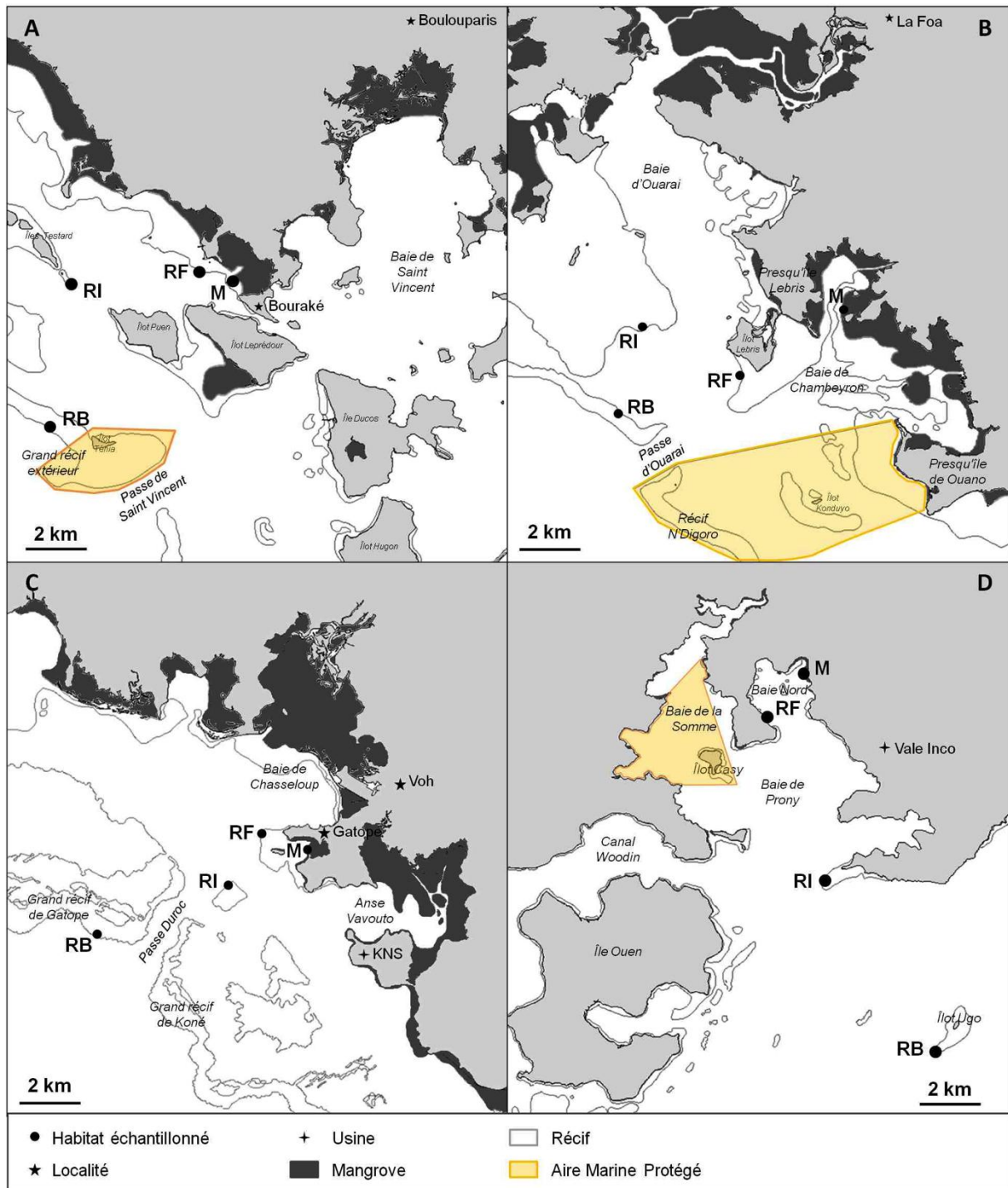


Figure 2.3 : Détails des quatre sites ateliers: (A) Saint Vincent, (B) Ouano, (C) Gatope et (D) Prony.

2. Plan d'échantillonnage

2.1. Campagnes d'échantillonnages

Trois campagnes d'échantillonnages ont été réalisées, une durant l'été austral (novembre à février 2009 ; Sigura 2009) et deux durant l'hiver austral (juin à septembre 2010 et 2011) afin d'intégrer de la variabilité saisonnière et interannuelle dans la caractérisation des signatures microchimiques. Lors de ces campagnes, les échantillonnages ne sont pas du même niveau et les objectifs attendus sont détaillés dans le tableau (Tab. 2.2).

Tableau 2.2 : Campagnes d'échantillonnages réalisées et objectifs scientifiques reliés.

Périodes	Sites	Prélèvements	Analyses	Chapitres	Objectifs
Eté 2009	2 sites	Toutes espèces (n = 44 esp)	Bords otolithes entiers	3, 4, 5, 6	Variabilité spatiale des signatures Elaboration des fonctions discriminantes
Hiver 2010	11 sites	Toutes espèces (n = 26 esp)	Bords otolithes entiers	3, 4, 5, 6	Variabilité spatiale des signatures Elaboration des fonctions discriminantes
			Transects microchimiques sur coupes transversales de <i>L. fulviflamma</i>	5, 6	Détermination de l'habitat juvénile Analyse détaillée des migrations
		Echantillons d'eau et de sédiment	Analyses microchimiques des échantillons d'eau et de sédiments	4	Corrélation microchimie environnement/otolithes
Hiver 2011	4 sites	Cibles (n = 4 esp)	Bords otolithes entiers	3, 4, 5, 6	Variabilité spatiale des signatures Elaboration des fonctions discriminantes

Deux niveaux d'échantillonnage sont réalisés suivant les axes de recherche de la thèse. Le premier niveau est composé d'un échantillonnage multi-spécifique large. Le second niveau est réduit à quatre espèces cibles, présentes dans le premier niveau.

2.2. Analyses multi-spécifiques

L'étude de la variabilité interspécifique des signatures microchimiques et la recherche des meilleures signatures multi-spécifiques requièrent un échantillonnage composé de plusieurs espèces. De même, la corrélation entre la microchimie de l'environnement et des otolithes étant susceptible de varier entre les espèces (Gillanders & Kingsford 2003, Swearer *et al.*

2003, Hamer & Jenkins 2007), son étude nécessite un échantillonnage multi-spécifique. Pour ces études, la microchimie à analyser est celle la plus récemment incorporée, révélée par l'analyse du bord de l'otolithe entier. Une seule analyse par otolithe (*i.e.* par individu) étant réalisée, le nombre d'individus et d'espèces inclus dans les analyses peut être élevé. Les échantillonnages multi-spécifiques larges ont été réalisés lors des deux premières campagnes (Été 2009 et Hiver 2010). Une liste d'espèces à collecter a été constituée sur la base de différents critères même si au final les aléas de la pêche ont défini les échantillons disponibles :

- Plusieurs espèces pour chacune des familles majeures (Acanthuridae, Chaetodontidae, Lutjanidae, Lethrinidae, Pomacentridae, Scaridae, Serranidae, Siganidae) afin de pouvoir former des groupes taxonomiques.
- Plusieurs espèces pour chacun des quatre groupes trophiques majeurs (piscivore, carnivore, herbivore-détritivore, planctonivore) définis selon la littérature (Kulbicki *et al.* 2005a, Kulbicki 2006, Chabanet *et al.* 2010, Guillemot *et al.* 2011) afin de pouvoir former des groupes fonctionnels (*i.e.* groupes trophiques).

Les regroupements taxonomique ou fonctionnel permettent de déterminer si des signatures multi-spécifiques regroupant des espèces proches (issues de la même famille ou ayant un régime alimentaire similaire) peuvent être utilisées comme de meilleurs proxy des signatures mono-spécifiques que des signatures multi-spécifiques globales, regroupant toutes les espèces analysées.

2.3. Espèces cibles

Parmi les espèces collectées lors des échantillonnages multi-spécifiques de 2009 et 2010, quatre espèces modèles ont été collectées à nouveau lors de l'échantillonnage hivernal 2011. Le choix s'est porté sur des espèces avec des traits de vie contrastés (niveau de mobilité, mode de reproduction, occupation des habitats, gamme de taille, etc.) détaillés ci-après.

Lutjanus fulviflamma (Forsskål, 1775) appartient à la famille des Lutjanidae (Fig. 2.4). Cette espèce présente une distribution étendue de l'Afrique de l'Est aux Samoa et du nord de l'Australie aux îles Ryukyu (Japon) (Carpenter & Niem 2001a, Allen *et al.* 2003). Les *L. fulviflamma* arborent une tâche noire caractéristique au niveau de la ligne latérale d'où la désignation commune en Nouvelle-Calédonie de « daurade à tâche noire » (Laboute & Grandperrin 2009). C'est une espèce carnivore dont le régime alimentaire est majoritairement

constitué de crustacés de toutes tailles et de poissons (Kulbicki *et al.* 2005a). D'après Loubens (1980), les *L. fulviflamma* présentent une période de reproduction comprise entre septembre et février en Nouvelle-Calédonie alors qu'ils sont caractérisés par une période de reproduction plus large (de août à mars) selon la FAO (Carpenter & Niem 2001a). Les individus adultes mesurent en moyenne 25 cm et la longueur totale maximale observée est de 35 cm (Carpenter & Niem 2001a). Cette espèce mobile évolue généralement en petits groupes constitués de 3 à 25 individus (Kulbicki 2006, Guillemot *et al.* 2011) cependant quelques individus peuvent être trouvés seuls, cachés au sein des anfractuosités des récifs. *L. fulviflamma* présente un cycle de vie complexe et il fréquente différents habitats au cours de son développement. Les adultes se trouvent sur les récifs coralliens à des profondeurs comprises entre 3 et 35m et peuvent aussi évoluer en eaux saumâtres, à la limite des eaux douces. En Nouvelle Calédonie, les juvéniles sont observés dans les zones côtières peu profondes telles que les mangroves (Thollot 1992a).

Ctenochaetus striatus (Quoy & Gaimard, 1825) appartient à la famille des Acanthuridae (Fig. 2.4). C'est une espèce abondante dont la distribution s'étend sur tout l'Indo-Pacifique (Allen *et al.* 2003). Elle est herbivore et détritivore, se nourrissant à la fois du film algal présent à la surface des substrats et de détritits (Carpenter & Niem 2001b, Laboute & Grandperrin 2009). C'est une espèce grégaire vivant en groupe de taille moyenne (25 à 50 individus) (Guillemot *et al.* 2011). *C. striatus* est une espèce ubiquiste récifale, habitante de récifs coralliens des lagons protégés aux zones exposés. Elle est qualifiée de sédentaire avec un home range restreint à une centaine de m², mais est soupçonnée être plus mobile (Claydon *et al.* 2012). Elle ne présente pas d'intérêt commercial en Nouvelle-Calédonie car elle est responsable de graves intoxications ciguatériques (Laboute & Grandperrin 2009). *C. striatus* est une des espèces les plus abondantes du lagon de Nouvelle-Calédonie.

Dascyllus aruanus (Linnaeus, 1758) appartient à la famille des Pomacentridae (Fig. 2.4). Elle présente un régime alimentaire de type planctonivore et évolue en groupe de taille moyenne (25 à 50 individus) (Kulbicki 2006, Guillemot *et al.* 2011). C'est une espèce présente dans l'ensemble du lagon qui est sédentaire, inféodée à des coraux branchus (type *Acropora* et *Pocillopora*) (Carpenter & Niem 2001a, Allen *et al.* 2003). C'est une espèce à œufs benthiques c'est-à-dire que lors de la ponte les œufs ne sont pas relâchés dans la masse d'eau mais collés au corail. Contrairement aux deux espèces précédentes, *D. aruanus* est une

espèce territoriale dont le home range se limite à quelques mètres carrés qu'elle défend contre les intrus (Sale 1971, Guillemot *et al.* 2011). Elle constitue le candidat idéal pour le rôle d'espèce contrôle car, pour un lieu donné, les variations de la microchimie des otolithes ne seront pas induites par le mouvement des individus mais uniquement par les variations temporelles de la microchimie des eaux environnantes.

Siganus lineatus (Valenciennes, 1835) appartient à la famille des Siganidae et est présent dans l'Indo-Pacifique Ouest (Fig. 2.4). C'est une espèce macro-herbivore, très mobile à l'âge adulte, grégaire se déplaçant en groupes composés de nombreux individus (> 50) (Guillemot *et al.* 2011). Les juvéniles évoluent au sein d'habitats côtiers peu profonds tels que les mangroves et herbiers alors que les adultes fréquentent les récifs coralliens et substrats rocheux peu profonds des eaux protégées lagunaires. Les individus adultes mesurent en moyenne 25 cm et la longueur totale maximale observée est d'environ 45 cm (Carpenter & Niem 2001b).

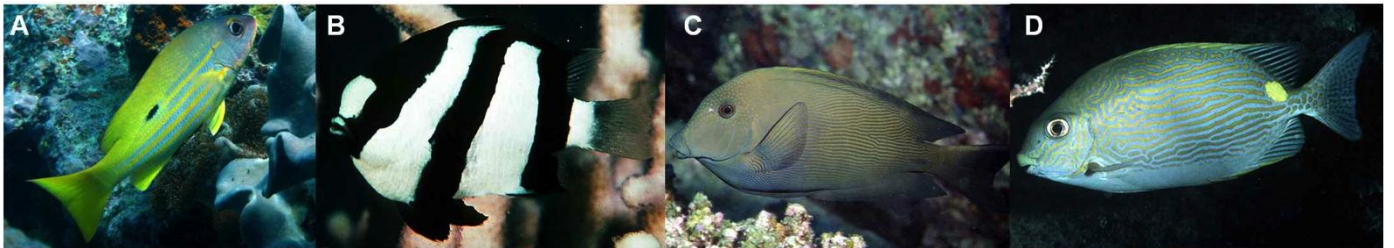


Figure 2. 4 : Photo des espèces modèles : (A) *Lutjanus fulvivittatus*, (B) *Dascyllus aruanus*, (C) *Ctenochaetus striatus* et (D) *Siganus lineatus* (Crédits photos Paillon, C. et Randall, J.E.).

3. Techniques de prélèvement

3.1. Mangroves

La méthode de prélèvement consiste à poser, à marée haute, un ensemble de filets maillants d'une longueur totale de 250 m le long des palétuviers et de récupérer les individus à marée basse (Fig. 2.5). Afin d'obtenir des espèces de tailles diverses ainsi que différents stades ontogéniques (juvénile et adultes), l'ensemble est composé de six filets de tailles de maille différentes : quatre filets de 50 m de longueur, d'1,5 m de chute et de 45 mm de taille de maille carrée auxquels s'ajoute deux filets de 25 m de long, d'1,5 m de chute et de 25 mm

de taille de maille carrée. Afin d'échantillonner les juvéniles dissimulés entre les racines de palétuviers, un pied isolé est entouré à marée haute de deux filets de 25 m de long, 1,5 m de chute et 7 mm de taille de maille. Une substance épaisse issue d'un mélange d'1 kg de poudre de roténone et d'eau de mer est versée à marée basse au niveau de la zone entourée. La roténone est une molécule toxique provoquant un empoisonnement des poissons présents dans la zone ciblée. Un laps de temps d'environ 15 minutes est nécessaire avant d'observer les premiers poissons remonter à la surface. Tous les individus empoisonnés sont récoltés à l'aide d'épuisettes.

3.2. Récifs

Les prélèvements sont réalisés en chasse sous marine, principalement au fusil harpon. Pour les poissons de petite taille (e.g. Chaetodontidae et Pomacentridae), la collecte est réalisée à l'aide de fléchettes fines propulsées par un élastique. *Dascyllus aruanus* est récupéré grâce à l'utilisation d'un mélange d'eugénol et d'alcool (Fig. 2.5). L'eugénol est un composé anesthésiant provoquant, à dose adéquate, un étourdissement des individus et permettant de les récupérer facilement à l'épuisette (Soto & Burhanuddin 1995, Munday & Wilson 1997).

Sur place, les échantillons récoltés sont stockés dans une glacière avant d'être congelés en chambre froide (-20°C) au laboratoire.



Figure 2.5 : Illustration des méthodes de prélèvements ; (A) au niveau des mangroves à l'aide de filets maillants et (B) au niveau des récifs au fusil harpon et à l'eugénol.

3.3. Echantillons de l'environnement

Lors de la campagne hivernale de 2010, trois prélèvements d'eau ont été réalisés sur chaque site et pour chaque habitat. En mangrove, les échantillons sont prélevés à marée basse, au niveau de la couche d'eau de surface. Pour les récifs, les prélèvements sont aussi effectués au niveau de la couche de surface, sans tenir compte de la marée. Chaque échantillon est prélevé avec une seringue de 40ml équipée d'un filtre à membrane en polyestersulfone (membrane PES de porosité 0,45µm) et uniquement 10ml de l'échantillon filtré est conservé pour analyse dans des tubes en polypropylène de 15 ml. Les échantillons sont ensuite fixés par ajout de 0,2 ml d'HNO₃ Suprapur® à 2% afin d'éviter le développement des organismes vivants présents dans l'eau. Ils sont conservés au laboratoire, à l'abri de la lumière.

Durant la même campagne, trois prélèvements d'1kg de sédiments de surface sont réalisés à chaque station. En mangrove, les prélèvements sont réalisés à marée basse et espacés de 10 mètres. Au niveau des récifs, ils sont effectués en apnée, sans tenir compte de la marée et également espacés de 10 mètres. Les échantillons sont stockés dans une glacière sur le terrain puis congelés dans une chambre froide (-20°C) du laboratoire.

Les paramètres environnementaux, température et salinité, sont également mesurés à trois reprises au niveau de la couche d'eau de surface pour chaque habitat échantillonné. La température est mesurée grâce à une sonde thermomètre Testo® d'une précision de 0,1°C. Un réfractomètre automatique Atago® d'une précision de 0.1‰ est utilisé pour les mesures de salinité.

4. Protocole de préparation des échantillons

L'ensemble de l'étude se déroule en suivant des précautions de non contamination métallique nécessaires pour les analyses de microchimie. Pour les extractions des otolithes, des pinces céramiques sont utilisées et la totalité du matériel employé est préalablement nettoyé à l'acide nitrique (HNO₃, 2%), rincé à l'eau ultra-pure et séché sous hotte à flux laminaire en conditions HEPA 100. De même, le matériel est rincé à l'eau ultra-pure avant et après dissection de chaque individu.

Les individus collectés sont mesurés au mm près selon la longueur à la fourche (LF) puis sont pesés au mg près. Les otolithes sont ensuite extraits puis plongés dans deux bains

consécutifs d'eau ultra-pure afin d'éliminer les éventuels tissus résiduels. Ils sont ensuite placés à sécher sous une hotte à flux laminaire (conditions HEPA 100), et conservés par paire dans des microtubes labellisés.

4.1. Otolithes *in toto*

Les analyses du bord des otolithes permettent de révéler la microchimie la plus récemment incorporée au sein des otolithes, caractéristique du lieu d'échantillonnage. Ces analyses permettent la détermination des signatures mono et multi-spécifiques, l'élaboration des fonctions discriminantes des sites et habitats, et l'étude de la relation microchimie otolithe/environnement.

Les otolithes entiers sont nettoyés en conditions ultra-propres suivant la procédure de Warner *et al* (2005) afin d'éliminer les contaminants de surface et les résidus de matière organique (Fig. 2.6). Lors de cette procédure, les otolithes sont individuellement déposés dans les puits d'une plaque en plastique. Un premier bain d'une heure est effectué dans une solution 50/50 d'eau oxygénée (qualité Suprapur® (H₂O₂, 30%)) et de soude (NaOH, 0,1 mol.L⁻¹). Durant les 5 dernières minutes, la plaque est déposée dans une cuve à ultrasons. Les otolithes sont ensuite rincés cinq fois cinq minutes dans des bains successifs d'eau ultra-pure. Ils sont ensuite placés à sécher sous une hotte à flux laminaire (conditions HEPA 100), et conservés jusqu'à analyses ICPMS dans des microtubes labellisés et préalablement décontaminés à l'acide.

4.2. Coupes transversales

Des coupes transversales d'otolithes sont préparées de manière à découvrir l'ensemble de la vie des poissons, du noyau jusqu'au bord de l'otolithe, puis sont montées en lames minces. La coupe transversale ne peut être réalisée directement et les otolithes doivent être inclus dans des blocs de résine (Fig. 2.6). La résine utilisée est une résine Araldite® 2020, composée d'une résine et d'un agent durcisseur mélangés en proportions égales (100g : 30g). Préalablement aux inclusions des otolithes, une petite quantité de résine est répartie dans les puits d'un moule en silicone. Ceci permet de réaliser les fonds qui sont laissés à sécher durant 12h au minimum. Ensuite, les otolithes sont déposés individuellement, face convexe sur le fond, et recouverts de résine. L'ensemble des blocs est placé sous une hotte pendant quelques heures avant d'être placé dans une étuve à 50°C durant 24h.

Une fois les blocs de résine secs, des sections transversales fines contenant le noyau de l'otolithe sont réalisées à l'aide d'une scie circulaire diamantée (Buehler®). La coupe fine réalisée est collée avec de la résine adhésive Crystalbond 509® sur une cale en verre, elle-même fixée sur une lame porte-objet (Secor 1992). La section est polie à l'aide de disques de polissage successifs aux grains de plus en plus fins (de 900 à 1200 grains/cm²). Le polissage s'affine avec des films abrasifs (de 9, 3 puis 1 µm) et deux solutions diamantées (1 et 0,3 µm) jusqu'à obtenir une section plane passant par le noyau de l'otolithe. Pour finir, les coupes sont nettoyées à l'eau ultra-pure, séchées sous la hotte à flux laminaire (HEPA 100) et conservées dans des boîtes plastiques.

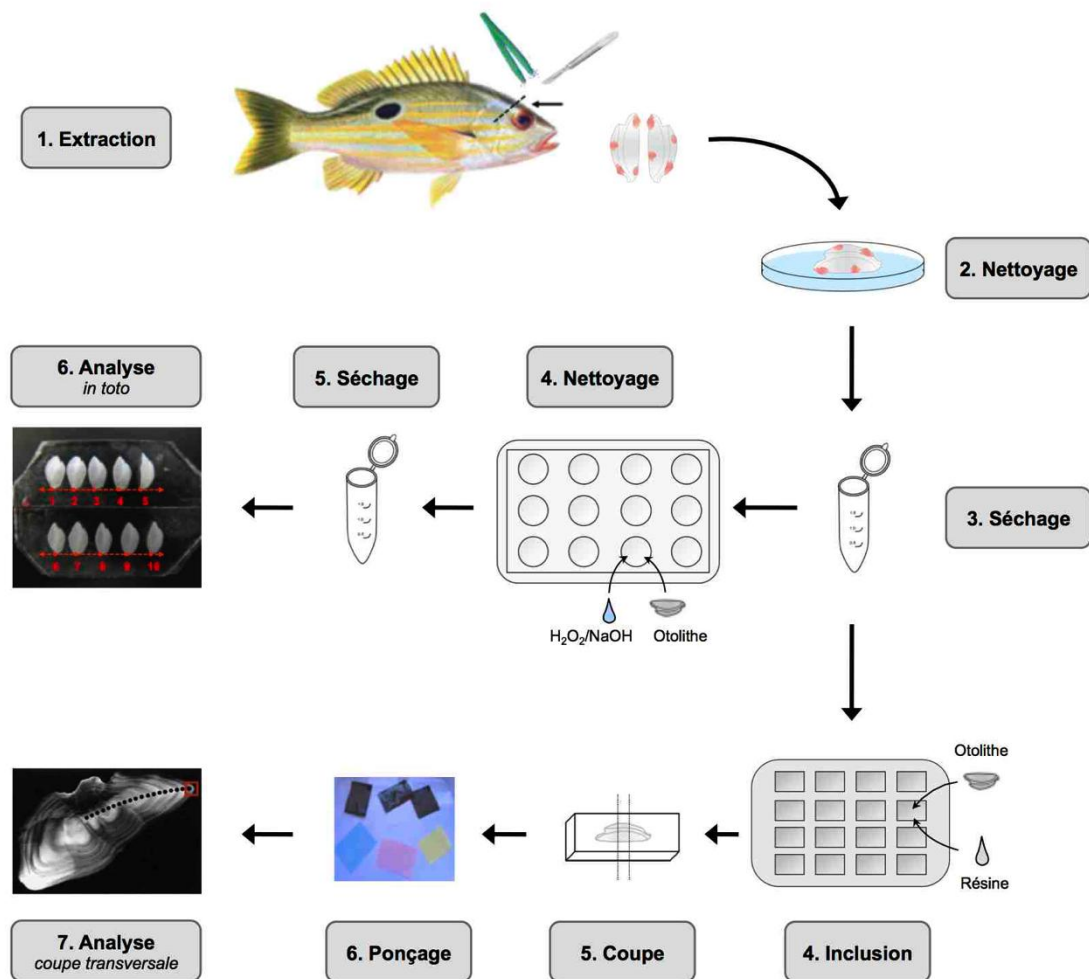


Figure 2.6 : Protocole de préparation des otolithes pour analyses *in toto* et analyses en transects.

4.3. Echantillons de sédiment

Le protocole de préparation des échantillons de sédiment suit le protocole de Totland *et al.* (1992). L'objectif est de récupérer la fraction fine ($< 63\mu\text{m}$) des sédiments car c'est au niveau de cette fraction granulométrique que la majorité des éléments sont biodisponibles.

Une fois décongelés, les échantillons sont tamisés sur une maille de $63\mu\text{m}$ à l'aide d'eau ultra-pure afin de récupérer uniquement la fraction fine du sédiment. La fraction fine et l'eau sont récupérées dans un bécher puis placées dans une étuve à 100°C jusqu'à complète évaporation de l'eau. Une fois sèche, la fraction fine est broyée au mortier en agate, conservée dans un sachet plastique propre et placée dans un endroit sec, à l'abri de la lumière.

5. Analyses microchimiques

5.1. Principes de fonctionnement de l'ICP-MS

Les signatures microchimiques sont caractérisées par méthode de spectrométrie de masse grâce à un spectromètre de masse à source plasma haute résolution ou ICP-MS (Inductively Coupled Plasma Mass Spectrometer) (Fig. 2.7). Cet appareil permet de déterminer la composition en éléments traces d'échantillons liquides en mode solution (SB- ICP-MS) ou solides en mode laser (LA- ICP-MS). L'analyse des échantillons par ICP-MS se divise en quatre étapes :

- Phase d'introduction et de nébulisation.
- Phase d'ionisation.
- Phase de séparation en masse et charge.
- Phase de détection.

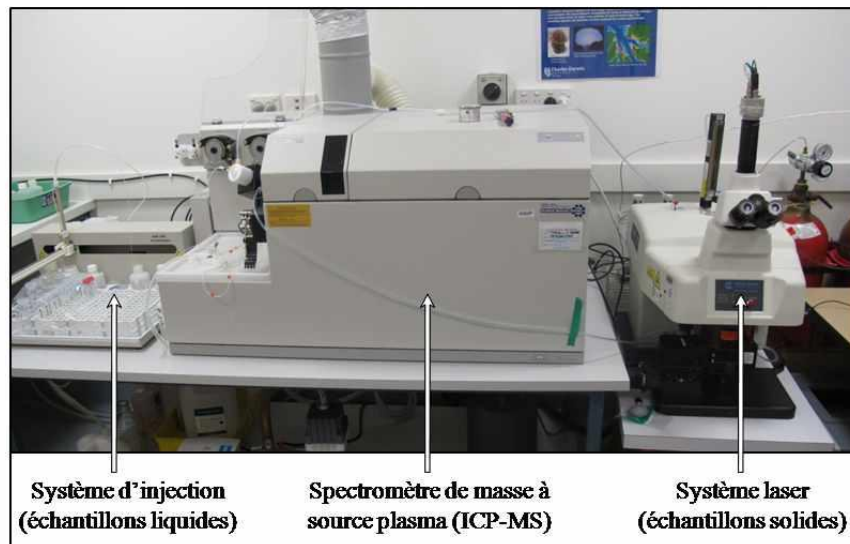


Figure 2.7 : Photo d'un système LA-ICP-MS, Environmental Analytical Chemistry Unit, Research Institute for the Environment and Livelihoods, Charles Darwin University.

La première phase dépend du type d'échantillons. Les échantillons liquides sont injectés dans le système par une aiguille de prélèvement automatique en téflon et entraînés à l'aide d'une pompe péristaltique jusqu'au nébuliseur qui permet de faire passer l'échantillon à l'état d'aérosol liquide (microgouttelettes de quelques μm). Pour les échantillons solides, l'appareil est couplé à un système d'ablation laser qui dégage les éléments traces en provoquant une ablation à la surface des otolithes. Ces éléments traces sous forme d'aérosols, sont véhiculés grâce à un courant d'argon et d'hélium. A partir de la phase d'ionisation, le principe de l'appareil est le même pour les deux types d'échantillons. Les aérosols passent au niveau d'une torche à plasma d'argon et sont vaporisés, dissociés, atomisés et ionisés sous l'effet d'une température élevée (entre 5000 à 8000°C). Pour la phase de séparation, une lentille ionique conduit les ions vers le spectromètre de masse quadripolaire où ils sont séparés selon leur charge (z) et leur masse (m). Seuls les ions sélectionnés au préalable sont transmis au détecteur dans lequel ils percutent un multiplicateur d'électrons à dynodes. Pour chaque ion qui percute la paroi des dynodes, ces dernières émettent des électrons. La série de dynodes de l'appareil va provoquer un effet « boule de neige » et les électrons émis heurtent au final un collecteur équipé d'un préamplificateur. C'est la phase de détection où les électrons émis sont dénombrés par le collecteur. Le signal se traduit en nombre de coups par seconde (nombre d'impulsions) et est directement proportionnel à la concentration de l'élément étudié dans l'échantillon. Pour finir, une interface informatique assure le transfert des données afin qu'elles soient traitées.

Les résultats en coups par seconde doivent être convertis en concentrations, exprimées en ppm (*i.e.* partie par millions) correspondant à un rapport de 10^{-6} ($\mu\text{g.g}^{-1}$). Les concentrations sont calculées à l'aide d'un standard interne dont la concentration dans l'échantillon est connue. Pour les échantillons solides carbonatés comme les otolithes, le standard interne utilisé est le calcium alors que pour les échantillons liquides, le standard interne est une solution d'Indium/Bismuth. De plus, pour les échantillons solides, un standard externe (NIST 612) est utilisé au début et à la fin de chaque procédure pour obtenir des courbes de calibration et palier la dérive temporelle de l'appareil. Pour les sédiments en solution, deux standards externes sont analysés en fin de procédure (UBR et BEN).

5.2. Choix des éléments analysés

Outre le Sr, le Ba et le Mn, des éléments tels que le Fe, Pb, Li, Mg, Cu et Ni sont susceptibles de servir de marqueurs caractéristiques de l'environnement (Campana 2005). Afin d'augmenter l'efficacité discriminatoire entre les sites, le maximum d'éléments possible est retenu pour les analyses d'où la notion d'« empreinte multi-élémentaire ». L'ICP-MS va permettre de déterminer la composition en éléments suivants : Li, B, Rb, Sr, Mo, Cd, Sn, Ba, Pb, Th, U, Mg, Ca, Ti, V, Cr, Mn, Ni, Cu, Zn via la mesure des isotopes suivants : ^7Li , ^{11}B , ^{85}Rb , ^{88}Sr , ^{95}Mo , ^{111}Cd , ^{117}Sn , ^{138}Ba , ^{208}Pb , ^{232}Th , ^{238}U , ^{25}Mg , ^{43}Ca , ^{44}Ca , ^{47}Ti , ^{51}V , ^{52}Cr , ^{55}Mn , ^{60}Ni , ^{65}Cu , ^{66}Zn .

5.3. Analyses chimiques des otolithes

5.3.1. Analyses laser sur otolithes *in toto*

Contrairement à la technique de dissolution des otolithes expérimentée dans plusieurs études de comparaisons interspécifiques (Gillanders *et al.* 2003, Swearer *et al.* 2003), la technique de l'ICP-MS couplé à un laser (LA-ICP-MS) permet de s'affranchir d'une différence d'incorporation des éléments due à une fréquentation d'environnements distincts au cours de la vie des espèces étudiées. La technique du laser permet de faire une ablation du bord de l'otolithe et donc une analyse des éléments les plus récemment incorporés au sein de l'otolithe alors que la dissolution analyse l'ensemble de la vie. De ce fait, la microchimie du bord des otolithes devrait refléter plus précisément l'exposition des poissons aux conditions environnementales du lieu de capture des individus et de la collecte des échantillons d'eau et de sédiment. Les analyses sont toujours réalisées au même site sur l'otolithe entier c'est-à-dire

au niveau du post-rostre (Fig. 2.8). Cette précaution est importante afin de s'assurer que les différences observées ne soient pas dues à des variations dans le site d'analyse (Hamer & Jenkins 2007). Une face d'une lame porte-objet est recouverte de scotch transparent double face sur lequel les otolithes sont collés. Ils sont agencés de manière à ce que les post-rostres soient alignés horizontalement. Cette démarche permet de simplifier la recherche du site d'analyse une fois la lame installée dans la chambre du laser. L'analyse par laser ICP-MS se constitue d'un tir laser sur le bord postérieur des otolithes (Fig. 2.8). La procédure d'analyse consiste en 30 secondes de blanc (laser non actionné) suivie de 2 minutes d'acquisition avec un faisceau laser de 90 μm de diamètre, 5 Hz de fréquence et 15 joules.cm^{-2} de puissance.

En moyenne, 80 ablations laser peuvent être réalisées par journée d'ICP-MS, soit 80 otolithes (ou individus) analysés par jour.

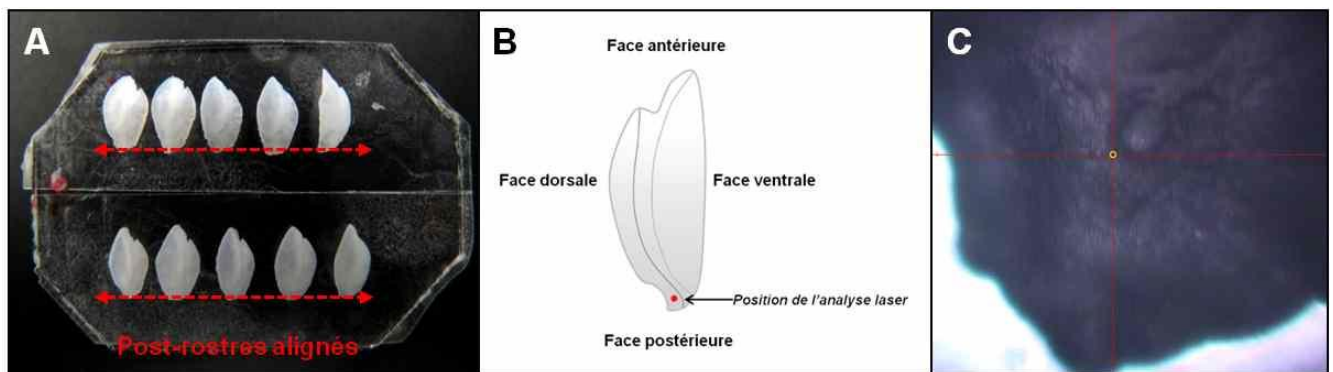


Figure 2.8 : Illustration de la méthode d'analyse *in toto*. (A) montage des otolithes entiers sur lame et (B et C) position de l'analyse laser sur otolithe *in toto*.

5.3.2. Analyses laser en transects

Cette méthode permet de reconstruire l'histoire de vie environnementale des poissons. Les otolithes destinés aux analyses laser par transects sont préparés en section transversale fine comme détaillée précédemment. Les transects se présentent sous la forme d'une succession d'ablation laser d'un diamètre de 90 μm tout les 120 μm , du noyau vers le bord de l'otolithe le long de l'axe principal de croissance (Fig. 2.9). La procédure d'analyse consiste en 30 secondes de blanc (laser non actionné) suivie de 2 minutes d'acquisition avec un faisceau laser de 90 μm de diamètre, 5 Hz de fréquence et 15 joules.cm^{-2} de puissance. Le nombre de transects réalisé en une journée d'analyse dépend de la taille de l'otolithe (i.e. distance noyau-bord). En moyenne, 3 transects constitués de 25 ablations chacun peuvent être effectués par jour (75 ablations par jour).

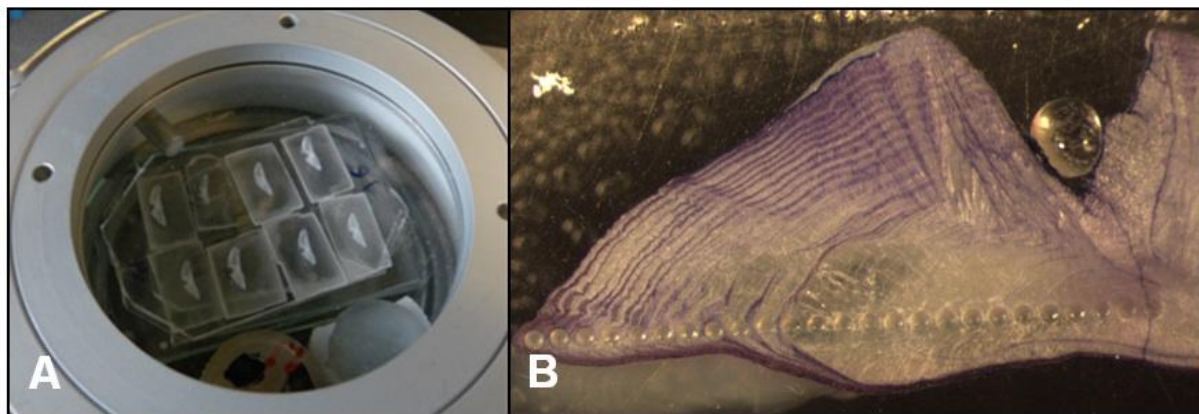


Figure 2.9 : Illustration de la méthode d'analyse en transects. (A) Coupes transversales positionnées dans la chambre du LA-ICP-MS et (B) transect sur une coupe transversale d'otolithe colorée au bleu de Toluidine pour ageage.

5.4. Analyses chimiques de l'environnement

Les échantillons d'eau et de sédiment sont analysés par ICP-MS en mode solution (SB-ICP-MS) et les éléments traces dosés sont les mêmes que pour les otolithes.

Avant analyse, les échantillons d'eau sont dilués par 40 afin d'obtenir une solution de salinité proche de 1‰ dans un milieu matriciel de 2,5% de HNO₃ contenant 1,0% d'Indium, standard interne qui permet de corriger la dérive de l'ICP-MS. Pour cela, 0,25 ml de l'échantillon d'eau de mer est dilué dans 9,4 ml d'H₂O ultrapure, 0,25 ml d'HNO₃ et 1 ml d'Indium.

Le passage à l'ICP-MS des sédiments nécessite une mise en solution. Plusieurs attaques acides successives sont réalisées dans des béchers en Téflon® sur 100mg de portion fine pour chaque échantillon (Fig. 2.10). Les acides utilisés sont de grade Suprapur®, l'eau utilisée est ultrapure et la totalité du protocole de Totland *et al.* (1992) se réalise sous hotte aspirante. La procédure consiste à deux phases de digestion acide forte à l'aide d'HNO₃ (69%) et d'HF (40%). Ensuite, deux phases de digestion acide modérées à l'HNO₃ (69%) uniquement. Une reprise est effectuée et diluée avec de l'H₂O. Un aliquote d'1/10 ml est réalisé. La solution finale de sédiment est diluée par 1000. Pour les analyses, la solution introduite dans l'appareil est constituée de 0,25 ml de la solution finale diluée avec 9,4 ml d'H₂O, 0,25 ml d' HNO₃ et 1 ml d'Indium.

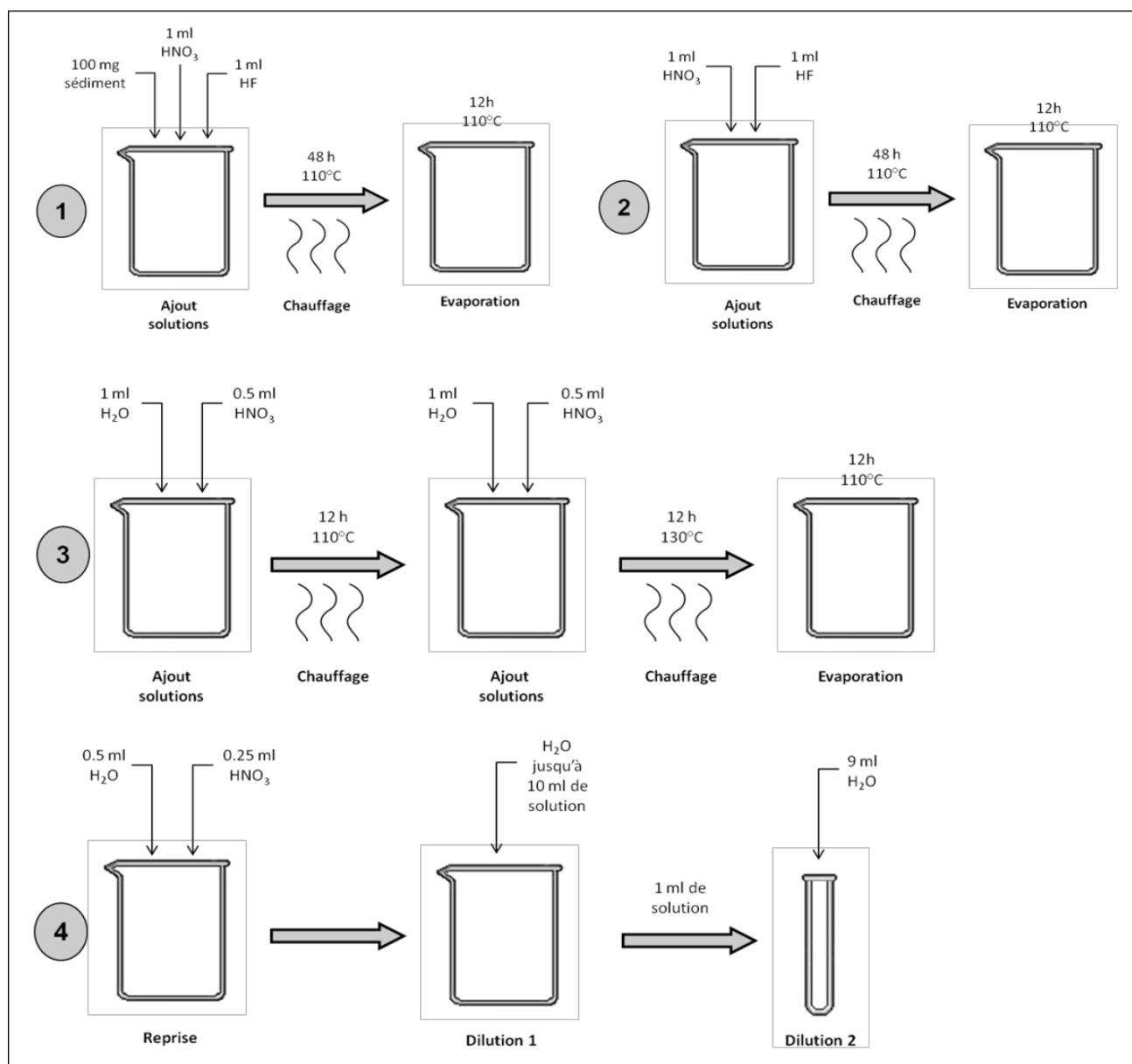


Figure 2.10 : Protocole de mise en solution des sédiments par acidification.

6. Traitement des données

6.1. Transformation des données microchimiques

Les données brutes issues de l'analyse ICP-MS sont exprimées en coups par seconde (cps) et doivent être transformées en concentrations exprimées en parties par millions (ppm). La méthode utilisée est celle de Longerich *et al.* (1996) et a été réalisée à l'aide d'un script élaboré sous le logiciel statistique R (R Development Core Team 2011). Pour chaque élément :

- Les données aberrantes (outliers ou données extrêmes), définies comme toutes valeurs supérieures à trois fois l'écart interquartile, ont été exclues (Tukey 1977). C'est une procédure classique en microchimie car les ICP-MS sont des appareils très sensibles qui produisent régulièrement des valeurs ponctuelles aberrantes appelées « spikes ».
- Les concentrations et les limites de détection (LOD) ont été calculées suivant la méthode de Longerich *et al.* (1996).
- Les éléments conservés pour les analyses statistiques ont été sélectionnés suivant deux critères : 1) les concentrations élémentaires dans les otolithes doivent être supérieure à la LOD dans 70% des analyses dans au moins un des habitats (ex. la mangrove) ou un des sites (ex. Ouano). 2) le coefficient de variation des concentrations mesurées au sein des standards externes (NIST 612) doit être inférieur à 10% pour chaque élément (Chittaro *et al.* 2004, Chittaro *et al.* 2006). Les quelques valeurs inférieures à la LOD et conservées dans les analyses ont été fixées à zéro.
- Dans le but de réduire les possibles variations causées par l'utilisation de deux ICP-MS différents, les concentrations élémentaires ont été standardisées par ligne (*i.e.* par échantillon) afin d'obtenir des compositions élémentaires relatives (en pourcentages de chaque élément dans l'échantillon).

6.2. Analyses statistiques

Les analyses statistiques utilisées sont adaptées à des données ne suivant pas les hypothèses de normalité et d'homoscédasticité, ainsi qu'à des plans d'échantillonnage non équilibrés. Toutes les analyses statistiques ont été réalisées à l'aide du logiciel R (R Development Core Team 2011).

6.2.1. Analyses descriptives

Des analyses de variances (ANOVA) à un et deux facteurs sont utilisées afin de tester les différences spatiales de compositions élémentaires (analyses multivariées MANOVA) et de concentrations élémentaires (analyses uni-variées ANOVA). Les ANOVA classiques exigent de respecter des conditions d'utilisation telles que la normalité des résidus et l'homogénéité des variances. Si les données ne répondent pas à ces postulats de base, elles sont en général transformées afin de ramener leur distribution à la normalité et d'homogénéiser la variation. A l'inverse, les méthodes d'ANOVA par permutations (PERMANOVA) permettent de se libérer de ces conditions d'utilisations. Elles construisent leur propre loi de distribution en utilisant

des permutations aléatoires du jeu de données (Legendre & Anderson 1999, Anderson 2001). La technique des PERMANOVA a été utilisée dans ce travail.

6.2.2. Discrimination spatiale

La méthode des Random Forest (forêts aléatoires en français) a été utilisée afin de discriminer les échantillons à différentes échelles spatiales à partir des compositions élémentaires des otolithes et de l'environnement. Il existe différentes méthodes de classification/prédiction utilisées dans les études de microchimie des otolithes : les Réseaux de Neurones (ANN) et les Analyses Discriminantes Linéaires (LDA) et Quadratiques (QDA). Dans une étude comparative, Mercier *et al.* (2011) ont cependant démontré que les Random Forest (RF) constituent la méthode statistique la plus adaptée aux données de microchimie des otolithes. Elle présente l'avantage de se libérer des conditions de normalité et d'homoscédasticité requises avec les autres méthodes statistiques. La pertinence de l'utilisation des RF a ensuite été démontrée au cours d'études de connectivité utilisant des jeux de données de microchimie des otolithes provenant des lagunes de la région du Golfe du Lion (Mercier *et al.* 2012, Tournois *et al.* 2013).

La méthode des Random Forest (RF) est basée sur la construction d'arbres de décision multiples. Chaque arbre de décision est construit à partir du tirage aléatoire d'un sous ensemble du jeu de données initial (classiquement les deux tiers), appelé « jeu de calibration ». Un arbre est constitué de nœuds correspondant à un partitionnement des observations en fonction de la valeur des variables. Pour les RF, la procédure de partitionnement aux nœuds est partiellement aléatoire. À chaque nœud, il y a un sous échantillonnage aléatoire des variables utilisées (ici les éléments chimiques) pour scinder les observations (ici les habitats ou les sites). Le tiers du jeu de données initial qui n'est pas utilisé pour la construction de l'arbre de décision est appelé « jeu test » car il est utilisé pour estimer la capacité de prédiction de cet arbre indépendamment du jeu de calibration utilisé pour le construire. Ainsi, la classe (*i.e.* habitat ou site) de chaque observation du jeu test est connue (*i.e.* lieu d'échantillonnage) et comparée à la classe prédite par l'arbre de classification compte tenu de la valeur des variables pour chaque observation. Ceci permet d'obtenir une valeur indépendante du taux d'erreur de l'arbre de classification des observations. Il y a donc deux procédures de tirage aléatoire dans les RF : chaque arbre est construit à partir d'un jeu aléatoire de calibration, et chaque nœud est calculé à partir d'un tirage aléatoire des variables

de partitionnement. Cette procédure est réalisée de manière itérative afin de construire une forêt d'arbres, le nombre d'arbres réalisés étant défini par l'utilisateur (dans ce travail, 5000 arbres sont construits à chaque RF). Cette construction d'arbres multiples permet de prédire la classe de chaque observation du jeu de données initial à plusieurs reprises, chaque arbre constituant un vote pour une classe donnée. La prédiction finale d'une observation correspond à la classe qui reçoit la majorité des votes sur le total des arbres de la forêt. De manière importante, le pourcentage de vote de cette prédiction est connu et constitue la probabilité associée à cette prédiction finale. Ainsi, les RF non seulement classifient les observations en fonction de la valeur des variables mais associent une probabilité (% vote) à chaque prédiction.

6.2.3. Echelles spatiales et organisationnelles

La méthode des RF a été utilisée afin de discriminer les échantillons à trois échelles spatiales à partir des compositions élémentaires des otolithes et de l'environnement. Dans un premier temps, les discriminations ont été réalisées à l'échelle **globale**. Cette échelle est constituée des données provenant des 11 sites autour de la Nouvelle-Calédonie et les différences ont été testées entre :

- Habitats (mangrove versus récif barrière interne).
- Sites pour un habitat donné (mangroves, récifs barrières internes).

Ensuite, les discriminations ont été réalisées à l'échelle **régionale**. Cette échelle comprend les données issues des 4 sites ateliers de la côte ouest et les différences ont été testées entre:

- Habitats (mangrove vs récifs frangeant, intermédiaire et barrière interne).
- Sites pour un habitat donné (mangroves, récifs frangeants, récifs intermédiaires, récifs barrières internes).

Enfin, les discriminations ont été réalisées à l'échelle **locale** définie comme l'échelle spatiale la plus fine. Cette échelle correspond à chaque site atelier et les différences ont été testées:

- Entre habitats (mangrove vs récifs frangeants, intermédiaires et barrière interne).

Ces analyses à différentes échelles spatiales ont été réalisées pour les niveaux organisationnels suivants :

- L'espèce.
- Multi-spécifique (toute espèce).
- Groupe taxonomique (famille).
- Groupe fonctionnel (régime alimentaire).

Lors de la construction d'arbres de classification, certains éléments chimiques sont informatifs car ils apportent de l'information utile à la classification, et d'autres éléments n'apportent que du bruit. L'élimination de ces éléments non-informatifs permet d'augmenter la précision des classifications tout en réduisant le nombre d'éléments constituant les signatures chimiques (Mercier *et al.* 2011) . A chaque échelle spatiale et organisationnelle testée, les RF ont donc été réalisées avec toutes les combinaisons d'éléments possibles. La meilleure combinaison d'éléments retenue est celle qui correspond au meilleur taux de classification correcte avec le plus petit nombre d'éléments.

Chapitre 3 : Quantification du pouvoir discriminant des signatures multi-élémentaires mono-spécifiques et multi-spécifiques dans le lagon de Nouvelle-Calédonie



Chapitre 3 : Quantification du pouvoir discriminant des signatures multi-élémentaires mono-spécifiques et multi-spécifiques dans le lagon de Nouvelle-Calédonie

Dans un milieu oligotrophe tels que les récifs coralliens, la discrimination spatiale des habitats par microchimie des otolithes est réputée limitée par l'absence de contraste environnemental et donc par une chimie de l'environnement relativement homogène (Patterson *et al.* 2004, Berumen *et al.* 2010).

En Nouvelle-Calédonie, la microchimie des otolithes a été étudiée à deux reprises et les résultats issus de ces premières études se sont avérés très prometteurs (Labonne *et al.* 2008, Sigura 2009). Il semblerait notamment que le potentiel informatif et discriminant de l'outil microchimique soit élevé sur le territoire. L'étendue géographique de la Nouvelle-Calédonie, sa spécificité géologique, la superficie de son lagon et l'hétérogénéité des habitats qu'il abrite seraient donc susceptibles d'induire des gradients environnementaux marqués. Ces gradients pourraient ensuite se traduire dans les otolithes de poisson par des signatures microchimiques caractéristiques des environnements traversés. Si tel est le cas, ces signatures pourraient être utilisées pour étudier la connectivité entre les différents environnements et habitats du lagon calédonien. Néanmoins, avant leur utilisation, il est nécessaire de démontrer l'existence et de caractériser le pouvoir discriminant de telles signatures en Nouvelle-Calédonie.

Ce chapitre s'insère dans l'axe méthodologique de la thèse et a pour objectif de caractériser les signatures microchimiques dans les otolithes de poisson en Nouvelle-Calédonie. Il s'agira notamment de :

- 1) Caractériser les variations spatiales de la microchimie des otolithes à l'échelle des sites et habitats de la Nouvelle-Calédonie.
- 2) Evaluer la précision des signatures microchimiques mono-spécifiques pour différentes espèces présentant des traits de vie contrastés.
- 3) Etudier la possible utilisation des signatures microchimiques multi-spécifiques comme proxy des signatures mono-spécifiques.

Ce travail de caractérisation de la microchimie des otolithes est primordial pour la suite de la thèse, et notamment pour le développement de l'axe écologique. Il fournit la base de la compréhension du pouvoir microchimique existant en Nouvelle-Calédonie et sera complété par le chapitre méthodologique suivant, les liens entre la microchimie de l'environnement et des otolithes.

Manuscrit A: Assessing the power of otoliths multi-elemental signatures in the coral reefs and mangroves of New Caledonia

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Abstract

Otoliths microchemistry is the only method available to reconstruct lifetime movements of fish and lifetime connectivity among habitats at individual level. Prior to use microchemistry as a tool to study connectivity, elemental fingerprints need to be characterized and their accuracy evaluated. The objectives of the present study were (1) to characterize spatial variations of elemental fingerprints in New Caledonia, (2) to assess the spatial scale at which correct site and habitat classifiers could be built using elemental fingerprints of numerous species, and (3) to evaluate the accuracy of multi-specific elemental classifiers as a proxy of mono-specific classifiers according to taxonomic or ecological similarity among species. Using Random Forest classification method, we found high correct classification rates at habitat level, specifically between mangrove (86.4%) and barrier reef (98.8%) for *Lutjanus fulviflamma*. Correct classification rates at the station level were lower than at the habitat level, except among mangrove stations (between 63 and 100% of correct classification).

Discriminations were enhanced by the chemical heterogeneity of coastal habitats and the chemical contrast between distant habitats. Use of multi-specific classifiers as substitutes of mono-specific classifiers was generally not relevant, even when multi-specific signatures were built with ecological or functional related species. However, when habitats were much contrasted, as it is the case for mangroves and barrier reefs, then multi-specific signatures appeared very relevant. Our results imply that studies estimating fish connectivity using otolith microchemistry require a sampling of all species concerned unless chemical contrast among habitats or stations is very high.

Key words: multi-element, LA-ICP-MS, otoliths, Random Forest, multi-specific

1. Introduction

Tropical seascapes harbor a high diversity of fish assemblages spread over multiple ecosystems such as mangroves, seagrasses and coral reefs. Actual increasing anthropogenic perturbations weaken these coastal ecosystems by threatening existing connectivity between local populations of fish within tropical seascapes. Therefore, identifying and quantifying connectivity in tropical seascapes has become a priority of conservation management (Almany *et al.* 2009, Sale *et al.* 2010). However, connectivity between fish populations remains a not fully understood ecological process, principally due to a challenging acquisition of empirical data of fish lifetime movements. Among methods for determining fish movements, otoliths microchemistry is the most promising to quantify connectivity by reconstructing lifetime movements (Fairclough *et al.* 2011, Mercier *et al.* 2012), identifying natal origins (Swearer *et al.* 2003, Standish *et al.* 2008), putative nurseries (Brown 2006b, Mateo *et al.* 2010) and habitat utilization (Forrester & Swearer 2002) of fish stocks.

Otoliths are paired calcareous structures located in the inner ear of all bony fishes, implicated in equilibrium and sound reception. They grow continuously by daily accretions of calcium carbonate (CaCO_3) originating from the surrounding waters. These deposits permit the incorporation of trace elements also originating from the surrounding waters. Otoliths being metabolically inert, the trace elements incorporated are never reworked or resorbed (Campana 1999). These key-properties of otoliths qualify them as natural recorders of water masses crossed by the fish during his lifetime. Microchemistry method consists in measuring the elemental composition of otoliths which, if it reflects the physicochemical properties of the surrounding water at a given time and area, will define an elemental fingerprint. Then, the chemical fingerprint can be used as a natural tag of a geographic location (Bath *et al.* 2000, Elsdon & Gillanders 2003a, Sturrock *et al.* 2012).

Prior to use otoliths microchemistry as a quantifier of actual connectivity, there is a need to define elemental signatures of species for a maximum of sites and to obtain a high level of discrimination between them (Hamer & Jenkins 2007). For a single species, spatial variations of elemental signatures could either allow good discrimination among study sites (Gillanders & Kingsford 2000, Swearer *et al.* 2003) or could not revealed significant differences (Patterson *et al.* 2004, Berumen *et al.* 2010). Benefit or loss of discrimination between locations seems to be related to the number of trace elements measured. Besides main trace

elements like Sr, Ba, Mg and Mn classically used in otolith microchemistry studies, Campana (2005) suggested that others elements such as Fe, Pb, Li, Cu, Ni, Al, Co, Zn, Ag, Cd, and Sn could also be effective markers of environment.

Unlike other coastal habitats with high terrigenous inputs (Gillanders & Kingsford 2000, Forrester & Swearer 2002, Brown 2006a, Cuveliers *et al.* 2010, Tournois *et al.* 2013), coral reefs are oligotroph systems with a limited variation of environmental chemistry of water masses (Patterson *et al.* 2004, Chittaro *et al.* 2006). Consequently, studying spatial variability of chemical fingerprints based exclusively on a limited number of trace elements is generally insufficient to discriminate different sites (Berumen *et al.* 2010). For example, Patterson and Kingsford (2004) studied spatial variability using Sr, Ba and Mn on the Australian Great barrier reef. Discrimination was possible at a large spatial scale (several hundred of km) but not at a smaller scale (ten km). They concluded that the coral reefs of the Great Barrier Reef could not be considered as different microchemistry units because of the low terrigenous influence leading to a lack of variation of environmental parameters. On the opposite, studying multi-elemental signatures provided a higher degree of discrimination (Chittaro *et al.* 2004, Labonne *et al.* 2008).

In this respect, New Caledonia lagoon is of special interest because it constitutes a tropical seascape including coral reefs close to coast and under high terrigenous influence, originating from numerous freshwater and anthropogenic inputs (Baille *et al.* 2012). Moreover, New Caledonia presents a mining history with past and actual exploitations of Ni, Co and Cr (Paris 1981). This specificity may generate heterogeneity in the environmental chemistry, related to the distance offshore, the habitat and the site (Labonne *et al.* 2008, Andréfouët *et al.* 2009).

Mono-specific elemental fingerprint can be difficult to obtain because of logistic constraints or conservation issues depending on the studied species. In this case, a multi-specific elemental fingerprint will be easier to obtain. However, heterogeneity of trace elements incorporation among species is not a well-understood process. Consequently, the use of multi-specific signature as a proxy should not be considered at random and should be carefully evaluated. Study of inter-specific variation of elemental signature is a way to adjust the accuracy of multi-specific signatures when selecting species by taxonomic or functional

similarity. Several studies focused on inter-specific variation of otoliths fingerprints (Gillanders & Kingsford 2003, Chittaro *et al.* 2006, Hamer & Jenkins 2007). Comparisons between elemental signatures of different species generally highlighted significant differences. However, similar patterns in spatial variations between species could be observed and revealed that elemental signatures were more similar between taxonomically closed species than distant ones (Swearer *et al.* 2003). Nevertheless, these taxonomically closed species have to share ecological traits such as diet and/or habitat.

The aims of this study were: (1) to describe the spatial variations of elemental compositions of otoliths in New Caledonia and the relative contribution of elements; (2) to assess the spatial scale at which elemental fingerprints can build correct classifiers for several species; (3) to evaluate the accuracy of multi-specific elemental classifiers as a proxy of mono-specific classifiers according to taxonomic or ecological similarity.

2. Material and methods

2.1. Sampling protocol

New Caledonia is a French archipelago located in the South West Pacific, 1500 km east of Australia (Fig. 3.1). The archipelago is composed of a main island and several groups of smaller islands. The main island is around 17 000 km², 400 km long and 60 km wide at its maximum. The main island is surrounded by the largest lagoon of the world (31 336 km²), delimited by a barrier reef of 1744 km long, the second longest in the world after the GBR (Andréfouët *et al.* 2009). The distance between the coast and the barrier reef varies from 1 to 65 km (Paris 1981) and some parts of the lagoon are registered on the Unesco heritage list since 2008. New Caledonia lagoon gathers several types of ecosystems constituting a tropical seascape such as mangroves, seagrasses, algal beds, bare soft bottoms and coral reefs (fringing, intermediate and barrier).

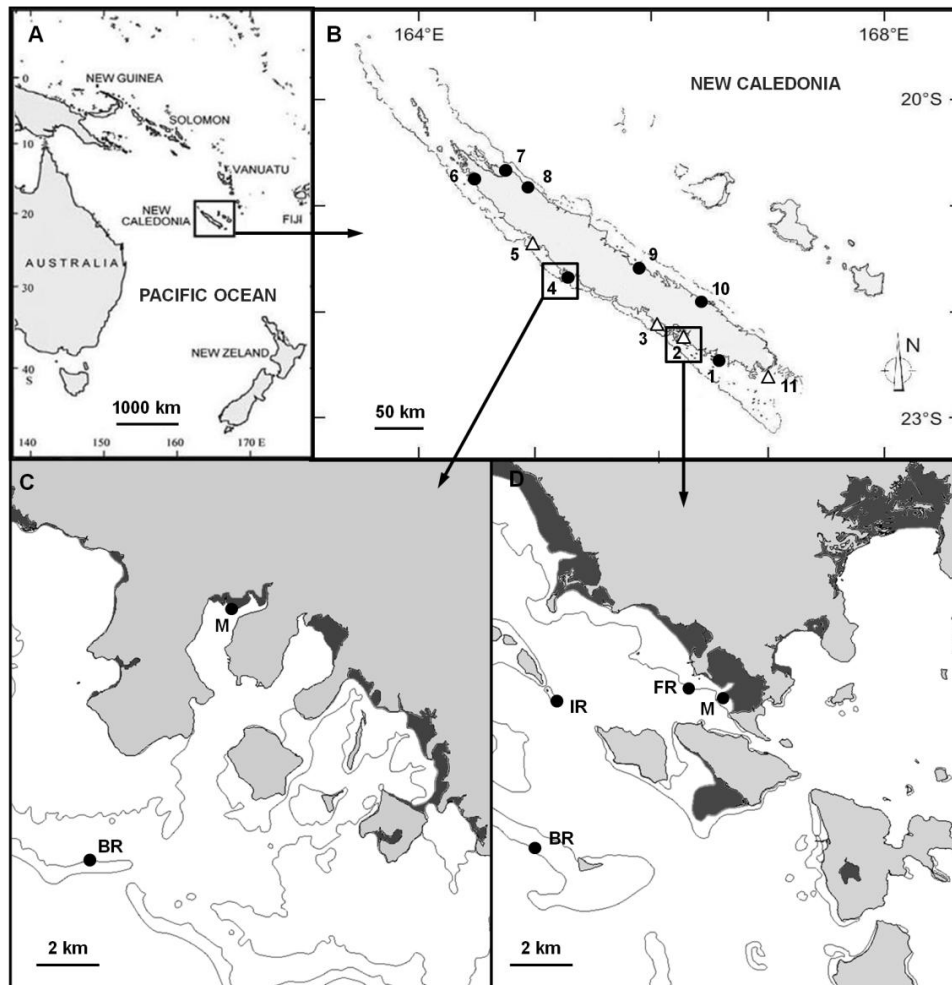


Figure 3.1: (A) New Caledonia geographic situation in the South West Pacific. (B) Study sites: circles and triangles indicate sites with 2 and 4 habitats sampled, respectively detailed in C and D (with M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier).

Fish were sampled for otolith analysis in different mangrove and reef habitats at 11 sites around the main land of New Caledonia in three years (2009, 2010 and 2011) (Fig. 3.1 and Tab. 3.1). In reef habitats, large species were collected by spear fishing and small species were anesthetized using clove oil and collected with handnets. In mangroves, gillnets were deployed at high tide along the mangrove maritime front and fish were collected with handnets in a few cm of water and/or on the ground at low tide. Different mesh sizes were used in order to catch species of different length and at different ontogenetic stage. Immediately after collection, fishes were stocked in a freezer (at -20°C) until dissection.

The first sampling took place during austral summer 2009 in the mangroves and inner barrier reefs of two sites (S2 and S5). The second sampling has been carried out during austral winter 2010 in the mangroves and inner barrier reefs of eleven sites around the main island. Fringing

and intermediate reefs were also sampled at four sites (S2, 3, 5 and 11). These four sites were designated as “west coast sites” and replicated during the third sampling in austral winter 2011. During the first and second samplings, a multi-specific collect has been carried out with respectively 44 and 25 species sampled (Tab. 3.1). The third sampling focused on four target species: *Dascyllus aruanus*, *Ctenochaetus striatus*, *Lutjanus fulviflamma* and *Siganus lineatus*.

Table 3.1: Sample size and otolith analyses; (PSO: Pôle de Spectrométrie Océan; RIEL: Resources Institute of Environmental Livelihoods).

Year	Site	Habitat	n stations	n species	LA-ICP-MS	n otoliths
2009	2	2 (M, BR)	4	44	PSO	209
2010	7	2 (M, BR)	14	25	PSO	114
	4	4 (M, FR, IR, BR)	16		RIEL	595
2011	4	4 (M, FR, IR, BR)	16	4	PSO	130
					RIEL	60

Multi-specific classifiers build thereafter included otolith elemental compositions of all species caught during the three samplings. Mono-specific classifiers were realized for the species showing the highest redundancy among the three samplings. They were built for the eight following species: *Dascyllus aruanus*, *Chaetodon lunulatus*, *Ctenochaetus striatus*, *Gerres oyena*, *Gnathodentex aureolineatus*, *Lutjanus fulviflamma*, *Scolopsis bilineata* and *Siganus lineatus*. Taxonomic and functional details of the eight species are summarized in table 3.2.

Table 3.2: Summary of species characteristic used to build mono-specific signatures (C: carnivore, MC: micro carnivore, H: herbivore, Z: zooplankton feeders).

Specie	Family	Home range	Diet
<i>Lutjanus fulviflamma</i>	Lutjanidae	Mobile	C
<i>Ctenochaetus striatus</i>	Acanthuridae	Sedentary	H
<i>Dascyllus aruanus</i>	Pomacentridae	Sedentary	Z
<i>Siganus lineatus</i>	Siganidae	Very mobile	H
<i>Chaetodon lunulatus</i>	Chaetodontidae	Sedentary	MC
<i>Gerres oyena</i>	Gerreidae	Mobile	C
<i>Gnathodentex aureolineatus</i>	Lethrinidae	Sedentary	C
<i>Scolopsis bilineata</i>	Nemipteridae	Mobile	C

2.2. Otolith preparation and chemical analyses

All material used for otolith handling was previously decontaminated in 5% ultrapure nitric acid bath during 24h, rinsed three times with ultrapure water (18.2 MΩ), dried and stored in clean plastic bag under a laminar flow hood (HEPA 100).

Before extraction of otoliths, fish samples were unfrozen and each individual was measured (Fork length, FL) to the nearest mm and weighted to the nearest g. Paired sagittal otoliths were extracted using ceramic tweezers and ultrapure water.

The chemical signatures of habitats were characterized by analyzing the chemical composition at the surface of otoliths. For mono-specific signatures, up to five otoliths were randomly selected in each sample of the eight following species: *Dascyllus aruanus*, *Chaetodon lunulatus*, *Ctenochaetus striatus*, *Gerres oyena*, *Gnathodentex aureolineatus*, *Lutjanus fulviflamma*, *Scolopsis bilineata* and *Siganus lineatus*. For multi-specific signatures, up to three otoliths were randomly selected for each species caught in each sample.

Otoliths were cleaned of adhering tissues following Warner *et al* (2005) method with a bath of 50/50 H₂O₂ (30%, Suprapur[®]) and NaOH (0.1 mol.L⁻¹, Suprapur[®]) during 1 hour. Otoliths were sonicated during the last 5 min of the bath, and then rinsed 5 times with milli-Q water for 5 min, dried under a laminar flow hood (HEPA 100) and stored in individual plastic vials.

Otoliths were chemically analyzed by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the Pôle de Spectrométrie Océan (Institut Universitaire Européen de la Mer, Brest, France) using a Thermo Element 2 coupled to a laser 193 nm CopexPro 102 Coherent, and at the Resources Institute of Environmental Livelihoods (Charles Darwin University, Darwin, Australia) using Agilent 7500ce coupled to a UP – 213 nm laser ablation system (Tab. 1). Both ICP-MS were operated at low resolution using argon as the carrier gas. Both laser systems parameters were set on a 90 μm laser beam diameter and a frequency of 5 Hz. Each analysis lasted 120 s including 30 s of background and 90 s of ablation (laser activated). To maximize discrimination between habitats, 20 isotopes were measured: ⁷Li, ¹¹B, ⁸⁵Rb, ⁸⁸Sr, ⁹⁵Mo, ¹¹¹Cd, ¹¹⁷Sn, ¹³⁸Ba, ²⁰⁸Pb, ²³²Th, ²³⁸U, ²⁵Mg, ⁴³Ca, ⁴⁷Ti, ⁵¹V, ⁵²Cr, ⁵⁵Mn, ⁶⁰Ni, ⁶⁵Cu and ⁶⁶Zn.

To reveal the latest elements incorporated from the habitat where the fish was caught, one laser ablation was done on the otolith surface. To standardize the analyses, the ablation was always done at the same location at the tip of the post rostrum on the otolith posterior side. Calcium was used as an internal standard to compensate possible variation due to differences in quantity of material ablated. To correct for instrument drift, an external standard (National Institute of Standards and Technology, NIST, 612) was analyzed twice at the beginning and at the end of each session and also every ten ablations.

Raw ICPMS data (counts per second, CPS) were cleaned of outliers, defined as values higher than three times the inter-quartile distance during both the blank and the ablation windows (Longerich *et al.* 1996, Heinrich *et al.* 2003). Cleaned data were then transformed in elemental concentrations (parts per million, ppm) and limits of detection (LOD) were calculated following Longerich *et al.* (1996). Any elemental concentrations lower than the LOD was set to zero. Only the elements that met the following two criteria were selected in statistical analyses: 1) elemental concentrations in otoliths had to be higher than the LOD in 70% of the otoliths in at least one habitat or one site and 2) the coefficient of variation of elemental concentrations in the NIST 612 had to be less than 10% (Chittaro *et al.* 2004, Chittaro *et al.* 2006). The use of two different ICP-MS and the time intervals between two sessions realized with the same ICP-MS may influence to some degree the absolute values of concentration. To address this potential issue, elemental concentrations were transformed in percent of all measured elements.

2.3. Statistical analyses

All statistical analyses were performed using R statistical software (R Development Core Team 2011). To describe spatial variation in multi-specific and mono-specific elemental composition of otoliths, non-parametric analysis of variance (PERMANOVA) and principal component analysis (PCA) were performed. Multivariate two-way PERMANOVA (with 999 permutations) based on Euclidian distance were performed to test the effect of site and habitat on the multi-elemental composition of otoliths (Legendre & Anderson 1999, Anderson 2001). Non parametric PERMANOVAs were used since the assumptions of normality and homogeneity of variance required by parametric MANOVAs were not met. The PCA was used to visualize which element characterized the spatial variations of the elemental

composition of otoliths. Elemental compositions were $\arcsin\sqrt{x}$ transformed in the PCA to equalize the variance and normalize the data.

Random forests (RF) algorithm (Breiman 2001) was performed to build habitat classifiers based on elemental concentrations of otolith. RF classification method allows freedom from normality and homoscedasticity assumptions (Breiman 2001) and is one of the most powerful methods for classification of otolith chemical signature . With the RF classification method, two-third of the data set is used to build a classification tree and the remaining one-third is classified along this tree. The RF algorithm builds 5000 trees to ensure that every individual gets predicted several times which permits to estimate classification accuracy (percent of correct classification). RF was performed for all possible element combinations in order to find the combination that showed the highest percent of correct classification. When several best combinations were found, the one with the smaller number of elements was retained.

RF was performed at different spatial scales and for several organizational levels. Three spatial scales were considered. The local scale focused on discriminations between habitats (mangrove, fringing, intermediate and barrier reef) within each site of the west coast. The regional scale focused on the west coast sites gathered and discriminations were done between habitats (mangrove, fringing, intermediate and barrier reef) and between stations of a specific habitat. The global scale focused on the 11 sites of the main island and discriminations were done at the level of habitat (mangrove versus barrier reef) and between stations of a specific habitat. Elemental signatures were explored at the species level for eight species (mono-specific signatures) and at the multi-species level with signatures calculated from data on 53 species, by grouping species from the same family and by grouping species with the same diet.

At all spatial scales, RF classifier were calculated for all 8 species for which we add enough information and their accuracy estimated from the percentage of individuals correctly classified into their actual location of collection. Then, classifiers were built with multi-specific elemental compositions using all species, species grouped by family and species grouped by diet. These multi-specific classifiers were used to predict the location of collection of the 8 species for which mono-specific signatures were calculated. The multi-specific predictions were then compared with the actual location of collection. That way, we could

indeed compare the accuracy of mono-specific and multi-specific classifier in predicting the location of collection of fish from 8 species. When a multi-specific classifier was build for comparison with a mono-specific classifier, the species under study was excluded from the multi-specific dataset in order to avoid circularity. For instance, *L. fulvivflamma* elemental compositions were predicted using multi-specific signatures calculated from all species, all Lutjanidae or all carnivores except *L. fulvivflamma*, and these multi-specific predictions were compared with predictions from mono-specific signatures calculated from *L. fulvivflamma* dataset only.

Pearson correlation test was performed to evaluate the correlation between classification accuracies obtained with mono-specific classifiers and accuracies obtained with multi-specific classifiers.

3. Results

3.1. Spatial characterization of otolith microchemistry

Twelve elements were retained in the statistical analyses: B, Ba, Cr, Mg, Mn, Pb, Rb, Sn, Sr, Th, U and Zn. Only spatial characterization of otolith chemistry obtained with the multi-species dataset and one mono-species dataset (*L. fulvivflamma*) are detailed below. Results for the remaining seven mono-species datasets are summerised below and detailed in Appendix 1 and 2.

Using multi-species otolith datasets of west sites, two-way multivariate PERMANOVA showed a significant interaction of factors (site x habitat, p-value = 0.001), indicating that differences among habitats were not consistent among sites (Tab. 3.3). Indeed, the PCA highlighted strong overlap among reef habitats (Fig. 3.2A) and sites (Fig. 3.2B). Similar results were obtained at species level with *L. fulvivflamma* otoliths datasets of west coast sites. Two-way multivariate PERMANOVA indicated a significant interaction between factors (site x habitat, p-value = 0.013) and a PCA highlighted strong overlap between habitats (Fig. 3.3A) and sites (Fig. 3.3B), with however mangrove relatively well separated from reefs (Fig. 3.3A and 3.3B).

For both multi-species and *L. fulvivflamma* analyses, mangrove was principally characterized by Mn, Mg and Ba whereas fringing, intermediate and inner barrier reefs constituted three close groups predominantly characterized by Sr (Fig. 3.2C and 3.3C). Importantly, however, the differences between mangroves and reefs were clearer when using mono-specific data (Fig. 3.3B) than multi-specific data (Fig. 3.2B).

Table 3.3: Multivariate results of PERMANOVA examining spatial variation in otolith elemental composition within site and habitat for multi-specific and *Lutjanus fulvivflamma* (***) $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

Species	Df	MS	F	p (>F)
Multi-specific				
Site	10	0.003065	1.994	0.006**
Habitat	3	0.075786	49.295	0.001***
Site x habitat	16	0.003374	2.194	0.001***
<i>L. fulvivflamma</i>				
Site	10	0.014207	3.2702	0.001***
Habitat	3	0.0049035	11.2864	0.001***
Site x habitat	14	0.0008362	1.9247	0.013*

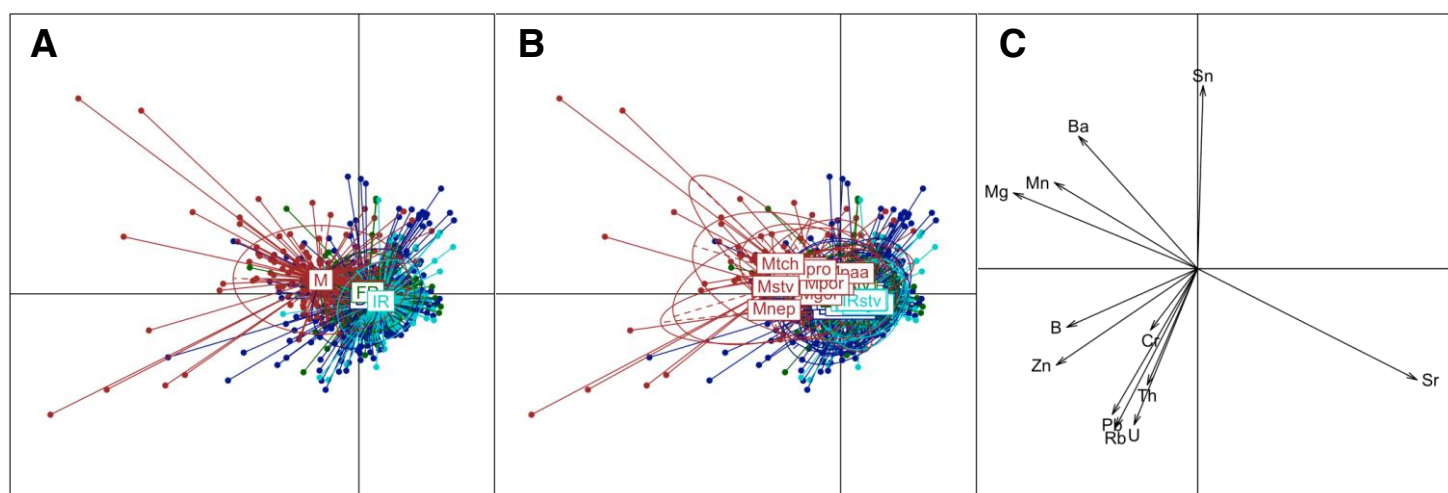


Figure 3.2: Plots of principal component analysis (PCA) of elemental compositions of multi-specific otoliths for habitats (A) and stations (B) and plot of contributions of elements in habitat and stations elemental compositions (C) (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

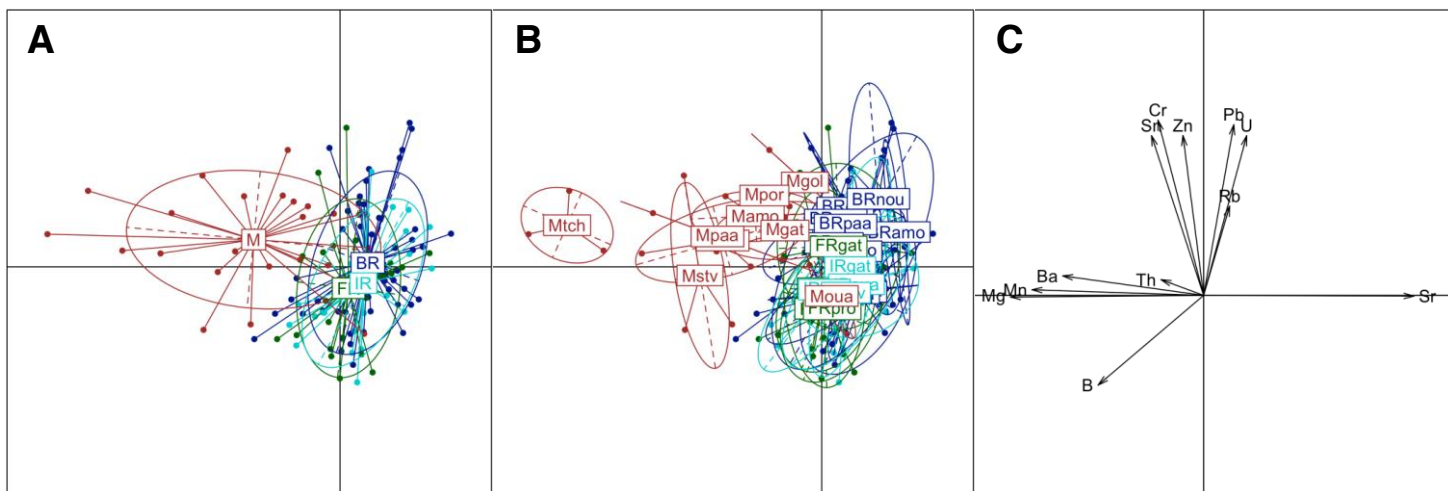


Figure 3.3: Plots of principal component analysis (PCA) of elemental compositions of *L. fulviflamma* otoliths for habitats (A) and stations (B) and plot of contributions of elements in habitat and stations elemental compositions (C) (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

Results of PERMANOVAs and PCAs obtained with the remaining seven species were mixed and are detailed in appendix (see Appendix 1 and 2). Differences between sites and habitats were generally less significant or even non significant when considering species collected only in reefs habitat (*D. aruanus*, *C. lunulatus*, *C. striatus* and *G. aureolineatus*) except for *S. bilineata*. Concerning species collected only in mangroves (*G. oyena* and *S. lineatus*), differences between stations were highly significant.

3.2. Accuracy of classifiers at different spatial scales

At the local scale (stations of the west coast sites), correct classifications of mangroves, fringing, intermediate and barrier reef habitats using multi-species otolith datasets showed moderate levels ranging between 49 and 69% depending on sites (Tab. 3.4). At the species level, rates were much higher. For *L. fulviflamma*, correct classification rates were high, ranging between 73 and 78% for all sites except Ouano where rate was only 43%. For *D. aruanus* correct classification rates were even higher, ranging between 74 and 85%, except at Prony where it reached 67%. For *C. lunulatus* rate of correct classification was 80% at Ouano and StVincent and 67% at Gatope and Prony. Percentages of correct classification were the highest for *C. striatus* with an average of 82% and a range of 71 - 96% depending on sites. There was no sufficient data to conduct these tests for the other species at the local scale.

Table 3.4: Summary of RF classification accuracies in percent of individuals correctly classified to the location they were collected. The highest classification accuracies ($\geq 80\%$) are represented in bold and italic (*Gat*: Gatope, *Oua*: Ouano, *Pro*: Prony, *StV*: StVincent; *M*: mangrove, *FR*: fringing reef, *IR*: intermediate reef, *BR*: barrier reef).

Species	Sample size (<i>n</i>)				Local				Regional					Global			
					Habitat				Habitat	Stations				Habitat	Stations		
					M/FR/IR/BR				M/FR/IR/BR	M	FR	IR	BR	M/BR	M	BR	
	M	FR	IR	BR	Gat	Oua	Pro	StV	4		4	4	4	4	11	11	11
Multi-specific	260	175	186	527	63	49	57	69	59		63	42	44	47	90	44	29
<i>L.fulviflamma</i>	37	38	35	86	74	43	78	73	67		100	63	54	48	95	65	29
<i>D.aruanus</i>	/	39	39	90	74	74	67	85	64		/	64	67	60	/	/	41
<i>C.striatus</i>	/	28	33	72	79	83	96	71	71		/	71	64	63	/	/	47
<i>C.lunulatus</i>	/	12	12	46	67	80	67	80	67		/	50	58	83	/	/	41
<i>S.lineatus</i>	41	/	/	/	/	/	/	/	/		84	/	/	/	/	63	/
<i>G.oyena</i>	33	/	/	/	/	/	/	/	/		83	/	/	/	/	67	/
<i>S.bilineata</i>	/	9	/	30	/	/	/	/	95		/	78	/	100	/	/	37
<i>G.aureolineatus</i>	/	/	/	29	/	/	/	/	/		/	/	/	80	/	/	24

At the regional scale (habitats of west coast sites), RF classification of the four habitats resulted in low percentages of correct classification for the multi-specific analysis (59%), ranging from 42% to 63% depending on the habitat (Tab. 3.4). For the four species tested, mono-specific analyses showed higher percentages: *L. fulviflamma* (67% of individuals correctly classified; range: 40% - 85% depending on the habitat), *D. aruanus* (64%; range 46% - 85%), *C. striatus* (71%; range 39% - 85%) and *C. lunulatus* (67%; range 25% - 91%) (Tab. 3.4). Classification of sites within a specific habitat at regional scale showed similar results. Mangrove stations showed a moderate correct classification rate for multi-specific signatures (63%) but a very high rate at the species level, ranging from 83% to 100% for three species (*G. oyena*, *L. fulviflamma* and *S. lineatus*). Individuals from fringing reef stations were correctly classified in 42% of cases using multi-specific signatures, and 50% - 78 % of cases with mono-specific signatures. Intermediate reef stations showed classification rates of 44% (multi-specific) and 54% - 67% (mono-specific). Correct classification of individuals from barrier reefs was 47% (multi-specific) and ranged from 47% to 100 % depending on the species (Tab. 3.4).

At the global scale (11 stations and 2 habitats around New-Caledonia), RF classifications accuracy of mangroves versus barrier reef was extremely high using a multi-specific signature (90% correct classification), and ranged from 81% in mangroves to 96% in reefs. At this scale, data was sufficient to test mono-specific signature only for *L. fulviflamma*. For this

species, 95% of individuals were correctly classified in the two habitats, with 86% correct classification in mangroves and 99% in barrier reefs. Within the mangrove habitat, fish were classified in the correct site in 44% of cases using the multi-specific signatures but in 63% - 67% of cases using mono-specific datasets (*G. oyena*, *L. fulviflamma* and *S. lineatus*). Within barrier reefs, correct classification of fish in their site of collection was poor for both multi-specific (29%) and mono-specific signatures (24% - 47%) (Tab. 3.4).

3.3. Characterization of chemical signatures

Among the 62 classifiers built with the RF method, 14 were above or equal to 80% of correct classifications (Appendix 3). B, Mn and Ba were the most recurrent element appearing in the best classifiers. Among these and at the global scale, multi-specific otoliths collected in mangrove and barrier reef of all stations were highly correctly classified (90%) with seven elements constituting the best combination: Ba, Mg, Mn, Pb, Rb, Sn and U (Appendix 3). Using only these elements, one-way multivariate PERMANOVA indicated a significant difference between the two habitats illustrated in the PCA (Tab. 3.5 and Fig. 3.4). At the regional scale, characterization of mangrove stations of the west coast sites with *S. lineatus* otoliths is a good example of high correct classification (84%). For this signature, the best combination of element was constituted of 2 elements: B and Mn (Appendix 3). Using these 2 elements, one-way multivariate PERMANOVA indicated a significant difference between sites (Tab. 3.5) and PCA showed a clear discrimination between the four mangroves of the west coast sites (Fig. 3.4). At the local scale, *C. lunulatus* otoliths collected in Ouano were correctly classified in 80% of cases using five elements (Ba, Mg, Mn, Rb and Zn) (Appendix 3). One-way multivariate PERMANOVA of these 5 elements indicated a significant difference between the three reefs illustrated in the PCA (Tab. 3.5 and Fig. 3.4).

Table 3.5: Multivariate results of PERMANOVA examining spatial variation in otolith elemental composition using elements from best combination only (Appendix 3) for multi-specific at global scale, *S. lineatus* at regional scale and *C. lunulatus* at the local scale of Ouano.

Species	Scale	Factor	Df	MS	F	p (>F)
Multi-specific	Global (11 sites)	Habitat	1	0.096	97.02	0.001***
<i>S. lineatus</i>	Regional (4 west coast sites)	Site	3	0.0006	20.261	0.001***
<i>C. lunulatus</i>	Local (Ouano)	Habitat	2	0.0002	8.0877	0.004**

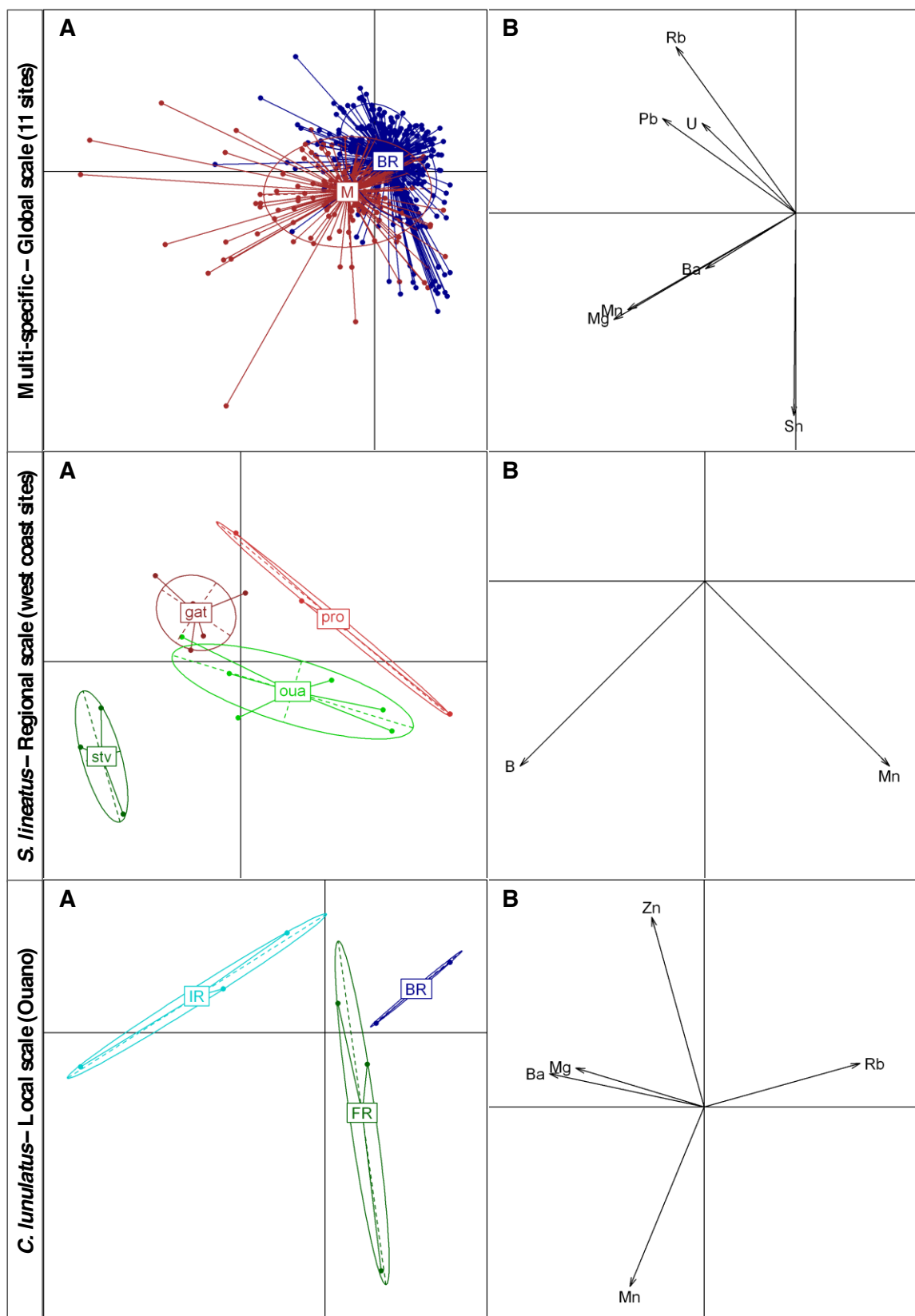


Figure 3.4: Plots of principal component analysis (PCA) of elemental compositions of otoliths using elements from best combination only (Tab. 3.5) for habitats or stations (A) and plot of contributions of elements in elemental compositions (B) (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef; gat: Gatope, oua: Ouano; pro: Prony; stv: St Vincent).

3.4. Multi-specific as a proxy of mono-specific

When multi-specific classifiers were used to predict habitat of single species, there was a significant loss in correct classification compared to mono specific classifiers (Fig. 3.5, Appendix 4). For instance, the rate of correct classification was 100% for *L. fulviflamma* individuals collected in mangroves of the west coast sites using mono-specific signature, and only 20% using global multi-specific signature. The only exception was observed at the global scale between mangrove and barrier reef with a high correct classification using mono-specific classifiers (95%) as well as using global multi-specific (90%) (Appendix 4). No correlation was observed between the rate of correct classification with mono-specific and multi-specific classifiers ($R = 0.09$, $p > 0.5$). This result remained when species were grouped by diet ($R = -0.39$, $p > 0.1$) or family ($R = -0.84$, $p = 0.01$).

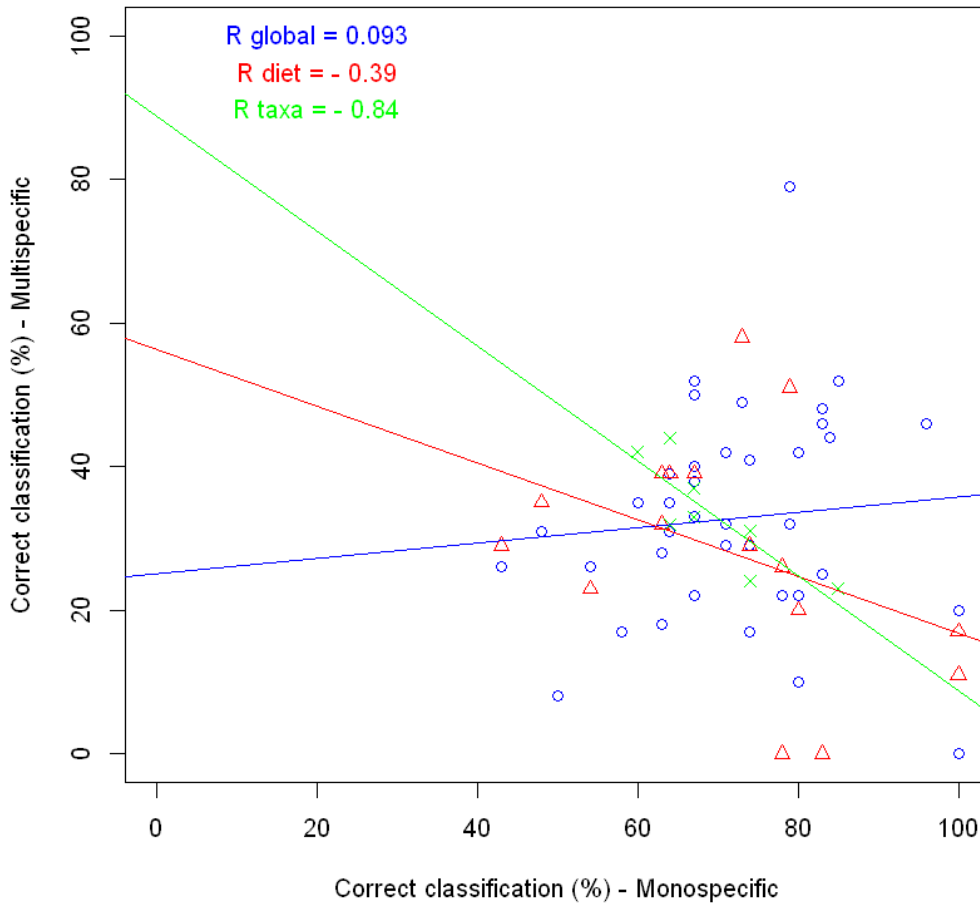


Figure 3.5: Plot representing correlations between percent of correct classifications obtained with mono-specific classifiers and percent of correct RF classifications obtained with corresponding global (blue circles), functional (red triangles) and taxonomic (green cross) multi-specific classifiers.

4. Discussion

4.1. Characterization and spatial scales of chemical signatures in otoliths

In New Caledonian seascape, percentages of correct classification were higher at the habitat level than at the station one. In fact, success in chemical distinction dropped down with the number of stations which lead to an increase of the variability on the one hand and a decrease in the number of replicates (individuals analyzed) on the other hand.

At the local scale, when habitats of west coast sites were taken individually, levels of discrimination between habitats varied according to the species but were predominantly high (>70% in most cases). Likewise, at the regional scale, when data of the four west coast sites were pooled, elemental fingerprints of the four species tested showed a slightly reduced but still high global level (>64%) of discrimination among habitats. Finally, at the global scale, *L. fulviflamma* element fingerprints were clearly distinct between mangrove and barrier reef (95% correct classification). At the regional and global scales, discrimination was lower at the station level than at the habitat level. However, correct classifications of stations belonging to the same habitat were quite high for mangrove species at the global level (>63%) and for both reef and mangrove species at the regional level (>63% in half of the cases).

Contrasted distant habitats were discriminated with a high success because of the different chemical influences from the coast to the barrier. A great discrimination was observed for mangroves stations because otoliths from mangroves presented a higher chemical heterogeneity than the reefs, mainly due to high concentrations of Mn. Mangroves receive higher and different terrestrial inputs such as urban or freshwater inputs from numerous estuaries along the New Caledonian coast (related to the characteristics of the catchments).

Fringing, intermediate and barrier reefs were chemically characterized by the presence of Sr, but they overlapped and were either moderately spatially discriminated or misclassified. These results could be explained by the spatial distribution of reefs habitats. Depending on the site, fringing reefs are spatially closer to mangrove being under a coastal influence and showing moderate heterogeneity of environmental chemistry. Terrestrial influences lead to distinguish fringing reefs from intermediate and barrier reefs, whereas intermediate reefs close to barrier reefs were misclassified as barrier reefs. In others sites, fringing reefs were not distinct from intermediate and barrier reefs because distance to the coast was higher and they

were under an oceanic influence through channels and passage in the barrier reef, leading to a more homogeneous chemical composition of the otoliths.

Likely, integration of the spatial distribution of habitats at local scale may improve the power of otolith fingerprints of reef habitats. For example some fringing and intermediate reefs can be pooled in an inshore reef category, and some intermediate and barrier reefs in an offshore category.

4.2. Inter-specific variations and multi-specific signatures

The present study also focused on the inter-specific variations of elemental compositions, the ability to use multi-specific classifiers as a proxy of mono-specific ones and to estimate if the use of taxonomic or ecologically closed species could enhance the potential as a proxy of mono-specific classifiers. The interest of such proxy is to compensate a lack of fish to build mono-specific classifiers when the studied species are difficult to collect (conservations issues, rarity, difficulty to sample, etc.). In the present study, the percent of correct classification obtained with mono-specific classifiers varied between species. Because elemental fingerprints varied between species, the use of global multi-specific signatures amplified the variability of the chemical signal and resulted in a high rate of misclassifications. This increasing chemical variation is reduced but remained important when multi-specific signatures are constituted of taxonomic closed species or species sharing the same diet. However, when chemical contrast among habitats is very high, as between mangrove and barrier reef then multi-specific signatures may prove useful.

Otolith microchemistry is based on the general assumption that their elemental composition is directly related to the physicochemical properties of the surrounding environment (Bath *et al.* 2000). Several exogenous factors are reported to affect element incorporation, such as water elemental compositions, temperature and salinity (Sturrock *et al.* 2012). For a given location, all species collected were not exposed to the same variations of exogenous factors because of their different ecology (mobility, diet, etc.). The mobility of species should be taken into account when studying spatial patterns of elemental fingerprints. For instance, at the site of Ouano, the success of classification of individuals was better for the least mobile species (*C. striatus*, *C. lunulatus* and *D. aruanus*) than with the mobile *L. fulviflamma*. For *L.*

fulviflamma, Ouano was the site where the discrimination between habitats was the lowest. This may be explained by frequent movements between habitats which were revealed in an acoustic study at the same location and for the same species (Chateau *et al.* 2012). Acoustic telemetry revealed patterns of movements at a lower temporal scale than the study of elemental fingerprints. These movements varied between fish and high level of movements between reefs habitats were observed, even several forays in mangrove for adult fish. Furthermore, Kaunda-Arara and Rose (2004b) showed in their study that *L. fulviflamma* could move over 1.5 km, which in some sites such as Ouano is sufficient to move between several habitats.

Unlike mobile species, species with high site fidelity and a small home-range may show lower variations in chemical composition of otolith. These fish will not be confronted to a change in chemical composition of ambient water during their movements (Chittaro & Hogan 2013). An acoustic study in Kimbe Bay (New Britain) revealed that outside the spawning season, the movements of *C. striatus* were limited, their feeding area being restricted to a mean maximum diameter lower than 13 m (Claydon *et al.* 2012). Furthermore, Mellin (2007) showed that this species showed no change in habitat use during their ontogeny in New Caledonia. *D. aruanus* and *C. lunulatus* present an extremely limited home range (Sale 1971, Guillemot *et al.* 2011). Nevertheless, even if a low mobility may enhance the success of discrimination, a sedentary species will still face temporal variations of the chemical composition of the ambient environment.

Besides exogenous factors, endogenous factors like diet, stress, growth rate or ontogenetic stage are suspected to influence element incorporation in the otoliths (Marohn *et al.* 2009, Sturrock *et al.* 2012). In the present study, the use of species with similar diet for multi-specific classifiers was not significantly more efficient than using data of all species. This result was unexpected because diet was suspected to be a major endogenous process involved in inter-specific variation of elemental fingerprints. However, studies testing if the variations in otolith chemistry could be influenced by diet showed contrasted results. A few studies found that variations of some elements were related to the food items ingested (Kennedy *et al.* 2000, Buckel *et al.* 2004, Walther *et al.* 2010). Buckel *et al.* (2004) found a significant difference in Ba and Sr ratios between juveniles *Pomatomus saltatrix* fed with invertebrate and finfish prey. However, they did not found a difference in Mn, Mg and Ca ratios. Walther

(2010) revealed significant effects of food on elemental concentrations of Ba and Sr but could not distinguish them from effects of growth rate and temperature. However, most studies revealed no significant difference in elemental concentrations between contrasted diets (Milton & Chenery 2001, Lin *et al.* 2007). For example, Marohn *et al.* (2009) found no variation in the concentration of Mg, Mn, Sr and Ba for European eels under eight different diets. In another study, Walther and Thorrold (2006) revealed that Sr and Ba concentrations in otoliths of juvenile *Fundulus heteroclitus* clearly reflect ambient Sr and Ba concentrations of the environment. In this study, the majority of multi-specific signatures were determined from carnivorous species and used to predict carnivorous species. However, the carnivorous group is an extended diet class composed of species feeding on diverse trophic levels and in different proportions (different size of mobile invertebrates ingested alone or mixed with a variable proportion of fish prey). The poor efficiency of carnivorous multi-specific signatures may therefore also be explained by the broad carnivorous class used in this study.

Before their incorporation in the otoliths, trace elements from the surrounding water have to pass by physiological barriers (blood plasma and endolymph). These processes are species specific, poorly understood and may result in inconsistent patterns of element incorporation among species (Campana 1999, Sturrock *et al.* 2012). In this study, prediction using multi-specific signatures of species from same family were realized under the hypothesis that influence of these processes could be reduced with taxonomically close species (Swearer *et al.* 2003). This hypothesis was not supported by our results with higher levels of misclassification when species were grouped by family compared with multi-specific signatures using all species. This implies that a precise knowledge of species specific physiological processes is required to fully understand the inter-specific differences of element incorporation into otoliths prior to using multi-specific signatures (Hamer & Jenkins 2007).

5. Conclusion

In New Caledonia, spatial scales of chemical discrimination with otolith fingerprints could be revealed between contrasted habitats (mangroves and reefs) at the scale of the island (global),

the west coast sites (regional) and within sites (local). Discrimination at the station level was lower and mainly related to the level of chemical heterogeneity within the habitats.

The results of this study showed that using multi-specific signatures as a proxy of mono-specific signature is generally not possible, even when grouping species by family or by diet. However, when studying highly contrasted habitats such as mangrove and barrier reefs, then multi-specific signatures can prove very useful. Prior to use multi-specific signatures as a proxy, future studies should quantify the effects of exogenous factors such as the mobility of species, and endogenous factors such as the physiological processes of elemental uptake, diet influence, growth rate and ontogeny.

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Le chapitre 3 a permis d'évaluer la précision de la classification spatiale des sites et habitats obtenue à partir de la composition élémentaire des otolithes de plusieurs espèces de poissons.

Le pouvoir discriminant de l'outil microchimique est important à l'échelle des habitats, particulièrement lorsqu'ils présentent des environnements contrastés comme par exemple les mangroves versus les récifs barrières. Les récifs frangeants et intermédiaires étant moins contrastés ou sous des influences terrigènes variables en fonction des sites, il en résulte une discrimination moins efficace. Néanmoins, il devrait être possible d'augmenter la précision des signatures en tenant compte de la distribution spatiale de ces deux habitats au sein de chaque site. La microchimie hétérogène au sein d'un habitat spécifique telle que celle constatée au sein des mangroves augmente le pouvoir de classification à l'échelle spatiale plus fine des stations.

Ce chapitre a également permis de tester l'utilisation des signatures multi-spécifiques comme substitut des signatures mono-spécifiques. Les résultats obtenus indiquent que dans la grande majorité des cas le multi-spécifique ne peut se substituer au mono-spécifique et ce même s'il est composé d'espèces taxonomiquement ou écologiquement proches de l'espèce constituant le mono-spécifique. Les différences interspécifiques d'incorporation des éléments au sein des otolithes sont vraisemblablement à l'origine de ce résultat. Ceci implique que les signatures microchimiques ne peuvent être développées qu'à partir de l'espèce étudiée. Des exceptions peuvent néanmoins se présenter, notamment lorsque les habitats à discriminer sont très contrastés. En effet, les mangroves et récifs barrières sont parfaitement discriminés par des signatures multi-spécifiques. Cela signifie qu'il est possible d'étudier la connectivité entre ces deux habitats même pour des espèces pour lesquelles un échantillonnage insuffisant n'a pas permis de construire des signatures microchimiques mono-spécifiques.

L'existence de fortes variations interspécifiques dans la chimie des otolithes implique que chaque espèce intègre les signaux environnementaux de manière singulière. Afin de mieux comprendre l'outil microchimie des otolithes, il apparaît donc nécessaire d'évaluer les interactions entre la microchimie des otolithes et de l'environnement. Ceci est l'objet du chapitre suivant.

Chapitre 4: Comparaisons entre les signatures multi-élémentaires de l'environnement et des otolithes en Nouvelle-Calédonie



Chapitre 4: Comparaisons entre les signatures multi-élémentaires de l'environnement et des otolithes en Nouvelle-Calédonie

La microchimie des otolithes est basée sur le principe que la composition élémentaire des otolithes reflète plus ou moins directement celle de l'environnement (Bath *et al.* 2000). Or, depuis le début de l'utilisation de la microchimie des otolithes en tant qu'outil de mesure de la connectivité écologique, d'aide à l'identification des stocks et de reconstruction de l'histoire environnementale des poissons, il a été montré que différents facteurs auraient une influence sur l'incorporation des éléments traces au sein des otolithes.

Ce chapitre s'insère dans l'axe méthodologique de la thèse et a pour objectif d'étudier la microchimie de l'environnement en Nouvelle-Calédonie et les corrélations avec la microchimie des otolithes. Il s'agira notamment de :

- 1) Caractériser les variations spatiales de la microchimie de l'environnement à l'échelle des sites et habitats de la Nouvelle Calédonie.
- 2) Evaluer la précision des signatures microchimiques de l'environnement, notamment dans l'eau et les sédiments, et examiner les différences entre ces deux compartiments.
- 3) Etudier les corrélations entre la composition élémentaires des otolithes, de l'eau, des sédiments, la salinité et la température.

Ce travail sur les relations entre la microchimie des otolithes et de l'environnement est utile pour mieux comprendre les différences et les points communs entre les signatures observées dans les otolithes de différentes espèces collectées dans les mêmes environnements.

Manuscrit B: Comparison of otoliths and environmental multi-elemental signatures in the coral reefs and mangroves of New Caledonia

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Abstract

Otolith chemistry is a method increasingly used to reconstruct environmental histories and to track lifetime movements of fish. However, it requires knowledge of relationships between the physicochemical properties of the environment and the elemental uptake into otoliths for each species of interest. We examined environmental (seawater and sediment) and otolith microchemistries of several species collected in mangroves and reefs around the main island of New Caledonia. We attempted to characterize the spatial variation of environmental microchemistry and to evaluate the level of spatial discrimination possible. We also tested the relationships between the elemental compositions of the otoliths and the environment, and with the variations of temperature and salinity. Our analyses indicated that sediment could not be chemically characterized at any spatial level. In contrast, seawater microchemistry showed

a high rate of correct classification at the level of habitat but not at the station level, except among mangrove stations because of their high chemical heterogeneity. Effects of environmental chemistry, temperature and salinity on otolith chemistry were complex and diverse depending on the species or the environmental factor considered. Ba and Sr showed classical variations with some species confirming their potential as indicators of past environments for these species, but this was consistent with all species. Individual influence of exogenous factors on Mn and Cr uptake were not clearly identified. However, these elements seem to be valuable indicators of coastal habitats used by fish. Our results indicated that precautions should be taken before using otolith chemistry as proxies for environmental conditions. Nevertheless, reconstruction of past habitats used by fish can still be realized.

Key words: environmental microchemistry, otoliths microchemistry, LA-ICP-MS, trace elements

1. Introduction

Otoliths are paired structures located in the inner ear of teleost fishes and are composed of calcium carbonate crystals, primarily aragonite, deposited within a protein matrix. Otoliths grow continuously throughout life with calcareous layers deposited daily on their external surface. Through these deposits, trace elements from the surrounding environment are incorporated into the otolith and are never reworked through time due to the metabolically inert nature of otoliths. Thanks to these key properties, otoliths can archive the life-history information of a fish with a great time resolution. And indeed, the elemental composition of fish otolith has become a powerful tool to reconstruct life history movements of fish (McCulloch *et al.* 2005, Milton *et al.* 2008, Mercier *et al.* 2012), to discriminate fish stocks (Campana *et al.* 2000, Ferguson *et al.* 2011), to assess connectivity between populations (Gillanders 2002, Cuveliers *et al.* 2010) and to evaluate the importance of nursery areas (Gillanders & Kingsford 2000, Brown 2006b, Tournois *et al.* 2013).

However, the use of otolith chemistry as a toolbox for the study of fish ecology is based on the principle that, to some extent, the chemical characteristics of the environment are transferred to otoliths (Elsdon & Gillanders 2003a, Sturrock *et al.* 2012). Furthermore, microchemistry of otolith not only can be used to study fish, but can also be used as an environment recorder. Indeed, if the relationships between otolith elemental composition and environmental conditions are known, then retrospective measures of environmental characteristics (elemental composition of seawater and sediment, temperature, salinity) and quality (events of metallic pollution) should be possible (Forrester & Swearer 2002, Elsdon & Gillanders 2003b). This is particularly interesting as species with different ecology, growth and longevity may provide complementary information on past environmental changes at different scales notwithstanding the analysis of archeological otoliths that may provide detailed information of the environment thousands of years ago (Carpenter *et al.* 2003, Disspain *et al.* 2011, Mercier *et al.* 2012).

Thus, the relationships between elemental composition of otoliths and physicochemical properties of ambient environment (seawater and sediment elemental composition, temperature and salinity) crossed by fish not only needs to be elucidated to reconstruct past changes in the environment but also to better understand the otolith microchemistry toolbox to study fish ecology (Elsdon & Gillanders 2003a, Elsdon *et al.* 2008, Sturrock *et al.* 2012).

Multiple factors are known to influence the uptake of trace elements in otoliths. There are both exogenous factors such as temperature (Elsdon & Gillanders 2002, Martin & Thorrold 2005), salinity (Elsdon & Gillanders 2002, Lin *et al.* 2007) and chemistry of surrounding waters (Elsdon & Gillanders 2004, Martin *et al.* 2013), and endogenous factors like ontogeny (De Pontual *et al.* 2003), somatic growth rate (Miller 2011) and diet (Buckel *et al.* 2004). Furthermore, trace elements from ambient environment have to cross a series of biological boundaries (gills, plasma and endolymph) before incorporation in otoliths. These poorly known physiological pathways of incorporation may influence trace element concentrations in otoliths. In fact, uptake of elements may be species-specific because species exposed to the same environmental conditions may show inter-specific variations of otolith fingerprint (Gillanders & Kingsford 2003, Swearer *et al.* 2003, Martin & Wuenschel 2006, Hamer & Jenkins 2007). In addition, the relationship between otolith and water chemistry may be influenced by the mobility of the fish between different locations at different times (Milton *et al.* 2008). Variations in trace element concentrations between the environment and the otoliths also depend on the elements considered (Bath *et al.* 2000, Milton & Chenery 2001, Elsdon & Gillanders 2003b). Yet, studies assessing the influence of exogenous and endogenous factors influencing elemental uptake generally focused on a limited number of trace elements like Sr, Ba, Mg and Mn (Bath *et al.* 2000, Elsdon & Gillanders 2002, Martin & Thorrold 2005, Martin & Wuenschel 2006). Consequently, no general rules can be applied (Elsdon & Gillanders 2003b) and it is difficult to use otolith chemistry as an archive of the chemistry of the ambient environment (Campana 1999).

The main objective of this study was to assess the relationships between the elemental concentrations in otoliths and the elemental concentrations in the environment, the temperature and the salinity for several coral reef and mangrove fish species of New Caledonia and for multiple elements. First, we characterized the environmental and otolith chemistries of habitats and sites. Second, we looked at correlations between salinity, temperature and concentration of 12 trace elements in seawater, sediments and otoliths of 9 coral reef and mangrove fish species. Third, we compared the ability of environment and otolith microchemistry to distinguish different habitats at different spatial scale. Then, chemical fingerprints present in the environment were compared with those present in otoliths in order to determine whether signals present in otoliths were a simple translation of signals

present in the environment or if major changes occurred due to exogenous and endogenous factors.

2. Material and methods

2.1. Sampling protocol

New Caledonia is a French territory located in the South West Pacific, 1500 km east of Australia (Fig. 4.1). The archipelago is composed of several small islands and a main island with a surface area of 17 000 km², 400km long and 60 km large at its maximum. Around the main island, the 1744 km long barrier reef delimits the largest lagoon of the world (31 336 km²) (Andréfouët *et al.* 2009). The distance between the coast and the barrier reef varies from 1 to 65 km (Paris 1981) and some parts of the lagoon are registered on the Unesco heritage list since 2008. New Caledonia tropical seascape is constituted of several habitats and ecosystems such as mangroves, seagrasses, algal beds, bare soft bottoms and coral reefs (fringing, intermediate and barrier).

Fish samplings were realized during three years (2009, 2010 and 2011) at eleven sites (Fig. 4.1 and Tab. 4.1). The first sampling took place during the austral summer 2009 in two sites (S2 and S5) where mangrove and inner barrier reef were sampled. The second sampling has been carried out during austral winter 2010 in eleven sites around the main island. Two habitats (mangrove and inner barrier reef) were sampled in seven sites and four habitats (mangrove, fringing, intermediate and inner barrier reefs) were sampled in four sites designated as “west coast sites” (S2, S3, S5 and S11). The third sampling took place during austral winter 2011 in the “west coast sites” within the same four habitats.

In reef habitats, large species were collected by spear fishing and small species were anesthetized using clove oil and collected with handnets. In mangroves, gillnets were deployed along mangrove maritime front at high tide and fish were collected at low tide. Different mesh sizes were used in order to catch fish of different size and at different ontogenetic stages. Immediately after collection, fishes were stocked in a freezer (at -20°C) until dissection.

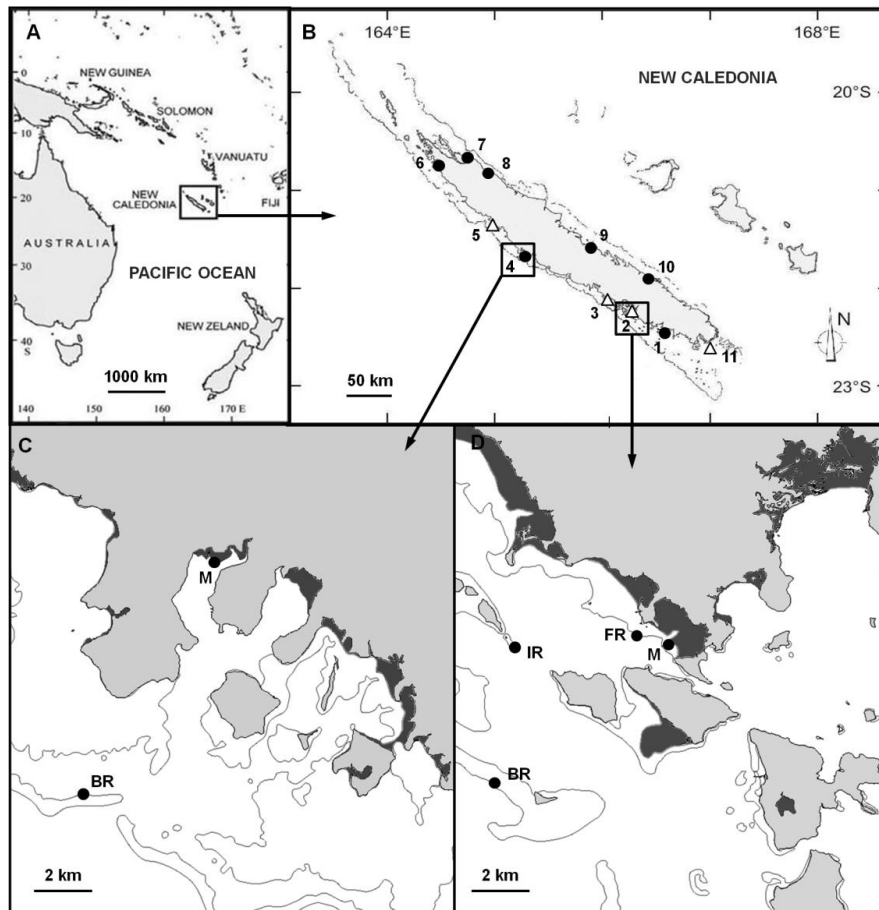


Figure 4.1: (A) Location of New Caledonia in the South West Pacific; (B) study sites: circles and triangles indicate sites sampled with 2 and 4 habitats respectively; (C and D) details of sites S4 and S2 (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

During the first and second samplings, a multi-specific collect has been carried out with respectively 44 and 25 species sampled (Tab. 4.1). The third sampling focused on three target species with contrasting diet and mobility level. *Lutjanus fulviflamma* (Lutjanidae) is a carnivorous Indo-Pacific snapper living in coastal habitats such as mangroves as juvenile and reefs as adults (Thollot 1992b, Carpenter & Niem 2001a). *Ctenochaetus striatus* (Acanthuridae) is an herbivore Indo-Pacific surgeonfish with a limited mobility and a home range restricted to a hundred square meters (Carpenter & Niem 2001b, Claydon *et al.* 2012). *Dascyllus aruanus* (Pomacentridae) is a planktivore and sedentary Indo-Pacific damselfish with a restricted home range to a few square meters (Sale 1971, Carpenter & Niem 2001a).

Three samples of seawater and two samples of sediment were collected in 2010 in each station (Tab. 4.1). Samples were collected at low tide in mangrove and independently of the

tide in reefs. In each station, replicates of seawater and sediment samples were 10 meters apart. Seawater was collected near the sea surface and 10 ml was filtered in a polypropylene tube for chemical analyses using a PES membrane filter (Polyestersulfone, porosity of 0.45 μm). To avoid development of aquatic organisms, seawater was immediately fixed with 0.2 ml of nitric acid (HNO_3 (2%), Suprapur®) and stocked in the laboratory in the dark. Each sediment replicate was constituted of 1 kg of surface sediment and immediately stocked in ice and then in a freezer until lab processing. Fine-grained sediments ($< 63 \mu\text{m}$) were collected under wet conditions using a 63 μm diameter sieve and ultrapure water. Mixtures of fine-grained sediments and ultrapure water were placed in a sterilizer (100°C) to evaporate. Dry fine-grained sediments were ground using an agate mortar and stocked in clean plastic bags, in the dark and dry atmosphere. Temperature (temperature sensor, 0.1°C) and salinity (automatic salinity refractometer, Atago®, 1‰) were measured three times in each station.

Table 4.1: Sample size and analyses realized (with M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef; B: Brest; D: Darwin; M: Montpellier).

Sampling	Year	<i>n</i> stations	<i>n</i> habitats	ICP-MS	<i>n</i> samples analyzed
Otolith	2009	2	2 (M, BR)	LA-ICP-MS	218 (B)
	2010	7	2 (M, BR)	LA-ICP-MS	122 (B) and 592 (D)
		4	4 (M, FR, IR, BR)		
	2011	4	4 (M, FR, IR, BR)	LA-ICP-MS	130(B) and 60 (D)
Seawater	2010	7	2 (M, BR)	SB-ICP-MS	90 (M)
		4	4 (M, FR, IR, BR)		
Sediment	2010	7	2 (M, BR)	SB-ICP-MS	58 (M)
		4	4 (M, FR, IR, BR)		

2.2. Otolith preparation and chemical analyses

All material used for otolith handling was previously decontaminated in 5% ultrapure nitric acid bath (24h), rinsed three times with ultrapure water (18.2 M Ω), dried and stored in clean plastic bags under a laminar flow hood (HEPA 100). At the lab, fish samples were unfrozen and each individual was measured (Fork length, FL) to the nearest mm and weighted to the nearest g. Paired sagittal otoliths were extracted using ceramic tweezers and ultrapure water.

The chemical signatures of sampling sites were characterized by analyzing the chemical composition at the surface of otoliths. To remove organic waste of otoliths surface, otoliths were cleaned following Warner *et al* (2005) method with a bath of 50/50 H₂O₂ (30%, Suprapur[®]) and NaOH (0.1 mol.L⁻¹, Suprapur[®]) solution during 1 hour, sonicated during the last 5 min of the bath, rinsed 5 times with ultrapure water for 5 min, dried under a laminar flow hood (HEPA 100) and stored in individual plastic vials until chemical analyses.

The elemental signatures in otoliths at species level were determined from the analysis of up to five otoliths randomly selected in each sample for each species. There were enough individuals in the samples to determine these mono-specific signatures for eight species: *Dascyllus aruanus*, *Ctenochaetus striatus*, *Chaetodon lunulatus*, *Gerres oyena*, *Gnathodenthes aureolineatus*, *Lutjanus fulviflamma*, *Scolopsis bilineata*, *Siganus lineatus*. However, samples from all 53 caught species were used to determine multi-specific signatures by randomly selecting up to three otoliths in each sample and for each species.

Otoliths were chemically analyzed using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the Pôle de Spectrométrie Océan (Institut Universitaire Européen de la Mer, Brest, France) using a Thermo Element 2 coupled to a laser 193 nm CopexPro 102 Coherent, and at the Resources Institute of Environmental Livelihoods (Charles Darwin University, Darwin, Australia) using a Agilent 7500ce coupled to a UP – 213 nm laser ablation system (Tab. 4.1). Both ICP-MS were operated at low resolution using argon as the carrier gas. Both laser systems parameters were set on a 90 µm laser beam diameter and a frequency of 5 Hz. Each analysis lasted 120 s including 30 s of background and 90 s of ablation (laser activated). The following 20 isotopes were measured: ⁷Li, ¹¹B, ⁸⁵Rb, ⁸⁸Sr, ⁹⁵Mo, ¹¹¹Cd, ¹¹⁷Sn, ¹³⁸Ba, ²⁰⁸Pb, ²³²Th, ²³⁸U, ²⁵Mg, ⁴³Ca, ⁴⁷Ti, ⁵¹V, ⁵²Cr, ⁵⁵Mn, ⁶⁰Ni, ⁶⁵Cu and ⁶⁶Zn.

One laser ablation on the otolith surface was done to reveal the latest elements incorporated from the habitat where the fish was collected. To standardize the analyses, the ablation was always done at the same location, at the tip of the post rostrum on the otolith posterior side. Calcium was used as an internal standard to compensate possible variation due to difference in quantity of material ablated. To correct instrument drift, an external standard (National Institute of Standards and Technology, NIST, 612) was analyzed twice at the beginning and at the end of each session and also every ten ablations.

Raw ICP-MS data (counts per second, cps) were cleaned of outliers defined as values greater than three times the inter-quartile distance during both the blank and the ablation windows (Tukey 1977, Longerich *et al.* 1996, Heinrich *et al.* 2003). Then, data were transformed in elemental concentrations (parts per million, ppm) and limits of detection (LOD) were calculated following Longerich *et al.* (1996). Elemental concentrations lower than the LOD were set to zero. Elements selected for statistical analyses had to meet the following two criteria 1) elemental concentrations in otoliths had to be higher than the LOD 70% of the time in at least one habitat or one site and 2) the coefficient of variation of elemental concentrations in the NIST 612 had to be inferior than 10% (Chittaro *et al.* 2004, Chittaro *et al.* 2006). The use of two different ICP-MS and the time intervals between two sessions realized with the same ICP-MS might influence the absolute values of concentration. To avoid the effects of possible variations, elemental concentrations were transformed in percent of all measured elements.

2.3. Sediment and seawater preparation and chemical analyses

Prior to analyses, successive acid digestions were realized in Teflon® beakers to dissolve sediment (Totland *et al.* 1992). For each sample, 100 mg of fine sediment were digested twice with strong acid attacks (Suprapur® grade HNO₃ at 69% and HF at 40%) for 12 hours. Then, the sediment was digested twice with moderate acid attacks using only HNO₃ (69%) for 12 hours. Sediment and acid mixture was evaporated on hotplate at 130°C after each digestion. A take-back was realized and diluted with ultrapure water and a 1/10 ml aliquot was collected. Both seawater and dissolved sediment were diluted in a final solution with a salinity of 1‰ in a matrix of 2.5% of HNO₃ and 1.0% of Indium for solution-based SB-ICP-MS. To achieve this, 0.25 ml of seawater sample was diluted with 9.4 ml of ultrapure water, 0.25 ml of HNO₃ and 1 ml of Indium. The same dilution was realized with sediment solutions. Indium was used as an internal standard to correct instrument drift.

All environmental samples were analyzed at the Laboratoire Géosciences (Université Montpellier II) using a SB-ICP-MS Agilent 7700x. Elements measured in seawater and sediments were the same than in otoliths; as for otoliths, absolute concentrations in ppm (parts per million) were transformed in percent of all measured elements.

2.4. Statistical analyses

All statistical analyses were performed using R statistical software (R Development Core Team 2011). To describe spatial variation in elemental composition of environmental samples, two-way (site x habitat) PERMANOVA (Legendre & Anderson 1999, Anderson 2001) with 999 permutations and principal component analysis (PCA) were performed.

Multivariate correlations between temperature, salinity, the chemical composition of the environment (seawater and sediment) and the otoliths were evaluated using Mantel correlation tests. Then, Spearman rank correlation tests were used to evaluate the relationships for each element.

Random Forests (RF) algorithms were performed to compare the spatial scales (site, habitat or station) of chemical signatures obtained from otolith and environmental elemental compositions. RF classification method allows freedom from normality and homoscedasticity assumptions (Breiman 2001) and is one of the most powerful methods for classification of habitats using otolith chemical signature (Mercier *et al.* 2011). With the RF classification method, two-third of the data set is used to build a classification tree and the remaining one-third is classified along this tree. The RF algorithm builds 5000 trees to ensure that every sample gets predicted several times which permits to estimate classification accuracy (percent of correct classification). All possible element combinations were tested to find the combination that showed the highest percentage of correct classification. When several best combinations were found, the one with the smaller number of elements was selected.

RF was performed at different spatial scales and for several organizational levels. Three spatial scales were considered; local, regional and global. The local scale focused on discriminations between habitats (mangrove, fringing, intermediate and barrier reef) within each site of the west coast. The regional scale focused on the west coast sites together and discriminations were done between habitats (mangrove, fringing, intermediate and barrier reef) and between stations of a specific habitat. The global scale focused on the 11 sites of the main island and discriminations were done at the level of habitat (mangrove versus barrier reef) and between stations of a specific habitat. Elemental signatures were explored at the species level for eight species (mono-specific signatures), at the multi-species level from 53 species and at the environmental levels on seawater and on sediment.

3. Results

In otoliths, twelve elements met our selection criteria and were thus used in the analyses: B, Ba, Cr, Mg, Mn, Pb, Rb, Sn, Sr, Th, U and Zn. In seawater and sediments analyses, all elements were always above the LOD but in order to compare chemistry of otoliths and environment, the same twelve elements were retained in the environmental data sets. Two samples from Amos seawater samples (S7) were removed from the data-set for all analyses because values of Zn were extreme, revealing a possible contamination of the sample.

3.1. Characterization of environmental chemistry

Temperature of seawater varied between 22 and 26.2°C and salinity varied between 20 and 38 on all stations sampled during the study (Tab. 4.2). Salinity was more variable in mangroves than in reefs with a much greater range of values and CV (Tab. 4.2). This was less clear for temperature with a CV only slightly higher in mangroves than in reefs. There was no correlation between the two variables ($R = -0.02$, $p = 0.928$).

At the global scale, with temperatures data of mangroves and barrier reefs of the eleven sites, two-way PERMANOVA showed a significant interacting factor site x habitat (p-value < 0.001). Temperatures were lower in mangroves than in barrier reefs within five sites and the difference was not significant within the remaining sites. At the regional scale, between the four types of habitat of the West coast sites, two-way PERMANOVA also showed a significant interacting factors site x habitat (p-value < 0.001). However, there was no clear pattern of temperature variation between the habitats and sites of the West coast. Concerning salinity, the two way PERMANOVA performed at global and regional scale resulted in significant interacting factors site x habitat (p-value < 0.001 for both). However, there was no clear pattern among habitat and sites at both scales.

Table 4.2: Mean temperature (°C) and salinity (S) by habitat with SE: standard error, CV: coefficient of variation, N: number of station, *n*: number of measures (M: mangrove, FR: fringing reef, IR: intermediate reef, BR: barrier reef).

	Temperature						Salinity					
	Mean	SE	CV	Range	N	<i>n</i>	Mean	SE	CV	Range	N	<i>n</i>
M	23.6	±1.0	4 %	22 - 25.3	8*	24*	33.6	±3.9	12 %	20 - 37	9*	27*
FR	23.7	±0.8	3 %	22.5 - 24.7	4	12	36.8	±0.6	2 %	36 - 38	4	12
IR	24.1	±0.6	3 %	23.3 - 25.6	4	12	36.7	±0.6	2 %	36 - 38	4	12
BR	25.3	±0.8	3%	23.8 - 26.2	11	33	35.7	±1.0	3 %	34 - 37	9*	27*

(*) Because of technical failures, temperature was not measured in the mangrove of three sites (S7, S9 and S10, see fig. 4.1) and salinity was not measured in the mangrove and barrier reef of two sites (S9 and S10).

Using seawater samples, two-way PERMANOVA showed non-significant interacting factor site x habitat (p-value = 0.99). Sites showed no significant differences (p-value = 0.43) whereas differences between habitats were significant (p-value < 0.01). Plots of PCA of seawater elemental compositions helped refine the significant habitat effect with mangroves clearly distinguished from the three reef types (Fig. 4.2A) and confirmed the lack of differences between sites (Fig. 4.2 B). Mangrove was mostly characterized by Mn and Cr, and to a lesser extent by Sn and Zn (Fig. 4.2 A and C). The three reefs were distinct from mangrove through the presence of Rb. Fringing and intermediate reefs formed two close groups and were mostly characterized by Mg. Barrier reefs were predominantly characterized by the presence of Sr and U.

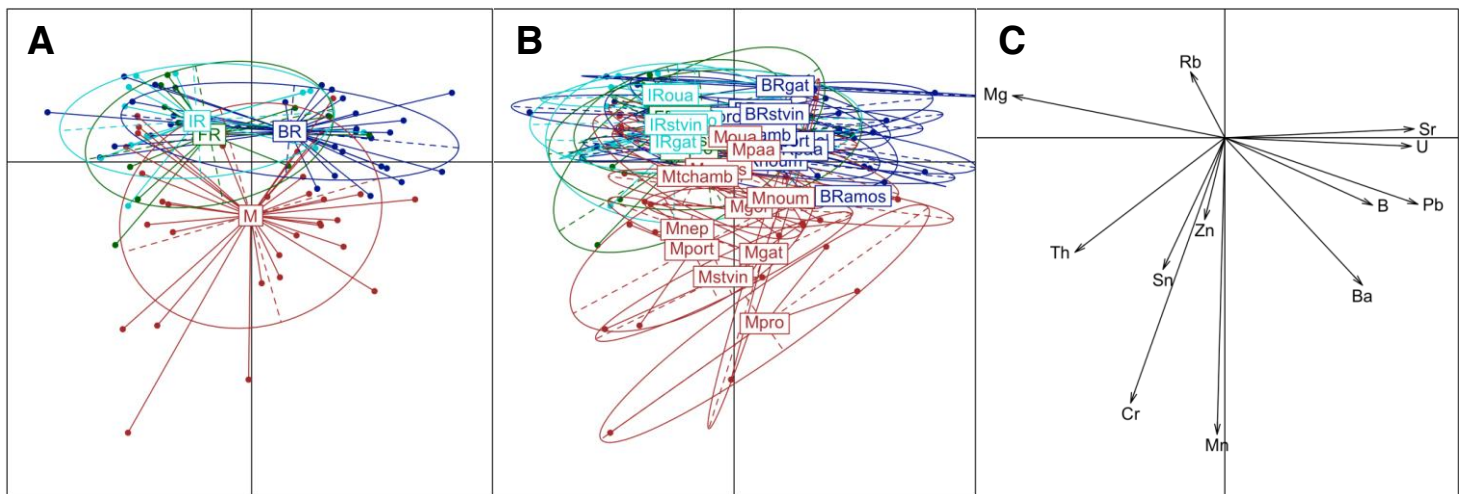


Figure 4.2: Plots of principal component analysis (PCA) of seawater elemental compositions for habitats (A) and stations (B) and plot of trace element contributions in habitat and stations elemental compositions (C) (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

Using sediment samples, a two-way PERMANOVA showed no significant site x habitat factor (p-value = 0.88). There were no significant differences between sites (p-value = 0.54) and between habitats (p-value = 0.65). PCA confirmed that environmental chemistry of habitats or sites could not be characterized using sediment samples (Fig. 4.3).

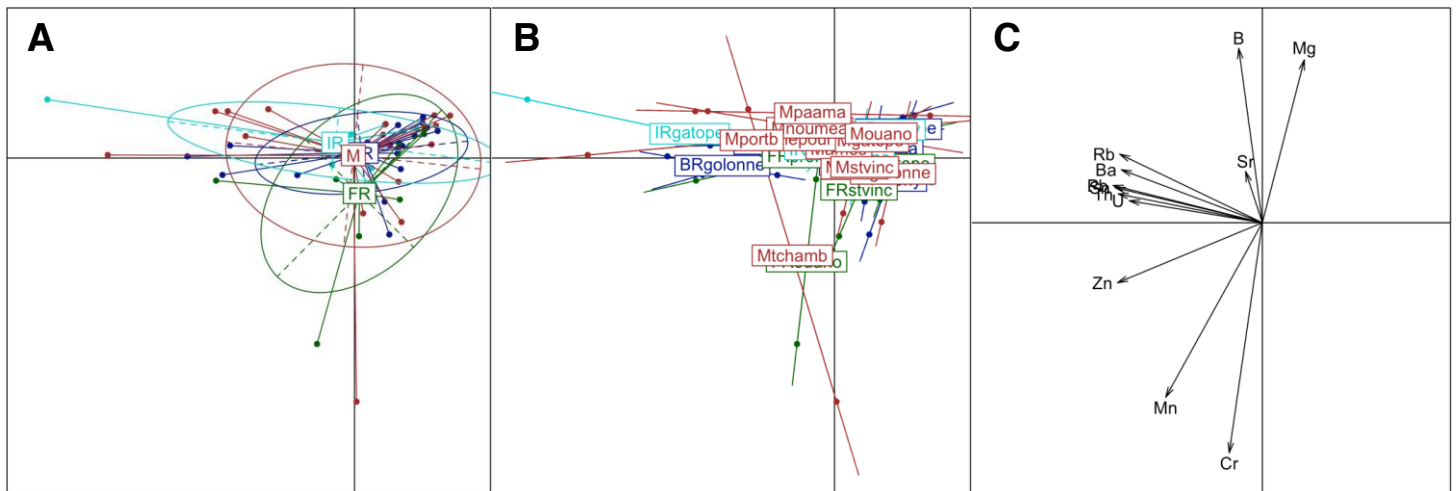


Figure 4.3: Plots of principal component analysis (PCA) of sediment elemental compositions for habitats (A) and stations (B) and plot of trace element contributions in habitat and stations elemental compositions (C) (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

3.2. Correlations between environment and otolith chemistry

Although seawater and sediment elemental compositions were not globally correlated with temperature or salinity (non-significant Mantel tests in Tab. 4.3), several elements showed significant relationships. In seawater, B and Pb were positively correlated and Cr, Mg, Rb and Th were negatively correlated with temperature. Mg was positively correlated and B, Ba, Pb, and U were negatively correlated with salinity. There were no significant correlations between elements in sediment and temperature. B and Sr showed a significant positive relationship and Mg a negative relationship between sediment and salinity. Although seawater elemental composition was not globally correlated with sediment elemental composition (non-significant Mantel tests in Tab. 4.3), Sr was the only one element which showed a significant positive correlation.

Table 4.3: Results of univariate (Spearman tests) and multivariate (Mantel tests) correlations between temperature, salinity and chemistries of seawater and sediment (“***” $p < 0.001$; “**” $p < 0.01$; “*” $p < 0.05$; “+” $p < 0.1$).

	Water Salinity		Water Temperature		Sediment Salinity		Sediment Temperature		Water Sediment	
	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p
B	-0.56	**	0.35	+	0.32	+	-0.06	NS	0.00	NS
Ba	-0.56	**	0.20	NS	-0.11	NS	-0.14	NS	-0.07	NS
Cr	-0.05	NS	-0.43	*	0.02	NS	-0.14	NS	0.25	NS
Mg	0.41	*	-0.38	+	-0.35	+	-0.09	NS	-0.16	NS
Mn	-0.23	NS	-0.32	NS	-0.06	NS	-0.15	NS	-0.07	NS
Pb	-0.38	*	0.44	*	-0.15	NS	-0.29	NS	-0.04	NS
Rb	0.29	NS	-0.40	*	-0.09	NS	-0.18	NS	0.19	NS
Sn	-0.32	NS	0.22	NS	-0.05	NS	-0.31	NS	0.19	NS
Sr	0.02	NS	0.28	NS	0.34	+	0.08	NS	0.31	+
Th	-0.04	NS	-0.44	*	-0.15	NS	-0.24	NS	0.23	NS
U	-0.54	**	0.27	NS	0.10	NS	-0.09	NS	-0.20	NS
Zn	0.07	NS	0.11	NS	-0.09	NS	-0.23	NS	0.06	NS
All	0.10	NS	0.04	NS	-0.10	NS	-0.10	NS	-0.04	NS

Concerning otolith chemistry, multivariate Mantel correlations were significant in several cases but univariate Spearman correlations were not always consistent between species (Tab. 4.4, see Appendix 5 to 8). With temperature, Mantel correlations were significant at the multi-specific and species levels (*L. fulviflamma* and *G. aureolineatus*). At multi-species and for several species, a positive relationship for Pb, Sr, Rb and U and a negative relationship for Cr, Mg, Mn and Zn were observed with temperature. With salinity, Mantel correlations were significant with otolith chemical compositions of *L. fulviflamma* and *C. striatus* only. At the element level, positive correlations were observed with Sr, Zn and U and negative correlations were observed with B, Cr, Mg, Mn, Sn and Zn for multi-species and several species. Mantel correlation tests between seawater and otolith chemistry were not significant except for *G. oyena*. However, spearman rank tests revealed a positive correlation for Mn, Pb, Th and U and a negative correlation for B, Sr and Th. Finally, significant Mantel correlation coefficients were observed between sediment and otolith chemistry for multi-species, *L. fulviflamma* and *G. oyena*. At the species levels, positive correlations were observed for Mn, Sn, Sr, Th and U and negative correlations were observed for Ba, Pb and Rb.

Table 4.4: Results of univariate (Spearman tests) and multivariate (Mantel tests) correlations between temperature, salinity, environmental chemistry and multi-specific otoliths (“****” $p < 0.001$; “***” $p < 0.01$; “**” $p < 0.05$; “+” $p < 0.1$).

	Otolith Salinity		Otolith Temperature		Otolith Seawater		Otolith Sediment	
	Rs	p	Rs	p	Rs	p	Rs	p
B	-0.40	*	-0.20	NS	0.10	NS	-0.25	NS
Ba	-0.31	NS	0.18	NS	0.23	NS	-0.20	NS
Cr	-0.37	*	-0.62	***	0.27	NS	0.05	NS
Mg	-0.38	*	-0.49	**	-0.19	NS	-0.16	NS
Mn	-0.35	+	-0.34	+	0.85	***	-0.08	NS
Pb	-0.27	NS	0.26	NS	0.36	+	-0.07	NS
Rb	0.10	NS	0.29	NS	-0.43	*	-0.27	NS
Sn	-0.56	**	0.09	NS	0.11	NS	0.12	NS
Sr	0.42	*	0.39	*	-0.12	NS	-0.10	NS
Th	0.07	NS	0.06	NS	0.05	NS	0.09	NS
U	-0.24	NS	0.50	**	0.08	NS	-0.06	NS
Zn	-0.41	*	-0.46	*	0.19	NS	-0.01	NS
All	0.11	NS	0.16	+	0.16	NS	0.46	*

3.3. Accuracy of environmental and otolith classifiers

At the local scale, the percentage of correct classification of 4 habitats within west coast sites using seawater dataset ranged between 50 and 79 % (Tab. 4.5). Prony showed the lowest percentage of correct classification (50%), and Ouano presented a moderate correct classification (67%) and Gatope (79%) and St Vincent (71%) showed good percentages of correct classifications. For the sediment, percentages of correct classification were low for St Vincent (43%) and Prony (50%), moderate for Gatope (63%) and high for Ouano (88%). In contrast, percentages of correct classification based on otolith data were high (>70% in most cases) for all species at all sites, except for *L. fulviflamma* and all species combined at the site of Ouano (Tab. 4.5).

At the regional scale, when pooling data of the 4 west coast sites within each habitat, seawater dataset permitted a high rate of correct classification (74%) of the four habitats (mangrove, fringing, intermediate and barrier reef) whereas classification accuracy based on sediment dataset remained low (42%). Similar to seawater, the analysis of elemental fingerprints of the four species tested showed a relatively high level (>64%) of discrimination among habitats at this scale. However, percentage of correct classification was lower (59%) when otoliths from many species were pooled in the RF analyses (Tab. 4.5). Within mangroves, RF analysis

showed an extremely high rate of correct classification (86%) of the four sites using seawater dataset. On the opposite, sediment samples were highly misclassified with only 25% of correct re-assignment. Within fringing reefs, both seawater and sediment showed a low rate of correct classification of the four sites (33% and 50% respectively). Within intermediate reefs, seawater presented a low rate of correct classification of the four stations (50%) unlike sediment (75%). Within barrier reefs, the four stations were misclassified both with seawater and sediment (37% and 57%). As for seawater, percentages of correct classification using otolith data were high in mangroves (83-100% depending on species). For reefs, accuracy of classification varied among species; it was high for *S. bilineata* (78%) and *C. striatus* (71%) in fringing reefs, *D. aruanus* (67%) in intermediate reefs, and *S. bilineata* (100%), *G. aureolineatus* (80%) and *C. lunulatus* (83%) in barrier reefs.

Table 4.5: Summary of RF classification accuracies in percent of environmental samples and otoliths correctly classified to the location they were collected. The highest classification accuracies ($\geq 80\%$) are represented in bold and italic (Gat: Gatope, Oua: Ouano, Pro: Prony, StV: StVincent; M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

Sample	Sample size (<i>n</i>)				Local				Regional				Global			
	M	FR	IR	BR	Gat	Oua	Pro	StV	M/FR/IR/BR	M	FR	IR	BR	M/BR	M	BR
Seawater	57	12	12	41	79	67	50	71	74	86	33	50	35	95	63	22
Sediment	22	8	8	22	63	88	50	43	42	25	50	75	57	76	23	30
Multi-specific	260	175	186	527	63	49	57	69	59	63	42	44	47	90	44	29
<i>L.fulviflamma</i>	37	38	35	86	74	43	78	73	67	100	63	54	48	95	65	29
<i>D.aruanus</i>	/	39	39	90	74	74	67	85	64	/	64	67	60	/	/	41
<i>C.striatus</i>	/	28	33	72	79	83	96	71	71	/	71	64	63	/	/	47
<i>C.lunulatus</i>	/	12	12	46	67	80	67	80	67	/	50	58	83	/	/	41
<i>S.lineatus</i>	41	/	/	/	/	/	/	/	/	84	/	/	/	/	63	/
<i>G.oyena</i>	33	/	/	/	/	/	/	/	/	83	/	/	/	/	67	/
<i>S.bilineata</i>	/	9	/	30	/	/	/	/	/	/	78	/	100	/	/	37
<i>G.aureolineatus</i>	/	/	/	29	/	/	/	/	/	/	/	/	80	/	/	24

At the global scale (eleven sites around the main land of New Caledonia), accuracy of RF classification of mangrove and barrier reef was extremely high with seawater (95%). Sediment also presented a high rate of correct classifications (76%). Within mangroves, the eleven stations were correctly classified with a moderate rate (63%) for seawater but not for sediment (23%). Within barrier reefs, the 11 stations were highly misclassified with both seawater and sediment (22% and 30% respectively). Similar to environmental datasets, classification accuracy of mangroves and reefs were very high with otolith datasets, 90% with

the multi-specific and 95% for the only species (*L. fulviflamma*) that was collected in both habitats. Similar to seawater, otoliths showed moderate rate of correct classification of mangrove stations (63-67% depending on species). As for environment, stations of barrier reefs were highly misclassified using otolith data (24-47% depending on species).

3.4. Comparison of environmental and otolith signatures

Seawater and sediment signatures obtained with RF classification method were generally constituted of fewer elements than otoliths signatures. Furthermore, elements constituting the otoliths signatures for a given scale varied according to species (see Appendix 3 and 9). Despite the fact that all elements constituting the otoliths signatures were not part of the environmental signatures, there were several common elements between both signatures. For instance, the *S. bilineata* otoliths signature obtained to classify the three reefs at the regional scale was constituted of B, Mn, Pb and Th, which were also observed in the environmental signatures (Tab. 4.6). Among the elements constituting the *C. lunulatus* otoliths signature distinguishing the barrier reefs at the regional scale, Ba and Pb were also found in seawater signature and Cr and Sr in sediment signature.

Table 4.6: Comparisons between elements constituting otoliths and environment best combinations for the highest otoliths RF classification accuracies (correct classification > 80%). Elements contributing to both otolith and environmental signatures are in bold. (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).

Specie	Scale	Habitat	Correct classification (%)	Combination of elements
<i>L. fulviflamma</i>	Global	M/BR	95	B Cr Mn Pb Rb Sn Th U
Multi-specific	Global	M/BR	90	Ba Mg Mn Pb Rb Sn U
Seawater	Global	M/BR	95	Mg Mn Zn
Sediment	Global	M/BR	76	B Mg Sr
<i>S. bilineata</i>	Regional	FR/IR/BR	95	B Mn Pb Sr Th
Seawater	Regional	FR/IR/BR	73	B Cr Pb Th
Sediment	Regional	FR/IR/BR	61	B Mn U
<i>L. fulviflamma</i>	Regional	M	100	Ba Cr Mg Pb Rb Sn Zn
<i>G. oyena</i>	Regional	M	83	B Sn Zn
<i>S. lineatus</i>	Regional	M	84	B Mn
Seawater	Regional	M	86	Cr Mg Th
Sediment	Regional	M	25	Cr
<i>C. lunulatus</i>	Regional	BR	83	Ba Cr Mg Mn Pb Sr
<i>G. aureolineatus</i>	Regional	BR	80	Ba
<i>S. bilineata</i>	Regional	BR	100	Mg Mn
Seawater	Regional	BR	35	Ba Pb
Sediment	Regional	BR	57	B Cr Sr
<i>C. lunulatus</i>	Local Ouano	FR/IR/BR	80	Ba Mg Mn Rb Zn
<i>C. striatus</i>	Local Ouano	FR/IR/BR	83	B Rb Sn U
Seawater	Local Ouano	FR/IR/BR	67	Mg Pb
Sediment	Local Ouano	FR/IR/BR	83	Cr
<i>C. striatus</i>	Local Prony	FR/IR/BR	96	B Cr Pb Rb Sn Sr Th Zn
Seawater	Local Prony	FR/IR/BR	33	B Pb
Sediment	Local Prony	FR/IR/BR	50	U
<i>D. aruanus</i>	Local StVincent	FR/IR/BR	85	B Sn Sr Zn
<i>C. lunulatus</i>	Local StVincent	FR/IR/BR	80	Ba Mn Rb
Seawater	Local StVincent	FR/IR/BR	77	Mn Pb
Sediment	Local StVincent	FR/IR/BR	60	Pb

4. Discussion

Otolith chemistry is a powerful tool frequently used in ecology to determine the environmental history of fish. The technique principally relies on the basic assumption that elements from the ambient environment incorporate in the otolith microstructure so that

otolith chemistry reflects the physicochemical properties of the environment. However, results of this study showed that otolith chemistry is more complex than a simple reflection of the chemistry of the environment.

4.1. Characterization of environmental chemistry and spatial scales of discrimination

Within New Caledonian seascape, elemental compositions of environment and otoliths showed similar trends of spatial structuration, nonetheless sediment generally exposed a less powerful rate of correct classification than seawater. Discriminations were higher between habitats when considering contrasted habitats. Mangroves and reefs were greatly differentiated and this difference was consistent through all sites. Discriminations between reefs habitats were lower because of their similar chemistries. Stations of the same habitat were not clearly discriminated except for mangroves stations at the regional scale for otoliths, seawater and sediment.

Mangrove was clearly distinguished from reefs at the local (within sites, 4 habitats), regional (west coast sites, 4 habitats) and global scales (all sites, 2 habitats) with otolith and environmental chemistries. These high discrimination levels could be explained by the specific chemical characteristics of New Caledonian mangroves, particularly high concentrations of Cr and Mn in seawater. Because of its natural configuration, the impacts of intensive mining activities and urbanization contribute to enhance the high natural soil erosion along the shoreline of New Caledonia. This leads to high terrigenous inputs with associated metals (Ni, Cr, Co and Mn) from the catchment to the lagoon (Ambatsian *et al.* 1997). Furthermore, numerous estuaries exist along the coast of New Caledonia, increasing the terrestrial influence through freshwater inputs. Therefore, a gradient of Cr and Mn was observed from the coast to the barrier reef, with higher concentrations in coastal habitats under terrigenous influence and lower concentration in reefs habitats under an oceanic influence.

The power of discrimination obtained from sediment chemistry was generally weaker than seawater resulting in a less obvious spatial structuration. However, the principal component of mangroves sediments were fine-grained sediments which have a high affinity with trace metals whereas reef sediment showed low level of this sediment-bound metal (Marchand *et al.* 2011). Indeed, mangroves could be chemically distinguished from reefs using chemistry of

sediment and constituted one of the highest level of discrimination obtained from sediment elemental composition in the present study.

Fringing and intermediate reefs chemically overlapped and were either moderately spatially discriminated or misclassified. Depending on their location along the west coast, these reefs were either under a high terrestrial influence (high terrigenous inputs) or an oceanic one (direct link to a passage in the barrier reef). When under terrestrial influence, fringing and intermediate reefs were generally grouped together and could be distinguished from both mangrove habitat and barrier reefs, which presented a clear oceanic signature. When under oceanic influence, fringing and intermediate reefs were generally misclassified with barrier reef.

Sr and U predominantly characterized seawater chemistry of barrier reefs. With otolith chemistry, Sr was also the major element characterizing reef habitats. Barrier reefs are distant from shore and under an oceanic influence with rapid renewal of waters (Migon *et al.* 2007). They were clearly distinguished from other reefs habitats in two west coast sites but were mixed with fringing and intermediate reefs in the two remaining sites. The distinction of barrier reef with fringing and intermediate mainly depends on the influence exerting on these last two (oceanic/terrestrial) and also depends on the distance of barrier reef from the coast.

4.2. Correlations between environment and otoliths chemistry

Otoliths are not in direct contact with the surrounding water as elements have to pass three main filters (brachial uptake, cellular transport and crystallization) before incorporating in the otoliths. These filters may either concentrate or dilute elements, meaning that otoliths do not directly reflect the elemental composition of surrounding waters (Campana & Thorrold 2001). Even, if otolith chemistry does not reflect the exact composition of surrounding waters, it may show proportional values and act as a proxy of environmental chemistry. Otolith chemistry would then allow reconstructing the past environment of fish, and trace metal concentrations in the otoliths may be related to the environmental exposure history of fish to contamination. Such a property would also allow using the otolith chemistry tool without the necessity of establishing an otolith chemistry baseline, by sampling directly the environment (Dorval *et al.* 2007). This would be a cost effective way to characterize otolith chemistry and retrace lifetime movement. But if otolith chemistry does not act as a proxy of environmental chemistry, then an otolith library must be established to adequately study life history of fish.

Furthermore, it would not be possible to use otoliths as potential indicators of past metallic contamination.

Before using otoliths to reconstruct environmental histories or define migratory patterns, it is therefore essential to validate the various factors influencing the uptake of trace elements. First, the general assumption was that the deposit of trace element metals in otolith reflected the proportions of dissolved elemental concentrations in the ambient waters (Bath *et al.* 2000). Then, several studies tended to show that elemental composition of otoliths was not only a function of water elemental composition, as salinity and temperature also could influence element uptake (Bath *et al.* 2000, Reis-Santos *et al.* 2013) indicating that the elemental composition of otolith was related to both physical and chemical properties of the surrounding environment, commonly qualified as exogenous factors affecting elemental incorporation (Walther *et al.* 2010).

In the present study, complex and diverse relationships between environment and otolith were observed, depending on the species or the environmental factor considered.

Concentrations of Ba and Sr in otolith generally showed the same relationships with environmental factors as indicated in previous studies. Sr and Ba are known to be incorporated proportionally to their concentration in the water (reviewed in Elsdon *et al.* (2008)). In fact, concentrations of Ba in otoliths (multi-specific and *L. fulviflamma*) showed a positive relationship with Ba in water, as observed in several studies (Elsdon & Gillanders 2002, 2003b, Martin & Thorrold 2005, Martin & Wuenschel 2006, Walther & Thorrold 2006). However, in the present study, Sr concentrations in otoliths did not show a significant relationship with Sr concentrations in seawater unlike indicated in many studies (Bath *et al.* 2000, Milton & Chenery 2001, Elsdon & Gillanders 2003b). This may be explained by the small range of salinity experienced in the present study since no estuary was sampled.

Ba concentrations in otoliths showed a positive relationship with temperature for only one species (*D. aruanus*) confirming the inconsistent results of previous studies indicating positive (Elsdon & Gillanders 2002, 2004) or non significant (Bath *et al.* 2000, Martin & Thorrold 2005, Martin & Wuenschel 2006) relationships. Temperature has generally a positive influence on concentrations of Sr in otoliths (Bath *et al.* 2000, Elsdon & Gillanders

2002, 2004, Martin & Wuenschel 2006), which was also found in our study with the multi-specific group. The effects of salinity on Sr and Ba in otolith are generally described as opposite with negative relationship on Ba (Bath *et al.* 2000, Elsdon & Gillanders 2002, 2003b, Martin & Thorrold 2005, Martin & Wuenschel 2006, Walther & Thorrold 2006) and positive relationship on Sr (Bath *et al.* 2000, Elsdon & Gillanders 2003b, Walther & Thorrold 2006, Miller 2011). In the present study, salinity showed a negative relationship with Ba in seawater but not in otolith as generally described. The positive relationship between salinity and Sr in otolith was found in our study with the multi-specific group.

Mn and Cr presented a coastal to ocean gradient concentrations with high concentrations in mangrove where temperature and salinity are slightly lower compared to reefs. The two elements showed similar relationship between concentrations in otoliths and environmental factors. A positive relationship between Mn in otoliths and in seawater was observed at the multi-specific level and for *L. fulvivflamma* and was also reported by Hamer *et al.* (2007) with the otoliths of the snapper *Pagrus auratus*. However, non significant links were reported by Elsdon and Gillanders (2003b) with otoliths of the Sparidae, *Acanthopagrus butcheri*. A positive relationship was observed between Cr in *L. fulvivflamma* otoliths and seawater. A negative relationship between Mn in otoliths and temperature was observed with the multi-specific group and the three species. This negative relationship was stronger for species sampled in mangrove (multi-specific and *L. fulvivflamma*). It was also found negative in the literature (Miller 2009) or non significant (Elsdon & Gillanders 2002). A negative relationship between Cr in otoliths and temperature was observed at the multi-specific level and for *L. fulvivflamma*, which is present in mangrove, and *C. striatus*, which is not present in mangrove. Finally, a negative relationship with salinity was observed with Mn in multi-specific otoliths only.

To our knowledge, no study focusing on factors affecting Cr incorporation in otolith was published. The multiple relationships described in this study could be related to the higher concentration of Mn and Cr in coastal habitats where temperature and salinity are lower. Consequently, our results combined to a lack of literature on both elements did not allow us to discriminate the effect of each factor on elemental incorporation and to identify which one may drive the incorporation of Mn and Cr in otoliths.

In the present study, Mg in seawater showed a positive relationship with salinity whereas it was negatively correlated with Mg in otoliths, the latter being also negatively correlated with salinity and temperature. The negative relationship of Mg in otoliths with temperature was observed at the multi-specific level and for *L. fulviflamma* and *D. aruanus*. The negative relationship with salinity was observed only with the multi-specific group. Our results contrasted with other studies focusing on exogenous factors driving Mg incorporation which did not found significant correlation with salinity or temperature (Elsdon & Gillanders 2002, Martin & Thorrold 2005, Dorval *et al.* 2007). Furthermore, Mg is not considered as a reliable environmental indicator because it is suspected to be physiologically regulated (Martin & Thorrold 2005, Hamer & Jenkins 2007, Miller 2011, Woodcock *et al.* 2012). Even if water was proved to be the primary source of otolith Mg, concentrations in the otolith did not change with its concentration in water (Dorval *et al.* 2007, Woodcock *et al.* 2012).

Zn is characteristic of anthropogenic inputs and can be a great indicator of past and actual urban pollutions (Bruland *et al.* 2003). In the present study, a negative relationship between Zn in otolith and temperature was observed at the multi-specific level and for *D. aruanus*. A positive relationship between Zn in otolith and salinity was observed for *D. aruanus*. In seawater, Zn follows nutrient-type distribution defined by relatively high concentrations in coastal waters compared to oceanic waters masses (Bruland *et al.* 2003). In the present study, Zn was not the major element distinguishing coastal habitat from offshore habitat but participated to mangrove distinction even so. Therefore, Zn concentrations were higher in mangrove habitat where temperature and salinity were lower compared to reef habitats. In their study, Ranaldi and Gagnon (2008) did not rely Zn concentrations in water with concentrations in otolith. In Thorrold *et al.* (1997) study, Zn concentration in otoliths was not related to Zn concentration in ambient water. On the opposite, Arai *et al.* (2007) stated that Zn appeared to be deposited in otoliths in relation to Zn concentration in the surrounding water. Both studies retrospectively determined Zn concentrations throughout life of fishes, from the core to the edge of otoliths. However, they did not possess retrospective data of temperature and salinity of waters crossed by fish. Hence, these authors could not take into account the possible influence of temperature and salinity on Zn uptake in otolith. Consequently, our results combined to previous studies focusing on Zn uptake did not allow us to discriminate individual effect of the three exogenous factors (water chemistry, temperature and salinity) on Zn incorporation in the otolith.

Positives correlations between concentrations of Pb in otoliths of the multi-specific group and of *C. striatus* were observed with temperature and with concentrations of Pb in seawater. The last relationship was previously described under experimental conditions in two studies (Geffen *et al.* 1998, Milton & Chenery 2001) where authors indicated that Pb concentrations in otolith were related to water concentrations.

In the present study, positive correlations between concentrations of Rb in otolith for the multi-specific group, *L. fulviflamma* and *C. striatus* were observed with temperature. On the opposite, negative relationships between concentrations of Rb in otoliths of the multi-specific group and of *L. fulviflamma* were observed with concentrations of Rb in seawater. A negative relationship between Rb in seawater with temperature was observed. Rb presents a conservative-type distribution in seawater, defined by concentrations that maintain a relatively constant ratio to salinity (Bruland *et al.* 2003). To our knowledge, only one study focused on variations of Rb concentration in otolith across an experimental salinity gradient (Hicks *et al.* 2010). The authors found a decrease of Rb concentrations in otoliths with salinity and no link between Rb concentrations in otoliths and in water despite high Rb concentrations in seawater. In the present study, we did not observe a relationship between salinity and concentrations of Rb in otoliths. However, the range of salinity in the present study was much smaller than in Hicks *et al.* (2010) study. Consequently, our results indicated complex relationships of Rb in otolith and Rb in seawater, and with temperature. It is difficult to identify which exogenous factors drive the uptake of Rb in otolith.

A negative relationship between concentrations of Sn in otoliths and temperature was observed at the multi-specific level and *L. fulviflamma* and *D. aruanus*. The links between concentration of Sn in otoliths and salinity were heterogeneous depending on the species considered. Relationship with salinity was negative with Sn in *L. fulviflamma* otolith but was positive with *D. aruanus* and *C. striatus* otoliths. Concentrations of B in otoliths showed a few and mixed correlations with environmental settings. None of them were consistent between species. Concentrations of Th and U in otoliths had no relationship with the environmental factors. To our knowledge, no published study focused on the relation between environmental settings and concentrations of Sn, B, Th and U in the otolith.

Relationships between elemental concentrations in sediment and elemental concentrations in otoliths, temperature and salinity were weak. However, *C. striatus* showed recurrent relationships between metal concentrations in otoliths and in sediment (positive with Mn, Mg and Sr; negative with B). *C. striatus* is a micro-herbivore with a significant detritus component in its diet. As it scrapes the sea bottom to collect microalgae the fish also ingests surface layer with diatoms and detritus (Carassou *et al.* 2013). Relationships with sediment chemistry may be related to its diet since it is known that food may have an effect on elemental incorporation (Buckel *et al.* 2004).

Beyond exogenous factors, several studies highlighted the role of endogenous factors on elemental uptake suspecting to be species-specific (Gillanders & Kingsford 2003, Swearer *et al.* 2003, Hamer & Jenkins 2007). Diet (Buckel *et al.* 2004), somatic growth rate, genetic (Barnes & Gillanders 2013), ontogenetic stage (Fowler *et al.* 1995) and even stress are suspected to influence elemental incorporation in the otoliths (Marohn *et al.* 2009, Sturrock *et al.* 2012). Endogenous factors are suspected to affect incorporation differently between species and variations may exist within species, depending on the ontogenetic stage or the somatic growth rate of individuals (Hamer & Jenkins 2007). In the present study, the effects of endogenous factors could not be taken into account.

In New Caledonia, there is a set of elements that appeared to provide the best discriminatory power for relating otolith metal concentration to the environmental history of the fish. Mn and Cr representative of coastal area, concentrations in otoliths showed relationship with water chemistry, temperature and salinity. We cannot identify separately which environmental factors drive the uptake of metal in otoliths.

According to literature, Mg is subject to strong physiological regulation susceptible to be specie specific (Martin & Thorrold 2005, Hamer & Jenkins 2007). The relationships of exogenous factors with Mg in otoliths were inconsistent except with temperature, which may be implicated in the physiological regulation. The relationships observed with concentrations of Sn, B, Th and U in otoliths were too mixed, balancing between no consistency between species (which could reflect an eventual specie-specific incorporation) or no relationship. For these elements, there is a lack of bibliography to interpret the inconsistency as a specie-specific process of incorporation.

5. Conclusion

Otolith was considered by Bath (2000) as an excellent record of the physicochemical properties of both present and past aquatic environment when focusing on Ba and Sr variations. This hypothesis was reassessed by other studies with contrasting results and by additional studies examining the influence of endogenous factors on elemental uptake (Buckel *et al.* 2004, Barnes & Gillanders 2013). The mechanisms driving the relationship between elemental concentration in otoliths and environment characteristics like water and sediment elemental compositions, temperature and salinity, or with endogenous factors are complex and not entirely understood (Martin & Wuenschel 2006). Consequently, precautions should be taken when using otolith chemistry as proxies for environmental conditions (Walther *et al.* 2010).

The present study highlights the necessity to test several elements as environmental markers and to not be limited on Ba and Sr, as elements like Mn and Cr are potentially indicative of coastal waters and potentially reflect water metal concentrations. In order to understand the driving mechanisms regulating elemental incorporation, there is a need to complete field studies by experimental designs with fixed control conditions reflecting a natural range of variations of environmental settings (temperature, salinity and water chemistry), along with a control of endogenous factors with fixed diet, measured of somatic growth rate and experimentation with individuals of the same species and ontogenetic stage (cohorts-specific). The experimental and field study need to be done for several species with contrasting ecological specificities (*i.e.* different diets) and taxonomically different (*i.e.* belonging to different families).

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Ce chapitre a permis de comparer le pouvoir discriminant des signatures microchimiques présentes dans les otolithes de poisson et dans leur environnement.

La structuration spatiale de la microchimie de l'eau correspond à la structuration obtenue à partir des otolithes. Le pouvoir de discrimination spatiale est fort à l'échelle des habitats à partir de la microchimie de l'eau, particulièrement entre la mangrove et le récif barrière. A l'échelle des stations, la discrimination est faible excepté pour les mangroves où la microchimie est plus hétérogène qu'au sein des récifs. Contrairement à l'eau, la discrimination spatiale à partir de la chimie des sédiments est généralement faible à l'échelle des sites et des habitats, excepté entre la mangrove et le récif barrière.

Les relations entre la microchimie des otolithes, celle de l'environnement et les paramètres physico-chimiques de la masse d'eau (température et salinité) sont complexes et ne sont pas toujours constantes entre les espèces. La chimie des otolithes n'est donc pas l'exact reflet de la chimie de l'eau car les otolithes et l'eau environnante ne sont pas en contact direct ; des éléments franchissent plusieurs barrières physiologiques avant d'être incorporés à l'otolithe (Campana 1999). Toutefois, la forte structuration spatiale observée à partir de la chimie de l'eau s'exprime de la même façon avec la chimie des otolithes. Ces résultats permettent l'utilisation logique de la microchimie des otolithes comme traceurs des environnements traversés par les poissons au cours de leur vie.

**Chapitre 5 : Importance du rôle de nurserie des mangroves de
Nouvelle-Calédonie pour un poisson corallien, *Lutjanus
fulviflamma***



Chapitre 5 : Importance du rôle de nurserie des mangroves de Nouvelle-Calédonie pour un poisson corallien, *Lutjanus fulviflamma*

La notion d'habitat juvénile ou « nourricerie » ou « nurserie » est essentielle à la dynamique des populations marines (Beck *et al.* 2001, Adams *et al.* 2006, Dahlgren *et al.* 2006, Layman *et al.* 2006, Sheaves *et al.* 2006). L'efficacité d'un plan de conservation ne peut être optimal si les habitats juvéniles essentiels pour les espèces protégées ne sont pas identifiés (Beck *et al.* 2001, Beck *et al.* 2003, Gillanders *et al.* 2003, Jones *et al.* 2010). Il est donc crucial d'identifier les habitats juvéniles et d'estimer leur contribution au renouvellement des populations adultes afin de cerner les habitats à protéger en priorité (Rooker *et al.* 2010).

La microchimie est un outil puissant couramment utilisé pour retracer l'utilisation des habitats au cours de la vie et donc pour l'identification des habitats juvéniles. Au cours de ce chapitre, le rôle de la mangrove comme habitat juvénile pour la dorade à tache noire *Lutjanus fulviflamma* en Nouvelle Calédonie est évalué à travers :

- 1) La quantification de la proportion d'individus adultes ayant occupés une mangrove comme habitat juvénile et l'estimation du temps passé au sein de cette mangrove.
- 2) L'évaluation de la distribution des mangroves calédoniennes en tant que déterminant principal de la distribution de l'espèce dans l'archipel.

Le travail méthodologique de caractérisation de la microchimie des otolithes réalisé dans les chapitres précédents permet de révéler l'habitat juvénile de *L. fulviflamma* et de statuer sur son caractère accessoire, essentiel ou obligatoire. Les données historiques sur l'abondance de l'espèce en Nouvelle-Calédonie combinées à la cartographie des mangroves permettent ensuite d'évaluer l'influence de cet habitat juvénile sur la distribution des populations adultes.

Manuscrit C: Essentialness of mangroves as nursery habitat for a coral reef fish in New Caledonia.

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Abstract

For many fish species, mangroves of the Western Atlantic have long been recognized as essential nurseries connected to coral reefs through ontogenetic migrations while there is still a debate about the role of mangroves in the Indo-Pacific. The present study attempted to estimate the importance of New Caledonian mangroves for juveniles of the blackspot snapper, *Lutjanus fulvivflamma*. Elemental composition of *L. fulvivflamma* otoliths was used to characterize the chemical signatures of mangrove and reef habitats of the main island of New Caledonia, and to estimate the proportion of adults inhabiting mangroves as juvenile. Then, the link between the distribution of *L. fulvivflamma* and mangroves in New Caledonia was tested using underwater visual census (UVC) and mangrove databases. Otolith elemental

compositions allowed a high percentage of discrimination between mangrove (83.8%; characterized by Ba, Cr, Mn and Sn) and reef ecosystems (98.7%; characterized by Rb and Sr). All *L. fulviflamma* adults analyzed presented a mangrove signature during their juvenile stage with 75% inhabiting mangrove during the entire juvenile part of otolith analyzed. Furthermore, the distributions of adult *L. fulviflamma* and mangroves were positively correlated ($r = 0.80$ for occurrence, 0.91 for density and 0.86 for biomass). Importantly, *L. fulviflamma* was absent from isolated islands of the New-caledonian archipelago where mangroves were absent. These results emphasize the essentialness of mangrove in the life cycle of *L. fulviflamma* in New Caledonia as an obligatory juvenile habitat for this species.

Key words: *Lutjanus fulviflamma*, otoliths, nursery, mangrove, coral reefs, UVC data

1. Introduction

Coral reefs and mangroves belong to the most diversified ecosystems of the world and have a high economic value (Bellwood & Hughes 2001). They are also considered as deeply impacted, with 30 % of coral reefs already severely impacted and a decrease of 50% of mangrove original distribution (Hughes *et al.* 2003, Mumby *et al.* 2004, Burke *et al.* 2011). Natural disturbances in conjunction with increasing human pressure lead to a dramatic loss of biodiversity and habitats for these systems (Nystrom *et al.* 2000, Pandolfi *et al.* 2003, Bellwood *et al.* 2004, Wantiez *et al.* 2006, Sandin *et al.* 2008, Mora & Sale 2011).

Coral reefs seascapes are fragmented ecosystems. These patchy structures imply a fragmentation of reef organisms in local populations connected by movements of individuals (Sale *et al.* 2010). Because most coral reefs species show a complex life cycle with a pelagic larval phase and a benthic juvenile and adult phase, connectivity can be achieved by movements of individuals at the larval, juvenile and/or adult stages. Typically, competent larvae return to the reef environment and settle in shallow-water habitats like mangroves, seagrass beds or reefs (Sale 1991). These shallow habitats may serve as nurseries where these juveniles may benefit from several advantages such as food and shelter from predators (Parrish 1989). As these fish grow, they move to coral reefs and recruit in the adult populations (Mellin *et al.* 2007).

In order to protect biodiversity, Beck (2001) pointed up the necessity to include nursery habitats in MPA networks. Identification of juvenile habitats for species with complex life cycle is therefore critical for the management and conservation of species (Chittaro *et al.* 2009, Sale *et al.* 2010). In particular, the direct measure of connectivity between juvenile and adult populations would allow managers to identify and protect the most productive nurseries (Adams *et al.* 2006). This is important especially when these nurseries are vulnerable to degradations and when connectivity between juvenile and adult habitats is threatened (Beck *et al.* 2001, Brown 2006b), which is the case for the mangrove – coral reef system.

One type of movement connecting mangrove to coral reefs is ontogenetic migration. Many of the coral reef species that use ontogenetic shift as life-history strategy use mangrove as juvenile habitat (e.g. Serranidae, Lutjanidae, Lethrinidae, etc.) (Jones *et al.* 2010). Mangrove

are thought to be adequate juvenile habitat because they can provide shelter from predation (complexity of roots, turbidity), supply food in abundance with an adapted size (zooplankton, little crustacean and fishes), and therefore increase the overall rate of survival for juveniles (Parrish 1989, Beck *et al.* 2001, Laegdsgaard & Johnson 2001, Jones *et al.* 2010). As a result, there can be strong relationships between fish abundance and the presence of mangroves. For example, numerous studies conducted in the Caribbean, showed greater biomasses of Lutjanidae in reefs close to mangroves (Nagelkerken *et al.* 2000, Mumby *et al.* 2004, Lugendo *et al.* 2007). For 17 species of Caribbean fish, Nagelkerken *et al.* (2000) showed a positive relationship between presence of mangrove and abundance on nearby reefs. For regions lacking mangrove, 11 of the 17 species were not recorded or were seen in low densities, highlighting the important role of mangroves in the life cycle of these species.

However, some species using mangrove during their juvenile life stage can also be observed in high density in other type of juvenile habitats (seagrass beds, soft bottoms, fringing reefs) (Mellin *et al.* 2007). Furthermore, Jones *et al.* (2010) showed that the absence of mangrove may have no significant impact on adult fish population densities on nearby reefs. Thus, mangroves may have a variable importance as nursery depending on the species and the region. Some species may use mangrove as obligatory juvenile habitat if they cannot complete their life cycle without mangroves. In this case, it is expected that these species are absent when mangroves are absent. For other species, mangrove may simply be an essential nursery habitat with a majority of juveniles using it. In that case, the species can be present when mangroves are not but fish abundances should increase with mangrove extent. Finally, mangrove may be an accessory nursery habitat with only a minority of juveniles found in mangroves (Adams *et al.* 2006). In this case, no particular relationship should be expected between mangrove extent and species abundance.

The connectivity between mangroves and reefs for ontogenetic shifters suggests that the distribution of juvenile habitats may be a primary determinant of species distribution when the juvenile habitat is an obligatory one. Because marine organisms disperse mostly by ocean currents as larval propagules, it has often been assumed that larval transport was the primary determinant of population connectivity and geographic range size. However, in a recent paper Luiz *et al.* (2013) showed that adult traits were equal or better predictors of geographic range size than larval traits, highlighting the importance of post-settlement processes likely to affect

the capacity of new colonizers to survive and establish reproductive populations. To some extent, the study by Luiz *et al.* (2013) converges with the concept of “realized connectivity” (Hamilton *et al.* 2008), which takes into account the post-settlement fitness of individuals with different larval traits. If larval and adult traits are important to the survival of individuals after their movement to a new habitat, and ultimately to population connectivity and geographic range size, then juvenile traits may also matters.

Testing whether the distribution of juvenile habitats is a primary determinant of species distribution therefore requires determining if habitats such as mangroves are obligatory, essential or accessories juvenile habitats. This implies back-calculating the proportion of adults that effectively used a given habitat as nursery. Methods used to directly measure connectivity between juvenile and adult populations require natural or artificial habitat-specific markers (Chittaro *et al.* 2004, Adams *et al.* 2006, Levin 2006). Among these methods, microchemistry of otoliths is one of the most powerful tools to identify juvenile habitats when adult and juvenile populations are spatially distinct (Gillanders & Kingsford 2000, Gillanders *et al.* 2003, Gillanders & Kingsford 2003, Gillanders 2005, Brown 2006b, Jones *et al.* 2010). Otoliths are paired calcified structures located in the inner ear of fishes. They show continuous growth allowing an incorporation of trace elements during the entire lifetime of a fish. Due to their inorganic nature, trace elements are not resorbed after incorporation. These key properties allow the use of otoliths as natural recorders of the habitats inhabited by fish throughout their life (Campana 1999). In particular, it is possible to analyze the chemical composition of the juvenile part of the otolith of an adult and retrospectively determine the habitats used by this adult when it was a juvenile.

The purpose of this study is to 1) investigate whether, in New Caledonia, mangroves are obligatory, essential or accessories juvenile habitats for *Lutjanus fulvivflamma* (Forsskal, 1775) (Lutjanidae) and 2) test whether the geographic distribution of mangroves in New Caledonia could explain the adult distribution of this species. To achieve this, we used otolith microchemistry to characterize the chemical signature of mangrove and reef habitats all around the island and to estimate the proportion of adults which used mangrove as a juvenile habitat. Then, we used an underwater visual census (UVC) database containing 2910 underwater transects to determine if the presence and/or the abundance of this species were correlated with the presence and/or the abundance of mangroves.

2. Materials and methods

2.1. Sampling protocol

New Caledonia is an archipelago located in the South West Pacific, 1500 km east of Australia, between latitudes 19°S and 23°S and longitudes 163°E and 168°E (Fig. 5.1). It is composed of a main island (Grande Terre), three groups of smaller islands (Loyalties, Isle of Pines and Belep) and some uninhabited islands and offshore reefs (Entrecasteaux, Chesterfield, Astrolabe, Petri, Matthew, Hunter and Walpole). The main island holds the largest lagoon of the world, with a linear barrier reef of 1744 km long and a surface area of 31 336 km² (Andréfouët *et al.* 2009). Distance between the main island and the barrier reef varies from 1 to 65 km (Paris 1981) and major parts of the lagoon are registered on the Unesco heritage list.

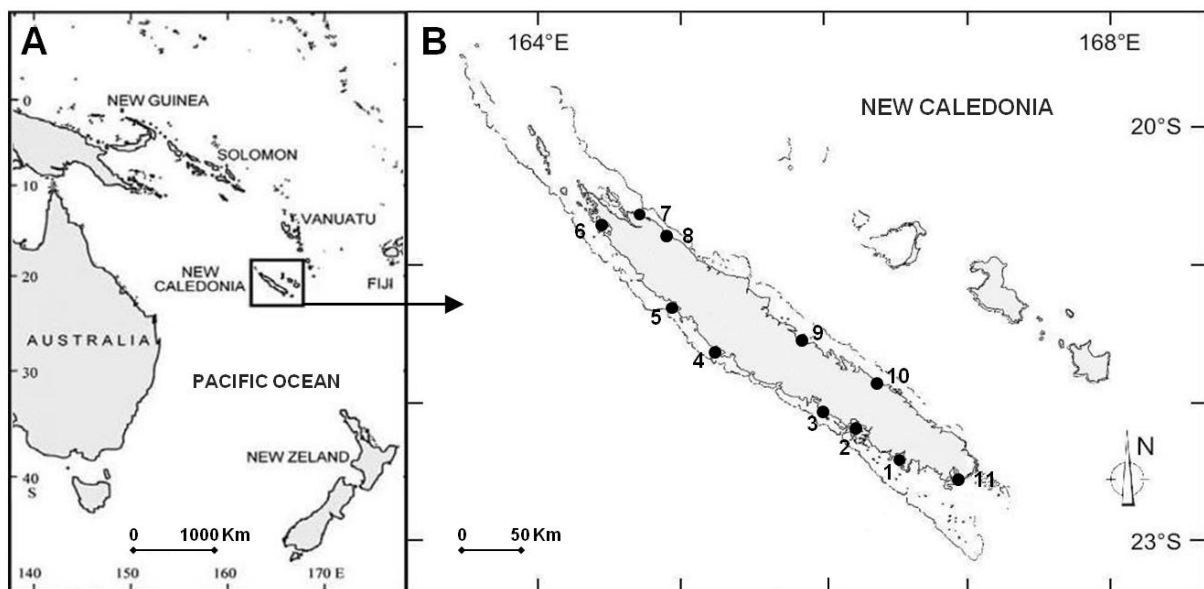


Figure 5.1: (A) New Caledonia geographic situation in the South West Pacific. (B) Study sites in New Caledonia: circles indicate sites of otolith microchemistry study

Lutjanus fulviflamma (Forsskål, 1775) or blackspot snapper (Family Lutjanidae) is a small Indo-Pacific snapper commonly targeted by subsistence fisheries. It is a carnivorous species with a mean size of 25 cm, found in different habitats according to its ontogenetic stage. Adults inhabit coral reefs between 3 to 35 m deep and juveniles are known to use shallow-waters habitats like mangrove (Loubens 1980, Thollot 1992a, Carpenter & Niem 2001a).

Fish were sampled for otolith analysis in different mangrove and reef habitats at 11 sites around the main land of New Caledonia over three years (2009, 2010 and 2011) (Fig. 5.1). Adults were collected in reef habitats by spear fishing and juveniles were collected in mangroves using small mesh (7 mm) gillnets surrounding an isolated mangrove tree. Gillnets were deployed at high tide and fish collected with handnets in a few cm of water and/or on the ground at low tide. Immediately after collection, fishes were stored in a freezer (at -20°C) until dissection. The first sampling took place during austral summer 2009 in the mangroves and inner barrier reefs of two sites (Gatope (2) and St Vincent (5)). The second sampling has been carried out during austral winter 2010 in the mangroves and inner barrier reefs of eleven sites around the main island. During this sampling, fringing and intermediates reefs were also sampled at four sites (Gatope (5), Ouano (3), Prony (11) and St Vincent (2)). Sampling in the mangroves, fringing, intermediate and inner barrier reefs of these 4 sites was replicated during austral winter 2011 (Tab. 5.1). Thus, our sampling included fish collected in different years, seasons and habitats, which is important as chemical signatures stored in fish otoliths may vary in space and time, and spatio-temporal variability needs therefore to be included in the characterization for robust chemical signatures in otolith (Mercier *et al.* 2012).

Table 5.1: Sampling design in order to: A. characterize reefs and mangrove chemical signatures by LA-ICPMS of the surface of otoliths and B. determine the juvenile habitat by LA-ICPMS of transverse sections of otolith along transects from core to 1260 μm

	Year	Site	Habitat	Laser analysis	<i>n</i> otoliths
A. Characterization of habitat signatures	2009	2	Mangrove and Reefs	Ablation on surface	20
	2010	11	Mangrove and Reefs	Ablation on surface	114
	2011	4	Mangrove and Reefs	Ablation on surface	62
B. Determination of juvenile habitats	2010	4	Barrier Reef	Transects	20

2.2. Otolith preparation

All equipment used for otolith handling was previously decontaminated in 5% ultrapure nitric acid bath during 24h, rinsed three times with ultrapure water (18.2 M Ω), dried under a laminar flow hood (HEPA 100) and stored dry in plastic bag under the flow hood.

At the lab, fish samples were unfrozen and each individual was measured (Fork length, FL) to the nearest mm and weighted to the nearest g. Paired sagittal otoliths were extracted using ceramic tweezers, cleaned with ultrapure water (18.2 M Ω) and stored dry in plastic vials.

The chemical signatures of reefs and mangroves were characterized by analyzing the chemical composition at the surface of otoliths of up to five fish randomly selected in each sample (Tab. 5.1A). Otoliths of these fishes were deeply cleaned of organic material following the Warner *et al.* (2005) method: otoliths were bathed in a 50/50 H₂O₂ (30%, Suprapur[®]) and NaOH (Suprapur[®] 0.1 mol.L⁻¹) solution during 1 hour, sonicated during the last 5 min of the bath, rinsed 5 times with ultrapure water for 5 min, dried under a laminar flow hood (HEPA 100) and stored dry in individual vials until microchemistry analyses.

The juvenile habitats actually used by adults were inferred from the analyses of chemical composition along transverse otolith sections of 20 adult fishes collected on the barrier reefs of Gatope, Ouano, Prony and St Vincent (5 fish per site, Tab. 1B). Transverse otolith sections including otolith's core were prepared by embedding right sagittal otoliths in resin (Araldite 2020[®]), sectioning transversely with a low speed saw (Buehler[®]), and polishing using sandpaper of decreasing grain (from 800 to 1600 grains/cm²), lapping film (9 to 1 μ m) and diamond-tipped polishing powders (Struers[®]). Final sections were rinsed in ultrapure water, dried under laminar flow hood (HEPA 100), mounted on a microscope slide (10 sections per slide) with double-side tape and stored in plastic boxes until microchemistry analyses.

2.3. Otoliths LA-ICP-MS analyses

Otoliths were chemically analyzed by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the Pôle de Spectrométrie Océan (Institut Européen Universitaire de la Mer, Brest, France) using a Thermo Element 2 coupled to a laser 193 nm CopexPro 102 Coherent, and at the Research Institute of Environment and Livelihoods (Charles Darwin University, Darwin, Australia) using an Agilent 7500ce coupled to a UP – 213 nm laser ablation system. Both ICP-MS were operated at low resolution using argon as the carrier gas, and with the laser system parameters set on a 90 μ m laser beam diameter and a frequency of 5 Hz. Each analysis lasted 120 s including 30 s of background and 90 s of

ablation (laser activated). To discriminate habitats, the following isotopes were measured: ^7Li , ^{11}B , ^{85}Rb , ^{88}Sr , ^{95}Mo , ^{111}Cd , ^{117}Sn , ^{138}Ba , ^{208}Pb , ^{232}Th , ^{238}U , ^{25}Mg , ^{43}Ca , ^{47}Ti , ^{51}V , ^{52}Cr , ^{55}Mn , ^{60}Ni , ^{65}Cu and ^{66}Zn .

To access the latest microchemistry signature representative of the habitat where the fish was caught, one laser ablation was done on the surface of the otolith of each subsampled fish (up to 5 individuals per sample). To standardize the analyses, the ablation was always done at the same spot at the tip of the post rostrum on the posterior side of otoliths.

To determine the juvenile habitat of adults, the juvenile part of otolith transverse sections were analysed by LA-ICPMS for each of the 20 adult fish collected in the barrier reefs of Gatope, Ouano, Prony and St Vincent. Because the otolith radius of the smallest juvenile caught in our samples was 1260 μm , we considered this radius as the juvenile part of otolith sections. For each adult, one LA-ICPMS transect was analysed. It consisted in ten successive laser ablations (point-by-point mode) from the core to 1260 μm by increment of 140 μm along the longest otolith growth axis.

To compensate possible variations due to differences in quantity of material ablated, calcium was used as an internal standard. An external standard (National Institute of Standards and Technology, NIST, 612) was analyzed twice at the beginning and at the end of each session (8 otoliths per session) and also after ten ablations for transect in order to correct instrument drift.

Raw ICPMS data (counts per second, CPS) were first cleaned of outliers, defined as any value greater than three times the inter-quartile distance, during both the blank and the ablation windows (Tukey 1977). Cleaned CPS were then transformed in elemental concentrations (parts per million, ppm) and limits of detection (LOD) calculated following Longerich *et al* (1996). Only the elements that met the following two criteria were kept in statistical analyses: 1) elemental concentrations had to be greater than the LOD in 70% of the otoliths in at least one habitat (e.g. mangrove) or one site (e.g. Ouano) and 2) the coefficient of variation of concentrations in the NIST 612 had to be less than 10% (Chittaro *et al.* 2004, Chittaro *et al.* 2006). When the concentration of an element was under the LOD, it was set to zero. By definition, this occurred in less than 30% of cases in at least one habitat or one site (otherwise

the element was removed from our analyses based on criterion 1). To reduce variation originating from analyses produced by two different ICP-MS, elemental concentrations were transformed in percent of all measured elements.

2.4. UVC surveys and mangrove area calculation

Fish diversity on the coral reefs of New Caledonia has been surveyed by IRD and UNC since 1986. Over the years, we built a database containing 2910 Distance-sampling Underwater Visual Census (D-UVC). The method consists in recording species name, abundance, body length and distance to transect line for each fish or group of fish observed along a 50 m transect. Distance-sampling method is fully described in Labrosse *et al.* (2002). Presence/absence of *L. fulviflamma* was directly extracted from our raw database and abundance calculated after truncation of original D-UVC datasets at a distance of 5 m on each side of the transect line (D'Agata *et al.* In press), effectively rendering D-UVC transects equivalents to 500 m² belt transects (50 m long x 5 m wide x 2 sides). Density of *L. fulviflamma* was therefore calculated by dividing the number of fish counted within the truncated D-UVC transects by 500 m². Biomass was calculated from density and length-weight coefficients (Kulbicki *et al.* 2005b). All transects were layed parallel to the reef between 0 to 15 meters depth and encompassed the full range of habitats presents in New Caledonia.

The presence, frequency of occurrence, average density and biomass of *L. fulviflamma* were calculated for the different lagoons of New Caledonia (as defined by Pelletier (2012)) and the remote reefs and islands of the archipelago for which we have data (Fig. 5.2). Surface of mangroves standardized by coast length (to account for the difference in size of the different lagoons) were calculated for the same lagoons, reefs and islands using the mangrove cartography of Virly (2007) available at www.georep.nc.

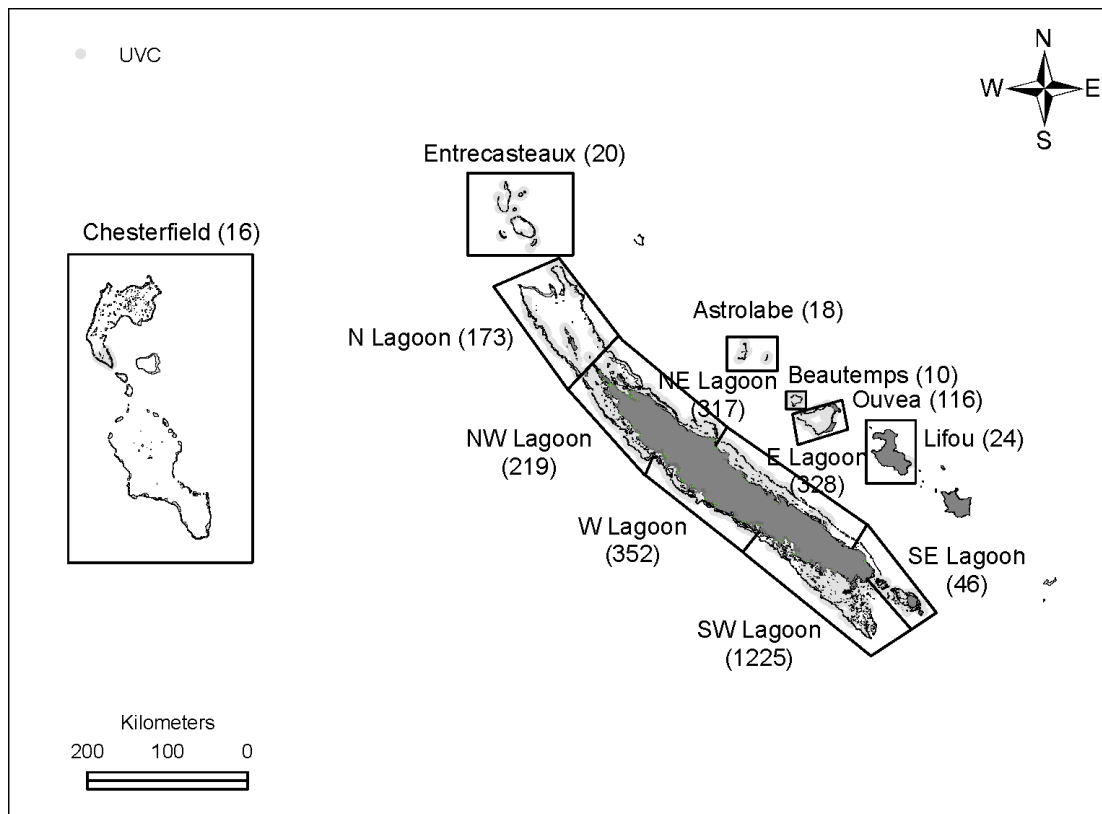


Figure 5.2: Position of the Underwater Visual Census transects (UVC) in the archipelago of New Caledonia. Polygons indicate the different lagoons, remote reefs and islands for which we have data; numbers indicate the number of transects.

2.5. Data analyses

One-way PERMANOVA (with 999 permutations) based on Euclidian distance (Legendre & Anderson 1999, Anderson 2001) was performed to test the effect of habitat (mangrove versus reef) on the multi-elemental composition of otoliths. This test was followed by one-way PERMANOVAs with 999 permutations in order to compare the chemical concentrations of each element between mangroves and reefs. Non parametric PERMANOVAs were used since the assumptions of normality and homogeneity of variance required by parametric MANOVAs were not met.

Random forests (RF) algorithm (Breiman 2001) was performed on elemental concentrations of otoliths surface to build an habitat classifier based on otolith microchemistry. This classification method allows freedom from normality and homoscedasticity assumptions and is the most appropriate for otolith microchemistry data (Mercier *et al.* 2011, Mercier *et al.* 2012). With the RF classification method, 75% of the dataset are used to build a classification

tree. Then, the remaining 25% are classified along this tree. The RF algorithm built 5000 trees to ensure that every individual gets predicted several times, which permits to estimate classification accuracy (percent of correct classification). Furthermore, we performed RF for all possible element combinations in order to find the combination that showed the greatest classification accuracy. The RF classifier based on this best combination of elements was then used to predict the juvenile habitats corresponding to the chemical composition of the different LA-ICPMS analyses performed along the otolith transverse sections of the 20 adults collected on the barrier reefs of Gatope, Ouano, Prony and St Vincent. A PCA was also used to further visualize the elemental composition of otoliths from fish collected in different habitats. Elemental compositions were $\arcsin\sqrt{x}$ transformed in the PCA to normalize the data.

The relationship between the presence of mangroves and *L. fulviflamma* in the different lagoons, remote reefs and islands of the Archipelago of New Caledonia was tested by a Chi-square test. Spearman rank correlations were calculated to test the relationship between mangrove and fish abundance (frequency of occurrence, density, and biomass).

3. Results

3.1. Building a RF habitat classifier from otolith microchemistry

Twelve elements met the criteria of selection and were retained for statistical analyses: B, Ba, Cr, Mg, Mn, Sn, Sr, Rb, U and Zn. A PERMANOVA showed that the overall elemental composition of otoliths was significantly different between mangrove and reef habitats ($F_{(1,194)} = 31.67$; $p < 0.01$). One-way PERMANOVA further revealed significant differences between habitats for six elements: Ba, Cr, Mn, Sn, Sr and Rb (Fig. 5.3). Elemental concentrations of Ba, Cr, Mn and Sn were significantly in greater concentrations in otoliths of individuals collected in mangrove than those collected in reefs. On the opposite, Rb and Sr were in lower concentration in otoliths from mangrove than in reef otoliths.

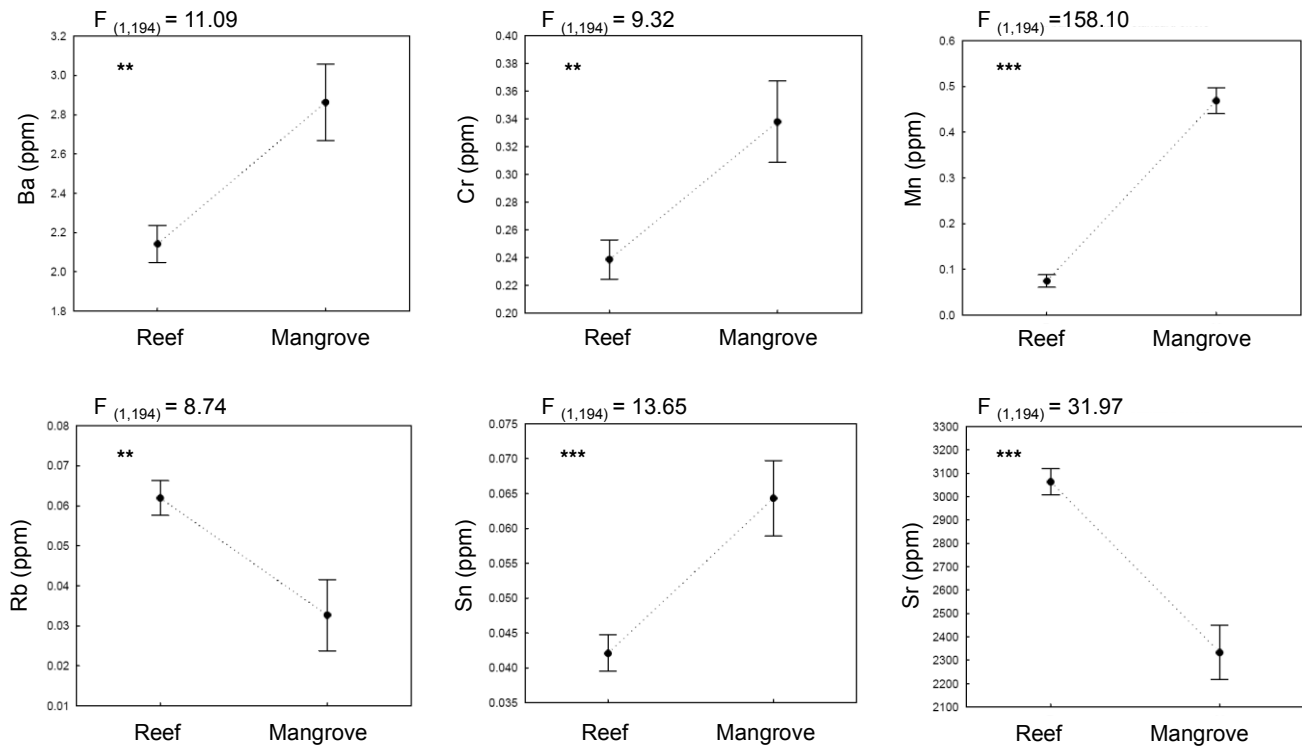


Figure 5.3: Otolith mean value of Ba:Ca, Cr:Ca, Mn:Ca, Rb:Ca, Sn:Ca and Sr:Ca ratios per habitat, (R) reefs and (M) mangroves. Standard errors ($\pm$ SE) are plotted. Results of one way PERMANOVAs are indicated in graphs with (***) $p < 0.001$ and (**) $p < 0.01$.

The best Random Forest (RF) classification was obtained with a combination of only three elements among the twelve tested: Mn, Sn and Rb. With these three elements, the RF was able to correctly classify 95.9% of the fishes in the habitat where they were collected. However, percentages of correct classification varied with the habitats. Fish from mangrove were correctly classified in their sampling habitat in 83.8% and fish from reefs were correctly classified in 98.7% (Tab. 5.2).

Table 5.2: RF classification matrix of habitats.

Habitats	Correct classification (%)	<i>n</i> fish classified in mangrove	<i>n</i> fish classified in reef	<i>n</i> fish in total
Mangrove	83.8	31	6	37
Reefs	98.7	2	157	159
Total	95.9	33		196

A PCA plot further showed the role of manganese (Mn), rubidium (Rb) and tin (Sn) in the discrimination of mangrove from the reef habitats with Mn and Sn characteristic of mangroves whereas Rb was characteristic of reefs (Fig. 5.3 and 5.4).

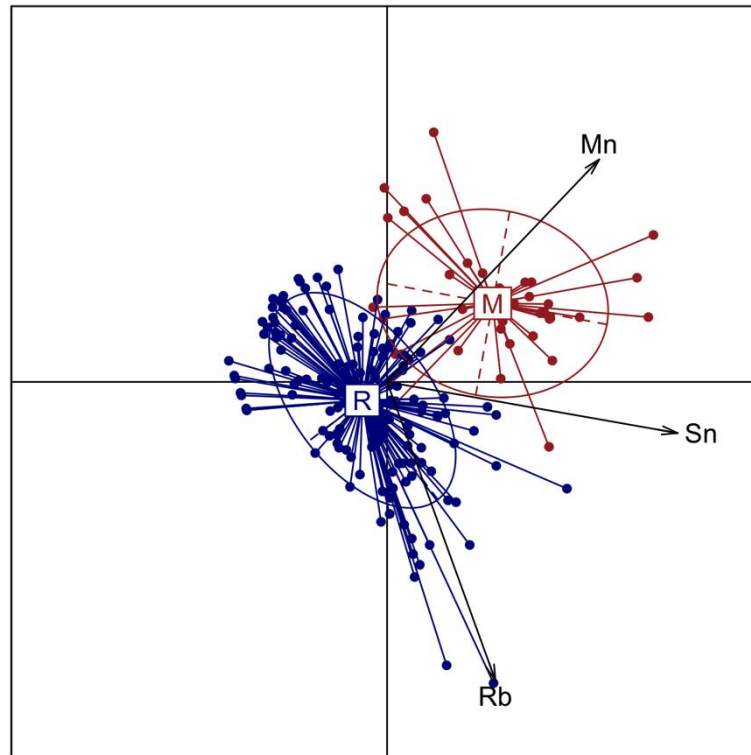


Figure 5.4: PCA biplot of individual fish collected in (M) mangroves and (R) reefs. Only concentrations of Rb, Sn and Mn in otoliths were used in the PCA.

3.2. Back-calculating juvenile habitats of adults

Applying the RF best classifier to all the chemical analyses done from the core to the distance of 1260 μm showed that all 20 adults *L. fulviflamma* collected on barrier reefs had a mangrove signature on the two first laser ablations (0 and 140 μm) (Fig. 5.5). A mangrove signature was revealed for 85 % of fishes (17 individuals) from 280 to the last analyses at 1260 μm .

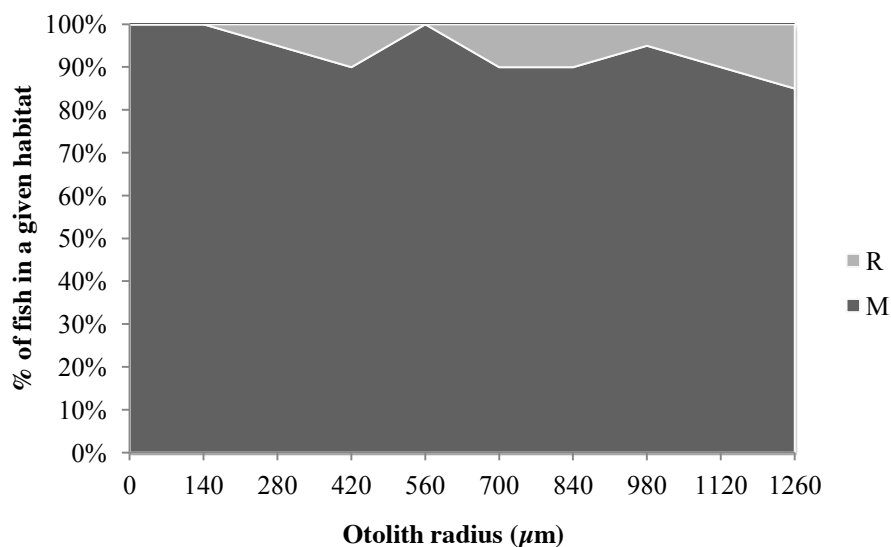


Figure 5.5: Prediction of habitats along the juvenile part of otolith transects of adults *L. fulviflamma* collected in barrier reefs. Dark grey area represents fish in mangrove whereas light grey area represents fish with reefs signatures (with M: mangrove and R: reef).

According to the classifier, only nly one fish used mangrove as juvenile habitat during a very short period of time (Fig. 5.6). This fish was collected at the site of Prony.

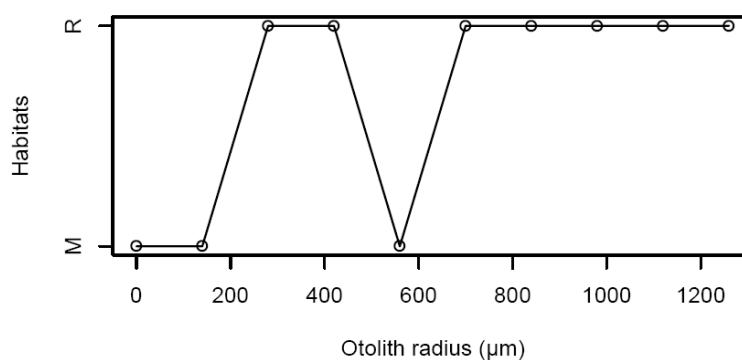


Figure 5.6: prediction of juvenile habitat from an otolith transversal section of an adult from Prony (site 11).

3.3. Relationship between mangrove and fish abundance

UVC surveys showed that the species was not recorded in places where mangroves are absent (Chesterfield, Entrecasteaux, Astrolabe, Beutemps-Beaupré, Lifou) and was always recorded in places where mangroves are present. Interestingly, some *L. fulviflamma* were recorded in the atoll of Ouvéa where mangroves are present but not in the nearby atoll of Beutemps-

Beaupré where mangroves are absent. The strong relationship between the presence of mangroves and *L. fulvivflamma* on reefs was formalized by a highly significant chi-square test ($\chi^2=13$, $df=1$, $P<0.0003$). Spearman rank correlations showed highly significant relationships between standardized mangrove areas and the frequency of occurrence ($R = 0.80$, $P<0.0009$), density ($R=0.91$, $P<0.0001$) and biomass ($R=0.86$, $P<0.0001$) of *L. fulvivflamma*. Fish abundance were 1) greater along the West coast of the mainland and in the NE lagoon where mangroves are well developed, 2) lower in the North Lagoon where reefs are very far away from the mainland, and the East, South East lagoons and Ouvéa island where mangrove are smaller, and 3) absent in the reefs, atolls and islands disconnected from the mainland and where mangroves are absent (Fig. 5.7).

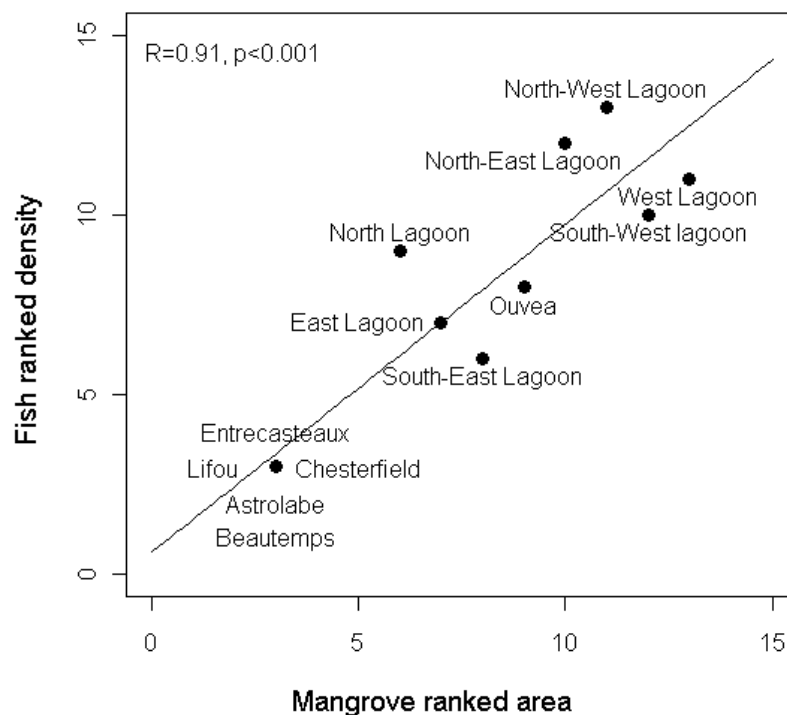


Figure 5.7: Relationship between standardized mangrove area and density of *L. fulvivflamma* in the different lagoons, reefs and islands of the archipelago of New Caledonia (see map on Fig. 2)

4. Discussion

The geographic distribution of species is a fundamental ecological parameter because it strongly influences species susceptibility to extinction. As such, understanding the drivers of

species geographic distribution is crucial for the development of conservation strategic plans. Up to now, it was believed that the main determinant of geographic range size of marine organisms was larval dispersion and a few studies have indeed reported a positive relationship between range size and planktonic larval duration (Mora *et al.* 2003, Lester & Ruttenberg 2005, Macpherson & Raventos 2006). However, many other studies found no relationship (Thresher & Brothers 1989, Victor & Wellington 2000). Very recently, Luiz *et al.* (2013) revised the larval dispersal paradigm and reported that adult traits were as important as larval traits to explain the geographic range size of reef fish species. To explain their findings, they proposed the following hypothesis: to found a new population, new colonizer not only must arrive on a new habitat as settling larvae, but they must also survive and find a mate to reproduce. And indeed, many adult traits such as large size or schooling behavior may diminish predation risks and consequently increase the probability of new colonizer to survive and establish new populations. If post-settlement survival is a crucial determinant of species geographic range size, then we hypothesized that the spatial distribution of habitats, which are obligatory for the survival of juveniles, should closely match the spatial distribution of species. In order to test this hypothesis, we selected a reef fish species known to use mangroves as nurseries, applied otolith microchemistry technique to determine whether mangroves were obligatory, essential or accessory juvenile habitats, and explored the relationship between species abundance and mangrove extent in the archipelago of New Caledonia.

4.1. Characterization of juvenile habitat

The first objective of this study was therefore to identify the role of mangrove as juvenile habitat for the blackspot snapper *Lutjanus fulviflamma* in New Caledonia using otolith microchemistry. We found that the otolith chemical composition of *Lutjanus fulviflamma* allowed discriminating almost perfectly mangrove from reef ecosystems. Six elements varied significantly between the two ecosystems. Ba, Cr, Mn and Sn were in greater concentrations in otoliths of fish from mangroves whereas Rb and Sr were in greater concentrations in otoliths of fish from coral reefs.

New Caledonia shows a peculiar geology context and a significant past and present mining activity. Among mining resources, past exploitations of Mn and Cr were found on the Main Island. Mn was extracted mainly on the west coast and is still present in basaltic complex

grounds. Cr was intensely extracted on the south and the north-west coast (Paris 1981). Moreover, Mn shows a scavenging profile type, which means higher concentrations in coastal waters and a tendency to decrease with distance from coastline (Bruland *et al.* 2003, Sturrock *et al.* 2012). Higher levels of Mn and Sn in mangrove could also originate from fertilizers used for agriculture and could be higher near areas with freshwater inputs (Dove & Kingsford 1998, Chittaro *et al.* 2009). Furthermore, Sn was present in antifouling paints until late 2012 in New Caledonia, which could also have contaminated coastal waters near harbors. Finally, differences in Ba concentrations between reefs and mangroves could be explained by Ba concentrations inversely related to Sr concentrations in estuarine system (Sturrock *et al.* 2012) where mangroves generally grow.

Our results show Sr in greater concentrations in otoliths from reefs and permit to discriminate reefs habitats from mangrove. In others studies, patterns of Sr between habitats are variable. In agreement with our study, Chittaro *et al.* (2004) reported Sr concentrations significantly greater in otoliths from reefs than mangroves in Bahamas. Sr/Ca ratios have also been positively correlated with salinity (Secor & Rooker 2000, De Pontual *et al.* 2003, Brown 2006a) and negatively correlated with temperature (Secor & Rooker 2000). However, Yamashita (2000) found the opposite patterns: Sr/Ca ratios were negatively correlated with salinity and positively correlated with temperature. Finally, Elsdon and Gillanders (2002) found interactive effects of temperature and salinity on Sr/Ca ratios in otoliths with Sr/Ca ratio negatively correlated with salinity, but that this effect was only present in medium (20°C) and high (24°C) temperature treatments. Finally, Rb is a trace element with a conservative profile, always in concentrations showing a constant ratio with salinity (Bruland *et al.* 2003).

The power of otolith microchemistry to identify juvenile and adult habitats resides in distinct chemical composition of otoliths collected from these different areas. In contrast to oceanic waters, coastal waters often show chemical heterogeneity due to different inputs (anthropogenic, fluvial, and upwelling) (Sturrock *et al.* 2012). Furthermore, coastal shallow-waters habitats are known to be important juvenile habitat (Parrish 1989, Beck *et al.* 2001). In temperate environment, several studies reported specific habitat tags which permit to identify juvenile habitat of fish (Forrester & Swearer 2002, Brown 2006a, Brown 2006b, Cuveliers *et al.* 2010). On the opposite, water masses in coral reefs environment often showed limited

chemical contrasts. Generally due to a lack of sources of trace elements, studies failed to detect strong differences among habitats at small scale (Chittaro *et al.* 2004, Chittaro *et al.* 2005, 2006). Nevertheless, some studies carried out at a small spatial scale in tropical environment, showed strong chemical differences between habitats (Lo-Yat *et al.* 2005, Lara *et al.* 2008, Mateo *et al.* 2010). In our study, lagoon habitats were discriminated from mangrove habitats at relatively small spatial scale (2 to 20 km). This classification accuracy is presumed to mainly derive from the environmental specificity of New Caledonia. In fact, the Main Island gets several sources of traces elements from freshwaters and terrigenous inputs linked to a singular geology context (Paris 1981). Thus, coastal shallow-waters habitats like mangrove can show contrasted chemical composition with reefs habitats.

4.2. Essentialness of mangrove

The second objective of our study was to determine whether mangroves were obligatory, essential or accessories juvenile habitats for *L. fulviflamma* in New Caledonia. To achieve this goal, we looked for elemental signature of mangrove in the juvenile part of otoliths from adults collected on barrier reefs. The results showed that all analyzed fishes had a mangrove signature at the beginning of their life. However, the duration of juvenile life in mangroves varied among individuals. Most fish inhabited mangrove during at least the entire juvenile part of otolith (1260 μm), which corresponds to about one year of life. One individual, however, used mangrove during a very short period of time, probably a few months during its early juvenile phase. This pattern may be explained by the particular morphology of the site where this fish was collected (Prony), an enclosed bay with fringing reefs adjacent to mangrove areas. Distinction between elemental signatures of reefs and mangroves may be less clear at this site.

Coastal shallow-waters habitats are recognized worldwide as important nursery areas for fish (Parrish 1989, Beck *et al.* 2001, Adams *et al.* 2006). For a given species, the importance of the nursery habitat may vary depending on the frequentation by juveniles. A habitat can be an obligatory nursery if juvenile cannot survive without it. It can be an essential nursery if a majority of juvenile uses this habitat and an accessory nursery when only a minority of juveniles uses it (Adams *et al.* 2006). Many studies, mostly conducted in the Caribbean region, established the nursery role of mangroves and their attractiveness for juveniles (Beck *et al.* 2001, Laegdsgaard & Johnson 2001, Cocheret de la Morinière *et al.* 2003a, Mumby *et*

al. 2004, Dorenbosch *et al.* 2006, Lugendo *et al.* 2007, Nagelkerken *et al.* 2008, Jones *et al.* 2010). Microchemistry studies also showed the importance of mangroves as nurseries (Chittaro *et al.* 2004, Chittaro *et al.* 2005, 2006, Mateo *et al.* 2010).

Our study took place in the South Pacific region where there is a lack of knowledge about mangrove-reefs interactions in comparison with the Caribbean region (Adams *et al.* 2006). In New Caledonia, previous studies showed that mangrove was used by blackspot snappers as one juvenile habitat among other shallow-water habitats (Thollot 1992a, Carpenter & Niem 2001a, Igulu *et al.* 2011). Our study reveals the true importance of mangroves for *L. fulviflamma*: all analyzed adults lived in mangroves as juveniles, which suggests that mangrove is likely to be an obligatory juvenile habitat for this species in New Caledonia.

Although a sampling of 20 adults is probably not sufficient to affirm with certainty that mangrove is indeed an obligatory habitat, the relationship that we found between the presence of mangroves and the presence of the species strongly support our conclusion from otolith microchemistry. New Caledonia is an archipelago with a large main island colonized by mangroves and surrounded by a complex reef ecosystem, and several islands and reefs offshore the mainland, some with mangroves and others without mangroves. *L. fulviflamma* was present all around the mainland, including on reefs very far away from the land (the northern and southern tips of the barrier reef), suggesting that individuals which completed their juvenile life in the mangroves of the mainland were able to swim long distances within an interconnected reef ecosystem and reach far away reefs. Furthermore, the species was present in offshore islands where mangroves are present, suggesting that planktonic larvae transported by oceanic currents can easily reach these islands and survive as juveniles in the mangroves. However, the species was not present at d'Entrecasteaux atolls, Astrolabe reef, Chesterfield banks, Beautemps-Beaupré Atoll and Lifou Island. One hypothesis to explain this absence is a lack of connectivity at the larval stage. However, this hypothesis is very improbable. For example, it would be very surprising that larvae can reach the atoll of Ouvéa where the species is present and not the nearby atoll of Beautemps-Beaupré or Astrolabe reef where the species is absent. Clearly, the most plausible explanation for the absence of the species at these reefs and islands is the absence of mangrove, implying that indeed mangroves are obligatory juvenile habitat for this species in New Caledonia. The strong relationship between *L. fulviflamma* abundance, either in terms of frequency of occurrence, density or

biomass, and mangrove area further strengthens the importance of mangrove in the life cycle of this species in New Caledonia.

Depending on the location, many species show considerable plasticity in habitat use as juveniles (McMahon *et al.* 2012). In our study, juveniles of *L. fulviflamma* used mangroves as an obligatory nursery area. In Zanzibar, Lugendo (2005) showed that juvenile of *L. fulviflamma* were found in different studied embayment habitats (mangroves, sand/mud flats and seagrass areas) and were therefore considered as a generalist species. In the Caribbean region, Mateo (2010) showed that almost 100% of schoolmaster (*Lutjanus apodus*) collected in mangrove and seagrass beds of St Croix and Porto Rico resided as juvenile in mangrove habitat. Juvenile habitat could possibly be used at different levels in different regions (Gillanders *et al.* 2003). This plasticity and variability in effectively used juvenile habitat could be significant enough to limit the general applicability of our results (Adams *et al.* 2006). Nevertheless, our study demonstrates that the spatial distribution of *L. fulviflamma* in New-Caledonia closely match the distribution of its juvenile habitat, which is in agreement with the study by Luiz *et al.* (2013) which proposes that post-settlement processes are main determinants of species geographic range. Our study encourages determining which habitats are obligatory or essential for fish juveniles in the different regions of the world because these habitats are probably crucial for the spatial distribution of species and would probably require some protection, and/or restoration for species maintenance and conservation.

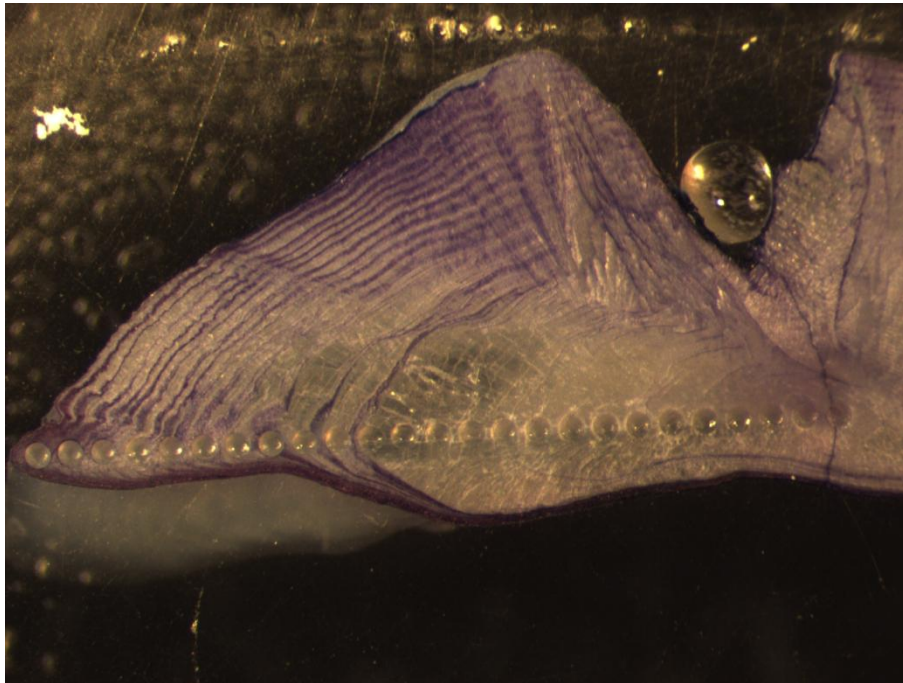
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Le chapitre 5 a permis d'identifier le rôle de la mangrove comme habitat juvénile obligatoire pour *Lutjanus fulviflamma* en Nouvelle-Calédonie. La reconstruction par microchimie des otolithes de l'histoire de vie environnementale d'adultes collectés sur le récif barrière a démontré que l'ensemble des individus présentait une signature élémentaire caractéristique de la mangrove dans la partie juvénile de leur otolithe. De plus, la distribution spatiale de l'espèce en Nouvelle-Calédonie s'est révélée être étroitement liée à la présence de mangrove.

Les mouvements ontogénétiques entre mangrove et récifs correspondent à un transfert unidirectionnel d'individus des habitats juvéniles vers les habitats adultes (Berkstrom *et al.* 2012). Cependant, les modalités de ce transfert sont peu connues. Les *L. fulviflamma* juvéniles quittent-ils les mangroves de manière définitive ou l'espèce y revient-elle à l'âge adulte ? La migration de la mangrove vers le récif barrière est-elle directe ou s'effectue-t-elle via l'occupation progressive d'habitats intermédiaires tels que les récifs frangeants et intermédiaires ? Ces questions font l'objet du chapitre suivant.

Chapitre 6 : Connectivité et patrons de mouvement d'une espèce de poisson corallien, *Lutjanus fulviflamma*



Chapitre 6 : Connectivité et patrons de mouvement d'une espèce de poisson corallien, *Lutjanus fulviflamma*

Les habitats constitutifs des paysages récifaux-lagonaires forment une mosaïque de patches interconnectés par des processus écologiques et biophysiques mais menacés par une pression humaine grandissante. La préservation de cette connectivité à travers tous les stades de la vie d'un poisson constitue un objectif clé dans la conservation des écosystèmes et se réalise notamment par la création de réseaux d'aires marines protégées (Almany *et al.* 2009, Sale *et al.* 2010). La compréhension des patrons de mouvements entre les habitats constitutifs des paysages récifaux-lagonaires est d'une importance primordiale pour le design de ces réseaux mais reste un phénomène mal caractérisé (Parrish 1989, Dorenbosch *et al.* 2005b, Lugendo *et al.* 2005, Lugendo *et al.* 2006).

La microchimie des otolithes est actuellement la technique la plus performante pour estimer la connectivité réelle entre les habitats à l'échelle de la vie entière d'un poisson (Campana 1999, Gillanders 2005, Thorrold *et al.* 2007, Rooker *et al.* 2010). En effet, la combinaison des études optiques et microchimiques des otolithes permet de déterminer rétrospectivement l'histoire de vie environnementale des individus, de comprendre les patrons de mouvements des poissons et d'étudier l'utilisation des habitats (Cowen *et al.* 2007, Elsdon *et al.* 2008). La reconstruction de l'histoire de vie environnementale par microchimie des otolithes consiste à associer les changements de composition chimique des otolithes présents le long de l'axe de croissance des otolithes avec les mouvements des poissons à travers différents environnements (Campana 1999).

Dans ce chapitre, l'utilisation des habitats et les patrons de connectivité au cours de la vie de *L. fulviflamma* sont étudiés via :

- 1) L'estimation du temps passé au sein des mangroves pour les juvéniles.
- 2) La caractérisation de la transition entre la mangrove et les récifs.
- 3) L'étude des patrons de mouvements au cours de la vie adulte.

Ce chapitre s'appuie sur le travail méthodologique de caractérisation de la microchimie des otolithes réalisé en amont et qui permet d'associer les changements de composition élémentaire avec les mouvements de ces poissons.

Manuscrit D: Migratory paths and connectivity for a coral reef fish, *Lutjanus fulviflamma*.

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Abstract

The increasing pressure exerted on tropical coastal ecosystems such as mangroves and coral reefs is leading to their degradation by threatening their resilience. A precise knowledge of existing ecological connectivity within tropical seascapes can provide an important contribution to enable the protection of these ecosystems. The present study used multi-element otolith fingerprints of the Indo-Pacific blackspot snapper *Lutjanus fulviflamma* to investigate the ecological connectivity between habitats of New Caledonia lagoon. We identified lifetime patterns of movements between the different habitats, characterized habitat use during juvenile and adult life stages, and described the transition between juvenile and adult habitats. Fish were captured in mangrove and reefs within four sites of New Caledonia Main Island. Using otolith elemental compositions and Random Forest classification method, the chemical signatures of mangrove, inshore and offshore reef habitats were characterized within each site with moderate to high accuracies (percentages of correct classification ranging from 50% to 100%). Then, signatures were used to predict habitat use during the lifetime of twenty *L. fulviflamma* adults. The prediction displayed a high level of global

accuracy (nearly half of total habitat predictions showed a probability ranging from 75% to 100%, the remaining half ranging from 50% to 75%). Reconstruction of environmental life history revealed that all individuals occupied mangroves when juvenile. This mangrove stage lasted at least a year, for a majority of the fish, to a maximum of 3 years. It also revealed three different lifetime patterns of movements between habitats: one with a single adult reef habitat, one with two adult reef habitats and one with multiple habitats use.

Key words: connectivity, patterns of movements, *Lutjanus fulviflamma*, otolith microchemistry, New Caledonia

1. Introduction

Tropical seascapes are spatially heterogeneous appearing as mosaics of fragmented habitats that include coral reefs, mangroves, seagrass beds, soft bottoms and several other habitats such as flats or embayments. This patchy nature results in fragmented local populations of marine organisms which are more-or-less connected (Almany *et al.* 2009). Connectivity defined as the exchange of individuals among local populations (Cowen *et al.* 2006, Crooks & Sanjayan 2006) can be measured in different ways (Calabrese & Fagan 2004). Structural connectivity is a metric that includes only the physical properties of the tropical seascapes while potential connectivity is a metric which embraces both physical properties and limited knowledge on mobility of organism. The direct quantification of movement and dispersal of organisms throughout the seascape refers to the metric of actual connectivity (Crooks & Sanjayan 2006, Moilanen & Hanski 2006, Nagelkerken 2009). This metric is ecologically highly relevant since it describes the real movement of organisms among local populations but it is the most challenging to measure.

For coral reefs fish, larval dispersal has long been considered as the major if not unique event of dispersion during life while juveniles and adults were considered as sedentary stages (Sale 1991). Yet, recent studies revealed that larval dispersal extent may be less than expected with rates of self-recruitment reported as high as 60% (Almany *et al.* 2007). On the opposite, dispersion by juvenile and adult movements may be more common than suggested (Kaunda-Arara & Rose 2004a, Grüss *et al.* 2011). Exchanges of individuals among local populations or connectivity at juvenile or adult stages have been classified in different types of movements operating at different spatiotemporal scales (Berkstrom *et al.* 2012): tidal linked movements, daily foraging and sheltering routine, spawning migrations and ontogenetic shifts. Through these movements, numerous reef fish species utilize multiple habitats during their life.

Connectivity between habitats enhances the resilience of ecosystems constituting tropical seascapes. The resilience of an ecosystem is defined as its capacity to absorb recurrent disturbances, regenerate or adapt to change without permanently switching to an alternative stable state (Mumby & Hastings 2008, Hughes *et al.* 2010). However, the continuous and increasing human pressure exerting on coral reefs is responsible for degradation and loss of habitats leading to a breakdown in connectivity within seascapes. This breakdown is threatening resilience of coral reef ecosystems (Mumby & Hastings 2008). Likewise, not only

coral reefs are threatened in the tropical seascapes but mangroves are also currently facing a high level of anthropogenic stress leading to a degradation and loss of these areas in the world (Valiela *et al.* 2001, Polidoro *et al.* 2010).

The twin degradation of coral reefs and mangroves in most tropical seascapes may have profound consequences for those numerous species that use mangrove as juvenile habitats and coral reefs as adult habitats (Nagelkerken *et al.* 2002, Cocheret de la Morinière *et al.* 2003a, Chittaro *et al.* 2004). In tropical seascapes, marine protected area (MPA) networks are established to preserve and maintain connectivity between fragmented habitats. Effective management of tropical seascapes must match the population biology and dispersal ability of target species but the patterns of actual connectivity and the scale at which they occur remain poorly understood. Identifying fish movements during their lifespan will contribute to a better understanding of actual connectivity in the tropical seascapes and is a key-objective in ecosystem conservation planning, by improving the knowledge required for the design of MPA networks (Almany *et al.* 2009, Sale *et al.* 2010).

Revealing movement patterns and uses of multiple habitats during entire fish life requires empirical studies of both larval and post larval dispersal, the latter consisting in juvenile and adult mobility patterns. Fish movements and habitat use can be estimated through different methods involving natural or artificial markers (Gillanders 2009, McMahon *et al.* 2011). Among methods of direct measurement of connectivity, microchemistry of otoliths is the only method which permits to identify and track back movements of fish during their entire life (Cowen *et al.* 2007, Elsdon *et al.* 2008).

Otoliths consist in calcareous structures located in the inner ear of fish. They continuously grow with successive deposits of calcium carbonate (CaCO_3) mainly originating from the surrounding water masses. During this calcification process, trace elements from the environment are incorporated and stored in their carbonate matrix. Therefore, otoliths constitute a natural recorder of waters masses occupied by fish during their life. Environmental history may then be revealed by the microchemistry signatures recorded along otolith growth, which in some case allows to reconstruct migratory paths of fish (Campana 1999, Elsdon & Gillanders 2003a).

Study of life history movements through microchemistry of otoliths requires chemical heterogeneity of water masses in order to chemically distinguish specific signatures of habitats or sites (Elsdon *et al.* 2008). That is why studies using microchemistry first focused on diadromous species (Secor 1992, Secor *et al.* 2001, McCulloch *et al.* 2005), and then on temperate marine species using distinct habitats during their ontogeny (Gillanders 2002, Brown 2006b, Shima & Swearer 2009). Studies that have tried to reconstruct the life history movements of coral reef fish using otolith microchemistry generally reported low rate or even sometimes an absence of chemical discrimination between reef habitats (Chittaro *et al.* 2006, Berumen *et al.* 2010), suggesting the technique may not be useful in coral reefs.

Lutjanus fulvivflamma is a common Indo-Pacific snapper which uses mangroves as nursery habitat and coral reefs as adult habitat (Thollot 1992b, Igulu *et al.* 2011, Berkstrom *et al.* 2013). This spatial segregation between adult and juvenile populations may imply distinct chemical signatures between life stages. Within the adult life stage, fish may use several types of reef, from the most coastal fringing reefs to the most oceanic barrier reefs, and perhaps some temporary use of mangroves as well. The identification of movements among reef types using microchemistry will depend on the chemical heterogeneity of reef habitats, and may thus be possible only in a few place of the world such as New-Caledonia, a continental island of the South-Pacific which possesses one of the world greatest nickel deposits and is characterized by a highly complex and heterogeneous geochemistry (Paris 1981, Ambatsian *et al.* 1997, Baille *et al.* 2012).

The aim of this study is to explore the patterns of movements and therefore, the use of multiple habitats during the lifespan, of *L. fulvivflamma* within the tropical seascapes of New Caledonia. More precisely, we used otolith microchemistry to investigate if all juveniles of *L. fulvivflamma* stayed in mangrove during a similar length of time or if the patterns varied among individuals or are site-specific. Then, we investigated if the transition from juvenile to adult habitat was direct to a single reef habitat or gradual with intermediate reef habitats. Finally, we studied whether adults moved permanently to reefs or if they returned at time in mangroves.

2. Materials and methods

2.1. Sampling protocol

New Caledonia is a French archipelago located in the South West Pacific, 1500 km east of Australia (Fig. 6.1). The archipelago is composed of a main island (Grande Terre) and several groups of smaller islands. The main island held the largest lagoon of the world, with a linear barrier reef of 1744 km long and a surface area of 31 336 km² (Andréfouët *et al.* 2009). Distance between the main island and the barrier reef varies from 1 to 65 km (Paris 1981) and some parts of the lagoon are on the Unesco heritage list since 2008.

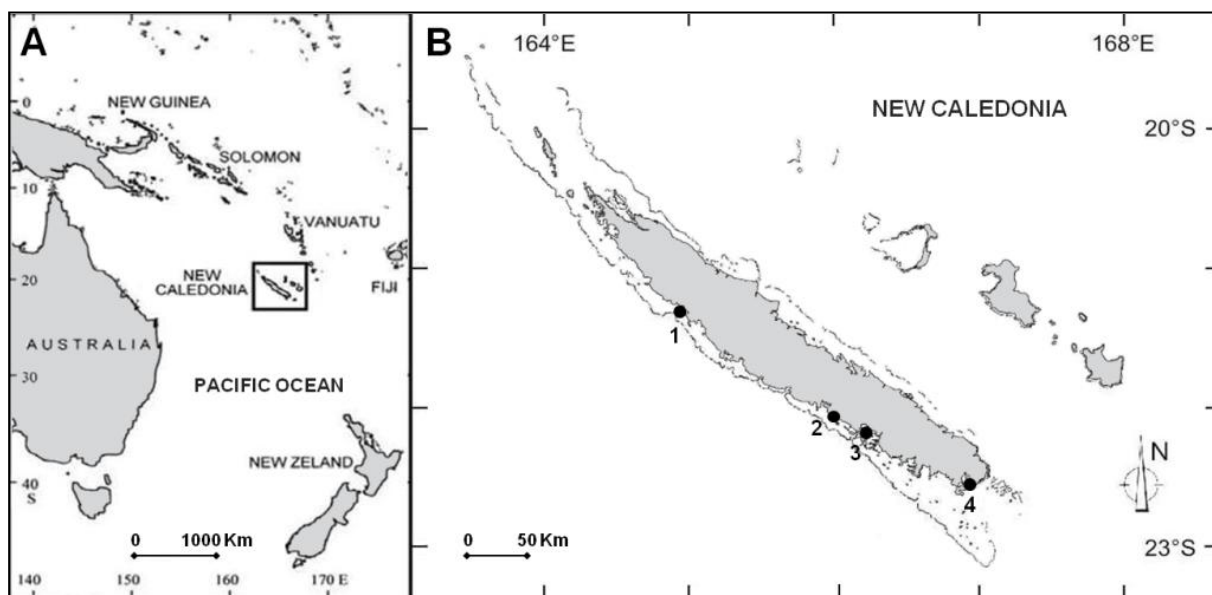


Figure 6.1: (A) New Caledonia geographic situation in the South West Pacific. (B) Study sites: Gatope (1), Ouano (2), St Vincent (3) and Prony (4).

The blackspot snapper *Lutjanus fulvivflamma* (Forsskål 1775, Lutjanidae) is a small snapper commonly targeted by subsistence fisheries throughout the Indo-Pacific. It is a carnivorous species with a maximum total length (TL) of about 35 cm, and a mean size of 25 cm (TL). This species has a complex life cycle and can be found in different habitats according to its ontogenetic stage. Adults inhabit coral reefs whereas juveniles use shallow-waters habitats, principally mangrove (Loubens 1980, Thollot 1992a, Carpenter & Niem 2001a, Igulu *et al.* 2011, Berkstrom *et al.* 2013). Because the movement of the species may partly blur the characterization of chemical signatures in otoliths, we used the highly sedentary damselfish *Dascyllus aruanus* (Cuvier 1829, Pomacentridae) as a control species. This small (maximum

TL of about 7 cm) damselfish lives in *Acropora* coral heads (Sale 1971) and its restricted home range combined with its strong site fidelity from settlement to adulthood may result in otoliths chemical signatures reflecting more accurately the chemistry of ambient water (Chittaro & Hogan 2013).

Fish were sampled for otolith analysis in different habitats at 4 sites around the main land of New Caledonia during three years (2009, 2010 and 2011) (Fig. 6.2). *L. fulviflamma* were collected in reef habitats by spear fishing and juveniles were collected in mangroves using gillnets with small meshes (7 mm) surrounding an isolated mangrove tree. Gillnets were deployed at high tide and fish collected with handnets in a few cm of water and/or on the ground at low tide. *D. aruanus* were anesthetized with eugenol and collected with handnets. Immediately after collection, fishes were stocked in a freezer (at -20°C) until dissection.

The first sampling took place during austral summer 2009 in the mangroves and inner barrier reefs of two sites: Gatope and St Vincent (Fig. 6.2A, 6.2C and Tab. 6.1). The second and third samplings have been carried out during austral winter 2010 and 2011 in the mangroves, fringing, intermediate and inner barrier reefs at four sites: Prony, St Vincent, Ouano and Gatope (Fig. 6.2). With fish collected in different years, seasons and habitats, our sampling included possible spatio-temporal variability of chemical signatures, which is essential to characterize robust chemical signatures in otolith (Mercier et al. 2012).

In order to characterize the environmental signatures of mangrove, fringing reef, intermediate reef and barrier reef habitats, up to five fishes from each sample were randomly selected for otolith analysis, with a total of 284 otoliths analyzed (Tab. 6.1A). Another five fishes were selected in the 2010 samples of the barrier reef habitat of each site in order to reconstruct fish environmental history over the entire life of 20 adults *L. fulviflamma* (Tab. 6.1B).

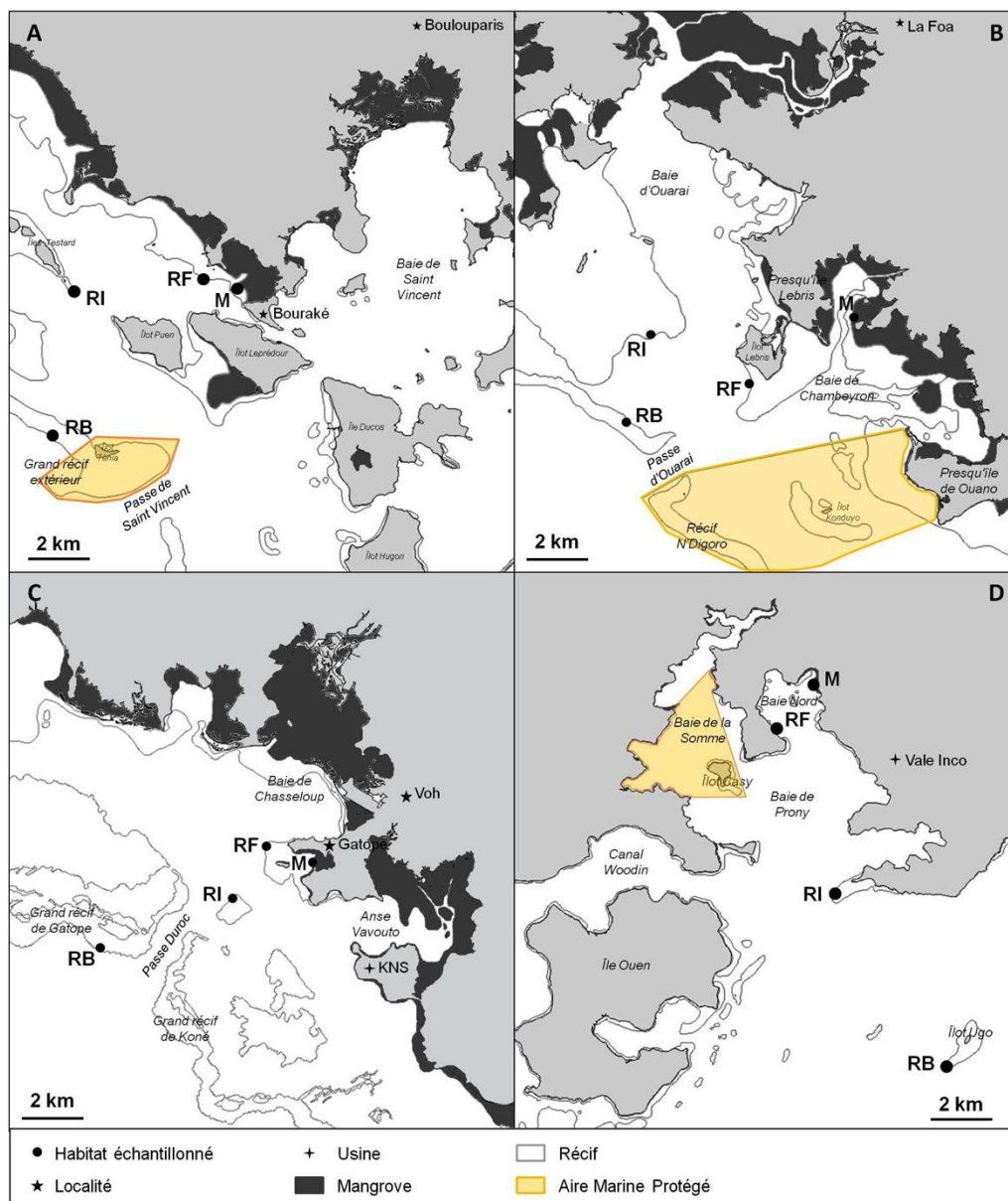


Figure 6.2: Details of habitats sampled in St Vincent (A) Ouano (B), Gatope (C) and Prony (D).

Table 6.1: Sample size to (A) characterize habitats chemical signatures by LA-ICPMS of the surface of otoliths and (B) determine the patterns of fish movements by LA-ICPMS of transverse sections of otolith along transects.

Objectives	Year	Site	Habitat	Laser analysis	Specie	n otoliths
A - Characterization of habitat signatures	2009	2	M, BR	Ablation on surface	<i>L.fulviflamma</i>	20
					<i>D. aruanus</i>	20
	2010 2011	4	M, FR, IR, BR	Ablation on surface	<i>L.fulviflamma</i>	125
					<i>D. aruanus</i>	119
B - Patterns of fish movements	2010	4	BR	Transects	<i>L.fulviflamma</i>	20

2.2. Otolith preparation

All material used for otolith extraction or preparation was decontaminated in 5% ultrapure nitric acid bath (24h), rinsed three times with ultrapure water (18.2 MΩ), dried and stored in plastic bag under a laminar flow hood (HEPA 100).

At the lab, fish samples were unfrozen and each individual was measured (Fork length, FL) to the nearest mm and weighted to the nearest g. Paired sagittal otoliths were extracted using ceramic tweezers, cleaned with ultrapure water, dried under a laminar flow hood (HEPA 100) and stored in individual plastic vials.

To characterize environmental signatures, otolith external surfaces of fish collected in various habitats (Tab. 6.1A) were analyzed with a Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Prior to analyses, otoliths were cleaned to remove any remaining tissue following the Warner *et al* (2005) method with a first bath of 50/50 H₂O₂ (30%, Suprapur[®]) and NaOH (0.1 mol.L⁻¹, Suprapur[®]) solution during 1 hour. They were sonicated during the last 5 min, then rinsed 5 times with ultrapure water for 5 min, dried under a laminar flow hood (HEPA 100) and stored in individual plastic vials until LA-ICP-MS analyses.

To predict the environmental life history and reconstruct the movements of 20 adults *L. fulviflamma* (Tab. 6.1B) transverse sections of otoliths were analysed by LA-ICP-MS along transects from core to edge. Right sagittal otoliths were embedded in resin (Araldite 2020[®]) and transverse sections including the otolith core were realized with a low speed saw (Buehler[®]). Sections were then polished using decreasing sandpaper (from 800 to 1600 grains.cm⁻²), lapping film (9 to 1 µm) and diamond-tipped powders (Struers[®], 1 to 0,3 µm), rinsed in ultrapure water, dried under laminar flow hood (HEPA 100), mounted on a microscope slide (10 sections per slide) with double-side tape and stored in plastic boxes until LA-ICP-MS analyses.

2.3. Otoliths chemical analyses

Otoliths of 2009 and 2010 samples were analyzed by LA-ICP-MS at the Pôle de Spectrométrie Océan (Institut Universitaire Européen de la Mer, Brest, France) using a Thermo Element 2 coupled to a laser 193 nm CopexPro 102 Coherent. Otoliths sampled in

2011 were analyzed at the Resources Institute of Environmental Livelihoods (Charles Darwin University, Darwin, Australia) using an Agilent 7500ce coupled to a UP – 213 nm laser ablation system. The following 21 isotopes were measured for habitat discrimination: ^7Li , ^{11}B , ^{85}Rb , ^{88}Sr , ^{95}Mo , ^{111}Cd , ^{117}Sn , ^{138}Ba , ^{208}Pb , ^{232}Th , ^{238}U , ^{25}Mg , ^{43}Ca , ^{44}Ca , ^{47}Ti , ^{51}V , ^{52}Cr , ^{55}Mn , ^{60}Ni , ^{65}Cu and ^{66}Zn . Both ICP-MS were operated at low resolution using argon as the carrier gas. The laser was set at 5 Hz frequency and 90 μm beam diameter. Each ICP-MS analysis consisted in 30 s background followed by 90 s ablation (laser activated).

To reveal the latest elements incorporated, and thus the chemistry corresponding to the habitat of collection, a single laser ablation was done on the external surface of selected otoliths (Tab. 6.1A). To standardize the analyses, the ablation was always done at the same spot at the tip of the post rostrum on the otolith posterior side.

To study patterns of movements during the entire life of each selected adult snapper (Tab. 6.1B), chemical transects were analyzed on fish otolith transverse sections. A transect consisted in successive laser ablations in point-by-point mode from the core to the edge of the otolith by increment of 140 μm and along the longest otolith growth axis.

To compensate for possible variation in quantity of material ablated, calcium was used as an internal standard. An external standard (National Institute of Standards and Technology, NIST, 612) was analyzed to correct instrument drift every ten ablations for transects and 10 otoliths for surface analyses.

Raw ICPMS data (counts per second, CPS) were transformed in elemental concentrations (parts per million, ppm) following Longerich *et al.* (1996) method. Any value greater than three times the inter-quartile distance were defined as outlier data and removed of data set (Tukey 1977). Limits of detection (LOD) were also calculated following Longerich *et al.* (1996) method and concentrations of elements inferior to the LOD were set to zero. Trace element selected for statistical analyses had to follow two further criteria 1) elemental concentrations had to be greater than the LOD in 70% of the otoliths in at least one particular habitat (*e.g.* barrier reef) or one particular site (*e.g.* Gatope) and 2) the coefficient of variation of elemental concentration in the NIST 612 had to be less than 10% (Chittaro *et al.* 2004, Chittaro *et al.* 2006).

Even if LA-ICP-MS parameters were identical, the use of two different instruments may induce variation in chemical analyses. To address this issue, all elemental concentrations were standardized in percent of all measured elements.

2.4. Age and growth back-calculations

In order to correlate fish movements with age and size, snappers were aged by counting the number of yearly increments (opaque bands) on otoliths and size-at-age were back-calculated from measurement of otolith radius at age using the Dahl-Lea back-calculation model (Lea 1910):

$$L_i = \frac{R_i}{R_{cpt}} L_{cpt}$$

where R_i and L_i are otolith radius and fish length at age i , R_{cpt} and L_{cpt} are otolith radius and fish length at capture. We assumed that one annulus is formed each year as it is the case for several Lutjanidae species closed to study species (Newman *et al.* 1996, Newman & Dunk 2002).

2.5. Data analyses

All statistical analyses were performed using the statistical R software (R development Core Team, 2011). To test the effect of habitat on the multi-elemental composition of otoliths, multivariate one-way PERMANOVA based on Euclidian distance were performed (Legendre & Anderson 1999). To compare variation in chemical concentrations of each element between habitats, univariate one-way PERMANOVA test were performed. For both tests, observations were permuted 999 times. When differences were significant, multiple comparisons among mean ranks were performed in order to identify which habitats were significantly different and for which element (Siegel & Castellan 1988). Non-parametric methods were used since the assumptions of normality and homogeneity of variance required by parametric MANOVAs were not met.

Random Forests (RF) algorithm (Breiman 2001) was performed on elemental composition of otoliths surface to build an habitat classifier based on otolith microchemistry. RF is a machine learning classification method allowing freedom from normality and homoscedasticity

assumptions. RF is also the most accurate method for classification of otolith chemical signature (Mercier *et al.* 2011, Mercier *et al.* 2012). With the RF method, a random subsample of about two-third of the dataset is used to build a classification tree and the remaining one-third is used to estimate classification accuracy of this tree. This process is reiterated many times to build a random forest of classification trees, 5000 trees in our case, in order to ensure that every individual gets predicted several times, which permits to estimate classification accuracy of the method (percent of correct classification). Here, all possible combinations of elements were tested by RF in order to find the combination that showed the highest percent of correct classification (Mercier *et al.* 2011, Mercier *et al.* 2012). In case of multiple best combinations, the one composed of the smaller number of trace elements was retained.

A PCA was also used to further visualize the elemental composition of otoliths from fish collected in different habitats. Elemental compositions were $\arcsin\sqrt{x}$ transformed in the PCA to normalize the data.

Paillon *et al.* (*In prep.*) (see Chapter 3) indicated strong differences in otolith chemistry between mangroves and reefs but possible confusions among reef types for *L. fulviflamma* in New Caledonia. They suggested that the latter may possibly be due to the intermediate position of patch reefs in the lagoon which, depending on sites, may be more influenced by coastal waters and therefore chemically closer to fringing reefs, or more influenced by oceanic waters and thus chemically closer to barrier reefs. Consequently, we adopted here a two-step strategy to predict habitat from otolith microchemistry. First, a RF habitat classifier was produced to distinguish mangroves from reefs using the chemical analyses of *L. fulviflamma* otoliths of all sites. Second, for each site, a RF classifier was built to distinguish inshore vs. offshore reefs, by either pooling fringing with intermediate reefs or pooling intermediate with barrier reefs. This procedure was based on the otoliths elemental compositions of the control species *D. aruanus* since this species is very sedentary and more prone to represent environmental conditions. The pooling that produced the highest percent of correct classification from the *D. aruanus* dataset was used to build RF classifiers for the same inshore and offshore pooling of reefs for each site with the *L. fulviflamma* dataset.

The two RF classifiers obtained for *L. fulviflamma* were then successively used on otolith transects to predict lifespan habitat use and movement of the species. First, each point of each transects were predicted either as mangrove or reefs using the first RF classifier. Then, the second RF classifier was used to predict whether reef predictions were inshore or offshore reefs. Because habitat predictions were obtained from a forest of 5000 trees, each tree providing a vote for a habitat, the analysis not only provided the most probable habitat (*i.e.* the habitat gathering the majority of the 5000 votes) but also the actual probability of the prediction (*i.e.* the % of votes).

3. Results

3.1. Habitat classification

Twelve elements responded positively to the criteria of selection and were selected for statistical analyses: B, Ba, Cr, Mg, Mn, Pb, Rb, Sn, Sr, Th, U and Zn. Multivariate PERMANOVA showed that elemental composition of *L. fulviflamma* otoliths differed significantly between mangrove and reefs ($F_{(1,143)} = 12.83$; $p = 0.003$). Univariate PERMANOVA revealed significant differences between habitats for five elements: Mn, Rb, Sn, Sr and U (Fig. 6.3). Mn and Sn were in greater concentrations in otoliths of individuals collected in mangrove. Rb, Sr and U were in greater concentrations in otoliths from reefs.

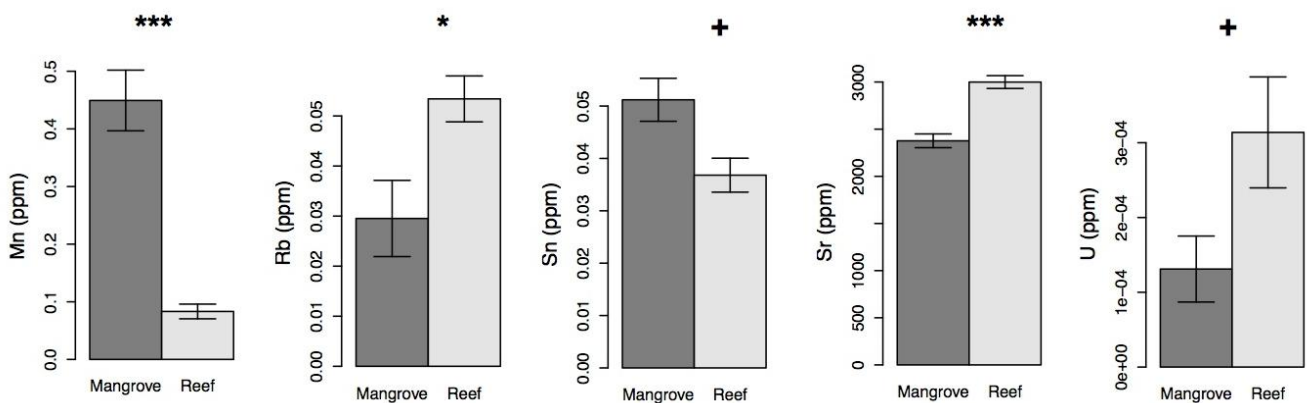


Figure 6.3: Otolith mean value of Mn:Ca, Rb:Ca, Sn:Ca Sr:Ca and U:Ca ratios per habitat (mangroves and reefs). Mean and standard error are plotted. Statistical significances of one-way anova results are indicated above graphs (“***” p-value < 0.001; “**” p-value < 0.01; “*” p-value < 0.05; “+” p-value < 0.1).

The best RF classifier of reefs and mangroves only used 3 elements: Mn, Pb, Rb (Tab. 6.2). With this combination, 92% of individuals were correctly classified. Percent of correct classification were 85% for fish collected in mangroves and 98% for fish collected in reefs. Mn and Rb were the strongest characteristics of mangroves and reefs, respectively (Fig. 6.4).

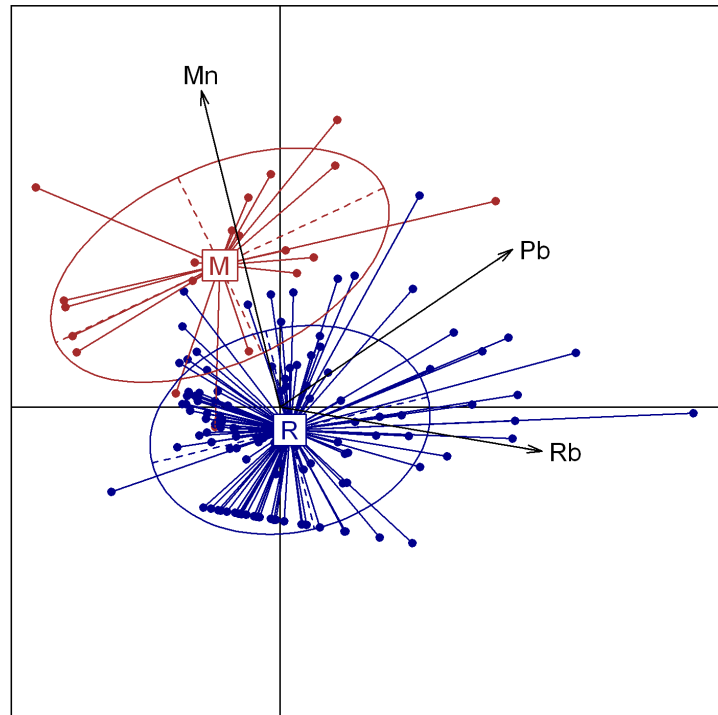


Figure 6.4: PCA biplot of individual fish collected in (M) mangroves and (R) all reefs. Only concentrations of Rb, Pb and Mn in otoliths were used in the PCA.

For Gatope, Ouano, and Prony, data on *D. aruanus* otoliths showed that RF classifications of reefs were best when intermediate and barrier reefs were pooled in an offshore reefs group. On the opposite, classification of reefs at St Vincent was most accurate when fringing and intermediate reefs were pooled in an inshore reefs group. The percentage of correct classification for fish collected in inshore reefs varied from 78% to 100% depending on the site (Tab. 6.2). For offshore reefs, the percentage of correct classification varied between 89% and 100%. Overall, between 90% (Prony) and 95 % (Gatope and St Vincent) of individuals were classified into the correct reef category for *D. aruanus* (Tab. 6.2).

Using the same reef grouping, the percentage of correct classification for *L. fulviflamma* varied from 70% at Ouano to 94% at Gatope (Tab. 6.2). Fish collected in inshore reefs showed a minimum of 50% correct classification at Ouano and a maximum of 80% at Gatope

and Prony. Percentage of correct classification for fish from offshore reefs ranged from 72% at St Vincent to 100% at Gatope.

Table 6.2: Random Forest classifications with details of the best combinations of elements and the percentage of correct classification for each analysis (M: mangrove; FR: fringing reef; IR: intermediate reef; BR: barrier reef).

Specie	Site	Discrimination	Optimal combination <i>Elements</i>	Correct classification (%)		
				<i>Individual</i>	<i>Mangrove</i>	<i>Reefs</i>
<i>L. fulviflamma</i>	All (4)	M vs Reefs	Mn, Pb, Rb	92	85	98
				<i>Individual</i>	<i>Inshore</i>	<i>Offshore</i>
<i>D. aruanus</i>	Gatope	FR vs IR/BR	Ba, Cr, Mg, Sr	95	78	100
	Ouano	FR vs IR/BR	B, Pb, Sn, Th	93	100	89
	Prony	FR vs IR/BR	Ba, Mg, Sr, U	90	80	95
	StVincent	FR/IR vs BR	B, Ba, Cr, Pb, Sn, U	95	100	90
<i>L. fulviflamma</i>	Gatope	FR vs IR/BR	Cr, Mn, Sn	94	80	100
	Ouano	FR vs IR/BR	Sn U	70	50	80
	Prony	FR vs IR/BR	Ba Mg Mn Sr	85	80	88
	StVincent	FR/IR vs BR	Cr Mg Pb Rb Sr Zn	75	78	72

3.2. Predictions of environmental life history

Reconstruction of microchemistry of entire life provided a high level of global accuracy. Nearly half of habitat predictions (42% of total habitat predictions) had a probability ranging from 75% to 100% of the votes. More than half of the predictions showed probabilities comprised between 50% and 75% (56% of total habitat predictions). The rest of the predictions had a probability ranging from 25% to 50% of the votes (2% of total habitat predictions). For fish collected in Gatope and Prony, the probabilities of RF habitat predictions were >75% for more than half of habitats predicted, the rest of the predictions were comprised between 50% and 75%. Ouano and St Vincent predictions showed inferior percentage of votes, with a majority of predictions comprised between 50% and 75% and less than half of the predictions >75% probabilities. In all sites, the probabilities of RF habitat predictions comprised between 25% and 50% constituted 2% of the total predictions realized which was considered as negligible.

The reconstruction of environmental life history revealed one main life history trait. All individuals had a juvenile phase in mangrove and an adult phase on reefs (see Appendix 12).

Three different patterns of movement were identified depending on the fish (Fig. 6.5). The first pattern showed four individuals evolving in two different habitats, mangrove as juveniles then offshore reef as adult. Two of these fish were collected in Gatope and two in Ouano (Fig. 6.5A). The second pattern showed fish evolving in three different habitats: mangrove as young, then inshore and offshore reefs as adults with no return to mangrove for adults but random movements between inshore and offshore reefs (Fig. 6.5B). This pattern was observed for five fishes: one from Gatope, two from Prony and two from St Vincent. The last pattern consisted of random movements between the three habitats (Fig. 6.5C). It was observed for eleven individuals, and was therefore the most common pattern. However, there were no obvious relationship between the site and type of patterns as an approximately equal number of fish displayed this pattern at each site (two at Gatope and three at each of the three other sites).

Fish ranged from 4 to 18 years in age and from 18 to 28 cm in fork length. Six individuals left the mangrove before the end of their first year with a mean size of 4 cm. Interestingly, all these six fish showed exclusively the third pattern of movements with some return in mangrove after first departure. Nine fish left the mangrove during their second year with a mean size of 14 cm. Among them, one individual displayed the first pattern of movements, four displayed the second and four displayed the third. Five fish left the mangrove during their third year with a mean size of 17 cm. Among them, three individuals displayed the first pattern of movements, one displayed the second and one displayed the third. There was no site-specific trend between the time spent in mangrove and the age or body length of fish.

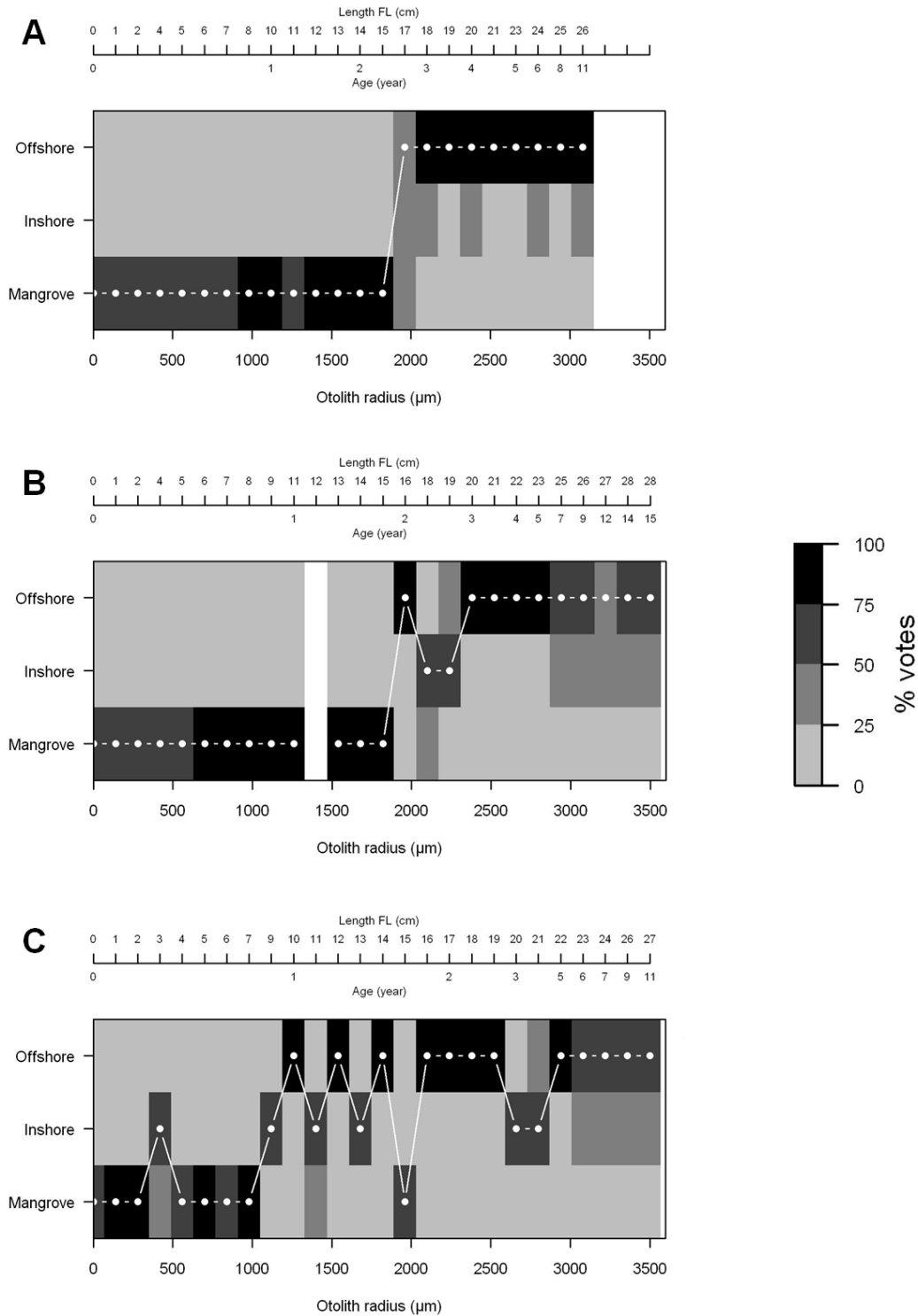


Figure 6.5: Representation of the three main patterns of lifespan movement for *L. fulvivflamma* using otoliths microchemistry and RF predictions. The x axis represents the otolith radius and the y axis the three possible habitats occupied during life. The second top x axis indicates the age and length of the fish (respectively below and above axis). The grey gradient shows the probabilities of habitat predictions (% of vote). The white line joins habitats predicted with the highest probability at each LA-ICPMS analysis.

4. Discussion

Several studies conducted in the Caribbean and the Indian Ocean have shown that many coral reef fish use mangroves as juveniles (Nagelkerken *et al.* 2002, Cocheret de la Morinière *et al.* 2003a, Chittaro *et al.* 2004, Dorenbosch *et al.* 2004, Dorenbosch *et al.* 2006). However, the pattern of use of mangrove in the South Pacific region is largely unknown (Thollot 1992a, Sheaves 2005, Unsworth *et al.* 2008). The high levels of mangrove and reef discrimination obtained with RF during this work permitted to study several characteristics of the blackspot snapper environmental life history and to understand the functional connectivity among reefs and mangrove in a continental island of the South Pacific, New Caledonia. First, we estimated the use of mangrove as juvenile habitats for this coral reef species as well as the patterns of transition between juvenile and adult habitats. Then, we investigated the different patterns of movements between mangroves and several reef categories showed by adults over their entire life.

4.1. Classification accuracy and the grouping of reefs

Inshore and offshore reef groups formed using *D. aruanus* otolith datasets may partly be explained by environmental characteristic of each site. For Gatope, Ouano, and Prony, classification accuracies of reefs were better when intermediate and inner barrier reefs were pooled in the offshore reefs group. On the opposite, St Vincent classification accuracies were better when fringing and intermediate reefs were pooled in the inshore reefs group. Even if Gatope intermediate reef was spatially closest to the fringing reef, it was environmentally closest to the inner barrier reef because of the proximity of a deep channel connecting the lagoon to the open ocean. For Ouano, the distinction between the three types of reefs was less obvious because they were all spatially close to one another with, in addition, a fringing reef probably under the influence of a channel bringing oceanic waters. Nevertheless, the intermediate reef was closest to the barrier reef than the fringing reef, suggesting that in Ouano, intermediate reefs are more influenced by oceanic waters than coastal ones. Prony is an enclosed bay with a fringing reef located deep inside the bay with strong influence of freshwater inputs and an intermediate reef located at the exit of the bay, equally distant to the fringing and barrier reefs, and under the influence of the same channel than the inner barrier reef. This particular spatial distribution of reefs in Prony may partly explain the environmental proximity of intermediate and barrier reefs at this site. Finally, in St Vincent,

fringing reefs are located along the shore of a series of high islands which extend inside the lagoon and are progressively replaced by coralline islands surrounded by intermediate reefs, thus making the environmental proximity of fringing and intermediate reefs logical in this site (Fig. 6.2).

The grouping of reefs in inshore and offshore categories from datasets collected in the otoliths of *D. aruanus*, a small and highly sedentary species, worked relatively well for *L. fulvivflamma*, a medium-sized relatively mobile species. For only one site, Ouano, inshore reef classification accuracy was high for *D. aruanus* (80%) and low for *L. fulvivflamma* (50%). This difference was also observed, but to a lesser extent, for the offshore reefs of this site (89 versus 80% correct classification), and may be explained by the spatial proximity of the reefs and the probable frequent movements among these reefs for the mobile species. This pattern was confirmed by a recent study of *L. fulvivflamma* movements using acoustic telemetry at Ouano (Chateau *et al.* 2012).

4.2. Pattern of juvenile habitat use and transition between juvenile and adult populations

As shown by many studies, mangroves are known to be a juvenile habitat with more or less importance depending on the geographical area and the species considered (Parrish 1989, Beck *et al.* 2001, Dorenbosch *et al.* 2005a, Adams *et al.* 2006). Mangroves provide shelters and abundance in small size prey for zoobenthivores juvenile snappers. In the Indo Pacific, mangroves are known to be an essential habitat for *L. fulvivflamma* juveniles (Thollot 1992a, Igulu *et al.* 2011, Olds *et al.* 2012, Berkstrom *et al.* 2013) and in New Caledonia they may even be obligatory (see Chapter 5). Except for one fish which stayed less than one year in mangroves as juvenile, all fish in this study stayed in mangrove during at least their first year, and for many individuals their first 2-3 years. Then, juveniles or sub-adults left the mangroves, definitely or for a variable length of time, to reach coral reefs before the end of their third year.

L. fulvivflamma movements between mangrove and coral reefs may be associated with an ontogenetic shift in diet (Berkstrom *et al.* 2013). In Tanzania, even if the main eaten preys were brachyurans for all size classes of *L. fulvivflamma*, there was a gradual ontogenetic shift

in diet for the secondary prey groups, mostly fish. Indeed, juvenile and sub-adult diets were mainly constituted of small shrimps and prawns which were replaced by demersal teleosts, the most abundant prey of the adult blackspot snapper (Cocheret de la Morinière *et al.* 2003b, Kamukuru & Mgaya 2004).

There was no site-specific trend for time spent in mangrove, or age or length of fish when they left their juvenile habitat. Nevertheless, all juveniles which left mangrove before their first year showed the third pattern of movement which involved visits to mangrove as adults. However, the transition between coastal juvenile habitats and adult reef habitats was generally direct toward offshore reefs. A few of the individuals showed a gradual transition, passing briefly through inshore reefs before reaching offshore reefs. This observation may be a real pattern or simply reflects an inshore reefs transition that was too brief to be chemically marked in otoliths, and/or an inshore reefs signature that was not distinct enough to be chemically discriminated from the offshore signature.

4.3. Life time movement patterns of the blackspot snapper

Reconstruction of environmental life history of the blackspot snapper revealed intra-specific variation in three different patterns of movement. The simpler one involved individuals using only two habitats during their life, mangrove as juvenile habitat and offshore reefs as adult habitat. The second pattern was more complex with the use of three habitats, only mangrove as juvenile habitat and both inshore and offshore reefs as adult habitats. In this pattern, some movements occurred between inshore and offshore reefs but there was no return to mangrove during the adult stage of the snapper. Finally, the third pattern of movement was the most common as it was observed in over half of the studied fish. This pattern consisted in multiple movements between reefs with short or long term returns to mangrove.

The intra-specific variation in patterns of movements was confirmed for *L. fulviflamma* in Ouano by a study using acoustic telemetry as artificial tag (Chateau *et al.* 2012). This study showed that *L. fulviflamma* presented multiple movements between mangroves and reefs (nearby reefs or distant reefs) as adults and that these movements' extent and duration varied according to individuals.

In their study, Hammerschlag-Peyer and Layman (2010) indicated that intra-specific movements and habitat use could significantly vary among individuals. These differences may be attributed to a polymorphism in behavioral traits, some phenotypic plasticity (bold or shy behavior), and/or different responses of individuals to competition for food and shelters.

In New Caledonia, the segregation between juvenile and adult habitats of the black-spot snapper was partial since some adult returned to mangrove. On tropical coasts, mangroves are used as foraging grounds by many fish from adjacent habitats because they provide trophic resources. This trophic role does not concern exclusively juveniles but also involves adults (Sheaves 2005). Mangroves may be an obligatory nursery habitat for juveniles of *L. fulviflamma* but they may be also a feeding habitat for sub-adults and adults. A gut content and stable isotopic analysis of *L. fulviflamma* in New Caledonia may provide details on activity of adult blackspot snappers visiting mangroves.

4.4. Limits of the method

Movements of adults and juveniles may occur at the local scale, between habitats within site (less than 10 km), to regional scale, between sites (more than 50 km) (Grüss *et al.* 2011). However, as Chittaro study (2005), environmental chemistry of New Caledonia tropical seascape was not distinct enough to identify site-specific chemical signatures for each habitat (ex. mangrove of Ouano). Estimate of movements between habitats have therefore been realized by taking each site independently and could not been studied between sites. This issue may not be a problem for our study as Kaunda-Arara and Rose (2004b) showed in their tracking study that *L. fulviflamma* could travel distances around 2 km corresponding to movements between patch habitats within a site and not among sites.

Furthermore, reconstruction of movement pathways with microchemistry could not provide a high temporal resolution due to the size of the laser ablation. We had to face a compromise between temporal resolution and the amount of material ablated. Using an extremely small laser ablation would have provided greater temporal resolution but will not have permitted the measurement of some important trace elements for habitat discrimination. In this context, daily movements linked to foraging activity could not be estimated with microchemistry. Other methods such as acoustic telemetry are complementary to otolith chemistry and can

provide information on connectivity at smaller spatial and temporal scales (Hitt *et al.* 2011, Chateau *et al.* 2012).

5. Conclusion

Functional links among habitats constituting a tropical seascape were mainly studied in the Caribbean region (Nagelkerken *et al.* 2000, Nagelkerken *et al.* 2002, Chittaro *et al.* 2004, Mateo *et al.* 2010), rarely in the Indo-Pacific region (Dorenbosch *et al.* 2005a, Nakamura *et al.* 2008, Unsworth *et al.* 2008, Unsworth *et al.* 2009, Berkstrom *et al.* 2013) and even less in the South Pacific (Thollot & Kulbicki 1988, Dorenbosch *et al.* 2005a, Sheaves 2005, Berkstrom *et al.* 2013). In this context, it has been suggested that coastal shallow-waters habitats such as mangrove and seagrass beds were less connected with coral reefs in the Indo-West Pacific region than in the Western Atlantic (Parrish 1989, Sheaves 2005). Besides, Thollot (1992a) showed that few interactions existed between mangroves and coral reefs in New Caledonia. The aim of this work was to demonstrate that biological connectivity between mangroves and coral reefs was effective for one species in New Caledonia. Indeed, mangroves and coral reefs of New Caledonia were interconnected for the blackspot snapper by ontogenetic migrations and by adult movements between the two ecosystems.

This work contributes to knowledge building on movement pathways and use of multi-habitats by coral reef fish in tropical seascapes. Collecting empirical data with direct measurements of movements is essential to improve fundamental knowledge of biological connectivity. This knowledge may provide powerful tools for spatial management planning, especially in the design of MPA networks which include coastal shallow-waters habitats such as mangrove as well as coral reefs in order to protect all habitats required by organisms to complete their life cycle. At present, most reserves fail to incorporate connectivity into their management frameworks (Almany 2009). For fish, the scales at which actual connectivity takes place at the different life stages are largely unknown (Di Franco *et al.* 2012).

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Le chapitre 6 a permis d'estimer la connectivité réelle entre les habitats du lagon de Nouvelle-Calédonie au cours de la vie de *Lutjanus fulviflamma*. Cette étude identifie une variabilité intra-spécifique des mouvements entre les habitats puisque trois patterns de mouvements aux échelles spatiales ascendantes ont pu être révélés. Un pattern à petite échelle spatiale où, une fois la mangrove quittée, les individus adultes évoluent au sein d'un habitat récifal unique. Un pattern à échelle spatiale intermédiaire où les individus se cantonnent à des mouvements restreints entre les différents habitats récifaux. Un pattern à échelle spatiale étendue où les individus montrent des déplacements de l'ordre de plusieurs kilomètres, alternant entre récifs barrières et retour vers la mangrove.

Par ailleurs, cette étude a aussi permis de préciser l'importance de la mangrove au cours de la vie de *L. fulviflamma*. Outre l'utilisation de la mangrove comme habitat juvénile obligatoire (Chapitre 5), la majorité des individus adultes effectuent des mouvements entre les récifs et la mangrove, ce qui indique le caractère essentiel des mangroves pour les adultes de cette espèce. Une étude parallèle par marquage acoustique suggère que les adultes de *L. fulviflamma* visitent les mangroves pour la recherche de nourriture (Chateau *et al.* 2012).

Les patterns de mouvements peuvent être influencés par la configuration du paysage marin constitué d'habitats continus ou fragmentés. Les habitats continus facilitent les mouvements sur de longues distances alors que les habitats fragmentés ont tendance à entraver les mouvements. Cependant, aucune tendance de mouvement spécifique aux sites et à leur géomorphologie n'a pu être observée au cours de notre étude. *L. fulviflamma* est une espèce qui de par sa taille, son home range et sa capacité à se déplacer sur de longues distances est susceptible de pouvoir franchir les barrières physiques d'un paysage fragmenté (Kramer & Chapman 1999, Kaunda-Arara & Rose 2004b, Sale *et al.* 2005). L'estimation de la mobilité des juvéniles et adultes et l'identification des habitats utilisés au cours de leur vie est d'une importance significative pour le fonctionnement des aires marines protégées car elle souligne la nécessité d'inclure de multiples habitats au cours de leur design (Grüss *et al.* 2011).

Chapitre 7 : Discussion générale et perspectives



Chapitre 7 : Discussion générale et perspectives

Les paysages marins tropicaux sont constitués de multiples habitats fragmentés qui abritent des populations locales reliées par des échanges d'individus. La perte et l'érosion de ces habitats menacent la résilience des écosystèmes en diminuant la connectivité écologique reliant les populations locales (Valiela *et al.* 2001, Hughes *et al.* 2003, Pandolfi *et al.* 2003, Duke *et al.* 2007). La préservation de la connectivité écologique, nécessaire au maintien de la résilience des écosystèmes, est donc un objectif clé pour la gestion efficace des écosystèmes côtiers tropicaux. Pour cela, il faut pouvoir quantifier l'importance des liens fonctionnels entre les habitats et comprendre leur structure (Mumby 2008, Cowen 2006). Cependant, il est remarquablement difficile de développer des estimations quantitatives de l'utilisation des habitats et des mouvements les connectant.

La microchimie des otolithes est l'outil le plus performant pour la mesure de la connectivité écologique au cours de la vie entière des poissons. Elle peut être particulièrement utile pour l'identification des nurseries c'est-à-dire des habitats juvéniles contribuant fortement au renouvellement des populations adultes, et pour l'estimation des habitats utilisés et des mouvements réalisés au cours de la vie des adultes. Avant d'utiliser la microchimie des otolithes pour quantifier la connectivité écologique, il est néanmoins nécessaire d'approfondir les connaissances acquises sur cet outil, notamment en établissant son potentiel et ses limites au sein des paysages marins tropicaux.

1. Axe méthodologique

Au cours de cette étude, le développement de l'outil microchimie des otolithes a été réalisé en plusieurs étapes visant à tester la fiabilité et le potentiel de cet outil en Nouvelle-Calédonie. Dans un premier temps, les variations spatiales de la microchimie des otolithes ont été caractérisées et les signatures spécifiques des habitats élaborées pour plusieurs espèces de poisson ainsi que pour l'environnement. Dans un second temps, l'utilisation de signatures multi-spécifiques comme proxy des signatures mono-spécifiques a été évalué. Enfin, dans un troisième temps, les liens entre la microchimie des otolithes et de l'environnement ont été explorés.

L'hétérogénéité spatiale de la microchimie est le facteur prépondérant influençant l'efficacité de la discrimination spatiale et l'obtention de bonnes classifications. Les résultats de ce travail ont permis de révéler un potentiel élevé de l'outil microchimie des otolithes en Nouvelle-Calédonie avec une discrimination spatiale importante à l'échelle des habitats, particulièrement des habitats fortement contrastés comme la mangrove et le récif barrière. Cependant, l'efficacité de la discrimination spatiale diminue au niveau des stations, particulièrement dans le cas des récifs qui présentent une microchimie relativement homogène entre eux.

Au cours du chapitre 3, il a été démontré qu'une variabilité interspécifique significative de la microchimie des otolithes existait au sein d'un même environnement. Par conséquent, l'utilisation de signatures multi-spécifiques en substitut de mono-spécifiques n'est en général pas possible même lorsque les signatures multi-spécifiques sont élaborées à partir de groupes d'espèces taxonomiquement ou fonctionnellement proches. Cette variabilité interspécifique pourrait résulter de traits de vie contrastés entre les espèces étudiées mais pourrait également être liée à une variation interspécifique des processus d'incorporation des éléments au sein des otolithes (Gillanders & Kingsford 2003, Swearer *et al.* 2003, Barnes & Gillanders 2013). Néanmoins, dans le cas d'habitats chimiquement très contrastés tels que les mangroves et récifs barrières, les signatures multi-spécifiques apparaissent comme substituables aux signatures mono-spécifiques. Ainsi, des similarités entre les espèces peuvent exister dans l'incorporation des éléments de l'environnement vers les otolithes même si des différences interspécifiques semblent être le cas général. De ce fait, l'étude des relations entre la microchimie des otolithes et de l'environnement abordée au cours du chapitre 4 a permis d'approfondir les connaissances sur les interactions existantes entre les otolithes et l'environnement. Ce chapitre indique qu'il existe de fortes relations entre la chimie des otolithes, de l'eau, de la salinité et de la température. Néanmoins, ces relations sont complexes et dépendent des éléments et des espèces considérées. Les résultats démontrent que la chimie des otolithes n'est pas l'exact reflet de la chimie de l'eau. L'influence spécifique de chaque facteur, exogène et endogène, sur l'incorporation des éléments au sein des otolithes est extrêmement difficile à caractériser pour tous les éléments et espèces étudiés. Sans ces connaissances, les interprétations écologiques issues de l'étude des signatures chimiques ne sont pas incontestables. Néanmoins, la forte structuration spatiale observée à partir de la

chimie de l'eau s'exprime au sein des otolithes, ce qui permet une utilisation cohérente de la microchimie des otolithes comme traceurs des environnements passés.

Au vu de la perte engendrée par l'utilisation de signatures multi-spécifiques, le mono-spécifique reste donc le classifiant ultime et ne peut être substitué excepté dans de rares cas. Ainsi, la reconstruction de l'histoire environnementale d'une espèce d'intérêt exige de caractériser les différents habitats à partir de la microchimie des otolithes de cette même espèce. L'échantillonnage devient problématique lorsque l'espèce étudiée est rare ou emblématique bénéficiant d'un statut de protection (aspect conservation) ou simplement difficile à échantillonner (aspect logistique). Au contraire, si l'espèce est abondante sans statut de conservation particulier, l'utilisation de la microchimie des otolithes pour reconstruire l'histoire environnementale des espèces permettra d'approfondir les connaissances sur leur biologie et d'estimer la connectivité réelle existante essentielle à la préservation des stocks d'adultes, notamment pour des espèces d'intérêt commercial. Cependant, il est important de souligner que les signatures multi-spécifiques caractérisent très bien les habitats contrastés tels que les mangroves et récifs barrières et peuvent donc être utilisées dans ce cas pour les espèces protégées et/ou difficiles à échantillonner en nombre suffisant.

2. Axe écologique

Estimer la connectivité réelle en déterminant les mouvements au cours de la vie nécessite de pouvoir tracer activement les mouvements d'individus entre les habitats ou d'identifier rétrospectivement les habitats fréquentés. La microchimie des otolithes utilise cette seconde approche et permet d'identifier les nurseries, de déterminer les habitats utilisés au cours de la vie des individus et les connexions existantes entre ces habitats.

Selon Beck (2001), un habitat est considéré comme une nurserie pour les juvéniles d'une espèce donnée si sa contribution à la production d'individus recrutant au sein des populations adultes est supérieure à la production d'autres habitats où les juvéniles sont également présents.

Au cours de cette thèse, l'analyse microchimique de la partie juvénile des otolithes de *Lutjanus fulviflamma* a démontré une fréquentation systématique des mangroves durant la phase juvénile. L'analyse de données de comptage et de la cartographie des mangroves a

montré que la distribution spatiale de l'espèce en Nouvelle-Calédonie est fonction de la présence des mangroves. Cet habitat est donc clairement identifié comme un habitat juvénile obligatoire pour *L. fulviflamma* en Nouvelle-Calédonie. La mangrove est assurément qualifiée de nurserie pour cette espèce en Nouvelle-Calédonie car elle assure le lien vital vers les récifs coralliens en transférant avec succès une biomasse de juvénile importante vers les populations adultes (Beck *et al.* 2001, Dahlgren *et al.* 2006). Néanmoins, il est important de réaliser qu'une certaine plasticité dans le choix des habitats juvéniles peut exister en fonction de la zone géographique. Outre la mangrove, *L. fulviflamma* utilise aussi d'autres types d'habitats juvéniles tels que les herbiers dans l'Océan Indien (Igulu *et al.* 2011, Kimirei *et al.* 2011, Berkstrom *et al.* 2013).

Les mouvements ontogéniques représentent des transferts de matière horizontaux et unidirectionnels entre la mangrove et les récifs coralliens (Berkstrom *et al.* 2012). La transition entre les deux écosystèmes peut être directe ou graduelle avec l'utilisation d'habitats intermédiaires identifiés comme les corridors spécifiques aux migrations ontogéniques. Au cours de cette étude, un faible nombre d'individus a montré une transition graduelle, ce qui suggère qu'une transition directe entre les habitats juvénile et adulte serait la règle pour *L. fulviflamma*. Cependant, il est aussi possible que la fréquentation d'habitats intermédiaires n'ait pu être identifiée en raison d'une fréquentation trop brève pour que les signatures microchimiques spécifiques des habitats intermédiaires soient marquées au sein de l'otolithe. En effet, il a été démontré que l'incorporation de la signature microchimique d'un environnement au sein des otolithes requiert un laps de temps de 2 semaines en moyenne, dépendant fortement du taux de croissance. Or, la microchimie des otolithes ne permet pas toujours d'analyser les mouvements à cette échelle temporelle. Ainsi, la résolution temporelle analysée va principalement dépendre du diamètre du faisceau laser de l'ICP-MS et du taux de croissance de l'individu. Plus le diamètre du laser sera important, plus la surface ablatée correspondra à un laps de temps important de la vie du poisson. De même, plus l'individu sera vieux, plus sa croissance sera faible, et donc plus la surface ablatée correspondra à un laps de temps important (Campana & Neilson 1985).

La connectivité est définie par les liens fonctionnels reliant les habitats et se traduit par les mouvements des individus entre habitats et populations locales. Les résultats obtenus durant cette thèse indiquent de remarquables mouvements pour *L. fulviflamma* avec trois

différents patterns de mouvements révélés. La multiplicité des patterns traduit une forte variabilité intra-spécifique empêchant d'établir un unique modèle de mouvement pour cette espèce.

Il n'y a pas de ségrégation spatiale stricte entre les populations juvéniles et adultes dans l'utilisation des habitats puisque au cours de ces travaux, il a été mis en évidence que les individus utilisent la mangrove à la fois en tant que juvéniles mais aussi à l'âge adulte. Malgré son utilisation au cours des deux stades de vie, la mangrove garde toutefois son statut de nurserie pour *L. fulviflamma* puisque les individus juvéniles ne sont pas présents au niveau des habitats adultes. Les mouvements constatés à l'âge adulte entre la mangrove et les récifs caractérisent des transferts de matière bidirectionnels entre ces deux écosystèmes.

3. Conclusion et perspectives de travail

La fragmentation du paysage marin tropical entraîne une rupture potentielle des échanges de gènes entre les habitats et tend à influencer la structure des métapopulations en augmentant l'hétérogénéité génétique. Dans un contexte de fragmentation croissante, les mouvements entre sous-unités sont donc cruciaux car ils permettent de connecter les populations locales et d'augmenter les échanges génétiques.

Cette étude apporte des connaissances nouvelles sur la connectivité réelle existant dans le lagon de Nouvelle-Calédonie qui sont essentielles à la gestion et la conservation des écosystèmes. Par la mise en évidence directe de connectivité réelle entre les mangroves et les récifs coralliens, ce travail de thèse souligne aussi l'importance du rôle des mangroves dans le Pacifique Sud. Cependant, ce travail a permis d'identifier l'importance des mangroves pour une unique espèce. Ces résultats encourageants incitent à de futures recherches ouvertes à d'autres espèces récifales afin d'estimer l'importance globale du rôle de nurseries des mangroves du Pacifique Sud. En effet, le rôle de nurseries des mangroves du Pacifique Sud reste méconnu par rapport aux mangroves de l'Atlantique Est (Caraïbes) et l'Océan Indien (Afrique de l'Est). Si les mangroves du Pacifique Sud s'avéraient effectivement posséder un rôle de nurserie essentielle pour de nombreuses espèces récifales, notamment pour des espèces commerciales, l'inclusion des mangroves lors du design des réseaux d'aires marines protégées allant de la côte vers le large devrait être encouragé.

Dans l'optique de futures recherches, notamment si elles s'ouvrent à d'autres espèces que *L. fulviflamma*, il serait intéressant d'ajouter l'étude des herbiers qui n'a pu être réalisée au cours de cette thèse pour des raisons logistiques. Les herbiers sont reconnus comme des nurseries pour de nombreuses espèces (Mellin *et al.* 2007, Unsworth *et al.* 2008). De plus, les herbiers sont souvent en bordure des mangroves et pourraient ainsi assurer un rôle important au sein du continuum mangroves - récifs coralliens en tant qu'habitat corridor lors des migrations ontogéniques ou des mouvements des adultes entre ces écosystèmes.

L'utilisation de plusieurs ICP-MS (Montpellier, Brest, Darwin) au cours de cette étude a été source de variations même si les réglages des différents appareils étaient identiques (fréquence, diamètre, puissance du laser, etc.). De même, l'intervalle de temps trop élevé entre deux sessions d'analyse sur le même appareil (Brest, 2009 et 2011) s'est aussi révélé être problématique. Pour palier ces problèmes d'inter-calibration, les données ont été standardisées et la plupart des analyses ont été réalisées non plus en concentrations absolues (en ppm) mais en concentrations relatives (en pourcentage de chaque élément mesuré). Cette transformation engendre une perte d'information non négligeable et dans l'optique de futures recherches, il serait préférable de travailler à l'aide d'un seul appareil et d'éviter un délai trop important entre deux sessions d'analyses.

La résolution temporelle analysée au cours des transects pourrait être affinée en diminuant le diamètre du faisceau laser mais au prix d'un temps d'analyse plus important et d'une baisse de la sensibilité de détection des concentrations. En effet, réduire le diamètre du faisceau afin d'augmenter la résolution temporelle des variations de concentration se fera au détriment de la quantité de matériel ablaté et donc du nombre d'élément supérieur aux limites de détection. Dans l'optique de futures recherches, l'obtention d'une résolution temporelle plus fine pour un temps d'analyse acceptable pourrait être abordée via la comparaison de deux méthodes d'analyse : le transect point par point (fixe) et le transect continu (à vitesse constante).

Dans le but d'augmenter la discrimination des habitats, il serait intéressant d'inclure lors de futures analyses les éléments traces appartenant au groupe des terres rares comme précédemment réalisés au cours de plusieurs études microchimiques (Dorval *et al.* 2007, Lara *et al.* 2008, Tournois *et al.* 2013). Plusieurs raisons permettent d'avancer leurs contributions à une caractérisation spatiale plus fine du lagon de Nouvelle-Calédonie. Dans un premier temps, ces éléments sont de bons indicateurs de l'environnement car ils ne sont pas altérés par

des processus métaboliques au sein de la masse d'eau. Ainsi, ils sont susceptibles de ne pas être régulés physiologiquement lors de leur incorporation au sein des otolithes et de présenter des concentrations dans les otolithes reflétant celles de l'environnement (Arslan & Paulson 2003). De plus, ces éléments sont aussi caractéristiques des apports terrigènes provenant des eaux douces et bassins versants et certains d'entre eux résulteraient des activités anthropiques (indicateur de pollutions ou autres) (Munksgaard *et al.* 2003, Nozaki 2009).

Certains ratios élémentaires et isotopiques, tels que les ratios Ba/Sr et $^{87}\text{Sr}/^{86}\text{Sr}$, diffèrent significativement entre le milieu marin et le milieu dulçaquicole (McCulloch *et al.* 2005, McMahon *et al.* 2013). Ils permettraient de retracer les migrations diadromiques en caractérisant la fréquentation de ces deux milieux ainsi que le passage au sein d'environnements de transition tels que les estuaires. La Nouvelle-Calédonie présente de nombreux estuaires le long de sa façade maritime et l'étude de ces ratios pourraient apporter de nouvelles connaissances sur les patterns de mouvements des poissons diadromiques calédoniens.

Dans l'optique d'études supplémentaires renseignant sur les possibles usages de la microchimie des otolithes au sein du paysage marin de la Nouvelle-Calédonie, il serait intéressant d'établir une première série de pistes quant à la potentialité de l'outil en tant qu'enregistreur d'impacts environnementaux. En effet, la microchimie des otolithes pourrait révéler les traces de l'influence anthropique passée et actuelle en Nouvelle-Calédonie telles que l'exploitation minière, le développement des zones urbaines ou des événements chimiques exceptionnels. Ces pistes ont pu être initiées au cours d'un programme d'étude de la microchimie des otolithes et de l'environnement de Nouvelle-Calédonie (Vigliola *et al.* 2013). Ce travail n'a pas permis de différencier les sites sous influence anthropique par l'analyse directe de la microchimie des otolithes de poisson. Cependant, l'outil de classification utilisé a permis de détecter ces sites en y associant une probabilité d'impact. Les résultats de cette étude préliminaire ainsi que l'ajout aux analyses microchimiques des terres rares, qui pour certains résulteraient directement des activités anthropiques, encouragent la réalisation d'études supplémentaires visant à approfondir le potentiel de l'outil microchimie en tant qu'indicateur d'impact.

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ANNEXES

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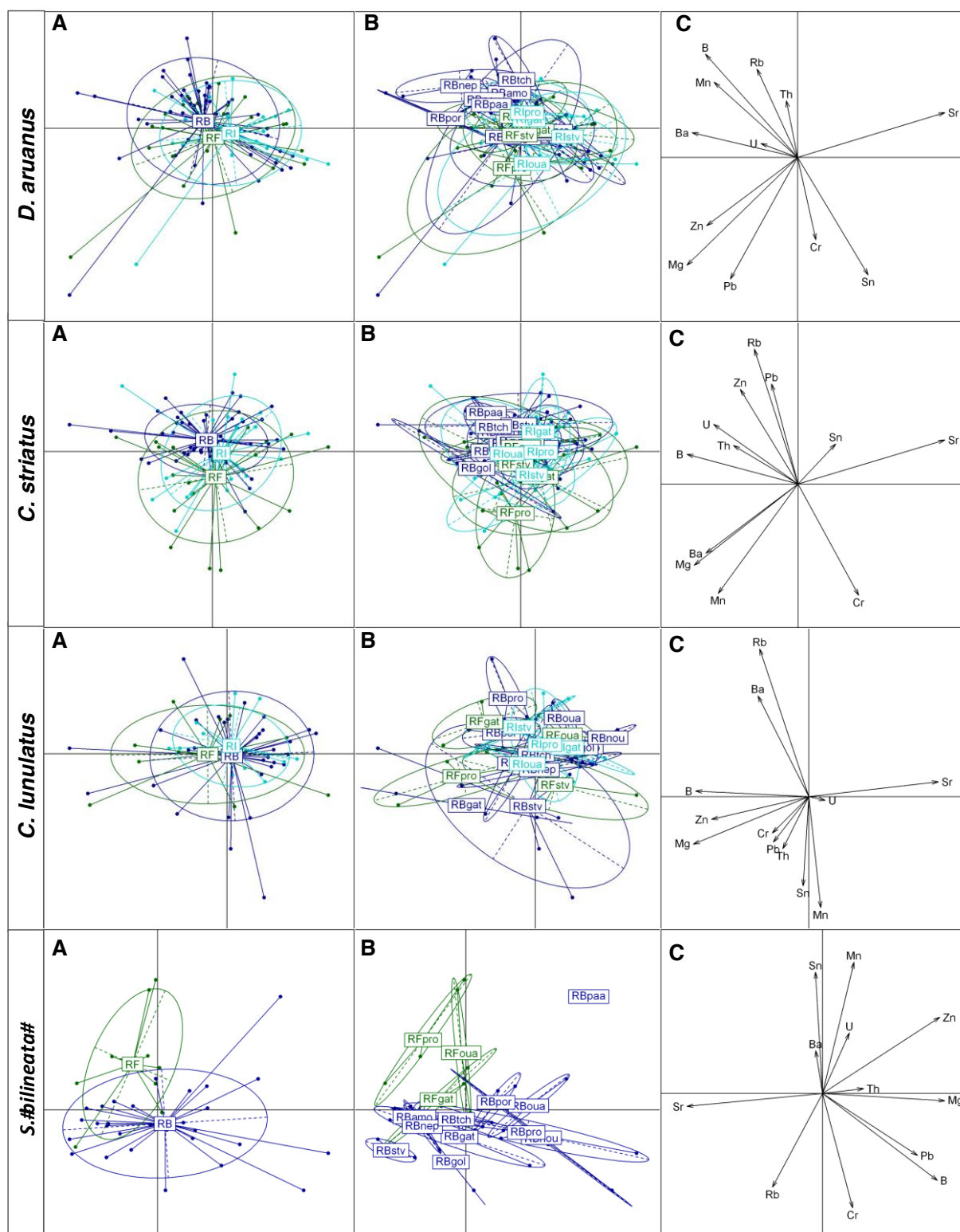
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Appendix 1: Multivariate results of PERMANOVA examining spatial variation in otolith elemental composition within site and habitat at the global scale for multi-specific group and the eight species.

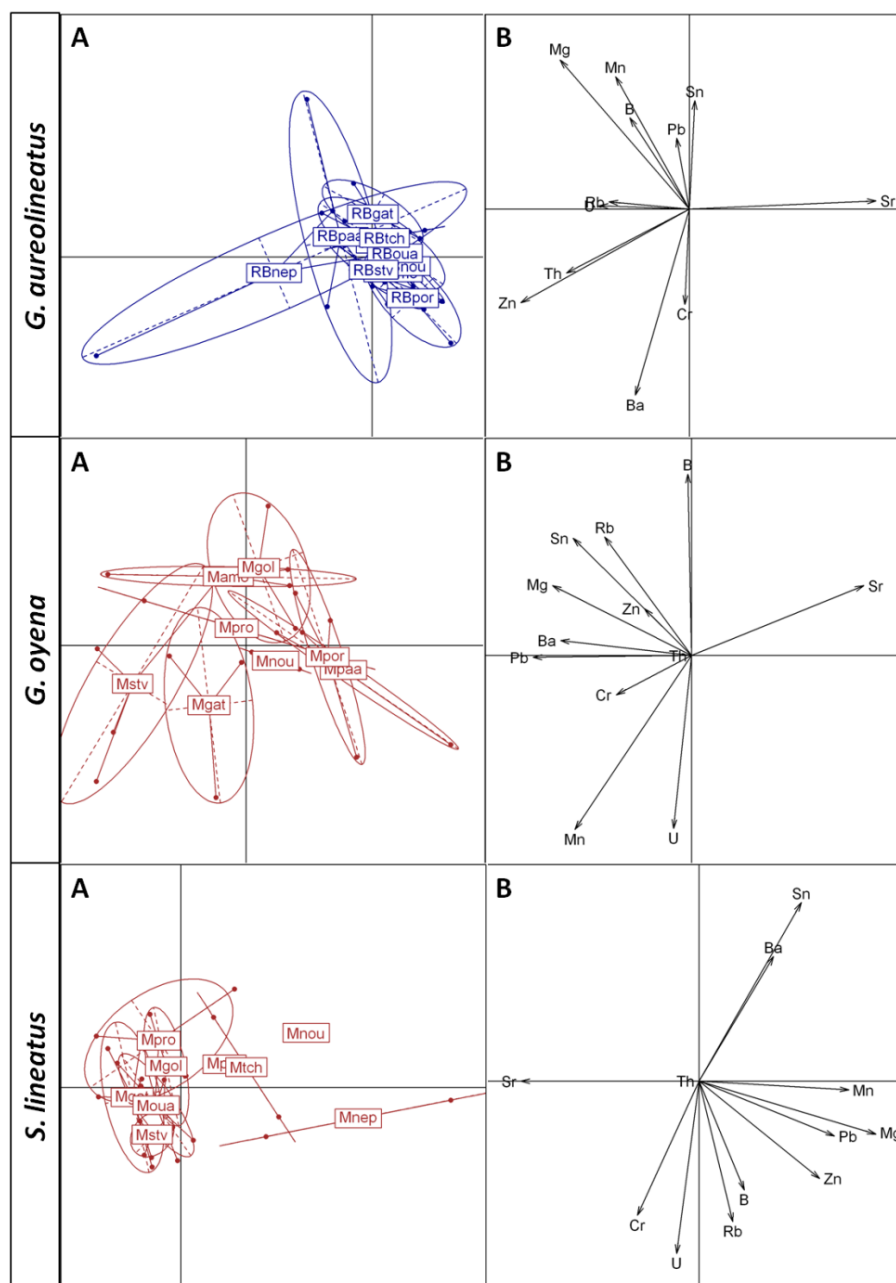
Species	df	MS	F	p
Multi-specific				
Site	10	0.003	1.99	0.006**
Habitat	3	0.076	49.29	0.001***
Site x Habitat	16	0.003	2.19	0.001***
<i>L. fulviflamma</i>				
Site	10	0.014	3.27	0.001***
Habitat	3	0.005	11.29	0.001***
Site x Habitat	14	0.0008	1.92	0.013*
<i>D. aruanus</i>				
Site	9	0.0023	1.96	0.04*
Habitat	2	0.0020	1.68	0.17
Site x Habitat	6	0.0018	1.49	0.11
<i>C. striatus</i>				
Site	10	0.0005	0.89	0.61
Habitat	2	0.0005	0.87	0.46
Site x Habitat	6	0.0005	0.98	0.46
<i>C. lunulatus</i>				
Site	10	0.006	2.15	0.026 *
Habitat	2	0.0003	0.91	0.44
Site x Habitat	6	0.0007	2.39	0.023 *
<i>S. bilineata</i>				
Site	10	0.006	2.0620	0.012*
Habitat	1	0.0022	7.3486	0.002**
Site x Habitat	2	0.0007	2.3191	0.063 +
<i>G. aureolineatus</i>				
Site	9	0.0006	1.3777	0.113
<i>G. oyena</i>				
Site	7	0.0031	3.8955	0.005**
<i>S. lineatus</i>				
Site	8	0.0036	4.8142	0.001***

(*** p < 0.001; ** p < 0.01; * p < 0.05; + p < 0.1)

Appendix 2: Plots of principal component analysis (PCA) of elemental compositions of otoliths.



Plots of PCA of *D. aruanus*, *C. striatus*, *C. lunulatus* and *S. bilineata* elemental compositions of otoliths for habitats (A) and stations (B) and plot of contributions of elements in habitat and station elemental compositions (C) (with FR: fringing reef; IR: intermediate reef; BR: inner barrier reef).



Plots of PCA of *G. aureolineatus*, *G. oyena* and *S. lineatus* elemental compositions of otoliths for stations (A) and plot of contributions of elements in habitat and station elemental compositions (B).

Appendix 3: Recap of the percentage of correct classification obtained by RF and the best combination of elements for the all classifiers tested.

Species	Scale	Site	Habitat	Level	Correct classification (%)	Combination of elements
Multi-specific	Local	Gatope	M/FR/IR/BR	Habitat	63	Ba Mg Mn Pb Rb Sn Zn
		Ouano	M/FR/IR/BR	Habitat	49	B Ba Mn Pb Sn Th Zn
		Prony	M/FR/IR/BR	Habitat	57	B Ba Mn Sn U Zn
	Regional	St Vincent	M/FR/IR/BR	Habitat	69	Ba Mg Mn Pb Rb U
		Pilot Sites	M/FR/IR/BR	Habitat	59	B Ba Cr Mg Mn Rb Sn
		Pilot Sites	M	Stations	63	B Ba Sn U
		Pilot Sites	FR	Stations	42	B Ba Mg Pb Rb Sn Zn
		Pilot Sites	IR	Stations	44	Ba Rb Sn Sr Zn
		Pilot Sites	BR	Stations	47	B Pb Sn Th U Zn
	Global	All	M/BR	Habitat	90	Ba Mg Mn Pb Rb Sn U
		All	M	Stations	44	B Ba Cr Mn Sn
		All	BR	Stations	29	B Pb Rb Sn Sr
<i>L. fulviflamma</i>	Local	Gatope	M/FR/IR/BR	Habitat	74	Ba Cr Mg Mn U Zn
		Ouano	M/FR/IR/BR	Habitat	43	B Mn Pb Zn
		Prony	M/FR/IR/BR	Habitat	78	B Ba Mg Mn Pb Sn
	Regional	St Vincent	M/FR/IR/BR	Habitat	73	B Cr Mn Pb Rb U
		Pilot Sites	M/FR/IR/BR	Habitat	61	Cr Mg Mn Pb Rb Sr
		Pilot Sites	M	Stations	100	Ba Cr Mg Pb Rb Sn Zn
		Pilot Sites	FR	Stations	63	B Mg Mn Pb Rb Sn
		Pilot Sites	IR	Stations	54	Ba Mg Mn Sr U
		Pilot Sites	BR	Stations	48	Cr Mn Pb Sn U Zn
	Global	All	M/BR	Habitat	95	B Cr Mn Pb Rb Sn Th U
		All	M	Stations	65	B Ba Mn Pb Sn Zn
		All	BR	Stations	29	Ba Cr Sn U Zn
<i>D. aruanus</i>	Local	Gatope	FR/IR/BR	Habitat	74	B Ba Cr Mn Rb Sr
		Ouano	FR/IR/BR	Habitat	74	B Ba Cr Mg Sn Th
		Prony	FR/IR/BR	Habitat	67	B Ba Mg Mn Rb Sr
	Regional	St Vincent	FR/IR/BR	Habitat	85	B Sn Sr Zn
		Pilot Sites	FR/IR/BR	Habitat	64	B Ba Cr Mg Mn Pb Rb Zn
		Pilot Sites	FR	Stations	64	B Mg Sr U
		Pilot Sites	IR	Stations	67	B Ba Th Zn
		Pilot Sites	BR	Stations	60	B Ba Pb Sn U
	Global	All	BR	Stations	41	B Ba Cr Mg Mn Rb Sn Sr Th U
<i>C. striatus</i>	Local	Gatope	FR/IR/BR	Habitat	79	B Mn
		Ouano	FR/IR/BR	Habitat	83	B Rb Sn U
		Prony	FR/IR/BR	Habitat	96	B Cr Pb Rb Sn Sr Th Zn
	Regional	St Vincent	FR/IR/BR	Habitat	71	Cr Mg Pb Th
		Pilot Sites	FR/IR/BR	Habitat	71	B Cr Mg Mn Pb
		Pilot Sites	FR	Stations	71	Cr Mn Rb Sr Th U
		Pilot Sites	IR	Stations	64	B Ba Rb U
		Pilot Sites	BR	Stations	63	B Mn Sr U
	Global	All	BR	Stations	47	Ba Mg Mn Pb Sn Sr Th
<i>C. lunulatus</i>	Local	Gatope	FR/IR/BR	Habitat	67	Cr Mn Pb Rb
		Ouano	FR/IR/BR	Habitat	80	Ba Mg Mn Rb Zn
		Prony	FR/IR/BR	Habitat	67	B Sn
	Regional	St Vincent	FR/IR/BR	Habitat	80	Ba Mn Rb
		Pilot Sites	FR/IR/BR	Habitat	67	Ba Rb Sn
		Pilot Sites	FR	Stations	50	Rb
		Pilot Sites	IR	Stations	58	Rb U
		Pilot Sites	BR	Stations	83	Ba Cr Mg Mn Pb Sr
	Global	All	BR	Stations	41	B Mn Pb Rb Sr
<i>S. bilineata</i>	Regional	Pilot Sites	FR/BR	Habitat	95	B Mn Pb Sr Th
		Pilot Sites	FR	Stations	78	Mg Pb Zn
		Pilot Sites	BR	Stations	100	Mg Mn
	Global	All	BR	Stations	37	Mn U
<i>S. lineatus</i>	Regional	Pilot Sites	M	Stations	84	B Mn
	Global	All	M	Stations	63	B Mn Rb U
<i>G. aureolineatus</i>	Regional	Pilot Sites	BR	Stations	80	Ba
	Global	All	BR	Stations	24	Ba Cr Sn Th
<i>G. oyena</i>	Regional	Pilot Sites	M	Stations	83	B Sn Zn
	Global	All	M	Stations	67	B Ba Co Cr Mn Ni U

Appendix 4: Percentage of correct classification obtained by RF with mono-specific and multi-specific classifiers.

Scale	Species	Habitat	Group	Mono-specific (%)	Multi-specific (%)
Local Gatope	<i>C.lunulatus</i>	FR/IR/BR	global	67	50
Local Gatope	<i>C.striatus</i>	FR/IR/BR	global	79	32
Local Gatope	<i>D.aruanus</i>	FR/IR/BR	global	74	41
Local Gatope	<i>D.aruanus</i>	FR/IR/BR	taxa	74	31
Local Gatope	<i>L.fulviflamma</i>	M/FR/IR/BR	global	74	29
Local Gatope	<i>L.fulviflamma</i>	M/FR/IR/BR	diet	74	29
Local Ouano	<i>C.lunulatus</i>	FR/IR/BR	global	80	22
Local Ouano	<i>C.striatus</i>	FR/IR/BR	global	83	48
Local Ouano	<i>D.aruanus</i>	FR/IR/BR	global	74	17
Local Ouano	<i>D.aruanus</i>	FR/IR/BR	taxa	74	24
Local Ouano	<i>L.fulviflamma</i>	M/FR/IR/BR	global	43	26
Local Ouano	<i>L.fulviflamma</i>	M/FR/IR/BR	diet	43	29
Local Prony	<i>C.lunulatus</i>	FR/IR/BR	global	67	22
Local Prony	<i>C.striatus</i>	FR/IR/BR	global	96	46
Local Prony	<i>D.aruanus</i>	FR/IR/BR	global	67	33
Local Prony	<i>D.aruanus</i>	FR/IR/BR	taxa	67	37
Local Prony	<i>L.fulviflamma</i>	M/FR/IR/BR	global	78	22
Local Prony	<i>L.fulviflamma</i>	M/FR/IR/BR	diet	78	26
Local St Vincent	<i>C.lunulatus</i>	FR/IR/BR	global	80	42
Local St Vincent	<i>C.striatus</i>	FR/IR/BR	global	71	42
Local St Vincent	<i>D.aruanus</i>	FR/IR/BR	global	85	52
Local St Vincent	<i>D.aruanus</i>	FR/IR/BR	taxa	85	23
Local St Vincent	<i>L.fulviflamma</i>	M/FR/IR/BR	global	73	49
Local St Vincent	<i>L.fulviflamma</i>	M/FR/IR/BR	diet	73	58
Regional	<i>C.lunulatus</i>	FR/IR/BR	global	67	52
Regional	<i>C.striatus</i>	FR/IR/BR	global	71	29
Regional	<i>D.aruanus</i>	FR/IR/BR	global	64	35
Regional	<i>D.aruanus</i>	FR/IR/BR	taxa	64	32
Regional	<i>L.fulviflamma</i>	M/FR/IR/BR	global	67	40
Regional	<i>L.fulviflamma</i>	M/FR/IR/BR	diet	67	39
Regional	<i>G.oyena</i>	M	global	83	25
Regional	<i>G.oyena</i>	M	diet	83	0
Regional	<i>L.fulviflamma</i>	M	global	100	20
Regional	<i>L.fulviflamma</i>	M	diet	100	11
Regional	<i>S.lineatus</i>	M	global	84	44
Regional	<i>C.lunulatus</i>	FR	global	50	8
Regional	<i>C.striatus</i>	FR	global	71	32
Regional	<i>D.aruanus</i>	FR	global	64	31
Regional	<i>D.aruanus</i>	FR	taxa	64	44
Regional	<i>L.fulviflamma</i>	FR	global	63	28
Regional	<i>L.fulviflamma</i>	FR	diet	63	32
Regional	<i>S.bilineata</i>	FR	global	78	22
Regional	<i>S.bilineata</i>	FR	diet	78	0
Regional	<i>C.lunulatus</i>	IR	global	58	17
Regional	<i>C.striatus</i>	IR	global	64	39
Regional	<i>C.striatus</i>	IR	diet	64	39
Regional	<i>D.aruanus</i>	IR	global	67	38
Regional	<i>D.aruanus</i>	IR	taxa	67	33
Regional	<i>L.fulviflamma</i>	IR	global	54	26
Regional	<i>L.fulviflamma</i>	IR	diet	54	23
Regional	<i>C.lunulatus</i>	BR	global	83	46
Regional	<i>C.striatus</i>	BR	global	63	18
Regional	<i>C.striatus</i>	BR	diet	63	39
Regional	<i>D.aruanus</i>	BR	global	60	35
Regional	<i>D.aruanus</i>	BR	taxa	60	42
Regional	<i>G.aureolineatus</i>	BR	global	80	10
Regional	<i>G.aureolineatus</i>	BR	diet	80	20
Regional	<i>L.fulviflamma</i>	BR	global	48	31
Regional	<i>L.fulviflamma</i>	BR	diet	48	35
Regional	<i>S.bilineata</i>	BR	global	100	0
Regional	<i>S.bilineata</i>	BR	diet	100	17
Global	<i>L.fulviflamma</i>	M/BR	global	79	79
Global	<i>L.fulviflamma</i>	M/BR	diet	79	51

Appendix 5: Results of Spearman and Mantel tests performed on environmental and otoliths elemental compositions with salinity

(“****” $p < 0.001$; “***” $p < 0.01$; “**” $p < 0.05$; “+” $p < 0.1$)

	Seawater		Sediment		Multispecific		<i>L. fulviflamma</i>		<i>D. aruanus</i>		<i>C. striatus</i>		<i>C. lunulatus</i>		<i>S. bilineata</i>		<i>G. aureolineatus</i>		<i>S. lineatus</i>		<i>G. oyena</i>	
	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p
B	-0.56	**	0.32	.	-0.40	*	-0.16	NS	0.26	NS	-0.34	NS	0.06	NS	-0.29	NS	-0.63	.	0.44	NS	-0.11	NS
Ba	-0.56	**	-0.11	NS	-0.31	NS	-0.17	NS	-0.38	NS	-0.14	NS	0.24	NS	0.24	NS	0.10	NS	0.06	NS	-0.38	NS
Cr	-0.05	NS	0.02	NS	-0.37	*	-0.51	**	-0.29	NS	0.44	.	0.19	NS	-0.04	NS	0.03	NS	-0.49	NS	-0.76	*
Mg	0.41	*	-0.35	.	-0.38	*	-0.26	NS	0.10	NS	0.04	NS	0.11	NS	-0.18	NS	0.34	NS	0.54	NS	0.05	NS
Mn	-0.23	NS	-0.06	NS	-0.35	.	0.11	NS	0.42	.	0.31	NS	0.04	NS	0.47	NS	0.25	NS	0.56	NS	0.14	NS
Pb	-0.38	*	-0.15	NS	-0.27	NS	-0.29	NS	-0.18	NS	-0.33	NS	0.30	NS	-0.02	NS	-0.12	NS	0.04	NS	-0.11	NS
Rb	0.29	NS	-0.09	NS	0.10	NS	0.11	NS	0.28	NS	-0.09	NS	-0.04	NS	-0.12	NS	-0.25	NS	-0.10	NS	-0.54	NS
Sn	-0.32	NS	-0.05	NS	-0.56	**	-0.35	.	0.08	NS	0.23	NS	-0.19	NS	0.18	NS	-0.25	NS	0.41	NS	-0.18	NS
Sr	0.02	NS	0.34	.	0.42	*	0.31	NS	0.10	NS	0.19	NS	-0.14	NS	0.07	NS	0.08	NS	-0.32	NS	-0.32	NS
Th	-0.04	NS	-0.15	NS	0.07	NS	0.11	NS	-0.01	NS	-0.37	NS	-0.07	NS	-0.02	NS	-0.13	NS	NA	NA	NA	NA
U	-0.54	**	0.10	NS	-0.24	NS	-0.19	NS	-0.33	NS	-0.03	NS	0.06	NS	0.53	.	0.72	*	0.12	NS	0.34	NS
Zn	0.07	NS	-0.09	NS	-0.41	*	-0.51	**	0.12	NS	-0.17	NS	0.13	NS	0.48	.	0.14	NS	0.67	.	-0.41	NS
All	0.10	NS	-0.10	NS	0.11	NS	0.23	.	-0.15	NS	0.30	*	-0.15	NS	-0.08	NS	-0.07	NS	-0.07	NS	-0.27	NS

Appendix 6: Results of Spearman and Mantel tests performed on environmental and otoliths elemental compositions with temperature

(“****” $p < 0.001$; “***” $p < 0.01$; “**” $p < 0.05$; “+” $p < 0.1$, NS: non significant, NA: no data)

	Seawater		Sediment		Multispecific		<i>L. fulvivflamma</i>		<i>D. aruanus</i>		<i>C. striatus</i>		<i>C. lunulatus</i>		<i>S. bilineata</i>		<i>G. aureolineatus</i>		<i>S. lineatus</i>		<i>G. oyena</i>	
	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p
B	0.35	.	-0.06	NS	-0.20	NS	-0.60	**	0.50	*	0.34	NS	-0.18	NS	0.19	NS	-0.26	NS	-0.12	NS	0.32	NS
Ba	0.20	NS	-0.14	NS	0.18	NS	0.10	NS	0.44	.	-0.06	NS	0.14	NS	0.34	NS	0.32	NS	0.70	NS	-0.29	NS
Cr	-0.43	*	-0.14	NS	-0.62	***	-0.36	.	-0.04	NS	-0.52	*	-0.03	NS	-0.31	NS	-0.10	NS	-0.61	NS	-0.32	NS
Mg	-0.38	.	-0.09	NS	-0.49	**	-0.40	*	-0.33	NS	-0.18	NS	-0.48	*	0.17	NS	-0.29	NS	0.06	NS	-0.46	NS
Mn	-0.32	NS	-0.15	NS	-0.34	.	-0.59	**	-0.17	NS	-0.43	.	-0.43	.	-0.07	NS	-0.25	NS	-0.38	NS	-0.17	NS
Pb	0.44	*	-0.29	NS	0.26	NS	-0.08	NS	0.02	NS	0.55	*	0.02	NS	0.64	*	-0.04	NS	0.12	NS	-0.38	NS
Rb	-0.40	*	-0.18	NS	0.29	NS	0.53	**	0.17	NS	0.59	**	0.15	NS	-0.21	NS	-0.31	NS	-0.29	NS	0.46	NS
Sn	0.22	NS	-0.31	NS	0.09	NS	-0.16	NS	-0.46	.	-0.21	NS	0.02	NS	-0.21	NS	-0.27	NS	0.52	NS	-0.41	NS
Sr	0.28	NS	0.08	NS	0.39	*	0.42	*	-0.09	NS	-0.03	NS	0.43	.	-0.32	NS	0.23	NS	-0.23	NS	0.23	NS
Th	-0.44	*	-0.24	NS	0.06	NS	-0.07	NS	0.24	NS	0.06	NS	-0.13	NS	0.06	NS	-0.60	.	NA	NA	NA	NA
U	0.27	NS	-0.09	NS	0.50	**	0.46	*	0.42	.	0.28	NS	-0.22	NS	0.37	NS	-0.61	.	-0.43	NS	-0.19	NS
Zn	0.11	NS	-0.23	NS	-0.46	*	0.22	NS	-0.53	*	0.02	NS	-0.03	NS	0.12	NS	-0.27	NS	-0.06	NS	0.46	NS
All	0.04	NS	-0.10	NS	0.16	.	0.34	**	0.10	NS	0.02	NS	0.12	NS	-0.06	NS	0.44	.	-0.26	NS	-0.35	NS

Appendix 7: Results of Spearman and Mantel tests performed on sediment and otoliths elemental compositions with seawater elemental composition

(“****” $p < 0.001$; “***” $p < 0.01$; “**” $p < 0.05$; “+” $p < 0.1$; NS: non significant, NA: no data)

	Sediment		Multispecific		<i>L. fulviflamma</i>		<i>D. aruanus</i>		<i>C. striatus</i>		<i>C. lunulatus</i>		<i>S. bilineata</i>		<i>G. aureolineatus</i>		<i>S. lineatus</i>		<i>G. oyena</i>	
	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p
B	0.00	NS	0.10	NS	-0.34	.	0.29	NS	0.29	NS	-0.15	NS	0.53	.	0.07	NS	-0.88	**	-0.12	NS
Ba	-0.07	NS	0.23	NS	0.29	NS	0.45	.	0.36	NS	-0.04	NS	-0.12	NS	0.12	NS	0.45	NS	0.74	*
Cr	0.25	NS	0.27	NS	0.20	NS	-0.08	NS	0.22	NS	0.11	NS	-0.32	NS	-0.10	NS	0.51	NS	0.55	NS
Mg	-0.16	NS	-0.19	NS	0.01	NS	0.08	NS	0.24	NS	0.07	NS	0.09	NS	0.18	NS	0.53	NS	0.55	NS
Mn	-0.07	NS	0.85	***	0.62	***	0.51	*	0.00	NS	-0.31	NS	0.46	.	0.24	NS	-0.03	NS	0.57	NS
Pb	-0.04	NS	0.36	.	0.18	NS	-0.13	NS	0.52	*	0.12	NS	0.53	.	-0.07	NS	0.20	NS	0.16	NS
Rb	0.19	NS	-0.43	*	-0.23	NS	-0.07	NS	-0.17	NS	-0.16	NS	0.34	NS	-0.36	NS	-0.43	NS	-0.57	NS
Sn	0.19	NS	0.11	NS	-0.12	NS	-0.06	NS	0.10	NS	-0.07	NS	-0.22	NS	-0.23	NS	0.20	NS	-0.08	NS
Sr	0.31	.	-0.12	NS	0.21	NS	-0.55	*	0.02	NS	-0.06	NS	-0.21	NS	-0.50	NS	0.18	NS	-0.24	NS
Th	0.23	NS	0.05	NS	0.33	.	-0.19	NS	0.08	NS	-0.31	NS	-0.12	NS	0.01	NS	NA	NA	NA	NA
U	-0.20	NS	0.08	NS	-0.09	NS	0.40	NS	0.25	NS	-0.04	NS	-0.32	NS	-0.20	NS	0.70	*	-0.35	NS
Zn	0.06	NS	0.19	NS	0.30	NS	0.01	NS	-0.34	NS	0.32	NS	0.43	NS	0.14	NS	-0.55	NS	-0.52	NS
All	-0.04	NS	0.16	NS	-0.02	NS	-0.02	NS	0.10	NS	-0.04	NS	-0.03	NS	-0.18	NS	0.01	NS	0.50	*

Appendix 8: Results of Spearman and Mantel tests performed on seawater and otoliths elemental compositions with sediment elemental composition

(“****” $p < 0.001$; “***” $p < 0.01$; “**” $p < 0.05$; “+” $p < 0.1$, NS: non significant, NA: no data)

	Seawater		Multispecific		<i>L. fulviflamma</i>		<i>D. aruanus</i>		<i>C. striatus</i>		<i>C. lunulatus</i>		<i>S. bilineata</i>		<i>G. aureolineatus</i>		<i>S. lineatus</i>		<i>G. oyena</i>	
	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p	Rs	p
B	0.00	NS	-0.25	NS	0.02	NS	0.19	NS	-0.07	NS	0.05	NS	-0.09	NS	-0.02	NS	-0.03	NS	-0.50	NS
Ba	-0.07	NS	-0.20	NS	-0.13	NS	0.08	NS	0.39	NS	-0.49	*	-0.31	NS	-0.08	NS	-0.37	NS	-0.64	.
Cr	0.25	NS	0.05	NS	0.06	NS	0.07	NS	0.10	NS	-0.23	NS	0.07	NS	-0.44	NS	-0.15	NS	0.29	NS
Mg	-0.16	NS	-0.16	NS	-0.13	NS	-0.38	NS	0.24	NS	-0.11	NS	0.06	NS	-0.08	NS	-0.08	NS	0.10	NS
Mn	-0.07	NS	-0.08	NS	0.00	NS	0.08	NS	0.41	.	-0.06	NS	0.09	NS	-0.13	NS	0.10	NS	-0.57	NS
Pb	-0.04	NS	-0.07	NS	-0.27	NS	-0.08	NS	0.28	NS	0.15	NS	-0.51	.	0.68	*	0.05	NS	-0.63	.
Rb	0.19	NS	-0.27	NS	0.05	NS	0.34	NS	-0.20	NS	-0.53	*	0.39	NS	0.43	NS	0.30	NS	-0.67	.
Sn	0.19	NS	0.12	NS	0.34	.	-0.04	NS	-0.27	NS	0.00	NS	0.03	NS	0.39	NS	0.10	NS	-0.62	NS
Sr	0.31	.	-0.10	NS	-0.01	NS	-0.34	NS	0.45	.	-0.16	NS	-0.05	NS	-0.09	NS	0.00	NS	-0.26	NS
Th	0.23	NS	0.09	NS	0.24	NS	0.38	NS	0.26	NS	-0.15	NS	-0.13	NS	0.60	.	NA	NA	NA	NA
U	-0.20	NS	-0.06	NS	-0.21	NS	-0.03	NS	0.28	NS	0.47	*	0.34	NS	-0.10	NS	-0.07	NS	0.00	NS
Zn	0.06	NS	-0.01	NS	-0.01	NS	0.13	NS	-0.07	NS	-0.12	NS	-0.25	NS	0.20	NS	-0.57	NS	-0.60	NS
All	-0.04	NS	0.46	*	0.52	*	-0.03	NS	0.05	NS	-0.11	NS	-0.07	NS	0.00	NS	-0.20	NS	0.51	*

Appendix 9: Summary of the percentage of correct classification obtained by RF and the best combination of elements for the all classifiers tested.

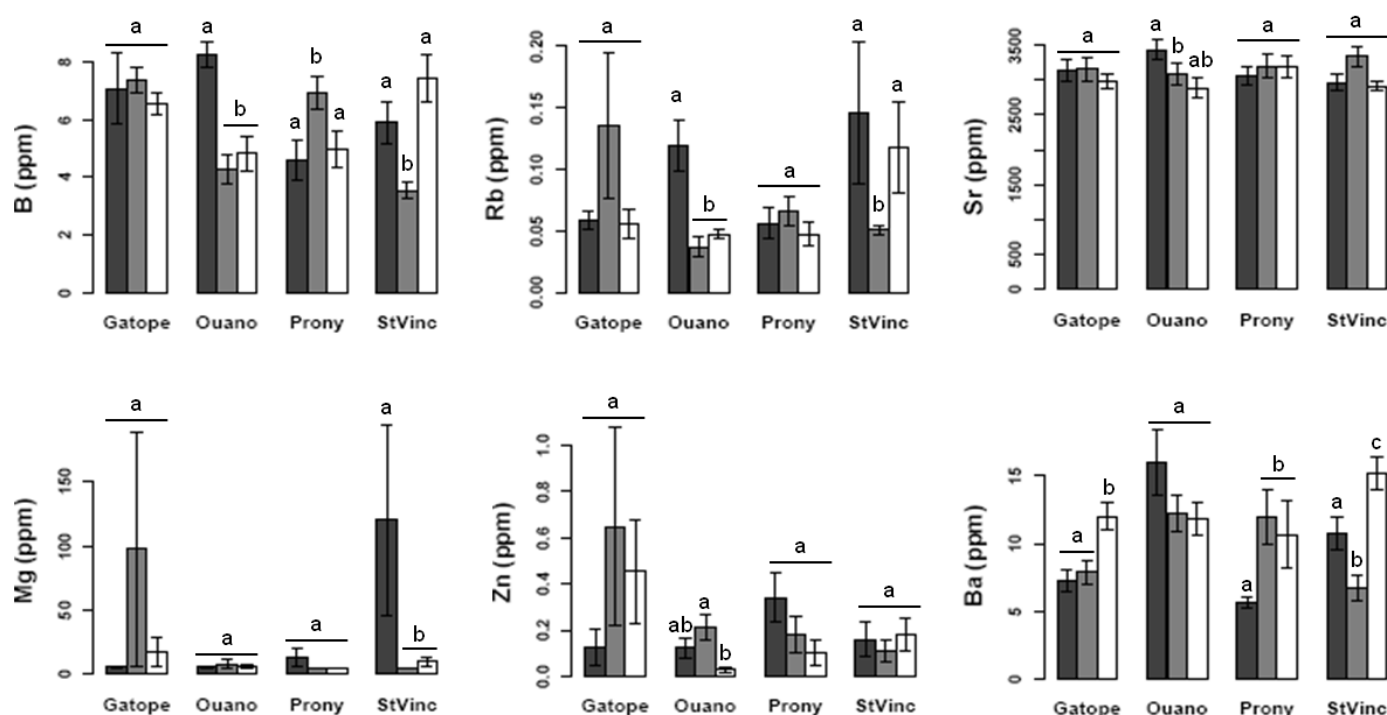
Species	Scale	Site	Habitat	Level	Correct classification (%)	Combination of elements
Seawater	Local	Gatope	M/FR/IR/BR	Habitat	79	Ba Cr Sn Zn
		Ouano	M/FR/IR/BR	Habitat	67	B Mg Mn
		Prony	M/FR/IR/BR	Habitat	50	Ba Mn U
		St Vincent	M/FR/IR/BR	Habitat	71	Mn Pb
	Regional	West coast	M/FR/IR/BR	Habitat	74	Mg Mn Rb Sn Sr
		West coast	M	Station	86	Cr Mg Th
		West coast	FR	Station	33	Th
		West coast	IR	Station	50	Zn
		West coast	BR	Station	35	Ba Pb
	Global	All	M/BR	Habitat	95	Mg Mn Zn
		All	M	Station	63	Cr Mg Mn Zn
		All	BR	Station	22	Pb
Sediment	Local	Gatope	M/FR/IR/BR	Habitat	63	Zn
		Ouano	M/FR/IR/BR	Habitat	88	Cr
		Prony	M/FR/IR/BR	Habitat	50	Pb U
		St Vincent	M/FR/IR/BR	Habitat	43	B
	Regional	West coast	M/FR/IR/BR	Habitat	42	Mg Mn Pb Sn
		West coast	M	Station	25	Cr
		West coast	FR	Station	50	Pb
		West coast	IR	Station	75	Pb
		West coast	BR	Station	57	B Cr Sr
	Global	All	M/BR	Habitat	76	B Mg Sr
		All	M	Station	23	Pb
		All	BR	Station	30	Cr Sr

(M: mangrove, FR: fringing reef, IR: intermediate reef, BR: barrier reef).

Appendix 10: Spatial variation in otolith fingerprints of *Dascyllus aruanus*

Multivariate PERMANOVA showed a statistical significant difference in otolith chemistry for fish from Ouano and St Vincent reefs (p-value = 0.04 and 0.02 respectively). There was no significant difference for otoliths from Gatope and Prony reefs (p-value = 0.6 and 0.8 respectively).

One-way ANOVA revealed significant differences in concentrations of B, Ba, Mg, Rb, Sr and Zn and post-hoc tests revealed between fringing, intermediate and barrier reefs which ones displayed these differences. These differences resulted in the following reef groups: intermediate and barrier reefs were pooled in the offshore reefs and fringing reefs constituted the inshore reefs in Gatope, Ouano and Prony. On the contrary, fringing and intermediate reefs were pooled in the inshore reef and barrier reef constituted the offshore reef in St Vincent.

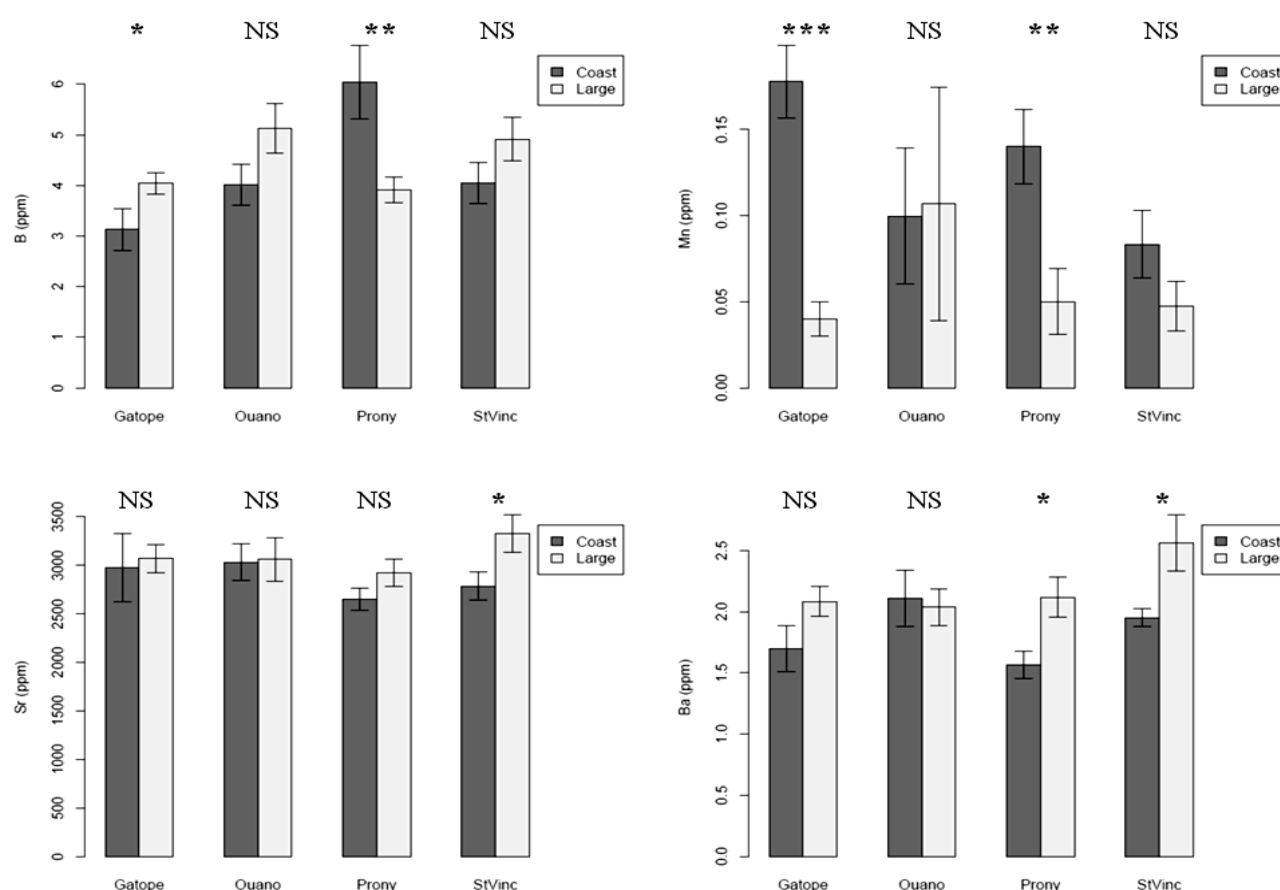


Dascyllus aruanus otolith elemental concentrations of B:Ca, Ba:Ca, Mg:Ca, Rb:Ca, Sr:Ca and Zn:Ca per reef habitat. Mean and standard error are plotted. Bar labelled with different letters (a, b, c) are statistically different from each other (Non parametric post-hoc tests, multiple comparisons of mean ranks for all groups (Siegel and Castellan, 1988)).

Appendix 11: Spatial variation in otolith fingerprints of *Lutjanus fulvivflamma*

Multivariate PERMANOVA showed a statistical significant difference between elemental compositions of otoliths inshore and offshore reefs from St Vincent (p-value = 0.03). There were no significant differences observed between reefs inshore and offshore reefs of Gatope, Ouano and Prony (p-value = 0.8, 0.9 and 0.2 respectively).

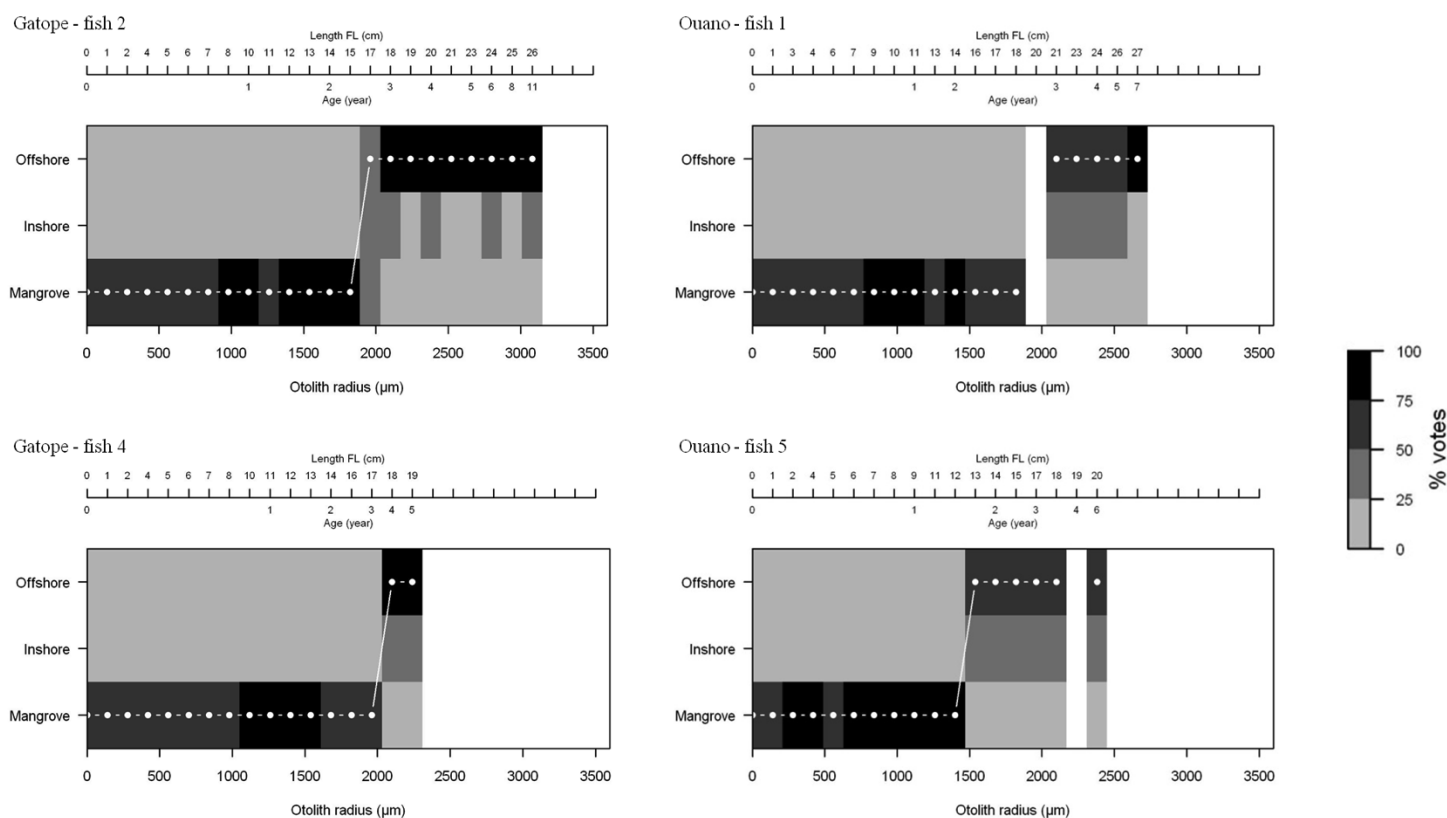
One-way ANOVA revealed significant differences in B, Ba Mn and Sr concentrations in otoliths between reefs. In Gatope, Mn concentrations were higher and B concentrations were lower in inshore reef compared to offshore reef. In Ouano, elemental concentrations of otoliths showed no significant differences between inshore and offshore reefs. In Prony, B and Mn concentrations were higher and Ba concentrations were lower in inshore reefs compared to offshore reefs. In St Vincent, concentrations of Ba and Sr were lower in inshore reefs compared to offshore reefs.



Lutjanus fulvivflamma otolith elemental concentrations of B:Ca, Ba:Ca, Mn:Ca and Sr:Ca per reef habitat (coastal vs large reefs). Mean and standard error are plotted. Statistical significances of one-way anova results are indicated above graphs (“***” p-value < 0.001; “**” p-value < 0.01; “*” p-value < 0.05).

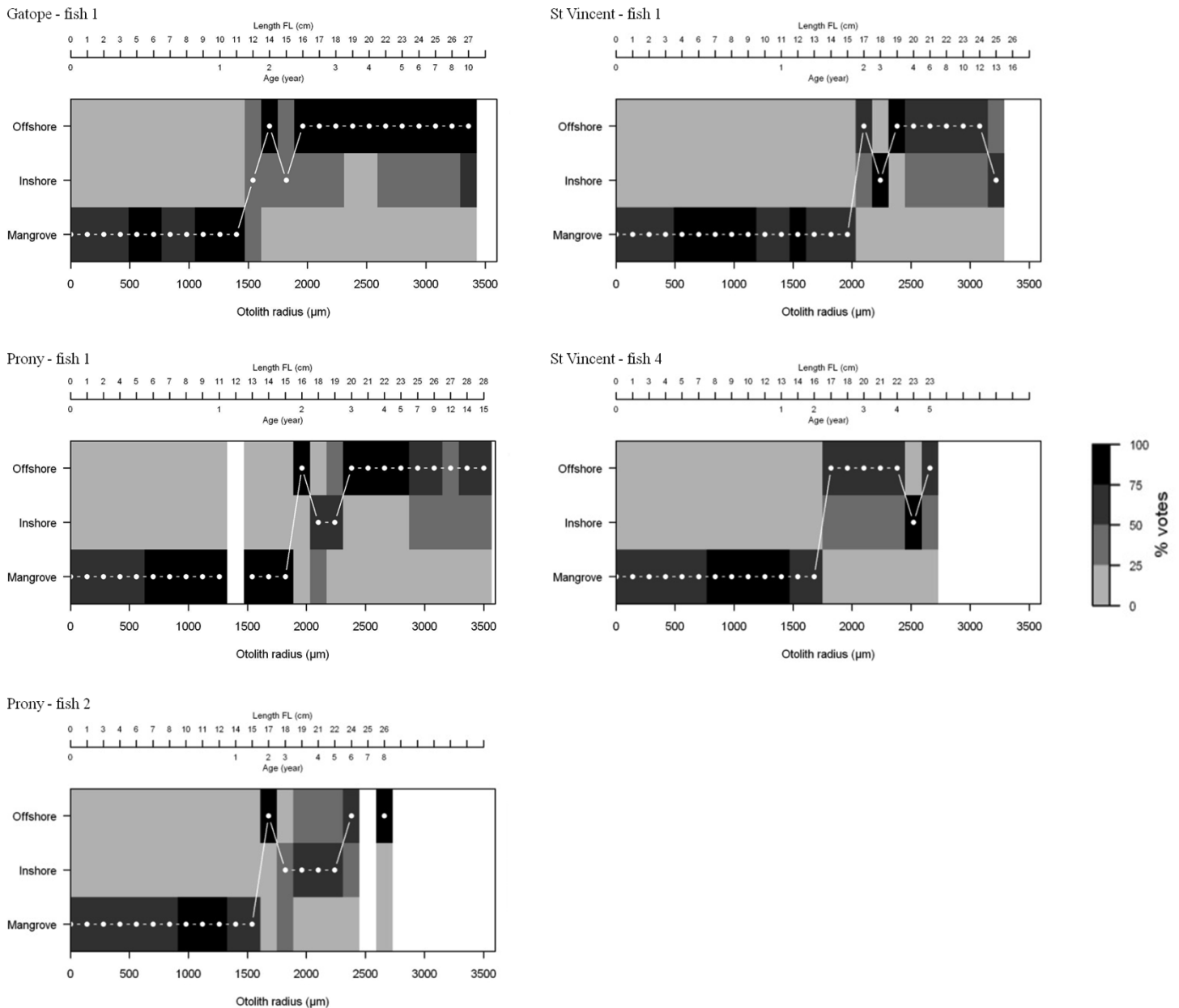
Appendix 12: Lifetime patterns of movements for *Lutjanus fulviflamma*

The first pattern showed four individuals evolving in two different habitats, mangrove as young then offshore reef as adult.



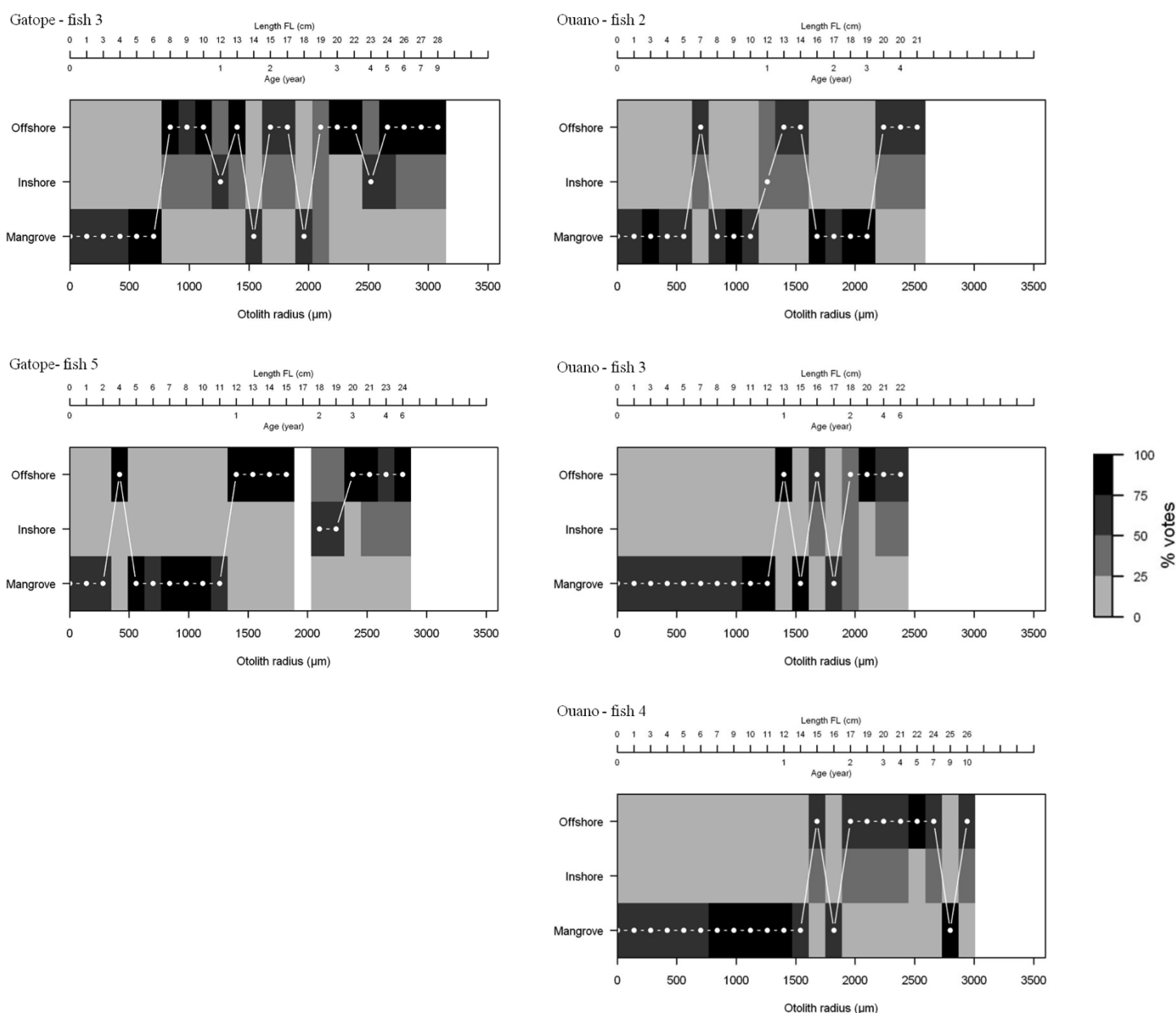
Representation of the first pattern of lifespan movement for *L. fulviflamma* using otoliths microchemistry and RF predictions. The x axis represents the otolith radius and the y axis the three possible habitats occupied during life. The second top x axis indicates the age and length of the fish (respectively below and above axis). The grey gradient shows the probabilities of habitat predictions (% of vote). The white line joins habitats predicted with the highest probability at each LA-ICPMS analysis

The second pattern showed five individuals evolving in three different habitats: mangrove as young, then inshore and offshore reefs as adults with no return to mangrove for adults but random movements between inshore and offshore reefs (Fig. 5).



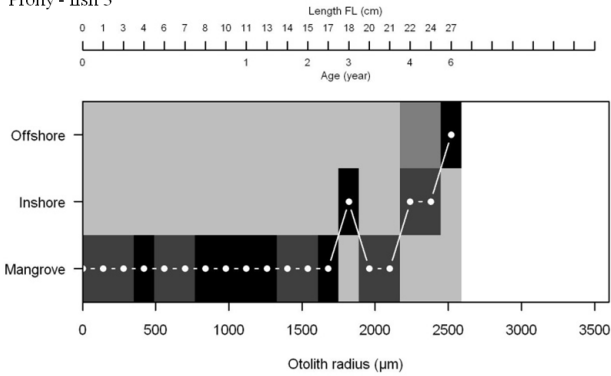
Representation of the second pattern of lifespan movement for *L. fulvivflamma* using otolith microchemistry and RF predictions. The x axis represents the otolith radius and the y axis the three possible habitats occupied during life. The second top x axis indicates the age and length of the fish (respectively below and above axis). The grey gradient shows the probabilities of habitat predictions (% of vote). The white line joins habitats predicted with the highest probability at each LA-ICPMS analysis

The third pattern showed eleven individuals evolving in three different habitats and presenting random movements between them: mangrove as young, then inshore and offshore reefs as adults with return to mangrove for adults (Fig. 6a and 6b).

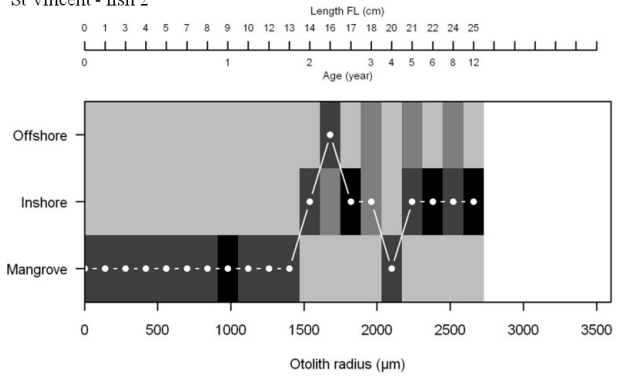


Representation of the third pattern of lifespan movement for *L. fulviflamma* using otoliths microchemistry and RF predictions. The x axis represents the otolith radius and the y axis the three possible habitats occupied during life. The second top x axis indicates the age and length of the fish (respectively below and above axis). The grey gradient shows the probabilities of habitat predictions (% of vote). The white line joins habitats predicted with the highest probability at each LA-ICPMS analysis

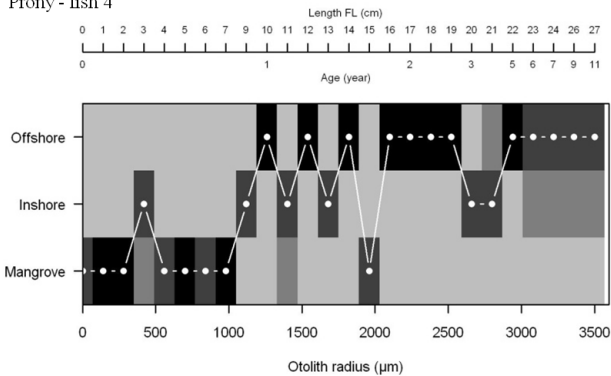
Prony - fish 3



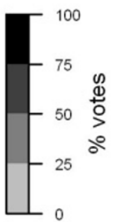
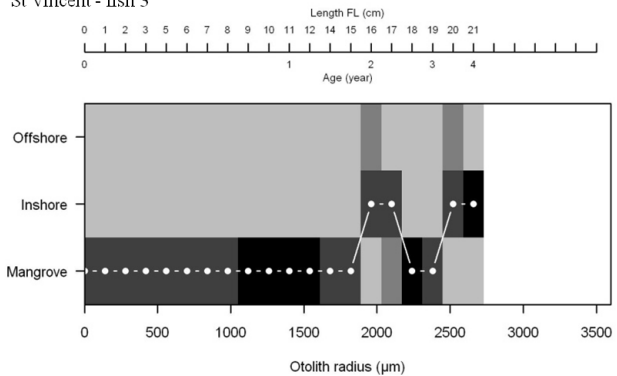
St Vincent - fish 2



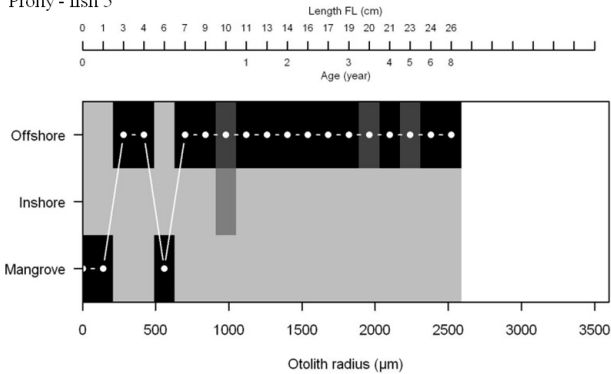
Prony - fish 4



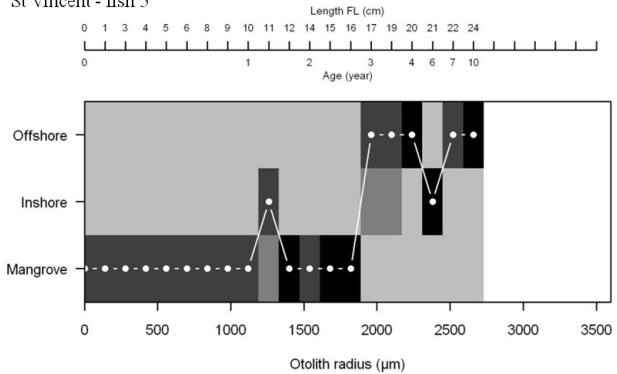
St Vincent - fish 3



Prony - fish 5



St Vincent - fish 5



Representation of the third pattern of lifespan movement for *L. fulvivflamma* using otoliths microchemistry and RF predictions. The x axis represents the otolith radius and the y axis the three possible habitats occupied during life. The second top x axis indicates the age and length of the fish (respectively below and above axis). The grey gradient shows the probabilities of habitat predictions (% of vote). The white line joins habitats predicted with the highest probability at each LA-ICPMS analysis.

Résumé - La connectivité écologique se mesure via l'estimation des mouvements réalisés par les organismes au cours de leur vie. Parmi les outils de mesure existants, seule la microchimie des otolithes est capable de reconstruire les mouvements des poissons au cours de leur vie entière, notamment lors des migrations ontogénétiques. Son utilisation au sein des milieux oligotrophes et chimiquement peu contrastés tels que les récifs coralliens reste cependant marginale. C'est dans ce contexte que s'inscrit ce travail sur la microchimie des otolithes des poissons des récifs coralliens et mangroves de Nouvelle-Calédonie. Il se divise en deux parties. La première, méthodologique, est axée sur l'estimation du potentiel de l'outil microchimie des otolithes en Nouvelle-Calédonie, avec un chapitre sur la détermination des signatures multi-élémentaires caractéristiques des différents habitats de la Grande Terre et un chapitre sur l'étude de la relation entre la microchimie de l'environnement et celle des otolithes. La seconde partie se concentre sur l'application de la méthodologie précédemment développée à des thématiques écologiques, avec un chapitre sur le rôle des mangroves sur le cycle de vie et la distribution géographique d'une espèce de Lutjanidae, *Lutjanus fulviflamma*, et un chapitre centré sur les mouvements réalisés au cours de la vie de cette espèce. Les résultats démontrent que la microchimie des otolithes présente un potentiel élevé en Nouvelle-Calédonie avec un fort pouvoir de discrimination entre la mangrove et les récifs coralliens. La relation entre la microchimie de l'environnement et celle des otolithes est complexe et dépend fortement des espèces considérées. Cependant, les contrastes chimiques marqués de l'environnement se retrouvent au sein des otolithes. Les résultats démontrent une connexion forte entre différents habitats pour *L. fulviflamma*, particulièrement entre la mangrove et les récifs coralliens avec une importance cruciale de la mangrove comme zone de nurserie. Trois différents patrons de mouvements entre habitats ont été identifiés. Cette diversité illustre une forte variabilité de la connectivité écologique entre les individus, avec pour certains d'entre eux, une fréquentation de l'habitat juvénile durant la phase adulte.

Mots clés : microchimie des otolithes ; poissons des récifs coralliens ; connectivité ; *Lutjanus fulviflamma* ; mangrove ; nurserie ; patrons de mouvements.

Abstract - Ecological connectivity is defined by organism movements between habitats. Among the tools used to measure connectivity, otolith microchemistry is the only one able to reconstruct the fish movement throughout their entire lifetime, and thus include ontogenetic migrations. However, it is seldom used in oligotrophic environments that typically show poor chemical contrasts such as coral reefs. In this context, this study focused on otolith microchemistry of coral reef and mangrove fishes of New Caledonia. This work comprises two parts. First, a methodological part to assess the potential of otolith microchemistry in New Caledonia, with one chapter on the determination of multi-elemental signatures of habitats from the Main Island and one chapter on the relationships between environmental and otoliths microchemistries. Second, the previously developed methodology was applied to ecological questions, with a chapter on the importance of mangrove on the life cycle and the spatial distribution of a Lutjanidae, *Lutjanus fulviflamma*, and a chapter on the lifetime movement patterns of this species. Results revealed a high potential of otolith microchemistry in New Caledonia with a high power of discrimination between mangroves and coral reefs. Relationships between environmental and otolith microchemistries were complex and species-specific. However, strong chemical contrasts in the environment were traduced in the otoliths. Results showed a strong connection between different habitats for *L. fulviflamma*, in particular between mangroves and reefs with a crucial importance of mangroves as nursery grounds. Three different patterns of lifetime movements among habitats were identified. This diversity illustrates a high variability of ecological connectivity patterns among individuals, with a possible return to the juvenile habitat during the adult stage.

Key words: otolith microchemistry; coral reef fish; connectivity; *Lutjanus fulviflamma*; mangrove; nursery; movement patterns.