Extent of Mangrove Nursery Habitats Determines the Geographic Distribution of a Coral Reef Fish in a South-Pacific Archipelago

Christelle Paillon1,2*, Laurent Wantiez2, Michel Kulbicki3, Maylis Labonne4, Laurent Vigliola1

1 Institut de Recherche pour le Développement (IRD), UR227 CoReUs, Laboratoire Excellence LABEX corail, Nouméa, New Caledonia, 2 Université de Nouvelle-Caledonie (UNC), LIVE (EA4243), Nouméa, New Caledonia, 3 Institut de Recherche pour le Développement (IRD), UR227 CoReUs, Laboratoire Excellence LABEX corail, c/o Laboratoire Arago, Banyuls sur Mer, France, 4 Institut de Recherche pour le Développement (IRD), LEMAR (UMR 6539), Institut Universitaire Européen de la Mer, Plouzané, France

Abstract

Understanding the drivers of species’ geographic distribution has fundamental implications for the management of biodiversity. For coral reef fishes, mangroves have long been recognized as important nursery habitats sustaining biodiversity in the Western Atlantic but there is still debate about their role in the Indo-Pacific. Here, we combined LA-ICP-MS otolith microchemistry, underwater visual censuses (UVC) and mangrove cartography to estimate the importance of mangroves for the Indo-Pacific coral reef fish Lutjanus fulviflamma in the archipelago of New Caledonia. Otolith elemental compositions allowed high discrimination of mangroves and reefs with 83.8% and 98.7% correct classification, respectively. Reefs were characterized by higher concentrations of Rb and Sr and mangroves by higher concentrations of Ba, Cr, Mn and Sn. All adult L. fulviflamma collected on reefs presented a mangrove signature during their juvenile stage with 85% inhabiting mangrove for their entire juvenile life (about 1 year). The analysis of 2942 UVC revealed that the species was absent from isolated islands of the New Caledonian archipelago where mangroves were absent. Furthermore, strong positive correlations existed between the abundance of L. fulviflamma and the area of mangrove (r = 0.84 for occurrence, 0.93 for density and 0.89 for biomass). These results indicate that mangrove forest is an obligatory juvenile habitat for L. fulviflamma in New Caledonia and emphasize the potential importance of mangroves for Indo-Pacific coral reef fishes.

Citation: Paillon C, Wantiez L, Kulbicki M, Labonne M, Vigliola L (2014) Extent of Mangrove Nursery Habitats Determines the Geographic Distribution of a Coral Reef Fish in a South-Pacific Archipelago. PLoS ONE 9(8): e105158. doi:10.1371/journal.pone.0105158

Editor: Candida Savage, University of Otago, New Zealand

Received March 27, 2014; Accepted July 18, 2014; Published August 20, 2014

Copyright: © 2014 Paillon et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This study was funded by the Developing Agency of New Caledonia (ADECAL), the Economic Zone of New Caledonia program (ZONECO) and the South Province of New Caledonia. Dive data from the remote reefs of New Caledonia were collected as part of the Pristine Reef project funded by the Total Foundation, the Pristine Seas project funded by the National Geographic Society, and the UNESCO World heritage survey funded by the Government of New Caledonia. The rest of the UVC data was collected by various projects funded by the Southern Province and the Northern Province, and by the Research Institute for Development (IRD) and the University of New Caledonia (UNC) core funding. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: christelle.paillon@gmail.com

Introduction

Coral reefs and mangroves are amongst the most productive ecosystems of the world and have high economic values [1]. They are also highly impacted by human activity, with 30% of coral reefs already severely impacted and a 50% decrease of mangrove area in the last 50 years [2–4]. Natural disturbances in conjunction with increasing human pressures have led to dramatic losses of quality and extent of these ecosystems [5–10]. Coral reef seascapes are fragmented ecosystems where local populations of reef organisms are connected by movements of individuals at the pelagic larval, and benthic juvenile/adult stages [11]. After a pelagic life of variable duration, larvae settle in shallow nursery habitats such as mangroves, seagrass beds or reefs [12] where juveniles may benefit from several advantages such as food availability and shelter from predators [13]. As they grow, individuals move to coral reefs and recruit into adult populations [14].

Mangrove forests are habitat for many juvenile coral reef fish species (e.g. Serranidae, Lutjanidae, Lethrinidae, etc.) as they provide shelter and food and therefore increase the overall rate of survival for juveniles [13,15–17]. As a result, there can be strong relationships between fish abundance and the presence of mangroves with, for example, greater biomass of Lutjanidae in reefs close to mangroves in the Caribbean [4,18,19]. However, some species using mangroves during their juvenile life stage can also be observed in high density in other juvenile habitats (e.g. seagrass beds, soft bottoms, fringing reefs) [14] and the absence of mangroves may have no significant impact on adult fish population densities on nearby reefs [19]. Thus, the importance of mangroves as nurseries may vary depending on the species and the region. As defined by Adams et al. [20], mangroves can be obligatory, important or facultative juvenile habitats. They are obligatory when species cannot complete their life cycle in the absence of mangroves. They are important when they make large contributions to adult populations. Finally, they are facultative...
nursery habitats when the species is abundant in numerous habitats as juveniles, and thus only a minority of juveniles is found in mangroves [20]. Following these three possibilities, the relationship between the abundance of the species and the extent of mangroves will be different.

The connectivity between mangroves and coral reefs for ontogenetic shifts suggests that the distribution of juvenile habitats may be a primary determinant of species’ distribution when the juvenile habitat is an obligatory one. It has often been assumed that larval transport is the primary determinant of population connectivity and geographic range size. However, in a recent paper Laize et al. [21] 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. Testing whether the distribution of a juvenile habitat (e.g. mangrove) is a primary determinant of a species’ distribution requires assessing if this habitat is obligatory, important or facultative. This implies the need to estimate the proportion of adults that effectively used the juvenile habitat as nursery. Methods used to directly measure connectivity between juvenile and adult populations require natural or artificial habitat-specific markers [20,22,23]. Among these methods, the microchemistry of otoliths is one of the most powerful tools to identify juvenile habitats when adult and juvenile populations are spatially distinct [16,24–28]. 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 lives [29]. 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 purposes of this study were to: 1) investigate whether, in New Caledonia, mangroves are obligatory, important or facultative juvenile habitats for Lutjanus fulviflamma (Forsskål, 1775) (Lutjanidae) and 2) test whether the adult distribution of this species is related to the geographic distribution of mangroves in the New Caledonia archipelago, using otolith microchemistry to characterize the chemical signature of habitats and an underwater visual census (UVC) database (2942 transects) to determine the relationship between the abundance of this species and the mangroves.

Materials and Methods

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. 1). It is composed of a main island (Grande Terre), three groups of smaller islands (Loyalties, Isle of Pins 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 [30]. Distance between the main island and the barrier reef varies from 1 to 65 km [31] and major mean adult 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 forests [32–34].

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. 1, Table S1). 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, fish were put on iced water (<5 °C) to be anesthetized. Afterwards, they were transported to the laboratory where they 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 and St Vincent). The second sampling was carried out during austral winter 2010 in the mangroves and inner barrier reefs of eleven sites around the main island (Fig. 1). During this sampling, fringing and intermediate reefs were also sampled at four sites (Gatope, Ouano, Prony and St Vincent). Sampling in the mangroves, fringing, intermediate and inner barrier reefs at these 4 sites was repeated during austral winter 2011 (Table 1). Thus, our sampling included fish collected in different years, seasons and habitats, which is important as chemical signatures recorded in fish otoliths may vary over space and time, and spatio-temporal variability needs to be included in the characterization for robust chemical signatures in otoliths [35].

Ethics and sampling approvals were provided by the Environmental Department of the Southern Province of New Caledonia for Nouméa, St Vincent, Ouano, Port Bouquet and Prony sites (permit #1103-2010/ARR/DENV), and by the Economic Development and Environmental Department of the Northern Province of New Caledonia for Néouipi, Gatope, Golone, Amos, Tchambouenne and Paama sites (permit #06912-2010/JJC). In this study, no protected species was collected and collections were not made in protected areas.

Otolith preparation

All equipment used for otolith handling was previously decontaminated in 5% ultrapure nitric acid bath during 24 h, rinsed three times with ultrapure water (18.2 MΩ), dried under a laminar flow hood (HEPA 100), and stored dry in plastic bags. At the lab, fish samples were unfrozen and each individual was measured (Fork length, FL) to the nearest mm and weighted to the nearest gram. Paired sagittal otoliths were extracted using ceramic tweezers, cleaned with ultrapure water (18.2 MΩ) 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 (outer edge) of up to five fish randomly selected in each sample (Table 1A). This method proposed by Warner et al. [38] allows characterizing the latest elemental signature recorded in the otolith, which corresponds to the habitat where the fish was caught [35–37]. Otoliths of these fishes were cleaned of organic material following Warner et al.’s [38] method: otoliths were bathed in a 50/50 H2O2 (30%, Suprapur grade) and NaOH (Suprapur grade, 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 used by adults were inferred from the analyses of the chemical composition along transverse otolith sections of 20 adult fish collected on the barrier reefs of Gatope,
Ouano, Prony and St Vincent (5 fish per site, Table 1B). Transverse otolith sections including otolith cores were prepared by embedding right sagittal otoliths in epoxy resin, sectioning transversely with a low speed saw, and polishing using sandpaper of decreasing grain (from 800 to 1600 grains/cm²), lapping film (9 to 1 mm) and diamond-tipped polishing powders. Finally, sections were rinsed in ultrapure water, dried under a 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.

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

![Figure 1. Map of New Caledonia with sampling sites for microchemistry (yellow circles) and underwater visual censuses (UVC, green circles). Polygons indicate the different lagoons, remote reefs and islands for which UVC data were collected; numbers indicate the number of UVC transects.](doi:10.1371/journal.pone.0105158.g001)

**Table 1.** Sampling design and chemical analyses.

| Objectives                  | Year | Number of sites | Habitat | Laser analysis | n otoliths |
|-----------------------------|------|-----------------|---------|----------------|------------|
| A. Characterization of habitat signatures | 2009 | 2 | M, BR | Ablation on surface | 20 |
|                            | 2010 | 7 | M, BR | Ablation on surface | 51 |
|                            | 2010 | 4 | M, FR, IR, BR | Ablation on surface | 63 |
|                            | 2011 | 4 | M, FR, IR, BR | Ablation on surface | 62 |
| B. Determination of juvenile habitats | 2010 | 4 | BR | Transects | 20 |

Sampling design and chemical analyses 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; M: Mangrove; FR: Fringing Reef; IR: Intermediate Reef; BR: Barrier Reef.

doi:10.1371/journal.pone.0105158.t001
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 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 discriminate habitats, the following isotopes were measured: \(^{7}\text{Li}, {^{11}\text{B}}, {^{18}\text{O}}, {^{25}\text{Mg}}, {^{45}\text{Ti}}, {^{47}\text{Ti}}, {^{50}\text{Mg}}, {^{51}\text{V}}, {^{52}\text{Cr}}, {^{53}\text{Mn}}, {^{55}\text{Mn}}, {^{60}\text{Ni}}, {^{65}\text{Cu}}, {^{66}\text{Zn}}\).

To measure the microchemistry signature of the habitat where the fish was caught, the surface of the otolith of each subsampled fish (up to 5 individuals per sample) was laser ablated following Warner et al. [39]. 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 parts of otolith transverse sections were analyzed 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 of ten successive laser ablations (point-by-point mode) from the core to 1260 μm by increments 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 each transect in order to correct for 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 [39]. Cleaned CPS were then transformed in elemental concentrations [parts per million, ppm] and limits of detection (LOD) calculated following Longerich et al [40]. 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% [23,41]. When the concentration of an element was under the LOD, it was set to zero. By definition, this occurred in less than 30% of the 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 to percent of all measured elements.

In order to correlate juvenile habitat use with age and size, the 20 adults were aged by counting the number of yearly increments (opaque bands) on otoliths and size were back-calculated from our raw database based on truncation of original D-UVC datasets at a distance of 5 m on each side of the transect line [46], effectively rendering D-UVC transects equivalents to 500 m² belt transects (50 m long ×5 m wide ×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 [47]. All transects were layed parallel to the reef between 0 to 15 meters depth and encompassed the full range of habitats present 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 [48]) and the remote reefs and islands of the archipelago for which we had data (Fig. 1). Surface area 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 [49] available at www.georep.nc.

**Data analyses**

One-way PERMANOVA (with 999 permutations) based on Euclidian distance [50,51] 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.

The random forests (RF) algorithm [52] was performed on elemental concentrations from otolith surfaces 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 [35,53]. 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,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).
Results

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. S1). Elemental concentrations of Ba, Cr, Mn and Sn were significantly greater in otoliths of individuals collected in mangroves than those collected in reefs. On the contrary, Rb and Sr were at lower concentrations in otoliths from mangroves than in reef otoliths.

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 fish in the habitat where they were collected. However, percentages of correct classification varied with the habitats. 83.3% of the fish from mangroves were correctly classified in their sampling habitat and 90.7% of the fish from reefs were correctly classified (Table 2).

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 characterizing mangroves whereas Rb was characteristic of reefs (Fig. 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 mm showed that all 20 adult *L. fulviflamma* collected on barrier reefs had a mangrove signature on their two first laser ablations (0 and 140 mm) (Fig. 3). A mangrove signature was predicted for 85% of fishes in age and from 18 to 28 cm in fork length. At an otolith radius of 1260 mm, 16 fish had one year, 4 had less than one year, and back-calculated body length ranged from 96 to 128 mm fork length (mean 112 mm ± 9 SD, n = 20).

Relationships between mangrove and fish abundance

UVC surveys showed that the species was not recorded in remote islands where mangroves are absent (Chesterfield, Entrecasteaux, Petri, Astrolabe, Beautemps-Beaupré, Lifou) and was always recorded in places where mangroves are present (Table S2). Interestingly, some *L. fulviflamma* were recorded in the atoll of Ouvéa where mangroves are present but not in the nearby atoll of Beautemps-Beaupré where mangroves are absent. The strong relationship between the presence of mangroves and the presence of *L. fulviflamma* was confirmed by a highly significant chi-square test ($\chi^2 = 14, df = 1, p < 0.0002$). Spearman rank correlations showed highly significant relationships between standardized mangrove areas and the frequency of occurrence ($R = 0.84, p < 0.0002$), density ($R = 0.93, p < 0.0001$) and biomass ($R = 0.89, p < 0.0001$) of *L. fulviflamma*. Fish abundance was 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 in the East, South East lagoons and Ouvéa island where mangroves are scarce, and 3) absent in the reefs, atolls and islands disconnected from the mainland and where mangroves are absent (Fig. 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 distributions is crucial for the development of strategic conservation plans. Up to now, it was generally 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 [54–56]. However, many other studies found no relationship [57,58]. Very recently, Luiz et al. [21] revised the larval dispersal paradigm and reported that adult traits were as important as larval traits in explaining the geographic range size of reef fish species. To explain their findings, they proposed the following hypothesis: to found a new population, new colonizers 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 colonizers to survive and establish new populations. If post-settlement survival is a crucial determinant of a species’ geographic range size, then we hypothesized that the spatial distribution of those habitats which are obligatory for the survival of juveniles should closely match the spatial distribution of the species. In order to test this hypothesis, we selected a reef fish species known to use mangroves as nurseries, applied otolith microchemistry techniques to determine whether mangroves were obligatory, important or facultative juvenile habitats, and explored the relationships between species abundance and mangrove extent in the archipelago of New Caledonia.

Characterization of juvenile habitat

The first objective of this study was to identify the role of mangroves 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 almost perfect discrimination of mangrove from

| Table 2. Classification matrix of habitats obtained from Random Forest analyses. |
|-----------------------------------------------|
| **Habitats** | **Correct classification (%)** | **Number of fish classified in** | **Mangrove** | **Reefs** | **Total** |
|---------------|-------------------------------|---------------------------------|-------------|-----------|-----------|
| Mangrove      | 83.8                          | 31                              | 6           | 37        |
| Reefs         | 98.7                          | 2                               | 157         | 159       |
| Total         | 95.9                          | 33                              | 163         | 196       |

doi:10.1371/journal.pone.0105158.t002
reef ecosystems using only three elements (Mn, Sn and Rb), and that 6 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. In agreement with our study, Chittaro et al. [23] reported Sr concentrations significantly greater in otoliths from reefs than mangroves in the Bahamas.

The power of otolith microchemistry to identify juvenile and adult habitats resides in the distinct chemical composition of otoliths collected from different habitats [39]. Mangroves grow in coastal areas often showing chemical heterogeneity due to different inputs (anthropogenic, fluvial and upwelling) compared with more oceanic coral reef environments. In addition, New Caledonia shows a peculiar geological context and a significant past and present mining activity with past exploitations of Mn and Cr on the Main Island [31]. Moreover, Mn shows a scavenging profile type, which means higher concentrations in coastal waters and a tendency to decrease with distance from the coastline [60]. Higher levels of Mn and Sn in mangroves could also originate from fertilizers used for agriculture and could be higher near areas with freshwater inputs [61]. 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

Figure 2. PCA biplot of otolith elemental concentrations from mangroves and reefs. Green and blue dots indicate the 37 fishes collected in mangroves (M) and the 159 fishes collected in reefs (R), respectively. Only concentrations of Rb, Sn and Mn in otoliths were used in the PCA. doi:10.1371/journal.pone.0105158.g002
Ba and Sr concentrations between reefs and mangroves may be explained by gradients in salinity and temperature between the two habitats [59].

Essentialness of mangrove

The second objective of our study was to determine whether mangroves were obligatory, important or facultative juvenile habitats for *L. fulviflamma* in New Caledonia. To achieve this goal, we looked for elemental signatures of mangroves in the juvenile parts of otoliths from adults collected on barrier reefs. The results showed that all analyzed fish had a mangrove signature at the beginning of their life.

Coastal shallow-water habitats are recognized worldwide as important nursery areas for fish [13,15,20]. Many studies, mostly conducted in the Caribbean region, established the nursery role of mangroves and their attractiveness for juveniles [4,15–17,19,62–64]. Our study took place in the South Pacific region where there is a lack of knowledge on mangrove-reef interactions [20]. In New Caledonia, previous studies showed that mangroves were used as one juvenile habitat among other shallow-water habitats [32,34,65]. Our study used otolith microchemistry, a powerful method to study the role of mangroves as nurseries [23,41,66,67] and revealed the true importance of mangroves for *L. fulviflamma*: all analyzed adults lived in mangroves as juveniles, which suggests that mangroves are probably an obligatory juvenile habitat for this species in New Caledonia.

Geographic distribution

Although a sample size of 20 adults is probably not sufficient to affirm with certainty that mangroves are indeed an obligatory juvenile habitat, the relationship that we found between the presence of mangroves and the presence of the species strongly supports our conclusion from otolith microchemistry. New Caledonia is an archipelago with a large main island colonized by mangroves and surrounded by a complex coral reef ecosystem, and several islands and offshore reefs with or without mangroves. *L. fulviflamma* was present all around the mainland, including on reefs very far away from the main land, suggesting that individuals who completed their juvenile life in the mangroves of the mainland were able to swim long distances within an interconnected reef ecosystem. Furthermore, the species was present in offshore islands where mangroves are present, suggesting that planktonic larvae transported by oceanic currents can reach these islands and survive as juveniles in the mangroves. However, the species was not present at d’Entrecasteaux atolls, Astrolabe reefs, Chesterfield banks, Petri atoll, Beaupré—Beaupré atoll and Lifou Island. One hypothesis to explain this absence is a lack of connectivity at the larval stage. However, this hypothesis is unlikely because models of larval dispersion indicate persistent connectivity among reefs in the

Figure 3. Proportion of adults *L. fulviflamma* occupying mangroves as juvenile. Results of RF prediction of habitats obtained along the juvenile part of otolith transects of the 20 adults *L. fulviflamma* collected in barrier reefs. For each fish and at each laser ablation along otolith transects, RF predict the habitat (mangrove or reef) corresponding to the chemical analysis. The green area represents the proportion of fish with a mangrove signature whereas the blue area represents the proportion of fish with a reef signature. Mean fish body length back-calculated from otolith radius are given in upper-x axis.

doi:10.1371/journal.pone.0105158.g003
archipelago of New Caledonia for species with pelagic larval duration (PLD) greater than 15 days [68], and *L. fulviflamma* has an average PLD of 23 days [21]. Furthermore, it would be very surprising that larvae can reach the atoll of Ouvéa where the species and mangrove are present and not the nearby atoll of Beaulains-Beaupré or Astrolabe reef where the species and mangrove are absent. Likewise, the hypothesis of overfishing seems unlikely because some of the sites where the species was absent are strictly protected while, on the contrary, the species was present at sites with the highest fishing pressure, like Nouméa, the capital city of New Caledonia. Clearly, the most plausible hypothesis for the absence of the species at these reefs and islands is the absence of mangrove, a probable obligatory juvenile habitat for this species in New Caledonia.

Depending on the location, many species show considerable plasticity in habitat use as juveniles [69]. In our study, juvenile *L. fulviflamma* used mangroves as a nursery area. In Zanzibar, Lugendo [70] showed that juvenile *L. fulviflamma* were found in mangroves but also in sand/mud flats and seagrass areas and were therefore considered as a generalist species. In the Caribbean region, Mateo et al. [67] showed that almost 100% of schoolmaster (*Lutjanus apodus*) collected in mangroves and seagrass beds of St Croix and Porto Rico resided as juvenile in mangrove habitat. This plasticity and variability in effectively used juvenile habitat could be significant enough to limit the general applicability of our results [20]. 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. [21] which proposes that post-settlement processes are main determinants of species geographic range. Our study encourages determining which habitats are obligatory or important for juvenile fish 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.

**Supporting Information**

**Figure S1** Comparison of otolith elemental concentrations between habitats. 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. Error bars represent standard errors (±SE). Results of one way PERMANOVAs are indicated with (***) p < 0.001 and (**) p < 0.01.

**Figure 4. Relationship between *L. fulviflamma* density and mangrove area.** Spearman-rank correlation between standardized mangrove area and density of *L. fulviflamma* in the different lagoons, reefs and islands of the archipelago of New Caledonia (see map on Fig. 1) is given. doi:10.1371/journal.pone.0105158.g004
Acknowledgments

The authors thank Mark Huxham and an anonymous referee for encouraging comments, helpful suggestions and the proofreading. We thank the staff of IRD, UNC, the Pôle de Spectrométrie Océan (Institut Européen Universitaire de la Mer, Brest, France) and the Research Institute of Environment and Livelihoods of Charles Darwin University. In particular, we express our gratitude to Gerard Mou-Tham, Joseph Baly, and Miguel Clarque for their invaluable field and laboratory assistance, Claire Bassoulet and Françoise Foti for their precious help with the LA-ICP-MS analyses.

Author Contributions

Conceived and designed the experiments: CP LV LW MK ML. Performed the experiments: CP LV LW MK ML. Analyzed the data: CP LV LW MK. Contributed reagents/materials/analysis tools: CP LV LW MK ML. Contributed to the writing of the manuscript: CP LV LW MK.

References

1. Bellwood RD, Hughes TP (2001) Regional-scale assembly rules and biodiversity patterns of reefs. Science 292: 1522–1524.
2. Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301: 929–933.
3. Burke LM, Reyer K, Spalding M, Perry A (2011) Reefs at risk revisited: World Resources Institute.
4. Mumby PJ, Edwards AJ, Arias-Gonzalez JE, Lindeman KC, Blackwell PG, et al. (2004) Mangroves enhance the biomass of coral reef fish communities in the Caribbean. Nature 428: 538–542.
5. Nystrom M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a human-dominated environment. Trends in Ecology & Evolution 15: 413–417.
6. Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjurto KA, et al. (2003) Baselines and Degradation of Coral Reefs in the Northern Line Islands. Plos One 3: 11.
7. Waniez L, Chateau O, Le Mouelle S (2006) Initial and mid-term impacts of cyclone Erica on coral reef fish communities and habitat in the South Lagoon Marine Park of New Caledonia. Journal of the Marine Biological Association of the United Kingdom 86: 1229–1236.
8. Bellwood DR, Hughes TP, Folke C, Nystrom M (2004) Confronting the coral reef crisis. Nature 429: 827–833.
9. Mora C, Sale PF (2011) Ongoing global biodiversity loss and the need to move beyond protected areas: a review of the technical and practical shortcomings of protected areas on land and sea. Marine Ecology-Progress Series 434: 251–266.
10. Sale PF, Van Lavieren H, Ablan Lagmac MN, Aurena J, Butler M, et al. (2010) Preserving Reef Connectivity: A Handbook forProtected Marine Area Managers. Connectivity Working Group, Coral Reef Targeted Research & Capacity Building for Management Program, UNU-INWEH.
11. Sale PF (1991) The ecology of fishes on coral reefs; Press A, editor. New York: Academic Press. 754 p.
12. Parrish JD (1989) Fish communities of interacting shallow-water habitats in tropical oceanic regions. Myers Ecology-Progress Series 58: 143–160.
13. Mellin C, Kuliwki M, Ponton D (2007) Seasonal and ontogenetic patterns of habitat use in coral reef fish juveniles. Estuarine, Coastal and Shelf Science 75: 481–491.
14. Beck MW, Heck KL, Abe KW, Chalders DL, Eggleston DB, et al. (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51: 633-641.
15. Jones DL, Walter JF, Brooks EN, Serafy JE (2010) Connectivity through ontogeny: fish population linkages among mangroves and coral reef habitats. Marine Ecology Progress Series 410: 245–256.
16. Laegdsgaard P, Johnson C (2001) Why do juvenile fish utilise mangrove environments? Journal of Experimental Marine Biology and Ecology 257: 229–253.
17. Laegdsgaard P, Johnson C (2001) Relative importance of mangroves as feeding habitats for fishes: a synthesis from optical remote sensing, coring and acoustic multibeam observations. Coral Reefs 28: 691–707.
18. Parrish JD (1989) Fish communities of interacting shallow-water habitats in tropical oceanic regions. Myers Ecology-Progress Series 58: 143–160.
19. Lea E (1910) On the methods used in the herring investigations. Publ Circ Cons Perm Int Explor Mer 53: 7–25.
20. Newman SJ, Dank HJ (2002) Growth, age validation, mortality, and other population characteristics of the red emperor snapper, Lutjanus sebae (Cuvier, 1828), off the Kimberley coast of north-western Australia. Estuarine Coastal and Shelf Science 57: 1049–1064.
21. Luiz OJ, Allen AP, Robertson DR, Floeter SR, Kulbicki M, et al. (2013) Adult and larval traits as determinants of geographic range size among tropical reef fishes. Proceedings of the National Academy of Sciences of the United States of America 110: 16498–16502.
22. Levan LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. Integrative and Comparative Biology 46: 282.
23. Chitarra PM, Frangipane T, Scaglia PF (2004) Discrimination of French grunts (Haemulon flavolabium, Desmarest, 1823) from mangrove and coral reef habitats using otolith microchemistry. Journal of Experimental Marine Biology and Ecology 308: 169–183.
24. Gillanders BM, Able KW, Brown JA, Eggleston DB, Sheridan PF (2003) Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. Marine Ecology Progress Series 247: 281–295.
25. Gillanders BM (2005) Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuarine, Coastal and Shelf Science 64: 47–57.
26. Brown JA (2006) Using the chemical composition of otoliths to evaluate the nursery role of estuaries for English sole Pleuronectes platessa populations. Marine Ecology Progress Series 306: 269–281.
27. Gillanders BM, Kingsford MJ (2000) Elemental fingerprints of otoliths of fish may distinguish estuarine “nursery” habitats. Marine Ecology Progress Series 201: 273–286.
28. Gillanders BM, Kingsford MJ (2003) Spatial variation in elemental composition of otoliths of three species of fish (family Sparidae). Estuarine Coastal and Shelf Science 57: 1049–1064.
29. Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188: 263–297.
30. Andréfousset S, Cahioch B, Flamand B, Pellentier B (2009) A reappraisal of the diversity of geomorphological and genetic processes of New Caledonian coral reefs: a synthesis from optical remote sensing, coring and acoustic multibeam observations. Coral Reefs 28: 691–707.
31. Paris JP (1861) Geologie de la Nouvelle-Caledonie; B.R.G.M. Ed, editor. Paris: Editions du R.G.M.
32. Carpenter KE, Niem VH (2001) FAO species identification guide for fishery purposes. The Living Marine Resources of the Western Central Pacific. Bon fishes part 3 (Moridae to Pomacentridae). Rome: FAO Fisheries Department. pp. 279–330.
33. Loubens G (1980) Biologie de quelques espèces de Poissons du lagon néo-Caledonien. II. Sexualité et reproduction. Cahiers de l’Indo-pacifique Volume 2: 41–72.
34. Tholet P (1992) Importance des mangroves pour la faune ichthyologique des récifs coralliens de Nouvelle-Caledonie. Cybium 16: 331–344.
35. Mercier L, Mousin D, Bruguiér O, Vuilbon L, Darnaude AM (2012) Multi-element otolith fingerprints unravel sea-lagoon lifetime migrations of gilthead sea bream Sparus aurata. Marine Ecology Progress Series 444: 175–194.
36. Chitarra PM, Finley KJ, Larinari PS (2009) Spatial and temporal patterns in the contribution of fish from their nursery habitats. Oecologia 160: 49–61.
37. Chitarra PM, Hogan JD (2013) Patterns of connectivity among populations of a coral reef fish. Coral Reefs 32: 341–354.
38. Warner RR, Sweater SE, Cassele JE, Sheehy M, Paradis G (2005) Natal trace- elemental signatures in the otoliths of an open-coast fish. Limnology and Oceanography 50: 1529–1542.
39. Lea E (1910) On the methods used in the herring investigations. Publ Circ Cons Perm Int Explor Mer 53: 7–25.
40. Newman SJ, Dank HJ (2002) Growth, age validation, mortality, and other population characteristics of the red emperor snapper, Lutjanus sebae (Cuvier, 1828), off the Kimberley coast of north-western Australia. Estuarine Coastal and Shelf Science 57: 67–80.
41. Chitarra PM, Usseglio-Polatera P, Fryer BJ, Sale PF (2006) Spatial variation in otolith chemistry of Latijus apodus at Turnelle Atoll, Belize. Estuarine Coastal and Shelf Science 67: 673–690.
42. Lea E (1910) On the methods used in the herring investigations. Publ Circ Cons Perm Int Explor Mer 53: 7–25.
46. D’Agata S, Mouillot D, Kulbicki M, Andrefouet S, Bellwood D, et al. (In press) Human-mediated loss of phylogenetic and functional diversity in coral reef fishes. Current Biology.

47. Kulbicki M, Guillemot N, Amand M (2005) A general approach to length-weight relationships for New Caledonian lagoon fishes. Cybium 29: 235–252.

48. Pelletier B, Jaffré F, Flamand B, Perrier J (2012) La bathymétrie des marges de la Grande Terre et des îles Loyauté. In: Bouvilloy J, editor. Atlas de la Nouvelle-Caledonie. Marseille (FRA), Nouméa: IRD, Congrès de la Nouvelle-Calédonie. pp. 33–36.

49. Virly S (2007) Typologies et biodiversité des mangroves de Nouvelle-Calédonie, Cartographie des mangroves. Nouméa, Nouvelle-Calédonie.

50. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

51. Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: Testing multspecies responses in multifactorial ecological experiments. Ecological Monographs 69: 1–24.

52. Breiman L (2001) Random forests. Machine Learning 45: 5–32.

53. Mercier L, Darnaude AM, Bruguier O, Vasconcelos RP, Cabral HN, et al. (2011) Selecting statistical models and variable combinations for optimal classification using otolith microchemistry. Ecological Applications 21: 1352–1364.

54. Macpherson E, Raventos N (2006) Relationship between pelagic larval duration and geographic distribution of Mediterranean littoral fishes. Marine Ecology Progress Series 327: 257–265.

55. Mora C, Chittaro PM, Sale PF, Kritzer JP, Ludsin SA (2003) Patterns and processes in reef fish diversity. Nature 421: 933–936.

56. Lester SE, Ruttenberg BI (2005) The relationship between pelagic larval duration and range size in tropical reef fishes: a synthetic analysis. Proceedings of the Royal Society B-Biological Sciences 272: 585–591.

57. Thresher RE, Brothers EB (1989) Evidence of intra-oceanic and inter-oceanic regional differences in the early life-history of reef-associated fishes. Marine Ecology Progress Series 57: 187–203.

58. Victor BC, Wellington GM (2000) Endemism and the pelagic larval duration of reef fishes in the eastern Pacific Ocean. Marine Ecology Progress Series 205: 241–248.

59. Sturrock AM, Trueman CN, Darnaude AM, Hunter E (2012) Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? Journal of Fish Biology 81: 766–795.

60. Brutland KW, Lohan MC, Holland HD, Turekian KK (2003) Controls of Trace Metals in Seawater. Treatise on Geochemistry. Oxford: Pergamon. pp. 23–47.

61. Dove SG, Kingsford MJ (1998) Use of otoliths and eye lenses for measuring trace-metal incorporation in fishes: a biogeochemical study. Marine Biology 130: 377–387.

62. Cocheret de la Morinière E, Pollix BJA, Nagelkerken I, Hemminga MA, Huiskes AHW, et al. (2005) Ontogenetic dietary changes of coral reef fishes in the mangrove-seagrass-reef continuum: stable isotopes and gut-content analysis. Marine Ecology-Progress Series 246: 279–289.

63. Nagelkerken I, Blaber SJM, Bouillon S, Green P, Haywood M, et al. (2008) The habitat function of mangroves for terrestrial and marine fauna: A review. Aquatic Botany 89: 135–145.

64. Dorenbosch M, Gro F, Nagelkerken I, van der Velde G (2006) Seagrass beds and mangroves as potential nurseries for the threatened Indo-Pacific humphead wrasse, Cheilinus undulatus and Caribbean rainbow parrotfish,Scarus guacamaia. Biological Conservation 129: 277–282.

65. Igulu MM, Nagelkerken I, Fraaije R, van Hilum R, Lijtenberg H, et al. (2011) The potential role of visual cues for microhabitat selection during the early life phase of a coral reef fish (Lutjanus fulviflamma). Journal of Experimental Marine Biology and Ecology 401: 118–125.

66. Chittaro PM, Usseglio P, Fryer BJ, Sale PF (2005) Using otolith microchemistry of Haemulon flavolineatum (French Grunt) to characterize mangroves and coral reefs throughout Turneffe Atoll, Belize: Difficulties at small spatial scales. Estuaries 28: 373–381.

67. Mateo I, Durbin EG, Appeldoorn RS, Adams AF, Juanes F, et al. (2010) Role of mangroves as nurseries for French grunt Haemulon flavolineatum and schoolmaster Lutjanus apodus assessed by otolith elemental fingerprints. Marine Ecology-Progress Series 402: 197–212.

68. Treml EA, Halpin PN, Urban DL, Patson LF (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landscape Ecology 23: 19–36.

69. McMahon KW, Berumen ML, Thorrold SR (2012) Linking habitat mosaics and connectivity in a coral reef seascape. Proceedings of the National Academy of Sciences of the United States of America 109: 15372–15376.

70. Leguado BR, Prouxter A, Cornelissen I, de Groene A, Nagelkerken I, et al. (2005) Habitat utilisation by juveniles of commercially important fish species in a marine embayment in Zanzibar, Tanzania. Aquatic Living Resources 18: 149–150.