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CARACTERISTIQUES BIOLOGIQUES, ECOLOGIE SPATIALE ET STRUCTURE DE LA POPULATION DE RAIES MANTA DE RECIF (*MOBULA ALFREDI*) DE NOUVELLE – CALEDONIE

***Thèse dirigée par* Dr Laurent Wantiez**

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JURY

Michel Kulbiki	Directeur de recherche, IRD	Rapporteur
Philippe Lenfant	Professeur UPVD	Rapporteur
Claude Payri	Directrice de recherche IRD	Présidente
Lydie Couturier	Chargée de recherche, FEM	Examinatrice
Eric Vidal	Directeur de recherche, IRD	Examinateur
Laurent Wantiez	Maitre de conférences HDR, UNC-ISEA	Directeur

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AVANT-PROPOS

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CHAPITRE 1

INTRODUCTION GENERALE

LES RAIES MANTA

Phylogénie

Les raies manta font partie des animaux les plus fascinants et charismatiques des océans. Ce sont les plus grands poissons batoïdes et parmi les plus grands élasmobranches se nourrissant de plancton (Marshall et al. 2009). Anciennement monospécifique (Notarbartolo di Sciara 1987), le genre *Manta* de la famille des Mobulidées se distingue en deux espèces à partir de 2009 (Marshall et al. 2009) : manta océanique, *Mobula birostris* (Atredi 1792) (Walbaum 1792), et manta de récif, *Mobula alfredi* (Krefft 1868). Le genre *Manta* (Bancroft 1829) était différentié des huit autres espèces du genre *Mobula* jusqu'en 2018, lorsque la famille des Mobulidées a été révisée comme étant monogénérique (White et al. 2018). En 2020, Hosegood et al. présentent des évidences d'une possible nouvelle espèce, la « raie manta caribéenne », occupant les eaux de la région des Caraïbes et qui, à ce jour, n'a pas encore été décrite.

Taille et reproduction

La raie manta de Récif, *Mobula alfredi*, a une envergure qui varie en fonction des populations et peut atteindre une taille maximum de 3.6 m (Hawaii, Deakos 2010 ; Maldives, Stevens 2016) à 5 m (Japan, Yano et al. 1999 ; Mozambique, Marshall et al. 2009 ; *in* Stewart et al. 2018). Les mâles sont en moyenne plus petits que les femelles. Le sexe est déterminé grâce à la présence, chez le mâle, de ptérygopodes sur la face ventrale au niveau des nageoires caudales (Fig. 1). Les femelles ont des organes génitaux internes. Comme tous les Chondrichtyens, les Mobulidées utilisent une fécondation interne pour se reproduire (Carrier et al. 2004, Pratt et al. 2005). La maturité d'un individu est déterminée par le développement des ptérygopodes chez les mâles et l'apparition de signes de gravidité (Fig. 2A) ou des premières marques de reproduction (Fig. 2B) chez les femelles (Clark 2010 ; Deakos 2010 ; Marshall and Bennett 2010, Stevens 2016 ; *in* Stewart et al. 2018). Ces dernières résultent de l'action du mâle qui mord l'une des nageoires pectorales de la femelle pour l'immobiliser lors de l'accouplement. L'âge de maturité des raies manta semble dépendre du sexe, les mâles atteignant en moyenne plus rapidement la maturité. Ils atteignent la maturité à une taille comprise entre 2,7 et 3 m, alors que les femelles sont matures avec une taille variant de 3,3 à 3,9 m (e.g., Deakos 2010, Marshall and Bennett 2010). Les estimations varient également selon les régions. Au Mozambique (Marshall et al. 2011) et à Hawaï (Clark 2010), les mâles sont matures entre 3 et 6 ans alors qu'aux Maldives la maturité est atteinte à 9 - 13 ans et 13 – 17 ans pour les mâles et les femelles, respectivement (Stevens 2016).

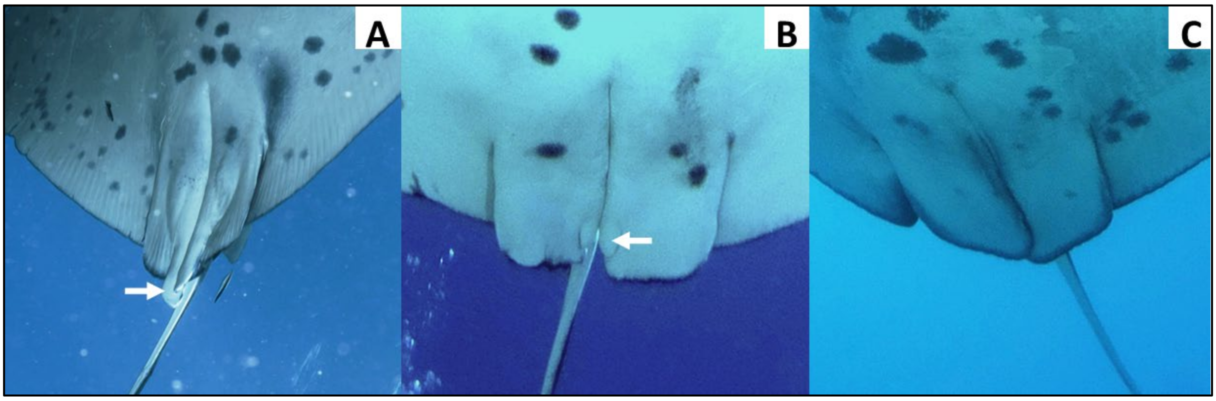

Figure 1. Identification du sexe chez la raie manta de récif (*M. alfredi*). A : mâle mature, B : mâle juvénile et C : femelle. Les flèches indiquent les ptérygopodes chez les mâles. *Photographie : Franck Bouilleret*

Le temps de gestation d'une raie manta de récif est d'un an et une femelle donnera naissance à un seul petit tous les 2 à 7 ans en fonction des conditions environnementales et de la disponibilité en nourriture (Marshall and Bennett 2010 ; Couturier et al. 2012 ; Deakos 2012 ; Stevens 2016).

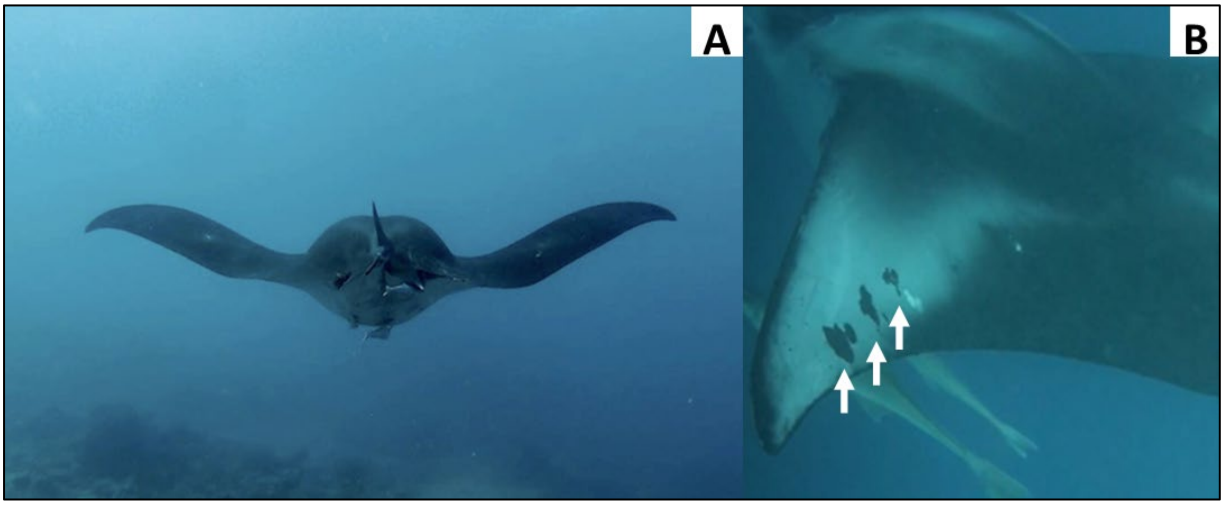

Figure 2. Signe de maturité d'une raie manta de récif femelle (*M. alfredi*). A : femelle gravide et B : marque de reproduction sur la nageoire pectorale gauche d'une femelle. *Photographie : Hugo Lassauce*

Pigmentation

Cette espèce a deux pigmentations différentes : blanche, dit « chevron », ou noire, aussi appelée « mélanique » (Fig. 3). Les individus chevrons ont la face dorsale noire avec des reflets blancs plus ou moins étendus et la face ventrale blanche avec des taches noires. Les raies manta noires ont la face dorsale totalement noire et la face ventrale noire avec des taches blanches (Fig. 3). Ces taches ventrales sont uniques à chaque individu (Marshall et al. 2009). Elles sont présentes dès la naissance et persistent tout au long de leur vie (Marshall et al. 2009). Le nombre et la taille de ces taches varient pour chaque animal (Marshall et al. 2009).

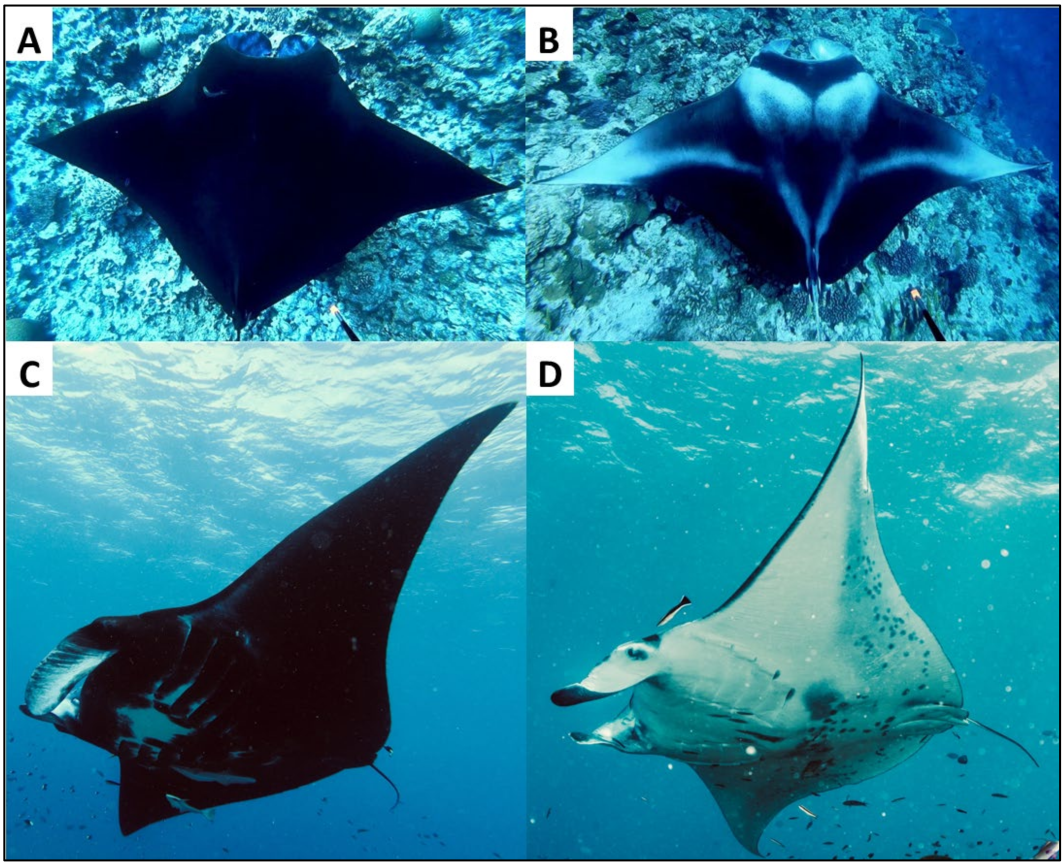

Figure 3. Types de pigmentations de la raie manta de récif (*M. alfredi*) en Nouvelle-Calédonie. A : face dorsale d’un individu noir, B : Face dorsale d’un individu chevron, C : Face ventrale d’un individu noir, D : face ventrale d’un individu chevron. *Photo A, B et C Hugo Lassauce. Photo D : Mark Erdmann.*

Les études de recensement et de suivis des populations démontrent une minorité d’individus mélaniques sans que les raisons qui expliqueraient cette différence de pigmentation ne soient connues (Venables et al. 2019). La proportion de raies manta de récif noires varie selon les régions du monde (Venables et al. 2019). Par exemple, le plus haut taux de spécimens mélaniques a été répertorié à Raja Ampat, Indonésie avec 40.7 % alors que les autres régions enregistrent moins de 10 %. Hawaii et les Maldives sont dépourvus de forme mélanique (Venables et al. 2019).

Abondance

La taille des populations de raies manta de récif dans le monde varie largement selon les régions. La plus grande population est recensée aux Maldives avec plus de 4000 raies manta (Stevens 2016). Les autres populations de l'océan Indien sont considérablement plus restreintes avec près de 1000 individus au sud du Mozambique (Venables et al. 2019) et sur la côte Ouest de l'Australie (Armstrong et al. 2020), et un peu plus de 250 individus aux Seychelles (Peel et al. 2020). En Indonésie, 624 raies manta ont été comptées à Bali (Germanov et al. 2019), 1176 aux Iles Komodo (Venables et al. 2019), et 1375 à Bird's Head Seascape (Setyawan et al. 2020). Dans l'océan Pacifique, plus de 300 raies manta ont été observées au sud du Japon (Kashiwagi 2014), en Polynésie Française (Carpentier et al. 2019) et à Hawaï (Deakoks et al. 2011).

Écologie spatiale

Les raies manta sont des filtreurs qui occupent principalement les habitats côtiers des eaux intertropicales du monde entier se nourrissant aussi bien dans les upwellings productifs des zones équatoriales que dans les milieux oligotrophes des zones tropicales (Couturier et al. 2012 ; Stewart et al. 2017). Les raies manta de récif sont observées en agrégation de plusieurs individus pouvant aller jusqu'à des centaines dans certaines régions comme les Maldives, le Mexique, ou au Mozambique, par exemple (Law 2010 ; Kitchen-Wheeler et al. 2010). Malgré le peu d'informations précises sur le comportement de ces animaux, les études existantes semblent attribuer cette distribution à une dépendance liée à la nourriture.

En se nourrissant essentiellement de zooplancton, les raies manta passent la majorité de leur temps à se nourrir ou à rechercher de la nourriture pour répondre à leurs besoins énergétiques (Sims et al. 2006 ; Jaine et al. 2012 ; Couturier et al. 2013 ; Weeks et al. 2015 ; Rohner et al. 2017 ; Barr et al. 2019). L'abondance et la distribution de cette ressource dépendent des conditions environnementales telles que les courants, la température, ou encore les apports terrigènes (Taniguchi 1973 ; Nair et al. 1992 ; Dagg et al. 2004). De ce fait, en fonction de la disponibilité en nourriture sur un site donné, les raies manta y seront plus ou moins fidèles (e.g., Dewar et al. 2008 ; Kitchen-Wheeler et al. 2010 ; Couturier et al. 2011, Jaine et al. 2012). Dans certaines régions, des agrégations de plusieurs individus peuvent être observées régulièrement sur de longues périodes lorsque la ressource est disponible. Des études utilisant la photo-identification ont démontré des taux de fidélité allant de 46.7 % en Australie de l'Est (Couturier et al. 2014) jusqu'à 90 % en Polynésie Française (Carpentier et al. 2019) sur des périodes pouvant s'étendre sur plus d'une décennie (e.g., à Hawaii, Deakos et al. 2011 ; en Polynésie Française, Carpentier et al. 2019, en Australie de l'Ouest, Armstrong et al. 2020 ; en Indonésie, Setyawan et al. 2020). D'autres régions ont observé une présence plutôt saisonnière des raies manta de récif avec des migrations liées aux variations de distribution de la ressource. Par exemple, Anderson et al. (2011a) ont attribué les mouvements saisonniers des raies manta aux Maldives aux changements de conditions environnementales causés par les moussons annuelles et Jaine et al. (2012) rapportent des pics de présence saisonniers liés à la dynamique océanographique de la côte Est Australienne. Cette hypothèse semble aussi se vérifier pour les déplacements verticaux puisque les raies manta sont capables de plongées profondes pour exploiter le plancton dans les couches mésopélagiques de la colonne d'eau. Dans les zones en bordure des plateaux continentaux, les raies manta sembleraient aussi profiter des migrations verticales de ce zooplancton (essentiellement euphausiacés et myctophidés) qui remonte des profondeurs mésopélagiques aux eaux épipélagiques pendant la nuit (Couturier et al. 2013 ; Braun et al. 2014). Les profondeurs maximums enregistrées à ce jour lors de ces plongées varient fortement en fonction de la région étudiée. Par exemple, les profondeurs maximums enregistrées sont de 360 m à Hawaï (Deakos et al. 2011), 294 m sur la côte Est de l'Australie (Jaine et al. 2014) et 432 m en Mer Rouge (Braun et al. 2014).

D'autres facteurs peuvent influencer le potentiel de dispersion des raies manta tels que des barrières géographiques. L'océan est un milieu qui semble continu où la présence de barrières limitant la dispersion n'est pas évidente. Dans ce contexte, le potentiel de dispersion des raies manta est difficile à prévoir. Les raies manta de récif ont la capacité de se déplacer sur de longues distances. Le long des côtes, cette espèce s'est déplacée sur des centaines de kilomètres allant jusqu'à 1150 km le long de la côte Est Australienne (Armstrong et al. 2019), 700 km sur la côte Ouest Australienne (Armstrong et al. 2020) ou encore 350 km au sud du Mozambique (Venables et al. 2020). A contrario, les vastes étendues d'eaux profondes pourraient limiter la connectivité. C'est l'hypothèse qui avait été émise par Deakos et al. (2011) pour expliquer l'absence de connectivité entre deux populations distantes de seulement 150 km mais séparées par des eaux de plus de 2000 m de profondeur à Hawaii. Des observations similaires ont été faites aux Seychelles (Peel et al. 2020), aux Maldives (Stevens 2016) et en Polynésie Française (Carpentier et al. 2019). Même si les eaux profondes constituent un facteur limitant la dispersion, il peut y avoir des échanges entre les populations dans les archipels et chaines d'îles. Par exemple, en Indonésie, des raies manta de récif ont enregistré des mouvements allant jusqu'à 450 km entre une chaine d'îles séparées par des étendues d'eaux profondes (Germanov and Marshall 2014).

Structure génétique des populations

La structure génétique d'une population est caractérisée par le degré de différentiation génétique des individus au sein de cette population. Une différenciation génétique apparait lorsque tous les individus ne se reproduisent pas de façon aléatoire (panmixie). Un brassage génétique hétérogène peut être le résultat de nombreux facteurs qui affectent le flux génique tels que des séparations physiques (distance ou barrières géographiques), des migrations, des processus évolutifs (e.g., sélection, dérive ou mutation) ou encore de variations démographiques ou comportementales (McVean 2001 ; Cardon et Palmer 2003).

Les populations de raies manta ont fait l'objet de peu d'études de leur structure génétique. Seuls, Hosegood (2020) and Venables et al. (2021) ont révélé une différentiation génétique entre des populations distantes de plus de 7000 km de part et d'autre de l'océan Indien. À l'échelle d'une seule population, ces études ont rapporté une absence de structure génétique entre les sous-populations des Maldives ainsi que celles du sud du Mozambique (Hosegood 2020 ; Venables et al. 2021, respectivement) témoignant de la capacité de cette espèce à maintenir un flux génique le long des côtes et entre des chaines d'îles interconnectées.

Menaces

Certaines caractéristiques telles qu'une petite population (Pimm et al. 1988), une aire de répartition géographique restreinte (Gaston 1994), une dépendance à un type d'habitat particulier (Brown 1995) ainsi qu'un cycle biologique suivant un modèle évolutif de type K (durée de vie longue, reproduction rare et tardive et faible fécondité) rendent une espèce plus vulnérable à l'extinction (MacArthur and Wilson 1967). Dans le milieu marin, les espèces de stratégie démographique K sont notamment, les mammifères marins et certains élasmobranches. Les raies manta ne font pas exception et ces caractéristiques réduisent considérablement leur capacité de résilience et de résistance face aux différentes menaces.

Les pêches

De nombreuses populations de raie manta ont été lourdement impactées par les pêches. Leurs branchies sont la cible d'une demande des marchés asiatiques (Croll et al. 2016 ; O'Malley et al. 2017). Des déclins importants et même des extinctions de population ont été enregistrés dans certaines régions du monde : Mexique (Homma et al. 1999 ; G Notarbartolo-di-Sciara 1995 ; White et al. 2006), Philippines (Alava et al. 2002), Indonésie (Dewar 2002 ; White et al. 2006) et Asie du sud-est (Marshall et al. 2018), par exemple. Par ailleurs, comme beaucoup d'espèces de la mégafaune marine, des prises accessoires de raies manta ont été rapportées le long des palangres, dans les sennes ou les filets dérivants de la pêche industrielle de thon, par exemple (SMMPM 2004 ; White et al. 2006 ; Hall et Roman 2013 ; Croll et al. 2016). En conséquence des déclins de populations lors de ces dernières décennies, les raies manta de récif sont classées « vulnérable à l'extinction » et en déclin dans la Liste Rouge de l'International Union for Conservation of Nature (IUCN) depuis 2011 (Marshall et al. 2018).

Autres menaces

Les milieux marins côtiers sont directement affectés par de nombreux facteurs notamment liés au développement des activités humaines incluant, entre autres, destruction, pollution ou augmentation des déversements terrigènes. Ces perturbations entrainent une perte de la biodiversité et les raies manta y sont particulièrement vulnérables étant donné leur affinité importante pour ces habitats (Dobson et al. 2006 ; Stewart et al. 2016).

Les agrégations régulières de raies manta sur des sites localisés près des côtes les rendent également attractives pour l'industrie du tourisme. Malheureusement, dans beaucoup de régions du monde, le tourisme de masse peu ou pas règlementé à un impact négatif sur les populations (Anderson et al. 2011b ; O'Malley et al. 2013).

Dans une moindre mesure, dans les régions où l'activité humaine est importante, les raies manta sont régulièrement victimes de collisions avec les bateaux ou encore d'enchevêtrement dans les lignes et filets de pêches abandonnés ainsi que dans les lignes de mouillage (Fig. 4) (Stewart et al. 2018 ; McGregor et al. 2019).

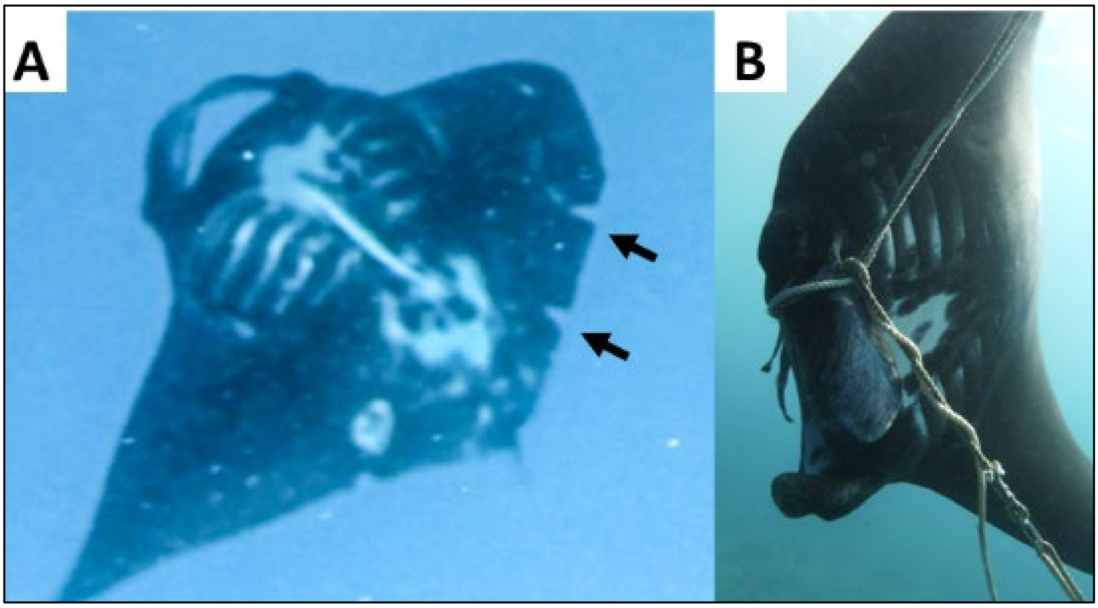

Figure 4. Raie manta de récif (*M. alfredi*), A : blessée par des hélices de bateau, B : prise dans une ligne de mouillage sur un site de plongée en Nouvelle-Calédonie. *Photos : A : Sandro Iannuzzella, B : Laurent Seveau*

LA NOUVELLE – CALÉDONIE

La Nouvelle-Calédonie est un archipel du Sud-Ouest du Pacifique dont la zone économique exclusive s'étend sur 1740 000 km^2 dans la mer de Corail entre l'Australie, les Îles Salomon, le Vanuatu et les Îles Fidji (Fig. 5). L'archipel compte une île principale, la Grande Terre, entourée d'îles plus petites, à l'Est les Îles Loyauté, au Sud l'Île des Pins, et au Nord les Îles Belep. La Grande Terre et les Belep sont entourées par une barrière de corail de 1660 km de long qui délimite un lagon de 16874 km^2 (Andréfouët et al. 2009). Le plateau continental est délimité par cette barrière de corail au-delà de laquelle les profondeurs atteignent rapidement plus de 2000 m. La bathymétrie autour des Îles Loyauté est caractérisée par un plateau continental très proche des côtes et des profondeurs au-delà des 2000 m qui les séparent de la Grande Terre. Au large de l'archipel, plusieurs récifs sont isolés dont les Chesterfield et Bellona à l'Ouest, les atolls Entrecasteaux au Nord, Petrie et l'Astrolabe au Nord-Est et les îles de Walpole, Matthew et Hunter au Sud-Est.

La Nouvelle-Calédonie avec ses récifs inscrits au patrimoine mondial de l'UNESCO (15.743 km^2 et 12.871 km^2 en zone tampon) et son Parc Naturel de la Mer de Corail (1.300.000 km^2) s'inscrit dans une importante dynamique de gestion et de conservation qui intègre les espèces emblématiques (GNC 2018). La raie manta a une importance culturelle et socio-économique pour le pays (Fig. 6). Dans ce contexte, elle pourrait jouer le rôle d'espèce parapluie - dont le domaine vital est assez large pour que sa protection assure celle des autres espèces appartenant à la même communauté (Ramade, 2002) - et être un catalyseur essentiel pour la conservation des habitats et des écosystèmes. Par ailleurs, un fort potentiel économique existe à travers des programmes écotouristiques autour des raies manta comme cela est déjà le cas dans beaucoup de régions du monde (Ris et al. 2017). Enfin, un partage des connais-

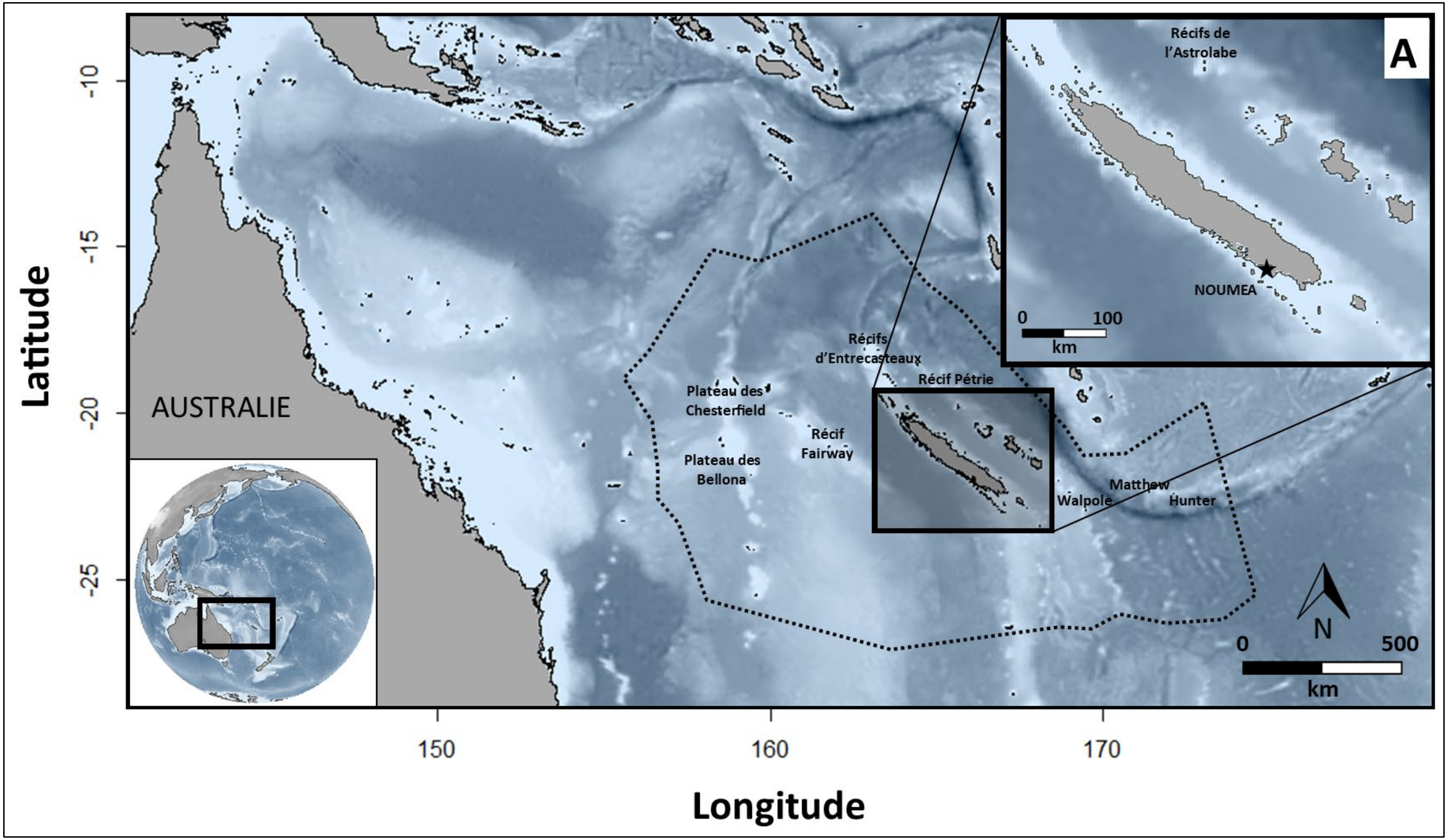

Figure 5. Carte de la région Pacifique Sud. Insert A : Nouvelle-Calédonie. La ligne en pointillée représente la Zone Economique Exclusive de la Nouvelle-Calédonie.

-sances au niveau régional permettrait une compréhension globale des enjeux auxquels les raies manta sont associées dans le but d'établir une gestion intégrée de ces espèces. Par exemple, comprendre les échelles de migration permettrait de connaitre certaines limites des protections en place à ce jour.

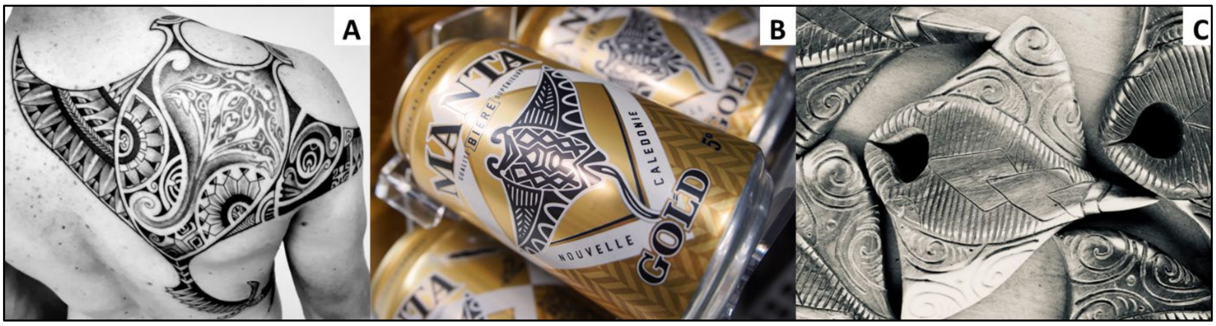

Figure 6. Importance de la **r**aie manta dans la culture océanienne. A : Tatouage polynésien (*artiste : Akhio Boy*), B : Marque de bière en Nouvelle-Calédonie, et C : Sculpture (artiste : *Mxm Woodworking*).

Dans ce contexte géographique, la population de raies manta de récif n'a encore fait l'objet d'aucune étude. En Nouvelle-Calédonie, les deux espèces de raies manta sont présentes. La raie manta océanique, *M. birostris,* n'est observée qu'à de rares occasions dans les eaux calédoniennes. Bien que son abondance, son utilisation de l'habitat et ses potentielles connexions avec les autres populations de la région Pacifique soient inconnues, étant donné le peu de rencontres, il semblerait que cette espèce ne soit que de passage dans la région. Par conséquent, ces travaux se porteront uniquement sur la population de raies manta de récif, *M. alfredi*. Ces dernières sont présentes sur l'ensemble de l'archipel. Ces animaux forment de petites agrégations le long des côtes, du récif ou dans les passes. La plupart des sites d'agrégations connus sont des stations de nettoyage dans les bordures de passes au niveau de la barrière de corail. Les raies manta s'y rendent pour se faire nettoyer, principalement par des labres nettoyeurs (*Labroides dimidiatus*), de leurs parasites et tissus nécrotiques ou morts provenant de blessures. Les autres sites d'agrégations sont des sites de nourrissage. Ils sont localisés dans les passes ou à l'intérieur du lagon et proches des côtes. Ces premières observations ont été collectées par des enquêtes auprès des différents utilisateurs de la mer : plaisanciers, plongeurs, pêcheurs et opérateurs touristiques. Cependant, ces informations sont insuffisantes pour déterminer les caractéristiques écologiques de cette population de raies manta. Combien sont-elles ? Comment occupent-elles l'espace des eaux calédoniennes ? Quelles sont les liens entre les différents groupes observés ? S'agit-il d'une seule population ou de groupes distincts évoluant dans le même paysage récifo-lagonaire ? Sont-elles capables de migrations dans les eaux océaniques ? Sont-elles fidèles à leur site ? Taille, distribution, connectivité, structure génétique, mouvements, utilisation de l'habitat sont autant de connaissances cruciales sur l'écologie de cette espèce pour connaitre l'état de santé de cette population et mettre en place des mesures de gestion et de conservation efficaces (Stewart et al. 2018). Aujourd'hui, la Nouvelle-Calédonie représenterait par sa taille, son contexte socio-culturel et sa législation, un sanctuaire pour les raies manta, ce qui en fait une référence rare à l'échelle mondiale. Il n'en reste pas moins que le développement économique et l'augmentation des usages de l'espace maritime calédonien représentent des pressions croissantes. Il est donc important d'améliorer les connaissances et d'encourager leur prise en compte pour faire évoluer les modèles de gestion existant en cas de besoin. Les informations apportées par ces travaux contribueront également à améliorer les connaissances sur l'espèce à l'échelle mondiale et serviront à sa conservation dans des régions où elle est plus vulnérable.

OBJECTIFS DE LA THESE

Cette thèse est structurée sur articles publiés ou en cours de publication et comprend un total de cinq chapitres. Les chapitres 1 et 5 ne sont pas destinés à la publication dans un journal scientifique contrairement aux chapitres 2, 3 et 4 qui sont ou seront publiés. Voici les détails bibliographiques et le statut de publication de chacun des chapitres :

Chapitre 1 : pas destiné à la publication. Auteurs : Lassauce H.

Chapitre 2 : soumis au journal *Coral Reefs.* Auteurs : Lassauce H., Chateau O., Erdmann MV. et Wantiez L.

Chapitre 3 :

- **Article 1 :** publié dans le journal *Plos One.* Auteurs : Lassauce H., Chateau O., Erdmann MV. et Wantiez L.
- **Article 2 :** sera soumis au journal *Marine Ecology Progress Series.* Auteurs : Lassauce H. et Wantiez L.

Chapitre 4 : en révision dans le journal *Endangered Species Research.* Auteurs : Lassauce H., Dudgeon CL., Armstrong AJ., Wantiez L. et Carroll EL.

Chapitre 5 : pas destiné à la publication. Auteurs : Lassauce H.

Le premier chapitre décrit le cadre contextuel nécessaire à la compréhension des travaux présentés dans les chapitres suivants et identifie les manques de connaissances qu'ils tentent de combler.

Le second chapitre utilise la science participative au travers de la photo-identification pour décrire les caractéristiques et l'utilisation de l'habitat de la population de raies manta. Ce chapitre présente la population de raies manta de Nouvelle-Calédonie et place ces résultats en perspective par rapport aux autres populations dans le monde. Les informations apportées dans ce chapitre représentent les premières bases de connaissance servant aussi à émettre les hypothèses testées dans les chapitres suivants.

Le troisième chapitre étudie l'écologie spatiale et le comportement de la population en utilisant la télémétrie satellitaire. A partir des résultats révélés dans le second chapitre donnant un aperçu de l'utilisation de l'habitat, cette étude tente de caractériser les différents profils de mouvements à une échelle spatio-temporelle fine dans un espace tridimensionnel avec le déploiement de balises satellites.

Le quatrième chapitre décrit la structure et la diversité génétique de la population. En s'appuyant sur les résultats des études précédentes, cette étude a pour objectif de mettre en évidence d'éventuelles similarités ou divergences génétiques entre les différents groupes présents dans les eaux calédoniennes. Ce chapitre place aussi ces résultats dans un contexte régional avec l'inclusion d'échantillons provenant de raies manta d'Australie de l'Est.

Le cinquième chapitre conclut le document et propose une synthèse de l'ensemble des résultats. Il aborde les perspectives d'études qui complèteraient ces travaux et contribueraient à mieux connaitre et protéger cette espèce.

CHAPITRE 2

USING CITIZEN SCIENCE TO INFER CHARACTERISTICS AND HABITAT USE OF REEF MANTA RAYS (*Mobula alfredi*) IN NEW CALEDONIA [1]

ABSTRACT

Reef manta rays (*Mobula alfredi*) are observed at several sites in all parts of the archipelago of New Caledonia. The species faces threats from human exploitation and activity in several regions of the world and crucial information on the ecology of the species is needed. This study is the first to investigate the population of New Caledonia and focuses on its characteristics and its habitat use. We used citizen science over four years (2015 – 2020) to collect 1741 photo-identifications from 11 sites. The unique ventral coloration patterns of the manta rays allowed the identification of 391 individuals. These results highlight the widespread distribution of the species in the archipelago with little connectivity between all aggregations sites, with only 5.4 % of the individuals observed at more than one site. Strong and long-term site fidelity was recorded at all studied sites through re-sighting rates and residency analysis. The population also records the highest known proportion of melanistic manta rays (43 %) to date, and a balanced male : female ratio (1.0 : 1.15). The analysis of injuries reported a low impact from anthropogenic stressors, with only 9.8 % of injuries judged to be of anthropogenic origin. Attempted predation rates were not higher than other location in the world with 29.7 % of individuals bearing bite-related injuries. Our study highlights the need for further investigations on the spatial ecology and structure of the

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[1] Lassauce H., O. Chateau, M. Erdmann et L. Wantiez (submitted). Using citizen science to infer characteristics and habitat use of reef manta rays (*Mobula alfredi*) in New Caledonia. *Coral Reefs.*

population to assess essential habitat and address potential localised threats, and thus, inform by conservation projects.

KEY WORDS: Site Fidelity ; Spatial Connectivity ; Photo-identification ; Injuries ; Population Size.

INTRODUCTION

The distribution patterns and habitat use of highly mobile marine species can be difficult to predict and understand. These animals are able to move and have the potential to disperse over large distances, although many use smaller home ranges than their dispersal capabilities suggest (Mannocci et al. 2017). Such observations have been made for numerous species including sea turtles (Bowen and Karl, 2007; Evans et al. 2019), marine mammals (Baker et al. 1990; Campbell et al. 2002; Baird et al. 2008), bony fish (Marnane 2000; Chateau & Wantiez 2009; Meyer et al. 2010) and elasmobranchs (Hueter et al. 2005, Bansemer & Bennett 2009, 2011, Chapman et al. 2015). In the marine environment, the importance of ecological and environmental factors that limit a given species' dispersal and result instead in long-term site fidelity patterns often remain unclear. For instance, habitat heterogeneity or topography (e.g., currents or deep-water channels) as well as habitat choice based on food resources or reproductive ecology can be the drivers of a fragmented population (Palumbi 1994, Bowen et al. 2016). The characterisation of a population range is crucial to identify effective management units for conservation purposes, especially for threatened species (Hueter et al. 2005).

Reef manta rays *Mobula alfredi* (Krefft 1868) are large, primarily coastal planktivores that are distributed in equatorial and tropical waters around the globe (Couturier et al. 2012). Similar to many other elasmobranchs, manta rays have conservative life history traits: low fecundity, slow maturity and high longevity, which make them particularly sensitive to overexploitation (Couturier et al. 2012; Stevens 2016). Since 2011, *M. alfredi* have been listed as Vulnerable to Extinction on the IUCN Red-List (Marshall et al. 2018) due to over-harvesting pressure as a result of the high commercial value of their gill plates for use in non-traditional Chinese medicine (O'Malley et al. 2016). In response to important declines of manta ray populations around the world (Ward-Paige et al. 2013), the number of studies on the ecology and behaviour of the species has increased considerably over the past decade (Stewart et al. 2018). A major part of previous research efforts focused on identifying population characteristics and structure, as well as spatial dynamics and habitat use patterns (Stewart et al. 2018). These studies have shown that reef manta rays are highly mobile species capable of movements over a thousand of kilometres along a continuous coastline (up to 1150 km, Armstrong et al. 2019) and hundreds of kilometres between island chains (up to 450 km, Germanov and Marshall 2014). Large-scale movements seem to be mainly conditioned by variations in food resources (Sleeman et al. 2007; Dewar et al. 2008; Anderson et al. 2011, Jaine et al. 2012; Couturier et al. 2018; Setyawan et al. 2018). On the contrary, when sufficient foraging opportunities persist within an area, reef manta rays tend to remain in or return to this area over extended periods of time, which is described as site fidelity or affinity. For example, strong evidence of site fidelity was observed in Hawaii (Deakos et al. 2011), Mozambique (Marshall 2008), the Seychelles (Peel et al. 2020), Indonesia (Setyawan et al. 2020), French Polynesia (Carpentier et al. 2019) and eastern Australia (Couturier et al. 2011). In these studies, individuals were re-sighted over decadal periods, confirming long-term, and potentially life-long use of aggregation sites (e.g., Clark 2010; Setyawan et al. 2020; Carpentier et al. 2019).

The spatial dynamics and habitat use patterns of animal populations have been studied using different methods (or a combination of them), including photographic mark-recapture methods, acoustic and satellite telemetry, or genomic techniques (Stewart et al. 2018). Most studies on manta rays use photo-identification (photo-ID) to describe population characteristics, assess connectivity and estimate population size (among others: Deakos et al. 2011; Marshall et al. 2011; Couturier et al. 2014). The cataloguing of individuals is made possible through the identification of each animal based on the variation in the ventral body pigmentation patterns (Marshall et al. 2011). Photo-ID offers an inexpensive, minimally invasive and widely accessible method to build large databases of individuals and conduct long-term monitoring of these populations. Importantly, submission of ID photographs by tourists or local enthusiasts (“citizen scientists”) can enable the collection of large quantities of data over extended geographic scales, while also insuring longevity of the monitoring program. This citizen science approach has proven highly effective for the study of manta rays because the species is very attractive for recreational divers, benefiting researchers with important contributions to their study (eg, Germanov et al. 2019).

New Caledonia is an archipelago located in the southern Pacific region where reef manta rays are sighted year-round at many sites. In New Caledonia, fisheries effort is low (harvesting on average 0.26t/km^2/year) in comparison to other Pacific islands, manta rays are not fished, and tourism development is still relatively insignificant (Guillemot et al. 2009). Anthropogenic influence on manta rays is presumed to be minimal, although manta rays are locally under no current legal protection. To date, the only published study on the reef manta rays of New

Caledonia is that of Lassauce et al. (2020), who used satellite telemetry to reveal unprecedented deep diving behavior for reef manta rays at all studied sites in New Caledonia, potentially indicating the presence of important food resources in deeper (300-700 m depth) waters. Moreover, ongoing genetic analysis has revealed small scale differentiation between studied sites within New Caledonia waters, suggesting limited connectivity and high site fidelity (Lassauce et al., in review). Nonetheless, information on the ecology and population dynamics of reef manta rays in New Caledonia remains scarce.

Our study aims to provide a first insight into the population size, sex ratio, incidence of melanism, injury and predation rates, as well as patterns of connectivity, site fidelity and residency of reef manta rays of New Caledonia. We analyse four years of compiled photo-ID data collected through citizen science from known aggregation sites around the archipelago.

METHODS

Study sites

Photo-IDs and observations on reef manta behavior were collected from known manta aggregation sites around New Caledonia. The archipelago is divided into a so-called Main Island surrounded by the second largest barrier reef in the world (1660 km in length) and islands in the south (Isle of Pines), east (Loyalty Islands) and north (Belep Island). We choose to focus our data collection efforts at three main study areas (Fig. 1): Noumea, Touho and Ouvea. These three study areas were selected among others based upon two factors. First, these were the areas with the most sightings of manta rays prior to our field work, also benefiting from the regular presence of tourist operators on site. Second, these areas, being distributed across New Caledonia, give a broad view of the connectivity within the archipelago. All other areas are defined as secondary in this study since data were only based on opportunistic collection of photo-IDs by citizen scientists, with no standardised surveys performed directly by the authors at these locations. Noumea is in the south-eastern part of the Main Island and includes two aggregation sites along the barrier reef, approximatively 20 km off the coast: Dumbea Channel and Boulari Channel (24 km apart). Several dive clubs and other tourist operators from Noumea visit these sites daily. Dumbea Channel is a feeding site for manta rays within the first 30 meters of the water column facing the reef crest of the southern tip of the reef channel. Manta rays are observed in groups of 5 to 10 individuals when the conditions are conducive with a high concentration of plankton year round. Boulari Channel is a cleaning station located on a 15-meter-deep reef flat on the North tip of the reef channel where manta rays are observed year-round. Touho is located in the northeast of the Main Island. The aggregation site is located on the barrier reef less than 5 km off the coast, at the northern tip of the Great Channel of Touho. This site is a 15- to 20-meter-deep reef flat with several cleaning stations aggregating manta rays year-round. A dive club was present almost daily on the site between 2014 and 2017 before its closure, after which nearly no visitors came to this site besides the authors. Ouvea is the most northerly of the Loyalty Islands, 90 km off the Main Island and separated by a 2000 m deep channel. Occurrence of reef manta rays are year-round at known aggregation sites that are located along the Southern and Northern reefs, called the Pleiades, that enclose the lagoon of Ouvea. All known sites are cleaning stations on the reef crest at a depth of 10 to 15 meters. Feeding is also reported occasionally in the proximity of these sites. Tourist operators regularly frequent these cleaning stations on Ouvea.

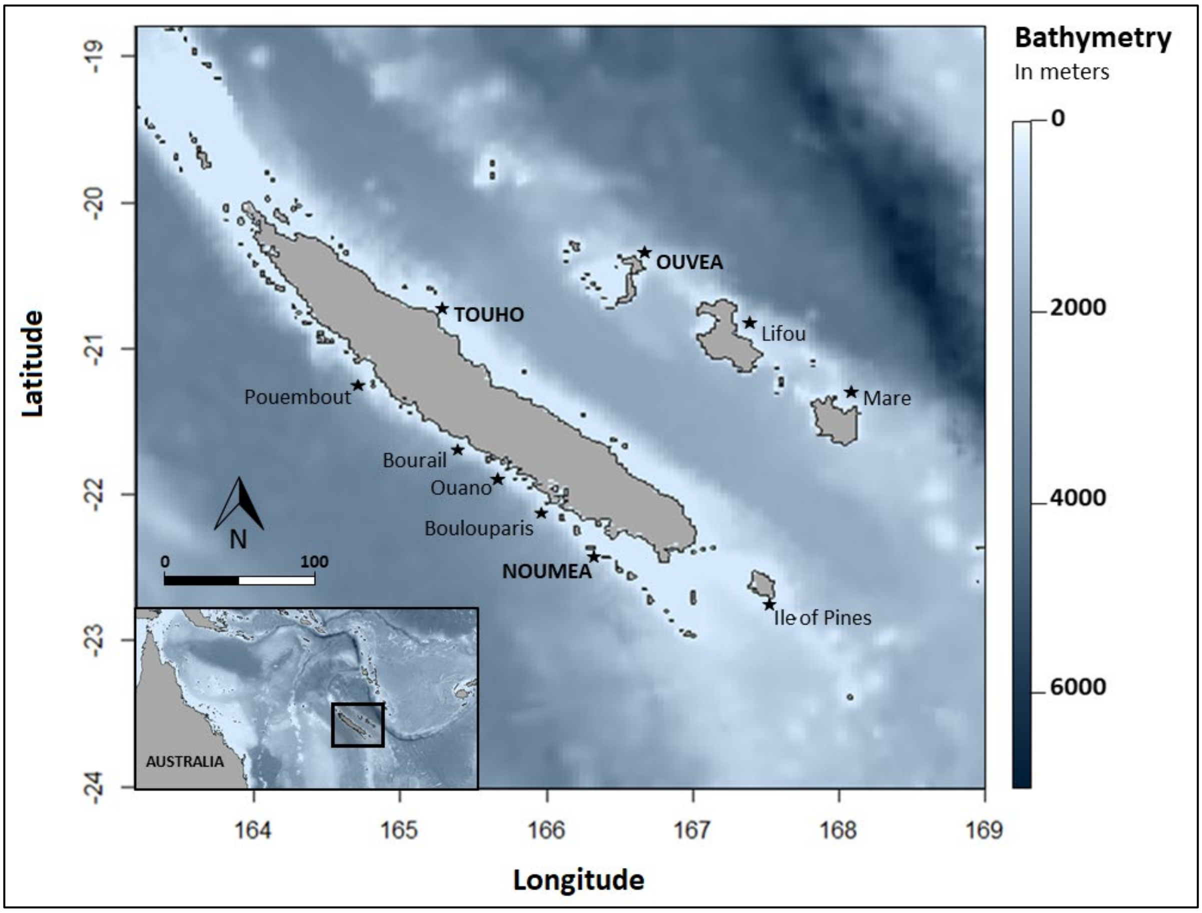


Figure 1. Sighting locations of reef manta rays (*Mobula alfredi*) in New Caledonia, South Pacific (inset) recorded by photo-identification between 1993 to 2020. Main studied areas are indicated with uppercase bold labels, while secondary study areas are in lowercase.

Data collection

Reef manta photo-IDs were collected from local tourist operators, dive clubs, fishermen and other users of the sea as well as the researchers during field operations from 2017 to 2020, with historical records reaching back to 1993. Images were captured while SCUBA diving or free diving using underwater digital cameras. Researchers also gathered photographs using a GoPro Hero4 action camera placed on the substrate within a cleaning station with the lens facing up and the configuration set on the “time lapse” mode, taking an image every 5 seconds. This system allowed us to gather photos during the day when the divers were not on site or between dives for a maximum duration of 3 hours prior to battery depletion

(Setyawan et al. 2020). Only photographs with a clear distinction of the spots and other patches located on the inter-brachial and sub-abdominal areas on the ventral surface of the animals were used for identification. Additional distinctive wound marks were also used to assist in the identification process. A single photograph of the ventral side of an individual was sufficient to constitute a positive identification in a sighting event. Re-sightings of a known individual were recorded when the identification occurred at least 24 hours after the previous identification (Marshall et al. 2008). The identification was processed visually by comparing the new photographs to the photo-IDs of each individual already recorded in the database. Once identified, distinctive characteristics such as the sex, the colour morph and injuries were recorded from all available images (following Marshall et al, 2011). The sex was determined by the presence or absence of claspers for male or female individuals, respectively (following Marshall et al, 2011) and marks of predators and of anthropogenic origin were distinguished among all injuries. Predation attempts were identified by the appearance of clear shark bites (Marshall and Bennett 2010a) and/or the tail cut, while damaged or missing cephalic fins, clear cuts on the frontal edge of the body and/or clear propeller marks (Carpentier et al. 2019) were identified as being of anthropogenic origin.

Surveys by the authors at the three main sites represent a total of 234 hours between 2017 to 2020 at different periods of the year (Table S2). Sampling was opportunistic based on boat and staff availability. The rest of the data was gathered from the citizen scientists via the ‘Manta Initiative in New Caledonia’ program (www.facebook.com/initiativemantaNC), a collaboration between The Manta Trust (www.mantatrust.org), Conservation International (www.conservation.org) and the Aquarium des Lagons (www.aquarium.nc) that aims to inform and promote citizen science. Citizen science has been used in many studies to gather information on population characteristics of reef manta rays around the world (Southern

Mozambique, Marshall et al. 2011; Hawaii, Deakos et al. 2011; East Australia, Jaine et al. 2012; Couturier et al. 2014; Indonesia, Germanov et al. 2019; French Polynesia, Carpentier et al. 2019). Opportunistic data collection results in inconsistent sampling efforts through time and locations (Setyawan et al. 2020). In order to account for this variability and enable comparisons between sites, we include an indication of sampling effort based on the number of days when at least one reef manta sighting was recorded.

The photographs that generated the data used for this study were collected from different periods depending on site. In Noumea, the earliest photograph was captured in 1993 and the latest was in December 2020. In Touho, the photographs were taken between January 2014 and August 2020. Photographs from Ouvea were dated from January 2005 to August 2020. Data collected at secondary sites ranged from 2017 to January 2020 in Mare, from January 2008 to November 2020 in Lifou, from 2003 to July 2020 in the Ile of Pines, from June 2019 and July 2020 in Boulouparis, from February 2015 and November 2017 in Bourail, from March 2010 and August 2019 in Pouembout, from March 2017 and 2019 in Ouano, and from September 2011 in Poindimie (Table S1).

Abundance estimations

Our approach to estimate the abundance of reef manta rays at each site is based on the method to estimate species richness through species accumulation curves. This approach requires to meet some assumptions to be valid: (1) observers continuously attempt to identify new individuals throughout the period; (2) all manta rays have equal chance to be identified; and (3) there are no significant additions to the population during the period that would cause true abundance to raise. In other words, the rate at which new individuals are discovered per unit effort should decline with time. We also assume that the number of manta rays that die during the period prior to being identified is negligible. Discovery curves of the cumulative number of individuals per sampling effort unit (days) were generated for each site using the *Sample rarefaction (Mao's tau)* function (Colwell et al. 2004) implemented in the PAST 4.03 software (Hammer et al. 2001). Non-linear models proposed within the *Non-linear fit* function in PAST were fit to our data by following Levenberg-Marquardt optimization and the best-fit model was chosen using the smallest AIC value (Table S3). Abundance estimates were indicated by the asymptote of the curve and 95 % confidence intervals were based on 1999 bootstrap replicates. Here, the New Caledonia dataset contains the combination of all data gathered from each of the 10 sites.

Site fidelity and residency analysis

'Site fidelity' is defined by Chapman et al. (2015) as the return of an individual to a location where it previously resided after an absence as long as or longer than the residency period. In the case of potential regular movements within a large home range including many sites, Couturier et al. 2011 applied the term 'site affinity'. Based solely on sightings, our data do not allow us to know whether an individual stayed at or left a site between two sightings. In our context, only a few movements were recorded between studied sites, suggesting a 'site fidelity' scenario rather than a 'site affinity' one, as described by the authors (Chapman et al. 2015, Couturier et al. 2011, respectively). Residency models, or models of lagged identification rates (LIR), defined as the probability of resighting an individual after a variable lag time, were fitted to our resighting data to compare patterns between sites. These statistical models are appropriate with the analysis of opportunistically sampled data, as it uses the recapture data to infer sampling effort (Whitehead 2001). Several studies used these statistical models on manta rays (Deakos et al. 2011; Germanov et al. 2019; Carpentier et al. 2019), whale sharks (e.g., Ramírez-Macías et al. 2012; Robinson et al. 2016; McCoy et al. 2018), and marine mammals (Whitehead 2001, Chabanne et al. 2017). We used the SOCPROG2.9 software (Whitehead 2009) to calculate LIR data and compare them to eight models with different closed and open population scenarios that includes variables such as emigration, mortality, and re-immigration (Table S5). In this case, the New Caledonia dataset was confined to only those periods when sampling effort was the highest and the most consistent: from 2017 to 2020 for Noumea and Ouvea, and 2014-2015 for Touho (Table S2). For each site, the best-fit model was chosen by selecting the lowest value from the Akaike information criterion (AIC), when no overdispersion occurs in the data, or the quasi-Akaike information criterion (QAIC) when overdispersion occurs in the data. The differences in AIC or QIAC values between the best-fit model and any other ones (ΔAIC or ΔQAIC) give an indication of how the data support other models based on the following results (Whitehead 2007): ΔAIC or ΔQAIC < 2 = substantial support; ΔAIC or ΔQAIC 4 – 7 = considerably less support; and ΔAIC or ΔQAIC > 10 essentially no support. All chosen models were then bootstrapped 1999 times to calculate standard errors (SE) and 95 % confidence intervals (CI).

Statistical analysis

The comparisons of sex ratio, ratio of normal chevron colored vs melanistic individuals for each site and potential sex bias in movements were tested using Chi-squared (χ^2) goodness of fit tests. Injury and attempted predation rate data were also compared using Chi-squared (χ^2) tests for contingency tables to assess the relationship between these variables and the sex at each site. Differences in mean numbers of re-sighting events, mean re-sighting period and re-sighting events per individual were tested using analysis of variance (ANOVA). Student t-tests (equal variances) and Welch's t-tests (unequal variances) were used for pairwise comparisons of means. Levene's tests were used to test the homogeneity assumptions.

RESULTS

Sampling effort

Sampling effort, as measured by number of survey days with at least one sighting event, was not evenly distributed through time and locations at all sites in New Caledonia. Data were collected within a total period of 654 survey days unevenly distributed from 1993 to 2020 (Table S1A). The largest sampling effort (91 % of all survey days) occurred at the three main study sites: Noumea (56.6 %), Touho (22.9 %) and Ouvea (11.5 %), while sampling effort at secondary sites ranges from 1 day at Poindimie and Bourail to 28 days at Mare. In Noumea (*N* = 370 days) and Ouvea (*N* = 75 days), most of the sampling effort was conducted between 2017 and 2020, with 55.7 % and 85.3 % of the total effort at these sites, respectively. In Touho (*N* = 150 days), sampling effort was the highest in 2014 and 2015, representing 85.3 % of the total effort. This sampling heterogeneity has been taken into account when analysing temporal and spatial patterns in our manta sightings as reported below.

Sightings distribution

A total of 1741 encounter records consisting of at least one photograph suitable for identification were collected from 1993 to 2020 for *M. alfredi*. Photographs were taken by recreational divers and snorkelers (85.6 %) between 1993 and 2020 and the authors (14.4 %) between 2017 and 2020 (Table S1B). These sightings originated from 11 sites around the archipelago of New Caledonia, although 94.4 % were recorded at the three main study sites of Noumea, Ouvea and Touho. Noumea had the highest percentage of sightings (48.9 % of total), with 851 sightings recorded in 370 days between 1993 and 2020. In Touho, 591 sightings were recorded in 150 days between 2014 and 2020, with 81 % of those sightings recorded in 2014 and 2015 when a tourism boat was operational there. In Ouvea, we recorded 206 sightings from 75 days between 2014 and 2020, with 80.1 % collected since 2017. At the eight secondary sites, a total of 93 sightings were recorded since 2003 in 59 days of successful survey effort. Mare and the Isle of Pines accounted for 37 and 27 sightings in 28 and 15 days, respectively, while the six other sites combined produced a total of 29 sighting events in 16 days. The average number of sightings per sampling effort unit was significantly different between sites ($F_{(3,653)} = 27.1$, $p < 0.001$). The highest rate was recorded in Touho with 3.9 individuals per day and the lowest was in Noumea with 2.3, while in Ouvea the rate was 2.7 individuals per day (Table 1).

Population characteristics

Sex ratio

Of the 391 reef manta rays recorded from New Caledonia, 50.4 % were identified as females, 44 % as males and 5.6 % were undetermined (Table 1). This male : female ratio (1.0 : 1.15) shows no significant bias ($\chi^2_{(1, N = 391)} = 2.84$, $p > .05$). Noumea ($N = 153$) is the only site with a significantly biased sex ratio towards females (1.0 : 1.47, $\chi^2_{(1, N = 153)} = 5.42$, $p < .05$), though we do note that 10 individuals from this area were unsexed. In Touho, Ouvea and all the remaining sites, we did not find any significant differences in the numbers of males to females. Touho had 34 males, 35 females and 3 unsexed individuals (1.03 : 1.0, $\chi^2_{(1, N = 72)}$ = 0.14, $p > .05$); Ouvea had 60 males, 50 females and 6 unsexed rays (1.2 : 1.0, $\chi^2_{(1, N = 116)}$ = 1.17, $p > .05$) and all the eight remaining sites combined had 22 males, 31 females and 3 unsexed individuals (1.0 : 1.41, $\chi^2_{(1, N = 68)}$ = 1.61, $p > .05$). When all data is pooled across New Caledonia, there was no significant difference in the number of males and females sighted ($\chi^2_{(1, N = 1741)}$ = 1.71, $p > .05$).

Melanism

Overall (N = 391 individuals), there are significantly more chevron-coloured reef manta rays (57.0 %) than melanistic ones (43.0 %) (1.33 : 1.0, $\chi^2_{(1, N = 391)}$ = 7.74, $p < .01$) (Table 1). However, this trend is significant only in the Noumea subpopulation, with 58.2 % chevron and 41.8 % melanistic manta rays (1.39 : 1.0, $\chi^2_{(1, N = 153)}$ = 4.08, $p < .05$), but not in Touho (1.25 : 1.0, $\chi^2_{(1, N = 72)}$ = 0.35, $p > .05$) and Ouvea (1.0 : 1.0, $\chi^2_{(1, N = 116)}$ = 0, $p = 1$), where there is no significant bias in the ratio of colour morphs.

Table 1. Summary of the characteristics of the population of reef manta rays (*M. alfredi*) in New Caledonia from photographic sightings dating from 1993 to 2020. M: Male, F: Female, C: Chevron, B: Melanistic, IDP: Isle of Pines.

Sites	No. of sites	No. of Individuals	No. of males	No. of females	Sex ratio (M : F)	No. of chevrons	No. of blacks	Colour morphs ratio (C : B)	No. of sightings	% of re-sighted individuals	No. of sightings per day **
Main study sites:											
Noumea	2	153	58	85	1.0 :1.47[b]	89	64	1.39 : 1.0[b]	850	59.5	2.3[c]
Touho	1	72	34	35	1.0 : 1.03	40	32	1.25 : 1.0	591	76.4	3.9[c]
Ouvea	2	116	60	50	1.0 : 1.22	58	58	1 .0 : 1.0	204	44.8	2.7[c]
Others:											
Mare	1	20	9	11	1.22 : 1.0	11	9	1.22 : 1.0	37	30	1.3
IDP	2	20	3	14	1.0 : 4.7[b]	12	8	1.5 : 1.0	27	45	1.8
Lifou	1	7	2	4	1.0 : 2.0	5	2	2.5 : 1.0	7	42.9	1.4
Pouembout	1	7	4	3	1.33 : 1.0	7	0	-	8	14.3	4
Boulouparis	1	6	4	2	2.0 : 1.0	5	1	5.0 : 1.0	6	33.3	3
Ouano	1	4	2	2	1.0 : 1.0	3	1	3.0 : 1.0	5	50	1
Bourail	1	1	1	0	-	1	0	-	2	0	2
Poindimie	1	1	0	1	-	0	1	-	1	100	1
Unknown	-	2	1	1	1.0 : 1.0	2	0	-	3	-	-
Total	**14**	**391***	**172[a]**	**197[a]**	**1.0 : 1.15**	**223**	**168**	**1.33 : 1.0[b]**	**1741**	**56.8**	**2.7**

* one or more individuals were also sighted in one or more different locations; these duplicates are only counted once in the New Caledonia total, but are nonetheless counted once within the table at each site where they were sighted.

** calculated only for those days with at least one sighting event.

[a] sex was undetermined for 22 individuals.

[b] indicates significantly biased ratio ($p < .05$).

[c] indicates significant difference among main study sites ($p < .05$)

Injuries and attempted predations (or bite marks)

Of the total of 391 identified reef manta rays in New Caledonia, 44.8 % had noticeable wounds or injuries (Fig. 2). Among these individuals ($N = 175$), the majority (67.4 %) showed marks of natural predation attempts, while 9.8 % had injuries assumed to be of anthropogenic origin. There was no significant difference in the proportions of males and females injured ($\chi^2_{(1,\, N = 170)} = 0.14$, $p > .05$), and neither was there a difference between sex in signs of attempted predation ($\chi^2_{(1,\, N = 118)} = 0.51$, $p > .05$). Between sites, significant differences were observed when comparing the proportions of injured ($\chi^2_{(3,\, N = 175)} = 22.7$, $p < .001$) and bitten ($\chi^2_{(3,\, N = 170)} = 48.6$, $p < .001$) manta (Fig, 2). Ouvea ($N = 116$) and Touho ($N = 72$) each had a significantly higher proportion of injured individuals with 54.3 % and 52.8 %, respectively, than Noumea ($N = 153$) with 38.6 %. The proportion of manta rays recorded with bite marks is significantly higher in Ouvea (43.1 %) than in Noumea (23.5 %) ($\chi^2_{(1, N = 86)}$ =11.6, $p < .001$). The differences in proportions of bitten individuals between Touho (33.3 %) and the two other sites were not significantly different. Pooling the data from the other remaining sites (*N* = 68) into an "Other Sites" category, both the proportion of injured manta rays (21.4 %) and the percentage of bitten individuals (10.7 %), are significantly the lowest compared to the three main sites.

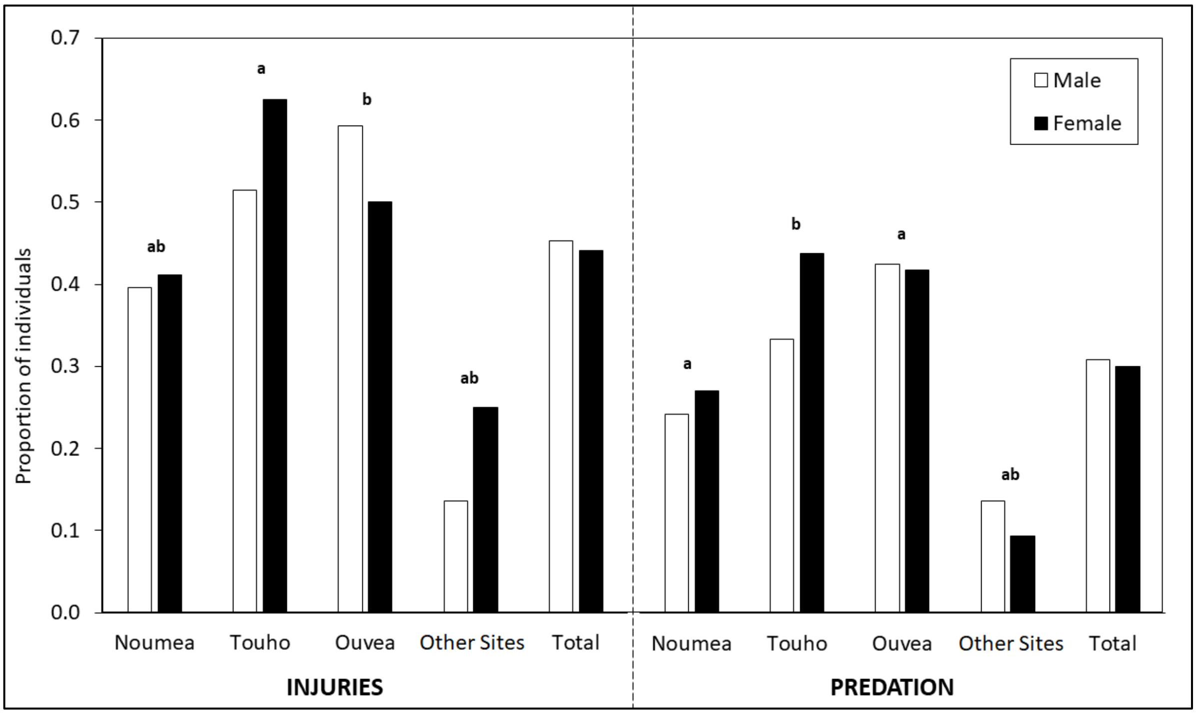


Figure 2. Percentages of males and females reef manta rays (*M. alfredi*) bearing one or more injuries (left) and attempted predation marks (right) at all sites in New Caledonia (*N* = 391): Noumea (*N* = 153), Touho (*N* = 72), Ouvea (*N* = 116) and Other sites (combining Mare, Isle of Pines, Lifou, Pouembout, Boulouparis, Ouano, Bourail and Poindimie, *N* = 68). Letters indicate pairwise significative differences (p < .05) in proportion of injured or bitten individuals between sites.

Individuals per site and estimated abundance

Using photo-ID we recorded 391 *M. alfredi* individuals throughout the 10 known aggregation sites in New Caledonia. In Noumea, we counted a total of 153 reef manta rays at two aggregation sites: 82.3 % at Boulari channel and 17.7 % at Dumbea channel, with 14.4 % of the Noumea individuals observed at both sites. In Touho, the total count was 72 reef manta rays, while in Ouvea, we recorded 116 individuals from two sites: Northern (16.5 %) and

Southern Pleiades (83.5 %) with 13.3 % observed at both sites. From the eight secondary sites we recorded a total of 66 individuals. Individual counts varied from one (in only one sighting) in Poindimie to 20 from both Mare and the Ile of Pines for 37 and 27 sighting events, respectively (Table S1).

The discovery curves describing the cumulative number of individuals recorded over time do not reach an asymptote for any of the sampling sites, indicating that these counts do not represent the whole population (Fig. 3). However, the curves for most sites, and especially for Touho, Noumea and the overall New Caledonia population, show a slight decline in accumulation rate, suggesting that extrapolation to an asymptote might provide an abundance estimate (Baker et al. 2006). The best-fitting model (Table S3) for the whole New Caledonia dataset was Hill's equation and reached its asymptote at 1347 individuals, 95 % CI [1225, 1413], which would indicate that only 29 % [27.7, 31.9] of the total number of reef manta rays in New Caledonia were sampled. In Noumea and Touho, the same model showed estimations of 441 [392, 462] and 151 [72, 163] individuals, suggesting that 34.7 % [33.1, 39] and 47.7 % [44.2, 100] of these groups were sampled, respectively. In Ouvea, the Michaelis-Menten model best-fitted the data and reached its asymptote at 246 [245, 248] individuals, suggesting that 47.1 % [46.9,47.3] of the population was sampled.

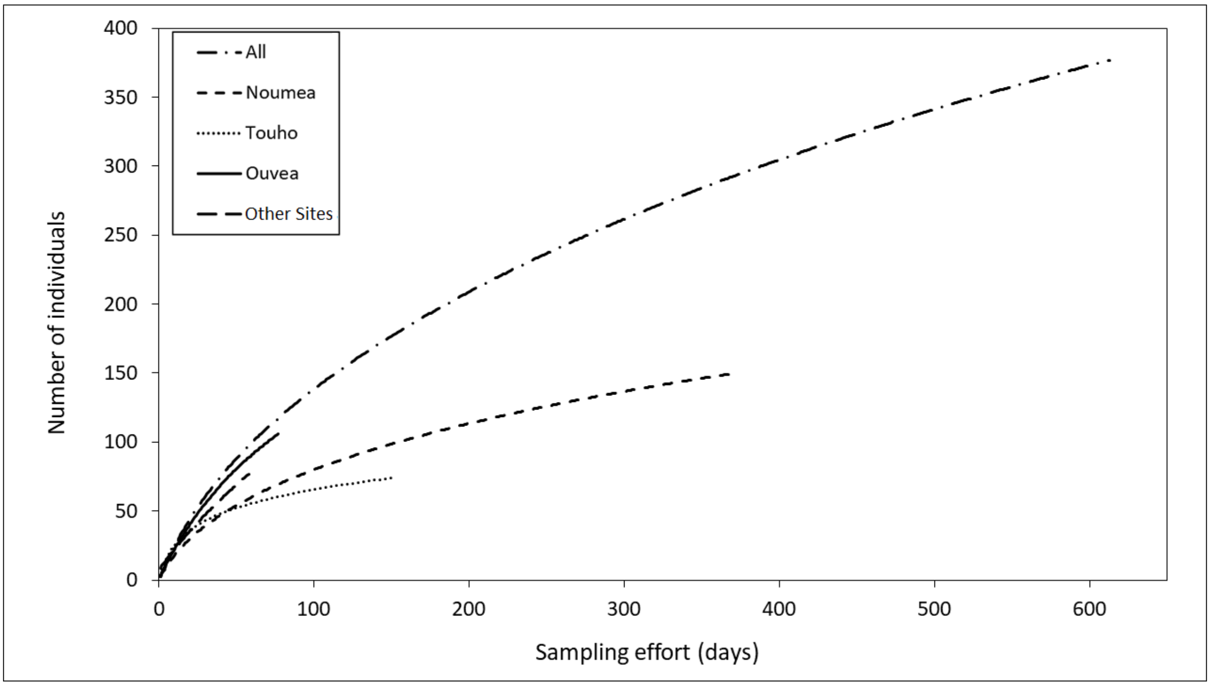


Figure 3. Discovery curves of the cumulative number of individuals of reef manta rays (*Mobula alfredi*) identified over the number of days of sighting events at all sites in New Caledonia: Noumea, Touho, Ouvea and Other Sites (combining Mare, Isle of Pines, Lifou, Pouembout, Boulouparis, Ouano, Bourail and Poindimie) between January 1993 and December 2020.

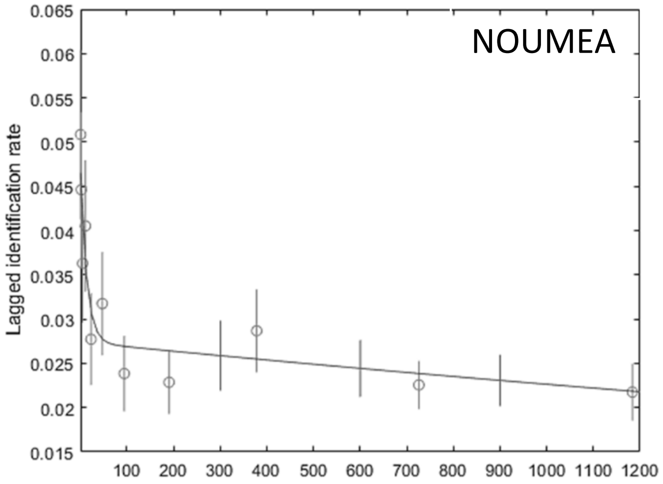
Connectivity

A total of 21 reef manta rays (5.4 %) have been observed at two or three different areas (Table S4). Overall, there were no distinguishable routine movement patterns. These movements were recorded by 10 males and 11 females, with no significant bias in the sex ratio (1.0 : 1.1, $\chi^2_{(1,\ N = 21)} = 0.04$, $p > .05$). The two areas with the highest number of rays connecting at least once were Noumea and Touho (approx. 320 km shortest distance through the water) with five individuals and time interval between re-sighting ranging from 141 to 2328 days. Two of them made the return trip from Noumea to Touho within a minimum time interval of 354 and 567 days. Three manta rays were sighted at both Touho and Ouvea, with the two areas being located approximatively 115 km apart and with a 2000-meter-deep channel in between. Two of them connected from Ouvea to Touho and one from Touho to Ouvea. Six manta rays were sighted at the Isle of Pines and either Touho ($N = 3$, approx. 300 km shortest distance through water) or Noumea (*N* = 3, approx. 100km). Connections between the Isle of Pines were observed in both directions with time intervals ranging from 89 to 446 days between sightings at the two areas. Only two individuals were recorded moving from Ouvea to Noumea (approx. 260 km). Only two individuals were sighted at 3 areas. One connected from Lifou to Ouvea to Noumea and the other moved from Poindimie to Touho to Noumea.

Re-sighting rate and residency

The overall site fidelity of all manta rays of New Caledonia was high, with 52.2 % of individuals re-sighted at least once (*N* = 391) (Table 1). The time interval between the first and last sighting event for a given individual (ie, re-sighting period as per Marshall 2008) is strongly influenced by sampling effort, which in our study is inconsistent through time and among areas (Table S1A). In New Caledonia, the average resighting period is 1,467 ± 1,507 days (approx. 4.0 ± 4.1 years). The longest re-sighting period was recorded for an individual sampled in Noumea with 9997 days (approx. 27.4 years) between the first observation in 1993 and the last in 2020. Among the three main study areas, the site fidelity was the highest in Touho (*N* = 72) with 75.4 % of individuals resighted at least once in 150 days of sampling and the lowest in Ouvea (*N* = 116) with 42.9 % of individuals resighted in 75 days (Table 1). In Noumea (*N* = 153) 59.7 % of individuals were resighted at least once in 370 days. On average each individual was resighted 9.1 ± 11.5 times, with a mean resighting period of 1947 ± 1776 days (approx. 5.3 ± 4.9 years). The individual with the most re-sightings was recorded 64 times, with a resighting period of 4953 days (approx. 13.6 years). In Touho (*N* = 72), 53 manta rays were observed more than once. In average, individuals from this site recorded 10.5 ± 9 re-sight events with a resighting period of 1,196 ± 764 days (approx. 3.3 ± 2.1 years). The most re-sighted individual recorded 35 encounters within a period of 4596 days (approx. 12.6 years). In Ouvea (*N* = 116), 45 manta rays were observed more than once. The number of re-sighting events was 3.3 ± 2.5 times on average and the mean re-sighting period of 1,021 ± 1,362 days (approx. 2.8 ± 3.7 years). The maximum number of sightings for an individual is 17 times within 202 days. Finally, of the eight remaining areas, Mare (*N* = 20) and Isle of Pines (*N* = 14) counted eight and seven individuals that were sighted more than once, respectively. The average of re-sighting events per individual was 3.1 ± 1.4 and 3.8 ± 2 times with mean re-sighting period of 366 ± 63 days and 1345 ± 1429 days (approx. 3.7 ± 3.9 years), respectively. The most re-sighted manta rays had eight and six sighting events within 428 and 1153 days (approx. 1.2 and 3.2 years), respectively.

The observed mean Lagged Identification Rate (LIR) shows the probability of re-sighting an individual after a variable lag time. Using the sightings data from each area, LIR reveal long-term residency as all trends indicate a levelling off over long-time lags (Fig. 4).



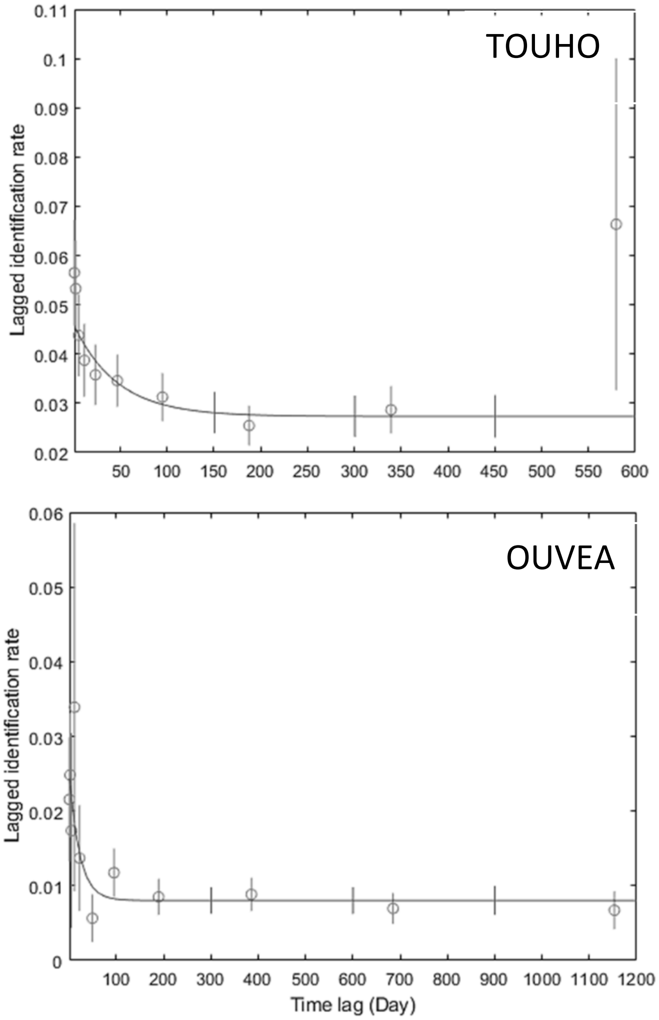


Figure 4. Lagged Identification Rates (LIR ± SE) of reef manta rays (*M. alfredi*) from each of the main study areas: Noumea (*N* = 104; 2017 – 2020), Touho (*N* = 61; 2014-2015) and Ouvea (*N* = 96; 2017-2020. Best-fitting models for each area are denoted in Table S5.

Lagged Identification Rate (LIR), calculated within each study area, showed a net decrease at time lags from 1 to approximatively 200 days at Touho and to approximatively 100 days at Noumea and Ouvea. This suggests that the manta rays stayed seasonally in the area. The LIR then remains stable at increasing time lags indicating the return of these individuals to the area in the following months. Models fitted to the LIR substantially supported the data based on the ΔQAIC (< 2) for Noumea and Ouvea (due to over-dispersion in the data) and the ΔAIC (< 2) for Touho (no over-dispersion in the data) (Table 2). The model H consisting of the following parameters: emigration, re-immigration and mortality (includes permanent emigration) was the best fit for Noumea and Touho. On average, a daily abundance of 20.83 (± SE = 3.72; 95 % CI = 14.93 – 29.94) and 22.40 (± SE = 4.08; 95 % CI = 12.16 – 28.01) manta rays may be found within the area of Noumea (*N* = 104) and Touho (*N* = 61), respectively. These individuals tend to occupy these areas for periods of 31.47 ± 15.52 and 139.57 ± 53.14 days and be away for periods of 23.68 ± 8.34 and 175.57 ± 686.69 days, respectively. The mortality rates were negligible for both areas since values were close to zero (< 0.001). Models E and F best fitted the data from Ouvea (*N* = 96). These models revealed a daily abundance of 39.79 (± SE = 13.94; 95 % CI = 18.89 – 73.84) manta rays. Residence times for individuals in Ouvea could not be estimated with precision as the sampling size was too small, and mortality was also negligible (< 0.001). Other models also explained the data to a lesser degree (ΔQAIC or ΔAIC between 4 – 7) and indicated similar results (Table S5).

Table 2. Maximum-likelihood values for parameters corresponding to each model fitting photographic sighting data of reef manta rays of New Caledonia for the three main study areas: Touho (2014 – 2015, Noumea (2017 – 2020) and Ouvea (2017 – 2020). Numbers between brackets indicate 95 % confidence intervals.

Study areas	Fitted models	Community size	Mean time in	Mean time out	Mortality	Emigration rate	Proportion of population in
Noumea (*N* = 104)	H (ΔQAIC = 0)	20.83 (14.94 - 29.94)	31.47 (9.03 - 72.62)	23.68 (10.24 - 43.70)	.00		
Touho (*N* = 61)	F (ΔAIC < 2)	21.7057 (13.73 - 29.21)	114.24 (9.34 - 318.54)	78.27 (10.05 - 275.10)			
	H (ΔAIC = 0)	22.4007 (12.16 - 28.01)	139.57 (7.04 - 171.04)	175.57 (5.74 - 959.69)	.00	.00	
Ouvea (*N* = 96)	C (ΔQAIC < 2)	77.90 (56.65 - 115.19)				.00	0.5
	D (ΔQAIC < 2)	77.9169 (56.71 - 115.26)	1168.42 (522.51 - 4621.88)				
	E (ΔQAIC = 0)					.00	
	F (ΔQAIC = 0)	39.79 (18.89 - 73.84)	32.54 (1.68 - 927.16)	69.48 (8.013 – 2.87 x 10^{24})			
	H (ΔQAIC < 2)	39.42 (13.09 - 63.77)	28.2164 (0.36 - 157.13)	42.50 (0.59 - 452.39)	.00		

DISCUSSION

This study offers the first insight into the population characteristics, habitat use and movement and occurrence patterns of reef manta rays in New Caledonia through the collection of photo-identifications provided in major part via citizen science. We report the presence of reef manta rays in all parts of the archipelago with the highest proportion of melanistic individuals documented worldwide to date. We also describe strong and long-term site fidelity among studied areas and limited connectivity between them.

Sampling effort and sighting distribution

Reef manta rays were sampled at numerous locations ($N = 10$) scattered around New Caledonia territory. While this provides an indication that manta rays are largely distributed in New Caledonian coastal waters, this only describes the data generated through photographs, and thus, does not account for all individuals located at areas that remained unsampled. New Caledonia consists in an Exclusive Economic Zone of 1 422 543 km^2 encompassing several islands and reefs that are potential habitats for reef manta rays. A campaign of aerial surveys (Laran et al. 2016) of marine megafauna (including manta rays) over New Caledonia waters, lead in 2014, confirmed the presence of manta rays at isolated reefs, such as Petrie Reef and Astrolabe Reefs, at other islands, such as Belep Islands, and other locations along the Main Island (Laran et al. 2016). Increasing and geographically extending our sampling effort by reaching more observers at these locations will help precise the distribution of the population.

Population characteristics

Sex ratio

The sex ratio (M : F) of the population of reef manta ray was equitable (1.0 : 1.15) which corresponds to previous findings in the Maldives (Stevens 2016), Hawaii (Deakos et al. 2011) and Japan (Kashiwagi 2014). Other studies reported female-biased distributions for populations in Mozambique (1.0 : 3.55, Marshall et al. 2009), east Australia (1.0 : 1.3) Couturier et al. 2014) and only in Nusa Penida, Indonesia, was a male-biased ratio found (1.4 : 1.0, Germanov et al. 2019). For manta rays, males provide less parental investment than female by only providing sperm while female investment for their offspring is much heavier (Stevens 2016). Following Trivers (1972), an operational sex ratio should be biased toward the gender with the lowest parental investment, hence, in this case a distribution biased toward males. Yet, sex distribution in reef manta rays might also depend on the use of the site. Areas adjacent to potential nursing ground might be frequented by more mature females than male (Marshall and Bennett 2010b) while males (smaller in size) may favor shallow coastal areas offering more protection from predator (McCauley et al., 2014; Stevens, 2016; Germanov et al. 2019). In New Caledonia, a female-biased ratio was only found in Noumea (1.0 : 1.47). There is no evidence for any nearby nursery area, thus further data collection recording the size, using standardized measurement techniques (Deakos 2010), and maturity stages would help provide a better understanding of the population structure. Another explanation for female-biased ratio could result from less bold attitude and smaller size of males that may diminish the likelihood of identification (Stevens 2016). Noumea is by far the most visited aggregation site by diving operators, which could be an important restricting factor for unadventurous male to frequent the area. In this scenario, males might occur in the deeper and outer part of the reef were they are less likely to be detected.

Melanism

The population of *M. alfredi* of New Caledonia has the highest proportion of melanistic individual reported to date (43.0 %) with locally 50.1 % in Ouvea. Melanistic individuals appear to be in minority with large differences in frequencies among populations around the world (Venables et al. 2019). The second largest proportion for melanistic individuals was recorded in Raja Ampat with 40.7 % while other studied locations recorded less than ten percent including Hawaii and the Maldives with no record of melanistic individuals (Venables et al. 2019). Within the southern Pacific region, the present work is, to date, the only published estimation, the population of Eastern Australia approximate 10 % of melanistic manta rays (Armstrong, pers. comm.). The differences in proportion of melanistic manta rays among populations seem to be due to neutral genetic processes alone since no evidence was found to support selection induced by predation (Venables et al. 2019). To date, no molecular studies investigated the genetic basis of melanism in manta rays.

Injuries and attempted predation rate

Most of the injuries recorded in the population were caused by natural attempted predation (67.4 %) and only few marks were of anthropogenic origin (9.8 %). This study shows that the reef manta rays in New Caledonia do not seem to be highly impacted by anthropogenic stressors as recorded in other parts of the world where manta rays are not directly targeted by fisheries. For example, in French Polynesia, Carpentier et al. (2019) reported 85 % and 53 % of injuries caused by fishing lines or boat propellers at Bora Bora and Maupiti, respectively. In western Australia 13.1 % of the population had injuries caused by vessel strikes. Anthropogenic stressors include boat/propeller strikes, cuts due to fishing hooks or entanglement in fishing lines/nets and anchor lines (Deakos et al. 2011; Carpentier et al. 2019). These sub-lethal wounds can have a severe impact on the population growth and distribution (Heithaus 2001, Deakos et al. 2011). Reproductive behaviour can be altered as the injured animal would spend more time and energy recovering, inducing a delayed sexual maturity (Harris 1989), or even causing early abortion in elasmobranch species (Adams et al. 2018). Manta rays have been recorded to spend more time at cleaning stations when injured to enhance the healing process, preventing them from foraging or mating (Marshall et al. 2011). In New Caledonia, inshore fishing remains relatively low (0.26 t/km^2/year) compared to other countries in the world, but the fast-growing population and the socio-economic rapid changes may result in a large increase in fishing pressure (Guillemot et al. 2009). In addition, the current pressure is not homogeneous since certain parts, including Noumea, are highly exploited and close to overfishing (Guillemot et al. 2009). In this context, further monitoring could be required to assess areas where potential high fishing pressure overlaps with the regular presence of manta rays.

The proportion of individuals bearing bite-related injuries (29.7 %) was close to those reported in Hawaii (24 %, Deakos et al. 2011) or east Australia (23 %, Couturier et al. 2014). The highest attempted predation rate was documented in Mozambique, representing 75 % of the population (Marshall and Bennett 2010a) and the lowest was in French Polynesia (only few individuals, Carpentier et al. 2019). Locally, we recorded a higher rate in Ouvea (42.9 %) which may suggest a higher abundance of predators at this location compared to all other areas in New Caledonia. The proportion of manta rays that die from natural predation remains unknown although such bite-induced wounds have the same impact on the individual (and the population) as with any other injuries (as aforementioned).

Individual count and estimated abundance

The overall individual count from data collected in New Caledonia between 2016 and 2020 (in 654 days) consisted of 391 reef manta rays. Based on these results, model projections estimate a population size at 1,347 individuals, 95 % CI [1,225, 1,413]. This numbers is an underestimation of the actual number of individuals as cumulated counts did not reach an asymptote and many areas have not been sampled. Locally, estimations of abundance were slightly over twice the current count for Touho and Ouvea. In comparison, the model estimation was three times higher in Noumea, although sampling effort at this area was two and five times higher than it was at Touho and Ouvea, respectively. These results combined with evidence of strong site fidelity (see relevant paragraph), suggest that the population could be structured into multiple partially independent groups. This prevents us from investigating manta ray population size of New Caledonia as a whole, sampling all aggregation sites in New Caledonia would require a sampling effort beyond our logistic capacities. Global effort to estimate individual counts vary greatly among location (Table 3) from the largest documented population in the Maldives with 4,411 individuals counted in 54,605 sightings over 12 years (Stevens, 2016) to a minimum of 305 reef manta rays in 11,111 sightings in Southern Japan (Kashiwagi 2014). In comparison to these previous findings, our results seem to indicate that New Caledonia is home for a rather small population, but this estimate could be in great part reviewed by increasing the sample size in this archipelago. Further efforts to estimate abundance and assess demographic dynamics should rather be concentrated at each known aggregation site using more robust modelling approaches based on regular and more intensive sampling effort (Deakos et al. 2011, Marshall et al. 2011, Couturier et al. 2014) or genetic analysis (Venables et al. 2021).

Table 3. Non-exhaustive list of reef manta ray (*Mobula alfredi*) population counts.

Location	Individual count	Sighting count	Years of monitoring	Reference
Maldives	4411	54605	12	Stevens 2016
Southern Mozambique	1176	-	-	Venables et al. 2019
Seychelles	252	-	-	Peel et al. 2020
Western Australia	1121	5146	17	Armstrong et al. 2020
Nusa Penida, Bali	624	5913		Germanov et al. 2019
Komodo National Park, Indonesia	1176	-	-	Venables et al. 2019
Bird’s Head Seascape, Indonesia	1375	4052	15	Setyawan et al. 2020
Southern Japan	305	11111	-	Kashiwagi 2014
French Polynesia	317	1347	16	Carpentier et al. 2019
Hawaii	309	1494	-	Deakos et al. 2011
New Caledonia	391	1741	4	This paper

Connectivity

Reef manta rays of New Caledonia seem to demonstrate site fidelity and little connectivity is observed between the studied areas. We reported only 5.4 % of individuals at more than one location over 6 years, reflecting limited movements between known aggregation sites. These records were also insufficient to detect any movement patterns. Individual movements may depend on local food availability in the absence of a significant anthropogenic influence (Dewar et al. 2008, Anderson et al. 2011; Armstrong et al. 2016; Jaine et al. 2014, Couturier et al. 2018). In fact, occurrence and movements of reef manta rays has been linked with zooplankton productivity. The archipelago of New Caledonia undergoes slight variations in environmental conditions with temperature varying from 22 to 24 °C in peak winter months and from 26 to 28°C in peak summer months (IFREMER, 2019) with little seasonal variation in chlorophyll *a* concentration (used a proxy for zooplankton abundance, Burgess 2017) (Dupouy 1990). In addition, all studied sites are in proximity of deep waters (> 2000 meter-deep), potential source of demersal zooplankton that may constitute an important part of the diet of the reef manta ray (Couturier et al. 2013). These parameters coupled with our findings suggest that reef manta rays seem to have sufficient food resource year-round at each of their respective aggregation sites. In other part of the world, seasonal presence and movements of manta rays was explained by monsoonal shift in Indonesia in the Komodo Marine Park (Dewar et al. 2008) and Raja Ampat (Setyawan et al. 2018), in Western Australia (Sleeman et al. 2007) and in the Maldives (Anderson 2011). Jaine et al. (2012) and Couturier et al. (2018) attributed the increase in abundance in winter to optimal oceanographic conditions for zooplankton productivity at Lady Elliot Island, East coast of Australia.

While only few movements were recorded, these connections (*N* = 30) link all parts of New Caledonia, between sites around the Main Island and Ouvea Islands, indicating that movements along continuous coastline (up to 325 km) and between island chain (up to 260 km) exist. Large scale movements have been documented in many locations around the world (Indonesia, Dewar et al. 2008; East Australia, Couturier et al. 2011, 2014; Maldives, Kitchen-Wheeler et al. 2012) with largest recorded distance along a continuous coastline being 1150 km (East Australia, Armstrong et al. 2019) and 450 km across archipelagos (Germanov and Marshall, 2014). This study also brings further evidence that deep-water channels do not seem to constitute a barrier as suggested by Deakos et al. 2011. Different fidelity rates between studied areas also indicate potential nuances in this supposition. Higher fidelity rates at a given area may reflect more optimal condition year-round than at other locations. In addition, further sampling effort to neighbouring areas may highlight additional connections, and thus, extend the range of occupancy around each of the known aggregation sites. In this sense, we found that almost a third of the manta rays that were observed at more than one area were sighted at the Isle of Pines where a total of 27 sightings were recorded in only 15 days of sampling. These connections were in majority with Noumea (approx. 100 km away), suggesting that more movements potentially exist between these adjacent locations. Surveys at aggregation sites along the west coast of the Main Island may show more connectivity with Noumea or same potential connections between Touho and other areas of the east coast or Ouvea with other Loyalty Islands.

Our analysis did not find a sex bias in the individuals that recorded these movements. This contradicts previous research that documented sex-based difference in habitat use (movements and site fidelity) induced by reproductive behaviour in populations of reef manta rays (Deakos et al. 2011; Stevens, 2016; Germanov et al. 2019). This observation is also true for many other marine species including other elasmobranchs such as the white shark (*Carcharodon carcharias,* Anderson et al. 2011), scalloped hammerhead shark (*Sphyrna lewini,* Daly-Engel et al. 2012) and blacktip reef shark (*Carcharhinus melanopterus,* Mourier and Planes 2013), but also sea turtles (Lee et al. 2007) and cetaceans (Engelhaupt et al. 2009). In fact, females tend to reside longer in an area with sufficient food resources while males tend to move between areas in search for mating opportunities (Stevens, 2016). In New Caledonia, genetic analysis showed limited gene flow and genetic differentiation between these areas (Lassauce et al., in review). This suggests little reproductive interactions between individuals from each area, indicating that the recorded movements might not be associated with reproductive behaviour but rather be related to searches for foraging ground. It is also possible that our analysis does not reflect the actual sex distribution of manta rays that were sighted at more than one area since based on only few observations (*N* = 21). Further monitoring efforts would be needed to better comprehend this behavioural pattern.

Site fidelity and residency

Site fidelity as defined by Chapman et al. (2015) or site affinity as defined by Couturier et al. (2014) is characterised by the return of an individual to a previously occupied area. This behaviour is common and has been documented for elasmobranchs (Bansemer and Bennett 2009, 2011; Hearn et al. 2010; Bessudo et al. 2011; Chapman et al. 2015). The habitat use and home range vary depending on the mobility capacity of a species (Chapman et al. 2015).

The present study shows high levels of site fidelity with rates ranging from 43 % to 75 % of the manta rays sighted more than once within an average re-sighting period of four years. The longest re-sighting period in this study reached over 27 years. This reveals a high tendency for reef manta rays to remain or return to a specific site. This observation was also supported by the lagged identification rate analysis which indicated that some individuals occupied an area following emigration and re-immigration patterns with the tendency to remain at or return to the same area (Whitehead 2009). Results of genetic analysis on this population also converge with these conclusions (Lassauce et al., in review). In fact, genetic differentiation was detected between the studied areas suggesting limited migration and assortative mating (Lassauce et al., in review). Previous research indicated that reef manta rays were able of large-scale movements across different types of habitats: continuous (along coastlines, e.g. Armstrong et al. 2019) or fragmented (between island chains, e.g. Germanov and Marshall 2014). However, these movements do not reflect a homogeneous use of the whole extend of the potential home range. Instead, *M. alfredi* tends to concentrate their movements within preferential areas (e.g., Hawaii, Deakos et al. 2011; Mozambique, Marshall 2008; Red Sea, Braun et al. 2015; Indonesia, Germmanov et al. 2019; Setyawan et al. 2020; French Polynesia, Carpentier et al. 2019). In comparison to our findings, the highest re-sighting rate was reported in Maupiti, French Polynesia, where 90 % of the individuals were sighted more than once (*N* = 51, Carpentier et al. 2019). In Nusa Penida, Indonesia, Germanov et al. (2019) recorded a re-sighting rate of 82 % (*N* = 624), in Maui, Hawaii, Deakos et al. (2011) found 73 %, in Bird's Head Seascape, Indonesia, Setyawan et al. (2020) counted 46.7 % and Couturier et al. (2014) documented 63 % at Lady Elliot, East Australia. These results indicates that the population of New Caledonia can be described as a metapopulation. As defined by Akcakaya et al. (2007), the term "metapopulation" indicates a set of geographically discrete sub-populations occupying the same region among which exchanges of individuals are limited. This hypothesis was also suggested by Setyawan et al. (2020) to describe the population of reef manta rays in Rajat Ampat, Indonesia.

Habitat selection is based on highest quality and suitability, thus, factors as the cost of changing territories and the heterogeneity in territory quality would result in a greater site fidelity (Switzer et al. 1993). Reproductive success is often the main driver to qualify high suitability of the habitat. As aforementioned (see previous paragraph), the life history of reef manta rays highly depends on their foraging success resulting in the species spending most of their time foraging or in search for foraging grounds (Dewar et al. 2008; Anderson et al. 2011; Jaine et al. 2012; Couturier et al. 2011, 2012, 2018, Armstrong 2016). Although our main studied areas are cleaning stations, high rates of site fidelity could be explained by sufficient year-long foraging opportunities at nearby sites (Deakos et al. 2011). In fact, Barr and Abelson (2019) demonstrated that *M. alfredi* chose foraging over cleaning when environmental conditions yield sufficient plankton concentrations, also implying a proximity between these cleaning and feeding sites. During our field operations, feeding events were observed near the cleaning station but were neither regular nor predictable, suggesting the potential existence of more prolific grounds. Given the geomorphology of the region, reef manta ray might also use deep offshore waters where the presence of plankton is possible. This is also favourited by the existence of upwelling events occurring sporadically during the summer in the south-west off the archipelago that bring nutrients and contribute to biological activity (Ganachaud et al. 2010). Recent records using satellite telemetry showed frequent and deep diving behaviour of reef manta rays in New Caledonia (Lassauce et al. 2020) suggesting foraging activity on demersal zooplankton (Couturier et al. 2013). More research using acoustic or satellite telemetry to identify important feeding grounds and environmental factors that influence visitation patterns would help understand the spatial ecology of the species and characterise the actual range pattern around each studied area. These information are essential to assess critical habitat and address potential localised threats, and thus, inform by conservation projects.

SUPPLEMENTARY MATERIAL

Table S1. Number of **A**: sampling effort units (days), **B**: sightings of reef manta rays (*Mobula alfredi*) per site and per year in New Caledonia recorded using photo-identification. Main sites represent locations where surveys were performed by the authors. Data at secondary sites were only collected by citizen scientists. Numbers in parentheses refer to the number of sightings recorded by the authors. Boxed sections indicate chosen periods to perform residency analysis. The dashed line indicates the beginning of data collection by the authors.

A	**1993**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**Total**
Main sites:																				
Noumea	1	1	0	0	1	5	2	7	8	14	28	14	23	22	38	41 (4)	77 (1)	42 (7)	46	370 (12)
Touho	0	0	0	0	0	0	0	0	0	0	0	0	33	95	3	3	6 (6)	7 (6)	3 (2)	150 (14)
Ouvea	0	0	0	2	0	1	0	0	0	1	0	1	1	4	1	15 (7)	16 (4)	17 (9)	16 (3)	75 (23)
Total main sites	**1**	**1**	**0**	**2**	**1**	**6**	**2**	**7**	**8**	**15**	**28**	**15**	**57**	**121**	**42**	**59 (11)**	**99 (11)**	**66 (22)**	**65 (5)**	**595 (49)**
Secondary sites:																				
Poindimie	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Boulouparis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2
Bourail	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
IDP	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	0	3	15
Pouembout	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	2
Lifou	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	2	5
Mare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	11	15	1	28
Ouano	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	5
Total secondary sites	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**7**	**17**	**22**	**7**	**59**
Total of all sites	**1**	**2**	**0**	**2**	**1**	**6**	**3**	**7**	**9**	**16**	**28**	**15**	**57**	**122**	**43**	**66 (11)**	**116 (11)**	**88 (22)**	**72 (5)**	**654 (49)**

B	Unk. date	1993	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total
Main sites:																					
Noumea	33	1	2	0	0	2	6	4	12	13	36	63	27	37	46	67	97 (16)	199 (20)	100 (32)	105	850 (68)
Touho	3	0	0	0	0	0	0	0	0	0	0	0	0	114	365	6	7	35 (35)	49 (47)	12 (10)	591 (92)
Ouvea	17	0	0	0	6	0	2	0	0	0	3	0	1	1	9	2	56 (34)	44 (25)	35 (25)	28 (7)	204 (91)
Total main sites	**53**	**1**	**2**	**0**	**6**	**2**	**8**	**4**	**12**	**13**	**39**	**63**	**28**	**152**	**420**	**75**	**160 (50)**	**278 (80)**	**184 (104)**	**145 (17)**	**1645 (251)**
Secondary sites:																					
Poindimie	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Boulouparis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	6
Bourail	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2
IDP	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	11	9	0	4	27
Pouembout	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	1	0	8
Lifou	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	1	2	7
Mare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	13	22	1	37
Ouano	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	5
Unk, site	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Total secondary sites	**3**	**0**	**3**	**0**	**0**	**0**	**0**	**3**	**0**	**7**	**1**	**0**	**0**	**0**	**1**	**1**	**14**	**22**	**29**	**12**	**96**
Total of all sites	**56**	**1**	**5**	**0**	**6**	**2**	**8**	**7**	**12**	**20**	**40**	**63**	**28**	**152**	**421**	**76**	**174 (50)**	**300 (80)**	**213 (104)**	**157 (17)**	**1741 (251)**

Table S2. Reef manta ray (*Mobula alfredi*) survey effort by the authors (in hours) at the three main study sites in New Caledonia.

Sites	2017	2018	2019	2020	Total per site
Noumea	14	20	28	0	62
Touho	0	27	18	6	51
Ouvea	16	15	82	8	121
Total per year	30	62	128	14	234

Table S3. Overview of the asymptotic models and evaluation of the goodness-of-fit using Akaike's Information Criterion (AIC) for discovery curves of the number of individuals per number of sighting events of reef manta rays (*Mobula alfredi*) at each site and for the whole population in New Caledonia.

			Noumea	**Touho**	**Ouvea**	**Others**	**NC**
Curve name	**Equation**	**Reference**	*AIC*	*AIC*	*AIC*	*AIC*	*AIC*
Von Bertalanffy	$Y = a(1 - be^{-cX})$	Brown & Rothery 1993	2116.7	519.07	12.208	7.9678	16578
Michaelis-Menten	$Y = \frac{a\,X}{b + X}$	Colwell & Coddington 1994 Tjørve 2003	3012.4	392.19	5.2285*	6.6533*	26492
Logistic function	$Y = \frac{a}{1 + be^{-cX}}$	Verhulst 1938 Tjørve 2003	11669	1492	857.67	240.79	114510
Gompertz	$Y = ae^{be^{cX}}$	Gompertz 1825 Tjørve 2003	6929.3	1035.6	363.18	104.16	65184
Hill	$Y = \frac{d + (a - d)}{1 + \left(\frac{b}{X}\right)^{c}}$	Hill 1910	19.909*	19.232*	8.6164	8.8224	107.18*
Abundance estimation (using best-fit model):			440.72 (392.1 – 461.6)	150.91 (25.64 – 163)	246.26 (245.4 – 247.5)	290.81 (284 – 299.1)	1346.7 (1225 – 1413)

** indicate the smallest value of AIC for each dataset. Numbers in brackets indicates 95% CI. NC = New Caledonia.*

Table S4. Summary of the connectivity between aggregation sites recorded for reef manta rays (*M. alfredi)* of New Caledonia using photographic sightings.

Manta ID	Sex	Location #1	Sightings #1	Location #2	Sighting #2	Location #3	Sighting #3	Location #4	Sighting #4	Location #5	Sighting #5
CD-MA-0195	♀	Isle of Pines	Dec-03	Nouméa	between Mar-17 and Dec-17 (*N* = 3)						
CD-MA-0203	♀	Isle of Pines	Jun-17	Nouméa	Apr-18						
CD-MA-0217	♂	Isle of Pines	Aug-17	Touho	between mar-19 and May-19 (*N* = 2)						
CD-MA-0027	♂	Lifou	Jan-08	Touho	between Sep-14 and Aug-20 (*N* = 33)						
CD-MA-0346	♀	Lifou	Dec-19	Ouvea	Aug-20	Nouméa	Jan-15				
CD-MA-0010	♂	Nouméa	Aug-06 and 07	Pouembout	Mar-10	Nouméa	between Feb-16 and Dec-20 (*N* = 32)				
CD-MA-0036	♂	Nouméa	between Apr-09 and Mar-15 (*N* = 5)	Touho	between Sep-15 and Oct-15 (*N* = 7)						
CD-MA-0053	♀	Nouméa	Apr-11	Touho	Sep-14 (*N* = 2)	Nouméa	between Jun-18 and May-20 (*N* = 11)				
CD-MA-0069	♀	Nouméa	between Apr-13 and Nov-14 (*N* = 2)	Touho	Jun-15 (*N* = 2)	Nouméa	between Mar-18 and Jan-20 (*N* = 3)				
CD-MA-0104	♂	Nouméa	between Nov-11 and Mar-17 (*N* = 4)	Bourail	Nov-17	Nouméa	Mar-18	Isle of Pines	between jun-18 and Jul-18 (*N* = 2)	Nouméa	May-19
CD-MA-0147	♀	Nouméa	Mar-16	Isle of Pines	Jun-17						
CD-MA-0148	♀	Nouméa	between Mar-16 and Feb-20 (*N* = 5)	Lifou	Jul-20						
CD-MA-0128	♀	Nouméa	Apr-12	Ouvea	2016	Touho	Sep-18				
CD-MA-0357	♂	Ouano	Mar-19	Boulouparis	Jul-20						
CD-MA-0103	♀	Ouvea	Aug-05	Noumea	between Mar-09 and Mar-20 (*N* = 24)						
CD-MA-0207	♂	Ouvea	between Jul-17 and May-18 (*N* = 3)	Touho	May-19						
CD-MA-0015	♀	Poindimié	Sep-11	Touho	between Jun-14 and Oct-15 (*N* = 18)	Nouméa	2016				
CD-MA-0290	♂	Pouembout	Mar-10	Boulouparis	Jul-20						
CD-MA-0030	♂	Touho	between Jul-14 and May-19 (*N* = 10)	Ouvea	Sep-20						
CD-MA-0070	♂	Touho	between Jan-15 and Feb-20 (*N* = 4)	Isle of Pines	May-20						
CD-MA-0187	♀	Touho	Jul-14	Isle of Pines	May-17						

Table S5. Model parameters and fits for reef manta ray (*M. alfredi*) sightings data from New Caledonia using Akaike Information Criterion (AIC) for Touho (2014 – 2015) and Quasi-Akaike information Criterion (QAIC) for Noumea (2017 – 2020) and Ouvea (2017 – 2020).

		Noumea		**Touho**		**Ouvea**	
Model	**Model explanation**	QAIC	ΔQAIC	AIC	ΔAIC	QAIC	ΔQAIC
A	Closed (1/a1=N)	25167.9307	35.6608	30939.5469	92.125	845.8863	3.9967
B	Closed (a1=N)	25167.9307	35.6608	30939.5469	92.125	845.8863	3.9967
C	Emigration/mortality (a1=emigration rate; 1/a2=N)	25146.9674	14.6975	30893.4986	46.0767	842.5976	0.708
D	Emigration/mortality (a1=N; a2=Mean residence time)	25146.9672	14.6973	30893.4985	46.0766	842.5976	0.708
E	Emigration + reimmigration (a1=emigration rate; a2/(a2+a3)=proportion of population in study area at any time)	25160.4114	28.1415	30913.6898	66.2679	841.8896	0
F	Emigration + reimmigration (a1=N;a2=Mean time in study area; a3=Mean time out of study area)	25137.5908	5.3209	30849.1546	1.7327	841.8896	0
G	Emigration + reimmigration + mortality	25150.9672	18.6973	30884.2822	36.8603	845.0502	3.1606
H	Emigration + reimmigration + mortality (a1=N; a2=Mean time in study area; a3=Mean time out of area; a4=Mortality rate)	25132.2699	0	30847.4219	0	842.6027	0.7131

N = population, res = residence.

CHAPITRE 3

AVANT-PROPOS

Le troisième chapitre étudie l'écologie spatiale et le comportement de la population en utilisant la télémétrie satellitaire. L'objectif est de caractériser les différents profils de mouvements à une échelle spatio-temporelle fine dans un espace tridimensionnel avec le déploiement de balises satellites. Le balisage satellite a été réalisé en plusieurs temps au cours des trois années de thèse en fonction des opportunités d'acquisition des balises. Une première partie de l'échantillonnage, avec de 11 balises sur un total de 23 déployées durant cette thèse, a permis de révéler un comportement de plongée unique pour l'espèce. Ces résultats préliminaires, ayant un intérêt majeur pour l'amélioration des connaissances de l'espèce, ont fait l'objet d'une publication dans la revue *PLoS ONE* présentée en première partie de ce chapitre. La seconde partie du chapitre présente l'ensemble des résultats obtenus par la totalité des balises satellites en adressant de manière plus complète aussi bien les mouvements horizontaux que les comportements de plongées de l'espèce en Nouvelle-Calédonie.

PARTIE 1

DIVING BEHAVIOUR OF REEF MANTA RAY (*MOBULA ALFREDI*) IN NEW CALEDONIA : MORE FREQUENT AND DEEPER NIGHT-TIME DIVING TO 672 METERS [2]

ABSTRACT

The interest in reef manta rays (*Mobula alfredi*) from the scientific community is growing in reaction to the major decline of populations around the world. Studies have highlighted the need to further investigate the spatial ecology of this species to inform conservation and management initiatives. Here we briefly report the results from nine SPLASH10-F-321A pop-off satellite archival tags (PSAT-tags) deployed in New Caledonia that recorded the world's deepest known dives for reef manta rays. All tagged individuals performed dives exceeding 300 m in depth, with a maximum depth recorded of 672 ± 4 m. Diel comparisons revealed that most of the deepest dives occurred during night-time. We hypothesize this deep-diving behaviour is employed to access important food resources at these depths during the night and may also indicate that zooplankton characteristics (e.g., abundance, nutritional quality, size, or dispersion) in the surface waters surrounding New Caledonian coral reefs are insufficient to sustain this megafauna. These results add new information on the habitat use of this species in a region where manta behaviour has not previously been studied, and increase the known depth range of *M. alfredi* by more than 200 m.

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[2] Lassauce H., O. Chateau, M. Erdmann et L. Wantiez (2020). Diving behavior of the reef manta ray (*Mobula alfredi*) in New Caledonia: more frequent and deeper night-time diving to 672 meters. *PLoS ONE* 15: e0228815.

INTRODUCTION

Reef manta rays (*Mobula alfredi*) are declining worldwide, in large part due to fishing pressure for their gill rakers [1,2]. Despite significant advances in our knowledge and understanding of this species in the past decade [3], more detailed information on the biology and the ecology of this species throughout its range is urgently needed [4]. Specifically, data on spatiotemporal dynamics and habitat use are necessary to develop concrete management plans and conservation actions [4] to prevent further declines of reef manta rays, now listed as “vulnerable” on the IUCN Red-List [5]. Satellite telemetry using pop-up satellite archival tags (PSAT tags) is one of the most effective methods to investigate fine scale horizontal and vertical movements and habitat use in manta rays [4,6,7–9], but until now there have been no such studies conducted in New Caledonian waters.

As planktivores, manta rays spend a major part of their time feeding or searching for foraging grounds [3,10–13]. Manta ray aggregations have been observed and monitored in multiple locations in tropical and sub-tropical waters around the world [3,12,14,16,17]. Seasonal or long-term presence of the species on a particular site is often associated with enhanced local productivity and increased food availability. For instance, seasonal migrations were found to be correlated with monsoonal shifts in the Indian Ocean [12,17]. As opportunistic feeders, manta rays are capable of undertaking relatively large-scale movements between productive areas (up to 750 km) [3,4,8,13–17]. Some studies have shown that reef manta rays are also able to explore substantial depths (up to 432 m), presumably to feed on deeper zooplankton and other food resources [8,10,18–20]. These foraging strategies remain unclear and more detailed information on this behaviour and the associated drivers are needed.

In New Caledonia, reef manta rays are not targeted by fishing, but have a highly fragmented distribution due to the specificity of their food resources and preferred habitat [8,13,16,21,22]. Environmental processes and conditions shape the distribution and the abundance of their zooplankton prey [21–24]. Nutrient enrichment is known to be the primary factor of phytoplankton proliferation, causing a subsequent increase in zooplankton abundance. Eutrophication benefits the development of phytoplankton upon which zooplankton feed [25,26].

Processes such as coastal upwellings and river run-off are both important sources of nutrient enrichment of coastal waters [26–28]. These processes, combined with tidal currents and bathymetry can support dense zooplankton concentrations and favourable feeding grounds for filter feeders such as *Mobula alfredi* [20]. Massive feeding aggregation of hundreds of reef manta rays have been observed targeting such dense zooplankton aggregations in the Maldives [29,30] and occasionally in the southern reaches of the Great Barrier Reef [22]. In New Caledonia, manta feeding grounds seem to be scattered, with aggregations never exceeding a dozen individuals (Lassauce, pers. obs.).

This short communication presents the first data collected on the diving behaviour of reef manta rays in New Caledonia. These data reveal an unexpected outstanding feature: the unique depths and high number of deep dives, which considerably extend the known depth range for *Mobula alfredi.*

MATERIAL AND METHODS

Ethic Statement

The tagging was conducted with authorizations from the Southern Province (permit no: 34584) and the Northern Province (permit no: 609011–33) of New Caledonia. In the Loyalty Islands Province, no permit was required by the competent authorities, though permission of the local customary representatives was granted.

Study area

A total of eleven tags were deployed on *M. alfredi* at three different locations in New Caledonia, an archipelagic nation consisting of a main island and three smaller islands off the east coast known as the Loyalty Islands (Fig 1).

One manta ray was tagged in Ouvea Island (20˚43'S, 166˚23'E) on the 4th of December 2015 (tagging depth = 10 m). Seven individuals were tagged in two channels of the barrier reef surrounding the Main Island, Boulari channel (BC, 22˚29'S, 166˚26'E) and Dumbea channel (DC, 22˚21'S, 166˚15'E) between the 31st of January and the 2nd of February 2017 (tagging depth range = 5–15 m). Three tags were deployed in Touho channel (location undisclosed) between the 27th and the 28th of November 2018 (tagging depth range = 5–15 m) (Fig 1). Apart from Dumbea channel where manta rays aggregate to feed near the surface, the other tagging sites are all manta ray cleaning stations [31].

Tagging process

This study used SPLASH10-F-321A PSAT tags (Wildlife Computers Inc., Redmond, Washington, USA) coated with Propspeed™ silicone coating to prevent fouling during the deployment period. These tags are equipped with a Fastloc-GPS receiver, allowing locations to be recorded even when the tag only surfaces for a brief period of time (0.2 second). All tags were programmed to archive light level, depth and sea temperature every 30 seconds and detach from the animal after a maximum of 180 days. Data were summarized every 12 hours and transmitted to the Argos satellite system (www.argos-system.org). Periods of 12 hours were chosen to represent daytime (from 7 am to 7 pm) and night-time (from 7 pm to 7 am). The twilight times varied from 5:04 am to 06:31 am and 6:58 pm to 5:42 pm at the time of the earliest deployment (04/12) and the latest release (16/06), respectively (civil twilight times). The maximum variation of the twilight times within the range of recorded movements is 9 minutes (https://meteogram.fr). Since the tag settings do not allow the precision to be able to discriminate crepuscular periods, we defined the daylight period from 7 am to 7 pm to ensure dusk is always included in the daytime period and dawn is always included in the night-time data.

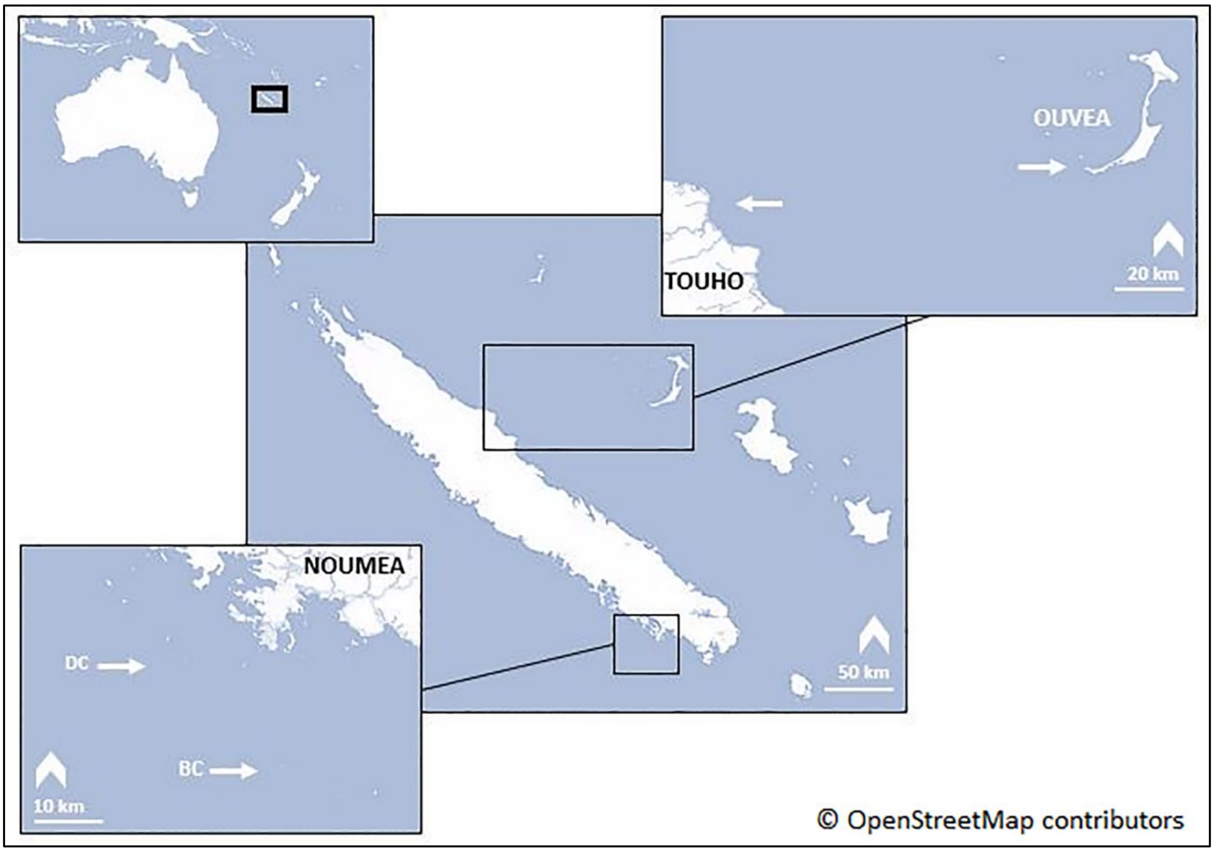

Figure 1. Tagging locations of *Mobula alfredi* in New Caledonia. Arrows indicate tagging locations in Boulari channel (BC) and Dumbea channel (DC) in Noumea (*n* = 7), Touho (*n* = 3) and Ouvea (*n* = 1). Source: *OpenStreetMap* contributors.

Tags were deployed while scuba diving. The tags are tethered by a 30 cm stainless steel cable to a titanium dart-tip that is applied into the dorsal musculature of the animal with a pole spear. Before being tagged, each manta ray was identified using photo-identification (except for tag #167754), its sex and maturity was determined, and its size was estimated (disc width DW to the nearest 10 cm) (Table 1). Maturity was assessed based on the presence of fully developed claspers for male individuals and the observation of either mating scars or pregnancy for female individuals.

Data analysis

Depths are presented as means (± SD) of the maximum depths and as maximum (± maximum accuracy) observed depths per period (day/night) over the total deployment duration. Maximum accuracy varied from 4 to 50 m with an average of 8.8 ± 9.9 m (n = 1099 dives). For diel comparisons for each individual, a Welch's t-test was used as a non-parametric test for samples with unequal variances and a Student t-test was used for samples with equal variances. A Levene's test was used to test the homogeneity assumption. A Kolmogorov-Smirnov test was run to compare the overall distribution of the number of dives per depth range during the night and the day. A Pearson's r test assessed the linear relationship between deployment duration and maximum depths recorded. Finally, a Fisher exact test evaluated the diel difference for each of these depth ranges. Temperature data are minimum temperatures recorded at corresponding depth readings within each 12-hour period over the total deployment duration of all tags.

Table 1. Summary of satellite tag deployment information and characteristics of the nine PSAT-tagged reef manta rays (*Mobula alfredi*) in New Caledonia that successfully transmitted data.

Manta ID	Sex	Estimated disc width (cm)	ARGOS PTT tag ID	Date of tagging	Site of tagging (latitude, longitude)	Deployment duration (days)	Data transmitted (%)
CD-MA-0109	Male, mature	300	#140916	04/12/2015	Ouvea (20°43'S, 166°23'E)	80	79
CD-MA-0004	Female, mature	330	#167755	30/01/2017	Noumea, BC (22°29'S, 166°26'E)	54	86
CD-MA-0166	Female, mature	350	#163079	31/01/2017	Noumea, DC (22°21'S, 166°15'E)	136	65
CD-MA-0167	Female, juvenile	240	#151348	31/01/2017	Noumea, DC (22°21'S, 166°15'E)	49	64
CD-MA-0168	Male, juvenile	260	#151349	31/01/2017	Noumea, DC (22°21'S, 166°15'E)	50	100
CD-MA-0000	Male, mature	330	#167754	31/01/2017	Noumea, BC (22°29'S, 166°26'E)	110	42
CD-MA-0036	Male, mature	300	#167756	01/02/2017	Noumea, BC (22°29'S, 166°26'E)	Failed	Failed
CD-MA-0170	Female, mature	400	#167757	02/02/2017	Noumea, DC (22°21'S, 166°15'E)	174	100
CD-MA-0026	Female, mature	340	#162378	28/11/2018	Touho (undisclosed)	Failed	Failed
CD-MA-0051	Female, mature	330	#162379	28/11/2018	Touho (undisclosed)	3	87
CD-MA-0047	Male, mature	320	#162380	29/11/2018	Touho (undisclosed)	5	85

All tags were SPLASH10-F-321A Fastloc GPS tags (Wildlife Computers Inc., Redmond, Washington, USA). Disc widths were visually estimated to the nearest 10cm. CD-MA-0036 and CD-MA-0026 failed to transmit data.

RESULTS AND DISCUSSION

Of the 11 tags deployed, two (#167756 and #162378) failed to transmit to the Argos system. The deployment duration of the functioning tags (*n* = 9) ranged from 3 to 174 days (73 ± 58 days). On average, 78 ± 19% of the data recorded by the tags was either transmitted by ARGOS satellite or downloaded from two tags recovered after deployment (Table 1). All nine individuals recorded dives deeper than 300 m (*n* = 78), and six of them performed dives deeper than 450 m (*n* = 22), including two exceptionally deep dives by two of the smaller tagged individuals (2.4 m female CD-MA-0167 and 3 m male CD-MA-0109) that reached maximum depths of 624 ± 4 m and 672 ± 4 m, respectively (Fig 2). This last dive extends the reported depth range for *M. alfredi* by more than 200 m, previously recorded as 432 m in the Red Sea [8]. A similar study in Indonesia using the same tags and tagging technique recorded a reef manta ray reaching a maximum depth of 624 ± 4 m in East Kalimantan (Erdmann, unpub.). In this study only 6 of the 30 tagged manta rays recorded dives deeper than 300 m, which indicate fewer deep dives compared to New Caledonia. In the Red Sea, 5 of the 7 tagged individuals dived deeper than 300 m [8] and none of the tracked manta rays in Eastern Australia reached these depths [7]. In New Caledonia, all individuals dived deeper than 300 m, representing 7.1% of all dives ($n = 1099$). The 200 m level was reached in 13% of all dives ($n = 1099$). The mean depth of all the dives was 103.1 ± 104.9 m ($n = 1099$) (Table 2). Maximum depths recorded were not correlated with deployment duration (Pearson's r test, $r = -0.19$, $n = 9$, $p > 0.5$). The dives recorded by the New Caledonian manta rays are thus both deeper in an absolute sense and more frequently exceeding the 200m mark than previously found in other parts of the world [7,8,32].

Difference in mean depths per individual between day and night were only significant for three manta rays. Regarding the diel comparison of the overall distribution of the number of dives per depth range, no significant difference ($p > 0.05$) was observed (Table 2).

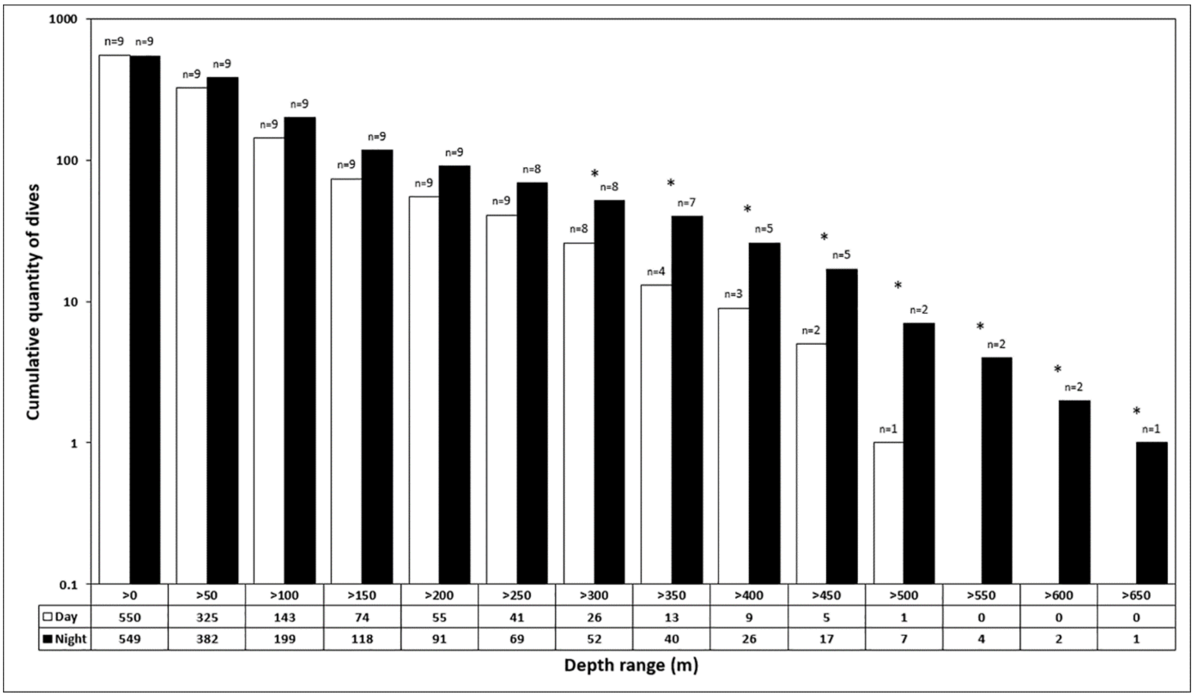

Figure 2. Comparison between day (white) and night (black) of the cumulative quantity of dives for all tagged reef manta rays (*Mobula alfredi*) in New Caledonia at different depth ranges (m). *n* = total number of individuals recorded in each depth range. * indicates a significant difference (Fisher exact test, $P < 0.05$) between number of day and night dives in a given depth range.

Table 2. Dive profiles between the nine PSAT-tagged reef manta rays (*Mobula alfredi*) in New Caledonia.

Manta ID	**Day Depth (m)**		**Night Depth (m)**		**% of dives with a maximum depth >300 m**	**Time at maximum depth during the deepest dive (min)**
	Mean ± SD	Max. ± Max. Accuracy	Mean ± SD	Max. ± Max. Accuracy		
CD-MA-0109	176.9 ± 95.4	512 ± 4	217.9 ± 134	672 ± 4	21.8	2.2
CD-MA-0004	100.2 ± 54.3	344 ±4	128 ± 77.9	472 ± 4	3.8	10.1
CD-MA-0166	71.9 ± 37.8 *	350 ± 50	105.6 ± 75.1 *	496 ± 4	5.8	10.8
CD-MA-0167	77.4 ± 55	464 ± 4	123.5 ± 115.6	624 ± 4	7.1	16.6
CD-MA-0168	95.8 ± 47.8 *	328 ± 4	146.6 ± 96.2 *	384 ± 4	9	6.5
CD-MA-0000	79 ± 57.3	450 ± 50	87.8 ± 54.1	350 ± 50	6.7	13
CD-MA-0170	54.6 ± 32.4 *	304 ± 4	65.9 ± 38.8 *	224 ± 4	0.3	1.4
CD-MA-0051	118 ± 77	272 ± 4	192 ± 122.7	376 ± 4	14.3	26.6
CD-MA-0047	257.6 ± 87.7	360 ± 4	252.8 ± 140.2	480 ± 4	40	14.4

* indicates $p < 0.05$. Means are averages of the maximum depths recorded for each dive.

Among all individuals, the number of deep dives (depth > 300 m) was significantly larger at night (7 pm– 7 am) than during the day (7 am– 7 pm) (Fisher exact test, $p = 0.010$) (Fig 2). This behaviour could be explained by the nocturnal exploitation, at night, of demersal food sources, which has been observed in reef manta rays [4,18,19], oceanic manta rays [33], other mobulid species [34–36], as well as whale sharks [37]. In this study, all manta rays spent a relatively short amount of time at maximum depth during their absolute deepest dive. Bottom time during each manta's absolute deepest dive averaged 11 ± 7 minutes, varying from 26.6 minutes at 376 ± 4 m to 1.4 minutes at 304 ± 4 m (Table 2). There was no significant correlation between maximum depth reached and the time spent at this depth (Pearson's *r* test, $r = - 0.06$, $n = 9$, $p > 0.5$). Analysis of dive profiles can provide valuable information on diving behaviour [38]. Classification of dive profiles has been mainly conducted on air-breathing marine animals such as seabirds [39], sea turtles [40], or seals [41], as well as a few studies focused on predatory fish [42,43]. These analyses revealed two main patterns that have been associated with distinct behaviours. Dives with very short or no bottom time, called "V-Shaped" dives, are possible indicators of travelling and/or prey searching behaviour [38–42]. By comparison, U-shaped or square-shaped dives profiles with distinctively longer bottom times suggest foraging activities [38–42]. Asymmetrical V-shaped dives were described for reef manta rays by Braun et al. [8]. These authors suggested that short bottom times with relatively slow descents and faster ascents reflected an optimized travelling behaviour using gliding [8]. In this study, three manta rays showed this type of profile with very limited time spent at maximum depth during their deepest dive (1.4 min at 304 ± 4 m, 2.2 min at 672 ± 4 m and 6.5 min at 384 ± 4 m) (Table 2). While travelling and/or prey searching could be an explanation for these particular dives, additional data on the velocity during ascent and descent would be needed to test this hypothesis. On the other hand, our results show that six manta rays remained at maximum depths for more than 10 minutes. These dive patterns are more akin to U-shaped profiles, suggesting the exploitation of aggregated prey [38,42]. As fishes, manta rays diving is not limited by the ability to store oxygen, but more probably by the low temperatures at these depths. During dives, temperatures were always colder than 20˚C below 300 m, with a minimum temperature of 7.6˚C recorded at the maximum depth of 672 ± 4 m (Fig 3). Manta rays are poikilothermic species with an optimal thermal range from 20 to 26˚C [3,7,32]. Previous studies have also shown that mobulid rays have the capacity to transmit warmth to the brain using a specific vesicular network in the pectoral fin that can function as a counter-current heat exchanger [44]. Consequently, basking in warm shallow water prior to diving and active swimming during descent and ascent could be used to increase the body temperature. This mechanism would allow manta rays to produce enough heat to reach demersal food resources and feed for a relatively short amount of time despite the cold temperatures of these depths. This behaviour has been observed for other mobulid rays and other fish such as tunas and sharks [33–37,44–47]. In order to fully support this hypothesis, more detailed data on the dive profile of these manta rays are necessary to confirm rapid descent and slower ascent directly followed by an extended period of basking in warm shallow water. If this last hypothesis can be verified, the identification of such comportment for the reef manta rays of New Caledonia highlights the probable presence of important demersal food resources at depth, resulting in significant foraging success that presumably compensates for the energetic costs. The unusual depths reached, and number of deep dives recorded suggest that foraging opportunities could be insufficient in the upper layer of the water column in New Caledonian waters, thereby forcing manta rays to explore deeper food resources. Detailed data on resource availability at varying depths and on the diet of manta rays in this region will help in determining the underlying drivers of their movements.

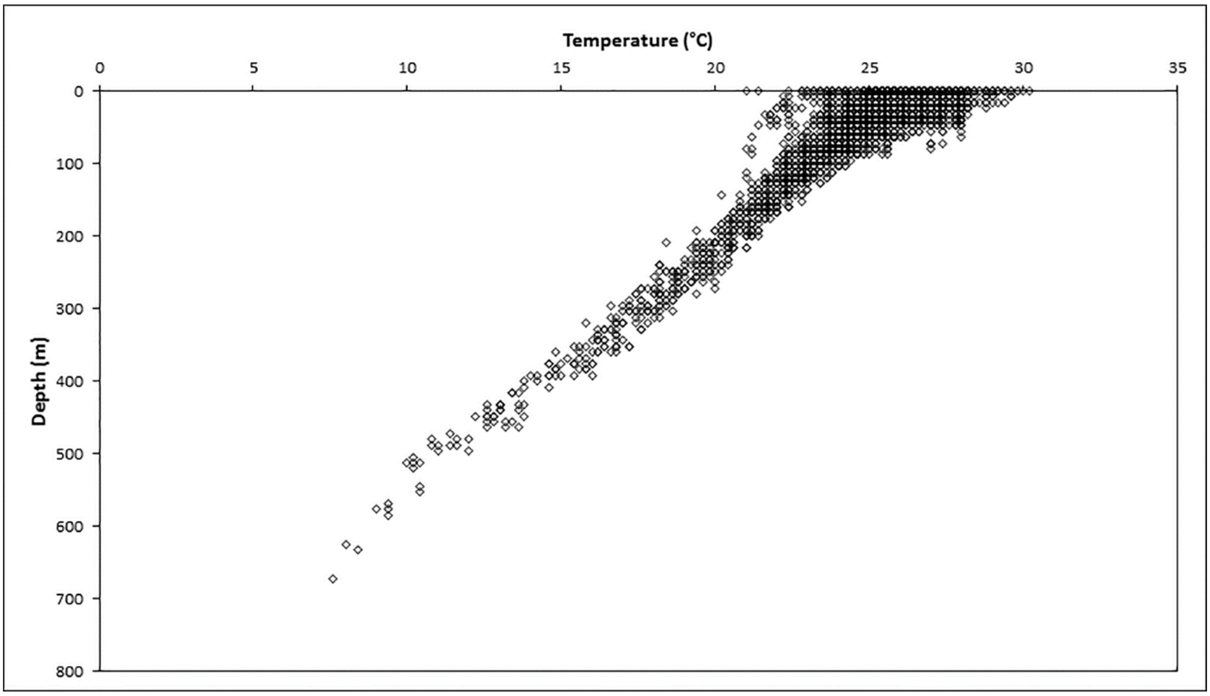

Figure 3. Relation between the minimum temperature (˚C) at corresponding depth (m) measurements (n = 3820) during the deployment of all tags (n = 9) on reef manta rays (*Mobula alfredi*) in New Caledonia.

These preliminary results extend the global knowledge on the depth range and, more generally, the habitat use of *M. alfredi*. In this case, these data appear to support previous findings that prey at mesopelagic depths (from 200 m to 1000 m) [48] are valuable, if not indispensable, food resources for reef manta rays [10,18,19]. A comprehensive knowledge of the distribution and the habitat use of the reef manta rays is necessary to inform conservation and fisheries management measures to ensure the long-term survival of the species [4]. Protective legislation has improved in recent years and numerous marine protected areas (MPAs) have been created throughout the known range of reef manta rays [49]; however, many of these MPAs are coastal in nature and do not extend into the deeper offshore waters used by reef manta rays. As deep-water fisheries are increasingly exploiting the mesopelagic zone [50], our study highlights the importance of incorporating offshore waters and deep-water foraging grounds in manta conservation initiatives.

PARTIE 2

SPATIAL ECOLOGY OF THE POPULATION OF REEF MANTA RAYS (*MOBULA ALFREDI*) IN NEW CALEDONIA USING SATELLITE TELEMETRY [3]

ABSTRACT

The reef manta ray (*Mobula alfredi*) is a highly mobile and plankton-feeding species that is classified vulnerable to extinction on the IUCN Red List for Threatened Species. Knowledge on the spatial ecology and the extent of their dispersal remain incomplete, especially within island-fragmented habitats as found in New Caledonia. Satellite telemetry was used in this study to investigate the horizontal movement ecology and the diving behaviour of reef manta rays in New Caledonia. A total of 21 manta rays were tagged with pop-up satellite archival transmitting tags (21 Fastloc and 2 MiniPAT) that remained deployed for a duration ranging from 3 to 180 days (mean ± SE = 76.7 ± 50.3). Our records allowed us to define home range and horizontal movement patterns. This analysis revealed strong site fidelity and an important affinity for coastal waters. Yet, long-distance migrations were also observed, mainly through coastal and shallow water paths. Deeper depths were recorded in New Caledonia compared to anywhere else sa far, these deep dives being also more frequent than elsewhere. All tagged individuals dived below 300 m with an overall frequency of one dive below 300 m every 4.8 days. The examination of the dive curves suggests that this behaviour is associated with the search and the exploitation of deep food resources despite the fact that

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[3] Lassauce H., O. Chateau, M. Erdmann et L. Wantiez (in prep). Spatial ecology of the population of reef manta rays (*Mobula alfredi*) in New Caledonia using satellite telemetry.

manta rays spend a major part of their time within the top layer of the water column (< 50 m). These results bring evidence on the greater use of shallow coastal waters by reef manta rays and that excursion into deep offshore waters are more episodic and associated with foraging opportunities. Our findings may assist the design of more appropriate management strategies for the species in New Caledonia and other regions worldwide.

KEY WORDS: Tagging ; Home Range ; Habitat Use ; Movement Patterns ; Diving Profile ; Deep Diving.

INTRODUCTION

The spatial ecology of a species is a key area of research for conservation-oriented studies. Understanding the processes that influence a species distribution and its dynamics is particularly relevant for conservation (Fletcher and Fortin 2018). Concretely, this type of information may assist in mapping biodiversity and ecosystem services, identifying effects and potential threats of environmental changes and anthropogenic activities, enabling effective prioritization of areas for biodiversity management. Ultimately, it provides crucial knowledge to develop tools and models used in conservation (Graham et al. 2012; Fletcher and Fortin 2018). To obtain a comprehensive view of these processes, the life history traits and the biology of the species as well as the environmental factors to which the species may respond should also be taken into account. The task is all the more challenging when studying highly mobile species in a constantly changing, three-dimensional and thus seemingly continuous environment like the ocean. Marine species that are capable of large-scale movements have a high dispersal potential especially in continuous environment where physical barriers are not obvious (Palumbi et al. 1994, 2003). Yet, several studies showed high residency and site fidelity patterns in these marine species where movements were influenced by environmental barriers and biological factors such as mating success, access to breeding grounds or access to consistent food resources. For instance, reef fishes show limited connectivity between reefs separated by large sand channels (Chateau and Wantiez 2009; Meyer et al. 2010), killer whales display intrinsic isolation of communities due to different food resources (Foot et al. 2010), bottlenose dolphins demonstrate restricted home range linked with productive habitats (Passadore et al. 2018) and oceanic whitetip sharks record high site fidelity driven by the consistent availability of prey (Madigan et al. 2015). To a greater extend, food resource is also a major factor that influence the movement of large-bodied filter-feeding species (Sims and Quayle 1998; Heyman et al. 2001, Graham et al. 2006). Yet, this resource depends on environmental factors with different variabilities between regions of the world. Consequently, spatial distribution and dynamics of these highly mobile filter-feeding species may be different from one region to the next.

Reef manta rays are filter-feeders found in tropical waters around the word. Populations are observed near coastal reefs and their movements have been documented to be driven by the availability of food resources. For instance, in the Maldives, seasonal peaks in productivity gather hundreds of individuals each year (Anderson et al. 2011). In Indonesia, reef manta rays perform long distance migration triggered by monsoon shifts and associated reduction in productivity (Germanov and Marshall 2014). In Australia, highly productive eddy events are likely to trigger offshore movements of individuals (Jaine et al. 2014) and seasonal variations in temperature appears to initiate latitudinal migration over hundreds of kilometres along the east coast of Australia (Jaine et al. 2012; Couturier et al. 2014). In contrast, when the resource is consistent throughout the year, reef manta rays seem to demonstrate strong residency patterns with only few connections between geographically close populations. In Hawaii, no connection was found over 10 years of photo-identification monitoring between two aggregation sites located only 150 km apart. Similarly, in Indonesia, acoustic telemetry suggested spatial segregation between populations that are only 150 km apart (Setyawan et al. 2018). These evidence suggest that the spatial ecology of reef manta rays can be difficult to predict and that localised investigation might be necessary to obtain comprehensive understanding of their movements. This is a crucial task as the species is heavily exploited in many regions of the world for their gill plates that are used in Asian medicinal trade (O’Malley et al. 2016). In addition to this fishing pressure, reef manta rays exhibit several conservative life history traits that exacerbate this vulnerability including small population size, low fecundity and fragmented distribution as well as a strong affinity for coastal shallow waters that augment their exposition to human activities. In fact, in addition to this pressure from targeted fishing, the species is also affected by other factors of disturbance such as bycatch fisheries (White et al. 2006), uncontrolled mass tourism (O’Malley et al. 2013; Venables et al. 2016), habitat degradation (Ward-Paige et al. 2013; Rohner et al. 2013; Croll et al. 2016; Lawson et al. 2017), boat strikes (McGregor et al. 2019) and fish nets entanglement (Stewart et al. 2018). Consequently, reef manta rays are classified vulnerable to extinction on the IUCN Red List of Threatened Species (Marshall et al. 2018). Providing robust information on the movement patterns and habitat use of reef manta rays would contribute to the design of effective management and conservation measures that are essential to protect the species.

Among methods that enable spatial analysis, satellite telemetry is now a common practice with an increasing number of studies deploying tags to track movements of a wide range of species including terrestrial species, marine birds, sea turtles, marine mammals, and elasmobranchs (Baird et al. 2011, Block et al. 2002, Hart et al. 2009; Mate et al. 2011; Graham et al. 2012; Phillips et al. 2018; Hofman et al. 2019; Haywood et al. 2020; Meyers et al. 2020). This tool allows researchers to investigate broad-scale and fine scale movements, diving behaviour as well as preferred depth and temperature, and sometimes revealing misconceptions or unexpected findings about the ecology of a species (Heupel et al. 2006; Hammerschlag et al. 2011; Crossin et al. 2017). Satellite tracking devices are effective tools to detect unsuspected large migrations in elasmobranchs such as the basking shark (Sims et al. 2005), the six gills shark (Andrews et al. 2010) and the white shark (Bonfil et al. 2005), for example. It also recorded unexpected deep dives (whale sharks, Brunnschweiler et al. 2009; Chilean devil rays, Thorrold et al. 2014, reef manta rays, Lassauce et al. 2020) and behaviour (basking sharks, Skomal et al. 2009).

In New Caledonia, reef manta rays have not been studied until recently (Lassauce et al. 2020; Lassauce et al., in prep; Lassauce et al., in review) and basic information on the population of this emblematic and threatened species are needed. Previous findings using four years of photo-identification monitoring revealed that the population of reef manta rays was distributed in all parts of the archipelago and exhibited high long-term fidelity rates at these sites. Connectivity was also limited but existent between all sites showing high dispersal potential (Lassauce et al., in prep). In addition, genomic analysis found genetic differentiation between aggregation sites which confirms the importance of site fidelity and exacerbated the lack of connectivity between geographically close habitats (Lassauce et al., in review). Another work presented the ability of the species to perform frequent deep dives reaching unprecedented mesopelagic depths (Lassauce et al. 2020).

On this basis, we use satellite tracking to investigate the spatial ecology of *M. alfredi* in the fragmented environment of the New Caledonia archipelago. Our objectives are to assess the fine scale habitat use and potential broad scale migration patterns as well as the use of mesopelagic depths by addressing the following points: quantify the use of offshore versus coastal waters, show the extend and limitation of the movements from aggregation sites with high fidelity rates, define the frequency and magnitude of the connectivity between studied sites, detect potential broad scale movements and the existence of unknown key habitats, describe the diving behaviour and vertical activity.

METHODS

Study sites

Satellite tags were deployed at three known aggregation sites in New Caledonia, South Pacific: Noumea, Ouvea and Touho. The archipelago of New Caledonia consists in a Main Island surrounded by a barrier reef of 1,660 km that enclose shallow waters of a 16,874 km^2 lagoon (Andréfouët et al. 2009). This barrier reef marks the limits of the continental shelf where the bathymetry drops to depths greater than 2000 m. Smaller islands with a relatively narrow continental shelf, named the Loyalty Islands, are located off the east coast of the Main Island separated by a 2000-meter-deep channel (Fig. 1).

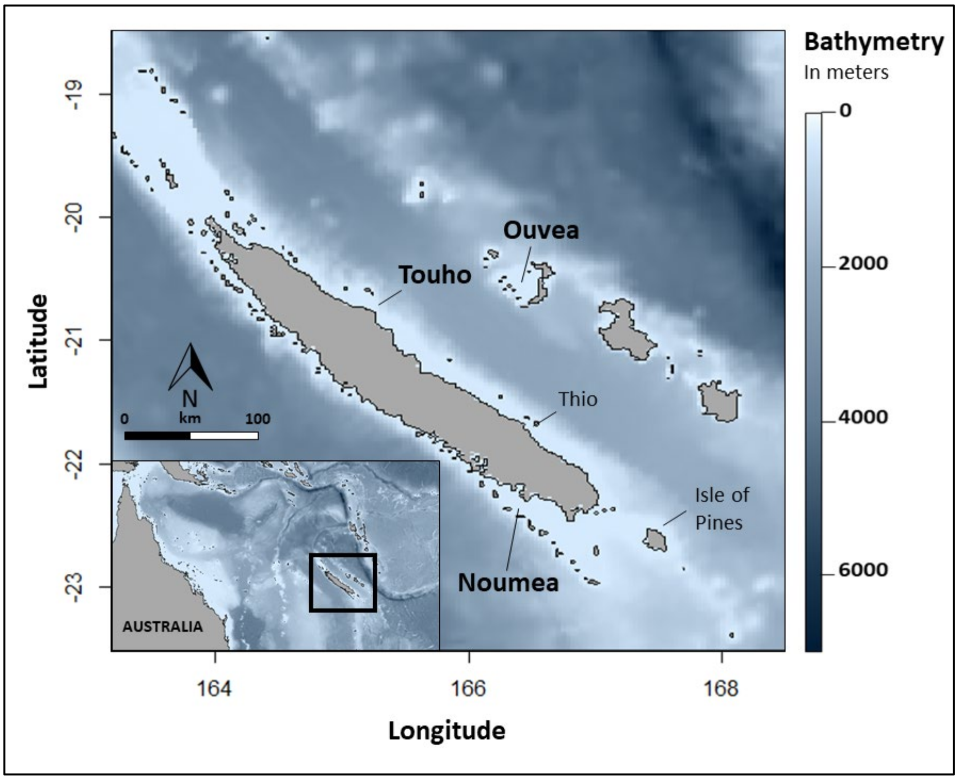


Figure 1. Locations of the deployment of pop-up satellite tags (SPLASH10 and MiniPAT) on reef manta rays (*Mobula alfredi*) in New Caledonia, South Pacific. Study sites (in bold): Noumea (*N* = 6), Ouvea (*N* = 4) and Touho (*N* = 6).

Reef Manta rays were tagged at two locations off the Main Island: Noumea in the southern part of the west coast and Touho in the northern part of the east coast. The other tagging site was off the northern island of the Loyalty Islands, Ouvea. In Noumea, tagging operations took place at two aggregation sites (24 km apart) on the barrier reef: the Boulari channel (22˚29'S, 166˚26'E) and the Dumbea channel (22˚21'S, 166˚15'E). The Boulari channel is a cleaning station on a 15-meter-deep reef flat on the north tip of the channel and the Dumbea channel is a feeding site within the 30 meters of the water column facing the reef crest of the south tip of the channel. In Touho (location undisclosed), the aggregation site is a cleaning station located at the north tip of the Great Channel of Touho on a 15-to-20- meter reef shelf less than 5 km off the coast. In Ouvea, the deployment of the tags occurred at two aggregation sites: the Northern Pleiades (20˚45'S, 166˚44'E) and the Southern Pleiades (20˚43'S, 166˚23’E). Both sites are cleaning station on the reef slope at a depth of 10 to 15 m on the continental shelf off the northern tip and southern tip of the island, respectively (Fig. 1).

Tagging

We deployed 21 SPLASH10-F-321A PSAT tags and two MiniPAT tags (Wildlife Computers Inc., Redmond, Washington, USA) coated with Propspeed™ silicone coating to prevent fouling during the deployment period. Tag deployments were timed to coincide with sightings peaks for all sites between December and February over 4 years from 2015 to 2020. Two additional tags were deployed opportunistically in Touho in November 2018. In Noumea and Touho tags were deployed while scuba diving, in Ouvea tagging was performed while free diving on free-swimming manta rays. The tag is tethered by a 30 cm stainless steel cable to a titanium dart-tip. A modified pole spear was used to apply dart into the dorsal musculature of the manta ray. All tags were programmed to remain attached for a maximum period of 180 days. Alternatively, detachment was programmed in case of the tag being recorded at a constant depth for more than 24 hours (in case of mortality). SPLASH10 tags collected external temperature, light level, and pressure (depth) data every 10 s. Data were transmitted via the ARGOS satellite system (www.argos-system.org) into 12-h periods. Prior to tagging, manta rays were identified using photo-identification, the sex and maturity was also determined, and the size (disc width) was visually estimated to the nearest 10 cm.

Data analysis

Locations were retrieved using the Wildlife Computers location processing systems (Fastloc GPS and GPE3). Location records are assigned with a quality rank based on the number of uplinks received per satellite pass for Argos data and on the number of connected satellites for Fastloc data. Argos data quality numbers rank from 3 to 0 and then A, B and Z, with 3 being the most accurate and Z being an invalid location. Fastloc data accuracy followed a gradient indicated by quality classes corresponding to the number of satellites, starting at 4 to 7 (Wildlife Computer, 2016). All location data were filtered according to the following steps. First, we manually removed duplicates and on-land locations, examined duplicates, and removed the less accurate ones. Second, argos-derived locations with an accuracy class inferior to A (no accuracy estimation) and Fastloc-derived locations inferior to 5 satellites were removed. Finally, we used the *sdafilter* function from the R package *argosfilter* (Freitas et al. 2008) to exclude improbable locations based on speed and distance where trips exceeding a given speed (2 m.s^{-1}) over a given distance (5000 m) were considered improbable.

Filtered datasets were input into the statistical programming language R version 3.6.3 (R Core Team, 2020) where all analysis were performed. Maps and bathymetry data were extracted from the *marmap* (Pante and Simon-Bouchet 2013) package in R.

Statistical analysis

Comparison of means between grouping factors were tested using an analysis of variance (ANOVA) and Tukey's post-hoc test. Welch's F tests were used when variance where unequal. Pairwise comparisons of means were tested with Student t-tests. The assumption of homogeneity was tested using Levene's tests. Chi-squared (χ^2) goodness of fit tests were used to examine the number of individuals between and among movement patterns. Z-tests of proportion were used to compare proportions. Finally, Kolmogorov-Smirnov test were used to compare distributions and Pearson correlation coefficient was used to test linear relationship between variables.

Ethic Statement

The tagging was conducted with authorizations from the Southern Province (permit no: 34584) and the Northern Province (permit no: 609011–33) of New Caledonia. In the Loyalty Islands Province, no permit was required by the competent authorities, though permission of the local customary representatives was granted.

RESULTS

Tagging

A total of 23 tags were deployed, 19 successfully transmitted data and four failed for unknown reasons (Table S1). Data from 16 tags were retrieved through the ARGOS server with a mean decoding rate of 83.9 ± 13.9 % (Mean ± SD), and three tags were physically recovered allowing 100 % of the data to be decoded. After the data filtering process, two tags were excluded from the analysis of horizontal movements and one tag from the analysis of vertical movements due to inaccurate data. The mean retention period is 76.7 ± 50.3 days (range from 3 to 180 days). Only the MiniPAT tag detached at the term of the programmed period (180 days), all other tag popped-up prematurely for unknown reasons. The majority of tagged individuals were females (57.9 %) including a pregnant one and two were juveniles (one of each sex). Finally, all tagged individuals recorded only a few locations per day of deployment with a total of one record every 1.5 days. This number varied between sites from one record every 5.5 days and 0.9 days in Ouvea and Noumea, respectively, while in Touho a location every 2.7 days of deployment was recorded.

Horizontal movements

Horizontal movements varied greatly in length and direction between individuals. Manta rays travelled an average of 4.6 ± 3.1 km d^{-1} (ranging from 0.2 to 12.3 km d^{-1}) with total track lengths varying from 23 to 688 km in 100 and 53 days, respectively. The latitudinal range extended from 7.1 to 224 km with an average of 76.7 ± 74.1 km. These movements remained mostly within a relative proximity of the site of deployment with 63.5 % of the recorded locations observed within 30 km from the deployment areas. The average distance from the tagging site was 41 ± 66 km with a maximum at 311 km. Manta rays remained close to shallow waters with limited offshore movements. Manta rays remained on average at distance of 3.5 ± 4.4 km from shallow waters (under 10-meter-deep) and only one individual went offshore up to 105 km from the nearest reef. During these movements, manta rays occurred at locations with highly varying bathymetry averaging 325 ± 411 m below the surface and a maximum recorded at 2720 m deep.

Movement patterns are defined using a threshold distance from the site of origin to determinate a home range area. This value corresponds to the distance beyond which the distribution of record frequencies shows a significant break. When combining all location data from all sites, the distribution of distance from the tagging site reveals a significant drop and levelling beyond the 50 km range (Fig. 2). This 50 km threshold from the site of origin will be used to identify movement patterns in and out of this range.

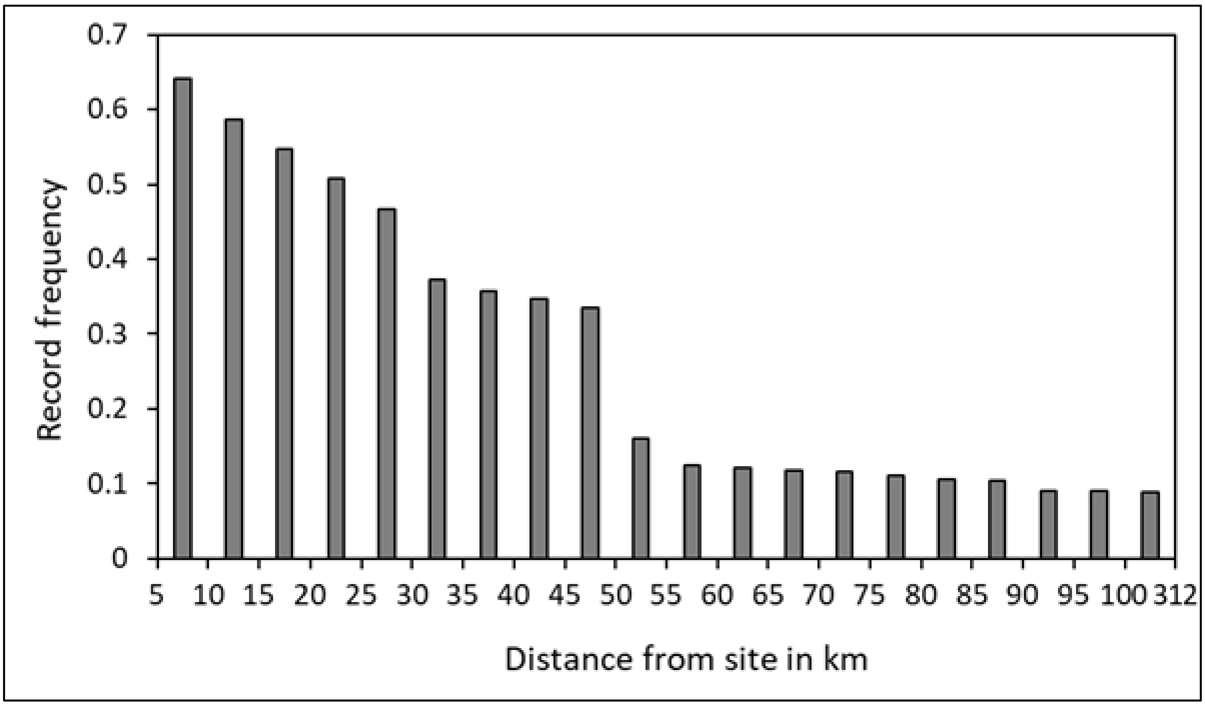


Figure 2. Frequency distribution of records of reef manta rays (*Mobula alfredi*) (*N* = 16) at different ranges of distance from the site of tag deployment, using satellite tags (SPLASH10), in New Caledonia.

Four different movement patterns were identified from the combined records of all sites (Fig. 3, Fig. 4, Table 1). First, *fidelity* when manta rays remained within 50 km from the location of tagging. This represents half of the individuals. Second, *excursion* when manta rays extended their movement beyond 50 km off the site of origin at least once but consistently returned to this area. Here, three individuals followed this pattern. The number of excursions per deployment varied from 1 to 16 for a maximum duration ranging from 1 to 9 days (Fig. S1). Third, the *relocation* pattern is described by manta rays that recorded more than half of all locations data at more than 50 km off the tagging site and remained outside this range until release of the tag. Only two individuals (12.5 %) relocated at distance ranging from 71 to 248 km for a maximum duration of 48 and 40 days before release of the tag, respectively. Finally, the fourth pattern, *fidelity + relocation*, represents manta rays that had more than half of their recorded location within the 50 km range before moving further away toward the end of the deployment. This last pattern describes 18.7 % of individuals. The counts of individuals were not significantly different between movement pattern categories ($\chi^2_{(3,16)} = 4.0$, $p > .05$).

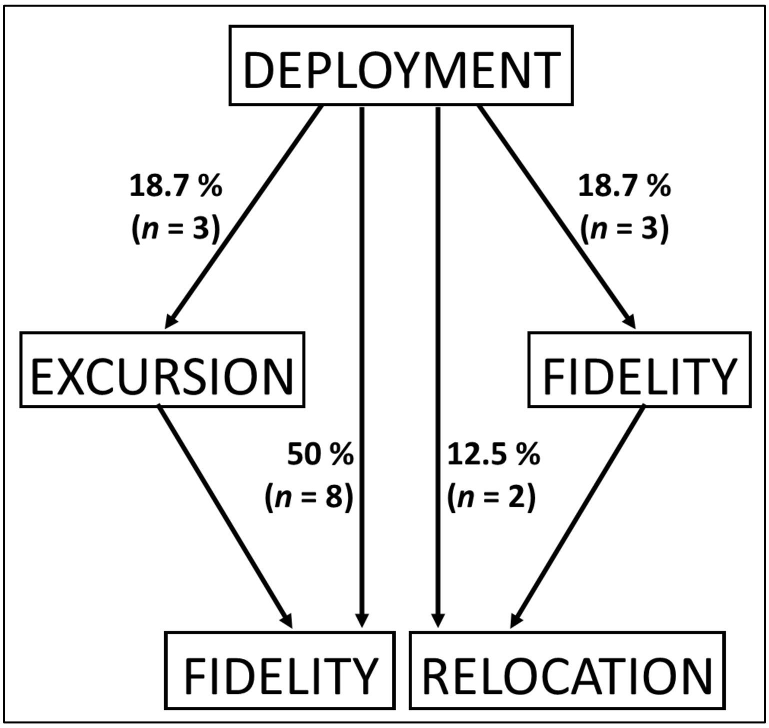


Figure 3. Movement patterns recorded for reef manta rays (*Mobula alfredi*) after deployment of the tag, using satellite tag (SPLASH10), in New Caledonia. Percentage based on the total number of individuals (*N* = 16).

Comparisons between each site reveal significantly different distributions of distance frequencies (Fig. S2, Table S2). Records of distance indicated smaller home ranges in Touho (*N* = 19 records) and Ouvea (*N* = 105) with a limit at 20 and 35 km from the tagging site, respectively. In Noumea (*N* = 626) the home range remained at 50 km (Fig. 4, Table 1). At this site, a third of the tagged manta rays remained within the home range perimeter and another third recorded excursions along the barrier reef up to 86.1 km north of the deployment location. The last third relocated at 96 (M4) and 250 km (M3) at the southern tip of the lagoon of the Main Island and on the east coast of the Main Island, respectively. In Ouvea, two individuals remained within the designated home range of 35 km from the site while another recorded an excursion halfway between the island of Ouvea and the Main Island over the 2000-meter-deep channel (Fig. 4, Table 1). The last manta ray relocated up to 225 km north toward the isolated reefs of Petrie and spent a maximum of 107 days outside its initial home range. In Touho, two manta rays remained within the 20 km home range (Fig. 4, Table 1). Two individuals recorded excursions, including one that moved 131 km down the coast for a maximum of 82 days outside its initial home range. Finally, the two other manta rays from Touho relocated. One travelled down to the Isle of Pines (311 km) to return later toward the tagging site where the tag detached 70 km south. The other relocated at the Isle of Pines. Differences in the number of individuals per movement patterns between site were not significant (Table S3).

Overlap in space occupation between individuals tagged at different sites was observed between manta rays from Touho and Noumea in the southern tip and the southern part of the east coast of the Main Island (Fig. 4). Among individuals tagged at the same site, overlap of movements outside the tagging sites only occurs off Noumea within an area located 50 km north of the Dumbea channel near the St-Vincent channel.

Sex

Females were recorded at significantly farther distance from the site of tagging than males ($t_{(781)} = 5.9$, $p < .001$). However, none of the gender was more mobile than the other with no significant difference in distances per day ($t_{(14)} = 0.46$, $p > .05$), total track lengths ($t_{(14)} = 0.14$, $p > .05$) or in the extend of the latitudinal range ($t_{(14)} = 1.01$, $p > .05$). The comparison of movement patterns among sex showed a significant difference in distributions of frequencies of recorded distance from sites (K-S: $D_{(21)} = 0.67$, $p < .001$). Females ($N = 9$ individuals) recorded a broader home range than males ($N = 7$) with a perimeter of 50 km against 35 km, respectively. Differences in the number of individuals per movement patterns between sex were not significant (*p-values* > .05 *by Fisher's exact test)*.

Table 1. Movement pattern characteristics and distribution between sites and sex of reef manta rays (*Mobula alfredi*) ($N = 17$) in New Caledonia using satellite telemetry (SPLASH10). HR: Home Range.

Movement Patterns	**Pattern explanation**	**Noumea**		**Ouvea**		**Touho**		**TOTAL**
		Male	Female	Male	Female	Male	Female	
FIDELITY	100 % records in the HR	1	1	0	3	1	1	7
EXCURSION	≥ 1 record outside the home range followed by ≥ 1 record within the HR	0	2	1	0	1	1	5
FIDELITY + RELOCATION	≥ 50 % records in the HR followed by ≥ 1 record outside at tag release	1	0	1	0	0	0	2
RELOCATION	≥ 50 % records outside the HR until tag release	0	1	0	0	1	1	3

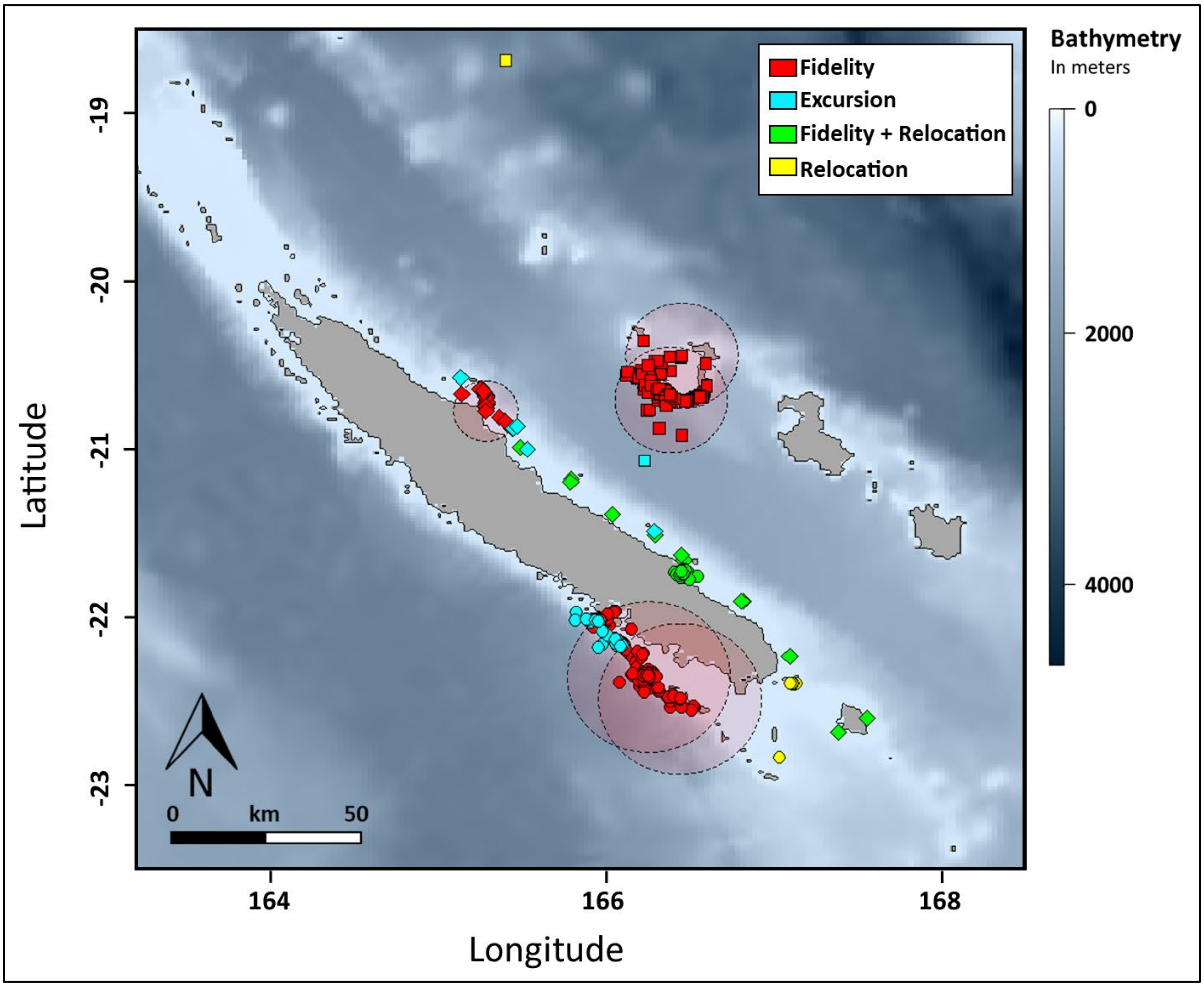


Figure 4a. Movement patterns of reef manta rays (*Mobula alfredi*) In New Caledonia using satellite telemetry (SPLASH10). Symbols shapes indicate site of origin: circle = Noumea (*N* = 6), square = Ouvea (*N* = 4) and diamond = Touho (*N* = 6).

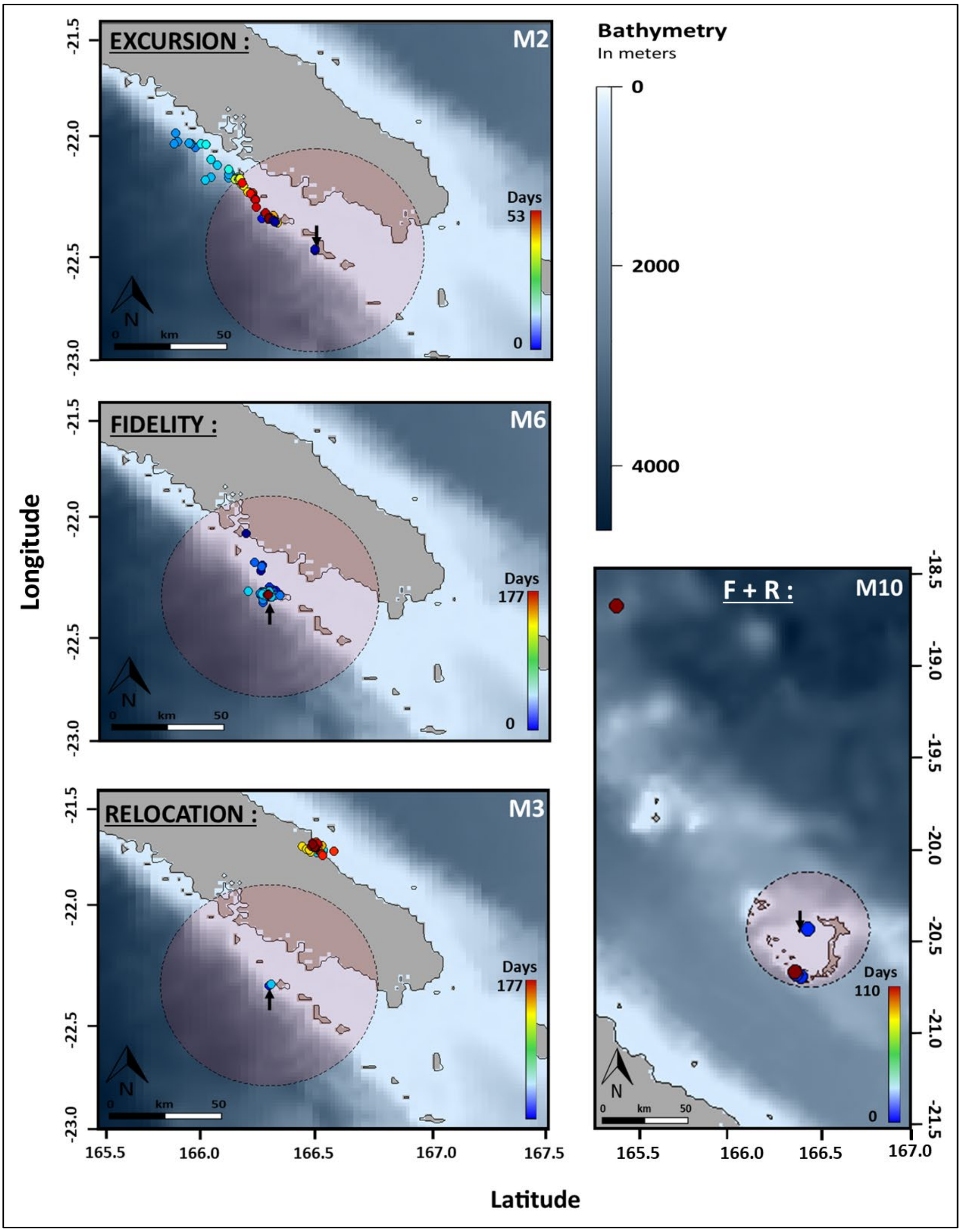


Figure 4b. Examples of movement patterns of reef manta rays (*Mobula alfredi*) In New Caledonia using satellite telemetry (SPLASH10). Circles indicate respective home range and arrows indicate deployment location. *F + R = Fidelity + Relocation* and *Mx* indicate individuals identification.

Diving behaviour

Reef manta rays of New Caledonia revealed frequent deep dive behaviour (Fig. S3, Table S4). All individuals recorded a dive below 300 m. Over the deployment period, individuals reached an average maximum depth of 490.6 ± 105.5 m, ranging from 320 ± 4 to 672 ± 4 m (Table S4). These deep dives occurred relatively regularly during the deployment duration with an average of one dive below 300 m every 4.8 days (ranging from more than one a day to one every 49 days). Among all deepest dives recorded each day, the proportion of dives below 300m reached 10.1% (n=3012). On average, a manta ray recorded a deep dive at 109.4 ± 110 m each day.

The vertical distribution shows a significantly greater use of the first 50 m of the water column by the manta rays with on average 82.9 ± 24.3 % of their time spent within the 0-50m depth range. In contrast, the tagged individuals only spent 0.3 ± 1.2 % of their time at depths below 300 m. Time at depth was significantly different between all depth ranges: 0 to 50 m, 50 to 100 m, 100 to 200 m, 200 to 300 m and > 300 m ($F_{(4,\ 14688)} = 3816$, $p = 0$). At maximum depth for each individual, the bottom time varied from 2.2 min at 672 and 416 m to 26.6 min at 376 m with an average of 9.1 ± 5.9 min. There was no significant negative correlation between maximum depth and time spent at depth ($r_{(21)} = -0.27$, $p > .05$).

A comparison of the dive behaviour between the four horizontal movement patterns revealed that manta rays exhibiting *excursion* and *relocation* patterns dived significantly deeper each day than individuals corresponding to *fidelity* and *fidelity + relocation* patterns ($F_{(3,2386)} = 90.6$, $p < .001$). Individuals showing a *fidelity* movement pattern recorded significantly shallower maximum depths per day. In addition, the proportions of dives below 300 m were significantly higher for individuals belonging to the *excursion* and *relocation* movement patterns with 13.2

% and 10.5 %, respectively, against 2.1 % and 3,9 % for the *fidelity* and *fidelity + relocation* patterns, respectively (Table S5). However, there were no significant difference in the distributions of time spent at different depth ranges between all movement patterns (Table S6).

Between sites, comparisons of diving behaviour were made among movement patterns (Fig. 5). The difference on average values of maximum depth per day between sites depends on the patterns ($F_{(4,\ 2380)} = 14.3$, $p < .001$) (Fig. 5). These values were significantly different amongst sites for all movement patterns, the lowest being recorded for Noumea (Fig. 5). Individuals that showed a *Fidelity* pattern recorded significantly higher daily maximum depth in Touho ($F_{(2,897)} = 95.5$, $p < .001$). The deepest daily average within the Excursion pattern was for Ouvea ($F_{(2,778)} = 38.3$, $p < .001$). Only individuals from Ouvea and Noumea exhibited a the *Fidelity + Relocation* pattern and mean values were significantly higher at Ouvea ($t_{(305)} = 2.2$, $p < .05$). Finally, only manta rays in Noumea and Touho described a *Relocation* pattern and the mean values of daily maximum depth were significantly higher in Touho ($t_{(121)} = 2.5$, $p < .05$). The proportion of dives below 300 m was significantly different only between sites among the *Fidelity* ($\chi^2_{(2,\ N\ =\ 900)} = 29.9$, $p < .001$) and *Excursion* ($\chi^2_{(2,\ N\ =\ 780)} = 31.9$, $p < .001$) patterns with the highest values recorded at Ouvea and the smallest at Noumea.

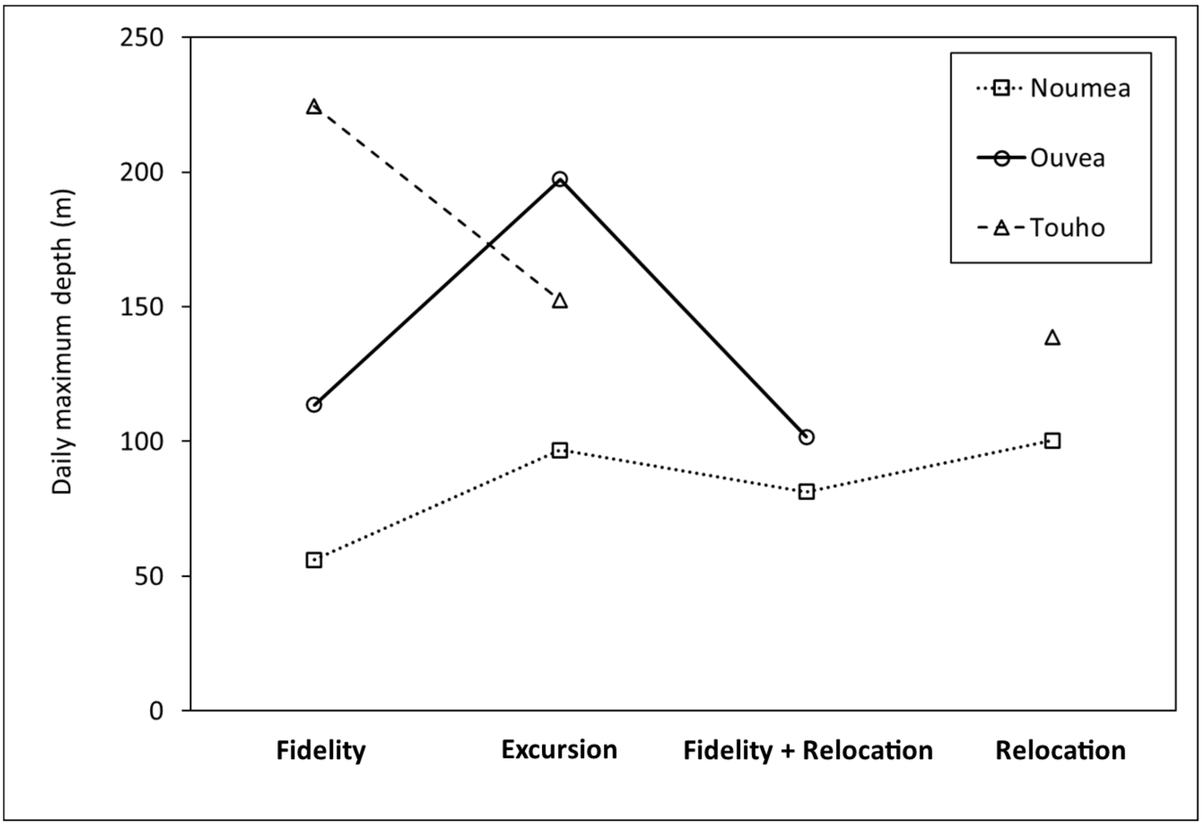


Figure 5. Interaction plot of average maximum depth recorded per day at each study site: Noumea (N = 6 individuals), Ouvea (N = 4) and Touho (N = 6), among four movement patterns: *Fidelity* (N = 900 records), *Excursion* (N = 780), *Fidelity + Relocation* (N = 309) and *Relocation* (N = 400) for reef manta rays (*Mobula alfredi*) using satellite telemetry (SPLASH10).

Three recovered tags (M4, M6 and M15) allowed the analysis of the diving behaviour into more details (Fig. 6). The manta ray M4 described a *Fidelity + Relocation* pattern, M6 corresponded to the *Fidelity* pattern and M15 could not be used in the horizontal movement analysis (due to all location data being filtered out). Overall, vertical movements were slow with ascents and descents peaking at 2.15 and 1.75 $m.s^{-1}$ and averaging 0.09 ± 0.07 and 0.08 ± 0.06 $m.s^{-1}$, respectively. Descents were significantly slower than ascents ($t_{(5682692)} = 128.96$, $p < .001$) (Fig. 7). Although vertical movements were slow, the average daily distance varied widely from 5313 ± 1275 to 8768 ± 3483 m for M6 and M15, respectively. All three manta rays travelled significantly different distances up and down the water column ($F_{(2,127.5)} = 90.4$, $p < .001$).

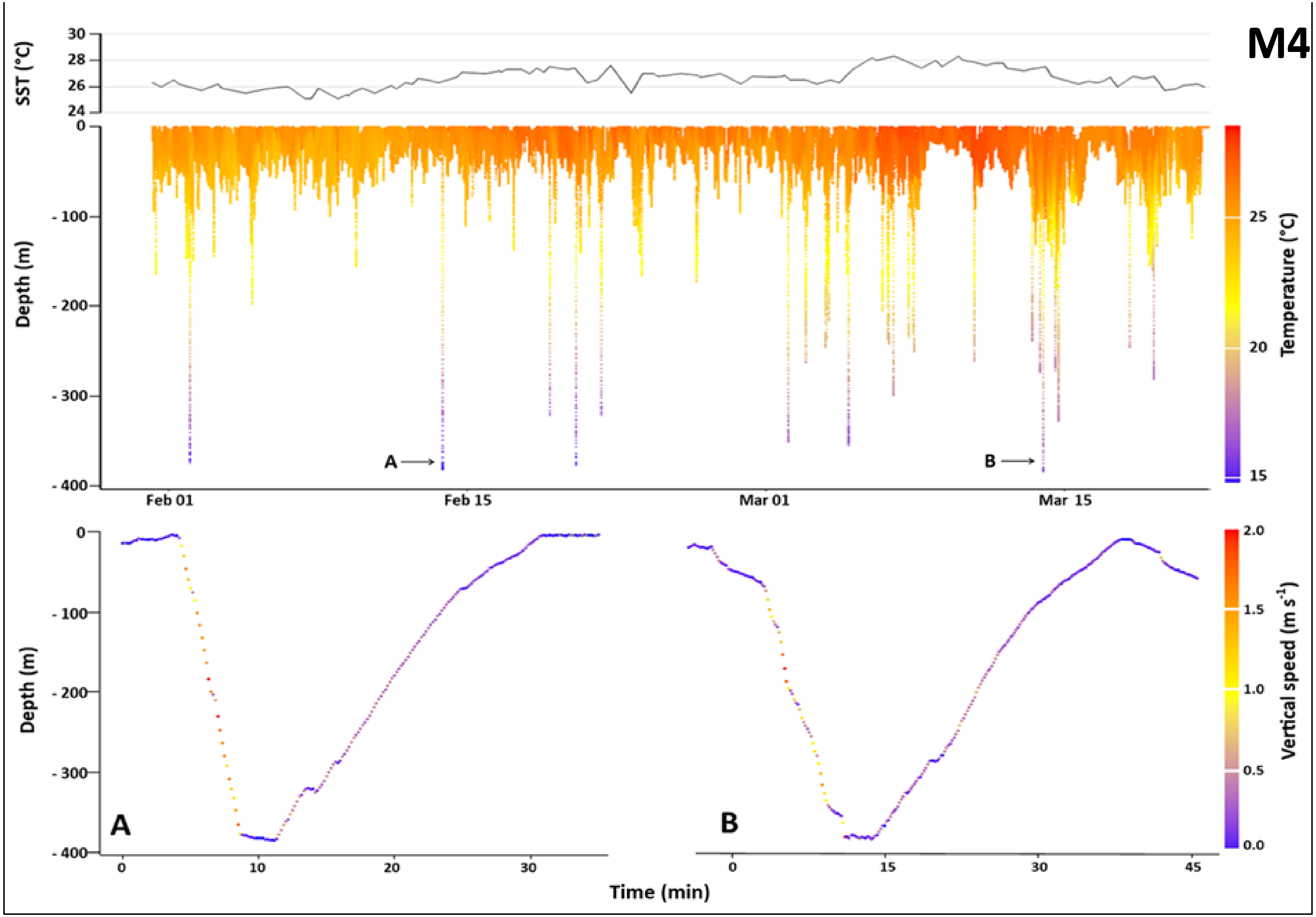


Figure 6. Dive profile of reef manta rays (*Mobula alfredi*) recorded using pop-up satellite tags (SPLASH10 and MiniPAT) in New Caledonia. STT indicates Sea Surface Temperature. A and B are the deepest dives.

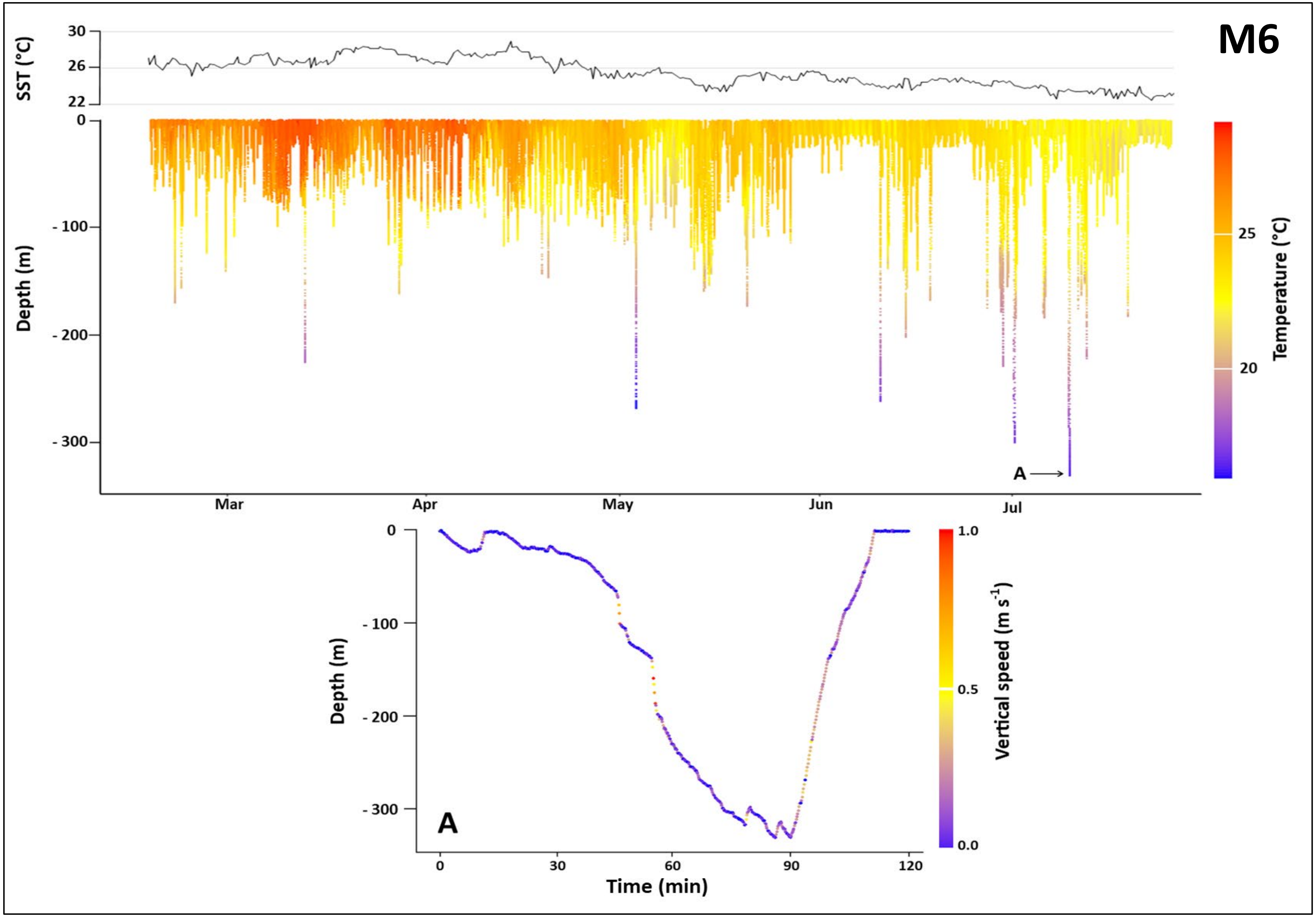


Figure 6 (continued).

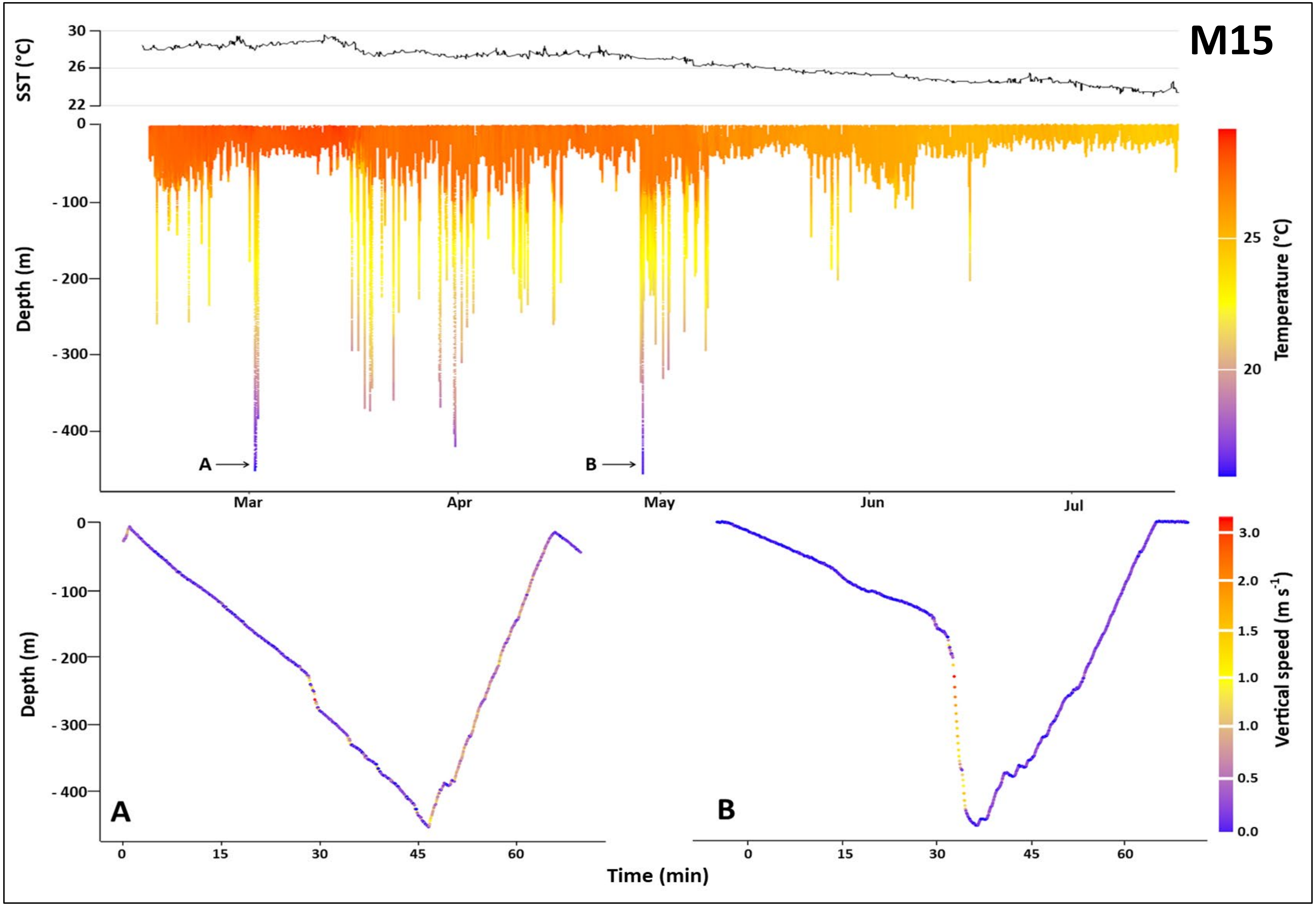


Figure 6 (continued).

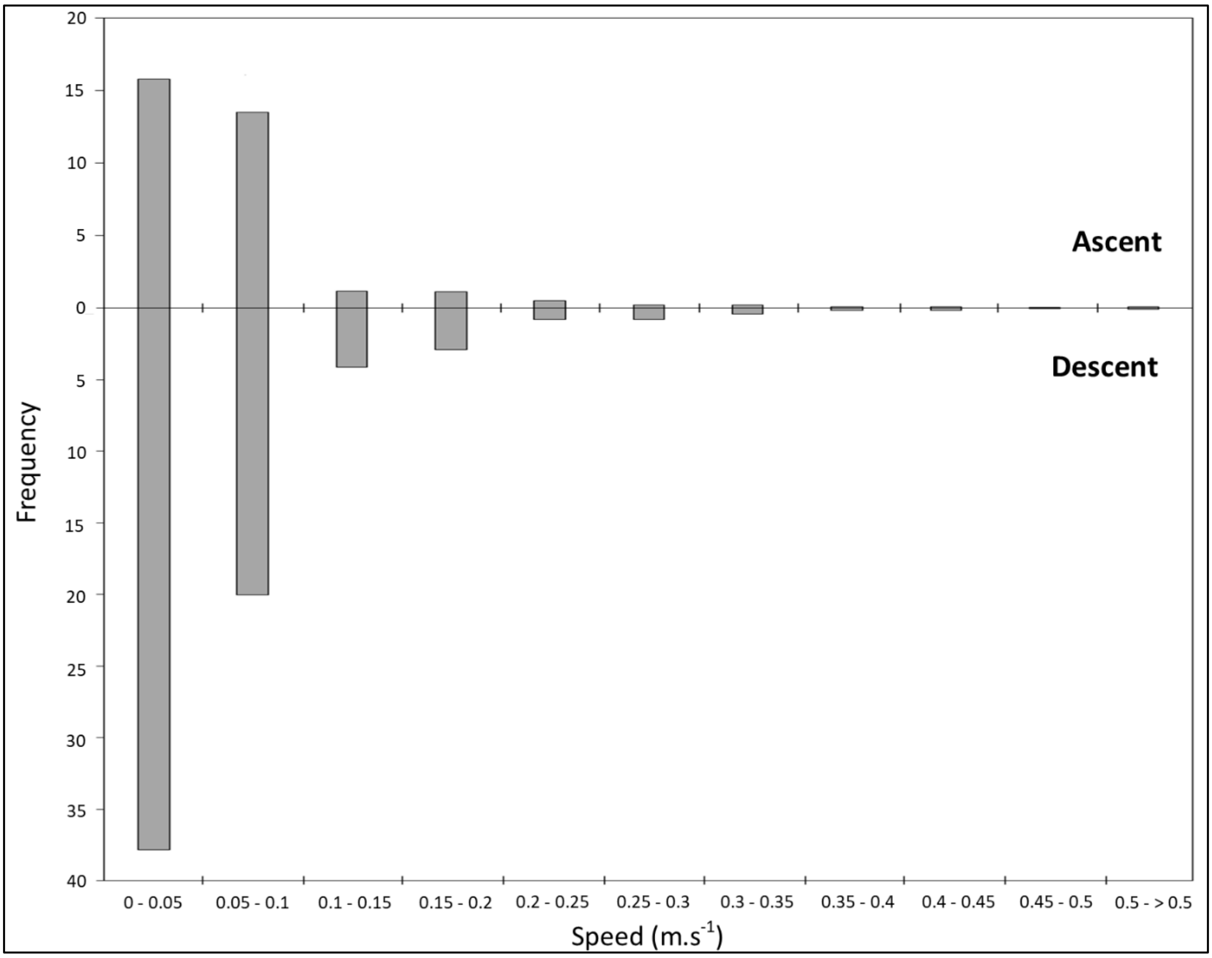


Figure 7. Frequency distribution of vertical speed during ascent and descent of reef manta rays (*Mobula alfredi*) (N = 3) recorded with satellite telemetry in New Caledonia.

The analysis of the deepest dives performed by these three individuals revealed significantly different profiles (Fig. 6, Table S7). M4 reached twice the maximum depth of 384 m within a 28 days interval and showed, during both dives, U-shaped profiles slightly skewed to the right. M6 reached a maximum depth of 336 m with a left-skewed U-shape profile. M15 exhibited V- shape dive profile for both of its deepest dives at 456 m. For these latest profiles, one was skewed right after an initially slow descent down to 200 m and the other to the right.

Temperature

Manta rays come across temperatures averaging 25.7 ± 2.1 °C with a minimum of 7.6 °C recorded at 672 m deep. Individuals spent most of their time (55.9 %) at temperature between 27 and 29 °C. The maximum reached depth per day was not correlated with the sea-surface temperature ($r_{(2150)} = 0.24$, $p > .05$) .

Sex

Maximum reached depths recorded during the whole deployment duration were no different between sexes ($t_{(17)} = 0.99$, $p > .05$). However, when looking at daily maximum depths, males tended to dive significantly deeper than females ($t_{(2794)} = 15.1$, $p < .001$) with on average 140.4 ± 126.3 m against 81.4 ± 83.5 m below the surface, respectively. The proportion of dives below 300 m is significantly higher for males than females with 12.8 % and 3.7 %, respectively ($z = 8.3$, $p < .001$).

The analysis of pattern in relation to daily maximum depths reveals that the difference in mean values between sex depends on the movement patterns ($F_{(2,2383)} = 19.5$, $p < .001$) (Fig. 8). Male were significantly recorded at higher daily maximum depths than females for all patterns, significantly (*Fidelity*: $t_{(121.5)} = 4.0$, $p < .001$; *Excursion*: $t_{(565.5)} = 10.5$, $p < .001$; *Relocation*: $t_{(397.3)} = 2.6$, $p < .01$). Regarding the proportions of dives below 300 m, males recorded significantly higher values than females among the *Excursion* ($z = 6.2$, $p < .001$) and *Fidelity* ($z = 6.0$, $p < .001$) movement patterns with 22 % and 9.7 % against 6.7 % and 1 %, respectively. There were no significant difference in the proportion of dives below 300 m between sex among the *Relocation* movement pattern ($z = 1.7$, $p > .05$).

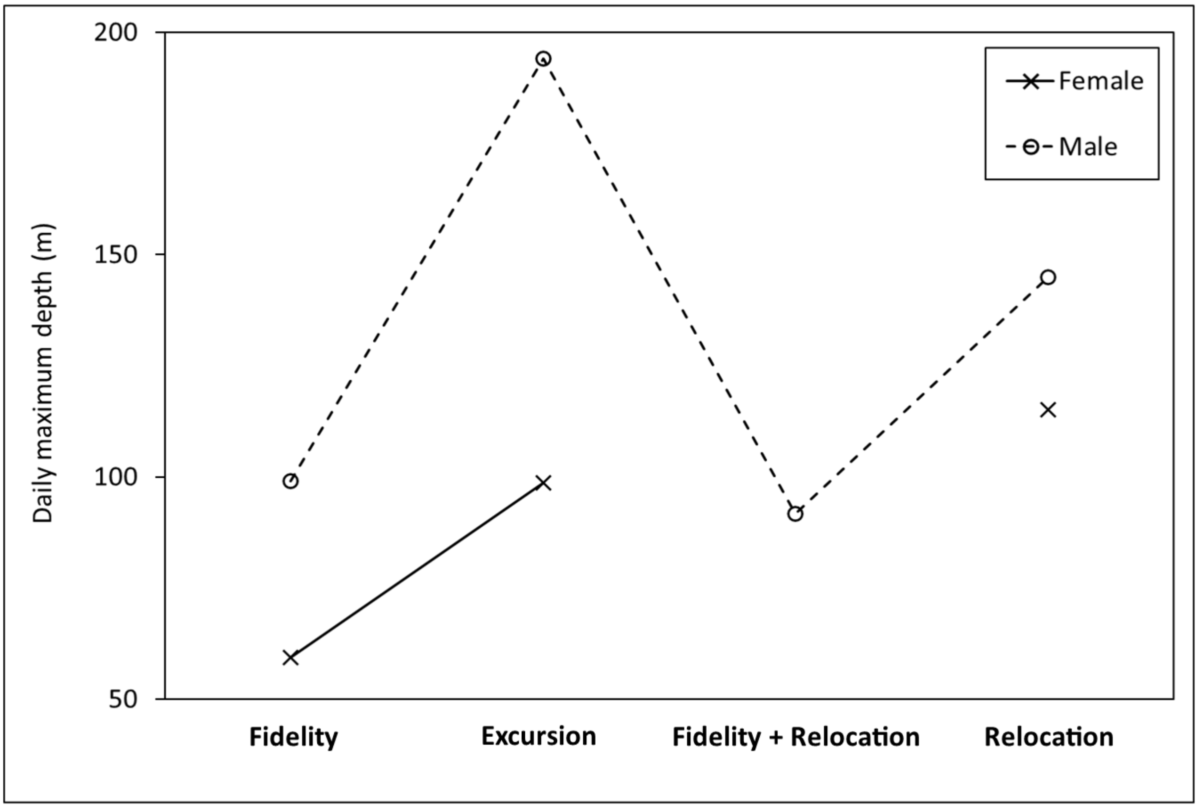


Figure 8. Interaction plot of average maximum depth recorded per day for each sex : Males (N = 7 individuals) and Females (N = 10), among four movement patterns: *Fidelity* (N = 900 records), *Excursion* (N = 780), *Fidelity + Relocation* (N = 309) and *Relocation* (N = 400) for reef manta rays (*Mobula alfredi*) using satellite telemetry (SPLASH10).

Diel comparison

Comparison of maximum depths showed no significant difference between day and night hours ($t_{(3010)}$ = 0.3, p > .05). Similarly, no significant difference was observed between proportion of dive below 300 m (z = 0.5, p > .05). Regarding occupation of the different depth ranges, reef manta rays tended to spend significantly more time within the first five meters during day hours ($t_{(2281)}$ = 4.9, p < .001), and inversely, occupied depths between 5 and 50 meters during slightly longer period at night-time ($t_{(2447)}$ = 3.4, p < .001). No significant diel differences were found when comparing time spent at other depth ranges. The distributions of occupation among different depth ranges were not different between day and night hours (K-S: $D_{(6)}$ = 0.17, p > .05). Diel variations in vertical distances were also compared using the data collected from the three retrieved tags. All three individuals showed different trends in the diel repartition of their vertical movements. M15 recorded greater vertical movements during day hours ($t_{(235.8)} = 7.0$, $p < .001$), M4 exhibited greater vertical movements during night hours ($t_{(80.4)} = 3.7$, $p < .001$) and M6 demonstrated no significant difference of vertical movements at night or during the day ($t_{(332.1)} = 0.1$, $p > .05$).

Diel comparison was also made among each movement patterns and no significant difference was observed for daily maximum depth (*Fidelity* : $t_{(899)} = 0.8$, $p > .05$; *Excursion* : $t_{(778)} = 0.4$, $p > .05$; *Relocation* : $t_{(398)} = 1.7$, $p > .05$; *Fidelity + Relocation* : $t_{(306)} = 1.2$, $p > .05$). Similarly, no significant difference was observed for the proportion of dives below 300 m among each movement patterns (*Fidelity* : $z = 0.1$, $p > .05$; *Excursion* : $z = 0.4$, $p > .05$; *Relocation* : $z = 1.8$, $p > .05$; *Fidelity + Relocation* : $z = 1.4$, $p > .05$).

DISCUSSION

Horizontal behaviour

Reef manta rays in New Caledonia tend to remain close to the coast and reefs. Except for one individual, all records were within 35 km off the closest reef. Several studies that documented movements of reef manta rays reported consistent use of coastal and reef areas in Australia (Jaine et al. 2014, Armstrong et al. 2020), in the Red Sea (Braun et al. 2015; Kessel et al. 2017), in the Seychelles (Peel et al. 2020), and in the British Indian Ocean Territory MPA (Andrzejaczek et al. 2020). The use of coastal habitat by reef manta rays and other planktivorous elasmobranchs has been associated with food availability (Dewar et al. 2008; Anderson et al. 2011; Couturier et al. 2011, 2018; Rohner et al. 2013; Armstrong et al. 2016, Mc Coy et al. 2018). Coastal areas of islands surrounded by deep waters are prone to assemble the conditions to generate primary productivity hotspots combining upwellings and land inputs and generating reliable food resources which might result in strong residency pattern in reef manta rays' behaviour (Peel et al. 2020, Setyawan et al. 2020). In New Caledonia, such conditions exist with little annual variations (Torreton et al. 2010, Le Borgne et al. 2010). Limited offshore movements are likely due to the foraging ground being coastal and the probable lower productivity of the adjacent oceanic waters (McCauley et al. 2014). Other than food resources, coastal areas and shallow waters of the lagoon offer more protection from predators such as large sharks and hence, also represent a suitable environment for potential nurseries. In New Caledonia, the detection of juvenile individuals remains sporadic, and neonates are even more scarce, although lagoons are places of nursery grounds for reef manta rays in Raja Ampat, Indonesia (Setyawan et al. 2020) and for other elasmobranchs (Heupel et al. 2007; Papastamatiou et al. 2009; Dale et al. 2011). Further investigations in suspected adequate nursery grounds using aerial surveys and accurate measurement methods to quantify maturation stages might lead to the discovery of nurseries in New Caledonia. In addition, coral reefs are also home to numerous cleaning stations that are essential to manta rays' well-being and health (Barr and Abelson 2019) as well as mating and socialization (Stevens 2016; Perryman et al. 2019). Sheltered waters also act as a thermal refuge where enclosed shallow waters have a significantly higher temperature than oceanic waters. In this study, manta rays were observed to favour relatively high temperatures (27 to 29 °C) but were also able to face much colder water for instance when deep diving (7.6 °C). Basking in warm shallow waters after a deep dive would allow regulation of the body temperature. Thermoregulation is an important part of the behaviour of mobulids (Thorrold et al. 2014; Stewart et al. 2016b) and other elasmobranchs (Thums et al. 2013; Speed et al.

2016) and the presence of warm-sheltered water in proximity of deep feeding grounds might be essential.

The use of offshore waters by one individual recorded in this study could be interpreted as a transiting trip toward potential alternative foraging grounds near isolated reefs. Presence of reef manta rays at isolated reefs have already been recorded during aerial surveys (Laran et al. 2016), further investigation in these areas might reveal new aggregations. Offshore movements by reef manta rays were also documented using satellite telemetry and were associated with foraging opportunities. In the east coast of Australia, Jaine et al. (2014) recorded most of the tagged rays in the offshore Capricorn Eddy Region and attributed this behaviour to be food related to the high productivity generated by the eddy. In the Red Sea, Braun et al. (2015) observed offshore excursions to deeper water at night where manta rays performed deep dives supposedly to exploit pelagic planktonic resources.

Despite their demonstrated coastal affinity, reef manta rays have been observed over waters up to 2720 m deep. The archipelago of New Caledonia has a relatively narrow continental shelf, especially on the east coast of the Main Island and around the Loyalty Islands, beyond which water depth drops rapidly. Even though manta rays spent most of their time within the first 50 meters, our results suggest that this species is able to use deeper water to transit between areas (e.g., to connect with an isolated reef) or to access to demersal food resources (see relevant *vertical behaviour* paragraph). This supports previous findings that recorded reef manta rays commuting between islands chain separated by deep water in Indonesia (Germanov and Marshall 2014, Setyawan et al. 2020) or transiting by deep water to access food resources in east Australia (Jaine et al. 2014) and the Maldives (Harris et al. 2020). However, even if there are evidence that deep water is not a strict barrier to their movements, large extenses of deep water might still be a restricting factor to connectivity. For instance, in this study, spatial occupancy overlapped only for individuals originating from sites sharing the same coastline and not for individuals that are separated by a 2000-meter-deep channel. Other evidence through different spatial and temporal scale using photo-identification (Lassauce et al., in prep) and genomics (Lassauce et al., in review) showed that these connections occur but were limited, highlighting the lack of connectivity over this deep-water channel. Other studies taking place in atolls were consistent with these observations with populations recording no or only few connections between islands separated by deep waters in Hawaii (Clark 2010; Deakos et al. 2011), in the Maldives (Kitchen-Wheeler et al. 2012), in French Polynesia (Carpentier et al. 2019) or in the Seychelles (Peel et al. 2020). On the contrary, reef manta rays showed long distance movements along continuous coastlines, up to 1150 km in east Australia (Armstrong et al. 2019), but also along the west coast of Australia (up to 700 km, Armstrong et al. 2020) or the coast of southern Mozambique (up to 350 km, Venables et al. 2020).

Horizontal movements were classified into four distinct patterns. The most dominant pattern was *Fidelity* where manta rays remained within their home range for the whole duration of the tag deployment. Manta rays displayed also excursions travelling, occasionally, relatively long distance as far as 131 km away from the site of origin. Moreover, total track lengths were relatively small (maximum of 688 km) in comparison to previous findings averaging 839 km in western Australia (Armstrong et al. 2020), 1169 km in east Australia (Jaine et al. 2014) and 1074 km in the Seychelles (Peel et al. 2020). Our results describe a behaviour that corresponds to site fidelity as defined by Chapman et al. (2015): the return of an individual to a location where it previously resided after an absence as long as or longer than the residency period. Additional evidence of such behaviour was found in New Caledonia using long-term photo-identification data to find high re-sighting rates for each site (Lassauce et al., in prep) and genomics analysis to detect genetic structure between sites (Lassauce et al., in review). Site fidelity is often motivated by consistent foraging opportunities over time within an area (Chapman et al. 2015). This behaviour has been largely documented for reef manta rays in Hawaii (Deakos et al. 2011), Mozambique (Marshall 2008), east Australia (Couturier et al. 2011), Indonesia (Germanov et al. 2019, Setyawan et al. 2020) or French Polynesia (Carpentier et al. 2019). Although most of the tagged individuals displayed constrained movements near the aggregation site of origin, manta rays of New Caledonia demonstrate the ability to potentially connect with all parts of the archipelago, at least parts that are linked by coastlines or shallow waters. In particular, almost a third of the individuals showed *Relocation* and *Fidelity + Relocation* patterns, connecting with sites up to 311 km from the site of origin. Coupled with return trips over 200 km away from the deployment location, these observations suggest that manta rays connect to intermediate sites. For instance, while no connection was observed between studied sites during this study, overlap occurred for individuals from Touho and Noumea at in-between sites along the east coast and at the southern tip of the Main Island. Sightings of manta rays using photo-identifications over five years revealed different aggregation sites throughout the archipelago with multiple re-sightings from individuals originating from different sites (Lassauce et al., in prep). The present results suggest the potential existence of additional aggregation sites where manta rays from different sites may encounter each other, although no field operation could be undertaken to confirm it.

The present results present a partial short-term overview of the spatial ecology of the reef manta rays of New Caledonia. The average deployment duration in this study was short (approx. 77 days) compared to what was achieved in other satellite telemetry studies on reef manta rays (e.g., 92 days, Jaine et al. 2014; 147 days, Braun et al. 2015; 116 days, Peel et al. 2020). This might also explain the relatively small track lengths recorded in this study in comparison to findings at other locations in the world. To complement this finding, other methods involving long term monitoring of the movements and geographically extended sampling effort based on these results might help decide whether these observations are sporadic or more frequent, whether these potential patterns are seasonal, to what extend long-term site fidelity is supported, as well as revealing new connections and new potential aggregation sites. To this regard, photo-identification offers such perspectives and acoustic telemetry may narrow the gap between photo-identification and satellite telemetry by generating spatially and temporally intermediate data. In addition, genomics would provide a greater picture of the evolutionary processes that shape the population. The combined use of such tools would provide detailed data to help achieve these goals.

Vertical behaviour

In New Caledonia, reef manta rays have been recorded deeper than anywhere else in the world and at a higher frequency. Lassauce et al. (2020) revealed part of these vertical behaviours, extending the previous known depth range of the species by more than 200 meters (Braun et al. 2014) with a maximum depth at 672 m. New data incorporated in this study (with the addition of 10 more tags) support this trend with all manta rays diving under 300 metres deep and more than 10 % of the daily maximum depth reaching below 300 m. These results reinforce the outstanding diving behaviour of the reef manta rays of New Caledonia in comparison to any other studied region worldwide.

Our present results describe frequent deep dives with one dive below 300 m every 4.8 days of deployment also reinforcing our previous findings in Lassauce et al. 2020. Deep dives recorded in other studies, relatively to the deepest ones, were not as frequent with only one dive below 150 m every 16.6 in the Red Sea (*N* = 1030 days, Braun et al. 2014) and 34.8 days in the Seychelles (*N* = 696 days, Peel et al. 2020). Several studies on elasmobranch species documented repetitive diving as a common behaviour (Graham et al. 2006; Weng et al. 2007; Gleiss et al. 2010, Queiroz et al. 2017). This behaviour was often associated with function such as foraging, horizontal movements (through gliding) and thermoregulation (Klimley et al. 2002).

Hypotheses on the function associated with a certain diving behaviour is rendered possible through the analysis of the different shapes of dive, and many studies used this method (e.g., Gleiss et al. 2011; Dragon et al. 2012; Viviant et al. 2014; Queiroz et al. 2017). The time spent at the deepest depth is one of the determinant factors to describe shape classes. U-shaped dives, with a distinct bottom phase, and V-shape dives, with little or no bottom phase, are the most commonly reported (Wislon et Block 2009; Queiroz et al. 2017). In addition, the speed of the animal during ascents and descents is another indicator of its behaviour and makes up the slope of the dive shape. These factors are also shaped by physiological constraints, such as the need to return to the surface for air breathing marine animal or to regulate the body temperature for ectotherms, and the investigation on the balance between gains and costs may help determine behavioural patterns. In our study, the analysis of the deepest dive profiles showed both U-shape and V-shaped dives. U-shaped dives were both skewed left and right while V-shaped dives were characterised by steeper ascent than descent. U-shaped dives were observed in several diving species and interpreted as foraging activity where the animal feed during the bottom phase on aggregated resources at depth (e.g., Wislon et Block

2009; Gleiss et al. 2010; Dragon et al. 2012; Viviant et al. 2014; Queiroz et al. 2017). V-shaped dives are thought to reflect exploration where the animal swim across the different depth layers of the water column to collect cues that propagate horizontally (Pade et al. 2009; Wilson and Block 2009; Gleiss et al. 2011, 2013; Queiroz et al. 2017). In addition, skewed-left dive profiles have been thought to represent slow exploration during descent following the sea floor before finding exploitable resources (U-shape) or not (V-shape) (Schreer and Testa 1996; Wilson and Block 2009). On the other hand, right-skewed dive profiles might indicate exploration during ascent (Schreer and Testa 1996; Wilson and Block 2009). Another hypothesis for left-skewed V-shaped dives was the optimization of horizontal movements using gliding during slow descent to save energy and active swimming in faster ascent (Weihs 1973; Braun et al. 2014). Our results suggest that the reason of these deep dives, illustrated by the analysis of the deepest ones, may be essentially associated with foraging activities. Reef manta rays would either explore the water column in search for feeding opportunities (V-shaped dives) or simply be foraging on demersal resources (U-shaped dives). Studies used stable isotope and signature fatty acid analysis to demonstrate that demersal zooplankton may be an important part of the diet of reef manta rays (Couturier et al. 2013). Similar results were also observed the oceanic manta ray (Burgess et al. 2016; Stewart et al. 2016b) and other filter-feeding elasmobranchs such as basking sharks (Sims et al. 2003, 2005) and whale sharks (Graham et al. 2006; Tyminski et al. 2015). Optimization of horizontal movements using gliding for reef manta rays would also be a plausible explanation as raised by Braun et al. (2014) and Lassauce et al. (2020), although, in this case, inconsistent speed during descent and ascent suggests patterns associated to foraging (Schreer and Testa 1996, Queiroz et al. 2017). Further investigations on the nature of the diet of reef manta rays in New Caledonia would help confirm these hypotheses. Non-lethal sampling and biochemical methods such as stable isotope or fatty acid analyses could assist in understanding the composition of the animal diet and help determine whether demersal zooplankton is part of it and in what proportion (Couturier et al. 2013).

Foraging and the search for food resources is an important driver of most behaviour of manta rays. The fact that reef manta rays dive deeper and more frequently may be triggered by the need to find alternative energy source due to limited or insufficient food within the upper layer of the water column in New Caledonia. One indication to corroborate limited use of near surface water to feed would be the fact that only few manta rays showed regular surface occupation during deployment. The number of satellite localizations reflect the number of times the animal is surfacing and high surface occupation within an area may be an indicator of surface feeding in reef manta rays (Jaine et al. 2014, Braun et al. 2015). Another indicator would be a diel partition in depth occupation since surface feeding is triggered by condition occurring during daytime (Armstrong et al. 2016). Unlike our previous findings (Lassauce et al. 2020), the addition of 10 supplementary tags in this study resulted in no diel difference in the time spent at depth ranges below 50 meters and in daily maximum depths. This does not allow us to indicate a preferential period of the day for reef manta rays to use deep waters which differs from previous findings where reef manta rays were recorded deeper at night (Braun et al. 2014; Peel et al. 2020).

The investigation of diving behaviour in relation to the different horizontal movement patterns indicates that manta rays that leave the home range (*Excursion* and *Relocation* patterns) tend to dive deeper. This supports other results that seem to link movements away from the home range and deep dives to a foraging behaviour. In addition, site fidelity within a home range could also indicate a higher availability of resources and an easier access to it, resulting in a lesser need to visit the deeper layers of the water column in this area.

CONCLUSION

This work used satellite telemetry to gather additional evidence that reef manta rays are resident to coastal water and show strong site fidelity. Yet, this species is capable of relatively long-distance migrations seemingly favouring, but not limiting them to, coastal and shallow water paths. Deep water might be a restraining factor but not a complete barrier to connectivity. Considerations highlighted by these findings are twofolded: 1) the strong affinity for coastal waters and high site fidelity attract to special concerns regarding threats associated with these behaviours such as habitat degradation and human exploitation and disturbance ; 2) the potential home range of this species may extend over greater distances than previously thought, especially in habitat fragmented by deep waters, which raises concerns regarding potential movements outside areas under protective jurisdictions. Spatial ecology of the reef manta ray seems highly driven by the availability of the food resource. In New Caledonia, this resource might be scarce within the upper layers of the water column pushing the species to dive deeper to possibly feed on demersal food. This finding brings additional supports that highlight the use of mesopelagic depths as an important part of the habitat of the reef manta ray. In New Caledonia, concerns regarding the species conservation are limited since reef manta rays are not targeted by fisheries and the human activity remains relatively low. Globally, such favourable context is scarce which makes the population of New Caledonia a reference to be preserved (O’Malley et al. 2016). Therefore, preventive precautions should be taken at local level where coastal development is rapidly expanding and might threaten critical habitats. This study shows the efficiency of the use of satellite telemetry to detect movement and behavioural patterns of reef manta rays. A long-term monitoring might reveal the existence of seasonal patterns and new key habitats, and an extended sampling to remote areas of the archipelago such as isolated reefs would offer a better understanding of the population distribution and connectivity. The combined use of other tools addressing different temporal scales, such as the photo-identification, acoustic telemetry and genomic analysis would help build a more complete representation of the spatial ecology of the species. In a global context, the reef manta ray is a species particularly sensitive to anthropogenic threats and concerns regarding their conservation demand an accurate understanding of its spatial ecology to efficiently reinforce management efforts worldwide.

SUPPLEMENTARY MATERIAL

Table S1. Summary of satellite tag deployments on reef manta rays (*Mobula alfredi*) in New Caleodnia.

Manta ID	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**	**M16**	**M17**	**M18**	**M19**
Individual information:																			
Sex	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Male	Male	Female
PTT	167754	167755	151348	151349	163079	167757	140916	196191	196192	196193	196194	162379	162380	167758	174667	182146	196864	196865	196866
Tracking information:																			
Tag Date	31/01/17	30/01/17	31/01/17	31/01/17	31/01/17	2/02/17	3/12/15	17/02/20	17/02/20	18/02/20	18/02/20	28/11/18	28/11/18	15/02/20	15/02/20	14/02/20	14/02/20	15/02/20	14/02/20
Duration (days)	110	54	49	50	136	174	80	22	30	131	50	3	5	54	181	105	67	88	68
Site	BC, Noumea		DC, Noumea				PS, Ouvea	PN, Ouvea				Touho							
Deployment location:																			
Latitude	-22.495	-22.495	-22.363	-22.363	-22.363	-22.363	-20.708	-20.446	-20.446	-20.446	-20.446	Undisclosed							
Longitude	166.441	166.441	166.256	166.256	166.256	166.256	166.388	166.453	166.453	166.453	166.453								
Decoded (%)	42	86	64	100	65	100	79	87	83	45	79	83	85	89	100	73	81	75	85

* indicates tags that were retrieved after release. BC, Boulari Channel. DC, Dumbea Channel. NP, Northern Pleiades. SP, Southern Pleiades. PTT, Platform Transmitting Terminal.

Table S2. Kolmogorov-Smirnov (K-S) test statistics of differences in distributions of frequencies of distance from sites records of reef manta rays (*Mobula alfredi*) (*N* = 16) in New Caledonia. P-values are above the diagonals and *D* statistics are below the diagonals.

Sites	Noumea	Ouvea	Touho
Noumea		0	0.004
Ouvea	0.714		0
Touho	0.524	0.714	

Sex	Male	Female
Male		0
Female	0.667	

Table S3. Chi-Square test statistics of differences number of individuals per movement patterns of reef manta rays (*N* = 16) in New Caledonia.

Variables	**Stat**	**Movement Patterns**			
		Fidelity	Excursion	Fidelity + Relocation	Relocation
Sex	*t*	1.17	0.08	4.75	1.78
	P	0.28	0.78	0.03	0.18
Sites	*t*	0.36	0.95	1.5	4
	P	0.84	0.95	0.47	0.14

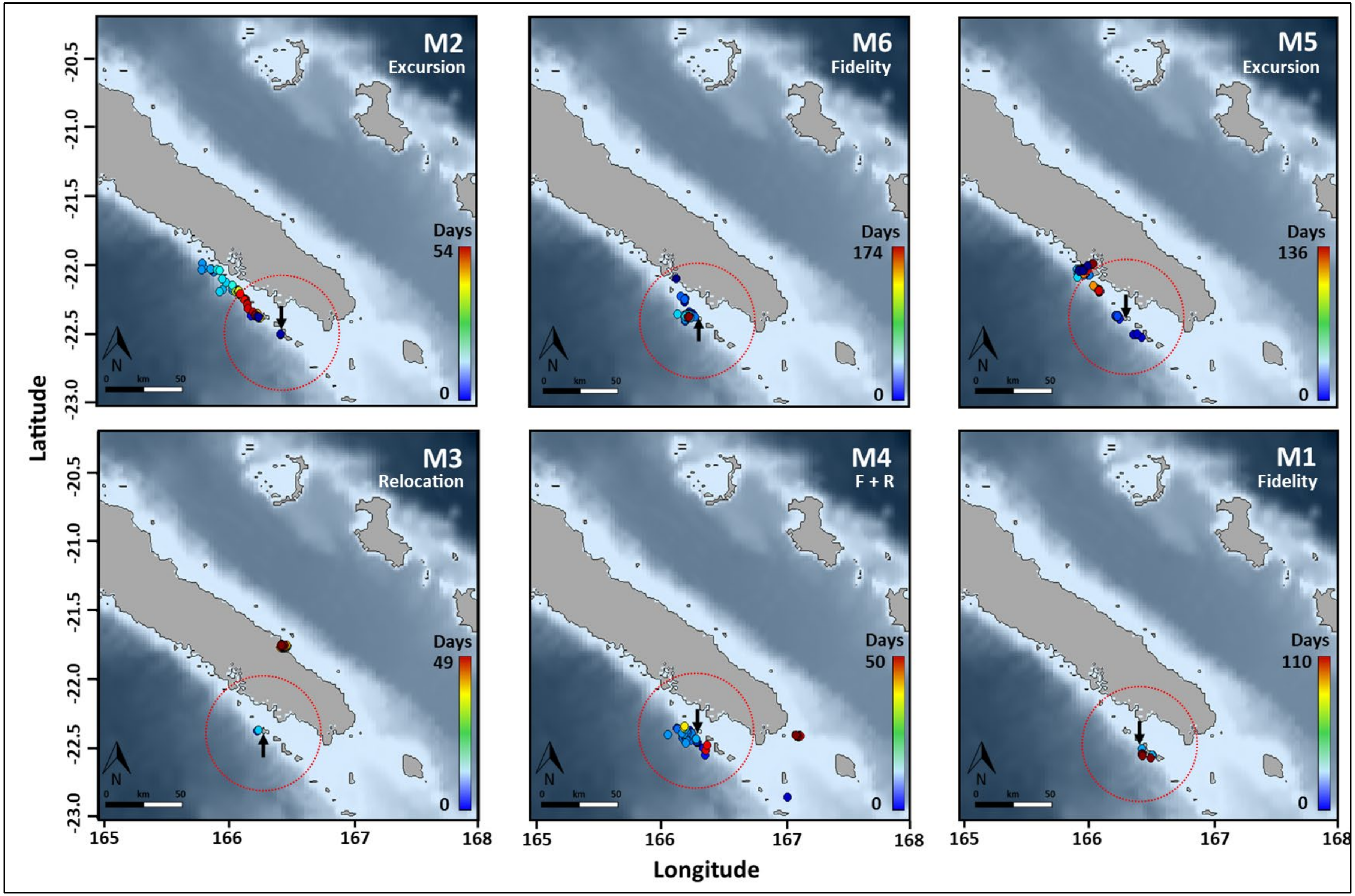


Figure S1. Horizontal movements of reef manta rays (Mobula alfredi) recorded using pop-up satellite tags (SPLASH10) in New Caledonia. Arrows indicates location of tag deployment. Red circle represents the home range area. *F + R* indicates the *Fidelity + Relocation* movement pattern.

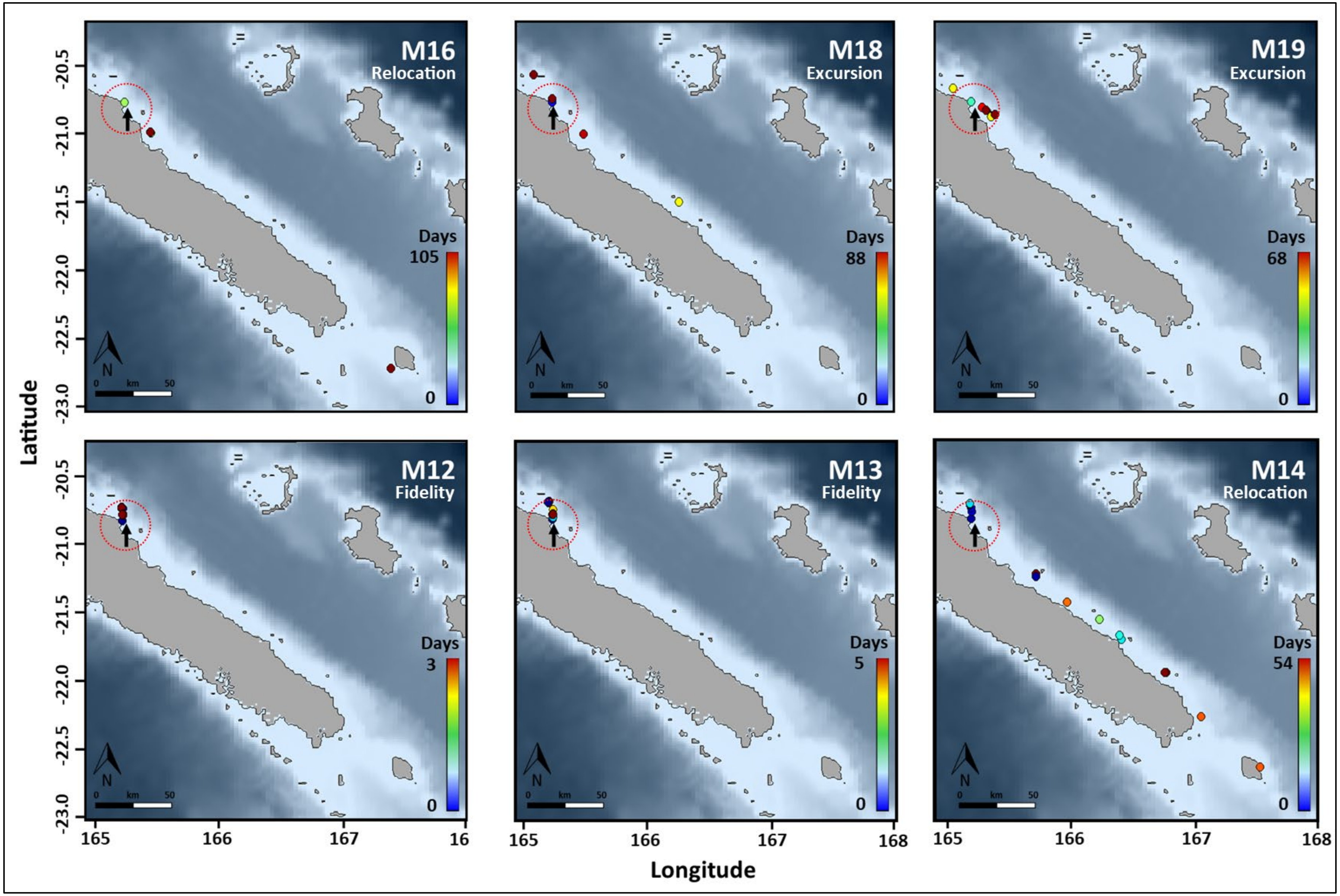
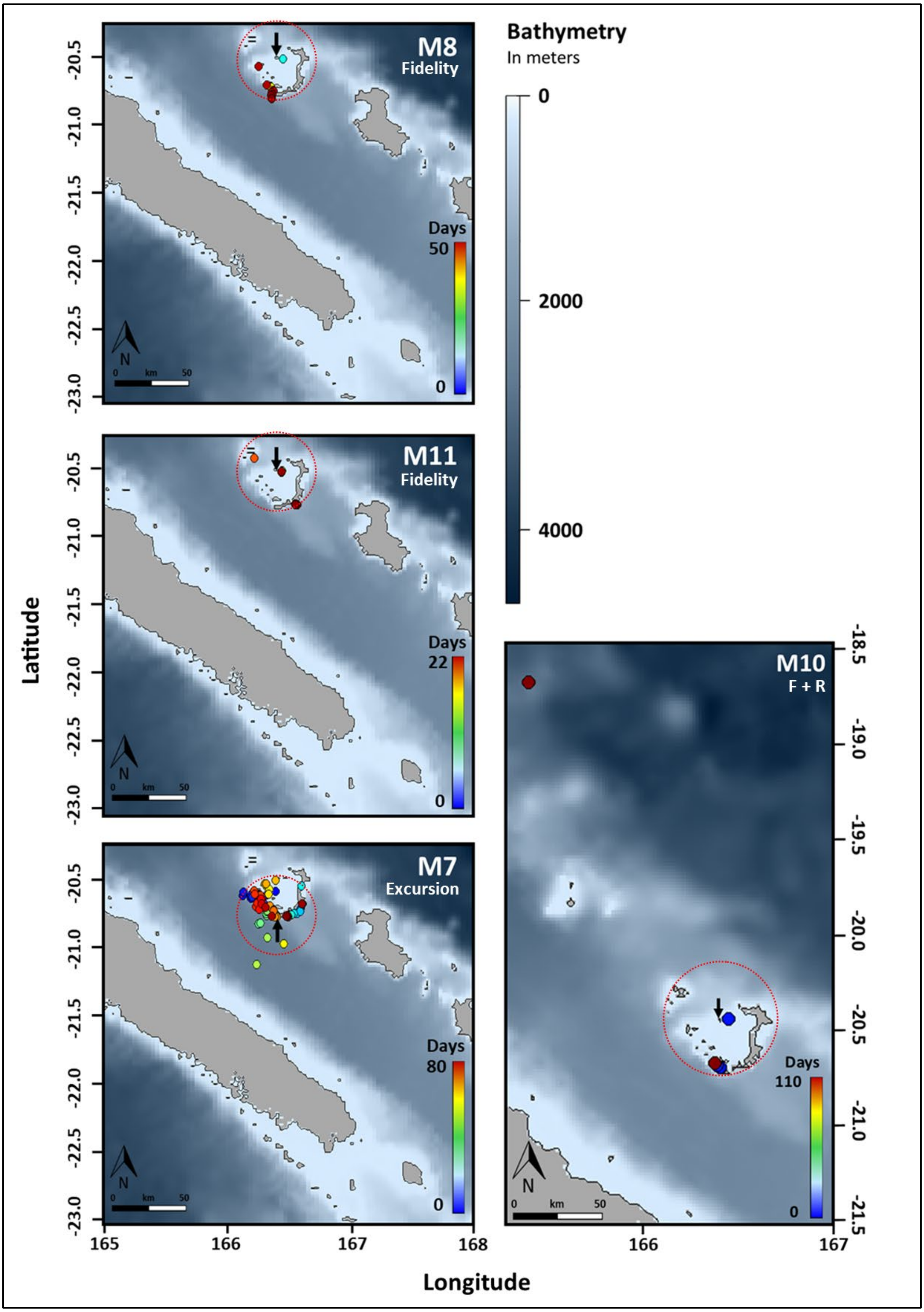


Figure S1 (continued).



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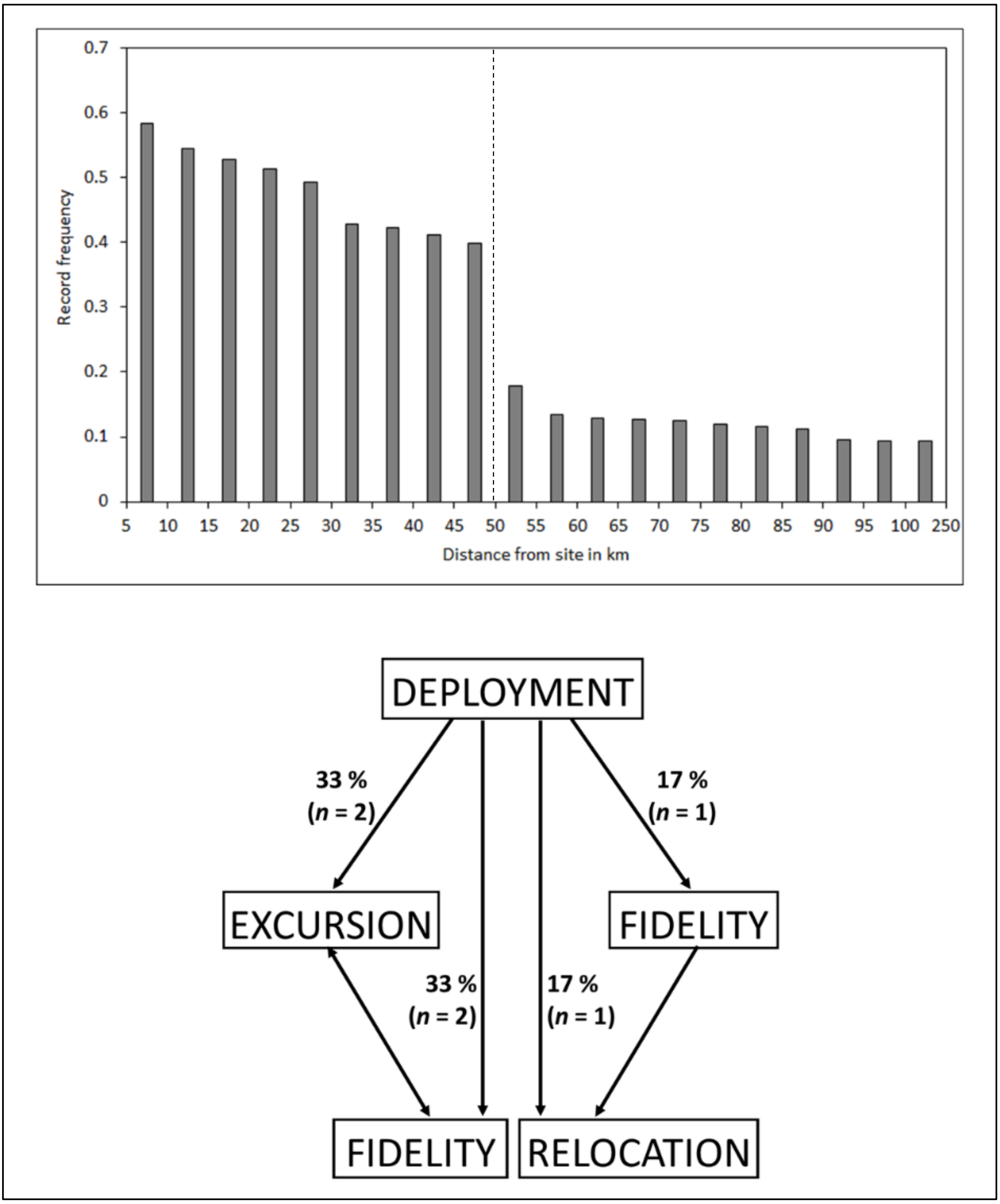

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Figure S2. Movement patterns recorded for reef manta rays (*Mobula alfredi*) after deployment of the tag, using satellite tag (SPLASH10), in Noumea, New Caledonia. Percentage based on the total number of individuals (*N* = 6). The Dashed line represent the home range limit.

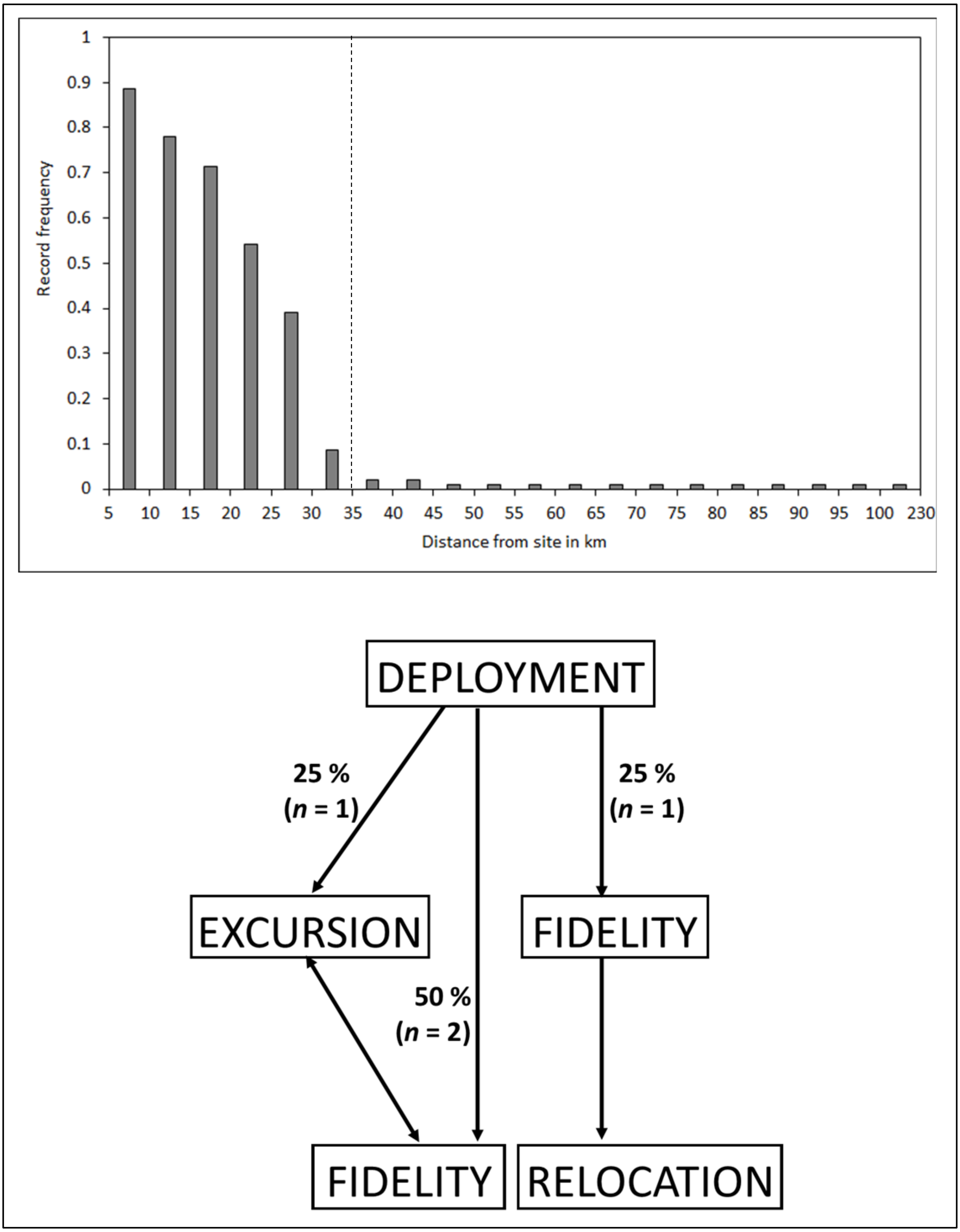

Figure S2. Continued. Ouvea (*N* = 4).

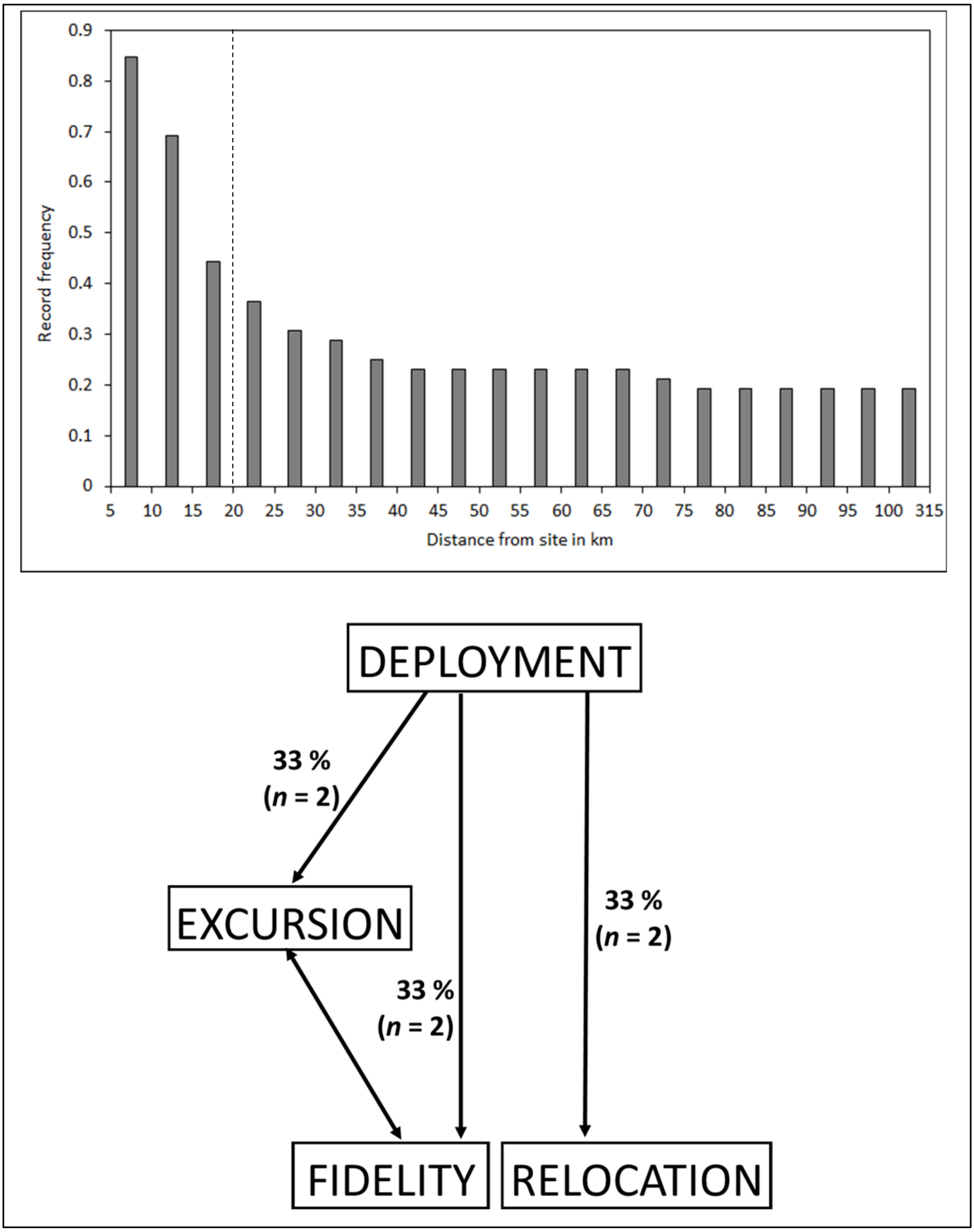

Figure S2. Continued. Touho (*N* = 6).

Table S4. Horizontal and vertical movement metrics for reef manta rays (*Mobula alfredi*) tracked using satellite tags in New Caledonia. Patterns are F : Fidelity, E : Excursion, F + R : Fidelity + Relocation and R : Relocation

Manta ID	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**	**M16**	**M17**	**M18**	**M19**
Individual information:																			
Site	Noumea	Noumea	Noumea	Noumea	Noumea	Noumea	Ouvea	Ouvea	Ouvea	Ouvea	Ouvea	Touho	Touho	Touho	Touho	Touho	Touho	Touho	Touho
Sex	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Female	Female	Male	Male	Male	Female
PTT	167754	167755	151348	151349	163079	167757	140916	196191	196192	196193	196194	162379	162380	167758	174667^{M}	182146	196864	196865	196866
Horizontal movements:																			
Pattern	F	E	R	F + R	E	F	E	F		F + R	F	F	F	R		R		E	E
Mean distance per day (km)	2.3	3.9	0.2	7.4	3.9	7.2	8.4	3.9	-	1.9	1.8	12.3	6.6	6.8	-	3	-	3.6	1.3
Total track length (km)	23.4	393.6	344.6	336	529.6	405.4	662	81.4	-	277.7	87.3	24.6	26.3	687.5	-	305.3	-	314.6	89.1
Latitudinal range (km)	34.1	215.9	7.1	57.5	58.9	71.2	68.7	32.8	-	224	37.4	11.7	15	56.8	-	212.4	-	101.3	22.7
Max. dist. from site (km)	10.1	86.1	249.7	96.3	51.8	33.2	43.7	34.4	-	225.1	30.2	11.8	15.3	311.4	-	304.5	-	131.5	22
Max. dist. from reef (km)	4.3	12.4	5.3	17	6.9	7.4	35	5.6	-	104.6	2.1	7	6.3	7	-	1.9	-	5.1	4.5
Vertical movements:																			
Maximum depth (m)	450	472	624	384	496	336	672	440	328	416	320	376	480	584	456	648	424	600	512
Deep dive frequency (days) *	15.6	13.3	8.0	7.0	11.3	43.3	2.3	10.5	9.7	26.0	49.0	3.0	1.3	5.9	11.3	3.9	0.6	2.2	4.8
Time at maximum depth (min)	13	10.1	-	6.5 - 9.4	10.8	18	2.16	7.2	8.64	2.16	3.6	1.25	14.4	13.68	2.9 - 7.2	3.6	10.1 - 6.5	9.36	4.32

* Frequency of dives below 300 m. ^{M} for MiniPAT tag, others are SPLASH10 tags. PTT, Platform Transmitting Terminal.

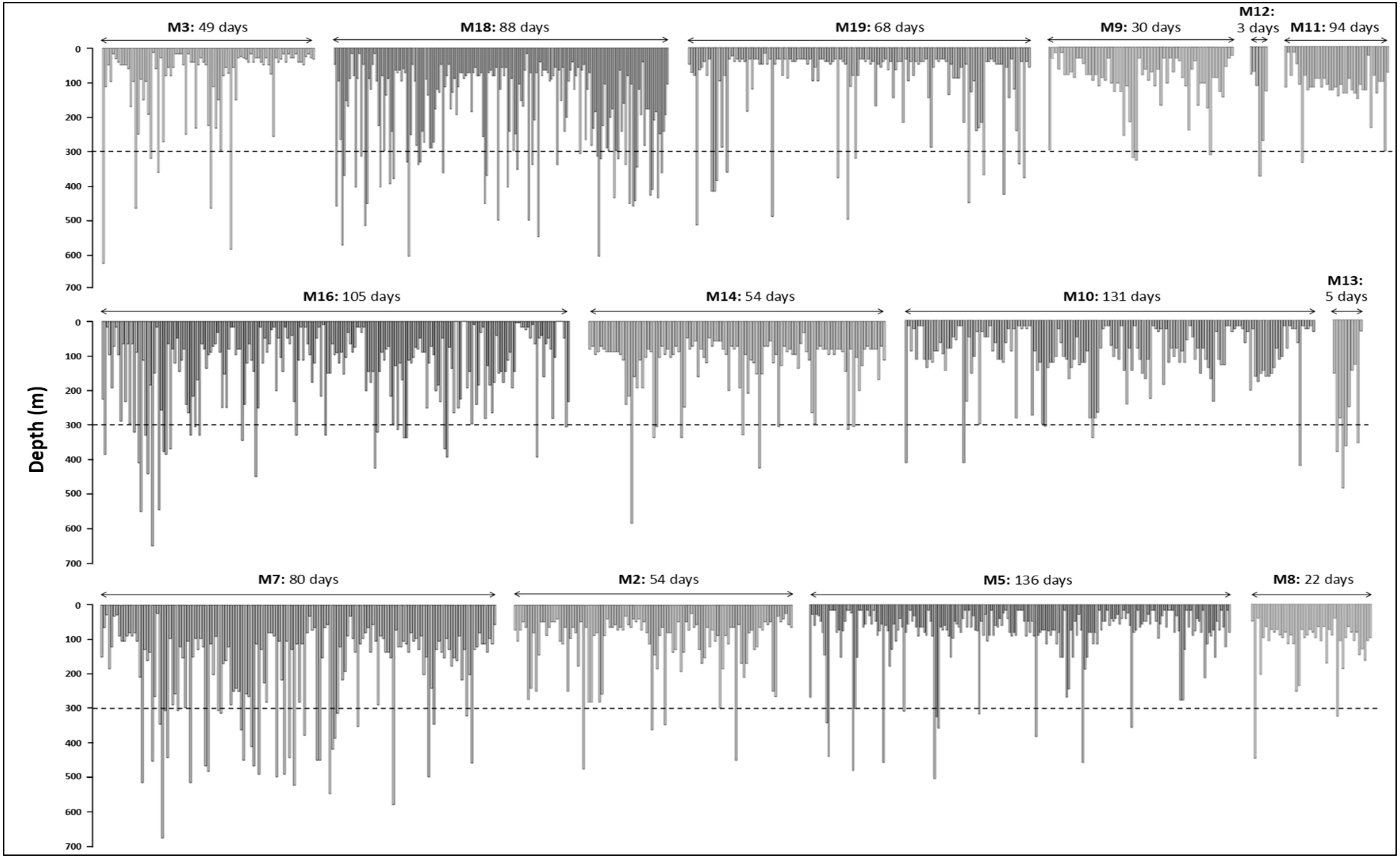

Figure S3. Daily maximum depth (m) for reef manta rays (*Mobula alfredi*) (*N* = 14) during deployment of pop-up satellite tags (SPLASH10) in New Caledonia. Days indicate the total duration of deployment for each individual (M)

Table S5. Z-test statistics of differences in proportion of dive below 300 m between movement patterns recorded for reef manta rays (*Mobula alfredi*) (*N* = 16) in New Caledonia. *P-values* are above the diagonals and *Z* statistics are below the diagonals.

Movement Patterns	*Relocation*	*Excursion*	*Fidelity*	*Fidelity + Relocation*
Relocation		0.18	0	0
Excursion	1.3		0	0
Fidelity	6.6	8.7		0.09
Fidelity + Relocation	3.3	4.5	1.7	

0 value indicates p < .0001.

Table S6. Kolmogorov-Smirnov (K-S) test statistics of differences in distributions of time spent at different depth ranges between movement patterns recorded for reef manta rays (*Mobula alfredi*) (*N* = 16) in New Caledonia. *P-values* are above the diagonals and *D* statistics are below the diagonals.

Movement Patterns	*Relocation*	*Excursion*	*Fidelity*	*Fidelity + Relocation*
Relocation		0.6974	0.9996	0.6974
Excursion	0.4		0.6974	0.6974
Fidelity	0.2	0.4		0.6974
Fidelity + Relocation	0.4	0.4	0.4	

Table S7. Kolmogorov-Smirnov (K-S) test statistics of differences in profiles of deepest dives of reef manta rays (*Mobula alfredi*) in New Caledonia. P-values are above the diagonals and *D* statistics are below the diagonals.

Individual		**M4**		**M6**	**M15**	
	Deepest dive	*A*	*B*	*A*	*A*	*B*
M4	*A*		0.0655	0	0.0114	0.0023
	B	0.125		0	0	0.0087
M6	*A*	0.1992	0.2201		0	0
M15	*A*	0.1311	0.164	0.2955		0
	B	0.1499	0.1132	0.1754	0.1938	

CHAPITRE 4

EVIDENCE OF FINE SCALE GENETIC STRUCTURE FOR REEF MANTA RAYS (*MOBULA ALFREDI*) IN NEW CALEDONIA [4]

ABSTRACT

Our understanding of the genetic connectivity of manta ray populations and the drivers that shape genetic structure is still limited. This information is crucial to identify the spatial boundaries of discrete populations and guide decisions on units to conserve. In this study, we use genome-wide single nucleotide polymorphisms (SNPs) to assess the genetic structure and diversity of reef manta rays (*Mobula alfredi*) at a local scale within New Caledonia and regionally in the western Pacific Ocean. We provide the first evidence of fine scale genetic differentiation in *M. alfredi,* found between the three cleaning station aggregation sites in New Caledonia (n=65) (N = 2676 SNPs, F_{ST} = 0.01, $p < 0.0001$). Furthermore, population structure was evident at the regional scale between individuals from New Caledonia (NC, n=73) and East Australia (EA, n= 19) on the basis of genetic differentiation statistics (3619 SNPs, F_{ST} = 0.096, $p < 0.0001$) and clustering algorithms, with unidirectional gene flow detected from east (NC) to west (EA). These results reveal that reef manta rays can form genetically distinct groups within a relatively small geographic range and highlights the need to consider genetic structure when designating management units for conservation action and planning.

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[4] Lassauce H., Dudgeon CL., Armstrong AJ., Wantiez L. et Carroll EL. (in review). Evidence of fine scale genetic structure for reef manta rays (*Mobula alfredi*) in New Caledonia. *Endangered Species Research.*

Keywords: Genetic Diversity ; Population Structure ; Management ; Conservation ; Genome-wide SNPs

INTRODUCTION

The metapopulation paradigm provides a useful framework to understand connectivity and population dynamics. In this context, local populations are viewed as discrete spatial entities that interact through gene flow and migration (Hanski & Gaggiotti 2004). Across species, the factors that influence the rate of gene flow between local populations are diverse and include dispersal or mobility, geographic distance between populations as well as life history traits (Allendorf et al. 2013). Environmental heterogeneity, and the resulting variation in habitat quality, is also a significant factor (Allendorf et al. 2013) as demonstrated by the impact of biogeographical barriers across different species: e.g., insects (Rabasa et al. 2008), amphibians (Smith & Green 2005), birds (Esler 2000), fishes (Schtickzelle et al. 2007) or mammals (Matthiopoulos et al. 2005). Such biogeographical barriers are frequent in the terrestrial environment and significantly partition the landscape creating a mosaic of ecosystems, such as forests, deserts, and grasslands (Briggs 1974).

One of the most challenging aspects of ecological studies is to identify population structure in apparently continuous ecosystems with no obvious physical barriers to dispersal, such as in the marine environment, and particularly for highly mobile species (Palumbi 1994). Over the past decade, studies have demonstrated that even when dispersal potential seems high, population structure can still occur in marine groups such as sharks (Geraghty et al. 2014, Vignaud et al. 2014), mammals (Andrews et al. 2010, Kershaw et al. 2017) and sea turtles (Roden et al. 2013, Read et al. 2015). In such cases, genetic population structure in the marine environment is shaped by an array of processes including oceanic conditions, currents, bathymetry, and geographical distances (Allendorf et al. 2013). Habitat choice, in particular site fidelity, is another key process that can drive population structure by limiting migration rates and encouraging assortative mating (Bowen et al. 2016). The main driver for site fidelity is often resource availability, as has been suggested for Hawaiian spinner dolphins (Andrews et al. 2010) and reef manta rays (Deakos et al. 2011, Couturier et al. 2014, Perryman et al. 2019).

To attain the key goal of conservation of protecting biodiversity by supporting the long-term persistence of viable, natural populations of wild species requires the identification of population units, and the geographic boundaries between them. This enables management and conservation programs to appropriately focus and prioritise efforts to maximize evolutionary potential and minimize extinction risks (Allendorf et al. 2013). Whilst the presence of population structures may increase genetic differentiation through local adaptation it may also lead to reproductive isolation and a reduction of genetic diversity (Futuyma 2019). Genetic isolation could diminish the resilience of a population or species and its capacity to adapt to a rapidly changing environment (Allendorf et al. 2013, Futuyma 2019). In addition, certain species are more vulnerable to extinction due to a combination of characteristics such as small population size (Pimm et al. 1988), limited geographic range (Gaston 1994), specialized habitat requirements (Brown 1995), and conservative life history traits (small litters, slow individual growth rate, late maturation, long inter-birth intervals, high survival rates, MacArthur & Wilson 1967). In the marine environment, these traits are found primarily, but not exclusively, in marine mammals and elasmobranchs.

The reef manta ray, *Mobula alfredi* (Krefft, 1868)*,* is a *K*-selected species and displays several traits that make it vulnerable in a changing environment (MacArthur & Wilson 1967). These characteristics include a large body size, a long-life expectancy, a low fecundity (Couturier et al. 2012), an often fragmented distribution (Couturier et al. 2011, Marshall et al. 2011, Rohner et al. 2013), and small local population sizes as estimated in East Australia (Couturier et al. 2014), Hawai'i (Deakos et al. 2011), Japan (Kashiwagi 2014), or Mozambique (Marshall et al. 2011). As planktivores, these animals depend on specific environmental conditions and processes that shape their preys' abundance and distribution (Rohner et al. 2013, Couturier et al. 2014). Food resource availability has been hypothesised to be the main driver of manta ray movements, often resulting in aggregations from a few to hundreds of individuals at feeding grounds and cleaning stations (Anderson et al. 2011, Couturier et al. 2011). Patterns of seasonal or year-round aggregations have been found to differ with locations. On the one hand, seasonal migrations were correlated with monsoonal shifts and productivity in Indonesia (Dewar et al. 2008), in the Maldives (Anderson et al. 2011, Kitchen-Wheeler et al. 2012, Harris et al. 2020) and in East Australia (Jaine et al. 2012, Couturier et al. 2014). Large-scale movements occur along continuous continental coastlines (up to 1150 km, Armstrong et al. 2019) and between island chains (Germanov & Marshall 2014). On the other hand, physical barriers such as open expanses of sea and deep-water channels are thought to be a factor that may reduce the chance of reef manta rays transiting even in between geographically close aggregation sites (e.g., 150 km, Deakos et al. 2011). Long-term residence patterns (spanning years to decades) have been recorded for reef manta rays at sites across the globe including: West Australia (Armstrong et al. 2020), East Australia (Couturier et al. 2011, 2014, Jaine et al. 2014), Hawai'i (Deakos et al. 2011), Mozambique (Marshall et al. 2011), the Red Sea (Braun et al. 2015), British Indian Ocean Territory (Andrzejaczek et al.

2020) and Indonesia (Setyawan et al. 2018, Setyawan et al. 2020). Such site-fidelity could leave reef manta rays even more exposed to anthropogenic pressure if activities such as targeted fishing and bycatch, coastal development, or unmanaged tourism occur at critical habitats (Anderson et al. 2011, Couturier et al. 2011, 2014, Stewart et al. 2018). Listed as Vulnerable to Extinction on the IUCN Red-List (Marshall et al. 2018), reef manta rays have been increasingly targeted by fisheries due to the high value of their gill-plates on the Asian market (Couturier et al. 2012, O'Malley et al. 2016). Globally, the last IUCN assessment reports a suspected population reduction of 30 – 49 % over the past three generation with further reduction predicted over the future generations (Marshall et al. 2018). More information is needed on population structure and the nature of the drivers that shape the movements and connectivity of manta rays to face these conservation challenges. Such information is essential to define management units and prioritize management efforts, for example the establishment of protected areas for genetically isolated groups.

Molecular tools provide opportunities for identifying species boundaries, patterns of gene flow, genetic diversity and spatial structure in elasmobranchs (Ovenden et al. 2018). To date, studies on manta rays using molecular tools have focused primarily on taxonomy (Kashiwagi et al. 2012, Hinojosa-Alvarez et al. 2016, White et al. 2018, Hosegood et al. 2020b). In the first application of genomic tools to examining population structure in manta rays, Stewart et al. (2016) applied ddRAD sequencing methods and found significant population structure in oceanic manta rays (*M. birostris*) at large geographic scales within the Indo-Pacific region. However, a more recent examination of a wider subset of *M. birostris* samples by Hosegood thesis (2020b), failed to find any population structure and suggested global panmixia for the species. This species is a close relative of *M. alfredi* and occupies mostly pelagic environments, demonstrating greater migration distances than reef manta rays (up to 1400 km, Hearn et al.

2014). In reef manta rays, genomic studies demonstrated population structure at large scales between the Indian and Pacific oceans (Hosegood et al. 2020a) and between regions separated by the Indian Ocean basin (Venables et al. 2020). To date, only one study has used genomic methods to investigate fine-scale population structure in reef manta rays and did not find significant genetic structure between reef manta rays sampled at aggregation sites separated by less than 100 km (within Mozambique) or less than 1000 km (between Mozambique and South Africa) (Venables et al. 2020). Molecular tools have also been used to assess structure in ecologically similar species: large filter-feeding elasmobranchs such as whale sharks (Vignaud et al. 2014) and basking sharks (Hoelzel et al. 2006), finding low levels of genetic differentiation among ocean basins.

Here we apply genomic methods to infer population structure and connectivity of reef manta rays (*M. alfredi*) at a local scale across New Caledonia, and compare them regionally within the Western Pacific Ocean to East Australia. The coastal waters of New Caledonia are listed as a UNESCO World Heritage Site, with six marine clusters representing a total of almost 16,000 km^2 of reefs, lagoons, and mangroves. Along with the Natural Park of the Coral Sea (1,292,967 km^2) these protected areas are part of an essential management and conservation process that integrates threatened species (GNC 2018).

In New Caledonia, reef manta rays are observed at different aggregation sites, used as cleaning stations or feeding grounds, along the outer slopes of the barrier reef of the archipelago (Fig. 1). These sites are either connected with continuous habitats, such as along coastlines and the barrier reef, or on isolated reefs and islands separated from other sites by deep open oceanic waters (> 2000 m). Preliminary observations using photo-identification (photo-ID) suggest that site fidelity is high but differs among sites, with some movements of individuals between sites (up to 330 km apart, H. Lassauce unpubl. photo-identification and satellite telemetry data). In the present study, we use single nucleotide polymorphisms (SNPs) from genotyping by sequencing (Kilian et al. 2012) to assess the genetic structure and diversity of the population of reef manta rays in New Caledonia. Specifically, we compare the genetic differentiation at a regional scale, with the inclusion of samples from East Australia, and at a local scale, levels of heterozygosity among sites in New Caledonia. In doing so, we provide the first estimates of genetic structure and diversity for this species in New Caledonia.

METHODS

Study sites

This study was conducted at four sites around New Caledonia, an archipelago principally consisting of a larger Main Island and three smaller islands approximatively 100 km off the east coast known as the Loyalty Islands (Fig. 1). The Main Island is surrounded by a 1600 km barrier reef that shelters the shallow waters of a 16874 km² lagoon (Andréfouët et al. 2009). The continental shelf ends at the barrier reefs, where it drops to depths greater than 2000 m. Bathymetry around the Loyalty Islands is similar, with a relatively narrow continental shelf and a deep channel (> 2000 metres) between the Loyalty Islands and the Mainland (Fig. 1).

Reef manta rays were sampled at three sites off the Main Island: Noumea in the South and Pouembout in the North are located on the west coast, while Touho is in the northern part of the east coast. One site, Ouvea, is located on the northern island of the Loyalty Islands (Fig. 1). At all sites, except Pouembout, manta rays are observed primarily on the reef slope (between 10 to 15 m deep) near reef channels, these sites are cleaning stations. In Pouembout, samples were collected opportunistically from an aggregation of manta rays that were found at the surface in shallow waters (< 12 m), feeding in nutrient-rich waters of the lagoon.

Additional specimens from two sites in Queensland, Australia (hereafter referred to as “East Australia”), were included in the study to serve as a reference group when determining the genetic population structure within New Caledonia.

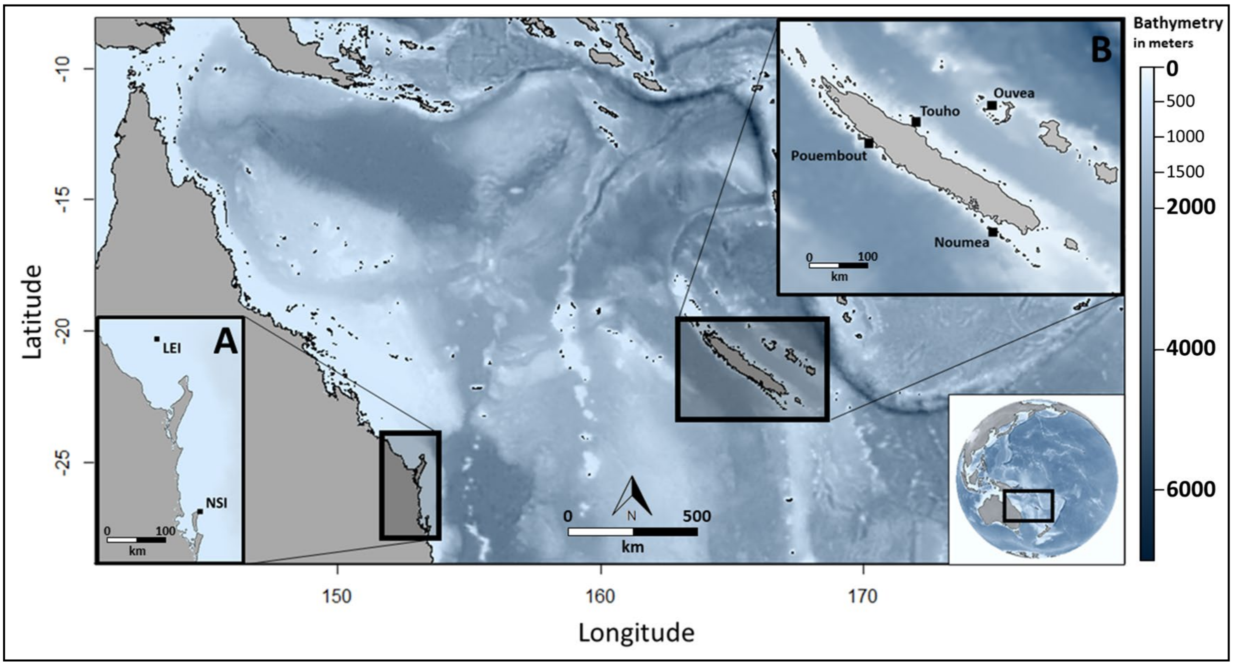


Figure 1. Sampling locations of reef manta ray (*Mobula alfredi)* within, inset A: Queensland, Australia (LEI: Lady Elliot Island, n = 9; NSI: North Stradbroke Island, n = 11) and, inset B: New Caledonia (Noumea, n = 24; Pouembout, n = 8; Ouvea, n = 18; Touho, n = 23).

Sampling Methods

Tissue samples were collected from the pectoral fin of reef manta rays using a biopsy tip attached to a 2-meter spear pole (Pneudart, Inc., Stewart et al. 2016). Sampling was conducted via either SCUBA diving or free diving, and samples were stored in 95-100% ethanol at -20°C. All individuals sampled were photo-identified and designated as either an adult male (on the basis of observation of fully developed claspers), adult female (on the basis of observations of mating scars or pregnancy), or a juvenile (defined by not fully developed claspers or the absence of mating scars). The exception to this were manta rays sampled from Pouembout, where the high turbidity of the water did not allow for photo-identification or confident observations of sex to be made. Sampling information is summarized in Table S1.

Laboratory Procedures

Genomic DNA was extracted from samples using a Qiagen DNAeasy Blood & Tissue Kit following the manufacturer’s instructions. DNA was quantified using the Qubit dsDNA Broad range assay (Thermo Fisher Scientific, CA, USA) and then diluted with Qiagen AE buffer to 50 ng/µL for use in genotyping by sequencing.

Development and genotyping of SNPs for all samples was undertaken following the standard DArTseq^{TM} protocol (Kilian et al. 2012). DArT (Diversity Arrays Technology Pty Ltd, Canberra, AUS) is a genotyping-by-sequencing platform that uses enzymatic double digest genome complexity reduction following Sansaloni et al. (2011) and next-generation sequencing on the Illumina HiSeq 2500 platform as described by George et al. (2018). In this study, the restriction enzymes *PstI* and *SphI* were used for the double digest of genomic DNA.

Data Analysis

Data quality check and cleaning

Sequencing reads were processed using proprietary DArT analytical pipelines (Jaccoud et al. 2001, Kilian et al. 2012). In the primary pipeline poor-quality sequences are filtered, applying more stringent selection criteria to the barcode region in comparison to the rest of the sequence. Identical sequences were then compiled into .fastq files and cleaned through the secondary pipeline using the DArT algorithm (DArTSoft14^{TM}, Kilian et al. 2012). This pipeline workflow first calls sequence clusters for all pooled samples and then for each individual. The DArT pipeline retained SNPs on the basis of the balance of average count for each SNP allele, the reproducibility values (>95%), the average count for each sequence or row sum (sequencing depth : ≥5) and the call rate (proportion of samples for which the marker is scored : >95%). Potential contamination was identified by comparing all reads to bacterial and viral sequences from GenBank and a custom DArT database. Lastly, we applied the following filters to generate the final dataset: <5% of individuals with missing data (call rate of >95%), average reproducibility of alleles at locus (>95%), across SNPs that share a sequence tag, only one random SNP was retained per locus, and monomorphic loci were removed. Individuals with a call rate of less than 80 % were also removed. The fine scale analysis of structure focused on the cleaning station sites, to the exclusion of Pouembout. This is because the relationship between cleaning and feeding aggregations is unclear, and feeding aggregations could contain manta rays from multiple cleaning stations and vice versa, decreasing any population genetic signals. Two datasets were generated, one including the outgroup (East Australia), referred to as the regional dataset, and the other excluding this outgroup called the local dataset (New Caledonia only). For the analysis of genetic diversity only, loci with Minor Allele Frequencies (MAF) > 0.05 were filtered out of these two datasets. All filters were applied using the “gl.filter” functions of the R package “dartR” (Gruber et al. 2018).

Genetic diversity

We calculated genetic diversity for each sampling site in New Caledonia, for New Caledonia as a whole, and for East Australia using the statistical programming language R version 3.6.2

(R Core Team, 2019). Specifically, we estimated observed (H_O) and expected (H_E) heterozygosity (Nei 1987) with the function "gl.basic.stat" of the R package "dartR" (Gruber et al. 2018). The inbreeding coefficient (F_{IS}) (Weir & Cockerham 1984) for each sample partition was also calculated using the "gl.basic.stat" function. The rarefacted allelic richness (A_R) was estimated for each sample partition using the function "allelic.richness" from the "adegenet" package in R (Jombart, 2008). Since sample partitions are different in sample size, the function uses the minimum number of individuals n = 19 for the regional dataset and n = 18 for the local dataset. Differences in the diversity between sample partitions were tested using analysis of variance (ANOVA) and t-tests (equal variance) or Welch's t-tests (unequal variance). Levene's tests were used to test the homogeneity of variances and a Shapiro–Wilks test assessed the normality of the data.

Population differentiation and structure

We inferred genetic differentiation and population structure at a regional scale between East Australia and New Caledonia and then at a local scale among our study sites. We also compared the genetic diversity at both, regional and local, scales.

Firstly, we estimated pairwise genetic differentiation indices for sample partition (F_{ST} and Nei's genetic distance D), and statistical significance through permutation tests (10,000) (Wright 1949), using the R package "stamppFst" function within the "STAMPP" (Pembleton et al. 2013).

We conducted genetic clustering using two complementary methods: Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010) and the program TESS3 (Caye et al.

2017). The outcome of the DAPC depends on the number of principal components (PCs) used in the analysis, and the number of clusters in the analysis. To assess the number of PCs to be used, we undertook an examination of the mean alpha score (difference in reassignment probabilities between observed and permuted clusters) to determine the appropriate number of PCs for each dataset, using the function “a.score” and “optim.a.score” of the R package “adegenet” (Jombart 2008). The outcomes of the two methods used to determinate the appropriate number of PCs were compared. First, alpha scores were calculated using the optimized-simulation-based method. This method generates an optimum number of PCs determined by the highest mean alpha-score generated through simulation. Second, alpha scores were calculated using the highest-recommended-number of PCs (N/3) method recommended by the developers of “adegenet” (Jombart 2008). An a-score close to one is a sign that the DAPC solution is both strongly discriminating and stable, while low values (toward 0) indicate either weak discrimination or instability of the results. The decision on the most appropriate method to choose the appropriate number of PCs was based on the examination of the mean a-score values for each population. The DAPC was then performed with the “dapc″ function of the R package “adegenet” (Jombart 2008) without assigning individuals to clusters a priori. This method derives a number of potential populations from the analysis and produces membership probabilities for each sample based on the data from all samples. Classification of a given sample to a cluster is based on the highest assignment probability. The expected classification rate for each population (prior) was compared to the number of correct classifications (posterior) generated using the DAPC.

Population genetic structure was further assessed using Bayesian clustering analysis of individual genotypes with the “TESS3R” package (Caye et al. 2016) in R to estimate individual ancestry coefficient assuming admixture of K ancestral genetic clusters in our data set. TESS3 implements a spatially explicit Bayesian clustering admixture model. By including information on individual geographic coordinates, TESS tends to perform better than nonspatial models implemented in programs using a similar algorithm such as STRUCTURE (Pritchard et al. 2000), when the ancestral level of differentiation is low (Durand et al. 2009, Francois & Durand 2010). It also performs better when the distribution of the studied species tends to be continuous with individuals geographically close and more prone to share ancestral genotypes (Durand et al. 2009). In the latter case, individuals are assigned to the most likely cluster on the basis of multi-locus genotypes sampled at distinct geographical locations without assuming predefined populations (Durand et al. 2009). Here, specified spatial prior is weak compared to the amount of information contained in the molecular dataset, and therefore, deviations from spatial smoothness are still allowed in the posterior inference (Corander et al. 2008). We added UTM coordinates for each individual and ran twenty replicates of the admixture model for each value of the maximal number of clusters (*K)*. The best value of *K* was examined from 1 to 10 with a maximum number of 10,000 iterations per run (20 repetitions) and a tolerance value of 10^{-7}. A cross-validation procedure with 10% of masked data was used to select the best value of K according to the asymptote in the plot of cross-validation scores (Caye et al. 2016). Given that fine scale population structure has not previously been described for this species, we did not have an a priori expectation of multiple genetic clusters within New Caledonia, and performed the analysis at both the local and regional scale. The analysis was processed for K-values from 2 to 5, although only the results for K = 2 and K= 4 are presented as only these clustering results warrant a biological interpretation.

Gene flow analysis

Relative directional migration rates were analysed using the “divMigrate” function of the “diveRsity” R package (Sundqvist et al. 2016). This function gives relative migration rates proportionally to the highest migration rate between two sites, which always equals one. We compared three differentiation metrics: “d” (Jost's D), "gst" (Nei's Gst), and "Nm" (Alcala et al. 2014). Migration rates were estimated with 10,000 bootstraps, and directional gene flow estimates with a rate under 0.2 were filtered out of the visual outcome. We used samples from New Caledonia (Noumea, Touho and Ouvea) and from East Australia for this analysis.

RESULTS

SNP dataset

The regional dataset initially comprised a total of 5148 bi-allelic SNP loci from 73 reef manta rays from four locations in New Caledonia and 20 individuals from East Australia (Table 1). One individual (MA138) from East Australia was removed due to a call rate of less than 80%. The local dataset had a total of 3973 bi-allelic SNP loci from 65 reef manta rays from three locations in New Caledonia. One of the sites (Pouembout) was removed from the fine scale analysis due to the small sample size and difference in habitat type compared with the rest of the sites (*n*=8). After the filtering, the total number of polymorphic SNP loci was 3619 for 92 individuals in the regional dataset and 2676 for 65 individuals in the local dataset. All filtering steps are summarized in Table 1.

Table 1. The number of single nucleotide polymorphism (SNP) loci initially found (Initial SNP dataset) using DArT sequencing in reef manta ray (*Mobula alfredi*) samples from East Australia and New Caledonia (Regional dataset) and only New Caledonia (Local dataset). Also shown is the number of loci and individuals retained as different quality control (QC) filters are applied to the dataset. The final datasets used in the subsequent analyses are shown in position 4.

	Regional dataset			**Local dataset**		
	Loci retained	Loci filtered	No. of individuals	Loci retained	Loci filtered	No. of individuals
Initial SNP dataset	5124	0	92	3973	0	65
Filter applied :						
1. < 5 % missing data	4454	670	92	3482	491	65
2. > 95 % repeatability	3680	774	92	2717	765	65
3. Remove secondaries	3619	61	92	2676	41	65
4. Remove monomorphic loci	3619	0	92	2676	0	65
In separate datasets :						
5. Minor Allele Frequency > 0.05	1990	1629	84	1843	833	73

Genetic diversity

Genetic diversity statistics for all sample partitions are shown in Table 2. The expected heterozygosity (H_E) was significantly higher for the east Australian outgroup ($t_{3978} = 6.35$, $p < 0.001$) compared with the overall New Caledonian dataset, while no significant difference was observed for the allelic richness (A_R) ($t_{3978} = 0.76$, $p > 0.05$) (Table 2). When considering the local dataset, there were no significant differences in any genetic diversity statistics between sample partitions within New Caledonia ($F = 0.05$, $p > 0.5$, Table 2).

Inbreeding coefficients (F_{IS}) are relatively high at all levels of population structure and all significantly different from zero ($p < 0.001$) (Table 2). At the sample site level, the average proportions of heterozygosity in individuals are significantly different ($t_{3816} = 2.18$, $p < 0.05$) between samples from East Australia ($F_{IS} = 0.0662 \pm 0.03020$) and individuals from all groups of New Caledonia in the regional dataset ($F_{IS} = 0.0844 \pm 0.02093$). Among groups from New

Caledonia, values ranged from 0.0699 ± 0.02762 in Noumea to 0.0812 ± 0.02918 in Ouvea with no significant differences among sites in the local dataset ($F = 0.93$, $p > 0.05$).

Table 2. Genetic diversity indices (± SD) of reef manta rays (*Mobula alfredi*) observed in regional and local datasets (MAF > 0.05) using 1990 and 1629 SNPs, respectively. n: sample size; m: number of males; f: number of females; un: number of individuals for which sex is unknown; A_R: allelic richness, H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: inbreeding coefficient.

	n/m/f/un	A_R	H_O	H_E	F_{IS}
Local dataset					
Noumea	23/8/15/-	1.9082 ± 0.1888	0.2879 ± 0.1698	0.3080 ± 0.1467	0.0699 ± 0.2762
Ouvea	18/5/13/-	1.9133 ± 0.1949	0.2819 ± 0.1687	0.3070 ± 0.1472	0.0812 ± 0.2918
Touho	24/10/14/-	1.9079 ± 0.1824	0.2844 ± 0.1664	0.3052 ± 0.1450	0.0702 ± 0.2805
Overall		1.9098 ± 0.1888	0.2847 ± 0.1683	0.3067 ± 0.1463	0.0717 ± 0.2828
Regional dataset					
New Caledonia	73/25/45/3	1.8815 ± 0.2072	0.2659 ± 0.1533 [a]	0.2878 ± 0.1447 [a]	0.0844 ± 0.2093 [a]
East Australia	19/-/-/19	1.8872 ± 0.2616	0.2971 ± 0.1897 [a]	0.3186 ± 0.1613 [a]	0.0662 ± 0.3020 [a]
Overall	92/25/45/22	1.8843 ± 0.2359	0.2815 ± 0.1731	0.3031 ± 0.1540	0.0710 ± 0. 2587

[a] indicates statistically significant difference between New Caledonia and East Australia values within the same column.

Population differentiation and structure

Pairwise F_{ST} and Nei's genetic Distance D

Statistically significant genetic differentiation was observed between all sampling sites (Table 3). The highest estimates of genetic differentiation are found between East Australia and New Caledonia ($F_{ST} = 0.0958$ ±SD 0.006, $p < 0.001$, Table 3). The degree of differentiation between New Caledonian sampling sites was lower but still statistically significant (pairwise $F_{ST} = 0.0116$ - 0.0165, Table 3). Overall F_{ST} values for the regional and local dataset are provided in Table 3. Nei's genetic distances followed the same pattern as the fixation indices.

Table 3. Pairwise F_{ST} values (above diagonal, with 95 % CI) and Nei's Genetic Distance (below diagonal) calculated for reef manta rays (*Mobula alfredi*) for regional and local datasets using 3619 and 2676 SNPs, respectively. n: sample size

Local dataset	n	Noumea	Ouvea	Touho
Noumea	23	-	0.0165 (0.0137 – 0.0194)	0.0155 (0.0131 – 0.0181)
Ouvea	18	0.0126	-	0.0116 (0.0089 – 0.0143)
Touho	24	0.0112	0.0110	-
Overall F_{ST}	65			0.0145

Regional dataset	n	New Caledonia	East Australia
New Caledonia	73	-	0.0958 (0.0896 – 0.1022)
East Australia	19	0.0270	-
Overall F_{ST}	92		0.0958

All F_{ST} values are significant p < 0.001.

Discriminant Analysis of Principal Components (DAPC)

Examination of the mean a-score values revealed a higher standard deviation in the optimum number of PCs, suggesting that the assignment of any individual to a population might be highly unstable (Fig. S1, Table S2, Fig. S2, Table S3). Thus, the appropriate number of PCs was 30 for the regional dataset and 21 for the local dataset, following the highest-recommended-number (N/3) method.

The DAPC showed individuals clustered into their country and sampling site of origin with high confidence (Table S4). Considering the regional dataset first, DAPC revealed a clear distinction across all samples from East Australia and New Caledonia, with PC1 providing clear discrimination (Figure 2). Using the classification algorithm, all individuals had a 100% probability of being assigned back to their country of origin (Table S5).

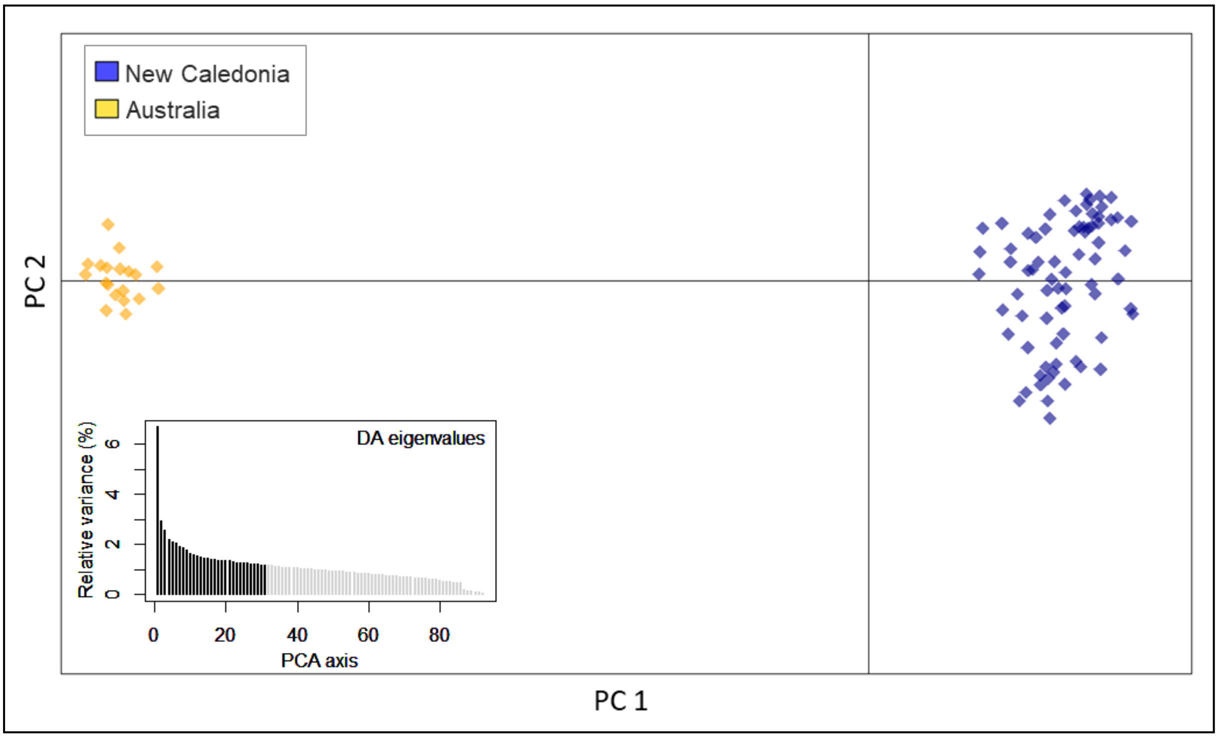


Figure 2. Inference of population structure of reef manta ray (*Mobula alfredi*) from East Australia and New Caledonia (regional dataset) using 3619 SNPs: scatter plot of individuals based on the first two principal components (PC) of the Discriminant Analysis of Principal Components (DAPC).

Considering the local dataset, the first of the two eigenvalues of DA represented most of the variation within this dataset (Fig. 3). The DAPC discriminated sites along both the north-south (PC1, discriminating Noumea) and east-west axes (PC2, differentiating Ouvea and Touho). Within New Caledonia, the highest assignment probabilities correspond to the population of origin for each pre-defined group (Table S5, Fig. S3). The average correct classification probability was highest for a sample from Noumea (94.12 ± 13.84), then Touho (85.43 ± 27.23), followed by Ouvea (79.79 ± 30.71) with only three (12 %) individuals per site likely to be incorrectly classified (Table S5, Fig. S3).

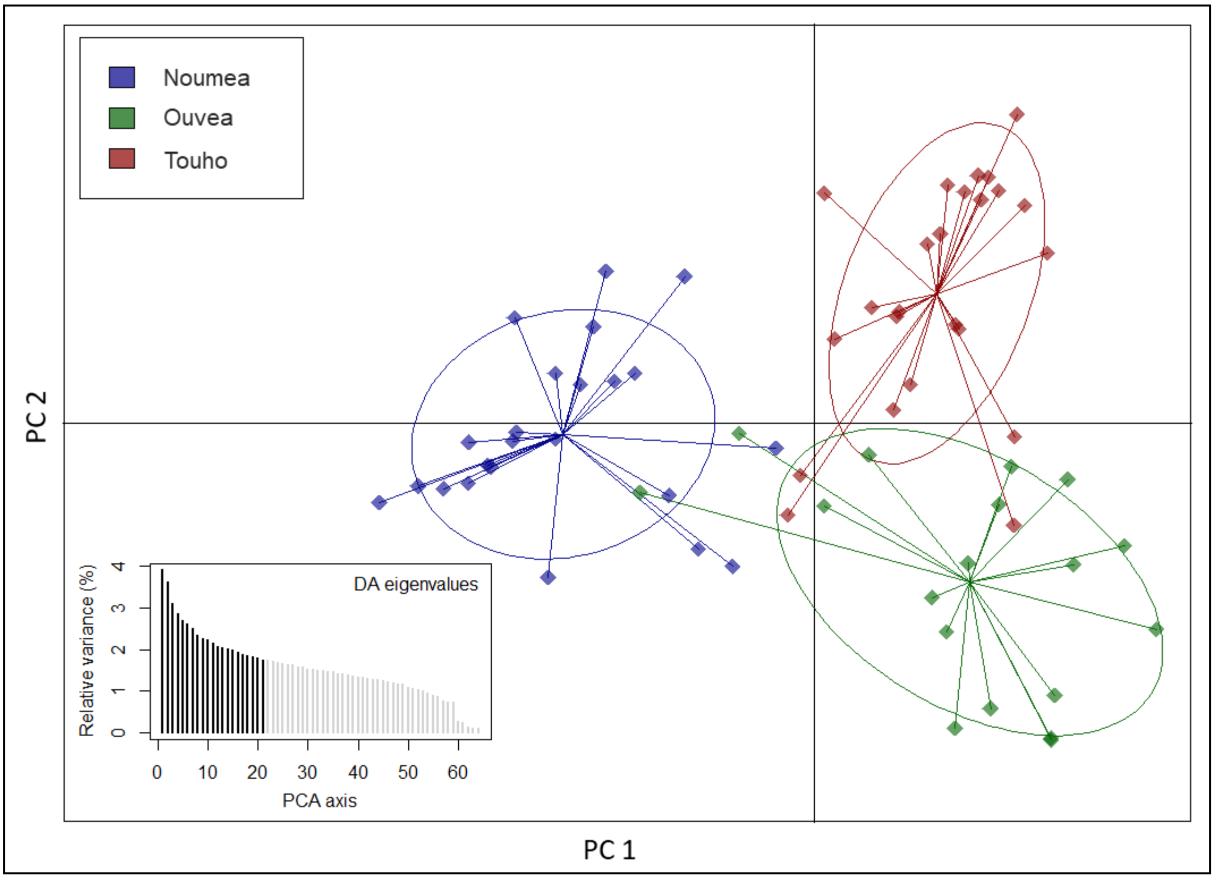


Figure 3. Inference of population structure of New Caledonian reef manta rays (*Mobula alfredi*) (local dataset) using 2676 SNPs: scatter plot of individuals based on the first two principal components of the Discriminant Analysis of Principal Components. The inertia ellipses describe the expected spread of genotype positions assuming a bivariate normal distribution.

Estimation of spatial structure: TESS3 analysis

For the regional dataset, the TESS3 analysis revealed the most likely number of ancestral populations/genetic clusters or best value of *K* as four (the cross-validation test exhibited a minimum value or a significant plateau at K=4) (Fig. S4), supporting subtle levels of population structure (Caye et al. 2016). At *K*=2, a strong distinction is revealed between Australian individuals and all the ones from New Caledonia with distinct admixture compositions with 2-5 % and 0-9 % ancestry proportions of each other's dominant cluster, respectively. At *K* = 4, each site has its own distinct admixture composition, with a dominant ancestral population (Fig. 4). On average, New Caledonia individuals have less than 1.4 ± 0.003 % ancestry proportions of the dominant East Australia cluster. The dominant ancestral populations comprise, on average, between 76 ± 0.006 % (Ouvea) and 84 ± 0.008 % (East Australia) of ancestry proportion per individual.

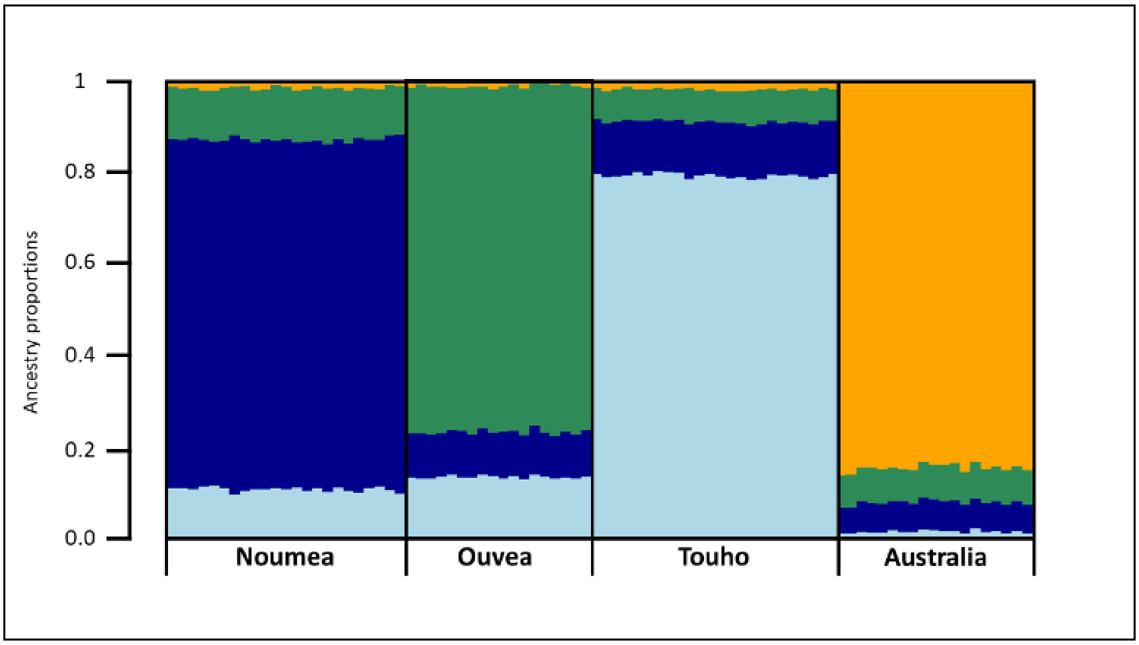


Figure 4. Individual admixture compositions of population structure inferred using TESS3 with $K = 4$, based on 3619 SNPs from 85 reef manta ray (*Mobula alfredi)* individuals across four sites with eighteen to twenty-four individuals per site. Black vertical lines separate the sample sites. Colours indicate ancestral populations.

Gene flow analysis

Relative directional migration rates were estimated using three differentiation metrics, but, as patterns were consistent, only Jost's *D*'s results are presented (Fig. 5). The values displayed in the figures do not represent the magnitude of each migration (as a measurement of the proportion of migrants per generation would) but compare migration rates proportionally to the highest, which always equals one.

The relative migration rates between East Australia and all New Caledonian sites are low but show statistically significantly higher migration rates from New Caledonia to East Australia than vice versa. Considering the local dataset, the highest bi-directional migration rate was found between Ouvea and Touho. Comparatively, the relative migration rates between Noumea and both Ouvea and Touho are slightly lower but also bi-directional.

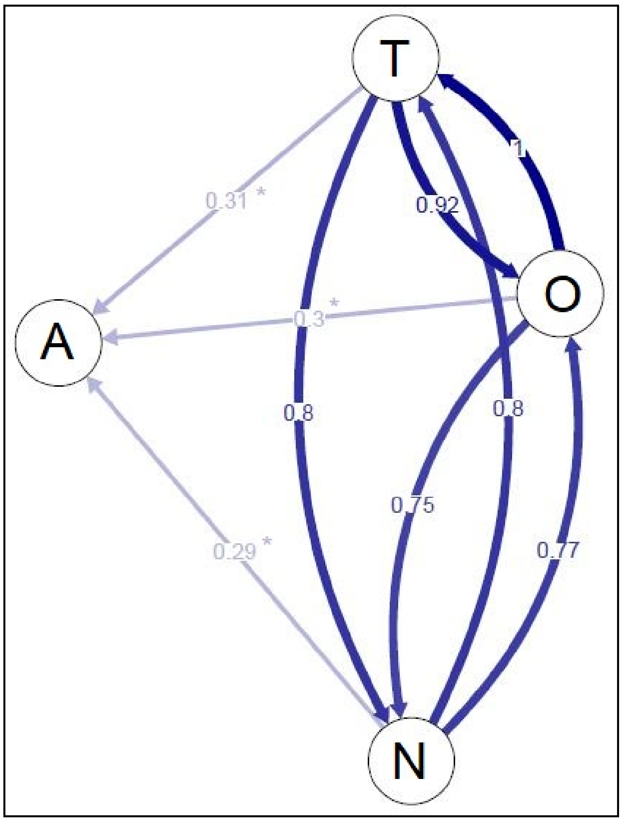


Figure 5. Population network and relative migration rates of reef manta rays (*Mobula alfredi*) based on Jost's *D* estimates of genetic differentiation for samples from East Australia and New Caledonia. A: the regional dataset (East Australia and New Caledonia), and B: the local dataset (only New Caledonia). The thickness of connecting lines is proportional to the relative rate of migration. Abbreviations are as follows: A, East Australia; N, Noumea; O, Ouvea; T, Touho. Statistically significant asymmetric rates are marked with an asterisk and migrations with a relative rate < 0.2 are not displayed.

DISCUSSION

Previous findings have indicated ocean-wide genetic differentiation and limited regional genetic connectivity in mobulid rays (Stewart et al. 2016, Hosegood et al. 2020a, Venables et al. 2020). Here we build on this work to provide evidence of fine scale population genetic structure of reef manta rays, highlighting new considerations for the management of the species. Despite many calls for studies such as ours to provide information on units to conserve and identify drivers of population structure in mobulids, only a few studies have investigated the population genetics of this taxon (Deakos et al. 2011, Stewart et al. 2016, 2018, Hosegood et al. 2020a, Perryman et al. 2019, Setwayan et al. 2018). The only comparable study, that investigated genetic structure of reef manta rays at a similar spatial scale (within hundreds of kilometres) and type of DNA marker (3057 SNPs) was conducted within southern Mozambique and found no evidence of genetic differentiation (Venables et al. 2020). Our findings provide evidence that fine scale population genetic structure can be observed for the species and may depend on its habitat use and the geographical context, highlighting the need to be assessed on a case-by-case basis.

Regional scale population genetic structure

We found strong genetic differentiation at the regional scale, which may indicate that large expanses of water lead to increased genetic differentiation in reef manta rays. This statistically significant population structure at a regional level is consistent with previous studies on the reef manta ray and its close relatives. For example, two studies reported clear genetic distinction at an ocean basin level (> 7000 km) for populations of *Mobula alfredi* between the Indian and Pacific oceans ($F_{ST} = 0.16$, Hosegood et al. 2020a, $F_{ST} = 0.38$, Venables et al. 2020). In comparison to our findings, the larger magnitude of F_{ST} in these papers may be due to the greater geographic distance between examined populations which can be an important driver of genetic differentiation as in other taxa (e.g., sharks, Geraghty et al. 2014, Vignaud et al. 2014, humpback and southern right whales, Rosenbaum et al. 2017, Carroll et al. 2020). Yet, further investigations would be required to validate this statement. While Venables et al. (2020) used a similar SNP discovery process to our study, F_{ST} discrepancies with Hosegood's study might be explained by the use of a different procedure. The use of common methods and sets of genetic markers would allow for comparison at a broader geographic extent and enable a better understanding of the genetic structure and diversity of the species (Domingues et al. 2017).

Stewart et al. (2016) discovered substantially lower levels of genetic population structure for the oceanic manta ray (*Mobula birostris)* ($F_{ST} < 0.004$) compared with the reef manta ray ($F_{ST} = 0.02$) across the Indian and Pacific oceans, using 25,040 SNPs from ddRAD sequencing based upon discovery and filtering processes different from ours. This discrepancy in protocol may explain the important F_{ST} difference between our study and Stewart's, preventing any comparison. However, we propose that interspecific difference in population structure found in Stewart et al. (2016) could be due to different biology and ecology. Reef manta ray undertake shorter migrations, has a narrower home range, and demonstrates higher site fidelity than oceanic manta rays (Couturier et al. 2012). The oceanic manta ray has been observed to undertake long distance travels between sighting locations (up to 1500 km, Hearn et al. 2014) and vagrants are occasionally recorded outside its known distribution (Couturier et al. 2015). Strong genetic differentiation has also been found in populations of other reef associated elasmobranchs separated by vast expanses of water (Vignaud et al. 2014, Momigliano et al. 2017). Physical barriers such as open expanses of sea and/or deep-water channel are thought to reduce the chance of reef manta rays (Deakos et al. 2011) and other reef associated species (Heupel et al. 2019) to transit even between geographically close aggregation sites (e.g., 150 km, Deakos et al. 2011, Setyawan et al. 2018).

Ancestral genetic clusters that are most common in individual from New Caledonia are found in greater proportion in the admixture composition of individuals from East Australia than the reverse. In addition, migration rates were higher from New Caledonia towards East Australia. This difference suggests that East Australia is a recipient of gene flow from New Caledonia. Previous studies on reef manta rays along the east coast of Australia have reported relatively high connectivity between sites separated by 380 km with a high proportion of individuals re-sighted at more than one site using photo-identification (Couturier et al. 2011, 2014) and detected with satellite (Jaine et al. 2014) and acoustic tagging (Couturier et al. 2018). In New Caledonia, satellite tagging does not indicate any offshore movements extending beyond New Caledonian waters (H. Lassauce unpubl. data) suggesting that migration is a rare event. Bi-directional migration between New Caledonia and Australia has been observed for several marine species. Tiger sharks (*Galeocerdo cuvier*) showed wide-ranging bi-directional migration between Australia and New Caledonia, partly driven by reproductive cycles with parturition taking place in coastal areas on the east coast of Australia (Werry et al. 2014). Bi-directional migrations have also been demonstrated for Green Turtles (*Chelonia mydas*) across the Coral Sea indicating that turtles foraging in New Caledonia nest on the Great Barrier Reef (Australia) and vice versa (Read et al. 2014). To the best of our knowledge, there is no evidence of unidirectional migration from New Caledonia toward Australia for any species having no planktonic life stage. Further research is needed on the genetic structure of other populations of reef manta rays in adjacent countries such as Vanuatu, Fiji or the Solomon Islands to extend our understanding of the species' regional connectivity.

Local scale population genetic structure

The present study is the first to report fine scale population structure for *M. alfredi.* In New Caledonia, genetic differentiation was detected between the three study sites: pairwise F_{ST} values were low but significant between all sites; DAPC distinguished three separate clusters and revealed high assignment probability rates for most individuals to their sampling location; and the TESS3 analysis revealed distinctive admixture compositions between samples from each site.

Population structure within such a relatively small area (maximum oceanic distance between sites is 335 km) has not been recorded for this species, and was not one of our a priori hypothesis. However, the low level of differentiation indicates a gene flow between the study sites. Therefore, we suggest that the New Caledonian reef manta ray exists as a metapopulation with at least three distinct but connected local populations. As far as we know, no other study has used a similar genotyping approach, which would allow the detection of slight genetic structures for any other marine species in New Caledonia.

Our understanding of these results is limited by the uncertainty in the spatial and reproductive ecology of reef manta rays, which appears to vary between regions of the world (Stewart et al. 2018). On the one hand, large scale movements reported from multiple locations suggest that populations of reef manta rays occupy large areas that include several key aggregation sites (Anderson and al. 2011, Marshall et al. 2011, Germanov & Marshall, 2014, Armstrong et al. 2019, Armstrong et al. 2020). On the other hand, reef manta rays have also been recorded to exhibit high site fidelity with sometimes clear segregation between geographically close aggregation sites around the world (Dewar et al. 2008, Deakos et al. 2011, Marshall et al. 2011, Couturier et al. 2014, Jaine 2014, Setyawan et al. 2018, Perryman et al. 2019, Setyawan et al. 2020). For instance, in Hawai'i, Clark et al. (2010) and Deakos et al. (2011) reported strong evidence through photo-ID of long-term, high site fidelity and no connection between two known aggregation sites less than 150 km apart. Similarly, in Indonesia, Setyawan et al. (2018) found high levels of site fidelity with acoustic telemetry and the absence of connection between study sites located only 150 km apart. High site fidelity has been documented in other regions of the world, such as in Mozambique (Marshall et al. 2011) and Indonesia (Setyawan et al. 2020). The habitat types or quality that could drive such differences in behaviour are not well understood. In New Caledonia, ongoing studies involving photo-identification and satellite telemetry seem to indicate high site fidelity and only a few connections between all three study sites (H. Lassauce unpubl. data). Only a few migrants per generation are needed to reduce genetic differentiation (Wang 2004). Therefore, it could be that long-term residency with limited movement is the norm with rare migration events linking local regions with gene flow.

Our results did not suggest that the deep ocean was a barrier to the dispersal of manta rays within New Caledonia as hypothesised by Deakos et al. (2011) in Hawai'i for *M. alfredi* , where no connection was discovered between two islands (150 km apart) separated by a 2000-meters-deep channel. In the context of our study, the continuous coastal environment, specifically the relatively shallow water between Noumea and Touho, could favour genetic connectivity (Couturier et al. 2011, 2014) while the deep-water channel separating these sites from Ouvea could act as a barrier discouraging gene flow (Deakos et al. 2011). In New Caledonia, deep diving behaviour of reef manta rays has been recorded using satellite telemetry (Lassauce et al. 2020). One individual travelled between Touho and Ouvea via a 2000 m deep channel and several animals made offshore foraging excursions (H. Lassauce unpubl. data), behaviours consistent with the research on other manta ray populations (Germanov & Marshall 2014, Jaine et al. 2014). These observations seem to indicate that deep waters may not be a barrier at a small spatial scale and in fact, other factors, possibly associated with the species ecology and behaviour, may drive fine-scale genetic differentiation.

Explanations for population structure include social behaviour and/or distribution of food resources. In New Caledonia, all our study sites (except Pouembout) are described as cleaning stations. Cleaning stations are critical habitats for manta rays. These sites are gathering points that enable social interactions between individuals and have been identified as essential areas for reproduction (Stevens et al. 2018) and other social behaviour (Perryman et al. 2019). The reproductive ecology of reef manta rays is still unclear, but some evidence suggests that females reside longer in areas with high mating potential and sufficient food resources while males move between aggregation sites (Marshall & Bennett 2010, Deakos et al. 2011, Stevens 2016, Stevens et al. 2018). For instance, the seasonal migration of *M. alfredi* in the Maldives was influenced by monsoon currents that promote phytoplankton blooms (Anderson et al. 2011). Migrations associated with food availability have also been documented in Indonesia (Dewar et al. 2008) and East Australia (Jaine et al. 2012). It is possible that high productivity attracts manta rays and variations in environmental conditions provoke migrations (Deakos et al. 2011, Setyawan et al. 2018), and so further work is needed to investigate correlations between migrations events and prey availability.

Genetic diversity

Genetic diversity of East Australia was significantly higher (Table 2) compared with New Caledonia, consistent with the former’s larger population size (Couturier et al. 2014) or its role as a recipient of gene flow. Indices of genetic diversity were similar among sites of New Caledonia (H_E ~ 0.31, Table 2). Although few studies have reported genetic diversity indices for elasmobranch populations, the results we present here are in the higher range of those reported. Venables et al. (2020) estimated genetic diversity for reef manta rays in Western Australia (H_E = 0.20) and Mozambique (all locations combined, H_E = 0.27) using a different panel of 3057 SNPs generated through DArT sequencing. For comparison with other elasmobranchs, lower levels of diversity were found in a population of bull shark (*Carcharhinus leucas*, H_E = 0.16, 1,494 SNPs*)* in Fiji (Glaus et al. 2020) as well as in a population of Galapagos shark (*C. galapagensis*) (H_E = 0.21, 7934 SNPS) in the Galapagos (Pazmiño et al. 2017) and across locations in the Pacific Ocean (from H_E = 0.20 to H_E = 0.24, 7784 SNPs) (Pazmiño et al. 2018). Similar levels of genetic diversity were also observed in populations of grey reef sharks (*C. amblyrhynchos:* H_E = 0.29 to H_E = *0.31, 4798 SNPs*) throughout the Indian and west Pacific Ocean (Momigliano et al. 2017) and for bonnethead sharks (*Sphyrna tiburo*) (H_E = 0.30 to H_E = 0.32, 5914 SNPs) in a ddRAD study on in genetically structured population in the Gulf of Mexico (Portnoy et al. 2018).

Inbreeding coefficients (F_{IS}) revealed significantly higher values in New Caledonia (F_{IS} = 0.084) than in East Australia (F_{IS} = 0.066). This suggests a higher proportion of inbreeding per generation in the population of New Caledonia than the Australian one, consistent with the latter's larger estimated population size (Couturier et al. 2014). This differs from the F_{IS} values not significantly different from zero presented in Venables et al. (2020) in Mozambique for the species using the same standard DArTseq protocol. Another study using a similar SNP-calling procedure found similar F_{IS} values (ranging from 0.065 to 0.070) for a highly migratory species of shark (*Galeorhinus galeus*) with no genetic structure at a regional level (Devloo-Delva et al. 2019). In contrast, Glaus et al. (2020) revealed lower inbreeding estimates for Bull shark (*Carcharhinus leucas*) populations from widespread locations across the Indian and Pacific Oceans. Additional work that employs next-generation sequencing is necessary to assess genetic diversity in other populations of reef manta ray. This would allow for a broader comparison and a better understanding of the genetic diversity of the species and its drivers at a global scale to achieve effective conservation and management.

Management and conservation

The challenge for conservation and management of mobile species is to identify the relevant management units. Information on the extent to which populations are genetically subdivided is crucial to establish effective conservation measures (Palsbøll et al. 2007). The findings presented here suggest that (1) the New Caledonian manta ray is a distinct population, and potentially a separate evolutionary significant unit (Waples & Gaggiotti 2006) from East Australia, and (2) New Caledonian manta rays exist in a metapopulation with sites within the reef system linked by gene flow. This work contributes to the use of genetic tools to identify ecological units for the creation of appropriate conservation measures rather than geographically or politically based legislation (Deakos et al. 2011, Stewart et al. 2016, 2018, Setwayan et al. 2018, Hosegood et al. 2020b, Perryman et al. 2019).

These findings could be used by management authorities when assessing conservation needs for the species in New Caledonia. Additional studies investigating the genetic connectivity with other nearby population such as Vanuatu and Fiji would also be an important contribution to set conservation needs. At a local scale, we recommend long-term monitoring of the populations to assess the impact of human activities. In New Caledonia, manta rays are not targeted by commercial or traditional fishing. Unsustainable practices such as trawling and dredging are prohibited in New Caledonian waters, mitigating marine megafauna bycatch (SMMPM, 2004). Yet this species is an important cultural and socio-economic asset in New

Caledonia and under no current legal protection. While direct impact seems relatively low, given the close proximity from the shore of the key habitat areas for this species, habitat degradation from mining (Bird et al. 1984), bush fires, and coastal development, could be important threats to manta rays in New Caledonia (Ris et al. 2017). Given this, the focus of protective measure must be directed toward identifying and monitoring key habitats for reef manta rays. Another potential action is for all three provinces and the New Caledonia government to protect mobulid species in the environmental code and add them to current monitoring plans. We also recommend further investigation to evaluate and monitor the extent of bycatch of mobulid species by commercial longline fishing in New Caledonia (Chapman 2001), and population viability analysis to assess the impact of possible threats. Monitoring could be done using citizen science with diving operators through partnerships with the ongoing Manta Trust project of New Caledonia. We also recommend that tourism, while currently low, be regulated in the future with a code of conduct or legal regulation to moderate impact.

PERMITS

Sample collection was conducted with authorizations from the Southern Province (permit no: 34584) and the Northern Province (permit no: 609011-33) of New Caledonia. In the Loyalty Islands Province, no permit was required by the local authorities, though permission of the local customary representatives was granted. Transportation of tissue samples from New Caledonia to New Zealand was conducted with the authorisation of the CITES authorities under the permit n°FR1998800088-E.

Additional specimens from two sites off the coast of Queensland, Australia, were collected under the following permissions: University of Queensland Animal Ethics #SBS/319/14/ARC/EA/LEIER, Great Barrier Reef Marine Park Authority permit #G16/37856.1, Department of National Parks, Sports and Racing QLD (Moreton Bay Marine Park) permit #QS2015/CVL1440, and Department of Agriculture and Fisheries QLD permit #199045.

SUPPLEMENTARY MATERIAL

Table S1 Reef Manta rays (*Mobula alfredi)* sampling information in New Caledonia (NC) and Australia.

Lab ID	Manta ID	Date	Location	Longitude	Latitude	Sex	Colour morph
T1	CD-MA-0034	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T2	CD-MA-0029	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T3	CD-MA-0031	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T4	CD-MA-0307	11/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T5	CD-MA-0128	12/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T6	CD-MA-0049	13/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T7	CD-MA-0039	13/09/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T8	CD-MA-0044	30/10/2018	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T9	CD-MA-0051	30/10/2018	Touho, NC	Undisclosed	Undisclosed	Female	Black
T10	CD-MA-0317	13/03/2019	Touho, NC	Undisclosed	Undisclosed	Female	Black
T11	CD-MA-0217	12/03/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T12	CD-MA-0033	28/11/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T13	CD-MA-0026	28/11/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T14	CD-MA-0067	30/11/2018	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T15	CD-MA-0042	28/05/2019	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T16	CD-MA-0207	28/05/2019	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
T18	CD-MA-0046	28/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T19	CD-MA-0065	29/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T20	CD-MA-0052	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T21	CD-MA-0088	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Black
T22	CD-MA-0030	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T23	CD-MA-0060	30/05/2019	Touho, NC	Undisclosed	Undisclosed	Male	Chevron
T24	Unknown	29/11/2018	Touho, NC	Undisclosed	Undisclosed	Female	Chevron
P1	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Unknown	Unknown
P2	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Unknown	Unknown
P3	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Male	Unknown
P4	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Male	Unknown
P5	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Unknown	Unknown
P6	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Female	Unknown
P7	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Female	Unknown
P8	Unknown	22/08/2018	Pouembout, NC	164.8	-21.18	Female	Unknown
B1	CD-MA-0078	25/10/2018	Noumea, NC	166.26	-22.29	Female	Black
B2	Unknown	25/10/2018	Noumea, NC	166.26	-22.29	Female	Black
B3	Unknown	17/04/2019	Noumea, NC	166.26	-22.29	Female	Black
B4	CD-MA-0140	17/04/2019	Noumea, NC	166.26	-22.29	Male	Black
B5	CD-MA-0076	17/04/2019	Noumea, NC	166.26	-22.29	Female	Chevron
B6	CD-MA-0153	13/05/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B7	CD-MA-0018	22/05/2019	Noumea, NC	166.26	-22.29	Male	Black
B8	CD-MA-0147	13/05/2019	Noumea, NC	166.26	-22.29	Female	Chevron
B9	CD-MA-0011	13/05/2019	Noumea, NC	166.26	-22.29	Female	Black
B10	CD-MA-0183	13/05/2019	Noumea, NC	166.26	-22.29	Male	Black
B11	Unknown	13/05/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B12	CD-MA-0036	13/05/2019	Noumea, NC	166.26	-22.29	Male	Black
B13	CD-MA-0313	20/06/2019	Noumea, NC	166.26	-22.29	Female	Black
B14	CD-MA-0009	20/06/2019	Noumea, NC	166.26	-22.29	Female	Chevron
B15	CD-MA-0230	9/07/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B16	CD-MA-0328	9/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B17	CD-MA-0330	9/07/2019	Noumea, NC	166.26	-22.29	Male	Chevron
B18	CD-MA-0006	9/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B19	CD-MA-0100	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B20	CD-MA-0134	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B21	CD-MA-0184	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B22	CD-MA-0083	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B23	CD-MA-0079	10/07/2019	Noumea, NC	166.26	-22.29	Female	Black
B24	CD-MA-0329	10/07/2019	Noumea, NC	166.26	-22.29	Male	Black
O1	CD-MA-0319	1/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Black
O2	CD-MA-0311	4/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Black

O3	CD-MA-0320	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O4	CD-MA-0321	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O5	CD-MA-0075	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Black
O6	CD-MA-0024	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Male	Black
O7	CD-MA-0322	5/05/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O8	CD-MA-0323	6/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Chevron
O9	CD-MA-0023	6/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Chevron
O10	CD-MA-0325	6/05/2019	PS, Ouvea, NC	166.39	-20.72	Male	Black
O11	CD-MA-0270	9/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Chevron
O12	CD-MA-0215	9/05/2019	PS, Ouvea, NC	166.39	-20.72	Female	Black
O13	CD-MA-0120	9/05/2019	PS, Ouvea, NC	166.39	-20.72	Male	Chevron
O14	CD-MA-0122	29/07/2019	PN, Ouvea, NC	166.44	-20.45	Female	Black
O15	CD-MA-0333	29/07/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
O16	CD-MA-0224	31/07/2019	PN, Ouvea, NC	166.44	-20.45	Male	Black
O17	CD-MA-0335	31/07/2019	PN, Ouvea, NC	166.44	-20.45	Male	Chevron
O18	CD-MA-0334	31/07/2019	PN, Ouvea, NC	166.44	-20.45	Female	Chevron
MA01	Unknown	13/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA08	Unknown	15/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA10	Unknown	15/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA13	Unknown	21/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA16	Unknown	23/01/2016	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA43	Unknown	9/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA49	Unknown	9/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA50	Unknown	10/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA53	Unknown	10/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA54	Unknown	10/06/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA58	Unknown	13/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA72	Unknown	16/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA78	Unknown	16/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA79	Unknown	17/09/2016	LEI, Australia	153.75	-27.42	Unknown	Unknown
MA136	Unknown	9/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA138	Unknown	9/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA147	Unknown	12/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA150	Unknown	14/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA152	Unknown	26/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown
MA158	Unknown	27/01/2017	NSI, Australia	142.72	-24.1	Unknown	Unknown

NC: New Caledonia, PS: Pleiades Sud, PN: Pleiades Nord, NSI: North Stradbroke Island, LEI: Lady Elliot Island.

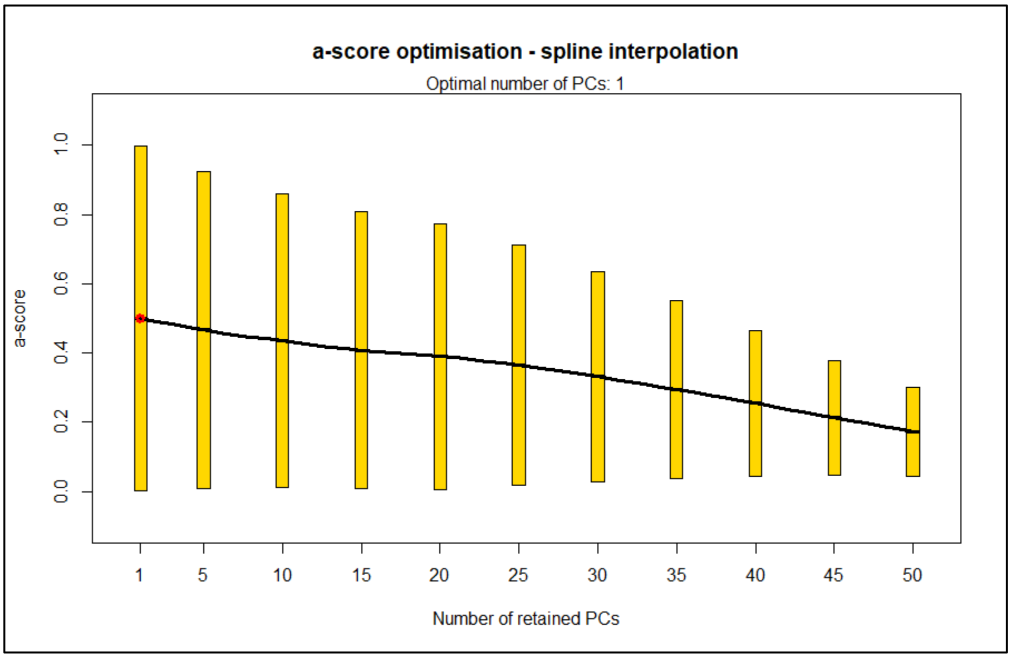


Figure S1 Alpha-score optimization for reef manta rays (*Mobula alfredi*) from East Australia and New Caledonia (regional dataset) using 3619 neutral SNPs – spline interpolation for PCs 1 through 50. Boxes show the overall mean and variance of individual population a-score for each of the PCs representing the regional dataset based on 1000 simulations.

Table S2 Alpha -scores simulated for testing hypotheses of structure for reef manta rays (*Mobula alfredi*) for East Australia and New Caledonia (regional dataset) using 3619 neutral SNPs.

Australia	New Caledonia	Mean	SD	#PCs	#DAs	Method for #PCs
0.62	0.03	0.33	0.31	30	10	N/3
1	0	0.50	0.70	1	10	Simulated

N/3 : indicates the highest-recommended-number method.
Simulated : indicates the optimized-simulation-based method.

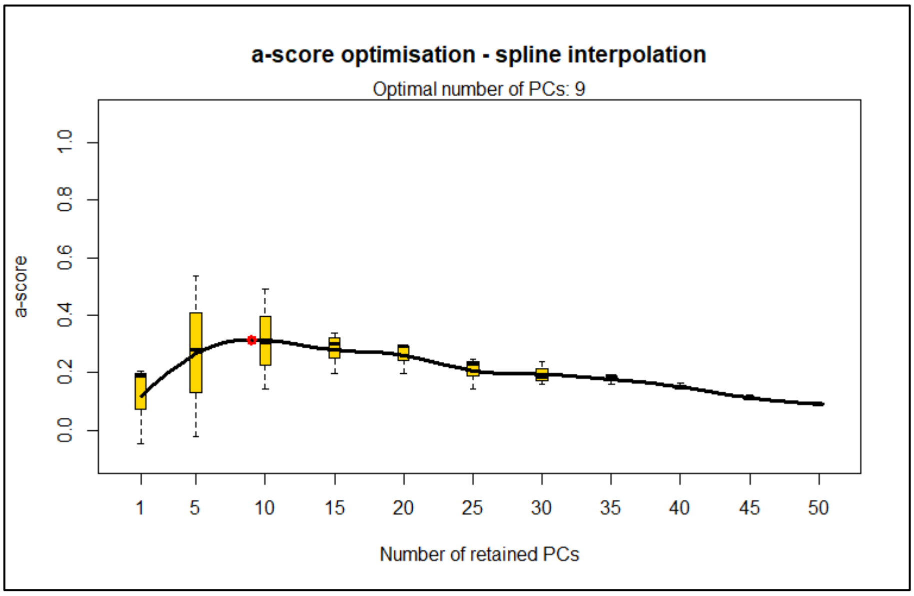


Figure S2 Alpha-score optimization for New Caledonian reef manta rays (*Mobula alfredi*) of (local dataset) using 2676 SNPs – spline interpolation for PCs 1 through 50. Box and whiskers show the overall mean and variance of individual population a-score for each of the PCs representing the regional dataset based on 1000 simulations.

Table S3 Alpha -scores simulated for testing hypotheses of structure New Caledonian reef manta rays (*Mobula alfredi*) (local dataset) using 2676 SNPs.

Noumea	Ouvea	Touho	Mean	SD	#PCs	#DAs	Method for #PCs
0.36	0.28	0.21	0.28	0.10	21	10	N/3
0.31	0.49	0.14	0.25	0.14	9	10	Simulated

N/3: indicates the highest-recommended-number method.
Simulated: indicates the optimized-simulation-based method.

Table S4 Confusion matrix showing predicted assignments for all individuals of reef manta rays (*Mobula alfredi*) of New Caledonia (local dataset) using 2676 SNPs the local based on DAPC using 2702 SNPs.

		Assigned Cluster				
		Noumea	Ouvea	Touho	**Prior**	**Posterior**
Group of origin	Noumea	23	0	0	0.3538	0.9411 ± 0.1384
	Ouvea	2	15	1	0.2769	0.7979 ± 0.3071
	Touho	0	3	21	0.3692	0.8543 ± 0.2723
				Overall	0.3382	0.8694

Table S5 Reef manta rays (*Mobula alfredi*) individuals average membership probability from the DAPC for each population from New Caledonia and East Australia (regional dataset) and within New Caledonia (local dataset) using 3619 and 2676 neutral SNPs.

Local dataset		**Assigned Cluster**		
		Noumea	**Ouvea**	**Touho**
Group of origin	**Noumea**	0.9412 ± 0.1384	0.0276 ± 0.0764	0.0312 ± 0.003
	Ouvea	0.1020 ± 0.2713	0.7979 ± 0.3071	0.1001 ± 0.1425
	Touho	0.0246 ± 0.0724	0.1211 ± 0.2293	0.8543 ± 0.2723
Regional dataset		**New Caledonia**	**Australia**	
Group of origin	**New Caledonia**	1	**0**	
	Australia	0	1	

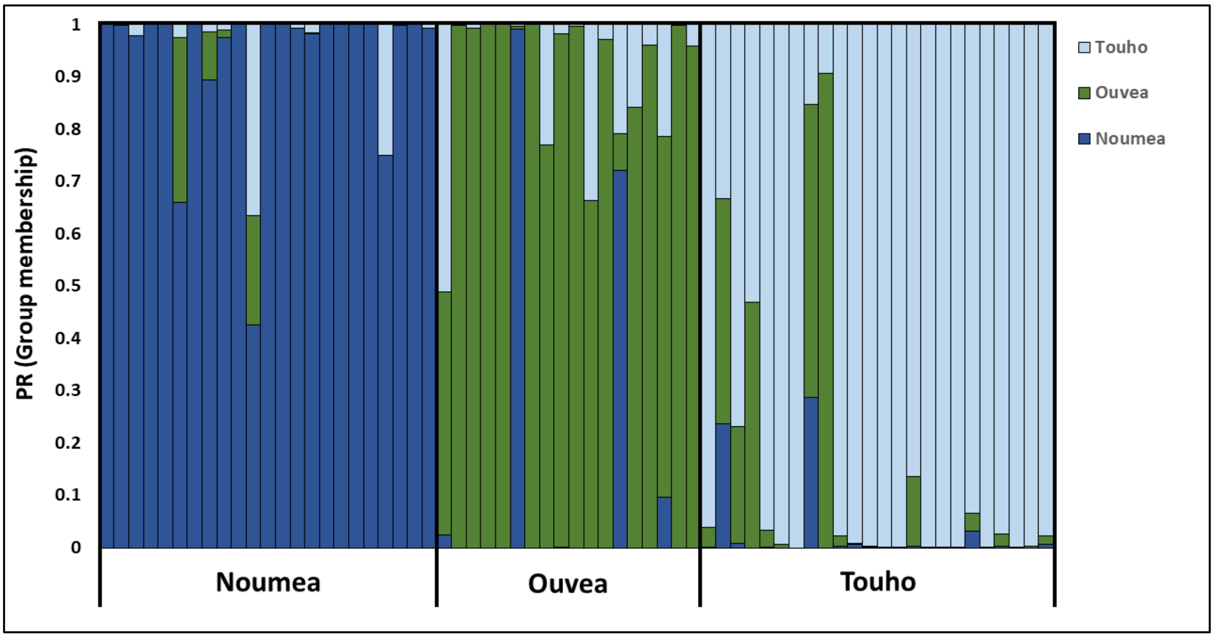
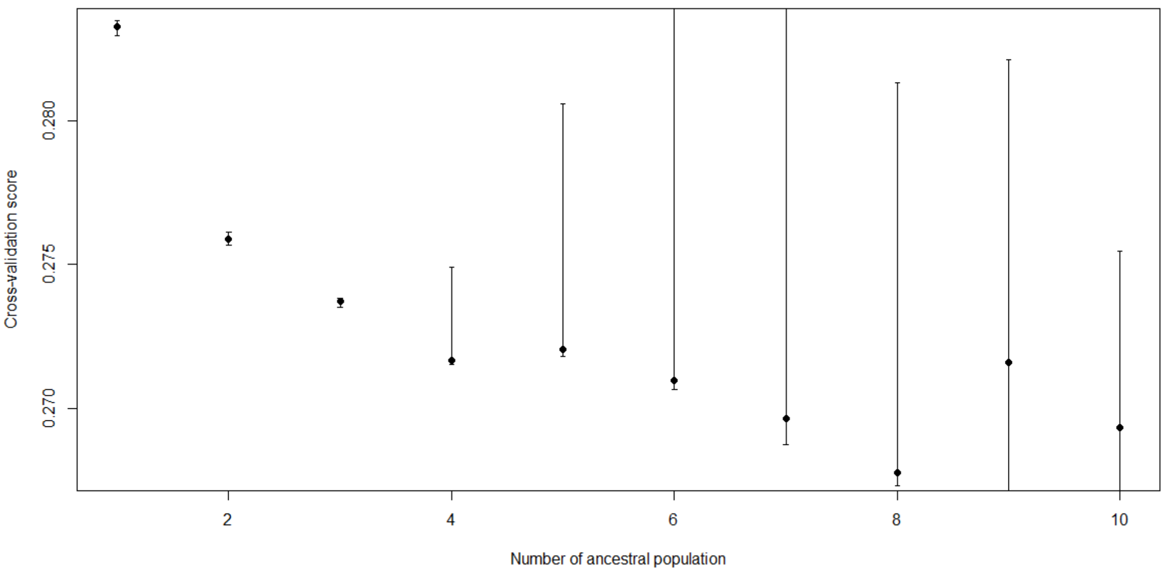


Figure S3 Probability of group membership for each individual reef manta ray (*Mobula alfredi*) within the four sampling locations from New Caledonia (local dataset) using 2676 neutral SNPs.



Supporting Figure S4 Cross-validation scores each K value from 1 to 10, with a maximum number of 100 iterations per run (20 repetitions) and a tolerance value of 10^{-7} for reef manta rays (*Mobula alfredi*) from East Australia and New Caledonia (regional dataset) using 3676 neutral SNPs.

CHAPITRE 5

Synthèse et perspectives

Les raies manta sont des espèces emblématiques ayant une importance culturelle en Nouvelle-Calédonie. Au-delà même de la culture, la présence de cet animal au plus proche des populations côtières, tisse un lien entre les communautés et le milieu marin. De nombreux témoignages rapportés durant ces travaux de terrain révèlent un attachement particulier pour les différents groupes de raies manta observés aux quatre coins de l'archipel. De plus, les raies manta représentent un attrait économique grandissant pour les opérateurs touristiques calédoniens. Ces valeurs iconiques et économiques sont des leviers importants pour la conservation de cette espèce mondialement menacée.

L'espace maritime de la Nouvelle-Calédonie est un bien naturel unique comprenant un des plus grands lagons du monde. Il détient un tiers des récifs coralliens les plus isolés et préservés de la planète avec plusieurs de ses lagons inscrits au patrimoine mondial de l'UNESCO (Wantiez et al. 2018). En tant que territoire développé et l'un des plus riches de la zone Sud-Ouest du Pacifique, la Nouvelle-Calédonie a un fort potentiel pour entreprendre des actions de recherche et de conservation que certaines autres nations insulaires d'Océanie n'ont pas. Dans ce contexte, certaines espèces emblématiques font déjà l'objet d'études scientifique et de protection telles que les tortues marines, les baleines, les requins ou les dugongs. En revanche, jusqu'à récemment les raies manta ne s'inscrivaient dans aucune initiative de recherche et de conservation. En réponse à ce manque, l'Initiative Manta en Nouvelle-Calédonie a été créée en 2016. L'objectif était d'étudier la population de raies manta afin de produire des données de références pour connaitre son statut et apporter des outils de conservation et de gestion. Ces travaux de thèse sont un des résultats de cette initiative et présentent donc les premières études académiques réalisées sur la population de raies manta en Nouvelle-Calédonie. Une combinaison de méthodes et de techniques a été employée pour tenter d'examiner les caractéristiques biologiques, l'écologie spatiale et la structure de la population de raies manta de récif du pays. Ces résultats contribuent à la connaissance globale sur l'écologie et le comportement de cette espèce vulnérable et constituent des bases de réflexions pour l'amélioration de sa protection en Nouvelle-Calédonie et dans la région du Pacifique intertropical.

CARACTERISTIQUES DE LA POPULATION

Les caractéristiques de la population sont l'effectif, le sex ratio, les proportions entre les différentes pigmentations, les taux de prédation et de blessure de nature anthropogénique. L'estimation de l'abondance issu d'une modélisation est probablement une sous-évaluation du nombre total de raies manta en Nouvelle-Calédonie. En effet, la population étant structurée en plusieurs groupes distincts, une estimation correcte à partir de la méthode de modélisation utilisée demanderait un effort d'échantillonnage plus important et prolongé de toutes ces sous-populations. Toutefois, en se basant uniquement sur les données collectées aux trois sites d'études, le nombre de raies manta en Nouvelle-Calédonie par rapport aux autres archipels isolés dans le monde semble être relativement élevé (Deakos et al. 2011 ; Peel 2019).

Un résultat spécifique à la Nouvelle-Calédonie est la présence de la plus grande proportion d'individus noirs au monde. Bien que cette observation ne semble pas avoir d'incidence sur l'écologie et la démographie de la population (Venables et al. 2019), cela peut être un argument d’attractivité touristique. Du fait de leur rareté, les raies manta noires sont plus recherchées par les communautés de plongeurs. Plusieurs clubs de plongées ont confirmé cette demande particulière de touristes, notamment japonais, qui viennent plonger en Nouvelle-Calédonie pour optimiser leurs chances de rencontrer une raie manta noire. Cette estimation de la proportion des différentes pigmentations est aussi la première de la région du Pacifique Sud. Elle est donc fondamentale pour une analyse globale de la variation des proportions de pigmentation de cette espèce et pour comprendre les processus évolutifs qui les génèrent. Une étude plus approfondie utilisant des méthodes moléculaires est maintenant nécessaire pour examiner la structure et la génétique de ce mélanisme (Venables et al. 2019).

Le taux de blessures causées par les activités humaines (équipements de pêche, collisions avec les bateaux/hélices, lignes de mouillage, par exemple) est faible dans le pays. Ce résultat révèle le peu de chevauchement entre les activités humaines et les espaces utilisées par les raies manta. Dans certains archipels ayant une densité de population plus importante tels que Hawaï (Deakos et al. 2011), Maupiti et Bora Bora en Polynésie Francaise (Carpentier et al. 2019), l’impact anthropogénique est beaucoup plus élevé. La Nouvelle-Calédonie offre donc un contexte relativement favorable pour la population de raies manta et pourrait ainsi être une référence pour la conservation de l’espèce. Cependant, étant donné l’évolution croissante du développement économique (Ris et al. 2017), une surveillance continue de l’évolution des activités côtières et de leurs impacts potentiels sur les raies manta serait nécessaire. Les raies manta étant attachées à certains sites, il serait d’autant plus pertinent d’établir des mesures préventives pour protéger ces sites dans les zones susceptibles d’être impactées par ce développement et d’investiguer les sites éloignés qui n’ont encore fait l’objet d’aucune étude. Concrètement, certaines pratiques pourraient être régulées dans des zones ciblées telles que les stations de nettoyage ou de nourrissage. Par exemple, les recommandations internationales dans ces zones sont de limiter la vitesse de croisière de bateaux, interdire la pratique de pêche impliquant l'utilisation de filets ou de lignes, limiter et modifier les lignes de mouillages suivant des alternatives adaptées mitigeant les risques d'enchevêtrement (The Manta Trust 2019).

ECOLOGIE SPATIALE

Fidélité au site

Les différents travaux de cette thèse montrent l'importance de la fidélité au site des raies manta en Nouvelle-Calédonie. Ce résultat est conforme à ce qui a été observé dans de nombreuses régions dans le monde. Dans cette étude, la fidélité au site a été observée à différentes échelles temporelles en combinant des méthodes complémentaires. Ainsi, les mouvements enregistrés sur une échelle de temps relativement courte (quelques mois) ont révélé une affinité importante aux habitats côtiers et plus particulièrement à des sites spécifiques. Sur une échelle de temps plus longue (plusieurs années), la présence régulière et répétée d'une proportion élevée de la population sur ces sites confirme encore un fois une utilisation durable de ces habitats. Finalement, cette étude est la première à démontrer les conséquences de ce comportement sur la génétique de la population avec notamment un léger degré de différentiation génétique à l'échelle locale.

La sélection de l'habitat se base sur des critères de qualité et de durabilité des apports nécessaires à la survie de l'espèce (Switzer et al. 1993). Suivant ces principes, une importante fidélité à un site témoigne d'une potentielle hétérogénéité de la qualité des habitats et de coûts bioénergétiques importants pour changer de site (Switzer et al. 1993). Les sites montrant un taux de fidélité élevé doivent être considérés comme étant clés pour l'espèce et les groupes de raies manta à ces sites doivent être traités comme une unité de conservation distincte. Cette affirmation semble d'autant plus pertinente dans les régions fragmentées tels que les archipels où le coût des déplacements inter-îles parait plus important. A ce titre, mesures et précautions devraient être prises pour évaluer et mitiger les possibles perturbations présentes et futures sur ces sites.

Connectivité

L'étude de la connectivité au sein de la population de Nouvelle-Calédonie montre que les raies manta sont capables de mouvements entre toutes les zones étudiées de l'archipel. Sur une échelle temporelle courte, les déplacements sur de longues distances étaient rares et surtout, restreints aux bords de côtes ou aux zones d'eau peu profondes pour la plupart. Ce constat renforce les hypothèses statuant que les eaux profondes pourraient constituer une barrière géographique limitant la connectivité pour cette espèce. À une échelle de temps plus longue, le nombre de connections observées entre les sites étudiés reste faible et ne concerne qu'une proportion limitée de la population. Le manque de connectivité entre les différents groupes étudiés est tel qu'il génèrerait un flux génétique insuffisant au sein de toute la population pour observer une panmixie totale.

L'écologie spatiale de ces animaux est fortement liée à la disponibilité en ressource. Des études approfondies faisant le lien avec la distribution et la dynamique de cette ressource amélioreraient considérablement la compréhension de l'utilisation de l'espace de cette espèce. Par exemple, un recoupement de données océanographiques permettant d'examiner les variations de concentration en chlorophylle-a (comme indicateur de zooplancton, Burgess

2017) et de données spatiales à long terme des raies manta pourrait être adéquat. Les résultats liés aux déplacements présentés dans cette étude n'ont pas permis de telles analyses. L'effort d'échantillonnage de photo-identification n'était pas suffisant pour obtenir des informations assez précises sur la temporalité de ces déplacements. En ce qui concerne la télémétrie satellite, l'échelle de temps suivie était trop courte pour associer les déplacements observés à d'éventuelles variations de la distribution de la ressource. En revanche, les données obtenues constituent une base permettant, par exemple, d'établir un plan d'échantillonnage adéquat pour l'utilisation de la télémétrie acoustique, une méthode permettant d'obtenir des résultats à des échelles temporelles et spatiales intermédiaires, plus appropriées à cet objectif.

Comportement de plongée

En Nouvelle-Calédonie, les raies manta de récif plongent plus profond et plus régulièrement qu'ailleurs dans le monde au regard des connaissances actuelles. Sa distribution verticale a été augmentée de plus de 200 m, ce qui souligne l'importance de l'utilisation des milieux mésopélagiques. L'analyse des profils de plongées semble indiquer que ce comportement est lié à l'exploitation de la ressource en profondeur. Les raies manta en retireraient donc un bénéfice qui surpasserait les coûts énergétiques engendrés par de telles plongées. Cette observation suggèrerait alors une concentration importante de ressources en profondeur et des opportunités de nourrissage insuffisantes dans les couches supérieures de la colonne d'eau de Nouvelle-Calédonie. Des recherches visant à définir l'alimentation de cette espèce dans le pays permettrait de vérifier la proportion de ressources alimentaires provenant des milieux profonds de celle exploitée proche de la surface. Des méthodes pertinentes et efficaces pour obtenir ce type de données seraient les analyses des isotopes stables et des signatures d’acides gras afin d’identifier la diététique des raies manta en la comparant avec du zooplancton de différentes origines (Couturier et al. 2013).

STRUCTURE GENETIQUE

Les premières évidences de différentiations génétiques entre des groupes de raies manta à des distances géographiques restreintes ont pu être démontrées à l’échelle de l’archipel de la Nouvelle-Calédonie, ce qui n’avait pas été possible dans d’autres régions où la fidélité au site est également importante et la connectivité limitée. Les méthodes et techniques en génomique évoluent rapidement et l’émergence de nouveaux outils moléculaires permet une analyse du génome plus fine. A ce jour, peu d’études génétiques ont été menées dans le but d’examiner la structure d’une population de raies manta et aucune structure entre les sous-populations n’a été révélée (Maldives, Hawaï) (Hosegood 2020). Ces résultats paraissent étonnants, notamment pour Hawaï où la connectivité inter-îles est très limitée voire absente. La disparité avec nos résultats peut venir de l’utilisation de processus de découverte de SNPs différents. Une méthode similaire à la nôtre ne révèle aucune structure au sein de la population de Mozambique du Sud. Dans ce cas d’étude, le résultat reflète une connectivité plus élevée le long des côtes favorisant la dispersion de flux génétiques. Sur des échelles plus vastes, entre des populations séparées par des océans, les résultats ont démontré une différenciation génétique plus nette (Hosegood et al. 2020 ; Venables et al. 2021). Une uniformisation des méthodes utilisées et une collaboration au niveau régional, et global, permettraient d’éclaircir les liens évolutifs et la capacité de dispersion de l’espèce dans différents contextes géographiques.

Une évaluation du niveau de structure génétique entre les populations de raie manta Nouvelle-Calédonie et de la côte Est de l'Australie a montré une connectivité génétique limitée. Ces travaux ont initié une dynamique de collaboration régionale qui aura pour objectif de compléter notre compréhension de la connectivité génétique et des liens évolutifs entre les populations de la région du Pacifique Sud. Il sera donc pertinent d'inclure d'autres populations de la région dans de futures études génétiques comme celles du Vanuatu, Fidji, les Iles Salomon ou même Wallis-et-Futuna. Par exemple, à moins de 250 km de la Nouvelle-Calédonie, la population de raies manta du Vanuatu n'a encore fait l'objet d'aucune étude. A cette distance, des liens avec la population de Nouvelle-Calédonie sont possibles.

CONCLUSION

Cette thèse s'inscrit dans une démarche d'amélioration des connaissances d'espèces emblématiques menacées. Les données obtenues lors de ces travaux constituent une base solide pour alimenter les réflexions visant à améliorer la conservation de l'espèce mais aussi à identifier les axes de recherche prioritaires. La raie manta de récif est une espèce vulnérable de par sa biologie mais aussi son écologie spatiale. En Nouvelle Calédonie, les préoccupations sont limitées puisque l'espèce n'est pas directement ciblée par les pêches et l'impact lié à l'activité humaine semble être relativement faible. Dans le monde, un tel contexte favorable pour la conservation de l'espèce est relativement rare, ce qui fait de la population de raies manta de Nouvelle-Calédonie une référence à l'échelle globale qui doit être préservée. Par conséquent, la population devrait tout de même être surveillée et des efforts pourraient d'ores et déjà être entrepris pour prendre en compte les menaces potentielles identifiées dans cette thèse. L'aspect culturel et iconique de l'espèce est un atout pour sa conservation, et mieux la connaitre ne fera que renforcer sa valeur affective auprès de la communauté et faciliter sa protection en Nouvelle-Calédonie et dans le reste du monde.

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APPENDIX 1 : LISTE DES PRESENTATION

- ***NOCTURNES RAIES ET REQUINS* (2017 -2018).** *Les raies manta de Nouvelle-Calédonie*. Aquarium des Lagons, Nouméa, Nouvelle-Calédonie.
- ***SOCIETY FOR CONSERVATION BIOLOGY OCEANIA* (2018).** *New-Caledonia, a sanctuary for Manta rays: A study on this unknown population.* Wellington, New Zealand.
- ***DOCTORIALES UNC* (2018).** *Raies Manta de Nouvelle-Calédonie : Etude de cette population inconnue.* Nouméa, Nouvelle-Calédonie.
- ***FIRST LATIN AMERICAN CONGRESS OF SHARKS, RAYS AND CHIMAERAS* (2019).** *Population characteristics and spatial ecology of manta rays of New-Caledonia (Mobula alfredi).* Playa del Carmen, Mexique.
- ***FESTIVAL INTERNATIONAL DE L'IMAGE SOUS-MARINE* (2019).** *Que savons des raies Manta ?* Nouméa, Nouvelle-Calédonie.
- ***SATO YAMA UMI PROJECT GENERAL WORKSHOP* (2020).** *Citizen science to study the population of reef manta rays of New Caledonia.* En ligne, Tokyo, Japon.
- ***DOCTORIALES UNC* (2020)*.*** *Structure et connectivité de la population de raies Manta de Nouvelle Calédonie.* Nouméa, Nouvelle-Calédonie.

Caractéristiques biologiques, écologie spatiale et structure de la population de raies manta de récif (*Mobula alfredi*) de Nouvelle – Calédonie

Résumé : Espèce emblématique et néanmoins menacée qui peuple les mers des régions tropicales et subtropicales du monde entier, la raie manta de récif (*Mobula alfredi*) est bien présente en Nouvelle-Calédonie. La population de l'archipel n'a cependant encore jamais été étudiée et la compréhension de sa biologie, son écologie, sa dynamique des populations et ses mouvements est encore très limitée à l'échelle globale. L'acquisition de connaissances de références pourrait jouer un rôle essentiel pour la conservation de l'espèce. Cette thèse tente de décrire la population, sa structure et l'écologie spatiale de raies manta de Nouvelle-Calédonie en utilisant des approches diverses combinant la science participative à la télémétrie satellite et la génomique. Un suivi de cinq ans permettant la collecte de 1741 photo-identifications de raies manta provenant de 11 sites répartis dans tout l'archipel a permis de connaitre les caractéristiques et la distribution de la population, d'estimer son abondance et d'obtenir un premier aperçu de son utilisation de l'espace et des potentielles source de blessures. L'utilisation de 21 balises satellites déployées à trois sites d'étude a permis d'obtenir des données de déplacements plus détaillés et d'observer les comportements de plongées. Finalement, le séquençage du génome de 92 échantillons dont 73 provenant de quatre sites en Nouvelle-Calédonie et 19 de deux sites sur la côte Est de l'Australie a permis de découvrir plus de 3000 SNPs (Single Nucleotide Polymorphism) et de notamment révéler l'existence d'une structure génétique à l'échelle régionale et locale. L'ensemble des résultats présentés dans cette thèse apporte les premières données sur la population de raies manta de récifs en Nouvelle-Calédonie et proposent les mesures et précautions qui devraient être prises pour évaluer et mitiger les possibles perturbations présentes et futures.

Mots clés : Génomique ; télémétrie satellite ; science participative ; photo-identification ; connectivité ; fidélité

Characteristics, spatial ecology and structure of the population of reef manta rays (*Mobula alfredi*) of New Caledonia

Abstract : Emblematic and threatened species distributed in the tropical and subtropical regions throughout the world, the reef manta ray is also represented in New Caledonia. The population of the archipelago has yet never been studied before and the understanding on the biology, ecology, populations dynamics and movements of the species remain globally limited. The acquisition of a knowledge of reference is essential for the conservation of the species. This thesis aims to describe the population, its structure, and the spatial ecology of reef manta rays of New Caledonia using diverse approaches combining citizen science, satellite telemetry, and genomic. A five-year monitoring allowed the collection of 1741 photo-identifications of manta rays from 11 sites distributed around the archipelago revealing the characteristics and the distribution of the population, an estimation of the abondance and a first insight into its habitat use and the potential source of injuries. The deployment of 21 satellite tags at three study sites recorded detailed data on horizontal movements and the vertical behaviour. Finally, the use of genome-wide single nucleotide polymorphisms (SNPs) discovered from of 92 samples, including 73 from four sites around New Caledonia and 19 from two sites on the east coast of Australia, revealed the existence of genetic structure at regional and local scales. The results presented in this thesis provide the first data on the population of reef manta rays of New Caledonia and suggest precautions and measures that should be considered to evaluate and mitigate the potential present and future disturbances.

Key words : Genomic ; satellite telemetry ; citizen science ; photo-identification ; connectivity ; site fidelity