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Cecile Berthe

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MINISTÈRE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE

ÉCOLE PRATIQUE DES HAUTES ÉTUDES

Sciences de la Vie et de la Terre

MÉMOIRE

Présenté

par
Cécile Berthe

pour l'obtention du Diplôme de l'École Pratique des Hautes Études

Première approche écologique, biologique et comportementale de la raie aigle à ocelles *Aetobatus ocellatus* en Polynésie française

soutenu le 10 octobre 2017 devant le jury suivant :

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École Pratique
des Hautes Études

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Sciences de la Vie et de la Terre

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First ecological, biological and behavioral insights of the ocellated eagle ray *Aetobatus ocellatus* in French Polynesia

CRIOBE USR3278

MUSÉUM NATIONAL D'HISTOIRE NATURELLE

« Mieux connaître la nature, c’est déjà l’aimer.

Et mieux l’aimer, c’est savoir qu’il faut la protéger »

Bernard Salvat (1974, in Thibault)

“We sweat and cry salt water,

so we know that the ocean is really in our blood”

Teresia Teaiwa

A **Christiane** Rivière, **Bernard** Evin et **Pierre-Marie** Delval

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Table of contents

Remerciements 7

Table of contents 10

Table of tables 13

Table of figures 14

Chapter I Résumé du diplôme EPHE en français 17

1. Introduction 17

Connaissance et conservation des espèces marines 17

Taxonomie et Description morphologique des raies aigle 18

Les raies aigle dans l'écosystème corallien : importance et vulnérabilité 19

Connaissances scientifiques sur les raies en Polynésie française 20

2. Problématique scientifique 21

3. Résultats obtenus 22

Etude 1/ Mise en évidence de la présence d'Aetobatus ocellatus (Myliobatidae) en Polynésie française 22

Etude 2/ Patterns de distribution de la raie aigle à ocelles selon des mesures biologiques et physiques sur deux sites de Moorea, Polynésie française. 23

Etude 3/ Comportement de déparasitage par frottement contre un banc de sable par la raie aigle à ocelles (Aetobatus ocellatus) 24

Etude 4/ Influence des bruits anthropiques sur le comportement de fuite des raies aigle Aetobatus ocellatus à Moorea (Polynésie française) 24

4. Conclusion et Perspectives 25

Perspectives en recherche fondamentale sur les raies aigle à ocelle 25

Perspectives de conservation des raies aigle 25

Perspectives de recherche sur les raies aigle en Polynésie française 26

Chapter II General Introduction 29

1. Knowledge and Conservation of marine species 29

2. Eagle rays in the world 29

3. Eagle rays in coral reefs: importance and vulnerability 32

4. Rays in French Polynesia 33

5. Scientific problematic 34

Chapter III DNA barcoding supports the presence of the cryptic Ocellated Eagle Ray, Aetobatus ocellatus (Myliobatidae), in French Polynesia, South Pacific 37

1. Introduction 38
2. Materials and methods 38
3. Results 40
4. Discussion 41

Chapter IV Distribution patterns of ocellated eagle rays along two sites at Moorea Island, French Polynesia 44

1. Introduction 45
2. Materials and methods 46
   Study site 46
   Photo-identification 47
   Underwater visual surveys and environmental data 47
   Benthic analyses 49
3. Results 49
   Spatio-temporal distribution 50
   Influence of environmental factors on the spatio-temporal distribution 51
   Benthic analyses 52
   Behavioral observations 52
4. Discussion 53
   Ontogeny and distribution patterns 54
   Energetics and distribution patterns 54
   Environmental factors and distribution patterns 55
5. Conclusions 56

Chapter V Chafing behavior on a patch of sandy bottom by ocellated eagle ray (*Aetobatus ocellatus*) 58

1. Introduction 59
2. Materials and Methods 59
3. Results and Discussion 60

Chapter VI Influence of Anthropogenic Noises on Escape Behaviour of White-Spotted Eagle Ray *Aetobatus ocellatus* at Moorea Island (French Polynesia) 62

1. Introduction 63
2. Materials & Methods 64

3. Results 67
4. Discussion 68
Chapter VII Conclusion & Perspectives 71
1. Perspectives in fundamental research on the ocellated eagle ray 71
2. Perspectives in conservation of eagle rays 73
3. Perspectives of research on eagle rays in French Polynesia 74
References 76

Table of tables

Table I-I : Classification of the Aetobatidae Family.....30

Table II-I: References of samples and COI sequences used for the genetic analysis. * Primarily recorded in BOLD Systems as Aetobatus cf. narinari 39

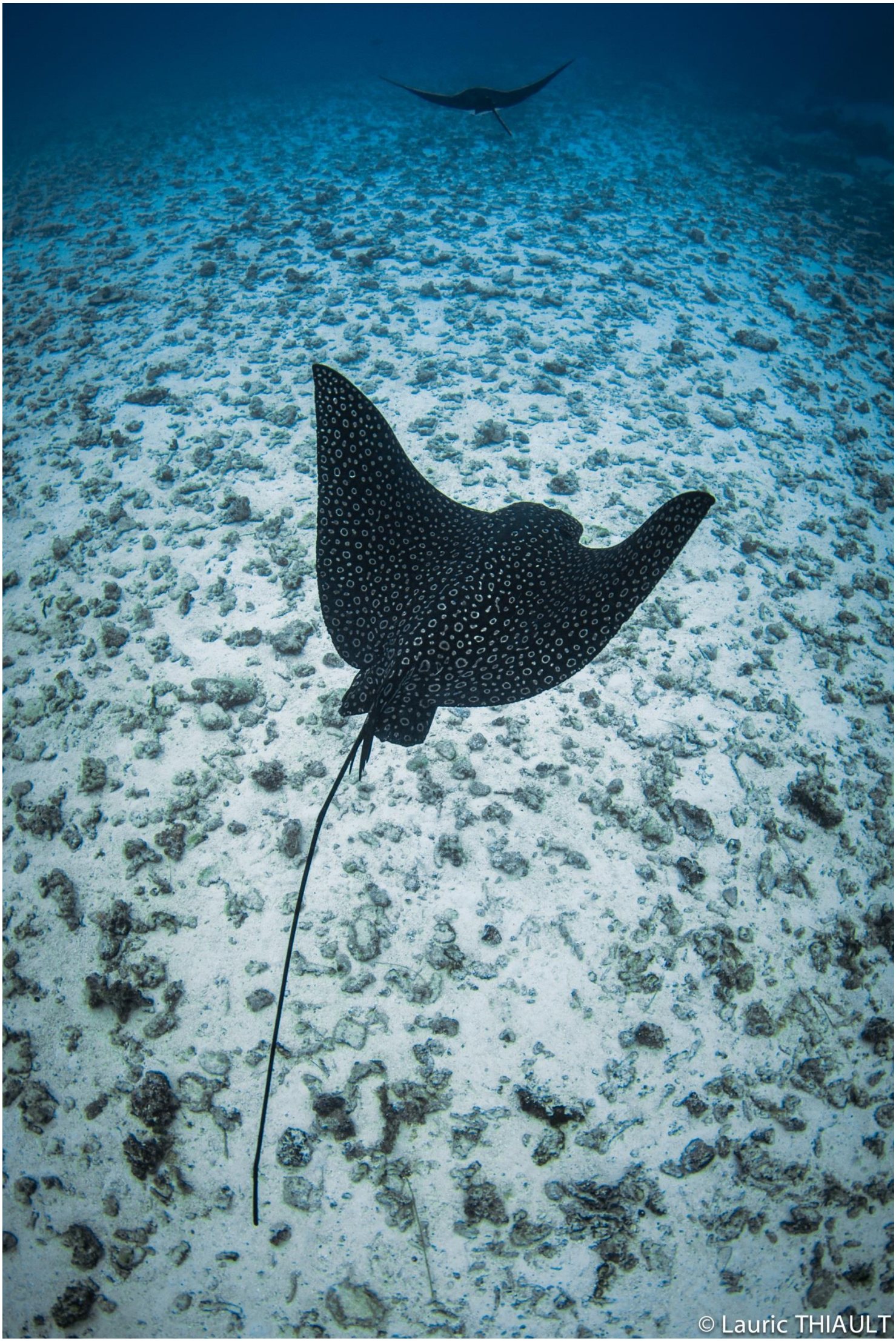
Table III-I: Different kind of behavior observed during the study, with their description and the number of individual involved in the behavior. 48

Table III-II: Numbers of eagle rays (Aetobatus ocellatus) that were observed; totals for adults are separated by sex. Juveniles column represents individuals under 70cm width, when sex from above was not possible to determine. Between parentheses is the number of identified individuals 49

Table of figures

Figure II-1: On the left, Myliobatis australis (©Australian National Fish Collection, CSIRO), ...31
Figure II-2: Morphology of ocellated eagle ray with the main external characteristics (from top to bottom: snout, eyes, spiracles, pectoral fins, pelvic fins, claspers - only for male - , stings and tail) that could be useful to identify the individual 31
Figure III-1: Locations for sampled *Aetobatus ocellatus* (grey circles) in French Polynesia and for comparative materials (circles in insert: Australia, New Caledonia, Indonesia, India, South Africa, Brazil, Japan, Korea). 39
Figure III-2: Neighbour-Joining distance tree (K2p model) of the partial COI sequences (652 bp, 'barcode region') revealing the placement of individuals of *Aetobatus ocellatus* from French Polynesia within the Aetobatus complex. Specimens are labelled with their BOLD Process ID. Bootstrap values over 75% are indicated above branches. 41
Figure IV-1: Map highlighting the two study sites on Moorea Island, French Polynesia, ...46
Figure IV-2 : Photo-identification using the I3S Pattern program on the right fin area. 47
Figure IV-3: Multiple Correspondance Analysis factor map (2 first components, 34.5% and 19.4%, respectively) representing the relationship between the study sites (ClubMed/Mareto), ontogenetic stage of the eagle rays (male/female/juvenile), seasons (warm/cold), and the time of the day (am/pm) 50
Figure IV-4: Multiple Correspondance Analysis scatter plots (2 first components, 18% and 13%, respectively) representing the relationship between sex (male/ female/juvenile), the site (ClubMed/Mareto) and the environmental factors: wave and wind direction (east/north/south/west), wind speed (high/medium/low) and current strength (no/light/medium/strong). 51
Figure IV-5: Abundance (number of macroinvertebrate individuals) and biomass (grams) for the two study sites. The boxes represent the first and third quartiles, black lines are the medians (second quartiles), and whiskers correspond to the range (min–max) of the distributions. An asterisk indicates statistically significant differences.....52
Figure IV-6: Percentage of observations for the ten different behaviors according to the study site: swimming, foraging, chafing, cruising, escaping, pre-mating, jumping, interacting with conspecific, interacting with heterospecific, interacting with snorkeler. See Table II for details on each behavior. 53
Figure V-1: Sites where chafing behaviors have been observed: on the left, Moorea island and the channel of ClubMed on the north. On the right Fakarava Atoll and the Tetamanu pass in the south. 59

Figure V-2 a, b, c: Sequence of an ocellated eagle ray (*Aetobatus ocellatus*) chafing on a patch of sandy bottom at Fakarava Atoll (June 2014) 60
Figure VI-1: Map of Moorea Island, overview of the study site at the Mareto public beach on the north coast of the island 64
Figure VI-2: Acoustic system used to test sound effect on eagle rays. a = the system in the field, b = the system itself with the waterproof box and the mp3 player, c = the speaker placed near a foraging eagle ray 65
Figure VI-3: Sound envelope of boat playback, white noise and the three single frequency tones (40 Hz, 6000 Hz, 1000 Hz) during 30 seconds with all sound calibrated at, at least 90 dB re 1 mPa RMS. .66
Figure VI-4: Eagle rays (*Aetobatus ocellatus*) behavior related to the control test (without sound–switched-off loudspeaker) and the tested sounds: a boat motor sound, a white noise and three single-frequency tones (40 Hz, 600 Hz, 1 kHz). Ten rays were tested for each sound type and nine rays for control test. Two possible responses of eagle rays were observed: no reaction (i.e. continue to forage) or escape behavior (i.e. swam away from the foraging site at, at least, 50 m). Stars indicate a significant difference between the number of rays showing escape behavior with one sound type and the control test (x2 test – P < 0.05). 68
Figure VII-1: The number of publications on social behavior increasing for animals in general since 2006. Bar chart on top show: data based on “animal” and “social behavior” as key word in Web Of Science. Bar chart at the bottom: data based on “shark” (not “shark bay”) or “elasmobranch” and “social behavior” in Web Of Science. (Adapted from Brown et al. 2011) 72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Chapter I Résumé du diplôme EPHE en français

L'objectif de mon Diplôme SVT EPHE est d'étudier pour la première fois les raies aigle à ocelles en Polynésie française. J'ai réalisé quatre études présentées dans les chapitres III (p.37) à VI (p.61), qui permettent d'en apprendre d'avantage sur la génétique, l'écologie et l'éthologie de cette espèce dans une région du monde où elle n'a jamais été étudiée et où son interaction avec des activités anthropiques est importante.

1. Introduction

Connaissance et conservation des espèces marines

La conservation des espèces marines est un enjeu mondial majeur, avec un nombre d'espèces menacées toujours grandissant (International Union for the Conservation of Nature, 2016). Pour protéger une espèce, son habitat et son écosystème, il faut d'abord connaître la taxonomie, la biologie, l'écologie, et l'éthologie de l'espèce elle-même. La taxonomie est le fondement de cette connaissance (White & Last 2012 ; Collares-Pereira et al. 2015) et des mauvaises identifications dues à une erreur ou une confusion taxonomique peuvent avoir de lourdes conséquences pour la gestion des stocks d'une espèce (Marko et al. 2004 ; Iglésias et al. 2010).

De manière générale, les espèces marines sont considérées comme moins vulnérables à l'extinction que les espèces terrestres (Powles, 2000). Cependant, certains groupes d'espèces marines sont beaucoup moins résilients que d'autres, comme le groupe des élasmobranches avec ses traits d'histoire de vie particuliers (ex. faible fécondité, maturité tardive, peu de petits par portée) (Reynolds et al. 2005). De plus, selon des évaluations de l'IUCN, comme la plupart de ces animaux sont pélagiques ou vivant en profondeur, ce qui rend leur suivi plus compliqué, le manque de données touche 47% de ces espèces (IUCN, 2008). Il est évident que ce groupe nécessite une attention particulière de la part des scientifiques pour améliorer leur conservation au niveau mondial, en augmentant les données sur leur biologie et leur écologie.

Le principal objectif de mon diplôme fut de collecter les premières informations sur la biologie, l'écologie et le comportement de la raie aigle à ocelles en Polynésie française afin d'améliorer l'appréciation de la protection dont pourrait nécessiter l'espèce dans cette partie du globe.

Taxonomie et Description morphologique des raies aigle

Les élasmobranches comprennent les requins avec 576 espèces et les batoïdes (raies, poisson-scies, guitares, torpilles, etc.) qui représentent le groupe le plus diversifié avec au moins 787 espèces (Eschmeyer et al. 2017). Ce groupe est divisé en 26 familles et a été décrit comme “the most taxonomically problematic of all elasmobranch groups” (Last et al. 2016). La famille des Myliobatidae est l’une de celles qui a nécessité l’attention des scientifiques récemment. Depuis 10 ans, le genre *Aetobatus* a été le sujet de découvertes importantes : un complexe d’espèces confirmé (Richards et al. 2009 ; Schluessel et al. 2010a), une nouvelle espèce décrite (*Aetobatus narutobiei*, White et al. 2013), et une espèce redécrite (*Aetobatus flagellum*, White & More 2013). Au final, la décision fut prise d’élever le genre Aetobatus au rang de famille sous le nom “Aetobatidae” (Table II-I, p.30).

La famille Aetobatidae comprend cinq espèces: *Aetobatus narinari*, *A. ocellatus*, *A. narutobiei*, *A. flagellum* and *Aetobatis laticeps*, communément appelées “raies aigle pélagiques”, caractérisées par leur forme oblongue, leur museau en forme de bec et leur capacité à se déplacer en pleine eau. La famille se distingue de la famille Myliobatidae (Figure II-1, p.31) entre autre par son dos recouvert de points, barres ou ocelles blanches, dont la répartition est propre à chaque individu. Sa partie ventrale est de dominance blanche avec des taches noires sur le bord des nageoires pectorales (Compagno & Last, 1999). Les raies aigle sont comprimées dorso-ventralement, avec la bouche et les cinq paires de branchies situées sur la face ventrale, deux spiracles sur la partie supérieure du corps, les yeux situés de chaque côté de la tête, et les nageoires raccordées à la tête. Ces nageoires assurent, en cas de fuite, une importante poussée par battement. La queue, parfois jusqu’à cinq fois plus longue que le corps, est fine et pourvue de dards venimeux. Les raies aigle sont vivipares matrotrophiques (échange de nutriments avec la mère à travers l’embryotrophe) avec de un à quatre petits par portée (Schluessel et al. 2010b). La période de gestation varie de 180 jours à une année et est fortement liée à la température de l’eau : plus l’eau est chaude, plus la période sera courte (Schluessel et al. 2010b; Harris, 1952 ; Wallman & Bennett, 2006).

Les raies aigle présentent plusieurs dimorphismes sexuels. Les mâles possèdent deux appendices sexuels externes, les pterygopodes (Figure II-2, p.31). Durant l’accouplement, le mâle insert un de ses deux appendices dans une posture ventre à ventre ou ventre contre dos avec la femelle. Les pterygopodes sont visibles depuis le dessus chez les mâles adultes. Les mâles deviennent matures à 130 cm d’envergure alors que les femelles le sont à 150-160 cm d’envergure (Schluessel et al. 2010b). Les femelles sont plus grandes que les mâles (Schluessel et al. 2010b).

Pour la recherche de nourriture, les raies aigle utilisent leur museau doté de deux excroissances rétractables, qu'elles déplient et utilisent comme une truelle pour retourner les fonds sableux. Leur mâchoire est composée de dents plates disposées en chevron, capables d'écraser les coquilles de mollusques (Summers, 2000 ; White et al. 2010). Schluessel et al. (2010b) ont analysé les contenus stomacaux d'individus d'Australie et de Taiwan et ont démontré une préférence alimentaire pour les bivalves, les gastéropodes et les malacostracés.

Les raies aigle sont des espèces dites « bentho-pélagiques » avec une distribution circumtropicale. Elles occupent une grande variété d'habitats, allant des lagons au grand large en passant par les eaux côtières et les estuaires. Elles peuvent être observés en large bancs de centaines d'individus mais aussi isolés (Bassos-Hull et al. 2014).

Les raies aigle dans l'écosystème corallien : importance et vulnérabilité

L'évaluation de la biodiversité est un élément central de la biologie de la conservation, pas seulement dans le but de déterminer la richesse spécifique mais également pour évaluer les différences entre les communautés occupant des espaces différents et évoluant dans le temps (Gauthier-Clerc et al. 2014). Actuellement, le changement climatique et les impacts humains plus localisés affaiblissent les espèces et engendrent des pertes profondes de biodiversité et des fonctions associées. Parmi les écosystèmes les plus fragiles de notre planète, les récifs coralliens ont toujours attiré l'attention grâce à leur biodiversité extrêmement élevée (25% de la biodiversité marine, alors qu'ils ne constituent que 0.2% des océans) et aux services (ex., source de nourriture, protection des côtes et cycle nutritif) qu'ils rendent à plus de 50 millions de personnes dans le monde (Costanza et al. 1997). Malheureusement, les récifs coralliens sont actuellement grandement menacés : 20% ont disparu depuis les vingt dernières années, 25% sont en grand danger et 25% seront menacés d'ici 2050 (Hughes et al. 2017 ; Chin et al. 2011).

Parmi les 4000 espèces de poissons décrites dans les récifs coralliens la conservation des raies aigle fait l'objet de préoccupations sérieuses de la part de l'IUCN qui a listé les espèces *A. narinari*, *A. ocellatus*, et *A. flagellum* réciproquement dans les catégories « presque menacé », « vulnérable » et « en danger ». En tant que mésoprédateurs, les raies aigle représentent un élément important de la complexité des écosystèmes coralliens et de la chaîne alimentaire, étant à la fois prédateur et proie. Leurs traits d'histoire de vie (croissance lente, maturité tardive, grossesse longue, peu de petits par portée, et ne se reproduisant pas chaque année) (Hoenig & Gruber, 1990 ; Schluessel et al. 2010b) les rendent particulièrement sensibles à de nombreuses activités anthropiques. Les raies aigles sont des captures accessoires des pêcheries vivrières dans la majorité

de leur aire de distribution. Leur habitat côtier et leur capacité de nage les rend particulièrement sensibles à une grande variété de méthodes de pêche (pêche au filet maillant, chalutage de fond ou pêche à la palangre). De plus l'alimentation les raies aigle et les Hommes sont localement en concurrence concernant la capture de bivalves. Certaines espèces de raies aigle sont alors considérées comme des espèces nuisibles à cause de l'impact notable qu'elles ont sur la diminution des bivalves.

Cependant, des études ont démontré que les régulations actuelles de population de raies aigle étaient réalisées sans réelle connaissance de l'écosystème et pouvaient avoir des conséquences inattendues (Yamaguchi et al. 2005). Selon Schluessel et al. (2010b), *A. ocellatus* ne se nourrit pas spécifiquement d'huîtres si les autres composants de son alimentation sont présents en quantité suffisantes dans son environnement.

Connaissances scientifiques sur les raies en Polynésie française

Sept espèces de raies myliobatiformes sont recensées en Polynésie française : deux espèces de Manta (*Manta birostris* et *Manta alfredi*), une espèce de raie aigle (*Aetobatus ocellatus*), une espèce de mobula (*Mobula tarapacana*), deux espèces de raies armées (*Taeniurops meyeni* et *Himantura fai*), et une espèce des profondeurs (*Plesiobatis daviesi*). En Polynésie française, les élasmobranches ont une forte valeur patrimoniale, au travers d'utilisations culturelles, religieuses et sociales par la société polynésienne depuis le début du 18ème siècle. Jusqu'en 2012, ils étaient également une source légale de nourriture. Depuis, toutes les espèces de requins et les raies Manta sont protégées par une loi de pays interdisant les activités de pêche ou le commerce des ailerons. Les élasmobranches sont aussi un atout primordial pour le tourisme, premier pilier économique de la Polynésie française (IEOM, 2015). Ils représentent une opportunité pour le territoire d'augmenter la fréquentation touristique au travers des activités de plongée libre et de plongée sous-marine ou le développement des activités d'observation de la vie sauvage en milieu naturel (shark ou ray watching, Torrente & Clua, 2017).

La raie aigle à ocelles, aussi appelée « Fai manu » en Tahitien (raie oiseau), est présente dans les cinq archipels de la Polynésie française (Bacchet et al. 2016). Elle ne bénéficie pas de protection juridique particulière. Sa chair est consommée localement de façon occasionnelle (pers.obs), mais l'espèce n'a jamais présenté un intérêt majeur dans les ressources alimentaires de la population polynésienne. Par contre, les raies aigle sont considérées comme des animaux nuisibles dans certains

atolls/îles de Polynésie française, où elles se nourrissent de bivalves (huitres perlières) à forte valeur commerciale (ISPF, 2014) (Planes et al. 2005).

De par leur attrait touristique important sur les sites de nourrissage de raies, appelés « ray feeding », les raies pastenagues ont fait l'objet de plusieurs études d'impact dont une thèse (Gaspar, 2008) à Moorea. En ce qui concerne les raies aigle à ocelles les connaissances scientifiques sont limitées en Polynésie française et nécessitent d'être approfondies.

2. Problématique scientifique

Ce diplôme a pour objectif de collecter les premières données biologiques et écologiques sur la raie aigle à ocelles en Polynésie française. Parmi les nombreuses inconnues, j'ai décidé de me concentrer sur quatre questions principales :

- Quelle espèce est présente en Polynésie française ?

Jusqu'aux travaux taxonomiques récents, l'espèce considérée comme évoluant dans les eaux polynésiennes était *Aetobatus narinari*, de la famille Myliobatidae. Aujourd'hui, le genre Aetobatus a été érigé au rang de famille et A. narinari considéré comme étant en réalité un complexe d'espèces. Le premier objectif du diplôme est donc de confirmer l'espèce ou les espèces présentes en Polynésie française.

- Comment les raies de Moorea utilisent-elles les différents habitats du lagon?

L'espèce n'ayant jamais été étudiée à Moorea, j'ai passé une année à observer deux groupes de raies et à rassembler des données sur la répartition spatio-temporelle des individus.

- Chez les raies aigle, comment s'effectue le déparasitage?

Cette question vient de l'observation opportuniste d'un comportement réalisée au cours du diplôme. L'écologie comportementale des poissons est une discipline scientifique complexe, nécessitant des compétences spécifiques pour être capable d'observer un comportement et d'en comprendre la connexion cognitive associée. Ce comportement pourrait aider à une meilleure compréhension de la biologie (dans le domaine du déparasitage) des raies aigle et ouvrir de nouvelles perspectives de recherche.

- Les sons d'origines anthropiques sont-ils susceptibles d'impacter le comportement des raies aigle?

Les raies aigle et leur emblématique proie polynésienne, l'huître perlière à lèvres noires *Pinctada margaritifera*, sont respectivement d'une grande importance touristique et commerciale pour le territoire. La déprédation des huitres perlières à forte valeur ajoutée par les raies aigles nécessite la mise en place de mesure de gestion afin d'assurer à la fois la préservation de l'espèce et de l'économie locale. Dans cette dernière partie de diplôme, j'ai testé un dispositif sonore pour connaître son impact sur les raies aigle. Le dispositif, s'il venait à être répulsif, pourrait être utilisé dans les concessions perlières pour diminuer la prédation des huîtres par les raies.

Toutes ces données apporteront de nouvelles informations sur l'écologie, la biologie, l'habitat et le comportement d'*A. ocellatus*, informations indispensables pour une meilleure connaissance de l'espèce afin d'améliorer les plans de gestion et de conservation qui pourraient être mis en place.

3. Résultats obtenus

Etude 1/ Mise en évidence de la présence d'Aetobatus ocellatus (Myliobatidae) en Polynésie française

Ce chapitre a fait l'objet d'une publication dans Cybium : Berthe Cécile, Mourier Johann, Lecchini David, Rummer Jodie L., Sellos Daniel Y., and Iglésias Samuel P. (2016a) DNA barcoding supports the presence of the cryptic ocellated eagle ray, Aetobatus ocellatus (Myliobatidae), in French Polynesia , South Pacific by. 40(2): 181–184.

Jusqu'aux travaux taxonomiques récents, l'espèce considérée comme évoluant dans les eaux polynésiennes était *Aetobatus narinari*, de la famille des Myliobatidae. En 2017, le genre *Aetobatus* a été érigé au rang de famille et *A. narinari* considérée comme étant en réalité un complexe d'espèces. Le premier objectif du diplôme est donc de confirmer à l'aide d'outils moléculaires l'espèce ou les espèces présentes en Polynésie française. L'échantillonnage des raies a été effectué dans les archipels des Tuamotu (Rangiroa et Manihi) et de la Société (Moorea et Maupiti) en 2012. Les prélèvements ont été conservés dans de l'éthanol à 98% à 4°C. L'ADN global a été extrait et l'analyse du cytochrome c oxidase sous-unité 1 (COI) a été réalisé par amplification par polymérisation en chaine (PCR). Les séquences COI obtenues ont ensuite été alignées avec des séquences d'autres spécimens de la famille *Aetobatidae* (anciennement genre *Aetobatus*) pour réaliser un arbre de Neigbourg-Joining (NJ). L'espèce du genre *Aetobatus* présente en Polynésie française a été identifiée

comme étant *A. ocellatus*, ou raie aigle léopard à ocelles. Tous les échantillons ont le même haplotype, suggérant que ce sont des conspécifiques et ils partagent une forte similarité génétique avec les spécimens d'Australie et de Nouvelle-Calédonie. La répartition complète de cette espèce ainsi que la présence d'autres espèces de la famille Aetobatidae en Polynésie française nécessiteraient plus d'études, en particulier dans les îles des Gambiers, des Marquises et des Australes.

Etude 2/ Patterns de distribution de la raie aigle à ocelles selon des mesures biologiques et physiques sur deux sites de Moorea, Polynésie française.

Ce chapitre fait l'objet d'une soumission dans le journal scientifique PlosOne : Berthe, Cécile, Besson, Marc, Latry, Lise, Bertucci, Frédéric, Lecellier Gaël, Iglésias Samuel and Lecchini David (2017) Distribution patterns of ocellated eagle rays from biological and physical measurements along two sites at Moorea Island, French Polynesia

Les raies aigle peuvent être vues en banc à plusieurs endroits autour de Moorea (centre de plongée, pers. obs.). En 2012, j'ai cherché des sites propices à l'observation régulière, mais nombre d'entre eux sont situés dans les passes autour de l'île, sujets aux courants, à une visibilité réduite et à un important trafic en surface. Deux sites se sont révélés appropriés à mon étude : le chenal de l'ancien centre touristique « Club Med » et la zone de mouillage de la plage publique Mareto, tous deux situés sur la côte nord de Moorea. Pendant une année, les données environnementales (date, heure, température de l'eau, marée, lune, direction et hauteur de houle, force et direction du vent, force du courant) et biologiques (présence ou non des raies, nombres, sexes, activités et autres espèces présentes) ont été collectées. Une analyse des correspondances multiples (MCA, Figure IV-3, p.50) a été effectuée pour identifier la relation entre le sexe et le stade ontogénique des raies et le site, la saison et l'heure de la journée. Les résultats montrent qu'il y a plus d'adultes (mâles et femelles) au chenal du Club Med le matin et plus de jeunes à la plage publique l'après-midi. Une analyse multi-facteur (MFA, Figure IV-4, p.51) a été effectuée pour relier la distribution spatio-temporelle des raies aux facteurs environnementaux. Les résultats montrent que les jeunes sont plus présents en l'absence de courant et les femelles en présence de vent du nord. Les autres variables environnementales n'entraînent pas de discrimination particulière. Les analyses démontrent aussi une prévalence pour la recherche de nourriture pour les jeunes de la Mareto, pendant que les adultes du chenal du clubmed adoptent un regroupement de type « repos ».

Etude 3/ Comportement de déparasitage par frottement contre un banc de sable par la raie aigle à ocelles (Aetobatus ocellatus)

Ce chapitre a fait l'object d'une publication dans Marine Biodiversity (2016b): Berthe C., Lecchini D. & J. Mourier J. (2016) Chafing behavior on a patch of sandy bottom by ocellated eagle ray (Aetobatus ocellatus). Marine Biodiversity: 8–11.

Héberger des parasites est couteux en énergie et de nombreux poissons des récifs coralliens sont connus pour utiliser les « stations de nettoyage » ou le frottement contre le sable, voire contre d'autres poissons (e.g., Losey et al. 1999; O'Shea et al. 2010) pour se déparasiter. Bien que les raies aigle soient connues pour héberger des ectoparasites (Marie et Justine 2006), la façon dont elles s'en débarrassent reste inconnue, car elles n'utilisent pas ces stations de nettoyage. J'ai rapporté ici deux observations de raies aigle à ocelles (*Aetobatus ocellatus*) (Berthe et al. 2016a) se frottant contre des bancs de sables en Polynésie française. La première observation a été faite à Moorea en mai 2011. La seconde observation a été réalisée dans l'atoll de Fakarava en juin 2014 (Figure V-1, p.59). Dans les deux cas, un individu est venu frotter sa partie ventrale (vraisemblablement au niveau des nageoires ou des branchies) contre le sable. Le comportement de frottement des raies aigle serait une stratégie dans la fonction de déparasitage ou en complément d'utilisation de station de nettoyage. Ce comportement a déjà été rapporté pour les requins de récif des Caraïbes (*Carcharhinus perezi*) et les requins pointe-noire (*Carcharhinus limbatus*) sur les ridules de sables (Ritter REF). De futures études devront être menées pour améliorer les connaissances sur ces différentes techniques de déparasitage et leur efficacité de nettoyage chez les élasmobranches.

Etude 4/ Influence des bruits anthropiques sur le comportement de fuite des raies aigle Aetobatus ocellatus à Moorea (Polynésie française)

Ce chapitre a fait l'objet d'une publication dans Comptes Rendus Biologies (2016c): Berthe C. & Lecchini D. (2016) Influence of boat noises on escape behaviour of white-spotted eagle ray Aetobatus ocellatus at Moorea Island (French Polynesia). Comptes rendus biologies 339(2): 99–103.

En 2012, le CRIOBE a mené un projet de recherche auquel j'ai participé pour développer des systèmes limitant la prédation de l'huître perlière par des durophages : les balistes, les poissons coffres, les napoléons et les raies aigle léopard (*Aetobatus ocellatus*). Des systèmes chimiques, électriques et acoustiques ont été testés à Moorea (archipel de la Société), et à Rangiroa et Manihi (archipel des Tuamotu). Comme le système acoustique a montré des signes intéressants de répulsions, j'ai poursuivi les tests sur les raies à Moorea. Le système est composé d'un boiter étanche

flottant comportant une batterie et un amplificateur, relié à un lecteur MP3 et un hydrophone. Les tests ont été réalisés en milieu naturel sur des raies adultes en train de manger dans la zone de mouillage de la plage publique Mareto. Différents sons pouvant perturber les raies pendant leur recherche de nourriture ont été testés : des bruits artificiels et des bruits de moteurs de bateau, tous vérifiés et calibrés grâce à un accéléromètre (Figure VI-3, p.66). Les résultats montrent que le bruit artificiel « White » ou des fréquences uniques (40 Hz, 600Hz ou 1 kHz) n'ont aucun effet sur les raies (100% des raies continuent de chercher leur nourriture dans le sable), tandis que l'enregistrement d'un moteur de bateau perturbe significativement les raies pendant cette activité (60% des individus ont montré un comportement de fuite, Figure VI-4, p.68). Les résultats de cette étude pourraient intéresser les professionnels de la perliculture, pour éloigner les raies aigle des concessions perlières et diminuer ainsi la prédation et les dommages matériels causés par cette espèce.

4. Conclusion et Perspectives

Perspectives en recherche fondamentale sur les raies aigle à ocelle

La dernière décennie a connu un intérêt grandissant pour la recherche sur le comportement animal. De nombreuses études ont été menées récemment pour décrire et comprendre les agrégations de requins et si ces agrégations supposent des comportements sociaux (Jacoby et al. 2014 ; Gallanger et al. 2014 ; Haulsee et al. 2016 ; Findlay et al. 2016). Newby et al. (2014) a exploré l'organisation sociale chez *Aetobatus narinari* et a mis en évidence l'absence de structure sociale liée à la parentalité. Pendant mon année d'observation à Moorea, j'ai pu identifier les individus et estimer les populations sur lesquelles j'ai travaillé. Des recherches futures pourraient utiliser ces données pour explorer les comportements au sein de ces populations, comme cela a déjà été fait chez les requins. Au sein de ces regroupements, les raies pourraient par exemple avoir des fonctions particulières ou présenter des préférences pour d'autres individus du groupe. Par ailleurs mes observations répétées d'un mâle adulte sur quatre années, permettent d'envisager la sédentarité de l'espèce, ou du moins de certains individus, au sein du lagon de Moorea et apportent une information importante pour la conservation de l'espèce.

Perspectives de conservation des raies aigle

Le statut actuel d'*Aetobatus ocellatus* sur la liste rouge de l'IUCN Red List est "Vulnérable" et des informations sur sa répartition, sa population et sa biologie sont toujours requises pour évaluer correctement un statut de conservation fiable de l'espèce (Kyne et al, 2016).

Osgood & Baum (2015) encouragent les chercheurs à porter leur attention sur les lacunes mentionnées par l'IUCN afin de combler ces manques, en taxonomie et données géographiques en

particulier. “The batoid fishes have long been regarded as the most taxonomically problematic group of all elasmobranch groups” (Last et al. 2016). La famille taxonomique Aetobatidae est un bon exemple de cette problématique taxonomique, au vu des nombreuses révisions qu’elle a nécessité ces dernières années. De plus, son ‘chemin de dispersion’ semble aller de la région Indo-Pacifique à l’océan atlantique, puis à l’océan pacifique à travers l’isthme de Panama (Richards et al. 2009 ; Schluessel et al. 2010a). Ceci place les îles Marquises à la fin de la boucle de dispersion. Situées à l’extrémité est du pacifique central, les Marquises sont connues pour leur exceptionnelle biodiversité avec un haut taux d’endémisme. Mon étude a permis de confirmer la présence d’*Aetobatus ocellatus* dans les eaux polynésiennes. Mais, elle n’exclut pas la présence d’autres espèces de la famille. Explorer l’ensemble des archipels apporterait des informations sur la répartition exacte de la famille, confirmerait ou non la présence d’autres espèces et ajouterait des connaissances sur la structure de la population d’*Aetobatus ocellatus* dans la région Indo-Pacifique.

Perspectives de recherche sur les raies aigle en Polynésie française

A Moorea, une étude de l’ensemble du lagon pourrait être réalisée afin d’identifier l’ensemble des habitats fonctionnels et de localiser les zones de nurserie ou de repos de l’espèce. Les zones de nurseries sont vitales pour la stabilité et la résilience des populations (Bonfil, 1997). Savoir où se situent ces zones est primordial pour la conservation de l’espèce. Deux principaux projets sur la gestion de la biodiversité sont actuellement en cours à Moorea: 1/ la révision du Plan de Gestion de l’Espace Maritime (PGEM) de l’île après 10 années de fonctionnement et 2/ le projet Moorea IDEA (Davies et al. 2016) dont le but est de modéliser le système socio-écologique entier de l’île. Utiliser des informations comme celles fournies par mon diplôme pourraient aider ces deux projets à tester leur viabilité et leur impact sur l’écosystème.

En Polynésie française, des études pourraient être menées pour mieux comprendre le chevauchement entre la répartition de l’habitat des raies et les activités humaines. Selon les perliculteurs les dommages causés peuvent variés d’un atoll à l’autre. Pourquoi certains atolls sont-ils plus touchés que d’autres ? Des informations sur les patterns de déplacement des raies aigle, la géomorphologie des atolls (fermés ou ouverts, passes étroites ou larges, etc.) ou la disponibilité en proies naturelles pourraient certainement aider à comprendre ces spécificités. Schluessel et al (2010b) ont démontré qu’*Aetobatus ocellatus* dans la baie de Moreton (Australie) ne se nourrit pas des huîtres élevées dans la baie et a présumé que c’était grâce à l’abondance de sources de nourriture naturellement présentes dans la baie. L’huître perlière à lèvres noires est-elle particulièrement attractive pour les raies aigle ? Est-ce que les sources de nourriture naturelle sont

moins abondantes dans les atolls concernés par les dommages ? Il est évident que les réponses à ces questions aideront à anticiper le comportement des raies aigle et à trouver une solution durable pour protéger deux espèces à fortes valeur ajoutées pour l'économie locale.

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Chapter II General Introduction

1. Knowledge and Conservation of marine species

Conservation of marine species is of great concern worldwide, with a steady increasing rate of threatened marine species (International Union for the Conservation of Nature, 2016). However, to protect a species, its habitats and ecosystems, people first need to understand the concerned species. Taxonomy is the foundation of this knowledge (White & Last 2012; Collares-Pereira et al. 2015) and taxonomic failure or confusion can cause mislabeling and thus can lead to dramatic loss (Marko et al. 2004; Iglésias et al. 2010). For example, the common skates *Dipturus cf flossada* and *Dipturus cf intermedia* were considered as a single species (*Dipturus batis*). Iglesia et al. (2010) identified some morphological and physiological characteristics which differentiated them. While *D. cf flossada* reaches sexual maturity at 130 cm, *D. cf. intermedia* reaches it at 180 cm. Had we not had this information, fishermen would have continued to catch the two species as if they were the same and to focus their effort on the larger individuals, which could have led to the extinction of *D. cf. intermedia*. Morphological and taxonomical studies can thus improve species management and conservation.

In general, marine species are thought to be less vulnerable to extinction than terrestrial species (Powles, 2000). However, some marine groups tend to be less resilient than others. The elasmobranch group is one of those due to its life-history traits (e.g. low fecundity, late maturity, few pups) (Reynolds et al. 2005). According to the IUCN assessment, 47% of the elasmobranch species are data deficient because most of them are pelagic or deep-sea species making them difficult to study. This group obviously requires greater attention from scientists to improve their conservation worldwide by collecting more data on their biology and ecology.

The main goal of my EPHE diploma was to gather the first biological, ecological and behavioral dataset on the ocellated eagle ray (*Aetobatus ocellatus*) in French Polynesia, in order to get a better understanding of the protection the species could need in this part of the world.

2. Eagle rays in the world

The Elasmobranches include sharks with 576 species and batoid fishes being the most diverse group with at least 787 species found in diverse habitats worldwide (Eschmeyer et al. 2017). This group is divided into 26 families and has been described as “the most taxonomically problematic of

all elasmobranch groups" (Last et al. 2016). Many species have recently been described and taxonomic work had to be done to evaluate the new classification of these species. The Myliobatidae family is one of those families which needed scientific attention. Over the last ten years, one of its genera, *Aetobatus*, was subjected to huge discoveries: one species complex was confirmed (Richards et al. 2009; Schluessel et al. 2010a), a new species was described (*Aetobatus narutobiei, White* et al. 2013), and a species was re-described (*Aetobatus flagellum,* White & More, 2013). These discoveries resulted in the re-definition of the genus *Aetobatus* into the Aetobatidae family (Table II-I).

Table II-I : Classification of the Aetobatidae Family

Kingdom	Animalia
Phylum	Chordata
Class	Chondrichthyes
Sub-class	Elasmobranchii
Superorder	Batoidea
Ordre	Myliobatiformes
Famille	Aetobatidae
Species	*Aetobatus narinari*
	Aetobatus ocellatus
	Aetobatis laticeps
	Aetobatus flagellum
	Aetobatus narutobiei

Aetobatidae family include five species: *Aetobatus narinari*, *A. ocellatus*, *A. narutobiei*, *A. flagellum* and *Aetobatis laticeps*, commonly named "pelagic eagle ray" in reference to their oblong shape, their spout-shape nose and their capacity to live in open-waters. The individuals from this family distinguishes themselve from Myliobatids among other things by their dark back with white dots, spots or bars (Figure II-1), which repartition is particular to each individual. Their ventral part is predominantly white with dark stains at the edges of the pectoral fins (Compagno & Last, 1999). Eagle rays are dorso/ventrally flattened, with their mouth and their five gills clefts located on the lower part of the body, two spiracles on the upper part, their eyes on each side of the head and their fins connected to the head. These fins give the species a huge thrust by flapping, allowing them to escape. Their tail, which can sometimes be five times longer than the body, is thin and encompass one to six venomous stings. As all cartilaginous fish, aetobatids are matrotrophic viviparous meaning that nutrients are exchanged with the mother through the embryotrophe, and they have only a few offspring per litter (Schluessel et al. 2010b).

Figure II-1: On the left, Myliobatis australis (©Australian National Fish Collection, CSIRO), on the right Aetobatus ocellatus(©C.Berthe)

The gestation period ranges from 180 days to one year and seems to be related to water temperature: the warmer the water the shorter the gestation period (Schluessel et al. 2010b; Harris, 1952; Wallman & Bennett, 2006). Yokota et al. (2006) studied the nursery areas of several elasmobranches. For *A. narinari*, they captured neonates during the first half of the year (January to June) and juveniles all year round. They then assumed a calving period at the beginning of the year.

Eagle rays show sexual dimorphisms. Males possess two external sexual appendixes, named pterygopodes or claspers (Figure II-2). During the copulation, the male insert one of its claspers in a belly to belly or belly to back posture with the female. Those sexual appendixes are visible from above for adult males, but a ventral observation is needed for non-mature individuals. Males reach sexual maturity when their disc width is about 130 cm whereas females reach it at 150-160 cm (Schluessel et al. 2010b). Eventually females are larger than males (Schluessel et al. 2010b).

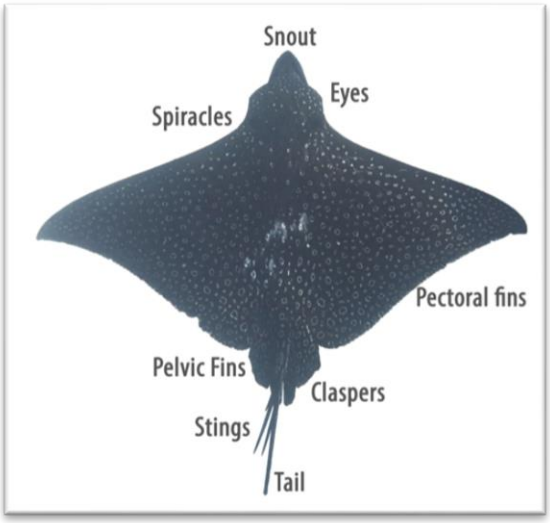

Figure II-2: Morphology of ocellated eagle ray with the main external characteristics (from top to bottom: snout, eyes, spiracles, pectoral fins, pelvic fins, claspers - only for male - , stings and tail) that could be useful to identify the individual

Eagle rays use their snout to forage. This snout consists in two retractable excrescences which can be spread out and use as a trowel to forage the sandy bottoms. Eagle rays have a series of flattened chevron-shaped teeth to crush hard-shelled molluscs (Summers, 2000; White et al. 2010). Schluessel et al. (2010b) analyzed stomach contents of individuals from Australia and Taiwan and showed a preferential diet for bivalves, gastropods and malacostraca.

Eagle rays are bentho-pelagic species with a circumtropical distribution. Therefore, they occupy a wide-range of habitats, including lagoon, coastal waters or open-seas. They can be observed in large school as well as alone (Bassos-Hull et al. 2014). Silliman et al. (1999) built up a behavioral catalogue of eagle rays in the Bimini. Among other behaviors they observed vertical movements during the swimming, school formations and jumps. Their study showed that schooling always take place at the same location and the same period of the day (according to tidal movement) and are organized (line- or diamond-shaped).

3. Eagle rays in coral reefs: importance and vulnerability

Biodiversity assessment is a central and urgent task in conservation biology, not only to determine species richness but also to evaluate differences between communities occupying different areas or changing with time. The impact of anthropogenic global environmental changes is so pervasive that some scientists consider that a global 6th extinction crisis, comparable to those that occurred over evolutionary time scale, has already started (Barnosky et al. 2011). Climate changes and more localized human impacts are undermining populations of vulnerable taxa and inducing profound losses of biodiversity and of associated functions. Thus, the rates of environmental change now being induced by human activity are well beyond those naturally experienced by most ecosystems. Among the most fragile ecosystems on Earth, which include for example the Polar regions and primary forests, coral reefs have always been under the spotlight of society due to their extremely high biodiversity (25% of the marine biodiversity while only constituting 0.2% of the ocean) and their services (e.g., food supply, protection of the coasts and nutrient cycling) provided to at least 50 million people worldwide (Costanza et al. 1997). Unfortunately, coral reefs are being severely threatened: 20% of coral reefs are already no more definable as such, another 25% are in great immediate threat and 25% will be threatened by 2050 (Hughes et al. 2017; Chin et al. 2011).

With more than 4,000 species of fish described, coral reefs support 25% of the world's fish biodiversity, and provide between 9 and 12% of the world's total fisheries. However, despite the huge numbers of studies on coral reef fish, some key species for the functioning of coral reefs are still

little studied. It is the case of eagle rays. As mesopredator, eagle rays represent an important link in the coral reef ecosystems complexity in general and especially in the trophic's food chain, being both predator and prey. Their life history traits (slow growing, late maturity, long pregnancies, few pups per litter, and may not reproduce every year) (Hoenig & Gruber, 1990; Schluessel et al. 2010b) make eagle rays very sensitive to many anthropic activities.

Eagle rays conservation is of great concerns according to the IUCN that listed the species *A. narinari*, *A. ocellatus*, and *A. flagellum* in the "near threatened", "vulnerable" and "endangered" categories, respectively. The last two species still need an evaluation of their conservation status. The species are likely to contribute to numerous inshore artisanal fisheries for most of their home range. Their coastal habitat and swimming capacities make them sensitive to a range of fisheries practices (gill-net fisheries, bottom trawl or demersal longline). Moreover eagle rays and human beings are locally in competition concerning the catch of bivalves. Some species of eagle rays are considered as pest because they move in mass migration into places where they have a huge impact on the decrease of the bivalve population (e.g., the eagle ray *A. flagellum* in Japan, Yamaguchi et al. 2005 or the cownose ray *Rhinoptera bonasus* in the Chesapeake Bay, California – Barker, 2006).

However some studies have demonstrated that current regulations of eagle ray populations are done without full knowledge of the ecosystem and that they have unexpected consequences. Indeed, Yamaguchi et al. (2005) explained the perturbation of the ecosystem after culling the ray to protect oyster farms: crabs which were also preyed on by rays saw their population growing, but it turned out that those crabs are also a major oyster predator. *A. ocellatus* don't feed on oysters if the other components of its diet are available in sufficient quantity in the environment (Schluessel et al. 2010b).

4. Rays in French Polynesia

Seven species of Myliobatiform rays are listed in French Polynesia: two species of Manta (*Manta birostris* and *Manta alfredi*), one species of eagle rays (*Aetobatus ocellatus*), one, species of Mobula (*Mobula tarapacana*), two species of stingrays (*Taeniurops meyeni* and *Himantura fai*), and one deep-sea stingray (*Plesiobatis daviesi*).

Elasmobranches had always played many roles in French Polynesia. Firstly, as previously mentioned, they are keys species in corals reef ecosystem and regulate the marine communities (Temple, 1987). Secondly, they have a huge patrimonial value for local population as they used to

have cultural, religious, social functions in the Polynesian society since the beginning of the 18th century and they were part of legal food resources. Since 2011, sharks and Manta rays are protected by a local law that prohibits fishing activities or fin trade with these animals. Lastly, elasmobranches are one of the greatest assets of the tourism industry, first economic pillar of French Polynesia (IEOM, 2015). They represent an opportunity for the territory as they could help increase the tourism frequentation, by supporting scuba-diving and snorkeling activities and by developing wild watching activities (Torrente & Clua, 2017).

Eagle ray also called « Fai manu » (*bird ray* in Tahitian) in French Polynesia is present in the five archipelagoes of French Polynesia (Bacchet et al. 2016). In the Society archipelagos, they evolve solitary in the lagoon or in the open-sea, and in school in the passes (pers. obs.). In the Tuamotu-Gambier islands, schools of hundreds of individuals have been seen during austral summer (pers.obs). In the Marquesas they benefits a preserved space thanks to the remoteness of the archipelagos (1500 km north-east from Tahiti – Taquet, 2016) and these rays are not under the local protection law. Some locals consume its flesh (pers.obs.) but the species has never been of great interest as a local food resource. As previously mentioned, eagle rays can be considered as pest animals in some locations in the world, where they migrate in numbers and consume until eradication bivalves that are locally of great value. In French Polynesia, eagle rays have never been reported migrating in mass. However they impact the Pearl industry, which is the second economic pillar of the territory (ISPF, 2014) by consuming the Black-lipped pearl oyster *Pinctada margaritifera*, and by huge damages in pearl farms of the Tuamotu islands (Planes et al. 2005).

5. Scientific problematic

As previously mentioned many species of the elasmobranch group are data deficient and need strong focus from scientists to better estimate their population and their resilience to environmental changes. In My EPHE diploma I aimed to gather information on the ocellated eagle ray in French Polynesia, a region where it has never been studied before. Among the many unknowns, I choose to explore four questions:

1. Which species of eagle ray occurs in Polynesian waters?

Before the recent genetic works, the species occurring in French Polynesia was considered to be *Aetobatus narinari*, from the Myliobatidae family. Since the genus *Aetobatus* has been erected at a family level and *A. narinari* defined as a species complex, the first objective of the diploma was to redefine the species occurring in French Polynesia waters.

2. How the eagle rays use the different habitats of the lagoon of Moorea Island?

As the species has never been studied in this part of the world before, I spent a year studying two groups of ocellated eagle rays to gather data on the spatiotemporal repartition of the species.

3. For eagle rays, what is the chafing behavior on sandy bottom for?

This problematic comes from an opportunistic observation of a behavior in the field. Fish behavioral ecology is a complex scientific domain that requires specific scientific skills to be able to observe behaviors and to relate them to specific cognition capacities. This behavior may help to better understand the biology of the eagle rays and lead to new research perspectives.

4. Does anthropic sound influence eagle ray's behavior?

Eagle rays and the Black-lipped Pearl Oysters *Pinctada margaritifera* are such valuable tourist attractions and commodities, respectively. The depredation of the valuable pearl oysters by eagle rays requires the implementation of management rules to preserve both the species and the economy of the French Polynesia. Here I explore a repellent system that pearl farmer could use to decrease the predation of their oyster by the eagle rays.

All of this collected data bring new insights to the ecology, the biology, the habitats, and the behavior of *A. ocellatus*. This set of information is crucial for a better understanding of the ocellated eagle ray and to improve management and conservation plans.

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Chapter III DNA barcoding supports the presence of the cryptic Ocellated Eagle Ray, Aetobatus ocellatus (Myliobatidae), in French Polynesia, South Pacific

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This chapter is based on the manuscript published in Cybium: *Berthe Cécile, Mourier Johann, Lecchini David, Rummer Jodie L., Sellos Daniel Y., and Iglésias Samuel P. (2016a) DNA barcoding supports the presence of the cryptic ocellated eagle ray,* Aetobatus ocellatus *(Myliobatidae), in French Polynesia , South Pacific by. 40(2): 181–184.* Nevertheless, I added here some additional information in order to better explain the study and better justify the link between the different studies in my EPHE Diploma. Locations of samples of ocellated eagle rays were chosen on an opportunistic strategy: samples were done in Rangiroa and Manihi during a mission with the DRMM on repellent system of oyster predators, in Maupiti during personal travel, and in Moorea by students working on sharks nurseries and who caught twice eagle rays in their net. Results support the presence of the ocellated eagle ray species in the Polynesian water. Complete home range of this species and presence of other species from the Aetobatidae family within the French Polynesia need further investigation, especially in the Gambier, Marquesas and Austral Archipelagoes.

1. Introduction

The spotted eagle ray *Aetobatus narinari* (Euphrasen, 1790) is a bentho-pelagic Myliobatidae species and until recently was considered to have a circumtropical distribution. Strong evidence based on genetic analyses suggested that the spotted eagle ray represented a species-complex with an Indo-Pacific lineage that was clearly distinct from the Central Atlantic and the Eastern Pacific lineages (Richards et al., 2009; Schluessel et al., 2010a; Ceyrutti-Pereyra et al., 2012). As a consequence, White et al. (2010), based on morphological and molecular data, split the species complex into two distinct cryptic species by resurrecting the Ocellated Eagle Ray, *A. ocellatus* (Kuhl, 1823), as a valid species occurring in the Indo-West Pacific. This left *A. narinari* restricted to the Atlantic and Eastern-Pacific.

This recent taxonomic revision with the division of *A. narinari* into two cryptic species and the distinction of populations with uncertainty concerning their range of distribution can have further implication for fisheries management and conservation (Dudgeon et al., 2012). *A. narinari* is listed as "Near Threatened" by the IUCN Red List (Kyne et al., 2006); whereas, the status of *A. ocellatus* has not yet been assessed. It is also not clear where in the Pacific Ocean the range of the three species separates or overlaps. Indeed, while samples have been compared between the Eastern Pacific and the Western Pacific, samples from the Central Pacific can clarify the geographic separation between the three species. The 6400 km oceanic expansion of the Pacific Ocean generally represents a genetic barrier between West and East Pacific for most neritic fish species (Schultz et al., 2008). Therefore, we hypothesize that the specimens from French Polynesia (Southern Central Pacific) likely belong to A. ocellatus. Overall, the present study aimed to identify the Aetobatus species present in French Polynesia using DNA barcoding. Our study contributes to a better understanding of the distribution of species in the Aetobatus complex, which is necessary for a correct evaluation of the conservation status of the different species.

2. Materials and methods

Tissue samples were collected from the atolls of Rangiroa and Manihi in the Tuamotu archipelagos and from the islands of Moorea and Maupiti in the Society archipelagos (Figure III-1). Details regarding collection data and photos of sampled individuals are available in the Barcode of Life Data System (BOLDSystems, http://www.boldsystems.org). A piece of tissue was removed by cutting a small fin clip (about 1 cm2) from captured individuals or using a modified spear gun with a biopsy tip mounted at the extremity allowing removal of a piece of tissue from the body of free-ranging individuals, a technique that has been successfully used on stingrays and sharks (Mourier et

al., 2013). Tissue samples were preserved in 98% ethanol at 4°C and are now registered with field numbers (TCB) and collection numbers at the Station de Biologie Marine de Concarneau of the Museum national d'Histoire naturelle (BPS) (Table III-I).

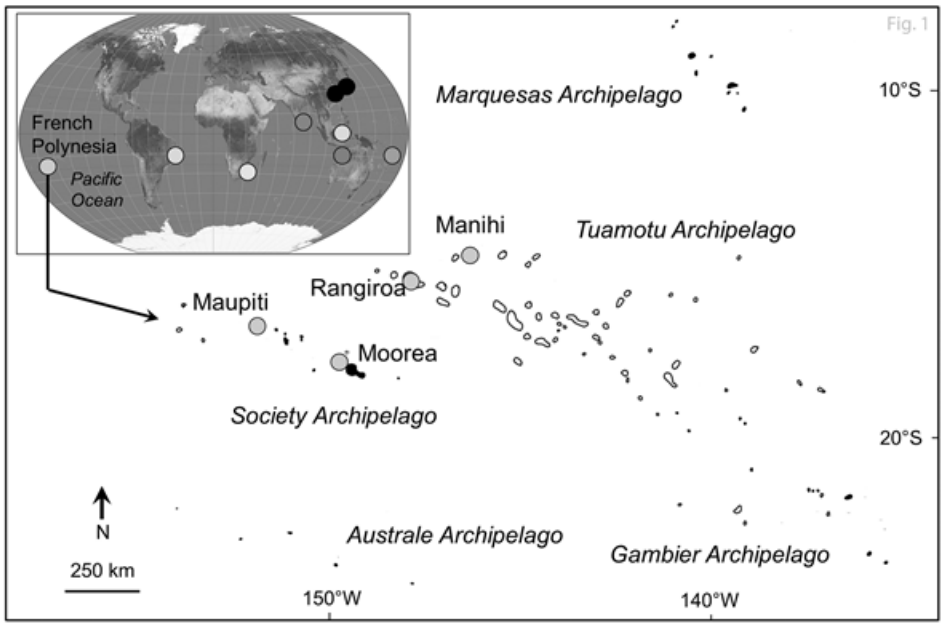


Figure III-1: Locations for sampled *Aetobatus ocellatus* (grey circles) in French Polynesia and for comparative materials (circles in insert: Australia, New Caledonia, Indonesia, India, South Africa, Brazil, Japan, Korea).

Table III-I: References of samples and COI sequences used for the genetic analysis. * Primarily recorded in BOLD Systems as Aetobatus cf. narinari

Locality	Tissue/DNA sample No.	GenBank Access. No.	BOLD Process ID	References
Aetobatus ocellatus				
French Polynesia, Manihi	TCB-001; BPS-2704	KT208286	AETO001-14	Present study
French Polynesia, Rangiroa	TCB-002; BPS-2705	KT208287	AETO002-14	Present study
French Polynesia, Moorea	TCB-004; BPS-2707	KT208288	AETO003-14	Present study
French Polynesia, Moorea	TCB-005; BPS-2708	KT208289	AETO004-14	Present study
French Polynesia, Maupiti	TCB-006; BPS-2709	KT208290	AETO005-14	Present study
French Polynesia, Maupiti	TCB-008; BPS-2711	KT208291	AETO006-14	Present study
Australia, Collier Bay	BW-A3015	EU398507	FOAF143-07	Ward *et al*., 2008
Indonesia, Lombok	BW-A2609	EU398508	FOAE397-06	Ward *et al*., 2008
India, Rameswaram	RMS-01	–	INELA022-12	Pavan-kumar *et al*., unpubl.
New Caledonia, Saint Vincent Bay	BPS-0656	–	ANCC001-13	Iglésias *et al*., unpubl.
Unknown locality	AU_41	JN184054	ANGBF2029-12	Aschliman *et al*., 2012
South Africa, Umhlanga	Smith 28.1 #3	JF492798	TZMSC556-06*	Steinke *et al*., unpubl.
Aetobatus narinari				
Brazil, São Paulo	LBPV53065	JQ365215	MFSP1964-11	Ribeiro *et al*., 2012
Brazil, São Paulo	LBPV53066	JQ365212	MFSP1965-11	Ribeiro *et al*., 2012
Brazil, São Paulo	LBPV53067	JQ365214	MFSP1966-11	Ribeiro *et al*., 2012
Brazil, São Paulo	LBPV53068	JQ365213	MFSP1967-11	Ribeiro *et al*., 2012
Aetobatus narutobiei				
Unknown locality	-	NC_022837	CYTC7664-15	Yang *et al*., unpubl.
Japan	-	AB291068	GBGC3822-07	Sasaki and Hamaguchi, unpubl.
Korea	FAfla050620-1	EU339362	GBGC6418-09	Yoon *et al*., 2009

Global DNA was extracted following the modified method with CTAB (Jones, 1953). Homologous fragments of 652 bp encoding the cytochrome c oxidase subunit 1 (CO1) were amplified via a Polymerase Chain Reaction using the TripleMaster® System (Eppendorf) following the manufacturer's instructions. Fragments were obtained with primer pairs of PCR S156/R249 and S156/R84 (Iglésias & Lévy-Hartmann, 2012). We used the following thermic cycle parameters: pre-denaturation at 94°C for 2 min, 45 cycles of denaturation at 94°C for 1 min, hybridization at 54°C for 1 min, and extension at 72°C for 3 min. At the end of electrophoresis, gel bands containing the PCR products were removed and purified using the QIAquick® gel extraction kit. Sequence reactions were realized using the BigDye® Terminator v3.1 (Applied Biosystems) sequence kit with different primers. The thermocycles were: pre-denaturation at 96°C for 2 min, 40 cycles of denaturation at 96°C for 0.5 min, hybridization at 50°C for 0.5 min, and extension at 60°C for 4 min. PCR and sequence reactions were conducted with a thermocycleur TGradient (Biometra). The sequencing was realized with the genetic analyzer ABI PRISMTM 310 (Applied Biosystems).

Connection of sequences, correction of electropherograms, and alignment of sequences were performed using Sequencher v4.2 (Gene Codes Corporation). Sequences contained no gaps or missing data. The final dataset involved 19 nucleotide sequences from the species *A. ocellatus*, *A. narinari* and *A. narutobiei* (White et al., 2013). The COI sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) and in the BOLDSystems under Accession No. and BOLD Process ID respectively listed in Table II-I. Sequences in the BOLDSystems are part of the project '*Aetobatus ocellatus* identification' (AETO). Evolutionary analyses were conducted in MEGA v5.05 (Tamura et al., 2011). The sequences from our specimens were aligned with available sequences from other Aetobatus specimens to construct a Neighbor-Joining (NJ) tree (Saitou & Nei, 1987) using a distance matrix of Kimura 2-parameter (Kimura, 1980) of genetic sequence similarity. Codon positions included were 1st+2nd+3rd. Bootstrap proportions for the NJ tree were determined with 10,000 pseudoreplicates to estimate each node's robustness. Sequences of the species *A. narutobiei* (according to the recent revision of White et al., 2013) were used as an outgroup to root the tree. The evolutionary model of Kimura 2-parameter (K2p) were also used to estimate the evolutionary divergence between sequences and the average evolutionary divergence over sequence pairs within and between groups.

3. Results

All DNA barcodes of eagle rays from samples obtained from the various locations in French Polynesia have a similar haplotype, suggesting they are all conspecifics. These individuals merge into

a cluster of species identified as *A. ocellatus* (**Error! Reference source not found.**). DNA barcoding provides strong support for species discrimination of *A. ocellatus* and *A. narinari* concordant with previous results (Richards et al., 2009; White et al., 2010). The intraspecific average evolutionary divergence was 0.9% for *A. ocellatus* and 0.3% for *A. narinari*, and the interspecific average evolutionary divergence was 23.7% for *A. ocellatus*/*A. narinari*. Individuals from French Polynesia share a strong genetic similarity with individuals from Australia and New Caledonia (Figure III-2). The highest intraspecific distance occurred between the individuals from French Polynesia and an individual from South Africa, corresponding to the most distant analyzed locations. The intraspecific divergence observed within *A. ocellatus* is about 78 times lower than the interspecific divergence observed between *A. ocellatus* and *A. narinari*, clearly matching the tenfold rule proposed by Hebert et al. (2004) and supporting *A. ocellatus* as a distinct species.

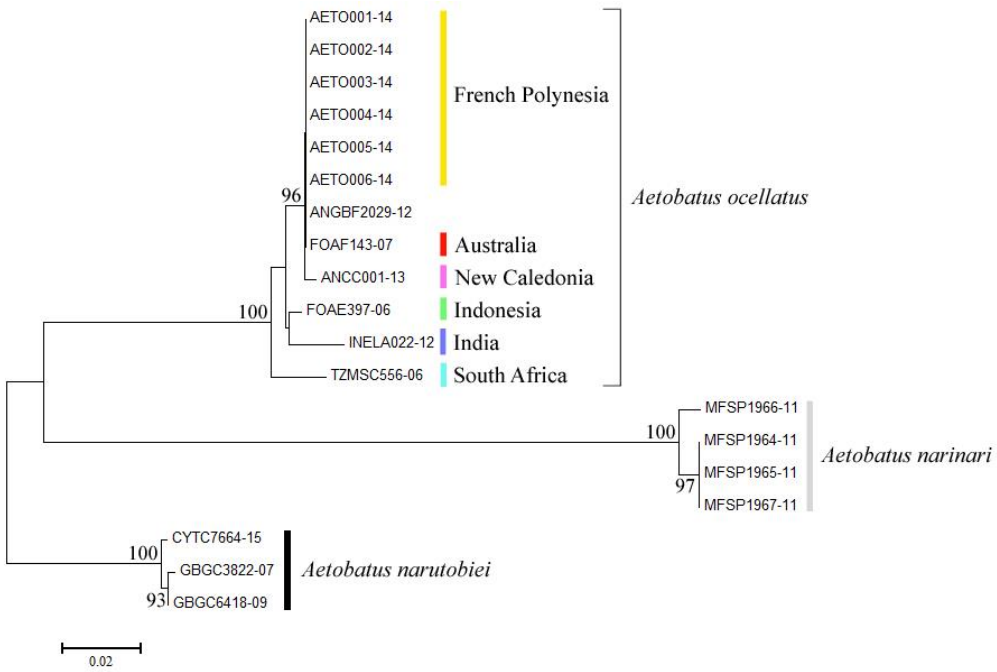


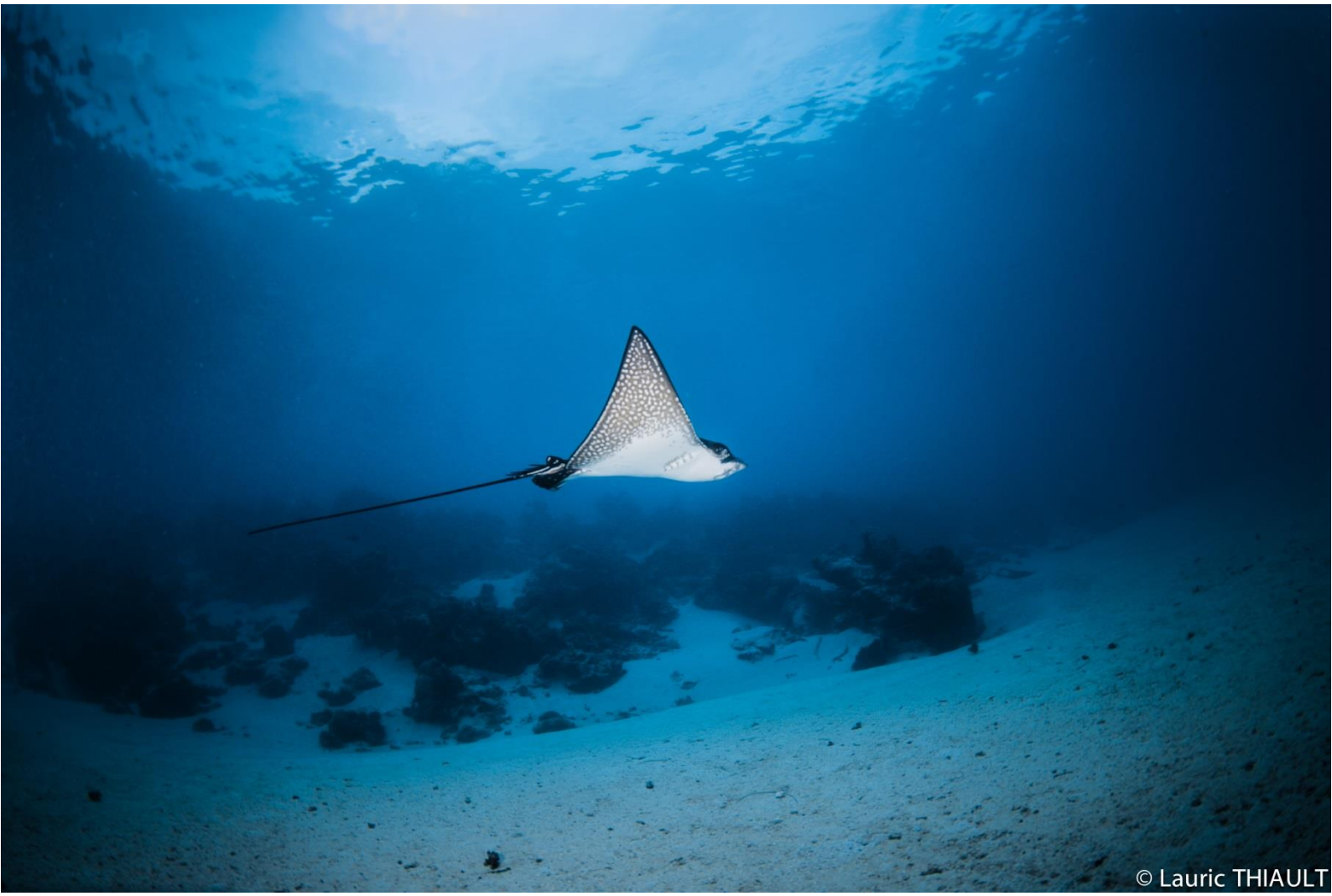
Figure III-2: Neighbour-Joining distance tree (K2p model) of the partial COI sequences (652 bp, 'barcode region') revealing the placement of individuals of *Aetobatus ocellatus* from French Polynesia within the Aetobatus complex. Specimens are labelled with their BOLD Process ID. Bootstrap values over 75% are indicated above branches.

4. Discussion

The present study supports the occurrence of the Ocellated Eagle Ray, *A. ocellatus*, in French Polynesia (Society and Tuamotu archipelagos). *Aetobatus ocellatus* is considered by the IUCN Red List (under the uncorrected name *A. narinari*) as "Vulnerable" in South-East Asia, and the species complex is considered "Near Threatened" globally (Kyne et al., 2006). The status of both *A. ocellatus* and *A. narinari* should therefore be reassessed, specifically because each species will be exposed to

different threats in their currently limited geographic range. Slow growth, late sexual maturity, and slow reproduction (over 12 months of gestation producing only a few pups) (Schluessel et al., 2010b) make Aetobatus species highly vulnerable to human fishing and other pressures. In French Polynesia, *A. ocellatus* is involved with two kinds of human activities. First, they are important for tourism activities, being commonly targeted for scuba diving and snorkeling activities mostly in channels of atolls and islands. At the same time, Planes et al. (2006) showed that eagle rays are one of the predators of the Black-lipped pearl oyster *Pinctada magaritifera* (Linnaeus, 1758). This oyster represents the second economic pillar of French Polynesia through pearl farming. Better knowledge of the Ocellated Eagle Ray could help determine new ways to protect the local economy while preserving the species.

Our results do not rule out the possibility that *A. narinari* could occur in Polynesian waters. A similar situation recently occurred within the genus Manta with both *Manta alfredi* (Krefft, 1868) and *M. birostris* (Walbaum, 1792) being present in the Marquesas Islands (Mourier, 2012), while *M. alfredi* is the dominant Manta the other archipelagos despite anecdotal sightings of *M. birostris*. Indeed, more samples need to be collected throughout the three other Polynesian archipelagos to complete the dataset initiated by the present study. However, results from this study have shown that the samples we collected belong to the same species and are genetically close to individuals from Australia and New Caledonia. Comparisons with samples from other areas such as Indonesia, India, or South Africa suggest that a particular genetic signature may distinguish populations. Increasing the number of samples and locations over a larger area would certainly increase our understanding of the population structure of both *A. ocellatus* and *A. narinari*.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Chapter IV Distribution patterns of ocellated eagle rays along two sites at Moorea Island, French Polynesia

This chapter is based on the manuscript submitted in PlosOne: *Berthe, Cécile, Besson, Marc, Latry, Lise, Bertucci, Frédéric, Lecellier Gaël, Iglésias Samuel and Lecchini David (2017) Distribution patterns of ocellated eagle rays from biological and physical measurements along two sites at Moorea Island, French Polynesia*. Nevertheless, I added here some additional information in order to better explain the study and better justify the link between the different studies in my EPHE Diploma. Eagle rays can be seen in school everywhere around the island (dive center obs, pers. obs.). In 2012 I started looking for locations where eagle rays can be easily observed. Most of them where located in the passes of the island, place with a lot of current, a bad visibility and important boats traffic. Two of them offered me a safer place to spend hours of observation: the channel near the former ClubMed hotel and the anchorage area of Tahiamanu also called “Mareto public beach”. One year observations lead to gather important information on spatiotemporal distribution on the north coast of Moorea. Further investigation at the Island scale would certainly provide more precise information on the habitats of the ocellated eagle ray.

1. Introduction

The ocellated eagle ray *Aetobatus ocellatus* (Kuhl, 1823) is a bentho-pelagic, large body-sized ray, occurring in the tropical area of the Indo-West Pacific (Schluessel et al 2010, Berthe et al, 2016a). It is a benthic feeder, foraging sand areas, with a preferential diet for bivalves, gastropods and malacostraca (Schluessel et al, 2010). The ocellated eagle ray occurs in the Society and Tuamotu archipelagos of French Polynesia (Berthe et al. 2016b) where they can be observed in large school as well as alone (Berthe et al. 2016b, Berthe and Lecchini, 2016).

Animals will choose a habitat that enhances fitness, a habitat that is best for their particular ontogenetic stage (Bonnet et al. 2015; Delaney, 2016), or sex (Delaney, 2016), with low predation risk and/or high prey availability (Glaudas and Rodríguez-Robles 2011). For example, white shark males and females have different foraging strategies which lead them to different habitats best suited for them (Bruce and Bradford 2015), and it is well documented that whale sharks and manta rays migrate according to plankton productivity (Wilson et al. 2001; Anderson et al. 2011). Little is known about the habitat selection of the ocellated eagle ray in French Polynesian lagoons. Have ocellated eagle rays in Moorea seasonal or tidal cycle? Have they any specific use of the lagoon of Moorea Island (e.g. nurseries area)? Are the groups of rays segregated by gender or ontogenetic stage?

Low reproductive rates (1–4 pups annually) combined with intense and unregulated inshore exploitation have led to the Aetobatus species complex being listed as ‘‘near threatened’’ globally and ‘‘vulnerable’’ in Southeast Asia. In French Polynesia waters, fishing for any of the 25 different species of sharks and rays has been prohibited since 2011. Elasmobranchs are important to the Polynesian economy in the form of eco-tourism activities, which bring in around USD$140 million annually for manta ray watching alone (O’Malley et al. 2013), and in the cultural history of Polynesia (Clua and Guiart 2015). Although *A. ocellatus* does not directly suffer from bycatch or fishing pressure in this region, it does face other anthropogenic stressors in their inshore habitats, such as habitat loss or habitat degradation because of increased noise, pollution or light (Musick 1999, Espinoza et al. 2014, Berthe and Lecchini 2016).

Here, we launched the first study on the ocellated eagle ray, in this region of the world to understand the distribution patterns of this species. Adults and juveniles were monitored during one year on two sites of the north coast of Moorea, the most inhabited part of the island. We were looking for habitat use and possibly correlations with environmental links. Our results could help

further conservation initiatives for managing this important species in Moorea lagoon, especially in the context of rising human impacts on the environment.

2. Materials and methods

Study site

Ocellated eagle rays were studied along the north coast of Moorea, French Polynesia. A preliminary investigation around Moorea identified sites where eagle rays are abundant. Most of them were located in the passes of the island, where there is a lot of current, a poor visibility and an important vessel traffic, but two sites in the lagoon were identified (Figure IV-1). The Mareto site, located on the east side of Opunohu Bay, is 350 meters long and 180 meters wide, with depths ranging from 2 to 7 meters. The ClubMed site, located on the west side of Opunohu Bay, between the main island and two small inlets, is 400 meters long and 180 meters wide, also with depths ranging from 2 to 7 meters. The two locations have the same topography, which consists of a sandy area, a channel, and a fringing reef. However, human influence between the two sites differs. Mareto is a public beach and a mooring area, with boats at low speed, while ClubMed is a channel where boat traffic is heavy (e.g., glass bottom boats, jet-skis, dive boats, transfer boat, etc.) year-round.

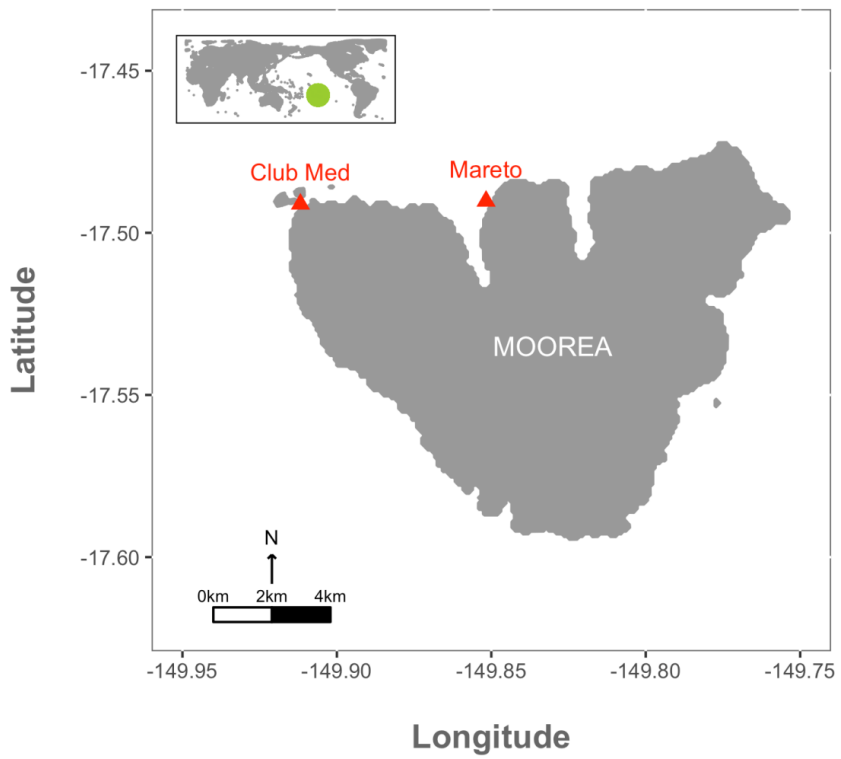

Figure IV-1: Map highlighting the two study sites on Moorea Island, French Polynesia, Clubmed on the left and Mareto on the right.

Photo-identification

Photo-identification has been used for many marine species, using marks such as mating scars or wounds (Vincent et al. 2001), body shape (Anderson and Goldman 1996) or even dorsal pattern (Brooks et al. 2010). This non-invasive method was used here to estimate the population size and to investigate the movements of individuals between the two study sites (Marshall and Pierce 2012, González-Ramos et al, 2017). We took >1000 photos of ocellated eagle rays between September 2014 and October 2015 using a Canon G16 in an underwater housing (Fantasea). Only photos where dorsal pattern was sharp were used for comparison. Photos were first manually analyzed using the entire dorsal pattern of the species. They were then analyzed using I3S Pattern software (v4.02, van Tienhoven et al. 2007) using the right fin pattern (Figure IV-2).

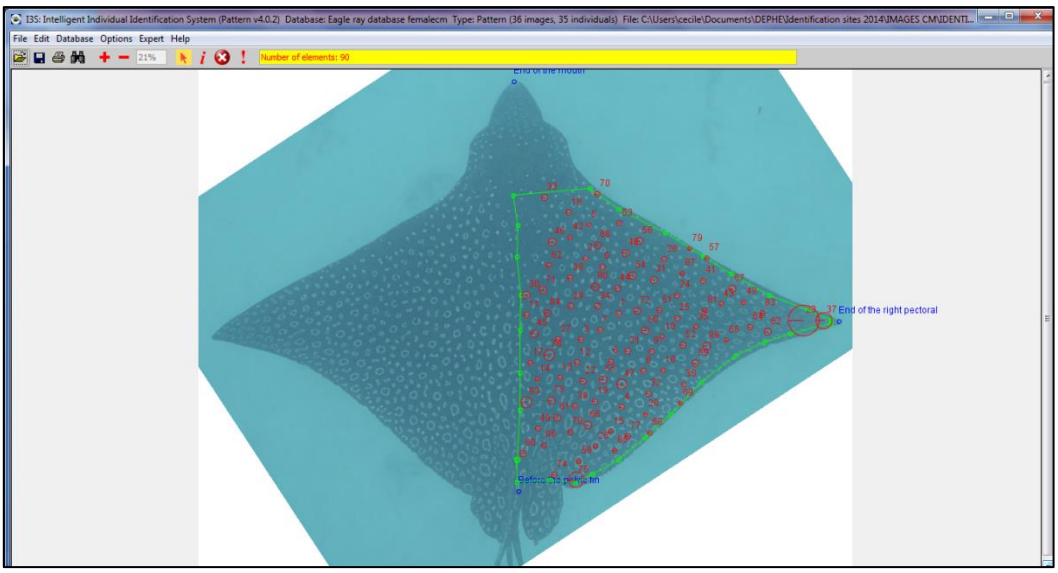

Figure IV-2 : Photo-identification using the I3S Pattern program on the right fin area.

Underwater visual surveys and environmental data

Visual surveys (n=89) were conducted to estimate records of ocellated eagle rays in the two study sites in the morning or afternoon from September 2014 to October 2015. To do this, a single observer would snorkel at the site with a mechanical flowmeter (General Oceanics, mechanical flowmeter model 2030 series) and a digital video camera (Canon G16). The observer recorded environmental parameters (see below) and counted boats and jet-skis at the site (Chapman et al, 2002; Planes et al, 2005). Rays were counted and sex/ontogenetic stage characterized when possible: Male = individual larger than 70 cm disk width (DW) with apparent claspers; Female = individual larger than 70 cm DW without claspers; and juvenile = individual smaller than 70 cm DW without apparent claspers (Berthe and Lecchini 2016). Moreover, the dominant behavior of the eagle rays

was also recorded: swimming, foraging, pre-mating, cruising, jumping, chafing, escaping, interacting with conspecific or heterospecifics, and interacting with snorkeler (see Table IV-I for details). A multiple correspondence analysis (MCA) coupled with general linear models (GLM) was conducted to identify the relationships between sex and ontogenetic stage of rays, site, season, and time of the day.

Table IV-I: Description of the different behaviors observed during the study

Behavior	Description of the Behavior
Swimming	Individual or group of individuals swimming against the current, like suspended in the water column, doing nothing special
Foraging	Searching into the substrate to eat
Chafing	Chafing against the substrate or using cleaning station in oder to remove exoparasites
Cruising	Moving individual or sub-group of individuals who swim by, swim to or out of the group
Escaping	swimming at least 50 m away from the foraging or swimming site when a boat passed by
Pre-mating	A male gripping a female on her back
Jumping	Jump out of the water
Interacting with conspecific	Other interaction than previous (e.g. two males fighting)
Interacting with heterospecific	Interaction with other species
Interacting with snorkeler	Swim to the observer

For each site, the date (day, month, and year) and the time (morning: from midnight to noon, afternoon: from noon to midnight) of the day were recorded. The wind speed (ordinal variable), wind direction (nominal variable), wave direction (nominal variable), wave height (ordinal variable), and tidal data (ordinal variable) were obtained via online forecast (http://fr.surf-forecast.com/). Prevailing current speed (ordinal variable) was recorded using a mechanical flowmeter (General Oceanics mechanical flowmeter model 2030 series). According to their frequency distribution, ordinal variable values were split into groups: low (0-15km/h), medium (16-30km/h) and high (31-45km/h) for wind speeds; no (no current), light (0-10cm/s), medium (10-20cm/s), and strong (more

than 20cm/s) for current strength. In order to link the spatial (Mareto vs. ClubMed) and temporal (September 2014 to October 2016) distribution of eagle rays to the six environmental parameters, a multiple factor analysis (MFA) was performed (Escoffier and Pages, 1994). The spatiotemporal distribution of males and females and between adults and juveniles and the six environmental variables were used as the first and second sets of variables for this analysis. The different clusters identified according to environmental variables provided resultant components that were analyzed by MANOVA, ANOVA and a post hoc Tukey's test for multiple comparisons.

Benthic analyses

A benthic sampling was conducted once at each site to determine the potential prey items on which the ocellated eagle rays would be foraging. To do this, an air pump and a 2mm mesh bag were used to excavate the upper 15cm² of sand from five random locations at each study site. Each sample was sieved through a 2mm mesh, and the macrobenthic fauna was then identified to the genus level (Carpenter and Niem 1998), counted, and weighed. Abundance (macroinvertebrate) and biomass were compared, between the two sites using Wilcoxon-Mann-Whitney rank sum tests. All statistical analyses were performed in R 2.15.0.

3. Results

Using photo-identification, 113 *Aetobatus ocellatus* unique individuals were identified: 40 males, 43 females, and 30 juveniles, of which 67 rays were identified at ClubMed, and 47 rays at Mareto (Table IV-II). Only one individual was observed at both sites, as a juvenile at the Mareto site in October 2014 and at the adult stage at the ClubMed site in September 2015. This instance was the only example of inter-site movement observed during the survey. Of all of the individuals identified at ClubMed, 99% were adult, while 64% of the individuals recorded at Mareto were juveniles.

Table IV-II: Count of eagle rays (Aetobatus ocellatus) that were observed; totals for adults are separated by sex. Juveniles column represents individuals under 70cm width, when sex was not possible to determine. Numbers in parentheses indicated identified individuals

Location	Males	Females	Juveniles	Total
ClubMed	244 (31)	145 (35)	1 (1)	390 (67)
Mareto	14 (9)	10 (8)	107 (30)	131 (47)
Total	**258 (40)**	**155 (43)**	**108 (30)**	**521 (113**

Spatio-temporal distribution

During the 89 surveys performed for this study, we made 521 ray observations. Of the 113 individual rays identified, 40% were observed only once, while 21% at least on 6 different occasions. No significant difference was observed in the number of surveys conducted during the dry vs. wet season or the morning vs. afternoon periods at each site (Pearson's chi-squared test with Yates' continuity correction, p-value > 0.20). Thus, a multiple correspondence analysis (MCA) coupled with GLM allowed us to highlight the relationships between ontogenetic ages of rays, sites, season, and time of the day (Figure IV-3). The MCA separated the ontogenetic stage of rays and the sites along the axis 1 (35% of the total variance). The axis 2 separated the season and the time of day (20% of the variance). The GLM showed that when one factor was analysed (ontogenetic stage, site, season, or time of day), all results were significant (e.g., factor "site": z value = 10.81, p-value<0.001). When the two-way interaction was analysed, all results were significant (e.g., for site and time of the day: z value = -7.27, p-value<0.001), except for the interaction between "site" and "season". When the interaction between three or all factors was analysed, results were not significant. Overall, the GLM and MCA analyses showed that, irrespective of season, there were significantly more adult males and females at the ClubMed site and more juveniles at the Mareto site, and there were significantly more rays in the morning at the ClubMed site and more rays in the afternoon at the Mareto site (Fig. 2).

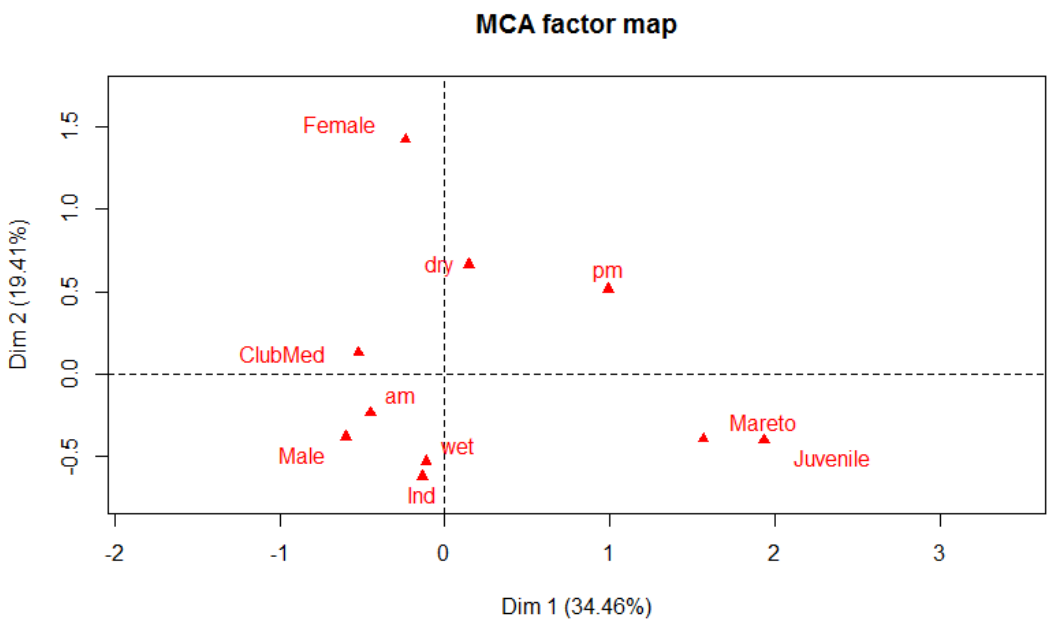

Figure IV-3: Multiple Correspondance Analysis factor map (2 first components, 34.5% and 19.4%, respectively) representing the relationship between the study sites (ClubMed/Mareto), ontogenetic stage of the eagle rays (male/female/juvenile), seasons (warm/cold), and the time of the day (am/pm)

Influence of environmental factors on the spatio-temporal distribution

Firstly, results from the MANOVA showed that wind speed, wind direction, wave direction, and current speed significantly influenced the abundance of rays between the two sites. Secondly, results from the MFA revealed a separation between the juvenile rays occupying the Mareto site and the adult male and female rays occupying the ClubMed site with three of the four environmental variables along the first axis (explaining 40% of variance;Figure IV-4). Thus, the juvenile rays were more abundant at the Mareto site when there was no current but an east or west wind (ANOVA and Tukey's test, all p-values <0.001). In contrast, the female adult rays were more abundant at the ClubMed site when the wind was coming from the north at a medium or high speed and when there was a westward or northward wave direction (ANOVA and Tukey's test, all p-values <0.05). The abundance of male adult rays at the ClubMed site did not correlate with any of the environmental variables measured. Lastly, the second axis of the MCA did not discriminate the spatial distribution of eagle rays according to the environmental variables.

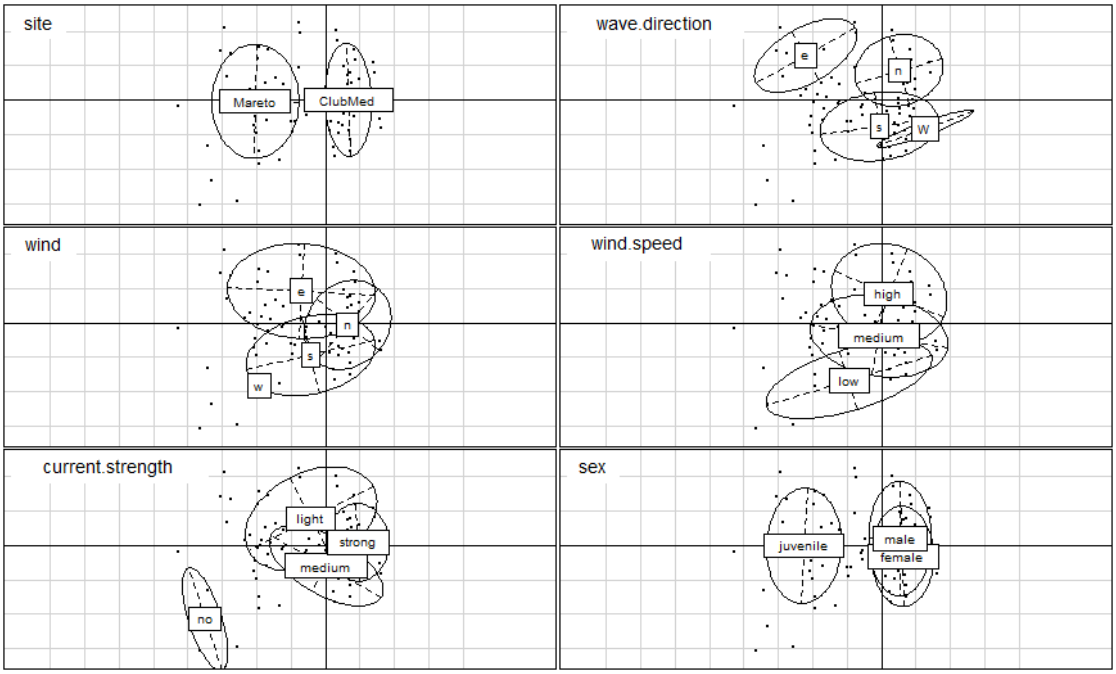

Figure IV-4: Multiple Correspondance Analysis scatter plots (2 first components, 18% and 13%, respectively) representing the relationship between sex (male/ female/juvenile), the site (ClubMed/Mareto) and the environmental factors: wave and wind direction (east/north/south/west), wind speed (high/medium/low) and current strength (no/light/medium/strong).

Benthic analyses

The comparison of the two study sites according to prey availability showed a significantly higher abundance of prey at Mareto site than at the ClubMed site (Wilcoxon-Mann-Whitney, W = 3, P-value < 0.05), with a density of 170 ind/m² and 91 ind/m² at each site, respectively. In contrast, the ClubMed site exhibited twice as much total biomass than the Mareto site (6.80 g. vs. 3.26 g, respectively; Figure IV-5), but the difference was not significant between the two sites (Wilcoxon-Mann-Whitney, W = 11, P-value = 1).

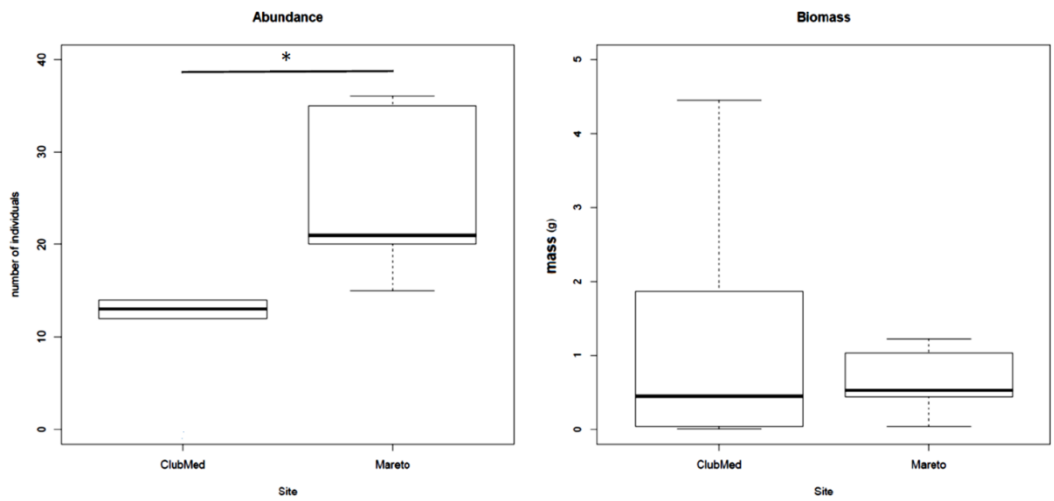

Figure IV-5: Abundance (number of macroinvertebrate individuals) and biomass (grams) for the two study sites. The boxes represent the first and third quartiles, black lines are the medians (second quartiles), and whiskers correspond to the range (min–max) of the distributions. An asterisk indicates statistically significant differences.

Behavioral observations

The behaviours of each individual eagle ray were observed during all surveys (Figure IV-6, Table IV-I). Among the 521 observations, four behaviors were frequently observed for rays at the ClubMed site: “swimming”, where an individual or group of individuals swam against the current, which occurred in 46% of the observations; “chafing”, where a ray was observed chafing against the substrate or using a cleaning station to remove ectoparasites (Berthe et al. 2016a), which occurred in 25% of the observations; “pre-mating”, where males were observed grasping on the back of females, which occurred in 7% of the observations; and “jumping”, where rays jumped out of the water into the air, which occurred in 5% of the observations. Two behaviors were frequently observed at the Mareto site: “foraging”, where rays were observed searching the substrate for food, which occurred in 79% of the observations and “swimming”, which occurred in 18% of the observations. Lastly, an

“escape” behavior was also observed in eagle rays at both sites, where a ray would be noted swimming at least 50 m away from the foraging or swimming site when disturbed by a boat passing by, and this was observed in 17% of the eagle rays at the ClubMed site but in only 3% of the eagle rays at the Mareto site.

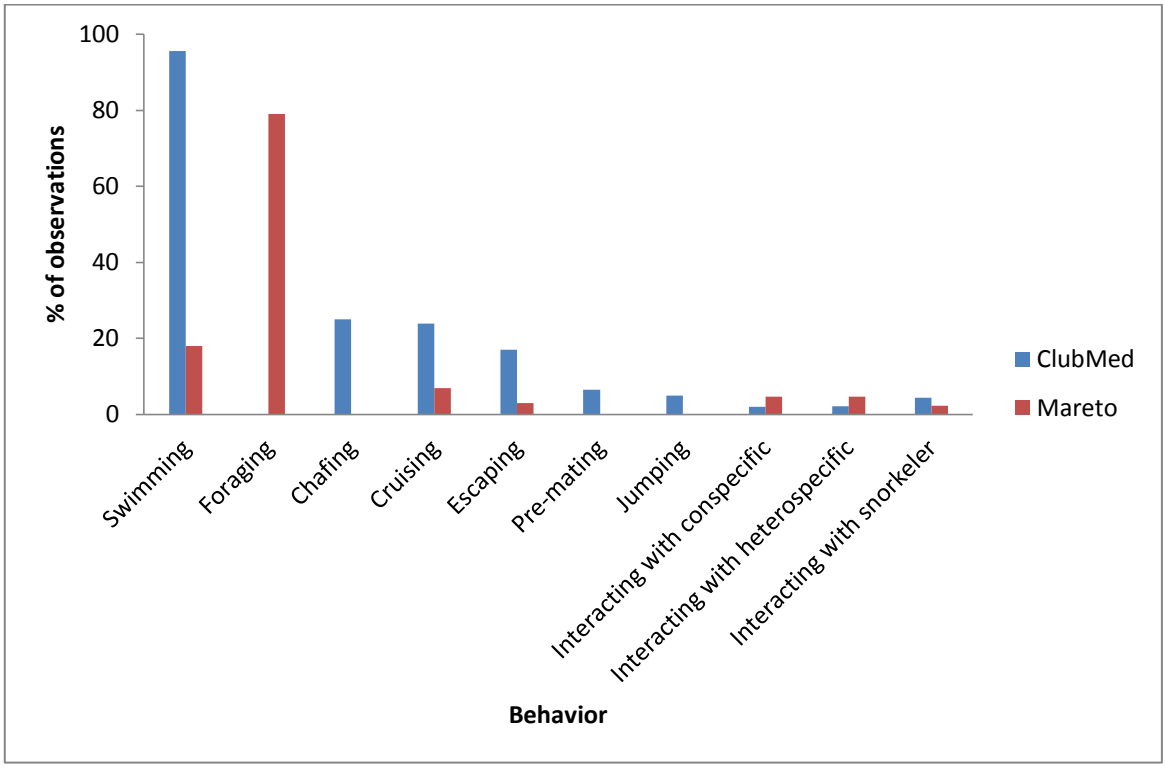

Figure IV-6: Percentage of observations for the ten different behaviors according to the study site: swimming, foraging, chafing, cruising, escaping, pre-mating, jumping, interacting with conspecific, interacting with heterospecific, interacting with snorkeler. See Table II for details on each behavior.

4. Discussion

Our study revealed the distribution patterns of eagle rays between two sites in the lagoon of Moorea Island, French Polynesia (Figure IV-4). The two study sites were similarly inhabited by the eagle rays. However the ontogenetic stage of individuals occurring at each site was different; adult rays were observed at ClubMed, while juveniles at Mareto. Others parameters were also different in the use of each site. While ClubMed was inhabited predominantly by individuals invested in an array of activities in the morning, Mareto was inhabited by individuals mostly engaged in foraging during the afternoon hours. Our results show that environmental drivers like wind speed and direction, wave direction and height, tidal stage, and ecological drivers like prey availability are all important in the spatio-temporal distribution of ocellated eagle ray populations. This spatio-temporal distribution need to be fully understood, in the context of a global increasing anthropogenic impact, already known to disturb health of elasmobranches (Dulvy et al. 2014).

Ontogeny and distribution patterns

Our findings support life history strategies found in several shark species with an ontogenetic partitioning of the habitats (Grubbs 2010). The ontogenetic stages induce different needs (e.g., specific diet or habitat), behaviours (e.g. solitaire or gregarious) or environmental adaptations (e.g., to low salinity, extreme temperature change, habitat shift). For example the broadnose sevengill shark (*Notorynchus cepedianus*) undergoes an important ontogenetic diet shift. Known to feed on chondrichthyans, it primarily consumes teleost fishes to avoid predation while a juvenile (Ebert, 2002). Similarly bull sharks (*Carcharhinus leucas*) are able to live in low salinity waters during early life history stages, which may offer them the capacity to live in rivers where their predators are absent but prey is abundant (Drymon et al. 2014). For eagle rays, no extreme shift in habitat or major differences between juvenile and adult stages are yet known. Neonate eagle rays are well developed at birth and have a similar morphology as adults, but obviously without the same body strength. The size of the neonate is about 36% the size of adult female (57cm DW for neonate, 160cm DW for adult female) (Schluessel et al. 2010). Given the difference in size, it is not surprising that, as neonates mature to juveniles and juveniles to adults, the diet shifts accordingly with the increase in body size and strength to bigger and harder prey items (Schluessel et al. 2010). We assumed that the energetic benefits of ontogenetic partitioning influence the distribution patterns. Indeed our results demonstrated that there were significant effects of three factors including current, wave direction, and prey availability (Figure IV-4) on the presence of eagle rays on each site at Moorea. Nevertheless, other abiotic factors like salinity and dissolved oxygen, as well as biotic factors, such as social behaviours will need more investigation in future studies to better understand drivers of eagle ray distribution patterns.

Energetics and distribution patterns

The energetic costs for some activities, like swimming or foraging, can be a key to explaining distribution patterns. Due to the low tidal variation around Moorea (30 cm), the currents are largely induced by waves. Conrath and Musick (2010) speculated that swimming against a strong current is energetically costly for young eagle rays, which could help to explain our findings. Juvenile rays forage at the Mareto site, which would be counterproductive if all of the energy gained from foraging was consumed swimming against a strong current, thus potentially explaining their low abundance at the site when there is a strong current. Like many other elasmobranchs, eagle rays need to get the oxygen from water through their gills by swimming or pulling in the water with their spiracles. We assumed that the group of adult rays at the ClubMed site takes advantage of swimming against the current to bring the oxygen directly to the gills, thus enabling them to rest. In addition to currents

and waves affecting habitat choices, prey availability drives this choice for many species (Schlaff et al. 2014). Our two study sites offered the same level of diversity in terms of prey items, but showed different patterns of prey abundance (Figure IV-5). The Mareto site provided a high abundance of small prey items, items that would suit juvenile eagle rays. The ClubMed site had bigger but fewer prey items, and indeed, foraging activities were not frequently observed at ClubMed. Here, and in other systems, ontogenetic partitioning induced by energetic needs may be an important adaptation that limits competition between life stages (Ajemian and Powers 2015), and we assumed that it was the main driver dictating how eagle rays use the lagoon around the island of Moorea. However, others factors like water temperature or sex are important too in elasmobranch distributions patterns (Schlaff et al. 2014), both of which were explored here along with the anthropogenic impact to which the north coast lagoon of Moorea is exposed.

Environmental factors and distribution patterns

Environmental factors (biotic or abiotic) play a role in influencing size class distribution patterns in elasmobranches (Schlaff et al. 2014). We explored season, sex, and the role of anthropogenic disturbance to explain the spatio-temporal distribution of eagle rays in Moorea. Seasons are known as environmental driver that can influence distribution of rays, as observed in A. narinari in Venezuela (Tagliafico et al. 2012) and the Gulf of Mexico (Bassos-Hull et al. 2014) and in A. flagellum in Japan (Yamaguchi et al. 2005). This lack of seasonal effect has been noted for eagle rays in other studies. For example, Schluessel et al. (2010) found that A. ocellatus was present year round in Queensland, Australia, which could be a general trend in locations with low temperature variation between wet and dry seasons, as in French Polynesia and northern Australia, and maybe the tropics in general. Sexual segregation has also been observed in several species and could be induced by foraging preference (Bruce and Bradford 2015) or reproductive behaviour (Wearmouth and Sims 2008). However, that was not the case in our study, as male and female rays were equally likely to be observed at both sites over the entire year. Similarly Silliman et al. (1999) observed no sexual segregation in eagle rays on Bimini Island, the Bahamas. Anthropogenic factors are also known to influence distribution patterns of marine species (Rossi-Santos 2015; O'Connor et al, 2016).

In a previous study on the north Coast of Moorea, Berthe and Lecchini (2016) showed that eagle rays stopped eating in the presence of boat noise. Anthropogenic noise is a form of pollution that increasingly contributes to natural soundscapes on a global scale (Kight and Swaddle 2011, Francis and Barber 2013, Simpson et al. 2016) and causes physiological, neurological, and hormonal problems (Bracciali et al. 2012; Buscaino et al. 2010; Cubero-Pardo et al. 2011). In the present study, escape behaviours were observed where eagle rays swam sharply away from their aggregation or

foraging site. Long-term studies are required in order to assess the chronic effects, if any, of anthropogenic disturbances on the spatio-temporal distribution and fitness of eagle rays.

5. Conclusions

Coral reef ecosystems are complex environments where all species are linked together and highly connected to human populations that are living near coastal areas. Understanding the drivers of habitat use by marine species is important to implement efficient conservation strategies in the context of the increasing anthropogenic impact that islands like Moorea – an attractive island for local residents and for tourism – will continue to face. Our study examined the spatiotemporal distribution of eagle rays, according to ontogenetic stage and energetic costs and benefits. Distribution patterns were explained predominantly (but not solely) by current, wave intensity, and prey availability at each coastline site (Mareto vs. ClubMed). The evolution of the Moorea coastline shows that, over the last twenty years, there has been a shift between embankments and sandy beaches, and construction now represents more than 50% of the coastline (Benet 2010). Habitat destruction and even just habitat alteration can have tremendous consequences for populations of elasmobranchs like disturbing abundance, distribution patterns, and the overall health of key species (Schlaff et al. 2014). Many sharks and rays species are thought to play an important role as predators in marine ecosystems. And rays are actually a major link in the food chain being at the same time prey and predator. Their decline could lead to an imbalance within communities of coral reef ecosystems (Heithaus 2008).

Although we acknowledge that this study has limitations, like only investigating two sites, our findings could be built on and could may then be useful for management purposes around the island. Indeed, findings from this study provide information about the most suitable habitats for rays, and especially for juveniles, that are not currently protected by the management plan of Moorea Island, PGEM ("Plan de Gestion de l'Espace Maritime", Leenhardt et al. 2015), but need to be prioritized in any further revision (Oh et al. 2016). Prohibiting construction, limiting boat speed or boat traffic, regulating tourism activities – especially in those areas – are some recommendations that could improve the management of this species.

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Chapter V Chafing behavior on a patch of sandy bottom by ocellated eagle ray (*Aetobatus ocellatus*)

This chapter is based on the short note published in Marine Biodiversity (2016b): *Berthe C., Lecchini D. & J. Mourier J. (2016) Chafing behavior on a patch of sandy bottom by ocellated eagle ray (*Aetobatus ocellatus*). Marine Biodiversity: 8–11.* Nevertheless, I added here some additional information in order to better explain the study and better justify the link between the different studies in my EPHE Diploma. Spending hours observing the ocellated eagle rays in Moorea offered me the opportunity to witness specific behaviors that has not yet been described. As my observations of a chafing behavior of eagle rays again the sand were supported by observations from a colleague, we decided to describe it and to submit it to a peer review.

1. Introduction

Hosting parasites is costly, and many coral reef fish species are known to clean themselves through chafing behaviors and/or using cleaning stations (e.g., Losey et al. 1999; O'Shea et al. 2010). In elasmobranchs, Papastamatiou et al. (2007) showed that sharks (*Carcharhinus amblyrhynchos*) use cleaning stations and are used as cleaners by smaller reef fish (rainbow runner—*Elagatis bipinnulata*). O'Shea et al. (2010) showed that manta rays use the cleaning stations in the Great Barrier Reef and in the Coral Sea. Although eagle rays are known to host ectoparasites (Marie and Justine 2006), how they remove them is not known as they have not been reported as regular users of cleaning stations.

2. Materials and Methods

Here, we report two observations of ocellated eagle rays (*Aetobatus ocellatus*) (Berthe et al. 2016a) chafing on patches of sandy bottom in French Polynesia. The first observation was made on Moorea Island (17°29' 28.39"S 149°54'41.70"W) in May 2011. A female was swimming in a channel, and then started to rub its body against the sand, chafing each side (presumably fins and/or gills), one after the other, over the course of about 30 seconds. The second observation was made on Fakarava Atoll (16°31'8.27"S 145°27' 37.13"W) in June 2014 (Figure V-1).

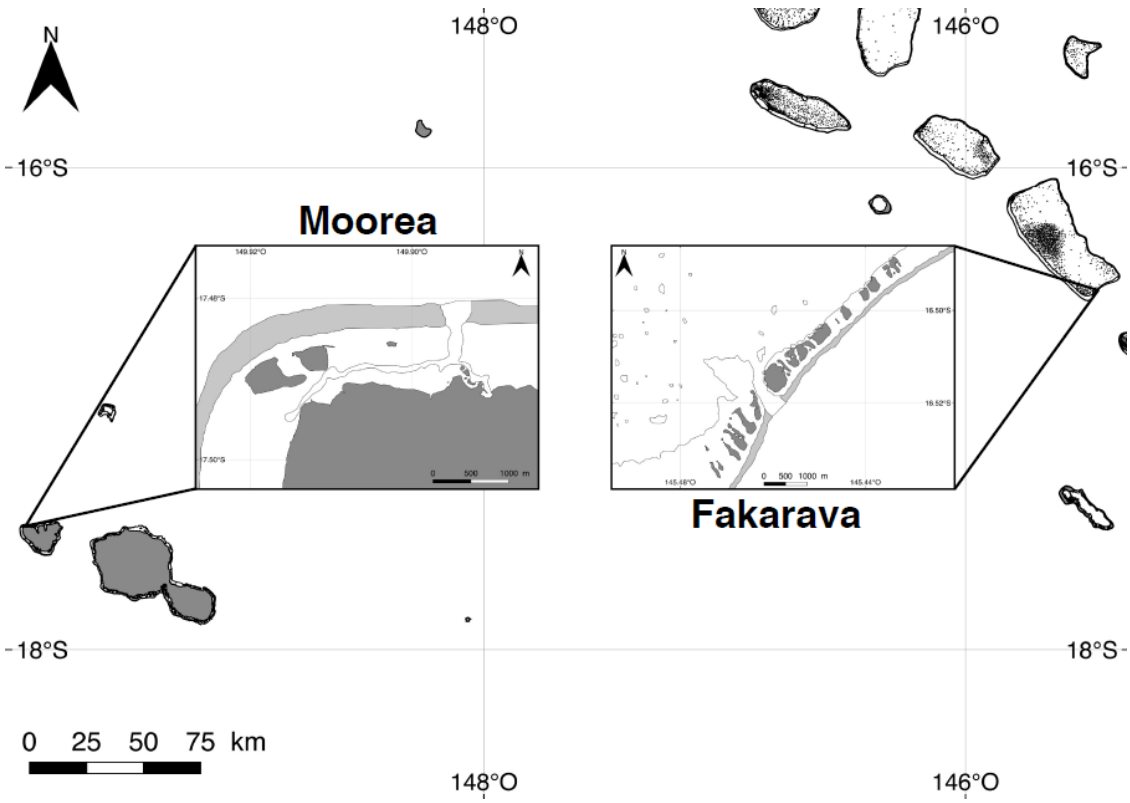


Figure V-1: Sites where chafing behaviors have been observed: on the left, Moorea island and the channel of ClubMed on the north. On the right Fakarava Atoll and the Tetamanu pass in the south.

3. Results and Discussion

Two female eagle rays were swimming together over a patch of sandy bottom at 15–20 m depth. One of them started chafing on the sand, first the left side of its body (presumably fins and/or gills—Figure V-2a), then the right (Figure V-2b), and left again (Figure V-2c) over the course of about 20 seconds. Overall, we propose that chafing behaviors in eagle rays may function to fulfil their maintenance requirements either as a primary strategy or in addition to using cleaning stations. Ritter showed that Caribbean reef sharks (*Carcharhinus perezi*) and blacktip sharks (*Carcharhinus limbatus*) chafed against sand ripples with either a parallel or perpendicular swim direction, rather than a transverse swim direction. Futures studies should be conducted to better understand the cleaning efficiency between chafing behavior and cleaning stations in elasmobranchs.

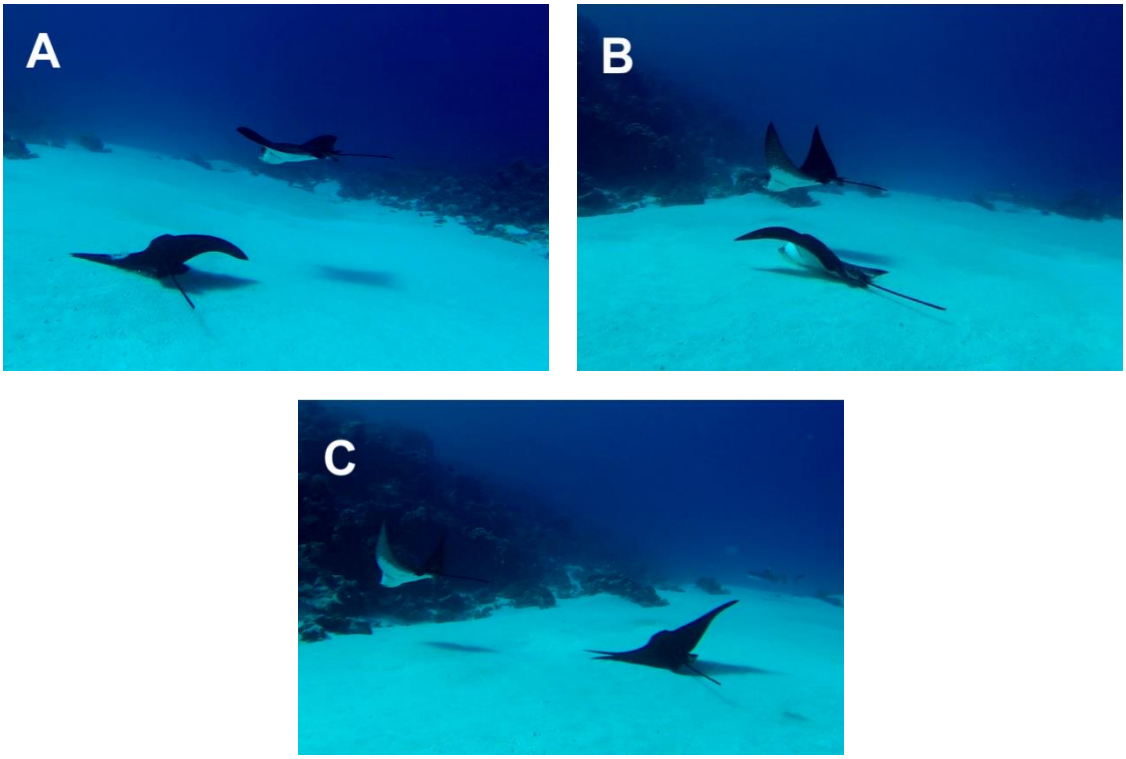
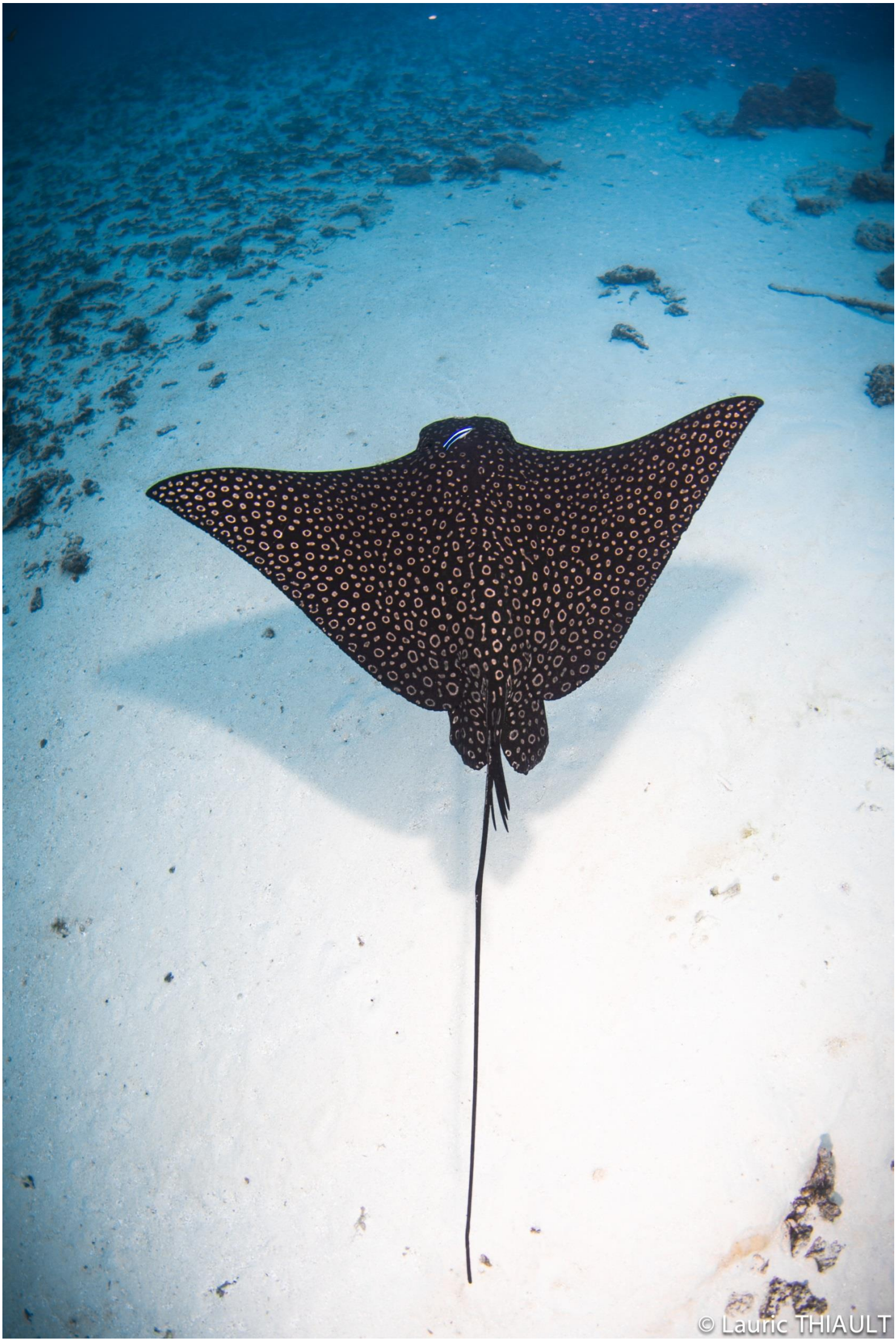


Figure V-2 a, b, c: Sequence of an ocellated eagle ray (*Aetobatus ocellatus*) chafing on a patch of sandy bottom at Fakarava Atoll (June 2014)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Chapter VI Influence of Anthropogenic Noises on Escape Behaviour of White-Spotted Eagle Ray *Aetobatus ocellatus* at Moorea Island (French Polynesia)

This chapter is based on the manuscript published in Comptes Rendus Biologies : *Berthe C. & Lecchini D. (2016c) Influence of boat noises on escape behaviour of white-spotted eagle ray* Aetobatus ocellatus *at Moorea Island (French Polynesia). Comptes rendus biologies 339(2): 99–103*. Nevertheless, I added here some additional information in order to better explain the study and better justify the link between the different studies in my EPHE Diploma. In 2012 I participated to a French Polynesian program of repellent systems tests on the predators of the black-lips pearl oyster. Among which are the trigger fishes, the puffer fishes, the humphead wrasses or the eagle rays. Chemical, electrical and acoustical systems were tested in Moorea Island (Society Archipelago), Rangiroa and Manihi Atolls (Tuamotu Archipelago). As the acoustical system seemed to have a positive effect on rays, I decided to pursue the tests long enough to have significant results.

1. Introduction

Anthropogenic (human-made) noise is a form of pollution that is contributing increasingly to natural soundscapes on a global scale (Kight et al. 2011, Francis et al. 2013). Anthropogenic noise causes physiological, neurological and endocrinological problems, increased risk of coronary disease, cognitive impairment and sleep disruption of many mammals, reptiles, fishes and invertebrates taxa (Slabbekorn et al. 2010, LePrell et al. 2012, Mora et al. 2014, Nedelec et al. 2014). For example, behavioural impacts on fishes (sharks and teleost) include lower feeding frequencies (Bracciali et al. 2012), increased movement and displacement (Buscaino et al. 2010, Cubero-Pardo, 2011), impaired orientation in larvae (Holles et al. 2013), and slower reaction times (Bracciali et al. 2012). However, anthropogenic noises produced by boat traffic could have some positive effects for marine aquaculture if these sounds could be used as a deterrent to repel the predators of oysters, mussels or other aquaculture taxa (Nash et al. 2000, Ross et al. 2001).

For marine aquaculture industries worldwide, depredation is an important and long-standing issue (Nash et al. 2000, Sanguinede 2001, Šegvić-Bubić et al. 2011). Farmers and researchers have been testing different techniques (e.g., magnetic or electric field, acoustic barrier, bubble curtain, cages) to reduce predation on marine aquaculture (Nash et al. 2000, Šegvić-Bubić et al. 2011, Planes et al. 2006). For example, mussels are a favorite prey item for diving ducks in Scotland and Canada, and farmers know that they can use boats to scare the birds. Ross et al. (Ross et al. 2001) reproduced the boat motor sound with an underwater playback system to avoid using real boats and to reduce costs, thus demonstrating an effective alternative deterrent when the farmers were absent. In French Polynesia, the pearl farming industry is the second economic pillar of the country, representing 60% of all exports (ISPF 2012). However, the oyster farmers convey that the industry has been heavily impacted by predation by teleost fishes, rays and other taxa for several years. Although other animals such as triggerfish, turtles, and pufferfish prey on the black-lipped pearl oyster *Pinctada margaritifera* in French Polynesia, the white-spotted eagle ray, *Aetobatus ocellatus*, is one of the most detrimental predators (Planes et al. 2006). The eagle rays were not only eating a large quantity of oysters, but they were also destroying oyster long-lines (Planes et al. 2006).

As many oyster famers observed that the presence of a boat near long-lines disturbed rays, in the present study, we focused on the use of an acoustic system to deter eagle rays from oyster farms. Specifically, our field experiments investigated behavioural responses of eagle rays to different sounds: boat motor's sound, white noise, and three single-frequency tones (40 Hz, 600Hz, and 1 kHz).

2. Materials & Methods

The present study was conducted on adults of white-spotted eagle rays *A. ocellatus* (range size: 1.0 to 1.3m) foraging under anchored boats on the north coast of Moorea Island, French Polynesia (17°29'23.09"S 149°51'6.06"W) from October 2012 to November 2013 (Figure VI-1). The acoustic system (Figure VI-2) used in field experiments consisted of a 7V battery (Yuasa NP2.1-1) connected to an amplifier (Formula F-102, CA, USA), connected to an mp3 player (Sansa Clip1, SanDisk, Milpitas, CA, USA). All the equipment was set up in a waterproof casing. The mp3 player was connected to an underwater loudspeaker (UW-30, frequency response 0.1–10 kHz, University Sound, Columbus, USA) with a 4m long cable to allow the placement of the speaker as close to the rays as possible (about 1 m above the animal – depth of site : 5 m). The acoustic system was surrounded by a buoy, for positive buoyancy and pushed in the field by an observer. The observer played sound as soon as a ray was spotted. The sounds were played only while the ray was in full view and foraging over sand. When an eagle ray finished foraging, was waiting for prey, or was resting, the experiment was not conducted. If the ray was not feeding, it left the area if an observer approached (Planes et al. 2006, C. Berthe, unpublished data).

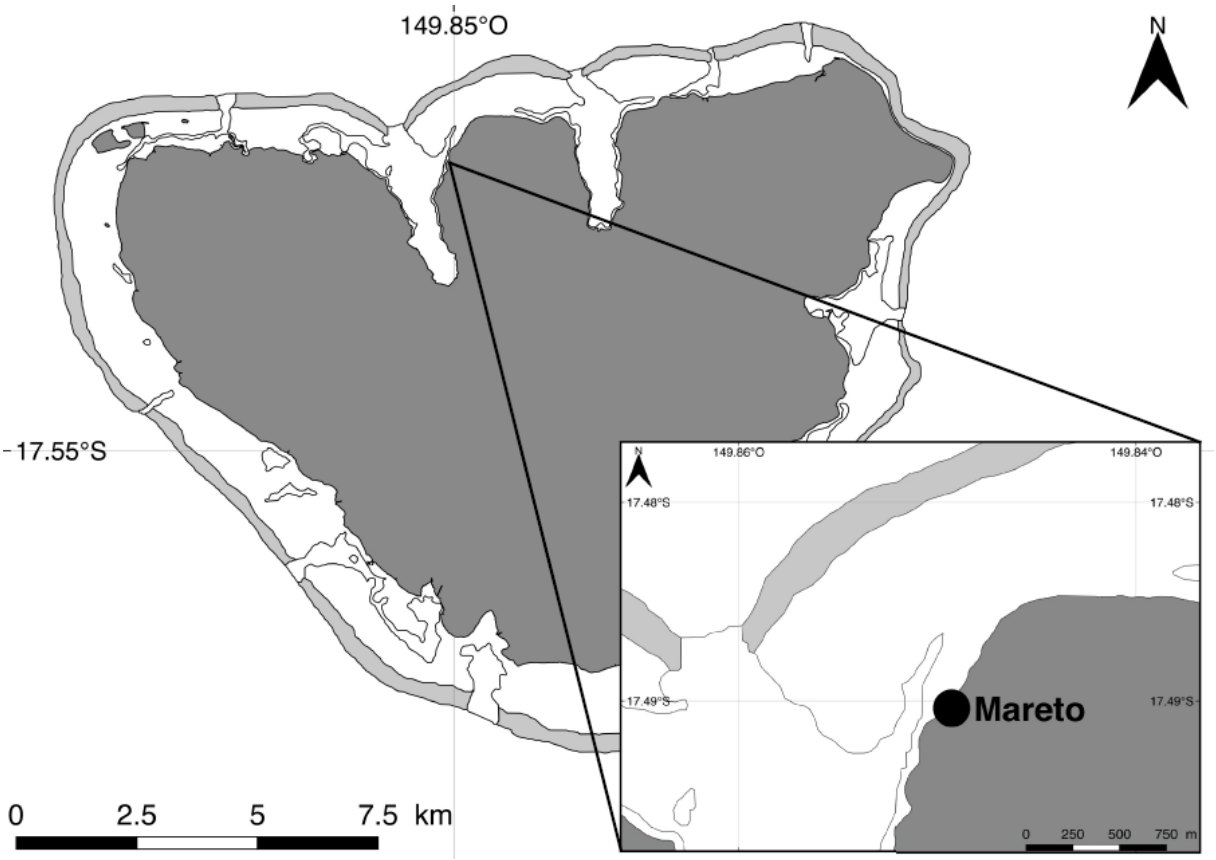

Figure VI-1: Map of Moorea Island, overview of the study site at the Mareto public beach on the north coast of the island

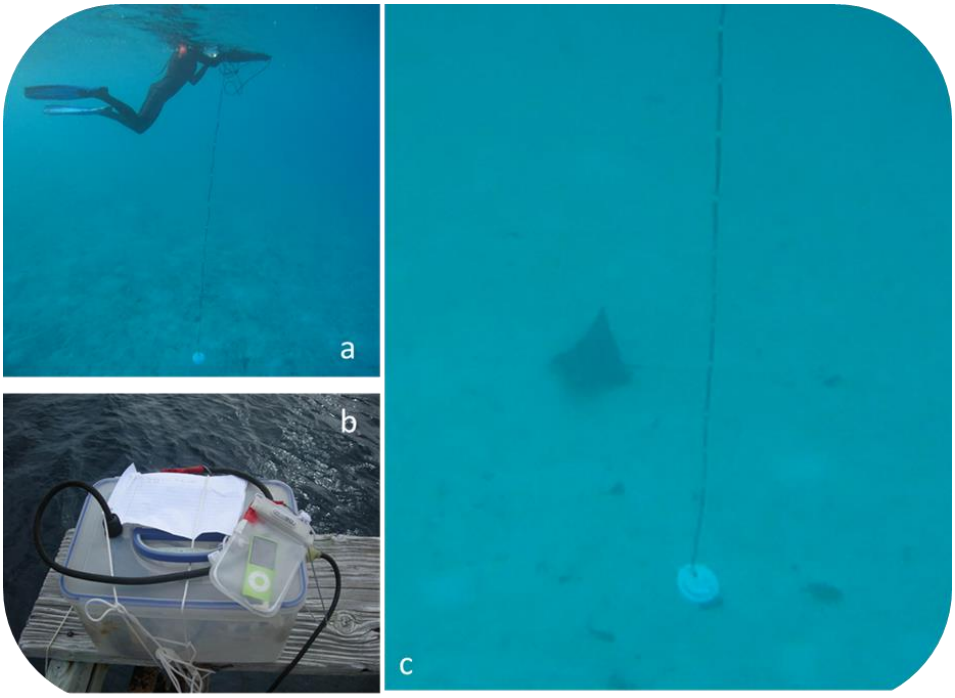

Figure VI-2: Acoustic system used to test sound effect on eagle rays. a = the system in the field, b = the system itself with the waterproof box and the mp3 player, c = the speaker placed near a foraging eagle ray

First, a control experiment was conducted on nine individuals in order to determine if the presence of the acoustic system and/or observer elicited flight behavior in white-spotted eagle rays when they foraged over sand. The system with the loudspeaker switched off was placed near the ray (about 1 m above the animal) without playing any sound, and the observer was positioned 5 m from the ray. The ray's behavior was noted during 5 minutes (i.e., escape or continue to forage).

Secondly, five sounds were tested: boat motor, white noise and three single-frequency tones (40 Hz, 600Hz, 1 kHz which correspond to, respectively, low, medium and high frequency perceived by elasmobranch fishes – hearing range from 20 or 40 Hz to 1 kHz (Casper 2006,Fay 2000). The artificial white noise (signal of which its frequencies are randomly distributed within a specified frequency range resulting in a constant power spectral density - sound waves extending over a wide frequency range: 10 Hz to 22 kHz) and the three single-frequency tones were created with Avisoft SasLab Pro (Holles et al. 2013, Parmentier et al. 2015). Ten 30-second replicate playbacks per sound (white noise, 40 Hz, 600Hz, and 1 kHz) were constructed.

For the boat motor sound, recordings were made at a depth of 5 m outside the barrier reef of Moorea (at 2 km away from the nearest reef) using a hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier; sensitivity -165 dB re 1 V/µPa; frequency range 2 Hz – 10 kHz; High Tech Inc., Gulfport MS) and a solid-state recorder (Edirol R-09HR 16-bit recorder; sampling rate 44.1 kHz; Roland

Systems Group, Bellingham WA). A boat with a 25 horse power Yamaha engine started 50 m from the hydrophone and drove past in a straight line for 100 m; passing the hydrophone at a closest distance of 10 m. The recorder was fully calibrated using pure sine wave signals generated in SAS Lab (Avisoft, Germany), played on an mp3 player, and measured in line with an oscilloscope (Figure VI-3). Recordings were clipped into 30 s samples so that when boat was present a whole pass was sampled. Ten 30-second replicate playbacks were then constructed.

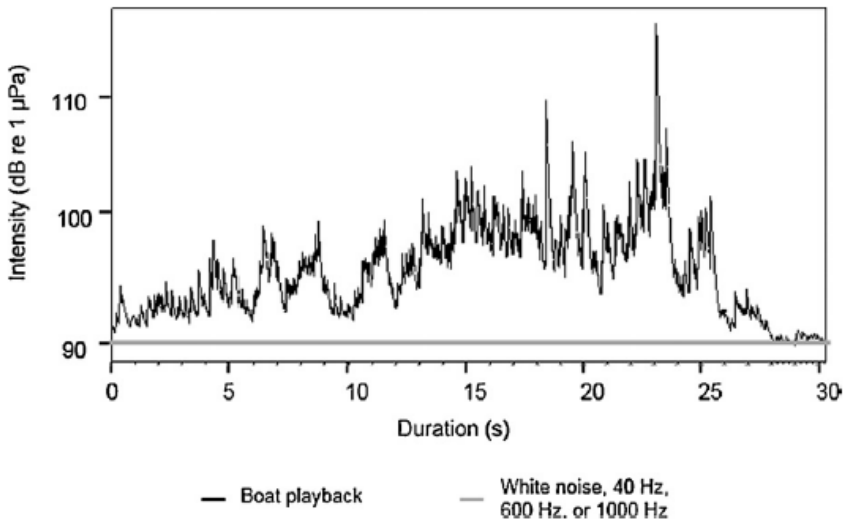

Figure VI-3: Sound envelope of boat playback, white noise and the three single frequency tones (40 Hz, 6000 Hz, 1000 Hz) during 30 seconds with all sound calibrated at, at least 90 dB re 1 mPa RMS.

As ambient noise at the experimental site was never above 80 dB re 1 µPa (across all frequency bands between 10 and 2000 Hz - Parmentier et al. 2015), the sound level of our five sound composites was calibrated at, at least 90 dB re 1µPa RMS by placing a hydrophone at 1 m of high-speaker (Figure VI-3) to ensure that it was above the local ambient noise floor. To check the quality of sound emitted by the loudspeaker, sound pressure and particle acceleration were measured for each sound tested (using the hydrophone described above and a M30 accelerometer, sensitivity 0–3 kHz, manufactured and calibrated by GeoSpectrum Technologies, Dartmouth, Canada; recorded on a laptop via a USB soundcard, MAYA44, ESI Audiotechnik GmbH, Leonberg, Germany). Acoustic analyses performed in MATLAB v2010a were described by Holles et al. (2013), Nedelec et al. (2014) and Parmentier et al. (2015).

Overall, 59 rays were tested using only one sound per ray (i.e., 10 rays per sound type and 9 rays for the control experiment), and the duration of sound exposure was 5 minutes. Two behavioral responses were recorded in real-time for each eagle ray: no reaction (i.e., ray continued to forage

over sand) or escape behavior (i.e., swam away from the foraging site at, at least, 50 m). Rays are easily distinguishable due to the individual pattern on their dorsal side (C. Berthe, unpublished data). Thus, no ray was tested twice during the experiment.

A first $\chi 2$ test was conducted on the five sound types in order to assess if rays had a significant homogeneous or heterogeneous behavior according to the tested sounds. Then, a second $\chi 2$ test was conducted to compare the number of rays showing escape behavior with one sound type compared to the control test (i.e., five $\chi 2$ tests conducted) to determine whether a particular sound had a significant effect (escape behavior) on the rays. The statistical analyses were conducted using R software v2.11.1 (R Development Core Team, 2010).

3. Results

During the control experiment, rays did not exhibit an escape behavior. The 9 tested rays did not move and continued to forage during the five-minute observation period (Figure VI-4). This result confirmed that rays were not disturbed by the presence of an observer.

During the sound tests, rays exhibited significant heterogeneous behavior depending on the sound tested ($\chi 20.05,4 = 18.2$, $p < 0.001$ - Figure V-4). For example, 60% of rays swam at least 50m away from the foraging site when the boat motor sound was played. Among the 6 rays that fled, 4 swan away in the 10 first seconds, 1 between 1 and 4 min and 1 during the last minute of boat playback. On the contrary, no ray exhibited an escape behavior when the 600Hz sound was played. Thus, none of the sounds at 40 Hz, 600 Hz, 1 kHz or the white noise resulted in altered ray behavior (i.e., comparison between sound and control tests - $\chi 20.05,1 <3.84$, $p>0.05$), and the rays continued to forage over sand (Figure VI-4). In contrast, the sounds of the boat motor elicited significantly more escape behaviors during foraging activity ($\chi 20.05,1 =37.7$, $p<0.001$).

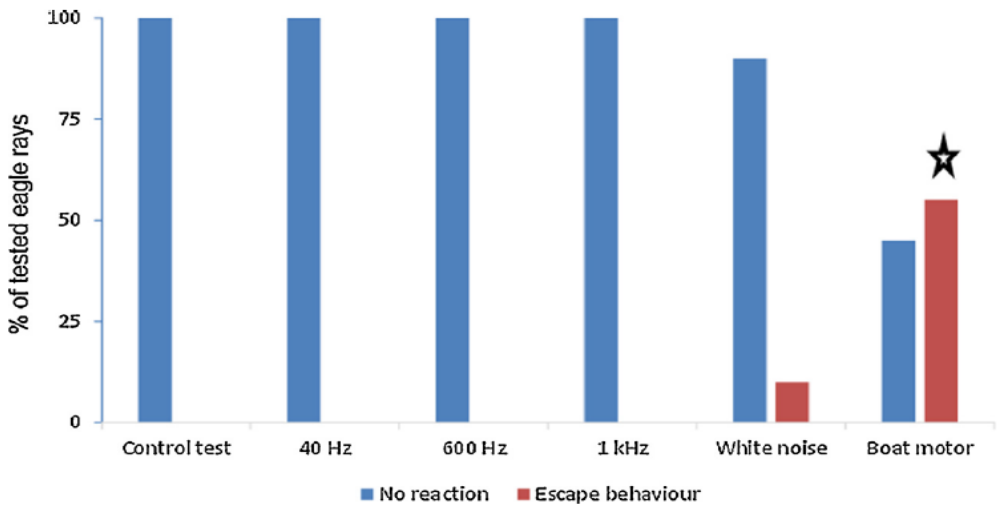

Figure VI-4: Eagle rays (*Aetobatus ocellatus*) behavior related to the control test (without sound–switched-off loudspeaker) and the tested sounds: a boat motor sound, a white noise and three single-frequency tones (40 Hz, 600 Hz, 1 kHz). Ten rays were tested for each sound type and nine rays for control test. Two possible responses of eagle rays were observed: no reaction (i.e. continue to forage) or escape behavior (i.e. swam away from the foraging site at, at least, 50 m). Stars indicate a significant difference between the number of rays showing escape behavior with one sound type and the control test (x2 test – P < 0.05).

4. Discussion

Our study showed the negative effect of anthropogenic noises on the foraging activity of eagle rays. Thus, 60% of the rays tested stopped foraging and fled the area when the boat motor sound was played (Figure VI-4). Local farmers in French Polynesia observed that eagle rays exhibited an escape behavior when a boat approached, and our findings confirmed this observation. Moreover, our field observations allowed us to identify that 66% of eagle rays fled between 1 to 10 seconds after the beginning of boat sound (sound characterized by low frequencies - < 350 Hz - at the beginning of recording). These field observations are consistent with those of Casper (2006), who demonstrated that elasmobranches mainly perceived the particle motion, which is more prevalent at low frequencies. Sharks are thought to be attracted to low frequencies because stressed prey emits low frequency sounds (Casper 2006, Fay 2000, Nelson 1963). Eagle rays are a favorite prey item of the hammerhead shark (Chapman, 2002) and could be more reactive to low frequencies to avoid shark' predation. This hypothesis should be, nevertheless, validated by future studies. Moreover, our study showed no escape behavior when only a single-frequency tone was played (40 Hz, 600 Hz or 1 kHz). Therefore, it may not be just a specific frequency that disturbs the eagle rays during their foraging activity, but rather a combination of sound frequencies and intensities.

Worldwide, a lot of research has been done on aquaculture predation (Ross et al. 2001, Littauer et al. 1991, Hutchings 1999, Fisher et al. 2011), but aside from use of anthropogenic noise, few

reliable and affordable solutions have been found (Ross et al. 2001, Pelton 2011). Our study confirmed that anthropogenic noise such as that produced by boat traffic can negatively affect the white-spotted eagle ray, similar to other marine species as indicated in earlier studies (Kight et al. 2011, Francis et al. 2013, Slabbekoorn et al. 2010, Nedelec et al. 2014). This negative effect on eagle rays could be used by humans to benefit marine aquaculture if such sounds deter the predators of pearl oysters (Nash et al 2000, Ross et al. 2001). Further experiments must be conducted *in situ*, near oyster long-lines and over a long-term period in order to determine if an underwater playback system could be used to efficiently deter eagle rays from oyster farms without negative effects on the growth of oysters and on the quality of the black pearls. Moreover, Newborough et al. (Newborough et al 2000) showed the problem of habituation effects with acoustic repulse systems that remove cetaceans from fishnets. To reduce the likelihood of habituation of eagle rays, local farmers would need to use different recordings, including sounds from different boat types or by using irregular temporal and spatial patterns when playing back sounds.

Overall, our study showed, for the first time, that anthropogenic noise is detrimental to the eagle rays, but this negative effect could be used by Human to deter the rays from oyster farms.

Chapter VII Conclusion & Perspectives

My diploma aimed was to gather the first biological, ecological and behavioral dataset on the ocellated eagle ray (*Aetobatus ocellatus*) in French Polynesia. Many studies have been done around the world on the gender Aetobatus (e.g., Bahamas: Silliman & Grueber, 1999; Korea: Oh et al. 2006; Mexico: Cuevas-Zimbrón et al. 2011; Japan: Fukuda & Zenitani, 2010; Australia: Scluessel et al, 2010; Venezuela: Tagliafico et al. 2012; USA: Bassos-Hull et al. 2014), but very few in the central Pacific (e.g., Hawaii: Richards et al. 2009). Thus, in French Polynesia we have little information on the biology and ecology of eagle rays, except the fact that they are predators of the black-lipped pearl oysters in the Tuamotu atolls (Planes et al. 2006). Since the genetic evidences of a species complex (Richards et al. 2009; Schluessel et al. 2010a), the genus *Aetobatus* needed a refreshing taxonomy and all species included in this genus needed an assessment of their ecology and home range for an accurate conservation status. Eagle rays play important roles in French Polynesia, as mesopredator in the coral reef ecosystem and because of their impact on the two local economic pillars (the tourism and the pearl industries). As no study has ever been done on eagle rays in French Polynesia, it became pretty clear to push the study beyond a simple taxonomic statement. I decided to launch a one year observation of schools of rays to better know the species ecology by understanding distribution patterns. This last chapter of my EPHE Diploma offers research perspectives on a poorly studied species in an area where its ecological and economic values are obvious.

1. Perspectives in fundamental research on the ocellated eagle ray

The last decade had witnessed an increasing interest for animal social behavior research; and even if the knowledge remains still very limited (Jacoby et al. 2011) elasmobranch groups do not slip out of this tendency (Figure VII-1). Many studies explored the shark aggregations and the social behaviors displayed in these aggregations (Jacoby et al. 2014; Gallanger et al. 2014; Haulsee et al. 2016; Findlay et al. 2016). Mourier (2011) showed the preference Blacktip reef shark *Carcharhinus melanopterus* has for other individuals and the importance of specific individuals within the entire group. Newby et al (2014) explored social organization in *Aetobatus narinari* and highlighted the lack of kin-structured sociality. During my year of observation, I could identify ocellated eagle rays at the individual level thanks to their unique dorsal pattern. This allowed me to estimate the populations that I was working on, and the movement patterns between the two populations (clubMed vs. Mareto). Further research could use this to explore eagle rays' behavior as it has been done for sharks. As the eagle rays gather in large groups, they may have specific function within the group and also display preferences to conspecific. As the elasmobranches do not communicate through sounds,

they have to use other ways to transmit information and a better understanding of the behavior between individuals within groups could help understand those ways of communication. Overall, the home range of a species is a crucial information for its conservation and management. My personal observations of one adult male during four years support the hypothesis that eagle rays, at least some individuals, are resident to Moorea Island. The year of observation, detailed in the chapter III, confirms the repeatedly presence of individuals throughout the year but need further investigations at a larger space and time scale to confirm, or not, the tendency to return in specific areas.

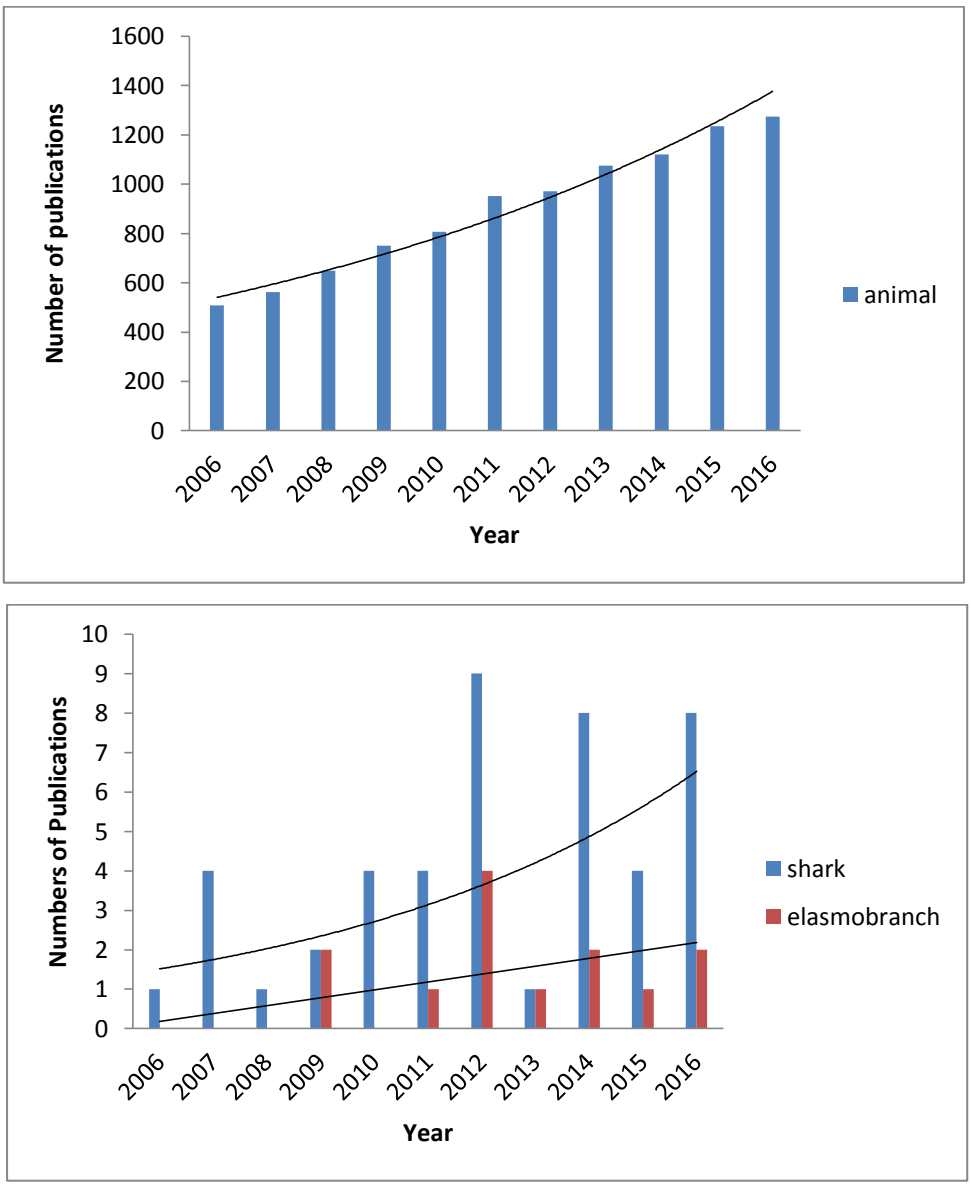

Figure VII-1: The number of publications on social behavior increasing for animals in general since 2006. Bar chart on top show: data based on "animal" and "social behavior" as key word in Web Of Science. Bar chart at the bottom: data based on "shark" (not "shark bay") or "elasmobranch" and "social behavior" in Web Of Science. (Adapted from Brown et al. 2011)

2. Perspectives in conservation of eagle rays

Aetobatus ocellatus current status in the IUCN Red List is "Vulnerable" and information about its home range, its population structure and its biology is still needed to properly assess an adapted conservation status (Kyne et al. 2016).

Integrated researches that combine ecological, biological, genetic and geographic data are needed to insure the effective conservation and management of elasmobranches species. Osgood & Baum (2015) encourage researchers to pay attention to data deficiency in the IUCN Red List and to fill those lacks for sharks and rays, especially in taxonomic and geographic scope. Moreover, White & Last (2012) explained the importance of taxonomic clarity to study and protect species because "it is the foundation of all the other life sciences". With more than 180 new species described in the last ten years, chondrichtyan taxonomy needed clarification and reassessment for a fundamental accuracy off species conservation. "The batoid fishes have long been regarded as the most taxonomically problematic group of all elasmobranch groups" (Last et al. 2016). The Aetobatidae family is a good example of this taxonomic problem, illustrated by all the taxonomic revision it needed. Otherwise the Aetobatidae family shows a high genetic structure despite high dispersal capabilities and a wide-range gene flow (Richards et al. 2009; Schluessel et al. 2010a). Its historical dispersal pathway seems to go from the Indo-Pacific region to the Atlantic Ocean, and then in the Eastern Pacific through the Isthmus of Panama. This places the Marquesas at the end of the loop. Located further east in the central Pacific than the rest of the French Polynesia, the Marquesan islands are known for their exceptional biodiversity with a high endemic species level. The presence of the two species of Manta rays (*M. birostris* and *M. alfredi)* and their home range overlapping have been showed by Mourier et al. in 2012. My study confirmed that *Aetobatus ocellated* occurs in the Polynesian Islands, but it does not exclude the presence of the other species of the family. Genetically exploring the archipelagoes of French Polynesia where no sample has been done yet (Gambier, Marquesas and Australs) could provide more information on the home range of the family, consequently confirming or not the presence of other species and adding knowledge of the population structure of *Aetobatus ocellatus* in the Indo-Pacific region.

Shifts in the fragile ecology of eagle rays have already conducted to extremities in some places of the world (see Introduction). Climate change is causing shifts in the ecology by inducing movement of populations around the planet (Pecl et al. 2017), related to water temperature changes, known to be a significant driver for some species migration to warmer or cooler waters. These movements by eagle rays population are likely to trigger major shifts in local ecosystems (i.e.:

Japan, Yamaguchi et al. 2005). My one year observation study demonstrated that movement of eagle rays in the lagoon of Moorea was driven by ontogenetic partitioning. No mass migration has been observed and never reported in French Polynesia. However, as it was a limited study, more investigations of the eagle rays movements over years and in other locations need to be done to fully understand all the drivers and improve the management of this species.

3. Perspectives of research on eagle rays in French Polynesia

In Moorea Island, more investigations could be done all around the island to identify the functional habitats in the lagoon and identify particular areas as nursery or resting areas. Nursery areas are vital for population stability and recovery (Bonfil, 1997). Knowing where those areas are located help to improve conservation plans. If the protection of nursery areas is essential, it is not sufficient and an ideal protection would be throughout all life stages. But this is not possible for sharks or rays due to their wide home range (Kinney & Simpfendorfer. 2009). Nevertheless, understanding the different life stages to provide the best protection is important and must be adapted to the species of concern. Data collected on specific habitats from my diploma and further data collection could help improve the efficiency of management plans if taken into account. Two main projects of the management of the biodiversity are currently undergoing on Moorea Island: 1/ the revision of the marine spatial planning (PGEM) of the island after 10 years of establishment and 2/ the Moorea IDEA project (Davies et al. 2016) which aims to build an entire digital socio-ecological system from the Moorea island. Using information like that provided by my diploma could help both projects to be more relevant when testing for the viability of future projects and their impact on the ecosystem.

In French Polynesia, more studies need to be launched to understand the overlap between eagle rays habitat uses and human activities. As we saw in the chapter V, pearl farming suffers damages from eagle rays feeding on the black-lipped pearl oyster (Planes et al. 2006). According to testimonies of perliculteurs damages vary from one atoll to another. Why are some atolls more affected than other? Information about movement patterns of eagle rays, the geomorphology of yhe atolls (closed or open, large or small pass, etc.) or the free-living prey availability could certainly help explain these specificities. Schluessel et al. (2010b) showed that *Aetobatus ocellatus* in Moreton Bay, Australia does not feed on the oysters raised in the bay and presume it is due to abundant food sources in general. Is the black-lipped oyster more attractive for eagle ray in French Polynesia? Are the natural food sources less abundant in some Tuamotu atolls? It is likely to think that the answers to these questions could help anticipate the behaviors of the eagle rays in the Tuamotu atolls and

complete research done in my diploma on repellent systems to prevent damages in the pearl oysters farms. The importance of finding answers to solve the predation problem lies in the fact that culling the eagle rays is not a sustainable solution. Overfishing of eagle ray or of its predator (apex predators like hammerhead or tiger sharks) likely induce a cascade effect on the entire ecosystem (Gray et al. 1997; Yamaguchi et al 2005). The predator-prey relationship is a complex process which is difficult to anticipate and need time to be fully understood and explained (Grubbs et al, 2016). Find a repellent system would be the solution to protect the both valuable species for the French Polynesia economy.

Ocellated eagle ray has significant impacts on the two economic pillars of the French Polynesia and each impact, the negative one on oyster farms and the positive one on tourism needs to be taken into account for a sustainable management of the species in the country.

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ÉCOLE PRATIQUE DES HAUTES ÉTUDES

SCIENCES DE LA VIE ET DE LA TERRE

First ecological, biological and behavioral insights of the ocellated eagle ray Aetobatus ocellatus in French Polynesia

BERTHE Cécile

October 10, 2017

Abstract

Marine species have, for a long time, been considered as less vulnerable to extinction than terrestrial species. Nowadays, they show an extreme sensitivity to global climate changes, with some groups far less resilient than others, as it is the case for the elasmobranchs group. In addition to specific life-history traits (i.e. low fecundity, late maturity, few pups per litter), most of elasmobranchs are pelagic or live in deep waters. Monitoring these species is incredibly difficult and data are missing for almost 50% of them. Elasmobranches need special attention from scientists to improve their conservation at a global scale by increasing knowledge on their biology and their ecology. My EPHE-SVT diploma, done in Moorea with the CRIOBE (USR3278 CNRS EPHE UPVD) aimed to gather the first biological, ecological and behavioral data of the ocellated eagle rays (*Aetobatus ocellatus*) in French Polynesia. It consisted in three main studies: Study 1/ A genetic study was first done after the discovery of the species complex among the *Aetobatus narinari* genus. It confirmed the presence of *Aetobatus ocellatus* in the Society and the Tuamotu archipelagoes. Study 2/ A year of monitoring highlighted the ontogenetic partitioning within the lagoon of Moorea, where the activity turned out to also be different between resting and foraging. This partitioning could allow avoiding intra-specific competition. Study 3/ Known as predator of the pearl oyster, eagle rays have a negative impact on the French Polynesian pearl farms. A study was conducted to determine whether artificial sounds (generated by computer or by boat motor) could ward this species off and be used as a repellent in the pearl farm industry. The boat motor sound was indeed efficient to stop individuals from foraging or to even scare them off. In conclusion, this diploma represents a first sept forward to improve the conservation of *Aetobatus ocellatus* in a region (i.e.: French Polynesia) where its impacts, both positive for the tourism and negative for the pearl farms, is undeniable.

KEYWORDS: Elasmobranches, ocellated eagle ray, *Aetobatus ocellatus*, COI, predation, habitat partitioning, protection, conservation, coral reefs

ÉCOLE PRATIQUE DES HAUTES ÉTUDES

SCIENCES DE LA VIE ET DE LA TERRE

Première approche écologique, biologique et comportementale de la raie aigle à ocelles *Aetobatus ocellatus* en Polynésie française

BERTHE Cécile

10 Octobre 2017

RÉSUMÉ

Les espèces marines ont longtemps été considérées comme moins vulnérables à l'extinction que les espèces terrestres. Mais elles présentent aujourd'hui une grande sensibilité aux changements climatiques actuels, avec certains groupes beaucoup moins résilients que d'autres, comme c'est le cas pour les élasmobranches. En plus de traits d'histoire de vie particuliers (ex. faible fécondité, maturité tardive, peu de petits par portée), la plupart de ces animaux sont pélagiques ou vivent en profondeur. Leur suivi s'avère compliqué et le manque de données touche presque 50% de ces espèces. Ce groupe nécessite donc une attention particulière de la part des scientifiques pour améliorer leur conservation au niveau mondial, en augmentant les données sur leur biologie et leur écologie. Mon diplôme EPHE-SVT, réalisé à Moorea au sein du CRIOBE (USR3278 CNRS EPHE UPVD) a pour objectif de rassembler les premières données biologiques, écologiques et comportementales sur les raies aigle à ocelles (*Aetobatus ocellatus*) en Polynésie française. Il est constitué de trois études principales : Etude 1/ Une étude génétique a d'abord été réalisée suite à la découverte du complexe d'espèce au sein d'*Aetobatus narinari* et a permis de confirmer la présence d'*Aetobatus ocellatus* dans les archipels de la Société et des Tuamotu. Etude 2/ Un suivi d'un an a permis de mettre en évidence une utilisation ontogénique de différents habitats du lagon de Moorea, habitats où l'activité s'avère également différente (entre repos et nourrissage). Cette répartition permettrait d'éviter une compétition intra-spécifique. Etude 3/ Reconnues comme prédateur de l'huître perlière, les raies aigle à ocelles ont un impact négatif sur la perliculture polynésienne. Une étude a donc été menée pour déterminer si des sons non naturels (sons générés par ordinateur ou sons de moteur de bateau) pourraient avoir un effet négatif sur cette espèce et présenter une possibilité de répulsif dans les concessions perlières. Les sons de moteurs de bateau ont effectivement montré une efficacité à stopper les individus en phase de nourrissage voire à les faire fuir. En conclusion, ce diplôme constitue un premier travail important pour améliorer la conservation de l'espèce *Aetobatus ocellatus* dans une région, la Polynésie française, où son impact, à la fois positif pour le tourisme et négatif pour la perliculture, reste indéniable.

MOTS-CLÉS : Elasmobranches, raie aigle à ocelle, *Aetobatus ocellatus*, COI, prédation, répartition de l'habitat, protection, conservation, récifs coralliens