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Jérémy Tornos

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# THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Ecologie et Biodiversité

École doctorale GAIA

## Approches intégrées en écologie de la conservation des oiseaux marins : cas de l'utilisation d'autovaccins pour la conservation des albatros sur l'île Amsterdam

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Le 6 juillet 2021

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UNIVERSITÉ  
DE MONTPELLIER



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## Publications et manuscrits présentés dans les annexes

- Annexe 1 : **Tornos Jérémy**, Forde Taya, Clessin Augustin, Bost Caroline, Lemberger Karin, Le Net Rozenn, Fléchet Aline, Gantelet Hubert, Gamble Amandine & Boulinier Thierry. Not only a single strain of *Pasteurella multocida* kills albatrosses on Amsterdam Island: insight into a community of bacterial pathogens affecting threatened seabirds. *In preparation*.
- Annexe 2 : **Tornos Jérémy**, Clessin Augustin, Gantelet Hubert, Fléchet Aline, Bely Marine, Gamble Amandine & Boulinier Thierry. Optimizing a vaccination approach against avian cholera to protect endangered albatrosses. *In preparation*.
- Annexe 3 : **Tornos Jérémy**, Ponchon Aurore, Delord Karine, Bely Marine, Clessin Augustin, Flechet Aline, Gantelet Hubert, Gamble Amandine & Boulinier Thierry. Prospecting by pre-breeders and failed-breeders in a highly site faithful albatross species: evidence and potential implication for the circulation of disease agents. *In preparation*.
- Annexe 4 : Aurore Ponchon, Amandine Gamble, **Jérémy Tornos**, Karine Delord, Christophe Barbraud, Justin MJ Travis, Henri Weimerskirch, Thierry Boulinier. Similar at-sea behaviour but different habitat use between failed and successful breeding albatrosses. *In revision*.
- Annexe 5 : Gamble A, Bazire R, Delord K, Barbraud C, Jaeger A, Gantelet H, Thibault E, Lebarbenchon C, Lagadec E, Tortosa P, Weimerskirch H., Thiebot J-B., Garnier R., **Tornos J.** & Boulinier T. 2020. Predator and scavenger movements among and within endangered seabird colonies: Opportunities for pathogen spread. *Journal of Applied Ecology* 57:367–378. doi:10.1111/1365-2664.13531.
- Annexe 6 : Lamb J., **Tornos J.**, Gamble A., Gantelet H., Keck N., Clessin A., Flechet A. & Boulinier T. Hanging out at the club: breeding status and territoriality affect individual space use and multi-species overlap at a seabird colony. *In preparation*.
- Annexe 7 : Gamble A, Garnier R, Jaeger A, Gantelet H, Thibault E, Tortosa P, Bourret V, Thiebot JB, Delord K, Weimerskirch H, **Tornos J.**, Barbraud C., & Boulinier T. 2019. Exposure of breeding albatrosses to the agent of avian cholera: dynamics of antibody levels and ecological implications. *Oecologia* 189:939–949. doi:10.1007/s00442-019-04369-1.
- Annexe 8 : Jaeger A, Gamble A, Lagadec E, Lebarbenchon C, Bourret V, **Tornos J.**, Barbraud C, Lemberger K, Delord K, Weimerskirch H, Thiebot J-B., Boulinier T., & Tortosa P. 2020. Impact of annual bacterial epizootics on albatross population on a remote island. *Ecohealth* 17:194–202. doi:10.1007/s10393-020-01487-8.

# Abréviations

Par ordre alphabétique :

AMA : Amsterdam Albatross, Albatros d'Amsterdam (*Diomedea amsterdamensis*)

*Er* : *Erysipelothrix rhusiopathiae*, bactérie responsable du rouget du porc

IYNA : Indian yellow-nosed Albatross, Albatros de l'océan indien (*Thalassarche carteri*)

MAT : Microscopic Agglutination Test

Multi-host MLST : Multi host Multi Locus Sequence Typing

NRP : Northern Rockhopper Penguin, Gourfou sauteur du nord (*Eudyptes moseleyi*)

ONU : Organisation des Nations Unies

*Pm* : *Pasteurella multocida*, bactérie responsable du choléra aviaire

RIRDC MLST : Rural Industries Research and Development Corporation Multi Locus Sequence Typing

RNN-TAF : Réserve Naturelle Nationale des Terres Australes Françaises

SKU : Brown Skua, Labbe subantarctique (*Stercorarius antarcticus*)

SOA : Sooty Albatross, Albatros fuligineux à dos sombre ou Albatros brun (*Phoebastria fusca*)

UICN : Union internationale pour la conservation de la nature

UNESCO : United Nations Educational, Scientific and Cultural Organization



# Introduction



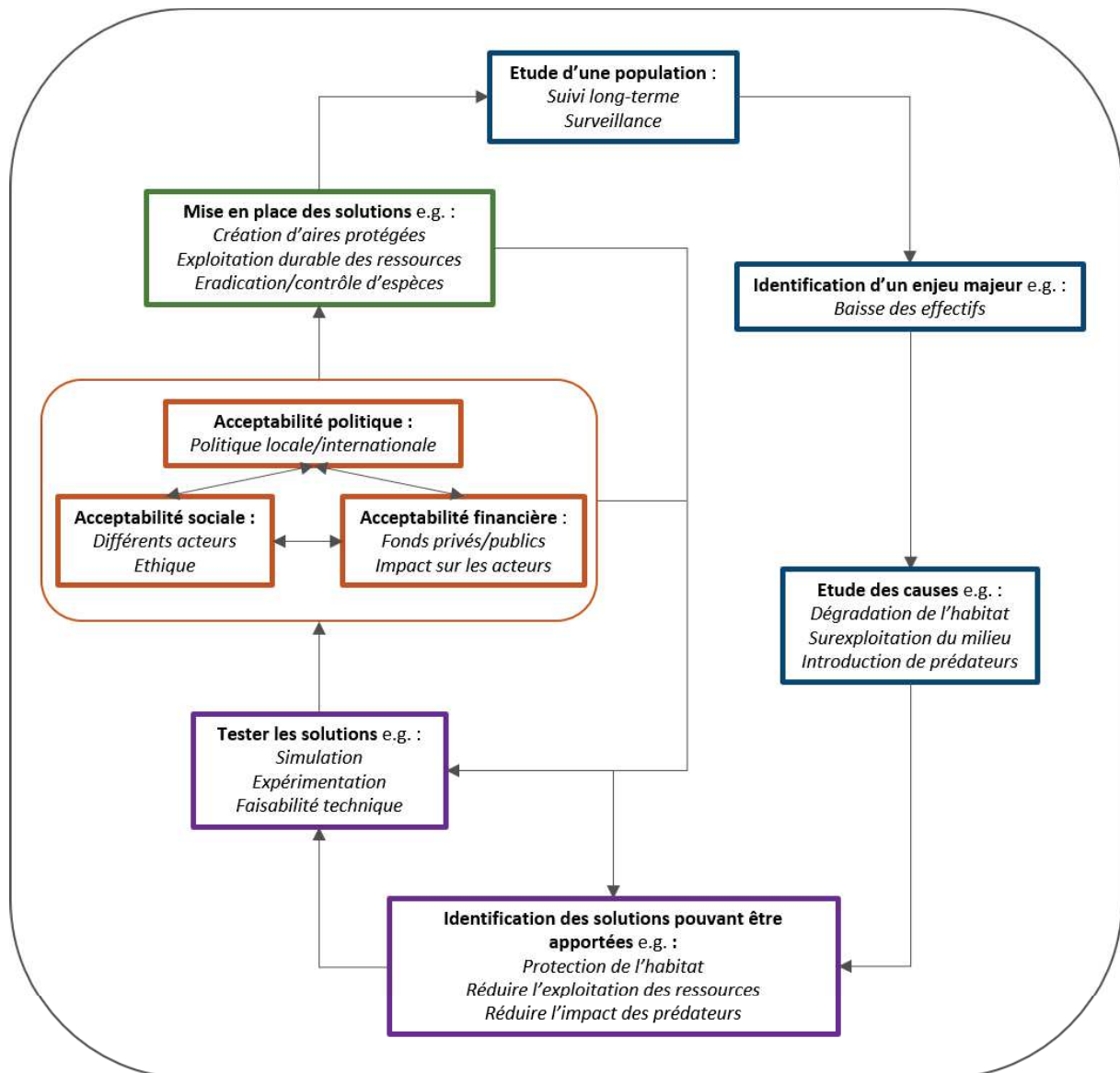


## La science de la conservation : principes et enjeux

La perte de la biodiversité au niveau mondial s'accélère (Tittensor et al. 2014; Ripple et al. 2017; Díaz et al. 2019), avec 18% des espèces considérées menacées (Atwood et al. 2020). Cet effondrement conduit à des transformations irréversibles des écosystèmes (ONU 2019). En effet, 75% de la planète montre des traces d'altération par l'Homme (Venter et al. 2016). La conservation de la biodiversité est devenue l'un des grands défis du XXI<sup>ème</sup> siècle et est, depuis 2010, au centre des objectifs de la Convention sur la Diversité Biologique d'Aichi (SCBD 2010, Japan). D'après Aichi Biodiversity Targets, d'ici à 2020, 17 % des zones terrestres et des eaux intérieures, et 10 % des zones côtières et marines, devaient être conservés par la mise en place d'aires protégées. A l'heure actuelle, 15.53% des zones terrestres et 7.65% des zones marines sont protégées (<https://www.protectedplanet.net/en>). Face à ce lourd constat, une récente discipline a vu le jour, comme une réponse de la communauté scientifique aux changements environnementaux massifs qui se produisent actuellement sur la planète, nommée « biologie de la conservation » (Orians and Soulé 2001). Cette discipline, apparue à la fin du XX<sup>ème</sup> siècle, a pour objectif la protection et la perpétuation de la diversité biologique (Soulé 1985). Une question est alors souvent soulevée : « Est-ce de la science ? Ou est-ce de la conservation ? ». La biologie de la conservation est bien une discipline à part entière, une science théorique et une science d'action. Cette science, plus récemment intégrée dans le plus large champ disciplinaire de la « science de la conservation » (Kareiva and Marvier 2012), combine ainsi de multiples approches, allant des sciences de la vie au sens large (écologie, biologie, biogéographie, génétique des populations, [...]), aux sciences économiques, sciences sociales, [...], ou encore les sciences politiques, de la communication ou de la santé ainsi que bien d'autres domaines (Kareiva and Marvier 2012). Elle est donc un ensemble de disciplines très variées où le fondamental et l'appliqué sont étroitement liés, voire même indissociables (Soulé 1985; Maris and Devictor 2014) et où des scientifiques, des décisionnaires et des gestionnaires sur le terrain seront associés pour répondre aux enjeux environnementaux auxquels ils font face (**Figure 1**).

Le principe de cette science est ainsi basé sur le constat que le changement global dû aux activités humaines impacte fortement les écosystèmes, à tous les niveaux (une perte de biodiversité, une dégradation de l'habitat, une disparition d'espèces...). Au niveau des populations d'espèces sauvages, la science de la conservation développe des approches fondamentales reposant sur l'acquisition de données par la surveillance et les suivis à long-terme des populations afin de mieux comprendre leur fonctionnement et les mécanismes de réponses aux changements de leur environnement (Nichols and Williams 2006). En réponse à cet état critique constaté, cette science a pour but de déterminer les causes des dynamiques en jeu, mais aussi de proposer des moyens d'y remédier. Les solutions proposées doivent pouvoir être testées et validées sur le terrain (Primack et al. 2012). Ainsi, la science

de la conservation se veut adaptative, évolutive et devra sans cesse se remettre en question afin d'apporter les réponses les plus adaptées (Gibbons et al. 2011; Restif et al. 2012).



**Figure 1:** Principes fondamentaux de la science de la conservation présentés de manière synthétique (adapté de Gibbons et al. 2011; Williams et al. 2020). Les exemples cités en italique ne sont pas exhaustifs. La science la conservation intègre de nombreuses approches, impliquant de la science fondamentale (encadrés bleus) ou appliquée (violets), des décisions sociales, politiques, économiques (orange) ainsi que des acteurs du terrain et de la conservation appliquée (verts). Il est important de noter que l'encadré orange implique à la fois de la science fondamentale (e.g., sciences économiques et sociales), de la communication et des pouvoirs décisionnaires, mais qu'il est ici schématisé et simplifié pour des raisons de lecture. Les couleurs et catégorisations sont à titre indicatif, toutes les étapes étant interconnectées, et il est souvent difficile d'en fixer les limites. La science de la conservation se veut adaptative : l'évaluation, à la fois sur le court, moyen et long terme des solutions proposées doit être continue afin de permettre un ajustement optimal. La diversité des compétences et des savoirs est essentielle et la science de la conservation ne peut être assurée que par une diversité d'approches d'acteurs : e.g., scientifiques, politiques, gestionnaires, entreprises

Face à l'accélération rapide de la disparition d'espèces et de la dégradation des écosystèmes (Johnson et al. 2017), la science de la conservation est une discipline qui a connu un essor considérable lors des dernières décennies et est encore à ce jour en pleine croissance (Williams et al. 2020). Les études menées sont nombreuses et variées, abordant une diversité de disciplines, concernant à la fois le niveau de menace des espèces cibles, mais surtout l'identification et la compréhension de ces menaces qui les touchent ainsi que les solutions pouvant être apportées pour protéger les espèces (Lawler et al. 2006; Kareiva and Marvier 2012). Les études menées portent sur de nombreux taxons (mammifères, oiseaux, amphibiens, végétaux...) à travers le monde (Lawler et al. 2006). Les menaces qui pèsent sur la biodiversité sont nombreuses : dégradation de l'habitat, introduction d'espèces exotiques, pollution, surexploitation des milieux ou encore la transmission de maladies (Lawler et al. 2006; Godet and Devictor 2018). Les solutions proposées, évaluées et testées sont tout aussi diversifiées : création d'aires protégées, mesures de gestion durable des ressources, restauration ou gestion de milieu, contrôle d'espèces introduites protection d'espèces pour n'en citer que quelques-unes (Godet and Devictor 2018). La science de la conservation est donc un domaine interdisciplinaire et évolutif qui nécessite un suivi temporel à long terme et qui ne peut être optimal que par l'intervention d'une diversité d'acteurs autant scientifiques, que politiques ou gestionnaires (Fisher et al. 2009). C'est dans ce contexte qu'a été développé la thèse. La question de conservation a été abordée par la combinaison de différentes approches, sans chercher à être exhaustif, mais en cherchant à avancer sur les questions importantes qui étaient soulevées.

### **Les oiseaux marins, un groupe particulièrement préoccupant : l'exemple des albatros**

Les oiseaux de mer sont des espèces pour lesquelles une large proportion de la population totale dépend d'un environnement marin pour au moins une partie de leur cycle (Croxall et al. 2012). Ils occupent une place centrale dans les écosystèmes marins, présents dans tous les océans, des zones côtières à la haute mer. Ils dépendent de l'écosystème terrestre essentiellement pendant leur période de reproduction, pendant laquelle ils vont former, souvent sur des îles à l'abri des prédateurs, des colonies de reproduction de quelques dizaines à plusieurs centaines de milliers de couples, pour élever leurs jeunes. Qui plus est, les oiseaux marins sont des prédateurs longévifs, pour la plupart au sommet de la chaîne alimentaire du milieu marin.

Du fait de leur mode de vie, à la fois terrestre et marin, ils sont soumis à de nombreuses pressions, tout au long de leur cycle de vie, ce qui font d'eux l'un des groupes d'oiseaux les plus menacés dans le monde (Croxall et al. 2012; Birdlife 2018), avec plus de 28% des espèces qui sont listés sur la liste rouge de l'UICN. Les oiseaux de mer font face à de nombreuses menaces, avec en tête de liste les espèces exotiques envahissantes, touchant plus de 45% des espèces (Dias et al. 2019). Les prises accidentelles (bycatch), associées aux activités de pêche, la chasse (ou piégeage) et les changements climatiques



touchent plus de 25% des espèces. Les maladies quant à elles, n'affectent que 4% des espèces mais ont en moyenne un impact très élevé et peuvent conduire à la disparition ou à l'extinction d'espèces (Smith et al. 2006; Descamps et al. 2012), notamment à cause de leur reproduction en colonies souvent denses. Parmi les oiseaux marins, les Procellariiformes (albatros et pétrels) et les Sphenisciformes (manchots) regroupent la plus grande part d'espèces menacées.

Les albatros (Diomedidae) plus particulièrement comptent 68% (15/22) de leurs espèces menacées ('En danger critique', 'En danger' ou 'Vulnérable') et les 32% restant considérés 'Quasi menacée' (5/22) et en 'Préoccupation mineure' (1/22) (IUCN 2019). Ces chiffres rendent les populations d'albatros particulièrement préoccupantes. Quatre-vingt pourcent des albatros sont notamment menacés par les prises accidentelles, ce qui constitue leur principale menace (Dias et al. 2019).

Les prises accidentelles générées par la pêche ont été particulièrement étudiées et constituent un exemple notable où la science de la conservation a sans équivoque aidé à répondre aux enjeux de conservation (**Figure 2**). Le déclin de la majorité des populations d'albatros a été au centre des préoccupations et le nombre d'études consacrées à cette problématique n'a cessé d'augmenter depuis les années 1980 (Croxall et al. 2012; Dias et al. 2019; Williams et al. 2020). Cet exemple illustre la diversité des disciplines qui ont été nécessaires à la fois pour comprendre, mais aussi proposer et mettre en œuvre des solutions. Les approches ont intégré de la science fondamentale (en particulier des suivis démographiques de population, des études du déplacement des individus), la recherche et l'évaluation de méthodes de prévention, une coopération internationale afin de communiquer et inciter les acteurs à la mise en place de mesures de conservation (par exemple, l'*Albatross Task Force, Agreement on the Conservation of Albatrosses and Petrels*). De plus, l'approche scientifique a permis de mettre en avant l'importance du rôle des politiques nationales et internationales dans la gestion et la conservation des espèces (Beal et al. 2021). Cet exemple donne un cadre pour considérer le développement d'une approche scientifique comparable sur un sujet relativement négligé jusqu'à présent, la prise en compte des menaces dues aux maladies infectieuses sur les populations d'oiseaux marins, et les moyens de lutter contre ces épidémies.



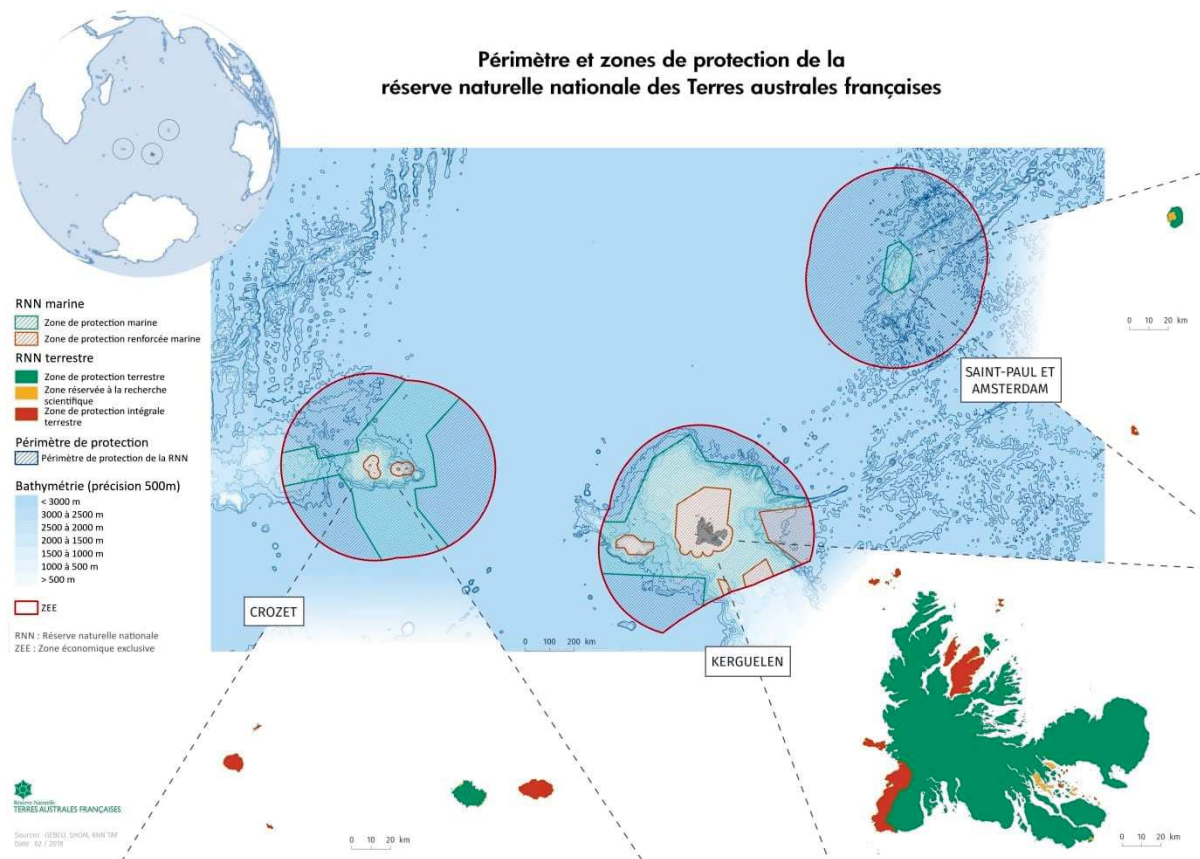
## Les maladies infectieuses : une problématique en biologie de la conservation ?

La prise en compte de la biodiversité dans les problématiques sur l'émergence et la gestion des implications des maladies infectieuses a pris une importance grandissante ces 20 dernières années (Daszak et al. 2000; Wobeser 2002; Young et al. 2017), même si les approches développées dans le cadre de l'écologie des maladies infectieuses (Grenfell et al. 1995; Hudson et al. 2002) sont encore relativement peu prises en compte dans les problématiques impliquant les espèces sauvages, notamment en France. Les appels pour des approches 'Une seule santé' ou 'One Health' vont dans le sens d'un rapprochement des disciplines impliquées, du domaine biomédical à l'écologie, la modélisation mathématique et la sociologie, et cela souligne notamment que des ponts entre disciplines sont encore à construire (Manlove et al. 2016). L'intérêt de coupler des approches d'écologie des maladies infectieuses à des approches biomédicales apparaît néanmoins évident lorsqu'il s'agit de proposer des solutions intégrées à des problèmes complexes faisant intervenir des dynamiques naturelles en relation avec les effets de changements environnementaux dus aux activités humaines (e.g., Holdo et al. 2009). Dans le cas des oiseaux marins, il est nécessaire de prendre en compte la question des maladies infectieuses, notamment à cause de la forte densité de leurs populations sur les colonies lors de la reproduction et des cas de mortalités massives qui ont pu être rapportées dans la littérature (Weimerskirch 2004; Phillips et al. 2016; Uhart et al. 2018).

## L'île Amsterdam : contexte biogéographique

Les Terres Australes et Antarctiques Françaises – TAAF, sont composées de 5 districts : les îles Crozet, les îles Kerguelen, le district de Saint Paul et Amsterdam, la Terre Adélie et les îles éparses. Ces territoires insulaires, sans habitants permanents, abritent une biodiversité exceptionnelle ayant évolué dans un isolement extrême. Cependant, et malgré cet isolement, la biodiversité de ces territoires subit les effets des changements globaux, en particulier des menaces directement liées aux activités humaines. Ainsi, afin de préserver ces régions sur le long terme, les Terres Australes Françaises (**Figure 3**), composées des îles Crozet, Kerguelen, Saint Paul et Amsterdam, sont classées depuis 2006 en réserve naturelle nationale (les îles éparses et la Terre Adélie bénéficiant d'autres statuts de protection particuliers). Cette réserve naturelle abrite parmi les plus fortes concentrations d'oiseaux et de mammifères marins au monde et l'étude, le suivi et la conservation de ces espèces est un des enjeux majeurs de son plan de gestion. En plus des enjeux de conservation, le caractère exceptionnel de ces îles en fait un terrain unique pour la recherche scientifique, notamment pour le suivi à long terme des populations d'oiseaux et de mammifères marins en milieu naturel, l'évolution et l'adaptation des espèces à la vie en milieu isolé et l'étude des effets des changements globaux, apportant des connaissances fondamentales sur la biologie des espèces. La Réserve Naturelle

Nationale des Terres Australes Françaises (RNN-TAF) a été inscrite en 2019 au patrimoine mondial de l'UNESCO du fait de ces phénomènes naturels d'une importance remarquables, ces processus écologiques, biologiques et évolutifs d'écosystèmes uniques et de l'importance de ces habitats pour la conservation d'une biodiversité exceptionnelle. Le district de Saint Paul et Amsterdam est constitué de deux îles volcaniques : l'île Amsterdam, d'environ 55 km<sup>2</sup> située au cœur de l'océan indien et sa voisine l'île Saint Paul, de 8 km<sup>2</sup> se situant 90 km plus au sud.



**Figure 3:** Position géographique des districts de la Réserve Naturelle Nationale des Terres Australes Françaises (RNN-TAF) ainsi que les périmètres et la nature des zones protections terrestres et marines. Source : plan de gestion de la RNN-TAF, ©TAAF.

L'île Amsterdam est l'une des îles les plus isolées au monde, située à plus de 3.000km de tout continent et à équidistance entre l'Afrique, l'Australie et l'Antarctique. L'île est un dôme volcanique s'élevant à 881 m d'altitude. Le sud-ouest est dominé par de hautes falaises abruptes, appelées falaises d'Entrecasteaux et atteignant jusqu'à 700 m d'altitude. Ces falaises s'élèvent jusqu'à un haut plateau central, appelé plateau des Tourbières. Une station scientifique permanente est située dans la partie nord de l'île et accueille environ 25 personnes, dont des scientifiques, des militaires et du personnel technique.

Les îles Saint Paul et Amsterdam sont les seules îles subtropicales de l'océan Indien, leur conférant donc un écosystème caractéristique unique dans cette région du monde, et présent sur seulement 2 autres complexes insulaires dans le monde, les îles Juan Fernandez dans le Pacifique Sud et les îles Tristan da Cunha en Atlantique Sud. Les îles Saint Paul et Amsterdam sont un des sites majeurs de reproduction de plusieurs espèces d'oiseaux marins emblématiques. Le district accueille notamment 3 espèces d'albatros, classées en danger d'extinction (**Figure 4**). L'île d'Amsterdam est en particulier le seul site de reproduction dans le monde de l'Albatros d'Amsterdam *Diomedea amsterdamensis* (nommé AMA dans la suite du document, pour Amsterdam Albatross). Cette espèce endémique ne compte qu'une cinquantaine de couples (Weimerskirch et al. 2018; Heerah et al. 2019) et fait l'objet d'une attention particulière au niveau international. Un Plan National d'Actions (PNA) en faveur de sa sauvegarde a notamment été mis en place dès 2010 et renouvelé en 2018 pour une durée de 10 ans. L'ensemble de la population d'AMA se reproduit uniquement sur le plateau central de l'île et reste largement isolé des autres espèces. Les couples se reproduisent en moyenne une fois tous les 2 ans sur des nids espacés de plusieurs dizaines à plusieurs centaines de mètres les uns des autres. L'Albatros à nez jaune de l'océan indien *Thalassarche carteri* (IYNA, pour Indian Yellow-Nosed Albatross) est l'espèce d'oiseaux marins la plus abondante se reproduisant sur l'île. Abrisant 65% de la population mondiale, l'île Amsterdam est un site clé pour cette espèce (Delord et al. 2013; Jaeger et al. 2018), les autres sites de reproduction étant : les îles du Prince Edouard (<18% de la population), les îles Crozet (<17% de la population) et les îles Kerguelen (<1% de la population) (Birdlife international 2021). Les IYNA se reproduisent annuellement en colonies denses le long des falaises d'Entrecatseaux, en groupes de quelques dizaines de couples jusqu'à plusieurs milliers. La troisième espèce d'albatros qui se reproduit sur l'île est l'albatros fuligineux à dos sombre *Phoebastria fusca* (SOA, pour Sooty Albatross), comptant quelques centaines de couples et représentant environ 4% de la population mondiale (Heerah et al. 2019). Les SOA se reproduisent également le long des falaises, mais en plus petites densités, de quelques individus isolés à une dizaine d'individus. Les IYNA et SOA ne se reproduisent pas en colonies mixtes même si ces derniers sont souvent trouvés en périphérie des colonies d'IYNA, avec quelques nids parfois en contact rapproché.

L'île Amsterdam, avec sa voisine l'île Saint Paul, représente également l'unique site de reproduction dans l'océan indien du Gorfou sauteur du Nord *Eudyptes moseleyi* (NRP, pour Northern Rockhopper Penguin), avec environ 12.000 couples reproducteurs sur l'île Amsterdam et 7.500 sur l'île Saint Paul (Barbraud et al. 2020). Cette espèce, seule espèce de manchot se reproduisant sur ces îles, ne se trouve que sur 2 autres sites situés dans l'océan Atlantique sud, l'archipel de Tristan et l'île Gough (Robson et al. 2011). Les colonies de NRP se trouvent au pied des falaises et sont majoritairement monospécifiques, mais il est très fréquent de trouver des individus nichant entre les nids des IYNA dans les parties supérieures des colonies.

Le Labbe subantactique *Stercorarius antarcticus* (SKU, pour Brown Skua) est une espèce charognarde et prédatrice qui se reproduit également sur l'île. Environ 60 couples sont répertoriés, nichant essentiellement sur le Plateau des Tourbières, mais peu de données sont à ce jours accessibles concernant cette espèce sur l'île Amsterdam.

En plus d'être un site clé pour ces nombreuses espèces d'oiseaux marins, l'île abrite une large colonie d'otaries à fourrure subantarctiques *Arctocephalus tropicalis* (SFS, pour Subantarctic Fur Seal).

Depuis le début du XIX<sup>ème</sup> siècle plusieurs espèces exotiques ont été introduites sur l'île (Micol and Jouventin 1995). Certaines espèces comme des bovins, des porcs ou des poulets ont été importées pour des raisons alimentaires, d'autres tels que le Chat domestique (*Felis catus*) ou l'Astrild ondulé (*Estrilda astrild*) ont été apportées en tant qu'animaux de compagnie et sont revenues à l'état sauvage par la suite. Enfin certaines espèces commensales non souhaitées ont débarqué sur l'île, avec les bateaux, de façon accidentelle comme le Rat brun (*Rattus norvegicus*) ou la Souris domestique (*Mus musculus*). Après l'éradication de trois de ces espèces au cours des dernières décennies, à savoir les bovins, les porcs et les poulets, l'île abrite encore 3 espèces de mammifères introduites, les chats domestiques (ou chats haret), les rats bruns et les souris domestiques, ainsi que les astrilds ondulés.

#### La situation critique de l'île Amsterdam : un enjeu majeur de conservation

Au cours des dernières décennies la population d'IYNA n'a cessé de diminuer sur l'île Amsterdam, et alors que les falaises d'Entrecasteaux comptaient 37.000 couples au début des années 1980, elles n'en comptaient que 22.000 en 2015 (Weimerskirch, H., Jouventin 1998; Heerah et al. 2019). Les activités de pêche, connues comme étant l'une des menaces majeures pour les albatros, causant une mortalité importante chez les adultes, ont été en premier lieu mises en cause de ce déclin de la population. Cependant, aucun lien direct n'a pu être établi entre la survie adulte et les activités de pêcherie. Des études complémentaires ont suggéré que ce déclin pourrait être dû à des épizooties régulières tuant massivement les poussins de cette espèce (Weimerskirch 2004; Rolland et al. 2009). La bactérie *Pasteurella multocida* (*Pm*) (**Encadré 1**), et dans une moindre mesure *Erysipelothrix rhusiopathiae* (*Er*), ont notamment été suspectées d'être à l'origine de ces épidémies récurrentes chez les poussins d'IYNA depuis les années 1980, entraînant un faible succès de reproduction et une diminution de la population (Weimerskirch 2004).



**Figure 4** (ci-contre)

**Encadré du haut :** Répartition spatiale des espèces principales se reproduisant sur l'île Amsterdam. D'après la carte ©IGN de l'île Amsterdam au 1:50.000. Les surfaces colorées représentent les zones sur lesquelles se trouvent les sites connus de reproduction des différentes espèces.

EN (Endangered) : espèce classée sur liste rouge, En Danger selon les critères de l'UICN.

LC (Least concern) : espèce classée en Préoccupation Mineure selon les critères de l'UICN.

Vert : zone dans laquelle se situent toutes les colonies d'YNA et SOA. Les configurations du site et des colonies ne permettent pas de distinguer les différentes colonies, ni les 2 espèces. Cette surface abrite environ 22.000 couples d'YNA et 600 couples de SOA.

Violet : principales colonies de NRP, regroupant environ 12.000 couples reproducteurs.

Bleu : zone de reproduction des AMA, regroupant une cinquantaine de couples reproducteurs. Chaque nid est espacé de plusieurs dizaines, voire centaines de mètres. (Photo de l'AMA, ©Nicolas Giraud)

En orange : zone de reproduction des SKU. La zone plus foncée représente le secteur avec la plus forte densité de nids, regroupant une soixantaine de nids, dans lequel un suivi a récemment été mis en place. La zone claire représente le secteur dans lequel des nids ont été repérés mais de façon plus diffuse.

Les otaries subantarctiques sont présentes sur tout le pourtour de l'île. Plus de 30.000 individus se reproduisent chaque année, représentant environ 11% de la population mondiale (Guinet et al. 1994; Hofmeyr 2015).

Enfin, plusieurs autres espèces d'oiseaux marins, dont la sterne antarctique *Sterna vittata*, le Pétrel noir *Pterodroma macroptera*, le Pétrel gris *Procellaria cinerea*, ou encore l'Océanite frégate (*Pelagodroma marina*) ou le Puffin à pieds pales (*Puffinus carneipes*) se reproduisent probablement sur l'île Amsterdam, mais leur nombre et leur localisation sont mal connus (Duriez and Delord 2012).

**Encadré en pointillé :** 3 espèces de mammifères introduits sont présents sur l'île, le Chat haret, le Rat brun et la Souris domestique. Peu d'informations sont disponibles à ce jour quant à la population et la répartition de ces espèces. Les rats et les souris sont toutefois présents dans les colonies d'oiseaux (données non publiées) et semblent être présents sur l'ensemble de l'île (des côtes au plateau central). Les chats sont régulièrement observés près de la station scientifique et des indices de présence sont détectés sur le plateau central. Bien que souvent observés jusqu'au début des années 2000 près des colonies d'études sur les falaises d'Entrecasteaux, aucune observation de chat n'a été effectuée depuis sur ce secteur (données d'observations personnelles).

**Encadré du bas :**

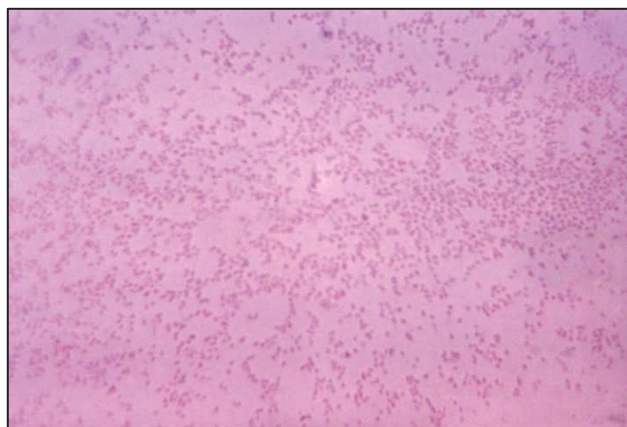
A – Exemple de cohabitation entre des couples d'YNA et de NRP. Les nids d'YNA surplombent les sites de reproduction des NRP mais la proximité entre les 2 espèces est très forte dans ces secteurs. Cette mixité ne se retrouve qu'en bordure de colonie, l'essentiel des colonies d'YNA et NRP étant monospécifiques.

B – Vue d'ensemble de la colonie de YNA dans laquelle est menée cette étude, environ 650 couples. Les sites de reproduction sont espacés de quelques mètres, la colonie est composée exclusivement d'YNA.

C – Exemple de proximité entre les SOA et les YNA. Les sites de reproduction de SOA sont indiqués par des ronds verts afin de mieux les visualiser. Certains sites de reproduction de SOA et YNA ne sont séparés que de quelques mètres. Les colonies de SOA ne sont composées que de quelques dizaines de couples maximum, réparties le long des falaises d'Entrecasteaux.

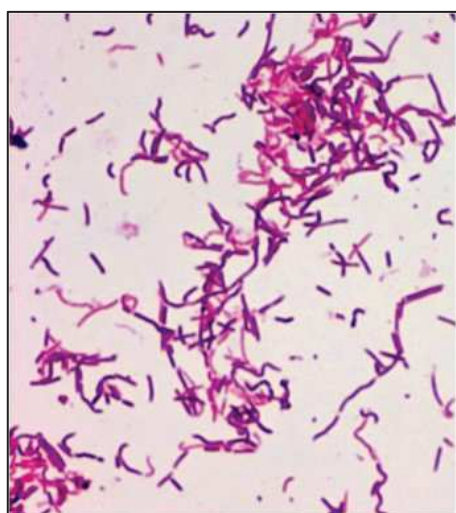


**Encadré 1** : Description de *Pasteurella multocida* et *Erysipelothrix rhusiopathiae*



***Pasteurella multocida*** est un coccobacille Gram négatif de la famille des Pasteurellaceae. Cette bactérie, agent responsable du choléra aviaire, est un commensal commun d'une très grande variété d'hôtes, sauvages, domestiques ou humains, mais est aussi un pathogène opportuniste hautement contagieux (Harper et al. 2006). Le choléra aviaire est une préoccupation majeure, particulièrement pour l'élevage de volailles mais également pour les élevages de bovins, porcins, ovins (entre autres) en raison de ses conséquences économiques mais aussi de santé publique (Harper et al. 2006). *Pm* est également un commensal très fréquent des félins, canins et rongeurs. La contamination par *Pm* peut se faire sous différentes formes : le contact direct entre les

hôtes, par des piqûres d'arthropodes ou de divers insectes, l'ingestion de carcasses ou de nourriture contaminées, d'eaux contaminées, voire même une contamination par aérosol dans les zones hautement contaminées (Friend and Franson 1999). Décrite chez plus de 180 espèces d'oiseaux (Samuel et al. 2007), elle constitue une préoccupation croissante pour la faune sauvage. Le choléra aviaire peut provoquer une mort rapide et peut tuer des milliers d'oiseaux en peu de temps (Samuel et al. 2007; Wilson and Ho 2013). En particulier, les changements globaux, les activités anthropiques et l'introduction d'espèces exotiques peuvent avoir joué un rôle dans la dissémination de l'agent pathogène chez les oiseaux sauvages, notamment dans les îles isolées (Weimerskirch 2004; Blanchong et al. 2006; Cooper et al. 2009; Bodenstern et al. 2015). Suite aux observations réalisées sur l'île d'Amsterdam, le choléra aviaire a été considéré comme la plus importante menace infectieuse pour les albatros (Phillips et al. 2016; Uhart et al. 2018). Elle peut par exemple, provoquer de graves épidémies chez les espèces de manchots (de Lisle et al. 1990; Cooper et al. 2009). Il est à noter que la bactérie est responsable d'infections chez l'homme, notamment lors de morsures de chiens ou chats (Crédit photo : Centers for Disease Control and Prevention, Office of the Associate Director for Communications, Division of Public Affairs, Dr. R. Weaver).



***Erysipelothrix rhusiopathiae*** est une bactérie Gram positif. Tout comme *Pm*, *Er* est un commensal commun mais aussi un pathogène opportuniste. Les volailles et le bétail sont particulièrement atteints mais *Er* est également décrit chez une très grande variété d'hôtes sauvages, y compris les oiseaux, les mammifères, les reptiles, les poissons et les arthropodes (Leighton 2008; Wang et al. 2010). De plus, *Er* a une large distribution géographique et a une longue persistance dans l'environnement (Brooke and Riley 1999). La bactérie représente donc un enjeu particulier sur le plan économique et sanitaire (Wood 1999). *Er*, connue pour provoquer de graves épidémies chez les volailles (Eriksson 2003) peut constituer une menace pour les oiseaux sauvages (Gartrell et al. 2005). Les voies de transmission d'*Er* ne sont pas bien documentées, notamment chez les oiseaux, mais il est suggéré que la contamination ai lieu via des abrasions cutanées, des plaies mais également via les muqueuses ou par des arthropodes (Brooke and Riley 1999; Chirico et al. 2003). Il est à noter aussi que cette bactérie est zoonotique, c'est-à-dire qu'elle peut infester l'homme et être responsable d'une maladie, le rouget. (Source image : Jones et al. 2019).

Des études plus récentes (Jaeger et al. 2018; Gamble et al. 2019; Jaeger et al. 2019; Barbraud et al. 2020) ont montré que la situation sur l'île d'Amsterdam s'est considérablement dégradée lors de la dernière décennie, avec des succès de reproduction extrêmement faibles accompagnés d'un déclin de la population non seulement chez les IYNA, mais aussi chez les 2 autres espèces nichant sur les falaises d'Entrecasteaux, avec une diminution de 17.4% entre 1994 et 2016 pour les SOA et de 79.8% entre 1972 et 2015 pour les NRP. Les travaux de Jaeger et al (2018), par l'isolement de *Pm* et la détection par PCR d'*Er*, confirment la suggestion antérieure d'un rôle de ces agents infectieux dans les mortalités massives de poussins des 2 espèces d'albatros, IYNA et SOA, mais peu d'éléments n'ont pu être apporté quant à leur rôle dans les mortalités chez les NRP. La caractérisation génétique des souches de *Pm* ayant été isolées sur quelques individus des 2 espèces d'albatros, durant 2 saisons de reproduction, suggère qu'il ne s'agit que d'une seule et même souche appartenant au Séquence Type (ST) ST61 dans le système RIRDC-MLST<sup>1</sup> (Jaeger et al. 2018). Ce ST, déjà présent dans la base de données génétique du système MLST (<https://pubmlst.org/organisms/pasteurella-multocida>) avait été reporté chez un canard au Danemark dans les années 1980 (Petersen et al. 2001). Cependant, bien que détectée par PCR, aucune souche de *Er* n'avait pu être isolée et donc identifiée.

Bien qu'il soit difficile de déterminer l'origine exacte de ces pathogènes, à cause de contraintes d'échantillonnage des populations sources probables et de la résolution des outils génétiques, ces épidémies ont été détectée de façon significative au cours des années 1980, laissant penser à une introduction ou une émergence récente. L'analyse par MLST, ayant conclu à une absence de diversité génétique des souches de *Pm* entre les individus, mais aussi entre les espèces hôtes (SOA et IYNA) et au cours du temps, suggère une introduction unique de *Pm* (Jaeger et al. 2018). La souche ST61 ayant été originellement reportée en Europe chez des espèces domestiques, une introduction via les espèces non-natives, connues pour être des porteuses communes de *Pm* comme de *Er* (voir encadré pour plus d'information sur ces agents pathogènes), est une des hypothèses principalement mise en avant (Jaeger et al. 2018). En l'absence d'informations plus détaillées pour *Er*, il est néanmoins difficile d'en déduire l'origine. La description d'épisodes épizootiques associés à *Pm* ayant affecté des populations d'oiseaux marins dans d'autres sites de l'hémisphère sud, notamment en Antarctique et subantarctique (Leotta et al. 2006; Cooper et al. 2009), montre néanmoins que l'origine du ou des

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<sup>1</sup> La RIRDC MLST (Rural Industries Research and Development Corporation MultiLocus Sequence Typing) est basée sur le séquençage de 7 gènes de ménage. L'ensemble des séquences obtenues pour une souche donnée correspond à un profil caractéristique représenté par un Séquence Type (ST). Le schéma RIRDC MLST, spécialement développé pour *Pm*, a été développé initialement pour étudier les isolats aviaires (<https://pubmlst.org/organisms/pasteurella-multocida>)

agents responsables des épizooties sur l'île d'Amsterdam n'est pas claire. Comme observé dans des élevages de volailles (Singh et al. 2013; Omaleki et al. 2020), plusieurs agents, concomitant ou se succédant, pourraient être responsables de ces épidémies.

La situation sur l'île Amsterdam est critique et constitue un enjeu majeur pour la conservation. Les épidémies menacent plus de la moitié de la population mondiale d'IYNA. Si la population d'AMA ne semble pas être affectée par des épidémies annuelles avec des mortalités massives et récurrentes (Jaeger et al. 2018), la présence de cette menace sur l'île est un enjeu majeur pour cette espèce. Si celle-ci venait à être affectée, la petite population de quelques dizaines de couples pourrait connaître un déclin, pouvant mener à son extinction à l'échelle mondiale. De plus, même si aucune piste n'avait jusqu'à récemment pu être explorée concernant les mortalités chez les NRP, l'hypothèse de l'implication de *Pm* ou *Er* reste forte. La population de NRP de l'île Saint Paul reste quant à elle en augmentation (Barbraud et al. 2020). Cependant, si les épidémies venaient à toucher cette colonie, l'ensemble de la population de l'océan indien serait alors affectée.

La présence de tels pathogènes est donc un danger pour de nombreuses espèces emblématiques, non seulement au sein de l'île Amsterdam, mais aussi à une échelle plus large.

### **Des essais de vaccination prometteurs**

La conservation des espèces est un des enjeux majeurs du plan de gestion de la RNN-TAF. De nombreuses mesures concernant les pêcheries, l'éradication d'espèces introduites ou encore la protection de l'habitat ont notamment été mises en place afin de limiter la mortalité des oiseaux marins. Face au constat alarmant du déclin des populations sur l'île d'Amsterdam, une réflexion a été menée sur les mesures pouvant être apportées afin de réduire les mortalités massives dues aux épidémies récurrentes. La vaccination est l'une des mesures de prévention très largement utilisées dans le monde de l'élevage (OIE 2012), afin de lutter contre les épidémies, qui ont des conséquences désastreuses sur le plan économique et sanitaire. De plus, la vaccination est considérée comme l'une des approches les plus courantes pour limiter l'impact des épizooties mortelles chez les animaux sauvages (Groner et al. 2016).

Cependant, la disponibilité de vaccins combinant à la fois innocuité et efficacité, contre les agents pathogènes de la faune sauvage, est limitée. La vaccination contre *Pm* et *Er* est néanmoins très courante dans les élevages (OIE 2012) et son efficacité sur de nombreuses espèces suggère que celle-ci pourrait être une solution adaptée pour certaines populations sauvages menacées. Outre le cas d'une petite population résiduelle élevée pour partie en captivité comme dans le cas du kakapo en

Nouvelle-Zélande (Gartrell et al. 2005; Livingston et al. 2013), l'utilisation d'une telle approche chez des oiseaux marins touchés par des épizooties, et en particulier des albatros, est tout particulièrement intéressant de par leur traits histoire de vie, qui permettent d'envisager une couverture vaccinale sur le terrain qui pourrait concerner une grande proportion d'individus sensibles : forte fidélité à leur site de reproduction où ils peuvent être vaccinés, forte longévité, et éventuellement forte persistance des anticorps maternels, susceptibles de pouvoir permettre une protection des jeunes plusieurs semaines après l'éclosion (Garnier et al. 2012). L'utilisation d'autovaccins, vaccins « sur mesure » préparés à partir de bactéries pathogènes isolées d'un sujet malade ou sain de la population concernée, est notamment très répandue contre le choléra aviaire dans l'industrie de la production de volailles (Giese 2016) et pourrait être une solution.

Depuis plusieurs années à présent, notre équipe de recherche évalue l'utilisation d'un autovaccin pour protéger les poussins d'YNA (espèce historiquement identifiée comme affectée par les épizooties) (Bourret et al. 2018; Gamble et al. 2019). Cet autovaccin a été développé par l'entreprise Ceva Biovac<sup>2</sup> à partir de l'une des souches isolées et caractérisées dans Jaeger et al (2018) et nommée D2C. Comme aucune souche d'*Er* n'avait pu être isolée et *Pm* étant suspectée être responsable d'une grande part des mortalités (Weimerskirch 2004; Jaeger et al. 2018), des premiers essais de vaccinations contre *Pm* sur des YNA ont été réalisés dès 2013, puis menés sur plusieurs années (Bourret et al. 2018; Gamble et al. 2019) (**Encadré 2**).

Les jeunes oiseaux étant apparus comme les plus touchés par les mortalités (Weimerskirch 2004; Jaeger et al. 2018) il était donc crucial de pouvoir les protéger au plus tôt. Deux approches expérimentales ont été ainsi évaluées afin de permettre une protection optimale des poussins :

- Une vaccination directe des poussins, dans les premiers jours suivant l'éclosion afin de les protéger au plus tôt. La vaccination directe des individus est la méthode la plus utilisée dans l'industrie de la volaille afin de lutter contre de potentielles épidémies impactant lourdement les élevages (OIE 2012). Elle est également possible avec de la faune sauvage et est parfois utilisée dans le cadre de mesures de conservation (e.g., Gartrell et al. 2005).
- Une protection suite à vaccination de leur mère. Le transfert d'anticorps maternels au poussin est un phénomène qui a été largement décrit chez des espèces d'élevage (Kowalczyk et al.

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<sup>2</sup> L'entreprise Ceva Biovac est spécialisée dans le développement d'autovaccin pour les animaux d'élevages, en particulier pour la volaille. Elle produit notamment des autovaccins contre *Pasteurella multocida*.

1985; Li et al. 1998) et a fait l'objet d'attentions particulières chez les oiseaux marins (e.g., Gasparini et al. 2002). Une étude menée notamment chez le puffin cendré, une espèce de Procellariiforme (Garnier et al. 2012), ordre auquel appartiennent les albatros, a montré que les anticorps maternels pouvaient persister plusieurs mois après l'éclosion chez les jeunes de cette espèce et pourraient ainsi conférer une protection au poussin lors de ses premiers mois. Contrairement à la première méthode qui impliquerait des campagnes annuelles de vaccination à large échelle de plusieurs milliers de poussins, cette seconde méthode offrirait l'avantage de pouvoir protéger plusieurs générations de poussins pour une seule mère vaccinée (Garnier et al. 2012). Cependant, cette méthode ne peut être efficace que si la persistance interannuelle des anticorps chez la mère est suffisante.

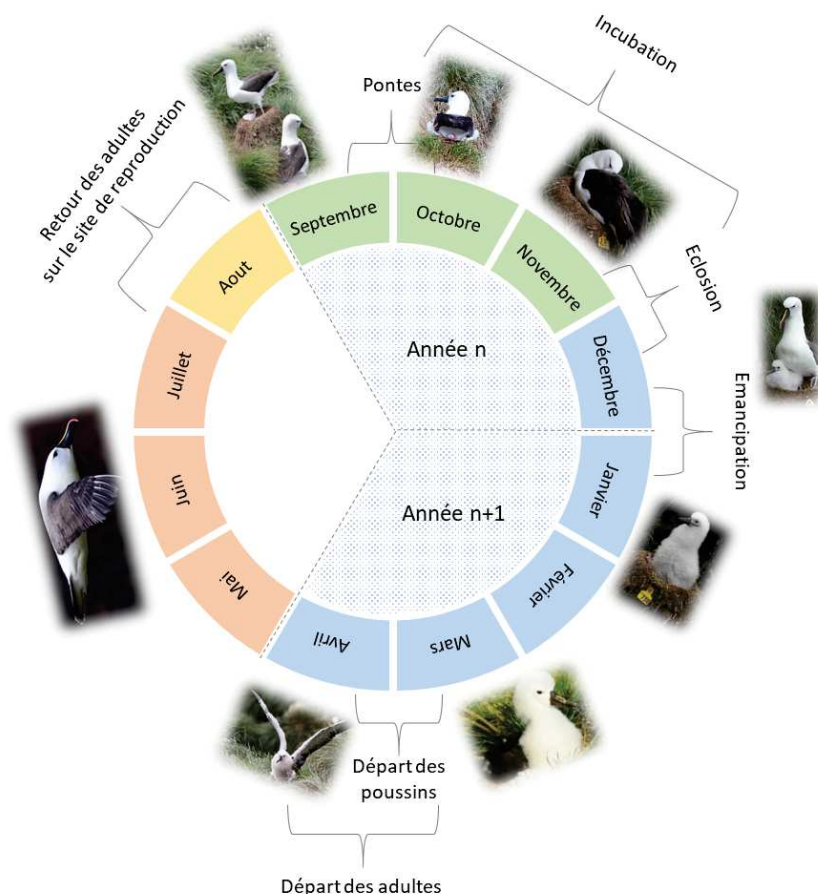
Une première étude de l'équipe (Bourret et al. 2018), menée sur 3 saisons de reproduction (2013-2014<sup>3</sup>, 2014-2015 et 2015-2016) a permis l'obtention de résultats encourageants sur l'effet de la vaccination sur les poussins d'IYNA (**Figure 5**). Les travaux montrent notamment qu'une vaccination des poussins à l'âge de 14 jours (+/-3 jours) leur permet de développer une réponse immunitaire et un effet protecteur, avec un risque de mortalité divisé par 2.4 comparé à des individus non vaccinés. Cependant, les travaux ont également mis en évidence qu'une vaccination plus précoce, à l'âge de 2 jours, ne permet pas de développer de réponse immunitaire et donc d'effet protecteur.

Les premiers travaux sur la protection via un transfert d'anticorps maternel dans ce système (Gamble et al. 2019, annexe 4) montrent que la vaccination des femelles induit bien une réponse immunitaire rapide des individus, avec des taux d'anticorps élevés quelques semaines après l'injection, mais le suivi annuel de ces individus a mis en évidence une faible persistance des taux d'anticorps dans le temps, avec des taux significativement diminués une année après la vaccination. Dans ce cas, cela ne permet pas le transfert d'une quantité suffisante d'anticorps au poussin pour offrir une protection. Ce résultat bien que décevant n'est pas surprenant, l'immunité naturelle pour le choléra aviaire n'est pas connu pour persister longtemps (Samuel et al. 1997) et la plupart des autovaccins sont produit essentiellement pour des espèces d'élevage, ayant une durée de vie relativement courte, ils ne sont pas spécifiquement conçus pour maintenir un taux d'anticorps élevé et donc une protection sur le moyen-long terme.

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<sup>3</sup> La saison de reproduction des IYNA commence en septembre de l'année n et finit en avril de l'année n+1. Une saison de reproduction sera donc toujours présentée sous le format saison n - n+1

L'entreprise Ceva Biovac a produit une nouvelle formulation du vaccin, dite 'water in oil', afin d'induire une réponse en anticorps élevée et plus durable après une seule immunisation (Jansen et al. 2005), il a ainsi été envisagé d'utiliser cette nouvelle formulation du vaccin dans le cas des albatros. Des résultats préliminaires suggèrent que des adultes ayant reçu une injection avec la première formulation et recevant une seconde injection avec la nouvelle formulation 2 ans après montrent une forte réponse immunitaire, avec notamment une meilleure persistance des anticorps une année après la seconde injection (Gamble et al. 2019). Cependant, ce test préliminaire, ne concernant que peu d'individus et confondant les effets d'un rappel et de la nouvelle formulation, ne permet pas de conclure si cette réponse est due au changement de formulation, à l'effet rappel d'une seconde injection ou, plus probablement, un effet combiné des 2 facteurs. De même, la faible taille d'échantillon n'a pas permis d'en tester le potentiel effet protecteur.



**Figure 5:** Cycle de reproduction annuel des IYNA. (Crédits photo Marine Bely, Augustin Clessin et Jérémy Tornos)

## Les enjeux et problématiques abordées dans la thèse

Les résultats des précédentes études ont apporté de nouveaux éléments afin de mieux comprendre la situation épidémiologique sur l'île Amsterdam et ont mis en évidence des solutions encourageantes. L'isolement d'une souche de *Pm* (Jaeger et al. 2018) a notamment permis de produire un autovaccin qui a nettement montré un effet protecteur sur le terrain dans certaines conditions (Bourret et al. 2018). Cependant, de nombreux points sont encore à approfondir afin d'ajuster le protocole de vaccination, mais également de mieux appréhender et décrire la situation éco-épidémiologique sur l'île. Les travaux de cette thèse vont aborder à la fois des questions appliquées sur l'optimisation de la vaccination mais aussi de questions plus fondamentales sur les processus éco-épidémiologiques sur l'île Amsterdam, développés dans trois axes :

- Le premier axe de cette thèse va explorer la diversité potentielle des agents infectieux présents sur l'île Amsterdam. Il est, en effet crucial, de pouvoir identifier si un seul pathogène ou une diversité d'agents infectieux sont impliqués dans les mortalités massives. L'identification de nouveaux agents pourraient notamment permettre de mettre à jour la composition du vaccin, et fournirait de plus, des éléments essentiels quant à leur potentielle origine.
- Le deuxième axe se concentrera sur l'optimisation du protocole de vaccination. Nous allons en particulier évaluer l'effet de l'âge de vaccination sur les poussins afin d'évaluer des stratégies possibles pour la mise en place d'une vaccination à grande échelle. De plus, nous allons évaluer les effets de la nouvelle formulation<sup>4</sup> du vaccin sur la persistance des anticorps chez les femelles et plus particulièrement sur le possible transfert chez les jeunes et l'effet sur leur survie. Enfin, nous allons tester un nouveau vaccin dont la composition<sup>5</sup> a été mise à jour suite à la découverte d'une nouvelle souche de *Pm*.
- Le troisième axe de recherche va explorer les mécanismes de circulation des agents infectieux entre et au sein des différentes espèces de l'île. L'obtention d'un vaccin et d'un protocole de vaccination est une étape importante d'une approche intégrée de la conservation, et l'acquisition d'éléments sur les mécanismes de circulation des agents infectieux est nécessaire pour comprendre et prédire les effets possibles et intérêt de de campagnes de vaccination à grande échelles. En particulier,

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<sup>4</sup> La formulation du vaccin concerne les ingrédients composants les adjuvants du vaccin et la méthode de production du vaccin. La nouvelle formulation est ici dite 'water in oil'.

<sup>5</sup> La composition du vaccin concerne les antigènes utilisés pour produire le vaccin. Le vaccin utilisé historiquement contient les antigènes de la souche D2C uniquement.

cet axe étudiera le rôle des individus de différentes espèces comme vecteurs potentiels de transmission à différentes échelles.

**Encadré 2** : Résumé des résultats de Bourret et al (2018) et Gamble et al (2019)

Les premiers essais de vaccination ont été effectués lors de la saison 2013-2014. Cinquante poussins ont été vaccinés à l'âge de 2 jours après l'éclosion afin de tester l'effet de la vaccination directe sur leur survie. Les 2 partenaires de 50 couples d'adultes reproducteurs ont également été vaccinés (car il était impossible de différencier les 2 sexes sur le terrain) afin d'évaluer la possibilité d'un transfert d'anticorps maternel vers le poussin via les œufs pondus les années suivantes et son potentiel rôle protecteur. Cependant, cette saison 2013-2014 a connu le plus haut succès de reproduction enregistré depuis 1997 (Jaeger et al. 2018) et les analyses n'ont montré qu'une très faible exposition des IYNA, adultes ou poussins, à *Pm*. Ceci suggérerait qu'aucune épidémie de grande ampleur n'avait pas eu lieu lors de cette saison, ne permettant pas de détecter d'effet protecteur de la vaccination (Bourret et al. 2018). Les adultes vaccinés ont montré quant à eux une réponse immunitaire suite à la vaccination (Gamble et al. 2019). Le potentiel transfert d'anticorps maternel aux poussins et son effet sur leur survie n'a pu être mesuré que lors de la saison de reproduction suivante.

Il est à noter que la méthode d'analyse sérologique n'était pas disponible les 2 premières années de l'étude, l'absence de réponse immunitaire n'avait pas pu être mesurée suite à la première année de l'essai, le protocole n'avait donc pas pu être mis à jour pour l'année suivante.

Suivant le même protocole, une nouvelle cohorte de poussins a été vaccinée lors de la saison 2014-2015. Les analyses ont suggéré cette fois une plus grande exposition à *Pm*, associée à une très forte mortalité des jeunes (Jaeger et al. 2018). Cependant, les analyses sérologiques n'ont montré aucune réponse immunitaire contre *Pm* chez les poussins vaccinés et de ce fait aucun effet positif de la vaccination n'a pu être détecté (Bourret et al. 2018). L'hypothèse d'une vaccination trop précoce a été soulevée. Le système immunitaire des poussins, ayant reçu une injection du vaccin 2 jours après l'éclosion, était probablement encore immature et incapable d'organiser une réponse protectrice (Mast and Goddeeris 1999). De plus, les taux d'anticorps mesurés chez les mères une année après la vaccination se sont révélés faibles, ne permettant pas un transfert d'anticorps suffisant pour obtenir un effet protecteur des jeunes (Gamble et al. 2019).

Suite à l'hypothèse d'une vaccination trop précoce pour les poussins, une nouvelle cohorte de poussins a reçu une injection du vaccin à l'âge de 14 jours (+/- 3 jours) lors de la saison 2015-2016. La vaccination plus tardive a cette fois permis une réponse immunitaire des poussins et, plus important encore, un effet protecteur (Bourret et al. 2018). Les analyses sérologiques contre *Pm* ont montré que peu des adultes montraient naturellement une réponse à *Pm*, ce qui peut suggérer qu'ils sont peu exposés à l'infection par la bactérie, du fait de leur présence sur la colonie seulement jusqu'au mois de Janvier, quand les poussins sont assez grands pour être laissés seuls sur le nid, et que les fortes mortalités n'ont pas encore lieu. Ces analyses faites sur les adultes vaccinés ont permis de montrer qu'ils répondaient fortement au vaccin dans les 15 jours suivant l'injection, puis suite à une injection de la nouvelle formulation du vaccin, mais qu'en revanche le taux d'anticorps ne persistait pas plus d'un an après une simple injection de la première formulation du vaccin (Gamble et al. 2019).





## Matériels et méthodes





## Procédures de terrain

Des mesures de biosécurité rigoureuses ont été respectées sur le terrain afin d'éviter la propagation de pathogènes parmi les colonies d'oiseaux mais aussi pour protéger le personnel contre toute infection potentielle. Cela repose notamment sur l'utilisation de vêtements spécifiques : pantalons imperméables, vestes et bottes en caoutchouc dédiées à chaque colonie d'oiseaux, port de masques et de gants lorsque nécessaire. Tout l'équipement ainsi que la tenue sont désinfectés après chaque manipulation ou expérimentation et à chaque fin de journée. Les gants, les masques et autre équipement jetable sont placés dans des poubelles dédiées. Toutes les autopsies ont été réalisées *in situ*, au plus proche du lieu de découverte de la carcasse, afin d'éviter la propagation de tout agent pathogène. Après les autopsies, les carcasses ont été laissées sur place.

## Catégorisation des agents infectieux

(Voir Annexe 1 pour plus de détails)

Un protocole de terrain spécifique et adapté aux conditions particulières du site d'étude a été développé avec l'aide de partenaires spécialisés dans le domaine. D'une part, le personnel de terrain a reçu une formation adaptée, élaborée en collaboration avec le Laboratoire Départemental Vétérinaire de l'Hérault (Nicolas Keck) et le réseau SAGIR (réseau de surveillance des maladies infectieuses des oiseaux et des mammifères sauvages terrestres, Office Français de la Biodiversité), en coordination avec Karine Lemberger et Rozenn Le Net du Laboratoire Vet Diagnostics, et Célia Lesage de la RNN-TAF, afin de réaliser des autopsies *in situ* et répondant spécifiquement aux conditions du terrain. D'autre part, l'entreprise Ceva Biovac a élaboré en collaboration avec l'équipe du CEFÉ un protocole de bactériologie adapté aux contraintes locales. Le personnel de terrain a notamment été formé à la culture bactérienne mais aussi à la reconnaissance des souches, tout particulièrement *Pm* et *Er*.

## Recherche de carcasses

Au cours de la saison de reproduction 2018/2019, une recherche active des carcasses a été mise en place dans les colonies d'étude d'IYNA et de SOA sur les falaises d'Entrecasteaux. Durant l'année 2019/2020, en plus de ces colonies, des efforts supplémentaires de recherche de carcasses de NRP ont été menés dans différentes colonies mais également sur les chemins d'accès entre la mer leurs colonies de reproduction.

### Échantillonnage des organes et diagnostic histologique

Après un examen externe et visuel, un diagnostic interne et un échantillonnage sur différents organes a été effectué. Autant que possible, 9 organes ont été prélevés et conservés dans du formaldéhyde à 4% : foie, cœur, poumons, rate, rein, thyroïdes, thymus, bourse de Fabricius et cerveau. Ces organes ont été spécifiquement ciblés car ils peuvent fournir des informations importantes sur une éventuelle infection. Tout autre organe présentant un aspect anormal (par exemple couleur, texture ou forme inhabituelles) a été de plus prélevé. Les échantillons ont été conservés à température ambiante jusqu'à leur expédition en France métropolitaine, entre 2 et 6 mois, selon les escales du navire.

Les échantillons destinés à l'histologie ont été sectionnés en coupes de 4 µm et colorés à l'hématoxyline et à l'éosine, puis examinés au microscope.

### Isolement bactérien

Lorsque cela était possible, un écouvillonnage a été effectué sur 4 organes cibles : poumon, foie, cœur et cerveau. Lorsque seul le squelette était trouvé, un écouvillonnage de la moelle osseuse du fémur ou du tibio-tarse a été effectué. Pour chaque organe, un écouvillon stérile a été brossé dans une partie interne et non contaminée de l'organe et directement stocké dans un milieu de conservation (eSwab media, Copan, Italia). Après chaque journée, tous les échantillons ont étéensemencés sur une gélose au sang (gélose de culture complétée par 5% de sang de mouton défibriné ; BIO-RAD, Hercules, Californie, USA). Le site n'étant pas équipé d'un système électrique suffisamment puissant, l'incubation n'a pas pu être réalisée dans un incubateur dans des conditions standards (37°C pendant 12-24h). Les géloses ont donc été incubées à température ambiante (~15°C) et vérifiées régulièrement jusqu'à l'observation de croissance de colonies bactériennes (entre 24 et 72h). Sur chaque gélose et pour chaque organe, toutes les colonies bactériennes ont été différenciées visuellement. Pour chaque organe, 1 ou 2 colonies de l'agent pathogène d'intérêt (en évitant les contaminants) ont été prélevées et stockées dans un milieu de conservation (Stock Culture Agar ; BIO-RAD, Hercules, California, USA). Les isolats dans les milieux de conservation ont été stockés à température ambiante jusqu'à leur expédition en France métropolitaine, de 2 à 6 mois, en fonction des escales du bateau.

### Caractérisation bactérienne

Après avoir été expédiés en France métropolitaine, les isolats ont été ensemencés sur une gélose au sang. Une sélection de colonies a ensuite été caractérisée par spectrométrie de masse (MALDI-TOF). Tous les agents pathogènes d'intérêt ont ensuite été conservés pour une caractérisation génotypique ultérieure (voir Annexe 1 pour les détails de la caractérisation génotypique).

### **Vaccination**

(voir annexe 2 pour plus de détails)

Deux expériences ont été mises en œuvre en parallèle, la première visait à évaluer la possibilité d'un transfert d'anticorps maternels et la protection potentielle du poussin pendant les premiers jours après l'éclosion. La seconde expérience visait à confirmer la protection des poussins via leur vaccination directe, comme décrit dans (Bourret et al. 2018), et à comparer deux âges de vaccination afin d'affiner l'âge ou la date idéale de vaccination. Il était aussi prévu de modifier la composition du vaccin si différentes souches de *Pm* étaient isolées à partir de carcasses d'oiseaux.

### Vaccination des adultes et transfert des anticorps maternels

110 couples d'adultes reproducteurs composant trois groupes ont été sélectionnés aléatoirement au sein de la colonie d'étude après la ponte fin novembre 2017. Le premier groupe d'adultes, '*Pm*-injected', était composé de 50 couples qui ont été vaccinés par voie sous-cutanée avec 0,5ml de vaccin autologue contre la souche D2C. Deux groupes contrôle ont été sélectionnés pour comparer leur survie avec celle des adultes '*Pm*-injected'. Le premier groupe contrôle, '*NaCl*-injected', était composé de 30 couples ayant reçu une injection de 0,5 ml de solution de chlorure de sodium (NaCl 0,9 %) et ayant connu les mêmes conditions de manipulation que les adultes '*Pm*-injected'. Le second groupe contrôle '*unmanipulated*', était composé de 30 couples dont les adultes et les poussins ont été strictement non manipulés afin d'évaluer tout effet indésirable de la vaccination ou de la manipulation. Les nids de tous les groupes ont été aléatoirement sélectionnés et repartis spatialement dans la colonie d'étude. Les individus adultes, sauf '*unmanipulated*', ont été capturés et échantillonnés avant l'injection pour mesurer leur niveau basal en anticorps puis annuellement pour le titrage des anticorps au cours du temps. Les poussins ont été manipulés et prélevés pour chaque couple, sauf '*unmanipulated*', pendant les saisons de reproduction 2018-2019 et 2019-2020 (respectivement un et deux ans après l'injection des deux parents) à environ 5, 10, 15, 30, 60 et 90 jours après éclosion pour mesurer la dynamique du

taux d'anticorps dans le sang. La survie des poussins a été évaluée régulièrement pendant la saison de reproduction, jusqu'à l'envol fin mars.

### Vaccination des poussins

Lors de la saison de reproduction 2017-2018, 45 poussins sélectionnés aléatoirement et spatialement répartis dans la colonie d'étude ont été vaccinés par voie sous-cutanée avec 0,5ml de vaccin contre la souche D2C : 22 à l'âge de 7 jours et 23 poussins à l'âge de 12 jours. Lors de la saison de reproduction 2018-2019, suivant le même design expérimental, 43 poussins ont été injectés par voie sous-cutanée avec 0,5ml de vaccin contre la souche D2C : 21 à l'âge de 7 jours et 22 poussins à 12 jours. Au cours de la saison 2019-2020, 50 poussins ont été injectés par voie sous-cutanée avec 0,5ml de vaccin à l'âge de 10 jours : 15 avec le vaccin contre la souche D2C, 15 avec le vaccin contre la souche A18Y007B1 (identifiée lors de la précédente saison) et 20 avec le vaccin bivalent contre les deux souches D2C et A18Y007B1.

### **Analyses sérologiques**

Les analyses ont été conduites par le laboratoire Ceva Biovac avec la technique de Micro Agglutination (méthode SEROPAST®, Ceva Biovac, France). Les titres d'anticorps mesurés correspondent à la dernière dilution pour laquelle une réaction positive a été observée. Les résultats sont exprimés sous forme de titres  $\log_2[\text{dernière dilution positive}/10] + 1$  (comme pour Gamble et al. 2019). Une analyse ANOVA à mesures répétées a été utilisée pour comparer les profils de séroconversion.

### **Analyse de la survie**

(voir annexe 2 pour plus de détails)

Toutes les analyses de survie ont été réalisées sur R (R Core Team 2020).

La survie des poussins des différents groupes a été suivie sur trois saisons de reproduction 2017-2018, 2018-2019 et 2019-2020. Comme dans Bourret et al. (2018), pour les saisons de reproduction 2017/2018 et 2018/2019, les poussins non manipulés et les poussins manipulés mais non injectés ont été utilisés comme groupes témoins. Pour la saison de reproduction 2019-2020, pour des raisons logistiques et compte tenu des résultats des précédentes saisons ne montrant aucun effet de la manipulation, seuls les poussins manipulés ont été utilisés comme groupe témoin. La survie des poussins en fonction du traitement, sur une saison de reproduction donnée, a été comparée à l'aide

d'un modèle Cox Hazard Ratio (Andersen & Gill 1982) suivant '*survie ~ traitement*' tel qu'implémenté dans le package 'survival' (Therneau 2020). Les graphiques ont été créés à l'aide du paquet 'survminer' (Kassambara et al. 2020) et la p-value des modèles a été calculée à l'aide de la méthode Long-Rank.

La survie des poussins vaccinés a été évaluée selon 2 méthodes. La première méthode, comparable à l'analyse de survie de Bourret et al. (2018), exclue tous les poussins morts avant d'avoir atteint l'âge de la vaccination. Dans une seconde analyse de survie, nous avons considéré tous les poussins morts de l'éclosion à l'envol.

Pour évaluer la survie des poussins issus de parents vaccinés, nous n'avons considéré que ceux pour lesquels les deux parents injectés sont restés et se sont reproduits ensemble l'année correspondante.

## Etude des déplacements

### Équipement GPS des IYNA

(voir Annexes 3 et 4 pour plus de détails)

L'étude de des déplacement des individus pré-reproducteurs ou en échec de reproduction est particulièrement difficile car elle concerne des individus qui ne sont potentiellement pas ou plus attachés à un site de reproduction et donc difficile à capturer et surtout à recapter. De plus les individus pré-reproducteurs sont particulièrement difficiles à trouver (dû à la faible production de jeunes lors des dernières décennies Jaeger et al 2018). Ce travail n'a donc été possible qu'à l'aide d'un suivi à long terme rigoureux de la population d'étude. Le statut reproducteur des individus a été suivi de façon très régulière afin de connaître les dates et stades d'échec. Les individus pré-reproducteurs sont tous d'âge connu (bagués au stade poussin), âgés entre 4 et 6 ans (aucun individu de moins de 4 ans n'a été observé), et n'ayant jamais été vu en reproduction auparavant. Afin d'optimiser les chances d'obtenir les données, des enregistreurs GPS ne nécessitant pas de recapture des individus ont été utilisés (GPS-UHF Harrier-L<sup>®</sup> et Kite-L<sup>®</sup>, Ecotone). Ces balises à permettent notamment une transmission des données sur une antenne réceptrice même si les individus sont distants de quelques centaines de mètres de celle-ci ou lors de leur survol de la colonie.

Etant plus facilement capturables, les individus en succès de reproduction ont été équipés d'enregistreurs GPS sans transmission des données distante (i-GotU GT-600, Mobile Action Technology, Taiwan), nécessitant donc une recapture de l'individu afin d'obtenir les données.

Les 2 catégories de GPS ont été programmés avec une fréquence d'acquisition de 5 minutes (avec arrêt durant les horaires nocturnes afin d'optimiser l'autonomie de la batterie). Les GPS ont été fixés aux



plumes du dos avec du ruban adhésif Tesa®. Les individus en succès de reproduction ont été recapturés 8 à 15 jours après le déploiement pour récupérer les enregistreurs et accéder aux données.

Au total, 65 oiseaux ont été équipés au cours de trois saisons de reproduction 2017-2018, 2018-2019 et 2019-2020, 20 individus en succès de reproduction, 30 en échec de reproduction et 15 pré-reproducteurs.

Les analyses statistiques réalisées pour l'étude des déplacements de prospection et des déplacements en mer sont décrites respectivement dans les annexes 3 et 4.

### Équipement GPS des SKU

(voir Annexes 5 et 6 pour plus de détails)

Lors des saisons 2015-2016 et 2016-2017, 18 individus reproducteurs ont été capturés sur le Plateau des Tourbières et équipés de GPS à transmission UHF (GPS-UHF Harrier-L®, Ecotone). Les GPS ont été programmés avec une fréquence d'acquisition de 2 à 5 min.

Lors des saisons 2017-2018, 2018-2019 et 2019-2020 des individus, au statut reproducteur inconnu, ont été capturés et équipés de GPS-UHF sur des zones de repos sur 2 secteurs de l'île : une zone de repos au pied des falaises sur la plage d'Entrecasteaux et une zone de repos au nord de l'île (appelée Mare aux éléphant). La capture d'individus sur les zones de repos permet notamment d'équiper des individus non reproducteurs. Les GPS ont été programmés avec une fréquence d'acquisition de 5 minutes (avec arrêt durant les horaires nocturnes afin d'optimiser l'autonomie de la batterie). Les GPS ont été fixés aux plumes du dos avec du ruban adhésif Tesa®.

Les analyses statistiques réalisées pour l'étude des déplacements des SKU sont décrites dans les annexes 5 et 6.

### **Contributions au projet**

Mes contributions dans le cadre de la thèse, mais également dans le projet global dans le cadre duquel cette thèse a été menée, ont été variées. Le travail que j'ai effectué a reposé sur une combinaison d'approches de terrain, d'analyses de laboratoire et d'analyses de données. J'ai été impliqué dans tout cela, avec l'aide de différents interlocuteurs du projet IPEV ECOPATH 1151 (Thierry Boulinier, Amandine Gamble) et d'équipes avec lesquelles nous collaborons. Pour le travail de thèse, j'ai effectué 3 missions de terrain dans les terres australes au cours des mois de novembre et décembre 2017, 2018 et 2019. A ces occasions, j'ai contribué en amont des missions et sur le terrain à la formation des personnels techniques (Volontaires Service Civil) du projet IPEV ECOPATH 1151 qui se sont succédés

pour des hivernages sur l'île d'Amsterdam ces années-là : Marine Bely (2017-2018), Augustin Clessin (2018-2019) et Aline Fléchet (2019-2020). Avant les départs sur le terrain, nous avons assuré la formation du personnel de terrain en nécropsie, microbiologie de terrain et déploiement et utilisation de GPS pour suivre les déplacements des oiseaux. Les formations aux nécropsies de terrain ont été effectuées en coordination avec Nicolas Keck, le responsable du Laboratoire Départemental Vétérinaire de Montpellier, Karine Lemberger et Rozenn Le Net du Laboratoire Vet Diagnostics, et Célia Lesage de la RNN-TAF. Sur le terrain, j'ai pu former le personnel aux différentes techniques utilisées, suivi de population, manipulation, prise de sang et prélèvements biologiques sur les différentes espèces manipulées. Chaque année, les discussions avec Hubert Gantelet de CEVA BIOVAC ont permis de déterminer les conditions de production des autovaccins en amont du terrain et de prévoir, suite au terrain, les analyses sérologiques anti-*Pasteurella multocida* (MAT, méthode SEROPAST, effectué par CEVA BIOVAC) et les caractérisations des isolats de bactéries. Les échantillons congelés sont organisés et gérés via le logiciel Collec-Science, avec l'aide des Plateformes Systèmes d'Information en Ecologie (Marie-Claude Quido) et Programmes à Long Terme (Christophe De Franceschi). J'ai réalisé les analyses statistiques sous R. Notre expertise nous a également amené à participer à l'expédition menée sur l'île aux Cochons (archipel de Crozet) en novembre 2019. Une équipe de scientifiques dont je faisais partie, a été envoyée sur place afin d'enquêter sur le déclin massif de la population de Manchots royaux (*Aptenodytes patagonicus*) de cette île (une baisse de 88% sur les 35 dernières années). L'une des hypothèses explorées est notamment l'émergence d'un ou plusieurs agents infectieux. Des prélèvements biologiques ont été effectués sur des espèces sentinelles afin de détecter par sérologie ou PCR des indices de présences de potentiels agents infectieux. Comme indiqué dans la discussion de la thèse, les premiers résultats obtenus montrent qu'une grande proportion de ces oiseaux ont été notamment exposés à l'agent du choléra aviaire.



## Résultats





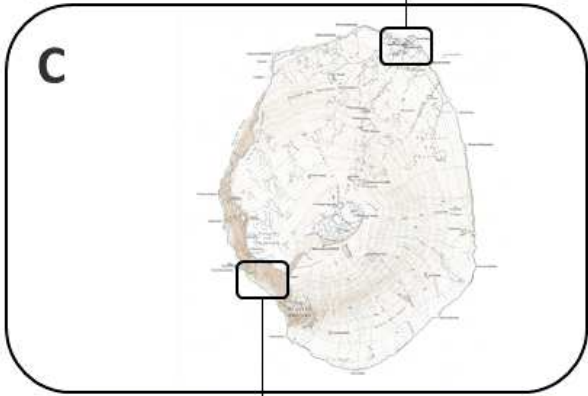
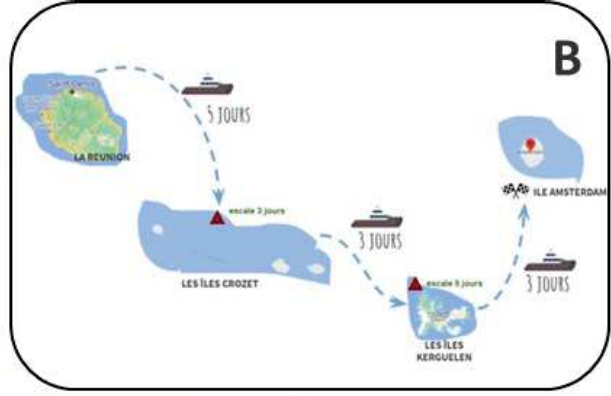
## Chapitre 1 : Un seul ou des pathogènes ? Exploration de la communauté d'agent infectieux affectant les oiseaux marins de l'île Amsterdam.

Le contenu de ce chapitre reprend les résultats du manuscrit en cours de préparation présenté en Annexe 1 :

### **Not only a single strain of *Pasteurella multocida* kills albatrosses on Amsterdam Island: insight into a community of bacterial pathogens affecting threatened seabirds**

Tornos Jérémy, Forde Taya, Clessin Augustin, Bost Caroline, Lemberger Karin, Le Net Rozenn, Fléchet Aline, Gantelet Hubert, Gamble Amandine & Boulinier Thierry.

Des épizooties sont responsables de fortes mortalités d'oiseaux marins sur l'île d'Amsterdam et un premier objectif de la thèse était de compléter l'exploration de la diversité des organismes pathogènes susceptibles d'être impliqués, préciser s'ils sont transmis entre les différentes espèces et si leur origine peut être identifiée. Du fait de la situation particulière de l'île d'Amsterdam, les conditions n'ont pas permis jusqu'alors un échantillonnage optimal sur le terrain pour répondre à ces questions (**Figure 6**), en particulier concernant la culture, l'isolement ou la conservation des potentiels pathogènes. Il est ainsi possible que certaines lacunes demeurent dans la description de la diversité des agents pathogènes au sein de l'île. Des lacunes persistent en particulier sur la description d'*Er*, isolée pour la première fois à la fin des années 1990 (Weimerskirch 2004) et identifiée comme appartenant au sérotype b. Bien que détectée par PCR dans les récents travaux (Jaeger et al. 2018), aucune isolation de souches d'*Er* n'avait pu être faite et sa caractérisation fine et son rôle dans les mortalités restaient encore mal connus. De plus, il n'est pas exclu que plusieurs souches de *Pm* ou *Er* circulent au sein ou entre les différentes espèces, ou bien que des souches aient pu évoluer au cours du temps (Wang et al. 2010; Orynbayev et al. 2019; Omaleki et al. 2020) et que celle-ci n'aient pas pu être isolées. La description de la diversité bactérienne associée aux événements de mortalités est pourtant un élément essentiel afin de mieux comprendre la situation éco-épidémiologique sur l'île. De plus, si les autovaccins induisent une protection efficace contre la souche avec laquelle ils sont produits, leur efficacité peut être réduite, voire nulle, contre une autre souche de la même espèce bactérienne, même très proche (Omaleki et al. 2020). L'exploration de la diversité des souches est un enjeu majeur afin d'optimiser et de mettre à jour les potentiels vaccins. Le premier axe de cette thèse s'est ainsi concentré sur la description de la diversité des potentiels pathogènes pouvant circuler sur l'île.



**Figure 6** (ci-contre) : L'isolement et la géographie particulière de l'île rend toute expérimentation particulièrement difficile. L'île n'est ravitaillée que 4 fois par an maximum par le navire ravitailleur le Marion Dufresne II, rendant complexe et long le rapatriement des échantillons vers le continent où les analyses seront réalisées, ceci peut être particulièrement contraignant pour les analyses bactériologiques.

- L'accès aux sites d'étude, au pied des falaises d'Entrecasteaux, nécessite un équipement spécifique, avec un accès par via ferrata, ainsi qu'une marche de plusieurs heures depuis la station de recherche.
- En période de reproduction, des séjours de plusieurs semaines sont effectués au pied des falaises dans une cabane aux conditions rudimentaires (accès à l'eau et l'électricité limité).
- La configuration des sites d'étude rend difficile la détection rapide des cadavres. Les carcasses sont souvent consommées ou altérées par les charognards (rats, labbes) avant d'avoir pu être autopsiées.
- Les sites de reproduction des SOA sont isolés et difficile d'accès pour la plupart. Seuls quelques nids accessibles ne permettent qu'un faible taux d'échantillonnage.
- Les conditions rudimentaires sur le site d'étude ne permettent pas d'avoir du matériel spécifique dédié à la bactériologie (e.g., incubateur, congélateur, hottes). Il est donc difficile de cultiver et conserver des potentiels pathogènes sans contamination.
- La plus grande partie du matériel scientifique peut être déposée par hélicoptère au pied du site d'étude. Cependant, une partie du matériel et certains produits spécifiques ne peuvent être transportés que lorsque le personnel se rend sur le site à pied.

A : La station scientifique Martin de Viviès accueille entre 25 et 30 personnes chaque année (scientifiques, personnel technique et administratif).

B : Trajet et escales du Marion Dufresne II. Une vingtaine de jours sont nécessaires pour atteindre l'île Amsterdam depuis l'île de la Réunion, avec des escales sur les îles Crozet et Kerguelen. 5 jours sont nécessaires pour rejoindre l'île de la Réunion depuis l'île Amsterdam.

C : Carte de l'île Amsterdam, d'après ©IGN 1:50.000. La station scientifique se trouve au nord de l'île. Le site d'étude se trouve au sud-ouest de l'île, au pied des falaises d'Entrecasteaux et n'est accessible qu'à pied depuis la base.

D-E : Passages techniques permettant de rejoindre le site d'étude.

F : Vue d'ensemble du site d'étude. La cabane (une partie vie commune et une partie laboratoire) est située au pied de la falaise. La zone verte indique la position et la surface de la zone d'étude des IYNA. Les zones violettes indiquent les positions des sites d'étude des NRP.

G : Zoom sur le site d'étude des IYNA, encadré en vert. Le site regroupe environ 650 couples reproducteurs. Les points verts situent la position approximative des quelques couples de SOA se reproduisant près du site d'étude.

En accord avec la Réserve Naturelle, un effort particulier a pu être mis en place lors de la période pendant laquelle les fortes mortalités ont généralement lieu, c'est-à-dire en Janvier-Février pour les IYNA et en Octobre-Novembre pour les NRP. Une forte présence sur le terrain d'un personnel qualifié est en effet nécessaire pour détecter les cas de mortalité avant que les espèces de charognards arrivent, empêchant la réalisation des autopsies.

### Des mortalités associées à des lésions de septicémie

Un suivi spécifique des mortalités, réalisé durant 2 saisons de reproduction pour les IYNA (2018-2019 et 2019-2020) et une saison pour les NRP (2019-2020), a permis de détecter et d'échantillonner un total de 105 cadavres des 3 espèces nichant sur les falaises d'Entrecasteaux (IYNA, SOA et NRP). Les observations d'événements de mortalité, avec la description de symptômes comparables, et les lésions macroscopiques observées sur certains organes lors de la réalisation des autopsies sur le terrain, appuient l'hypothèse de l'intervention d'agents pathogènes dans les mortalités (**Figure 7**).



Les analyses histo-anatomo-pathologiques des organes lésés et de certains tissus cibles viennent également conforter cette hypothèse (Jaeger et al. 2020, **Figure 8**), montrant pour une grande part des analyses de tissus prélevés des signes de septicémies bactériennes et des lésions caractéristiques compatibles avec une forme aiguë de choléra aviaire.

### *Pasteurella multocida* comme principal agent pathogène en cause

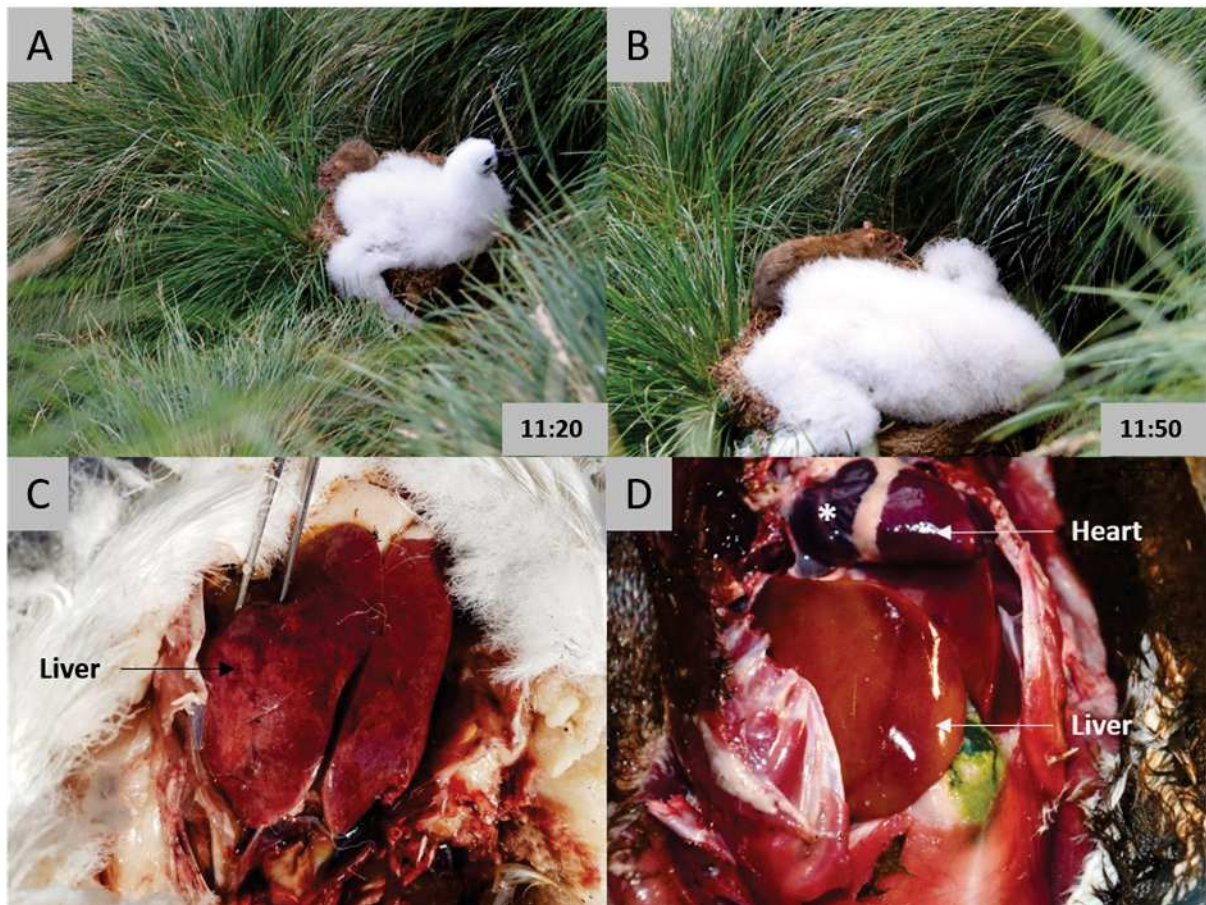
Des prélèvements bactériens sur les tissus lors de l'autopsie ont été mis en culture sur gélose au sang. *Pm* a ainsi été suspectée dans 93% des autopsies, toutes espèces et saisons confondues, d'après les caractéristiques macroscopiques<sup>6</sup> des colonies (forme, odeur et couleur des colonies dans les milieux de culture, **Figure 9**). *Er* a quant à elle été suspectée dans seulement 3% des cadavres et uniquement chez des IYNA au cours de la saison 2018-2019, mais n'a pas été détectée au cours de la saison suivante. L'isolement et la conservation des colonies bactériennes a permis une caractérisation génétique plus fine des souches. Une première approche a été réalisée par le typage des souches avec le système multi-host MLST<sup>7</sup>, couramment utilisé avec *Pm*.

Au cours de la première saison 23 autopsies d'IYNA ont été réalisées, et ont permis d'identifier 2 souches différentes de *Pm*. Une de ces souches, la plus répandue parmi les isolats (91% des autopsies), correspond à la souche D2C précédemment décrite par Jaeger et al (2018). Le suivi rigoureux a néanmoins permis l'identification d'une seconde souche de *Pm*, isolée sur 2 cadavres de poussins de IYNA. Cette souche, nommée AY18007B1, en plus d'être décrite pour la première fois sur Amsterdam, est également nouvelle dans la base de données du schéma multi-host MLST. Les 2 souches sont étroitement apparentées (**Figure 10**), mais le séquençage total de leurs génomes a montré une distance significative entre elles, avec une différence de plus de 12000 SNP. Ce résultat indique que les deux souches ne se sont pas différenciées l'une de l'autre récemment, ce qui suggère deux événements d'introduction, concomitant ou indépendants. De plus, la découverte de cette nouvelle souche, non décrite auparavant, apporte des résultats fondamentaux quant à la diversité de *Pm*.

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<sup>6</sup> *Pm* et *Er* ont été reconnues par identification macroscopique (validé par spectrométrie de masse à posteriori) à chaque fois qu'elles étaient présentes lors d'essais préliminaires portant sur 72 cultures (voir Annexe 1).

<sup>7</sup> La MLST (MultiLocus Sequence Typing) est basée sur le séquençage de 7 gènes de ménage. L'ensemble des séquences obtenues pour une souche donnée correspond à un profil caractéristique représenté par un Séquence Type (ST). Le schéma multi-host MLST, spécialement développé pour *Pm*, couvre les isolats provenant de divers hôtes, notamment les bovins, les ovins, les porcins et les oiseaux (<https://pubmlst.org/organisms/pasteurella-multocida>).

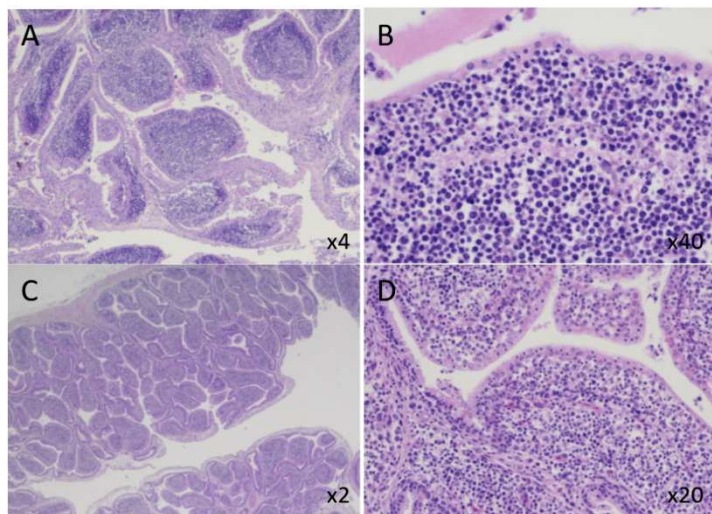


**Figure 7:** Symptômes caractéristiques observés sur les poussins, avant le décès ou lors de l'autopsie. Selon l'âge de l'individu ou l'état de conservation du cadavre les symptômes ne sont pas toujours observés, ou partiellement.

- A- Un poussin IYNA faible sur son nid, avec les ailes pendantes hors du nid et respirant difficilement (11:20am). Un rat brun visitait le nid (©Augustin Clessin).
- B- Le poussin décède 30min plus tard (11:50am). Un rat est aussi observé sur le nid, des blessures ont été détectées sur l'abdomen du poussin, probablement causées par des morsures de rats, ayant eu lieu avant la mort de l'individu d'après les hématomes et les saignements observés (©Augustin Clessin).

Des lésions caractéristiques sur certains organes sont observées sur de nombreux cadavres.

- C- Lésions caractéristiques sur le foie d'un poussin IYNA autopsié. L'aspect hétérogène et granuleux du foie peuvent correspondre à une infection bactérienne. (*Pm* a été isolée sur ce tissu) (©Augustin Clessin).
- D- Lésions caractéristiques sur le foie et le cœur d'un poussin de NRP autopsié. L'aspect hétérogène et granuleux du foie accompagné d'un épanchement sanguin péricardique (étoile blanche) peuvent correspondre à une infection bactérienne. (*Pm* a été isolée sur ce tissu) (©Caroline Bost).

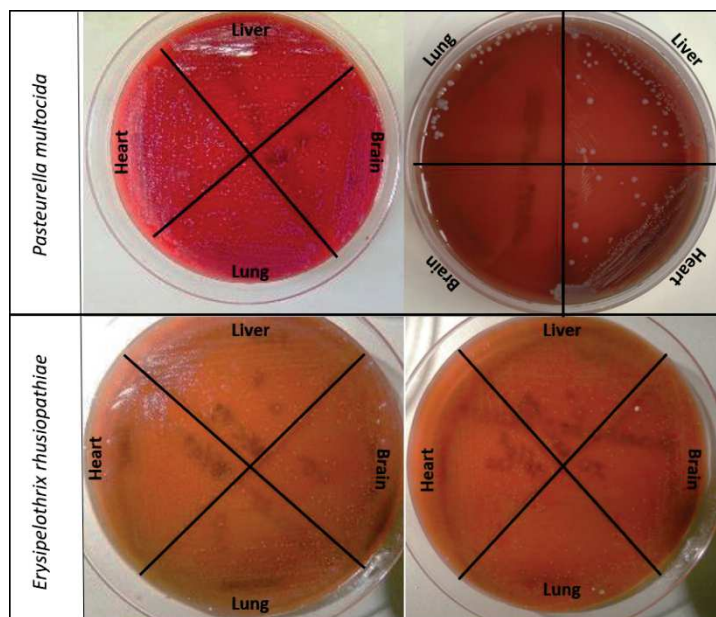


**Figure 8** : Lésions caractéristiques observées, en coloration Gram, lors de l'analyse histo-anatomopathologique de la bourse de Fabricius de 2 poussins de IYNA autopsiés.

A et B : Nécrose et érosion superficielles avec des bactéries dans les cryptes et lymphocytolyse. Grossi respectivement 4x et 40x. La souche D2C de la bactérie *Pm* a été isolée sur ce poussin.

C et D : Apoptose et lymphocytolyse, avec des bactéries dans les cryptes. Grossi respectivement 2x et 20x. La souche A18Y007B1 de la bactérie *Pm* a été isolé sur ce poussin.

(©Karin Lemberger/Rozenn Le Net)



**Figure 9** : Croissance bactérienne typique sur gélose au sang de mouton. Dans la mesure du possible, 4 tissus ont été ciblés pour chaque autopsie : foie, cerveau, poumon et cœur.

Encadré du haut : deux cultures positives de *Pm* ; colonies ovoïdes grises (~2mm) avec une odeur caractéristique (©Augustin Clessin).

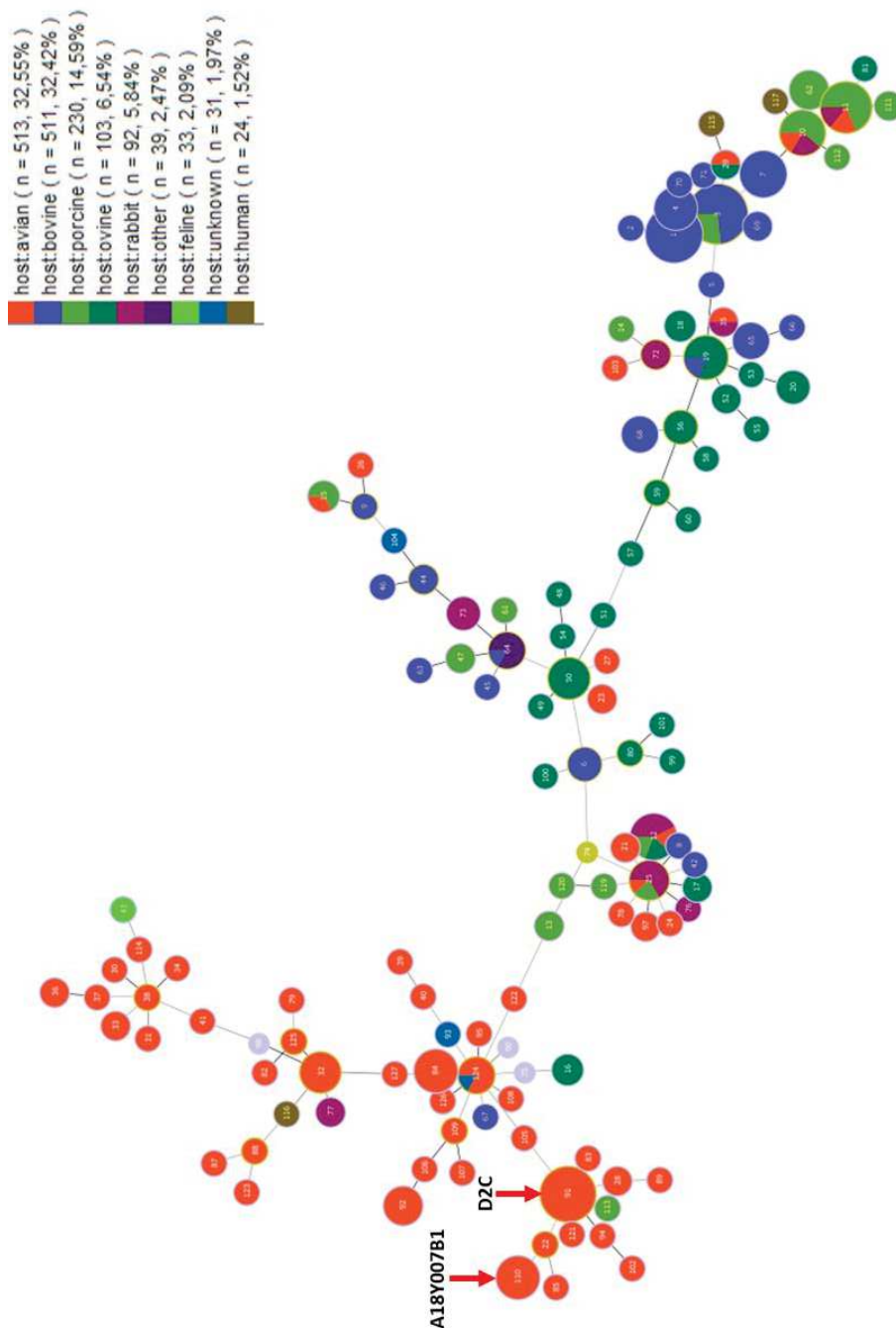
Encadré du bas : deux cultures positives de *Er* ; nombreuses petites colonies blanches transparentes (~1mm) (©Augustin Clessin).

Dans la majorité des cas, la croissance bactérienne a été détectée dans plusieurs organes échantillonnés et un seul type de colonie a été identifié. De plus, la même souche a toujours été détectée dans tous les organes infectés, ce qui suggère que l'infection et la septicémie sont dues à une seule souche et non à une co-infection de différentes souches ou agents pathogènes.

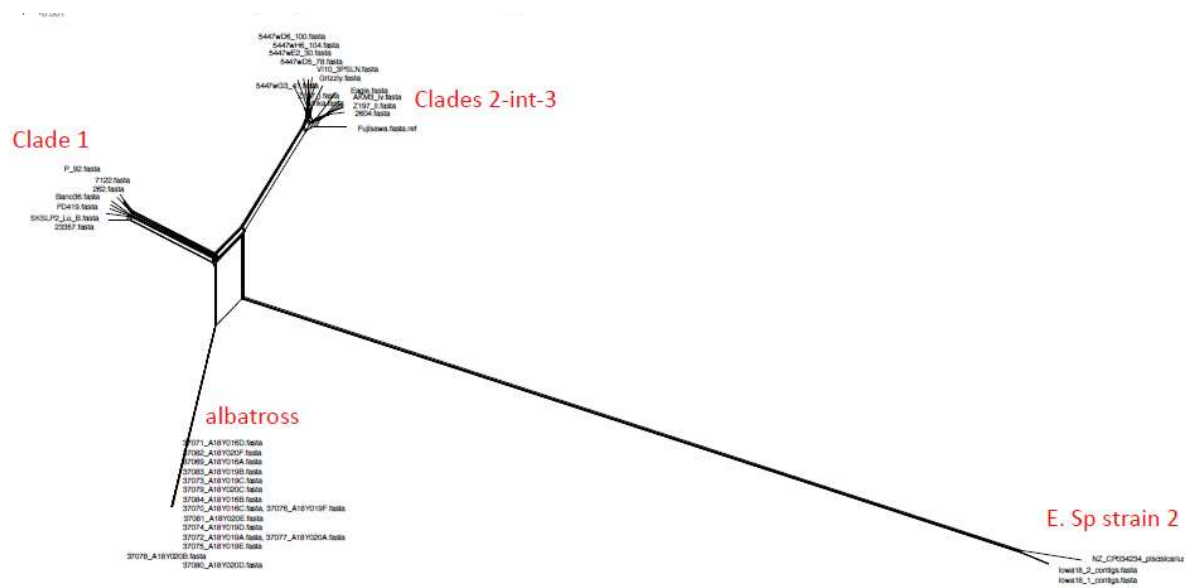
### Caractérisation d'un nouveau clade de l'agent du rouget *Erysipelothrix rhusiopathiae*

Le suivi des mortalités a permis d'isoler *Er* sur 3 cadavres de IYNA lors de la saison 2018-2019. Tous les isolats ont pu être conservés et caractérisés. Le séquençage du génome entier de ces isolats montre qu'ils appartiennent tous à la même souche (**Figure 11**). Cependant, ces *Er* isolés sur les IYNA de l'île Amsterdam semblent appartenir à un nouveau clade au sein du groupe des *Er*, regroupés habituellement en trois clades distincts (Forde et al. 2016). Il s'agit de la première caractérisation génétique d'*Er* sur l'île d'Amsterdam. Importante pour des raisons de conservation, la découverte du « clade des Albatros » est également intéressante car elle apporte de nouveaux éléments sur la diversité globale de *Er* (Forde et al. 2016).

Cette étude confirme le rôle des maladies dans la situation critique de l'île d'Amsterdam et révèle qu'une diversité d'agents pathogènes est impliquée. L'agent pathogène le plus répandu est la souche D2C de *Pm* qui avait été isolée dans le système plusieurs années auparavant (Jaeger et al. 2018; Jaeger et al. 2020), mais au moins une autre souche de *Pm*, la souche AY18007B1 et une de *Er*, sont associées aux mortalités. Leur caractérisation génomique montre qu'elles sont bien différenciées de la souche locale prédominante de *Pm*, et d'autres souches connues. Ces résultats sont importants pour des raisons écologiques et appliquées, mais ils ne permettent pas pour l'instant de déterminer l'origine de ces souches et les processus qui pourraient expliquer leur présence et leurs effets. La caractérisation de l'agent pathogène est essentielle pour produire des vaccins autogènes afin de protéger les oiseaux des infections mortelles auxquelles ils auront une forte probabilité d'être exposés. Enfin, l'étude souligne l'utilité pratique de combiner les nécropsies de terrain et les travaux de microbiologie avec des méthodes génomiques pour répondre à des questions pertinentes pour la conservation de la biodiversité. En parallèle de l'exploration de la diversité des pathogènes dans le système, le travail s'est aussi porté sur une approche vaccinale. Les essais de vaccination pourront apporter dans ce contexte des éléments essentiels, d'une part ils permettront de tester *in situ* dans une population sauvage une méthode de protection et de conservation, mais apporteront également des éléments plus quantitatifs quant au rôle des agents infectieux dans les mortalités.



**Figure 10:** Minimum spanning tree des sequence type (STs) de *Pasteurella multocida* basé sur le schéma multi-host MLST (<http://www.pubmlst.org/pmultocida/>). Chaque cercle représente un ST (les numéros de référence sont à l'intérieur des cercles). ST91 et ST110 sont spécifiés par une flèche rouge verticale. Le code couleur indique l'origine animale des isolats séquencés disponibles dans la base de données, n représente le nombre de ces isolats pour chaque groupe animal. (Annexe 1)



**Figure 11:** Structure de la population d'*Erysipelothrix rhusiopathiae* basée sur une analyse de réseau. Le réseau phylogénétique d'*Er* a été estimé en utilisant l'analyse Neighbor-Net telle qu'implémentée dans SplitsTree. L'analyse comprend le clade des albatros, le clade 1, les clades 2, 3 et intermédiaires et la souche 2 d'*Erysipelothrix sp* (espèce proche d'*Er*). (Annexe 1)



## **Chapitre 2 : Optimisation de la vaccination contre le choléra aviaire chez l'albatros à bec jaune : effet de l'âge à la vaccination, du transfert d'anticorps maternels et des souches utilisées**

Le contenu de ce chapitre correspond principalement à un manuscrit en cours de préparation, présenté en Annexe 2 :

### **Optimizing a vaccination approach against avian cholera to protect endangered albatrosses**

Tornos Jérémy, Clessin Augustin, Gantelet Hubert, Fléchet Aline, Bely Marine, Gamble Amandine & Boulinier Thierry

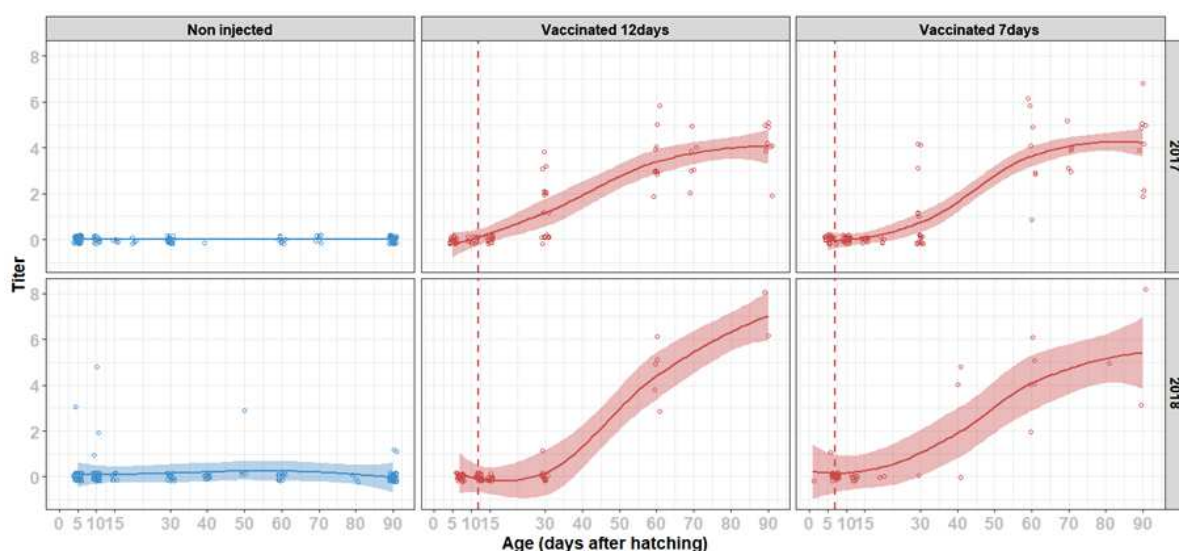
Les résultats de Bourret et al. (2018) suggèrent que, malgré l'effet protecteur encourageant de la vaccination directe des poussins contre l'agent du choléra aviaire isolé sur l'île d'Amsterdam, le choix de l'âge est un critère essentiel et qu'une vaccination trop précoce n'offre pas de réponse immunitaire et donc aucune protection. Cependant, il est essentiel d'avoir l'effet protecteur de la vaccination le plus tôt possible afin de prévenir les potentielles épidémies pendant les stades précoces des poussins. Il est par ailleurs important de s'assurer d'une persistance entre années du taux d'anticorps chez les adultes si l'on veut permettre une éventuelle protection des poussins via un transfert maternel d'anticorps (Gamble, Garnier, et al. 2019). Le second axe d'étude de cette thèse va ainsi s'articuler sur l'ajustement de l'âge idéal de vaccination des poussins afin d'offrir une protection optimale si une vaccination à grande échelle devait avoir lieu, et la poursuite de l'exploration d'une stratégie de vaccination des adultes reproducteurs pour protéger leurs poussins. La configuration des sites de nidification ainsi que la taille de la population ne permettent pas d'avoir un suivi fin de toute la population de IYNA, il serait utile d'envisager une stratégie ne nécessitant pas une vaccination à un âge précis, tout en optimisant l'âge de vaccination. Le cycle de reproduction des IYNA sur l'île Amsterdam est très synchronisé et constant entre les années (avec une date moyenne d'éclosion identique sur les 7 dernières années (de 2013 à 2019) se trouvant le 5 décembre, avec 75% des poussins ayant éclos le 8 décembre), il serait donc possible d'envisager une date ou une période durant laquelle une vaccination pourrait être effective sur une très grande majorité de poussins. Compte tenu des éléments apportée par Bourret et al. (2018), nous avons exploré l'effet de la vaccination sur les poussins à 7 et 12 jours après l'éclosion au cours de 2 saisons de reproduction. Ainsi, en complément des éléments apportés par la précédente étude nous pourrions explorer un gradient d'efficacité de la vaccination sur les poussins à différents âges : 2 jours (pas de réponse humorale et pas d'effet



protecteur détectés), 7 jours (à tester), 12 jours (à tester) et 14 jours (réponse humorale et effet protecteur détectés). Deux cohortes de 25 poussins ont ainsi chacune été vaccinées à l'âge de 7 jours ou 12 jours lors de 2 saisons de reproduction (2017-2018 et 2018-2019). Ces cohortes ont été comparées à 2 groupes contrôles afin de tester l'effet de la vaccination.

### Effet de l'âge de vaccination des poussins et interférence des rats

Les résultats montrent que la vaccination des poussins induit une réponse immunitaire identique qu'ils soient vaccinés à 7 ou 12 jours après l'éclosion (**Figure 12**), et de façon comparable les 2 années. L'étude suggère néanmoins que malgré une réponse immunitaire identique, l'âge de la vaccination a une influence sur l'effet protecteur (**Figure 13**). Lors de la saison 2017-2018, la vaccination à 12 jours offre un effet protecteur significatif contrairement à la vaccination à 7 jours.

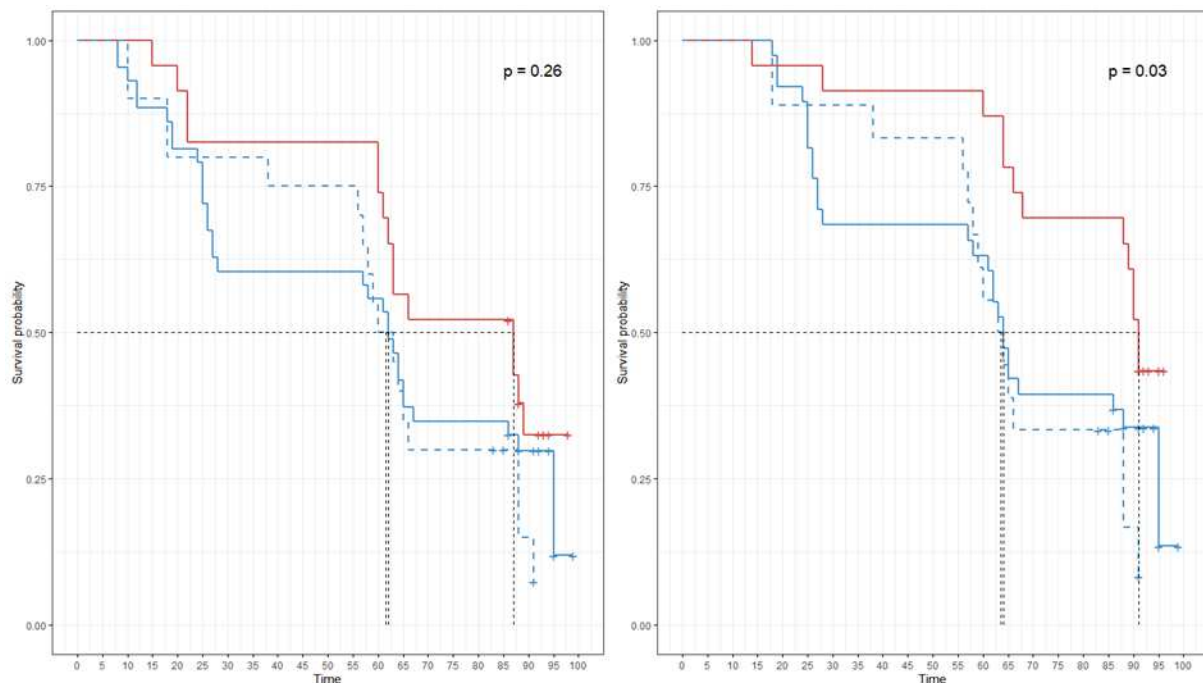


**Figure 12** : Quantification des anticorps anti-Pm chez les poussins IYNA vaccinés au cours de la saison 2017/2018 (encadrés du haut) et 2018/2019 (encadrés du bas). Not injected : poussins non vaccinés mais manipulés et échantillonnés selon la même procédure que les poussins vaccinés ; Vaccinated 12days : poussins vaccinés 12 jours après l'éclosion ; Vaccinated 7days : poussins vaccinés 7 jours après l'éclosion. Les lignes en pointillés indiquent le moment de la vaccination. Chaque point est une valeur unique de titre pour un poussin à un âge donné. Les points sont volontairement séparés pour plus de lisibilité.

Le risque de décès des poussins vaccinés à 12 jours est réduit de 78% (HR=0.322; CI: 0.148-0.7013;  $p < 0.01^{**}$ )<sup>8</sup> par rapport au groupe témoin non manipulé et de 50% (HR=0.5047; CI: 0.2555-0.9969,

<sup>8</sup> HR : Hazard Ratio, dans le cas de l'étude HR représente le risque de décès d'un individu à un instant donné. CI : intervalles de confiance supérieurs et inférieurs à 95 % pour le HR. p : Significativité statistique globale du modèle.

$p < 0.05^*$ ) par rapport au groupe témoin manipulés (**Figure 13**). La survie finale passe à plus de 45% pour les vaccinés contre moins de 15% chez les individus témoins. Les résultats montrent également qu'en considérant tous les poussins vaccinés, quel que soit l'âge de l'injection et de l'éclosion à l'envol, le risque de décès reste significativement réduit de 49% (HR=0.5138 ; CI: 0.3247-0.8129;  $p < 0.01^{**}$ ) comparé aux poussins manipulés. Ce résultat suggère que, même si l'on ne connaît pas l'ensemble des paramètres démographiques de la population à vacciner, il est possible de mettre en œuvre un plan de vaccination à grande échelle sur une fenêtre temporelle, de 7 à 12 jours, et de diviser par 2 le risque de décès des individus.



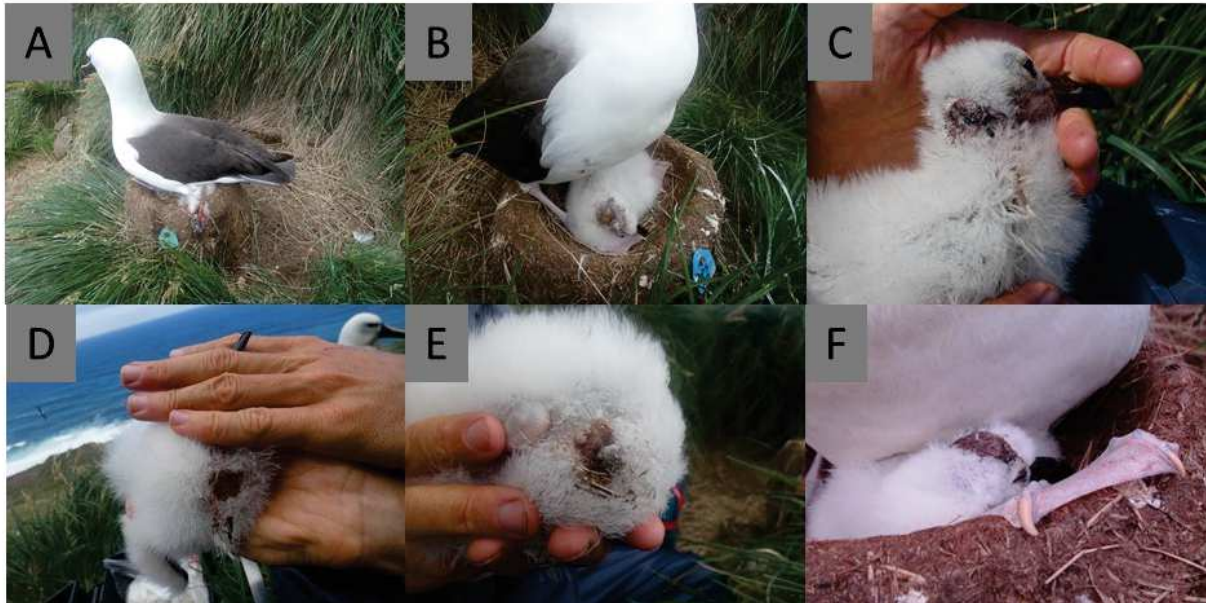
**Figure 13** : Courbes de survie pour la saison de reproduction 2017/2018. Courbes de survie du panneau de gauche pour les poussins vaccinés à 7 jours : ligne unie rouge pour les poussins vaccinés (n=23) ; ligne unie bleue pour les poussins manipulés et échantillonnés mais non injectés (n=43) ; ligne pointillée bleue pour les poussins non manipulés (n=20). Courbes de survie du panneau de droite pour les poussins vaccinés à 12 jours : ligne unie rouge pour les poussins vaccinés (n=22) ; ligne unie bleue pour les poussins manipulés et échantillonnés mais non injectés (n=38) ; ligne pointillée bleue pour les poussins non manipulés (n=18). Les axes X (Temps) correspondent aux jours après l'éclosion. La p-value a été mesurée selon la méthode du log-rank. La ligne pointillée noire représente les valeurs médianes du temps de survie. La survie est évaluée à partir de la vaccination : les échecs ayant eu lieu avant 7 jours pour l'encadré de droite et 12 jours pour celui de gauche ne sont pas considérés dans l'analyse pour tous les groupes.

Lors de la saison 2018-2019, alors que le protocole expérimental était le même que celui de la saison 2017-2018, aucun effet bénéfique sur la survie des poussins n'a été observé, quel que soit leur âge de vaccination. Bien que décevant, ce résultat apporte pourtant des éléments essentiels à l'optimisation

de la vaccination et souligne en particulier que l'efficacité d'un vaccin peut varier dans le temps. De nombreuses raisons peuvent expliquer ce résultat. Notamment, l'isolement de la nouvelle souche A18Y007B1 au début de cette même saison de reproduction (voir Chapitre 1) est une piste à explorer, car le vaccin utilisé lors de cette expérience avait été développé à partir de la souche historique D2C. Même si A18Y007B1 a été découverte dans une proportion plus faible que D2C, la protection contre cette dernière a pu être moindre si les deux souches circulaient de manière concomitante dans la colonie étudiée. D'autre part, une prédation par les rats plus importante que d'habitude a été observée aux jeunes stades des poussins. Dans de nombreux cas, des blessures sévères ont été observées sur les poussins, notamment sur la tête et le cou (**Figure 14**). L'efficacité du vaccin pourrait ainsi avoir été minimisée en raison du taux de blessure et de prédation élevés. Malheureusement, il est très difficile de quantifier précisément la part de la mortalité due aux rats. En plus d'être responsables de prédatons directes, les rats jouent potentiellement un rôle important de réservoir et de disséminateur de *Pm* (Heddleston et al. 1964; Gregg et al. 1974), ce qui peut aggraver les épidémies en augmentant considérablement le nombre d'individus infectés pouvant ainsi diminuer l'efficacité de la vaccination (Perelman et al. 1990; Rees et al. 2013; Langwig et al. 2019; van Dijk et al. 2021). L'exposition à la bactérie par inoculation lors de morsures est notamment susceptible d'entraîner une plus forte mortalité (Gregg et al. 1974).

#### Exploration de la possibilité d'une protection par transfert d'anticorps maternels

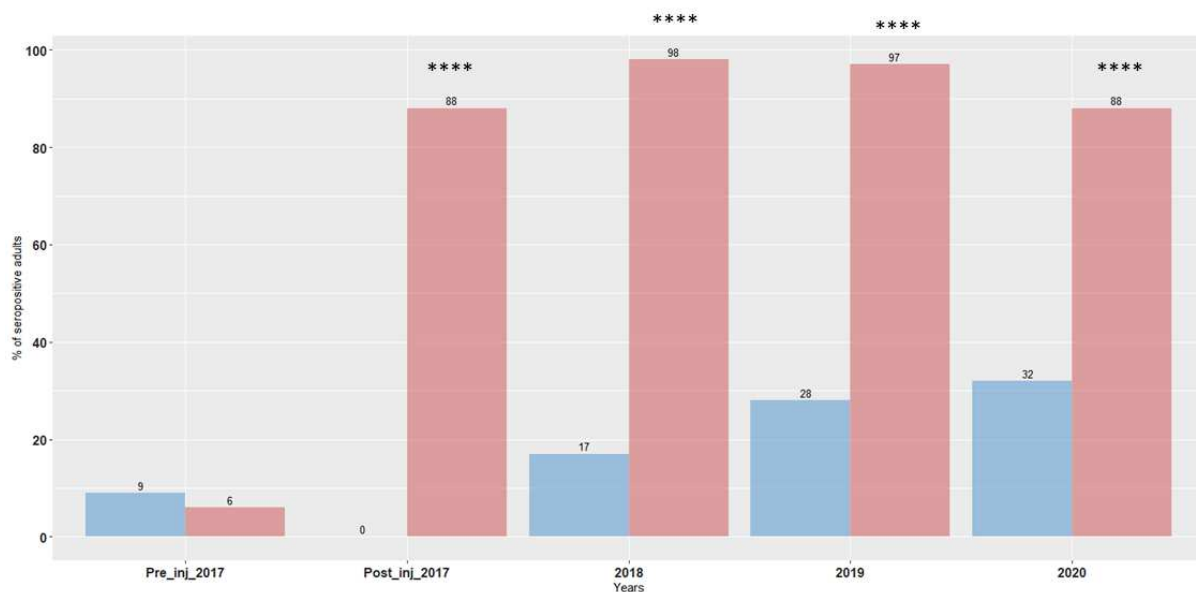
En plus de fournir des éléments sur l'âge ou la période efficace pour la vaccination, nous avons également exploré la possibilité d'une protection des jeunes via transfert d'anticorps maternels, qui n'a jusqu'alors pas pu être mis en évidence. Suite aux résultats préliminaires de Gamble et al. (2019), une nouvelle cohorte d'adultes a été vaccinée (en 2017-2018) avec la nouvelle formulation du vaccin dite 'water in oil'. Ces individus ont été suivis sur plusieurs années afin d'évaluer la persistance des anticorps et plus particulièrement leur potentiel transfert chez les jeunes. De plus, la survie des poussins issus des couples vaccinés a pu être suivie sur 2 saisons de reproduction (1 et 2 années après la vaccination des adultes, *i.e.* lors des saisons 2018-2019 et 2019-2020) afin d'évaluer l'effet protecteur du transfert d'anticorps.



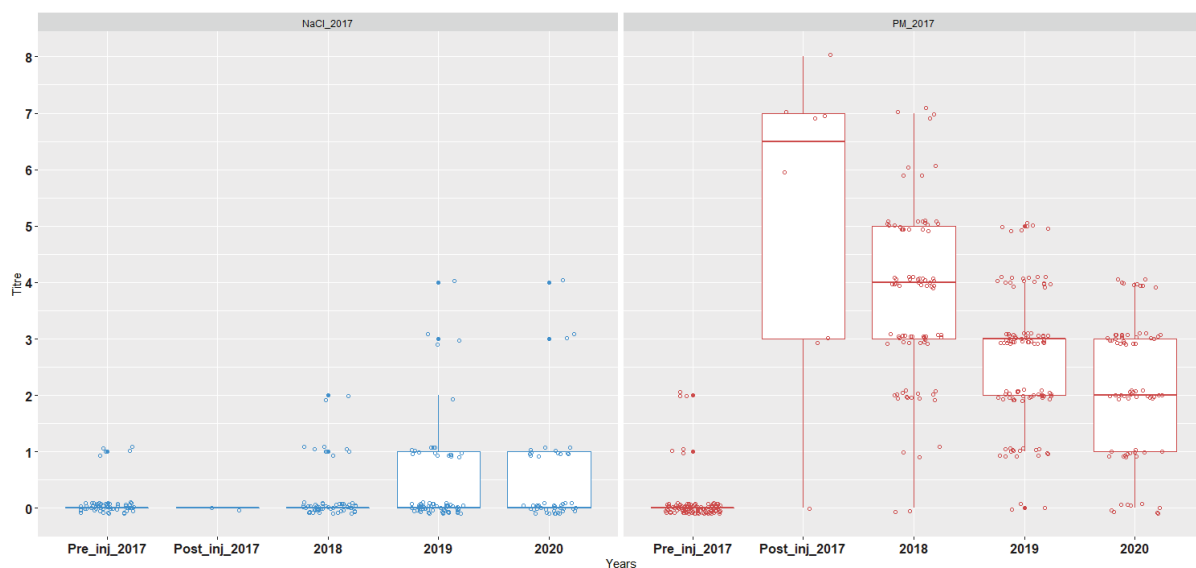
**Figure 14:** Photographies de poussins de IYNA blessés ou tués par des rats (20 premiers jours après l'éclosion) pendant la saison de reproduction 2018/2019. A : poussin ayant reçu des morsures mortelles au niveau du cou et de la tête par des rats alors qu'il était sous la surveillance d'un parent (âgé de 6 jours). B : poussin blessé à la tête, l'os du crâne est visible, alors qu'il était sous la surveillance d'un parent (âgé de 7 jours). C, D, E : poussins (<15 jours) blessés par des rats respectivement au cou, à la poitrine et sur la partie inférieure de l'abdomen. F : poussin blessé à la tête par un parent, l'os du crâne étant visible. B à F : tous les poussins sont morts dans la semaine suivant l'observation de leur blessure.

Les résultats montrent que la nouvelle formulation du vaccin induit, chez les adultes, une réponse immunitaire persistante sur plusieurs années, contrairement à la première utilisée dans Gamble et al. (2019). La proportion d'individus séropositifs est significativement supérieure chez les adultes vaccinés (88%) comparés aux adultes non vaccinés (32%) 3 ans après la vaccination (**Figure 15**) et les taux d'anticorps sont encore significativement supérieurs malgré une décroissance au cours des années (**Figure 16**).

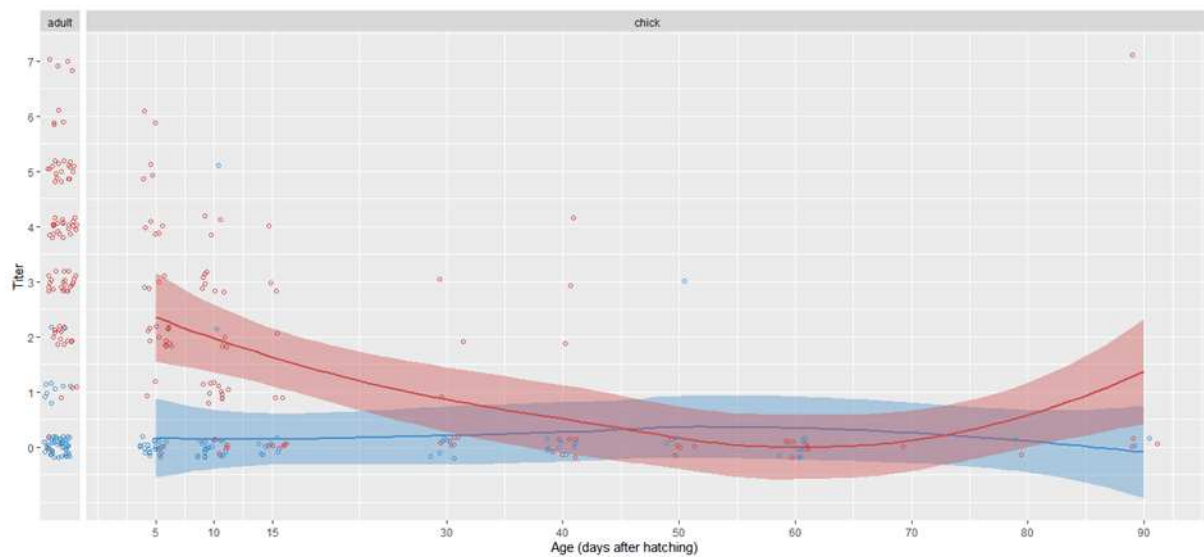
La réponse immunitaire des adultes est de plus associée à un transfert d'anticorps vers les poussins, une année après vaccination (**Figure 17**) et les résultats suggèrent que les anticorps peuvent persister chez les poussins jusqu'à 50 jours après l'éclosion. Malheureusement, pour des raisons logistiques (délais de retour des échantillons), il n'a pas encore été possible de mesurer les taux d'anticorps des poussins 2 et 3 années après vaccination. Cependant, la forte corrélation entre les taux mesurés chez la mère et chez le poussin lors de la saison 2018-2019 nous laisse supposer que le transfert a bien eu lieu jusqu'à 3 années après vaccination.



**Figure 15:** Pourcentage d'adultes séropositifs avant et jusqu'à 3 ans après l'injection. Bleu : adultes injectés avec 0,5ml de NaCl 0,9% en décembre 2017. Rouge : adultes injectés avec 0,5ml de vaccin contre la souche D2C en décembre 2017. Pre\_inj\_2017 : au moment de l'injection en 2017 ( $n_{\text{nacl}}=58$  et  $n_{\text{pm}}=104$ , respectivement pour le nombre d'individus échantillonnés injectés avec du NaCl et vaccinés). Post\_inj\_2017 : 2 à 3 semaines après l'injection ( $n_{\text{nacl}}=2$  et  $n_{\text{pm}}=8$ ). 2018 ( $n_{\text{nacl}}=58$  et  $n_{\text{pm}}=88$ ), 2019 ( $n_{\text{nacl}}=71$  et  $n_{\text{pm}}=104$ ) et 2020 ( $n_{\text{nacl}}=47$  et  $n_{\text{pm}}=72$ ) respectivement 1, 2 et 3 ans après injection. Le chiffre au sommet des barres indique le pourcentage d'adultes séropositifs pour chaque période. \*\*\*\* signifie que la différence entre NaCl injecté et vacciné au cours d'une saison est significativement différente ( $p < 0,01$ ).

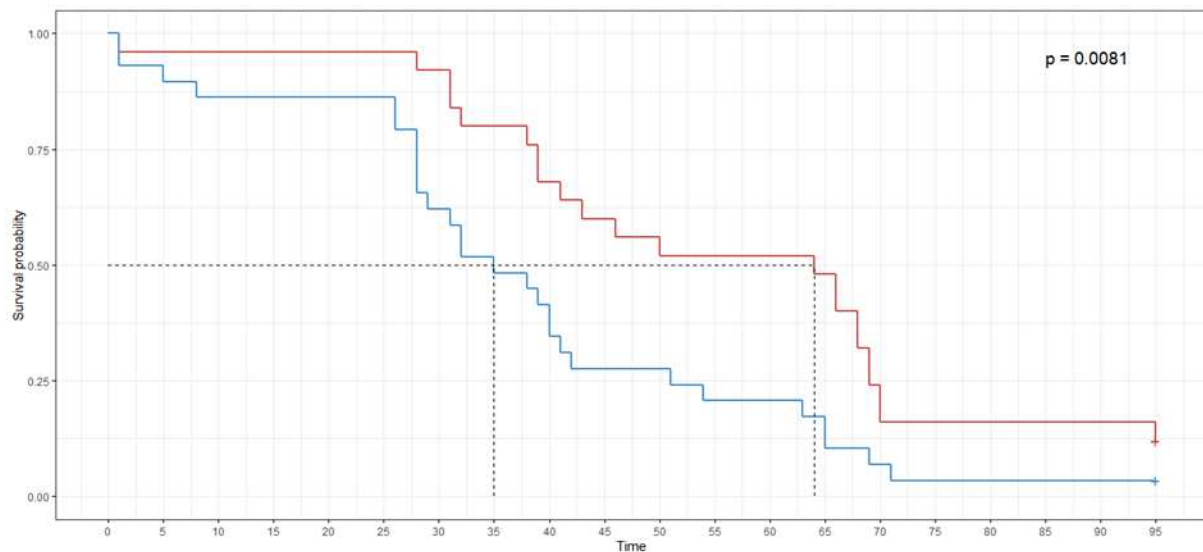


**Figure 16:** Quantification, en échelle logarithmique, des anticorps anti-*Pm* par micro-agglutination (MAT) chez les adultes IYNA injectés en décembre 2017. NaCl\_2017 (panneau de gauche en bleu) : adultes injectés avec 0,5ml de NaCl 0,9%. PM\_2017 (panneau de droite en rouge) : adultes injectés avec 0,5ml de vaccin contre la souche D2C. Pre\_inj\_2017 : au moment de l'injection en 2017. Post\_inj\_2017 : 2 à 3 semaines après l'injection. 2018, 2019 et 2020 respectivement 1, 2 et 3 ans après injection. Chaque point correspond à une seule valeur de titre pour un individu. Les points sont volontairement séparés pour des aspects visuels.



**Figure 17:** Quantification, en échelle logarithmique, des anticorps anti-*Pm* par MAT mesurés chez les poussins en fonction de leur âge (en jours après l'éclosion) 1 an après l'injection des adultes en 2017-2018. Panneau de gauche (adult) : titre d'anticorps chez les parents lors de la saison 2018-2019. Panneau de droite (chick) : titre d'anticorps chez leurs poussins respectifs en 2018-2019. Bleu : parents injectés avec une solution de NaCl 0.9% et leurs poussins respectifs. En rouge : parents injectés avec le vaccin contre D2C et leurs poussins respectifs. Chaque point est une valeur unique de titre pour un individu. Les points sont volontairement séparés pour des aspects visuels. La courbe suit une régression « loess ».

Lors de la saison 2018-2019, aucun effet protecteur du transfert d'anticorps n'a pu être détecté. Bien que décevant, cela conforte les résultats obtenus avec la vaccination directe des poussins lors de cette même saison et montre que la protection contre la souche D2C n'a pas été effective cette année-là, très probablement pour les raisons évoquées précédemment (circulation d'une seconde souche, forte prédation des rats). Les résultats obtenus lors de la saison 2019-2020, 2 années après la vaccination des adultes, sont néanmoins prometteurs en montrant que la survie des poussins issus des adultes vaccinés est supérieure à celle des groupes témoins, avec un risque de décès réduit de 53% ( $HR=0.4743$ ;  $CI: 0.2680-0.8396$ ;  $p=0.01^*$ ) comparés aux poussins issus de parents injectés avec du NaCl (**Figure 18**). La nouvelle formulation du vaccin apporterait donc un effet protecteur des poussins 2 années suivant la vaccination des adultes.



**Figure 18** : Courbes de survie des poussins pendant la saison de reproduction 2019/2020. Courbe rouge : poussins manipulés et prélevés issus de parents injectés avec le vaccin contre D2C (n=25). Courbe bleue : poussins manipulés et prélevés issus de parents injectés avec la solution de NaCl (n=29). L'axe des abscisses (Time) correspond aux jours après l'éclosion. La p-value a été mesurée selon la méthode du log-rank. Les lignes pointillées noires correspondent aux valeurs médianes du temps de survie (50% de mortalité).

### Vaccins contre les souches circulantes de *Pasteurella multocida*

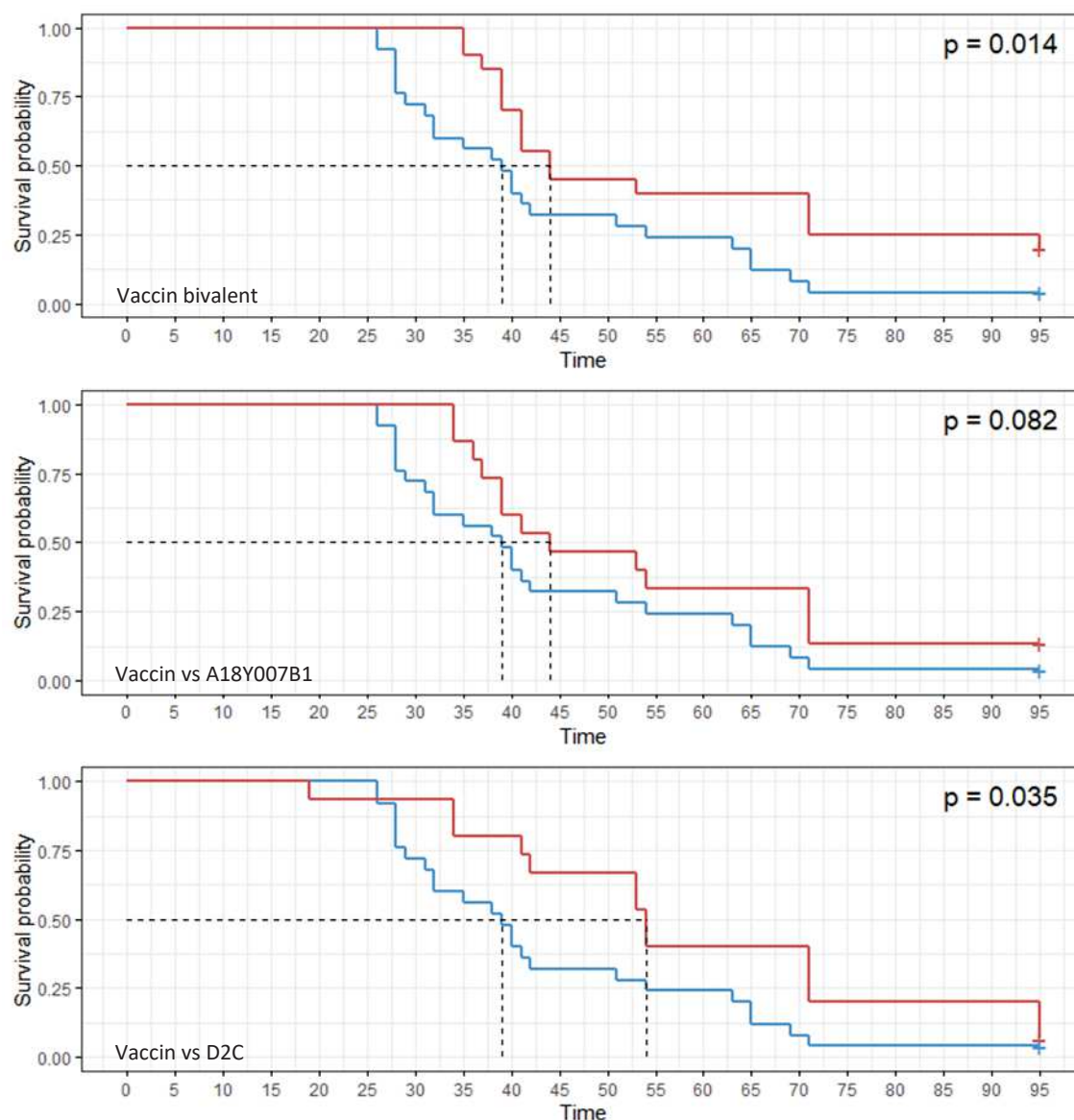
Les résultats précédents confirment que la protection par la vaccination est une méthode prometteuse pour protéger les individus contre des épidémies de choléra aviaire chez l'espèce considérée. Cependant, ils montrent également que l'efficacité peut varier dans le temps et qu'un suivi long terme et surtout une meilleure compréhension des processus éco-épidémiologiques sont indispensables. Jusqu'alors, le vaccin n'a été produit qu'à partir de la souche historique seule D2C, mais la découverte d'une nouvelle souche de *Pm* apporte des informations nouvelles et pourrait notamment permettre une mise à jour du vaccin. Lors de la saison 2019-2020, afin d'évaluer l'intérêt d'ajuster le vaccin aux souches détectées, 2 nouveaux autovaccins ont été produits en plus de celui produit jusqu'alors, un autovaccin produit à partir de la nouvelle souche A18Y007B1 isolée lors de la précédente saison et un autovaccin bivalent, c'est-à-dire produit à partir des 2 souches D2C et A18Y007B1.

Durant cette saison, 3 cohortes de poussins ont été vaccinées avec les 3 lots de vaccins produits et leur survie évaluée tout au long de la saison de reproduction (**Figure 19**). Comme pour la saison 2018-2019, une forte prédation des rats a été observée, cependant, un effet protecteur de la vaccination a tout de même pu être mis en évidence, même si celui-ci a probablement été diminué. Les résultats montrent que la vaccination contre la souche A18Y007B1 n'a pas eu un effet protecteur, confortant l'hypothèse

que celle-ci circule de façon minoritaire dans la population. La vaccination contre la souche D2C, historiquement utilisée, a quant à elle entraîné la détection d'un effet protecteur, confirmant les résultats du Bourret et al. (2018) et ceux de la saison 2017-2018, avec un risque de décès réduit de 51% ( $HR=0.4944$ ;  $CI: 0.2563-0.9950$ ;  $p<0.05^*$ ) comparé aux poussins manipulés mais non vaccinés. Confortant ce résultat, le vaccin bivalent offre également un effet protecteur avec un risque de décès réduit de 54% ( $HR=0.4578$ ;  $CI: 0.2390-0.8764$ ;  $p<0.05^*$ ).

Cette étude souligne que l'intégration de nombreux facteurs doivent être pris en compte afin de mettre en œuvre une vaccination dans une population sauvage. Nous avons notamment montré que le suivi de la population est essentiel, et nous avons mis en évidence l'importance de l'âge de vaccination des poussins et le fait qu'une approche par vaccination des adultes pourrait permettre une protection des jeunes. Nous montrons également que la formulation et la composition du vaccin est cruciale à considérer. La recherche et la mise à jour de nouveaux pathogènes, et la détermination de leur importance épidémiologique, est pour cela essentielle. Cependant, le suivi sur plusieurs années de la vaccination a également permis de mettre en évidence que les épizooties, sur les dernières années, ne sont pas le seul facteur de mortalité et que la prédation par les rats est un facteur à prendre en compte.





**Figure 19:** Courbes de survie pour la saison de reproduction 2019/2020. Encadré supérieur : poussins injectés avec le vaccin bivalent à l'âge de 10 jours. Rouge pour les poussins vaccinés (n=19) et bleu pour les poussins manipulés et échantillonnés mais non injectés (n=25). Encadré central : poussins ayant reçu le vaccin contre A18Y007B1 à l'âge de 10 jours : Rouge pour les poussins vaccinés (n=15) et bleu pour les poussins manipulés et échantillonnés mais non injectés (n=25). Encadré inférieur : poussins injectés avec le vaccin contre D2C à l'âge de 10 jours. Rouge pour les poussins vaccinés (n=15) et bleu pour les poussins manipulés et échantillonnés mais non injectés (n=25). L'axe des abscisses (Time) correspondent aux jours après l'éclosion. La p-value a été mesurée selon la méthode du log-rank. Les lignes pointillées noires correspondent aux valeurs médianes du temps de survie (50% de mortalité).

### **Chapitre 3 : L'étude de la circulation des pathogènes au sein des populations**

Ce chapitre reprend essentiellement l'article en cours de préparation en Annexe 3 mais aussi les articles en cours de préparation ou publiés des annexes 4 à 6 :

La vaccination à l'échelle d'une population repose dans beaucoup de cas sur le principe d'immunité collective, c'est-à-dire que la proportion d'individus immunisés atteint un seuil suffisant permettant la diminution, voire l'interruption de la transmission de l'agent pathogène (Keeling and Rohani 2007). Une protection indirecte des individus non vaccinés contre un agent pathogène peut donc ainsi se produire lorsque la couverture vaccinale est suffisante (Fine et al. 2011). Cependant, l'immunité collective peut varier dans le temps et dans l'espace en raison des processus démographiques (Peel et al. 2014), par exemple, la reproduction chez les IYNA induit une entrée importante et annuelle dans la population de poussins non immunisés et en contrepartie le vieillissement de la population entraîne la mortalité d'individus immunisés. Par ailleurs, en utilisant un vaccin qui ne bloque pas la transmission, l'effet principal recherché est de protéger des cohortes d'individus afin d'améliorer les paramètres démographiques de la population (Garnier et al. 2012). Il est donc important de considérer comment les processus démographiques peuvent affecter les stratégies de vaccination. Dans cette thèse nous avons notamment donné des éléments de réflexion sur la protection des jeunes, nouveaux entrants dans la population, dès le plus jeune âge et de façon régulière, par la vaccination directe de ceux-ci ou via une protection transmise par la mère. Cependant, l'entrée de nouveaux individus non immunisés ne se fait pas uniquement par les naissances mais également par la circulation ou l'arrivée d'autres hôtes potentiels au sein d'une population locale. Les déplacements d'individus entre populations ou sous-populations sont essentiels à considérer pour comprendre la dynamique des populations subdivisées (Brown and Kodric-Brown 1977; Hanski 1998). Les déplacements d'individus infectés ou non, et la transmissibilité de l'agent pathogène, sont des éléments clés pour les dynamiques épidémiologiques, et donc lors de l'élaboration d'un plan de vaccination (Haydon et al. 2006).

La compréhension du réseau social au sein des populations et entre les populations est donc très importante pour concevoir des stratégies visant à atténuer la transmission des agents pathogènes (Rogers et al. 1998; Cross et al. 2005; Weiss et al. 2020) et l'étude des déplacements des hôtes potentiels est primordiale afin d'optimiser la structuration spatiale de la vaccination. En raison de leurs variétés possibles de voies de propagations, la circulation de *Pm* et *Er* est particulièrement difficile à évaluer, elle est néanmoins à considérer. La proximité de 3 espèces hôtes d'oiseaux marins dans les falaises d'Entrecasteaux peut s'avérer critique. Si les différentes colonies et les différentes espèces

sont en contact régulier, une vaccination par secteur ou par espèce pourrait ne pas être efficace. De plus, même si les AMA, qui ne se reproduisent pas dans les falaises d'Entrecasteaux, ne se trouvent pas dans une proximité directe et ne semblent pas affectés par les épidémies récurrentes, il est important d'évaluer le risque de transmission des pathogènes vers cette espèce menacée. Le dernier axe de cette thèse va donc reposer sur l'étude des déplacements de certains hôtes potentiels au sein et entre les populations.

Différents types de déplacements peuvent influencer la circulation des agents de maladies infectieuses, et si la migration est un exemple souvent étudié (Altizer et al. 2011; Bauer and Hoyer 2014; Fritzsche McKay and Hoyer 2016), elle ne constitue pas le seul moyen potentiel de cette circulation. D'autres déplacements souvent négligés constituent pourtant un risque potentiel, tels que les comportements de dispersion ou la prospection des hôtes (Boulinier et al. 2016). Dans cet axe de recherche nous allons principalement nous intéresser aux déplacements de prospection, définis comme la visite de zones de reproduction où un individu pourrait potentiellement s'installer pour se reproduire mais qui ne se reproduit pas actuellement (Ponchon et al. 2013). Ce déplacement, ayant lieu au sein de la saison de reproduction et à fine échelle entre les colonies, représente en effet un risque de dissémination d'agents infectieux. Ces déplacements de prospection peuvent notamment être réalisés par de jeunes individus immatures (pré-reproducteurs) mais également des individus reproducteurs, en échec de reproduction (Cadiou et al. 1994; Ponchon et al. 2017), ces derniers étant particulièrement nombreux dans le contexte de mortalités massives de poussins sur l'île d'Amsterdam et surtout en contact rapproché avec les pathogènes responsables de ces mortalités. Contrairement aux individus en succès de reproduction, qui ne se déplacent probablement qu'entre leur site de nidification et la haute mer afin de s'alimenter, les individus en échec de reproduction ou les immatures pourraient jouer un rôle dans la dissémination d'agents pathogènes s'ils devaient visiter des zones de reproduction (Boulinier et al. 2016). Notre objectif principal a donc été de déterminer si de tels déplacements entre colonies ou sous-colonies pouvaient être détectés et quantifiés chez une espèce très fortement fidèle à son site de reproduction tel que l'YNA, et si ces déplacements étaient effectivement majoritairement effectués par les individus immatures ou en échec de reproduction, et non par des individus en succès de reproduction.

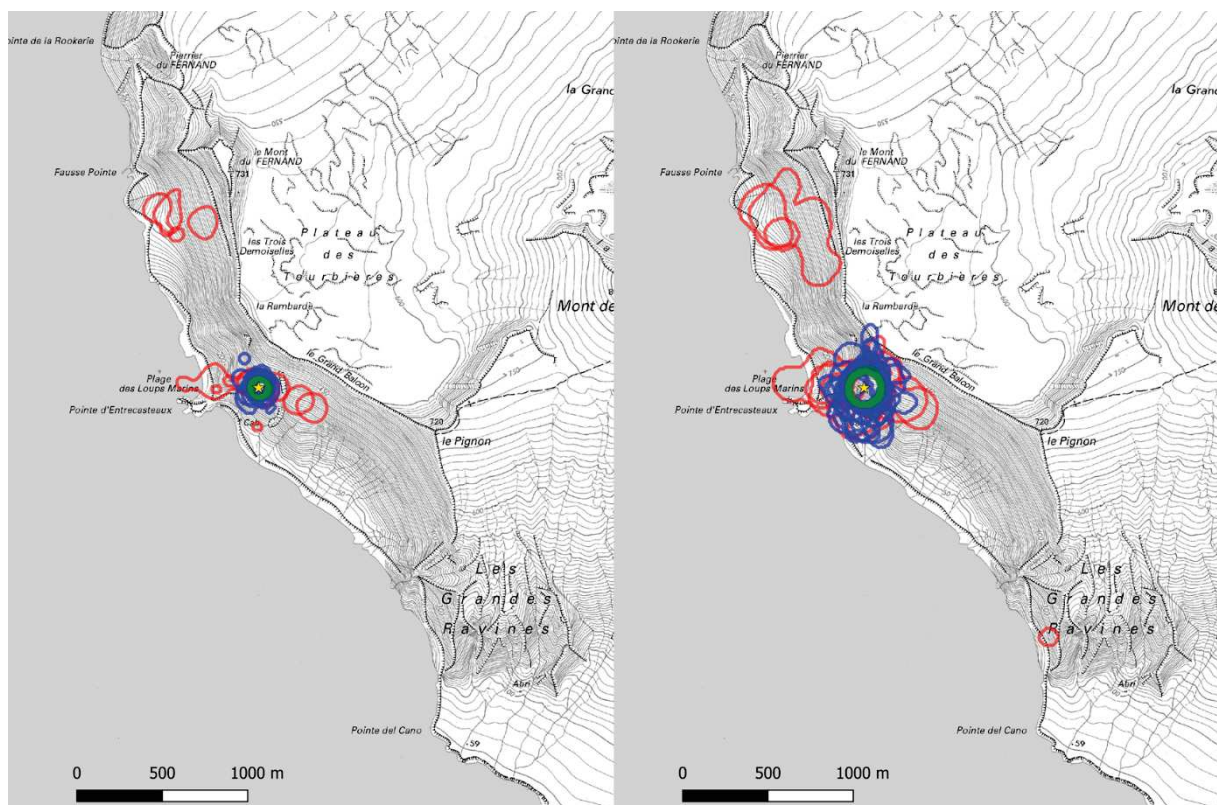
Les résultats obtenus montrent que les individus immatures âgés de 4 ans visitent<sup>9</sup> de façon significative plus de sites différents que les autres groupes d'individus (**Table 1**), avec une moyenne de 15 sites visités par individu, contre 3.5 et 1.7 respectivement pour les individus en échec et succès de reproduction (les individus de 5 et 6 ans ayant des comportements proches des individus en échec de reproduction). De plus, les individus de 4 ans visitent de façon significative des sites plus espacés que les autres groupes, avec une distance moyenne entre les sites de plus de 1300 mètres contre 105 et 33 mètres pour respectivement les individus en échec et en succès de reproduction. L'aire utilisée par les individus de 4 ans (basée sur l'analyse d'intensité de Kernel) est également supérieure, avec une moyenne, pour l'utilisation principale (core area 50%), de plus de 63.000 m<sup>2</sup> contre 15.000 et 8.000 m<sup>2</sup> pour respectivement les individus en échec et en succès de reproduction (**Figure 20**). Les résultats suggèrent également que dans une moindre mesure, les individus en échec de reproduction visitent en moyenne plus de sites que les individus en succès de reproduction, avec des distances entre les sites et une aire d'utilisation de l'habitat sensiblement supérieures.

**Table 1:** Distance moyenne entre les sites visités et nombre de sites différents visités pour les individus des 3 statuts considérés, SB : individus en succès de reproduction, FB : individus en échec de reproduction et PB : immatures, 4y, 5y et 6y âgés respectivement de 4 ans, 5 ans et 6 ans. n est le nombre total d'individus suivis. Tous les groupes sont significativement différents du groupe PB 4y ( $p < 0.01$ ).

Status	Age	n	distance between sites (m)		nb sites visited	
			mean	sd	Mean	sd
PB	4y	4	1,319	795	15	8.21
PB	5y	2	80.5	16.3	3	1.41
PB	6y	7	261	349	3.86	2.19
FB	adult	27	105	84	3.52	2.33
SB	adult	19	33	61.4	1.74	1.28

Si nous considérons que l'aire utilisée par un individu reflète le nombre d'individus susceptibles d'être en contact avec celui-ci, la distance entre les sites et le nombre de sites reflètent la capacité d'un individu à disperser un pathogène dans l'espace, nous pouvons supposer que les jeunes individus, et de manière plus minoritaire les individus en échec de reproduction, peuvent jouer un rôle important en tant que disséminateur de pathogènes et que le facteur spatial est un élément important en prendre en considération pour un plan de vaccination. Cette observation est néanmoins à pondérer par

<sup>9</sup> Une visite est estimée dans l'étude comme un individu s'étant posé pendant au moins 15 minutes dans une zone d'un rayon de 20 mètres (correspondant à la précision du GPS)



**Figure 20** : Mouvements à petite échelle basés sur un noyau de cinquante pour cent (à gauche) et de quatre-vingt-dix pour cent (à droite) de l'intensité d'utilisation de l'habitat. Vert : reproducteurs ayant réussi ; bleu : reproducteurs ayant échoué ; rouge : pré-reproducteurs ; étoile jaune : centre de la colonie d'étude où les oiseaux ont été équipés.

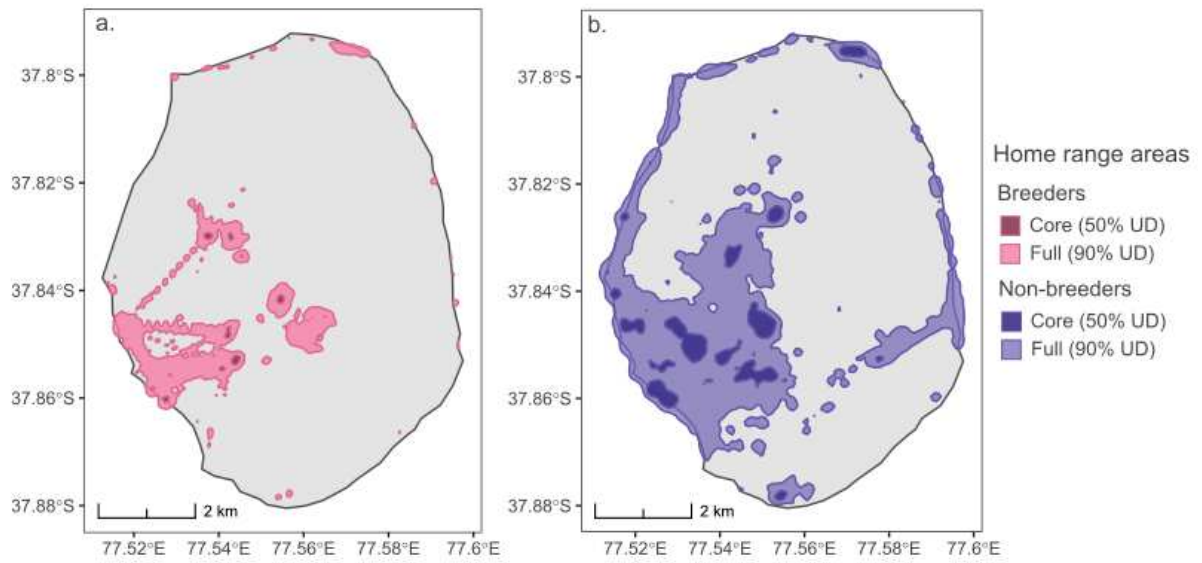
le fait que ces individus sont peut-être moins exposés aux agents infectieux car ils fréquentent moins assidument un nid. Les comportements en mer des différents groupes, pourraient aussi jouer un rôle dans la transmission d'agent pathogènes, entre individus de la même espèce ou d'espèces différentes. Ces résultats font l'objet d'un manuscrit en cours de révision (Ponchon et al, accepted sept 2021, annexe 4).

L'étude de la circulation des agents infectieux à une échelle plus globale, au sein et entre les différentes espèces présentes, est un enjeu particulier étant donné le contexte de l'île. Les déplacements des IYNA sont essentiels à considérer mais d'autres hôtes peuvent représenter des ponts potentiels entre les différentes zones de reproduction des IYNA mais aussi avec les autres espèces. Dans cette dernière partie, nous avons porté une attention particulière aux déplacements des labbes subantarctiques (SKU). En effet, le comportement alimentaire de cette espèce, à la fois prédatrice et charognard, la conduit à consommer des cadavres de poussins d'IYNA, mais aussi d'autres espèces, potentiellement morts des suites d'une infection létale, et la rend donc tout particulièrement à risque d'être exposée

à *Pm* (Boulinier et al. 2016). Cette espèce représente donc un hôte potentiel d'intérêt et l'étude de son comportement est un élément essentiel pour la compréhension de la propagation d'agents infectieux sur l'île d'Amsterdam.

En combinant différentes approches, incluant de la sérologie, de la biologie moléculaire ainsi que des suivis de déplacements de SKU reproducteurs par pose de GPS, la première étude que nous avons menée sur des individus reproducteurs nous a permis de montrer que cette espèce pourrait jouer un rôle de disséminateur entre différentes espèces, incluant les IYNA, les SOA et les NRP, mais également les otaries (Gamble et al 2020, Annexe 5). Cependant, les résultats obtenus ne suggéraient pas que les SKU représentaient un risque majeur pour les AMA, les individus suivis ne fréquentant que très peu la zone de reproduction des AMA, malgré la proximité géographique de leurs zones de reproduction respectives, sur le Plateau des tourbières.

Néanmoins, cette première étude ne concernait que des individus reproducteurs, capturés, sur des territoires de reproduction situés en marge sud de la zone du Plateau des tourbières, à cause des contraintes d'accès à certaines zones (Annexe 5). Dans un deuxième temps, nous nous sommes donc intéressés aux déplacements d'une gamme plus large d'individus en effectuant les captures sur des repaires fréquentés par plusieurs catégories d'individus, notamment des SKU reproducteurs et non reproducteurs. Nous avons mis en évidence des différences de comportements assez nettes entre individus en relation avec leur attachement avec un territoire de reproduction au cours de la saison (Lamb et al, en préparation, annexe 6). Plus particulièrement, les résultats suggèrent que les individus non reproducteurs sont plus mobiles et que leurs interactions avec les différentes espèces d'oiseaux marins et les otaries de l'île sont plus importantes que les individus reproducteurs. Les résultats confirment également que les individus reproducteurs n'utilisent que très peu les zones de reproduction des AMA. Cependant, les individus non reproducteurs semblent utiliser de façon plus importante ce secteur (**Figure 21**), faisant d'eux un risque potentiel de pont d'agents infectieux vers cette espèce. Les suivis sérologiques montrent que près de 100% des individus équipés, reproducteurs ou non, sont séropositifs aux anticorps anti-*Pm*, confirmant que cette espèce est particulièrement exposée à *Pm* (Annexe 5 et 6). Les SKU, peuvent représenter un pont épidémiologique majeur pour les autres espèces de l'île Amsterdam, et doivent être considérés dans les mesures de conservation prises. Un effort de vaccination de cette espèce pourrait par exemple être envisagé afin de réduire la potentielle dissémination de l'agent pathogène et éventuellement contribuer à une immunité globale.



**Figure 21** : Domaines vitaux centraux (zones sombres) et totaux (zones claires) des SKU reproducteurs (rose) et non reproducteurs (bleu) sur l'île Amsterdam, 2017-2020. D'après Lamb et al, in prep, ©Juliet Lamb

# Discussion





L'ensemble des travaux menés dans cette thèse a permis de fournir une variété de résultats importants pour la conservation des albatros sur l'île Amsterdam. Nous avons montré dans un premier temps qu'une diversité de pathogènes étaient impliqués dans les mortalités récurrentes survenant dans les populations de trois espèces d'oiseaux marins en danger d'extinction se reproduisant sur l'île d'Amsterdam, deux espèces d'albatros (IYNA et SOA) et une espèce de gorfou (NRP), en particulier une souche de *Pasteurella multocida* (Annexe 1). Nos travaux expérimentaux sur le long terme ont permis de mettre en évidence que la vaccination contre cette souche était une solution prometteuse, mais dont les résultats peuvent encore être améliorés, en partie à cause de l'impact négatif fort des rats, présents en abondance et actifs comme prédateur, charognard et sans doute responsable d'une amplification de la transmission de la maladie et de ses effets (Annexe 2). Nos essais de vaccination des poussins de IYNA suggèrent que l'âge de vaccination est un facteur important à prendre en compte, mais que des campagnes de vaccination sur des créneaux de dates resserrés (pour une vaccination de la majorité des poussins autour de 10 jours) permettraient de protéger un nombre significatif de poussins. La nouvelle formulation du vaccin, dite 'water in oil' induit une meilleure persistance des anticorps chez les adultes jusqu'à au moins trois ans après vaccination. Les anticorps maternels ont été détectés chez les jeunes IYNA une année après vaccination des mères et les résultats suggèrent un effet protecteur deux années après la vaccination des femelles. La découverte d'une nouvelle souche de *Pm* a permis de produire un autovaccin bivalent, incluant les 2 souches de *Pm* connues, montrant un effet protecteur durant l'année d'expérimentation. Enfin, nos travaux ont permis de mettre en évidence l'importance de considérer les déplacements des hôtes potentiels dans la dissémination des agents infectieux. Nous avons montré que les jeunes individus pré-reproducteur de IYNA étaient susceptibles de visiter différentes parties des colonies de leur congénères et pourraient ainsi favoriser la circulation de pathogènes au sein de l'île (Annexe 3). De plus, nos résultats montrent que les labbes (SKU) sont fortement exposés à *Pm* et que leurs activités pour s'alimenter les amène à se déplacer d'une façon active au sein et entre les groupes de reproducteurs de plusieurs espèces, incluant, mais dans une mesure limitée, la petite population endémique de l'Albatros d'Amsterdam (AMA) (Annexes 4 et 5) ; ils sont donc susceptibles de jouer un rôle clé dans la circulation des agents infectieux.

Il est toutefois important de souligner qu'au sein de chaque année, les nombres d'individus dans les différents traitements ont été limités à quelques dizaines. Un tel effort nous a néanmoins paru raisonnable étant données les effets attendus et les contraintes éthiques concernant l'expérimentation sur les animaux, ce type de vaccins n'ayant jamais été utilisés dans de telles

conditions, en population naturelle et chez ces espèces sauvages. Le travail sur une seule zone limite la possibilité de généralisation des résultats, mais le fait que les individus des différents groupes de traitement étaient mélangés aléatoirement au sein de cette zone permet d'être confiant dans la valeur des résultats obtenus. Le fait que nous ayons pu suivre les dynamiques de réponse en anticorps des différentes catégories d'individus est aussi un élément important. Il est à noter que les traits d'histoire de vie de l'espèce considérée, avec sa forte longévité, sa forte fidélité au site de reproduction et le fait que les individus puissent être facilement capturés à la main, ont permis l'obtention de données exceptionnelles, à la fois au sein des saisons de reproduction (suivi de la dynamique de réponse en fonction de l'âge par exemple), mais aussi entre saisons de reproduction (suivi de la persistance des anticorps sur plusieurs années par exemple).

Nos travaux confirment donc le rôle important des agents infectieux, et notamment de *Pasteurella multocida*, responsable du choléra aviaire, dans les mortalités massives de plusieurs espèces menacées de l'île Amsterdam. La souche de *Pasteurella multocida*, nommée D2C et connue historiquement (Jaeger et al. 2018), apparaît comme étant le pathogène majoritairement impliqué dans les mortalités massives de poussins d'albatros à bec jaune. Néanmoins, un suivi rigoureux des mortalités a permis l'identification d'une seconde souche de *Pm*, non détectée précédemment sur l'île et non décrite jusqu'alors dans le système de classification utilisé (multi-host MLST). Le suivi a permis également de confirmer la présence d'*Erysipelothrix rhusiopathiae* et son rôle, bien qu'apparaissant plus minoritaire, dans les mortalités de jeunes oiseaux. Les travaux sur la description des souches apportent des éléments fondamentaux qui permettent de mieux décrire la situation éco-épidémiologique sur l'île. Mais l'identification de ces souches permet également d'enrichir nos connaissances sur la diversité des pathogènes de façon beaucoup plus globale. La découverte d'un nouveau clade chez *Er* est en particulier une découverte intéressante dans le contexte de ce qui est connu dans le domaine (Forde et al. 2016). La poursuite des travaux sur ces deux agents, par des analyses de génomique sur les souches isolées, devrait donc permettre d'obtenir de nouveaux résultats importants. Il est à noter que ces bactéries d'intérêt vétérinaires sont responsables de zoonoses.

La découverte d'une nouvelle souche de *Pm* a permis la mise à jour du vaccin, qui n'était initialement produit que contre la souche D2C historiquement connue. Cet autovaccin a montré des résultats prometteurs. Les autovaccins ne permettent pas toujours une protection croisée contre plusieurs souches de la même espèce bactérienne, et cela peut être particulièrement le cas chez *Pm* (Omaleki et al. 2020). Il est donc primordial d'assurer un suivi des mortalités afin de surveiller l'émergence

potentielle de nouvelles souches ou de nouveaux pathogènes, et de continuer les essais au cours des années à venir. Les travaux de Omaleki et al (2020) suggèrent également que les approches usuelles de caractérisation des souches, notamment par MLST, ne permettent pas toujours une identification assez fine et que des approches moléculaires plus fines, tel que le séquençage total ou la caractérisation des SNP, sont nécessaires. Il serait donc important dans la suite des travaux d'utiliser ces approches pour explorer la variabilité spatiale et temporelle de l'exposition des individus, notamment des différentes espèces hôtes présente sur l'île. Ceci est d'autant plus important que la vaccination, si elle était appliquée massivement, pourrait éventuellement favoriser l'émergence et la circulation d'autres souches, éventuellement présentes mais minoritaires par exemple (Martcheva et al. 2008; Omaleki et al. 2020). La description des potentielles co-infections et la prévalence de chaque souche dans l'espace et dans le temps sont des paramètres indispensables à prendre en compte afin d'apprécier au mieux le risque de remplacement de souches (Martcheva et al. 2008). Une approche par modélisation, couplée aux données sur les souches détectées, sera nécessaire pour cela.

La description des souches est également importante pour des raisons philosophiques et éthiques. La mise en place d'une vaccination à grande échelle comme mesure de conservation de la biodiversité serait plus ou moins justifiée en fonction de l'origine des souches. L'origine naturelle ou anthropogénique des souches sera notamment une question à soulever afin de justifier ou non l'intervention humaine dans la conservation de ces espèces. Des travaux de génétique des populations, permis par un échantillonnage adéquat, pourrait alors apporter des éléments importants. Si la souche D2C caractérisée par le schéma MLST a déjà été décrite sur un canard en Europe, suggérant une introduction due aux activités humaines, A18Y007B1 et la souche de *Er* ne sont pas connus dans les systèmes de classifications utilisés. Bien qu'absents des bases de données actuelles disponibles, il n'est pas possible à ce stade d'exclure que ces pathogènes ont été introduits dans l'écosystème par des espèces amenées par l'homme. Les travaux complémentaires sur l'analyse du génome des différentes souches isolées sur le terrain, et de nouvelles souches susceptibles d'être isolées chez les mammifères de l'île, pourront permettre de mieux les situer par rapport aux autres souches conspécifiques et ainsi apporter des éléments de réponse quant à leur origine naturelle ou anthropogénique. Il est à noter que si *Pm* n'a pour l'instant pas pu être isolée à partir de prélèvements effectués par l'équipe sur des rats et souris sur l'île d'Amsterdam, la totalité (7/7) des chats de l'île Amsterdam sur lesquels des écouvillons buccaux ont été réalisés en 2020 se sont révélés porteurs de *Pm*. La caractérisation de ces isolats a néanmoins permis d'identifier 2 nouvelles souches de *Pm*, différentes des D2C et A18Y007B1, appartenant de plus à la sous espèce *P. m. sceptica*.

Bien que les essais de vaccination soient prometteurs, ils ne sont pas encore pleinement concluants. La vaccination des poussins d'IYNA permet en effet d'améliorer significativement leur survie certaines années, multipliant par 2 ou plus le nombre de poussins à l'envol. Cependant, le taux global de survie des poussins jusqu'à l'envol reste faible, ne dépassant pas 50% pour les poussins vaccinés et 20% pour les témoins (

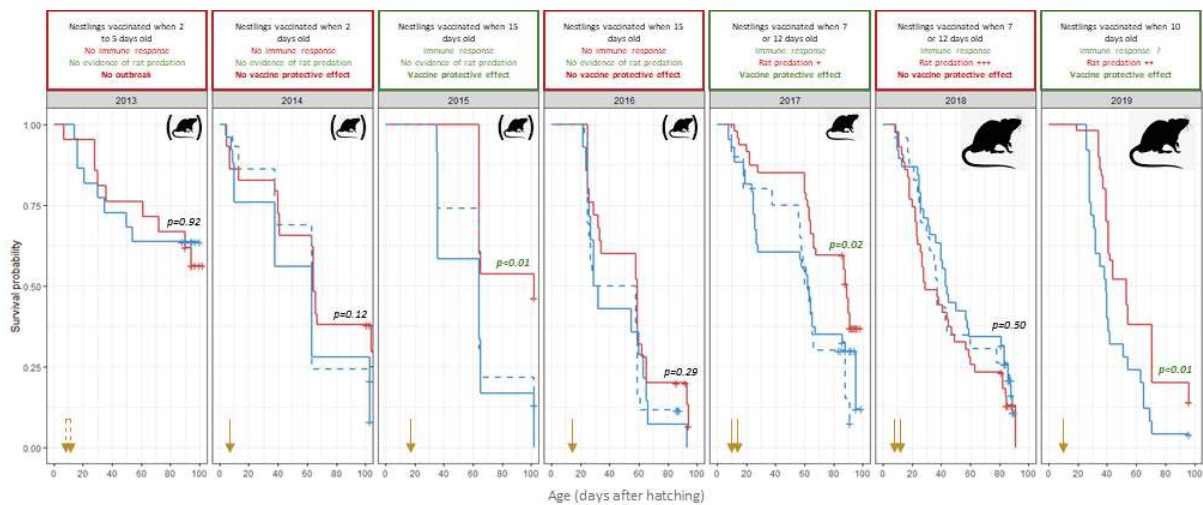
**Figure 22)** alors que des taux supérieurs à 85% pourraient être attendus en condition optimale pour cette espèce (Cuthbert et al. 2003). Ce plus faible taux de survie pourrait être expliqué par la présence des rats qui sont en particulier suspectés d'être responsables d'une partie des mortalités chez les poussins d'IYNA, mais aussi très probablement de SOA et NRP. Leur rôle dans les mortalités peut intervenir directement, par prédation ou morsures mortelles des jeunes, mais également indirectement avec une contribution dans la circulation et la transmission des pathogènes. En effet, l'exposition et la quantité relative locale du pathogène sont des facteurs pouvant limiter l'efficacité de la vaccination (Langwig et al. 2019) et les rats pourraient jouer le rôle à la fois de réservoir de *Pm*, maintenant dans le temps et l'espace de fortes concentrations du pathogène, mais aussi jouer un rôle clé dans sa circulation en se déplaçant entre et au sein des colonies et en contaminant d'autres hôtes par contact ou morsures. Des travaux préliminaires suggèrent notamment que les rats seraient fortement exposés à *Pm* (d'après des analyses PCR et sérologiques ; résultats non publiés), principalement lorsqu'ils sont capturés dans les colonies de reproductions pendant les épisodes de mortalités. Dans le cadre d'un projet de restauration des écosystèmes, la RNN-TAF prévoit d'éradiquer le rat brun, ainsi que la souris et le chat, de l'île Amsterdam dans les prochaines années (début de l'éradication à large échelle prévue à l'automne 2023). En assurant la poursuite des suivis éco-épidémiologiques et des essais de vaccination durant et après l'éradication, il sera possible d'obtenir des éléments clés pour mieux comprendre le rôle des rats. En particulier, les suivis de survie permettront de mieux comprendre les interactions possibles entre la mortalité directe provoquée par prédation et la mortalité indirecte due à la circulation du pathogène par les rats. Nous pouvons par exemple envisager 3 scénarios pouvant se produire après l'éradication (**Figure 23)** :

- (i) Les rats sont responsables uniquement de mortalités dues à de la prédation directe. Dans ce cas, l'éradication des rats devrait améliorer la survie des témoins et des individus vaccinés dans les mêmes proportions (effet additif)
- (ii) Les rats, en plus de causer de la mortalité directe, contribuent à la circulation de *Pm*, augmentent l'exposition des hôtes et diminuent l'effet de la vaccination (Langwig et al.

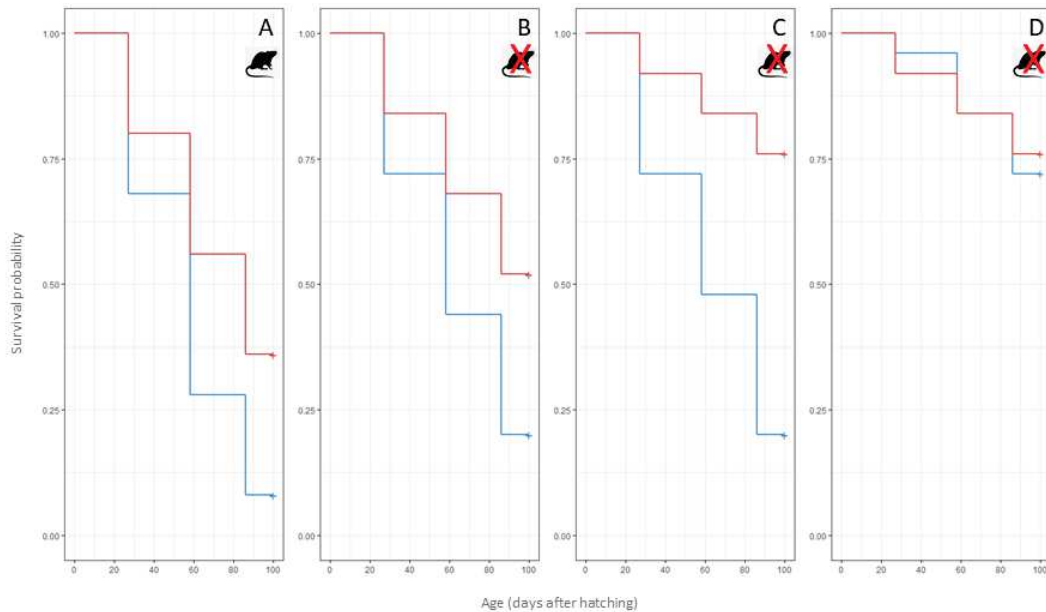
2019). Après l'élimination des rats, la survie des individus vaccinés serait alors d'autant plus améliorée par rapport à celle des témoins (interactions d'un effet des rats et de *Pm*)

- (iii) Les rats sont responsables de la quasi-totalité des mortalités, directes ou indirectes, étant responsables à eux seuls de la prédation, du maintien de *Pm* dans le milieu et de sa circulation. Dans ce derniers cas, l'élimination des rats conduirait à une survie améliorée mais identique pour les individus vaccinés ou témoins (effet confondant)

L'éradication des rats pourrait permettre également de disposer d'une situation bien meilleure pour évaluer le rôle éco-épidémiologique du transfert d'anticorps maternels. Bien qu'important pour les perspectives de mesures de gestion pour la conservation des espèces impliquées, ces travaux sont également fondamentaux pour la compréhension du rôle des anticorps maternels et leur potentiel effet protecteur dans des populations naturelles en général (Garnier et al. 2012). Si des travaux suggèrent un effet protecteur en condition de laboratoire (Gomez-Chamorro et al. 2019), aucune approche expérimentale en milieu naturel n'a montré cela à notre connaissance.



**Figure 22:** Suivi de l'effet protecteur apporté par le vaccin depuis le début des essais de vaccination (saison 2013-2014) et jusqu'à la dernière saison complète suivie (2019-2020). Courbes rouges : survie des poussins vaccinés, courbes bleues : groupes témoins (traits pleins pour les poussins manipulés, traits pointillés pour les poussins non manipulés). Les flèches indiquent l'âge de la vaccination. Le symbole représentant un rat entre parenthèse signale la présence des rats mais sans circulation ou prédation importantes observées. Les rats sans parenthèses indiquent la présence des rats avec une circulation et une prédation observées de façon flagrante, l'importance de la prédation observée étant proportionnelle à la taille du symbole. Lors des saisons 2013-2014 et 2014-2015, les poussins ont été vaccinés trop jeunes, ce qui n'a induit une réponse immunitaire quantifiable, ni un effet protecteur. Lors de la saison 2016-2017, bien que vaccinés plus tardivement, aucune réponse immunitaire n'a été observée, probablement dû à un problème de conservation du vaccin lors du transport, l'ayant rendu inefficace. Lors des saisons 2015-2016, 2017-2018, 2018-2019 et 2019-2020, les poussins ont montré une réponse immunitaire et la vaccination a été associée à un effet protecteur 3 saisons sur 4. La forte prédation des rats et la détection d'une autre souche de *Pm* lors de la saison 2018-2019 pourrait expliquer l'absence de détection d'un effet protecteur du vaccin cette année.



**Figure 23** : Prédiction de l'effet de la vaccination après l'éradication des rats selon différents scénarios possibles quant au rôle des rats dans les mortalités des poussins d'IYNA. Courbes rouges : survie des poussins vaccinés, courbes bleues : survie des poussins non vaccinés. A – Situation actuelle avec la présence des rats. B – scénario (i), effet additif : les rats étaient responsables uniquement de prédation directe. C – scénario (ii), interaction des effets : les rats, en plus de la prédation directe, jouaient un rôle dans la circulation et la transmission de *Pm*. D – scénario (iii), effet cumulatif : la circulation et la transmission de *Pm* n'étaient uniquement due qu'aux rats et ces derniers étaient responsables de l'ensemble des mortalités, directes ou indirectes.

Cette étude souligne également le rôle potentiel joué par les différents hôtes. Les déplacements d'individus au sein et entre les colonies sont des facteurs essentiels à prendre afin de planifier des campagnes de vaccination à grande échelle. Nos résultats suggèrent notamment que les différentes colonies, mais aussi les différentes espèces de l'île ne sont pas compartimentées. Des ponts existent entre groupes de reproducteurs, par les déplacements d'individus conspécifiques pouvant prospecter au sein de différents sites de reproduction, mais également par les déplacements d'hôtes prédateurs ou charognards, tel que le labbe subantarctique qui visite de nombreuses colonies d'espèces différentes et spatialement éloignées.

Les résultats obtenus permettent d'envisager différents scénarios de dynamiques éco-épidémiologiques et d'efficacité de stratégies de vaccination. Pour cela, différentes approches de modélisation pourraient être utilisées. Les stratégies vaccinales pourraient notamment être évaluées en utilisant des modèles basés sur les réseaux de transmission d'agents infectieux qui peuvent exister entre et au sein des espèces (Craft et al. 2008; Woodroffe and Donnelly 2011; Robinson et al. 2018). Le choix des individus à vacciner pourraient notamment être exploré. Si les IYNA semblent être très

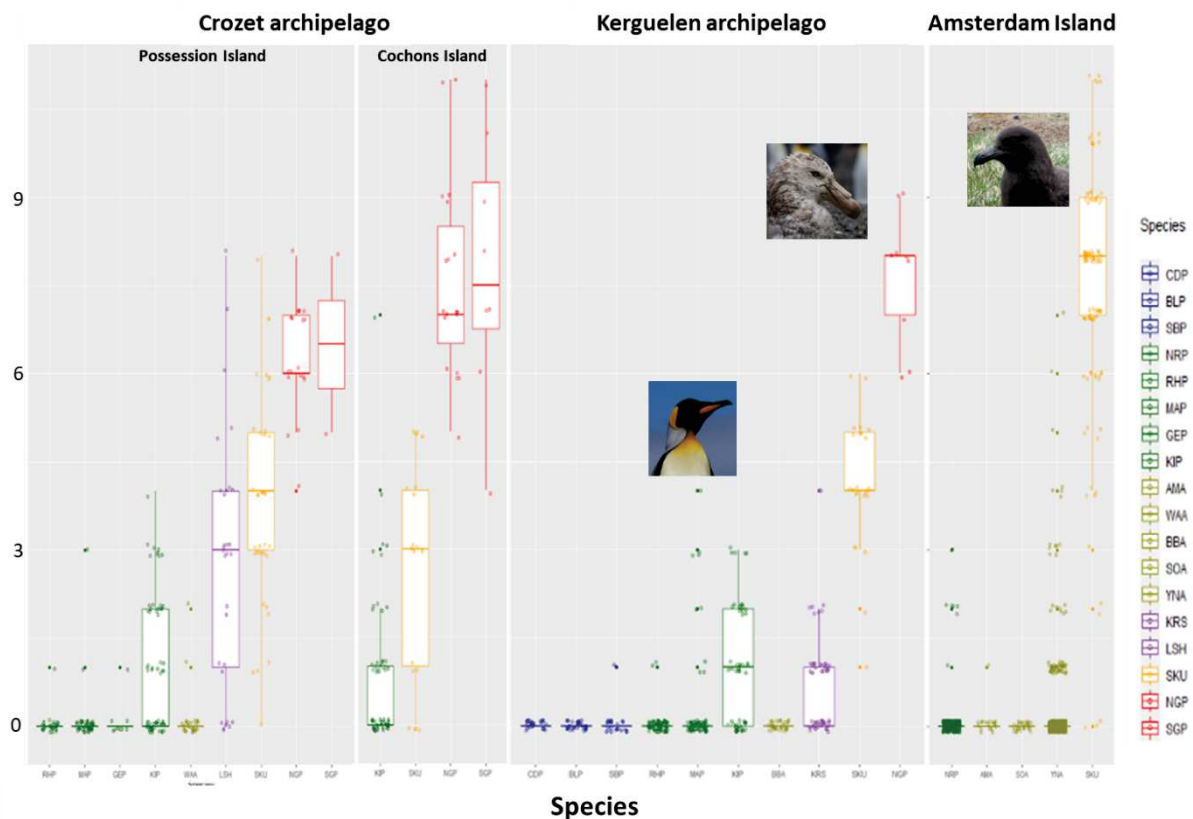
impactés par les pathogènes, la stratégie vaccinale pourrait également se concentrer sur des individus fortement disséminateurs, tels que les SKU, qui bien que non impactés par les fortes mortalités, jouent un rôle probable dans la transmission et le maintien d'agents pathogènes. La population des SKU présente d'autant plus l'avantage d'être petite, ne comptant que quelques centaines d'individus. Il faudrait néanmoins déterminer dans quelle mesure la vaccination pourrait diminuer les risques de dissémination par les SKU, sachant qu'une partie de la dissémination doit se faire via des contacts directs lors des activités d'alimentation (transport de cadavres ou de restes de poussins, contacts avec le bec des individus s'étant alimenté sur des cadavres). La vaccination de la petite population d'Albatros d'Amsterdam pourrait également présenter un intérêt : cette population n'est généralement pas touchée par les mortalités associées au choléra aviaire (une mortalité anormale a cependant été observée lors des années 2000 et 2001 ; Jaeger et al. 2018), mais elle est à risque, en particulier étant donnée sa petite taille : une très forte couverture vaccinale pourrait notamment être obtenue pour les poussins, étant tous manipulés chaque année dans le cadre du suivi démographique. Le choix des population à vacciner va également dépendre de la capacité du vaccin à réduire la transmission de l'agent pathogène, à réduire la probabilité d'être infecté, ou encore à éliminer l'agent pathogène (Barnett and Civitello 2020).

L'émergence des maladies infectieuses est un problème pour la conservation (Daszak et al. 2000; Wobeser 2002; Young et al. 2017). Plus qu'un problème de conservation d'espèce menacées, les maladies infectieuses représentent également une menace pour la santé publique (Smith et al. 2006; Jones et al. 2008). La surveillance sanitaire des populations sauvages est donc un élément essentiel afin de prévenir et agir de façon efficace. Depuis quelques années à présent, la RNN-TAF collabore avec notre équipe et le réseau SAGIR (Office Français de la Biodiversité) afin d'assurer une surveillance des mortalités suspectes dans les différents districts. Le suivi d'espèces sentinelles est également une approche efficace afin de répondre à des questions de surveillance éco-épidémiologiques (Halliday et al. 2007) et notamment les suivis sérologiques de ces espèces (Gamble, Ramos, et al. 2019). Le SKU, de par son comportement alimentaire, pourrait ainsi représenter une espèce sentinelle sur l'île Amsterdam, mais aussi à plus large échelle dans tous les districts des TAAF, voire des îles subantarctiques dans le monde. Des résultats préliminaires sur les données sérologiques (**Figure 24**, non publiés) suggèrent notamment que cette espèce serait très largement exposée à *Pm* sur tous les districts de la RNN-TAF, même si aucune épidémie de grande ampleur n'a été détectée ailleurs que sur l'île Amsterdam et que les titres en anticorps mesurés sont plus faibles. Ces résultats soulèvent des

questions importantes quant à la situation éco-épidémiologique à plus large échelle, des épizooties de choléra aviaires ayant été décrites en Antarctique et sur l'île Marion (Leotta et al. 2006, Cooper et al. 2009). Les résultats des analyses sérologiques de plusieurs autres espèces permettent de plus de mettre en évidence que certaines sont exposées à *Pm* sur les différentes îles de la RNN-TAF (**Figure 24**).

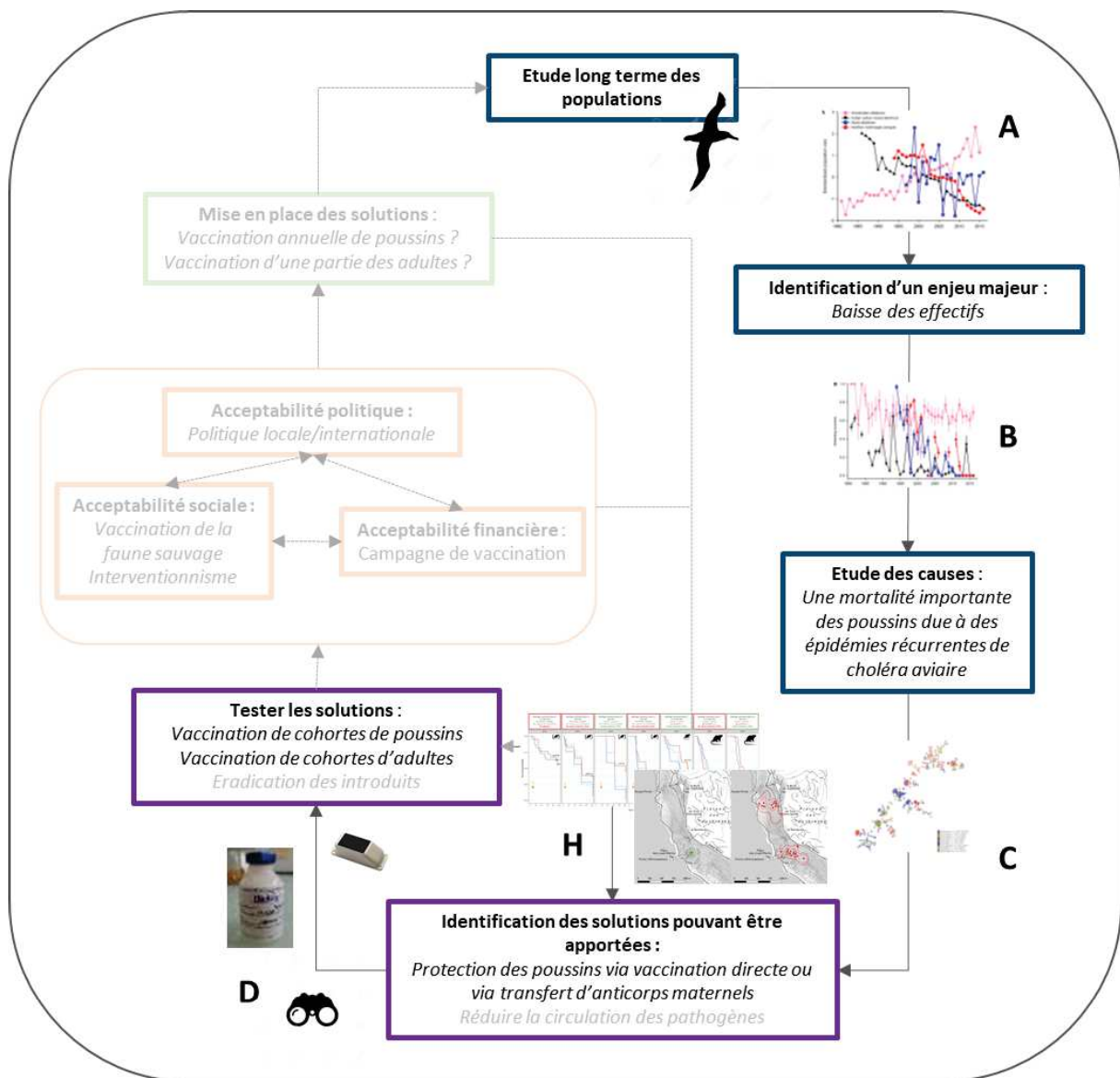
Cette étude met donc en évidence les difficultés auxquelles qui peuvent être rencontrées lors de la programmation et de la mise en œuvre d'un plan de vaccination, de surcroît lorsqu'une protection à grande échelle est requise dans une population sauvage. Si des étapes apparaissent essentielles, tel que la production de la bonne formulation, la composition ou encore l'innocuité du vaccin, de nombreux autres paramètres nécessitent d'être pris en compte pour maintenir l'efficacité d'un plan de vaccination dans le temps et l'espace. Des variants, des souches ou autres évolutions peuvent apparaître et rendre rapidement inefficace un vaccin (Koyama et al. 2020; Omaleki et al. 2020). Les déplacements individuels, les paramètres géographiques, météorologiques ou démographiques sont autant de paramètres essentiels à considérer dans une approche vaccinale (Haydon et al. 2006; Rees et al. 2013; Barnett and Civitello 2020). La crise sanitaire mondiale que nous vivons actuellement face à la maladie de la Covid-19 est un exemple évident de la complexité de tels programmes. Malgré la mobilisation internationale de scientifiques, politiques et industriels, l'évaluation de l'efficacité de vaccins reste difficile (Olliaro et al. 2021) alors que 96 vaccins sont actuellement à l'essai.





**Figure 24** : Résultats préliminaires suggérant l'exposition de différentes espèces d'oiseaux marins à *Pm* au sein des différentes îles de la RNN-TAF, archipels de Crozet, Kerguelen et île Amsterdam. Les titres d'anticorps anti-*Pm* sont mesurés par MAT et affichés en échelle logarithmique. On peut notamment noter que le labbe subantarctique (SKU, jaune) et les pétrels géants (NGP, rouge, et SGP, orange), autres espèces au comportement prédateur et charognard présentent des titres élevés ; les manchots (KIP, RHP, MAP, GEP, NRP, vert) présentent des titres globalement faibles ; un charognard non migrateur (LSH) présente des titres élevés sur Crozet. L'interprétation de tels résultats ouvre des perspectives importantes (Boulinier, Gamble, Tornos, Gantelet et al. Non publié). Liste des espèces : CDP = Common Diving petrel (*Pelecanoides urinatrix*), BLP = Blue Petrel (*Halobaena caerulea*), SBP = Slender Billed Petrel (*Pachyptila belcheri*), NRP = Northern Rockhopper penguin (*Eudyptes moseleyi*), RHP = Rockhopper penguin (*Eudyptes chrysocome*), MAP = Macaroni Penguin (*Eudyptes chrysolophus*), GEP = Gentoo Penguin (*Pygoscelis papua*), KIP = King Penguin (*Aptenodytes patagonicus*), WAA = Wandering Albatross (*Diomedea exulans*), BBA = Black Browed Albatross (*Thalassarche melanophris*), KRS = Kerguelen Shag (*Leucocarbo verrucosus*), LSH = Lesser Sheathbill (*Chionis minor*), NGP = Northern Giant Petrel (*Macronectes halli*) et SGP = Southern Giant Petrel (*Macronectes giganteus*)

Dans cette thèse nous avons montré la nécessité d'intégrer de nombreuses approches dans les mesures de conservation et pour aborder les dynamiques éco-épidémiologiques. Ce travail n'a pas pour prétention d'apporter de solution définitive à ce stade car de nombreux aspects sont encore à explorer. Néanmoins, comme illustré dans la **Figure 25**, nos travaux apportent des éléments clés nécessaires à la compréhension des processus infectieux sur l'île Amsterdam et fournissent donc des éléments de réflexion quant à la mise en œuvre de mesures de conservation. Plus spécifiquement, la **Figure 25** illustre comment se positionne les résultats obtenus dans le cadre de cette thèse dans un schéma général d'une approche intégrée de la conservation de populations d'oiseaux marins affectées par un agent pathogène. Des approches de modélisation permettront d'explorer les intérêts relatifs et conséquences potentielles des différentes mesures de gestion envisageables. Ces approches pourraient notamment mettre en évidence les lacunes qui persiste dans notre connaissance du système et donc de pouvoir anticiper les prochaines orientations de recherche (Restif et al. 2012). Les approches de modélisation permettraient de plus de prévoir les stratégies vaccinales pertinentes (Haydon et al. 2006; Garnier et al. 2012).



**Figure 25** : Principes fondamentaux de la science de la conservation appliqués à nos travaux sur la compréhension des processus infectieux. A - Les suivis long terme des populations ont pu mettre en évidence une baisse des effectifs et un faible succès de reproduction sur plusieurs espèces sur l'île Amsterdam (Weimerskirch 2004; Rolland et al. 2009; Jaeger et al. 2018). B – Des agents infectieux sont responsables des fortes mortalités des poussins de IYNA mais aussi de NRP (Weimerskirch 2004; Jaeger et al. 2018; Jaeger et al. 2020). C – Différentes souches des agents infectieux ont pu être caractérisées génétiquement (Jaeger et al. 2018, Annexe 1). D – Suite à de premiers essais (Bourret et al. 2018, Annexe 7), plusieurs campagnes de vaccinations avec un autovaccin produit à partir de souches isolées sont en cours d'essais. En parallèle, des études sur l'écologie des hôtes et des pathogènes sont menées afin de fournir des éléments essentiels à la mise en place de mesures de conservation (Annexe 2 à 6). H – Les suivis long terme et les essais de vaccination sur plusieurs années permettent d'optimiser le protocole de vaccination. Les informations concernant le déplacement des hôtes permettront d'optimiser les stratégies de vaccination.

Les parties transparentes représentent les phases restant à explorer. Notamment la partie sociale et financière d'une approche de conservation par vaccination. Le futur plan d'éradication des espèces introduites apportera également des éléments clés.

La combinaison d'approches et de disciplines diverses est ici essentielle, tant sur le plan fondamental qu'appliqué.

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## Annexe 1

Not only a single strain of *Pasteurella multocida* kills albatrosses on Amsterdam Island: insight into a community of bacterial pathogens affecting threatened seabirds.

**Tornos Jérémy**, Forde Taya, Clessin Augustin, Bost Caroline, Lemberger Karin, Le Net Rozenn, Fléchet Aline, Gantelet Hubert, Gamble Amandine & Boulinier Thierry.

*In preparation.*



**Not only a single strain of *Pasteurella multocida* kills albatrosses on Amsterdam Island: insight into a community of bacterial pathogens affecting threatened seabirds**

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## Abstract

Seabirds are widely threatened due to several factors, especially fishing activities, invasive species, pollution and climate change. Albatrosses and penguins in particular are species that are concerned by those threats at broad scales. On Amsterdam Island, in the French Southern Territories, the breeding population of several seabird species has been declining over the last decades. If several elements points to the responsibility of recurrent outbreaks of avian cholera (caused by the bacteria *Pasteurella multocida* – *Pm*) leading to high rates of mortality of nestlings as the main cause of decline of the locally abundant but threatened population of Indian yellow nosed albatrosses - IYNA (*Thalassarche carteri*), it is still unclear whether a diversity of pathogens strains and species has been involved, notably also the agent of erysipelas (*Erysipelothrix rhusiopathiae* – *Er*). The goal of this study was thus to explore the potential diversity of pathogens associated with mortalities of native seabirds in an attempt to better understand the eco-epidemiological dynamics at play and the potential usefulness of management interventions. To do so, we organized the search and on-site necropsy of fresh carcasses over 2 breeding seasons. This resulted in the analysis of 105 chicks and adults. Necropsies were conducted in situ with collection of organs in formaldehyde 4% for histopathology analyses and swabs from targeted organs for culture on agar media. *Pm* was highly suspected in 93% according to colonies shape and color in culture media and was confirmed by mass spectrometry (Maldi-tof) in 60% of mortalities (respectively 86% and 50% for the two breeding seasons). During the first breeding season, we identified 2 different strains of *Pm*, based on a multi-host MLST scheme, circulating on the island. One of those strains, which was clearly the most prevalent among the isolates, is not different by MLST from the strain that had been isolated before on the island and correspond to a strain previously described in ducks in Europe. However, the second strain, isolated from 2 dead IYNA chicks, is new to the multi host MLST scheme. The 2 strains are closely related, but the sequencing of their whole genomes showed a significant distance between them, with more than 12000 SNPs difference, stressing that they are distantly related, which might suggest a different origin. *Er* was isolated from several organs of 3 dead albatrosses. The whole genome sequencing of those *Er* isolates suggests that they all belong to the same strain. However, those *Er* appear to belong to a new clade within *Er*, clustering separately from the three distinct clades commonly found. This study confirms the role of diseases in the critical situation on Amsterdam Island and reveals that a diversity of pathogens is involved. The most prevalent pathogenic agent is the strain of *Pm* that had been isolated in the system several years before, but at least another strain of *Pm*, and one of *Er*, are associated with mortalities.

Their genomic characterization show that they are well differentiated from the prevalent local strain of *Pm*, and from other known strains. Those results are important for ecological and applied reasons. The pathogen characterization may be useful for producing autogenous vaccines to protect birds from lethal infections. They also stress that more sampling and analyses are needed to identify the origin and cause of emergence of the pathogens. Finally, the study highlights the practical usefulness of combining field necropsy and microbiology work with genomic methods to address questions of relevance for biodiversity conservation.

## Introduction

Seabirds are one of the most threatened groups of birds (Croxall et al. 2012; Birdlife 2018). Albatrosses are particularly concerned with 77% of species (17/22) considered threatened by IUCN (Phillips et al. 2016) and often facing multiple threats (Dias et al. 2019). If the main threats are likely invasive alien species, bycatch, climate change or overfishing, diseases appear to have in average a very high impact on the few species affected (Dias et al. 2019) and can lead to extirpation or extinction of species (Smith et al. 2006; Descamps et al. 2012). On Amsterdam Island, a remote volcanic island in the southern Indian ocean, recurrent outbreaks occur since the middle of 1980's and kill thousands of Indian yellow-nosed albatross (*Thalassarche carteri*) chicks annually (Weimerskirch 2004).

*Pasteurella multocida*, the causative agent of avian cholera, was identified as the major cause of such mortalities (Weimerskirch 2004). Further studies have also identified the pathogen as responsible of mortalities for two other endangered species hosted on the island (Jaeger et al. 2018), the dark mantled sooty albatrosses (*Phoebastria fusca*) and the northern rockhopper penguins (*Eudyptes moseleyi*). *Pasteurella multocida* is a common commensal (Harper et al. 2006) but is also an opportunistic highly contagious pathogen that causes a rapid death and can kill thousands of birds in a short time (Samuel et al. 2007; Wilson and Ho 2013). Avian cholera is a major concern for poultry because of both economic and public health consequences (Harper et al. 2006). But *Pasteurella multocida* has been reported in more than 180 bird species (Samuel et al. 2007) and is a growing concern for wildlife. In particular, global changes, anthropogenic activities and introduction of alien species may have play a role in the dissemination of the pathogen in wild birds, particularly in isolated island (Weimerskirch 2004; Blanchong et al. 2006; Cooper et al. 2009; Bodenstern et al. 2015). Also, avian cholera was considered the most severe global infectious threat to albatrosses (Phillips et al. 2016; Uhart et al. 2018) and can cause severe epidemics among penguin species (de Lisle et al. 1990;

Cooper et al. 2009). The situation on the island is particularly critical because of the emblematic species nesting there.

A second pathogen, *Erysipelothrix rhusiopathiae*, was also identified as potentially involved in the mortalities (Weimerskirch 2004). As for *P. multocida*, *E. rhusiopathiae* may be a commensal or an opportunistic pathogen for both human and animals. The bacterium is able to infect a large number of host species and have a long persistence in the environment (Brooke and Riley 1999). Also, *E. rhusiopathiae* have a large geographical distribution and is found in a wide variety of wild and domestic animals, including birds, mammals, reptiles, fish and arthropods (Leighton 2008; Wang et al. 2010). The pathogen is commonly found in swine and is a particular issue for economics and health (Wood 1999). Erysipelas also causes severe disease outbreaks in poultry (Eriksson 2003) and may be a threat for wild birds (Gartrell et al. 2005). The presence of *E. rhusiopathiae* may be thus a particular threat for the threatened species nesting on Amsterdam Island.

It is not clear how those pathogens arrived on the island but since 1980 diseases has had a devastating impact on Amsterdam Island (Weimerskirch 2004; Jaeger et al. 2018). However, chickens, pigs, cats, rats, cattle and other alien species were introduced on the island. All of those species are known to be carrier (healthy or not) of a diversity of pathogens, and notably *E. rhusiopathiae* and *P. multocida* (Harper et al. 2006; Wang et al. 2010). The presence of such species on the island may have play a role in the introduction of the pathogens, but do not exclude a natural circulation. Identify pathogens origin is an important step to understand the epidemiological situation on the island. However, the remote situation of Amsterdam Island, and furthermore, the very difficult access to bird colonies makes challenging any bacteriology study.

Previous works have been done on Amsterdam Island to identify and characterize the pathogens diversity. A first study, Weimerskirch 2004, identified two isolates of *E. rhusiopathiae* from two dead Indian yellow-nosed albatross chicks. Both isolates were identical and were characterized to belonging to serotype 1b. The study also identified several isolates of *P. multocida* but no further characterization could be done at the time. A more recent study, Jaeger *et al* 2018, identified 6 isolates of *P. multocida* from carcasses of sooty and Indian yellow-nosed albatrosses. All 6 isolates were identical and belonged to the same sequence type (ST) 61 in the RIRDC MLST scheme. If the presence of *E. rhusiopathiae* was confirmed by PCR analysis, the pathogen was not isolated on culture media and thus couldn't be characterized. *P. multocida* ST61, also called strain D2C, was previously identified in a duck in Denmark in 1985 and support the hypothesis that the introduction of the strain may be anthropogenic. However, it is still impossible to provide any clues on the potential origin of *E. rhusiopathiae*.

Those works showed a hint of the diversity of pathogens potentially circulating in seabird populations on Amsterdam Island. However, the remote situation of Amsterdam, combined with the rough field conditions made difficult the sampling and the conservation of live pathogens. Therefore, some gaps remain in their characterization. Firstly, it would be important to go further in the characterization of *E. rhusiopathiae* to infer the potential origin, natural or anthropogenic, of the pathogen. Secondly, the few sample sizes of carcasses and the difficulty to cultivate bacteria *in situ* could have led to a lack in the diversity of pathogens. It is not excluded that several strains of *P. multocida* or *E. rhusiopathiae* circulate within or between the different species, or that strains may have mutate among time (Wang et al. 2010; Orynbayev et al. 2019; Omaleki et al. 2020).

The goal of this study is to described the diversity of pathogens circulating on Amsterdam Island. We developed an ambitious protocol in order to isolate a maximum of pathogens strains in this extreme remote study site. Special attention will be given to *Pm* to screen any potential strain diversity and emergence of new strains. A particular focus will also be devoted to isolating *Er* to characterize the strain(s) circulating on the island. This work aims to better understand the mechanisms involved in the recurrent epidemics.

## **Materials & Methods**

### Study site:

Amsterdam Island, situated in the southern Indian ocean is one of the most isolated islands in the world. The island, part of the French Southern and Antarctic Lands, is Located equidistant Australia, Africa and 3,100 km from Antarctica, the island is a volcanic dome rising up to 881 m. The south west of Amsterdam Island is dominated by high steep cliff, up to 700 m, called Entrecasteaux cliffs, rising up to a. central plateau (Plateau des Tourbières). A permanent scientific station is located on the northern part of the island and hosts about 25 people, including scientists and technical staff. Amsterdam Island hosts four emblematic, but threatened, seabird species, listed as Endangered: the Indian yellow-nosed albatross (IYNA) *Thalassarche carteri*, the Sooty albatross (SOA) *Phoebetria fusca*, the Amsterdam albatross (AMA) *Diomedea amsterdamensis* and the Northern rockhopper penguin (NRP) *Eudyptes moseleyi* (BirdLife International 2021). Also, Amsterdam island was classified as a National Nature Reserve since 2006 and was listed in the UNESCO world heritage in 2019. The island is a particular critical site as it hosts 100% of the world population of the endemic AMA, 65% of the IYNA world population (Heerah et al. 2019). Other seabird species also breed on the island including the Brown skua (*Stercorarius antarcticus*), and other smaller petrel species (Duriez and Delord 2012). Several alien

species were introduced over time, whether for food (cattle, pigs, chickens), convenience (feral cats, common waxbill) or accidental (brown rats, house mice) reasons. After eradication of three of those species over the last decades, including cattle, pigs and chickens, three introduced mammal species remain on the island, feral cats (*Felis catus*), brown rats (*Rattus norvegicus*) and house mice (*Mus musculus*) and the only introduced bird species, common waxbill (*Estrilda astrild*).

The seabird species are spatially distributed on the island. The whole population of AMA, composed of less than 60 breeding pairs (Heerah et al. 2019), breeds only on the central plateau while other albatrosses and penguins breed only in the south-west in the Entrecasteaux cliffs. About 22,000 breeding pairs of IYNA breed in dense colonies along the Entrecasteaux cliffs. 650 pairs of SOA also breed along the cliffs, but in smaller densities, from some isolated individuals to tenth individuals. IYNA and SOA do not breed in mixed colonies but SOA are often found at the periphery of IYNA colonies, at a very close distance. Few colonies of NRP remain on the island with about 12,000 breeding pairs. If the main colonies of NRP are monospecific, it is very usual to find NRP nesting between IYNA nests in the upper parts of the colonies. The proximity between species is thus important to consider in the potential circulation and diversity of pathogens within or between colonies and species.

The particular geography of the island makes any specific experimentation particularly challenging. Access to Entrecasteaux cliff colonies required specific equipment and a long walk from the main station. Also, during breeding season, stays of several weeks are done at the bottom of the cliffs with only a hut with rudimentary conditions. Furthermore, the island is only supplied maximum 4 times a year by a boat, making it difficult and long to ship back the samples to the continental France, where analyses can be conducted and can be particularly restrictive especially for bacteriological analysis.

#### Carcasses survey:

During 2018/2019 breeding season, a specific search of carcasses was implemented in the study colonies of IYNA and SOA in Entrecasteaux cliffs. During 2019/2020, in addition to those colonies, an intense search of carcasses of NRP was conducted in different colonies and on the path between their landing area on the Entrecasteaux beach and their breeding colonies (Figure 1). All carcasses, from very fresh dead bird to only skeleton, were included in the study as long as it was possible to sample at least bone marrow. During breeding seasons, scavengers as brown skuas or brown rats are very active, also small carcasses may be taken away very rapidly after death. An active survey during day time was thus implemented in order to find and sample as many carcasses as possible, notably dead chicks.

During 2018/2019 breeding season, 29 necropsies, including 27 chicks and 2 adults, of IYNA were performed between early December until late March. During 2019/2020 breeding season, 71 necropsies were carried out between late September and late March. 11 carcasses of IYNA were necropsied, including 6 chicks and 5 adults, and 60 carcasses of NRP, including 21 chicks and 39 adults.

#### Field procedures:

All field assistants received a dedicated formation for wildlife necropsies, in collaboration with a veterinary lab and the French national agency for wildlife. The formation was specifically developed in accordance with field conditions, species and constraints of Amsterdam Island.

Rigorous biosecurity measures were observed in the field to avoid spreading the disease among bird colonies and to protect sampler from any potential infection. These involved the use of dedicated clothing consisting of a different set of waterproof leggings, jackets, and rubber boots for each bird colony, in addition of face masks and gloves for individual protection. Waterproof overalls, equipment and hands were disinfected after each necropsy, respectively using hydrogen peroxide, commercial disinfectant wipes, and hydroalcoholic gel. Gloves, face masks and any other disposable equipment (e.g., needles, scalpel blades, wipes) were placed in dedicated trashes. All pieces of clothing were thoroughly cleaned and disinfected again at the end of each day, and necropsy equipment was disinfected with detergent.

Furthermore, all necropsies were conducted *in situ* at the exact place, or very close, from where the carcass was found to avoid the spread of any pathogens within or between colonies. After necropsies, carcasses were left in place.

#### Organ sampling and histological diagnostic:

A general necropsy protocol was implemented in the ideal case of a very fresh carcass. However, because carcasses were often partially eaten by brown rats or brown skuas, or because the weather conditions do not permit an ideal sampling, necropsies had to be adapted according to the carcass condition and field constraints. After an external and visual examination of the carcass, the carcass was opened for an internal diagnostic and sampling. When possible, 9 organs were systematically examined, collected (a piece or the whole organ depending on size and origin) and stored in formaldehyde 4% buffer: liver, heart, lungs, spleen, kidney, thyroids, thymus, bursa of Fabricius and brain. Those organs have been specifically selected as they may give important information about a potential infection. In addition, any other organ looking abnormal was collected. Samples in

formaldehyde were stored at room temperature until they were shipped to continental France, from 2 to 6 months depending on boat's stopover.

All samples for histology were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 4  $\mu\text{m}$  and stained with hematoxylin and eosin and then examined microscopically. Special stains were used when needed.

#### Bacterial isolation from seabird carcass samples:

When possible, swabbing was performed on 4 targeted organs: lung, liver, heart and brain. When only skeleton was found, swabbing of the bone marrow of femur or tibiotarsus was performed. For each organ, a single sterile swab was brushed in an inner and not contaminated part of the organ and directly stored in a conservation media (eSwab media, Copan, Italia). At the end of each days, all samples were streaked onto a blood agar plate (blood agar base supplemented with 5% defibrinated sheep blood; BIO-RAD, Hercules, California, USA). Because no power is available in the hut, incubation could not be done in an incubator in standard conditions (37°C for 12-24h). Therefore, plates were left at room temperature (~15°C) and the growth of bacteria was monitored very regularly until colonies of sufficient size and number were visible. On each plate and for each organ, all bacterial morphospecies were visually differentiated. For each organ 1 or 2 colonies of pathogen of interest (i.e., avoiding obvious contaminants) was randomly collected and stored in conservation media (Stock Culture Agar; BIO-RAD, Hercules, California, USA). Isolates in conservation media were stored at room temperature until they were shipped to continental France, from 2 to 6 months, depending on boat's stopover, for further analyses.

#### Bacterial characterization:

After being shipped to continental France, isolates stored in conservation media were grown on blood agar plate. Colonies from the new growth media was then randomly selected and a speciation was performed, using matrix assisted laser desorption/ionization – time of flight (MALDI-TOF). To estimate the probability to detect the causative pathogen and not a random contaminant, during 2018/2019 breeding season, for each isolate from 7 necropsies, 3 to 6 colonies that grew from the new growth media were selected and analyzed (105 colonies in total). Then, only 1 colony was analyzed for each organ of each necropsy for the 22 other carcasses (63 colonies in total). Considering the good rate of detection during the 2018/2019 season, only 1 isolate for each necropsy among all organs sampled was selected during 2019/2020 breeding season and only 1 colony was analyzed (79 colonies in total).

In order to maximize chance to select the causative agent, isolates from bone marrow or brain were selected. Those organs might be infected only if septicemia happened and which external contamination is unlikely (scavengers usually eat organs in abdomen first). All interesting pathogens were then stored for further genotypic characterization.

#### *Pasteurella multocida* genotyping:

All isolate of *Pm* from IYNA characterized using MALDI-TOF was genotyped using a set of seven housekeeping genes (*adk*, *aroA*, *deoD*, *gdhA*, *g6pd*, *mdh*, *pgi*), following the multi-host multi-locus sequence typing – multi-host MLST (<http://pubmlst.org/pmultocida>). Unfortunately, only 6 isolates identified as *Pm* from NRP could be analyzed. The strain D2C, identified in Jaeger *et al* 2018 and corresponding to the ST 61 in RIRDC MLST scheme, was also analyzed following multi-host MLST scheme for comparison with isolates analyzed during those 2 breeding seasons. Allele numbers and sequence types (ST) were identified by comparison with reference strains deposited in the *P. multocida* MLST database sited at the University of Oxford (<http://pubmlst.org/pmultocida/>). A minimum-spanning tree was built using goeBURST Full MST algorithm (<http://online.phyloviz.net/index>), by concatenating all seven MLST genes found in various hosts worldwide.

One selected isolate from each strain characterized was analyzed by whole genome sequencing. Total bacterial DNAs was extracted from an isolate on a KingFisher automat (Thermo Fisher Scientific) using magnetic beads (QIAamp cadior MagAttract Kit, Qiagen), and subjected to full NGS sequencing using the Fast DNA Library Prep Set (NEB) on an IonTorrent S5 sequencer (Thermo).

Genomic DNA from each sample was first enzymatically fragmented. Fragments with sizes between 100 and 500 bp were purified and marked with a specific genetic barcode (IonCode). The resulting libraries were then mixed and sequenced.

The raw data were analyzed using CSI Phylogeny 1.4 (<https://cge.cbs.dtu.dk/services/CSIPhylogeny/>).

#### *Erysipelothrix rhusiopathiae* genotyping:

DNA was extracted from clonal populations by suspending 1 loopful of colonies in 200 µl of phosphate buffered saline (PBS), and then using the DNeasy Blood and Tissue Kit (Qiagen, Mississauga, ON, Canada) following manufacturer's instructions, with the exception that DNA was eluted in a smaller volume (50 µl) of the provided elution buffer to yield a higher DNA concentration.



## Results

### External and internal symptoms:

The intensive survey of the mortalities permitted to observed symptoms of birds, from few hours to few minutes before dying. It is impossible to know when exactly started an infection, but IYNA and NRP died within hours after first visual symptoms. Birds showed consistent symptoms, confirming first observations described in 1996 (Weimerskirch 2004): breathing difficulties, opening widely their beak and suffocating (Figure 2); looking weak, IYNA hanging down their wings out of the nest and NRP laying on the ground. Birds did not show any defense or escape reaction when approaching. Birds died in few hours following first signs (Figure 2). Those symptoms were observed on 1 NRP female adult, 4 NRP chicks and 3 IYNA chicks for the 2 breeding seasons. Weak birds are an easy prey for predators or scavengers, also those symptoms could be only described if an observer found and stayed close to the dying bird.

The necropsies also revealed consistent symptoms on internal organs (Figure 2). Liver, heart and lungs showed characteristic symptoms, as heterogeneous and granular aspect, blood effusions or hemorrhages. Both external and internal lesions support the hypothesis that pathogens may have cause the death of a significant part of the birds.

The hypothesis is also confirmed by histological analysis carried out on tissues sampled and stored in formaldehyde 4% (Figure 3). Because necropsies are often not complete (e.g., organs partially or totally eaten by scavengers, decayed organs), it was not possible to have a systematic identification of symptoms and lesions for each organ. However, microscopic examination of the tissues of the majority of the carcasses revealed histological lesions consistent with systemic bacterial infections. It is likely to be the primary cause of death or a facilitating factor (weakening of the individual facilitating predation). Histological analysis included one or more of the following lesions: presence of circulating bacteria in the vessels and/or within the filter organs with occasional associated thrombosis, multi-organ congestion and hemorrhage with intravascular necrotizing hepatitis and/or fibrino-necrotic splenitis with intralesional bacteria. Less predominantly, bacterial vasculitis, necro-suppurative inflammation of the thymus or pulmonary oedema has also been speculatively attributed to the bacterial infection directly or secondary to the associated septic shock. These lesions were compatible with an acute form of avian cholera, but remained non-specific and needed be completed with bacteriological culture.

#### *Pasteurella multocida* isolation:

During 2018/2019 breeding season, after bacterial growth on agar plate, *Pasteurella multocida* was suspected in 85% (23/27) of the carcasses of IYNA chicks and 100% (2/2) of IYNA adults (Table 1). The MALDI-TOF speciation confirmed *Pasteurella multocida* in all cases it was suspected demonstrating that the detection of *P. multocida* based on macroscopic characteristics on agar media was 100%. Despite a very high mortality rate on SOA chicks, no carcass was found.

Results from the 7 necropsies, for which 3-6 colonies were analyzed for each organ, showed that the isolation of the relevant pathogen, and not a contaminant, for characterization is quite accurate. Of the 13 isolates that were confirmed of being *Pm*, only 2 (15%) were not a pure colony but was contaminated with respectively *Enterococcus faecalis* and *Staphylococcus aureus*, both common commensals, in particular in rat mouth (Marcotte and Lavoie 1998). A total of 114 organs were sampled for the 25 carcasses positive to *Pm*. But *Pm* was confirmed in 76 organs (67%). Common commensal bacteria were characterized in other organs (e.g., *Enterococcus sp.*, *Staphylococcus sp.*, *Proteus sp.*) and suggests a contamination by rats or skuas. This result suggests that even if *Pm* is suspected and likely present (same macroscopic characteristics than other organs), 2/3 of times a wrong isolate, probably a contaminant, was selected on the culture media. This is important to consider as it suggests that the detection of *Pm* by MALDI-TOF might have been under evaluated in other samples where only 1 isolate from 1 organ was analyzed.

During 2019/2020 breeding season, *Pasteurella multocida* was suspected in 67% (4/6) of IYNA chick necropsies and were all confirmed by MALDI-TOF analyses (Table 1). *Pm* was suspected in 100% (5/5) of adults necropsied, but was only confirmed in 40% (2/5) using MALDI-TOF. *Pm* was suspected in 100% of necropsies of NRP chicks (21/21) and adults (39/39). However, *Pm* was confirmed respectively in 43% (9/21) and 51% (20/39). Other bacteria characterized are common commensal (e.g., *Enterococcus sp.*, *Staphylococcus sp.*, *Serratia sp.*, *Escherichia coli*) and suggest a contamination when sampling or by scavenger.

Because only 1 colony was analyzed by MALDI-TOF, the probability of detection was reduced during 2019/2020 breeding season (67% the previous season). Furthermore, NRP breed in dense vegetation patches making it difficult to detect carcasses. Also, carcasses were in most of the cases partially eaten by scavengers and agar media were frequently contaminated making difficult the isolation of pure colony of *Pm*. However, considering the results of the previous season, we could expect that macroscopical determination gave an accurate rate of infection by *Pm*.

*Pm* was suspected in 100% of SOA chick carcasses (4/4) but it could not be confirmed by MALDI-TOF because none of the isolate selected resulted in bacteria growth before being analyzed. The only SOA adult necropsied was a carcass mostly eaten by scavenger. Few organs could be sampled and all bacterial culture showed only obvious contaminants (e.g., yeasts, proteus, enterococcus). It was thus impossible to infer any cause of death either by external or internal observation of the carcass, or with bacterial culture.

#### *Pasteurella multocida* genetic characterization:

The analysis of strain D2C, corresponding to the ST61 in RIRDC MLST scheme according to Jaeger *et al* 2018, showed that it belongs to ST91 following multi-host MLST scheme (Table 2).

For each carcass, all *Pm* isolates were identical, meaning that in all organs and all replicates the same strain was identified. This result suggest that death is due to an infection of only 1 strain and not a co-infection of different strains.

For 2018/2019 breeding season, of the 25 carcasses (23 chicks and 2 adults) were *Pm* was characterized using MALDI-TOF, 23 (21 chicks and 2 adults) corresponded to the ST91 following multi-host MLST scheme (Table 3), identical as strain D2C previously identified. 2 carcasses of IYNA chicks corresponded to a new combination of housekeeping genes that was not described in the multi-host MSLT scheme. The sequence was registered on the MLST database (<http://pubmlst.org/pmultocida/>) and was attributed ST110. The refence strain of the new ST110, selected in the brain of an IYNA chick, was called A18Y007B1 (Table 2). Both strains D2C and A18Y007B1 belong to Capsular A, Serotype 1, *ssp P. multocida gallicida*.

The genetic relationships of those STs with other strains are shown on a minimum spanning tree built using data from 1576 isolates available on *Pasteurella multocida* MLST database (Figure 5). Phylogenetic analysis based on genome-wide single-nucleotide polymorphisms revealed that ST91 and ST110 are divergent for 12,610 SNPs. Considering genetic relationship and SNPs divergence, ST91 and ST110 might be considered as 2 different former groups.

For 2019/2020 breeding season, all isolates characterized as *Pm* from IYNA chicks (4/4) and adults (2/2) and from NRP chicks (3/9) and adults (3/20) were identified as ST91. ST110 was not identified during this breeding season. However, ST110 was detected in only 9% of the 25 isolates during the previous season. Also, considering that only 6 samples were analyzed for both NRP and IYNA, the probability to detect it was lower and the circulation of ST110 was not excluded.

#### *Erysipelothrix rhusiopathiae* isolation:

During 2018/2019 breeding season, after bacterial growth on agar plate, *Er* was suspected in 11% (3/27) of the carcasses of IYNA chicks but not of IYNA adults (Table 4). The MALDI-TOF speciation confirmed *Er* in all 3 cases it was suspected demonstrating that the detection of *Er* based on macroscopic characteristics on agar media was also very accurate. However, *Er* was neither suspected nor characterized in any of the IYNA and NRP isolates during the 2019/2020 season.

Interestingly, the 3 carcasses of IYNA chicks being infected by *Er* were found during a short time period (one on the 13<sup>th</sup> of January and the 2 others on the 16<sup>th</sup> of January) and were located in the same sub-area within one monitored colony.

#### *Erysipelothrix rhusiopathiae* genetic characterization:

From the 3 necropsies from which *Er* was characterized, 15 strains were identified as *Erysipelothrix rhusiopathiae* and 1 as *Erysipelothrix sp.* The whole genome sequencing of all 16 strains indicated that all isolates were very closely related (Figure 6). This result suggest that all strains were identical.

The core genome sequencing showed that the 16 strains clustered separately from the commonly described *Er* clades (Figure 6 and 7).

#### **Discussion:**

In this study we illustrated that implementing a protocol that integrate several technics to determine the cause of the death and identify potential pathogens is a powerful approach. Combining histological analysis, bacterial characterization and genetics was very accurate and provided very important information to better understand the epidemiological situation on Amsterdam Island and provided essential results for basics and applied reasons.

We confirmed, following previous studies (Weimerskirch 2004; Jaeger et al. 2018; Jaeger et al. 2019), that *Pm* is responsible of a large part of the mortalities in IYNA chicks, but also in NRP and SOA chicks. *Pm* was identified to be the causative agent for the death of 85% and 67% of IYNA chicks during respectively 2018/219 and 2019/2020 breeding seasons and was suspected for 100% of the NRP and SOA chicks during 2019/2020 breeding seasons. This confirmed that avian cholera became an endemic disease on Amsterdam Island, affecting significantly at least 3 threatened bird species The population trends for IYNA, NRP and SOA are particularly alarming, with a decrease of >80% since 1980s for IYNA and NRP (Jaeger et al. 2018) and avian cholera is the main cause of decline.

7 IYNA adults were necropsied and *Pm* was highly suspected in all carcasses. Role of *Pm* in NRP adult survival was not described to date. In this study we necropsied during 1 breeding season 39 carcasses of NRP adult and all were highly suspected to be infected by *Pm*. If avian cholera is likely to affect massively IYNA chicks and in a more minority adults (Rolland et al. 2009; Gamble et al. 2019), it is clear that adults are affected by *Pm* infection.. Also, adult mortality for such long-lived seabird may be dramatic in the critical situation of Amsterdam Island.

A new strain of *Pm*, nor previously characterized on Amsterdam Island neither in the multi-host MLST database was discovered on 2 IYNA chicks. This result is important for basics and conservation reasons. *Pasteurella multocida* is an important pathogen because of its implications in economy and conservation. While many studies focus on domestic animals (e.g., poultry, cattle, swine), gaps remain in wildlife. The discovery of A18Y007B1 (ST110) enhance and complete the genetic database of *Pm* and therefore the knowledge on the diversity of strains. Furthermore, the identification of another strain circulating in the population had applied consequences. Protect endangered species on Amsterdam Island is a major concern and vaccination of IYNA chicks against *Pm* is currently being studied, with an autologous vaccine developed from strain D2C (ST91). If first results were very encouraging and showed a protective effect of vaccination (Bourret et al. 2018), recent studies (Tornos *et al* in prep) showed that vaccination efficacy may be minimized some years. It was not excluded that the circulation of another strain was involved and the discovery of A18Y007B1 allowed the autologous vaccine to be updated. Preliminary results suggest a better protective effect when vaccinated against both strains (Tornos *et al* in prep). We illustrated in this study that a long-term survey of the epidemics, with an integrated approach, is essential to optimize.

A18Y007B1 was identified in only 7% of IYNA carcasses (2/29) and during 1 breeding season, suggesting that D2C previously identified, is the main pathogen causing mortalities. It is not excluded that strain A18Y007B1 circulate in the population for several years or decades. However, only the intensive survey of mortalities during the 2 breeding seasons permitted to detect it and would explain why it was not detected previously.

Genetic characterization of D2C suggested that the strain was probably imported through anthropogenic activities (Jaeger et al. 2019). D2C was previously described in ducks in Europe and introduction of species on Amsterdam Island, such as chickens, may be at the origin of epidemics. However, A18Y007B1 had never been described before in the multi-host MLST scheme and was not referenced in any other hosts since it was deposited in the database on July 2019. The genetic divergence between D2C and A18Y007B1 excluded the hypothesis that one evolves from the other.

However, it is hardly possible to infer the origin of A18Y007B1, anthropogenic or natural, without further genetic analysis.

*Erysipelothrix rhusiopathiae* was detected in 10% of IYNA carcasses (3/29) and only during 1 breeding seasons. Although in a minority cause of the mortalities, *Er* could be detected thank to a dedicated monitoring. Albeit also probably present, *Er* was not detected nor in NRP neither in IYNA carcasses during 2019/2020 breeding season. As predicted in other studies (Jaeger et al. 2018; Jaeger et al. 2019), *Er* do not appear to be the major threat for seabirds on Amsterdam Island but can still be responsible of a part of the mortalities and is additive to other threats facing populations.

Genetic characterization of *Er* provided interesting new elements concerning the diversity the pathogen. All isolates belonged to same group but interestingly clustered separately from main clades of *Er* commonly described. Genetic clustering of Amsterdam strains suggest that a natural exposure is more likely than a human introduction. The “Albatross clade” is a very interesting discovery. Also, further sampling, on other species and sites, would be interesting to determine if this clade is endemic to Amsterdam Island, or albatross species or even found in other seabird species.

The origin of A18Y007B1 and *Er* remains unclear. If an anthropogenic introduction was suspected, the genetic analysis does not exclude a natural exposure. In contrast, presence of D2C is likely due to introduced species. However, it clearly appears that mortal epidemics are mainly due to infection of D2C and even if birds would be naturally exposed to *Er* and A18Y007B1, they should not be a major threat. This results also suggest that even if wildlife is naturally exposed to pathogens, introduction of similar pathogens (e.g., variants, mutants, strains) may be a severe threat. Such information is important for conservation measures as we pointed out the responsibility of human activities in a potential species extinction.

The role of rodents is not clear in the circulation of pathogens on the colonies. Rats are known to be common carrier of *Pm* (Harper et al. 2006), and likely play a role in epidemics of avian cholera in poultry (Haq and Abdullah 2015). Further studies are currently implemented to better understand the role of rats in epidemics on Amsterdam Island. Preliminary results (data not published) showed PCR positive rats to *Pm* in mouth of individuals sampled in Entrecasteaux colony and a high seroprevalence for antibodies anti-*Pm* in rat plasma. Also, many carcasses showed ante-mortem wounds caused by rat bites and it is not excluded that a part of infections may have been caused by direct transmission through bites (Curtis et al. 1980). Strain identification of *Pm* found in brown rats would help to understand their role in epidemics.

Interestingly, the 2 chicks infected by A18Y007B1 were observed few days before dying with the exact same wounds. Both chicks were injured at the head, with typical rodent tooth prints on the skull, probably due to brown rats. Death after *Pm* infection was confirmed but rat bites may have played a role in the infection of A18Y007B1. Many other carcasses showed wounds (usually on the abdomen, or wings, or breast), but it was the only 2 with such typical head injuries.

More than a threat for birds, *P. multocida* and *E. rhusiopathiae* also appear to cause severe diseases in marine mammals (Crawford et al. 2019) and furthermore in subantarctic fur seal (*Arctocephalus tropicalis*) breeding in numerous on Amsterdam Island. Fur seal population trend is nowadays good on Amsterdam Island, but the presence of such pathogens could be a threat if epidemics occur in the population.

#### **Authors' contributions**

TB conceived the idea of this work; TB, JT, AG and AC designed the study; JT, AC, CB and AF collected the data; KL and RL analyzed performed the histological analysis; TF led the genotyping of *Erysipelothrix rhusiopathiae* and HG of *Pasteurella multocida*; JT, AG and TB led the writing of the manuscript. All authors contributed substantially to the drafts and gave final approval for publication.

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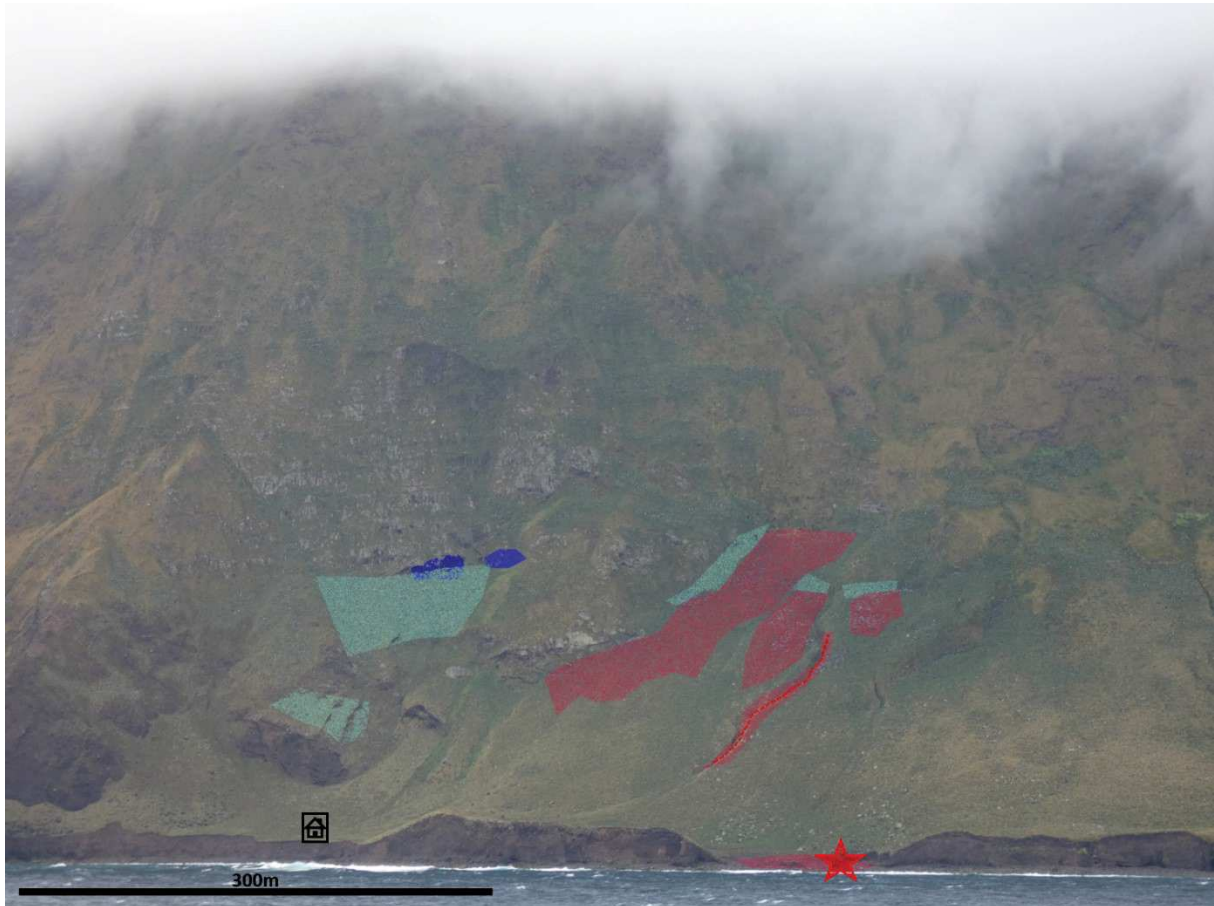
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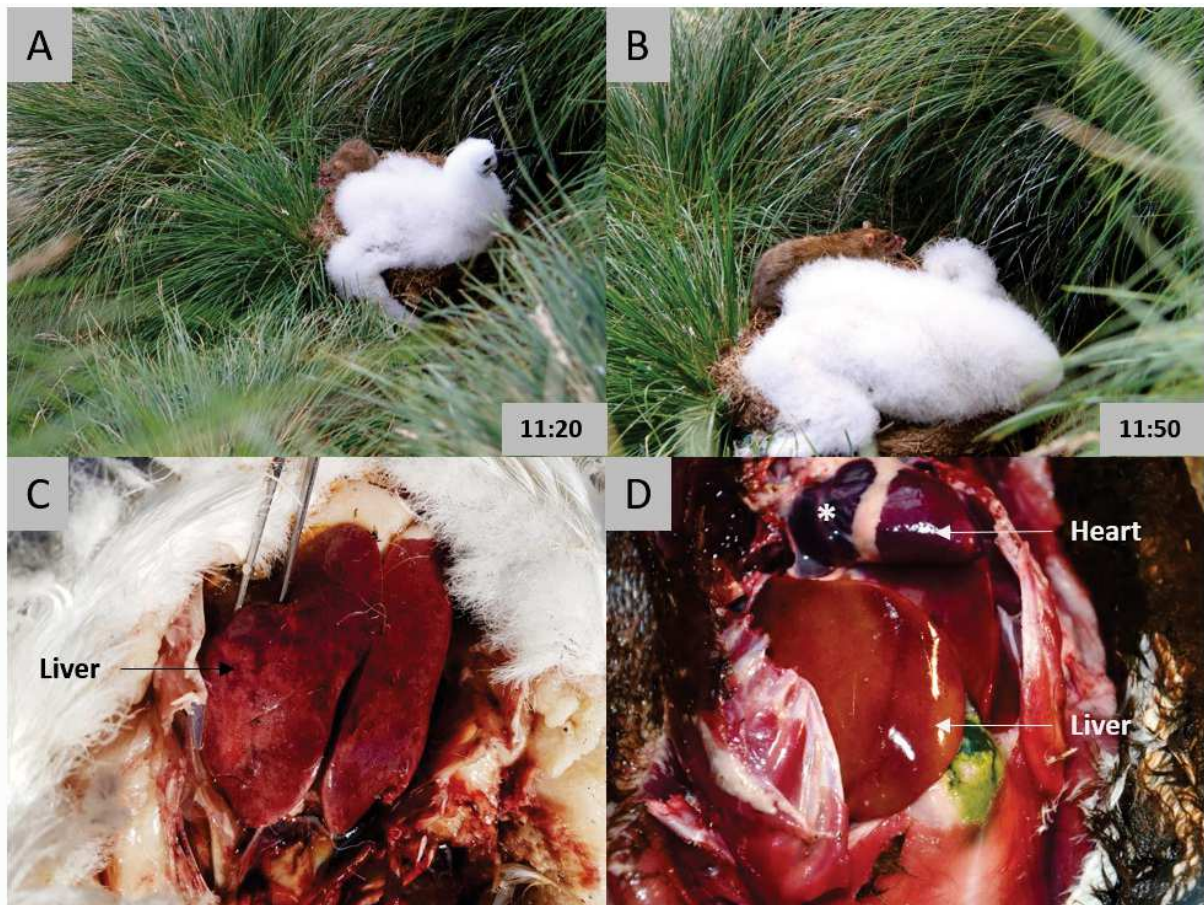
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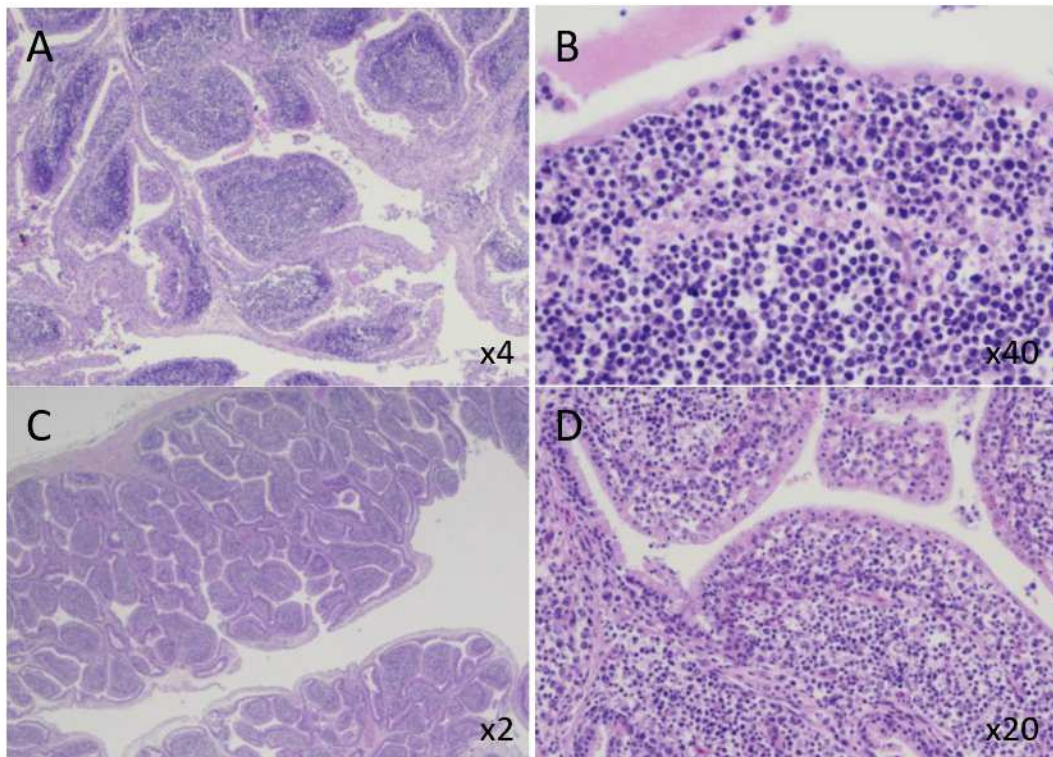
Not only a single strain of *Pasteurella multocida* kills albatrosses on Amsterdam Island: insight into the community of bacterial pathogens affecting seabirds – Figures –



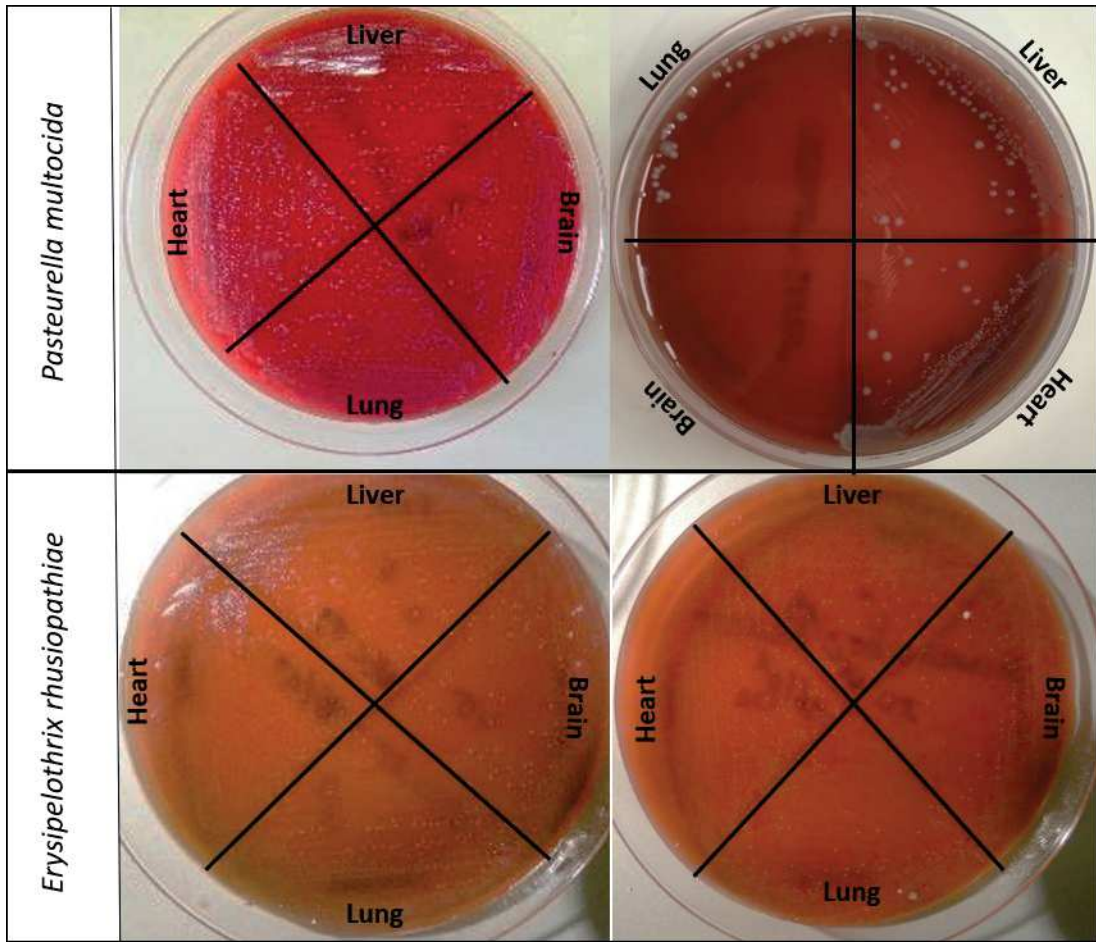
**Figure 26: Picture of a part of the Entrecasteaux cliffs**, indicating study sites and areas for search of carcasses. Light blue, colonies of IYNA; dark blue, colonies of SOA; and red, areas where were implemented specific search of NRP carcasses. The red star indicates the landing area of the NRP and the dotted line the path between landing area and colonies. The black square with a pictogram indicates the location of the hut. The cliff is about 600 meters high over the hut.



**Figure 27: Dead chicks show consistent pathological symptoms.** A- a diseased and weak IYNA chick on its nest, with wings hanging down out of the nest and hardly breathing, 11:20am; a brown rat was visiting the nest. B- 30 minutes later the chick is dead, 11:50am; a rat is also on the nest, wounds were detected on the abdomen of the chick, probably caused by rats ante mortem considering hematomas and bleeding. C- typical liver lesions caused by *Pasteurella multocida* infection on an IYNA chick: heterogeneous and granular appearance. D- typical liver and heart lesions on a NRP chick, caused by *Pasteurella multocida*: heterogeneous aspect of the liver and pericardiac blood effusion (white star).



**Figure 28: Typical lesion on the bursa of Fabricius** observed with histopathological analyses. A and B: Gram coloration from a IYNA chick dead from an infection of the Pm strain D2C; superficial necrosis and erosion with bacteria in the crypts and lymphocytolysis. C and D: Gram coloration from a IYNA chick dead from an infection of the Pm strain A18Y007B1; apoptosis and lymphocytolysis.



**Figure 29: Typical bacterial growth on sheep blood agar media.** When possible, 4 tissues were targeted for each necropsy, liver, brain, lung and heart. Upper part: two positive cultures of Pm; grey ovoid colonies (~2mm) with a strong typical odor. Lower part: two positive cultures of Er; numerous small transparent white colonies (~1mm). In the majority of cases, bacteria growth was detected in several organs sampled and only one single type of colony identified. Furthermore, the same strain was always detected in all organs infected suggesting that infection and septicemia is due to one strain and not a co-infection of different strains or pathogens.

**Table 2: Proportion of *Pm* suspected and characterized** in necropsies carried out during 2 breeding seasons on 2 species, NRP- Northern Rockhopper Penguin (*Eudyptes moseleyi*), IYNA- Indian Yellow-nosed Albatross (*Thalassarche carteri*). *Pm*- *Pasteurella multocida*. N is the total number of necropsies. The number of suspected identifications is based on the characteristics of the colonies (shape, color, odor) after 24-48h incubation at 37°C on sheep blood agar media. Bacteria were characterized by mass spectrometry (MALDI-TOF). Numbers with \* mean that there might be more necropsies with actually *Pm*, but this could not be detected because of sampling bias.

Species	Season	Stage	N	Necropsies with <i>Pm</i> suspected (% of total necropsies)	Necropsies with <i>Pm</i> characterized (% of total necropsies)
YNA	2018/2019	Chick	27	23 (85%)	23 (85%)
		Adult	2	2 (100%)	2 (100%)
	2019/2020	Chick	6	4 (67%)	4 (67%)
		Adult	5	5 (100%)	2* (40%)
NRP	2019/2020	Chick	21	21 (100%)	9* (43%)
		Adult	39	39 (100%)	20* (51%)
SOA	2019/2020	Chick	4	4 (100%)	0*
		Adult	1	0	0*

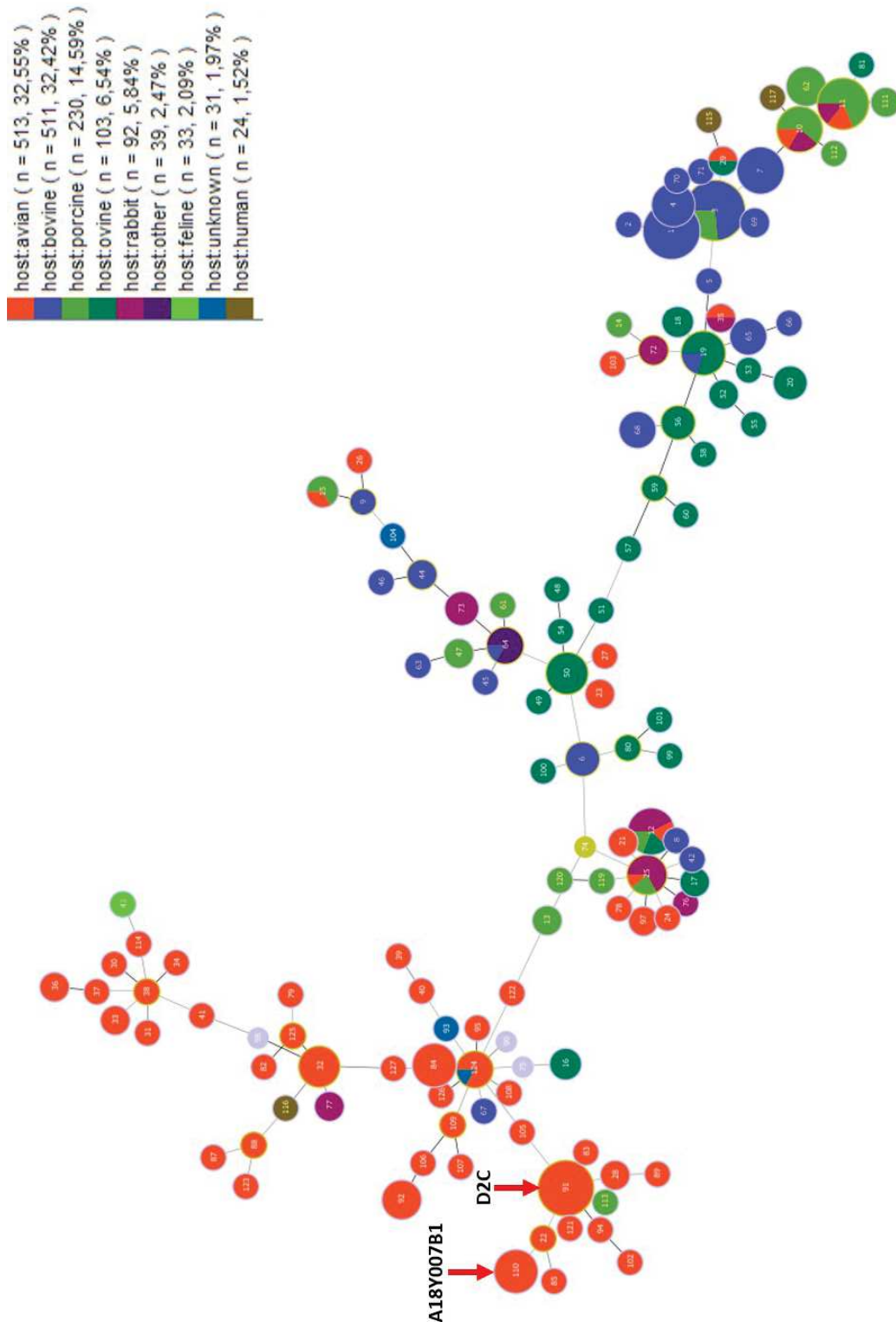
**Table 3: *Pasteurella multocida* strains** with corresponding allele numbers demonstrated for the 2 strains characterized on Amsterdam Island and number of each ST in the multi-host MLST scheme.

Strain	ST	<i>mh_adk</i>	<i>mh_aroA</i>	<i>mh_deoD</i>	<i>mh_gdhA</i>	<i>mh_g6pd</i>	<i>mh_mdh</i>	<i>mh_pgi</i>
D2C	91	10	33	19	6	25	12	32
A18Y007B1	110	12	47	31	35	35	12	44

**Table 4: Proportion of both strains of *Pasteurella multocida* D2C and A18Y007B1** within the two species sampled among the two breeding seasons. NRP-Northern Rockhopper Penguin (*Eudyptes moseleyi*), IYNA- Indian Yellow-nosed Albatross (*Thalassarche carteri*). N (MLST): number of samples analyzed in the multi-host MLST scheme. Numbers with \* mean that there might be more necropsies with strain A18Y007B1 because only a selection of samples were analyzed.

Species	Season	Stage	Total number of samples	Samples analyzed in MLST scheme	D2C (ST91)	A18Y007B1 (ST110)
IYNA	2018/2019	Chick	23	23	21 – 91%	2 – 9%
		Adult	2	2	2 - 100%	0
	2019/2020	Chick	4	4	4 – 100%	0
		Adult	2	2	2 – 100%	0
NRP	2019/2020	Chick	9	3	3 – 100%	0*
		Adult	20	3	3 – 100%	0*

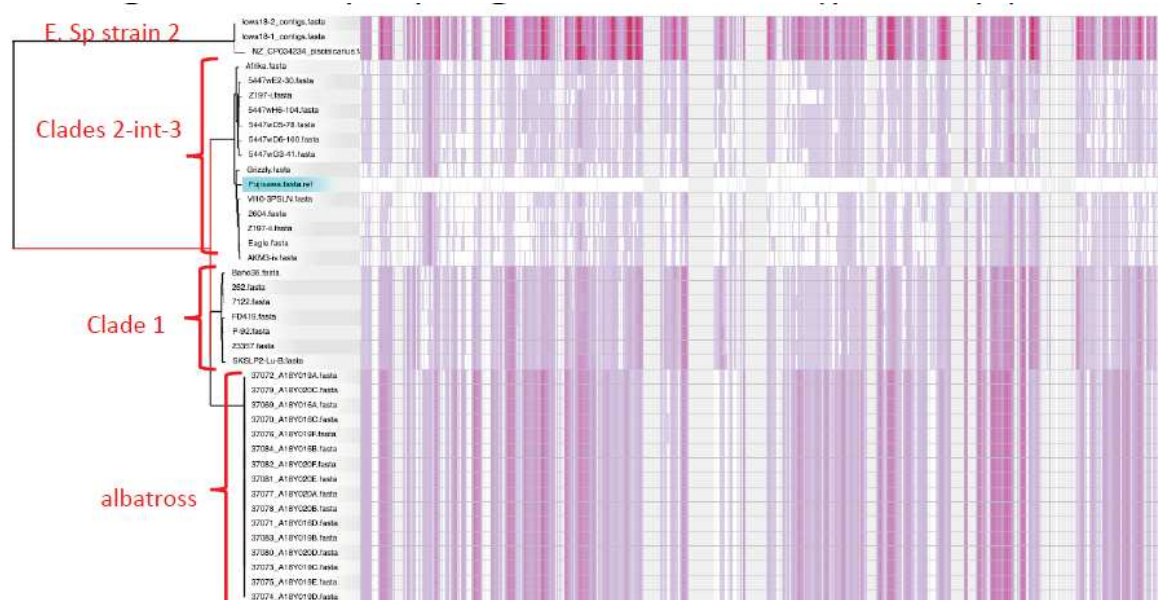




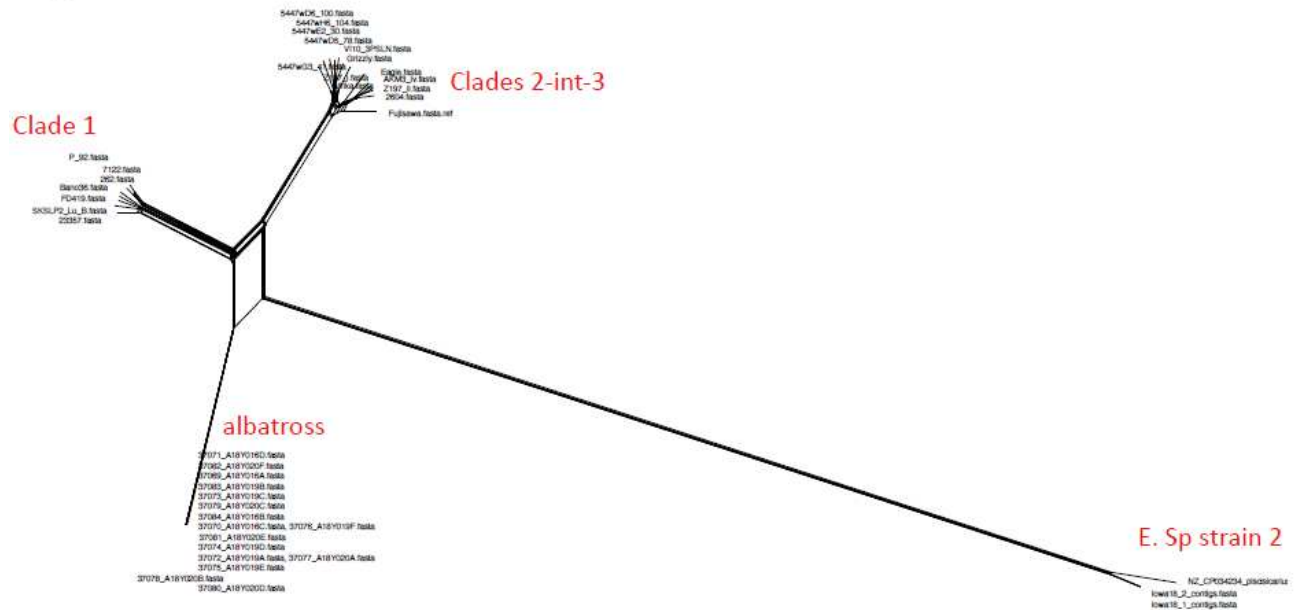
**Figure 30: Minimum-spanning tree** of *Pasteurella multocida* sequence types (STs) based on the multi-host MLST Scheme (<http://www.pubmlst.org/pmultocida/>). Each circle represents a ST (reference numbers are inside circles). ST91 and ST110 are specified by a vertical red arrow. The color code indicates the animal origin of sequenced isolates available in the database, n represents the number of these isolates for each animal group.

**Table 5: Proportion of *Er* from necropsies carried out during 2 breeding seasons on 2 species, NRP- Northern Rockhopper Penguin (*Eudyptes moseleyi*), IYNA- Indian Yellow-nosed Albatross (*Thalassarche carteri*). *Er*- *Erysipelothrix rhusiopathiae*. N is the total number of necropsies. The number of suspected identifications is based on the characteristics of the colonies (shape, color, size) after 24-48h incubation at 37°C on sheep blood agar media. Bacteria were characterized by mass spectrometry (MALDI-TOF).**

Species	Season	Stage	N	Necropsies with <i>Er</i> suspected (% from total necropsies)	Necropsies with <i>Er</i> characterized by mass spectrometry
YNA	2018/2019	Chick	27	3 (11%)	3 (11%)
		Adult	2	0	0
	2019/2020	Chick	6	0	0
		Adult	5	0	0
NRP	2019/2020	Chick	21	0	0
		Adult	39	0	0



**Figure 31: Core genome sequencing of 16 strains of *Er* isolated from 3 carcasses of albatross chicks. “Albatross clade” appears to cluster separately from the main branches commonly described (clade 1, and clades 2, 3 and intermediate).**



**Figure 32: Population structure of *Erysipelothrix rhusiopathiae* based on network analysis.** A phylogenetic network of *E. rhusiopathiae* was estimated using Neighbor-Net analysis as implemented in SplitsTree. Analysis include "Albatross clade, clade 1, clades 2, 3 and intermediates and *Erysipelothrix* sp strain 2 (the closets species to *Erysipelothrix rhusiopathiae*).

## Annexe 2

Optimizing a vaccination approach against avian cholera to protect endangered albatrosses.

**Tornos Jérémy**, Clessin Augustin, Gantelet Hubert, Fléchet Aline, Bely Marine, Gamble Amandine & Boulinier Thierry.

In preparation.



# Optimizing a vaccination approach against avian cholera to protect endangered albatrosses

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## **Abstract**

1. Infectious diseases have become a growing concern for wildlife conservation and in some cases, management approaches based on the use of vaccines have been proposed. Prior to their potential implementation, a thorough understanding of the eco-epidemiological dynamics is necessary, as well as field tests.

2. Among the issues that are required to be investigated before proposing the use of a vaccine, it is important to assess which life history stage, age and timing of vaccination would be most suitable and practical in relation to the species ecology and field situation. In a disease affecting offspring, the age and timing of vaccination are especially important to optimize because of the need to account for the expected timing of immune protection, notably function of the development of the immune system, and the timing of epizooties. Moreover, it is important to determine against which strain(s) the vaccine need to be used, notably in the case of bacterial pathogens known to occur in diverse strains in populations of wild or farmed animals. In the case of natural populations of long-lived species threatened by an identified pathogen killing young animals and found in domesticated species, it has been suggested that specifically tailored, so-called, autologous killed vaccines could provide an efficient, safe and practical approach, notably if breeding females could be vaccinated to protect their young offspring.

3. Following an earlier study on the use of such a vaccine against avian cholera in threatened albatrosses, it was determined that the vaccine developed against a single strain isolated in one of the local threatened host populations could be efficient at protecting nestlings some years, but not all, and that persistent levels of antibodies could not be obtained among years in adults, apart after the injection of a novel formulation of the vaccine in previously vaccinated individuals.

4. In order to address further these issues, we implemented a follow up study in which we used the novel formulation of the vaccine on a new cohort of adults, set up two age groups of vaccination of nestlings, and investigated the potential effect of bacterial strain diversity.

5. The results show that the novel formulation of the vaccine lead to persistent inter-year level of antibodies in adults, associated with a transferred protective effect on the nestlings. The protection of vaccinated nestlings compared to controls was confirmed 2 of the 3 years, with very comparable results for the two age of vaccination. Close monitoring of the cause of death of nestlings enabled us to document heavy pressure by introduced rats and to isolate and characterize a previously

undetected strain of the agent of avian cholera, in addition to the most prevalent one, isolated earlier. A bivalent vaccine led to protection of the chicks the year it was used.

6. *Synthesis and application.* The eco-epidemiological situation on Amsterdam Island is critical for the conservation of threatened albatross species and the vaccination approach represents a potentially useful management tool. The results nevertheless underline that interactions with other environmental factors, such as introduced mammals, need to be explicitly considered.

**Keywords:** prospecting, breeding failure, immature, *Thalassarche carteri*, Procellariiformes, individual behavior, infectious diseases

## Introduction

Infectious diseases are a major concern for human health, especially since the global coronavirus disease crisis of 2020. But emerging infectious diseases have also become a growing issue over the past few decades for wildlife conservation (Daszak et al. 2000; Smith et al. 2009) and the increase of their occurrence in wildlife populations is a particular concern for wildlife managers (Harvell 1999; Daszak et al. 2000; Dobson and Foufopoulos 2001; Kilpatrick 2011). In some cases, management approaches based on the use of vaccines have been proposed (Haydon et al. 2006; Leendertz et al. 2017; Flies et al. 2020), although they pose many challenges (Plumb et al. 2007; Barnett and Civitello 2020). Prior to their potential implementation, a thorough understanding of the eco-epidemiological dynamics is notably necessary, as well as field tests to address specific issues.

Among the issues that are required to be investigated before proposing the use of a vaccine, it is important to assess which life history stage, age and timing of vaccination would be most suitable and practical in relation to the species ecology and field situation (Saiada et al. 2019). In a disease affecting offspring, the age and timing of vaccination are especially important to optimize because of the need to account for the expected timing of immune protection, notably function of the development of the immune system, and the timing of epizooties. Moreover, it is important to determine against which strain(s) the vaccine need to be used, notably in the case of bacterial pathogens known to occur in diverse strains in populations of wild or farmed animals (Orynbayev et al. 2019; Omaleki et al. 2020). In the case of natural populations of long-lived species threatened by an identified pathogen killing young animals and found in domesticated species, it has been suggested that specifically tailored, so-



called, autologous killed vaccines could provide an efficient, safe and practical approach, notably if breeding females could be vaccinated to protect their young offspring (Garnier et al. 2012; Bourret et al. 2018).

Seabirds are among the most threatened groups of species, and even if diseases do not appear to be a threat affecting many seabird species (4,2% affected), they have on average a high impact on the few species affected (Dias et al. 2019). Avian cholera in particular has been identified as the main disease causing severe threats to albatrosses (Phillips et al. 2016), with the particular case of Amsterdam Island, in the Indian Ocean, where regular massive die offs of nestlings have been reported over the last decades and associated with this bacterial infectious disease (Weimerskirch 2004; Jaeger et al. 2018; Jaeger et al. 2019). Following an earlier study on the use of a vaccine against avian cholera in threatened albatrosses of Amsterdam island, it was determined that the vaccine developed against a single strain isolated in one of the local threatened host populations could be efficient at protecting nestlings some years, but not all (Bourret et al. 2018), and that persistent levels of antibodies could not be obtained among years in adults, apart after the injection of a novel formulation of the vaccine in previously vaccinated individuals (Gamble et al. 2019). In order to address further these issues, we implemented a follow up study in which we used the novel formulation of the vaccine on a new cohort of adults, set up two age groups of vaccination of nestlings, and investigated the potential effect of bacterial strain diversity.

Amsterdam Island hosts four threatened seabird species, listed as Endangered: Indian yellow-nosed albatross (IYNA) *Thalassarche crateri*, Sooty albatross (SOA) *Phoebastria fusca*, Amsterdam albatross (AMA) *Diomedea amsterdamensis* and Northern rockhopper penguin (NRP) *Eudyptes moseleyi* (BirdLife International 2021). The island is a particular critical site as it hosts 100% of the world population of the endemic AMA, 65% of the IYNA world population and is one of the only two breeding sites of NRP in the Indian ocean (Heerah et al. 2019). Since the early 1980's chicks from three of the four species, NRP, IYNA and SOA, are experiencing high mortality rate due to recurrent epizootics caused mainly by *Pasteurella multocida*, the avian cholera causative agent, and to a lesser extent by *Erysipelothrix rhusiopathiae* (Weimerskirch 2004; Jaeger et al. 2018; Jaeger et al. 2019; Tornos et al., unpublished). The adult survival does not seem to be affected (Gamble et al. 2019), but appeared to be lowered for some years (Rolland et al. 2009). Combining this high chick mortality rate with a survival of the adults lessened, the population of IYNA on Amsterdam Island has been declining dramatically on the last decades, from 37,000 pairs in the 1980's to 27,000 pairs in 2006 and 22,000

pairs in 2015 (Weimerskirch, H., Jouventin 1998; Rolland et al. 2009; Heerah et al. 2019). This critical situation in a National Nature Reserve is a major concern for managers and scientists. Investigating epidemiological dynamics in wildlife is often challenging but remote oceanic island offers a simpler ecosystem as a mainland would, with a limited richness of wildlife, interaction and movements. The difficulty to decipher such pathosystems is thus reduced (Wilson and MacArthur 1967) and provides an opportunity for scientists to explore an eco-epidemiological situation. It is furthermore an opportunity for managers to investigate potential conservation measures.

Several studies have thus been carried out to understand the eco-epidemiological situation on Amsterdam Island (Weimerskirch 2004; Jaeger et al. 2018; Gamble et al. 2019; Jaeger et al. 2019). It has been clearly proven that *Pasteurella multocida* (*Pm*) is responsible of the major significant part of the chick mortality (Weimerskirch 2004; Jaeger et al. 2018). A survey of mortalities on IYNA and SOA colonies during two breeding seasons has permitted to identify one strain of *Pm*, named D2C, corresponding to sequence type (ST) 61 in the RIRDC MLST scheme (Jaeger et al. 2018) and ST 91 in multi host MLST scheme (Tornos et al., unpublished). One approach that was considered to limit the impact of epizootics in IYNA population was the vaccination. An earlier study conducted on Amsterdam Island provided promising results and showed an encouraging solution with a vaccination to protect chicks of IYNA (Bourret et al. 2018). The use of vaccines in wildlife is not a new approach (Groner et al. 2016) but the implementation of vaccination protocols in wildlife and in particular with field constraints requires different aspects to be considered closely together (Haydon et al. 2006). Notably, following the study of Bourret *et al* 2018, several aspects of the vaccination design were discussed, important for academic and conservation reasons.

The life history stage to be vaccinated and the timing of vaccination are important to consider (Garnier et al. 2012; Saiada et al. 2019), as discussed in Bourret *et al* 2018. The study showed that an early vaccination protocol (2 days after hatching), resulted in a lack of seroconversion of chicks. The chick immune system being probably still immature and unable to mount a protective response (Mast and Goddeeris 1999). In contrast, vaccinating chicks 14 days after hatching (+/-3) proved an effective response, resulting in a seroconversion and a protective effect of the vaccine (Bourret et al. 2018). However, offering a protective effect of vaccination as early as possible may be important as it could avoid epidemics during the early stage of chicks. Nevertheless, for logistical reason it could not be possible to vaccinate at an exact age every year as hatching date could not be always known. Furthermore, if a more extensive vaccination procedure was carried out in different colonies, it would

be important to evaluate a date or a window when vaccination could be the more effective, i.e., when average in age of chicks is close to the ideal age of vaccination. The degree of synchrony of hatching will thus affect the ability to vaccinate chicks at comparable ages. IYNA on Amsterdam Island are very synchronized and consistent among years, the mean hatching date was on the 5<sup>th</sup> of December for the 3 breeding seasons concerned for this study, with 75% of chick having hatched on the 08<sup>th</sup> of December. It is therefore possible to consider a date or a window of days when vaccination could occur having a significant part of the chicks with an effective age of vaccination. Considering those elements, in the current study we explored vaccination effect on chicks at 7 days and 12 days after hatching. We will then have effectiveness of the vaccination on chicks having 2 days old (ineffective), 7 days old, 12 days old and 14 days old (effective).

Secondly, the vaccine used in Bourret *et al* 2018 was an autologous vaccine against a single strain (strain D2C, characterized by RIRDC MSLT). D2C was isolated in one of the local threatened host populations and it was injected to both young nestlings, for their immediate protection, and adults, in an attempt to enable a recurrent transfer of protection by females to their young offspring. But it is possible that multiple strains of *Pasteurella multocida* circulate concomitantly or not within the population (Orynbayev et al. 2019). The strain D2C had been isolated from 6 carcasses, 1 SOA and 5 IYNA, over two breeding seasons (Jaeger et al. 2018) which may not have allowed a representative sampling of the varieties of strains circulating in the populations of hosts. Epizooties might be due to a variety of strains circulating simultaneously or alternatively within a season, or taking turns one year to another. However, if the autogenous vaccine induced an effective protection against the strain they are produced with, they might not be effective against another strain, even very closely related (Omaleki et al. 2020). Also, a protocol to detect and necropsy dead birds was implemented during 2018-2019 and 2019-2020 breeding seasons. Bacteriological analysis permitted to identify another strain of *Pasteurella multocida* in a fresh dead IYNA chick, named A18Y007B1 (Tornos et al., unpublished). With other strains circulating in the population, we can easily suppose that the vaccination against only one strain may have relative efficiency, varying in time and space, depending on the circulation rate of each strain. In this study we thus implemented a new vaccination design including the new strain. Expecting that vaccination only against strain D2C might not be effective every year, and optimizing composition of the vaccine could overcome potential loss in efficiency. Also, we can expect that a vaccination against a strain circulating in a minority in the population would not have a significant effect as a multivalent vaccine targeting a diversity of strains would have.

Effectiveness of vaccination depends on the immunity level acquired by the population (Rees et al. 2013; van Dijk et al. 2021). Vaccination of the chicks showed encouraging results but a high level of immunity in the population could only be established after several generations of chicks vaccinated and only if a sustained immunity is acquired. Annual vaccination campaigns should be carried out over several years or decades and therefore might be costly and time consuming. In birds system, antibodies can be transferred from mother to chick via the egg yolk (Gasparini et al. 2002; Garnier et al. 2012). Vaccination of females could therefore confer a possible protection of offspring during the young stages (Pastoret et al. 1998) and over several breeding seasons for long-lived seabird species. The third aspect we explored in the current study is the potential protection afforded to chicks via the vaccination of mothers. A previous study on Amsterdam Island showed that the transfer of anti-*Pm* antibodies from mother to chick is possible (Gamble et al. 2019). However, the low antibody level detected in mothers two years after the injection of the vaccine did not result in a sufficient quantity of antibody transferred to the chick (Gamble et al. 2019) and therefore did not confer a protective effect (Grindstaff 2010). Considering those results, a new formulation of the vaccine was implemented to induce a higher and longer immune response and vaccination of adults was carried out to test the ability to protect chicks via maternal antibodies transfer.

Based on previous encouraging results of the efficacy of an autologous vaccine to protecting chicks from lethal infections (Bourret et al. 2018) and the potential transfer of antibodies from mother to the chick (Garnier et al. 2012; Gamble et al. 2019), we investigated several approaches to refine the vaccination design. This study therefore focused on three major axes: (1) defining an ideal age of vaccination, (2) updating vaccine composition integrating two strains of *Pm*, and (3) testing the potential protection of offspring via maternal antibody transfer.

## **Methods**

### Study site and population:

Amsterdam Island is a 55km<sup>2</sup> volcanic island and is part of the French Southern Lands National Nature Reserve since 2006 and inscribed in the world heritage list (UNESCO) since 2019. In addition to containing areas of exceptional natural beauty and aesthetic importance, the nature reserve contains threatened species of outstanding universal value from the point of view of science or conservation. The island hosts about 22 000 pairs of the endangered Indian yellow-nosed albatross (Weimerskirch *et al.* 2018), representing about 65% of the world breeding population (Birdlife 2018). The whole

breeding colony on Amsterdam Island is situated along stretches of the large Entrecasteaux cliffs, on the south-west coast of the island. The study plot is a patch of breeders hosting about 650 pairs nesting on those cliffs with an area of about 5000 meters square (Figure S3).

Indian yellow-nosed albatross nests colonially and breeds annually. Females lay a single egg in September-October, hatching occurs in November-December after approximately 70 days of incubation. Rearing is carried out by both parents and chicks fledge in March-April, which make short feeding visits to the nestling after it is left most of the time alone on its nest, from early January.

The study plot has been monitored since 2013 during each breeding seasons, with data and samples collected as part of capture-mark-recapture and breeding survey protocols involving marked nests, adults and chicks (Bourret et al. 2018; Gamble et al. 2019; Jaeger et al. 2019). In particular, a specific effort was made since 2018 to visit very regularly the area at the time of epizooties (January-February) to detect nestling death before scavengers, in order to conduct field necropsies and microbiology work to attempt isolation of bacterial strains.

Capture and handling of the birds have been carried out in accordance with regulation of the National Nature Reserve of the Southern French Territories and permits from the animal care authority (Ministry of Research). Breeding adult individuals were only manipulated before laying or after chick hatching on to avoid any disturbance when incubating. The vaccination design was carried out in the study plot.

#### General vaccination designs

Two experiments were implemented in parallel, the first one was to assess the possibility of a maternal antibody transfer from the mother to the chick and the potential protection of the chick during the early days after hatching, when massive mortalities occur. The second experiment was to confirm the protection of the chicks via their direct vaccination, as described in (Bourret et al. 2018), and to compare two age of vaccination in order to refine the ideal age or date of vaccination. A possible modification of the vaccine was considered if different strains of *Pm* would be isolated from dead birds.

#### Vaccine preparation

As in Bourret et al. 2018, the vaccine preparation relied on the production of autologous vaccines, i.e., vaccines made of killed bacteria strain(s) isolated from the field. The *Pm* strain D2C, which had been used by Bourret et al. 2018, had been isolated from a dead dark mantled sooty albatross collected on

Amsterdam Island during the 2011–2012 breeding season (Bourret et al. 2018; Jaeger et al. 2018). A second *Pm* strain, A18Y007B1, was isolated from a dead Indian yellow-nosed albatross chick necropsied during the 2018-2019 breeding season (Tornos et al., unpublished). Strains D2C and A18Y007B1 were grown separately on solid soybean-casein digest agar with yeast extract in a 500 cm<sup>2</sup> tissue culture flask at 37°C for approximately 22 hours. The bacteria were then collected in 20 mL of phosphate buffered saline (PBS), concentrations were estimated using optical density at 580 nm, and they were inactivated (killed) with a 34–38% formaldehyde solution at room temperature (20°C ± 5°C). After centrifugation at 2,934 × g for 30 minutes, the bacterial pellets were resuspended in PBS. Mineral oil adjuvants were used to obtain water-in-oil emulsions with 60% v/v adjuvant and containing the equivalent of 1e9 bacteria/ml, i.e., 1e9 bacteria/ml for monovalent vaccines (against respectively strain D2C or strain A18Y007B1) and 0.5e9 bacteria/ml/strain for the bivalent vaccine (against both strains). Water-in-oil emulsions have proven to induce strong immunity adjuvants and are extensively used for the formulation of inactivated avian vaccines (Hilgers et al. 1998; Jansen et al. 2005; Jansen et al. 2006). Endotoxin content was assessed using the kinetic chromogenic method of the European Pharmacopoeia and were less than 5,000 EU/mL for the 2017-2018, 2018-2019 and 2019-2020. Several innocuity and efficacy tests conducted on chickens, turkeys, and ducks had indicated that these were appropriate antigen doses. The inactivated bacteria were also used to set up an agglutination assay (Bourret et al. 2018 for more details) to detect antibodies.

#### Vaccination of adults and maternal antibodies transfer:

110 pairs of reproductive adults composing three groups were randomly selected within the study plot after egg laying in late November 2017. The first group, named adults *Pm*-injected cohort, was composed of 50 pairs that were injected subcutaneously with 0.5ml of autogenous vaccine against D2C strain (at this stage the strain A18Y007B1 was still unknown). Both individual for each pair were vaccinated, except for one pair which only one adult received the injection because it was in failure before capturing the second individual. Two control groups were used to compare their survival with that of the adults *Pm*-injected cohort. The first control group, adult NaCl-injected cohort, experienced the same protocol and handling conditions that the adults *Pm*-injected cohort. Both partner of 30 pairs were injected with 0.5ml of sodium chloride solution (NaCl 0.9%), except for one pair which only one adult received the injection because it was in failure before capturing the second individual. The second control group, unmanipulated cohort, was composed of 30 pairs that adults and chicks were left strictly unmanipulated to assess any potential adverse effect of vaccination or handling. Nests from

all groups were mixed and dispersed within the study plot. Adult individuals were captured and sampled before injection to measure their basal level in antibodies. Because adults leave the chick alone 3 weeks after hatching and only come back for feeding, making captures difficult, only 8 vaccinated and 2 NaCl injected adults were sampled in the few weeks following vaccination (2 to 4 weeks) to measure induced or natural seroconversion. Adults were then sampled one to two times a year for antibodies titration. Chicks were handled and sampled for each pair during the 2018-2019 and 2019-2020 breeding seasons (respectively one and two years after injection of both parents) at approximately 5, 10, 15, 30, 60 and 90 days old to measure and assess the dynamic of antibodies level in blood. Age of chicks is most of the time exactly known, but it may be estimated for some chicks when hatching date is missing (estimated with tarsus and wing measurement). Plasma samples were anonymized and randomized before serological testing. Chick survival was assessed very regularly among the breeding season, until fledging in late March.

#### Vaccination of chicks:

50 chicks from 50 pairs of reproductive adults were randomly selected, spatially spread within the study plot, after chick hatching. During the 2017-2018 breeding season, to optimize bird handling the 50 chicks were respectively the chicks of the adult *Pm*-injected cohort. Adults being injected after chick hatching, no maternal antibody have been transferred to the chick, also there was no interaction between adult's seroconversion and chick's serological status. 22 chicks were injected subcutaneously with 0.5ml of autogenous vaccine against D2C strain at 7 days old and 23 chicks at 12 days old (4 chicks being dead before receiving an injection and 1 chick did not receive the right volume of vaccine and was not considered in the study). During the 2018-2019 and 2019-2020 breeding season, 50 new pairs with a chick were randomly selected, independently of the adult *Pm*-injected cohort. In 2018-2019 the same design as the previous year was carried out, 21 chicks were injected subcutaneously with 0.5ml of autogenous vaccine against D2C strain at 7 days old and 22 chicks at 12 days old (7 chicks being dead before receiving an injection). In 2018-2019, a new strain of *Pm* named A18Y007B1 was identified on two dead chicks. Also, two new autogenous vaccines, one against strain D2C and one bivalent against both strains, were developed in addition of the one previously used and were tested in the following season 2019-2020. During this last season, considering results from previous seasons, 50 chicks were injected subcutaneously at 10 days old: 15 with 0.5ml of autogenous vaccine against strain D2C, 15 with 0.5ml of autogenous vaccine against strain A18Y007B1 and 20 with 0.5ml of the bivalent autogenous vaccine against both strains D2C and A18Y007B1. Chicks from each pair were handled and

sampled for the three breeding seasons at approximately 5, 10, 15, 30, 60 and 90 days old to measure and assess the dynamic of antibodies level in blood. For the three breeding seasons chick survival was assessed very regularly until fledging in late March. Chicks from the Adult NaCl-injected cohort and unmanipulated cohort control groups previously described were used to compare their survival, representing respectively a control group with chicks manipulated following the same frequency of captures and sampling as vaccinated chicks and a control group with chicks left unmanipulated.

#### Field Procedures:

Rigorous biosecurity measures were observed in the field to avoid spreading the disease among bird colonies and among individual birds within the colonies. These involved the use of dedicated clothing consisting of a different set of waterproof leggings, jackets, and rubber boots for each bird colony. Waterproof overalls, any other equipment (e.g., caliper, ruler) and hands were disinfected between contacts with any two birds, respectively using hydrogen peroxide, commercial disinfectant wipes, and hydroalcoholic gel. All pieces of clothing were thoroughly cleaned and disinfected again at the end of each day.

#### Serological analysis

As in Gamble et al. 2019, a specific antibody titration by agglutination (SEROPAST® method, Ceva Biovac, France) was carried out. The aim of this microagglutination method (MAT) is to titrate antibodies against *Pasteurella multocida* in bird plasma by slow agglutination on microplates. Ninety-six well, U-bottomed microplates are used. Each plasma undergoes serial twofold dilutions in sterile physiological water on the first 11 wells of a row (the last well being used as a negative control containing only physiological water). A hen serum sample positive to the Heddleston 1 type is used as a control. An inactivated antigenic suspension of *P. multocida*, from strain D2C strain or strain A18Y007B1 is then added to all wells, resulting in final serum dilutions ranging from 1:10 to 1:10240. Plates are read following incubation at 37°C for 18 to 24 hours. Sedimentation of the antigen (resulting in the presence of a pellet) indicates a negative reaction. Agglutination of the antigen (resulting in a cloudy solution) indicates a positive reaction. Antibody titer is the reciprocal of the last dilution where a positive reaction is observed. Individuals were considered seropositive if agglutination was observed at the tenfold or more dilution. Results are expressed as titers  $\log_2[\text{last positive dilution}/10] + 1$  (Gamble et al. 2019). A set of plasma samples (35 chicks and 133 adults) vaccinated against strain D2C was tested with both antigenic suspension (strain D2C or A18Y007B1) to assess the potential specificity



of the method and thus impacting titration of antibodies produced avec injection of each of the vaccine or even the potential specificity of each vaccine. Two-way repeated measures ANOVA analysis were used to compare seroconversion profiles. The antigenic solution for MAT experiment needs to be prepared independently for each serial of tests. Also, to assess any bias in titration and to be sure that titration can be comparable between years, antibodies level from 19 samples of the 2018/2019 breeding season (including vaccinated, NaCl injected or not injected individuals) were tested independently with the antigenic solution prepared in 2018/2019 and the antigenic solution prepared in 2019/2020. We measured a very positive correlation between both titration (*Pearson's correlation coefficient*  $cor=0.9942$ ,  $p<<0.01^{****}$ , *formula*  $\sim y=0.91x-0.03$ ) confirming that titration by MAT can be compared within or between years.

### Survival analysis

The survival of the chicks from treatment and control groups was studied for the three breeding seasons 2017/2018, 2018/2019 and 2019/2020. Different treatment groups were considered for the analyses: chicks unmanipulated (named unmanipulated); chicks manipulated and sampled from parents injected with NaCl solution (named manipulated); chicks manipulated, sampled and vaccinated (named vaccinated) and chicks manipulated and sampled from parents vaccinated. As in Bourret et al. 2018, for 2017/2018 and 2018/2019 breeding seasons chicks unmanipulated and chicks manipulated but not injected were used as control groups. For 2019/2020 breeding season, because of logistical reasons and considering previous results, only chicks manipulated were used as control group. Chick survival as a function of treatment in a given reproductive season was compared using a Cox proportional hazards model (Andersen & Gill 1982) of the form  $survival \sim treatment$  as implemented in the 'survival' package (Therneau 2020). All analyses were computed using R (R Core Team 2020). Plot were created using 'survminer' package (Kassambara et al. 2020) and p-value of models were calculated using Long-Rank method.

The survival of chicks vaccinated was evaluated following 2 methods. The first method, comparable to the survival analysis of Bourret et al. 2018 excluded all chick dead before having reached the age of vaccination for all groups. To estimate survival of the chicks vaccinated at 7, 10 or 12 days after hatching, chicks from vaccinated groups and both control groups dead before respectively 7, 10 and 12 days old were not considered in the survival analysis. However, survival estimation considering chicks dead since hatching could be relevant because those will count for the calculation of the growth

rate of the population. Also, in a second survival analysis we considered all chicks being dead from hatching to fledging.

To evaluate the survival of the chicks from parents vaccinated, we only consider those for which both parents injected stayed and reproduced together the corresponding year (*i.e.*, we did not take into account pairs whose parents were separated and reproduced with an extra partner, not vaccinated).

## Results

The breeding success of both control groups was very low (<15%) during 2017/2018 and 2018/2019 breeding season (Supplement, Figure S1). No significative difference was observed between control groups for the 2 breeding seasons (Supplement, Figure S1 & Table S1), meaning that there is no significant adverse effect of handling. A global season effect was detected in survival between the 2 breeding seasons (Supplement, Table S1) with a risk of death 1.4 times higher during 2018/2019 breeding season.

### Age of vaccination

For the two groups of chicks that were vaccinated respectively at 7 or 12 days after having hatched, the serological analysis of the plasma samples collected showed a seroconversion of all the chicks, whatever the age of vaccination, for both breeding season (Figure 1, Table 1). By contrast, non-injected chicks did not show a dynamic of seroconversion. We can notice that during 2018/2019 breeding season, 6 chicks were naturally seropositive at some time points. 2 were naturally seropositive at 10 days old but were not seropositive at 5 (titer respectively 5 and 1). 1 chick was seropositive at 5 and 10 days old (titer respectively 3 and 2) but not after 10 days old. None of the parents of those 3 chicks were seropositive when sampled, excluding the hypothesis of antibodies transmitted by the mother. 1 chick was seropositive at 50 days old but was not tested seropositive before or after. 2 chicks were slightly seropositive at 90 days old. We did not detect any difference in the serological profiles of the different groups between the two seasons: there was no difference in the seroconversion profile whether chicks were vaccinated at 7 or 12 days old ( $F(1, 308) = 0.006, p = 0.94$ ). However, we detected an association between the age at vaccination and their survival probability. For the 2017/2018 breeding season (Figure 2), we did not detect a positive effect of vaccine injection on survival for the group that had been injected at 7 days old (compared to manipulated chicks or *~manipulated*; Hazard Ratio  $HR=0.7038$ ; Confident Interval  $CI: 0.3810-1.300, p=0.26$ ), while chicks that had been injected at 12 days old showed a higher survival probability compared to manipulated (*~manipulated*,  $HR=0.5047$ ;

CI: 0.2555-0.9969,  $p < 0.05^*$ ), with a risk of death compared to manipulated chicks divided by 2 and divided by 3.1 compared to unmanipulated chicks (~unmanipulated,  $HR=0.322$ ; CI: 0.148-0.7013;  $p < 0.01^{**}$ ).

For the 2017/2018 breeding season, including chicks being dead before 7 days old for all groups we did not find any effect of vaccination at 7 days ( $HR=0.592$ ; CI: 0.3332-0.1052;  $p=0.07$ ). However, for a vaccination at 12 days old the risk of death stayed reduced, divided by 2.2 compared to manipulated chicks (~manipulated;  $HR=0.4597$ ; CI:0.2547-0.830,  $p < 0.01^{**}$ ) including chicks being dead before 12 days old. Importantly, considering all chicks vaccinated, whatever the age of injection, from hatching to fledging, the risk of death is divided by 1.9 (~manipulated;  $HR=0.5138$ , CI: 0.3247-0.8129;  $p < 0.01^{**}$ ).

During the 2018/2019 breeding season, the same design was implemented. No positive effect of vaccination effect was detected (Figure 3), whether the chicks were vaccinated at 7 days old (~manipulated;  $HR=1.609$ ; CI:0.8920-2.874;  $p=0.12$ ) or at 12 days old (~manipulated;  $HR=1.243$ ; CI: 0.6907-2.238;  $p=0.47$ ). Many reasons may explain this result and are explored in the discussion. One interesting point is the isolation of the new strain A18Y007B1 from dead albatrosses early that season. Even if this strain was discovered in a lower proportion (Tornos et al., unpublished) than the historical known strain D2C, it may explain the lower effect of a vaccination against D2C if both strains are circulating concomitantly in the study colony. Furthermore, a higher predation of rats was noticed during the early stage of chick rearing than usually observed: in many instances, severe bite wounds were observed on the chicks, notably on the head and neck (Supplement, Figure S4). These hypotheses were comforted by the higher mortality rate measured during this season compared to the previous season.

#### Change in the composition of the vaccine

Due to logistical reasons, plasma samples from 2019-2020 season could not be analyzed. However, considering serological profile from previous seasons we could expect a seroconversion of most of the chicks. As expected, because the strain was suspected to be in a minority, the monovalent vaccine against strain A18Y007B1 did not show a significant effect (Figure 4) on global survival (~manipulated;  $HR=0.5579$ ; CI: 0.2810-1.1079;  $p < 0.1$ ). In contrast to the previous season 2018/2019, the monovalent vaccine against strain D2C showed a significant effect on global survival (Figure 4). The model showed a risk of death divided by 2 when vaccinated compared to manipulated chicks (~manipulated;  $HR=0.4944$ ; CI: 0.2563-0.9950;  $p < 0.05^*$ ). This result confirmed the protective effect of the vaccine but

emphasized that the effect may vary over time. Finally, and as expected, injection with the bivalent vaccine showed a significant effect on survival compared to control group. The risk of death was divided by 2.2 compared to manipulated chicks when vaccinated with the bivalent vaccine ( $\sim$ manipulated;  $HR=0.4578$ ;  $CI: 0.2390-0.8764$ ;  $p<0.05^*$ ).

#### Maternal antibodies transfer and protection of the chicks

The majority of the adults injected with the vaccine against strain D2C became seropositive (Figure 5) and that seroconversion occurred within a month following injection. 88% (7/8) adults showed high titer in antibodies when sampled between 2 to 4 weeks after being injected (Figure 5), with a significant higher titer in antibodies than controls (*mean titer difference*  $+5.04$  [ $\pm 1.18$ ],  $p<0.01^{****}$ ). 98%, 97% and 88% of adults vaccinated were seropositive when sampled respectively 1-, 2- and 3-years following vaccination (Figure 6) when only 6% of those were seropositive before vaccination. The mean titers in antibodies were still significantly higher up to 3 years after injection (Figure 6) compared to the same individuals before injection (*mean titer difference respectively*  $+3.61$  [ $\pm 0.47$ ],  $p<0.01^{****}$ ,  $+2.55$  [ $\pm 0.45$ ]  $p<0.01^{****}$  and  $+2.08$  [ $\pm 0.49$ ],  $p<0.01^{****}$ ) (Supplement, Table S2). The mean titers in antibodies were significantly higher 3 years after injection for individuals vaccinated compared to NaCl injected (*mean titer difference*  $+1.69$  [ $\pm 0.60$ ],  $p<0.01^{****}$ ). Also, it is important to highlight that a decrease in antibody titer from one year to the next was detected after the initial seroconversion (Supplement, Figure S2). Adults injected with the NaCl solution did not show a trend for seroconversion after injection compared to vaccinated birds.

Serological profile of chicks during the 2018/2019 breeding season (*i.e.*, 1 year after parents' injection) showed a significantly higher titer in antibodies 5 days after hatching for chicks from vaccinated parents compared to chicks from parents injected with NaCl solution (*mean titer difference*  $+2.68$  [ $\pm 1.21$ ]  $p<0.01^{****}$ ). As expected, those results demonstrate that we do have a transfer of maternal antibodies from the mother to chick (Figure 7), with 93% seropositive chicks from vaccinated parents versus 4% from NaCl injected parents (Figure 8). The antibody levels in females showed a positive correlation with chick levels 5 days after hatching (*Pearson's correlation coefficient*  $cor=0.92$ ,  $p<0.01^{***}$ ,  $n=11$  pairs mother/chicks) and mean antibodies titer are comparable (paired Student test,  $p=0.78$ ). Mean antibodies titer is still significantly higher 10 days after hatching for chicks from vaccinated parents (*mean titer difference*  $+1.37$  [ $\pm 1.25$ ]  $p=0.01^*$ ) compared to chicks from NaCl injected parents, with respectively 82% of chicks seropositive and 14%. 50% of sampled chicks from

vaccinated parents were still seropositive at 15 days old and 33% at 40 days old. None of the chicks from both groups was seropositive 50 days after hatching. One chick from vaccinated parents showed a high level in antibodies at 90 days old. This chick was seropositive at 40 days old but was seronegative at 60 days old. Likely impossible to determine the cause of a such immune response, however it could have been induced by a natural exposure of the chick to *Pasteurella multocida* in late stages. The presence of maternal antibodies in the early stages might have induced a high immune response.

Unfortunately, due to logistical reasons chick samples from 2019/2020 breeding season could not be analyzed. Considering the significantly lower titer in antibodies in 2019/2020 for adults vaccinated compare to 2018/2019 and the positive correlation mother/chick in antibodies level, we could expect a lower titer for chicks at 5 days old and thus a sooner decay compared to those of 2018/2019.

Considering the relative fast decay in antibodies level for chicks and the only protection they have against strain D2C, we did not expect a significantly high effect of maternal antibodies in survival. As supposed and confirming results from direct vaccination of chicks against D2C, during 2018/2019 breeding season (Supplement, Figure S3) we did not detect any positive effect in chicks' survival (*~manipulated*;  $HR=0.8285$ ;  $CI: 0.5313-1.2912$ ;  $p=0.4$ ). However, maternal antibodies transfer showed a positive effect on chicks' survival during 2019/2020 breeding season (Figure 9) while a lower level in antibodies titer for chicks was expected. The risk of death was divided by 2.1 compared to the chicks from NaCl injected parents (*~manipulated*;  $HR=0.4743$ ;  $CI: 0.2680-0.8396$ ;  $p=0.01^*$ ). Those results reinforced previous results and confirmed the loss in efficiency of the protection against D2C during 2018/2019 breeding season, whether via direct vaccination of chicks or via maternal antibodies. But nevertheless, those results are encouraging as we did have transfer of antibodies from the mother to the chick, and maternal antibodies showed an effective protection of the chick during 2019/2020 breeding season which might not be neglected.

## **Discussion**

In this study we explored multiple aspects of a vaccination design in a wild population of Indian yellow-nosed albatrosses. Previous encouraging results showed the high potential of an autologous vaccine to protect chicks from death (Bourret et al. 2018). The results show that the protection of vaccinated nestlings compared to controls was confirmed 2 of the 3 years. We showed that age of vaccination is an important factor that have to be considered, but in the case of very synchronized birds like

albatrosses, implementing a large-scale vaccination in a short window of time is possible. The study highlighted the need of a close monitoring to detect any variation in pathogens community to update vaccine if needed and keep the protecting effect of the vaccination. Finally, we showed that the new formulation of the vaccine led to persistent level of antibodies in adults with a seroprevalence still high 2 years after vaccination. Vaccinated adults offered protective effect on the nestlings via maternal antibodies transfer 2 years after being vaccinated.

This study confirmed previous results and showed that the used of autogenous vaccines is a very encouraging solution to protect chicks of IYNA from death due to avian cholera diseases. The results showed that the age of injection plays a role in the efficiency of the protection and complemented previous results. Vaccination of chicks leads to a seroconversion whether chicks are 14 (from Bourret *et al* 2018), 12 or 7 days old, but not at 2 days old. However, efficacy in protecting chicks depends also on the age of injection and the results suggested that the later the injection, the better the protection. Nonetheless, those results compared survival only after vaccination and a key point to be considered is that the effectiveness of a vaccination protocol may be evaluated including chicks being dead before injection because those will count for the calculation of the growth rate of the population. For the 2017/2018 breeding season, including chicks being dead before 12 days old, results showed a risk of death reduced. Importantly, considering all chicks vaccinated, whatever the age of injection, from hatching to fledging, the risk of death stayed significantly reduced. This result is important as it suggests that, even if the whole demographic parameters of the population are not known, it is possible to implement a large-scale vaccination design on a time window. IYNA being very consistent among years in their breeding phenology, on December 18th of most years, 75% of chicks would be 10 days old or more, thus acting on a short window at about this date could be used as a rule of thumb to expect a positive effect on the number of chicks surviving.

If autologous vaccine showed encouraging solution, the study also highlighted that the efficacy of a vaccine may vary in time. Vaccination against D2C was effective in 2015/2016 (Bourret *et al.* 2018), 2017/2018 and 2019/2020. However, we did not detect any positive effect on survival for chicks vaccinated in 2018/2019. While disappointing, this result emphasized the complexity of vaccination and the need of an integrated approach to refine efficacy. Two important matters are to be considered regarding the 2018/2019 breeding season.

First, Amsterdam Island hosts 3 invasive species, brown rats (*Rattus norvegicus*), house mice (*Mus musculus*) and feral cats (*Felis catus*) that are known to be threats for many seabird species (Croxall et al. 2012; Dias et al. 2019). Interestingly, if those alien species are found on the island for many decades now, none of them was described as a major threat for IYNA and no obvious and extended predation was described since the beginning of the monitoring several decades ago. However, a close monitoring of the cause of death of nestlings enabled us to document heavy pressure by introduced rats during 2018/2019 breeding season (Supplement, Figure S4). During the first weeks of chick rearing, many attacks were documented on young chicks. Chicks were attacked while alive and protected by one parent. Rats killed directly or most of the time caused fatal injuries leading to the death of the chick within few days. First observations of such attacks were documented during 2017/2018 breeding season but the impact did not seem to be significant (personal observation). The predatory behavior seems to be established now as it was observed every year since. The reasons of the shift remain poorly understood (only few years of hindsight) but the specific behavior of rodents on such island are well documented (Adler 1994; Russell et al. 2011). Considering this, a stronger effort was implemented the following years to document this behavior. Further studies, with specific and appropriate materials are in process (camera trap, specific trapping, CMR...) and tend to confirm that rats now play a major role in chick mortality. Also, even if the vaccination showed positive effect during 2017/2018 and 2019/2020 season, we have to take into consideration that the effectiveness of the vaccine might be minimized due to the high predation rate. Unfortunately, it is hardly possible to quantify precisely and explain the part of the mortality due to the rats (most of the dead chicks simply disappeared from nests and cause of death could not be identified in all cases). More than responsible of a direct predation and death, rats may play an important role as reservoir and moreover disseminator of *Pasteurella multocida* (Heddleston et al. 1964; Gregg et al. 1974). Furthermore, ongoing studies show that rats in Entrecasteaux cliffs, and especially in the study colony, have a high prevalence in DNA of *Pm* measured in oral swabs making them potential carrier and spreader of the bacteria. In maintaining a high rate of transmission of *Pm*, by circulating intensively between nests, visiting nests or biting birds, rats may play a significant role in the epidemics. However, efficacy of a vaccine depends on the percentage of individuals being vaccinated in the population and in most cases only a high level of population immunity makes vaccine effective (Perelman et al. 1990; Rees et al. 2013; van Dijk et al. 2021). With less than 15% of chicks vaccinated in the study colony (50 chicks vaccinated over ~400 chicks in the study colony) representing about 0.2% of the whole Amsterdam colony, the efficacy of vaccine might be reduced. The very near coming eradication plan for the 3 invasive species is a promising

conservation plan. Combining eradication and vaccination should maximize the survival of chicks of endangered IYNA and are very encouraging measures.

Secondly, repeated outbreaks of *Pasteurella multocida* on Amsterdam Island are probably not due to the re-emergence of the same strain, or to a single strain circulating alone (Kock et al. 2018; Crawford et al. 2019). Confirming this hypothesis, during 2018/2019 season a close monitoring of the cause of death of nestling allowed us to identify a new strain of *Pm* (Tornos et al. in prep) differing from the historical identified strain D2C (using multi-host MLST identification). However, recent studies showed that immunization acquired following injection with an autologous vaccine might not be effective against another strain (Omaleki et al. 2020). Furthermore, this study suggests that even in a very same strain (same MLST sequence type), a more closely difference only identifiable by whole genome sequencing, might minimize the efficacy of an autologous vaccine. Our results showed that by updating the composition of the vaccine, including the new strain, we improved the vaccination efficacy. Also, it would be important to go further in the identification and search for more precise clades to ensure and optimize if needed the vaccination. Moreover, we demonstrate that a long-term and integrated monitoring of the population, including IYNA but also other species as rats, is necessary to maintain the efficacy of vaccination and potentially plan conservation measures.

In this study we showed that the new formulation of the vaccine induced a longer persistence of antibodies in blood in vaccinated adults compared to the first formulation used (Gamble et al. 2019) with a large proportion of adults seropositive 3 years after injection and a significant higher titer in antibodies in blood. However, antibodies level decreased significantly one year to another and we could expect adults to be closely seronegative in few years. The study in Gamble *et al* 2019 showed very interesting results when injecting a booster 2 years after primo-injection with a significantly higher immune response and a potential longer persistence of antibodies in blood. Also, it would be interesting to implement a second injection for adults on the next years (4 or 5 years following primo-injection).

The results also confirmed that we do have maternal antibodies transfer from mother to chick as described for other seabird species (Garnier et al. 2012; Gamble et al. 2019). Our work suggests that despite a relatively fast decay of maternal antibodies, a protection of the offspring might be offered when vaccinating female. Unfortunately, when adults were vaccinated the strain A18Y007B1 was not yet identified and adults and chicks are only protected against strain D2C. The protective effect of



maternal antibodies could thus have been minimized considering the contrasted effect of direct vaccination of chicks against only strain D2C.

Finally, this study illustrated the promising interest of vaccination to protect an endangered species. However, we highlighted that the application on wildlife might be challenging and several parameters have to take into account before implementing a large-scale design. Albeit very promising, vaccination could be ineffective or even more damaging for a population if the vaccination campaign was not well designed. Efficacy of a vaccination may be compromised, by selecting more virulent strains, or promoting spillover (Barnett and Civitello 2020). Also, we recommend an integrated approach before implementing vaccination in wild populations. Approaches may include the study of the host-pathogen system, rigorous monitoring, field trials and modelling.

#### **Authors' contributions**

TB conceived the idea of this work; TB, JT and AG designed the study; JT, MB, AC and AF collected the data; JT performed statistical analyses; JT and TB led the writing of the manuscript. All authors contributed substantially to the drafts and gave final approval for publication.

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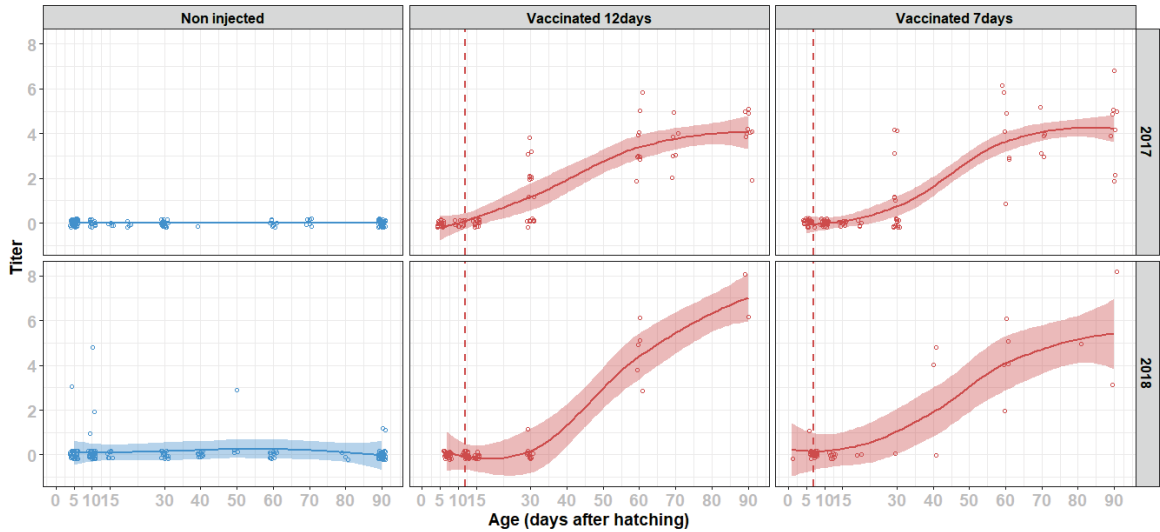
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# Optimizing a vaccination approach against avian cholera to protect endangered albatrosses – Figures

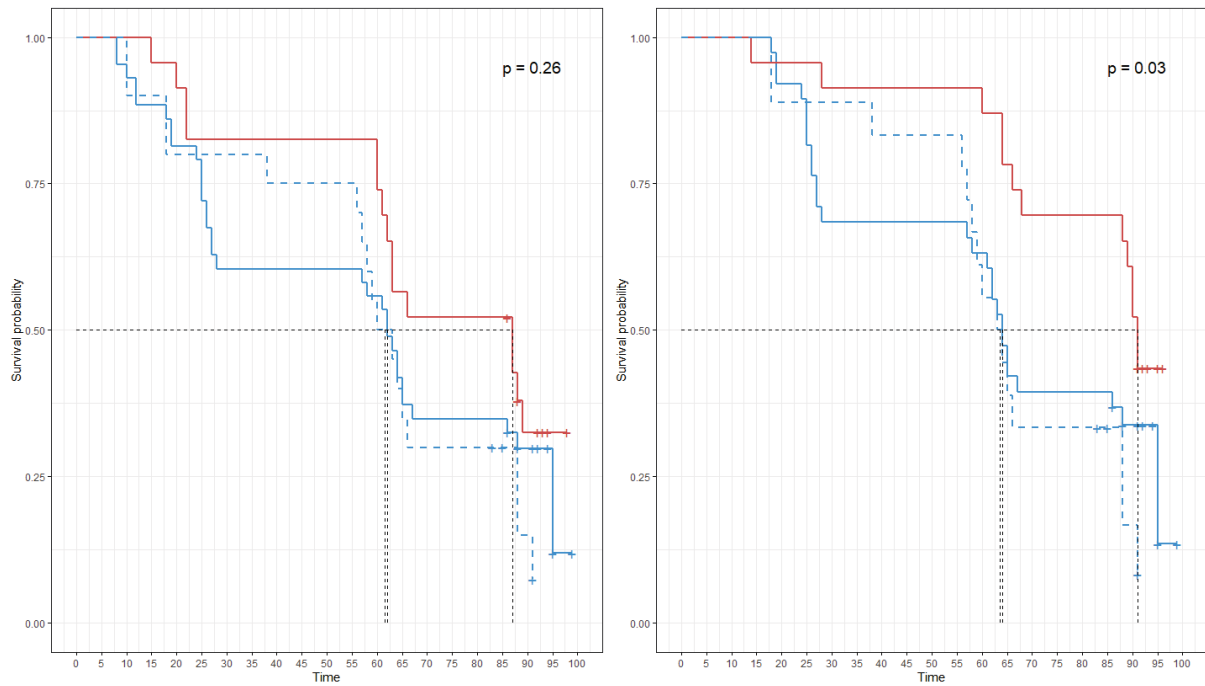


**Figure 1:** Immune response of chicks following vaccination. Quantification of anti-Pm antibodies by MAT in IYNA chicks vaccinated during the 2017/2018 (upper panels) and 2018/2019 (lower panels) season. Not injected: chicks not injected but manipulated and sampled following the same design as vaccinated chicks; Vaccinated 12days: chicks vaccinated 12 days after hatching; Vaccinated 7days: chicks vaccinated 7 days after hatching. Dotted lines indicate time of vaccination. Each dot is a single value of Titer for one chick at a given age. Dots are voluntary jittered for visual aspect

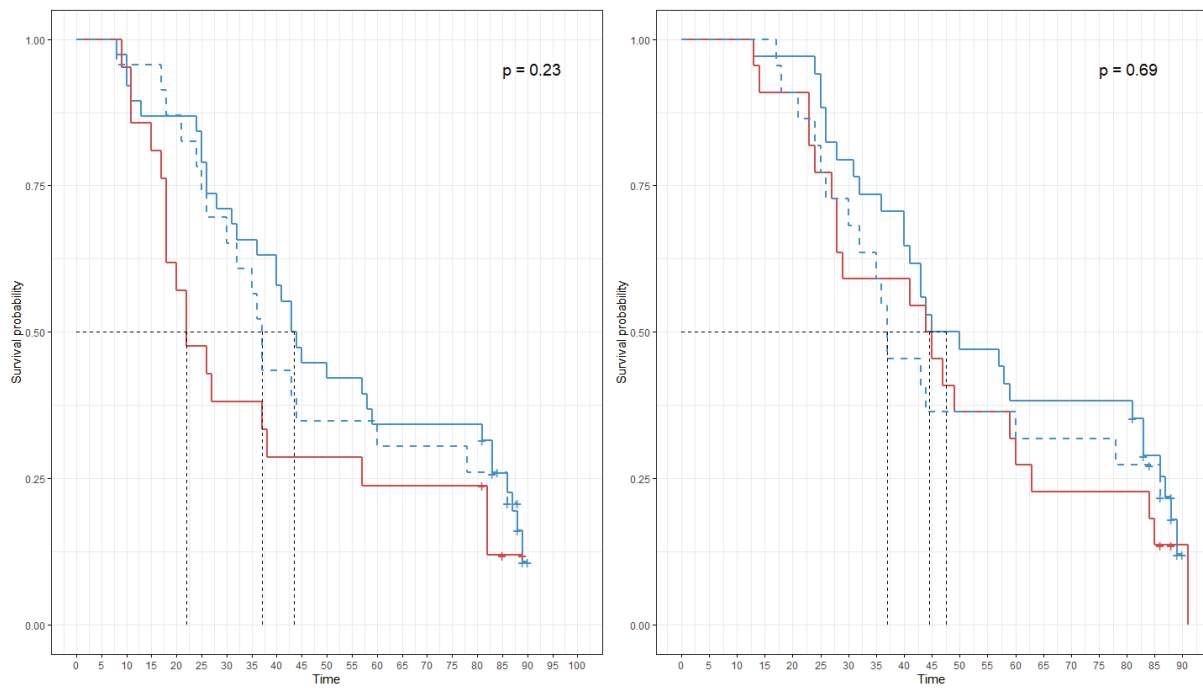
**Table 1:** details for figures 1 and 2. Number of chicks sampled considering season, group and age of sampling. Sd: standard deviation; se: standard error; ci: confident interval. Age may be sometimes an estimation.

Season	Treatment	Age (days after hatching)	Number sampled	mean Titer	sd	se	ci
2017/2018	Not injected	5	34	0	0	0	0
		7	1	0	NA	NA	NaN
		10	12	0	0	0	0
		15	4	0	0	0	0
		20	4	0	0	0	0
		30	24	0	0	0	0
		40	1	0	NA	NA	NaN
		60	8	0	0	0	0
		70	7	0	0	0	0
	90	40	0	0	0	0	
	Vaccinated 12days	5	20	0	0	0	0
		10	7	0	0	0	0
		12	3	0	0	0	0
		15	14	0	0	0	0
		30	20	1.2	1.2814466	0.2865402	0.5997355
		60	9	3.6666667	1.2247449	0.4082483	0.9414223
		70	6	3.5	1.0488088	0.4281744	1.1006574
	90	10	4.1	0.875595	0.2768875	0.626363	
	Vaccinated 7days	5	20	0	0	0	0
		7	3	0	0	0	0
		10	22	0	0	0	0
		15	9	0	0	0	0
		20	4	0	0	0	0
		30	21	0.7142857	1.3093073	0.2857143	0.5959896
		60	7	4	1.8257419	0.6900656	1.6885296
	70	5	3.8	0.83666	0.3741657	1.0388506	
	90	8	4.25	1.6690459	0.5900968	1.3953573	
2018/2019	Not injected	5	36	0.0833333	0.5	0.0833333	0.1691757
		10	30	0.2666667	0.980265	0.1789711	0.366037
		15	8	0	0	0	0
		30	13	0	0	0	0
		40	11	0	0	0	0
		50	4	0.75	1.5	0.75	2.3868347
		60	13	0	0	0	0
		80	3	0	0	0	0
		90	37	0.0540541	0.2292434	0.0376874	0.0764336
	Vaccinated 12days	7	25	0	0	0	0
		12	21	0	0	0	0
		15	12	0	0	0	0
		30	13	0.0769231	0.2773501	0.0769231	0.167601
		60	5	4.6	1.1401754	0.509902	1.4157148
	90	2	7	1.4142136	1	12.706205	
	Vaccinated 7days	1	1	0	NA	NA	NaN
		5	1	1	NA	NA	NaN
		7	21	0	0	0	0
		12	8	0	0	0	0
		20	2	0	0	0	0
		30	2	0	0	0	0
40		3	3	2.6457513	1.5275252	6.5724106	
60		5	4.2	1.4832397	0.663325	1.8416853	
80		1	5	NA	NA	NaN	
90		2	5.5	3.5355339	2.5	31.765512	

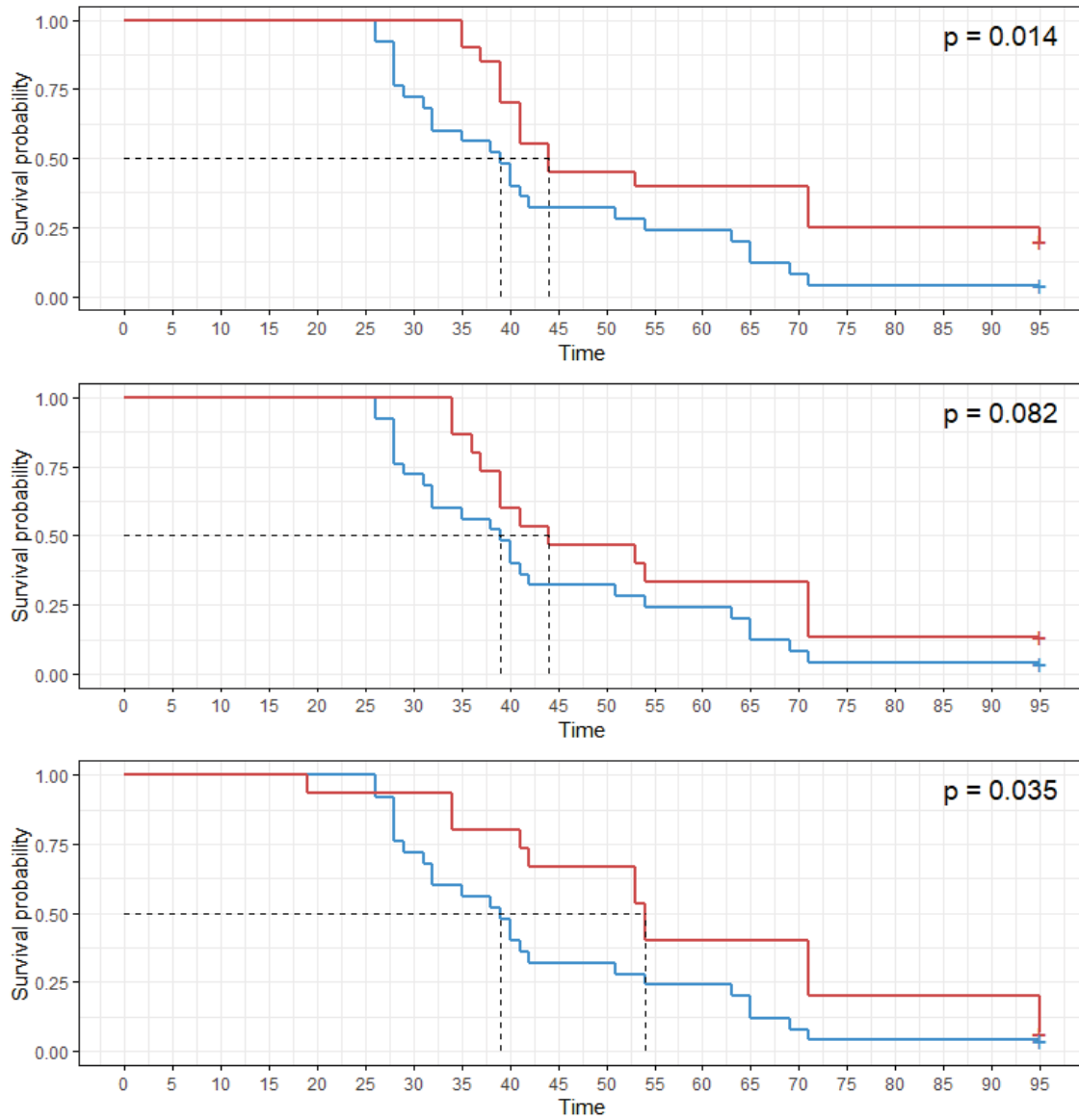




**Figure 2:** Survival curves for 2017/2018 breeding season. **Left panel** survival curves for chicks vaccinated at 7 days old: red plain line for the chicks vaccinated (n=23); blue plain line for chicks manipulated and sampled but not injected (n=43); blue dashed line for the unmanipulated chicks (n=20). **Right panel** survival curves for chicks vaccinated at 12 days old: red plain line for the chicks vaccinated (n=22); blue plain line for chicks manipulated and sampled but not injected (n=38); blue dashed line for the unmanipulated chicks (n=18). X-axes (Time) correspond to days after hatching. Log-rank method for p-value. Black dashed line are the median survival time values

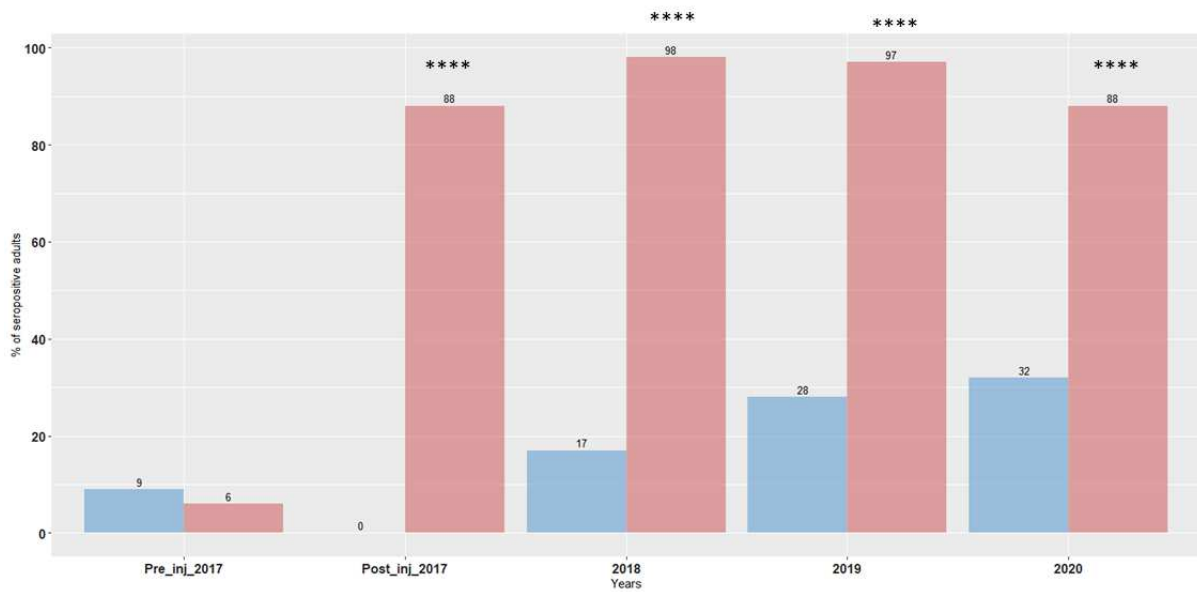


**Figure 3:** Survival curves for 2018/2019 breeding season. **Left panel** survival curves for chicks vaccinated at 7 days old: red plain line for the chicks vaccinated (n=21); blue plain line for chicks manipulated and sampled but not injected (n=37); blue dashed line for the unmanipulated chicks (n=23). **Right panel** survival curves for chicks vaccinated at 12 days old: red plain line for the chicks vaccinated (n=22); blue plain line for chicks manipulated and sampled but not injected (n=33); blue dashed line for the unmanipulated chicks (n=22). X-axes (Time) correspond to days after hatching. Log-rank method for p-value. Black dashed line are the median survival time values

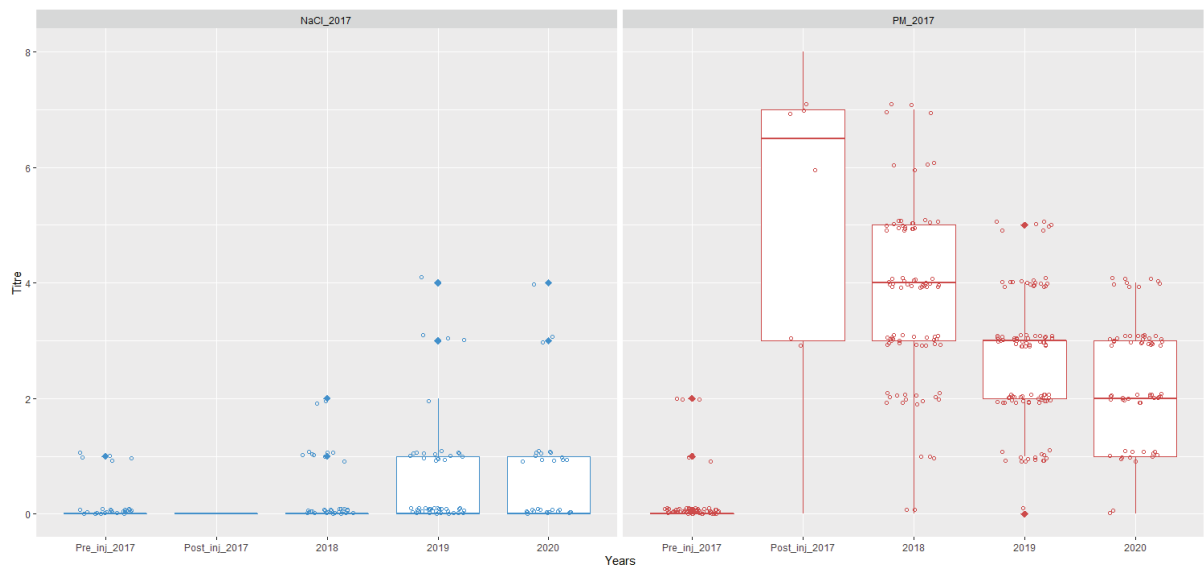


**Figure 4:** Survival curves for 2019/2020 breeding season. **Top panel** survival curves for chicks injected with bivalent vaccine at 10 days old: red plain line for the chicks vaccinated (n=19); blue plain line for chicks manipulated and sampled but not injected (n=25). **Middle panel** survival curves for chicks injected with vaccine against A18Y007B1 at 10 days old: red plain line for the chicks vaccinated (n=15); blue plain line for chicks manipulated and sampled but not injected (n=25). **Bottom panel** survival curves for chicks injected with vaccine against D2C at 10 days old: red plain line for the chicks vaccinated (n=15); blue plain line for chicks manipulated and sampled but not injected (n=25). X-axes (Time) correspond to days after hatching. Log-rank method for p-value. Black dashed line are the median survival time values

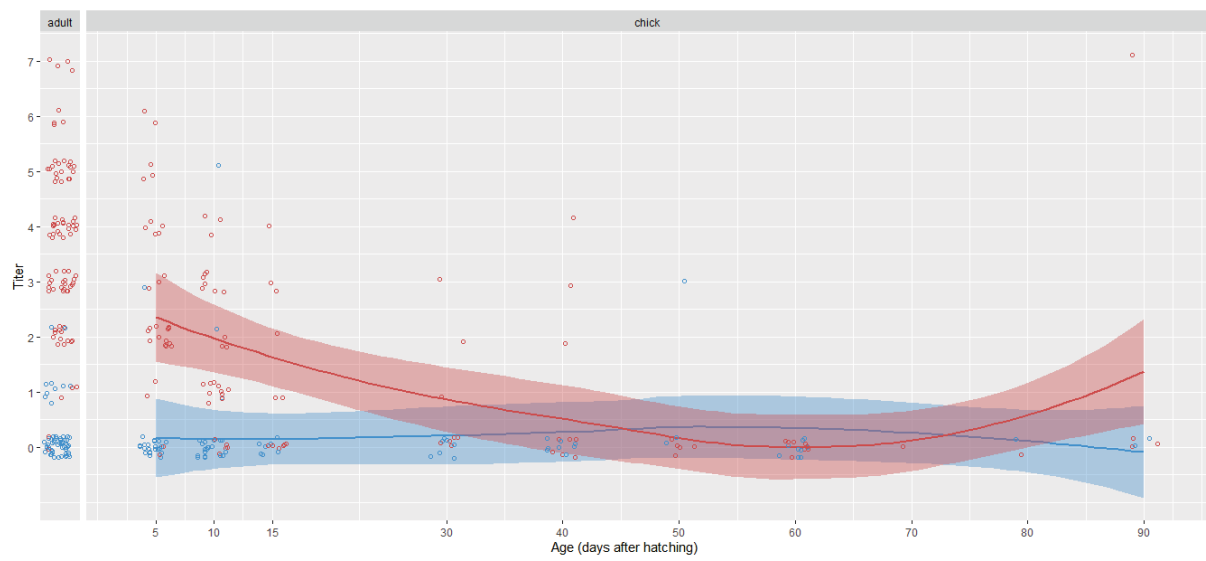
Treatment	Season	Number of samples	Number of seropositive samples (percentage of total)	Titer (log)	sd	se	ci
NaCl_2017	Pre_inj_2017	58	5 (9%)	0.09	0.28	0.04	0.07
	Post_inj_2017	2	0 (0%)	0.00	0.00	0.00	0.00
	2018/2019	58	10 (17%)	0.21	0.49	0.06	0.13
	2019/2020	71	20 (28%)	0.42	0.84	0.10	0.20
	2020/2021	47	15 (32%)	0.47	0.88	0.13	0.26
PM_2017	Pre_inj_2017	104	6 (6%)	0.09	0.37	0.04	0.07
	Post_inj_2017	8	7 (88%)	5.13	2.80	0.99	2.34
	2018/2019	88	86 (98%)	3.69	1.50	0.16	0.32
	2019/2020	104	101 (97%)	2.63	1.19	0.12	0.23
	2020/2021	72	63 (88%)	2.16	1.22	0.14	0.29



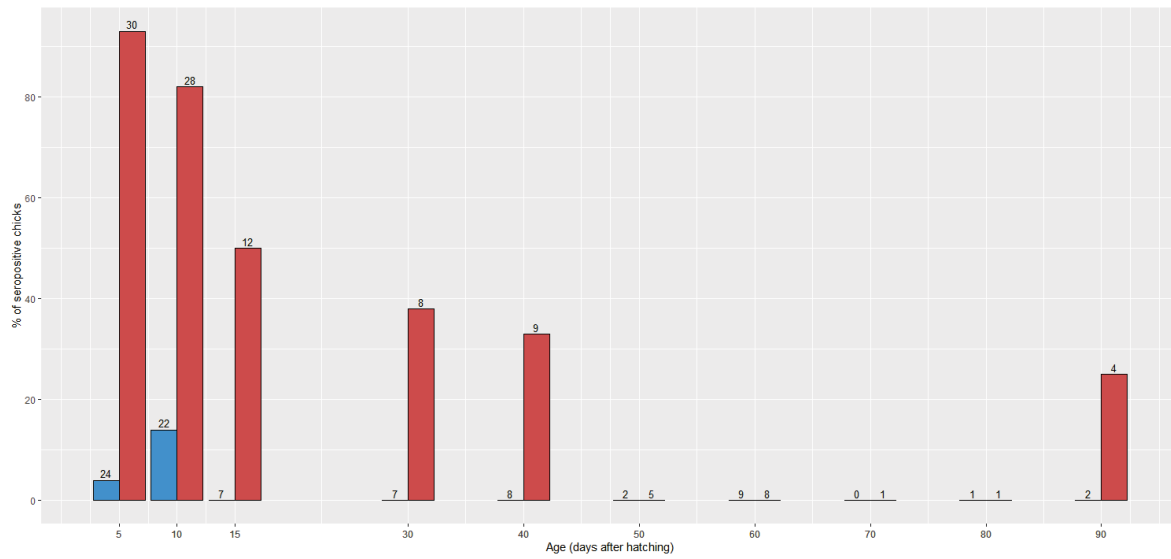
**Figure 5:** percentage of adult individuals seropositive and mean antibodies titer (log scale) in plasma samples before and up to 2 year after injection. Upper table: NaCl\_2017: adults injected with 0.5ml of NaCl 0.9% and sampled before being injected (pre\_inj\_2017) injected in December 2017 and 2 to 3 weeks after injection (post\_inj\_2017), then during 2018/2019, 2019/2020 and 2020/2021 breeding season; PM\_2017: adults injected with 0.5ml of vaccine against strain D2C before injection (Pre\_inj\_2017) and 2 to 4 weeks after injection (post\_inj\_2017 then during 2018/2019, 2019/2020 and 2020/2021 breeding season. Bottom figure: percentage of adult individuals seropositive. Blue: adults injected with 0.5ml of NaCl 0.9 in December 2017; Red: adults injected with 0.5ml of vaccine against strain D2C in December 2017. Number on top of the bar is the percentage of seropositive adults for each period. \*\*\*\* when difference between NaCl injected and vaccinated within a season is significantly different with  $p < 0.01$ .



**Figure 6:** Immune response of adults following vaccination. Quantification of anti-Pm antibodies by MAT in IYNA adults injected in December 2017. NaCl\_2017 (left panel): adults injected with 0.5ml of NaCl 0.9% and sampled before being injected (pre\_inj\_2017) injected in December 2017 and 2 to 3 weeks after injection (post\_inj\_2017), then during 2018/2019 breeding season and during 2019/2020 breeding season and 2019/2020 breeding season; PM\_2017 (right panel): adults injected with 0.5ml of vaccine against strain D2C before injection (Pre\_inj\_2017) and 2 to 4 weeks after injection (post\_inj\_2017), then during 2018/2019 and 2019/2020 breeding season. Each dot is a single value of titer for one individual. Dots are voluntary jittered for visual aspect.

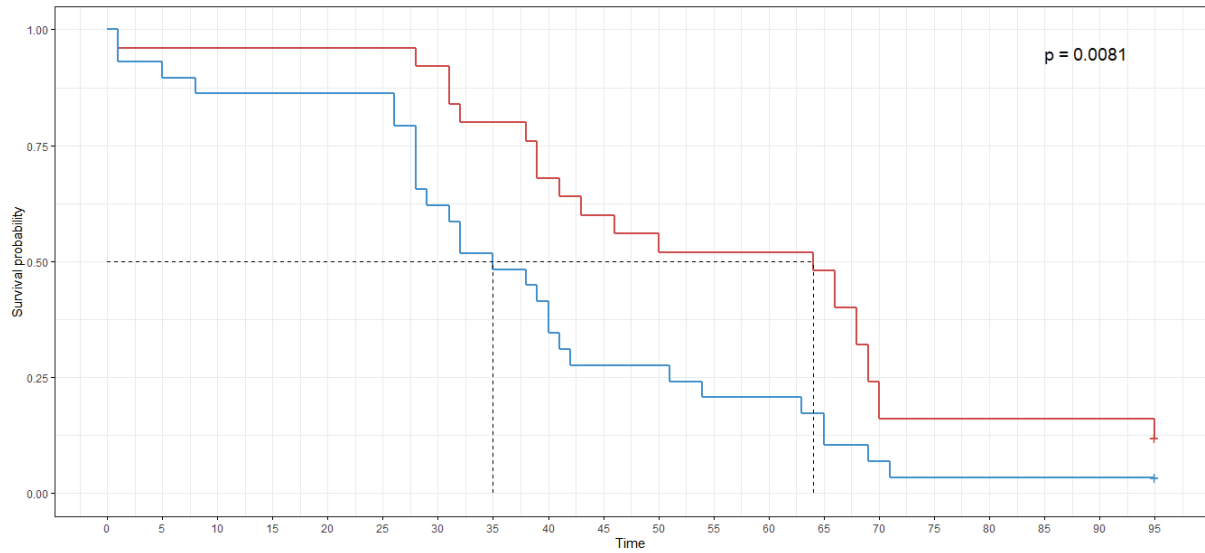


**Figure 7:** Quantification of anti-Pm antibodies by MAT in IYNA chicks and parents 1 year after parents have been injected. Left panel: antibodies titer for parents measured in 2018/2019 breeding season. Right panel: antibodies titer for respective chicks. Blue: parents injected with NaCl solution. Red parents injected with vaccine against D2C. Each dot is a single value of titer for one individual. Dots are voluntary jittered for visual aspect.



	Age (days)	5	10	15	30	40	50	60	70	80	90
<b>PM_2017</b>	Total chicks	30	28	12	8	9	5	8	1	1	4
	% seropositive	<b>93%</b>	<b>82%</b>	<b>50%</b>	<b>38%</b>	<b>33%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>25%</b>
<b>NaCl_2017</b>	Total chicks	24	22	7	7	8	2	9	0	1	2
	% seropositive	<b>4%</b>	<b>14%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	-	<b>0%</b>	<b>0%</b>

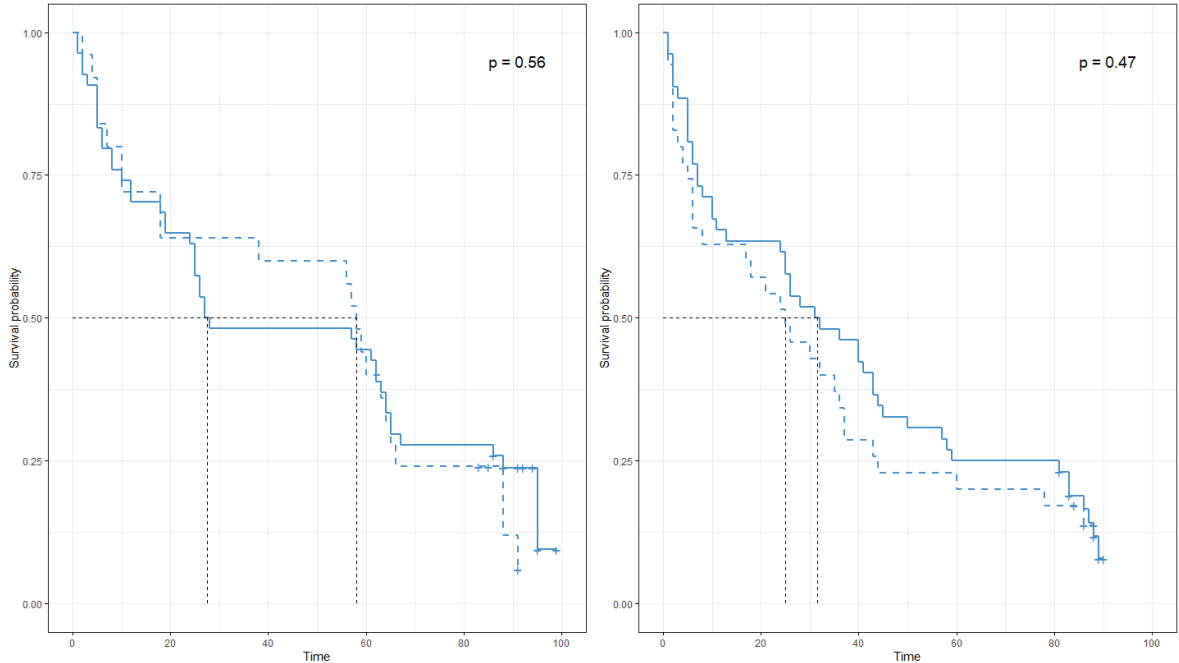
**Figure 8:** percentage of chick individuals seropositive among time during 2018/2019 breeding season. Blue: chicks from parents injected with 0.5ml of NaCl 0.9 in December 2017; Red: chicks from parents injected with 0.5ml of vaccine against strain D2C in December 2017. Number on top of the bar is the number of chicks sampled. The table gives the number of chicks sampled and the percentage of seropositive chicks for each age in the two groups.



**Figure 9:** Survival curves for chicks during 2019/2020 breeding season. Red plain line for the chicks manipulated and sampled from parents injected with vaccine against D2C (n=25); Blue plain line for chicks manipulated and sampled from parents injected with NaCl solution (n=29). X-axes (Time) correspond to days after hatching. Log-rank method for p-value. Black dashed line are the median survival time values.



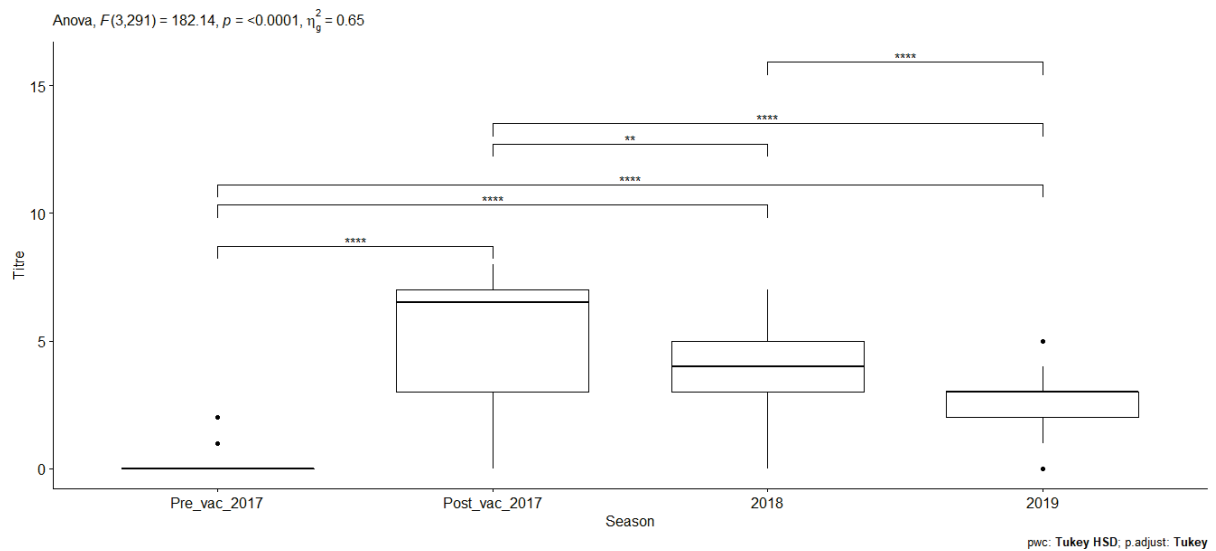
# Optimizing a vaccination approach against avian cholera to protect endangered albatrosses – Supplements



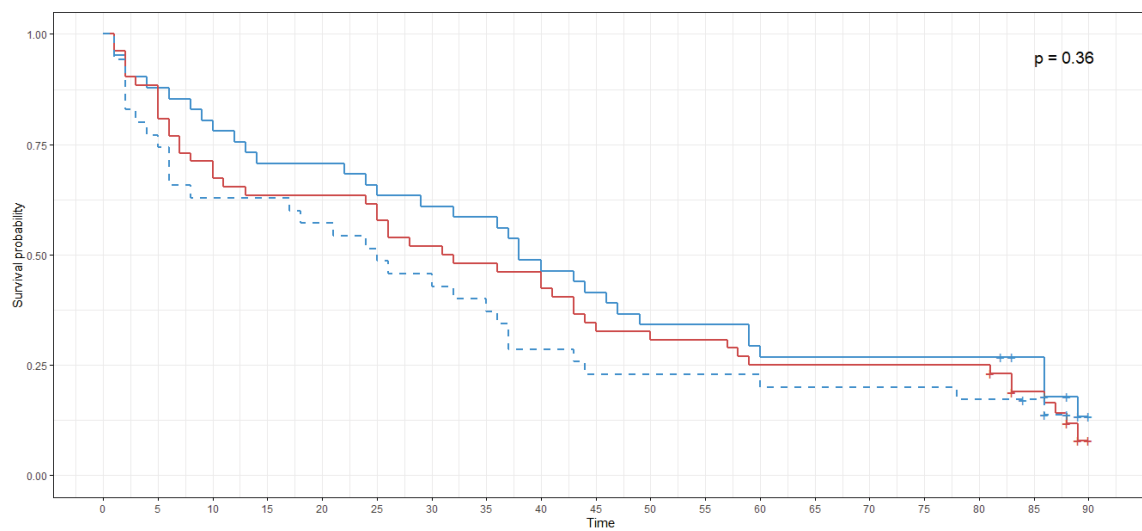
**Figure S1:** Survival curves for chicks from both control groups for 2017/2018 and 2018/2019 breeding seasons. Plain line for the chicks manipulated and sampled, dashed line for chicks left unmanipulated. **Left panel** 2017/2018 breeding season, **right panel** 2018/2019 breeding season. X-axes (Time) correspond to days after hatching.

**Table S1:** resume of Hazard Ratio cox model results for control groups for the 2017/2018 and 2018/2019 breeding seasons.

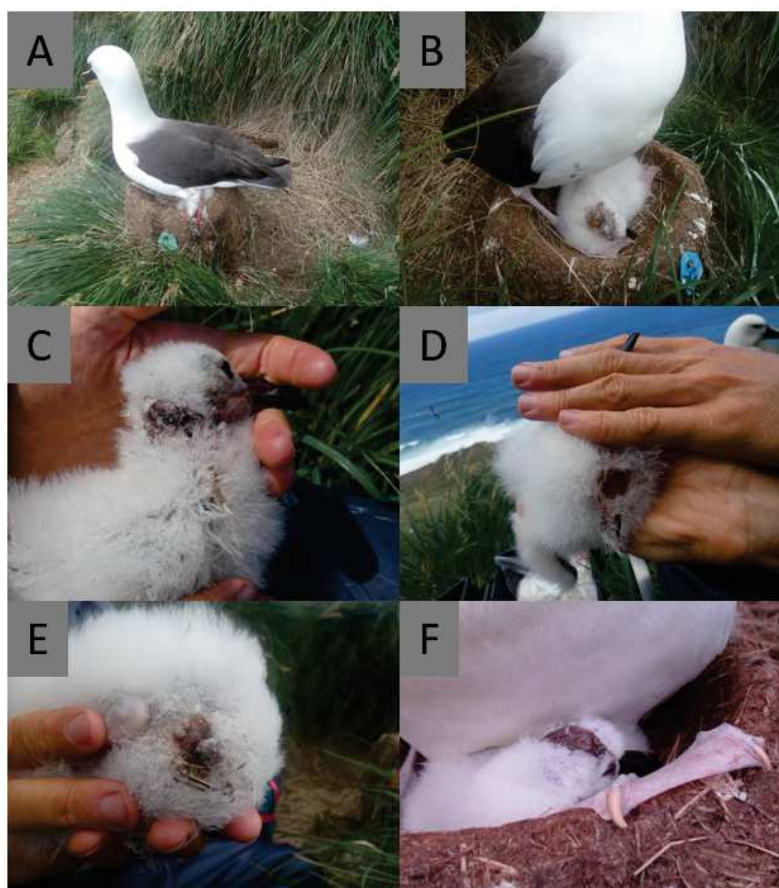
	Treatment	Total number of chicks	Number of events	Hazard Ration [HR]	Confident Interval [CI]	Pr(> z )
2017/2018	manipulated ~ unmanipulated	79	66	1.173	0.6973 - 1.972	0.548
2018/2019	manipulated ~ unmanipulated	87	76	1.185	0.7459-1.884	0.472
All seasons	Treatment effect	166	142	1.151	0.8154 - 1.625	0.42
All treatments	Season effect	166	142	1.407	1.003-1.972	0.05*



**Figure S2:** Immune response of adults following vaccination. Quantification of anti-Pm antibodies by MAT in IYNA adults injected in December 2017. Adults injected with 0.5ml of vaccine against strain D2C before injection (Pre\_inj\_2017) and 2 to 4 weeks after injection (post\_inj\_2017), then during 2018/2019 and 2019/2020 breeding season. Each dot is a single value of titer for one individual. Antibodies titer are significantly higher even 2 years after injection compared to the basal titer before injection. We note a significant decrease in antibodies titer from one year to the next.



**Figure S3:** Survival curves for chicks from vaccinated parents during 2018/2019 breeding season. Red plain line for the chicks manipulated and sampled from parents injected with vaccine against D2C (n=41); Blue plain line for chicks manipulated and sampled from parents injected with NaCl solution (n=52); Blue dashed line for the unmanipulated chicks (n=35). X-axis (Time) correspond to days after hatching. Log-rank method for p-value.



**Figure S4:** pictures of chicks killed or injured during early stages (first 20 days after hatching) during 2018/2019 breeding season. A: chick killed by rats while attended by parent (6 days old). B: chick injured on head, with skull bone visible, while attended by parent (7 days old); the chick died within a week. C, D, E: chicks (<15 days old) injured by rats respectively on the neck, on the breast and on the lower abdomen; all chicks died within a week after being injured. F: Chick injured on the head while attended by parent, with skull bone visible.

**Table S2:** ANOVA table for the linear mixed model and the significance of each group and season tested. Only significative values were left, all other interactions not presented in the table are not significative.

group1	group2	group interaction	null.value	estimate	conf.low	conf.high	p.adj	p.adj.signif
Season	Pre_inj_2017	Post_inj_2017	0	4.0135802	3.1109949	4.9161656	4.19E-10	****
Season	Pre_inj_2017	2018	0	2.2217994	1.9057005	2.5378983	4.19E-10	****
Season	Pre_inj_2017	2019	0	1.6507231	1.348714	1.9527322	4.19E-10	****
Season	Pre_inj_2017	2020	0	1.4177125	1.0848809	1.7505441	4.19E-10	****
Season	Post_inj_2017	2018	0	-1.7917808	-2.697237	-0.8863246	8.85E-07	****
Season	Post_inj_2017	2019	0	-2.3628571	-3.2634913	-1.462223	4.40E-10	****
Season	Post_inj_2017	2020	0	-2.5958678	-3.5073003	-1.6844352	4.20E-10	****
Season	2018	2019	0	-0.5710763	-0.8815599	-0.2605927	6.34E-06	****
Season	2018	2020	0	-0.8040869	-1.1446269	-0.463547	2.57E-09	****
Traitement	NaCl_2017	PM_2017	0	1.8729479	1.7079945	2.0379013	4.19E-10	****
Season:Traitement	Pre_inj_2017:NaCl_2017	Post_inj_2017:PM_2017	0	5.0387931	3.8262437	6.2513425	4.19E-10	****
Season:Traitement	Pre_inj_2017:NaCl_2017	2018:PM_2017	0	3.6069749	3.0632146	4.1507353	4.19E-10	****
Season:Traitement	Pre_inj_2017:NaCl_2017	2019:PM_2017	0	2.5484085	2.0215266	3.0752904	4.19E-10	****
Season:Traitement	Pre_inj_2017:NaCl_2017	2020:PM_2017	0	2.0759553	1.5121313	2.6397793	4.19E-10	****
Season:Traitement	Post_inj_2017:NaCl_2017	Post_inj_2017:PM_2017	0	5.125	2.5832873	7.6667127	1.42E-08	****
Season:Traitement	Post_inj_2017:NaCl_2017	2018:PM_2017	0	3.6931818	1.3941162	5.9922475	1.99E-05	****
Season:Traitement	Post_inj_2017:NaCl_2017	2019:PM_2017	0	2.6346154	0.3394831	4.9297477	1.08E-02	*
Season:Traitement	2018:NaCl_2017	Post_inj_2017:PM_2017	0	4.9181034	3.7055541	6.1306528	4.19E-10	****
Season:Traitement	2018:NaCl_2017	2018:PM_2017	0	3.4862853	2.9425249	4.0300456	4.19E-10	****
Season:Traitement	2018:NaCl_2017	2019:PM_2017	0	2.4277188	1.9008369	2.9546008	4.19E-10	****
Season:Traitement	2018:NaCl_2017	2020:PM_2017	0	1.9552656	1.3914416	2.5190896	4.20E-10	****
Season:Traitement	2019:NaCl_2017	Post_inj_2017:PM_2017	0	4.7024648	3.5034464	5.9014831	4.19E-10	****
Season:Traitement	2019:NaCl_2017	2018:PM_2017	0	3.2706466	2.7577681	3.7835251	4.19E-10	****
Season:Traitement	2019:NaCl_2017	2019:PM_2017	0	2.2120802	1.7171321	2.7070282	4.19E-10	****
Season:Traitement	2019:NaCl_2017	2020:PM_2017	0	1.739627	1.2055235	2.2737304	4.20E-10	****
Season:Traitement	2020:NaCl_2017	Post_inj_2017:PM_2017	0	4.6569149	3.4272865	5.8865433	4.19E-10	****
Season:Traitement	2020:NaCl_2017	2018:PM_2017	0	3.2250967	2.6442479	3.8059455	4.19E-10	****
Season:Traitement	2020:NaCl_2017	2019:PM_2017	0	2.1665303	1.6014511	2.7316095	4.19E-10	****
Season:Traitement	2020:NaCl_2017	2020:PM_2017	0	1.6940771	1.0944043	2.2937499	4.20E-10	****
Season:Traitement	Pre_inj_2017:PM_2017	Post_inj_2017:PM_2017	0	5.0384615	3.8588642	6.2180589	4.19E-10	****
Season:Traitement	Pre_inj_2017:PM_2017	2018:PM_2017	0	3.6066434	3.1409725	4.0723142	4.19E-10	****
Season:Traitement	Pre_inj_2017:PM_2017	2019:PM_2017	0	2.5480769	2.102231	2.9939228	4.19E-10	****
Season:Traitement	Pre_inj_2017:PM_2017	2020:PM_2017	0	2.0756237	1.5866743	2.5645731	4.19E-10	****
Season:Traitement	Post_inj_2017:PM_2017	2018:PM_2017	0	-1.4318182	-2.6190506	-0.2445858	5.48E-03	**
Season:Traitement	Post_inj_2017:PM_2017	2019:PM_2017	0	-2.4903846	-3.669982	-1.3107872	2.51E-09	****
Season:Traitement	Post_inj_2017:PM_2017	2020:PM_2017	0	-2.9628378	-4.1593925	-1.7662832	4.20E-10	****
Season:Traitement	2018:PM_2017	2019:PM_2017	0	-1.0585664	-1.5242373	-0.5928956	4.91E-10	****
Season:Traitement	2018:PM_2017	2020:PM_2017	0	-1.5310197	-2.0381117	-1.0239276	4.20E-10	****



## Annexe 3

Prospecting by pre-breeders and failed-breeders in a highly site faithful albatross species: evidence and potential implication for the circulation of disease agents.

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In preparation.



**Prospecting by pre-breeders and failed-breeders in a highly site faithful albatross species: evidence and potential implication for the circulation of disease agents**

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**Lay summary:**

Finding the best place for breeding is an important choice for animals, especially for those faithful to their breeding site for life. Visiting different places prior to site selection is thus a crucial step, but such movements may also result in the spread of pathogens or parasites. Using movement analyses, we show that pre- and failed breeders of Indian yellow nosed albatrosses, a long-lived endangered seabird, are prospecting spatially distant parts of a large colony in which recurrent disease outbreaks occur.

**Abstract:**

Prospecting, defined as the visit of breeding areas where an individual could potentially settle to breed but does not currently breed, is a difficult behavior to study as it requires tracking the movements of individuals that are not attached to a breeding site at the time of study. In general, individuals of highly site faithful species may not be expected to prospect for breeding sites outside of the habitat patch where they were born or currently reproduce, but in the context of global environmental change, a better knowledge of prospecting movements is particularly important as they could inform us about the spatial scale at which dispersal events could take place. They could also lead to the dispersal of parasites and infectious agents among groups of breeding hosts. On Amsterdam Island, southern Indian Ocean, *Pasteurella multocida* (*Pm*), the bacteria responsible for avian cholera, has been causing for the last three decades recurrent massive mortalities of nestlings of albatrosses. Using GPS-UHF tracking devices and detailed analyses of movements of the locally abundant but threatened Indian yellow-nosed albatross *Thalassarche carteri*, we report how young pre-breeding individuals, but also to a lesser extent failed breeder, visited breeding patches of conspecifics. Those birds explored different conspecific subcolonies on the island at a hierarchy of spatial scales. By connecting spatially distant (up to 2000 meters) breeding patches within a season, the behavior of the birds, notably pre-breeders, might play a role in the local spread of *Pasteurella multocida*. Our study highlights that further careful characterization of prospecting movements could be important for the dynamics of subdivided populations, but also spatial disease ecology.

**Keywords:** conspecific attraction, informed dispersal, metapopulation dynamics, spatial disease ecology

## Introduction

A strong motivation for studying the movement of species is that global environmental change may dramatically affect animal populations and their habitats at various geographic scales. A better knowledge of how it does could allow us to predict or mitigate such effects (Morales et al. 2012; Travis et al. 2013). The link between individual movements and population responses is nevertheless not as obvious as it could seem because the spatial and temporal scales of movement and demographic processes are often not the same and, in many species, individuals are highly faithful to their breeding site. While the mechanistic links between animal movement and population dynamics are ecologically logical, it is much less clear when knowledge of animal movement is a prerequisite for understanding and predicting population dynamics (Morales et al. 2010). Moreover, our understanding of how individuals integrate different sources of information in order to make recruitment and dispersal decisions is still rudimentary (Reed et al. 1999; Clobert et al. 2009; Cooper and Marra 2020a).

In this context, prospecting, defined as the visit of breeding areas where an individual could potentially settle to breed but does not breed currently, is an important but difficult behavior to study as it requires tracking the movements of individuals that are potentially not attached to a breeding site at the time of study (Boulinier et al. 1996; Ponchon et al. 2013; Cooper and Marra 2020b). A better knowledge of such movements is nevertheless particularly important as they could inform us about the spatial scale at which dispersal events could take place (Cadiou et al. 1994; Boulinier and Lemel 1996; Calabuig et al. 2010; Ponchon, Chambert, et al. 2015; Ponchon et al. 2017), with implications for population dynamics and genetics (Hanski and Gaggiotti 2004). They could also lead to the dispersal of parasites and infectious agents among groups of breeding hosts (Boulinier et al. 2016). Prospectors can indeed interact with other individuals, adults and offspring, and could thus contaminate a breeding site or group, or be contaminated (Boulinier et al. 2016; Evans et al. 2020).

Colonial breeding species, such as seabirds, are convenient to study those behaviors because they breed at clear hierarchies of spatial scales, with the possibility to detect movements of individuals among and within breeding patches. Such long lived species are nevertheless highly philopatric and site faithful, and thus may not be expected to prospect for breeding sites outside of the habitat patch where they were born or currently reproduce (Burger and Schreiber 2001; Inchausti and Weimerskirch 2002). At the same time, it would be unexpected that they do not prospect at all during their lifetime (Boulinier and Danchin 1997; Reed et al. 1999; Doligez et al. 2004; Votier et al. 2011; Campioni et al. 2017). Little data is available on prospecting behavior on particularly site faithful species such as albatrosses (Campioni et al. 2017), despite their potential importance. Conspecific attraction to

potential breeding sites has nevertheless been used in such species for conservation purposes, but the origin and status of the attracted individuals are rarely known (Podolsky 1990; VanderWerf et al. 2019). The data available on prospecting individuals of the relatively widespread black-browed albatross show movements in the vicinity of colonies at various distances from the birth colony, but little documentation is yet available of actual prospecting behavior involving the landing of individuals from known colony or subcolony of origin. For instance, prospecting visits to a conspecific colony by black-browed albatrosses were defined in Campioni et al. (2017) as tracks that entered an area within 1 km of a conspecific breeding colony. It is important to also determine at finer spatial scales whether individuals in such species may visit different areas within a breeding season, by landing on breeding patches and wandering around, and whether they do so recurrently. Given earlier observation of patterns of recruitment in a spatial context in seabirds, prospecting movements are expected to involve young, pre-breeding individuals, and to a lesser extent failed breeders (Cadiou et al. 1994), and the extent of their movements is expected to decrease as a function of their age.

While migration and risk of disease spread are often considered together, the link between disease spread and other types of animal movements, such as those associated with dispersal and prospecting, is much less appreciated (Boulinier et al. 2016; Daversa et al. 2017). Infectious diseases can be a threat for social vertebrates, and the spatial ecology of these diseases is important to explore in addition to their temporal dynamics (Craft et al. 2011; Boulinier et al. 2016; Gamble et al. 2019; Scherer et al. 2020). On Amsterdam Island (southern Indian Ocean), avian cholera causes recurrent massive mortalities of albatross chicks. The Indian yellow-nosed albatross *Thalassarche carteri*, a locally abundant but threatened species (Rolland et al. 2009), has notably been recurrently affected by epizooties for the last three decades (Weimerskirch 2004; Jaeger et al. 2018; Jaeger et al. 2019; Jaeger et al. 2020). In this context, different types of movements of individuals, from foraging to migration and prospecting, could play a major role in the circulation of the infectious agent at different scales (Boulinier et al. 2016; Gamble et al. 2020). This motivated the implementation of a movement ecology study, in parallel to eco-epidemiology investigations, to evaluate whether potential within-season prospecting movements of Indian yellow-nosed albatrosses could contribute to the dissemination of *Pasteurella multocida*, the bacteria responsible for avian cholera, among subcolonies of Amsterdam island. As opposed to successful breeders which are expected to mainly move between their nest site and the sea to forage, failed-breeders or pre-breeding individuals could play a role in the dissemination of pathogens if they were to visit different patches where conspecifics breed (Boulinier et al. 2016).

Such movements could be especially important for disease spread if they would occur between relatively isolated patches and be repeated within a breeding season.

In order to explore the spatial and temporal patterns of habitat use on the colony by individuals as a function of their breeding status during the reproductive period, we used GPS-UHF loggers which do not require the recapture of individuals. We predicted contrasted patterns for successful breeders, failed breeders, and young, pre-breeding, individuals.

## **Materials and methods**

### *Study site and population:*

Amsterdam Island (37.8°S, 77.6°E), French Southern and Antarctic Lands, is a ~55 km<sup>2</sup> isolated volcanic island that hosts the majority of the world breeding population of the endangered Indian yellow-nosed albatross, about 22 000 pairs (Weimerskirch et al. 2018), the only two other significant breeding populations (> 50 pairs) being on Prince Edward Island and Crozet Islands, more than 2000 km east. On Amsterdam Island, the species breeds along stretches of the large Entrecasteaux cliffs, on the south-west coast of the island. The birds come back to breed on the island in September, eggs hatch at the beginning of December and the nestlings fledge in April (Gamble et al. 2019). The whole breeding colony is made of patches of breeders (i.e., subcolonies) of varying sizes, from several thousands to tens of pairs, delimited by topographic structures (Figure S3). Two large parts of the colony host most of the breeding pairs, with more or less continuous subcolonies spread over strips of about 500 m in width and 100 m in height, while smaller isolated patches of breeders are distributed in the same area of the island, but up to 2 km away (Del Cano cliff). The birds we tracked were all captured in a monitored subcolony of about 650 pairs nesting over an area of 5,000 square meters (Figure S3).

### *GPS equipment:*

The deployment of GPS was focused on the monitored area of the breeding colony where daily visits around the time of hatching enabled to choose the equipment of different categories of birds. Pre-breeders were birds of known age (ringed as nestlings with metal and Darvic encoded rings in previous years) that had not been detected breeding. Equipment occurred during three consecutive breeding seasons, 2017-2018, 2018-2019 and 2019-2020. In order to find as many pre-breeder birds as possible, captures started early in the season and lasted during several weeks, from October 25<sup>th</sup> to December 15<sup>th</sup>. Pre-breeding birds of known age, besides being rare due to the high mortality rates of nestlings most breeding seasons over the last decades (Jaeger et al. 2018), are more present in the colony in the

early season (data not published). At this time, successful breeders are incubating, and they spend most of the time on the nest incubating (shifts of 2-12 days) or foraging at sea (ACAP 2009; Heerah et al. 2019). Incubating individuals are sensitive to disturbance and their eggs can notably be exposed to predation if they are captured at that time. Consequently, successful and failed breeder birds were captured during the post hatching period, between December 2<sup>nd</sup> and December 15<sup>th</sup> (date depending on field logistics). Failed-breeder were individuals previously identified (nest tagged and/or individual leg ring) and seen incubating an egg and later seen on an empty nest. A total of 65 birds were equipped over the three seasons, 20 successful breeder, 30 failed-breeder and 15 pre-breeder birds (Table 1, Table S1). Birds were regularly resighted during the tracking period by opportunistic sightings and regular dedicated sessions of resighting in the study area. Failed-breeders were expected to prospect and to do so more likely late in the season if the behavior relates to their breeding location for the next breeding season (Boulinier et al. 1996; Reed et al. 1999), thus, as for pre-breeders, this group of individuals was tracked with GPS devices that did not require to recapture the individuals.

Failed and pre-breeders were equipped with 13 grams solar-powered GPS-UHF loggers: Harrier-L in 2017-2018 and Kite-L in 2018-2019, Ecotone, Poland (both loggers have the same characteristics of weight and dimension), which remotely download data to a small base station linked to an antenna set up on the margin of the subcolony. Devices were attached to the birds' back feathers with Tesa® tape, which allow their spontaneous detachment after a few weeks. Opportunistic retrieval of the loggers started after 12 days of tracking but if the birds were not catchable anymore, the loggers were left to fell off naturally. In 2017-2018 and 2019-2020, GPS-UHF loggers were programmed with a 5 minutes acquisition frequency, depending on weather conditions and battery charge. Indian yellownosed albatrosses do not visit colony at night (Weimerskirch et al. 2001). Consequently, to optimize the duration of the battery and the duration of data recording, all those loggers were set to stop acquiring location during night time (from 10pm to 5 am local time). In 2018-2019, because of a software malfunction, GPS-UHF loggers were programmed with a 15 minutes acquisition frequency and worked 24/7.

Because they can be easily recaptured on their nest during the breeding season, successful breeders were equipped with battery-powered GPS devices (i-GotU GT-600, Mobile Action Technology, Taiwan) that required the loggers to be recovered to access the data. Those GPS devices were programmed with a fixed acquisition frequency of 5 mins and stopped during night time. The loggers were also attached to the back feathers with Tesa® tape. Successful breeders were recaptured 8 to 15 days after deployment to retrieve the loggers and access the data.

### *Analyses and representation of movement data:*

The tracking data were explored using two approaches, one that focused on documenting the intensity of use of different areas, and the other to detect and locate landings. The distribution of habitat utilization was computed using the BRB|MKDE program, for Biased Random Bridges for Movement-based Kernel Density Estimation (Benhamou 2011). Parameters were set up identically for the 3 seasons and both logger's types (GPS and GPS-UHF). Settings were chosen accordingly to the less accurate data, 15 min acquisition frequency on the GPS-UHF loggers from the season 2018-2019. GPS location accuracy was evaluated by placing 4 GPS (2 solar-powered-devices and 2 battery-powered devices) on fixed sticks in the colony during ~24h. All locations recorded were in a radius of 20m around each stick, representing a circle 40m wide. The barycenter of all locations was calculated for each stick and corresponded to the stick location (when plotting barycenter location on orthophotos). Intensity distribution, defined as the time spent by a bird in a limited area, was computed (Benhamou and Riotte-Lambert 2012). Non-overlapping virtual circles of 20 m in radius (based on the mean accuracy of devices) were used to estimate the intensity. A bird had to stay at least 45 min (3 times acquisition frequency of GPS-UHF loggers in 2018-2019) in the area to count as a visit and a new visit was counted each time the bird re-entered it after a time lag spent outside the area longer than 45 min (3 times acquisition frequency). 50% (i.e., core area) and 90% (i.e., overall distribution) kernel intensity use of habitat were considered using a smoothing parameter of 40m for prospecting. We assumed that the area of the kernels could reflect the number of individuals possibly in contact, or close to the bird. Areas were thus evaluated and compared using ANOVA analysis. Results are presented as mean (standard deviation sd). Maps are visualized with QGIS 3.0.3.

The kernel analysis informs us about the intensity of use of habitat, but does not give information about behavior of birds, notably if it is flying over the area or landed in specific site. We implemented a program in R version 3.0.5 (Team R Development Core 2018) to identify potential landings of birds using all data points recorded within the perimeter of the island. For this, we considered as 1 landing 3 successive locations distant for 20 m or less (based on mean accuracy of GPS), assuming that the probability of recording 3 successive closed locations if a bird is flying over is very low. The program follows this step: When 2 successive locations are distant less than 20m, a barycenter is recorded. If the third location is distant less than 20m from this barycenter, a new barycenter is calculated considering the 3 last locations and this new barycenter is considered as a landing site. Then, following the same process, all successive locations are compared to the previous barycenter and a new barycenter is calculated until a location is more than 20m distant. If 3 or more locations are out of the

radius of 20m from the last barycenter, the bird is considered as having left the landing site. The last barycenter calculated is recorded as the position of 1 landing site and 1 landing is recorded. At the end, all landing sites calculated in a perimeter of 40 m are considered as the same site. Because we have different duration of tracking among the groups (<15 days for successful breeders; up to 2 months for failed or pre-breeders), data were also compared on the first 8 days after equipment. However, analyses were extended to the whole tracking period in all groups to detect any prospecting movements. Statistical analyses were conducted in R version 3.5.0 (Team R Development Core 2018) to estimate the number of areas visited, the number of visits for each area and the distance between areas visited. As the GPS locations were likely affected by a degradation of the signal related to the height of the 800 m cliff at the study site and the parameters we had to set up for the analyses, we might have missed or overestimated a few landings and visited areas. We nevertheless are confident that we detected most of the landings of the birds; this was notably confirmed by comparing the visited areas when looking at the raw data on a map with that of the kernel intensity use of habitats (Figure 5). Results were also consolidated by visual observations. Pictures of the cliff from the boat were taken to detect and validate the subcolonies potentially visited.

#### *Field Procedures:*

Capture and handling of the birds were carried out in accordance with regulation of the National Nature Reserve of the Southern French Territories and permits from the animal care authority (French Ministry of Research). Rigorous biosecurity measures were observed in the field to avoid spreading infectious agents among birds and areas. These involved the use of dedicated clothing consisting of a different set of waterproof leggings, jackets, and rubber boots for each bird colony (Bourret et al. 2018). Waterproof overalls, any other equipment (e.g., caliper, ruler) and hands were disinfected between contacts with any two birds, respectively using hydrogen peroxide, commercial disinfectant wipes, and hydroalcoholic gel. All pieces of clothing were thoroughly cleaned and disinfected again at the end of each day.

## **Results**

#### *Data recovered:*

Data from 59 birds over the 65 equipped were recovered and analyzed (Table 1). 93% of the failed-breeders (25/27 with retrieved data, 3 birds were missing) have more than 10 days of tracking, with data up to 47 days (supplement Table S1). 85% of the pre-breeders (11/13, 2 birds were missing) have

more than 6 days of data and up to 54; two pre-breeders have only less than 2 days of tracking data recovered. 84% of the successful breeders have more than 5 days of tracking (16/19, 1 bird was missing) and up to 21 days of data.

As expected for such a seabird species, all birds did large scale movements at sea (results not shown). Colonies in the Southern Indian Ocean are situated in 4 island groups: Amsterdam & Saint Paul, Kerguelen, Crozet and Marion & Prince Edward Islands (ACAP 2009; Weimerskirch et al. 2018). The 3 other groups are distant more than 1400km and up to 3300km from the Amsterdam colony. We did not detect any prospecting movement on or close to any of those colonies. We thus focused the exploration of potential movements over land at relatively small scales, within and between subcolonies on Amsterdam Island. Tracks were analyzed within the island in order to detect any prospecting movement, determining all sites where birds could have landed.

#### *Prospecting movements:*

All results presented here are considering the whole tracking period for all individuals. The comparison between birds as a function of their age and breeding status gave the exact same conclusions when carried out using data of the 8 first days of tracking (Supplement Table S2, Figure S1 and Figure S2). As predicted, successful breeders used a limited area over land, staying mostly in a small area around their nesting site (Figure 1). For those birds, the mean area of the fifty percent kernel intensity use of the habitat (core area) was 8,818m<sup>2</sup> (sd 642m<sup>2</sup>) (Table 1). Most of the failed breeder birds came back to the same site they were equipped, but using a larger area than successful breeders, with a mean area of the fifty percent kernel intensity use of the habitat of 15,293m<sup>2</sup> (sd 5,837m<sup>2</sup>). Even if not significantly higher (Figure 2), this result suggests that they landed and visited sites in the same subcolony, but further from their initial nesting site or close from the nesting subcolony. Interestingly, pre-breeder birds showed different behaviors according to their age. Individuals of 5- and 6-years old used a comparable area than failed-breeders, with a mean area of the fifty percent kernel intensity use of the habitat of respectively 15,879m<sup>2</sup> (sd 5,408m<sup>2</sup>) and 16,692m<sup>2</sup> (sd 5,244m<sup>2</sup>), while 4 years old birds used significantly a higher area, with a mean area of the fifty percent kernel intensity use of the habitat of 63,721m<sup>2</sup> (sd 33,058m<sup>2</sup>). Total number of visited sites was significantly higher for 4 years old individuals (Table 3), with a mean of 15 (sd 8) visited sites per individuals and a maximum of 26 sites (Table S1). As for core area, 5- and 6-years old individuals as well as failed-breeders have comparable results, with a mean of respectively 3 (sd 1.4), 3.9 (sd 2.2) and 3.5 (sd 3.3) visited sites per individuals. Successful breeders have the lowest mean of visited sites with 1.7 (sd 1.3) and a maximum of 5 sites. Individuals of 4 years old showed a significant higher number of visited sites (Figure 3). The maximum distance



between 2 visited sites was 198m for 1 successful breeder, with a mean of 33m (sd 61). On the opposite, the mean distance was significantly higher, 1,319m (sd 795), for the 4 years old individuals (Figure 4) with a maximum of 2,157m (Table S1).

## **Discussion**

Detailed tracking of the movements of pre- and failed breeders have been advocated to shed light on the processes of exploration and settlement, with possible implication for predicting the dynamics of the spatial distribution of individuals (Morales et al. 2010; Ponchon et al. 2013; Ponchon, Garnier, et al. 2015), but little data exist, especially compared to the very large knowledge acquired on foraging and migratory movements (Carneiro et al. 2020). Here, we show that in a highly site faithful long-lived seabird species (Bried et al. 2003), the Indian yellow-nosed albatrosses, young pre-breeders individuals, and to a lesser extent older pre-breeder and failed-breeders, explored different conspecific subcolonies within a very large colony, while successful breeders limited their movements to going back and forth from their nest site to the sea. This has implications for the scale at which recruitment may occur, but also for the potential spatial scale at which individuals may spread pathogens among breeding patches. The spatial scale considered is relatively small (within few kilometers), especially compared to the range of the foraging of the birds (thousands of kilometers), but very little is known about such movements on land among subcolonies.

Various movements can be involved in eco-epidemiological dynamics and multi-host/parasite systems and their careful characterization is important for fundamental and applied reasons (Boulinier et al. 2016). This study considered movement occurring within a breeding season, but movements in-between years can also occur. The breeding site selection every year is very important for long lived birds (Jones 2001) even for a site faithful species. In the critical situation of Amsterdam Island where regular massive mortalities occur during breeding season, the neighborhood information could play an important role in the selection of breeding site (Kildaw et al. 2005; Boulinier et al. 2008; Pärt et al. 2011). This is probably even more important if such colonies are decreasing in size and number of breeding pairs, driving birds to move toward bigger colonies (Dittmann et al. 2005) and in this way increase inter-annual movements. Those movements might occur in different scales, within the island but also between islands. Indian yellow-nosed albatrosses breed in 4 island groups in the Indian ocean: Amsterdam & Saint Paul, Kerguelen, Crozet and Marion & Prince Edward Islands (ACAP 2009; Weimerskirch et al. 2018). Such birds have a high potential of dispersal and they could in some cases colonize or establish new colonies far away from their initial colony (Munilla et al. 2016). But in most

of cases movement at a such scale are more difficult to characterize, detecting such individuals is technically difficult as they are likely breeding in another not monitored colony. For logistical reasons (steep and inaccessible cliffs) and biosecurity aspects (to avoid pathogens spreading between colonies) it was not possible to access other colonies on Amsterdam Island, but some individuals equipped with GPS-UHF loggers could be detected by placing UHF antennas in the range of other colonies on the island. Since 2017, it was possible to access the study subcolony early in the breeding season, and the preliminary data (not published) show that some individuals, in particular pre-breeders, are detected early in the season (pre-laying and early incubation period) and not detected later on, suggesting that some of the birds might have moved to another subcolony.

Behavior during long-distance travel is experienced differently through life stages (Péron and Grémillet 2013) and such movements can be an important perspective. Indian yellow-nosed albatross migrate during the non-breeding period and use pelagic water in the subtropical region (Delord et al. 2011), but may not seem to visit other colonies. Low accuracy of geolocators commonly used for migratory studies, did not permit to determine if birds can be in contact with each other, especially during feeding where they can be grouped at sea. At this time pathogens might be transmitted between individuals from different colonies or different island. Some case of birds temporally maintained in animal care center are also reported, mainly in Australia, after bycatch or grounding. Animal care center are also hosting several species of animals, including birds and can be a source of contamination, *Pm* being commonly found in wild and domestic animals (Xiao et al. 2015).

Because of its various ways to be transmitted into a host's system, *Pm* can be highly successful in its spread. Direct bird-to-bird contact, through arthropod or various insect bites, ingestion of contaminated carcasses or contaminated food or water, also in highly contaminated area *Pm* can potentially be aerosolized (Friend and Franson 1999). Natural exposure of albatrosses to the pathogen likely vary in time, during the breeding season but also between years (Gamble et al. 2019), but is difficult to quantify. Studying an epidemiological situation is important for basic and applied reason, above all in the situation of Amsterdam Island where several endangered species breed and are vulnerable to avian cholera outbreaks. Understanding the social contact network within and among those population is very important to design strategies to mitigate pathogen transmission and effects (Rogers et al. 1998; Cross et al. 2005; Gamble et al. 2020; Weiss et al. 2020).

The finding of the occurrence of frequent prospecting movements to different areas of the large colony of the studied albatross species is interesting also in the context of earlier and ongoing work on the use of conspecific attraction in restoration and translocation projects (Podolsky 1990; Parker et al. 2007;

Jones and Kress 2012; VanderWerf et al. 2019). In such management projects, decoys of albatrosses (or other species) have been used to encourage the settlement of individuals in areas where breeding was not occurring, and it is interesting to confirm that mostly pre-breeders, but also failed breeders, might be attracted to such areas (Munilla et al. 2016). The tracks of prospecting individuals can also be useful to locate potential breeding patches that may be overlooked in population surveys in inaccessible areas. This has been the case for a small patch of breeders on a very vertical part of the very large cliff of Entrecasteaux (above the Baie du Loup), not visible from land, where repeated static GPS locations of a potential prospecting individual focused our attention to an area where we eventually detected a group of nests on a photograph taken from the sea (pers. obs.). Our study thus highlights the usefulness of tracking devices for exploring not only foraging or migratory movements, but also under-documented prospecting movements (Ponchon 2013). Such an approach has also been used to detect movements among molting sites for non-breeding penguins, which is notably of interest with regards to spatial variability in exposure to various risks, and mixing among groups (Baylis et al. 2020). Although movements not directly related to foraging or migration may occur at relatively small spatial scales, they can have direct consequences for our ability to understand the spatial dynamics of populations as well as eco-epidemiological dynamics.

#### **Authors' contributions.**

TB conceived the idea of this work; TB, JT and AG designed the study; JT, MB, AC and AF collected the data; JT analyzed the tracking data; JT, AG and TB led the writing of the manuscript. All authors contributed substantially to the drafts and gave final approval for publication.

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**Prospecting by pre-breeders and failed-breeders in a highly site faithful albatross species: evidence and potential implication for the circulation of disease agents - Figures**

**Table 1:** Summary of the tracked Indian yellow-nosed albatrosses and retrieved data. FB, failed-breeders; PB, pre-breeders and SB, successful-breeders.

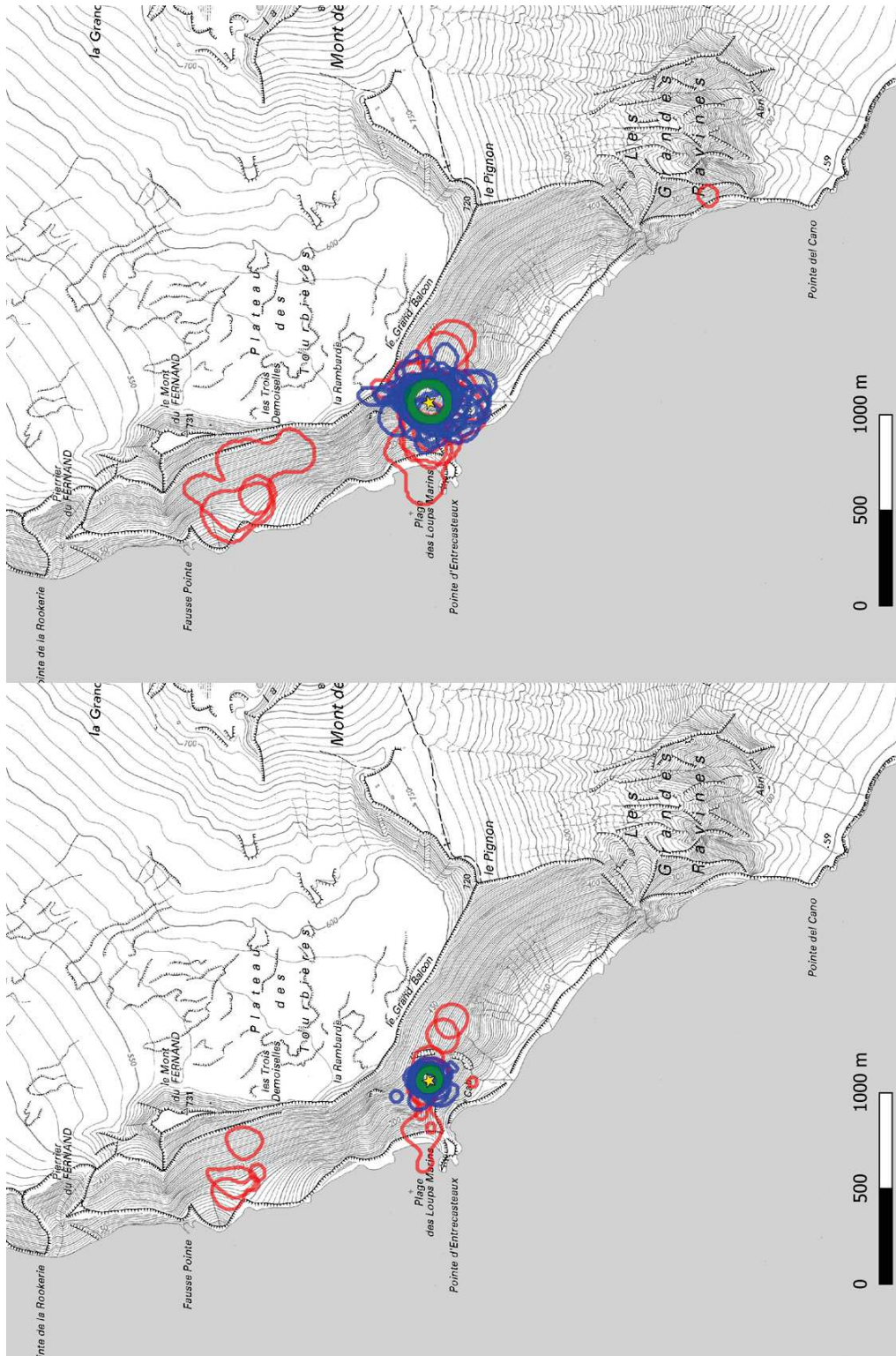
Season	Status	Number of equipped individuals	Number of individuals with acquired data	Mean tracking period (days)	Min tracking period (days)	Max tracking period (days)
2017/2018	FB	13	11	19.4	4.1	28.9
	PB	2	2	6.2	6.1	6.2
	SB	0	0	-	-	-
2018/2019	FB	12	11	17.6	1.9	47.3
	PB	3	2	48.3	42.6	54.0
	SB	10	10	7.6	1.9	21.3
2019/2020	FB	5	5	24.5	20.4	26.6
	PB	10	9	15.7	1.1	43.3
	SB	10	9	7.8	2.7	20.2
	Total	65	59			

**Table 2:** Mean area of the fifty and ninety percent kernel of intensity use of habitat on the breeding cliffs for the individual Indian yellow-nosed albatrosses of the 3 considered status: SB, successful-breeder; FB, failed-breeder and PB, pre-breeder.

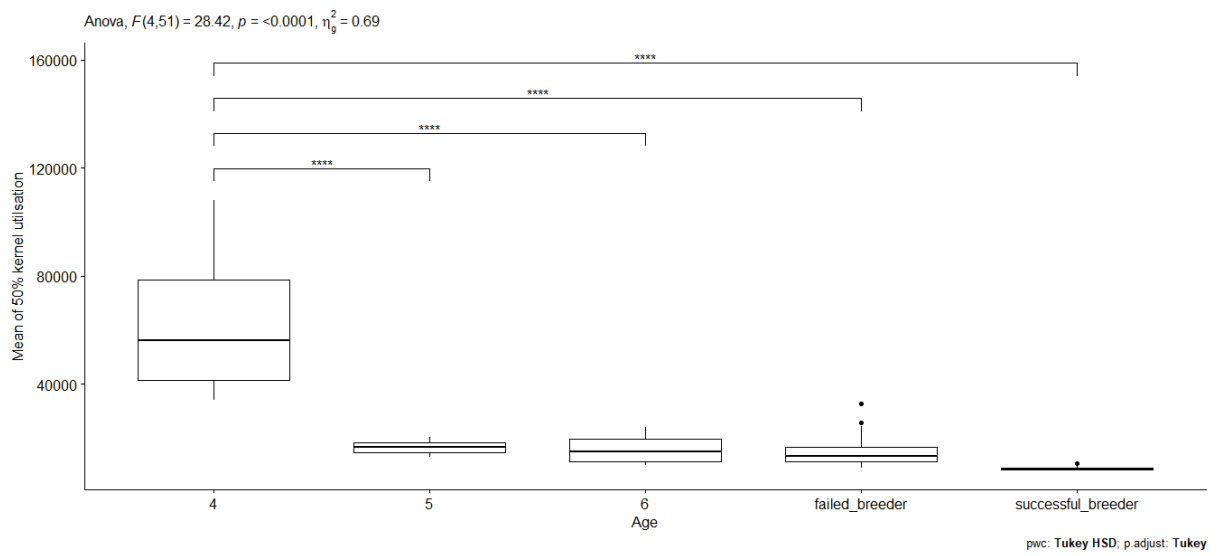
Status	Age	n	area 50% kernel (m <sup>2</sup> )		area 90% kernel (m <sup>2</sup> )	
			mean	sd	mean	sd
PB	4y	4	63,721	33,058	140,600	96,591
PB	5y	2	16,692	5,244	83,400	46,740
PB	6y	7	15,879	5,408	59,543	19,876
FB	adult	28	15,293	5,837	74,099	62,636
SB	adult	15	8,818	642	28,900	3,371

**Table 3:** Mean distance between visited sites and number of different visited sites on the breeding cliffs for the individual Indian yellow-nosed albatrosses of the 3 considered status: SB, successful-breeder; FB, failed-breeder and PB, pre-breeder.

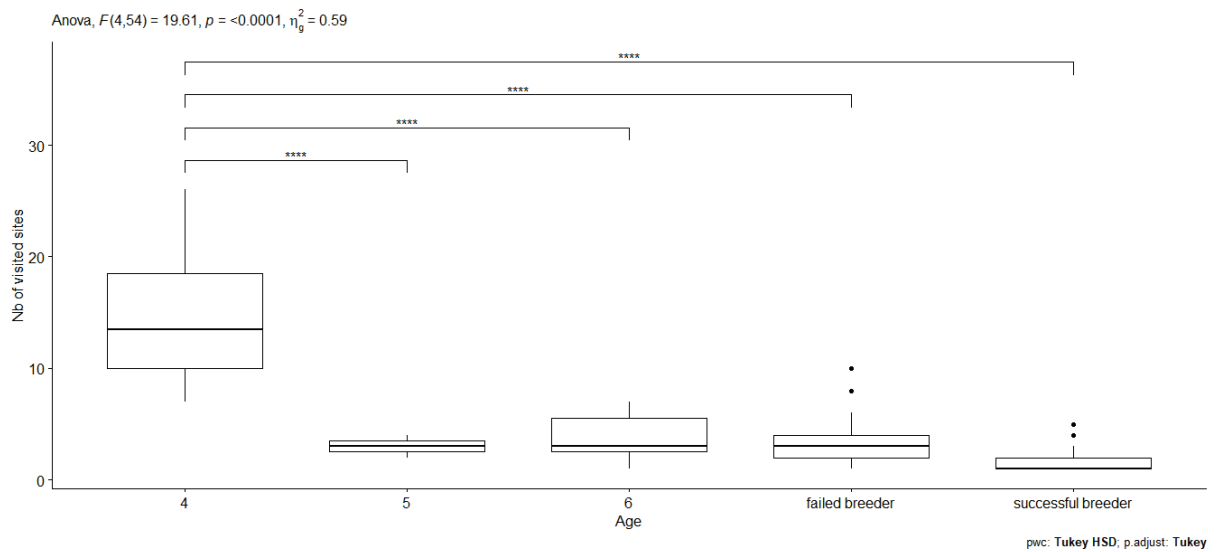
Status	Age	n	distance between sites (m)		nb sites visited	
			mean	sd	mean	sd
PB	4y	4	1,319	795	15	8.21
PB	5y	2	80.5	16.3	3	1.41
PB	6y	7	261	349	3.86	2.19
FB	adult	27	105	84	3.52	2.33
SB	adult	19	33	61.4	1.74	1.28



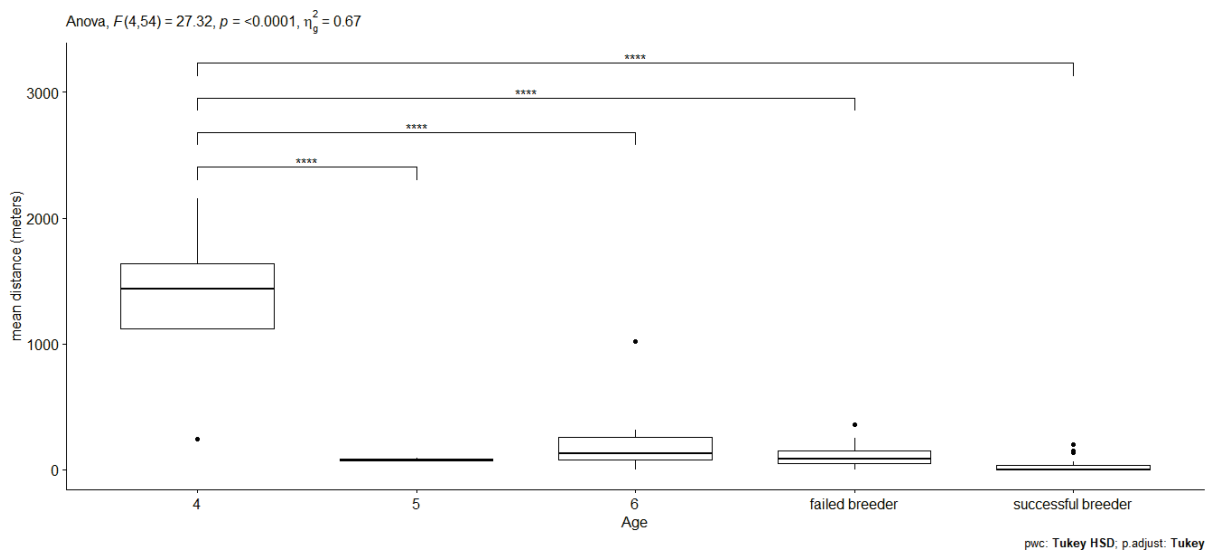
**Figure 1:** Small-scale movements based on fifty percent (left) and ninety percent (right) kernel of the intensity of use of the habitat. Green: successful breeders; Blue: failed-breeders; Red: pre-breeders; Yellow star: center of the study colony where birds were equipped



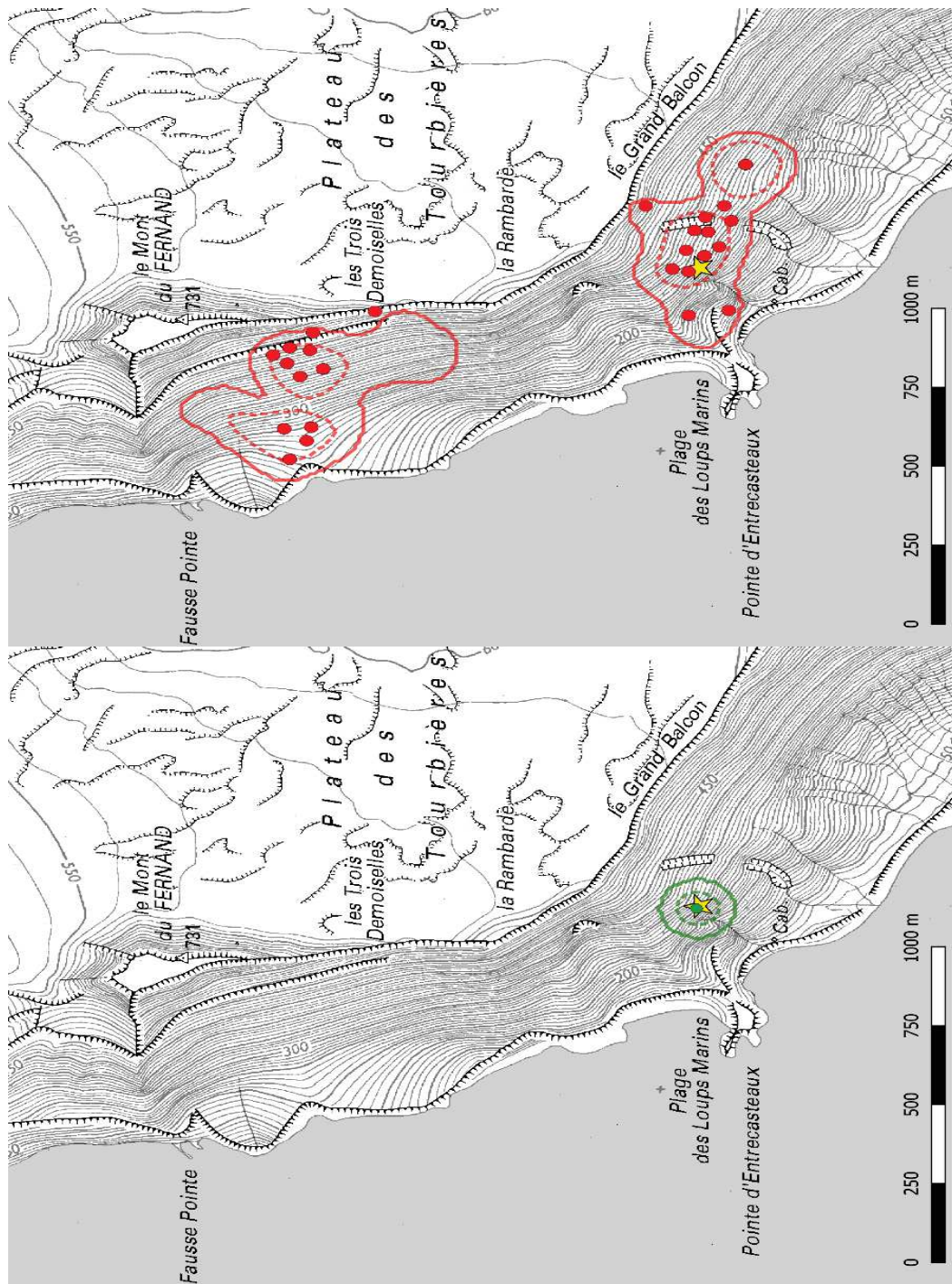
**Figure 2:** Mean of 50% kernel intensity area according to the age and status of individuals. Individuals of 4 years old have a significantly higher core area ( $p < 0.001$ \*\*\*\*) than all other individuals.



**Figure 3:** Mean number of visited sites and number of different visited sites. Individuals of 4 years old have a significantly higher core area than all other individuals.



**Figure 4:** Mean distance between visited sites and number of different visited sites during the first 8 days of tracking. Individuals of 4 years old have a significantly higher core area ( $p < 0.001$ \*\*\*\*) than all other individuals.



**Figure 5:** Illustration of kernel intensity use of habitat and detected visited areas for typical Successful-breeders and Pre-breeders. Plain line, 90% kernel; Dash line, 50% kernel. Each dot is a 40m diameter circle (determined as a landing site; see text). In green, a Successful-breeder; Red, a Pre-breeder. The figure on the left shows an example of 1 successful-breeder with a small range of area use(having the minimum 90% kernel area). The figure on the right 1 shows a pre-breeder with a large range of area use (having the maximum 90% kernel area).

**Prospecting by pre-breeders and failed-breeders in a highly site faithful albatross species: evidence and potential implication for the circulation of disease agents – Supplements**

**Table S1:** Movement parameters of tracked individuals. ID\_Bird: individual code on the plastic ring; Status: FB, Failed-breeders; PB, Pre-breeders and SB, Successful-breeders. For each bird, the table presents for the whole period of tracking and for the first 8 days: the number of landings, the number of sites visited and the maximum distance in meters between 2 visited sites. CP1\* are data from the whole tracking period for that individual and CP1\*\* data are after failure removed for the analysis for that individual.

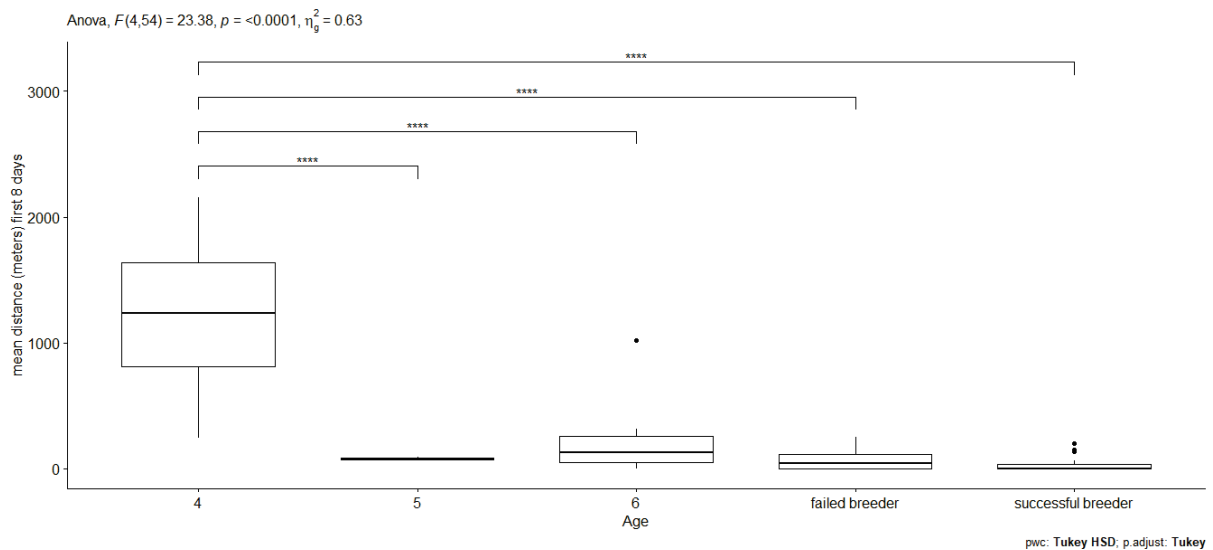
ID_Bird	Status	Season	Date of equipment	Tracking duration (days)	Number of total sites with landing	Number of total landings	Max distance between 2 sites with landing (m)	Number of sites visited - first 8 days	Number of visits - first 8 days	Max distance between 2 visited sites - first 8 days (m)
CB4	FB	2018/2019	02/12/2018	1.9	2	10	67.92	2	10	67.92
DK2	FB	2017/2018	02/12/2017	4.1	4	40	89.71	4	40	89.71
BG2	FB	2018/2019	02/12/2018	10.0	1	35	0.00	1	35	0.00
CB1	FB	2018/2019	02/12/2018	10.2	2	12	39.64	2	7	39.64
Y87	FB	2018/2019	02/12/2018	10.4	3	27	106.43	3	25	106.43
CB3	FB	2018/2019	02/12/2018	10.6	2	32	49.46	1	19	0.00
CB2	FB	2018/2019	02/12/2018	11.2	4	38	151.69	4	22	151.69
DR0	FB	2018/2019	02/12/2018	11.7	1	26	0.00	1	25	0.00
DS5	FB	2018/2019	02/12/2018	11.8	3	40	57.41	3	39	57.41
Z03	FB	2017/2018	02/12/2017	13.5	3	15	103.80	3	13	103.80
C10	FB	2017/2018	20/11/2017	14.4	2	8	29.95	0	0	0.00
DK0	FB	2017/2018	02/12/2017	16.7	2	9	33.27	2	9	33.27
DK1	FB	2017/2018	02/12/2017	18.4	2	31	44.46	2	16	44.46
AW0	FB	2017/2018	21/11/2017	19.6	6	32	200.80	0	0	0.00
DN7	FB	2019/2020	25/11/2019	20.4	5	21	160.02	4	8	125.54
DF8	FB	2017/2018	02/12/2017	22.9	8	139	178.40	5	40	117.48
BW6	FB	2017/2018	02/12/2017	23.3	10	75	355.97	5	38	239.10
DJ9	FB	2017/2018	02/12/2017	24.0	8	26	204.07	6	16	200.74
DH4	FB	2019/2020	25/11/2019	24.2	6	29	253.88	6	14	253.88
DH9	FB	2019/2020	25/11/2019	24.5	2	27	53.65	1	9	0.00
DR6	FB	2019/2020	25/11/2019	26.6	4	11	154.02	4	11	154.02
DM2	FB	2019/2020	25/11/2019	26.6	4	27	119.94	1	6	0.00
DK3	FB	2017/2018	02/12/2017	27.3	2	20	72.99	1	5	0.00
U69	FB	2018/2019	02/12/2018	28.0	2	36	62.98	2	10	62.98
BG9	FB	2017/2018	01/12/2017	28.9	4	17	146.25	2	6	53.47
Z21	FB	2018/2019	02/12/2018	40.8	2	135	85.58	1	11	0.00
BF3	FB	2018/2019	02/12/2018	47.3	1	116	0.00	1	17	0.00

ID_Bird	Status	Season	Date of equipment	Tracking duration (days)	Number of total sites with landing	Number of total landing	Max distance between 2 sites with landing (m)	Number of sites visited - first 8 days	Number of visits - first 8 days	Max distance between 2 visited sites - first 8 days (m)
AT7	PB	2019/2020	03/11/2019	1.1	1	14	0.00	1	14	0.00
AP6	PB	2019/2020	17/10/2019	1.8	3	13	1,017.53	3	13	1,017.53
AT1	PB	2017/2018	02/12/2017	6.1	7	16	240.85	7	16	240.85
DJ8	PB	2017/2018	31/10/2017	6.2	16	40	2,157.08	16	40	2,157.08
AM8	PB	2019/2020	03/11/2019	6.9	7	42	202.84	7	42	202.84
AN7	PB	2019/2020	03/11/2019	10.1	3	11	99.64	3	11	99.64
AU2	PB	2019/2020	03/11/2019	14.6	6	17	315.88	6	15	315.88
BU1	PB	2019/2020	25/11/2019	19.9	26	95	1,464.47	16	42	1,464.47
BL9	PB	2019/2020	25/11/2019	21.7	4	24	91.99	4	19	91.99
AP3	PB	2019/2020	02/11/2019	22.2	2	27	63.47	1	3	0.00
AM3	PB	2018/2019	02/12/2018	42.6	2	60	69.00	2	23	69.00
No code	PB	2019/2020	03/11/2019	43.3	5	53	126.37	5	22	126.37
BA9	PB	2018/2019	02/12/2018	54.0	11	33	1,415.14	3	5	1,003.80
ID_Bird	Status	Season	Date of equipment	Tracking duration (days)	Number of total sites with landing	Number of total landing	Max distance between 2 sites with landing (m)	Number of sites visited - first 8 days	Number of visits - first 8 days	Max distance between 2 visited sites - first 8 days (m)
CB7	SB	2018/2019	04/12/2018	1.9	1	13	0.00	1	13	0.00
CP6	SB	2019/2020	12/12/2019	2.7	1	6	0.00	1	6	0.00
CC3	SB	2018/2019	04/12/2018	4.1	1	4	0.00	1	4	0.00
CC2	SB	2018/2019	04/12/2018	5.0	1	13	0.00	1	13	0.00
CP5	SB	2019/2020	12/12/2019	5.2	4	26	197.96	4	26	197.96
CN9	SB	2019/2020	12/12/2019	5.5	5	28	138.99	5	28	138.99
CC4	SB	2018/2019	04/12/2018	6.0	1	15	0.00	1	15	0.00
CB8	SB	2018/2019	04/12/2018	6.1	2	12	29.18	2	12	29.18
CC1	SB	2018/2019	04/12/2018	6.6	3	17	64.43	3	17	64.43
CP7	SB	2019/2020	12/12/2019	6.7	1	18	0.00	1	18	0.00
CP3	SB	2019/2020	12/12/2019	7.2	1	47	0.00	1	47	0.00
CC0	SB	2018/2019	04/12/2018	7.7	1	19	0.00	1	19	0.00
CP4	SB	2019/2020	12/12/2019	7.7	1	16	0.00	1	16	0.00
CP2	SB	2019/2020	12/12/2019	7.7	1	15	0.00	1	15	0.00
CN8	SB	2019/2020	12/12/2019	7.7	1	25	0.00	1	25	0.00
CC5	SB	2018/2019	04/12/2018	8.1	1	22	0.00	1	19	0.00
CB6	SB	2018/2019	04/12/2018	9.3	4	29	151.96	4	28	151.96
CP1*	SB	2019/2020	12/12/2019	20.2	2	75	44.85	2	34	44.85
CP1**	SB	2019/2020	12/12/2019	7.5	2	34	31.47			
CB9	SB	2018/2019	04/12/2018	21.3	1	54	0.00	1	24	0.00

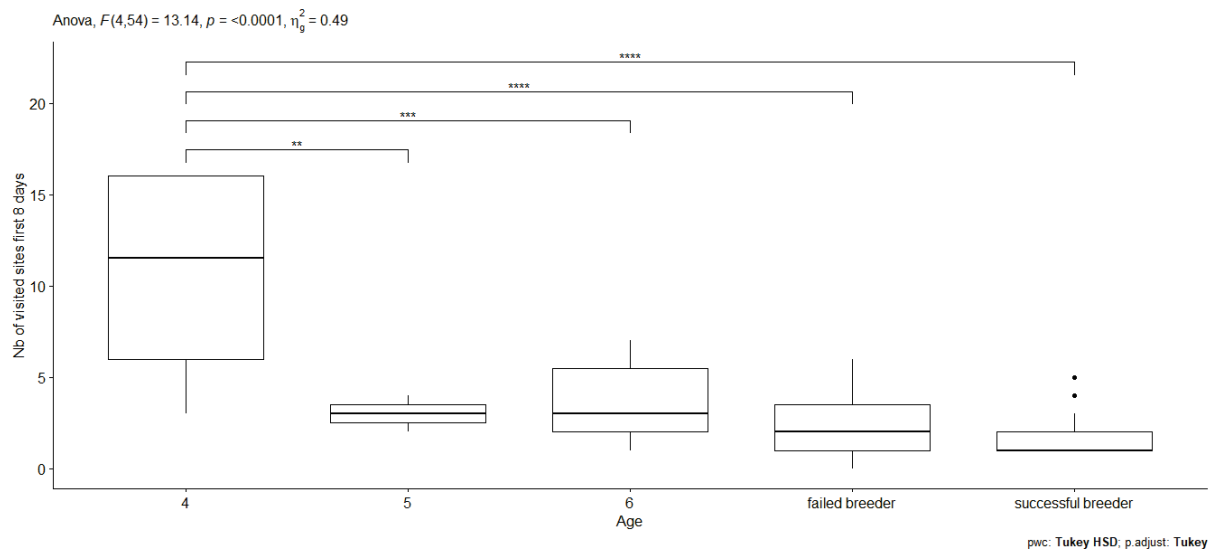


**Table S2:** Mean distance between visited sites and number of different visited sites during the first 8 days of tracking for the individuals of the 3 considered status, S, successful-breeder; FB, failed-breeder and PB, pre-breeder.

Status	Age	n	distance between sites (m) first 8 days		nb sites visited first 8 days	
			mean	sd	mean	sd
PB	4y	4	1217	805	10.5	6.56
PB	5y	2	80.5	16.3	3	1.41
PB	6y	7	252	356	3.71	2.36
FB	adult	27	67.8	78.1	2.37	1.78
SB	adult	19	33	61.4	1.74	1.28



**Figure S1:** Mean distance between visited sites and number of different visited sites during the first 8 days of tracking. Individuals of 4 years old have a significantly higher core area ( $p < 0.001$ \*\*\*\*) than all other individuals.



**Figure S2:** Mean number of visited sites and number of different visited sites during the first 8 days of tracking. Individuals of 4 years old have a significantly higher core area than all other individuals.



**Figure S3:** Picture of the study site from the top. Indian Yellow-nosed breed in dense patches from few tenth to thousands of individuals. The birds we tracked were all captured in a monitored subcolony of about 650 pairs nesting over an area of 5,000 square meters.



## Annexe 4

Similar at-sea behaviour but different habitat use between failed and successful breeding albatrosses.

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*In revision, Marine Ecology Progress Series*



## **Similar at-sea behaviour but different habitat use between failed and successful breeding albatrosses**

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*Running head: At-sea distribution and habitat use of YNA*

**Abstract:**

Breeding failure is expected to induce behavioural changes in central place foragers. Indeed, after failing their reproduction, breeding individuals are relieved from having to come back to their breeding site for reproductive duties and thus are less constrained than successful breeders in their movements during the remaining of the breeding season. Accordingly, they are expected to adjust their behaviour, travelling longer in distance and/or time to reach foraging grounds. They are also expected to use different foraging areas to decrease local intra-specific competition with successful breeders. We compared the at-sea behaviour and habitat use of successful and failed Indian yellow-nosed albatrosses nesting in Amsterdam Island, Southern Indian Ocean, during two chick-rearing seasons. Failed breeders exhibited the same at-sea foraging behaviour, travelling as far and as long as successful breeders. They also spent the same amount of time on their nest between at-sea trips. Nevertheless, habitat models revealed a partial spatial segregation of failed breeders, which used specific foraging areas characterized by deeper and colder waters, in addition to the ones they shared with successful breeders. Our study shows the importance of combining a range of analytical methods (spatial analysis, behavioural inferences with advanced movement models and habitat models) to infer the at-sea behaviour and habitat use of seabirds. It also stresses the importance of considering individual breeding status when aiming at understanding the spatial distribution of individuals, especially when they likely have conservation implications.

**Keywords:** breeding failure, behavioural state, foraging behaviour, *Thalassarche carteri*, Procellariiformes, habitat models, inter-individual variability

## 1. Introduction

Seabirds are currently one of the most threatened groups of birds, with nearly 50% of all species showing a decline in population size in recent years (Croxall et al. 2012, Dias et al. 2019). Commercial fisheries are one of the greatest threats to seabirds through both competition with fisheries (Grémillet et al. 2018) and incidental mortality in fishing gear (Anderson et al. 2011, Phillips et al. 2016). Other threats include pollution, pathogens, climate change, and invasive predators, such as rats, mice, and cats (Croxall et al. 2012, Phillips et al. 2016). Due to their extreme life history traits, Procellariiformes, which include albatrosses and petrels, are particularly vulnerable: they are long-lived, exhibit delayed sexual maturity, and lay at most once a year a single-egg clutch that cannot be replaced. Moreover, Procellariiformes are wide-ranging and their foraging range frequently covers hundreds to thousands of kilometres (e.g., Nicholls et al. 2002, Pinaud & Weimerskirch 2007, Mackley et al. 2010, Gutowsky et al. 2014a), inevitably overlapping with fisheries (Phillips et al. 2016). Hence, understanding the spatial ecology of Procellariiformes is crucial for identifying potential threats they may face when at-sea.

These last decades, the development of miniaturized tracking devices recording individual locations have provided detailed knowledge on the at-sea distribution and the foraging ecology of hundreds of seabird species (Oppel et al. 2018). Notably, it has been shown that seabird at-sea distribution is largely driven by the distribution and abundance of prey, which is in turn determined by the biophysical environment (Weimerskirch 2007). However, at-sea distribution also strongly varies among species, among populations of the same species and among individuals of the same population (Phillips et al. 2017a). Intra-specific variation may depend on sex (Patrick & Weimerskirch 2014, Edwards et al. 2016), age (Lecomte et al. 2010, Péron & Grémillet 2013, Riotte-Lambert & Weimerskirch 2013, Gutowsky et al. 2014b, Weimerskirch et al. 2014) and/or other inter-individual differences (Gutowsky et al. 2015). One major factor likely to affect the at-sea distribution and foraging



behaviour of seabirds is breeding status, namely whether an individual is currently succeeding or failing at reproduction (Phillips et al. 2017a). Indeed, during the breeding season, whereas successful breeders may allocate the majority of their time to foraging for themselves and their offspring, multiplying trips between the colony and foraging areas, failed breeders are not restricted in such a way and may be free to engage in longer and/or further at-sea trips (e.g. Fijn et al. 2014, Ponchon et al. 2017). Likewise, breeding failure may lead individuals to leave the colony earlier and use different wintering grounds, leading to spatial segregation during the non-breeding season (Phillips et al. 2005, Catry et al. 2013, Clay et al. 2016), with potential negative consequences on subsequent reproductive success (Desprez et al. 2018). Yet, the short and long-term consequences of breeding failure are still overlooked in movement studies because failed and non-breeders are generally more challenging to capture and track. Indeed, they are not as attached to their nesting site as successful breeders and may leave their colony earlier (e.g. Catry et al. 2013, Ramos et al. 2018).

Identifying important seabird areas for prioritizing conservation efforts has been usually based on tracking data collected from successful breeders (e.g., Delord et al. 2014, Lascelles et al. 2016, Soanes et al. 2016, Dias et al. 2017, Heerah et al. 2019), and it is only recently that other life stages have been tracked (Péron & Grémillet 2013, Gutowsky et al. 2014b, Weimerskirch et al. 2014, de Grissac et al. 2016). However, there is still little information on movements at sea of breeders that have failed at the egg or chick stage. In terrestrial ecosystems, several recent studies have demonstrated that conservation actions were more effective when inter-individual variation was taken into account. For example, accounting for individual differences in seasonal movement behaviours of African elephants *Loxodonta africana* resulted in the design of very different conservation corridors to enhance connectivity (Osipova et al. 2019). On the other side, accounting for individual breeding performance in noctule bats *Nyctalus noctula* (Mackie & Racey 2007) and sage grouse *Centrocercus urophasianus* (Smith et al. 2018) highlighted the need to protect distinct areas, as failed and successful

breeders did not use the same habitats. Gaining information on the behaviour and habitat use of failed breeding individuals may thus help determine whether they use similar areas to successful breeders or whether they are spatially segregated and behave differently.

The Indian yellow-nosed albatross (*Thalassarche carteri*) inhabits the Southern Indian Ocean. On Amsterdam Island, failed breeders represent a large proportion of the population, which has been recurrently hit by avian cholera outbreaks (Weimerskirch 2004, Jaeger et al. 2018). The disease, caused by the bacterium *Pasteurella multocida*, appears to be responsible for the death of a large proportion of albatross chicks (Bourret et al. 2018, Jaeger et al. 2018, Jaeger et al. 2020). Introduced rodents likely also contribute to the low breeding success of the population (Micol & Jouventin 1995), as observed in other seabird populations worldwide (Cuthbert & Hilton 2004, Jones & Ryan 2009, Caravaggi et al. 2019, Holmes et al. 2019).

As breeding failure is overlooked in tracking studies compared to other factors such as sex or breeding stage within the breeding season (incubation vs. chick rearing), we investigated the at-sea behaviour and habitat use of Indian yellow-nosed albatrosses nesting on Amsterdam Island based on their breeding status during two chick-rearing seasons, in 2015-2016 and 2018-2019. We predicted that failed breeders would perform longer trips than successful breeders as they do not need to regularly return to the colony to feed their chick. These may be longer trips in terms of maximum distance to the colony or trip length, and/or in terms of trip duration since failed breeders could spend more time foraging in areas closer to the colony rather than travelling further (Votier et al. 2017). Failed and successful breeders were also predicted to forage at least partly in distinct areas that may differ in their oceanographic characteristics, with failed breeders being able to target areas of high productivity that may be too far away from the colony for successful breeders (Antolos et al. 2017). Failed and successful breeders were also predicted to exhibit different activity budgets, with failed breeders spending more time resting on the water and less time flying than successful breeders

(Ponchon et al. 2019). Moreover, we expected failed breeders to spend more time on their nest compared to successful breeders to maintain pair bond (Hedd & Gales 2005).

## **2. Material & Methods**

### *2.1. Study area and species*

The study was carried out in a colony of Indian yellow-nosed albatrosses on Amsterdam Island (37°86' S, 77°52' E) during two breeding seasons: December 2015 - January 2016 and November 2018 - January 2019 (hereafter 2015 and 2018 respectively). This population holds two-third of the world population, with *ca.* 22 000 pairs breeding each year (Heerah et al. 2019). The species annually lays a single clutch of one egg in September-October that hatches in late November or early December. Chicks fledge in March-April. The average adult apparent annual survival rate is 0.90 (Gamble et al. 2019), the average annual breeding success is 0.16 (Jaeger et al. 2018) and chick survival rate varies annually between 0.08 and 0.58, low chick survival being correlated with the occurrence of avian cholera outbreaks (Jaeger et al. 2020). The Indian yellow-nosed albatross is currently classified as Endangered on the IUCN Red List of Threatened Species, as the population has decreased by 86.6% between 1981 and 2016 (Jaeger et al. 2018).

### *2.2. GPS Equipment*

Each study year, two groups of adult Indian yellow-nosed albatrosses were tracked during the early chick-rearing period. One consisted of failed breeders which had lost their eggs or young chicks (n=30), and the other consisted of successful breeders (n=18). Failed breeders were equipped with solar-powered GPS-UHF loggers (Harrier-L or Uria, Ecotone, Poland) which were attached to the birds' back feathers with Tesa® tape after breeding failure. The devices were programmed with an initial acquisition frequency of 15 min but this could be modified to 5, 10 or 20 min, depending on battery

charge and weather conditions. Location data were automatically downloaded by an antenna placed at the edge of the study colony when the birds were in range. Failed breeders were tracked between 9 December 2015 and 11 January 2016 and between 30 November 2018 and 19 January 2019. The loggers naturally fell off after some time when the birds could not be recaptured to retrieve the loggers.

Because they could be more easily recaptured, successful breeders were equipped with GPS devices (i-GotU GT-600, MobileAction Technology, Taiwan) programmed with a fixed acquisition frequency of 5 min. The loggers were attached to the back feathers with Tesa® tape. Successful breeders were tracked between 5 and 20 December 2015 and 30 November to 26 December 2018. In 2018, GPS devices had a duty cycle of 17h on and 7h off at night, providing locations from 5am to 10pm UTC (GMT+5). All individuals were recaptured to retrieve the loggers and access the data.

### *2.3. Data Analysis*

As only 3 failed breeders were tracked beyond 26 December over the 2 study years, these data were excluded from the analysis and the study periods were bounded between 5 to 21 December 2015 and 30 November to 26 December 2018 to match the tracking period of successful breeders. Moreover, because of the important time gaps due to duty cycles in successful breeders in 2018, two separate datasets were used.

#### *2.3.1. Trip characteristics and colony attendance*

The first dataset included all complete trips, which were composed of a minimum number of 12 consecutive locations further than 1km from the colony for a total duration of at least 2h, regardless the duration of time gaps within trips (Supplemental material S1). It was used to calculate the general trip characteristics (maximal distance to the colony, total trip duration and total distance travelled), to

retain as much data as possible and avoid discarding the longest trips, which generally had the longest time gaps in failed breeders. Trip characteristics were calculated for each trip and then, averaged for each individual and eventually averaged by breeding status and year. Note that because of the presence of large time gaps in successful breeders in 2018 and failed breeders, the total distance travelled is likely underestimated. Time spent in the colony between two at-sea trips was averaged for each individual and then averaged for each groups. As the minimum trip duration for successful breeders in 2018 was 30h, it is unlikely that colony attendance was overestimated due to the non-detection of nocturnal trips occurring during the off period of the loggers.

Linear mixed models were used to test the effects of breeding status (fixed), year (fixed) and individuals (random) on trip characteristics and colony attendance, which were square-root-transformed to improve the normality of the residuals. Additional F-tests were conducted to compare variances between failed and successful breeders.

2.3.2. Individual at-sea behaviour and distribution A second dataset was used to analyse individual distribution and at-sea behaviour. First, data from failed and successful breeders in 2015 as well as data from failed breeders in 2018 were filtered to remove all trips which had time gaps > 5h. Data from successful breeders in 2018 were filtered with a different time threshold of 8h, to avoid discarding all trips. Then, all trips from all the 4 groups were linearly interpolated to 15-minute intervals to address the issue of uneven sampling in time intervals with the *pastecs* R package (Ibanez & Grosjean 2018).

The R package *moveHMM* (Michelot et al. 2016) was used to fit a three-state Hidden Markov model (HMM) to the at-sea interpolated location data for each year separately. The states reflected three different activities at sea: 1) resting, characterised by a small step length and low turning angle, 2) travelling, characterised by a long step length and low turning angle, and 3) foraging, characterised

by an intermediate step length and a large turning angle (Grecian et al. 2018). A set of different initial parameters was used to ensure that the global minimum in negative log-likelihood had been reached. The model was validated with the visual inspection of pseudoresiduals. The Viterbi algorithm was used to classify the most likely behaviour at each time step (Fig. 1). Finally, all locations occurring between 10pm and 5am were excluded for all 4 groups to avoid overestimating resting and travelling activities due to linear interpolation.

The proportion of time spent in each activity per trip and for periods between 5am and 10pm was calculated. Mixed effects logistic regressions were then used to test breeding status and year as fixed effects and individual identity as random effect on the proportion of time spent in each of the three identified states.

### 2.3.3. Spatial analysis

At-sea distribution was inferred separately for successful and failed breeders and for each year from the calculation of the 50% and 90% Utilization Distribution (UD) contours using the *adehabitatHR* package (Calenge 2006) with a cell size of 500m and a common smoothing factor  $h$  fixed at 70km. A saturation plot showed that the tracking sample sizes for each group reached a plateau, suggesting sufficient representativeness of the different groups (Supplemental Material Figure S2).

Spatial overlaps between the 50 and 90% UD of successful and failed breeders tracked the same year were estimated with a randomization procedure. An initial spatial overlap matrix was calculated with the *kerneloverlap* function of the *adehabitatHR* package, using the Utilization Distribution Overlap Index (UDOI; Calenge 2006) for each pair of individuals, regardless of their breeding performance for a given year. A second “membership” matrix indicating whether a pair of individuals had the same breeding performance (coded 0) or a different breeding performance (coded 1) was built. After removing diagonals from both matrices, a Pearson correlation coefficient  $R_{obs}$  was

calculated between the two matrices. Then, the distribution of each individual was randomly and independently rotated around the colony location. A new spatial overlap matrix and a new membership matrix were built, and a new Pearson's correlation coefficient  $R_{rand}$  was calculated. This randomization procedure was repeated 1000 times to obtain a distribution of correlation coefficient  $R_{rand}$  representing the null hypothesis of no difference in the spatial distribution of the two groups (see Cecere et al. 2018 for the detailed procedure). All statistical analyses were carried out using R v.3.6.0 (R Core Team 2017).

#### 2.4. Habitat modelling

Initially, 8 biological and oceanographic variables likely to affect seabird distribution were selected for the Southern Indian Ocean. Data for bathymetry, Sea Surface Temperature (SST), Chlorophyll a concentration (CHLA), and wind speed were obtained from ERDDAP (<https://coastwatch.pfeg.noaa.gov/erddap>) while Sea Level Anomalies (SLA) and Eddy Kinetic Energy (EKE) were obtained from Copernicus Marine Environment Monitoring Service (CMEMS, <http://marine.copernicus.eu/>). The environmental variables, which were available daily or weekly, were averaged over the two study periods (5 December 2015 – 21 December 2015 and 30 November 2018 – 26 December 2018). SST and CHLA gradient (respectively gSST and gCHLA) were computed from the corresponding environmental layers using the *terrain* function in the *raster* package (Hijmans 2019). The final resolution of the layers was 0.1°. Prior to modelling, strongly correlated predictors were identified by estimating all pair-wise Spearman rank correlation coefficients. High correlation coefficients ( $r > 0.7$ ) were found between SLA and SST in both 2015 and 2018 and between wind speed and SST in 2015. Therefore, SLA was excluded for 2018 and both SLA and wind speed were excluded for 2015. In 2018, gSST values were negligible ( $<0.001$ ) so this variable was also excluded. The

remaining environmental layers were bathymetry, CHLA, gCHLA, SST, EKE and gSST for the 2015 models and bathymetry, CHLA, gCHLA, SST, EKE and wind speed for the 2018 models.

The presence probabilities were predicted separately for failed and successful breeders and separately for 2015 and 2018 using the software Maxent v. 3.4.0 (Phillips et al. 2017b). Overall, 4 habitat models were built. Only locations associated with resting or foraging states were used. The *spatially rarify occurrence data* tool in the python-based toolbox *SDMtoolbox 2.0* for ArcGIS 10.6 (Brown et al. 2017) was used to filter the tracking data at 5 km to reduce spatial autocorrelation. Duplicated records were not removed to give more weight to cells used by several individuals. The models were replicated 50 times for each group and validated using bootstrap sampling. The predictive performance of the models was assessed using the Area Under the receiver operating characteristic Curve (AUC), which evaluates how well model predictions discriminate locations where observations are present from background locations.

### **3. RESULTS**

#### *3.1. Trip characteristics and behaviour*

Breeding failure was not associated with major individual behavioural changes and this absence of change was consistent between the two years of tracking (Tables 1, 2, 3; Fig. 2). There was no significant difference between failed and successful breeders in terms of maximum distance to the colony or trip duration although failed breeders presented greater inter-individual variability (F-test for max distance to the colony:  $F_{42,69} = 0.21$ ,  $p < 0.001$ ; F-test for trip duration:  $F_{42,69} = 0.10$ ;  $p < 0.001$ ; Fig. 2). Likewise, the proportion of time spent foraging and resting between 5am and 10pm, was similar (Table 2) as well as the time spent in the colony between at-sea trips (Table 1).. Nonetheless, failed breeders tended to travel shorter total distances during a trip and both successful and failed breeders



spent slightly more time flying although those differences were weakly significant ( $p=0.05$  and  $p=0.04$  respectively; Tables 1, 2, 3).

### *3.2. Observed at-sea distribution and predicted habitat use*

Failed and successful breeders were not strongly spatially segregated, neither in their 50% nor 90% kernel UD (Fig. 3; Table 4) and this remained consistent between the two years of tracking (Table 4). Nevertheless, the bearing of the two groups when at-sea was different. In 2015, successful breeders mainly used areas South of the colony while failed breeders mainly used foraging grounds situated south-west of the colony (Fig.1, 3). In 2018, successful breeders used habitats East of the colony while failed breeders had a more variable bearing, mostly directed East and South (Fig. 1, 3).

The four habitat models built from the foraging and resting locations of failed and successful breeders achieved AUC values  $>0.91$ , indicating excellent model predictive abilities. They showed that failed and successful breeders had different habitat preferences (Fig. 4; Table 5). Failed breeders differed from successful breeders, especially in 2015, when 3 individuals used habitats with colder SST (around  $10^{\circ}\text{C}$ ) that successful breeders and failed breeders in 2018 did not use (Fig. 4a). Failed breeders also generally used habitats characterised by deeper waters, with one 2700m deep and another one 4500m deep, when other groups favoured depths of 1500m (Fig. 4b). Both successful and failed breeders used habitats characterized by higher CHLA values in 2018 (Fig. 4c). While bathymetry was the most important variable for successful breeders in 2015, SST was still significant. On the contrary, the two most important variable for failed breeders were SST and CHLA (Table 4). SST and CHLA gradients, EKE and wind speed contributed for  $< 5\%$  to individual distribution (Table 5).

The predictions obtained from the habitat models showed that failed and successful breeders were likely to be encountered in different areas (Fig. 5). In 2015, while successful breeders were predicted to be found mostly East of the colony, up to 1200 km but also in a 300 km range around the

colony except West (Fig. 5a), failed breeders were predicted to substantially favour areas up to 300 km North-East of the colony, as well as areas West and South-West of the colony, up to 1200 km (Fig. 5b). In 2018, successful breeders were still predicted to use areas East and North-East of the colony but presence probabilities were slightly lower (Fig. 5c). On the contrary, failed breeders were predicted to be found in a larger area than in 2015 but with lower probabilities (Fig. 5d). Nevertheless, they were still predicted to visit the same area 1200 km South West of the colony, an area that was never used by successful breeders.

## 4. Discussion

### 4.1. At-sea behaviour

Contrarily to our predictions, we did not find any major difference in the at-sea behaviour of failed and successful breeders, neither in 2015 nor 2018. The tracked failed individuals shared the same trip characteristics, and the same amount of time foraging, travelling or resting on the water as successful breeders. A recent study on black-browed albatrosses *Thalassarche melanophris* reported a difference in the time spent in foraging, resting and flying activities between successful and failed breeders when at-sea, especially during daylight (Ponchon et al. 2019). However, this difference appeared later in the breeding season in that species, when the chicks were > 3 months. Here, our tracking period mostly covered the early chick-rearing, while chicks were < 2 months. This suggests that during our study period, breeding individuals may have not yet paid strong cost for reproduction and thus they did not have to forage more compared to failed breeders to be able to both feed their chick and maintain their own body condition. Alternatively, the foraging conditions were potentially good enough to prevent detecting any effect of reproductive cost on the variables we measured at that time of the breeding season.

Moreover, we had expected failed breeders to spend more time at-sea, resting on the water or exploring foraging areas situated further away from their colony. But they did not. They only slightly travelled shorter total distances over a trip but this is likely an underestimation due to the presence of long gaps that have been interpolated. Although they did not have to invest in reproduction anymore, failed breeders did not change their at-sea behaviour and they spent the same amount of time at sea as successful breeders. Nevertheless, they tended to go at-sea more frequently than successful breeders, as they spent 28% less time on their nest between at-sea trips on average. Maintaining some nest attendance even after breeding failure shows that the birds are still attached to their nesting site, possibly due to the necessity of maintaining some pair bond with the mate.

#### *4.2. Spatial distribution and habitat use*

Failed and successful breeders were not significantly spatially segregated in either year. All the tracked birds mostly foraged in the same areas, situated on average 300-500 km away from the colony. This range is more restricted than the ones measured during incubation in successful breeders (Pinaud & Weimerskirch 2007, Antolos et al. 2017), but range contraction is frequent among albatrosses between incubation and chick-rearing (Weimerskirch et al. 1993, Awkerman et al. 2005, Heerah et al. 2019). Habitat models nevertheless revealed differences in the habitat preferences, and thus the predicted at-sea distribution, between failed and successful breeders. Although the tracked birds shared most of their foraging areas, failed breeders had a larger predicted distribution. In particular, failed individuals in 2015 differed from the three other groups as they used foraging areas characterized by colder and deeper waters, possibly associated with the Subtropical and Subantarctic Fronts (Pinaud et al. 2005). Successful breeders mainly foraged in habitat of  $\sim 15^{\circ}\text{C}$ , which aligns with the findings of a previous study tracking individuals from the same colony (Antolos et al. 2017). Likewise, depth, SST and CHLA showed the strongest correlation with Indian yellow-nosed albatrosses at-sea distribution during the

chick-rearing season, as in successful Indian yellow-nosed albatrosses breeding in Prince Edward Island (Makhado et al. 2018). On the contrary, EKE, SST gradient and CHLA gradient, which are generally used as proxies for indicating the presence of fronts and eddies (Bost et al. 2009), were weakly correlated. This suggests that the birds were not specifically attracted by areas showing such oceanographic features, assumed to be important for marine top predators (Bost et al. 2009, Scales et al. 2014).

Overall, the at-sea distribution and behaviour of Indian yellow-nosed albatrosses nesting on Amsterdam Island were consistent between years. An absence of clear spatial segregation suggested that the marine habitats used by Indian yellow-nosed albatrosses were productive enough to provide food for the whole colony, regardless of their breeding status and that individuals favoured foraging areas close to their colony (Pinaud et al. 2005). Nevertheless, failed breeders showed some flexibility, as they also used areas that successful breeders did not reach. Interestingly, those areas are not situated further away from the colony so they would presumably still be accessible to successful breeders. At the same time, as the levels of breeding failure are high in our study colony, most of successful breeders becomes failed breeders over the breeding season, thereby decreasing competition between successful and failed breeders. Further information on diet, i.e. through stable isotopes, should be investigated to determine whether failed and successful breeders just use different habitats but feed on the same prey species or whether each breeding group partly segregate both in space and diet.

Although we did not initially find any major difference in the at-sea behaviour nor distribution between failed and successful breeders, we did find a difference in habitat preferences, revealed by the habitat models. Failed breeders were predicted to have larger distributions compared to successful breeders because they could use additional foraging areas characterized by deeper and colder waters. Those results show the importance of combining a range of analytical methods (spatial analysis, behavioural inferences with advanced movement models, habitat models) to infer the at-sea

behaviour and habitat use of seabirds. Here, habitat models brought crucial additional information that behavioural and statistical analyses alone would have not highlighted.

Our study presents some limitations, though. We don't have information on the exact age or sex of the tracked individuals, except that they are sexually mature and breed. It is thus possible that the differences observed in the predicted distribution might be due to another individual factor. However, no study has shown so far that sex or age led to a spatial segregation in Indian yellow-nosed albatrosses (Pinaud et al. 2005, Antolos et al. 2017, Makhado et al. 2018). Another limitation is the time gap in the GPS data. Apart from the 7h time gaps due to the duty cycles in successful breeders in 2018, data in failed breeders had several long-time gaps. We limited biases by removing all trips containing gaps longer than 5h. Nevertheless, fitting a movement model on interpolated data can lead to overestimations in resting or travelling, as interpolated trajectories are straighter than non-interpolated data. Moreover, the distributions presented here correspond to areas used mostly during daylight, between 5am and 10pm so some foraging and resting areas might have been overlooked. Nevertheless, the UD kernels and the predicted distributions are relatively homogeneous, suggesting that the identified areas are likely used as much at night as during day.

#### *4.3. Conservation implications*

In the present study, we only investigated the effects of breeding status during early chick-rearing, when individuals are still bounded to their colony. Further studies are needed to determine whether breeding failure has longer term effects on individual behaviour and habitat use, especially regarding carry-over effects on the subsequent non-breeding season and future reproductive performance. So far, some studies have concurrently tracked failed and successful breeding procellariiforms over winter and each have provided contrasting results both in terms of spatial distribution and at-sea behaviour (Phillips et al. 2005, Catry et al. 2013, Clay et al. 2016, Desprez et al. 2018, Ramos et al. 2018, Ponchon et al. 2019). This poses major challenges in terms of conservation

given the spatial scales procelariiforms are able to cover during the non-breeding season (Lascelles et al. 2016, Carneiro et al. 2020). Moreover, breeding failure is very common in our study population and the proportion of failed breeders can rapidly increase over the breeding season, sometimes leading to complete breeding failure of the colony. Nowadays, food shortage, major climatic extreme events and disease outbreaks episodically lead seabird populations to massive breeding failures (Ponchon et al. 2014, Barbraud et al. 2015, Jaeger et al. 2018, Ropert-Coudert et al. 2018, Piatt et al. 2020). Punctual breeding failures may not directly affect population persistence. Nevertheless, on the long-term, several consecutive breeding failures may modify individual at-sea behaviour and distribution, which may expose individuals to greater threats encountered at sea (Heerah et al. 2019). It may also induce carry-over effects and may ultimately affect population dynamics and viability through reduced productivity and annual survival. This is why it becomes urgent to address more thoroughly inter-individual variability by notably including individual breeding status in addition to breeding stage (juveniles, immatures or adults) or sex into tracking studies to better predict individual spatial distribution and at-sea behaviour and ultimately mitigate threats that the entire population would face.

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## **Data availability**

GPS data are deposited on the Movebank website (program ECOPATH, Indian yellow-nosed albatross, managed by T. Boulinier) and all statistical analyses can be found on the GitHub repository [https://github.com/auponchon/yna\\_15\\_18\\_analysis](https://github.com/auponchon/yna_15_18_analysis).

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Table 1: Sample size, at-sea trip characteristics and nest attendance of successful and failed Indian Yellow-nosed albatross breeders in 2015 and 2018 based on the raw location dataset. Results are shown as mean  $\pm$  standard error (range).

<b>Breeding status</b>	<b>Success</b>	<b>Fail</b>	<b>Success</b>	<b>Fail</b>
<b>Year</b>	2015		2018	
<b>Number of individuals</b>	8	13	9	14
<b>Total number of trips</b>	24	29	19	42
<b>Maximal distance from the colony (km)</b>	340 $\pm$ 40 (155 - 853)	470 $\pm$ 70 (29 - 1230)	376 $\pm$ 24 (233 - 631)	393 $\pm$ 24 (24 - 1406)
<b>Total distance travelled (km)</b>	1099 $\pm$ 141 (417 - 3253)	754 $\pm$ 112 (58 - 2313)	1058 $\pm$ 93 (578 - 2185)	1063 $\pm$ 171 (59 - 4776)
<b>Trip duration (h)</b>	53 $\pm$ 6 (17 - 171)	72 $\pm$ 10 (4 - 175)	58 $\pm$ 3 (31 - 89)	91 $\pm$ 14 (5 - 413)
<b>Time at nest between at-sea trips (h)</b>	42 $\pm$ 6 (14 - 116)	31 $\pm$ 6 (1 - 100)	41 $\pm$ 7 (5 - 76)	33 $\pm$ 6 (0.3 - 115)

Table 2: Sample size and percentage of time spent in different behavioural states based on the reduced dataset including locations occurring only between 5am and 10pm for successful and failed Indian Yellow-nosed albatross breeders in 2015 and 2018. Results are shown as mean  $\pm$  standard error.

<b>Breeding status</b>	<b>Success</b>	<b>Fail</b>	<b>Success</b>	<b>Fail</b>
<b>Year</b>	2015		2018	
<b>Number of individuals</b>	8	10	9	12
<b>Number of trips</b>	23	20	18	32
<b>Time spent foraging per trip during day (%)</b>	32.0 $\pm$ 3.2	27.1 $\pm$ 4.9	35.3 $\pm$ 2.5	44.3 $\pm$ 3.5
<b>Time spent resting per trip during day (%)</b>	20.5 $\pm$ 3.0	25.0 $\pm$ 3.3	25.5 $\pm$ 5.9	25.9 $\pm$ 2.2
<b>Time spent travelling per trip (%)</b>	55.6 $\pm$ 4.1	65.3 $\pm$ 3.8	56.7 $\pm$ 5.8	47.8 $\pm$ 1.8

Table 3: Results from the models testing the effects of breeding status and year on trip characteristics (linear mixed models), nest attendance and time spent in three states (logistic regressions). Individual identity is included as random effect. Significant results are in bold

Response variable	Explanatory variable	F or z value	df	p-value
<b>Maximal distance to the colony</b>	Breeding status	0.03	110	0.86
	Year	0.31	110	0.58
<b>Trip length (km)</b>	Breeding status	4.33	110	<b>0.05</b>
	Year	0.67	110	0.41
<b>Trip duration (h)</b>	Breeding status	1.18	110	0.28
	Year	0.73	110	0.40
<b>Time at nest between trips (h)</b>	Breeding status	3.23	29	0.08
	Year	0.09	58	0.77
<b>Proportion of time spent foraging per trip</b>	Breeding Status	0.03	35	0.97
	Year	0.88	35	0.38
<b>Proportion of time spent resting per trip</b>	Breeding Status	0	35	0.99
	Year	0	35	0.99
<b>Proportion of time spent travelling per trip</b>	Breeding Status	0.12	34	0.90
	Year	-2.08	34	<b>0.04</b>

Table 4: Observed and randomized overlap (UDOI method) of 50% and 90% UD<sub>s</sub> between failed and successful breeders tracked the same year or between individuals with the same breeding status tracked in different years. P-values > 0.05 indicate no significant spatial segregation between the two groups tested.

	50% UD			90% UD		
	<i>r<sub>obs</sub></i>	<i>r<sub>rand</sub></i>	p-value	<i>r<sub>obs</sub></i>	<i>r<sub>rand</sub></i>	p-value
<b>Success vs. failed in 2015</b>	-0.14	-0.08 ± 0.06	0.18	-0.14	-0.10 ± 0.04	0.22
<b>Success vs. failed in 2018</b>	-0.07	-0.09 ± 0.06	0.67	-0.03	-0.09 ± 0.04	0.92
<b>Success 2015 vs. success 2018</b>						
<b>Failed 2015 vs. failed 2018</b>	-0.20	-0.13 ± 0.05	0.08	-0.19	-0.17 ± 0.04	0.34

Table 5: Relative contribution (in %) of each environmental variable in individual distribution from 5am to 10pm based on resting and foraging locations obtained from a jackknife resampling. The most important variable for each group is indicated in bold.

Breeding status	Successful		Failed	
	Successful	Failed	Successful	Failed
<b>Year</b>	2015		2018	
<b>SST</b>	26.8	<b>30.1</b>	<b>40.5</b>	<b>32.9</b>
<b>CHLA</b>	18.5	28.5	20.8	30
<b>Bathym</b>	<b>48.4</b>	21.6	30.6	20.1
<b>Wind speed</b>	-	-	6.6	15.2
<b>gCHLA</b>	1.7	8.3	0.5	0.6
<b>gSST</b>	2.4	6.4	-	-
<b>EKE</b>	2.2	5.2	1	1.2

Figure 1: At-sea locations between 5am and 10pm associated with foraging and resting states for successful (left panel) and failed Indian Yellow-nosed albatross breeders (right panel) nesting on Amsterdam Island (black triangle) for 2015 (upper panel) and 2018 (lower panel). Tracks are superimposed with Sea Surface Temperature (in °C).

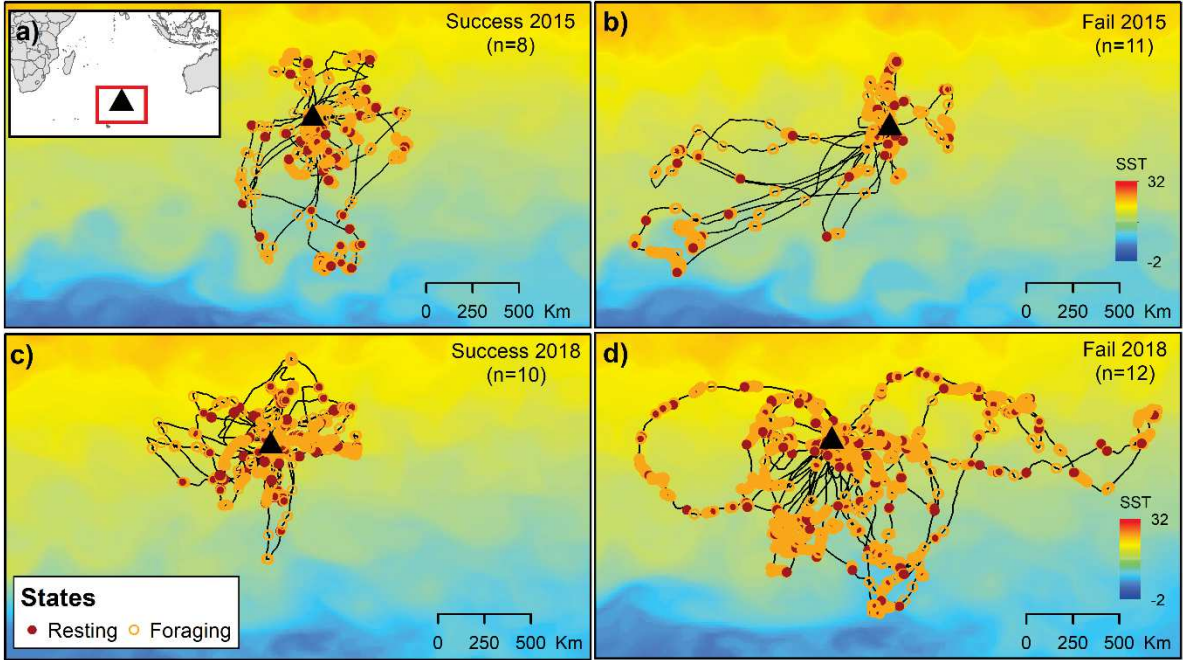


Figure 2: Boxplots of the trip characteristics of Indian yellow-nosed albatrosses by breeding status and year. Each dot represents a trip. A) Maximal distance to the colony, b) total distance travelled, c) trip duration and d) nest attendance.

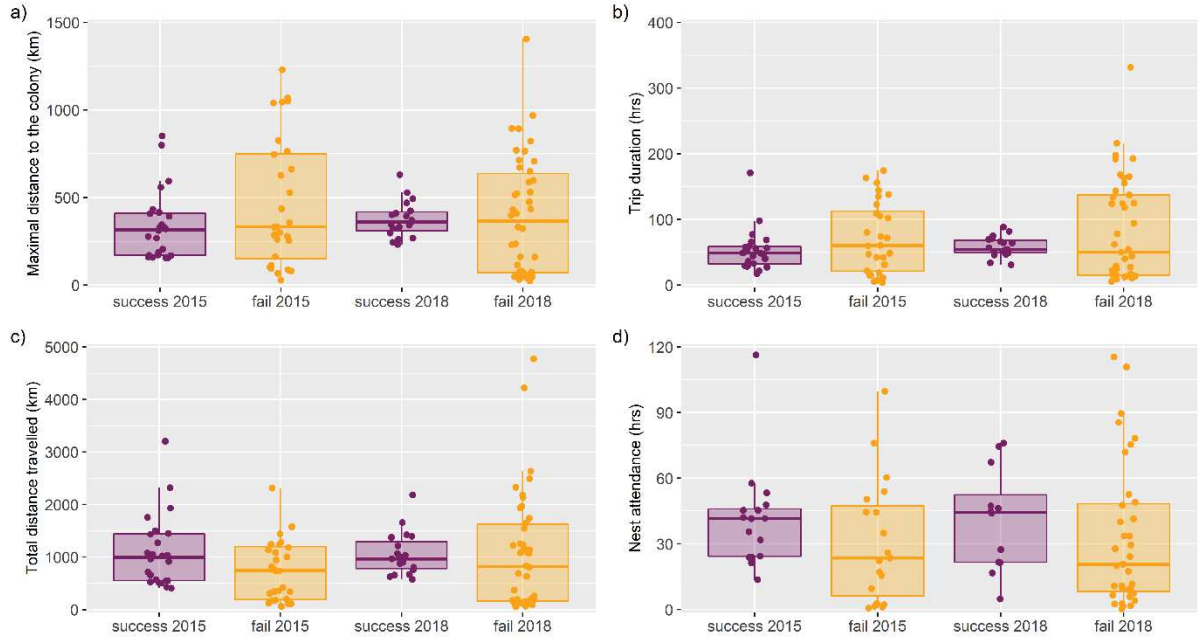


Figure 3: (a-b) Kernel uniform distribution of foraging and resting areas used between 5am and 10pm by successful (purple) and failed Indian yellow-nosed albatross breeders (orange) nesting on Amsterdam Island (black triangle) in 2015 and 2018. (c-d) Rose diagram showing the directions of foraging and resting locations between 5am and 10pm for successful and failed breeders in 2015 and 2018.

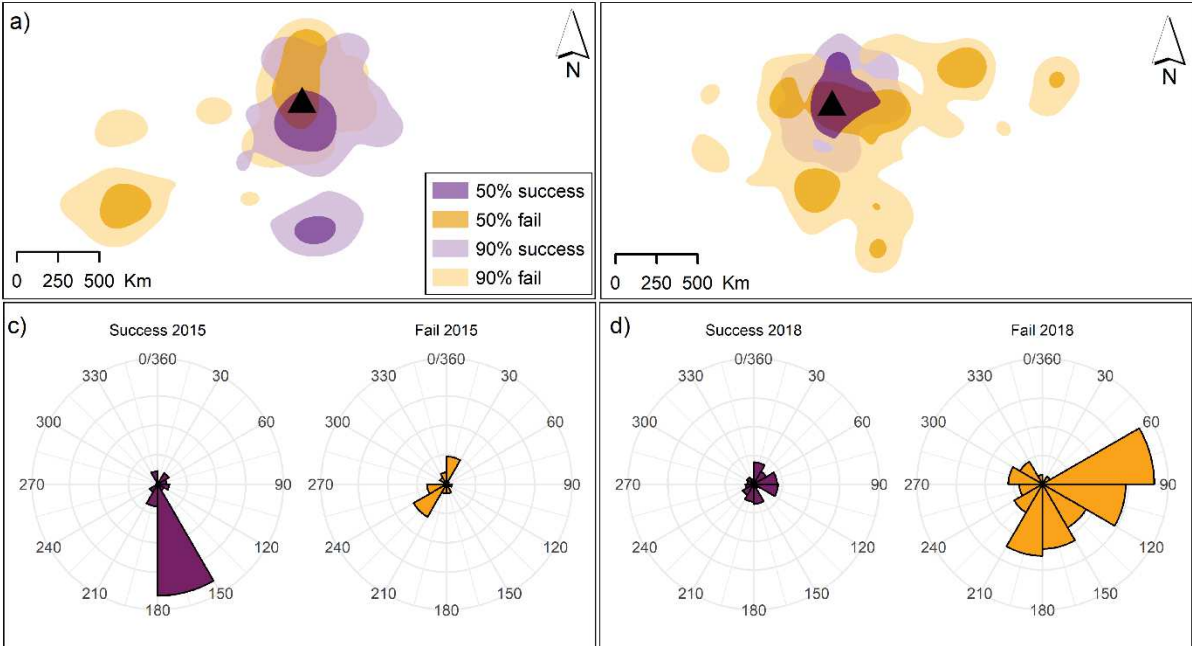




Figure 4: Response curves obtained from the habitat models for each environmental variable for successful (purple) and failed Indian yellow-nosed albatross breeders (orange) in 2015 (solid lines) and 2018 (dashed lines). Models were built from resting and foraging locations between 5am and 10pm. (a) Sea Surface Temperature SST in °C (b) Concentration of chlorophyll a CHLA in  $\text{g}\cdot\text{m}^{-3}$  (c) Bathymetry in m (d) SST gradient (e) CHLA gradient and (f) Eddy Kinetic Energy EKE in  $\text{m}\cdot\text{s}^{-1}$  (g) Wind speed in  $\text{m}\cdot\text{s}^{-1}$

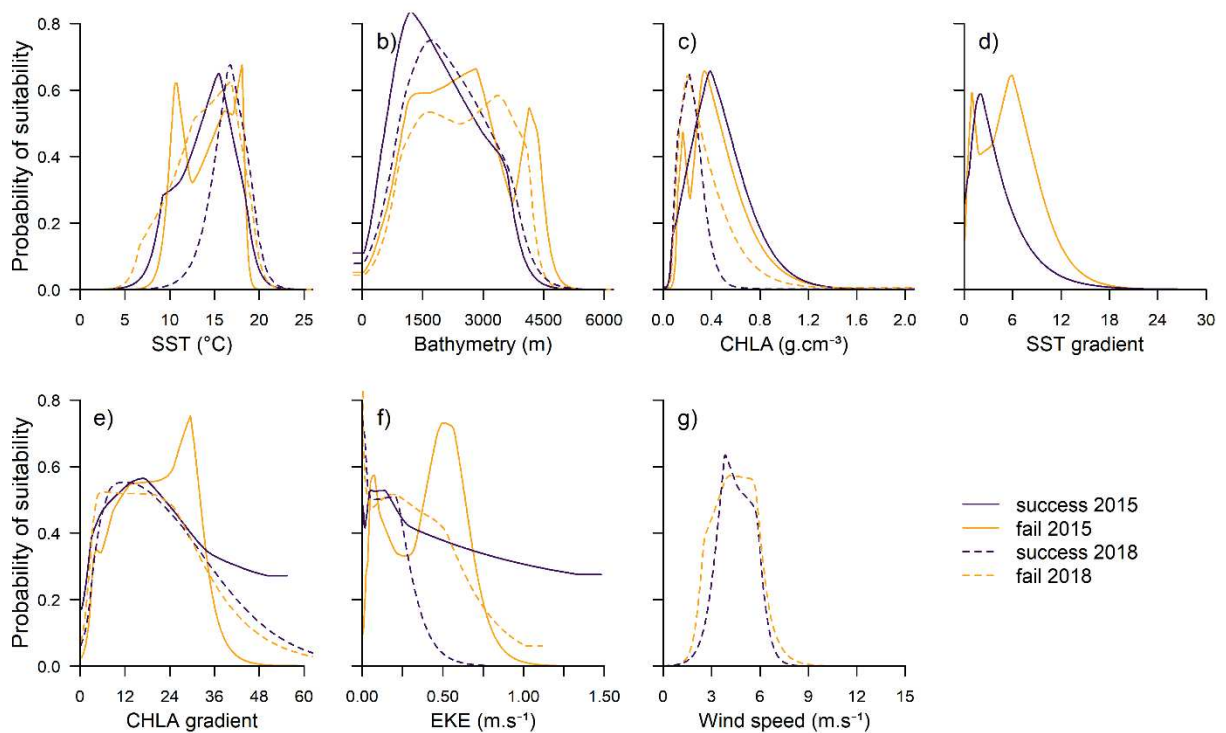
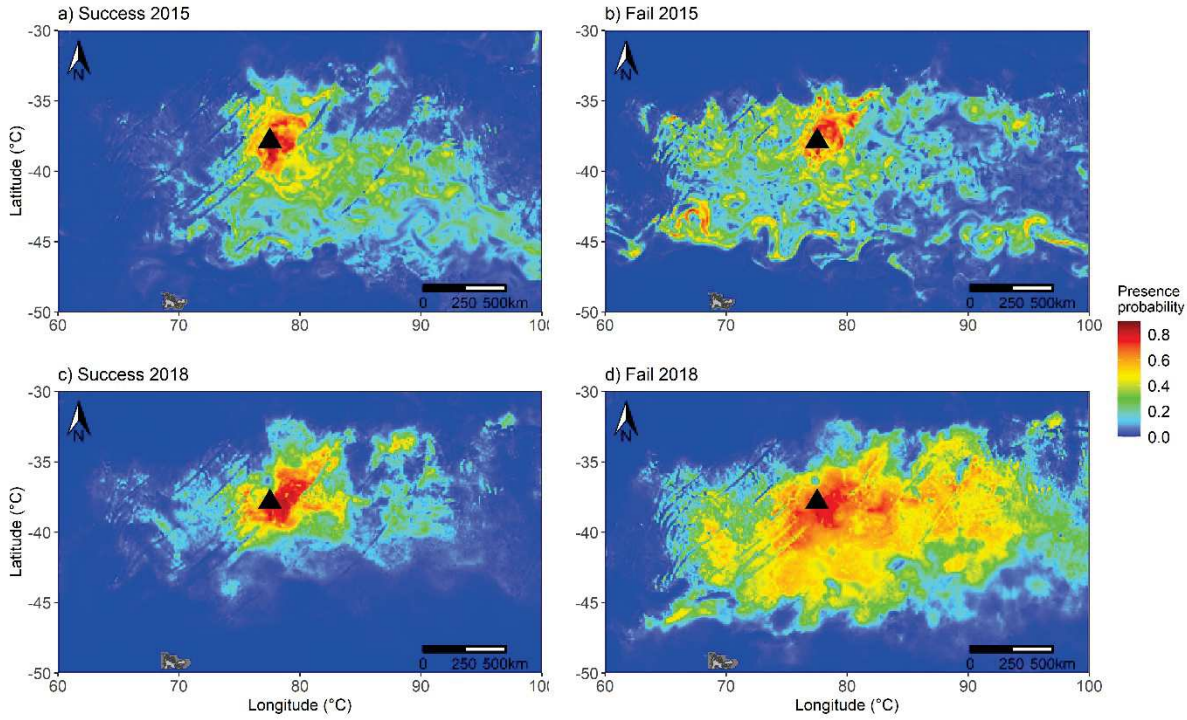
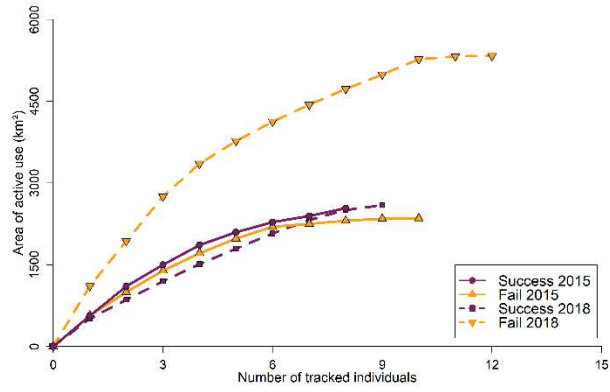


Figure 5: Predictions of foraging and resting habitat suitability obtained from the habitat models built for successful (left panel) and failed Indian yellow-nosed albatross breeders (right panel) in 2015 (upper panel) and 2018 (lower panel). The black triangle represents the nesting colony on Amsterdam Island.



Supplemental Material

**Figure S1:** Representativeness of the number of tracked individuals used to calculate UD kernels. This sample size is based on a dataset which contains locations recorded only between 5am and 10pm.



## Annexe 5

Predator and scavenger movements among and within endangered seabird colonies: Opportunities for pathogen spread.

Gamble A, Bazire R, Delord K, Barbraud C, Jaeger A, Gantelet H, Thibault E, Lebarbenchon C, Lagadec E, Tortosa P, Weimerskirch H., Thiebot J-B., Garnier R., **Tornos J.** & Boulinier T.

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# Predator and scavenger movements among and within endangered seabird colonies: Opportunities for pathogen spread

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## Abstract

1. The spatial structure of host communities is expected to constrain pathogen spread. However, predators and/or scavengers may connect distant host (sub) populations when foraging. Determining whether some individuals or populations play a prominent role in the spread of pathogens is critical to inform management measures.
2. We explored movements and epidemiological status of brown skuas *Stercorarius antarcticus*, the only avian terrestrial consumer native of Amsterdam Island (Indian Ocean), to assess whether and how they could be involved in the spread of the bacterium *Pasteurella multocida*, which recurrently causes avian cholera outbreaks in endangered albatross and penguin species breeding on the island.
3. High proportions of seropositive and DNA-positive individuals for *P. multocida* indicated that skuas are highly exposed to the pathogen and may be able to transmit it. Movement tracking revealed that the foraging ranges of breeding skuas largely overlap among individuals and expand all along the coasts where albatrosses and penguins nest, but not on the inland plateau hosting the endemic Amsterdam albatross *Diomedea amsterdamensis*.
4. Considering the epidemiological and movement data, skua movements may provide opportunity for pathogen spread among and within seabird colonies.
5. *Synthesis and applications.* This work highlights the importance of considering the behaviour and epidemiological status of predators and scavengers in disease dynamics because the foraging movements of individuals of such species can potentially limit the efficiency of local management measures in spatially structured host communities. Such species could thus represent priority vaccination targets to implement efficient management measures aiming at limiting pathogen spread and also be used as sentinels to monitor pathogen circulation and evaluate the effectiveness of management measures.

## KEYWORDS

conservation biology, disease ecology, dynamic space utilization, individual heterogeneity, movement ecology, *Pasteurella multocida*, sentinel species, serology

## 1. INTRODUCTION

Infectious diseases threaten populations of many endangered wild species and are now recognized as a main threat relevant to conservation biology (Young et al., 2017). The structure of contact networks among hosts of infectious agents can strongly impact epidemiological dynamics (Craft, Hawthorne, Packer, & Dobson, 2008; Strona, Carstens, Beck, & Han, 2018), hence extinction probabilities (Cleaveland et al., 2002). Some individuals or populations may occupy key positions in contact networks and contribute more than others to spreading infectious agents through their behaviour (Dougherty, Seidel, Carlson, Spiegel, & Getz, 2018; Lloyd-Smith, Schreiber, Schreiber, Kopp, & Getz, 2005; Paull et al., 2012), notably in multi-host systems (Craft et al., 2008). Such individuals or populations may thus constitute particularly relevant targets for disease control protocols aiming at interrupting transmission chains (Pepinet et al., 2016; Robinson et al., 2018; Rushmore et al., 2014).

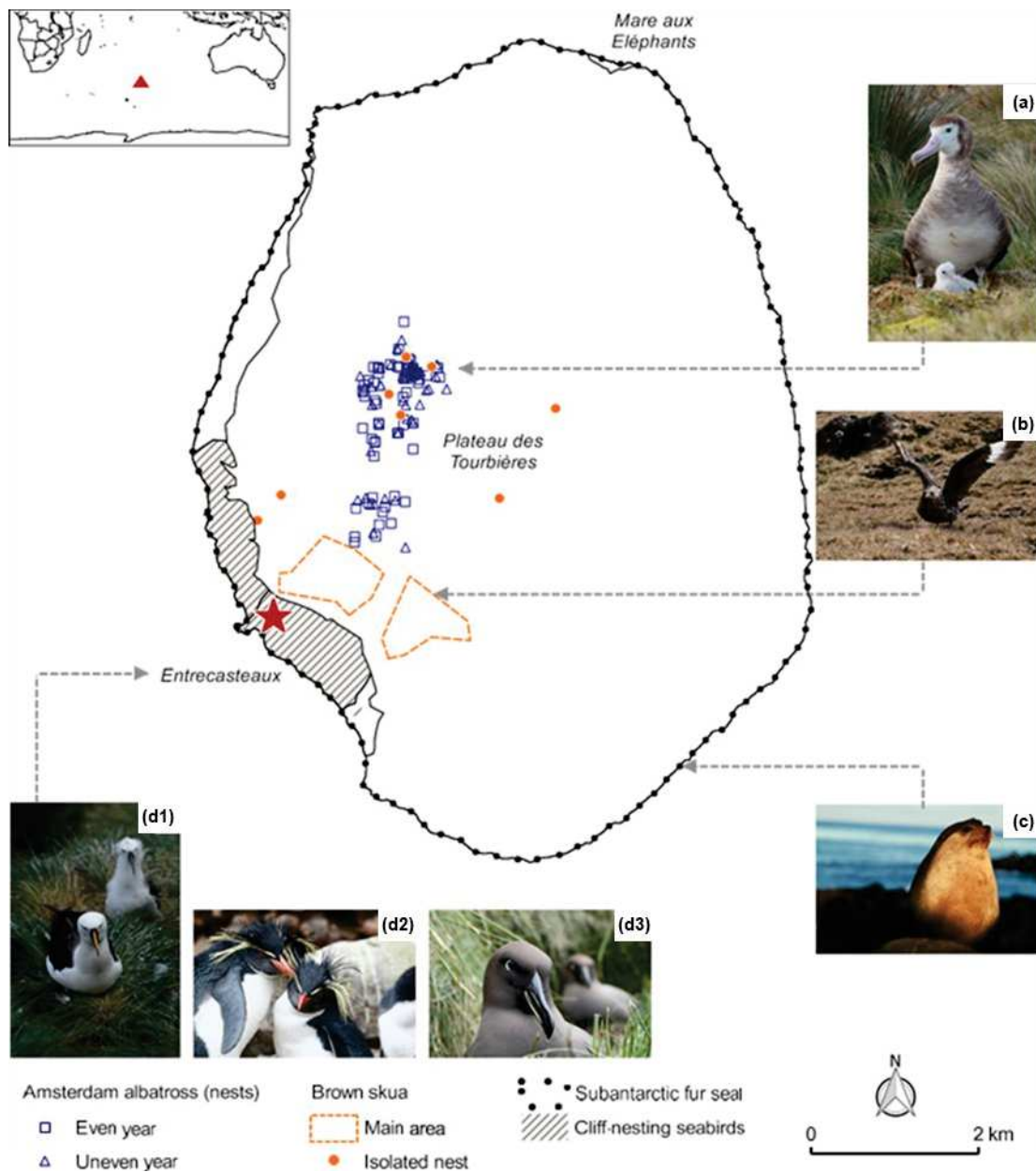
Terrestrial predators and scavengers may effectively connect otherwise isolated colonies or social groups when foraging, potentially contributing to pathogen spread with conservation (Craft et al., 2008), public health (Navarro et al., 2019) or fundamental implications (Boulinier et al., 2016). In wild communities subject to infectious disease outbreaks, deciphering the contact structure and examining the hosts' respective contributions in epidemiological dynamics are thus critical to develop control measures. The role of predator and/or scavenger species as potential spreaders of infectious agents is poorly documented because it requires targeted field efforts at the often unpredictable time of outbreaks (Daversa, Fenton, Dell, Garner, & Manica, 2017). For instance, the role of scavengers has been suspected in avian cholera, caused by the bacterium *Pasteurella multocida* (*Pm*), outbreaks (Wille et al., 2016), but their actual contribution as spreaders still requires proper examination.

The recurrent outbreaks of avian cholera in seabirds on remote Amsterdam Island (Indian Ocean, 37°49'S, 77°33'E; Jaeger et al., 2018; Weimerskirch, 2004) provide a unique opportunity to better understand the potential epidemiological role of a predator and scavenger species within a relatively simple host community (Figure 1). Avian cholera is a widespread disease severely threatening the viability of several avian populations (Descamps, Jenouvrier, Gilchrist, & Forbes, 2012; Samuel, Takekawa, Baranyuk, & Orthmeyer, 1999). On Amsterdam Island, Indian yellow-nosed albatrosses *Thalassarche carteri* have been recurrently hit by avian cholera outbreaks since the mid-eighties, potentially following the introduction of the pathogen through past human activities, such as animal farming, or accidental rodent introduction from visiting ships (Jaeger et al., 2018; Micol & Jouventin, 1995). Infection by *Pm* causes septicaemia in nestlings, leading to their rapid death (Bourret et al.,



2018; Jaeger et al., 2018) with important consequences on the breeding success of the local yellow-nosed albatross population. For instance, on Gough Island (which is rodent infested but considered avian cholera-free), the breeding success of the Atlantic yellow-nosed albatross *Thalassarche chlororhynchos* is as high as 70% (Cuthbert, Ryan, Cooper, & Hilton, 2003), while it has been below 10% most of the past twenty years on Amsterdam Island for the Indian yellow-nosed albatross (Jaeger et al., 2018). This example and others (e.g. Sebastiano, Eens, Pineau, Chastel, & Costantini, 2019) illustrate that infectious diseases can represent an important, but often neglected, threat to seabird population viability. Avian cholera outbreaks on Amsterdam Island not only affect this globally significant yellow-nosed albatross population (Weimerskirch, 2004), but are also suspected to cause mortality in two other endangered species: the sooty albatross *Phoebastria fusca* and the northern rockhopper penguin *Eudyptes moseleyi* (Jaeger et al., 2018).

Despite the strong impact of avian cholera outbreaks on the endangered seabirds of Amsterdam Island (Jaeger et al., 2018) and the availability of a vaccine proven to protect albatross nestlings (Bourret et al., 2018), no control measure has been implemented besides basic biosecurity measures. Indeed, relying on vaccination of nestlings to maintain the local yellow-nosed albatross population to its current size (~22,000 pairs; Heerah et al., 2019) would require to vaccinate thousands of nestlings every year and may thus not represent an efficient conservation strategy. In contrast, interrupting transmission chains by treating individuals responsible for the among-colony circulation of the bacterium may help to protect some colonies with minimal efforts. Since *Pm* transmission occurs primarily through an oro-faecal route (Samuel, Shadduck, Goldberg, & Johnson, 2003), it is unclear how *Pm* circulates locally. Indeed, seabirds on Amsterdam Island breed in spatially structured, mostly mono-specific, colonies (Figure S1.A.1) and albatrosses and penguins forage exclusively at sea (Heerah et al., 2019). Hence, although contacts may be frequent within dense seabird colonies, contacts between individuals from different colonies may thus be rare besides potential limited prospecting movements (Boulinier et al., 2016).



**Figure 1:** Breeding sites of colonial vertebrates on Amsterdam Island (37°49'S, 77°33'E). Amsterdam albatrosses (a) nest on the northern part of the inland plateau; brown skuas (b) mostly nest on the southern part on the inland plateau; subantarctic fur seals (c) breed all around the island; Indian yellow-nosed albatrosses (d1), northern rockhopper penguins (d2) and sooty albatrosses (d3) nest in the high cliffs laying south-west of the island. The red star denotes the monitored yellow-nosed albatross subcolony. Photos: Romain Bazire, IPEV. Map background: Réserve Naturelle Nationale des Terres Australes Françaises

Terrestrial predators and scavengers could, however, spread *Pm* across the island while foraging among seabird colonies undergoing outbreaks. Notably, the small population of brown skuas *Stercorarius antarcticus*, the sole terrestrial vertebrate predators and scavengers native to the island, could disseminate the bacterium when foraging through shedding and/or by moving infected albatrosses

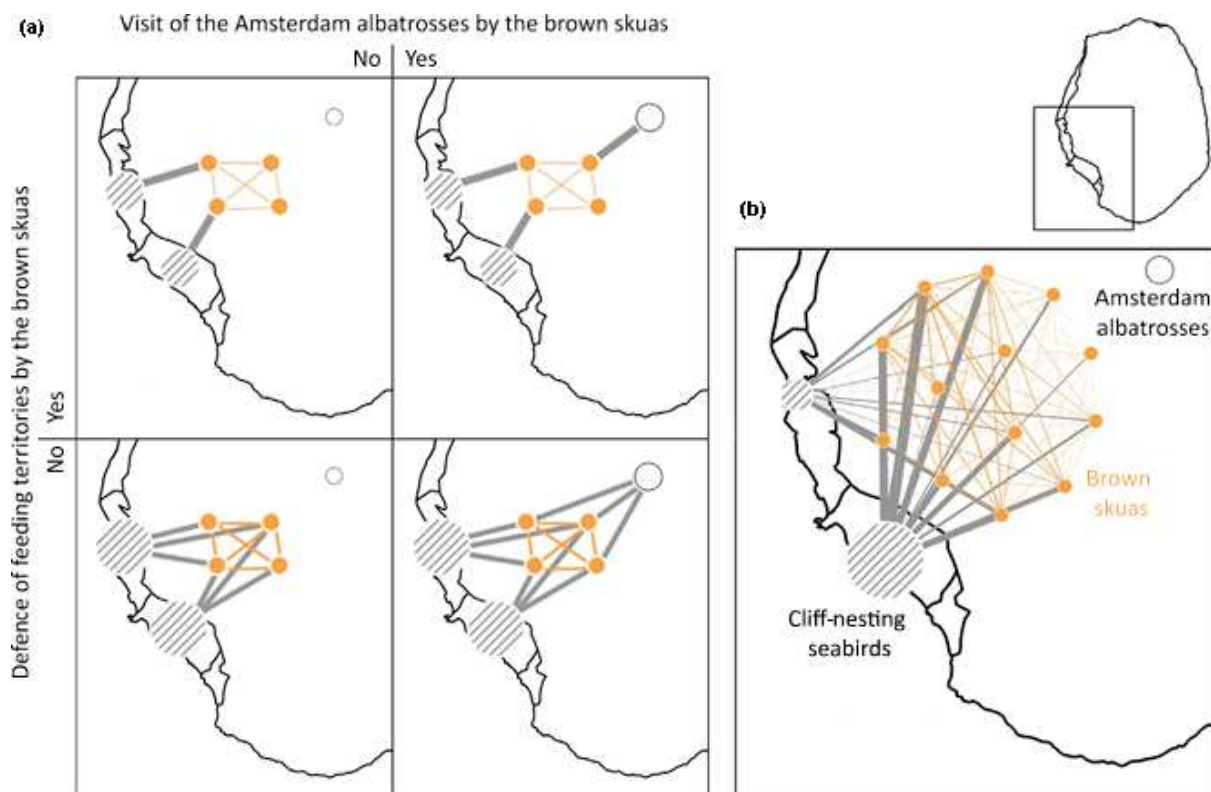
and penguins (Pietz, 1987). Introduced brown rats *Rattus norvegicus* and house mice *Mus musculus* (Micol & Jouventin, 1995) could also carry and shed the bacterium, notably as they prey and scavenge on seabird chicks (Figure S1.A.2; Thiebot, Barbraud, Delord, Marteau, & Weimerskirch, 2014), although the distribution of resources and geographical barriers created by the island relief probably constrain the spatial scale of their contribution. In addition, the endemic Amsterdam albatross *Diomedea amsterdamensis* could also be at risk of exposure to *Pm* via foraging skuas because it nests a few kilometres inland from the yellow-nosed albatross colonies, although nestling die-offs have seldom been recorded in this species (Jaeger et al., 2018).

Considering the wide range of feeding habits of skuas and the potential heterogeneity of foraging strategies among individuals (Furness, 1987), some individuals could play a prominent role in the circulation of infectious agents. In the skua population of Amsterdam Island, we examined the extent to which the individuals (a) are exposed to *Pm*, (b) forage on coastal versus inland areas and (c) exploit exclusive feeding territories within the seabird colonies. We expected the skuas to forage mostly on the coasts where prey species breed in dense colonies (Figure 2a, scenarios 1 and 3), maximizing feeding opportunities (Figure S1.A.2a), but also exposure risks to *Pm*. Further, we expected skuas to hold exclusive individual feeding territories (Figure 2a, scenarios 1 and 2; Pietz, 1987; Trivelpiece, Butler, & Volkman, 1980; Votier, Bearhop, Ratcliffe, & Furness, 2004), with restricted movements of some individuals potentially inducing heterogeneity in exposure to *Pm* and limited contacts among skuas (outside pairs, contacts are expected to occur mostly on foraging sites and clubs, i.e. sites where individuals gather outside breeding territories; Klomp & Furness, 1990). Testing these hypotheses should bring new insights on pathogen circulation in spatially structured host communities and help managers to design and implement efficient disease control protocols.

## 2. MATERIALS AND METHODS

### 2.1 Study population

Around 60 pairs of brown skuas breed at low density yearly on Amsterdam Island's 'Plateau des Tourbières' (PDT; Figure 1). Breeders generally lay two eggs in October/November, and nestlings hatch in late November/early December and fledge 40–50 days later. Skuas attend clubs notably in the north ('Mare aux Éléphants' [MAE]) and the south-west (bottom of 'Entrecasteaux' cliffs). Amsterdam Island holds important populations of Indian yellow-nosed albatrosses, sooty albatrosses (~400 pairs; Heerah et al., 2019) and northern rockhopper penguins (~12,000 pairs), all breeding in mostly monospecific



**Figure 2:** Hypothetical (a) and observed (b) epidemiological networks linking seabirds on Amsterdam Island based on breeding brown skua movements. Each orange node represents an individual skua. The hatched and white nodes represent grouped subpopulations of cliff-nesting birds (i.e. yellow-nosed albatrosses, rockhopper penguins and sooty albatrosses) and Amsterdam albatrosses, respectively.

Edge widths between skuas and other species represent the relative time spent in each potential foraging area. Panel b was built based on data collected on skuas breeding in the southern part of PDT and presented in Figure 4. Edge widths between two skuas represent the probability of two individuals being present in the same potential foraging area at the same time (see Appendix S1.F for calculation details)

colonies on the south-west coastal cliffs between August and May (Figure 1). The 40–50 pairs of Amsterdam albatrosses nest biennially further inland, on the northern part of the PDT (Figure 1). A few small *Procellariiformes* are also present on the island in very low numbers (Micol & Jouventin, 1995). All around the island, subantarctic fur seals *Arctocephalus tropicalis* calve on the beaches in December (Guinet, Jouventin, & Georges, 1994), also providing food opportunities for skuas (placentas and dead pups). Introduced brown rats and house mice are often observed in seabird colonies (Figure S1.A.2). Feral cats *Felis catus* were also introduced on the island (Micol & Jouventin, 1995), but are rarely observed in seabird colonies.

## 2.2 Field sampling

During three breeding seasons (2011–2012, 2015–2016 and 2016–2017), blood samples (1 ml from

the metatarsal vein using heparinized syringes) and cloacal swabs (using sterile cotton tips) were collected from 66 adults and 9 nestlings of brown skuas captured on their nests in the southern part of PDT or in clubs at Entrecasteaux and MAE between November and January (Table 1). Breeders handled in 2015–2016 and breeders and club attendants handled in 2016–2017 were marked with leg rings for individual identification. Red blood cells and plasma were separated by centrifugation a few hours after collection. Swabs were stored in 0.5 ml of a lysis buffer (RNA NOW<sup>®</sup>, BIOGENTEX, USA, in 2011–2012; Longmire buffer the following years; Longmire et al., 1988). Samples were kept at –20°C in the field, and then stored at –20°C (plasma) or –80°C (swabs) until analysis.

**Table 1:** Proportions of brown skuas from Amsterdam Island seropositive according to MAT and ELISA and excreting *Pm* DNA according to PCR or RT-PCR from cloacal swabs. Clopper-Pearson 95% confidence intervals are indicated between brackets and numbers of positive/tested individuals between parentheses.

Site	Stage	2011-2012			2015-2016			2016-2017		
		MAT	ELISA	PCR	MAT	ELISA	RT-PCR	MAT	ELISA	RT-PCR
Plateau des Tourbières	Breeding adults	1.00	1.00	0.06	1.00	1.00	0.10	1.00	1.00	0.80
		[0.79;1.00]	[0.79;1.00]	[0.00;0.30]	[0.69;1.00]	[0.69;1.00]	[0.00;0.45]	[0.69;1.00]	[0.69;1.00]	[0.44;0.97]
	(16/16)	(16/16)	(1/16)	(10/10)	(10/10)	(1/10)	(10/10)	(10/10)	(8/10)	
	Nestlings	0.00	0.00	0.00	-	-	-	0.17	0.17	0.50
		[0.00;0.71]	[0.04;0.78]	[0.00;0.31]	-	-	-	[0.00;0.64]	[0.00;0.64]	[0.12;0.88]
		(0/3)	(0/3)	(0/10)				(1/6)	(1/6)	(3/6)
Entrecasteaux	Club attendants	-	-	-	1.00	1.00	0.33	1.00	1.00	0.20
					[0.69;1.00]	[0.69;1.00]	[0.07;0.65]	[0.69;1.00]	[0.69;1.00]	[0.03;0.56]
					(10/10)	(10/10)	(3/10)	(10/10)	(10/10)	(2/10)
Mare aux Éléphants	Club attendants	-	-	-	-	-	-	0.80	0.75	0.00
								[0.44;0.97]	[0.35;0.97]	[0.00;0.31]
								(8/10)	(6/8)	(0/10)

### 2.3 Immunological assays

*Pm*-specific antibody levels in plasma samples of skuas were measured using two immunoassays in order to ascertain past exposure to *Pm*: an enzyme-linked immunosorbent assay (ELISA; ID Screen<sup>®</sup> *Pasteurella multocida* Chicken and Turkey Indirect, IDvet, France; with the positivity threshold determined following Garnier et al., 2017) and a microagglutination test (MAT; SEROPAST<sup>®</sup>, Ceva Biovac, France). Technical details are given in Appendix S1.B.

## 2.4 Molecular detection of *Pm*

Total nucleic acids were extracted following the RNA NOW<sup>®</sup> isolation and purification protocol for skua cloacal swabs preserved in RNA NOW<sup>®</sup>, and with the QIAamp cadof Pathogen Mini<sup>®</sup> Kit (QIAGEN) for skua cloacal swabs preserved in Longmire lysis buffer. *Pm* DNA was detected with a real-time polymerase chain reaction (RT-PCR) targeting the strain previously detected in a dead sooty albatross on Amsterdam Island (Jaeger et al., 2019). Technical details are given in Appendix S1.B.

## 2.5 Movement tracking

In 2015–2016 and 2016–2017, 18 breeding skuas captured on the southern PDT were also equipped with solar-powered GPS-UHF devices with a remote data download link (GPS-UHF Harrier-L<sup>®</sup>, Ecotone). The GPS acquisition frequency was set at 2 to 5 min, and altitude above sea level was also recorded in 2015–2016 (see Appendix S1.C for more details). Loggers were deployed during the early chick rearing period (late November/early December), when skuas' energetic needs are at their highest (Furness, 1987) and avian cholera outbreaks occur in albatross colonies (Bourret et al., 2018; Weimerskirch, 2004). Sufficient data for spatial analysis (>24 hr) were collected from 13 individuals, with data spanning 12–102 days (median [25 and 75% quartiles] = 54 [47; 85]; Table S1.C.1), covering most of the chick-rearing period for the majority of the tracked individuals. A bootstrap analysis estimated this sample to adequately represent space use of the skua population breeding on southern PDT during this period (Lascelles et al., 2016; see Appendix S1.C and Figure S1.C.1).

Using the GPS data, the intensity (spatial distribution of the mean residence time per visit of each area, i.e. areas where an individual tends to spend more time) and recursion (spatial distribution of the number of visits in each area, i.e. areas that an individual tends to repeatedly visit) distributions were computed to get a dynamic picture of space utilization (Benhamou & Riotte-Lambert, 2012). The overlap of the patches recursively used by different individuals was visually examined to inform on the existence of exclusive feeding territories in this population. To estimate the intensity and recursion distributions, non-overlapping virtual circles of 50 m in radius (referred to as 'areas') were delimited. A new visit to an area occurred each time the tracked individual re-entered it after a time-lag spent outside the area longer than 10 min and remained in that area for at least 15 min (to exclude cases in which birds went through an area without using it) using the BRB|MKDE program (for Biased Random Bridges for Movement based Kernel Density Estimation; Benhamou, 2011). All data were analysed and represented after exclusion of locations recorded within the nesting area of the tracked individuals unless indicated otherwise. Resulting distributions were visualized in QGIS 2.18.21.

## 2.6 Monitoring of yellow-nosed albatrosses

In order to assess whether skuas were present in coastal seabird colonies during avian cholera epizootics, the proportions of yellow-nosed albatross nestlings surviving and excreting *Pm* DNA in a subcolony of Entrecasteaux were used as a proxy for the progress of the epizootics. *Pm* DNA excretion was assessed using the same design used in brown skuas. Details and sample sizes are given in Appendix S1.D.

## 2.7 Statistical analyses

All statistical analyses were run using R 3.6.1 and the script available in Appendix S3. Seroprevalences were calculated as the proportion of seropositive individuals among tested individuals each year. Differences in antibody levels among locations within a year or among years in a location were investigated using Wilcoxon tests with a Bonferroni correction and a 5% significance level.

# 3. RESULTS

## 3.1 Detection of *Pm* and anti-*Pm* antibodies

Specific antibodies were detected in all but two plasma samples from adult brown skuas by both MAT and ELISA, resulting in seroprevalence varying between 80% and 100% depending on the site and year (Table 1). In 2016–2017, based on the ELISA results, antibody levels were significantly lower in MAE than in Entrecasteaux and PDT (both  $p < .01$ ; Table S1.B.1). All other pairwise comparisons were not statistically different (Figure 3). The same results were obtained from the MAT data. *Pm* DNA was detected in cloacal swabs of adults from PDT and Entrecasteaux, but not from MAE (Table 1). The proportion of *Pm* positive samples was generally low ( $\leq 33\%$ ), except for PDT in 2016–2017 with 8/10 positive individuals. Regarding nestlings, neither specific antibodies nor *Pm* DNA was detected in samples from 2011 to 2012. In 2016–2017, *Pm* DNA was detected in three seronegative nestlings; one other nestling was seropositive but not excreting *Pm* DNA (Table 1; Figure S1.E.1).

## 3.2 Individual movement tracking

Both years, breeding skuas moved along the western coast (south to north-east) of the island, where fur seals, albatrosses and penguins breed (Figure 4). Some individuals visited the whole western coast within 24 hr (Figure S1.C.3, Appendix S2). Breeding skuas visited the surroundings of Entrecasteaux and MAE clubs. When travelling along the cliffs, skuas exploited a large altitudinal gradient, suggesting that they potentially visited fur seals, penguins and albatross colonies (Figures S1.C.2, S1.C.3).

However, none of the tracked individuals visited the Amsterdam albatross breeding area.

Recursively exploited areas largely overlapped among the tracked individuals (Figure 5; Figure S1.C.4).

Entrecasteaux cliffs were intensively and recursively exploited by all the tracked individuals

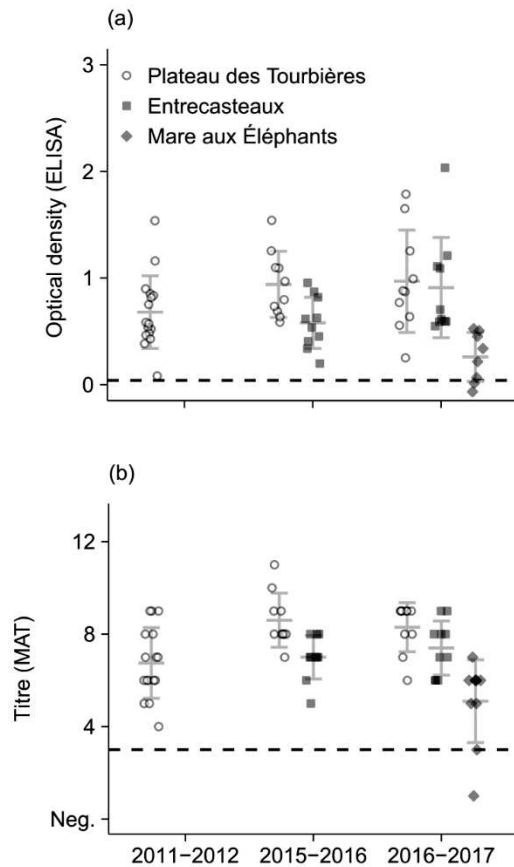


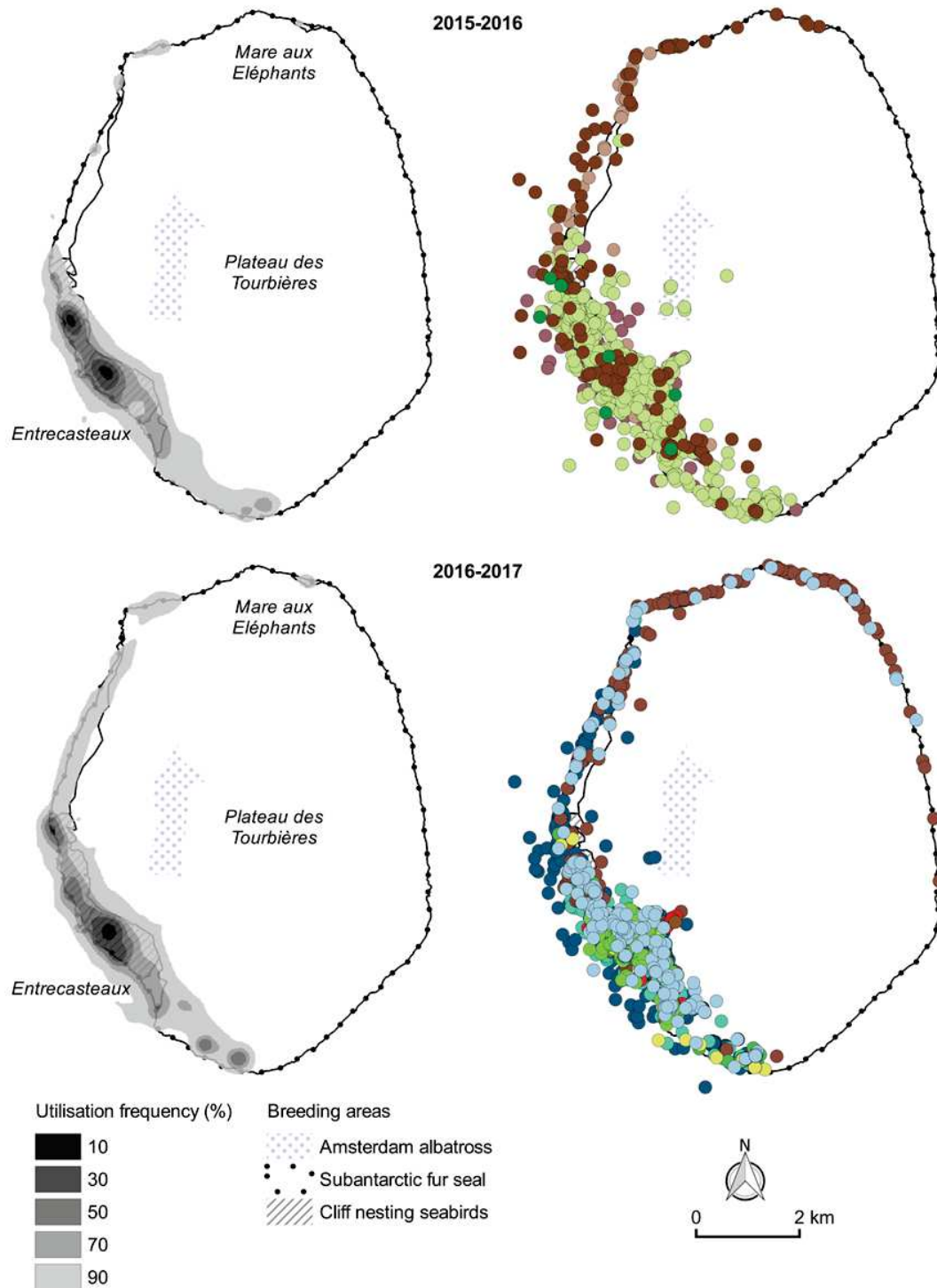
Figure 3: Evidence of high exposure of brown skuas to *Pasteurella multocida*: anti-Pm antibody levels for skuas measured by ELISA (a) and MAT (b) over three years on Plateau des Tourbières (the main skua breeding ground on Amsterdam Island), and two clubs (Entrecasteaux and Mare aux Éléphants). Mean  $\pm$  standard deviation are shown in grey. The dashed lines represent the seropositivity thresholds. For ease of visualization, data points were horizontally jittered

(Figure 5, Figures S1.C.4, S1.C.5, Table S1.C.2), notably during avian cholera outbreaks (Figure S1.D.1), and represented the only recursively visited site for four of them. Other sites recursively visited included the northern part of the south-west cliff, harbouring large seabird and fur seal colonies, the north coast, hosting another large fur seal colony, and the south plain where seabirds and fur seals are rare, but rodent populations may be present. Note that all the tracked individuals were seropositive and Pm DNA was detected in 8/13 birds (Table S1.C.2) at the time of logger deployment.

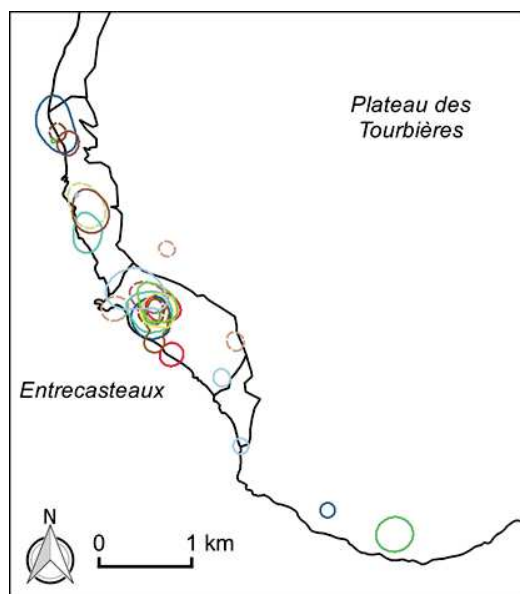


#### **4. DISCUSSION**

Here, we explored different scenarios of foraging strategies of a predator and scavenger, the brown skua, in relation to the circulation of a pathogen within a spatially structured community of endangered seabirds. Movement data suggest that skuas may interconnect several seabird colonies during avian cholera epizootics, potentially contributing to pathogen spread. Our study allowed to characterize biological processes relevant to pathogen circulation, such as the fact that skuas do not hold foraging territories, a necessary first step to build mechanistic models of eco-epidemiological dynamics and explore potential control measures (Daversa et al., 2017; Dougherty et al., 2018).



**Figure 4:** Space utilization by breeding brown skuas. Left: distribution of space utilization outside of their breeding area by all individuals equipped with GPS-UHF loggers during the chick-rearing period on Amsterdam Island in 2016–2017 and 2015–2016. Right: corresponding unfiltered raw locations; each colour represents an individual



**Figure 5:** Recursive space utilization by breeding brown skuas equipped with GPS-UHF loggers during the chick-rearing period on Amsterdam Island. The contours correspond to the individual 50% recursion distribution isopleths; each colour represents an individual with dashed and solid lines for individuals tracked in 2015–2016 and 2016–2017, respectively. See Figure S1.C.4 for individual data

#### 4.1 Skua exposure and infection by *Pm* and implications for epidemiological surveillance

We report the first detection of anti-*Pm* antibodies in skuas and show that the population of brown skuas from Amsterdam Island is highly exposed to this bacterium. The proximity of Entrecasteaux cliffs, where skuas may be exposed to *Pm* through feeding on infected albatrosses and penguins (Figure S1.A.2a), may intensify exposure and explain the high specific antibody levels measured in individuals sampled at the Entrecasteaux club or breeding on PDT (Pepin et al., 2017). Because detailed antibody kinetics are not available, it is not possible to estimate the timing of infection from serological data. Nevertheless, the detection of PCR-positive birds at Entrecasteaux club or breeding on PDT reveals ongoing infection at the time of sampling. In contrast, skuas attending the MAE club may forage preferentially on nearby fur seal colonies, potentially minimizing their exposure to *Pm*, which would explain their lower antibody levels and the absence of PCR-positive birds at this site. More generally, the important proportion of seropositive but PCR-negative adult skuas suggests that specific antibodies may persist past the infection period, as commonly observed in acute infections (Pepinet al., 2017). Jaeger et al. (2018) reported the circulation of a unique *Pm* strain among sooty and yellow-nosed albatrosses, but technical constraints for field microbiology (e.g. low availability of fresh carcasses, difficulty to obtain bacterial isolates from non-invasive approaches) limited the sample sizes. While it is not possible to confirm that the epizootic *Pm* strain killing albatrosses is circulating in skuas, our current understanding of the system supports the circulation of a common *Pm* strain circulating in the different species of the island. Future works should aim at sequencing more *Pm* isolates from different species of the island to

confirm this hypothesis.

Interestingly, detection of specific antibodies in the majority of PCR-negative adults also suggests that a large proportion may survive *Pm* exposure, contrasting with the high mortality rate reported during previous outbreaks in Antarctica (Leotta, Chinen, Vigo, Pecoraro, & Rivas, 2006; Parmelee, Maxson, & Bernstein, 1979). This heterogeneity is not surprising given the known variability of *Pm* virulence in different hosts (Christensen & Bisgaard, 2000). Moreover, the low mortality rate of adult skuas on Amsterdam Island is similar to that of adult yellow-nosed albatrosses on that island (Gamble, Garnier, et al., 2019; Rolland, Barbraud, & Weimerskirch, 2009), but the higher seroprevalence in skuas suggests different rates of exposure and/or persistence of antibody levels. If skuas are highly exposed but resistant or tolerant to infection by *Pm*, they could also be involved in the maintenance of the pathogen on the island. This result also shows that skuas are potentially good sentinels to detect the circulation of *Pm*, especially using serology (Halliday et al., 2007). Hence, implementing long-term serosurveys targeting such species can be especially useful for the acquisition of baseline data on (pathogenic and non-pathogenic) infectious agent circulation and for the early detection of pathogen circulation. Indeed, in long-lived species such as seabirds, monitoring protocols based only on breeding pair counts can take several years before capturing juvenile mortality events because of late recruitment.

#### **4.2 Movements and disease transmission in structured communities**

Apex predator movements have been well studied in relation to foraging during the breeding season, but their implications for infectious agent circulation have only recently been considered (Boulinier et al., 2016; de Souza Petersen et al., 2017). As observed elsewhere (Carneiro, Manica, & Phillips, 2014; Pietz, 1987), skuas breeding on Amsterdam Island foraged almost exclusively onshore, mostly along the coastal cliffs harbouring dense seabird colonies with high feeding opportunities. This population may thus regularly be in contact with yellow-nosed and sooty albatrosses, rockhopper penguins and subantarctic fur seals during avian cholera outbreaks. Interestingly, several individuals recursively used different patches along the western coasts, corresponding to different seabird and fur seal colonies: this may reflect particular opportunities for the dissemination of infectious agents. In addition, as opposed to other populations (Trivelpiece et al., 1980; Pietz, 1987; Votier et al., 2004; but see Carneiro et al., 2014), breeding skuas on Amsterdam Island did not seem to hold individual-exclusive feeding territories, as supported by the large space utilization overlap among individuals. The high food availability in large breeding colonies of vertebrates may explain the absence of such territories on Amsterdam Island. Accordingly, the absence of individual-exclusive feeding territories associated with

high seroprevalences suggests that, if breeding individuals contribute to *Pm* circulation on the island, their role could be rather homogeneous (Figure 2), contrary to other systems (Dougherty et al., 2018; Marchand et al., 2017). However, other possible sources of heterogeneity among individuals remain to be explored, such as variations in the duration or intensity of shedding.

We did not observe breeding skuas foraging in the small and low-density breeding population of the Amsterdam albatross, suggesting that they may not connect this population to others (Figure 2b). This is consistent with the high breeding success of this albatross population over the last decades (Jaeger et al., 2018). Although a few skua pairs nest among Amsterdam albatrosses (Figure 1), contacts between the two species may be rare if skuas forage only on the coast, as movements around the nests are usually limited. However, considering the flexibility of skuas' foraging behaviour and potential behavioural differences between breeders and non-breeders, some individuals may occasionally visit this colony, especially if environmental conditions and food availability change (Carneiro, Manica, Trivelpiece, & Phillips, 2015). Such changes, modifying the host space utilization, can have important consequences on the dynamics of infectious agents (Giles et al., 2018; Merkle et al., 2017). Long-term monitoring of these dynamics is essential to better understand pathogen circulation and design robust management options.

#### **4.3 Maintenance and circulation of infectious agent**

The population of brown skuas may play a key role in the circulation of *Pm* on Amsterdam Island, but the complete maintenance community (i.e. the set of connected host populations that together can maintain the pathogen over the long term, notably in winter when most seabirds are absent of the island; Viana et al., 2014) remains to be functionally characterized. Rodents may be good candidates as local maintenance hosts of *Pm*, but little data are yet available to examine this hypothesis. Rodents can reach high densities and are present year-round; they may feed on dead seabird nestlings and are preyed upon by skuas (Figure S1.A.2b,d). In addition, as observed in poultry (Curtis, 1983), rodents may directly transmit *Pm* through movements within colonies and biting of live nestlings (Thiebot et al., 2014; Figure S1.A.2c). Hence, spatio-temporal aspects of rodent exposure to *Pm* and subsequent shedding need to be explored from an eco-epidemiological standpoint (Guzzetta et al., 2017; Lloyd-Smith, Cross, et al., 2005). Because the home range radius of rodents is likely much smaller than that of skuas, rodents could be important in the within-colony spreading of *Pm*, while skuas may play a key role at a larger spatial scale. These elements suggest potentially complex epidemiological networks involving several bridge hosts (Caron, Cappelle, Cumming, Garine-Wichatitsky, & Gaidet, 2015) linking cliff-nesting seabirds to Amsterdam albatrosses. Skuas could move *Pm* from the coastal cliffs to PDT, where a few breed in

proximity to Amsterdam albatrosses. Foraging rodents could then move the bacterium from those skuas to the Amsterdam albatrosses. More generally, this stresses the importance of considering processes occurring at nested spatial scales and epidemiological networks in their entirety when exploring chains of transmission (Boulinier et al., 2016; Daversa et al., 2017). In the case of Amsterdam Island, future work should examine the role of introduced rodents in the maintenance and small-scale circulation of avian pathogens.

#### **4.4 Implications for conservation**

Our study revealed that the movements of potential bridge hosts (here, skuas and potentially rodents) may reduce the efficiency of local actions aiming at controlling multi-host infectious agents. For instance, locally intensive vaccination programmes targeting albatrosses (e.g. Bourret et al., 2018; Gamble, Garnier, et al., 2019) combined to rodent population control would seem feasible in accessible seabird colonies. However, such attempts would likely fail to control the pathogen because, although the system appears highly spatially structured, skuas could re-introduce the pathogen from inaccessible, hence non-treated, seabird colonies. In such a system, efficient disease control measures would likely need to interrupt the transmission network by targeting bridge hosts by, for instance, vaccinating native terrestrial predators (here skuas) with a vaccine blocking transmission and eradicating introduced rodents. However, we do not recommend culling native terrestrial predators as there are growing evidences that scavenging contribute in disease controls (e.g. Le Sage, Towey, & Brunner, 2019) and the culling of a native species would raise strong ethical issues. In the case of avian cholera on Amsterdam Island, an autogenous vaccine has proven efficient to protect yellow-nosed albatross nestlings (Bourret et al., 2018), but whether it blocks transmission remains to be investigated. The direct negative impact of introduced rodents on seabird populations is well documented, and their eradication is seen as a priority for seabird conservation (see Duron, Shiels, & Vidal, 2017 for a review). In contrast, their role in epidemiological dynamics has rarely been explored, but its potential is highlighted by our results. Implementing the eradication of introduced rodent populations on Amsterdam Island in parallel to the epidemiological monitoring of seabird populations would provide a unique opportunity to semi-experimentally assess their role in the eco-epidemiological dynamics while representing a significant management action in itself.

When deciding what management measure to implement in a situation such as the one on Amsterdam Island, modelling approaches can enable the stakeholder to clearly outline the management objectives, the available means to reach them and the level of uncertainty in the parameters underlying the dynamics of the system. Indeed, comparing the potential benefits of different management strategies is not as

straightforward as it may seem and we encourage stakeholders to work hand in hand with disease ecologists and modellers, even in an apparently simple situation such as that on Amsterdam Island. For instance, compartmented epidemiological models represent attractive options to assess the necessary level of rodent population control (Mariën et al., 2019) or vaccination coverage and protocols (Baker, Matthiopoulos, Müller, Freuling, & Hampson, 2019; Haydon et al., 2006) to efficiently benefit endangered species while accounting for their particular life-history traits (Garnier et al., 2012). Network models based on (direct or indirect) contact networks at the interspecific (Craft et al., 2008; Woodroffe & Donnelly, 2011) and intraspecific levels (Pepin et al., 2016; Robinson et al., 2018; Rushmore et al., 2014) could be used to explore the benefits of targeting potential super-spreading individuals or group of individuals for vaccination or population control. Models also allow to explore sources of heterogeneity in pathogen maintenance and transmission (Dougherty et al., 2018; Marchand et al., 2017; Robinson et al., 2018). Finally, management should ideally be conducted in an adaptive dynamic framework (Keith, Martin, McDonald-Madden, & Walters, 2011), requiring a design allowing to track eco-epidemiological variables, notably through the monitoring of sentinel species (Halliday et al., 2007), before and after interventions (Viana et al., 2014). The present paper provides key elements to fuel such modelling approaches.

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#### **AUTHORS' CONTRIBUTIONS**

T.B. conceived the idea of this work; T.B., J.T., H.W., K.D. and C.B. designed the study; J.T., A.G. and J.-B.T. collected the data; H.G. and E.T. developed the MAT; A.G., A.J., C.L., P.T. and E.L. performed the serology and molecular analyses; A.G. analysed the epidemiological data; A.G., R.B. and J.-B.T. analysed the tracking data; A.G. and T.B. led the writing of the manuscript. All authors contributed substantially to the drafts and gave final approval for publication.

#### DATA AVAILABILITY STATEMENT

Data are available on the OSU-OREME Repository <https://doi.oreme.org/b4b4a2e1-3d00-45b7-b3b2-2ca05d054346> (Gamble, Bazire, et al., 2019).

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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## Annexe 6

Hanging out at the club: breeding status and territoriality affect individual space use and multi-species overlap at a seabird colony.

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T.

*In preparation.*





## Introduction

Many of the key processes underlying wildlife population dynamics are spatially structured. These include habitat and resource selection (Aebischer et al. 1993), intra- and interspecific interactions (Laundré 2010), migratory connectivity (Amstrup et al. 2004), disease transmission (White et al. 2017), and exposure to anthropogenic and environmental risk (Hays et al. 2019). Ultimately, linking demographic outcomes to spatially-explicit processes requires detailed knowledge of individual variation in movement patterns and distribution (Morales et al. 2010). Due the high cost, labor, and disturbance required for obtaining individual movement data, however, the sample of individuals for which such data can be collected often represents only a small fraction of the overall population. To minimize bias in inferences and maximize power to describe population-level variation, tracking studies must therefore sample a sufficiently diverse population to effectively capture patterns and processes of interest.

In order to directly link habitat conditions and movement decisions to demographic outcomes, studies of wildlife movement frequently target known breeders (e.g., Hinam and St. Clair 2007, Schofield et al. 2010, Rushing et al. 2016). In addition, breeding individuals are obligated to defend and return to a predictable location during the breeding season and may return to the same site year after year, which makes them easier to capture and follow over time than individuals of unknown breeding status (Votier et al. 2011, Cooper and Marra 2020). However, non-breeders are often present at high rates in wildlife populations, and their presence and distribution indirectly affect multiple aspects of population dynamics (Penteriani et al 2011, Pardo et al. 2013). Individuals to obtain breeding territories may enhance survival or reproductive success of breeders (Branconi et al. 2020) or buffer against decreases in adult survival (Klomp and Furness 1992). Conversely, non-breeders attending the breeding grounds may increase density-dependent pressures such as resource competition, aggression, and disease transfer, which in turn may depress reproductive success and survival (Bretagnolle et al. 2008). Assessing the relative

importance of each of these positive and negative effects generally requires understanding the prevalence, distribution, and interactions of non-breeders with the breeding population.

There is growing evidence that non-breeders differ systematically from breeding individuals in their movement patterns and habitat use. Non-breeders attending breeding areas interact directly with breeders in shared habitat, and may in fact range more widely and interact with more individuals than breeders whose movements are limited by the energetic constraints of raising young or defending territories (Webb et al. 2011, Mayer et al. 2017). Individuals present at colonies but not attending nests, which may include non-breeding adults and sub-adults at the start of the season and failed breeders as the season progresses, tend to make more frequent long-distance movements and visit more different breeding areas than do established or successful breeders (Votier et al. 2011, Ponchon et al. 2015). In addition, non-breeders may occupy entirely different foraging habitats both during (McInnes et al. 2016, Borghello et al. 2019) and after breeding (Clay et al. 2016). Thus, individuals that do not currently hold a territory where successful breeding is in progress may play important roles in transferring information, propagules, contaminants, genes, and pathogens among otherwise disjunct locations within and between breeding populations (Boulinier et al. 2016). Movement studies that underrepresent such individuals therefore risk mischaracterizing movement patterns and distribution of the overall population, potentially influencing the evaluation of demographic parameters and risk factors (Cooper and Marra 2020).

In some instances, direct access to breeding areas for studying breeders is limited because those areas are difficult to locate (Zharikov et al. 2006) or because restrictions are in place to reduce disturbance at sensitive times or there is a risk of introducing infectious agents, such as when there is evidence of nearby circulation of pathogenic agents (Gamble et al. 2020). In such cases, it may be attempted to capture breeding individuals when they are attending non-breeding sites, such as when they are at foraging or resting sites, e.g. at sea in front of breeding areas in the case of the secretive marbled murrelet, a small seabird which breeds in old growth forests (Zharikov et al. 2006). In some species, on-land loafing

areas, or so called 'clubs', could also be a place where individuals of unknown breeding status could be captured to be tracked back to their potential breeding sites. Such areas exist near breeding colonies of many seabird species, but little is known about the behavior of the individuals attending them (Klomp & Furness 1992), despite their potential contribution to key ecological processes such as recruitment to the breeding population and trophic interactions near the colonies (Klomp & Furness 1990, Furness 2015).

The brown skua (*Stercorarius antarcticus*), a colonially-breeding seabird, provides a unique opportunity to compare movement patterns between breeding and non-breeding individuals occupying the same breeding area. Both breeding and non-breeding brown skuas may attend mixed-species breeding colonies and defend territories, making it possible to follow non-breeders as well as known breeders (Borghello et al. 2019). Brown skuas feed on other seabirds via predation, scavenging, and kleptoparasitism (Furness et al. 2017), bringing them into frequent contact with a variety of native and introduced bird and mammal species as well as anthropogenic discards (Hemmings 1990). At the same time, as scavengers, skuas may act as reservoirs for the accumulation and transmission of pathogens (Miller et al. 2008, Vicente and VerCauteren 2019). Brown skuas have been implicated as potential spreaders of pathogenic agents in remote seabird colonies, notably avian cholera bacteria *Pasteurella multocida* on Amsterdam Island, where recurrent outbreaks threaten endangered seabird populations (Jaeger et al. 2018). Thus, understanding the interactions of skuas with sympatric seabird species is important to accurately measuring and predicting dynamics of interspecific disease transmission and spread (Gamble et al. 2020). However, inferences about brown skua movement and interaction with other species are currently limited to breeders, whose movements are necessarily constrained by the need to incubate eggs and feed and protect nestlings (Carneiro et al. 2014, 2015, Gamble et al; 2020).

To assess how skua movement patterns and habitat use vary with breeding status, we captured and tracked brown skuas attending a sub-tropical breeding colony at either nest sites (i.e., known breeders) or clubs (i.e., loafing areas used by individuals of unknown breeding status). We fitted both

groups with GPS transmitters to measure movement frequency and distance, habitat use, and overlap with breeding areas of seabird and marine mammal species of concern. Our principal research question was whether we could use the tracking of birds captured at clubs to infer their breeding status and their potential contributions to important ecological interactions among individuals and species. More specifically, we investigated 1) whether movement patterns and distribution differed between breeding and non-breeding skuas; and 2) whether the breeding status of skuas affect the frequency or intensity of their use of breeding areas occupied by other species, and thus their potential role as sentinels and spreaders of infectious agents.

## Methods

### *Study site*

We conducted all fieldwork on Amsterdam Island (37°49'S, 77°33'E), a 55 km<sup>2</sup> volcanic island in the southern Indian Ocean hosting large populations of emblematic but globally threatened seabird species (Heerah et al. 2019). The island consists of a mountainous plateau 500 – 800 m in elevation whose western edge is defined by sheer cliffs. Seabird breeding habitat is concentrated in the southwest quadrant of the island, with seabird species breeding in largely monospecific sub-colonies within the larger colony area. The central plateau provides nesting habitat for brown skuas (~60 pairs), along with the entire population of the endemic and endangered Amsterdam albatross *Diomedea amsterdamensis* (40-50 pairs) (Gamble et al. 2020; Figure 1). The beaches and cliffs are used by three additional species of endangered seabirds: yellow-nosed albatrosses *Thalassarche carteri* (~22000 pairs), sooty albatrosses *Phoebastria fusca* (~400 pairs), and northern rockhopper penguins *Eudyptes moseleyi* (~12000 pairs) (Figure 1). Sub-Antarctic fur seals (*Arctocephalus tropicalis*) breed in large numbers (~6400 pups; Guinet et al. 1994) along the perimeter of the island (Figure 1). Several species of non-native predators occur

throughout the island, including house mice (*Mus musculus*), brown rats (*Rattus norvegicus*), and feral cats (*Felis catus*).

Brown skuas breed annually on Amsterdam Island between October and January. The breeding period includes a month-long incubation period (October – November) followed by a 40-50 day chick-rearing period beginning in late November (Furness et al. 2017). Nests are scattered throughout the central plateau (Plateau des Tourbières), with the densest aggregations in the southwestern quadrant of the plateau (Figure 1). Away from nest sites, breeding and non-breeding skuas congregate throughout the breeding season in communal loafing areas (clubs) at the base of the western cliffs (Entrecasteaux), close to small lakes on the central plateau, and in the northeast quadrant of the island (Mare aux Eléphants; Figure 1).

#### *Capture and tagging*

We captured skuas during three breeding seasons using noose poles, leg nooses, or by hand. Cohorts 1 (austral summer 2017-18) and 2 (austral summer 2018-19) consisted of adult skuas of unknown breeding status captured at the Mare aux Eléphants and Entrecasteaux loafing sites (Figure 1) during the late chick rearing period (18 December – 20 January). Cohort 3 (austral summer 2019-20) included individuals from both loafing areas in addition to breeding skuas captured on nests on the Plateau des Tourbières (Figure 1) during late incubation and early chick-rearing (8 November – 8 December).

Upon capture, we collected physical measurements and blood samples, verified nest contents for breeding birds, banded birds on both legs with unique identifying markers (a metal ring and a Darvic ring with an alphanumeric code), and attached remote-downloading, solar-powered GPS transmitters (GPS-UHF Harrier-L, Ecotone Telemetry, Gdynia, Poland; 13 g) to scapular feathers – i.e., the upper back between the wings – using waterproof cloth adhesive tape (tesa Extra Power: Hamburg, Germany). Transmitters were set to record locations every 5 minutes during daylight hours (0000 – 1700 UTC) and

lasted between 0 and 100 days (average: 48 days). Receiving antennas were set in the field near the two loafing sites where captures were made and on the margin of the skua breeding area in the central plateau. To limit bias from small sample sizes and residual behavioral effects of capture and transmitter attachment, we excluded transmitters that lasted less than one week (i.e., 6 days or fewer) from further analysis. Because of the difficulties to access the main skua breeding area situated in a strictly protected area, the breeding success of breeding individuals could not be directly monitored; however, tracking data confirmed that all individuals captured at nest sites continued attending the same nest sites after GPS attachment.

#### *Data processing*

We conducted all data analysis in R 4.2.0 (R Core Team 2020). We initially cleaned data by regularizing tracks to consistent five-minute intervals during each daylight tracking period using the *ltraj* function in package *adehabitatLT* (Calenge et al. 2006) and removed erroneous locations using a speed filter of 12 km/hour, or 1 km between successive locations (vanBemmelen et al. 2018).

To determine the breeding status of skuas captured at non-breeding sites, we used the *find\_nests* function in the *nestR* package (Picardi et al. 2020). We first filtered the dataset for optimal processing, retaining every 4<sup>th</sup> location (i.e., 20-minute frequency). We then defined a breeding site as an area of at least 60 points within 15 m of one another located in or near core skua breeding habitat areas on the Plateau des Tourbières (Figure 1). Onsite tests indicated an error rate of ~20 m in GPS fixes taken at the base of the cliff; we chose a slightly smaller radius to optimize definition of nest sites in the more open plateau habitat. We further specified that the area should be visited at least once per day for three or more consecutive days within the week following capture. We tested the function on the tracks of known breeders ( $n = 7$ ) to ensure that it correctly identified known nest locations before applying it to individuals of unknown status captured at loafing sites ( $n = 32$ ). We considered sites that fit the parameters to be

breeding sites if they occurred in known breeding areas during the breeding season (1 November – 1 February); otherwise, we classified them as non-breeding territories.

To separate areas used for nesting and loafing from areas used for active foraging to compare the behavior of breeders and non-breeders, we processed individual tracks using a two-state Hidden Markov Model in package *moveHMM* (Michelot et al. 2016). This allowed us to assign each location to an underlying movement state, which in our model included either stationary (movement characterized by turning angles close to zero and short distances between points), or mobile (movement characterized by wide turning angles and longer distances) (Figure S1).

#### *Multi-species overlap*

To compare overall habitat use by skuas, we calculated separate composite kernel density estimates by group for breeders and non-breeders (i.e., individuals not attending apparent nest sites during the tracking period) as well as for each tracked individual using the *kde* function in the *ks* package (Duong 2020). We used a plugin bandwidth estimator and set the grid size to approximately  $0.001^\circ$ . We then generated polygons representing the core (50% isopleth) and full (90% isopleth) individual and composite home ranges. We projected the isopleths using a Lambert Azimuthal Equal Area projection centered on the island and calculated their areas ( $\text{km}^2$ ).

We evaluated the overlap of core (50% isopleth) and full (90% isopleth) habitat areas for each individual skua as a percentage of total breeding habitat areas for Amsterdam albatrosses (Figure 1, green area), cliff-nesting seabirds (Figure 1, purple area), and southern fur seals (Figure 1, yellow area). For each individual, we also calculated the proportion of all GPS locations within each of the three habitat areas (Amsterdam albatross, cliff nesters, and seals) classified as mobile (i.e., active foraging or commuting behavior) by the HMM.



## *Modeling*

To ensure that tracking duration was comparable among groups, we initially compared tracking duration between breeders and non-breeders using a single-factor ANOVA. We then constructed generalized linear mixed models (GLMM) in the *nlme* package (Pinheiro et al. 2020) to assess overall differences in behavior and home range size between breeding and non-breeding skuas. We constructed separate models for each of three different response variables: proportion of locations classified as mobile (0-1), core home range area (50% UD; km<sup>2</sup>), and full home range area (90% UD; km<sup>2</sup>). All models used a Gaussian distribution and identity link and included fixed effects of breeding status (breeding or non-breeding) as well as a nested random effect of capture site and cohort. In all cases, we also tested models with an additional fixed effect of tracking duration; however, since models including duration were not supported for any of the response variables (i.e., increased the AIC value by > 2 points relative to models without duration; Burnham and Anderson 2004), our final models included breeding status as the only fixed effect. We considered the response variables to differ between breeders and non-breeders if the 95% confidence interval of the coefficient estimate for breeding status did not overlap zero.

To compare the probability of interaction with other species between breeders and non-breeders, we also extracted the subset of individual locations and home range polygons that overlapped of each of the three nesting areas (Amsterdam albatross, cliff nesters, and seals) and modeled each response variable within the overlap areas (core home range area, full home range area, and proportion mobile locations) as a function of breeding status (fixed) and year-site interaction (random) as described above.

To test the influence of differences in territoriality among non-breeders, we ran additional models of the full set of location data using three breeding groups (breeders, territorial non-breeders, and non-territorial non-breeders) as a fixed factor and year-site as a random factor, and compared their AIC values to those of the models without territoriality. We considered the additional territorial classification to be valuable if AIC values improved by > 2 points relative to models without territoriality. We further assessed

differences among groups using post-hoc general linear hypothesis tests in the *multcomp* package (Hothorn et al. 2008).

Finally, to assess seasonal changes in movement patterns across all breeding groups, we measured each of the three movement parameters (core home range size, full home range size, and proportion of active locations) across a five-day moving window centered on each day of the season. We then added day of season to the GLMM structure described above to test for seasonal trends and between-group differences.

#### *Determination of epidemiological status*

Upon capture, blood samples (1 mL from the metatarsal vein using heparinized syringes) and cloacal swabs were collected to explore the epidemiological status of the individuals skuas with regards to the agent of avian cholera *Pasteurella multocida* (*Pm*), as conducted in a previous study in the same population (Gamble et al. 2020). Red blood cells and plasma were separated by centrifugation a few hours after collection. Swabs were stored in 0.5 mL of a lysis buffer (Longmire buffer). Samples were kept at -20°C in the field, then stored at -20°C (plasma) or -80°C (swabs) until analysis.

In order to assess past exposure to *Pm*, specific antibody levels against the bacteria were measured in plasma samples of skuas using a microagglutination test (MAT; SEROPAST®, Ceva-Biovac, France; see Gamble et al. 2020 for more details). Moreover, the presence of bacteria DNA in cloacal swabs was assessed: total nucleic acids were extracted and *Pm* DNA was detected with a real-time polymerase chain reaction (RT-PCR) targeting the strain previously detected in a dead sooty albatross on Amsterdam Island (Jaeger et al., 2019; see Gamble et al. 2020 for details).

## Results

We deployed transmitters on a total of 50 individuals over 3 breeding seasons (2017-2020). Of these, seven collected no data, and six collected data for < 7 days. The remaining 37 transmitters lasted an average of 56 days (range = 10 – 100) and included eight breeders and 29 non-breeders (Table 1). Tracking duration was similar between breeders (mean  $\pm$  SD: 60.3  $\pm$  26.8 days) and non-breeders (55.4  $\pm$  29.4 days) ( $F_{1,35} = 0.16$ ,  $p = 0.69$ ).

We identified potential nest sites on the Plateau des Tourbières for seven (of seven) known breeders, as well as four (of 32) additional individuals of unknown breeding status. Three of the additional sites were located in known breeding areas during the breeding season. The remaining site was in a loafing area with no known nests; thus, we presumed it to be a non-breeding territory (Figure S2). The model also identified multiple locations that fit the selection criteria in or near the loafing areas where captures had been made (Figure S2). Since these areas do not contain breeding habitat, we classified them as non-breeding territories and these individuals as territorial non-breeders (Table S1).

Behavioral patterns and overall distributions differed among breeders, territorial non-breeders, and non-territorial non-breeders. Although the three groups used broadly similar areas, composite core and full home range sizes for the non-territorial non-breeding group were substantially larger than for either the breeding or territorial non-breeding group (Figure 2). Individually, core home range sizes were similar between groups, but full home range areas were smaller for breeders than for non-breeders (Table 2; Figure 3). Breeders also spent more time stationary and less time mobile than either non-breeding group, and territorial non-breeders were significantly less mobile than non-territorial non-breeders (Figure 3).

Areas used by non-breeders included larger proportions of the breeding habitats of seabird cliff nesters (90% UD) and sub-Antarctic fur seals (50% UD) than the home ranges of breeding individuals (Table 2). Compared to breeders, non-breeders were more likely to be mobile when using Amsterdam

albatross breeding areas, while mobility rates did not differ between breeding groups in cliff-nester or seal habitat (Table 2; Figure 4).

During the breeding season, core and full home range sizes remained relatively consistent for breeders but declined substantially in both territorial and non-territorial non-breeders (Figure 5). Home range sizes of territorial non-breeders converged with those of breeders toward the end of the breeding season, while those of non-territorial non-breeders remained larger. The proportion of locations classified as active for the breeding group peaked around the time of hatching and then declined, while the proportion of active locations for both non-breeding groups declined throughout the season but remained higher than in the breeding group. (Figure 5)

*[Pending final results] Finally, 100% of the skuas were seropositive to Pm, while Pm DNA was detected among xx% [waiting for final results] of the sampled skuas. Breeders were [similar/different] to non-breeders, with seropositivity rates of xx% in breeders and xx% in non-breeders.*

## **Discussion**

Our results show substantial differences between the distribution and behavior of breeding and non-breeding brown skuas during the breeding season. Non-breeders occupied larger areas, spent more time moving among sites, and overlapped more with nesting areas used by species of concern.

The fact that non-breeding skuas ranged throughout the island increases previous estimates of space use derived from breeding skuas alone (Gamble et al. 2020) and reinforces the species' likely role as a vector for the spread of disease between discrete habitat areas (Jaeger et al. 2018, Gamble et al. 2020). Notably, while earlier work had not detected any breeding skuas that overlapped with both the breeding area of the critically small population of Amsterdam albatross and the cliff nesting seabird colonies where the avian outbreaks are recorded, several skuas were recorded to do so in the current study. Larger home ranges of non-breeders could result from the lack of spatial and energetic obligations

associated with nesting. Without the need to return to a central nest site and provision chicks, non-breeders may be more able to explore prospective feeding areas and adapt to seasonal changes in resource distribution, resulting in wider-ranging movements. Larger home ranges could also result if breeders are more socially dominant than non-breeders. Unlike most seabirds that feed on ephemeral marine resources, the skuas in this study foraged almost exclusively in terrestrial habitats with relatively stable distributions of prey. Thus, more dominant individuals may be able to exclude less dominant skuas from preferred foraging areas, forcing subdominant birds to range more widely in search of food, although, confirming the results of Gamble et al. 2019, we found no evidence of the occurrence of feeding territories in the study population (contrary to what is found in other populations, e.g., Hahn & Bauer 2008).

We observed greater mobility of non-breeders compared to breeders in our study, which suggests more time spent foraging or moving among sites. This is somewhat surprising given that the energetic needs of non-breeders are likely lower than those of breeders provisioning chicks. Non-breeders could potentially be exploiting more movement-intensive foraging strategies (e.g., kleptoparasitism vs. predation and scavenging). Mobile behavior could also result from territorial exclusion – if non-breeders are unable to control a single productive feeding area, they may need to spend more time foraging to meet their resource needs. Differences in behavior between groups provide insight into the intensity of interactions between and within species, and could play an important role in spatially mediated processes such as disease transfer (Han et al. 2015), genetic connectivity (Cushman and Lewis 2010), and risk exposure (Jacobson et al. 2016). In our study, higher mobility rates likely bring non-breeders into potential contact with a greater number of individuals and species than breeders; however, those contacts may be less intense or shorter in duration compared to those of more stationary breeders.

The larger and more mobile movements of non-breeders broadly resulted in greater overlap with species of concern, although specific patterns of overlap varied by species. In Amsterdam albatross

breeding areas, non-breeders occupied similar proportions of shared habitat, but they were more mobile within shared habitats than breeders. Although some skua nests were located within Amsterdam albatross breeding areas, breeders appeared to travel to the colony edges to feed, consistent with previous observations (Gamble et al. 2020). In contrast, non-breeders traveled to or across Amsterdam albatross breeding areas rather than remaining at the colony edges. Thus, breeders primarily used shared habitat areas for less movement-intensive behaviors (e.g., chick-rearing), while non-breeders used these areas for foraging or traveling between foraging patches. In contrast, non-breeders shared larger portions of their home ranges with seals and cliff-nesting seabirds more than did breeders, although behavioral patterns within shared areas did not differ between groups. This suggests that the frequency of interspecific interactions in these habitats, but not their nature or intensity, differs between breeding and non-breeding skuas.

Interestingly, tracking also revealed differences among non-breeders in territorial behavior, and non-breeders with clearly defined territories showed movement and distributional patterns intermediate between the wide-ranging movements of non-territorial non-breeders and the more constrained movements of breeders. The ability to defend a territory is often related to social dominance, which may be a function of sex, age, body size, or a combination of these factors (Piper 1997, Hahn et al. 2008). Although we did not measure individual correlates and therefore cannot speculate on the underlying drivers of territoriality in this system, our results show that there is considerable heterogeneity among non-breeders. In contrast, there was limited variability in movements of breeders, which occupied territories at or near their breeding sites throughout the breeding season. In a simulation study, McClure et al. (2020) found that increasing variability of home range sizes was associated with increasing rates of disease transmission in a mammalian system, presumably because only a few “super-spreaders” are needed to introduce propagules into new areas. The observation that 100% of the skuas are seropositive

to *Pm* in the population suggest that they are all exposed via their feeding activities, but some individuals could nevertheless play more important role as spreaders (Gamble et al. 2020).

The systematic differences that we observed in space-use of non-breeders relative to breeders could be particularly important to predicting effects of both interannual variation and long-term changes in environmental conditions. When food resources or nest sites are limited, non-breeders attending breeding sites likely serve as a pool of potential recruits in the event of mortality affecting the breeding population (Porter and Coulson 1987, Klomp and Furness 1992). Thus, while the breeding population remains relatively stable over time, the number of non-breeders is likely to fluctuate as interannual changes in resource availability affect adult survival and recruitment. Our study suggests that any environmental changes that increase in the number of non-breeding skuas would substantially increase the potential for both intra- and interspecific interactions, with potentially deleterious effects on sympatric seabird species of concern. Actions that reduce supplemental food availability (e.g., eradicating introduced rodents) could potentially reduce the number of non-breeding skuas, but might also increase predation pressure on other seabirds. The high exposure of skuas to *Pm* and the planned eradication of introduced mammal species from Amsterdam Island highlight the need for demographic data on that skua population, as well as further work on their use of space in relation to foraging and epidemiological dynamics.

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Table 1. Capture and tracking details for skuas on Amsterdam Island, 2017-2020.

Cohort	Year	Number of tracked skuas	Capture dates	Duration (mean $\pm$ SD)	Fixes (mean $\pm$ SD)	Breeders	Non-breeders (with territories)
1	2017-18	7	22 December - 20 January	22 $\pm$ 21	3079 $\pm$ 3293	0	7 (1)
2	2018-19	19	18 December - 10 January	60 $\pm$ 28	4539 $\pm$ 2824	2	17 (3)
3	2019-20	17	8 November - 8 December	47 $\pm$ 34	3050 $\pm$ 3231	8	9 (4)

Table 2. Coefficient values for models of core home range area (50% utilization distribution), full home range area (90% utilization distribution), and proportion of locations classified as active for all locations and locations overlapping other seabird species for breeding and non-breeding brown skuas on Amsterdam Island, 2017-2020.

	y	Intercept		Non-territorial non-breeders (vs Breeders)		Territorial non-breeders (vs Breeders)	
		$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI
All	Core home range	0.03	[-0.36 - 0.42]	<b>0.54</b>	<b>[0.09 - 0.99]</b>	0.08	[-0.49 - 0.65]
	Full home range	1.20	[-1.66 - 4.06]	<b>4.54</b>	<b>[1.21 - 7.87]</b>	1.25	[-2.89 - 5.39]
	Proportion active	<b>0.30</b>	<b>[0.22 - 0.38]</b>	<b>0.26</b>	<b>[0.16 - 0.36]</b>	0.09	[-0.03 - 0.21]
Overlap							
Amsterdam albatross	Core home range	0.01	[-0.01 - 0.03]	<b>0.02</b>	<b>[0.01 - 0.04]</b>	0.01	[-0.03 - 0.05]
	Full home range	0.04	[-0.06 - 0.14]	0.08	[-0.04 - 0.20]	-0.04	[-0.18 - 0.1]
	Proportion active	<b>0.29</b>	<b>[0.07 - 0.51]</b>	<b>0.36</b>	<b>[0.11 - 0.61]</b>	0.30	[-0.01 - 0.61]
Cliff nesters	Core home range	0.01	[-0.05 - 0.07]	0.06	[-0.02 - 0.14]	0.03	[-0.05 - 0.11]
	Full home range	<b>0.16</b>	<b>[0.01 - 0.32]</b>	<b>0.38</b>	<b>[0.20 - 0.56]</b>	<b>0.33</b>	<b>[0.11 - 0.55]</b>
	Proportion active	<b>0.60</b>	<b>[0.44 - 0.76]</b>	0.02	[-0.18 - 0.22]	<b>-0.26</b>	<b>[-0.48 - -0.04]</b>
Sub-Antarctic fur seals	Core home range	0.01	[-0.01 - 0.03]	<b>0.02</b>	<b>[0.01 - 0.04]</b>	0.01	[-0.01 - 0.03]
	Full home range	0.05	[-0.03 - 0.13]	<b>0.12</b>	<b>[0.02 - 0.22]</b>	0.03	[-0.09 - 0.15]
	Proportion active	<b>0.53</b>	<b>[0.39 - 0.67]</b>	-0.02	[-0.18 - 0.14]	<b>-0.24</b>	<b>[-0.42 - -0.06]</b>

Figure 1. Seabird breeding sites and brown skua capture locations (2017-2020) on Amsterdam Island.

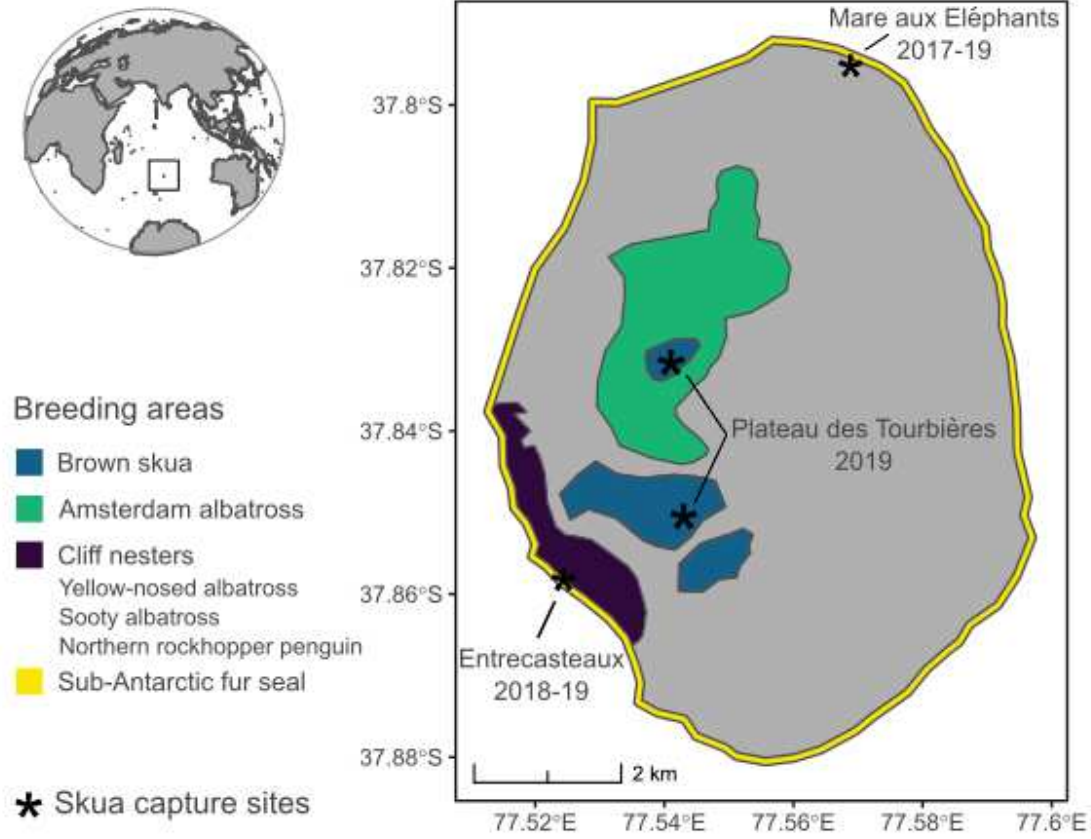




Figure 2. Movement parameters for breeding (pink), territorial non-breeding (green), and non-territorial non-breeding (blue) brown skuas on Amsterdam Island, 2017-2020: a) core home range area (50% utilization distribution); b) full home range area (90% utilization distribution); and c) proportion of locations classified as active.

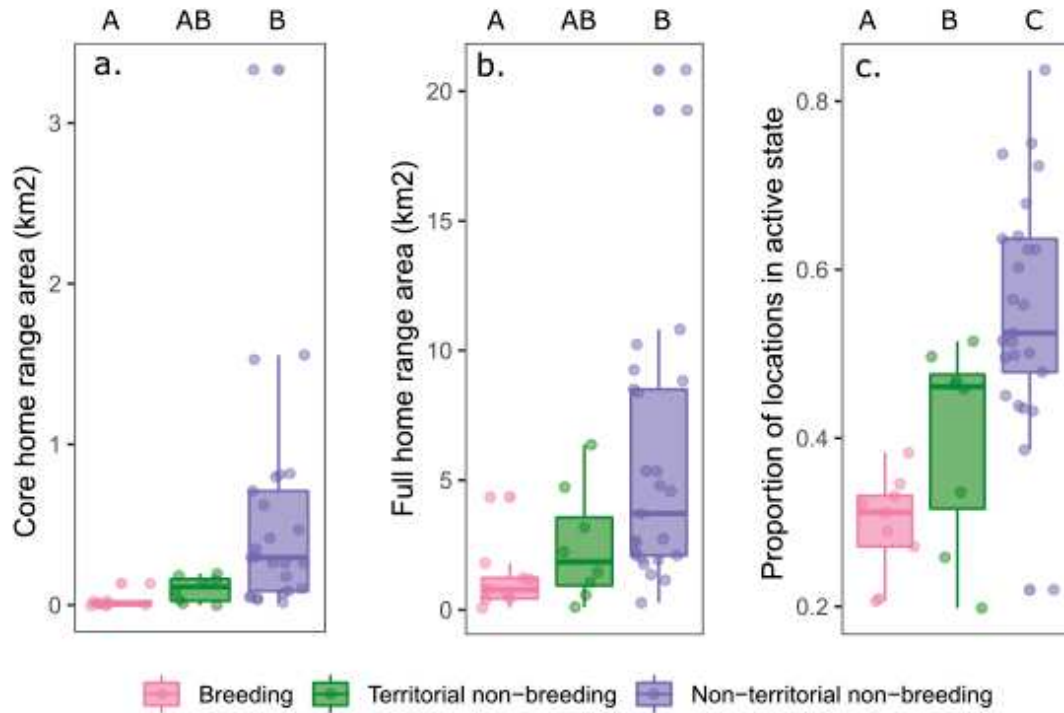


Figure 3. Sizes of core (dark areas) and full (light areas) home ranges for breeding (pink), territorial non-breeding (green), and non-territorial non-breeding (blue) brown skuas on Amsterdam Island, 2017-2020.

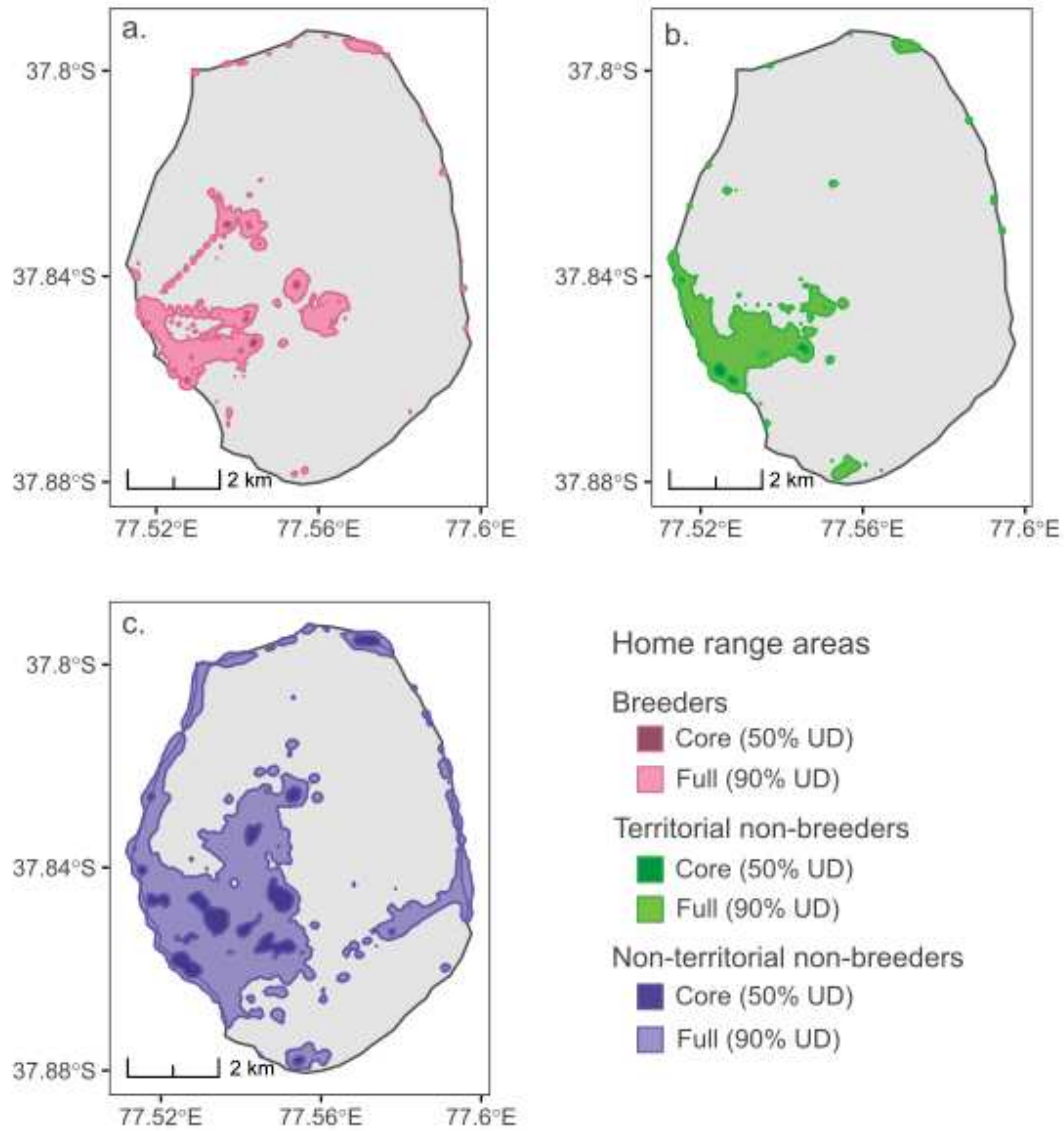


Figure 4. Area of core home range (50% utilization distribution; a-c); full home range (90% utilization distribution; d-f); and proportion of mobile locations in habitat overlapping species-specific breeding areas for Amsterdam albatrosses (green: a, d, g), cliff-nesting seabirds (purple: b, e, h), and sub-Antarctic fur seals (yellow: c, f, i) for breeding, territorial non-breeding, and non-territorial non-breeding brown skuas tracked on Amsterdam Island, 2017-2020.

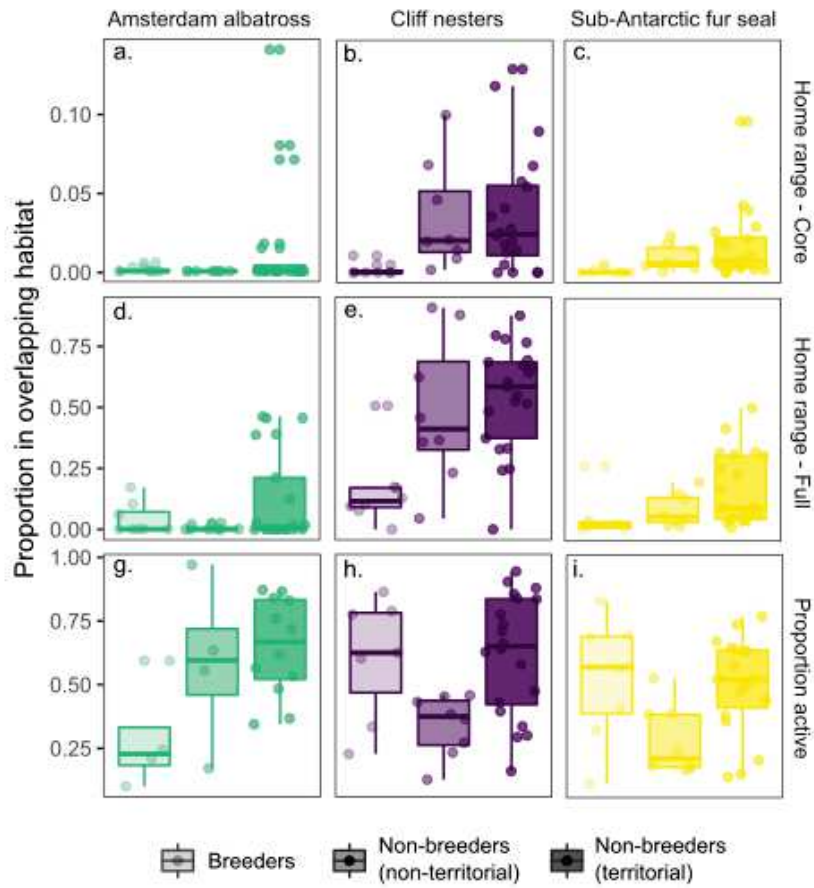
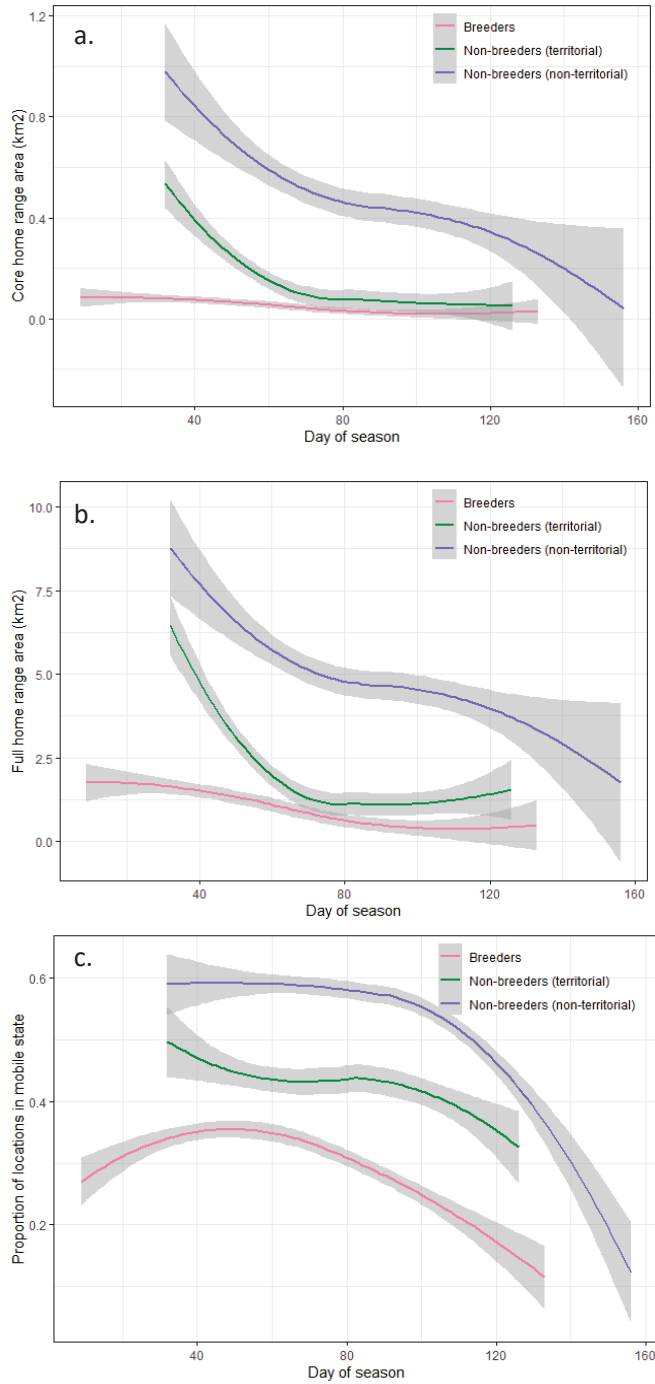


Figure 5. Changes over the tracking period in a) core home range area (50% utilization distribution); b) full home range area (90% utilization distribution), and c) proportion of mobile locations in breeding (pink), territorial non-breeding (green), and non-territorial non-breeding (blue) brown skuas on Amsterdam Island, 2017-2020. Shaded areas represent 95% confidence intervals.



SUPPLEMENTAL INFORMATION

Table S1. Territory sites and attendance patterns for brown skuas on Amsterdam Island, 2017-2020. A territory site was defined as any area of at least 60 locations within 15m of one another visited during  $\geq 3$  consecutive days.

Lon	Lat	First date visited	Last date visited	Total visits	Days visited	Consecutive days visited	% days visited	Maximum % daily locations	Site type
77.525	-37.858	2018-12-26	2019-01-31	495	37	37	100	38	Non-breeding
77.555	-37.842	2019-01-08	2019-01-31	95	18	9	75	20	Breeding
77.543	-37.830	2018-12-26	2019-01-31	66	20	5	54	31	Breeding
77.543	-37.830	2018-12-26	2019-01-30	49	18	3	50	14	Non-breeding
77.525	-37.858	2018-12-18	2019-01-19	66	13	13	39	18	Non-breeding
77.528	-37.860	2018-01-14	2018-01-29	53	12	6	71	20	Non-breeding
77.524	-37.858	2018-12-18	2019-01-19	260	22	22	67	79	Non-breeding
77.538	-37.830	2019-11-08	2020-01-31	559	60	27	71	68	Breeding
77.537	-37.830	2019-11-09	2020-01-31	185	35	22	42	48	Non-breeding
77.541	-37.855	2019-11-16	2019-12-30	515	36	36	80	73	Breeding
77.541	-37.855	2019-12-13	2020-01-02	23	6	3	29	22	Non-breeding
77.542	-37.852	2019-11-16	2019-11-19	76	4	4	100	67	Breeding
77.544	-37.853	2019-11-17	2020-01-25	537	55	34	77	67	Breeding
77.544	-37.853	2019-11-17	2020-01-31	171	37	11	49	37	Non-breeding
77.544	-37.852	2019-11-17	2020-01-24	131	44	28	64	40	Non-breeding
77.542	-37.848	2019-11-17	2020-01-30	340	45	29	60	80	Breeding
77.542	-37.847	2019-12-19	2020-01-23	169	25	11	69	53	Non-breeding
77.565	-37.845	2019-11-21	2020-01-22	207	35	12	56	49	Breeding
77.528	-37.860	2019-11-28	2020-01-31	206	17	3	26	70	Non-breeding
77.571	-37.795	2019-12-20	2020-01-02	108	12	11	86	56	Breeding
77.565	-37.845	2019-11-24	2020-01-17	17	10	3	18	6	Non-breeding
77.545	-37.834	2019-11-22	2019-12-16	315	19	19	76	67	Breeding
77.525	-37.858	2019-12-02	2020-01-31	274	51	36	84	33	Non-breeding
77.525	-37.858	2019-12-08	2020-01-31	59	10	3	18	30	Non-breeding
77.545	-37.853	2020-01-04	2020-01-31	190	16	10	57	43	Non-breeding
77.525	-37.858	2019-12-07	2020-01-16	148	19	6	46	33	Non-breeding
77.525	-37.858	2019-12-08	2020-01-26	171	26	21	52	42	Non-breeding
77.525	-37.858	2019-12-10	2020-01-10	132	23	16	72	34	Non-breeding

Figure S1. Properties of Hidden Markov Models used to classify locations of brown skuas on Amsterdam Island (2017-2020) as stationary or mobile: a) step lengths by state; b) turning angles by state; c) example track (one individual over one day) showing stationary and mobile locations; and d) number of locations by state for each tracked individual.

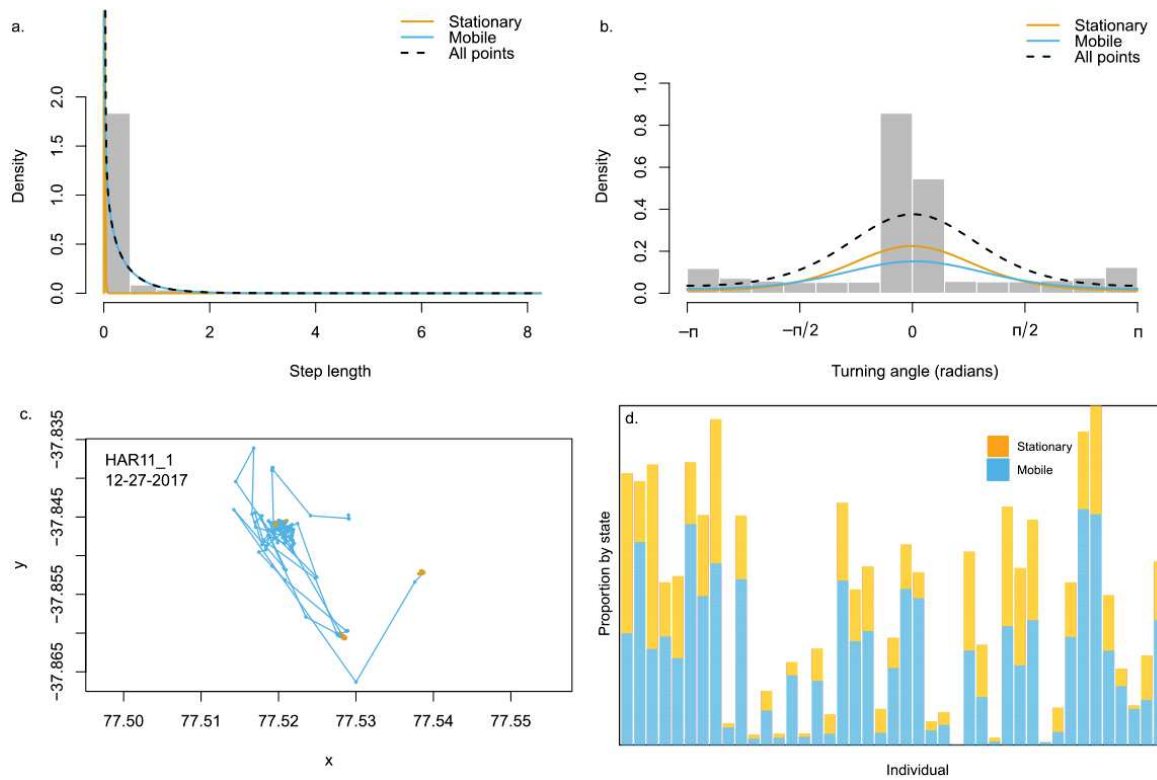
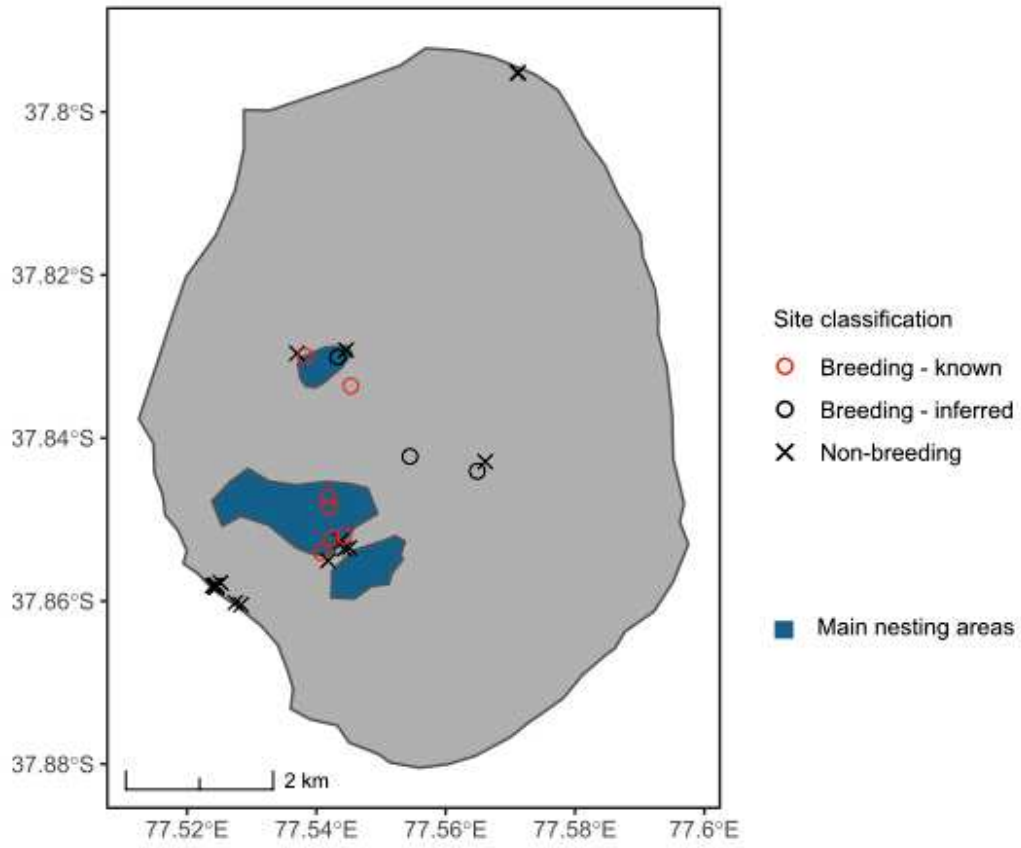


Figure S2. Locations identified as potential nest sites based on attendance patterns of brown skuas on Amsterdam Island, 2017-2020. Sites corresponding to nests where breeding birds were captured were classified as known nest sites, sites used by birds captured on clubs and located in known breeding areas during the breeding period were classified as inferred breeding sites, and sites located in areas not known to contain nesting skuas or attended only after the breeding period were classified as non-breeding territories.



## Annexe 7

Exposure of breeding albatrosses to the agent of avian cholera: dynamics of antibody levels and ecological implications.

Gamble A, Garnier R, Jaeger A, Gantelet H, Thibault E, Tortosa P, Bourret V, Thiebot JB, Delord K, Weimerskirch H, **Tornos J.**, Barbraud C., & Boulinier T.

2019, *Oecologia*





# Exposure of breeding albatrosses to the agent of avian cholera: dynamics of antibody levels and ecological implications

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## *Abstract*

Despite critical implications for disease dynamics and surveillance in wild long-lived species, the immune response after exposure to potentially highly pathogenic bacterial disease agents is still poorly known. Among infectious diseases threatening wild populations, avian cholera, caused by the bacterium *Pasteurella multocida*, is a major concern. It frequently causes massive mortality events in wild populations, notably affecting nestlings of Indian yellow-nosed albatrosses (*Thalassarche carteri*) in the Indian Ocean. If adults are able to mount a long-term immune response, this could have important consequences regarding the dynamics of the pathogen in the local host community and the potential interest of vaccinating breeding females to transfer immunity to their offspring. By tracking the dynamics of antibodies against *P. multocida* during 4 years and implementing a vaccination experiment in a population of yellow-nosed albatrosses, we show that a significant proportion of adults were naturally exposed despite high annual survival for both vaccinated and non-vaccinated individuals. Adult-specific antibody levels were thus maintained long enough to inform about recent exposure. However, only low levels of maternal antibodies could be detected in nestlings the year following a vaccination of their mothers. A modification of the vaccine formulation and the possibility to re-vaccinate females 2 years after the first vaccination revealed that vaccines have the potential to elicit a stronger and more persistent response. Such results highlight the value of long-term observational and experimental studies of host exposure to infectious agents in the wild, where ecological and evolutionary processes are likely critical for driving disease dynamics.

**Keywords** Capture–mark–recapture · Disease ecology · Immuno-ecology · Maternal antibodies · Seabird · Serological dynamics · Survival

## Introduction

The threat infectious diseases represent for biodiversity has recently been acknowledged and the ability to predict the effect of a pathogen on host populations is an important challenge for conservation (Smith et al. 2009; Young et al. 2017). However, it requires understanding the transmission dynamics, which can directly depend on the capacity of hosts to survive infection and to mount an immune response (Keeling and Rohani 2008). An often neglected but critical compartment of the host population is thus the proportion of individuals surviving infection. These individuals may, for instance, constitute a reservoir compartment (Viana et al. 2014) or limit transmission through a herd immunity effect (Iverson et al. 2016). For instance, breeding females can mount a protective immune response and passively transmit it to their offspring (Boulinier and Staszewski 2008). Protection could thus potentially be transferred from surviving individuals to potentially highly susceptible ones. Immunological, and particularly serological, data can be especially useful to track past exposure and quantify epidemiological dynamics (e.g., Pepin et al. 2017; Borremans et al. 2016) while informing on host responses, notably when collected in longitudinal settings (i.e., repeated sampling of individuals; Cizauskas et al. 2014; Buzdugan et al. 2017). Such data can thus be used to estimate the proportion of individuals that survived the previous exposure to infection.

Bacterial agents known for their pathogenicity in domesticated animals are commonly encountered in the wild, but variations in susceptibility among host populations and compartments of host populations are largely underestimated, especially when several strains circulate (Benskin et al. 2009). For instance, avian cholera, caused by *Pasteurella multocida* (*Pm*), has been repeatedly implicated in mass mortality events of adult waterfowl and seabirds in the northern hemisphere (e.g., Descamps et al. 2012; Österblom et al. 2004; Wille et al. 2016), while it seems to principally affect nestlings in the seabird community of Amsterdam Island (37°49'S, 77°33'E, southern Indian Ocean) where recurrent epizootics have been recorded since the mid-1980s (Weimerskirch 2004; Bourret et al. 2018; Jaeger et al. 2018). In addition, some evidence suggest that exposure of waterfowl to *Pm* results in a short-lived immune response (Samuel et al. 2003, 2005a, b), contradicting the suggested, but not fully explored, herd immunity effect leading to avian cholera epizootics fadeout in a population of common eiders (*Somateria mollissima*; Iverson et al. 2016). Hence, despite avian cholera being an important pathogen for both domestic (Christensen and Bisgaard 2000) and wild birds, basic knowledge on host responses to these bacteria is insufficient to be able to predict its impact on wild populations.

On Amsterdam Island, avian cholera outbreaks recurrently affect seabirds, providing a unique opportunity to gain insights on the responses of long-lived hosts to such a pathogenic agent in the wild. Nestling die-offs appear to particularly affect the locally abundant but globally endangered Indian yellow-nosed albatross (*Thalassarche carteri*; Weimerskirch 2004; Jaeger et al. 2018). In contrast, impact of the pathogen on adult albatrosses appears limited, with no associated effect on the

population (Rolland et al. 2009) despite a few infected individuals found dead (Jaeger et al. 2018). A potential disease-induced mortality of adults could have a strong demographic effect in such a long-lived species. However, it is not clear if adult birds are even exposed to the infectious agent. Notably, the pathogen also threatens the endangered endemic Amsterdam albatross (*Diomedea amsterdamensis*; Rivalan et al. 2010). Indeed, the entire population of this endemic species breeds in relative close proximity to the affected yellow-nosed albatross population, and predating and scavenging brown skuas (*Stercorarius antarcticus*) may act as epidemiological bridges between the two populations (Boulinier et al. 2016).

In long-lived species such as albatrosses, antibody responses could be expected to persist longer than in waterfowl, because Procellariiformes are slow-living animals, characterized notably by a long lifespan and a long chickrearing period (Lee 2006; Garnier et al. 2013; Ramos et al. 2014). The maintenance of high individual levels of specific antibodies over several breeding seasons could strengthen herd immunity, but also allow the long-term transfer of potentially protective antibodies from breeding females to offspring via the egg yolk (Gasparini et al. 2002; Garnier et al. 2012). Vaccination against *Pm* is widely considered the most effective way to protect poultry flocks (Ahmad et al. 2014) and autogenous vaccines are routinely used to reduce the risk of emergence of *Pm* and the mortality it causes (Christensen and Bisgaard 2000). On Amsterdam Island, vaccination has proven efficient to protect yellow-nosed albatross nestlings after direct vaccination (Bourret et al. 2018), while non-vaccinated nestlings appear to die without mounting an immune response. If vaccination of adults led to the maintenance of high antibody levels over several breeding seasons, it could open important conservation perspectives by considering the vaccination of breeding females to protect offspring via maternal antibody transfer (Garnier et al. 2012).

In this context, the goal of this study was to improve our understanding of the role of birds that have survived exposure to *Pm*. This was done by assessing (1) whether adult yellow-nosed albatrosses show serological evidence of exposure to *Pm*, and if yes, (2) whether they are able to survive subsequent exposure, and (3) whether they can mount and maintain an immune response leading to the transfer of antibodies from breeding females to their offspring. Based on the previous observations suggesting no impact of the epizootics on adult survival in the recent years (Rolland et al. 2009), we explored predictions from two scenarios. In a first scenario, only a small proportion of adults is exposed to the bacteria, for instance, if outbreaks happen in the colony when attendance by adults is low (late in the breeding cycle). In that case, we would expect a low-to-null seroprevalence in non-vaccinated adults, independently of any potential disease-induced mortality. In a second scenario, a significant proportion of adults is exposed, survive exposure because disease induced mortality is low, and mount a detectable immune response. In that case, we would expect a moderate-to high seroprevalence in non-vaccinated adults. Both scenarios would lead to a high annual survival of adults, with no effect of vaccination on survival, but different seroprevalences. In addition,

if high antibody levels are maintained several years following vaccination, maternally inherited antibodies could be detected in young nestlings. To explore these scenarios, we assessed the proportion of adults mounting a humoral immune response to *Pm* exposure, the persistence of antibody levels over several years, adult annual survival, and the transfer of these antibodies to nestlings in both naturally exposed and vaccinated adult yellow-nosed albatrosses breeding on Amsterdam Island. To do so, we set up a capture–recapture program including the monitoring of both non-vaccinated and vaccinated breeders in a colony recurrently affected by avian cholera outbreaks. In addition to the recording of individual resighting histories, breeding adults and their offspring were screened for anti-*Pm* antibodies for 4 consecutive years using two different immunoassays. Considering the threat avian cholera can represent for wild bird populations (Descamps et al. 2012; Phillips et al. 2016; Uhart et al. 2017), the results of the present study may have important implications from basic eco-immunology of host–parasite interactions (Anderson and May 1991) to applied conservation.

## **Materials and methods**

### Study population

Fieldwork was carried out on Amsterdam Island, in Entrecasteaux cliffs, where approximately 20,000 pairs of yellow-nosed albatrosses nest between September and March each year (Rolland et al. 2009; Electronic Supplemental Material [ESM] 1, Fig. S1.1). Yellow-nosed albatrosses lay one egg per year in the early September and eggs hatch in late November–mid December (Jouventin et al. 1983). Nest attendance by adults is high until January, but drops thereafter. Nestlings are thus on their own on their nest until fledging in April, except for punctual feeding visits from their parents. No albatross is present in Entrecasteaux cliffs between April and August. The study was focused on a naturally delimited subcolony of approximately 250 breeding pairs of yellow-nosed albatrosses.

### Vaccine development

Following a process designed for the poultry industry, an autogenous vaccine was produced from a *Pm* strain isolated from the carcass of a sooty albatross nestling found dead in Entrecasteaux cliffs in 2011–2012 (strain reference D2C; Jaeger et al. 2018). This strain was characterized as serotype Heddleston 1, Namioka 7 (Bourret et al. 2018). The vaccine was constituted from killed bacteria of this strain mixed with a mineral oil adjuvant to obtain a water-in-oil emulsion (Ceva Biovac, France). This vaccine has been available since 2013. The formulation was modified in 2015 to induce a higher and more durable response. More details on the vaccine production and its use in albatross nestlings can be found in Bourret et al. (2018).

### Field sampling and vaccination design

During the late incubation and the early chick-rearing periods (November–December) in 2013, 134 breeding adults from 70 nests were captured, blood-sampled (0.5–1 mL from the metatarsal veins), and marked with metal and alphanumeric Darvic<sup>®</sup> plastic leg bands allowing individual identification without recapture. Each captured adult individual received a subcutaneous injection of 0.5 mL in the back of the neck of either the vaccine (67 “vaccinated” individuals) or a placebo (0.9% sodium chloride solution; 67 “NaCl-injected” individuals). When observed during the following weeks, birds from either group were recaptured and blood-sampled to assess their response to injection (37 NaCl-injected and 34 vaccinated individuals). These birds then also received a second injection of either NaCl or the vaccine. A short-time booster effect was detected by MAT 1 year but not 2 years after vaccination (ESM 1, Fig. S3.1) and no effect on raw return rate 1 year after vaccination was recorded. Birds injected twice in 2013 were grouped with birds injected once in the following analyses to be conservative with regards to the inter-annual persistence of antibody levels following vaccination. When both partners from a nest were captured, they received the same treatment. Birds from both treatment groups were spatially mixed and distributed throughout the study subcolony.

All banded individual observed within the study sub-colony during the three following breeding seasons (2014–2015, 2015–2016, and 2016–2017) were recaptured and blood-sampled to monitor the inter-annual variations of specific antibody level. Most of the captures occurred during the early chick-rearing period and no individual was sampled more than three times within a season.

In 2015, after obtaining the first results of the immunoassays quantifying the anti-*Pm* antibody response and interannual antibody persistence (see “Results”), it was decided to attempt to boost the anti-*Pm* antibody levels of the birds that had been vaccinated 2 years before. Thirteen individuals were injected in December 2015 with a new formula of the vaccine, while keeping 13 others as control (injecting them with NaCl). The use of the new formula of the vaccine, although preventing our ability to disentangle a potential booster effect from an effect of the new vaccine formulation, was motivated by the aim to maximize the potential to obtain the persisting levels of antibodies.

Nestlings of injected successful breeders of the 2014–2015, 2015–2016, and 2016–2017 cohorts were blood-sampled at approximately 5, 10, 30, 60, and 90 days of age to study the transfer and decay of maternal antibodies. In the absence of sexual dimorphism, adult birds were sexed using a molecular technique (Fridolfsson and Ellegren 1999) for comparison of mother’s and nestling’s antibody levels at hatching. The age of nestlings for which hatching date was not known was estimated using their tarsus length (ESM 1, Fig. S1.2).

Captured individuals were handled on site for only a few minutes. Biosecurity measures were followed to avoid spreading infectious agents between individuals (ESM 1). Within hours of collection, blood samples were centrifuged and plasma extracted. Plasma was kept at 4 °C in the field, then at –20 °C in the laboratory until analyzed. Sample sizes of the different treatment groups are summarized

in ESM 1, Table S1.1.

### Immunological assays

Detection of antibodies in plasma samples was used as an indication of exposure to *Pm* antigens (natural or vaccination). Until now, a single strain of *Pm* has been repeatedly isolated from dead birds, rather supporting a single introduction event of the bacterium on the island (Jaeger et al. 2018); seropositivity against *Pm* is thus likely to inform on exposure to this strain, although the circulation of other, potentially less pathogenic, strains cannot be excluded. Given the classical caveats of the use of serology in wildlife (Gilbert et al. 2013; e.g., test sensitivity and specificity below unity, individual heterogeneity in response), anti-*Pm* antibody levels in plasma samples were measured using two complementary immunoassays detecting different immunoglobulins to maximize the probability to detect past exposure events. First, specific agglutinating antibodies (mostly IgM and IgY) were measured using a microagglutination test (MAT) developed from the *Pm* strain D2C isolated on Amsterdam Island (SEROPAST<sup>®</sup>, Ceva Biovac, France; see Bourret et al. 2018). Individuals were considered seropositive if agglutination was observed at the tenfold or more dilution. Results are expressed as titers ( $\log_2[\text{last positive dilution}/10] + 1$ ). Second, a commercial indirect enzymelinked immunosorbent assay (ELISA) detecting chicken IgY against the Heddleston serotypes 1, 3, and 4 (ID Screen<sup>®</sup> Pasteurella multocida Chicken and Turkey Indirect, IDvet, France) was used. Results are expressed as optical densities (ODs). The use of anti-chicken IgY to detect yellow-nosed albatross IgY has previously been validated using an experimental design and the positivity threshold was adapted using a method relying on fitting a mixture of normal distributions to the values of ODs (see Garnier et al. 2017). The two assays, thus, only partly overlap in their targeted immunoglobulins: they detect different isotypes, directed against different epitopes, which may or may not be agglutinating. In addition, the MAT was manufactured using the *Pm* strain isolated from the field and does not rely on an intermediate conjugate; as such it is expected to be more sensitive and more specific than the ELISA to detect exposure of albatrosses to *Pm* on Amsterdam Island (ESM 1). Overall, 697 samples collected from 134 adults and 83 nestlings were analyzed (ESM 1, Table S2.1).

### Immunological data analyses

Annual variations in seroprevalences in “non-vaccinated individuals” (individuals before vaccination and NaCl injected individuals) were assessed based on the data collected during the late incubation and early chick-rearing period using logistic regressions with serological status (seronegative or seropositive) as the response variable and breeding season as an explanatory variable. As many individuals were sampled several times and a large proportion of individuals were partners, we used generalized linear mixed models in the ‘lme4’ R package (Bates et al. 2015), with the individual and the



nest as random effects; likelihood ratio test (LRT)  $\chi^2$  are reported. The Wilcoxon rank-sum test with a Bonferroni correction was used for comparisons of antibody levels between groups of different treatments and between periods. One individual was removed from all the analyses because of consistent surprisingly high ODs (ESM 1, Fig. S2.3). For the figures, the seroprevalences were calculated as the proportion of individuals tested seropositive at least once among all tested individuals during a given period. Sample sizes are reported in the corresponding figures. All statistical analyses were conducted in R 3.3.3 (R Development Core Team 2017) and codes are available in ESM 2.

### Capture–recapture analysis

To explore a potential effect of vaccination on the annual survival of breeding adults, resightings of banded individuals were recorded between the breeding season 2013–2014 and 2016–2017 on the study site and analyzed in a capture–recapture framework (Lebreton et al. 1992). To increase the power of the analysis, 109 non-injected individuals newly marked in December 2014 or December 2015 as part of a parallel study (Bourret et al. 2018) were included. Marked individuals were thus divided in three treatment groups: NaCl-injected, vaccinated, and non-injected. The capture histories of marked individuals were built as series of 0 s (not observed during the breeding season; i.e., dead or alive but not seen) and 1 s (observed at least once during the breeding season; i.e., alive and seen). Each pair received a different code and a random-effect capture–recapture model (Choquet et al. 2013) was used, allowing to account for the potential dependence of fates (survival and resighting) between the members of pairs. The use of such approaches allows to account for potentially heterogeneous resighting probabilities and thus limits the risk of biased estimations of survival probabilities. First, we used U-CARE to assess the goodness-of-fit of the data set to a Cormack–Jolly–Seber (CJS) model (Choquet et al. 2009a). Then, individual resighting histories were analyzed by running a CJS model in E-SURGE (Choquet et al. 2009b). Our initial model included a fixed vaccination effect, a fixed breeding season effect plus their interaction and a pair random effect on resighting probability, and a fixed vaccination effect and a pair random effect on survival probability (Table 1).

Model	Survival	Resighting	#Id.Par.	Deviance	AICc	AICc weight
1	<i>trt+pair</i>	<i>trt.t+pair</i>	14	603.03	631.65	0.030
2	<i>trt+pair</i>	<i>trt.t</i>	13	624.73	651.27	0.000
3	<i>trt+pair</i>	<i>trt+pair</i>	8	615.17	631.38	0.034
4	<i>trt+pair</i>	<i>t+pair</i>	8	614.97	631.18	0.038
6	<i>trt</i>	<i>trt.t+pair</i>	13	604.22	630.76	0.047
7	<i>c</i>	<i>trt.t+pair</i>	11	604.65	627.04	0.302
8	<i>c</i>	<i>trt+pair</i>	5	616.67	626.76	0.347
9	<i>c</i>	<i>t+pair</i>	5	617.76	627.85	0.201

**Table 1** Model selection for survival and resighting probabilities of yellow-nosed Albatrosses  
 Fixed effects considered: *c* = constant, *trt* = treatment (non-injected, NaCl-injected and vaccinated), *t* = breeding season.  
 Random effect: *pair*. #Id.Par. is the number of identifiable parameters of the model. Additive effects are denoted by a plus symbol (+) and interactions by a dot (.)

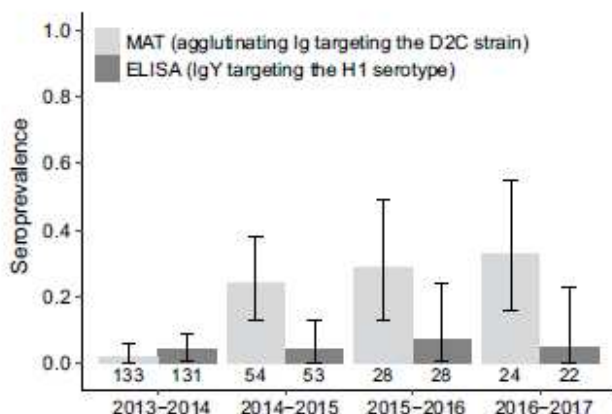
Survival probability was constrained to be constant between seasons to allow parameter identifiability (Little et al. 2010). The among pair variation in survival or resighting ( $\vartheta$ ) for the  $i^{\text{th}}$  individual of pair  $j$  was modeled as follows:  $\log \text{it}(\vartheta_{ij}) = \beta_0 + b_j$ , where  $\beta_0$  is the mean survival or resighting probability on the logit scale and the  $b_j$ 's are independent and identically distributed as an univariate normal distribution  $N(0, \sigma^2)$  where  $\sigma^2$  is the variance among pairs (Choquet et al. 2013). Parameters were estimated by maximization of the likelihood (Lebreton et al. 1992) and models including different fixed effects were then compared using AIC corrected for small sample size (AICc; Burnham and Anderson 2002). Random effects of pair were tested using LRTs at the 5% significant level following Choquet et al. (2013). In total, 243 individuals from 130 nests were included in the analysis, corresponding to 670 resighting events.

## Results

### Immune response after natural exposure

Evidence of natural exposure of adults to *Pm* was found. Seroprevalence according to MAT in non-vaccinated adults was low during the 2013–2014 breeding season (2%, 3/133; Fig. 1) and significantly higher during the three following seasons (LRT  $\chi^2 = 14.21$ ,  $p < 0.01$ ; ESM 1, Tables S3.1–2), reaching 33% (8/24) in 2016–2017. After 2014–2015, 7/23 (observed/potential) seroconversion events were observed (ESM 1, Fig. S3.2). Seroprevalence according to ELISA was low and stable during the 4 years of the study, varying between 4 and 7% (LRT  $\chi^2 = 2.04$ ,  $p = 1.00$ ; ESM 1, Table S3). As expected, the MAT was thus more sensitive compared to the ELISA for detecting natural exposure.

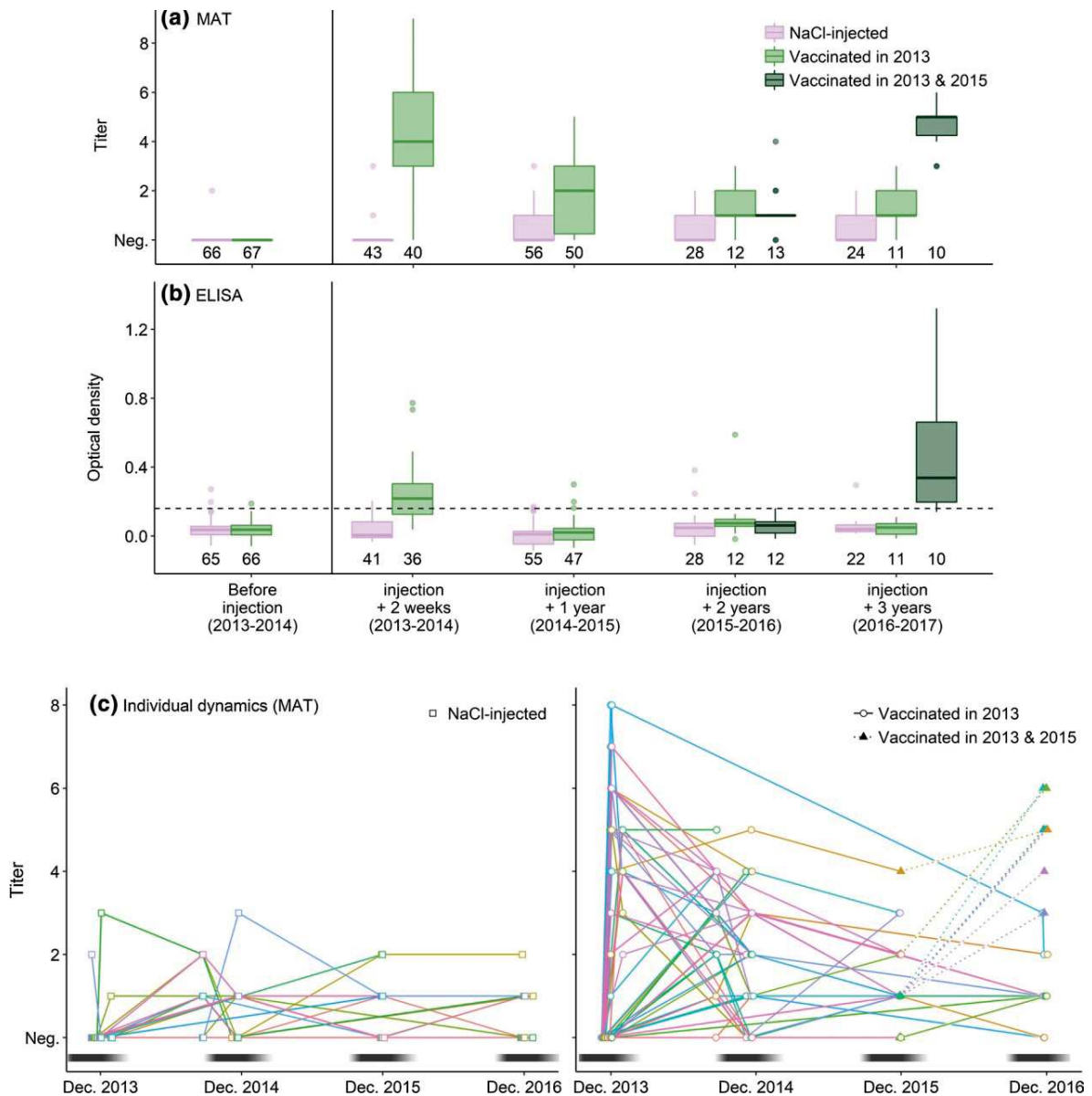
Most NaCl-injected individuals that seroconverted were resighted the following years (Fig. 2c; ESM 1, Fig. S3.2). Regarding the persistence of specific antibody levels following natural exposure, after 2014–2015, 6/10 seroreversion events were observed based on the MAT results (Fig. 2c; ESM 1, Fig. S3.2). Specific antibody response after natural exposure may thus be short-lived at the inter-annual scale. Overall, these results suggest some exposure to *Pm* in 2014–2015 and the following years.



**Fig. 1** Prevalence of anti-*Pm* antibodies in non-vaccinated adult yellow-nosed albatrosses after egg-laying measured using an MAT and an ELISA. Sample sizes are reported below the bars; bars represent the 95% CI.

### Immune response after vaccination

The initial vaccine injection (received in 2013–2014) induced a strong short-lived immune response. Most individuals responded to vaccination during the weeks following injection (MAT: 85%, 34/40; ELISA: 61%, 22/36; ESM 1, Fig. S3.3). Notably, all the individuals recaptured 3 weeks or more after vaccination were MAT-positive (21/21). Positive individuals after vaccination showed higher antibody levels in the weeks following injection than naturally exposed individuals (i.e., positive non-vaccinated individuals) during the whole study (MAT:  $W = 1249$ ,  $p < 0.01$ ; ELISA:  $W = 168$ ,  $p = 0.04$ ; Fig. 2a, b). Thus, vaccination induced a strong and immediate-specific immune response detected by both MAT and ELISA. One year after vaccination (i.e., 2014–2015), 76% (38/50) of the recaptured vaccinated individuals had specific antibodies levels detectable by MAT, with a higher proportion among those that had received two injections of the vaccine (17/20 for the birds that received two injections in 2013 versus 14/27 for the birds that received one). At the same time, seroconversion was observed in 30% (17/56) of the recaptured non-vaccinated individuals (Fig. S3.2). Assuming similar exposure to the bacterium of vaccinated and non-vaccinated individuals and 100% seroconversion of vaccinated individuals, vaccinated individuals had thus a 0.66 probability to remain MAT-positive 1 year after vaccination (see detailed calculation in ESM 1). In contrast, seroprevalence and antibody levels measured by ELISA 1 year after vaccination were not higher in vaccinated individuals than in NaCl-injected individuals (Fig. 2a, b; ESM 1, Fig. S3.3), independently of the numbers of injections received (ESM 1, Fig. S3.1), suggesting no persistence of ELISA-detected antibody levels over a year after the initial vaccination. More precisely, the modeled vaccine response would suggest a peak  $OD \pm SD$  of  $0.29 \pm 0.08$  reached  $26 \pm 9$  days after vaccination and a return to seronegativity by  $61 \pm 23$  days after vaccination (ESM 1, Fig. S3.5).



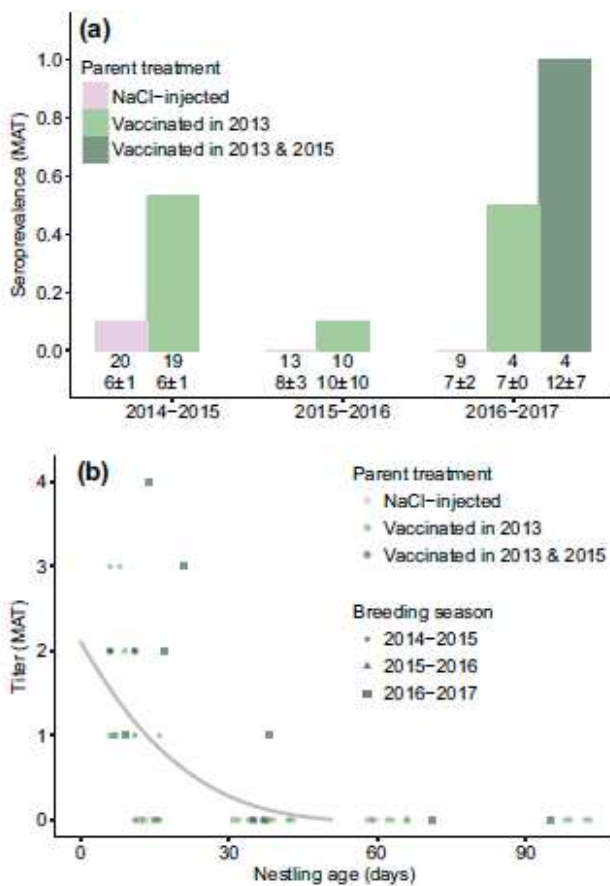
**Fig. 2** Quantification of anti-*Pm* antibodies by MAT (a) or ELISA (b) before and after vaccination and individual antibody-level dynamics (c) in adult yellow-nosed albatrosses after injection of NaCl or of an autogenous vaccine. a, c MAT non-null titers were considered positive. b ELISA positivity threshold is denoted by a dashed line; sample sizes are reported below the boxes. c Titers were measured by MAT; each line and color represents an individual. Nest attendance by adults is schematized by the black and white line, the darker the line, the higher is the attendance.

The new vaccine injection received 2 years after the initial vaccination induced a longer immune response. All individuals that received that injection in 2015–2016 mounted an immune response detectable by MAT and ELISA 1 year later (i.e., 2016–2017; Figs. 2c, S3.4). These individuals had higher specific antibody levels than 1 year after the initial vaccination (MAT: paired  $W = 36$ ,  $p = 0.05$ ,  $n = 10$ ; ELISA: paired  $W = 55$ ,  $p < 0.01$ ,  $n = 10$ ), and than other individuals that had received two injections in 2013–2014 1 year after vaccination (MAT:  $W = 147$ ,  $p < 0.01$ ,  $n = 27$ ; ELISA:  $W = 140$ ,  $p < 0.01$ ,  $n = 24$ ). NaCl-injected and individuals vaccinated in 2013 but not in 2015–2016 (independently from the number

of injections received in 2013) had lower levels of specific antibodies in 2016–2017 (Fig. 2a, b). This suggests that the injection of the newly formulated vaccine received 2 years after the initial vaccination induced a stronger and more persistent immune response than the first vaccination.

### Maternal transfer of antibodies

Vaccination of breeding females led to the transfer of maternal antibodies to their offspring. Specific antibodies were detected by MAT in the first blood sample of a high proportion (> 0.50) of nestlings of parents vaccinated the previous year (i.e., in the 2014–2015 cohort after vaccination of parents in 2013 and the 2016–2017 cohort after parents had received a new injection in 2015, Fig. 3a).



**Fig. 3** Prevalence of anti-*Pm* antibodies in the first blood sample of yellow-nosed albatross nestlings (a) and antibody-level decay after hatching (b) measured using MAT. **a** Sample sizes and mean  $\pm$  SD nestling age are reported below the boxes. **b** The gray line represents the fitted value of the generalized additive mixed model linking antibody level to age with nestling identity as a random effect (ESM 1); only nestlings with detectable levels of antibodies in their first blood sample are represented

Notably, within the 2014–2015 cohort, 8/9 nestlings of females that had received two vaccine injections the previous year (versus 2/10 for females that had received one) and, within the 2016–2017 cohort, 4/4 nestlings of females that received a new vaccine injection the previous year were detected as seropositive. A low proportion of the other nestlings were detected seropositive at their first blood sample (from zero for nestlings of non-vaccinated adults in 2014–2015 and 2015–2016 to

2/4 for nestlings of vaccinated adults 3 years after vaccination). In seropositive nestlings, antibody titers were low at hatching, ranging from 0 to 4, and decayed after hatching, with most nestlings becoming seronegative by 21 day; only one maintained a detectable antibody level for at least 38 days (Fig. 3b). Of all nestlings, specific antibodies were detected in only two of the 2016–2017 nestlings using ELISA (OD = 0.54 and 0.81, aged of 6 and 14 days, respectively). These individuals were associated with females vaccinated in 2013 and 2015 who had high ODs in December 2016 (OD = 0.47 and 1.32, respectively).

#### Capture–recapture analysis

Capture–recapture modeling suggested that adult survival was high and independent of vaccination against *Pm*. In more detail, the goodness-of-fit test suggested no lack of fit of the data to a CJS model ( $\chi^2 = 7.14, p = 0.21$ ). Excluding a pair random effect on resighting probability (Table 1, model 2) markedly increased the AICc value of the initial model. For resighting, the assumption of an absence of variance among the pairs ( $\sigma = 0$ ) was rejected ( $\sigma \pm SE = 5.939 \pm 2.743, p < 0.001$ ), indicating high heterogeneity among pairs in resighting probability. Removing the effects of treatment or season on resighting (models 3 and 4 versus 1) greatly increased the deviance, but resulted in large model uncertainty based on AICc values. Including a pair random effect on survival only slightly improved the deviance of the model compared with the treatment survival model (model 1 versus 6). For survival, the assumption  $\sigma = 0$  could not be rejected ( $\sigma \pm SE = 0.794 \pm 1.098, p = 0.137$ ). Therefore, survival probabilities of individuals associated in pairs were independent. Removing the vaccination effect on survival (model 7) markedly decreased the AICc and a vaccination effect on survival was 5.7 less likely than a constant survival, suggesting that adult survival was independent of vaccination against *Pm*. Model averaged resighting probabilities were estimated between 0.806 and 0.913 for all years and treatments, and survival probability [CI 95%] was estimated at 0.924 [0.824; 0.965].

#### **Discussion**

Despite critical implications for the understanding of disease dynamics and the implementation of potential management strategies, temporal patterns of exposure to bacteria and features of host humoral responses are often unknown in wild populations. Here, we provide novel information on these issues in a system involving *Pm* in a population of a long-lived and threatened species, by having repeatedly sampled breeding individuals over a 4-year period. We found evidence of adult exposure to the pathogen despite high annual survival, highlighting that adults are able to survive exposure. Indeed, the adult survival of Indian yellow-nosed albatrosses estimated in this study is close to the adult survival of Atlantic yellow-nosed albatrosses (*Thalassarche chlororhynchos*) estimated on Gough Island (mean  $\pm$  SE 0.92  $\pm$  0.01), and higher than the one estimated on Tristan Island (0.84  $\pm$

0.02; Cuthbert et al. 2003), from where avian cholera has never been reported to our knowledge. Immune response after exposure or vaccination was not life-long but long enough to provide information on the recent epizootics dynamics, especially when using the MAT assay specifically developed with the locally isolated *Pm* strain, suggesting that serology can be a useful tool to track the patterns of *Pm* exposure in the wild. In addition, the detection of specific antibodies in nestlings 1 year after vaccination of breeding females provides insight into possible applications of these tools for conservation, although more work is needed to understand the mechanisms involved in antibody-level persistence.

#### Immune response after natural exposure

Our study shows that, despite high annual survival, a large proportion of adult yellow-nosed albatrosses from Amsterdam Island are exposed to the agent of avian cholera. This is consistent with results in geese, suggesting that adult birds can survive exposure to *Pm* (Samuel et al. 2005a, b), although it had not been quantified. It, however, contrasts with the other waterfowl and seabird populations in which avian cholera induced high adult mortalities (Leotta et al. 2006; Descamps et al. 2012; Österblom et al. 2004; Wille et al. 2016). This heterogeneity is not surprising given what is generally known of the variability of *Pm* virulence depending on the strain and the host (Christensen and Bisgaard 2000). On Amsterdam Island, the previous work suggests the circulation of a unique strain of *Pm* among seabirds (Jaeger et al. 2018), although more work is needed to confirm the lack of *Pm* diversity, notably by considering potential non-pathogenic strains circulating among healthy animals that could induce cross-reacting immune responses. Individuals surviving infection to a pathogenic agent can play an important role in its maintenance (Viana et al. 2014) and circulation at different spatial scales (Boulinier et al. 2016) by potentially carrying it.

Observations of seroconverting and seroreverting individuals each season since 2014–2015 suggest that the high seroprevalences observed these years correspond to several outbreaks, and not just to the maintenance of antibodies after the 2014–2015 outbreak. Years of high seroprevalences (2014–2015, 2015–2016, and 2016–2017) correlate with years of low fledging success recorded in yellow-nosed albatrosses on Amsterdam Island (Bourret et al. 2018; Jaeger et al. 2018), indicating that seroprevalence may be used to track outbreaks. In addition, low seroprevalences were observed in yellow-nosed albatrosses in the first year of the study despite the fact that *Pm* outbreaks and low fledging success had been recorded in the previous years (Jaeger et al. 2018). This suggests that antibody levels reached in response to exposure to *Pm* may not persist over successive breeding seasons. Similarly, levels of antibodies produced by ducks after experimental infection wanes within a few months (Samuel et al. 2003). Short-lasting immunity against *Pm* may, thus, impact the dynamics of epizootics but not be sufficient to lead to fadeouts. These results contradict what has been suggested, but not assessed, in eider ducks exposed to *Pm* in the Canadian Arctic (Iverson et al. 2016).

The short-lasting humoral response observed also suggests that individuals might not maintain *Pm* infection over the years and that the contribution of chronically infected adults to the inter-annual maintenance of *Pm* may be limited, unless it occurs in seronegative birds. Carrying of the bacteria by seronegative birds has been reported in previous studies on other species (Samuel et al. 2003), but duration is not known (Wobeser 1997 for a review). The main affected species may also not be the sole reservoir. Studies on the roles of brown rats (*Rattus norvegicus*; Curtis 1983) and brown skuas, which may be year-round residents on the island and probably highly exposed to *Pm* through foraging, are, hence, needed. Brown skuas may notably play a key role as spreaders of *Pm* within the island (Boulinier et al. 2016).

#### Immune response after vaccination

Vaccination of adult yellow-nosed albatrosses induced an immune response, but detectable antibody levels dropped to below detection within a year in a large proportion of individuals. However, a new injection 2 years after the initial one using a new vaccine formulation induced high levels of antibodies that were maintained over the following year. Considering the frequency of the outbreaks (indicated by low fledging success; Jaeger et al. 2018) and the high seroprevalence in non-vaccinated individuals, it is likely that most of the individuals have been exposed prior to vaccination. The observed difference in antibody-level maintenance between the first and second vaccine injection is thus less likely to be due to a booster effect than to an effect of a difference in the vaccine formulation, although natural (re)exposure or vaccination are likely inducing different responses. Overall, our results open perspectives regarding the maintenance of anti-*Pm* antibody levels for several years after vaccination.

#### Maternal transfer of antibodies

The detection of specific antibodies in nestlings of vaccinated females suggests that, as observed in other seabird host-infectious agent systems (e.g., Gasparini et al. 2002, Garnier et al. 2012), anti-*Pm* antibodies can be transferred from mother to offspring via the egg yolk. However, low antibody levels in mothers the years following the initial vaccination prevented the transmission of sufficient quantity of antibody for a potentially protective level to be maintained (Grindstaff 2010). Indeed, avian cholera outbreaks are usually recorded several weeks after hatching (Weimerskirch 2004; Bourret et al. 2018). Future work should, thus, focus on testing different vaccination designs in breeding adults (notably based on different vaccine formulations and injection schedules) to assess if it is possible to induce a strong long-lasting immune response. Such response could lead to the transfer of sufficient antibody levels to nestlings over several breeding seasons, as observed in another procellariiform species (Ramos et al. 2014), to ensure potentially protective levels at the time of the outbreaks.



### Perspectives for disease surveillance and management

Vaccination of wild populations, despite numerous constraints and keeping in mind the importance of ethical issues, represents a powerful conservation tool (Plumb et al. 2007). Vaccination programs can be developed with different purposes, including elimination of the infectious agent(s) or direct protection of a target population. On Amsterdam Island, attention is strongly focused on the rare endemic Amsterdam albatross, but other species, potentially involved in *Pm* circulation or maintenance, also need to be considered. Avian cholera appears to kill thousands of nestlings of the locally abundant yellow-nosed albatross and their immunization, active by direct vaccination (previously proven in Bourret et al. 2018), or passive by maternally transferred antibodies, could thus be a potentially important management tool if maintained over series of years to protect several cohorts. Vaccination of parts of the yellow-nosed albatross local population to limit negative demographic effects and vaccination of brown skuas to limit the spread of the bacteria within the island or direct precautionary vaccination of Amsterdam albatrosses are possible options that could be considered. The use of vaccination for conservation in Procellariiformes would benefit from the longevity and faithfulness of individuals to their breeding site, which should help to strengthen potential local herd immunity effects. In any case, a priori modeling would be a useful step to determine which components of the community may be more pertinent to vaccinate and with which potentially optimal design (Haydon et al. 2006; Garnier et al. 2012). The results of the current study thus clearly open perspectives, but empirical work is still required regarding the potential effects of vaccination on shedding and nestling protection via maternal antibody transfer.

In addition, our study revealed that serology could be useful to track the circulation of a supposedly highly lethal disease agent in the wild. This approach is not available for all systems and interpretation of the results depends on the temporal persistence of antibodies following infection (Gilbert et al. 2013; Garnier and Graham 2014; Metcalf et al. 2016). However, recent examples have illustrated that serology could be useful to detect individuals surviving to supposedly highly lethal infectious agents (e.g., Mulangu et al. 2018 for the Ebola virus, Cizauskas et al. 2014 for the anthrax bacillus). In addition, when serological data are rigorously analyzed or combined with other data sources, they can bring valuable information on epidemiological dynamics and recent advances show that, despite being an old tool, it still show promise (Borremans et al. 2016; Buzdugan et al. 2017; Pepin et al. 2017).

Overall, our results highlight the value of detailed observational and experimental studies of the dynamics of host exposure to infectious agents in the wild, where ecological and evolutionary processes are likely critical for driving disease dynamics, and such knowledge may be useful to inform management plans.

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## **Author contribution statement**

TB and RG conceived the idea of this work; TB, RG, AG, JT, KD, HW, and CB designed the study; HG and ET conceived the vaccine and the MAT. JT, TB, AG, AJ, KD, VB, and JBT collected the data; AG ran the ELISA and analyzed the serological data; AG and CB ran the capture–recapture analyses; AG led the writing of the manuscript and all authors contributed substantially to the drafts and gave final approval for publication.

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## Compliance with ethical standards

**Conflict of interest** We declare no conflict of interest.

**Permits** The experimental design was approved by the Comit   de l’Environnement Polaire (TAAF A-2013-71, A-2014-134, A-2015107, and A-2016-80) and the French Ministry of Research (04939.03).

**Data accessibility** Supplementary data associated with this article can be found on the OSU OREME online repository at <https://doi.org/10.15148/c8256ec7-b5e1-4cc4-8429-7bf6cc79263c>.

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## Annexe 8

Impact of annual bacterial epizootics on albatross population on a remote island.

Jaeger A, Gamble A, Lagadec E, Lebarbenchon C, Bourret V, **Tornos J**, Barbraud C, Lemberger K, Delord K, Weimerskirch H, Thiebot J-B., Boulinier T., & Tortosa P.

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# Impact of Annual Bacterial Epizootics on Albatross Population on a Remote Island

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## Abstract

The reduced species richness typical of oceanic islands provides an interesting environmental setup to examine *in natura* the epidemiological dynamics of infectious agents with potential implications for public health and/or conservation. On Amsterdam Island (Indian Ocean), recurrent die-offs of Indian yellow-nosed albatross (*Thalassarche carteri*) nestlings have been attributed to avian cholera, caused by the bacterium *Pasteurella multocida*. In order to help implementing efficient measures for the control of this disease, it is critical to better understand the local epidemiology of *P. multocida* and to examine its interand intra-annual infection dynamics. We evaluated the infection status of 264 yellow-nosed albatrosses over four successive breeding seasons using a real-time PCR targeting *P. multocida* DNA from cloacal swabs. Infection prevalence patterns revealed an intense circulation of *P. multocida* throughout the survey, with a steady but variable increase in infection prevalence within each breeding season. These epizootics were associated with massive nestling dies-offs, inducing very low fledging successes ( $\leq 20\%$ ). These results suggest important variations in the transmission dynamics of this pathogen. These findings and the developed PCR protocol have direct applications to guide future research and refine conservation plans aiming at controlling the disease.

**Keywords:** Bacteria, Disease ecology, Eco-epidemiology, Molecular biology, Seabird, Wildlife

## INTRODUCTION

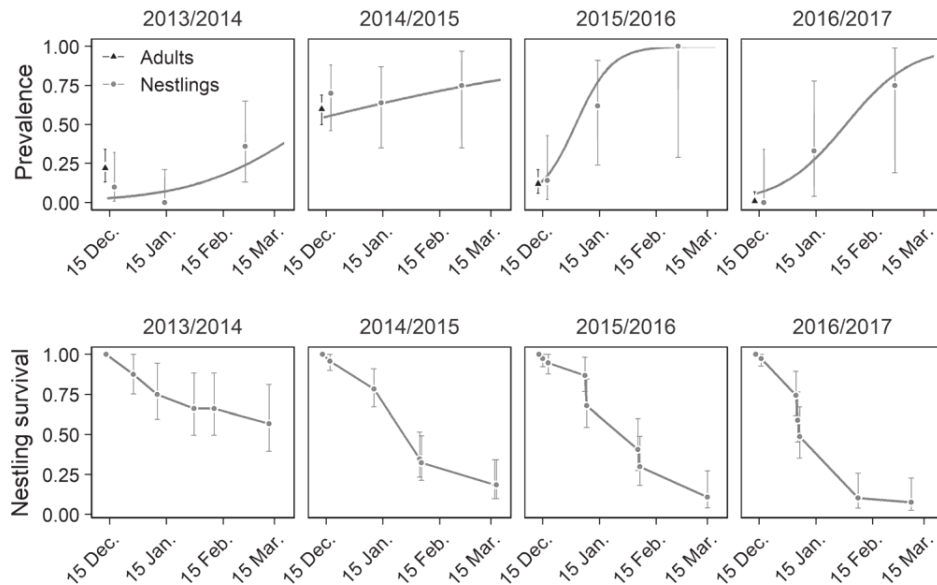
Emerging infectious diseases are listed among the top five drivers of species extinction (Daszak 2000). However, investigating infection dynamics *in natura* and identifying emergence factors are challenged by the number of animal species potentially involved in the maintenance and transmission of such pathogens. Insular ecosystems are particularly appropriate for such studies because species richness on islands, especially young and remote oceanic ones, is generally lower than on mainland sites with comparable climate (MacArthur and Wilson 1967; Kier et al. 2009). Furthermore, geographic isolation facilitates the identification of migratory or introduced taxa that may add complexity to a given pathosystem (Tortosa et al. 2012).

Amsterdam Island (37° 49' S, 77° 33' E) is a 53 km<sup>2</sup> volcanic island lying in the southern Indian Ocean, more than 3000 km away from any continent. To help preserving its remarkable wildlife, the island has been designated as part of the National Nature Reserve of the French Southern Territories in 2006 and as part of the French Austral Lands and Seas UNESCO World Heritage site in 2019. Amsterdam Island notably hosts several endangered seabird species, including the northern rockhopper penguin (*Eudyptes moseleyi*) and three albatross species: the Indian yellow-nosed (*Thalassarche carteri*), the sooty (*Phoebastria fusca*), and the endemic Amsterdam (*Diomedea amsterdamensis*) albatrosses (IUCN 2018). Local numbers of yellow-nosed albatrosses have severely declined over the past 30 years, mirroring recurrent die-offs of the nestlings from infectious diseases (Jaeger et al. 2018), while adults do not appear to be affected (Rolland et al. 2009; Gamble et al. 2019b). Two microbiological studies have substantiated the link between nestling mortalities and infection by the Gram-negative bacterium *Pasteurella multocida* (hereafter *Pm*; Weimerskirch 2004; Jaeger et al. 2018). Isolation of the etiological agent from two distinct bird species on Amsterdam Island and identification of a single genotype support the hypothesis of a clonal infection, suggesting the recent introduction of this agent (Jaeger et al. 2018), potentially as a consequence of poultry and/or rodent introduction to the island (Micol and Jouventin 1995). The causal link between *Pm* infection and nestling mortality has been further addressed through experimental vaccination, which significantly increased nestling survival during an avian cholera outbreak (Bourret et al. 2018).

Avian cholera is a worldwide infectious disease with major economic impact and conservation concern for several host species (Crawford et al. 1992; Wobeser 1997; Österblom et al. 2004; Leotta et al. 2006; Descamps et al. 2012; Singh et al. 2014; Wille et al. 2016; Iverson et al. 2016). However, *Pasteurella multocida* is notorious for exhibiting distinct patterns of pathogenicity across hosts species (Blanchong et al. 2006a). For instance, avian cholera seems to strongly affect adult survival in several systems such as eider ducks and gulls in North America (Wille et al. 2016; Iverson et al. 2016), guillemots in Northern Europe (Österblom et al. 2004), cormorants in South Africa (Crawford et al. 1992), or skuas and Adelie penguins in Antarctica (Leotta et al. 2006). In contrast, some sparse evidence suggests that some

populations or species may exhibit lower mortality rates and support chronic carrying of Pm, such as some geese species in North America (Samuel et al. 2005), or as generally considered in poultry (Glisson 2013). This heterogeneity in host response to Pm infection hence does not allow making predictions regarding the system investigated on Amsterdam Island. Deciphering the epidemiological dynamics of this emerging infectious disease is a first step towards understanding the dynamics of maintenance and transmission of a pathogen likely introduced to this ecosystem.

Although a handful of commercial tools are available for the screening of this disease (including ID-Vet® indirect ELISA and a RT-PCR kit recently developed by Bioeksen®), we developed and validated a real-time PCR approach targeting the Pm strain isolated from Amsterdam Island (Gamble et al. 2019b). We used this PCR scheme to carry out high-throughput screening of *P. multocida* DNA and examine the infection dynamics of adult and nestling yellow-nosed albatrosses during four successive breeding seasons and in relation to the nestling survival. The screening further aimed at identifying the periods during which more intensive sampling should be conducted in order to quantify the case fatality rate of the disease and its spatial progression in the colony. Lastly, this screening aimed at testing a number of hypotheses regarding transmission patterns. Pm infections are highly heterogeneous among bacterial strain and host species, and among individuals within a species (Wobeser 1997; Glisson 2013), some individuals developing acute and lethal diseases (*e.g.* Iverson et al. 2016), while some other develop chronic diseases or becoming asymptomatic carriers (*e.g.* Samuel et al. 2005). Our hypotheses were thus generated from previous demographic, serological, and experimental data collected in the study system rather than knowledge acquired in other systems. First, we predicted that Pm would be detected in both adults and nestlings, as a previous serosurvey revealed that adults were also exposed to the bacterium (Gamble et al. 2019b). Second, considering the high mortality rate of the disease in nestlings (Weimerskirch 2004; Bourret et al. 2018), we predicted that Pm would be detected at high prevalence years of large nestling die-offs, in addition to lesions suggestive of avian cholera such as necrosis and bacterial colonization of several organs following septicaemia, but not in years of high fledging success. Finally, nestlings were expected to die following infection, while adults were expected to either clear out the infection or chronically carry it, as suggested in other wild systems (Samuel et al. 2005) and as generally admitted in poultry (Glisson 2013).



**Figure 1.** Proportion of yellow-nosed albatrosses shedding *Pm* DNA (a) and nestling survival (b) during the four breeding seasons on Amsterdam Island. Raw prevalence is represented by black triangles (adults) and grey dots (nestlings). The temporal variation in prevalence in nestlings (grey line) was predicted based on the generalized linear mixed model that fitted best to nestling data (prevalence~breeding season: day; Table S3). Bars (a, b) and shaded area (b) represent the corresponding 95% confidence intervals; sample sizes are given in Table S2.

## METHODS

### Bird Sampling

Fieldwork was conducted between December 2013 and March 2017, in the Entrecasteaux cliffs (south-western coast of Amsterdam Island) where approximately 20,000 pairs of yellow-nosed albatrosses nest from September to March (Rolland et al. 2009). Yellow-nosed albatrosses lay a single egg in early September that hatches between late November and mid-December (Jouventin et al. 1989). Nest attendance by adults is high until January. Nestlings are then mostly on their own in their nest until fledging in April, except during feeding visits by their parents (Jaeger et al. 2018). We surveyed a naturally delineated subcolony of approximately 250 albatross pairs where bird exposure to *Pm* has been monitored since the 2013/2014 breeding season (Bourret et al. 2018; Gamble et al. 2019b). Monitored nests were georeferenced and marked with alphanumeric tags to individually identify the nestlings within a breeding season. Adults were marked with alphanumeric rings, allowing individual identification.

During the four studied seasons, cloacal swabs were collected from adults ( $n = 197$  individuals, including 100 with two samples or more) during the early chick-rearing period (late November to early January) using sterile applicators (one to two samples per breeding season, per individual) and from nestlings ( $n = 67$  individuals, including 50 with two samples or more) between hatching and fledging (up to five samples per breeding season, per individual). Sample sizes per sampling occasion and per

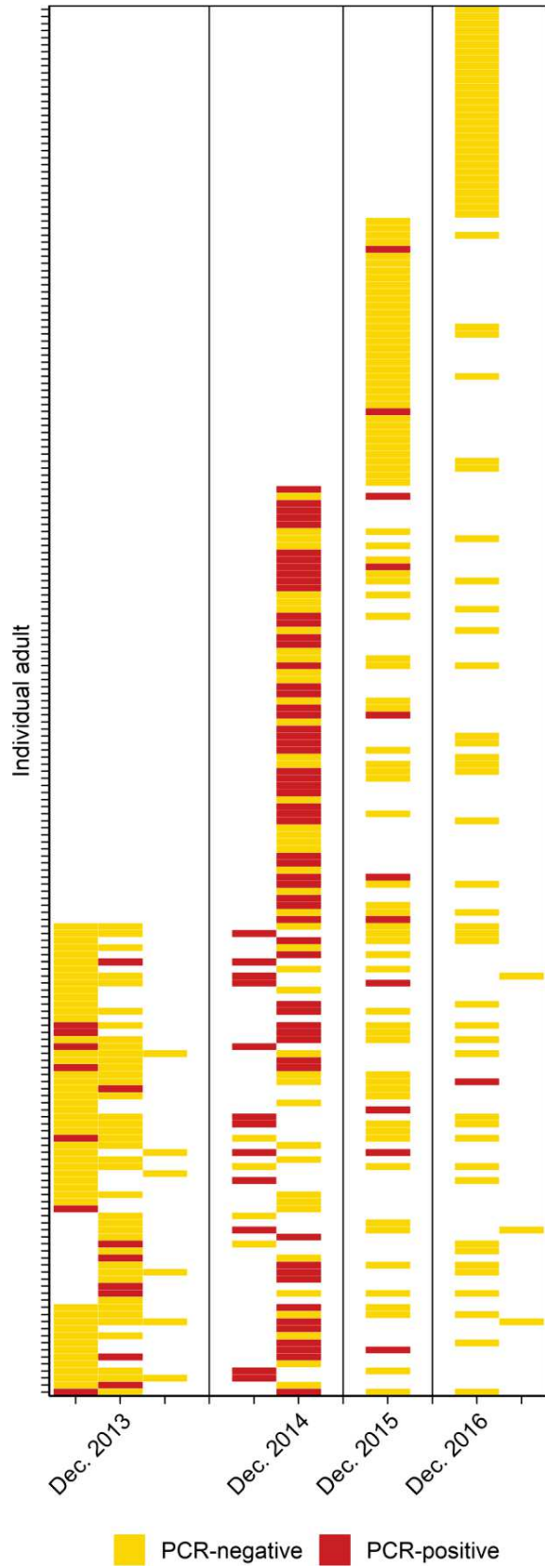
individual are detailed in Figures 2 and 3, and Table S2 of the Electronic Supplementary Material (ESM), respectively. Swabs were conserved in 0.5 mL Longmire lysis buffer (Longmire et al. 1988) and kept at 0–4°C in the field, and then at 20°C when brought back to the main station, and eventually stored at 80°C at the research facilities until analysis. In addition, when apparently fresh and intact dead birds were opportunistically found on the colony, necropsies were carried out and tissue samples were collected (n = 21 individuals). Samples were stored in 4% formaldehyde, routinely processed, and stained with haematoxylin and eosin for histological examination essentially as previously described (see <https://www.protocols.io/view/haematoxylin-eosin-h-e-staining-ihxcb7n>).

Additional Gram staining was used for bacterial characterization (see ESM for additional details).

Concomitantly, nestling survival was monitored during four monthly visits to the subcolony per year, between early December (following hatching peak) and late March (before fledging). Survival monitoring was focused on the sampled nestlings, in addition to up to 30 unmanipulated (*i.e.*, visually monitored without any handling) nestlings in 2014–2015, 2015–2016, and 2016–2017. Because sampling of the nestlings appeared to have no detectable effect on their survival (Figure S1; Bourret et al. 2018), the two groups (sampled and unmanipulated) were pooled together in the survival analyses. Additional details are given in ESM.

#### **Nucleic Acid Extraction and PCR Detection of *P. multocida***

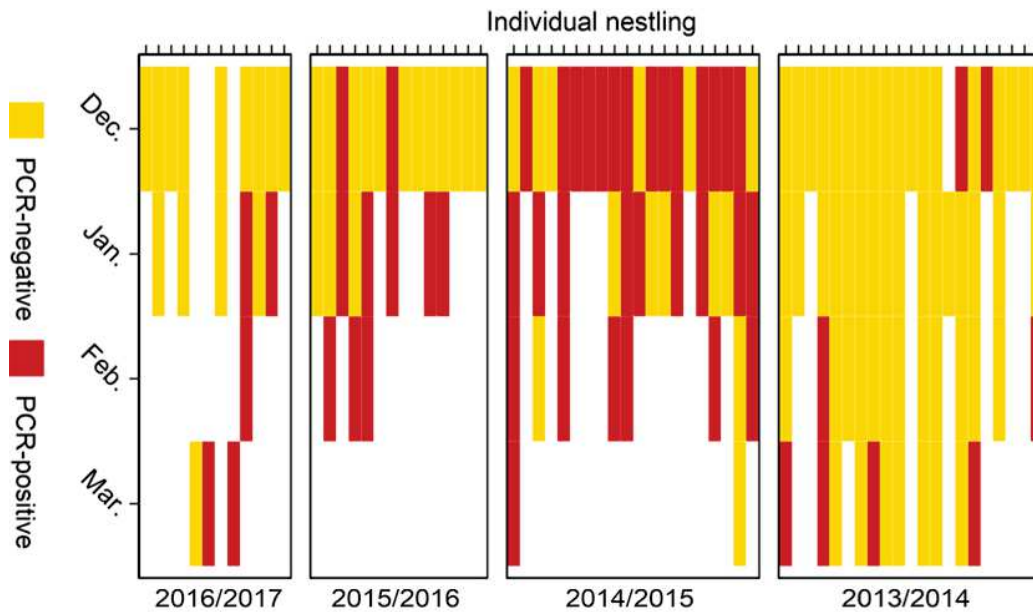
Nucleic acids were prepared from cloacal swab samples using manual QiaAmp cadon pathogen Mini kits (Qiagen, Courtaboeuf, France) following the manufacturer's protocol. A real-time probe-based PCR protocol was developed using Pm-for (5'-ACGGCGCAACTGATTGGACG-3') and Pm-rev (5'-GGCCATAAGAAACGTA ACTCAACA-3') primers allowing the amplification of a 116 nucleotides amplicon within KMT1 gene, a locus routinely used for the detection of Pm through end-point PCR (Townsend et al. 1998). Amplification was monitored in a Stratagene MX3005P (Agilent Technologies, Santa Clara, USA) thermocycler using the fluorescent Pm-probe (5'FAM-TCAGCTTATTGTTATTGCCGGT3'BHQ1).



**Figure 2.** Individual histories of *Pm* detection by PCR in cloacal swab samples of adult yellow-nosed albatrosses. Each line represents an individual and each column a month. Individuals sampled several times within a month were considered PCR-positive if at least one of these samples tested positive



Amplifications were performed in 25  $\mu\text{L}$  final volume containing 12.5  $\mu\text{L}$  of Absolute Blue real-time PCR Low Rox Mix (Thermo Scientific, Waltham, MA, USA), 0.4  $\mu\text{M}$  of each primer, and 0.2  $\mu\text{M}$  of *Pm*-probe. The PCR conditions included a first Taq polymerase activation step (95°C for 15 min), followed by 40 cycles each composed of a denaturation (95°C for 15 s.), an annealing (54°C for 30 s.) and an extension (72°C for 30 s.) step. The sensitivity of the PCR was measured by serially diluting genomic DNA prepared from the D2C *Pm* strain (Jaeger et al. 2018). The assay specificity was not addressed, as previous microbiological analyses suggested the occurrence of a single *Pm* clone in albatrosses from Amsterdam Island (Jaeger et al. 2018) and the seasonal dynamics of this clone were the focus of this study.



**Figure 3.** Individual histories of *Pm* detection by PCR in cloacal swab samples of yellow-nosed albatross nestlings. Each line represents an individual and each column a month. Individuals sampled several times within a month were considered PCR-positive if at least one of these samples tested positive.

### Statistical Analyses

Prevalence (with 95% Clopper–Pearson confidence interval) was calculated as the proportion of adults or nestlings testing positive among all sampled individuals during a given period. Variation in prevalence was quantified using logistic regressions, with *Pm*-PCR status (negative or positive) used as the response variable, and breeding season (categorical), day within the season (continuous), and their interaction as potential explicative variables. The best model was selected using Akaike Information Criterion (AIC) (Burnham and Anderson 2002). As many individuals were sampled several times and a large proportion of adult individuals were partners, we used generalized linear mixed models in the “lme4” R package (Bates et al. 2015), with the individual and nest (for adults only) as random effects. Likelihood ratio (LR) tests are reported between parentheses, and effect sizes, odds ratios, and AIC are reported in Tables S3-5. Because the timing of presence in the colony differed between adults and nestlings, two distinct models were used. The potential effects of handling and breeding season on nestling survival were investigated by fitting a Cox proportional hazards model (Andersen and Gill 1982) to the data using the “survival” package (Therneau and Lumley 2019). Finally, a potential

association between *Pm*-PCR status and fledging probability at the individual or nest level was investigated. To do so, we classified as PCR-positive all the nestlings from which *Pm* DNA was detected in at least one sample (individual level), or that belonged to a nest from which *Pm* DNA was detected in at least one sample (*i.e.* including its parents; nest level). We then fitted logistic regressions, with fledging success used as the response variable, and *Pm*-PCR status (negative or positive), breeding season (categorical), and their interaction as potential explicative variables. All statistical analyses were conducted in R 3.3.3. Additional methods and scripts are provided as ESM.

## RESULTS

The sensitivity of the developed RT-PCR scheme showed a positivity threshold of 9 DNA molecule templates per reaction. We screened 391 samples from 197 adults and 192 samples from 67 nestlings. *Pm* DNA was detected in 157/ 583 tested samples (Table S2).

In adults, which were sampled during the early chick-rearing period only, prevalence varied significantly among breeding seasons (LR  $v_2 = 79$ ,  $P < 0.01$ ; Tables S3-4), reaching its maximum in 2015 (0.60 [0.50; 0.69]) and minimum in 2017 (0.01 [0.00; 0.07]; Fig. 1a). In nestlings, the model with an effect of the breeding season (LR  $v_2 = 35$ ,  $P < 0.01$ ), day (LR  $v_2 = 13$ ,  $P < 0.01$ ) and their interaction (LR  $v_2 = 7$ ,  $P = 0.07$ ) on prevalence was selected, indicating variations among and within breeding seasons (Fig. 1a; Tables S3 and 5). Notably, prevalence was generally low at the beginning of the chick-rearing periods ( $\leq 0.14$ ), except in 2014/2015 when prevalence was high already in December (0.70 [0.46; 0.88]), mirroring prevalence in adults during the same period. Each year, prevalence tended to increase throughout the season (see Table S2 for numerical data). Remarkably, prevalence in nestlings was maximal in 2014/2015, 2015/2016, and 2016/ 2017, corresponding to seasons with very low fledging success ( $\leq 0.20$ ) while fledging success reached 0.57 [0.40; 0.81] in 2013/2014 (Fig. 1b).

The longitudinal survey of a subset of birds also revealed that some individuals testing positive at a given time point may test negative at one following time point (47/191 adults, and 9/50 nestlings; Figs. 2, 3), suggesting that a fraction of the birds could possibly clear out the infection. In 2014/2015, 2015/2016, and 2016/2017, all the fledged nestlings sampled longitudinally from hatching were detected as positive at least once although we did not detect any association between infection status and survival at the considered monthly sampling scale (Figure S6). We did not detect any spatial structuration in *Pm* infection status among nests either (Figure S5). Lastly, a histological analysis carried out to assess the extent of tissue damage resulting from *Pm* infection and to rule out other underlying processes revealed necrotic lesions in the heart, spleen, and/or liver together with Gram-negative bacterial sepsis in nine of 21 necropsied albatross nestlings (Figures S2-4), together with hepatic and pulmonary congestion as well as haemorrhagic myocarditis (not shown).

## DISCUSSION

This study reports the first multi-year investigation of *Pm* infection in an albatross population, hence contributing to fill the important knowledge gap regarding the impact of infectious diseases on one of the most rapidly declining bird taxa (Phillips et al. 2016). The results reveal that yellow-nosed albatrosses on Amsterdam Island have been facing *Pm* infection at their breeding sites every year throughout the study duration, even when the fledging success is relatively high. Such epizootics are in line with the massive nestling die-offs that have been recorded over the last three decades on the island, especially in yellow-nosed albatrosses (Weimerskirch 2004; Jaeger et al. 2018).

### Temporal Variations in *P. multocida* Prevalence

Infection prevalence was overall high, but varied among and within breeding seasons. As expected considering the high pathogenicity of *Pm* in the study system (Weimerskirch 2004; Bourret et al. 2018; Jaeger et al. 2018; and this study), very few nestlings ( $\leq 20\%$ ) fledged in 2014/2015, 2015/2016, and 2016/2017, corresponding to the years with highest *Pm* prevalence. Interestingly, all the fledged nestlings sampled longitudinally during these three breeding seasons from hatching were detected as positive at least once. In contrast, in 2013/2014, prevalence was minimal (but non-null) and nestling survival exceeded 50%. A salient pattern highlighted here is the temporality of the infection. Indeed, prevalence was most often low at the beginning of the chick-rearing period and then increased onwards until fledging. The estimates obtained for 2014/ 2015 do not follow this trend, though: prevalence was already high upon the first sampling date (*i.e.*, at the time of egg hatching), with over 50% of the birds testing positive, and remained high throughout the season. During 2014/ 2015 season, the epizootic may thus have started earlier for reasons that remain to be elucidated. The magnitude of the infection prevalence together with its temporal dynamics suggests that epizootics occur quasi-annually in this subcolony.

### Enlightening Mechanisms of Pathogen Maintenance

The recurrence of avian cholera outbreaks on Amsterdam Island raises the question of the compartments involved in pathogen maintenance between two successive breeding seasons, when most seabirds are absent from the island. We can hypothesize (H1) that some of the infected birds surviving the infection become chronic shedders, hence reinfesting the island every year as suggested in other wild systems (Samuel et al. 2005). The role of chronic shedders may be especially important to explain outbreak recurrence in a system such as Amsterdam Island where a high proportion of individuals, adults in particular, seems to survive exposure, contrasting with other systems where adult survival is strongly affected by the disease (*e.g.* Crawford et al. 1992; Österblom et al. 2004; Leotta et al. 2006; Descamps et al. 2012; Wille et al. 2016; Iverson et al. 2016). Alternatively, the pathogen may

persist on the island during winter in the absence of yellow-nosed albatrosses, either in the environment (Samuel et al. 2004) or in one of the resident avian/rodent species (H2). These non-exclusive hypotheses may be tested by screening (1) environmental samples, (2) adult birds at their return to the island in September, and (3) the putative reservoir species that permanently settle on the island. Indeed, *Pm* circulation on Amsterdam Island is not restricted to yellow-nosed albatrosses. Notably, predators and scavengers such as brown skuas (*Stercorarius antarcticus*) and introduced rodents (*Rattus norvegicus* and *Mus musculus*) may contribute to the maintenance and/or circulation of the pathogen (Curtis 1983; Boulinier et al. 2016; Gamble et al. 2019a). Screening the environment and the resident animal species, especially before the return of yellow-nosed albatrosses, will improve our understanding of the whole epidemiological network. The novel PCR protocol we developed here is pivotal in this perspective. Overall, the results presented herein will help to design field studies in an iterative process, as future sampling protocols designed to elucidate the transmission and maintenance cycles will need to be updated (Restif et al. 2012; see below).

### **Methodological Implications**

Avian cholera outbreaks on Amsterdam Island are characterized by a rapid epidemiological process, as highlighted by the steep increase in *Pm* prevalence and decrease in nestling survival throughout the breeding season, although some inter-annual variation exists. Hence, a finer temporal scale of sampling is needed to precisely quantify key epidemiological parameters, such as the case fatality rate and the probability to clear out the infection, as well as to assess the presence of chronic shedders, as suggested by the longitudinal survey. Indeed, the discrepancy between the within-year temporal scales of the sampling (once a month) and the epidemiological process (*Pm* being believed to cause death within a few days; Wobeser 1997) prevented us from quantifying these parameters in this study (see Figure S7 for detailed explanation). In addition, future studies could include repeated sampling (*e.g.* successive collection of several swabs from a given individual at a given time point) in order to account for detection probability issues. Indeed, most molecular assays have a sensitivity below unity, and repeated samples can allow to estimate the protocol detection probability and ultimately account for it by analysing the data using the patch occupancy framework (McClintock et al. 2010; DiRenzo et al. 2018). Designing the optimal sampling protocol requires preliminary knowledge on test sensitivity, but also infection prevalence (Mackenzie and Royle 2005), which our study brings here. Finally, the reported inter-annual variations in outbreak onset support the need of a monitoring scheme covering the full breeding season to identify the determinants of outbreak onset.

### **CONCLUSION**

In this study, we showed the relevance of a multi-scale (inter- and intra-seasonal) approach to better understand the infection dynamics of *Pm* in endangered insular wildlife. Altogether, the results

highlight the need for a comprehensive monitoring of *Pm* infection spanning different biotic and abiotic compartments possibly involved in pathogen maintenance on the Island. This study will guide future research on Amsterdam Island and other sites affected by avian cholera and comparable diseases (e.g., Crawford et al. 1992; Österblom et al. 2004; Blanchong et al. 2006b; Leotta et al. 2006; Descamps et al. 2012; Wille et al. 2016; Iverson et al. 2016). Eventually, it may also guide stakeholders to refine conservation measures and target the most relevant compartments of the pathosystem for the control of severe diseases affecting endangered wildlife populations.

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## **AUTHOR CONTRIBUTIONS**

AJ, CL, and PT set up the quantitative PCR. TB, CB, and HW are responsible of the field research programs. AJ, TB, KD, JT, VB, JBT, and AG implemented the study in the field. AJ and EL implemented the molecular analyses. KL conducted the histological analyses. AG managed the data. AG and AJ conducted the data analyses. PT and AG led the writing of the manuscript. All authors contributed to the final version of the manuscript.

## **COMPLIANCE WITH ETHICAL STANDARDS**

**CONFLICT OF INTEREST** The authors declare that they have no conflict of interest.

**STATEMENT OF ANIMAL ETHICS** The experimental design was approved by the Comit'e de l'Environnement Polaire and Comit'e National de Protection de la Nature (TAAF A-2013-71, A-2014-134, A-2015-107 and A2016-80) and the French Ministry of Research (licence #04939.03).

**DATA AVAILABILITY** The datasets and R code used in the current study are available on the OSU OREME online repository:

<https://data.oreme.org/doi/view/e34e5542-f819-4c88-a048-655f77e3668e>.

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**Résumé** - L'Albatros à nez jaune de l'océan indien (*Thalassarche carteri*) est une espèce menacée d'extinction dont la population principale, qui se reproduit sur l'île Amsterdam (Terres Australes et Antarctiques Françaises), connaît un déclin et une très forte mortalité des jeunes individus depuis plusieurs décennies. La bactérie responsable du choléra aviaire, *Pasteurella multocida*, est suspectée comme étant à l'origine de ces mortalités et notre équipe évalue depuis plusieurs années maintenant l'utilisation d'un vaccin pour protéger les poussins, via leur vaccination directe, mais aussi celle des femelles reproductrices susceptibles de transmettre des anticorps protecteurs à leur poussin. Le vaccin utilisé est un vaccin autologue (autovaccin) spécifiquement développé par Ceva-Biovac contre une souche de la bactérie *Pasteurella multocida* isolée sur cadavre d'albatros. Suite à l'obtention de premiers résultats prometteurs, les objectifs de la thèse étaient d'ajuster le protocole de vaccination, mais également de mieux appréhender et décrire les processus éco-épidémiologiques en jeu au sein des populations de vertébrés de l'île Amsterdam. Ceci a reposé sur l'analyse fine du suivi de couples reproducteurs année après année, mais aussi sur l'utilisation de données éco-épidémiologiques expérimentales complémentaires, notamment une nouvelle formulation du vaccin. Une diversité de pathogènes responsables de mortalités a pu être caractérisée. L'âge optimal de vaccination des poussins paraît être vers 10 jours et la vaccination des femelles reproductrices semble pouvoir protéger les poussins pendant plusieurs années, mais la forte densité de rats (*Rattus norvegicus*) actifs dans les colonies a rendu difficile l'identification d'un effet protecteur fort du vaccin. Les résultats obtenus améliorent la compréhension des mécanismes de circulation d'agents infectieux chez des espèces à reproduction coloniale et permettront de comparer les bénéfices relatifs attendus de différents scénarios de vaccination. Le travail montre l'importance d'intégrer des approches complémentaires, notamment du domaine biomédical et de l'écologie des populations, pour aborder la problématique de l'émergence de maladies infectieuses dans le contexte des changements environnementaux actuels.

**Abstract** - The largest population of the endangered Indian yellow-nosed albatross (*Thalassarche carteri*) is breeding on Amsterdam Island (French Southern and Antarctic Lands), where it has been declining and experiencing a very high mortality of nestlings over the last decades. *Pasteurella multocida*, the causative agent of avian cholera, is suspected to be responsible for these mortalities and the use of a vaccine to protect albatross nestlings has been evaluated for a few years. Direct vaccination of the nestlings has been tested, but also a potential protective effect via a transfer of maternal antibodies. The vaccine used was a specifically tailored vaccine (autologous vaccine) developed by Ceva Biovac against a strain of *Pasteurella multocida* isolated from a dead albatross on the island. Based on promising initial results, the objectives of the thesis were to adjust the vaccination protocol and to improve our knowledge of the eco-epidemiological processes involving vertebrate populations breeding on Amsterdam Island. The work has been based on a close monitoring of breeding individuals within and between years, and on novel experimental data, in particular with the use of a new formulation of the vaccine. We found that a diversity of pathogens was involved in the mortalities and that an optimal age for the vaccination of nestlings is at about 10 days after hatching. We also found evidence that the vaccination of breeding females could protect nestlings against avian cholera over several years. However, the high density of brown rats (*Rattus norvegicus*) in the bird colony prevented a strong protective effect of the vaccine. These results improve our knowledge of the mechanisms of pathogen circulation in colonial breeding species. In addition, they provide useful elements for assessing the relative benefits expected from different vaccination scenarios. This thesis shows the importance of integrating complementary approaches, particularly from the biomedical field and population ecology, to address issues related to the emergence of infectious diseases in the current context of environmental change.