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ETIOLOGIE ET IMPACT DES LESIONS CORNEENNES CHEZ LE THON ROUGE DU SUD D'ELEVAGE, *THUNNUS MACCOYII* (CASTELNAU, 1872)

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ABT Atlantic Bluefin Tuna

ASBTIA Australian Southern Bluefin Tuna Industry Association

BCI Body Condition Index

CCSBT Commission for the Conservation of Southern Bluefin Tuna

CL Corneal Lesion

EL Eye Lesion

FL ForkLength

GAB Great Australian Bight

HOGG Head On Gill-and-Gutted weight

LC Lésion Cornéennes

OR Odds Ratio

PBT Pacific Bluefin Tuna

SBT Southern Bluefin Tuna

TRS Thon Rouge du Sud

VAD Vitamin A Deficiency

YT Yellowfin Tuna

1. RÉSUMÉ EN FRANCAIS

INTRODUCTION (voir section 2, p.27)

L'organisation des Nations unies pour l'alimentation et l'agriculture (FAO) définie l'aquaculture comme étant la culture de tout organisme aquatique, incluant les poissons, mollusques, crustacés et plantes aquatiques. La consommation mondiale de poisson a évolué rapidement lors des dernières décennies. Alors que l'aquaculture était presque inexistante dans les années 1950, l'aquaculture représentait près de 47% de la production mondiale de poisson avec 80.0 millions de tonnes de poisson produites (FAO, 2018b). Dans un contexte global de gestion des écosystèmes marins, l'aquaculture pourrait représenter un moyen de limiter la pression du secteur de la pêche sur les stocks de population sauvage. De nombreuses innovations dans l'industrie de la pêche se développent pour être plus sélectives et s'associent avec certaines mesures, comme l'autorisation de la pêche de certaines espèces uniquement durant des périodes et des localisations précises. Néanmoins, il semble complexe à l'heure actuelle de pouvoir comprendre toutes les interactions du biotope, et ces mesures pourraient toujours modifier la structure des populations en termes d'âge, de distribution, de démographie et interférer avec leur rôle dans l'écosystème (Tveterås et al., 2017). Ainsi, l'aquaculture pourrait représenter une alternative intéressante si elle se développe de manière durable (Godfray et al., 2010; Osmundsen et al., 2020) puisque, comme tout industrie de production animale, elle possède un impact environnemental non négligeable (Alongi, 2002; Amirkolaie, 2008; Gross, 1998; Kümmerer, 2009).

Après la seconde guerre mondiale, le gouvernement d'Australie du Sud commença un sondage sur les populations sauvages de poissons migrant à travers la Grande Baie Australienne. La pêche industrielle du thon rouge du sud (TRS), *Thunnus maccoyii* (Castelnau 1972), se développa dans les décennies qui suivirent, jusqu'à atteindre des prises annuelles de 21 000 tonnes en 1982. La recherche dans le domaine de l'élevage de thon rouge démarra dans les années 1960, avec une mise en place pratique et effective dans les années 1990 à la suite de la mise en place de quotas par la Commission de Conservation du Thon Rouge du Sud (CCSBT) en 1989. Ces quotas furent mis en place afin de permettre aux stocks sauvages de se reconstituer. En Australie, l'élevage du TRS se rapproche plus d'un système de grossissement que d'un système d'élevage à proprement parler. Les compagnies capturent des thons sauvages de 2 à 3 ans, migrant à travers la Grande Baie Australienne, qui sont ensuite ramenés près de la côte de Port Lincoln. Les thons sont ensuite transférés dans des enclos marins de 45m de diamètre et 10 mètres de fond, contenant entre 1100 et 7000 poissons. Ils sont ensuite engraissés avec des poissons fourrages pendant quatre à six mois avant d'être vendus, principalement sur le marché Japonais. Le cycle du TRS n'étant pas fermé dans ce système, cela oblige les compagnies à capturer de nouveaux poissons chaque année. Cependant, cela permet également d'éviter l'installation de nombreuses maladies et parasites. Les animaux sont uniquement traités avec du praziquantel à la suite de leur arrivée pour lutter contre le trématode *Cardicola forsteri*. L'industrie du TRS tolère un taux de mortalité cumulée global de 1%. L'Australian Southern Bluefin Tuna Industry Association (ASBTIA) travaille avec les élevages de TRS, principalement dans la recherche et le développement, pour améliorer son élevage et surveiller la santé des poissons.

Lors des premiers mois de grossissement en 2017, certaines compagnies ont subi des pertes allant de 3% à 8%. De plus, de nombreux poissons parmi ces morts montraient des lésions oculaires, avec un blanchiment de l'œil ou le développement de tissu cicatricielle jusqu'à la perforation totale de l'œil dans certains cas. Ces lésions étaient présentes de manière unilatérale comme bilatérale. Lors du mois de Mars, le Future Fisheries Veterinary Services (FFVS) a entrepris des examens cliniques sur certains poissons présentant ces lésions oculaires. La plupart avaient des marques d'abrasion sur les flancs avec une perte d'écailles, associée à une absence de contenu stomacal et à une bile verte et foncée. Cet aspect de bile est souvent signe d'une anorexie d'au moins 24 à 48h. Certains poissons étaient infestés par des parasites externes, principalement des poux de mer du genre *Caligus spp*. Les plongeurs chargés de l'entretien intérieur des enclos rapportaient un comportement altéré des poissons atteints de lésions oculaires présentant une nage erratique, lente, parfois ne pouvant pas suivre le mouvement du banc principal et une difficulté pour se nourrir. Des prélèvements réalisés dans les organes tels que le foie, les reins et la rate n'ont révélé aucun agent pathogène. Il était donc peu probable que les lésions oculaires soient associées à une infection systémique.

L'industrie du TRS de Port Lincoln en 2017 était composée de dix compagnies, toutes de tailles différentes possédant de 3 à 18 enclos. La Table 1 (p.37) résume les caractéristiques de l'industrie.

Une analyse descriptive de la mortalité en 2017 a mis en évidence une hausse de la mortalité à 1,67% (n=10), qui a poussé l'industrie à entreprendre un projet de recherche pour essayer de comprendre le problème qu'avait subi le secteur cette année-là. L'analyse mis en évidence une atteinte locale à l'échelle des compagnies avec certaines ayant plus souffert que d'autres d'une mortalité croissante, ainsi qu'un probable phénomène de cluster à l'échelle de l'enclos. Les compagnies 1, 3, 6 et 10 possédaient respectivement 4,5%, 4,3%, 2,6% et 5,1% de mortalité cumulée. Les compagnies qui enregistraient une incidence de lésions oculaires croissantes ont permis de mettre en évidence un lien possible entre ce symptôme et l'augmentation de la mortalité (Figure 12, p.40).

Une étude descriptive des lésions, macroscopiques et histologiques (voir section 2.4, p.41), ainsi qu'une analyse par diagnostic différentiel, laissent à penser que ces lésions touchent en premier lieu la cornée, avec probablement un ulcère cornéen pouvant mener à une kératite puis à une cicatrisation du tissu ou à une perforation totale de l'œil sans guérison des tissus. Inspiré de précédentes descriptions faites par Hayward et al. entre 2008 et 2010 (Hayward et al., 2008, 2009a, 2010), une échelle évolutive des lésions a été créé pour décrire différentes observations allant d'un score 0 (œil sain) à un score de 4 (œil totalement cicatrise ou perforé avec perte très probable de sa fonction de vision, voir Table 3 p.46).

Les objectifs de cette étude sont :

1. **Déterminer si les lésions cornéennes (LC) représentaient un problème important pour l'industrie du TRS de Port Lincoln.**

Pour cela la première étape fût de chercher une association entre la mortalité des poissons et la présence des LC, en réalisant une étude cas-témoin sur les années 2017 et 2018, puis de voir les conséquences de ces lésions sur l'état général des poissons en termes de poids, taille et d'index d'état corporel (index utilisé dans l'industrie du thon pour évaluer le taux de graisse et la composition de la chair). Ces résultats ont permis ensuite de réaliser une étude financière du manque à gagner engendré par ce problème.

2. **Identifier la ou les étiologies des LC.**

La première hypothèse étant la présence de poux de mer, la première étude a consisté à suivre les populations de parasites en 2018 et 2019 pour estimer le facteur de risque lié à leur présence. Enfin, une macroanalyse de l'industrie du TRS comprenant des données d'élevage et environnementales a été menée pour estimer d'autres facteurs de risques pouvant mener au développement des LC.

IMPACT DES LESIONS CORNEENES SUR LA SANTE DU THON ROUGE DU SUD D'ELEVAGE ET SUR LE PROFIT DES ELEVAGES (voir section 3, p.51)

Les LC étaient déjà observées par les éleveurs de TRS, mais ne semblaient pas présenter de problème majeur. Au cours des années 2017 et 2018, les poissons de certains enclos (n=12 en 2017 et n=4 en 2018) furent observés au moment de la récolte afin d'estimer le score de lésion de leurs yeux. Les deux yeux de chaque poisson étaient notés, 11 973 poissons furent ainsi inclus dans l'étude. Chaque poisson étant identifié par un numéro avant la vente, le poids éviscéré, la taille et l'index d'état corporel de chaque poisson pouvaient être mis en lien avec le score attribué à leurs yeux. Ainsi l'impact sur les caractéristiques corporelles des poissons fut calculé avec une analyse transversale à l'échelle de l'individu. L'impact sur la mortalité fut calculé avec une analyse cas-témoin rétrospective à l'échelle de l'individu dans les 16 enclos utilisés pour la première étude. Un 'cas' était définit comme un TRS mort pendant le grossissement, alors qu'un témoin était un poisson ayant survécu jusqu'à la récolte. L'association entre LC et mortalité a ensuite été estimé par le calcul d'odds ratio avec un modèle utilisant une régression logistique à modèle mixte, incluant l'effet de cluster au niveau de la compagnie et de la cage. L'impact financier a été estimé uniquement avec le manque à gagner représenté par la mortalité, la perte de poids et la baisse du prix d'achat entraîné par le fait d'avoir de poissons visuellement moins attractifs du fait de la présence des LC. Le manque à gagner a été calculé à l'échelle du poisson, puis de la cage et estimé pour chaque augmentation de 1% de poisson atteint de LC au sein d'une cage.

Parmi tous les poissons observés, 2.72% présentaient des LC sévères (*i.e.* aveugle d'un ou des deux yeux), la prévalence générale de LC variant de 0.24% pour les enclos les moins atteints à 9.37% pour les plus touchés (Table 4). Pour les comparaisons des caractéristiques physiques des TRS, les valeurs ont été centralisées par rapport à la moyenne de l'enclos dans lequel ils étaient présents afin d'effacer des effets aléatoires liés à l'enclos, à la compagnie ou à l'année. Les résultats sont résumés dans la Table 5. Le poids éviscéré des individus aveugles d'un ou des deux yeux était respectivement inférieur de 3.07 Kg (95% CI : 2.55 – 3.59) et 3.19 Kg (95% CI : 1.65 – 4.74) comparé au reste de l'enclos. Leur index d'état corporel était respectivement inférieur de -0.88 unités (95% CI : -1.04 – -0.72) et -1.91 unités (95% CI : -2.39 – -1.42). L'index d'état corporel de ces animaux sévèrement touchés avait 6 fois plus de chance de se trouver dans le 5^{e} centile du bas de l'enclos (OR = 5.63, 95% CI : 4.30-7.38) (Figure 20). Aucune différence statistiquement significative n'a été mise en évidence entre les caractéristiques des TRS ayant des cornées saines et ceux avec de légères lésions.

Une association forte entre les LC et la mortalité a été mise en évidence. En prenant en compte les éventuels effets aléatoires liés à l'année, à l'élevage ou à l'enclos, le risque de mourir pour les TRS atteints de LC était 17 fois supérieur par rapport aux poissons non atteints (OR = 16.98, 95%CI : 14.50 - 19.90, Table 6).

Il a été estimé que chaque poisson atteint de LC et ayant survécu jusqu'à la récolte, représente en moyenne une perte de $AUD89,87 (95% *CI = $65,70 – $118,42)* du fait de la perte de poids et de leur aspect moins attractif, ce qui permet aux acheteurs de diminuer le prix d'achat de 10%. Chaque poisson mort représente une perte potentielle de $AUD460,26 (95% *CI = $224,69 – $741,32)*. En utilisant les données de prévalences estimées précédemment, il était possible d'estimer la perte financière engendrée par le problème des LC au niveau de l'enclos. Ainsi, ces années-là, chaque pourcent de poisson aveugle représentait un manque à gagner moyen de $AUD 7 957 (95% *CI = $2 722 – $17 241)* (Figure 21). Avec une prévalence moyenne de 4,64% sur les enclos atteints, la perte moyenne était de $AUD 37 557 (95% *CI = $12 872 – $81 294)* par enclos.

Cette étude a montré un important biais de sélection, du fait que certaines compagnies ne souhaitaient pas être inclues alors que celles qui l'étaient le plus avaient d'importants problèmes de LC. Une seule compagnie possédait peu de problèmes et nous a laissé observer leurs poissons. La population inclue dans cette étude n'était donc pas représentative de la population de toute l'industrie. Lors de l'observation des poissons au moment de la récolte, beaucoup ne pouvaient pas être inclus du fait de la rapidité du passage de chaque poisson sur la chaîne de transformation et l'impossibilité de l'observateur de ralentir la chaîne de transformation. Comme de nombreux phénomènes de lésions biologiques, certains yeux étaient difficiles à classer de manière catégorique avec le système de score développé ici et se situaient parfois entre deux scores. Cependant, ce biais fût effacé par une redéfinition *post hoc* des grades, ensuite utilisée pour réaliser l'analyse statistique. De même, chaque année, le même observateur était choisi pour noter les lésions lors de la récolte, excepté pour quelques jours où l'observateur ne pouvant pas être présent sur différents sites, une deuxième personne formée par l'observateur principal aidait à noter les lésions. Bien qu'un grand nombre de poissons aient été observés, le faible nombre de cas dans la catégorie aveugle (n=33) participe au problème d'imprécision sur certains intervalles de confiance. La différence statistiquement significative entre les poissons non atteints, atteints de manière unilatérale et atteints de manière bilatérale conforte l'hypothèse que les LC ont d'importantes conséquences sur les animaux ainsi qu'une intensité croissante avec la sévérité des lésions. L'hypothèse principale qui pourrait expliquer ces phénomènes serait que les poissons aveugles ne seraient plus assez compétitifs, ne verraient plus la nourriture et souffriraient donc d'anorexie. En utilisant un modèle à plusieurs niveaux, le risque de mourir pour les poissons aveugles était passé de 19.6 à 17. Cela met en évidence un effet de la compagnie sur le problème. Cela peut être dû à des différences d'environnement, de localisation où se trouvent les enclos des différentes compagnies, ou à des différences de gestion d'élevage. Cette question sera abordée dans la partie suivante. Enfin, l'analyse financière a montré que ce problème peut ne pas être négligeable pour les compagnies et que la perte engendrée par ce phénomène était réelle. Cependant, la perte étant liée à la prévalence de LC, les faibles prévalences observées ici ne montrent pas de problème majeur pour l'économie des élevages. Nous avions considéré ici uniquement le manque à gagner. Cela ne comprenait les autres pertes annexes, lié au coup du transport des poissons invendus, à l'alimentation gaspillé ou consommée par d'autres poissons mais certainement moins valorisée du fait que la croissance atteint généralement un plateau. Chaque poisson a également un coût lié aux droits de pêches et au quota instauré par le gouvernement. Si la prévalence des LC venait à augmenter dans le futur, ce problème pourrait représenter de lourdes pertes pour les compagnies.

INVESTIGATION DU ROLE PUTATIF DES POUX DE MER DANS LE DEVELOPPEMENT DES LESIONS DE LA CORNEE CHEZ LE THON ROUGE DU SUD D'ELEVAGE (voir section 4, p.63)

Les élevages de poissons en milieu marin souffrent pour beaucoup de parasites regroupés sous le nom de « poux de mer ». Ces derniers ont été régulièrement décrits comme agents étiologiques de lésions cutanées (S. Johnson et al., 2004), raison pour laquelle ils ont fait l'objet d'une attention particulière dans notre étude. Ils ont notamment été décrits comme agents étiologiques des LC chez le TRS (Hayward et al., 2008). Cependant, Hayward et al. ont présenté cette conclusion suite à une étude transversale qui ne peut pas écarter une hypothèse de causalité inverse, ce qui laisse un doute sur le fait que l'infestation par les poux de mer ait été antérieure à l'apparition des LC. Pour vérifier cette hypothèse, deux modèles de régression logistique ont été réalisés dans notre étude. Le premier utilisait comme prédicteurs l'intensité de l'infestation par les poux de mer, la présence de lésions cutanées et de marques de dents au même jour que l'intensité de LC considérée (analyse transversale). Le deuxième utilisait les mêmes prédicteurs, mais décalés sur une période précédant l'observation des LC dans une période de 3 semaines pour mettre en évidence une corrélation temporelle éventuelle. Les données utilisées pour l'étude au moment du transfert ont été obtenues lors du contrôle aléatoire de certains poissons par le gouvernement sur les critères imposés et pour le respect des quotas. Cent poissons par enclos de tractation étaient choisis aléatoirement. Les poux de mer prélevés étaient ensuite placés directement dans une solution de formol à 10%. L'identification de l'espèce était ensuite réalisée sous loupe binoculaire et microscope optique grâce à la description de l'espèce *C. chiastos* (Lin et Ho en 2003). Les compagnies participantes à cette étude étaient volontaires, car cela demandait une modification de fonctionnement de l'élevage du fait de la nécessité de la présence de plongeurs pour prendre les animaux en photo. Toutes les photos étaient ensuite analysées par la même personne, qui décrivait pour chaque individu présent sur les photos, la présence de poux de mers, de LC, et de lésions tel que : frottements, éraflures et entailles sur le corps, lésions cutanées au niveau du nez, et les marques de dents (Figure 15).

Dans notre étude, seuls des poux de mer de l'espèce *C. chiastos* ont été trouvés sur les TRS. La plupart étaient des femelles adultes et quatre spécimens de stades antérieurs ont été également trouvés. Une première observation avait été réalisée lors du transfert des poissons sauvages dans les enclos de grossissement. Sur les cinq enclos de tractation, trois possédaient déjà des poissons avec des poux de mer, mais à des prévalences faibles, allant de 4% à 11% et des intensités très faibles, de 2 à 2,2. En 2018, lors de la période de grossissement, 1 704 TRS pris en photo ont été inclus dans notre étude pour le suivi de la présence de poux de mer. La prévalence moyenne était de 43,9% avec une intensité d'infestation moyenne de 3,5 sur toute la population, le nombre de parasites variant de 0 à 32 par poisson (Table 8). Simultanément, une faible prévalence de LC dans la compagnie 1 avait été enregistrée et une prévalence presque nulle pour la compagnie 2. Concernant les enclos présentant des LC chez la compagnie 1, l'intensité de l'infestation par les poux de mer variait légèrement avec des périodes de chute entre fin avril et début mai pour remonter légèrement début juin (Figure 26). Au contraire, la prévalence restait haute avec un pic en avril et juin simultanément à l'augmentation de l'incidence de LC parmi les morts. A partir du mois de mai, l'incidence de mortalité augmentait lentement, jusqu'à la récolte alors que l'incidence de poissons avec des LC diminuait. Lors de la saison 2019, seule la compagnie 1 a été surveillée avec un total de 508 TRS provenant de cinq enclos différents. La prévalence moyenne de poux de mer était légèrement supérieure (47,6%) ainsi qu'une intensité de 4,2 (Table 10). La tendance de variation de l'évolution de la prévalence de poux de mer et de LC était similaire lors de la saison 2019 (Figure 27). Bien que la prévalence de LC fût plus faible qu'en

2018 pour la compagnie 1, le taux observé restait supérieur à la médiane de la population. Ces observations furent donc défavorables à l'hypothèse d'un lien entre poux de mer et LC, puisque la compagnie présentait une grande différence de prévalence de LC alors que la charge de poux de mer était similaire. Le premier modèle de régression logistique à modèle mixte réalisant une analyse transversale semblait mieux expliquer le lien entre poux de mer et LC (log likelihood=-74,5 ; p<0.001) comparé au modèle successif prenant en compte les parasites et lésions cutanées précédant les LC (log likelihood=-78,7 ; p=0.002). Les résultats sont présentés dans la Table 13. Ainsi, un modèle final a montré qu'avec un modèle de régression logistique mixte, les TRS infestés par une intensité légère de poux de mer ($intensité \in [2,6]$) avaient 1,1 fois plus de risque de développer des LC et que les poissons moyennement infestés ($intensité \in [8,32]$) avaient 1,24 fois plus de risque de développer des LC, par comparaison avec des poissons non parasités (Table 12). Le travail d'identification nous a également permis de mettre en place une clé d'identification simplifiée de l'espèce *C. chiastos*, utilisable par le laboratoire de l'ASBTIA (Figure 24).

Des biais de sélection concernant les photos prises par les plongeurs ont pu entraîner une sélection avantagée des TRS malades, car ces derniers nageaient généralement moins en profondeur, plus lentement et voyaient moins facilement les plongeurs. Il est possible que les plongeurs aient pris en photo les animaux qu'il était plus facile pour eux d'attraper, plutôt que de prendre des échantillons vraiment aléatoires. De plus, un grand nombre de photos n'étaient pas exploitables du fait de leur qualité car l'eau parfois trouble, le manque de luminosité et le courant rendaient les photos floues. Il semble que les TRS sauvages étaient très peu infestés par les poux de mer à leur arrivée, avec un rapide développement de la population de parasites dans les premiers mois de grossissement. Ce développement pourrait être facilité par une plus grande sensibilité des animaux aux parasites, du fait d'une baisse du système immunitaire lié à la tractation, les TRS semblent s'acclimater au lieu de grossissement dans une période de 30 jours (Kirchhoff et al., 2011). La contamination pourrait être due à d'autres espèces de poissons vecteurs présents dans l'environnement (Hayward et al., 2011). L'intensité observée dans notre étude était très inférieure aux intensités précédemment décrites dans la littérature (max = 495 poux de mer, C. J. Hayward et al., 2010) et par rapport avec les intensités considérées comme dangereuses ou létales dans d'autres espèces, les faibles intensités observées ici était très probablement peu significatives pour la santé d'un thon de 15 à 40 kg. Nos modèles n'ont pas permis de mettre en évidence un lien de temporalité entre l'apparition des parasites et des LC, ce qui suggère une apparition simultanée des deux problèmes. Etant donné que l'hypothèse de l'implication causale des poux de mer ne pouvait pas expliquer le développement des LC, d'autres facteurs de risques ont été étudiés, cette fois-ci à l'échelle de la cage, comparant les données pré- et post-transfert des poissons pour le grossissement.

ANALYSE DES FACTEURS DE RISQUES DANS LE DEVELOPPEMENT DES LESIONS DE LA CORNEE CHEZ LE THON ROUGE DU SUD D'ELEVAGE (voir section 5, p.83)

Cette étude avait pour but d'étudier différents critères environnementaux et de gestion d'élevage pour estimer leur potentielle influence sur le développement des LC. Comme de nombreux troubles sanitaires, il est probable que les LC aient une origine multifactorielle. Ainsi, de nombreuses variables pourraient se combiner pour installer un environnement propice au développement des LC. Le facteur de risque principal selon les compagnies de TRS était la présence des poux de mers. Cette hypothèse a été écartée à la suite d'une étude réalisée précédemment dans ce projet. L'analyse a été réalisée sur les données de cinq compagnies l'année 2017 et sur trois compagnies qui avaient continué à enregistrer les données de LC sur les TRS morts en 2018 et 2019. L'analyse a été réalisée au niveau de l'enclos. L'analyse préliminaire de l'évolution de la mortalité et du développement des LC a été réalisée avec des graphiques en série temporelle chronologique des données de mortalité et de LC cumulées. Des graphiques en boîte ont été utilisés pour étudier la présence de clusters au niveau de l'enclos, de la compagnie et de l'enclos de tractation des TRS du site de capture au site de grossissement. Les enclos étaient classifiés comme un 'enclos cas' si la mortalité cumulée dépassait 1%, et la prévalence de LC supérieure à 14% (valeur médiane à l'échelle de la population totale) parmi les morts. Une régression logistique à modèle mixte a ensuite été réalisé pour estimer l'association potentielle entre les facteurs de risques et le développement des LC.

Après les premières semaines de stockage, la mortalité augmentait toujours légèrement, probablement du fait de l'adaptation des poissons sauvages à leur nouvel environnement. Il semble qu'un cluster au niveau de la mortalité et des LC soit présent au niveau de la compagnie, et plus précisément au niveau de l'enclos (Figure 28, Figure 29 et Figure 32). Cependant, il ne semblait pas exister de cluster au niveau de l'enclos de tractation des bancs de poissons après la capture (Figure 30 et Figure 31). Suite aux demandes d'accès aux données d'élevage, les variables que nous avons été autorisé à étudier furent : le nombre initial de TRS dans l'enclos de tractation, le diamètre de l'enclos de tractation, le nombre de jour de tractation, le nombre initial de TRS dans les enclos de grossissement, le type de matériaux utilisé pour les filets, la proximité des enclos de grossissement avec un récif, le fait que les TRS présents dans un enclos de grossissement proviennent de bancs différents et le passage des poissons au cours de la tractation dans des eaux froides lié au phénomène de remontée d'eau (upwelling). Au total, 98 enclos ont été inclus dans l'analyse dont 17 étaient considérés comme des 'enclos cas', 12 en 2017, 3 en 2018 et 2 en 2019. Le modèle a mis en évidence un facteur de risque concernant le développement de LC dans les 'enclos cas' comparés aux autres enclos : la localisation de l'enclos à proximité d'un récif (valeur continue) (OR = 0,87 ; 95% CI : 0,80 – 0,95, Table 16) signifiant que pour chaque kilomètre d'éloignement d'un 'enclos cas' des récifs alentours le risque de développement de LC pourrait être réduit de 13%.

La limite principale dans cette étude a été l'obtention de certaines données d'élevage, car certaines compagnies ne souhaitaient pas fournir des informations par souci de confidentialité. Un biais de sélection était également à considérer étant donné que toutes les compagnies n'enregistraient pas la présence de LC chez les morts, ce qui avait limité la population disponible pour l'étude. Comme il a été mentionné précédemment, le développement des LC était probablement lié à de nombreux facteurs s'additionnant. Le but de cette étude n'était donc pas de trouver une cause mais des facteurs favorisants. En aquaculture, les LC observées chez d'autres espèces étaient souvent liées à une abrasion mécanique qui pourrait se soigner dans des conditions environnementales optimales, mais pouvant aussi fortement se dégrader dans le cas inverse. Dans notre analyse, le facteur de risque ressortant était la proximité avec un récif. Cependant, les enclos affectés étaient physiquement regroupés par compagnies. Un facteur confondant, pourrait donc entraîner ce résultat concernant le récif. En effet, tout enclos affecté serait ainsi à une distance similaire d'un récif, à l'intérieur d'une même compagnie. De plus, il n'y a biologiquement pas de raison que la proximité avec un récif facilite le développement de LC. Cela aurait pu être le cas si nous avions mis en évidence un lien avec les poux de mer car ces derniers sont présents sur des poissons habitants ces récifs. Cependant, l'hypothèse du lien avec les poux de mer a été écarté dans le chapitre précédant

Si les animaux se trouvent dans les enclos à une densité trop importante, cela pourrait entraîner des comportements agressifs, accompagnés le plus souvent de morsures au niveau des yeux (Ferguson et al., 2006). Bien que l'excès d'aliment ait été relié à une baisse de l'agressivité (Sunde et al., 1998), il se pourrait que le système de distribution n'ait pas été optimal et bien qu'une quantité suffisante soit donnée, une mauvaise répartition ne permet pas à tous les animaux de se nourrir correctement. Lors de la tractation des TRS, l'alimentation était limitée et les animaux arrivaient généralement au site de grossissement affamés. Lors du premier repas, l'agitation des animaux pourrait entraîner des mouvements abrasifs à l'origine des LC, car la peau et les nageoires des TRS sont très durs et les dents acérées, ou par collision avec l'enclos. Il a été montré chez d'autres espèces de poissons telles que les salmonidés, que des lésions similaires pourraient se développer suite à une déficience en vitamine A (Ferguson et al., 2006; Kitamura et al., 1967; Poston et al., 1977). Cette déficience était généralement accompagnée d'autres symptômes non observés chez les thons, mais cela pourrait être expliqué par l'importante différence physiologique entre les espèces et l'âge des animaux. L'hypovitaminose A pourrait participer au développement des LC si certains individus ne peuvent plus se nourrir et n'auraient donc plus d'apport en vitamine A.

CONCLUSION ET RECOMMANDATIONS (voir section 6, p.95)

Pour conclure, les poissons atteints de LC ont un plus grand risque de mourir avant la fin du grossissement, par rapport aux poissons sains et arrivent en moins bonne condition physique s'ils survivent jusqu'à la récolte. Ce problème est important sur un plan de la santé animale, mais pourrait l'être aussi d'un point de vue économique pour les compagnies. Au vu de la faible prévalence de LC au cours des trois dernières années, ces problèmes n'ont pas eu un impact fort sur les bénéfices des entreprises. Il devrait tout de même être considéré si les prévalences de LC augmentent dans les années à venir, car il pourrait représenter de plus importantes pertes financières. La présence des poux de mer était l'hypothèse principale de l'industrie pour expliquer le développement des LC. Cette hypothèse n'a pas été vérifiée. Cela met en évidence le caractère multifactoriel de l'étiologie des LC. Ces parasites étant déjà très problématiques chez d'autres espèces d'élevage, il est tout de même primordial de continuer à surveiller les populations de TRS pour pouvoir mettre en place un système de contrôle le plus rapidement possible, si ces parasites venaient à s'installer de manière cyclique et intense dans la région de Port Lincoln.

Un seul facteur de risque a pu être mis en évidence, la proximité des enclos avec un récif. Cependant, il semble avoir une faible influence sur le risque, qui serait d'autant plus non représentatif de la réalité, du fait de la présence d'un facteur confondant étant que chaque compagnie garde ses enclos proches les uns des autres, à un même endroit. Bien que notre étude n'ait pas pu mettre en évidence la ou les étiologies du développement des LC, ou des facteurs favorisant leur développement, il est possible d'émettre quelques recommandations pour essayer de diminuer le risque d'émergence des LC telles que :

1. Limiter la densité dans les enclos de grossissement et de commercialisation,
2. Transférer les poissons dans les enclos de grossissement le plus rapidement possible à la suite de la capture,
3. Amarrer les enclos de grossissement le plus loin possible des récifs alentours pour limiter les interactions avec les espèces sauvages,
4. Nourrir progressivement les animaux à leur arrivée sur le site de grossissement, pour éviter tout comportement agressif et favoriser la reprise de l'alimentation.

Une étude plus compète des facteurs de risque devrait être entreprise pour comprendre les mécanismes liés au développement des LC, qui semble être multifactoriel.

2. INTRODUCTION

2.1. The aquaculture in the world, stakes for the future of food consumption and veterinary medicine

2.1.1. Global situation of aquaculture

The Food and Agriculture Organization of the United Nations (FAO), defined the aquaculture (Edwards & Demaine, n.d.) as:

“*the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resources, with or without appropriate licences, are the harvest of fisheries.*”

In 2016, the global aquaculture production reached 110.2 million tonnes with 80.0 million tons of food fish and 30.1 million tonnes of aquatic plants (FAO, 2018b). Fish production stayed far behind the meat production which produced 326.8 million tonnes in 2016 (FAO, 2018a), but grew every year while the meat industry remained stable. Aquaculture was one of the fastest growing sector in the food industry as this practice was almost not existent in 1950 with less than 1 million ton of production compared to the fisheries, while in 2016 the aquaculture production represented 47% of the total production of fish in the world (FAO, 2018b) (Figure 1).

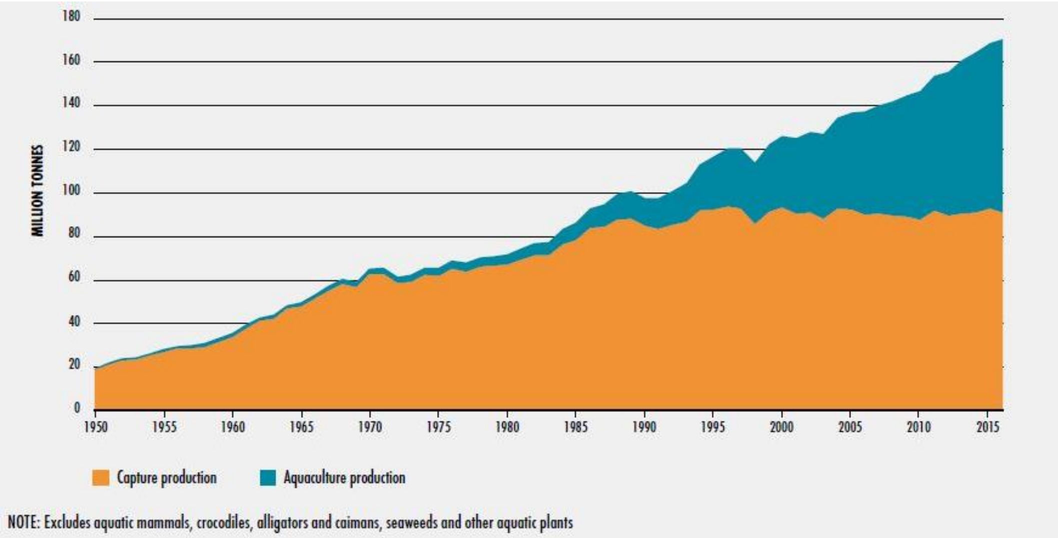


Figure 1. World capture fisheries and aquaculture production (FAO, 2018a)

2.1.2. Sustainability of aquaculture and concern of the future of aquatic wild stocks

A major preoccupation in today's ecology is the protection of the wild stock of any species. Fisheries showed a great impact on several aquatic species leading sometimes to risks of extinction. Since 2002 the number of marine fish species registered by the International Union for Conversation of Nature (IUCN) on the Red List, which gives information on the global conservation of animals, plant and fungi, had burst and particularly in categories such as Vulnerable (VU), Endangered (EN) and Critically Endangered (CE) (Figure 2, Davies and Baum, 2012).

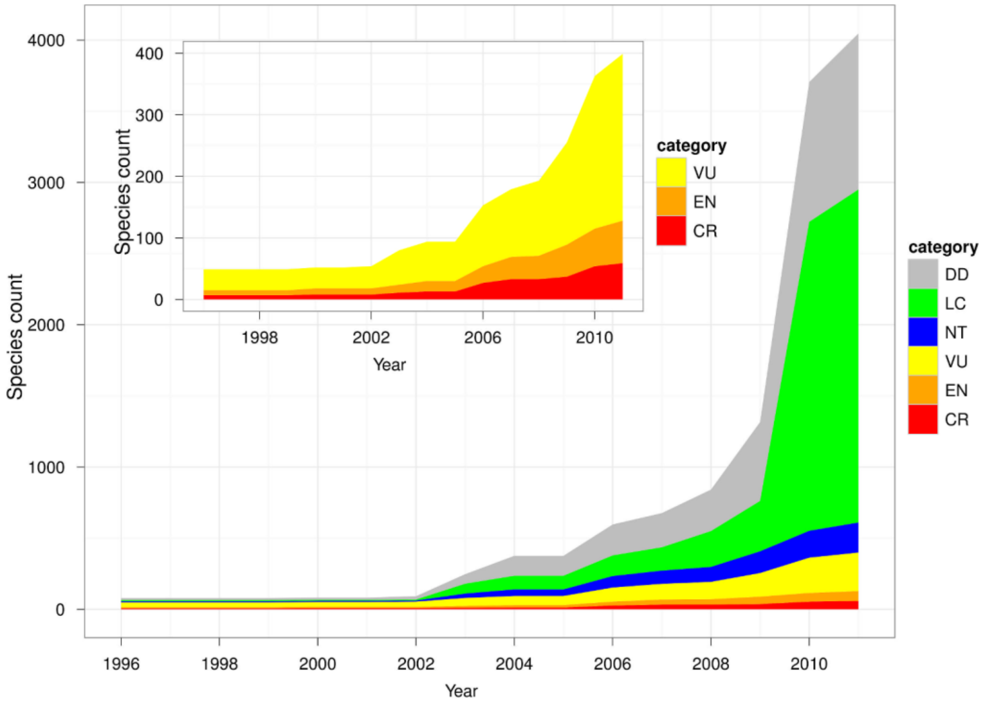


Figure 2. Total number of marine fish species on the IUCN Red List each year by category. Red List categories are Data Deficient (DD), Least Concern (LC), Near Threatened (NT), or one of the three threatened categories, Vulnerable (VU), Endangered (EN), Critically Endangered (CR). Inset is expanded view of the species listed in threatened categories: vu, en, or cr (Davies & Baum, 2012).

The reduction of the wild stock is tackled by the reduction of the number of fished animals with the implementation of restriction quotas, permitted fishing periods, and in defined fishing zones considering reproduction and migratory behaviours. Nevertheless, such selective technics could severely influence the structure of the populations in term of age, distribution, demography and their role in the ecosystem which could still be detrimental for the biodiversity and the environment (Tveterås et al., 2017). Tveterås et al. also discussed how to improve the current state of fisheries, but the solutions seem extremely complex with the need of a high level of understanding of the role and the influence of every species in the ecosystem and how they are constantly modified by climate change, which in reality did not seem achievable in the coming decades. That is why aquaculture could reduce the burden of fisheries on wild stock and present a more sustainable way of producing fish (Godfray et al., 2010).

Like all animal production systems, aquaculture also presents environmental impacts, for instance, by the accumulation of antibiotics in the water and the sediments (Kümmerer, 2009), the destruction of natural aquatic habitat like the tropical mangroves (Alongi, 2002), the pollution by waste waters (Amirkolaie, 2008) or genetic pollution (Gross, 1998). However, this industry being relatively new compared to others, a lot of investments are currently made in the development of more sustainable farms with a reduced impact on the surrounding environment (Osmundsen et al., 2020), with yields corresponding to the growing human consumption.

2.1.3. Veterinary medicine in aquaculture

The interest in aquaculture in the veterinary community is lacking. In 2018 in France, over the 18,548 registered veterinarians, only 14 (0.08%) were registered as fish veterinarians (Minitère de l'Agriculture et de l'Alimentation, 2018). The aquaculture sector offers many opportunities for veterinarians such as field interventions, development of sustainable aquaculture, fish welfare issues or research. Aquaculture is facing many challenges in health management and biosecurity. High mortality diseases are severely affecting populations like the white spot syndrome virus (WSSV) in shrimp farms, furunculosis caused the bacteria *Aeromonas salmonicida* in many fish species or parasites like sea lice which are as much a deadly threat for the fish as a welfare issue. Disease management in aquaculture implies great issues on an economic point of view because of the high density of production, and the diseases that might imply to cull the entire population, but also by the reduction of the quality of the meat and all the social consequences that come with these problems (Lafferty et al., 2015).

Veterinarians in aquaculture for the food industry must deal with these problems with complex protocols as the individual administration of drugs is almost impossible, expensive or represent a stress for the fish which goes against animal welfare standards. The medication of fish would most of the time be done through the water by bathing the fish, raising the question of the treatment of the water post administration and the environmental impact of it. The limit between responsible use of veterinary medicine and necessity of the treatment can be hard to draw. However, the use of veterinary medicines such as antibiotics or antiparasitic improved significantly the biosecurity and the control of diseases on farm sites. On the other hands, the aquaculture industry showed that many health problems can be resolved without the use of drugs but only by understanding the environment with the analysis of the water quality, the structure of the population or the behaviour of the fish. Many diseases and syndromes described in fish are still poorly understood and not effectively taken in charge. There is a strong need of veterinarians in aquaculture to be able develop the industry, working hand in hand with farmers and aquatic biologist to lead it towards sustainable and welfare friendly systems before reaching too large and intensive scale that slows down any attempt of change.

2.2. Tuna farming overview

2.2.1. The southern bluefin tuna – *Thunnus maccoyii*

The southern bluefin tuna (SBT), *Thunnus maccoyii*, (Castelnau, 1872), is a marine fish with a bullet-like shape, able to fold its dorsal and pectoral fins into grooves area which allows better hydrodynamics when diving in deeper areas. Its dark metallic blue back with silvery flanks is covered of small scales, has almost a round section and a pointy nose (Figure 3). Its average length ranges between 40cm to 1.5m for an average weight ranging between 20 to 50kg but some individuals can go up to 2.5m and weight 400kg (Burgess, 2019). It is classified Critically Endangered on the IUCN Red List of Threatened species (IUCN, 2009).

Figure 3. Underwater picture of a juvenile southern bluefin tuna, *Thunnus maccoyii*, in a grow-out cage from the tuna industry of Port Lincoln. It is recognisable with the dark metallic blue back and the silvery flanks, a bullet-like shape of the body with a pointy nose and a groove under the pelvic fin. Photography taken by Dr. Charles Caraguel

They have a long migration pattern from the South-Ouest Atlantic near South Africa, to the Indian ocean, to the South Pacific Ocean, and pass through the Great Australian Bight (GAB) during the Australian summer to feed at the age of one to five years (Figure 4). Only in the GAB, juvenile SBT stay closer to the surface, in the top 20m, to warmer temperatures and stay slightly deeper in mid-summer when the underlying water levels mix and warms up, or when the moon is bright and that SBT can have a better visibility (Great Australian Bight Research Program, 2017). On the opposite, outside the GAB, they dive more frequently and deeper than 200m, up to 600m during the day from the age of three. The particularity of SBT is that they are endotherms fish that will keep their body temperature higher than the water temperature, allowing high swimming performances and optimal digestion rates (Great Australian Bight Research Program, 2017).

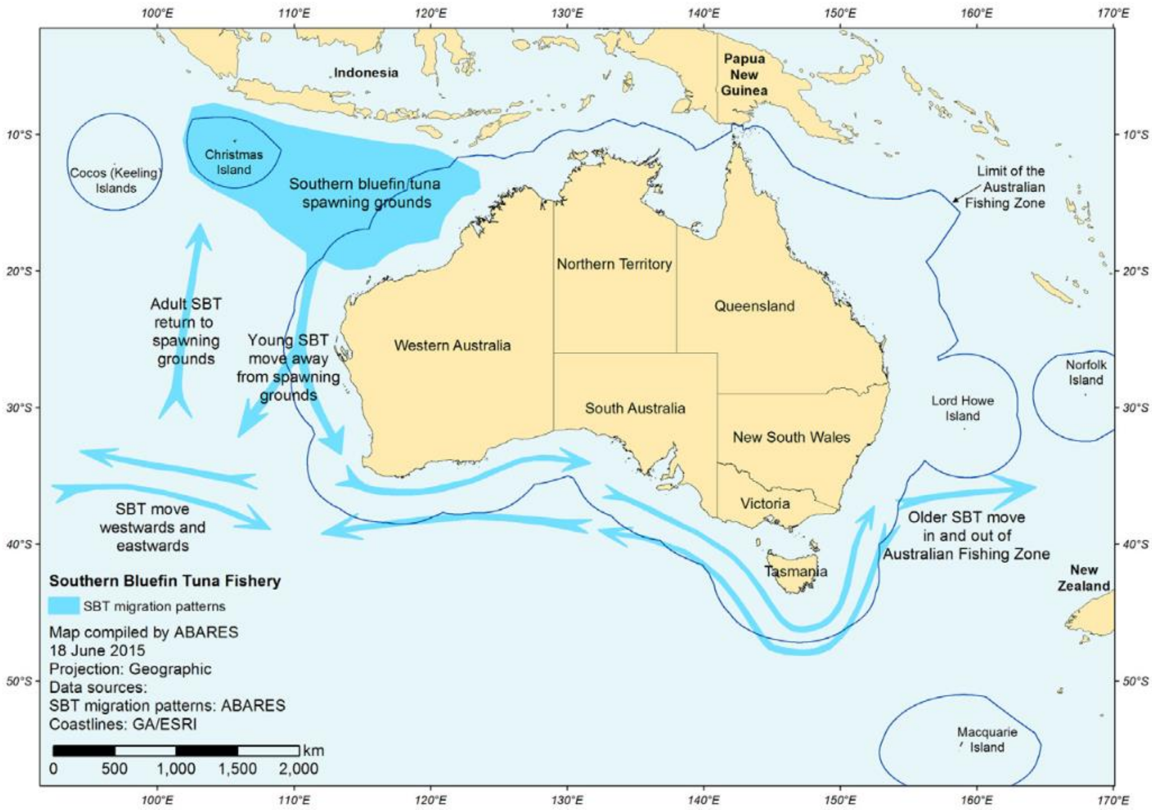


Figure 4. Southern bluefin tuna migration patterns. Map provided by ABARES (*Australian Bureau of Agricultural and Resource Economics and Sciences*) (Ellis & Kiessling, 2016).

2.2.2. Tuna farming: history, process and global situation (Benetti et al., 2016)

Research in tuna farming started in the 1960s, but it was only in the 1990s that the production reached an industrial scale. Only recently the cycle of tuna farming had been closed, but hatcheries are only established in Japan or in small scale productions in Europe. The tuna aquaculture includes only bluefin tuna with the pacific bluefin tuna (PBT), *Thunnus orientalis* (Temminck & Schlegel, 1844), in Japan and Mexico, the Atlantic Bluefin Tuna (ABT), *Thunnus thynnus* (Linnaeus, 1758), in the Mediterranean Sea and the SBT in Australia (Figure 5). Yellowfin Tuna (YT), *Thunnus albacares* (Bonnaterre, 1788), was cultured in Mexico and Oman but the productions stopped.

Figure 5. Tuna cage from Port Lincoln holding SBT. A diver's boat and a feeding boat are anchored next to the cage. Farmers can move around the cage by walking on a buoyant plastic circle.

Consumers commonly refer to “tuna” including many species from the *Scombridae* family such as PBT, ABT, SBT and YT but also skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758), bullet tuna, *Auxis rochei* (Risso, 1810), frigate tuna, *Auxis thazard* (Lacapède, 1800), bonitos (*Sarda*), and big eye tuna, *Thunnus obesus* (Lowe, 1839). The genius *Thunnus spp.* represents the high-value tunas while the other ones are low-value tunas. The high value of the genius *Thunnus spp.* led to a decrease of the wild stocks making species like the SBT characterized “Critically endangered” by the International Union for Conservation of Nature and Natural Resources (IUCN, 2009), meaning that the risk of extinction of this species in the wild was at high risk. This was the main issue bringing the industry to develop tuna farming. The word “farming” is commonly used to describe this type of production. However, “ranching” is more accurate as the SBT industry uses adults or subadults which are fattened for several month, adding market value to the fish. Tuna ranching is at the edge between aquaculture and fisheries. With the wish to reduce the pressure on wild stocks, five intergovernmental organisations were created from the 1950s to the 2000s to control and implement sustainable measures in the tuna industry: the Inter-American Tropical Tuna Commission (IATTC), the Western and Central Pacific Fisheries Commission (WCPFC), the Commission for the Conservation of Southern Bluefin Tuna (CCSBT), the Indian Ocean Tuna Commission (IOTC) and the International Commission for the Conservation of the Atlantic Tunas (ICCAT). Their main missions concern stock management, sustainability of the production and biotic or abiotic factors that could impact wild stocks and human populations.

The global tuna catch approximated 7.5 million metric tons in 2014 (FAO, 2018b) but only a third were from bluefin tuna, yellowfin tuna and big-eye tuna. The aquaculture production of bluefin tuna (PBT, ABT and SBT) was of 51,000 metric tons in 2016 (Tveterås et al., 2017) with a production growth of between 9 to 13% each year. The high value of the bluefin tuna is mainly due to the Japanese consumption, usually as sashimi and sushi, which is of 400,000 to 500,000 metric tons per year. The average price varies between 40-100 USD/kg for high-grade bluefin tuna and 10-20 USD/kg for low grade bluefin tuna. The price record was made in January 2019 with a 278kg wild bluefin tuna bought by Kiyoshi Kimura ("Tuna King") for USD3.1m or 11,151USD/kg.

2.2.3. The southern bluefin tuna ranching industry of Port Lincoln

After the second world war, the South Australian government started surveys of tuna fish stocks in the Great Australian Bight and realised the potential resource represented by the tuna school migrating. European immigrants, mostly from Croatia participated to the expansion of the fishing industry in Port Lincoln. The fishing techniques improved quickly in the 1970s evolving from pole fishing (Figure 6) to purse seine fishing and led to mass catch, peaking to 21,000 tonnes in 1982.

Figure 6. Fisherman from Port Lincoln catching tuna by pole fishing, between 1950s and 1960s (credits: Tacoma preservation society).

SBT is a quota restricted wild fishery where the global allowable catch is shared between 14 different countries and is regulated by the CCSBT. Quotas were implemented in 1989, to help SBT wild stocks to recover from overexploitation by fisheries. It led the ranching engineering to improve in order to keep the tuna industry of Port Lincoln (South Australia) as much profitable as it has been so far. Every year, the CCSBT calculates a new quota corresponding to the current stocks. For the last three years, the global total allowable catch for SBT was of 17,647t with 6,165t allocated to Australia, the latter being almost exclusively held by the tuna industry of Port Lincoln.

The ranching of SBT, starts with the capture of 2 to 3 years-old wild fish using purse seine nets in the Great Australian Bait (Figure 7). For this technic, the boat releases one end of the net and then surrounds the tuna school until reaching back the net. The bottom of the net is weighted to sink while the top is buoyant. After the catch, the bottom is closed to prevent the fish to escape.

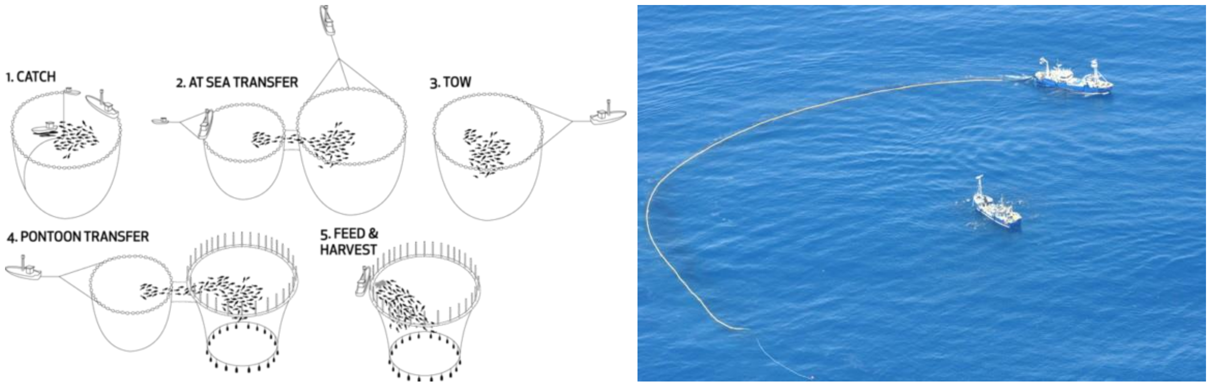


Figure 7. Purse seine fishing technique method (on the left) in the case of southern bluefin tuna. On the right, a picture of the catching (picture taken by the ASBTIA). The school of fish are spotted from a helicopter, caught, transferred to a tow cage and towed to Port Lincoln where they will be transferred to grow-out cages, fed for four to six month and then harvested.

The fish are then towed to the farms. SBT schools are targeted by helicopter and specifically chosen regarding specific criteria, making the use of purse seine more specific and reducing the by-catch issues usually associated with this technic. The school of SBT is then towed usually for 15 days back near the coast. The fish are kept in cages anchored at 10 to 20km from the shore off Port Lincoln (Figure 8), where they will be fattened for four to six months. The diameter of the cage is of 45m and the net is 10 meters deep, containing 1100 to 7000 fish. The cycle of production for SBT in Port Lincoln is not closed, leading farmers to capture wild fish every year. After several month of ranching, all the fish are harvested and most of them are sold to the Japanese market. Thanks to this short farming cycle this industry suffers not much from parasitism or diseases. The companies only treat the fish with praziquantel once a year to fight against blood flukes, *Cardicola forsteri* (Cribb, Daintith & Munday, 2000), by injecting the chemical in pilchards, used to feed the fish.

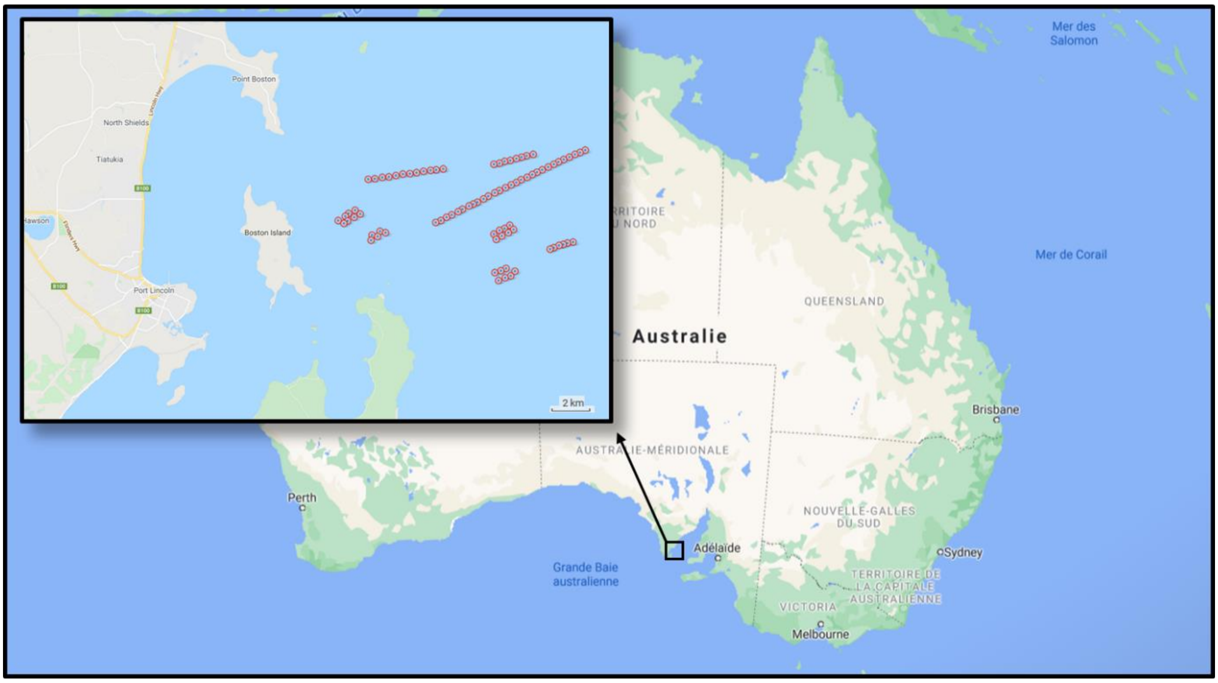


Figure 8. Location of the cages of the tuna industry in Port Lincoln, South Australia. Each red circle represents a tuna cage, which are grouped by companies.

The tuna industry of Port Lincoln targets a mortality rate across all the companies of 1%. In 2017, some cages experienced 3% to 8% of mortality with most of the mortalities showing evidences of eye lesions. These lesions were recognizable by the eye becoming cloudy, fully white or black and sometimes perforated. The Australian Southern Bluefin Tuna Industry Association (ASBTIA) works with all the tuna companies of Port Lincoln, especially for Research and Development to improve the ranching of the SBT and monitor the health of the fish.

2.3. Descriptive analysis of mortality across the tuna industry of Port Lincoln in 2017

2.3.1. Preliminary observations

During the 2017 ranching season, several companies reported a rise of mortality in some cages with up to 90% of the mortality collected, showing unilateral or bilateral ocular lesions. Ocular lesions ranged from local or complete opaqueness to perforated eyes.

The 23rd of March 2017 the Future Fisheries Veterinary Services (FFVS) performed clinical examinations on random tunas. The first tuna killed on the boat weighed 24kg, had its left eye perforated and no lesions were visible on the right eye. The fish was in poor general condition and had abrasions on both sides of the body. No food was found in the stomach, but a dark green bile was found in the gall bladder meaning that the fish did not eat for at least 24 to 48 hours. No external parasites were found on the fish, and no blood flukes, *C. forsteri*, were found in the heart (adult stage) or in the gills (eggs). The second fish killed on the boat weighed 25kg and had an ulcer on half of the left eye with a complete opacity of the cornea and no lesion observed on the right eye. No food was found in the stomach, but a dark green bile was found in the gall bladder. This fish was suffering from a severe skin abrasion on both sides of the body with scale losses. Two individuals of *Caligus spp.* (Caligidae, Burmeister 1835) were found on the left side of the head and six *Euryphorus sp.* (Caligidae) at the base of the gill arch. No blood flukes were found in the heart or in the gills and no other parasitic organisms on the skin.

On the 28th of March 2017, Kirsten Rough, marine biologist at the ASBTIA, observed a clinically affected fish with both eyes showing severe eye lesions and more than 50 *Caligus spp*. on it. During a tagging trial none of the fish caught had eye lesions but the prevalence of *Caligus spp.* on the fish was estimated at 27% with intensity of infestation ranging from 0 to 10 per fish. During the tagging of a second cage of the same company no fish had eye lesions but the prevalence of *Caligus spp.* was estimated at 53% with intensity of infestation ranging from 0 to 20 per fish. The divers reported that the clinically affected fish with visible eye lesions swam slowly and were unlikely to feed when the lesions were severe. They also had an erratic swimming with the inability to show a schooling behaviour.

The lesions on the eyes seemed to be located on the cornea and more likely to be a traumatic injury as no bacteria or protozoa were find. The depletion of the lymphoid tissue in the spleen revealed a chronic stress and an inflammatory challenge for the fish. No bacteria or protozoa were found in the other organs, the damage of the eye did not lead to a systemic infection. The primary hypothesis for the causation of the eye lesions was that the external parasitic sea lice, *Caligus spp.,* caused pruritis leading the tuna to rub on hard surfaces causing a trauma on the cornea. No evidence suggested a systemic infection, but most of the fish were anorexic. No registered treatment exists for *Caligus spp.* in farmed SBT.

2.3.2. Descriptive analysis of the mortality data

The tuna industry of Port Lincoln was composed of ten companies, all members of the ASBTIA. All the companies recorded the mortality of the fish through the ranching season but the frequency of eye lesions (EL) within the mortality was not always recorded. The industry had companies of various sizes from small scales, owning three cages and farming less than 14,000 fish per year, to large scales, owning 18 cages and producing more than 60,000 fish per year (Table 1).

Table 1. Description of the Southern bluefin tuna industry of Port Lincoln by a summary of stocking data from 2017

Season	2017
Number of companies	10
Number of cages	86
Number of fish	268,299
Cage per company	3-18
Fish per company	11,492 - 62,034
Fish per cage	1,139 - 6,997

In 2017 the global mortality rate increased to 1.67% while the target of the industry without major events was to stay below 1% of cumulative mortality. Previously, two major events led to an increase of mortality. The first and most important one was the infestation by the "blood fluke", *C. forsteri*, in the heart ventricle of the fish, from 2007 to 2010, with a peak of mortality higher than 12% across the whole industry (Aiken et al., 2006). The second event was due to extremely rough weather conditions leading to the unmooring of tuna cages that increased the mortality to more than 3% (Kirsten Rough, personal communication, 2017). The mortality history of the tuna industry of Port Lincoln from 1997 to 2017 is presented on the Figure 9.

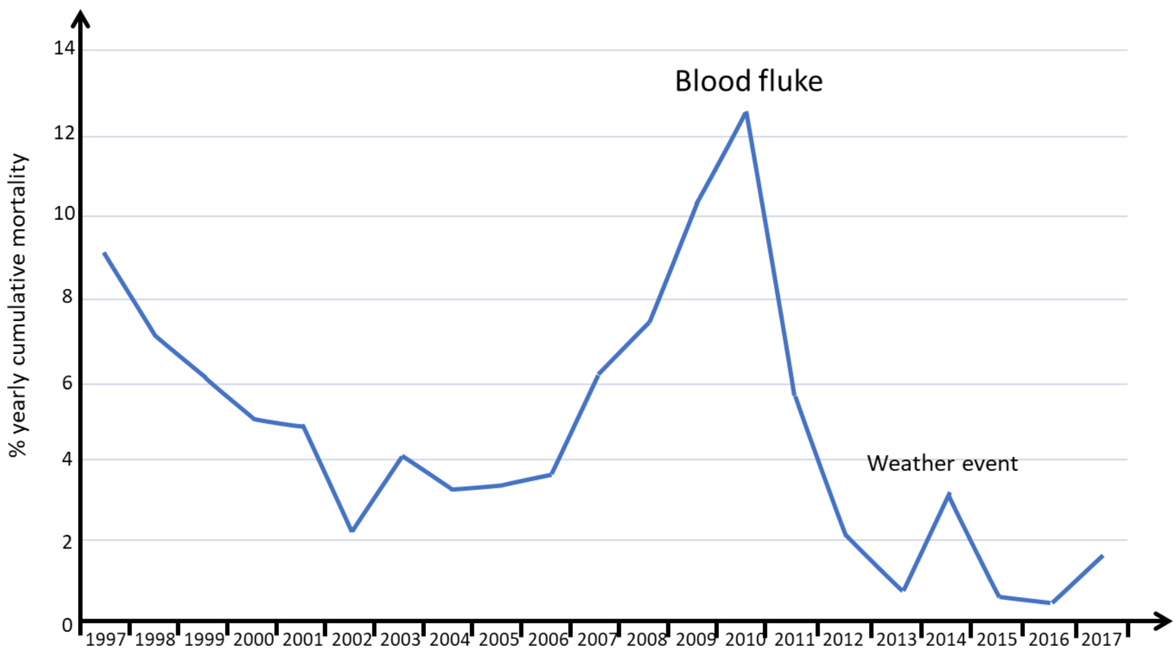


Figure 9. Mortality history of the southern bluefin tuna in the tuna industry of Port Lincoln (SA, Australia) from 1997 to 2017.

The increase of cumulative mortality to 1.67% (n=10, σ=1.9) in 2017 alarmed the ASBTIA which decided to start an investigation to understand the reasons of this rise. This percentage was calculated for the entire industry, as it could be assumed with the high standard deviation, the high mortality could be attributed to several companies only.

At the scale of a ranching season, the mortality peaked on short periods (Figure 10). In 2017 the mortality increased at three moments. The first and most intense event happened at the beginning of March, the second one at the beginning of April and the third one in June.

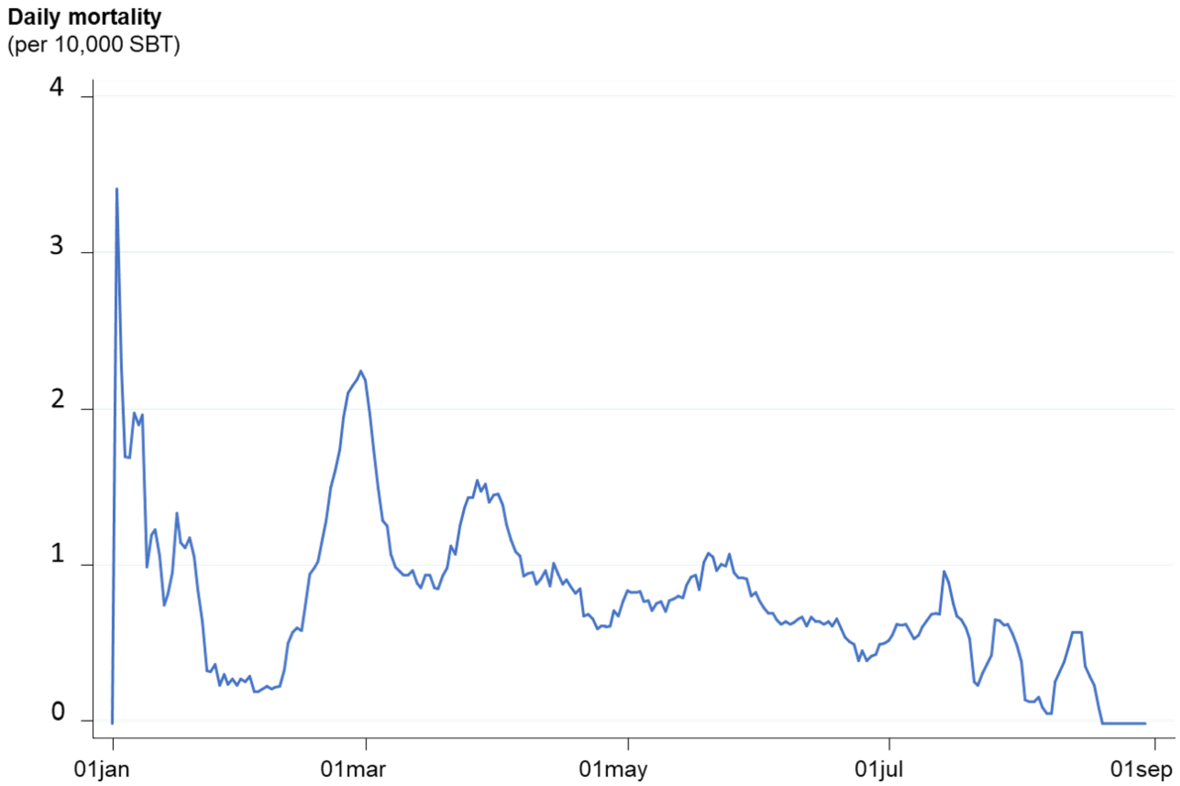


Figure 10. Daily mortality through the 2017 ranching season. The number of deaths is given for 10,000 fish.

The Table 2 shows the broad picture of the cumulative mortality and the prevalence of dead fish bearing EL during the 2017 ranching season. The high global cumulative mortality was associated with four companies. Companies 1, 3, 6 and 10 had respectively 4.5%, 4.3%, 2.6 and 5.1% of cumulative mortality. EL were a clinical sign that was already described by the SBT farmers but always as anecdotal observations. However, in 2017 some companies that experienced a high level of mortality were also experiencing a high level of EL.

Table 2. Cumulative mortality during the 2017 ranching season at the level of the company. Negative inferior limit for the confidence intervals were reported as 0 as a negative mortality is not possible; *Company with a high cumulative mortality; **Company with high cumulative mortality and high prevalence of eye lesions

	Cumulative mortality (%)		Eye lesions (%)	
Company	Mean	Confidence interval	Mean	Confidence interval
1**	4.5	0 – 10.9	1.1	0 – 3.5
2	0.4	0 – 0.9	0.09	0 – 0.39
3**	4.3	2.0 – 6.7	2.2	1.0 – 3.4
4	0.8	0 – 1.9	0.03	0 – 0.16
5	0.4	0 – 0.8	0.08	0 – 0.31
6*	2.6	1.0 – 4.2	N/A	.
7	0.5	0 – 1.0	N/A	.
8	0.5	0 – 1.1	N/A	.
9	0.2	0 – 0.5	N/A	.
10*	5.2	2.3 – 8.1	N/A	.

It seemed that the high cumulative mortality at the level of the company could be attributed to some cages (Figure 11). The cages with more than 1% of mortality represent 37.2% of the total number of cages, showing evidences of a clustering at the cage level.

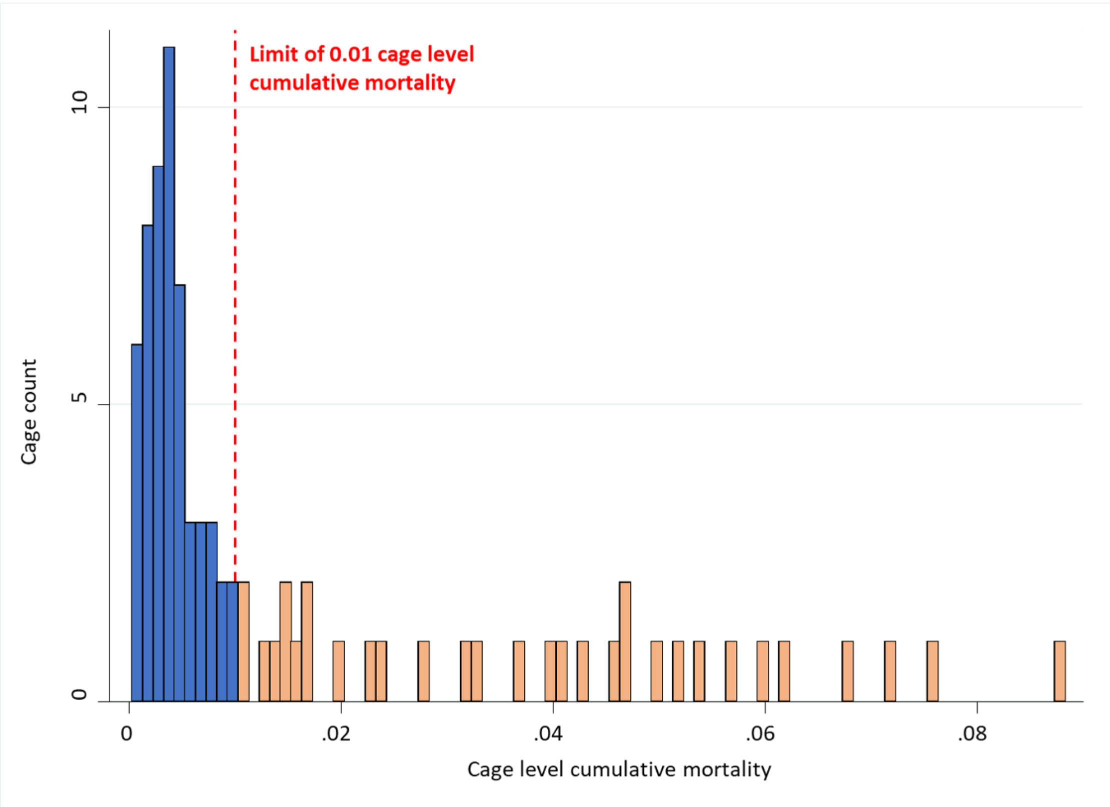


Figure 11. Cumulative mortality distribution across cages. Blue columns represent the cages under the 0.01 cumulative mortality; the orange columns represent the cages with a cumulative mortality higher than 0.01.

As already mentioned before, EL were more frequently observed in companies with a high mortality. Therefore, the companies started to collect data about eye lesions within the mortalities while others were already monitoring them. A linear relation between the cumulative mortality with EL and the cumulative mortality within the cages was brought to light (Figure 12), showing a first evidence of correlation between the mortality and this ophthalmic symptom. By defining the limit at 1% for the total mortality and the limit at 0.11% (median percentage of cumulative mortalities bearing CL across the five companies in 2017) for the blind mortality four zones can be defined. Still few cages are in the top-right zone showing high mortality and high corneal lesion while most of the cages are in the bottom-left zone representing the expected "good" condition of the fish defined by low mortality and low or no eye lesions. However, many cages shifted in the top-left zone showing a higher prevalence of eye lesions with a low mortality but still getting closer to the limit. Hypothesis could be made to describe this as the harvest happening before the mortality peak, with a late development of the eye lesions or a moderate affect allowing some fish to heal and surviving until harvest.

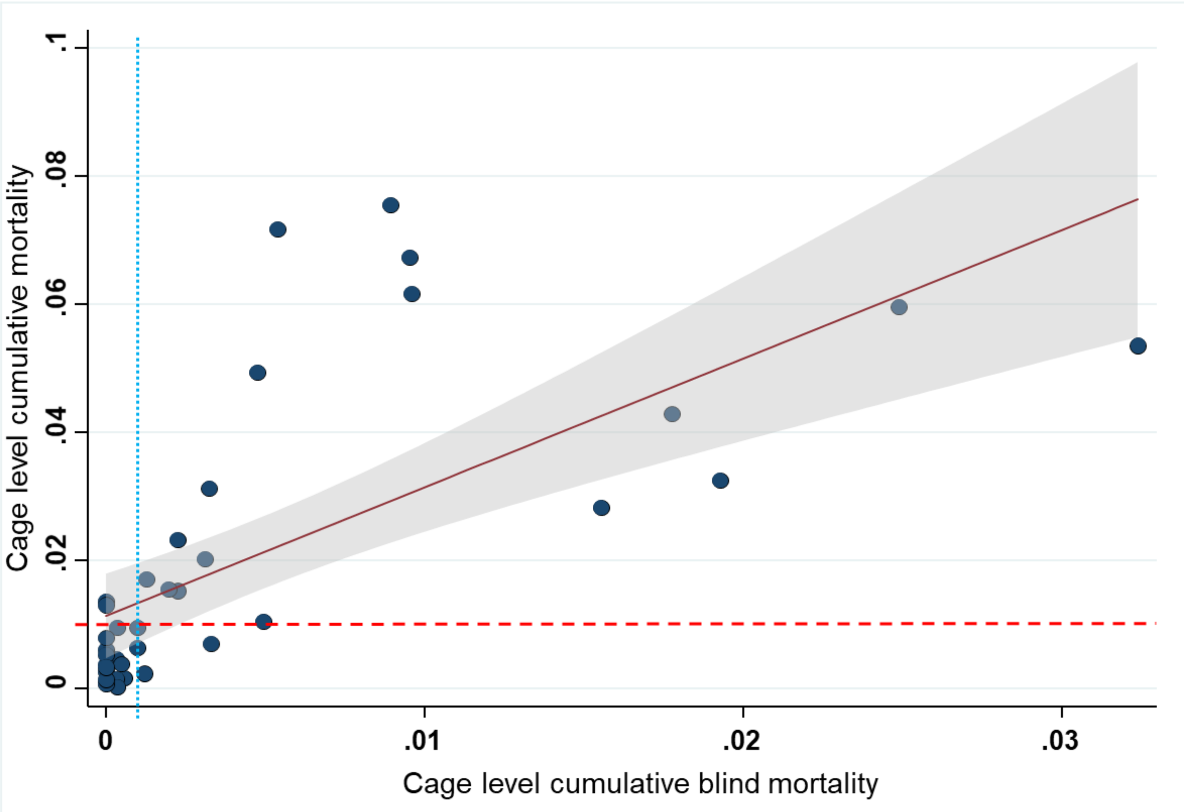


Figure 12. Relation between the cumulative blind mortality and the total cumulative mortality within the cages in the companies 1, 2, 3, 4 and 5. The dashed red line represents the 0.01 limit of cumulative mortality tolerated by the industry; the dotted blue line represents the 0.0011 limit of cumulative blind mortality that was chosen as definition of a problem of eye lesions in a cage.

All these first evidences in 2017 lead the ASBTIA and the companies to start investigating the potential link between the mortality and the increase of EL. The same analysis on 2018 and 2019 strengthened this hypothesis (see below, section 5.3.1 p.86). Moreover, it allowed us to look for potential patterns in the affection.

To conclude this affection seemed to be clustered to some cages with a mortality correlated with fish suffering from eye lesions. This affection could have great consequences on the companies as some cages reached more than 6% cumulative mortality during the ranching season and several were around 4% of cumulative mortality.

2.4. Anatomical pathology of the corneal lesions

2.4.1. Anatomy and physiology of the eye in fish

(adapted from R. R. Dubielzig, 2010; Fath El-Bab, 2004; Kern & Colitz, 2013; Roberts, 2012)

The anatomy of the eye in *Scombridae*, and more precisely in tuna was not described yet in the literature. The following will present the general anatomy of the eye in teleost fish which is the infraclass including *Scombridae* and most of the known fish, the other infraclass being the elasmobranchs.

Most structures in the eye of teleost fish are similar to those of other vertebrates (Figure 13). The external structure, the sclera, bears the insertions of the oculomotor muscles and has the particularity compared to mammals to have a cartilaginous support. Usually in mammals, the lens is an ellipsoid structure with the ability to change its shape with the contraction of the ciliary muscle giving a variable refraction index. In fish the lens is almost a perfect sphere with a poor ability to change the refraction index and a short focal length. A small accommodation can be made by the contraction of the *retractor lentis* muscle and the *falciform process* (Figure 13) moving the lens towards the retina and usually protruding through the iris giving a large angle of view and a very narrow anterior chamber. The choroid has a function of nutrition for the retina providing oxygen and nutrients by the mean of a dense capillary network. The retina has a similar organization as other vertebrates, composed of rod and cone receptors cells. The colour vision in teleost fish vary according to the environment in which they live (freshwater/sea water, shallow water/deep waters, etc.).

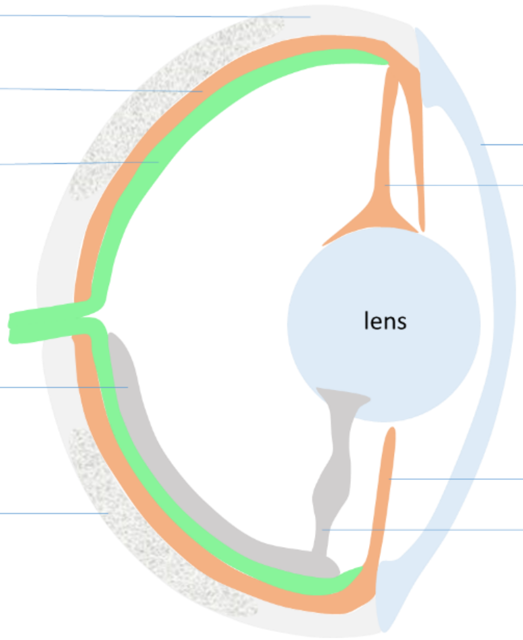


Figure 13. Diagrammatic representation of the teleost eye (adapted from Fath El-Bab, 2004)

The cornea is following from the sclera at the front of the eye. It is a transparent medium with a similar refraction index to water, meaning that the light is not refracted when passing through the cornea. It is composed of five layers: the epithelium, the Bowman's layer, the stroma, the Descmet's membrane and the endothelium. Compared to mammalians, the cornea is thicker, and hydration is made by the epithelium. The thickness differs according to the environment of the fish. Freshwater fish seem to have a thicker cornea and the thickness increases with age, body length and corneal diameter.

The corneal epithelium has first a mechanical function in preventing the entrance of pathogens as a physical barrier. The layer is composed of three types of cells: the squamous cells, non-keratinized, organized in multiple layers, the number of layer depending on the species of fish; the wing cells which are polyhedral cells; the basal cells, a single layer connecting the epithelium to the underlying basal membrane (Figure 14). The Bowman's layer is a thin layer having a great importance in the healing of corneal damage. It is mainly composed of type IV collagen, laminin and fibronectin. The stroma in a lamellar rigid structure, with the function of keeping the structural integrity of the eye to avoid shape modification which would imply a change in optical properties (Figure 14). This tissue is avascular, and its clarity will be modified by any physical modification in its composition. It is mainly composed of cells, collagen and glycosaminoglycans. The Descemet's membrane is the basal lamina of the corneal endothelium (Figure 14). The endothelium is a single layer cell structure. Its function is to keep stable the hydration of the stroma by pumping water out to the anterior chamber to maintain the pressure gradient and the optic characteristic of the medium (Figure 14).

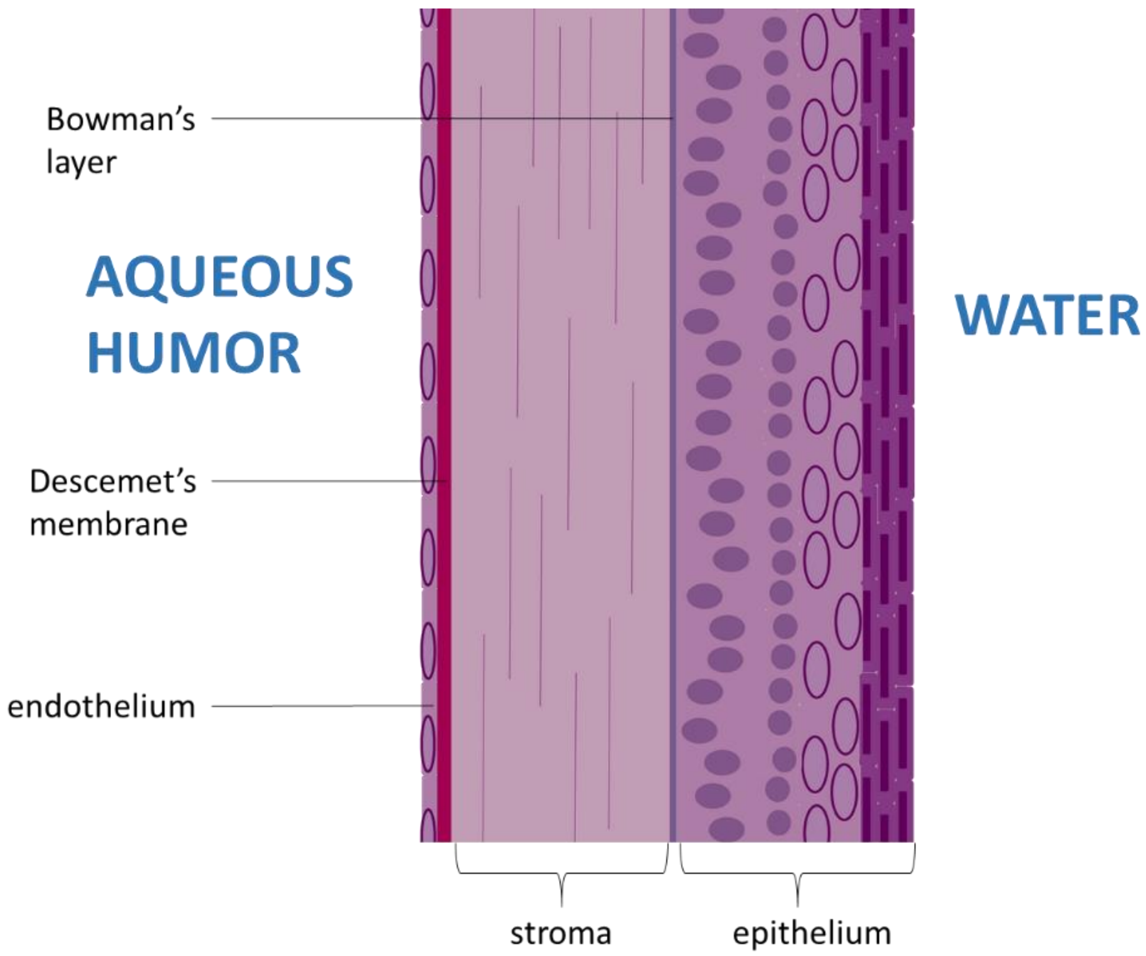


Figure 14. Diagrammatic representation of the cornea in teleost fish.

2.4.2. Macroscopic description of the lesions

EL were observed in tuna farming for many years (Hayward et al., 2008; Rough, 2000; Rough et al., 1999). However, no precise aetiology in the literature could be found and no description of the anatomical pathology either. The aim of this short study was to give a probable explanation of the pathological mechanism in order to: (1) define the type of lesion and (2) understand how these lesions could occur.

When observed alive in grow-out cages, fish bearing EL had a white or partially white eye, sometimes with scratches on the nose, the head or the body, easily spotted as white discoloration of the skin, and most of the time on the same side than the affected eye (Figure 15). They had an erratic swimming, were usually slower, sometimes at counter-current from the school, and bumped into obstacles such as peers or nets. The photos below were on the boat from moribund or dead fish, but the behaviour and the discoloration could be spotted on several fish from the boat, next to the cages.

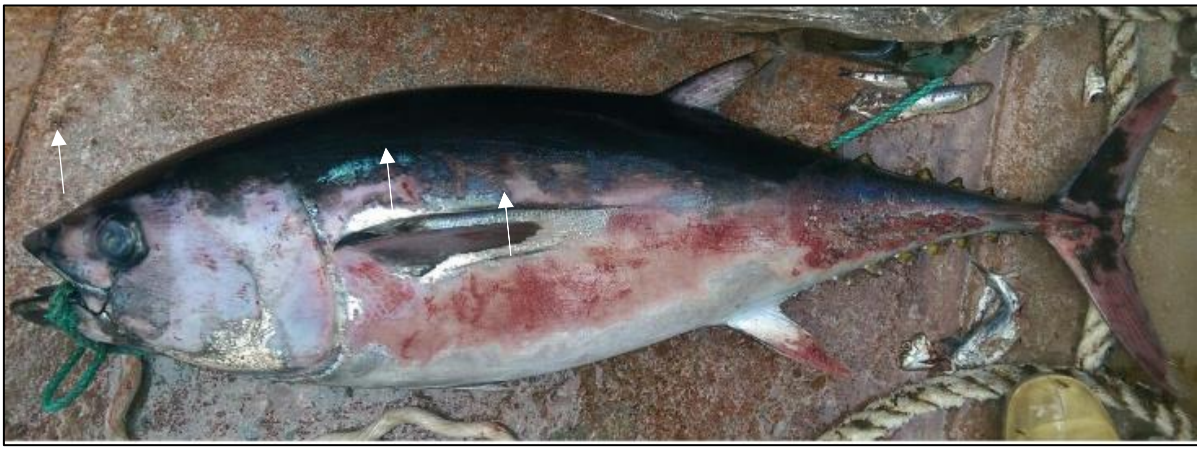


Figure 15. Moribund tuna ranched in Port Lincoln (South Australia), with perforated eyes. White discolorations and rashes are visible on the skin. Photography from the Future Fisheries Veterinary Services.

A complete perforation of the central part of the eye could also be spotted in some individuals, with a surrounding redness of the sclera and opacity of the remaining cornea or partial ulceration of the cornea (Figure 16).

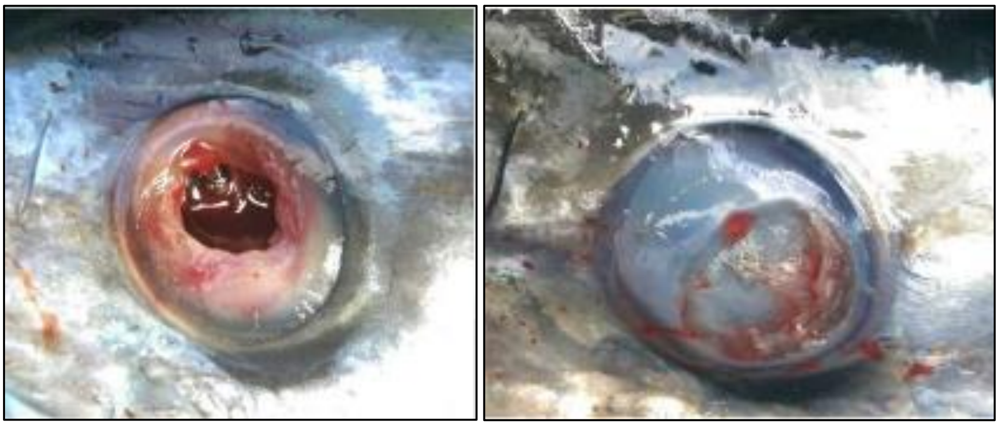


Figure 16. Complete perforation of the eye (on the left) and severe ulceration of the cornea with complete opacity of the surrounding cornea. Photography from the Future Fisheries Veterinary Services.

When the eyes were dissected a clear difference could be made between unaffected eyes and severely affected eyes, the latter showed marked disruption to the structural organisation of the internal tissues (Figure 17). On the left picture we could see a clear cornea, an intact lens, a translucent aqueous humor, and delineation of the retina and the sclera, whereas on the right picture, the cornea was opaque, thicker and internal structure were not readily identifiable, replaced and effaced by chronic inflammation and scarring.

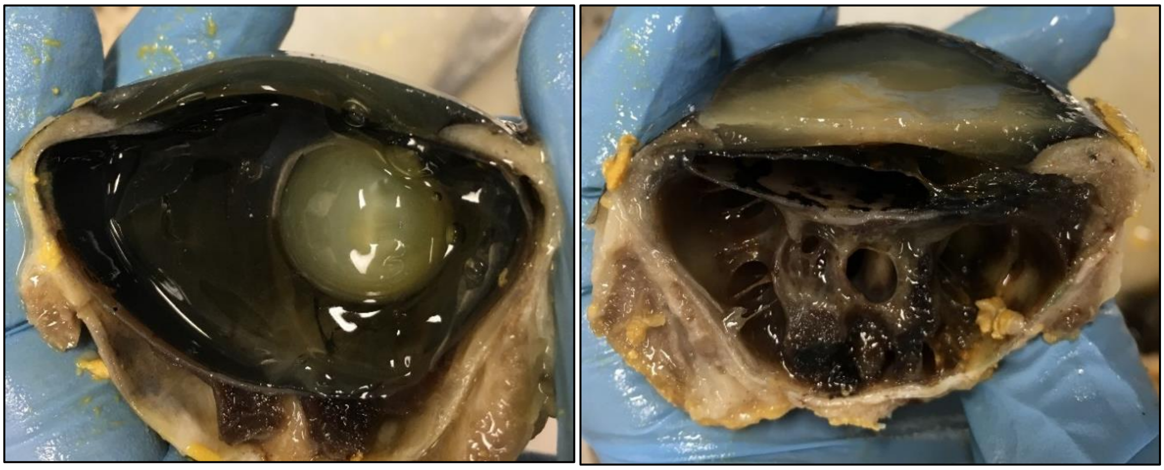
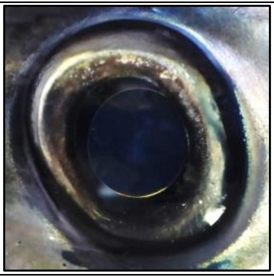
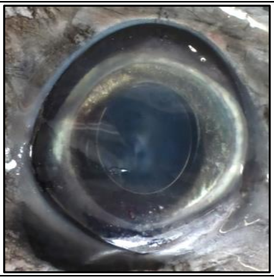
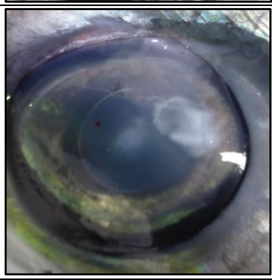
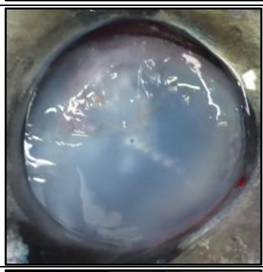
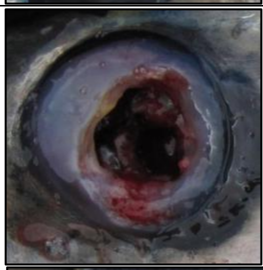


Figure 17. Dissected tuna eyes fixed in 10% buffered formalin. Unaffected eye on the left and severely affected eye on the right. On the left picture we could see a clear cornea, an intact lens, a translucent aqueous humor, and delineation of the retina and the sclera. On the right, the cornea was opaque, thicker and internal structure were not readily identifiable, replaced and effaced by chronic inflammation and scarring.

These observations suggested a chronic inflammatory response leading to structural tissue injury, loss and scarring, and subsequent complete loss of function of the cornea. From here, the EL will be now considered as corneal lesions (CL). A modified version of the 5-scale CL scoring system developed by Hayward et al. (2008) was made to score the eyes of the SBT (Table 3):

Table 3. Scoring scale for corneal lesion (CL) in southern bluefin tuna modified from Hayward et al. (2008).

Corneal lesion score	Visual aspect	Definition
Score 0		eye with a fully transparent cornea
Score 1		eye with part(s) of the cornea appearing cloudy (milky but transparent enough to let the light go through and to see the pupil)
Score 2		eye with part(s) of the cornea appearing thickened, white, and opaque (not transparent enough for the light to go through and to see the pupil)
Score 3		eye with a fully thickened, white, and opaque (not transparent enough for the light to go through and to see the pupil) cornea but with a normal (ellipsoidal and flat) shape of the eye
Score 4	 	eye with a fully thickened, white, and opaque (not transparent enough for the light to go through and to see the pupil) cornea with or without redness (neo-vascularisation) and/or black (melanin deposit) and with a deformed shape in depression of the eye. In severe cases, the cornea is perforated

2.4.3. Histopathology examination of corneal lesions

The general anatomy of the fish eye and particularly of the cornea was described in Section 2.4.1 (p.41). The histological slides for the normal eye and the severely affected eye were made from the eyes presented in Figure 17. During the harvest, some fish were beheaded before being sold. Eyeballs from these fish were taken to be observed at the laboratory of the ASBTIA and of the University of Adelaide. The samples were first open in the sclera to enable the liquids to enter the eye then kept in 10% buffered formalin.

Normal eye

The epithelium was pluristratified with usually 4 or 5 cells in thickness (Figure 18). The cells were attached to each other and to the Bowman's membrane. Areas showed breaks in the epithelium. Yet this might be due to sectioning artefact as the breach is clear-cut and no sign of inflammation or abnormal tissue was visible. The wide stroma was composed of laminated eosinophilic collagen fibrils interspersed with few flattened nuclei (presumptive fibroblast or fibrocytes). The collagen fibres were well differentiated, the separation between some of them was due to the histological technique.

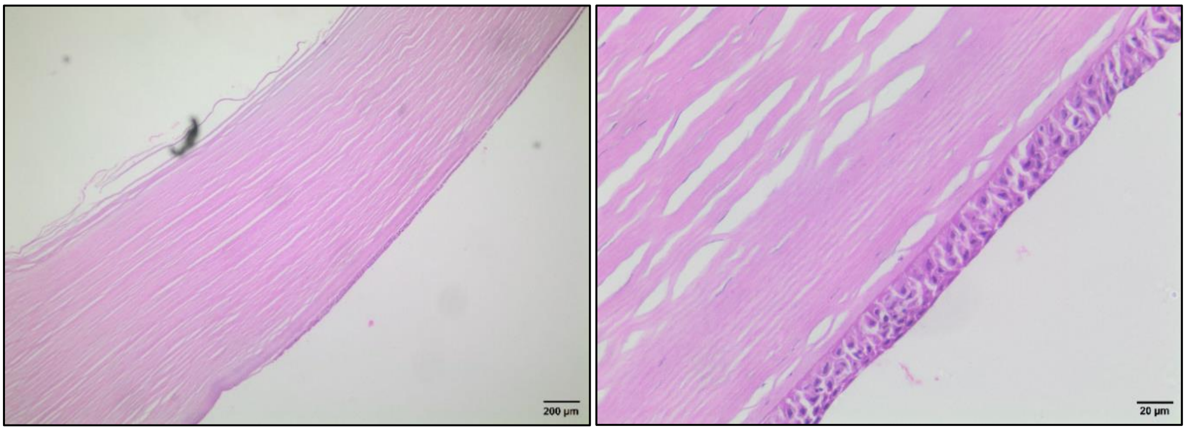


Figure 18. Histological observation of the cornea of a tuna with a normal eye.

Severely affected eye

The Figure 19 showed pathological modifications of the eye structures. First, the corneal epithelium was thickened compared to a normal eye, the thickness of the sructure exceeded 20 cells. The epithelium seemed still attached to the corneal stroma, but this latter had completely lost its layered structure. There was a marked increase in cellularity of the corneal stroma, largely attributable to fibrocollagenous proliferation, inflammatory cell infiltration and blood cells associated with neovascularisation, with capilaries fenestrating the stroma.

After the stroma, a large cavity should be present (the anterior chamber of the eye), filled by aqueous humor, and usually showing no cells or tissues on histology. Here we could see a tiny gap compared to a normal eye, immediately followed by an other biological structure. Due to many structural modifications this second part was hardly identifiable. It could be post inflammatory tissue, modified iris, retina or choroid.

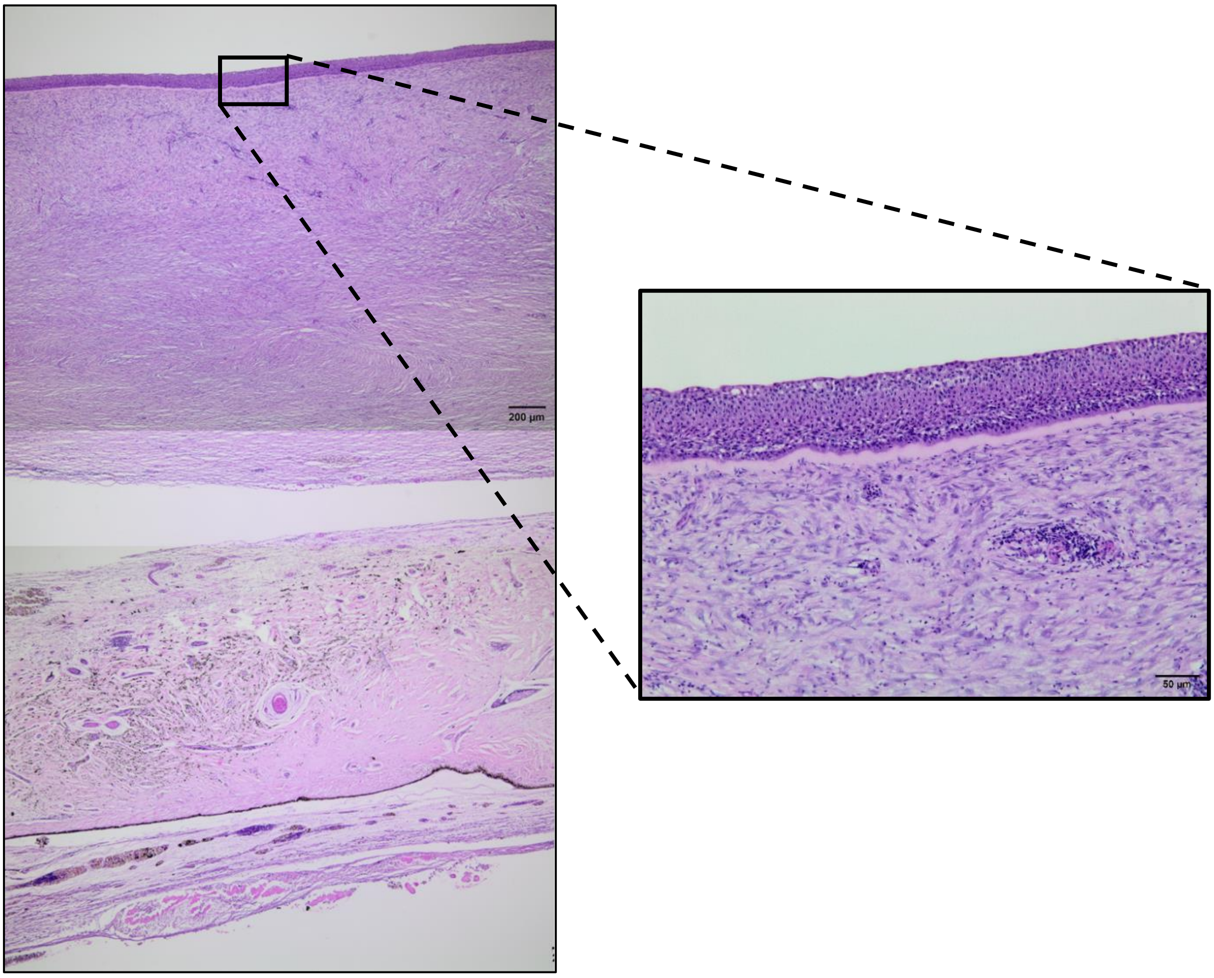


Figure 19. Histological observation of the cornea of a tuna with a severely affected eye.

2.4.4. 'Cloudy eye' disease in fish – Differential diagnosis

Many ophthalmic diseases in fish refer as 'cloudy eye', as one or more tissue will become whitish. This modification can appear within different tissues as the cornea, the anterior chamber or the lens.

Ophthalmology in tuna is still poorly documented. However, some corneal or lens cloudiness had already been described in some species of tuna. Traumatic injuries are common in farmed tuna by rubbing against the nets of the cages or to other individuals as these fish have tough skin and fins, leading to cataracts, corneal ulcers or eye loss (Munday et al., 2003). Parasitic diseases seemed also to be involved in some ophthalmic alterations as external parasites such as *Caligus chiastos* (Hayward et al., 2011), *Caligus elongatus* (Rough et al., 1999) could graze directly on the cornea or make the fish rub against hard surfaces and cause corneal ulcers.

Other diseases leading to whitening in the eye were described in farmed or aquarium fish. UV irradiation was described as possible aetiology to cataract (Munday et al., 2003; Roberts, 2012). In some species of fish traumatic injuries could be due to contact with jellyfish sting or fighting (Roberts, 2012). White cataracts could be observed as a result of the infestation by metacercaria of *Diplostomum spp.* (Shariff et al., 1980). *Cryptocotyle spp.* infecting marine fish and *Ichtyophthirius multifiliis* infecting freshwater fish, respectively known as "marine ich" and "freshwater ich", led to skin and gills lesions. An infection on the cornea would then be at the origin of a keratitis with the risk of a secondary bacterial or fungal infection resulting in ulceration. Corneal ulceration was described in wild plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) following the infection of the lymphocystis virus (Russell, 1974).

Alimentation is the base of health and welfare in all farmed animals. Understanding and managing the alimentation is a great challenge, especially in aquaculture as many sectors still feed with baitfish. Deficiencies in micronutrients could be the source of ophthalmological lesions. A deficiency of riboflavin (vitamin B2) will result in a detachment of the corneal epithelium and corneal oedema (Hughes et al., 1981) while zinc deficiency could result in cataracts lesions (Ketola, 2018). Hypovitaminosis A was also associated with hyperplastic changes in the cornea of farmed fish species like salmonids (Ferguson et al., 2006). A systemic granulomatous disease in farmed sea bream (*Sparus aurata*) was first described in the 1970' and was then associated with dietary and environmental factors blocking the metabolism of tyrosine (Paperna et al., 1980). One of the symptoms of this disease is the accumulation of whitish materials in the anterior chamber, usually affecting only one eye (Roberts, 2012; Smith, 2019).

Finally, the "gas bubble" disease, an environmental disease due to high saturation of dissolved gas in the water such as nitrogen and oxygen cause the formation of bubble in the eye potentially leading to the rupture of the cornea and the collapsing of the eye.

Neoplasia lesions were never described in the cornea or the lens of fish, but only in the retina (Roberts, 2012).

2.4.5. Discussion on the diagnostic of the corneal lesions observed in the tuna industry

The principal hypothesis after such observation would be the setting of a corneal oedema, probably after a mechanical wound causing a corneal ulcer. This pathological pathway is common is other species as in mammals and was frequently described (R. Dubielzig et al., 2010). After a mechanical abrasion of the epithelium, the cornea is weakened leading to structural and functional modifications part of the healing process. In the epithelium, acanthosis which is the hyperplasia of the epithelium cells, leading to the thickening of the tissue and could be also associated with melanosis that would explain the blackened aspect observed in several score 4 eyes. The main inflammatory response usually happens in the stroma. First, neovascularisation grows from the limbus, enabling the immune cells infiltration (keratitis), then blood plasma would leak with the movement of the cells and settle the oedema. Another hypothesis to explain the severity of the oedema would be that the breach in the physical separation between the stroma and the sea water would lead to osmoregulation issues. The sea water, highly concentrated in Na^+ and Cl^- ions could cause stromal dehydration, leading to a severe fluid leakage from the neovascularisation capillaries and from the anterior chamber to the stroma.

Even if the origin of the wound was not clearly identified, neither the pathological ways of the lesion development or the healing process, the observations led to strengthen the assumption of the evolution of lesions in the cornea. In the following parts, we will refer to the lesions as corneal lesions (CL).

2.5. Aims of the study

The aims of this study were:

1. to determine if CL represented a significant issue for the tuna industry of Port Lincoln.

To answer this question the analysis started with investigating the frequency of CL across the industry, then the potential link between the CL outbreak and the mortality by conducting a case-control study over the 2017 and 2018 ranching seasons, the impact of the CL on the fish that survived until the harvest was estimated using physiological indicators. Using these results, a financial analysis of the impact of eye lesions on the tuna industry was carried out in order to have hindsight on the relevance of the situation.

2. to identify the aetiology (or aetiologies) of the CL.

The main hypothesis from the tuna industry being the presence of sea lice, our first investigation on the aetiology consisted in monitoring the burden of the sea lice *C. chiastos* over the 2018 and 2019 ranching seasons, and to estimate the potential risk that this parasite could represent in the development of CL in SBT. The last stage consisted in carrying out a macroanalysis of the tuna industry, through the management of the companies, the features of each site of production and their environment in order to find the other potential risk factors facilitating the development of CL.

Finally, the main results will be the base of our recommendations to try preventing any new CL outbreak.

3. HEALTH AND ECONOMIC IMPACT OF CORNEAL LESION IN RANCHED SOUTHERN BLUEFIN TUNA

3.1. Introduction

The employees of the tuna farms who first described the eye lesions were talking about a white or cloudy stain of the eye. A lot of pathological lesions can lead to this appearance in the transparent structures of the eye. As described in the section 2.4.1 (p.41), the aetiology for the commonly described “cloudy eye” in fish is wide. This affection was observed since the early times of Southern bluefin tuna (SBT), *Thunnus maccoyii*, farming in Port Lincoln (Rough, 2000) and described recently as being corneal lesions (CL) but was not a health issue yet. In the early 2000’, the emergence of a new copepod parasite, *Caligus chiastos*, led to an epidemiologic monitoring that showed numerous SBT bearing gross eye injuries (Hayward et al., 2008). However, CL were not the centre of interest of these study as they did not seem to cause any global impact on fish growth and mortality across the industry. In 2017, as described in the section 2.3 (p.35) the SBT industry faced an increase of cumulative mortality that was particularly striking some companies. In parallel, farmers were describing a more frequent observation of CL. This chapter presents the impact of the CL on tuna metrics such as their weight, length and condition index (a common index used in tuna markets, explained more in detail in section 3.2, p.51). Then we investigated if this affection could be associated with the increase of mortality and finally how it could impact the SBT industry on a financial level.

3.2. Material and methods

3.2.1. Data access and collection

Mortality data

Stock entry and daily mortality counts were accessed from three companies in 2017, and one company in 2018. These companies were selected because (i) they recorded the count of dead SBT with visible CL as reported by divers and (ii) were willing to participate. For each company, the investigation focused on cages that experienced increased mortality with CL as well as one additional cage per company that had no apparent CL concerns for reference. Overall, 12 study cages in 2017 (company 1: n=5, company 2: n=2, company 3: n=5) and four study cages in 2018 (all from company 1) were investigated.

Harvest data

SBT from study cages that 'survived' until harvest were assessed during processing. On the processing line, both eyes of each SBT were scored using the modified version of the 5 scale CL scoring system developed by (Hayward et al., 2008) described above (Table 3).

The head on gill-and-gutted (HOGG) weight (kg) and the forklength (FL) (m) of each fish were recovered retrospectively and linked to the observed eyes' score using SBT individual processing tag number.

Financial data

The study is based on the observation and measurement of 33,200 fish from 11 net cages (8 from the 2017 production season and 3 from the 2018 production season, across 2 companies. The Australian Southern Bluefin Tuna Industry Association (ASBTIA) provided all the data about SBT price per kilogram and grading of the fish. The HOGG weights used to estimate the revenue loss were taken from the results of the impact on harvest fish metrics (see section 3.3.1, p.54).

3.2.2. Analysis

Impact on productivity

A cross-sectional analysis was conducted at the individual fish level using harvest data only. The full bodyweight (BW) was back calculated by adding 12% weight to HOGG plus 1 kg for each fish (*BW = HOGG x 1.12 +1*) as per the Office of Parliamentary Counsel recommendations (*Fisheries Management Regulations 2019*, 2019). For each SBT, the body condition index (BCI) was calculated from their BW and FL as follow:

$$BCI = \frac{BW}{FL^3} \qquad \textit{(Eq. 2)}$$

Individual fish metrics (HOGG, FL and BCI) were centralised by withdrawing the cage average from the individual value (e.g. $HOGG_{centralised} = HOGG_{individual} - HOGG_{cage\ average}$). This centralisation of the fish metrics facilitated comparisons and reporting by removing any cage, company and year marginal effects. The vision of each individual SBT was categorised according to both eye CL scores as follow:

Normal vision - SBT with both eyes scored as ≤ 1;

Impaired vision - SBT with one eye scored as 2 and the other eye scored as ≤ 2;

One-eyed - SBT with one eye scored as ≥ 3 and the other eye scored as ≤ 1; and

Blind - SBT with one eye scored as ≥ 3 and the other eye scored as ≥ 2.

Centralised fish metrics were compared across vision categories using simple linear regression. Bonferroni correction was used to adjust for multiple pairwise comparisons between vision categories. The level of significance α is adjusted to α/m where *m* is the number of possible pairwise comparisons (*m* = 6).

Impact on mortality

A retrospective case-control analysis was conducted at the individual fish level using mortality and harvest data from the 16 study cages. A case was defined as an SBT that died during the ranching season (before harvest) while a non-case (control) was defined as an SBT 'alive' until harvest. The exposure of interest was presence (or absence) of CL in the case and in the control SBTs. CL were observed and reported by divers during the season or scored and recorded by one of the investigators (T. Dumond) at processing. At processing, an SBT found with at least one eye with a grade ≥ 3 was classified as CL positive.

The association between CL and mortality was investigated by estimating the odds ratio between the two variables using a simple logistic regression in which CL was the only predictor. To account for the clustering of fish within a cage, cages within a company, random effect were respectively added for 'company' and 'cage-within-company' into the model (i.e. mixed effect model). The direct outputs of the model's coefficients, ignoring random effects, provided company- and cage-specific estimates of association. To be relevant to the industry as an all, population-averaged estimates of the odds ratios (OR), and their 95%CI limits, were converted using the following approximation formula (Dohoo et al., 2009):

$$\text{OR} = exp\left(\frac{\beta_{CL}}{\sqrt{1+0.346\times(\sigma^2_{company}+\sigma^2_{pen})}}\right) \qquad \textit{(Eq. 1)}$$

where β_{CL} is the model fixed effect coefficient estimate for CL and $\sigma^2_{company}$ and σ^2_{pen} are the model's variance estimates for the random effects.

Analyses for mortality and productivity impacts were implemented in the statistical package STATA v.15.1 (StataCorp Ltd, Texas, USA).

Impact on revenues

A retrospective case-cohort analysis was conducted using mortality and harvest data only from study cages that experienced substantial CL (>1% of CL) to estimate the revenue loss attributable to CL at the fish, cage, and company level.

At the fish level, if a CL affected SBT died, the loss of revenue ($R_{dead\ CL}$) was calculated as:

$R_{dead\ CL} = ((HOGG_{death} + HOGG_{gain}) * Market\ price$ (Eq. 3)

where $HOGG_{death}$ represents the HOGG of the SBT at the time of death ('actual' product loss), $HOGG_{gain}$ represents the gain in HOGG between time of death and hypothetical harvest if the SBT did not died and ('opportunity' product loss) and *Market price* is the price offered per kg of HOGG SBT. The addition $HOGG_{death} + HOGG_{gain}$ is the HOGG at harvest of an SBT not affected by CL. If a CL affected SBT remained alive until harvest, the loss of revenue ($R_{harvested\ CL}$) was calculated as:

$R_{harvested\ CL} = [HOGG_{loss} + (HOGG_{CL} * 10\%)] * Market\ price$ (Eq. 4)

where $HOGG_{loss}$ represents the HOGG difference between CL and non-CL SBT at harvest (estimated from the analysis of impact on productivity), $HOGG_{CL}$ is HOGG at harvest of a SBT affected by CL, and '10%' reflects the down pricing applied by buyers for 'lower aesthetic' product.

At the individual cage level, the loss of revenue was calculated as:

$R_{cage} = N_{Cage} * \%_{CL} * (\%_{dead/CL} * R_{dead\ CL} + (1-\%_{dead/CL}) * R_{harvested\ CL})$ (Eq. 5)

where N_{Cage} is the SBT count in the cage, $\%_{CL}$ is the cumulative proportion SBT affected by CL in the cage and $\%_{dead/CL}$ is the cumulative proportion of CL fish that died in the cage. At the company level, the loss of revenue was then calculated by multiplying R_{cage} by the number of cages in a company affected by CL.

To account for the inherent variability of some input parameters (e.g. $HOGG_{loss}$), a stochastic modelling approach was used by allocating a distribution for each varying parameter. Input parameters values were sampled from the distributions to calculate the value of our outcome of interest. This step was repeated using Monte Carlo iterative process to obtain a distribution reflecting the variability and uncertainty around the final outcome. The simulation was run for 100,000 iterations and the outcomes' distribution were reported with the mean, 2.5th and 97.5th percentiles. The calculations were implemented in MS Excel using the PopTools Excel add-in v3.2 (PopTools 2011).

3.3. Results

3.3.1. Consequences of corneal lesions of fish surviving until harvest

Across 2017 and 2018, 11,973 out of 72,866 harvested SBT (16.4% coverage) from the 16 study cages (12 from 2017 and 4 in 2018) were assessed at processing (23,946 eyes scored). Not all SBT could be assessed at processing due to logistical constrains (i.e. study cages from separate companies harvested on the same day at different locations, SBT harvested from a same cage were processed in separate lines or at different locations). Between 164 and 2,606 fish were inspected from each study cage, which represented between 7.30% to 70.9% coverage of the harvested fish per cage. Overall, 2.72% of the fish were found with severe CL (one-eyed or blind) (Table 4). The CL prevalence was consistent between the two ranching seasons but varied between 0.24% and 9.37% across cages.

Table 4. Count (N) and percentage (%) of southern bluefin tuna assessed at harvest in 2017-18 across vision categories.

	2017		2018		Total	
Vision categories	N	%	N	%	N	%
Normal vision	6,044	94.5%	5,335	95.6%	11,379	95.0%
Impaired vision	170	2.66%	98	1.76%	268	2.24%
One-eyed	160	2.50%	133	2.38%	293	2.45%
Blind	20	0.31%	13	0.23%	33	0.28%
Total	6,394		5,579		11,973	

Table 5 summarises the comparison in HOGG, FL and BCI across the four vision categories. Centralisation of the SBT metrics successfully cancelled any year, company or cage effects (see almost nil random effect variance estimates in Table 5). The HOGG of both one-eyed and blind fish at harvest was significantly lighter by respectively 3.07 Kg (95% CI: 2.55 – 3.59) and 3.19 Kg (95% CI: 1.65 – 4.74) compared to the rest of their cage. The FL of one-eyed fish at harvest was significantly shorter by 2.8 cm (95% CI: 2.1 – 3.5) compared to the rest of their cage while blind fish did not differ significantly from the rest of their cage. One-eyed and blind fish had significantly lower centralised BCI, -0.88 units (95% CI: -1.04 – -0.72) and -1.91 units (95% CI: -2.39 – -1.42), respectively. Blind fish also had a significantly lower BCI compared to one-eyed fish (Table 5). Overall, one-eyed and blind fish tended to be towards the lower sized fish within a cage (Figure 20). The BCI of these fish was also 6 times more likely to be in the bottom 5th percentile of the cage (OR = 5.63, 95%CI: 4.30-7.38).

Table 5. Outputs of mixed effect models comparing head on gill-and-gutted weight (HOGG in Kg), forklength (FL in cm) and body condition index (BCI) across vision categories (fixed effects) compared to the cage average (centralised values). Within a fixed effects column, categories sharing the same upper letter were non-significantly different from each other using a Bonferroni adjusted P-value.

Metrics	**Centralised HOGG**	**Centralised FL**	**Centralised BCI**
Fixed effects	*Mean (95%CI)*		
Normal vision	0.10[a] (0.01 – 0.18)	0.08[a] (-0.03 – 0.20)	0.03[a] (0.001 – 0.05)
Impaired vision	-0.31[a] (-0.85 – 0.23)	-0.39[a] (-1.12 – 0.35)	0.03[a] (-0.14 – 0.20)
One-eyed	-3.07[b] (-3.59 – -2.55)	-2.76[b] (-3.46 – -2.05)	-0.88[b] (-1.04 – -0.72)
Blind	-3.19[b] (-4.74 – -1.65)	-1.28[ab] (-3.38 – 0.83)	-1.91[ab] (-2.39 – -1.42)
Random effects	*Variance (95%CI)*		
Year	5.90×10^{-9} (8.19×10^{-24} – 4.24×10^{6})	6.18×10^{-9} (1.62×10^{-23} – 2.36×10^{6})	7.44×10^{-10} (5.66×10^{-25} – 9.77×10^{5})
Company	2.49×10^{-9} (9.27×10^{-18} – 0.67)	2.47×10^{-9} (5.64×10^{-18} – 1.08)	3.23×10^{-10} (1.17×10^{-18} – 0.09)
Cage	6.33×10^{-9} (3.19×10^{-14} – 0.001)	7.07×10^{-9} (1.76×10^{-14} – 0.003)	7.96×10^{-9} (3.33×10^{-14} – 0.001)

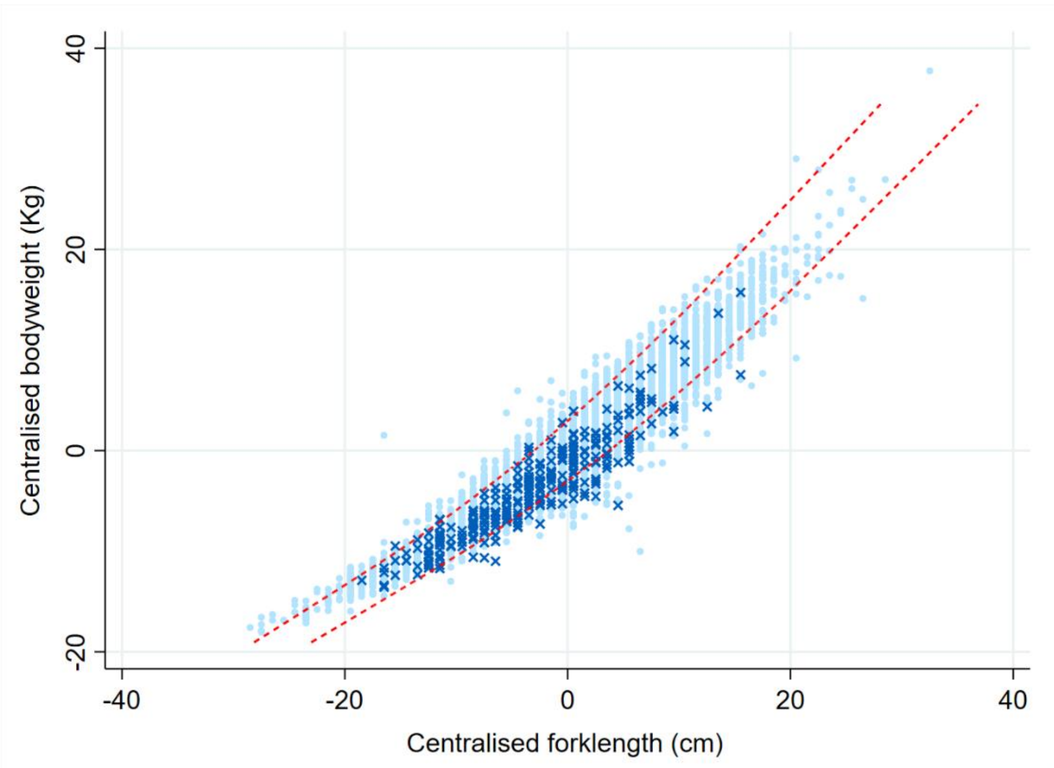


Figure 20. Scatter plot of centralised bodyweight and forklength of one-eyed or blind (blue cross, n = 326) and normal or impaired vision (blue dot, n = 11,647) southern bluefin tuna at harvest (total n = 11,973) in 2017 and 2018. The red dashed lines represent the 5th (lower line) and 95th (upper line) percentile of body condition index across all fish.

3.3.2. Risk of mortality for fish presenting corneal lesions

A strong and significant association between CL and mortality was found when comparing the frequency of CL in dead and in harvested SBT from the 16 study cages. After accounting for company and farm effects, the odds of dying were 17 times higher for CL fish than for non-CL fish (OR = 16.98, 95%CI: 14.50 - 19.90, Table 6). The mortalities and therefore this association varied greatly across cages within a company. Almost no year or company effect was found because of the small number of years (n = 2) and companies participating (n = 3).

Table 6. Multilevel model of the association between mortality and blindness of the fish.

Variables	Estimates	95%CI	p-value
Fixed effect	*Odds ratio*		
Corneal lesion	16.98	14.50 – 19.90	p<0.001
Random effects	*Variance*		
Farm	1.80×10^{-14}	-	
Cage	1.035	0.511-2.094	

3.3.3. Economic impact of corneal lesions in the tuna industry

Financial impact at the fish level

Among the monitored fish the average weight of fish in the non-blind group was 28.3 (±13.4) kg. The average weight in blind fish surviving until harvest was lower by 3.17kg than the non-blind fish. The average price per kilo in the industry is $AUD15/kg. The blind fish usually attract a lower price because they are downgraded by the traders and are sold at 90% of the full price. Therefore, each harvested blind fish represented a revenue loss of $AUD89.87 (95% *CI = $65.70 – $118.42)*. Each dead blind fish represented a revenue loss of $AUD460.26 (95% *CI = $224.69 – $741.32)*.

Financial impact at the cage level

The probability for a blind fish to die was approximated with the ratio of blind mortalities within the total of mortalities at 0.3694 (95% *CI = 0.3440 – 0.3948)*. The average number of fish in a grow-out cage across the industry was 3,100. Among the monitored cages, those with blind fish issues had on average a prevalence of 4.64% blindness.

On average, the revenue loss for a given cage with a higher incidence of blind fish was estimated at $AUD 37,557 (95% *CI = $12,872 – $81,294)*. A way to better understand the possible revenue loss created by blind fish is to represent the revenue loss of a cage as a function of the percentage of blind fish in the cage (Figure 21). The revenue loss of a cage increases rapidly as evident from Figure 21. The average revenue loss increased by $AUD 7,957 (95% *CI = $2,722 – $17,241)* with increase in proportion of blind fish by 1%.

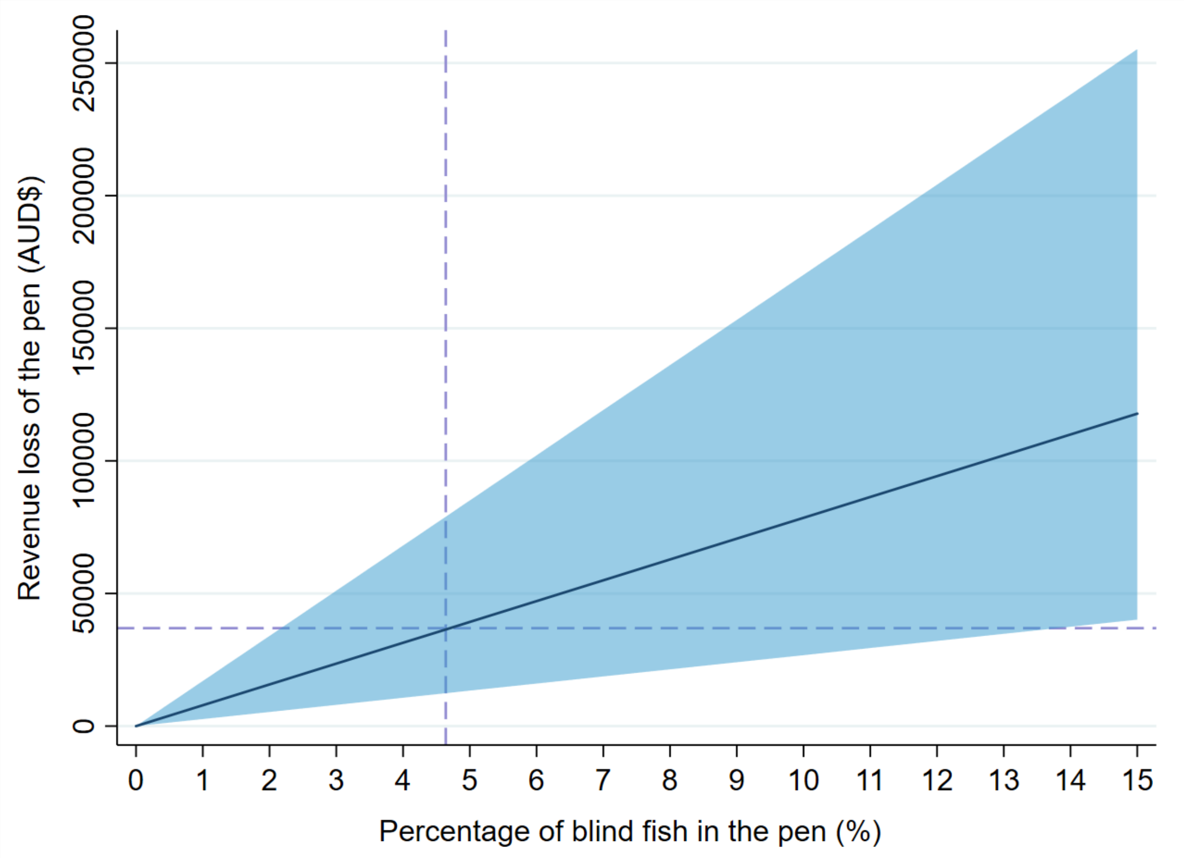


Figure 21. Revenue loss of a pen (in AUD$) associated to the proportion of fish with CL within a pen. The blue shaded area represents the confidence interval at 95% of the loss; the dashed lines show the revenue loss corresponding to the 4.64% average prevalence of fish with CL observed in 2017 and 2018.

3.4. Discussion

The first concern about the blind fish was if they were more likely to die than non-blind fish. The case-control study undertaken on the monitored fish showed that the blind fish were 17 times more likely to die during the farming season compared to non-blind fish in the same cages (OR = 16.98, 95%CI: 14.50 - 19.90, Table 3).

One-eyed and blind fish present at harvest appeared to be in lower condition than expected compared with non-blind fish. One-eyed were lighter by 3.1 Kg (95% CI: 2.6 – 3.6) and blind fish by 3.2 Kg (95% CI: 1.6 – 4.7) leading respectively to an average loss of -0.9 unit (95% CI: -1.0 – -0.7) and -1.9 units (95% CI: -2.4 – -1.4) of condition index. One-eyed were shorter by 2.8 cm (95% CI: 2.1 – 3.5) compared to the rest of the cage.

Finally, the question was to estimate if the increases in mortality and the lower condition of fish at harvest have critical consequences on the profits of the companies. In the cages monitored in 2017 and 2018, each 1% of blind fish in the cage represented on average a revenue loss of $AUD 7,957 (95% *CI = $2,722 – $17,241)*. The average percentage of blind fish in the monitored cages with a higher incidence of blindness was 4.64%, representing a $AUD 37,557 (95% *CI = $12,872 – $81,294)* revenue loss for the company.

During the design of the study the monitoring targeted companies and cages with a known blind problem while other companies were unwilling to be monitored during the harvest season. The population in the study was therefore not representative of the entire industry. The monitoring was always made during the processing of the fish just after the harvest of the same day. The observer was grading the CL while one employee from the company was washing the fish on both sides allowing the observer to look at both eyes. Many fish were not graded due to the rapidity of processing and employees were sometimes inspecting the fish on only one side making the monitoring of the second eye impossible. During the harvest and the processing, a lot of sharp tools could accidentally injure the fish on the cornea making the grading very uncertain and requiring the exclusion of these fish from the study.

Monitoring of the fish at harvest was done by a field investigator from the University of Adelaide while the monitoring of the dead fish over the entire farming season was done by the divers who removed mortalities from the cages. Different observers could mean that the recording of blind fish is not consistent between cases and controls. However, a blind fish was easily identifiable as its eye(s) are obviously altered in appearance. The analysis was done on the comparison of blind fish and non-blind fish, the probability of mistake in blind identification was very low.

At the beginning of the project a description of the different eye lesion grades had been made. Four grades were described after the first month of monitoring. During the monitoring it appeared that some lesions were in between two grades. The grading in this situation was more subjective and observer dependent. Two people were monitoring the fish in 2018 and one of these did the entire monitoring in 2017. The pre-determined picture eye scoring system and the single observer of most fish meant monitoring was consistent. The in between situations were rare and were usually concerning grades 1 and 2 which were later merged during the analyse which was conducted on a binary system comparing non-blind fish (group 0, 1 and 2) and blind fish (group 3 and 4).

The lower HOGG weight for blind fish was statistically significant. The imprecision in the confidence intervals of the blind fish was due to the lower sample size in this group with only 33 fish in total (Table 2). However, there was not enough fish in this group to make a difference with the one-eyed group.

For the same reason, the blind fish had a large confidence interval in their fork length. Even if there were not many fish in this group, they all had approximately a normal size. In the same way, even if the confidence interval of the normal fish and the impaired vision fish were cross-checking, the size of the impaired vision fish was slightly higher than the size of the normal fish. It is possible to draw some hypothesis to explain this chaotic evolution of the size: (1) the slightly increasing size of impaired fish could be due to the fact that many blind fish already died, the fish remaining could be stronger or late affected fish which have grown as the normal fish ; (2) the time that the lesion takes to perforate the eyes is still unknown, blind fish observed at harvest could be fish affected by the lesions at a late stage a few weeks before harvesting ; (3) the tuna could get infected randomly and the lack of energy could avoid their growth (4) it could also be the weak tuna, i.e. smaller tuna, getting infected, the blindness having no consequence on the size, the fish would not have grown more anyway. We cannot make any conclusion about a link between CL and the forklength of the tuna.

Blind fish had a significant lower condition index. The condition index was decreasing with the severity of the CL. This comforts the idea that blind fish were in lower condition than one-eyed as the difference could not be made only by the body weight. The shifting of the BCI of both one-eyed and blind fish (figure 2) was a good indication to confirm the association between blindness and poor condition. After earlier reports associating sea lice prevalence and poor condition (Hayward et al., 2008, 2009a), the present study showed that CL and poor condition were associated. Hypothesis could explain these observations, as a lack of competition for one-eyed and blind fish with normal fish leading to feeding issues and possibly starvation. Another explanation could be that blind fish have to use more energy to be competitive with normal fish and fight against physiological misfunction, inflammation or infections. CL were widely observed in aquaculture and were sometimes due to parasites as in sea bass, *Dicenthracus labrax,* (Vagianou et al., 2006). In this case, CL led to corneal ulcers or blindness and caused, anaemia, slow growth rate and systemic infections. In the present study, no bacterial investigations were made. Deficiencies like hypovitaminosis A were described in salmonids as an aetiology of CL, associated with poor growth and higher risk of mortality as in our situation (Ferguson et al., 2006).

In the case-control study blind SBT were still observed at harvest. It is unclear whether they survived regardless of their lesion or whether they got injured at a later stage in the season compared to the ones that died. This was an unexpected finding and might be worth exploring further if the incidence of blindness and downgrading continues to be a concern for the industry.

With the multilevel model in the case-control study, the odds of dying for blind fish compared to normal fish decreased from 19.6 to 17, showing an effect of the farm on the likelihood of blindness. This could be due to differences of operational practices and / or location within each farm. SBT were caught in the wild and brought back to Port Lincoln by tow cages which in some seasons share the tuna between several farms. If one of the shared tow cages is treated differently to others amongst any individual operator, this could be another explanation. Finally, the mortality rate of each farm is different and creates a background noise which can misrepresent the attribution of mortality due to blindness.

In the financial analysis, only the revenue loss was estimated. The actual loss for the farms is greater as the number of dead SBT also contributes to the cost. During the feeding, the food fed before the fish died is money spent for no return. Every SBT also has a cost to have access to the fishing rights and quota allocation, as well as the physical logistics to catch them and transport them from the fishing areas to the farming areas (this includes fixed costs of aeroplanes, vessels, people and infrastructure involved in the capture and transfer of the fish). Costs also include those related to fishing rights, fishing practices and storage of feed, which is entirely based on another quota restricted wild fishery. Even if the revenue loss can seem important as thousands of dollars would be lost, most cages represent a benefit bigger than $AUD 1 millon, meaning that revenue fraction that the loss represent might seem high at the fish level but is small when brought back to the cage level and more over to the company level.

Blind fish is a common phenomenon in aquaculture but less apparent in the tuna ranching industry. While tuna ranching does occur at several locations around the world this study cannot be generalised to other bluefin tuna ranching operations. Moreover, this study was targeted to an identified problem so cannot be generalised for the entire tuna industry of Port Lincoln.

4. INVESTIGATING THE PUTATIVE ROLE OF SEA LICE IN CAUSING CORNEAL LESIONS IN RANCHED SOUTHERN BLUEFIN TUNA

4.1. Introduction

Previous reports (Hayward et al., 2007, 2008, 2009a, 2010, 2011; B. F. Nowak et al., 2012; Barbara F Nowak, 2004; Rough et al., 1999; Rough, 2000), including FRDC projects No 2003/225) (Nowak et al., 2007;) identified sea lice as a differential cause of corneal lesions (CL) in ranched Southern bluefin tunas (SBT), *Thunnus maccoyii*. Sea lice are a highly diverse group of copepodid ectoparasites belonging to the Caligidae family (World of Copepods, 2020) which infest the integument of a wide range of marine host species. Sea lice of the genus *Caligus* spp. includes 430 described species (Ju-Shey Ho et al., 2016) and infests a wide range of host finfish species according to their geographical distribution. The first species of copepods described in SBT was *Caligus elongatus* in 1995 when an outbreak occurred in SBT associated with corneal ulceration and panophthalmitis (Rough et al., 1999; Rough, 2000). In 2004, *Euryphorus brachypterus* and *Pseudocycnus appendiculatus* were reported in SBT, the latter as being the most prevalent with a progressing burden over the ranching season, however, no outbreak *sensus scricto* were reported (prevalence of 44.8% but a low intensity of 3.77 lice per infested fish) (Hayward et al., 2007). In 2005, an outbreak of the caligid sea lice *Caligus chiastos* , Lin & Ho, 2003, occurred with a higher prevalence early in the season, when the water temperature was warmer (ca. 17°C), and then progressively decreasing as the water temperature dropped until harvest (ca. 14°C), however, the parasite intensity remained low with an intensity of 5.77 lice per infested fish (range: 0-42 lice per infested fish) with an overall prevalence of 57.3% across the season (C. J. Hayward et al., 2008; C. J. Hayward et al., 2009). These previous events led the tuna industry to the hypothesis of sea lice as being the principal risk factor in the development of CL. That is the reason why we started to investigate sea lice burden first.

Although sea lice infestations have been reported in overseas bluefin tuna farming industry (Japan, Mediterranean or Mexico), burdens were relatively low and rarely associated to major health concerns or death (Balli et al., 2016). In ranched SBT, sea lice were associated with a rise of fish stress level revealed by an increased in plasma cortisol and glucose concentrations (Hayward et al., 2010). The exact causal pathway between the ectoparasite infestation and the genesis of corneal lesions has not been documented but hypothetical pathogeneses involve a primary ulcer of the cornea caused by either (i) a physical trauma resulting from the SBT flashing against the cage's net to dislodge itchy ectoparasites, (ii) the parasite feeding directly on the corneal epithelium or (iii) a combination of both. Although Hayward et al. (2008) found an association between the severity of CL and the intensity of *C.*

chiastos infestation at the individual SBT level, their cross-sectional investigation (i.e. at a single point in time, i.e. without follow-up) could not rule-out reverse causation. Therefore, it remains uncertain if the sea lice infestation precedes the apparition of corneal lesions or if SBT with corneal lesions are more susceptible to be subsequently infested by the ectoparasite. Investigating the progression of individual SBT corneal lesion relative to sea lice infestation would require individual identification and repeated handling (at least twice) which is logistically unrealistic. However, without demonstrating a chronological succession of the two events at the fish level, the monitoring of the infestation and of the development of CL at the cage level could strengthen this hypothesis. This project aimed to monitor sea lice burden across two companies through the 2018 and 2019 ranching seasons in order to see if a temporal correlation could be made between sea lice infestation and the incidence of corneal lesions.

4.2. Material and methods

4.2.1. Data access and collection

Sea lice burden and speciation at transfer

In an effort to assess the sea lice burden of wild caught stocks before to be ranched, a subset of SBT being weighted and measured for quota monitoring at the time of transfer between tow cages and grow-out cages were visually examined. For a given tow cage, a total of 100 SBT were caught using a hook-and-line baited with a fresh whole sardine or herring. During weighting and fork-length measurement, the accessible side of the fish was visually examined for presence of sea lice at the surface of the skin. Individual fish sea lice counts were recorded and, when found, sea lice were collected and transferred into 2mL microtubes filled with seawater and stored at room temperature. The same day after landing, collected parasites were transferred into 2mL microtubes filled with 70% ethanol and stored at 5 °C until microscopic identification.

Microscopic identification - Collected sea lice were individually examined at low magnification under a dissecting microscope. The speciation of the sea lice followed the standard copepods taxonomic identification keys (Parker et al., 1968). Briefly, the three main segments of the sea-lice body were recognised - the cephalothorax, genital and abdomen segments (Figure 22). For each of these segments, the general anatomical features were identified and compared to the standard identification keys. Special attention was given to compare our specimens with *C. chiastos* which was previous reported in ranched SBT by Hayward et al. (2008). Following the original description of *C. chiastos* made by Lin & Ho (2003), the 2nd and 4th thoracopod were dissected and examined at high magnification to count *spines* and *setae* on each of the *segments* of the *endopod* and *exopod* (Figure 23).

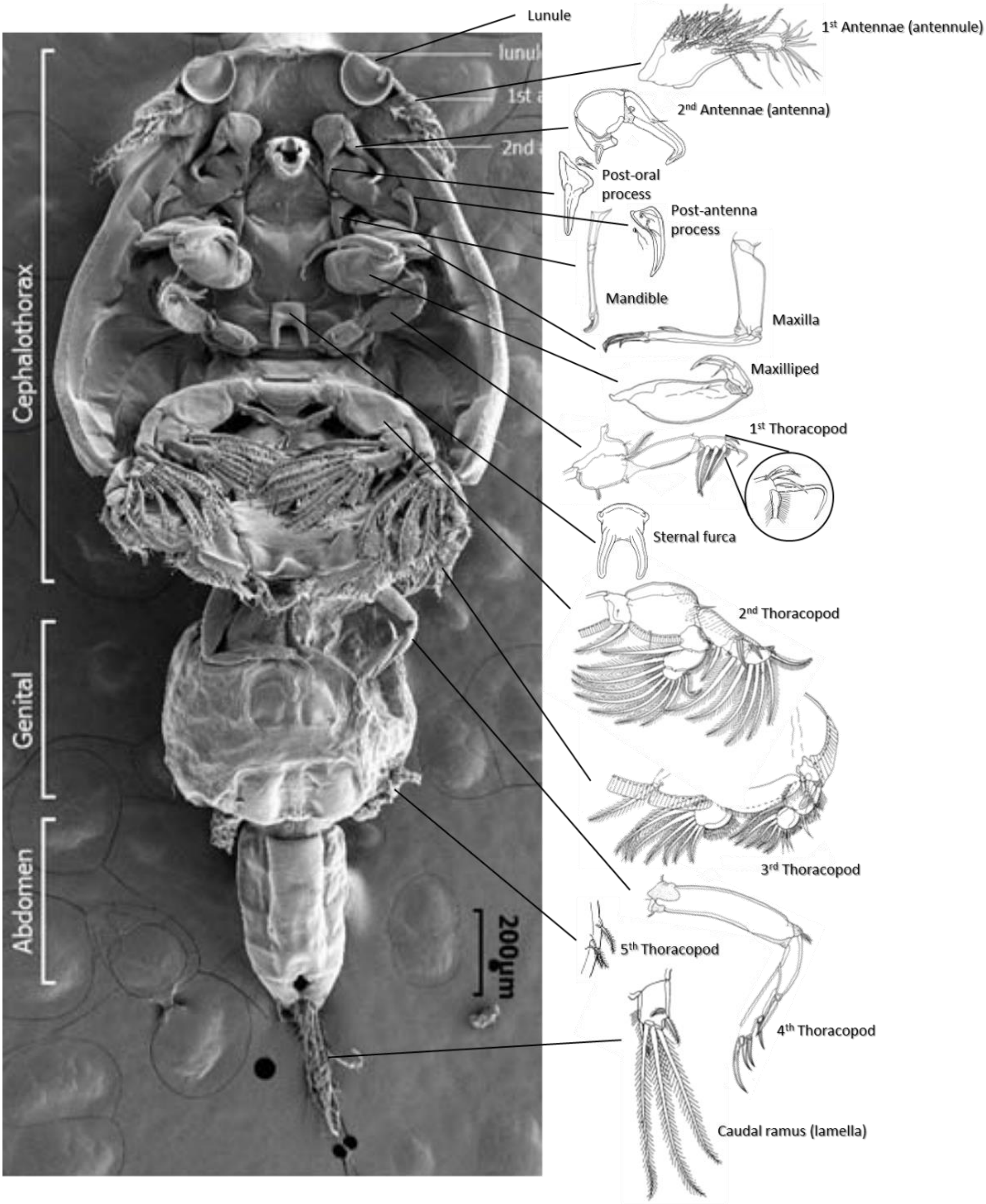


Figure 22. Adult female *Caligus chiastos* Lin and Ho, 2003, without egg strings, viewed under scanning electron microscope. The three anatomical segments of a sea louse are identified on the left side and the appendices are identified on the right (adapted from Hasmi, 2013)

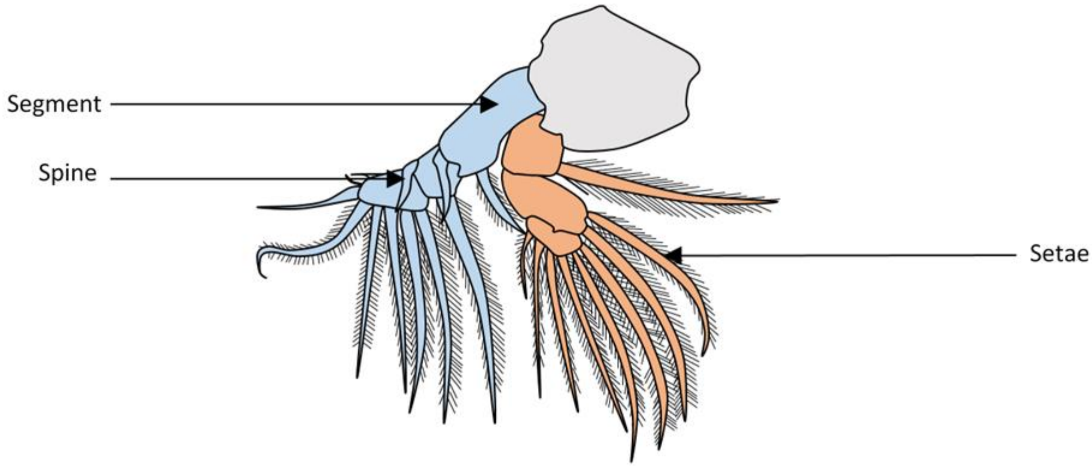


Figure 23. Anatomical features of the 2nd thoracopod of *Caligus chiastos* Lin and Ho, 2003. This appendix is composed of a basipodite (in grey) which carries an *exopod* (in blue) and an *endopod* (in orange). The exopod and endopod are divided into *segments* which can carry *spines* (hard and short spine-like structures, usually at the distal end of a segment) and/or *setae* (long bristle-like structure bearing numerous hair-like structures, usually on the margin of a segment). For identification, each part of the appendix and its segments is described with a numerical transcription of the position and the number of spines and setae. Within a segment, the spine count is reported using Roman numbers while setae count is reported using Arabic number. Within a exopod or endopod, the segments are separated by a semi-colon, while commas separate subgroups of spines or setae within a segment. For instance, the 2nd thoracopod of *C. chiastos* would be coded as – *Exopod: I-1; I-1; II,I ,5* and *Endopod: 0-1; 0-2; 6*. Other appendices may only have a vestigial exopod or endopod and would not bear any spine or setae.

Sea-lice burden monitoring in growing cages

Two ranching companies that experienced corneal lesions (CL) in 2017 were approached to volunteer in monitoring the burden of sea lice in their cages. Companies' divers were supplied with a GoPro® HERO6 Black camera and trained for underwater captures. Up to twice a week across the season, divers were asked to take bursts of photos (30 frames in one second, JPEG format, 4000x3000 resolution) of at least 10 tunas per cage not deeper than 5 meters to have enough light and no more than 2 meters from the fish to distinguish the sea lice. The camera cards were collected, and batteries changed as necessary and the bursts were collated and examined individually for quality on a large high-resolution computer screen. For an individual fish, a suitable burst required a clear view of the head, back, side and upper belly. Suitable bursts were examined by the same person to count sea lice as well as apparent cutaneous injuries observed on the surface of the fish using the following six categories:

- 'corneal lesions', white area on the surface of the eye or complete white or black coloration of the eye or obvious perforation of the cornea;
- 'teeth mark', pinpoint and/or linear skin marks with no apparent breach of the skin layer and matching the teeth pattern of a bite print;
- 'scratch', linear skin marks with no breach of the skin layer and no teeth pattern;

- ‘rubbed’, extended skin discoloration suggestive of scale loss but with no breach of the skin barrier;
- ‘gash’, linear breach of the skin layer with apparent subcutaneous tissues; and
- ‘nose lesion’ white or black erosive lesions on the tip of the snout.

4.2.2. Data analysis

Sea lice burden data preparation for analysis

When relevant, sea lice counts were doubled as an approximation of the sea lice count on the whole fish to account for the fact that only one side of the fish was examined. The burden of the sea lice population was measured and reported using:

- the *prevalence*, the proportion of individual infested with at least one sea louse;
- the *abundance*, the average count of sea lice per fish across in the entire cage population; and
- the *intensity*, the average count of sea lice per fish among infested fish only.

The occurrence of CL was reported as the proportion of examined tuna suffering from CL at the time of captured (i.e. prevalence) either (i) in live SBT photographed weekly or (ii) in dead SBT collected after each mort dives. Daily surface water temperature was recorded in degrees Celsius using a logger attached to cage in the middle of ranching zone.

The data were analysed as time series to describe the temporal variation during the ranching season. To have a better representation of the trends, a moving average was calculated on a 20 days period for sea lice prevalence, abundance, intensity and CL prevalence and on a 15 days period for the incidence of mortality and incidence of CL within the mortalities. Analyses for mortality, CL, prevalence, abundance and intensity were implemented in the statistical package STATA v.15.1 (StataCorp Ltd, Texas, USA).

Inferential analysis

We first investigate the cross-sectional (outcome and predictor data matched at the same point in time) association between sea lice burden, skin damages and CL at the individual SBT level. The different damages “scratch”, “rubbed”, “gash” and “white nose” as described before were gathered under the same variable “skin damage” as they could all be part of the cause or be a consequence of CL. Based on individual fish data collected during grow-out, we built a multifactorial logistic regression with the presence of CL as outcome, the intensity of sea lice, the skin damage of the fish, and the teeth marks as predictors. In this model the sea lice intensity was divided into three categories: none (0 sea lice), mild (2-6 sea lice per affected fish) and moderate (8-32 sea lice per affected fish). To investigate a potential temporal relationship between sea lice infestation the presence of CL and the model was made at the cage level, comparing the presence of CL (outcome) in the cage on a given day to the intensity of sea lice infestation, the skin damage and the teeth marks (predictors) in the whole cage during a previous period. The year, the company and the cage were entered as random effects. The intensity was calculated by adding the number of sea lice observed during the period divided by the number of fish infested during the same period. The prevalence of skin damages was calculated by adding the number of damages during the period divided by the number of fish observed during the same period. The period considered was a 2-week period starting 21 days before the observation of the CL and ending 7 days before the observation. It was settled considering that new sea lice present in the 7 days before the observation of CL would not be able to damage the fish enough to lead to grade 3 or grade 4 CL and that after 21 days, the sea lice would have leave the fish according to their life-cycle (González & Carvajal, 2003). This model was compared to a similar model run on the same observations but with the intensity and the skin damage calculated on the same day as the presence of CL. The intensity was chosen as a predictor as the damage made by sea lice is depending on the number of parasites infecting the fish. The prevalence would be a good predictor for the extension of the infestation, but in our case, studying the CL, the intensity is more relevant. The year, the company and the cage were entered as random effects. The model created for inferential statistics were implemented in the statistical package STATA v.15.1 (StataCorps Ltd, Texas, USA).

The association between CL, sea lice intensity, skin damage and teeth marks were investigated by estimating the odds ratio between the four variables using a simple logistic regression in which sea lice intensity, skin damage and teeth marks were the predictors. To account for the clustering of cages within a company and companies within a year, random effects were respectively added for 'year', 'company-within-year' and 'cage-within-company-within-year' into the model (i.e. mixed effect model). The direct outputs of the model's coefficients, ignoring random effects, provided year-, company- and cage-specific estimates of association. To be relevant to the industry as an all, population-averaged estimates of the odds ratios (OR), and their 95%CI limits, were converted using the following approximation formula (Dohoo et al., 2009):

$$\mathrm{OR} = exp\left(\frac{\beta}{\sqrt{1+0.346\times\left(\sigma^2_{year}+\sigma^2_{company}+\sigma^2_{cage}\right)}}\right) \qquad \textit{(Eq. 1)}$$

where β is the model fixed effect coefficient estimate for either mild sea lice intensity, moderate sea lice intensity, skin damage or teeth marks and σ^2_{year}, $\sigma^2_{company}$ and σ^2_{cage} are the model's variance estimates for the random effects.

Analyses for mortality and productivity impacts were implemented in the statistical package STATA v.15.1 (StataCorp Ltd, Texas, USA).

4.3. Results

4.3.1. Sea lice identification

A single species of sea lice was almost exclusively found – *C. chiastos*. One chalimi and three nauplius stages were collected but the majority were adults, mostly mature gravid females. The main keys for identifying this species were (Figure 24):

(1) a sub-circular genital segment,

(2) a shorter abdominal segment,

(3) two long caudal teeth on the sternal *furca*,

(4) on the exopod of the 2nd thoracopod, the spine of the first proximal segment is longer than the spine of the second segment, and

(5) the axis of these two spines crosses over posteriorly,

(6) on the exopod of the 4th thoracopod (no endopod), the first proximal segment bears a long outer spine, and

(7) the distal segment a first proximal outer spine followed by three outer spines (i.e. an armature I-0; I, III).

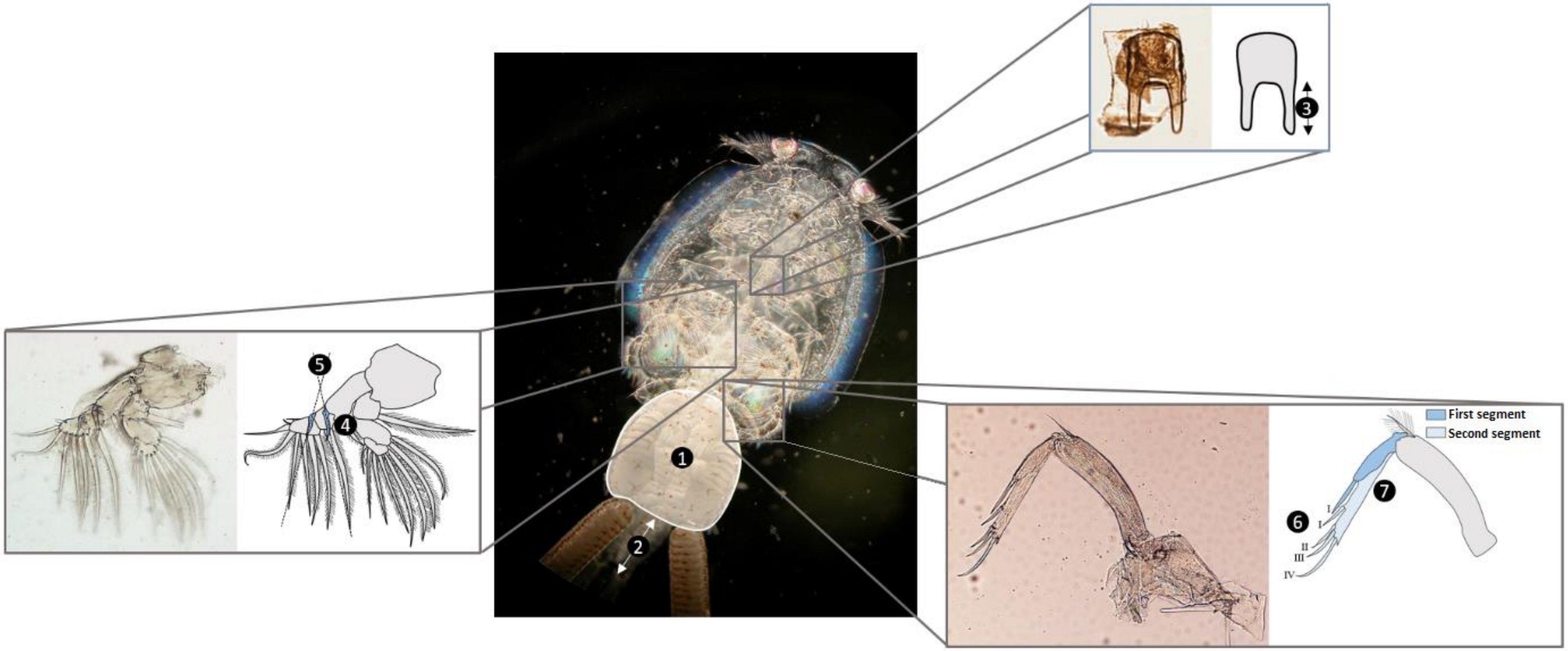


Figure 24. Main identification features of *Caligus chiastos* Lin and Ho, 2003. This sea lice species can be identified at low magnification by its a sub-circular genital segment (1), a shorter abdomen segment (2), two long teeth on the sternal furca (3), the first external spine on Th2 is longer than the next one (4) and (5) cross over it posteriorly, (6) a 2-segmented exopod bearing an armature of "I, IV" on leg 4 with (7) a long first outer spine.

4.3.2. Sea lice burden at transfer

Across March 2018, a total of 500 SBT were visually examined for sea lice from five separate tow cages from three different ranching companies. Sea lice were found in three of the tow cages and from two of the companies with prevalence ranging from 4.0% to 11.0%, an abundance ranging from 0.1 to 0.2 sea lice per SBT and an intensity ranging from 2.0 to 2.2 sea lice per infested SBT, after doubling (Table 7). None of the examined SBT showed corneal lesion (CL) at the time.

Table 7. Burden of sea lice and corneal lesion at transfer of wild caught Southern bluefin tuna from tow cage to grow-out cage during March 2018.

			Sea lice			Corneal Lesion
Company	**Tow cage**	**n**	**Prevalence**	**Abundance**	**Intensity**	**Prevalence**
A	A1	100	11.0%	0.2	2.2	0.0%
B	B1	100	0.0%	0.0	0.0	0.0%
C	C1	100	0.0%	0.0	0.0	0.0%
	C2	100	5.0%	0.1	2.0	0.0%
	C3	100	4.0%	0.1	2.0	0.0%

4.3.3. Sea lice burden monitoring during the ranching period

Burden trends in 2018

A total of 51,150 camera captures were collected in 2018 of which 1,704 SBT were identified and screened for sea lice and skin lesions. The company 1 was monitored at 19 instances between February and June 2018 with two to six diving surveys per month, each separated by between 24h to 21 days (Figure 25). The company 2 was monitored at 15 instances between February and May 2018 with two to six diving surveys per month, each separated by between 24h to 26 days (Figure 25).

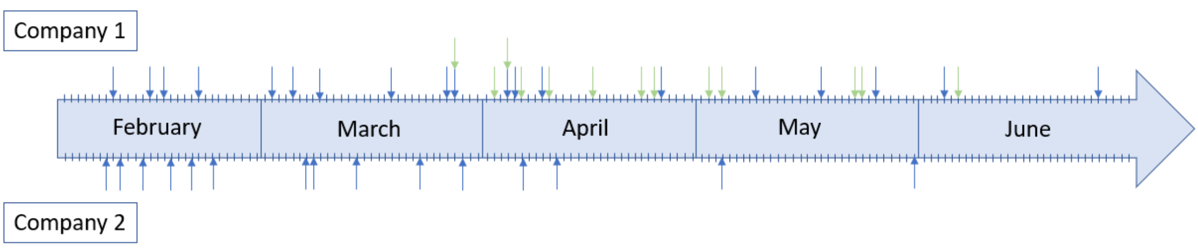


Figure 25. Frequency of camera capture to survey corneal lesions, sea lice burden and skin lesions in ranched Southern bluefin tuna. The arrows on the top represent the diving sessions for company 1, the arrows on the bottom represent the diving sessions for company 2. Blue arrows: surveys from 2018; green arrows: surveys from 2019.

Across the 2018 ranching season, sea lice were observed on 43.9% of the surveyed SBT and individual SBT count of sea lice ranged between 0 and 32 per fish, after doubling. We found no evidence of differing sea lice burden between the two companies (Table 8).

Table 8. Summary of sea lice burden in Companies 1 and 2 across the 2018 ranching season.

Parameters	**Company 1**	**Company 2**	**Overall**
SBT count	754	950	1,704
SBT ≥1 louse	343	405	748
Sea lice count range	0-32	0-28	0-32
Sea lice prevalence (% affected SBT)	45.5%	42.6%	43.9%
Abundance (average count/SBT)	1.7	1.4	1.5
Intensity (average count/ affected SBT)	3.7	3.3	3.5

On a daily level, the sea lice burden, CL prevalence, the skin lesions prevalence, the number of fish surveyed, the daily incidence of mortalities and of mortalities with CL are presented for both companies in the Table 9. SBT with corneal lesions (CL) were observed during the ranching season in most of the cages from the Company 1 (~80%), with a daily mean CL prevalence of 7.4% (ranging from 0 to 50%) and less than half of the cages from the Company 2 (~40%), with a daily mean prevalence of CL of 0.5% (ranging from 0 to 2.9%).

Table 9. Summary of daily sea lice burden, corneal lesions and skin lesions for Companies 1 and 2 on a day-level. The prevalence is the percentage of affected SBT on a day, abundance and intensity are count of sea lice respectively per SBT and per affected SBT on a day.

Parameters	**Company 1**				**Company 2**			
	Mean	*Median*	*Min*	*Max*	*Mean*	*Median*	*Min*	*Max*
Daily sea lice prevalence	49.3%	48.6	18.2%	100.0%	45.1%	45.7%	21.2%	100.0%
Daily sea lice abundance	1.9	1.7	0.4	6.0	1.5	1.4	0.6	3.0
Daily sea lice intensity	3.7	3.6	2.0	6.0	3.3	3.1	2.6	4.6
Daily CL prevalence	7.4%	2.1%	0.0%	50.0%	0.5%	0%	0.0%	2.9%
Daily skin lesion prevalence	24.7%	22.2%	0.0%	63.6%	36.6%	37.5%	8.0%	100.0%
Daily number of fish captured on camera	40	39	6	80	63	61	4	116
Daily incidence of mortalities (per 10,000 SBT)	1.25	0.88	0	13.4	0.25	0	0	5.1
Daily incidence of mortalities with CL (per 10,000 SBT)	0.36	0	0	2.52	0.026	0	0	0.84

In 2018, all the cages of both companies were monitored, but only three cages from company 1 were considered of 'CL concern' (i.e. more than 1% of cumulative mortality and more than 20% of the mortalities with CL). No cage from the Company 2 were considered of concern regarding CL. The overall progression of the sea lice burden, CL and mortality of all the cages from Company 1 and 2 across the

2018 ranching season is presented on the Figure 26. Both companies had similar prevalence of sea lice at the beginning of the ranching season (~50%-60%) but it decreased by half in the Company 2 while it relatively stables in Company 1 at around 40%-50% during the season. The sea lice intensity (Company 1: 2.0-6.0; Company 2: 2.6-4.6) and abundance (Company 1: 0.4-6.0; Company 2: 0.6-3.0) were comparable in both companies, maybe slightly higher in the Company 1. However, a clear difference can be seen on the CL prevalence where company 1 experienced CL from late March to late April, with an overall prevalence staying between 20 to 30% while almost none were observed in the company 2. Moreover, most of the mortalities from the Company 1 showed CL during the first half of the season, which was not the case in the company 2. In both companies the sea lice burdens were comparable while CL were almost only present in the Company 1.

The Figure 27 presents the progression of the sea lice burden, CL and mortality of all the cages from Company 1 across the 2018 and 2019 ranching seasons, comparing the cages deemed of 'CL concern' to cages of 'no CL concern'. During the whole month of April, almost all the mortalities were bearing CL. The first event of sea lice infestation in April with the increasing incidence of dead fish bearing CL happened just before the rise of CL surveyed on live fish. From May, the incidence of mortality rose until the harvest but the proportion of mortalities with CL within the mortalities was decreasing. The prevalence and intensity of sea lice were quite stable with short variation through the season, even when the water temperature decreased.

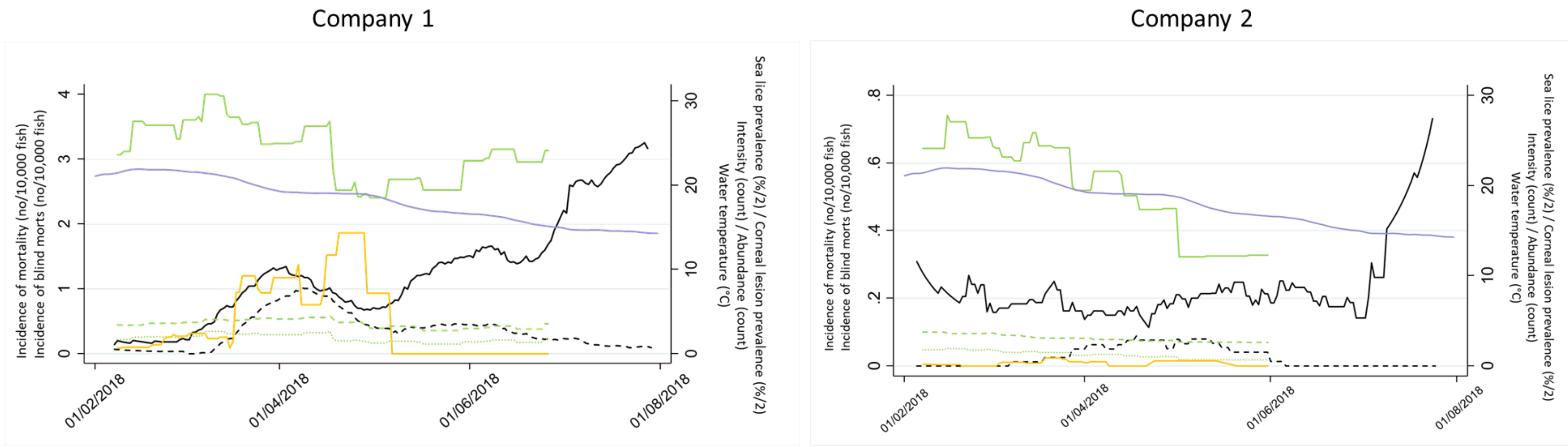


Figure 26. Progression of the sea lice burden, corneal lesion (CL) and mortality of all the cages from Company 1 and 2 across the 2018 ranching season. Continuous green line - sea lice prevalence (halved), dashed green line - sea lice intensity (average count of lice per infested fish, doubled), dotted green line - sea lice abundance (average count of lice per surveyed fish, doubled), orange line - CL prevalence (halved), continuous black line - incidence of mortality (proportion of dead per stock present in the cage), dashed black line - incidence of dead fish with CL (proportion of dead with CL per stock present in the cage), purple line – surface water temperature (°C). Sea lice prevalence, abundance, intensity and CL prevalence were calculated with a moving average on a 20 days period, the incidence of mortality and incidence of CL within the mortalities were calculated with a moving average on a 15 days period.

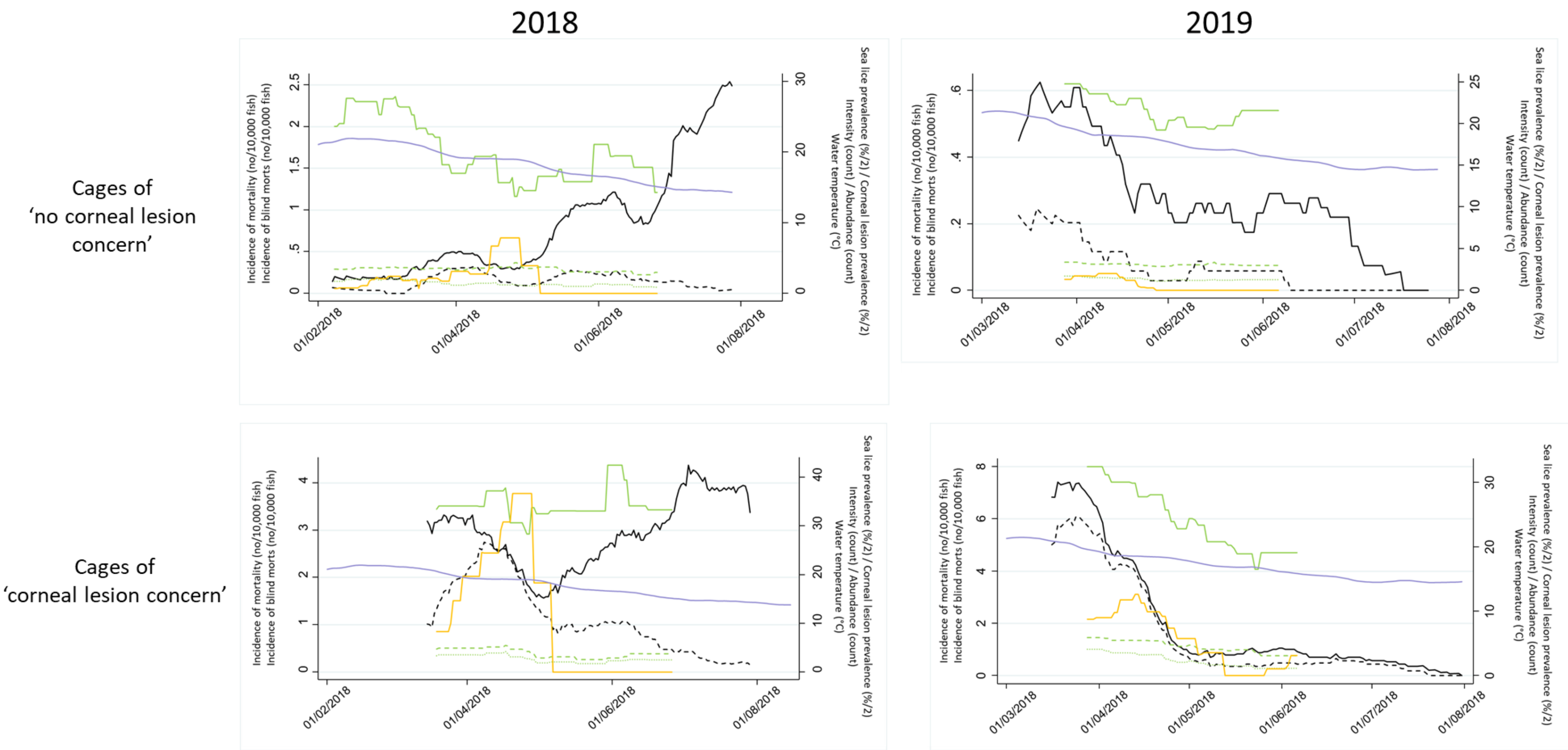


Figure 27. Progression of the sea lice burden, corneal lesion (CL) and mortality into three 'of CL concern' cages from Company 1 across the 2018 ranching season and two from the 2019 ranching season, compared to the progression in the remaining cages 'of no CL concern'. Continuous green line - sea lice prevalence (halved), dashed green line - sea lice intensity (average count of lice per infested fish, doubled), dotted green line - sea lice abundance (average count of lice per surveyed fish, doubled), orange line - CL prevalence (halved), continuous black line - incidence of mortality (proportion of dead per stock present in the cage), dashed black line - incidence of dead fish with CL (proportion of dead with CL per stock present in the cage), purple line – surface water temperature (°C). Sea lice prevalence, abundance, intensity and CL prevalence were calculated with a moving average on a 20 days period, the incidence of mortality and incidence of CL within the mortalities were calculated with a moving average on a 15 days period.

Burden trends in 2019

During the 2019 ranching season, only Company 1 was monitored. A total of 508 SBT were surveyed across five cages. The company was monitored across 13 instances between March and June 2019 with one to seven diving survey per month, each separated by between 24h to 18 days (Figure 25). The prevalence and burden of sea lice was similar between years with a lice count range slightly narrower (0-18 sea lice per fish) (Table 10).

Table 10. Summary of sea lice monitoring from company 1 in 2018 compared to 2019

Parameters	Company 1	
	2018	2019
SBT count	754	508
SBT ≥1 louse	343	242
Sea lice count range	0-32	0-18
Prevalence (% affected SBT)	45.5%	47.6%
Abundance (average count/SBT)	1.7	2.0
Intensity (average count/ affected SBT)	3.7	4.2

On a daily level, the sea lice burden as well as the corneal lesion prevalence, the skin damages prevalence, the number of fish monitored, the incidence of daily mortalities and the incidence of daily SBT with CL within the mortalities are presented for both companies in the Table 11.

Table 11. Summary of daily sea lice burden, corneal lesions and skin lesions across the companies 1 on a day-level. The prevalence is the percentage of affected SBT, abundance and intensity are count of sea lice respectively per SBT and per affected SBT.

Parameters	**Company 1**			
	Mean	*Median*	*Min*	*Max*
Daily sea lice prevalence	47.0%	42.1%	28.6%	81.3%
Daily sea lice abundance	2.0	1.7	0.9	6.0
Daily sea lice intensity	4.0	3.9	2.5	7.4
Daily CL prevalence	7.1%	5.0%	0.0%	20.6%
Daily skin damage prevalence	27.7%	20%	2.4%	62.5%
Daily number of fish monitored	39	37	31	52
Daily number of mortalities (per 10,000 SBT)	0.47	0.21	0	3.4
Daily incidence of mortalities with CL (per 10,000 SBT)	0.27	0	0	3.2

In the cages with a 'CL concern' in 2019 the curve of the incidence of SBT with CL is extremely close to the curve of the incidence of mortality showing that most of the mortalities had CL (Figure 32). The highest sea lice prevalence was observed during the first two month of ranching in the cages with a 'CL concern' reaching more than 60% and decreasing until June under 40%. On the opposite hand, the cages with 'no CL concern' in 2019 the incidence of mortality is almost ten times lower and 30 times lower for the number of CL within the mortalities with almost no CL within the live fish. However, the intensity of sea lice was similar in both types of cages. The trends of sea lice prevalence and CL prevalence varied similarly through the ranching season, more obviously in the cages with a 'CL concern'. In 2019, the global mortality incidence in the cages with 'no CL concern' was lower compared to 2018 and showing an opposite trend with an increase at the beginning of the ranching season then always decreasing until harvest. The sea lice burden trend in 2019 in the cages with 'CL concern' was different from what the company 1 experienced in 2018 as the prevalence was staying high through the whole ranching season. Finally, the prevalence of CL in 2019 in the cages with a 'CL concern' compared to the observation of 2018 was lower but still higher than what is commonly expected in the industry.

Temporal association between sea lice burden and corneal lesions

First, we investigated the 'cross-sectional' association (i.e. at one point in time) between the presence of CL and either the burden of sea lice or presence of skin damages at the individual fish level. The final mixed effect logistic regression revealed weak associations between CL and sea lice intensity, skin damage and teeth marks (Table 12). The confidence intervals between the odds ratio of mild and moderate intensity of infection, enhancing a significative difference between the two results. Moreover, it was not possible to ascertain beyond doubt that the model predictors proceeded temporally the outcome.

Table 12. Final mixed effect logistic regression model for corneal lesion at the individual Southern Bluefin tuna level.

Fixed effect	*Odds ratio*	*95% Confidence interval*	*P*-value
Lice count category			
None	1.00	-	-
Mild	1.10	1.05 – 0.15	P<0.001
Moderate	1.24	1.17 – 1.30	P<0.001
Skin damage	1.09	1.05 – 1.13	P<0.001
Teeth marks	1.26	1.13 – 1.40	P<0.001

Random effect	*Variance*	*Confidence interval*
Year	9.51×10^{-33}	-
Company	3.27	0.60 - 17.9
Cage	1.27	0.48 – 3.34

Two additional logistic regressions were conducted at the cage level to explore the temporal associations between CL and these predictors (Table 13). The first model aimed at investigating a ‘successive’ association by forwarding (i.e. delaying) the CL prevalence of the cage relative to the sea lice burden or the skin damage, while the second model investigated the ‘cross-sectional’ association. Based on the log values of the likelihood function, the cross-sectional model revealed a better fitness for data and a stronger association between CL and the sea lice intensity when compared to the successive model (Table 13). Both models used the same dataset which allowed for a direct comparison of the likelihood function values. Skin damage and teeth mark were not significantly associated with CL in both models and were, therefore, withdrawn from the model.

Table 13. Comparison of the final mixed effect logistic regressions for corneal lesion in Southern Bluefin tuna at the cage level exploring either a ‘successive’ or a ‘cross-sectional’ association.

	‘Successive’ model *Log likelihood = -78.7*			**‘Cross-sectional’ model** *Log likelihood = -74.5*		
Fixed effect	*Odds ratio*	*95% Confidence interval*	*P-value*	*Odds ratio*	*95% Confidence interval*	*P-value*
Lice intensity	1.29	1.10 – 1.51	P=0.002	1.55	1.26 – 1.91	P<0.001
Random effect	*Variance*	*95% Confidence interval*		*Variance*	*95% Confidence interval*	
Year	4.4×10^{-34}	-		6.75×10^{-35}	-	
Company	0.23	4.3×10^{-3} – 12.3		0.10	2.2×10^{-4} – 42.2	
Cage	0.92	0.19 – 4.4		0.34	0.02 – 5.57	

4.4. Discussion

Even if the species of sea lice was not monitored through the entire season, the monitoring at transfer enhanced that the only sea lice infesting farmed SBT in Port Lincoln in 2018 were adults *C. chiastos*, as reported by Hayward et al. in 2008. The anatomy of the sea lice being complex, this study offered a quick method of species identification for *C. chiastos* with evident characteristics easily visible under a dissecting microscope and/or an optical microscope.

During the transfer from the tow cages to the grow-out cages, SBT seemed to be almost free of sea lice, with a low prevalence but it increased quickly in the first two month of ranching, which is coherent with previous investigations from Hayward et al. (Hayward et al., 2008, 2011). The infestation absent in the wild and starting during growing could be because of the high density of fish that facilitated the host finding for juvenile sea lice (Costello, 2006) or because SBT ranching uses an open system and sea lice as well as other pathogens can freely enter and exit the sea cage. The potential for sea lice hosts other than SBT is highly diverse and likely. The genus Caligus spp., for instance, is known to easily cross the host species barrier. Indeed, *C. chiastos* has been found in common local finfish species such as big eye trevally (*Caranx sexfascinatus*), Australasian snapper (*Pagrus auratus*), and Crescent sweetlips (*Plectorhinchus cinctus*) (World of Copepods, 2020b). The life cycle of caligid sea lice was mostly direct, but the few individuals from juveniles stages found in our study and from previous study (Hayward et al., 2011) suggested that *C. chiastos* might develop a two-host life cycle by being confronted to a species that it would not encounter in the wild, SBT. This phenomenon was already described in other cultured fish like red sea bream, *Pagrus major*, in Japan and Korea as only adults stages from the *Caligidae* family were found on the fish, yet, no intermediate host could be identified so far (J.-S. Ho et al., 2004; Venmathi Maran et al., 2012). Hayward et al. (2011) suggested that the Degen's leatherjacket (*Thamnaconus degeni*) could act as a reservoir for *C. chiastos* because they are regularly observed around and inside the SBT cages and were also found infested with the sea lice. This idea corroborates another observation made in Japan where Pacific bluefin tuna, *Thunnus orientalis*, would have been infested by *Caligus macarovi*, another species of Caligid sea lice, because of the migration of *Cololabis saira* (Nagasawa, 2011). Even if at the transfer from the tow cage to the grow out cages SBT are said to be still wild, they sometimes waited in tow cages for several days in the bay of Port Lincoln in shallow waters, close to grow out cages and wild fish which can be vector of *C. chiastos* (Hayward et al., 2011). It is hard to confirm if the sea lice monitored at transfer were on the SBT before they were caught.

The increase of parasite at the beginning of the ranching season could be because of a higher susceptibility due to a drop in the immune system of SBT after the distance they swam from the catching area to ranching area ; it seemed that SBT acclimate to ranching condition quickly, within a month (Kirchhoff et al., 2011), which corresponded to the period of high prevalence in our study.

In 2018, the monitoring of sea lice across two companies (1 and 2) showed that fish were infested by sea lice early in the ranching season with a mean prevalence of 43.9% but with low mean intensity that did not exceed 3.7 parasites per fish daily and a maximum observation of 32 parasites on one individual. In 2019 observations showed a similar outbreak of sea lice reaching a mean prevalence of 81.3% in April with a daily mean intensity that did not exceed 4 sea lice per infected fish. The observed prevalence could be considered high as the majority of the monitored fish were infested even if previous report of sea lice infestation in SBT were facing most of the time a prevalence of 100% (Hayward et al., 2010). However, the intensity was considerably lower than what was observed by Hayward et al. as in their study SBT were infected by 295 parasites on average with the maximum count reaching 495 sea lice on one individual. Sea lice infestation in SBT are not common and no record of reference value exist for this species. These parasites are a common issue in fish farming and particularly in salmonid farming with the sea lice *Lepeophtheirus salmonis* (Pike & Wadsworth, 1999). In the salmon industry, farmers use specific figures to describe an infestation which are usually heavier than those observed in our study. The limit considered as harmful for sea lice is 0.05 lice/cm^2 of skin, which represent around 7 lice for a fish of 100g, and a deadly risk at 0.12 lice/cm^2 of skin. Compared to the weight of the SBT held in the sea cages, the number of sea lice to be harmful for the fish should be greater than what was observed in 2018 and 2019. One way that could explain an impact at such low intensity would be if the SBT skin was over-reacting to the presence of parasite and create an intense inflammatory reaction leading the SBT to scratch intensively or lead to systemic bacterial infections. The severity of diseases and lesions are associated with the number of parasitic sea lice, but depends on the size and the age of the fish, the general state of health, the species of copepod and the developmental stage present (Pike & Wadsworth, 1999). In salmon farms, treatment start when fish are infected by 1 to 5 female sea lice for a fish under 500g and infected by more than 5 female sea lice for bigger fish (Eithun, 2000). In the present study, the very low intensity of parasitism regarding the size and weight, between 15 and 40 kg, of the fish let suppose that SBT would need more parasites to be severely injured or dead. Sea lice can also lead to secondary infections, open wounds, osmoregulatory failure and respiratory deficiency (Brandal & Egidius, 1979; S. C. Johnson et al., 1996; Pike & Wadsworth, 1999; Wootten et al., 1982). Bacteria as *Aeromonas* sp. or *Vibrio* spp. had been reported as potentially vectored by *Caligus elongatus* (Munday et al., 2003) and can be implied in gross eye pathology as CL.

A primary hypothesis on CL was to attribute them to sea lice grazing on the cornea of the SBT as it was the case for *Caligus elongatus* (Rough, 2000). A mechanical break in the corneal epithelium would appear with SBT rubbing against nets or each other in order to attempt to remove the sea lice. The sea lice burden monitoring showed that the Companies 1 and 2 were showing a similar sea lice burden but different levels of CL. Moreover, when comparing the 2018 and 2019 ranching season from the Company 1, the sea lice burden was also of same intensity from a year to the other but the CL prevalence in 2019 was lower. This observation tends to show that sea lice are not participating in the development of CL. To investigate this point further, the successive model that considered as predictors the intensity of sea lice in the previous period before the observation of CL should have been fitting better than the cross-sectional model. Yet, the best fit was for the cross-sectional mixed-effect logistic regression. No temporal relationship could be evidenced which suggested that it was more likely that the sea lice infestation happened simultaneously to the emergence of CL, the sea lice certainly targeting the fish with CL which might be less competitive to fight parasites and swim slowly and represent easier targets. As sea lice could not explain the development of CL alone, it was decided to investigate other risk factors inherent to the companies (see section 5, p.83). The sea lice intensity was weakly correlated with the prevalence of corneal lesions (CL) in our final cross-sectional mixed-effect logistic regression. The significative difference between the odds ratio of mild and moderate sea lice intensity tends to show that the more sea lice are infesting the fish, the higher the odds to have CL. However, the tiny odds show that these parasites did not represent a main risk for the development of CL in our case. These results do not rule out Hayward's hypothesis as sea lice being a cause of CL, but they enhance the multifactorial aetiology of CL.

Some bias could have altered some of the results. All the pictures were not taken by the same divers everyday across the season. A lack of consistency is made by the change of operators, the appreciation of the depth and the distance of the fish was also different but crucial for the observation of sea lice on the pictures. To try to reduce as much as possible this bias, a form explaining how to take the pictures had been made to have more consistency between divers. Pictured SBT should be randomly chosen, but blind fish usually come closer to divers and are more likely to be isolated and near the surface. All the pictures were analysed by the same person for consistency in the detection of sea lice skin damages on the pictures. Checking the pictures underwater was difficult so it was not possible for the divers to be sure that their pictures were clear, and many were blurry when loaded on the computer, leading the operator to remove them from the study.

5. RISK FACTORS ANALYSIS FOR CORNEAL LESIONS IN RANCHED SOUTHERN BLUEFIN TUNA

5.1. Introduction

The first part of this study showed what were the consequences of the corneal lesions (CL) for the survival rate of southern bluefin tuna (SBT), *Thunnus maccoyii*, for their physical condition when they survive until harvest, and for the companies on a financial side. After this first step, the need was to identify risk factors that could lead to CL or ease its development in the population. It is very likely that these CL were initiated by a physical trauma of the corneal epithelium followed by either scaring of the wound of infection by other pathogens and worsening of the wound (see section 2.4.5, p.49). The first hypothesis made by the industry to explain a potential physical trauma was the burden of the sea lice *Caligus chiastos*, which could graze on the surface of the eye, creating a corneal ulceration. However, our investigation (see section 4, p.63) showed that sea lice alone failed to explain the development of CL, leading to the need to investigate other potential risk factors which may increase the risk of CL. As most disorders, the outbreak of CL is likely to happen with multifactorial causes as described by Snieszko (1974), when the hosts meet the pathogen in a favourable environment. However, this description was made for infectious diseases but could be extended to other factors, and here the word “pathogen” must be understood as the etymological Greek definition of what “produces suffering” and not only micro-organisms or parasites but detrimental events too. The short pathological study that was done was not enough to speculate about any specific causes. But many pre- and post-transfer factors vary between the companies and could participate in facilitating the development of CL.

A retrospective analysis of mortality patterns was expected to provide clues about the cause(s) and risk factors associated with SBT mortalities and/or CL. It was hypothesised that potential risk factors such as stock source, towing and grow-out conditions may contribute to the increase in CL and therefore the increase in mortality. The aim of this study was to highlight risk factors in the development of CL and help the farmers to reduce the CL prevalence for the coming ranching seasons as our first investigation (see section 3, p.51) showed a substantial financial impact of this issue.

5.2. Materials and methods

5.2.1. Study populations

The Table 14 presents the stock characteristics of the population which recorded information of mortalities with corneal lesions during the 2017, 2018 and 2019 ranching season. Only three company recorded them for three consecutive years, representing 39% of the population in 2017, 30% in 2018 and 29% in 2019.

Table 14. Structure of the population which recorded information of mortalities with corneal lesions for 2017, 2018 and 2019 ranching seasons.

Season	2017	2018	2019
Number of companies	5	3	3
Number of cages	38	29	31
Number of fish	101,157	97,398	100,686
Fish per cage	1,416 – 6,997	1,956 – 4,398	1,378 – 4,347

5.2.2. Data access and collection

Mortality and corneal lesions data

Mortality records and associated CL findings for the 2017, 2018 and 2019 ranching seasons were accessed from participating companies. Daily or every second day, except weekends, divers collected the dead SBT and recorded total count of mortalities and the presence of CL (within the mortalities) for each individual cage. There was no scoring system and recording standard for CL, and CL classification was scorer-dependent (subjective). Half (n=5) of the companies provided CL records in their mortalities in 2017, three companies in 2018 and three companies in 2019.

Stocking and management data

Cage level factors data was accessed from the industry and collated into a separate MS Excel spreadsheet for all the participating cages from 2017, 2018 and 2019 ranching seasons. Information provided by the industry included towing conditions, grow-out cage parameters, grow out cage coordinates, SBT numbers and stocking and harvest dates.

5.2.3. Data analysis

Mortality and corneal lesion descriptive analysis

Individual grow-out cages stock, mortality and CL data were accessed and collated within an MS Excel spreadsheet. Time series plots were generated to display cumulative mortality patterns and CL data and smoothed with a seven-day window to explore trends over time. Dot plots were generated to investigate the presence of cluster of mortalities or CL at the cage and at the tow cage level. A box plot was generated to explore the potential clustering of mortalities at the tow cage level. All the graph were generated using the statistical package STATA v.15.1 (College Station, TX, USA).

Risk factor analysis

A grow-out cage was classified as a 'mortality case' if its cumulative mortality during the entire ranching season was greater than the historical accepted level of 1%. Among the companies that recorded CL in their mortality, a cage was classified as a 'CL case' if at least 14% of their mortality showed CL (median percentage of mortalities bearing CL across the industry). A mixed-effect logistic regression to estimate unconditional association between potential risk factors and $Case_{mort}$ or $Case_{CL}$. Towing cage cross-classified with company was added to the model as random effects to account the lack of independence amongst cage-level data. Analysis outputs were interpreted at the 5% level of significance.

The association between CL and the risk factors was investigated by estimating the odds ratio between the variables using a simple logistic regression in which the risk factors were the predictors. To account for the clustering of cages within a company and companies within a year, random effects were respectively added for 'year', 'company-within-year' and 'cage-within-company-within-year' into the model (i.e. mixed effect model). The direct outputs of the model's coefficients, ignoring random effects, provided year-, company- and cage-specific estimates of association. To be relevant to the industry as an all, population-averaged estimates of the odds ratios (OR), and their 95%CI limits, were converted using the following approximation formula (Dohoo et al., 2009):

$$\text{OR} = exp\left(\frac{\beta_{RF}}{\sqrt{1+0.346\times\left(\sigma^2_{year}+\sigma^2_{company}+\sigma^2_{cage}\right)}}\right) \qquad \textit{(Eq. 1)}$$

where β_{RF} is the model fixed effect coefficient estimate for the considered risk factor and σ^2_{year}, $\sigma^2_{company}$ and σ^2_{cage} are the model's variance estimates for the random effects.

The proximity of the cages to surrounding reef or cost was calculated using the GPS coordinates (latitude and longitude), provided by the companies for the tuna cages and from the website Mapcarta (https://mapcarta.com) for the location of Berlin rock, Davidson rock and Donington reef. The distance between each rock and each cage was then calculated using the formula:

$$d = \cos^{-1}(\sin\left(Lat_1 * \frac{\pi}{180}\right) * \sin(Lat_2 * \frac{\pi}{180}) + \cos(Lat_1 * \frac{\pi}{180}) * \cos(Lat_2 * \frac{\pi}{180}) * \cos(Lon_2 * \frac{\pi}{180} - Lon_1 * \frac{\pi}{180})) * R_{Earth}$$

With *d* being the distance between the point 1 and 2, $Lat_{1/2}$ and $Lon_{1/2}$ respectively being the latitude and longitude of the points, and R_{earth} the radius of earth at the equator (6,378.137 km).

The routes of the towing boats were drawn using their GPS positions provided by the ASBTIA and reported on the website Maptive (https://www.maptive.com/). The routes were then used to find any upwelling event during the journey using the satellite water temperature maps from the website IMOS OceanCurrents (http://oceancurrent.imos.org.au/index.php).

5.3. Results

5.3.1. Descriptive of mortalities across 2017, 2018 and 2019

In 2017, the companies 1, 3, 6 and 10 were responsible for the high cumulative mortality at the industry level while in 2018 the companies 1, 5, 6 and 10 were (Figure 28). Only the company 10 in 2019 had a cumulative mortality higher than the expected 1%. From this observation, the mortalities seem to be clustered at the company level.

The high cumulative mortality that was observed in specific companies was now expressed at the cage level (Figure 29). Each year small groups of cages compared to the total number of cages in the industry were showing a high cumulative mortality, showing evidences of clusters at the cage level. In 2019, the few cages that stand out from the others in term of cumulative mortality are all from the same company which did not provide any data on mortalities with CL.

At the tow cage level, the high level of mortality can be attributed to specific tow cages which could enhance the existence of a cluster in the tow cages (Figure 30). However, a tow cage is rarely shared and all the fish from a specific tow cage are usually destined to go in only one company. This cluster could be a bias of the cluster at the company level.

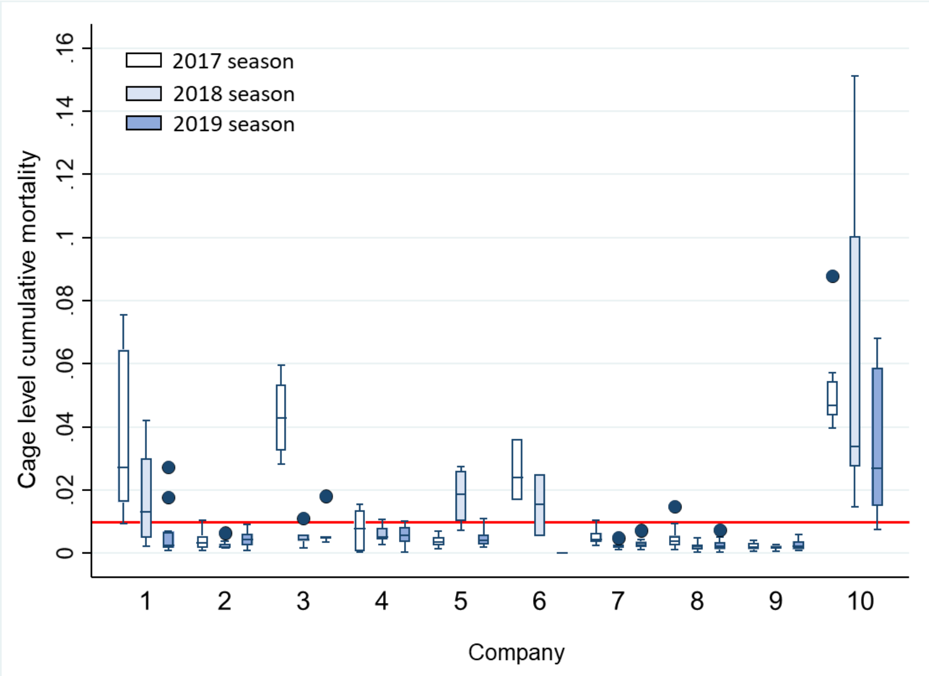


Figure 28. Cage level cumulative mortality from the 10 companies of the tuna industry across 2017, 2018 and 2019 ranching seasons. The red line represents the 1% accepted yearly cumulative mortality.

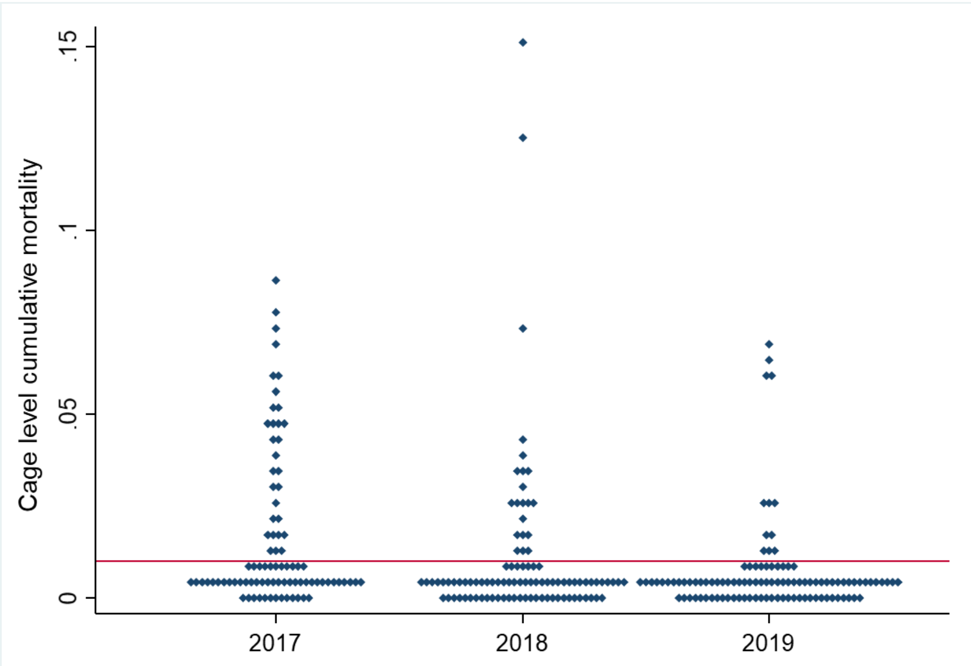


Figure 29. Cumulative mortality distribution across cages. The red line represents the expected limit of 1% of cumulative mortality. Each dot represents a cage.

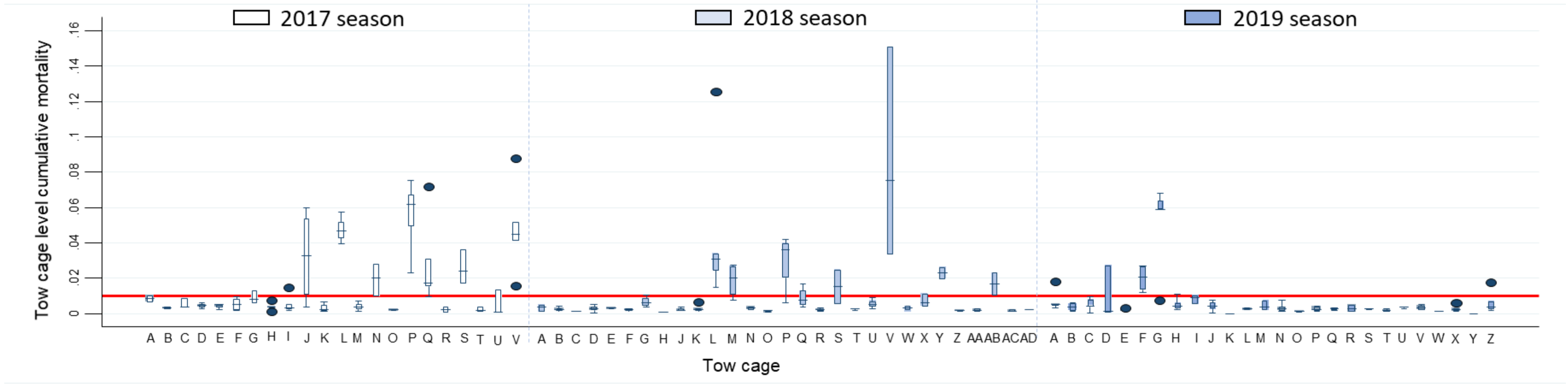


Figure 30. Tow cage level cumulative mortality from the 10 companies of the tuna industry across 2017, 2018 and 2019 ranching seasons. The red line represents the 1% accepted yearly cumulative mortality.

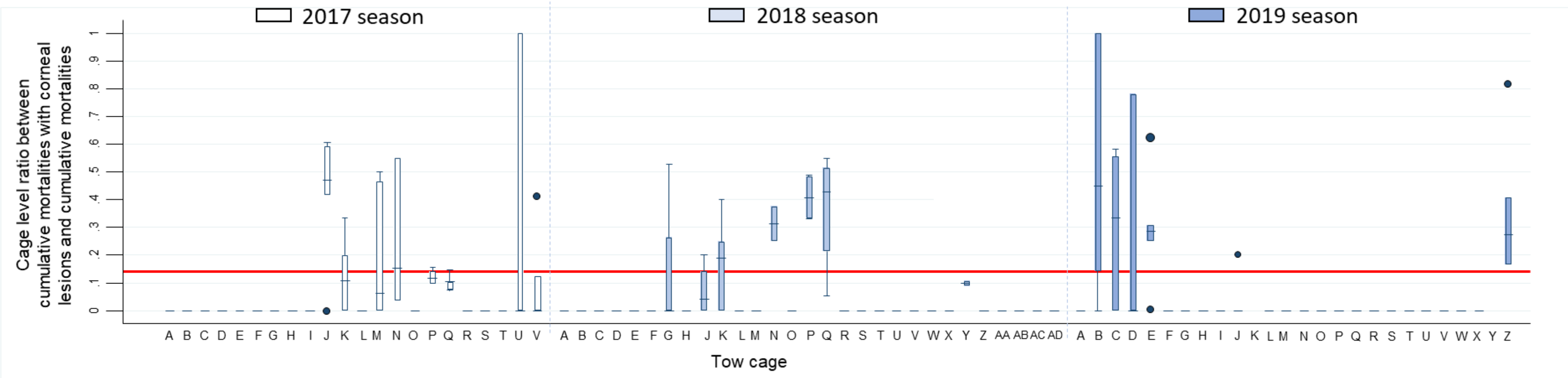


Figure 31. Tow cage level cumulative mortalities with corneal lesions from the 5 companies that recorded them in 2017, and the three companies that recorded them during the 2018 and 2019 ranching seasons. The red line represents the 0.14 median of the ratio of mortalities with corneal over the global mortalities across the population.

5.3.2. Descriptive of mortality with CL across 2017, 2018 and 2019

The Figure 32 presents with a box plot the ratio between the cumulative mortalities with CL and the global cumulative mortality for each company during the 2017, 2018 and 2019 ranching seasons. This graphic shows well the lack of data that this analysis suffered from as many companies did not record the information of CL within the mortalities. In 2017, five companies recorded the CL, the company 3 being the most impacted by the issue. However, this latter decided not to continue recording the CL. It seems that the companies which had a significant level of CL 2017 also had in 2018 and 2019. The CL incidence could be clustered at the level of company. However, when focusing on the company 4, it seems that overall, this company did not have a CL issue as defined before, but only few cages did. This evidence the better possibility to see the CL issue clustered at the cage level. This figure only represents ratios and so must be put in perspective with the Figure 28 as for instance, the company 4 had low cumulative mortality. However, even if there overall cumulative mortality shows that this company did not suffer from the CL issue, a great part of their mortality was due to CL and might still have been avoided.

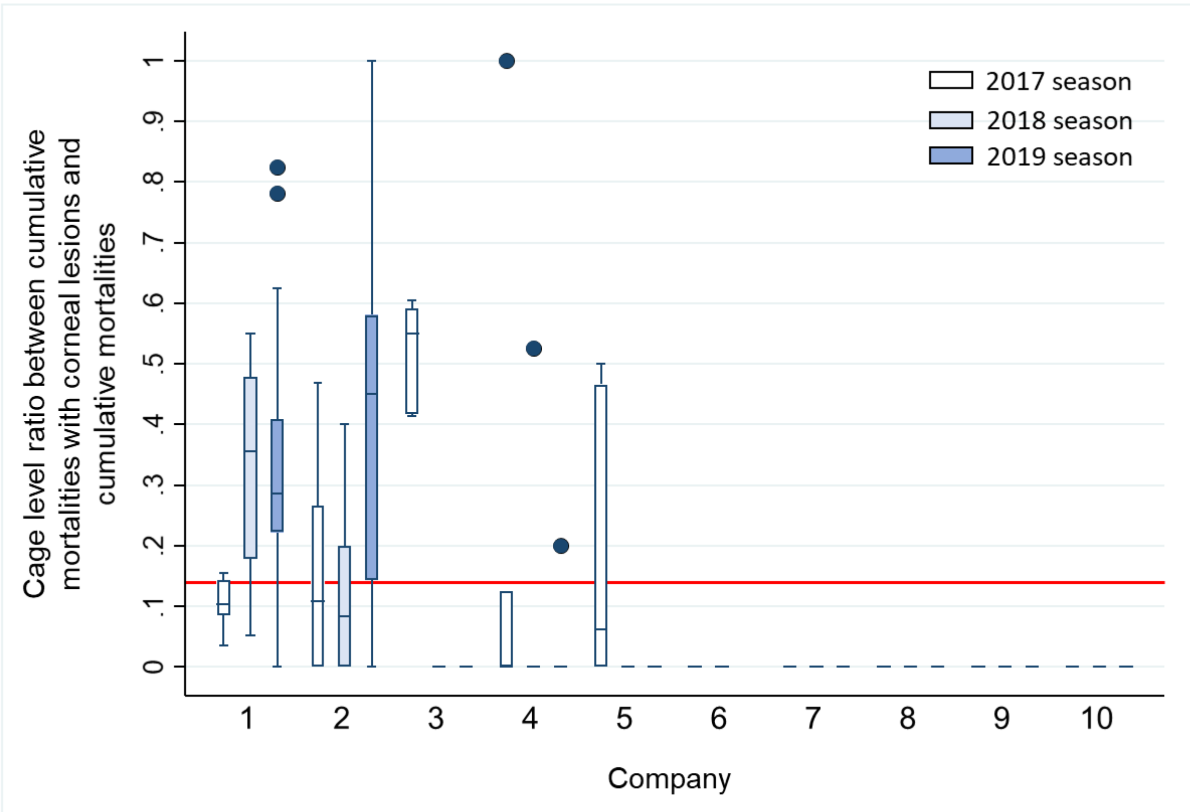


Figure 32. Cage level ratio between cumulative mortalities with corneal lesions and cumulative mortalities in the companies that recorded corneal lesions within the mortalities (2017: n=5; 2018: n=3; 2019: n=3) across the 2017, 2018 and 2019 ranching seasons. The red line represents the median of mortalities bearing corneal lesions across the industry (0.14).

When looking at the tow cage level (Figure 31) it seems that CL could be clustered to specific tow cages. But as mentioned earlier, tow cages are rarely shared and most of the time a tow cage is only used by one company. It is then very likely that an unknown confounding bias could exists at the level of the company, giving the impression of a cluster at the tow cage level.

5.3.3. Risk factors analysis for 2017-19 cumulative mortalities with corneal lesions

CL were not recorded by every company each year, five did it in 2017 and only three continued for the 2018 and 2019 ranching seasons. All the cages in which CL were recorded within the mortalities were used to analyse risk factors in the development of CL, by comparing variables through three consecutive years. The Table 15 describes the cage-level data for each for the 2017 to 2019 ranching seasons. The companies changed the number of cages they own from a year to another, so the total number of cages varied 29 in 2018 to 31 in 2019. A lower number of observations could be seen for tow cages data as some cages were holding fish from different tow cages, no specific value could be attributed to these cages. In total, this study included 98 cages across the three years, 17 cages (17.4%) were considered as a case (>1% cumulative mortality and >14% mortalities with CL), 12 cages in 2017, 3 cages in 2018 and 2 cages in 2019. The other cages were considered as a negative control.

Table 15. Descriptive summary of variables collected in the risk factor analysis for the cage-level cumulative corneal lesion in Southern bluefin tuna in Port Lincoln during the 2017, 2018 and 2019 ranching seasons.

	2017				**2018**				**2019**			
Variables	**Obs.**	**Mean**	**Median**	**Min-Max**	**Obs.**	**Mean**	**Median**	**Min-Max**	**Obs.**	**Mean**	**Median**	**Min-Max**
Initial number of SBT in tow cage	38	12,855	12,273	7,673 – 17,673	28	12,828	13,179	6,464 – 15,998	29	16,938	14,964	10,572 – 23,880
Tow cage diameter (m)	38	46	45	45 - 50	28	46.3	45	45 - 50	29	46.2	45	45 - 50
Number of days towing	38	103	11	5 - 18	28	15.8	13	9 - 34	29	14.0	15	8.0 – 18.0
Initial number of SBT in grow-out cage	38	2,662	2,940	1,416 – 3,162	29	3,359	3,281	1,956 – 4,398	31	3,248	3,296	1,378 – 4,347
Reef proximity (km)	38	5.8	6.5	1.4 – 10.7	29	5.6	6.6	1.4 – 8.1	31	5.5	6.5	1.3 – 8.0
		Count (%)				**Count (%)**				**Count (%)**		
SBT from tow mix	38	3 (8.9%)			29	1 (3%)			31	2 (6%)		
Towed through upwelling	38	24 (63.2%)			25	7 (28%)			0	.		.
Net material	38				12				N/A	.		.
Nylon		5 (13.2%)				0 (0%)						
Polyester		33 (86.8%)				12 (100%)						

The results from the mixed-effect logistic regression analysis are presented in the Table 16. The only factor that fitted the model the best was the proximity of the cages considered as a ‘case’ to a reef. The odd of becoming a cage with CL and mortalities as defined by the ‘case’ definition could decrease by 13% for every extra km of distance from a reef. The odds ratio for the other risk factors investigated were not reported here as they were found to be at no risk or not statistically significant.

Table 16. Mixed effect logistic regression model of cage-level risk factor variables for cage- cases (> 1% cumulative mortality and >14% of mortalities with CL) from 87 cages in 3 companies across the 2017, 2018 and 2019 ranching season.

Fixed effect	*Odds ratio*	*Confidence interval*	*P*-value
Reef proximity (km)	0.87	0.80 – 0.95	P=0.001
Random effect	*Variance*	*Confidence interval*	
Year	0.81	0.03 – 21.7	
Tow cage	2.24	0.30 – 16.7	
Company	7.34×10^{-33}	-	

5.4. Discussion

This analysis of the characteristics of the environment, either surrounding the cages or the ranching management by the different companies, aimed to highlight factors that could facilitate the development of CL. The results showed that the odd of a cage to become a case with a high cumulative mortality (<1%) and high ratio of cumulative mortality with CL over the global cumulative mortality (<14%) could be reduced by 13% for every extra kilometre of distance to a reef for cages considered as cases compared to non-case cages.

The main limitation in this study was the data collection. Ranching companies could be reluctant to share some management data as those could be used by rival companies for their personal interest. All the data that were requested were not shared by the companies and the analysis was then done on the available data. As it was mentioned earlier, the recording of CL in mortalities was not performed by all the companies which also reduced the sample of cages that could be used for the risk factor analysis in the development of CL.

The purpose here was not to find the cause of the development of CL as it was very unlikely to find strong evidences of a unique cause. In aquaculture, the development of corneal ulcers was mostly found to be caused by mechanical abrasion that could originate from many sources, which could heal rapidly in optimal conditions or deteriorate with bacterial or fungal opportunistic infection and lead to the rupture of the cornea (Ferguson et al., 2006). That is why this study tried to highlight the variables that could make that ranched SBT were not in optimal conditions. The only risk factor that was evidenced by our study was the proximity of the considered cages to a reef. But the companies gather their cages on a same area for each company. A confounding bias due to a cluster at the company level could have made up this result. Moreover, the proximity to a reef does not seem biologically justified in facilitating the development of CL. This could have been the case if the sea lice were shown to be participating in the development of CL as many fish infested by sea lice can live in these reefs. However, this was not the case in our situation as sea lice could not explain the CL (see section 4, p.63). The density of fish in the grow out cages or in the tow cages was not found as a risk factor in our study. However, a high density could lead to aggressive behaviour between fish, with a well know behaviour in aquaculture called "eye snapping", were dominant fish bite directly the eye of other conspecifics (Ferguson et al., 2006). Even if frequent and excess feeding was shown to reduce aggressions (Sunde et al., 1998), the wild SBT could injure themselves during the first feeding in the grow-out cages. While the fish are towed from the catching site to the ranching area, they are fed, depending on the weather, and in lower quantity than their need. It was described by the industry that the wild SBT were voracious during the first feeding. Such agitation could lead to messy movements, the fins, skin, and teeth of SBT being tough and sharp, accidents of collisions with the eyes of conspecifics could injure the cornea of the fish. The high quantity fed at the arrival has the purpose of boosting the growth to start the ranching (Benetti et al., 2016). However, excess feeding after an almost fasting period during towing might not be the more efficient way of feeding regarding the welfare and the behaviour of wild caught SBT. Usually, blocs of bait fish are dropped in the centre of the cage. Another way of delivery which could distribute the feed more equally could reduce detrimental behaviours. In other species like salmonids (Salmonidae), the development of hyperplastic changes in the corneal epithelium and the increase of stromal thickness was associated with vitamin A deficiency (VAD) (Ferguson et al., 2006). VAD was also associated with other symptoms observed in the tuna industry like poor growth, high mortality, eye lesions with oedematous eye and maybe when seeing the dissected eye (see section 2.4.2 p.43) displaced lens and degeneration of retina (Poston et al., 1977). However, other symptoms described in salmonids like anaemia, twisted gill opercula and haemorrhages in the eye or at fin base (Kitamura et al., 1967) were not described in the tuna industry for fish with CL. But these latter symptoms were described on rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), fingerlings, which physiology is highly different from adults SBT and could explain the different expression of symptoms of VAD. This deficiency could not be investigated in our study. However, this hypothesis stayed probable considering a feeding issue. The SBT were only fed bait fish, being there only source of nutrients. If a part of the population was not properly fed during towing and then excluded by dominant fish during the first days of feeding in the grow-out cages, a sub-population could suffer VAD. Finally, commonly described cause of CL in fish was the infestation by sea lice, like in Atlantic salmons with *Lepeophtheirus salmonis* (Krøyer, 1837), which could pierce the cornea and was associated with corneal opacity (Ferguson et al., 2006). The hypothesis of sea lice causing the CL in our case was investigated and could not explain the development of CL in this situation (see section 4 p.63). Further investigations on risks factors for the development of CL should be undertaken, maybe with a particular focus on feeding behaviours.

6. CONCLUSION AND RECOMMENDATIONS

Corneal lesions (CL) in Southern bluefin tuna (SBT) were already observed for several year as an anecdotal report on mortalities. With a growing number of fish showing this symptom in 2017 we were able to highlight that fish bearing these lesions were more likely to die before harvest and were in poorer condition in terms of weight and body condition index. Even if no temporal correlation could be made between the development of the corneal lesions and the loss of weight and body condition index, the literature reported that most ocular diseases in fish were associated with poor growth and high mortalities which support our conclusions. A financial analysis of the loss of revenue that can be attributed to the CL showed that the companies should not take this problem slightly. A cage with a high percentage of blind fish will quickly represent a loss of revenue and the figures showed here were not including the potential loss due to the investment done for each fish. However, the low prevalence of CL that most cages showed in 2017, 2018 and 2019, alleviate these conclusions and show that this affection is not a problem currently, but could if the prevalence increases.

The industry was mostly concerned about the emergence of sea lice in the SBT cages. As sea lice were described in other aquaculture systems to be at the origin of CL, an analysis of their burden and the risk due to their presence showed that they should not be the cause of the current CL. The attempt to show a temporal correlation between the outbreak of CL and the outbreak of sea lice failed to confirm the hypothesis that sea lice would infest the fish before the emergence of the lesions. However, it is well known that sea lice are a heavy burden as much for the welfare and the health of the fish as for the cost they represent in other aquaculture systems (Costello, 2006). Therefore, we suggest that the monitoring of sea lice in the tuna industry should be maintained to try to prevent any outbreak. The species *Caligus elongatus* was already described from several years in the tuna industry, but now the species *Caligus chiastos* seemed to settle in the area. We provided in this study a simple key to identify *C. chiastos* which can be used only with a dissecting microscope. They might not be a problem yet in the tuna industry, but sea lice should be considered as a serious problem if numbers increase from a year to another. It would be easier and cheaper to tackle any outbreak before it would become a regular issue with a heavy burden that make the control more complicated. After a better understanding of the dynamic of the sea lice population in the tuna industry, sustainable control measures could be considered as biological or mechanical controls.

A global analysis of the fish environment from their catch in the wild to the ranching period showed only one factor that could participate in facilitating the development of CL. Our main hypothesis was that corneal ulcers would break the integrity of the eye, by mechanical abrasion, which could be due to different origins (scratching against nets, bad handling, collision between conspecifics, aggressive behaviour against weak fish, competition for food, jelly fish, external parasites). These types of lesions according to the literature could heal in perfect environmental conditions. However, risk factors could avoid the healing process and lead to corneal oedema, keratitis and infection of the eye and possibly to systemic infections. The only factor evidenced in our study was the proximity of the cages to a reef, but it did not seem to be a realistic risk factor as it was very likely to be associated with a confounding bias. The aetiology could not be found with our study, neither strong risk factors but recommendations could still be made:

1. The density of fish in the grow-out cages and towing cages should be carefully limited,
2. The towing of the wild SBT from the catch area to the grow-out cages should be as short as possible, avoiding keeping the SBT in the tow cages for days near the catch area or in the ranching area,
3. Mooring the grow-out cages as far as possible from the shore and reefs to limit interactions with wild species,
4. Feeding fish progressively, maybe by dividing the school in smaller groups or by spreading bait fish in several areas to avoid detrimental behaviours due to voracious feeding of the tuna after towing.

Further investigations on the risk factors should be undertaken to understand the mechanisms of development of CL as it seems to be a multifactorial affection. Regarding the current prevalence of CL across the monitored companies, the CL issue should not represent a main problem. But understanding the affection would help to tackle any bigger outbreak quickly, which would be crucial for the companies' profits.

AGREMENT SCIENTIFIQUE

En vue de l'obtention du permis d'imprimer de la thèse de doctorat vétérinaire

Je soussigné, Jean-Luc GUERIN, Enseignant-chercheur, de l'Ecole Nationale Vétérinaire de Toulouse, directeur de thèse, certifie avoir examiné la thèse de **Thomas DUMOND** intitulée **« Etiologie et impact des lésions cornéennes chez le thon rouge du sud d'élevage, *Thunnus maccoyii* (Castelnau, 1872) »** et que cette dernière peut être imprimée en vue de sa soutenance.

Fait à Toulouse, le 16/10/2020
Enseignant-chercheur de l'Ecole Nationale Vétérinaire de Toulouse
Professeur Jean-Luc GUERIN

J.L. G...

Vu :
Le Directeur de l'Ecole Nationale Vétérinaire de Toulouse
M. Pierre SANS

Ecole Nationale Vétérinaire de Toulouse - Direction de l'Enseignement -

Vu :
La Présidente du jury
Professeure Charlotte VAYSSE

Vaysse

Vu et autorisation de l'impression :
Le Président de l'Université Paul Sabatier
M. Jean-Marc BROTO

UNIVERSITE PAUL SABATIER - TOULOUSE

Le Président de l'Université Paul Sabatier,
La Vice-Présidente de la CFVU
Fabienne ALARY

M. Thomas DUMOND
a été admis(e) sur concours en : 2015
a obtenu son diplôme d'études fondamentales vétérinaires le: 09/07/2019
a validé son année d'approfondissement le: 15/10/2020
n'a plus aucun stage, ni enseignement optionnel à valider.

Université de Toulouse

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Toulouse, 2020

NOM : DUMOND **PRENOM** : Thomas

TITRE : Etiologie et impact des lésions cornéennes chez le thon rouge du sud d'élevage, *Thunnus maccoyii* (Castelnau, 1872)

RESUME :

Les lésions de la cornée (LC) peuvent se développer jusqu'à la perte d'un ou des deux yeux chez le thon rouge du sud (TRS, *Thunnus maccoyii*). L'impact sur la santé et sur les finances de ce problème émergeant dans les élevages Australiens reste inconnu. Premièrement, une étude hybride rétrospective des données de mortalité et un sondage lors de la récolte des thons a été menée sur les années 2017/18 pour évaluer les potentiels conséquences des LC. Ensuite, une macroanalyse des variables environnementales et de gestion d'élevage a été menée pour explorer les potentiels facteurs de risques. Enfin, l'étude s'est focalisée sur les populations de *Caligus chiastos* qui infestaient les TRS, les poux de mers étant connu pour endommager la peau des poissons, ils étaient une préoccupation majeure dans le développement des LC. Les TRS aveugle d'un ou deux yeux étaient 17 fois plus enclin à mourir et un TRS mort représentait une perte de revenus de AUD$ 460.26 dans les enclos étudiés. Les TRS affectés survivant jusqu'à la récolte étaient plus légers de 3kg comparé au reste de leur cage. Cela se traduisait par une perte de revenus de AUD$ 89.87 par poisson affecté. Les enclos étudiés affectés possédaient en moyenne 4,72% de poissons aveugle d'un ou des deux yeux, amenant à une perte moyenne de AUD$ 37 557au niveau de l'enclos. Peu de facteurs pouvaient être identifiés comme menant à un risque majeur dans le développement des LC. Les enclos à forte densité ou proche d'un récif étaient respectivement 12,7 et 29,9 fois plus enclin à posséder une mortalité supérieure à 1%. Chaque kilomètre supplémentaire de la localisation d'u enclos par rapport à un récif pourrait réduire la chance de développer des LC de 28% et chaque jour supplémentaire de transport depuis le site de capture jusqu'au site de grossissement pourrait augmenter la chance de développer des LC de 24%. Enfin, *Caligus chiastos* infestait la plupart des enclos étudiés avec une prévalence moyenne de 42,6% mais une faible intensité moyenne de 12 poux de mer. Cependant, aucune corrélation temporelle n'a pu être mis en évidence entre l'infestation par les poux de mer et le développement des LC, signifiant qu'il était impossible de conclure que *C. chiastos* étaient la cause des LC. Il semble que les LC causent d'important dommages aux TRS avec des pertes non négligeables pour l'industrie.

MOTS-CLES : lésion cornéenne, thon rouge du sud, œil, épidémiologie, poux de mer, caligus

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TITLE: Aetiology and impact of corneal lesions in farmed southern bluefin tuna, *Thunnus maccoyii* (Castelnau, 1872)

ABSTRACT:

Corneal lesion (CL) can develop into the loss of one or both eyes of southern bluefin tuna (SBT, *Thunnus maccoyii*). The health and financial impact of this emerging condition in the Australian ranching industry remains unknown. First, a hybrid design of retrospective mortality records and prospective harvest surveys was conducted of the 2017/18 ranching seasons to assess these potential impacts. Then, a macroanalysis of variables from the environment and the farm management was conducted to explore potential risk factors. Finally, the study focused on *Caligus chiastos* populations that were infesting the fish, as sea lice are known to damage the skin of the fish, they were a major concern in the development of CL. One-eyed or blind SBT were 17 times more likely to die and a dead SBT represented a revenue loss of AUD$ 460.26 in the studied cages. Affected SBT that survived until harvest were significantly lighter by 3 kg than the rest of their cage. This translated into a revenue loss of AUD$ 89.87 per affected SBT. Studied affected cages had an average of 4.72% of one-eyed or blind SBT resulting into an average cage-level revenue loss of AUD$ 37,557. Few factors could be identified as major risks in developing CL. Cages with higher densities or closer to a reef were respectively 12.7 and 29.9 times more likely to develop a mortality higher than 1%. Each extra kilometre of the cage location from a reef could reduce the odds to develop CL by 28% and each extra day of towing from the catching site to the grow-out area could increase the odds to develop CL by 24%. Finally, *Caligus chiastos* was infesting most of the monitored cages with a mean prevalence of 42.6% but a low mean intensity of 12 sea lice. However, no temporal correlation could be evidenced between the infestation of sea lice and the development of CL, meaning that it was not possible to conclude that *C. chiastos* was the cause of the CL. It appears that CL are causing great damage to SBT and are valuable for the tuna industry.

KEYWORDS: corneal lesions, southern bluefin tuna, eye, epidemiology, sea lice, caligus