Understanding variation in ecology and physiology of marine migratory species.

By

JENNY BORTOLUZZI

BSc Marine Biology with Oceanography, University of Southampton 2015

MRes Marine Biology, University of Plymouth and Marine Biological Association, 2018

A thesis submitted in partial fulfilment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

School of Natural Sciences
(Zoology)

Trinity College Dublin

March 2023

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

I consent / do not consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

Bortoluzzi

Jenny Bortoluzzi

Summary

As the world faces the threats of multiple compounded and worsening crises, scientists are racing to gather the knowledge necessary to safeguard entire ecosystems and species. Technological advances are continuously facilitating more in-depth studies to understand the mechanisms driving species functioning and variations among and within populations, communities, and individuals. New methods are providing insights into difficult to access environments and species such as the open ocean. The following thesis uses theoretical and empirical approaches to understand the drivers of variation in ecological niches of large marine predators. One of the methods commonly used to study a species resource use is stable isotope analysis (SIA). However, the relationship between variation in stable isotope values in the tissues of consumers and their diet is often misconstrued or over-simplified. Here, we lay out the underlying factors that influence stable isotope ratios and how these can be accounted for when designing an ecological study. I then review new advances in stable isotope technologies and how compound-specific SIA can be used to ask questions about the life-history of a broad range of species. Having shown the value in combining biochemical methods with other disciplines, I apply this approach to study the ecology of large marine vertebrates in Ireland. By using fatty acid analysis in combination with reproductive hormone analysis, biologging, morphometrics and observations, I investigate the ecology and physiology of blue sharks, *Prionace glauca*, in Ireland. This population of predominantly female individuals in varying stages of maturity show indications of a seasonal change in resource use between the June and November – possibly indicating opportunistic foraging on abundant gelatinous or planktonic prey. Finally, I present the results from

our tagging efforts on mature female porbeagle sharks, *Lamna nasus*, caught in Donegal (Ireland) in April 2022. As I have followed these two individuals over the past nine months, both have crossed many environmental and jurisdictional boundaries but have displayed high inter-individual variation. I argue the need for increased and continued cross-country collaboration in the Northeast Atlantic to study and manage this critically endangered species. The work carried out here emphasises the value in moving our field from uni- and multi-disciplinary approaches towards more holistic interdisciplinary approaches, particularly when our aim is the sustainable preservation of large oceanic migrators.

Acknowledgements

As I think is expected, when I started this PhD, I had my doubts as to whether I was making the right decision. I had chosen what would seemingly be a heavily computational and statistical topic and which, as someone who many describe as shark-obsessed, may not have been the obvious choice. But as I sat jetlagged in front of my computer interviewing for the position (who interviewed who, we'll never really know) it was clear to me that I would be lucky to land Dr Andrew Jackson as a supervisor. I was right. Andrew has not only entertained but encouraged my ideas and always found ways to make them work, letting me shape this PhD into something I was truly passionate about. His response to my wildest suggestions was always "Do it!". The support, advice and care he showed for me as a supervisee and a person is what makes him a great supervisor and someone that academia is lucky to count in its ranks. Add to this team Dr Nick Payne whose Aussie humour and habit for shortening every word and name under the sun made for entertaining meetings and fieldwork days. When PhD despair hit, Nick always showed the enthusiasm for my work and results that I couldn't and pulled me out of writer's block more than once. Having Andrew and Nick as a supervisory team is like the perfect mix of cynicism and positivity. Thanks also to the Jackson and Payne lab groups for all the fieldwork help and stimulating meetings.

Of course, the backdrop of the fabulous Zoology department certainly helped, and I am so thankful for the wonderful community within these (very old) walls and the various cohorts of postgrads and staff who have offered listening ears over the years. I was so lucky to start my journey with Mo, Sam and Alain's guiding advice and hope I have been able to keep their legacy going for new cohorts. The office across the hall have been so gracious in dealing with my impromptu appearances when avoiding my work and Tallulah, Emma, Simon and Grace A have never complained even when they could have! Testament to our great workplace are our numerous tearoom breaks always echoing with laughter, none more imbued into these walls and sorely missed than Aoibheann Gaughran's. I am so pleased to see pre-covid traditions and new ones brought back by the enthusiasm of our new cohorts of RAs and Postgraduates. Of course, this department wouldn't run without the amazing staff members who give everything they can to allow us to carry out our scientific visions. Alison, Sinead, Rebecca and Fiona have been my fairy godmothers, and I am once again so grateful for their continued advice and support.

When I moved to Dublin and started this PhD, I found it hard to make friends. How do you do that as an adult? The answer to that turned out to be simple: drink wine and eat cheese. And it turns out, there is no one better to do that with than the incredible four: Floriane, Andrew, Laura and Paula. So, here's to Friday night wine, raclettes, spontaneous concert-

booking and 20-minute voice-notes for many many more years. They have been the most supportive bunch, listening to all my ups and downs week after week. I couldn’t have done this without them.

Some friendships just come easily, and the instant connections I formed with Aedín and Abby are a testament to the truly wonderful women and friends that they are. Fen, Ellen and Kim have been there for me since our master’s, and I cannot imagine having better cheerleaders and friends than them. Women lifting other women up are the best kind, Alex and Chelsea understood that assignment so perfectly. All these women are doing incredible things and I can’t wait to see what they all achieve in the future.

Après onze ans d’amitié avec Manon, Margaux and Lauranne, je sais que peu importe mes longues absences, elles seront toujours au bout du téléphone et j’espère qu’elles savent combien je leur en suis reconnaissante.

I am fur-ever grateful to my four-legged emotional support system for the many snuggles: my dear Elfie, who was my best friend for 13 years, our gorgeous Penny, and our Cat’s Aid fosters past, present and future.

Papa et Maman, depuis toujours vous nous avez encouragés à poursuivre nos passions, à apprendre et à saisir toutes les opportunités qui se présentent à nous. Je n’en serais pas là où je suis sans votre soutien constant, vos sages conseils et tout l’amour avec lequel vous avez rempli ma vie (et bien plus encore!). Merci pour tout ce que vous avez fait et faites pour moi, Robin et Michael chaque jour.

To Marlowe, and all my potential future niblings: I cannot wait be your cool auntie, to share my love of the ocean and outdoors with you, to take you diving with sharks and teach you all about them. This PhD was just one step for me towards playing my part in making sure you get to live in a world where these amazing animals are still swimming around in healthy oceans. I also cannot wait to see what passions you will find in your lives and learn from you about whatever it is that brings a smile to your face.

Last but not least, of course, I cannot thank you enough, Kevin, for absolutely everything that you are, everything that you’ve done and for moving to Dublin to be with me 3 years ago. I can’t imagine life without you anymore (despite how mad your mess can sometimes drive me). More than anything else, thank you for cheering me on always, holding me when I need and believing in me even when I don’t. I hope we keep jumping into cold seas together everywhere we go.

Table of Contents

List of Tables

List of Figures

Publications

Bortoluzzi, J., Darby, J. and Jackson, A. L. 2021. Technological advances in stable isotope analysis provide ever more insights into animal ecology. *Journal of Animal Ecology,* 90**,** 1394-1397.

Contribution: I led the writing of the publication.

Arnoldi, J.F., **Bortoluzzi, J.R**., Rowland, H., Harrod, C., Parnell, A.C., Payne, N., Donohue, I. and Jackson, A.L., 2023. Identifying the limits where variation in consumer stable isotope values reflect variation in diet. *bioRxiv*, pp.2023-04.

Contribution: This preprint is the result of work carried out for Chapter 2 and in subsequent months. I co-wrote the manuscript, contributed to the study design and data modelling.

Harding, L., Gallagher, A., Jackson, A., **Bortoluzzi, J**., Dolton, H.R., Shea, B., Harman, L., Edwards, D. and Payne, N., 2022. Capture heats up sharks. *Conservation Physiology*, *10*(1), p.coac065.

Contribution: I co-led fieldwork organisation and data collection for the fieldwork carried out in Ireland, assisted with data collection in the Bahamas and reviewed the manuscript.

Conferences talks

- TCD Botany-Zoology Postgraduate Symposium (2019, 2020, 2021)
- Sharks International Valencia (2022)
- Ecology and Evolution Conference supported by the Irish Ecological Association and BES (2023)

Science Communication and Outreach

Public Talks	Irish Wildlife Trust Shark Webinar Presentation Sharks4Kids School presentations from 2019 to present Green Schools Marine Week 2021 MISS Elasmo Week 2020 (Irish Basking Shark Group video) MARECO workshop presentation (2021 and 2022)
TV	RTE 1 EcoEye Blue Horizons episode
Radio	The Louise McSharry Show on RTE Radio 2 BBC Radio Foyle
Podcasts	The Critter Shed The Whole Tooth Podcast by Save our Seas Foundation TCDSU Diversity In Stem

Chapter 1 General Introduction

Intertwined with the climate crisis the world faces today is the rapid and accelerating loss of biodiversity and ecosystems, often referred to as the “biodiversity crisis” or “sixth mass extinction” (Barnosky *et al.*, 2011, Ceballos *et al.*, 2015, Gonçalves-Souza *et al.*, 2020). Through land-use, habitat degradation and fragmentation, overexploitation, pollution and more, humans have brought about a catastrophic decimation of the Earth’s biosphere (Tilman *et al.*, 2017). In 2022, a report by Birdlife International revealed almost half of the world’s bird species have declining populations and over a fifth are considered to be of significant conservation concern (Birdlife International, 2022). According to the IUCN Red List, of the more than 42,100 species assessed, at least 41% of all amphibians, 27% of all mammals, 21% of all reptiles and 36% of all reef corals are threatened with extinction (IUCN, 2023). Plants do not fare any better, with estimates of up to 36% of all trees (Ter Steege *et al.*, 2015) and up to 40% of vascular plants (Nic Lughadha *et al.*, 2020) being threatened with extinction. Ireland is no exception to these global trends and, since the early 2000s, assessments have consistently shown that Ireland’s biodiversity levels are in decline with 91% of the 58 habitats listed under the EU Habitats Directive having been found to be ‘inadequate’ or ‘bad’ in status (Morrison and Bullock, 2018). According to the National Biodiversity Data Centre, 31,000 species are known to occur on and around the island yet only 10% of those are Red List assessed and one fifth of those that are, are threatened with extinction (National Biodiversity Data Centre, 2019). Additionally, of the 58 Irish species of cartilaginous fish (sharks, rays and chimaeras) assessed (out of a total of 71), 29% were determined to be threatened with extinction (Clarke *et al.*, 2016). Many species and taxa such as fungi, mosses, marine invertebrates, chimaeras, or insects remain under-represented in extinction risk assessments, often due to the difficulty of studying them (e.g. difficulty of access) or applying assessment criteria (McKinney, 1999, Dahlberg and Mueller, 2011, Nic Lughadha *et al.*, 2020, Finucci *et al.*, 2021).

When considering biodiversity loss and extinction risk, large-bodied species and/or those with slower life-histories, such as chondrichthyans and marine mammals, are known to be particularly vulnerable (García *et al.*, 2008, Hutchings *et al.*, 2012, Pardo *et al.*, 2016). For sharks, rays, and chimaeras, this vulnerability has resulted in over one third of all species being threatened with extinction (Dulvy *et al.*, 2021). In the last half century, this group has been subjected to excessive mortality, primarily from overfishing, resulting in estimated declines of over 70% in oceanic species (Pacoureau *et al.*, 2021); the complete depletion of reef sharks on almost 20% of surveyed reefs (MacNeil *et al.*, 2020); and the reduction of sawfishes presence to around 59% of their historical distribution (Yan *et al.*, 2021). Yet, knowledge gaps remain a challenge to the conservation of these species with an estimated 12.9% of assessed chondrichthyan species still classed as data deficient (Dulvy *et al.*, 2021) and population level assessments are likely even lower (Jorgensen *et al.*, 2022).

With large gaps in our current understanding of the biosphere still remaining, scientists today are working against the clock to improve our knowledge of environments' and species' existence, populations and ecologies both to monitor the damage being done and provide the tools necessary to mitigate it (Bar-On *et al.*, 2018). Technological advances have provided many tools to undertake this work, but many challenges still remain. The sheer scale of the marine environment combined with its diversity, complexity and opacity create inherent logistical and technical challenges along with persisting funding barriers to research and monitoring (Hussey *et al.*, 2015, Jorgensen *et al.*, 2022). This is particularly true of oceanic systems and wide-ranging migratory species whose geographical ranges span multiple jurisdictional boundaries such as Exclusive Economic Zones (EEZs) as well as Areas Beyond National Jurisdiction (ABNJs) such as the high seas (Dulvy *et al.*, 2008, Lascelles *et al.*, 2014, Dulvy *et al.*, 2017). Along with an estimated 829 other species, marine vertebrates such as blue and porbeagle sharks, are considered 'very highly migratory', 'highly migratory' or 'migratory' species (Lascelles *et al.*, 2014) and are sometimes capable of trans-oceanic movements (Da Silva *et al.*, 2010, Cameron *et al.*, 2018), leading them to regularly, predictably and often cyclically cross these boundaries (Lyster, 1989). During their migrations, these species also cross multiple physical and environmental boundaries such as varying temperatures, depths or habitat types with significant implications for their ecologies and physiologies (Rasmussen *et al.*, 2007, Saunders *et al.*, 2010, Campana *et al.*, 2011, Francis *et al.*, 2015, Whitlock *et al.*, 2015). These cross-boundary, often rapid movements expose marine migratory species to a wide-range of pressures and present a challenge for their conservation (Lascelles *et al.*, 2014) and study.

In order to understand the life-history, ecology and physiology of pelagic sharks, and identify their roles in the different systems they occupy, scientists have employed a variety of developing methods. Historically, studies relied on direct observation using tools such as mark-recapture, with often low recapture success rates (at their highest generally around 20%; Kohler *et al.*, 2002, Queiroz *et al.*, 2005, Costa *et al.*, 2012, Madigan *et al.*, 2015); or controversial lethal sampling of individuals to obtain information on diet (stomach content analysis), reproductive status (examination of internal reproductive organs), or age and growth rates (enumeration of vertebral growth zones) (Cortés, 1997, Heupel and Simpfendorfer, 2010, Hammerschlag and Sulikowski, 2011). More recently, the advancement of electronic tag technology – telemetry – has allowed for more precise and long-term tracking of marine vertebrate movements (Hussey *et al.*, 2015). Acoustic tracking, a fine-scale method providing information on the presence of a tagged animal, relies on the presence of fixed or mobile (by vessel pursuit) receivers to detect individuals (Hussey *et al.*, 2015). This results in limitations such as possible disturbance to the behaviour of the animal by a moving vessel; relying on the presence of the animals within range and unknown movements outside of the receiver array; high cost of equipment (particularly receivers, of which multiple are needed to cover the area of study effectively); and limited battery life of tags for example (Arnold and Dewar, 2001, Hussey *et al.*, 2015, Crossin *et al.*, 2017). Despite the development of smaller acoustic tags, which can now sometimes last up to 10 years and be equipped with environmental (e.g. temperature or depth) or physiological (e.g. heart rate) sensors, acoustic tracking (passive or active) may not be the most appropriate tool, in isolation, to study far-ranging, oceanic species (Arnold and Dewar, 2001). In contrast, satellite telemetry can be a highly effective tool to track long distance movements and can provide high resolution locations, however, their deployments are often shorter (weeks to months) and more expensive (Crossin *et al.*, 2017). There are two main methods of data recovery in satellite telemetry: pop-up archival tags (PSAT) which transmit light-inferred geolocations upon release and surfacing of the tag; and satellite transmitting tags (SPOT) which transmit data every time the mounted tag breaks the surface (Hazen *et al.*, 2012). While ARGOS and GPS locations obtained from satellite transmissions are more precise and allow for almost real-time tracking, they may not be suitable for species that rarely, if ever, surface but may be particularly suitable for species that spend a significant amount of time at the surface. On the contrary, PSATs may be most useful for species that rarely surface but are also capable of collecting and storing a large amount of environmental and behavioural information such as depth, temperature and energy expenditure (Hazen *et al.*, 2012). Combination of both technologies could result in optimal data collection (more precise, longer-term tracks, combined with in-depth environmental use insights), if appropriate and the welfare of the animal is not seriously compromised which may be the case for smaller individuals or species and lead to biased interpretations of results. Additionally, while this technology provides invaluable information on movement behaviours, a large part of the picture remains unexplored.

Trophic ecology and reproductive behaviour questions, for example, cannot be answered using telemetry directly. As discussed, these fields have historically relied on lethal sampling of individuals, such as gut content analysis or dissection of gonads, but have themselves seen many advances allowing for ever deeper insights into these behaviours. Remaining in the electronic realm, biologging refers to the use of miniaturised devices for logging animal's behaviours, physiologies or environment (Rutz and Hays, 2009). These loggers can include depth and temperature sensors, internal body-temperature sensors, heartbeat sensors, accelerometers, cameras and more (Rutz and Hays, 2009, Hazen *et al.*, 2012, Payne *et al.*, 2014). The insight from these loggers, particularly in packages comprising multiple sensors, can provide unprecedented levels of detail into the ecology (Papastamatiou *et al.*, 2022), evolution (Harding *et al.*, 2021) and physiology (Ste-Marie *et al.*, 2022) of pelagic migrators (Block, 2005). However, just like telemetry, biologging faces limitations in the form of cost and battery life, and significant considerations have to be made for the welfare of any animal being tagged, particularly when it comes to the amount of drag created by the device(s) (Kay *et al.*, 2019). Additionally, attachment of these devices often require capture of the animal, a stressful event which can result in several hours of ‘stress response’ post-release (Iosilevskii *et al.*, 2022). In the case of sharks, this response can last 6-9 hours before a return to regular behaviours (Iosilevskii *et al.*, 2022) and biologging device batteries may only last a few hours themselves. Studies of behaviours captured by biologging must, therefore, take this recovery time into consideration and make a cost-benefit judgment call.

Of course, telemetry and biologging only represent a small part of the tools available to us to study pelagic migrators and, beyond lethal sampling, biochemical methods have taken centre stage in many studies into the trophic, reproductive and habitat ecology of marine migratory species. One such method is the use of stable isotope analysis (SIA). Bulk SIA has been increasingly used by ecologists to answer questions of resource and habitat use by consumers (Fry, 2006, Michener and Kaufman, 2007, Newsome *et al.*, 2007). Put simply, bulkSIA measures the ratio of heavy to light isotopes of different elements in the tissues of consumers and provides an indication of the feeding habitat and/or trophic level of said consumers (Chouvelon *et al.*, 2011). However, while it can provide insight into the movement or trophic ecology of consumers, particularly in the marine environment (Chouvelon *et al.*, 2011, Trueman and Glew, 2019), limitations exist to this approach, in particular the number of confounding factors that can influence conclusions drawn from the data. For this reason, careful planning must be made by scientists attempting to use this tool including sampling of source isotope values, knowledge of tissue turnover rates, as well as sampling of possible prey items when possible (Araújo *et al.*, 2007, Hoeinghaus and Zeug, 2008, Graham *et al.*, 2010, MacKenzie *et al.*, 2014). Importantly, prey taxon or species cannot be obtained from stable isotope data and, once again, bulkSIA is best used in combination with other methods such as those discussed previously or other biochemical methods. In addition, just as telemetry and biologging methods have seen great strides made to improve them, so has SIA. The development of compound-specific stable isotope analysis (CS-SIA) now enables us to differentiate isotope ratios not just of bulk tissues but of compounds within those tissues such as amino acids (AA-CSIA) or fatty acids (FA-CSIA); and, as such, trace the origins and routes of these elements before and after they enter the tissues of the consumer (Evershed *et al.*, 2007, Jochmann and Schmidt, 2015, Whiteman *et al.*, 2019). This can provide information about the animal’s diet (Manlick and Newsome, 2022) and its physiology, such as nutrient allocation, tissue turnover or reproductive strategies for example (Gannes *et al.*, 1998, Whiteman *et al.*, 2021) and has great potential to untangle life-history strategies of many species (Bortoluzzi *et al.*, 2021). However, while bulkSIA has become a fairly cheap and accessible method, CS-SIA is still in its relative infancy and access to laboratories capable of running it is more difficult and expensive. As a result, ecologists may choose to submit fewer samples for analysis in an already often small sample size of wild animals. Until costs are optimised, access to CSIA remains limited for many ecologists.

Other ecological tracers such as lipids and fatty acids are also available to ecologists and have been since the 1960s, but their use in marine ecology has recently started to grow in popularity (Pethybridge *et al.*, 2018, Meyer *et al.*, 2019). They can be used to quantify energy flows in food webs (Pethybridge *et al.*, 2018); identify key processes underpinning ecosystems (Dalsgaard *et al.*, 2003); assess the physiological status of an organism (Dalsgaard *et al.*, 2003); and determine and distinguish environmental conditions and habitats utilised by organisms (Meyer *et al.*, 2019). With a large scope of application in trophic and habitat marine ecology, lipid and fatty acid analysis can be a powerful tool for scientists to explore but confounding factors exist such as environmental variables (e.g., temperature), secondary consumption or species-specific rates of bioconversion (Meyer *et al.*, 2017, Meyer *et al.*, 2019) and care must be taken when drawing conclusions from results in isolation. Their real strength comes when combined with other biochemical tracers and methods such as SIA (Hebert *et al.*, 2006) or reproductive hormone analysis (Rangel *et al.*, 2021).

The latter, reproductive biochemical tracers, is used as a non-lethal way of assessing the reproductive status of organisms such as elasmobranchs through sex steroid hormone analysis of the blood, or reproductive endocrinology(Awruch, 2013, Awruch *et al.*, 2014). However, due to the range of reproductive strategies in the elasmobranch lineage, generalising hormonal control from one reproductive mode to the other is inappropriate and validation of this method has to be carried out for each reproductive mode by prior visual examination of gonads (requiring lethal sampling) combined with hormonal level (Awruch, 2013, Fujinami and Semba, 2020). Once validated, circulating levels of sex steroid hormones, such as testosterone, progesterone or oestradiol, can be effectively used to assess reproductive maturity and status with minimal handling of the animal (Awruch, 2013). Once again, the combination of this method with others such as ultrasonography can also increase its validity and depth of insight into the ecology of marine animals (Awruch, 2013, Sulikowski *et al.*, 2016, Rangel *et al.*, 2022).

While one’s access to funds and tools may limit the number of methods available to them, collaborations among the scientific community can provide highly beneficial opportunities to fill these gaps in access and knowledge. Additionally, scientists studying marine migratory species are likely to want to optimise the small sample sizes to capture as much information in as much detail as possible. Combining any or many of the above methods can be a really powerful way to obtain more robust insights and evidence into the lives of these animals and subsequently inform conservation and management measures. Of course, this only represents a selection of a wide range of available methods and many more (e.g. genetics, eDNA, toxicology, heavy metal tracers, local and indigenous ecological knowledge) are not included here but can and should be considered.

1.1 Research Outline

In this thesis, I provide both theoretical and empirical arguments and examples for the effectiveness of combining disciplines and methods to complete our ecological knowledge of marine migratory species and their life-history traits – information essential to our ability to manage and conserve them.

- Chapter 2: Identifying the limits where variation in stable isotopes reflect variation in diet.

Stable isotopes have become ubiquitous in ecological studies, particularly trophic ecology, either as a standalone method or in combination with others. However, the true relationship between variation in diet and variation in stable isotope ratios in the tissues of consumers is often misunderstood and misinterpreted. Here, I use mathematical models and simulations to tease apart this relationship and show that important factors such as source isotope values, number of resources, and tissue and diet turnover rates can confound the results and conclusions of these studies. I propose simple corrections and considerations to apply to both study design and result interpretation to infer accurate and appropriate conclusions.

- Chapter 3: Technological advances in Stable Isotope Analysis provide ever more insights into animal ecology.

While bulk stable isotopes have been used for several decades now, the use of compound-specific stable isotope analysis (CS-SIA) is a more recent development in the field and presents some new and exciting opportunities. I review and contextualise a study by Whiteman et al., (2021) who examined the allocation of nutrients in breeding emperor penguins using amino-acid CS-SIA and redefined the concept of capital vs income breeding as a spectrum. I explore the potential of CS-SIA to investigate life-history strategies – knowledge of which is often essential, but lacking, to the appropriate conservation and management of species – in finer and clearer detail. I conclude that the methods used by the authors are powerful and applicable to a range of taxa.

- Chapter 4: Hungry (and horny) teenagers: seasonal variations in diet and reproductive status of blue sharks, *Prionace glauca*, in the Irish sea (Northeast Atlantic).

Large, migrating, marine species are often both particularly vulnerable to anthropogenic pressures and challenging to study. Blue sharks, who carry out transatlantic migrations, are seasonal visitors to Irish waters where they play an important role in local economies as targets of recreational sport fishing activities. But they are also the most commercially fished species of shark in the world and much of their ecology, biology and life-history traits still need to be understood. I undertake a one-year study of the reproductive physiology and feeding ecology of blue sharks in the Irish Sea. Using a combination of biochemical and biologging methods, I show that the population of blue sharks off the coast of Cork are majority females and subadults during the summer with larger individuals present in the early autumn. I also provide evidence for a shift in resource use between the sharks' arrival in Ireland and the end of the season, identifying this as a potential move towards partial foraging on abundant gelatinous prey items.

- Chapter 5: Rapid transboundary movements of endangered porbeagle sharks, *Lamna nasus,* highlight need for cooperative management approaches.

As a Critically Endangered species in the northeast Atlantic, porbeagle sharks have, in recent years, been the subject of many management measures resulting in a stabilisation of their population. Knowledge of habitats critical to their life-history such as potential pupping or foraging grounds is key to ensuring their continued survival and a recovery of their population. I provide data from the movements across physical and jurisdictional boundaries of two mature female individuals tagged off the northwest coast of Ireland. These data highlight the opportunities for growing international collaborative partnerships to fill knowledge gaps and can inform future research questions.

- Chapter 6: Discussion

Finally, I close with a discussion on the crisis faced by the ocean today; the role of Ireland in addressing biodiversity conservation; and the increasing need for ecological research to address areas of conservation priorities and fill knowledge gaps. I present growing evidence of the benefits of moving from uni- or multidisciplinary to interdisciplinary and transdisciplinary research and address the challenges and barriers faced by those working towards it today.

Chapter 2 Identifying the limits where variation in stable isotopes reflect variation in diet.

***Authors**:* Jenny Bortoluzzi, Jean-Francois Arnoldi, Hugh Rowlands, Chris Harrod, Andrew Parnell, Nicholas Payne, Ian Donohue, Andrew L Jackson

Author contributions: ALJ, JB, HR, CH and J-FA conceived the study. JB, J-FA and ALJ led the work. J-FA and ALJ designed and wrote the methodology. ALJ conducted the simulations for among individual variation in R with input from JB and HR. J-FA derived the mathematical models and conducted the simulations for within individual variation in Python. JB, J-FA and ALJ wrote the manuscript. All authors made significant contributions to the manuscript and approved the final manuscript for publication.

Status: This manuscript is being prepared for publication following a first submission and review in the Journal of Animal Ecology. Based on reviews, additional work is being carried out and the new target journal is Methods in Ecology and Evolution.

2.1 Abstract

To answer key ecological questions about resource use in ecological communities, it is necessary to understand how individual niche use varies both among individuals and within individuals over time. Analysis of stable isotopes is used commonly to study such questions. There is, however, considerable debate regarding how isotopic values relate to diet, which can undermine their utility. We develop an analytical mathematical model alongside simulations to examine the relationship between variation in diet and stable isotope values both among individuals and within individuals over time. We show that dietary variation is directly proportional to variation in the stable isotope values of consumers normalised by the variation in resource isotope values and further scaled by the number of resources in the diet. We also find that the relationship between dietary and isotopic values strengthens when the number of (independent) tracers grows. Additionally, when considering temporal diet variation within a consumer we identify the interplay between diet turnover rates and tissue turnover rates that controls the sensitivity of stable isotopes to detect diet variation. Our work demonstrates clearly that variation in the stable isotope values of consumers reflect variation in their diet. However, this relationship can be confounded with other factors to the extent that they may mask the signal coming from diet. We show how simple quantitative corrections can recover the correlation in some situations, and in others we can adjust our interpretation in light of the new understanding arising from our models. Our framework provides guidance for the design and analysis of empirical studies where the goal is to infer niche width from stable isotope data.

2.2 Introduction

Over the years, much of the field of ecology has focused on quantifying species' niches, though the concept itself is controversial (Pocheville, 2015). Of particular interest has been understanding how niches vary over scales from individuals (Bolnick *et al.*, 2002, Matthews and Mazumder, 2004, Araújo *et al.*, 2007, Araújo *et al.*, 2011, Bolnick *et al.*, 2011, Layman *et al.*, 2012) through to populations (Newsome *et al.*, 2012) and communities (Bolnick *et al.*, 2011) over time and space. Understanding the factors driving variation in niche use across levels of biological organisation is central to understand ecological concepts such as resource use, geographic diversity, community composition and structure, and the effects that events such as competition, extinction or invasive species will have for conservation biology and applied ecology (Newsome *et al.*, 2007). In addition, the study of niche variation among individuals represents an opportunity to understand the evolutionary selection of traits and the advantages and trade-offs of generalist and specialist strategies. This can be applied to understanding the past and predicting the future impacts of anthropogenic influences on population dynamics and structure. Clearly, niche variation within and among individuals can be highly influential in determining the structure and survival of populations. Many tools are used, alone or in combination with others, to explore these questions. Among them, stable isotope analysis is an established and cost-effective method to quantify niche occupancy. The technique is, however, not flawless and is frequently misused, due primarily to lack of clarity regarding the implicit and explicit assumptions made during its application.

The use of stable isotopes to characterise community structure and niche width has become increasingly common since the late 1990s (Jackson *et al.*, 2011, Shipley and Matich, 2020). Particular ratios of heavy to light stable isotopes are found in the tissue of consumers after accumulating through the food web via the process of assimilation (either through primary production or consumption) and metabolic incorporation. They have, therefore, been proposed as a way to quantify the niche space of individuals or populations, resulting in the concept of "isotopic niche" (Jackson *et al.*, 2011, Phillips *et al.*, 2014), via the "you are what you eat" premise (Deniro and Epstein, 1976). The isotopic niche—that is, the area occupied by a group of measurements in isotopic space—is driven by differences among individuals in isotopic values (Newsome *et al.*, 2007). While the isotopic niche is often equated to the dietary niche, it has also received criticism for the very same reason. Hette-Tronquart (2019), for example, argued that trophic and isotopic niche spaces must be kept conceptually distinct and the relationship between the isotopic niche and niche overlap among individuals is complicated and unknown. The authors also point out that discrimination factors and individual variation at the physiological level impact consumer isotope values. For example, the turnover rates of elemental pools affect stable isotope ratios in consumers, which vary depending on the tissue and on the animal and translate into an isotopic niche more or less closely correlated to the ecological niche of an individual, population or community. It is, therefore, essential to acquire knowledge of turnover rates specific to focal species of interest and to carefully select tissues and preservation methods appropriate to the research question (Jackson *et al.*, 2011).

In addition, ideally in ecological studies the study of populations should relate to that of populations of comparable individuals (*e.g.* adults of a species; juveniles of a species; geographically isolated populations, *etc*.). If this is true, it can be assumed that on a broad level their physiologies are similar and that ecological conclusions can be drawn from the differences in their isotopic values. However, in addition to diet, environmental, behavioural and physiological factors also affect isotopic variance among individuals (Cherel *et al.*, 2005, Phillips *et al.*, 2014, Shipley and Matich, 2020). It is, therefore, important that they are considered in analyses. Such factors include: baseline isotopic values; the number of resources available and the variation in their own isotopic values; the proportion and quality of those resources being consumed and how this changes over time (Hoeinghaus and Zeug, 2008, Shipley and Matich, 2020).

Here, we explore the general biomass incorporation model that forms the basis of our understanding of stable isotope ecology from a statistical perspective (Fry, 2006, Boecklen *et al.*, 2011, Yeakel *et al.*, 2016). We use a series of vector algebra methodologies to determine the key drivers of variation in stable isotopes within and among individuals over time and examine whether there is a quantitative relationship between diet variation and stable isotope variation. In addition, we identify processes and features of the system that can confound this relationship including the number of food sources and variation in their stable isotope values (that is, baseline variation *sensu,* Jackson *et al.,* 2011). We offer both quantitative and conceptual approaches for mitigating these often-unwanted sources of variation. We assess the performance of the analytical model by comparing it with *in silico* process-based simulations. We begin with a general model that considers the dynamic incorporation of stable isotopes over time from a dynamically changing diet across a population of individuals (Navarro *et al.*, 2013, Rodriguez and Gerardo Herrera, 2013). We then separately consider (1) the simpler case of variation among individuals at equilibrium with their diet (that is, constant diet over time); and (2) variation within a single individual over time where diet varies. We conclude by integrating these two scenarios into the general framework where both are considered simultaneously. We discuss implications for empirical study-design, statistical analyses and the assumptions made in this theoretical model.

2.3 Methods and Models

Ultimately, from an ecological perspective, we are interested in describing the variation in stable isotope values and linking them to underlying biological and environmental processes with the goal of illustrating how well this variation represents variation in diet.

We start by imagining a dataset comprising stable isotope data $y = (y(t_w, w), w = 1, \ldots, W)$ measured over a population of W individuals (w) at multiple time points (t_w) where we are interested in the statistical variance[1] of the stable isotope data $\mathrm{Var}(y)$. In order to understand how this variance relates to an underlying variation of diet across and within individuals, we will develop analytical approximations for variance among individuals assuming no variation within individuals, and variance within a single individual over time. The analytical predictions are illustrated by comparison with simulated data. Both will show that the isotopic variance is related to a specific

---

[1] For any given data set $X = (X_1, \ldots, X_K)$ we define the (empirical) mean, which we denote either as $\bar{X}$ or as $\langle X \rangle$ as

$$\bar{X} = \langle X \rangle = \frac{1}{K}\sum_{i=1}^{K} X_i$$

and the (empirical) variance as

$$\mathrm{Var}(X) = \frac{1}{K}\sum_{i=1}^{K} (X_i - \bar{X})^2$$

statistics of diet variation normalised by a baseline isotopic variance across food sources. In the case of within individual isotopic variance in time, this relationship has an additional component, reflecting the interplay between tissue turnover and the autocorrelation of diet variation in time. We conclude by explaining why the complete population variance can be decomposed, under simplifying assumptions, as a sum of the two variances.

We will consider n-food sources which have distinct (and fixed) isotopic signatures x_i, $i = 1, \dots, n$. Henceforth, we denote n-dimensional vectors by bold symbols. Thus $x = (x_i)_{i=1,\dots,n}$ is the vector of isotopic values of the n food sources. Likewise, $f(t,w) = \big(f_i(t,w)\big)_{i=1,\dots,n}$ is the vector of diet proportions of individual w at time t with respect to the n food sources. We will consider the following classic model for the dynamics of isotopic content $y(w,t)$ of an individual w:

$$\frac{dy(t,w)}{dt} = \lambda(\langle f(t,w)|x\rangle - y(t,w) + \psi), y(0,w) = y_0(w) \tag{1}$$

Where $\langle \cdot \mid \cdot \rangle$ denotes the scalar product[2] and ψ is the additive fractionation factor of the stable isotope considered (but it will play no role in what follows). The tissue turnover rate λ models the assimilation rate of stable isotopes into the measured tissues of the organism. $y_0(w)$ sets initial conditions at time $t = 0$ (but in subsequent sections we will consider stationary states, independent of initial conditions).

---

[2] Thus $\langle u|v\rangle = \sum_{i=1}^{n} u_i v_i$

2.3.1 Variation among individuals

In this section, we neglect temporal variation of an individual's diets and assume that their isotopic content is at equilibrium (we relax this assumption in the next section). We therefore drop the dependency on time from the elements of the data y. Given Eq. (1), assume that the isotopic content $y(w)$of individual w satisfies:

$$y(w) = \langle \boldsymbol{f}(w)|x\rangle + \psi, \tag{2}$$

Importantly, because the diet is expressed in proportions, we have that $\langle \boldsymbol{f}(w)|\mathbf{1}\rangle = 1$, where $\mathbf{1} = (1, \dots, 1)$. Let $\bar{y} = \frac{1}{W}\sum_{w=1}^{W} y_i$ be the isotopic mean of the population. Likewise let $\bar{\boldsymbol{f}}$ be the vector of mean, over the population, of diet proportions. Then by linearity of Eq. (2), we have that

$$y(w) - \bar{y} = \left\langle \boldsymbol{f}(w) - \bar{\boldsymbol{f}} \middle| x \right\rangle = \langle \Delta\boldsymbol{f}(w)|\boldsymbol{x}\rangle, \tag{3}$$

Where $\Delta\boldsymbol{f}(w) = \boldsymbol{f}(w) - \bar{\boldsymbol{f}}$. Note that $\langle \Delta\boldsymbol{f}(w)|\mathbf{1}\rangle = 0$, which means that the difference vector $\Delta\boldsymbol{f}(w)$ lies on a plane orthogonal to $\mathbf{1}$. Let P be the orthonormal projector on that plane. Then

$$\Delta\boldsymbol{f}(w) = P\Delta\boldsymbol{f}(w) \Rightarrow \langle \Delta\boldsymbol{f}(w)|\boldsymbol{x}\rangle = \langle P\Delta\boldsymbol{f}(w)|\boldsymbol{x}\rangle = \langle \Delta\boldsymbol{f}(w)|P\boldsymbol{x}\rangle. \tag{4}$$

With $P\boldsymbol{x} = \boldsymbol{x} - \langle \boldsymbol{x}\rangle\mathbf{1}$ where $\langle \boldsymbol{x}\rangle = \frac{1}{n}\sum x_i$ is the mean isotopic value of food sources (note that the mean is not taken over the population but over the n different food sources). In particular,

$$||P\boldsymbol{x}||^2 = \sum_{i=1}^{n}(x_i - \langle \boldsymbol{x} \rangle)^2 = n\mathrm{Var}(\boldsymbol{x}), \tag{5}$$

where Var($\boldsymbol{x}$) is the isotopic variance of food sources, which defines the baseline isotopic variation. With these notations, the population isotopic variance reads

$$\begin{aligned} \mathrm{Var}(y) &= \frac{1}{w}\sum \ \langle \boldsymbol{x}|\Delta \boldsymbol{f}(w)\rangle \quad \langle \Delta \boldsymbol{f}(w)|\boldsymbol{x}\rangle \\ &= \ \langle \boldsymbol{x}|C_f \boldsymbol{x}\rangle \\ &= \ \langle P\boldsymbol{x}|C_f P\boldsymbol{x}\rangle \end{aligned} \tag{6}$$

where C_f is the $n \times n$ diet covariance matrix:

$$\left(C_f\right)_{ij} = \frac{1}{W}\sum_{w} \Delta f_i(w)\Delta f_j(w)\,; i,j = 1,\dots,n \tag{7}$$

In deriving Eq. (7), we used the fact that $C_f = PC_fP$. Indeed $C_f\mathbf{1} = 0$, so it does not have full rank, and acts only on vectors in the plane orthogonal to $\mathbf{1}$. We can decompose C_f in terms of its $n-1$ eigenvalue-eigenvector pairs $(\sigma_\alpha^2, \boldsymbol{u}^{\boldsymbol{\alpha}})$, with the eigenvectors $\boldsymbol{u}^\alpha$ all lying in the plane orthogonal to $\mathbf{1}$. This leads us to the expression:

$$\mathrm{Var}(y) = \sum_{\alpha=1}^{n-1} \sigma_\alpha^2 |\langle \boldsymbol{u}^\alpha | P\boldsymbol{x}\rangle|^2 \tag{8}$$

Using Eq. (8), we deduce upper and lower bounds on the isotopic variance

$$n \min_{\alpha} \sigma_{\alpha}^{2} \mathrm{Var}\,(\boldsymbol{x}) \leq \mathrm{Var}(y) \leq n \max_{\alpha} \sigma_{\alpha}^{2}\, \mathrm{Var}(\boldsymbol{x}) \tag{9}$$

where we see appearing the baseline isotopic variation, as well as the smallest and largest eigenvalues of C_f, representing respectively the length of the

smallest and largest axis of variation of the multivariate diet distribution.

We can deduce a statistical expectation if we assume that $P\boldsymbol{x}$ is a random vector in the hyperplane (of dimension $n-1$) orthogonal to the vector $\mathbf{1}$.

In this case the expected projection of $P\boldsymbol{x}$ on a vector $\boldsymbol{u}^{\alpha}$, will be, on average over random realisations of $P\boldsymbol{x}$ given by

$$E_x |\langle u^{\alpha}|Px\rangle|^2 = \frac{1}{n-1} \|Px\|^2 = \frac{n}{n-1} \mathrm{Var}(x) \tag{10}$$

We thus get an approximate expectation for the isotopic variance

$$\mathrm{Var}(y) \approx \frac{n}{n-1} \mathrm{Var}(\boldsymbol{x}) \mathrm{Tr}(C_f) \tag{11}$$

Where $\mathrm{Tr}(\boldsymbol{C_f}) = \sum_{\alpha} \boldsymbol{\sigma_{\alpha}^{2}}$ is the trace of $\boldsymbol{C_f}$ (the sum of its diagonal elements) which is, given Eq. (7) evaluated at $\boldsymbol{i} = \boldsymbol{j}$, also the sum of diet variances $\sum_{\boldsymbol{i}=\mathbf{1}}^{\boldsymbol{n}} \mathrm{Var}(\boldsymbol{f_i})$. Expression Eq. (11) is exact if $\boldsymbol{C_f}$ is proportional to the identity matrix (all its eigenvalues are equal), which corresponds to the case where we have an isotropic distribution of diet vectors.

In other words, the more anisotropic the diet distribution (i.e. the more unequal the eigenvalues of $\boldsymbol{C_f}$ and hence variances of each diet proportion) the more variation we expect around the above expression.

2.3.1a Simulation of data

We tested the above analytical results on simulated data. Simulations were performed in the R statistical language and full code is provided in the Supplementary Material (currently available from https://github.com/AndrewLJackson/isoVarEcol). Ignoring trophic discrimination (enrichment) factors (ψ) which provide only additive effects in our conceptualisation and are therefore irrelevant to the calculation of variance, we generate a population of consumer isotope values as

$$\boldsymbol{y} = F\boldsymbol{x} \tag{12}$$

where $F = \left(F_{w,i}\right)$ is the matrix of diet proportions $F_{w,i} = f_i(w)$ and $\boldsymbol{x} = (x_i)$ is the vector of food source isotope values x_i for i from 1 to n.

For any given population, n food sources are drawn from a uniform random distribution from 2 to 20, and their corresponding isotope values drawn by $x_i \sim$ Unif$(0,10)$. A vector of diet proportions for each individual w is then drawn from a Dirichlet distribution $\boldsymbol{f}(w) \sim$ Dir$\left(\alpha_1, \alpha_j, \dots, \alpha_n\right)$ where each α_j are drawn according to $log_{10}\left(\alpha_j\right) \sim$ unif$(-1,2)$ such that a wide range of diet proportions are possible with high densities close to the boundary of the simplex possible with concave probability densities possible with $\alpha < 1$, convex when $\alpha > 1$ and the probability density function is flat when all $\alpha_j = 1$. This procedure is repeated for the case of more than one isotope such that $\boldsymbol{x}$, and hence $y(w)$, has more than one column corresponding to the number of isotope tracers. Finally, the entire process is replicated across 1000 populations and the relationship between the simulated diet and isotope values then compared with the analytical predictions from our model.

2.3.2 Variation within individuals

We now consider the variation in isotopes over time of a single individual and ask how it may relate to a variation in time of its diet. As above, we allow this individual to consume a variety of n resources. The data considered thus takes the form of $y = (y(t_k), k = 1, \dots, K)$ where the t_k are the K time points at which the isotopic content of the individual has been sampled. We assume that $y(t)$ is stationary, meaning that, if t is unknown, $y(t)$ behaves as a random variable of fixed mean and variance (noted $V\big(y(t)\big)$ and $E\big(y(t)\big)$, respectively), and that the data consists of (many) independent samples of $y(t)$. We thus assume that the empirical mean and variance $\bar{y}$ and Var(y) are unbiased estimates of the abstract quantities $V\big(y(t)\big)$ and $E\big(y(t)\big)$.

Given our model Eq. (1) we can express the isotopic variation $z(t) = y(t) - E\big(y(t)\big)$ around the stationary mean as

$$\frac{dz}{dt}(t) = \lambda\big(\langle \Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle - z(t)\big), \tag{13}$$

where $\Delta\boldsymbol{f}(t) = \boldsymbol{f}(t) - E\big(\boldsymbol{f}(t)\big)$ is the vector of dietary variations around their stationary mean. A key parameter here is λ which represents the tissue's turnover rate, and hence temporal integration interval of the isotopic signal.

To compute the stationary variance $V\big(y(t)\big)$, we start from

$$\frac{dz^2}{dt}(t) = 2z(t)\frac{dz}{dt}(t) = 2\lambda\big(z(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle - z^2(t)\big) \tag{14}$$

Thus, if we take an expectation over the sampling time t we have that

$$\begin{aligned}\mathbb{E}\frac{dz^2}{dt}(t) &= 2\lambda\left(\mathbb{E}z(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle - \mathbb{E}\big(z^2(t)\big)\right)\\ &= 2\lambda\left(\mathbb{E}z(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle - \mathbb{V}\big(y(t)\big)\right)\end{aligned} \tag{15}$$

The stationary variance $V\big(y(t)\big) = E\big(z^2(t)\big)$ must therefore satisfy

$$V\big(y(t)\big) = E(z(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle) \tag{16}$$

Which we now compute. To do so, we can analyse the dynamics of $z(t)\langle\Delta f(t)|x\rangle$, where

$$\frac{dz\langle\Delta\boldsymbol{f}|\boldsymbol{x}\rangle}{dt}(t) = \frac{dz}{dt}(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle + z(t)\left\langle\frac{d\Delta\boldsymbol{f}}{dt}(t)\middle|\boldsymbol{x}\right\rangle \tag{17}$$

At this point, we need to model the dynamics of the dietary proportions $\Delta\boldsymbol{f}(t)$. We will consider here a simple stochastic system of the form of an Ornstein-Uhlenbeck process, where the diet varies around a mean according to a Brownian motion process (Uhlenbeck and Ornstein, 1930)

$$\frac{d\Delta\boldsymbol{f}}{dt}(t) = -\gamma\Delta\boldsymbol{f}(t) + \boldsymbol{\xi}(t) \tag{18}$$

Here $\xi(t)$ is a white noise term which may be correlated across food sources, but not in time, thus stochastically driving changes in diet. The rate γ models the tendency of the diet to return to its temporal mean, and its inverse is the autocorrelation period of the diet time-series. Given this model, we can now go back to Eq. (17) and see that

$$\begin{gathered}\frac{dz\langle\Delta\boldsymbol{f}|\boldsymbol{x}\rangle}{dt}(t) = -(\gamma+\lambda)(z(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle) + \lambda\langle\boldsymbol{x}|\Delta\boldsymbol{f}(t)\rangle\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle \\ +z(t)\langle\boldsymbol{\xi}(t)|\boldsymbol{x}\rangle\end{gathered} \tag{19}$$

Taking a temporal expectation then gives

$$E\,\frac{dz\langle\Delta\boldsymbol{f}|\boldsymbol{x}\rangle}{dt}(t) = -(\gamma+\lambda)E(z(t)\langle\Delta\boldsymbol{f}(t)|\boldsymbol{x}\rangle) + \lambda\left\langle\boldsymbol{x}\middle|C_f^t\boldsymbol{x}\right\rangle \tag{20}$$

Where C_f^t is the $n\times n$ dietary temporal covariance matrix, whose components are

$$\left(C_f^t\right)_{ij} = E\left(\Delta f_i(t)\Delta f_j(t)\right), i,j = \mathbf{1},\dots,n$$

which, in practice, we may assume to be well approximated by the empirical covariance matrix computed from the data $y = (y(t_k), k = 1,\dots,K)$. To deduce Eq. (20) we used the fact that $E(z(t)\langle\boldsymbol{\xi}(t)|\boldsymbol{x}\rangle) = 0$, which follows from the fact that the forcing term $\boldsymbol{\xi}(t)$ is a random term uncorrelated through time, and consequently independent of the isotopic value of the individual. Thus, in the stationary state we have that

$$0 = -(\gamma + \lambda) E_t(z(t)\langle \Delta \boldsymbol{f}(t) | \boldsymbol{x} \rangle) + \lambda \left\langle \boldsymbol{x} \middle| C_f^t \boldsymbol{x} \right\rangle \tag{21}$$

and thus, from Eq. (16)

$$V\big(y(t)\big) = \frac{\lambda}{\lambda + \gamma} \left\langle \boldsymbol{x} \middle| C_f^t \boldsymbol{x} \right\rangle \tag{22}$$

Based on the reasoning of the previous section (that led us to Eq. (11), we can deduce an expectation over random realisations of the food sources isotopic vector $\boldsymbol{x}$, given that $\bar{y} = E\big(y(t)\big)$ and $\text{Var}(y) = V\big(y(t)\big)$:

$$\text{Var}(y) \approx \frac{\lambda}{(\lambda + \gamma)} \frac{n}{(n-1)} \text{Var}(\boldsymbol{x}) \text{Tr}\big(C_f^t\big) \tag{23}$$

This result indicates that the relative rate at which diet varies through time (γ) and the rate at which tissue turnover (λ) incorporates the isotopic signal from the sources is key to controlling how much variance from dietary or source baseline isotope values is identifiable in the variance over time in the consumer. Ideally, λ would be much larger than γ, in which case prefactor $\left(\frac{\lambda}{\lambda+\gamma}\right)$ goes to 1. At the other end of the spectrum, if $\gamma \gg \lambda$ then the pre-factor goes to 0 and all diet variance is lost. When $\gamma = \lambda$, then the sources of diet and source variance are exactly halved when viewed as the isotopic variance in the consumer. How much shrinkage of this variance is acceptable is a decision for the empirical team given knowledge of their study system. The key assumptions that we have made to derive this result are:

(i) temporal stationarity of the first moments of time series;

(ii) no underlying dynamical structure in the diet change (no interactions between diet proportions);

(iii) perhaps most importantly, no temporal correlations in the driving force (modelled as white noise $\boldsymbol{\xi}$) of diet change.

Assumption (i) is the implicit premise for a notion of variance to be meaningful. Assumption (ii) can be relaxed, in which case the autocorrelation period of dietary time-series would not be uniform. If the diet changes in some direction it will tend to quickly return to the mean (a short autocorrelation period), while, if it changes in another direction, it might tend to return to the mean much more slowly (a long autocorrelation period). We would then have to replace C_f in Eq. (23) by a more complex matrix that encodes this anisotropy of the autocorrelation. Finally, assumption (iii) would not hold, if for instance, diet changes are forced by seasonal effects. In this case, we could replace the stochastic model by a periodically forced one, which is not harder to handle. Our results would then hold by simply replacing the autocorrelation of diet variation by the frequency of diet oscillations. This means that if those frequencies are very large compared to tissue turnover rate, diet oscillations will be severely dampened in the isotopic signal. On the other hand, if diet oscillations are slow enough, the tissue turn-over rate will cease to play a confounding role.

We can finish by noting that we can use this calculation of temporal variance within an individual to compute the variance of $y = (y(w, t_w), w = 1, \ldots, W)$ without having to neglect, as we did in the previous section, the temporal variation of individuals' diets. Indeed, assuming identical autocorrelation time and tissue turnover for all individuals in the population, and assuming that the sampling events t_w of the various individuals are independent —or that individuals feeding dynamics are independent— we get that:

$$\mathrm{Var}(y) = \langle \boldsymbol{x} | C_f \boldsymbol{x} \rangle + \frac{\lambda}{\lambda + \gamma} \langle \boldsymbol{x} | \overline{C_f^t} \boldsymbol{x} \rangle \tag{24}$$

where C_f is the population covariance matrix of temporal mean diet proportions, while $\overline{C_f^t}$is the population mean temporal covariance matrix of the individuals' diet dynamics. The expectation over a random realisation of isotopic resource vector x thus takes the form

$$\mathrm{Var}(y) \approx \frac{n}{n-1} \mathrm{Var}(\boldsymbol{x}) \times \left(\mathrm{Tr}(C_f) + \frac{\lambda}{\lambda + \gamma} \mathrm{Tr}\left(\overline{C_f^t}\right) \right) \tag{25}$$

We see here that, with this interpretation of the population covariance matrix C_f, the results of the first section do not, in fact, require temporal fluctuations of diet to be negligible. Rather, the tissue turnover rate is small enough to buffer those fluctuations.

2.3.2a Simulation of data

For a given individual we uniformly chose the number of its food sources between 3 and 15 and chose its mean diet from a Dirichlet distribution, following the procedure used for the across individual case outlined in the previous section. To generate dietary dynamics, it is convenient to first model consumption dynamics $c(t) = \left(c_i(t)\right)$ (which need not sum to one and from which diet is deduced as $f_i = c_i / \sum_j c_j$). We identified the mean diet vector of the individual, drawn from a Dirichlet distribution, with the individual's mean consumption vector $\bar{c}$ (in general the two differ by a positive multiplicative constant, but it plays no role for our purposes). We modeled the fluctuations of consumption around the mean as a discretised Ornstein-Uhlenbeck process with autocorrelation rate γ, bounded from below to ensure non-negative consumption. More precisely, after having chosen a time step $dt = 10^{-3}$, starting from $\boldsymbol{c}(0) = \bar{\boldsymbol{c}}$, we defined

$$\boldsymbol{c}(t + dt) = max\left\{\boldsymbol{c}(t) - \gamma\left(\boldsymbol{c}(t) - \bar{\boldsymbol{c}}\right)dt + \mu R\boldsymbol{\xi}\sqrt{dt}, 0\right\} \tag{26}$$

where $\boldsymbol{\xi} = (\xi_i)_{i=1,\ldots,n}$ with the elements ξ_i are independently drawn from a standard normal distribution. This term models Brownian motion, $\mu > 0$ controls the intensity of the forcing (chosen uniformly between 0 and 2) and R is a matrix that models its correlation structure across food sources. In particular, if it has rank one, the motion is unidirectional in consumption space whereas if R is proportional to the identity matrix I, the motion is isotropic. To consider a range of possible behaviours we model R as

$$R = (1 - \sigma)I + \sigma M \tag{27}$$

with M a random matrix with elements independently drawn from a standard normal distribution (but many other modelling choices are possible). We drew σ uniformly between 0 and 0.5. From consumption we could define diet (sum to one) and simulate isotopic content of the individual according to Eq. (1). Individual's rate of tissue turnover λ was fixed a 1 while γ was chosen uniformly between 0.1 and 2, thus spanning cases where diet variation is slow compared to tissue turnover rate, to case where it is fast. We simulated the dynamics over the interval [0, 100] and, to avoid transient effects, computed the variance of the time series on the last 90% of its duration.

2.4 Results and Discussion

The key result in both models is that variation in diet both over time and within an individual manifests as variation in the stable isotope values of the consumer. Furthermore, this relationship is directly proportional in all scenarios of our model such that, all other variables being constant, a doubling in diet variation will lead to a doubling in the stable isotope variation observed in the consumer. It is clear from the models that other processes and features, as have been articulated in conceptual terms in the literature (Cabana and Rasmussen, 1996, Post, 2002, Sweeting *et al.*, 2005, Araújo *et al.*, 2007, Layman *et al.*, 2007, Hoeinghaus and Zeug, 2008, Yeakel *et al.*, 2016, Hette-Tronquart, 2019), also drive variance in the consumers' stable isotope values in a similar 1:1 manner. Specifically, these are the number of food sources (expressed as $n/(n-1)$), the variance in the underlying stable isotope values of the food sources $\mathrm{Var}(x)$, and the relationship between tissue turnover and the rate of change in the diet for the case of the temporally dynamic model $\frac{\lambda}{\lambda+\gamma}$. We explore these in more detail, and knowing this, can offer advice in the design of empirical studies to mitigate these potentially confounding features and provide quantitative corrections that could be used in the data analysis phase to remove their effect.

2.4.1 Variation among individuals

As proposed conceptually by Bearhop *et al.* (2004), we find that the mechanistic model of stable isotope incorporation supports directly the idea that variation in diet is inferable through observing variation in the stable isotope values of consumers (Eq 11). The 1:1 relationship between Var(y) and Tr$\left(C_f\right)$ means that, in a situation where there were two populations of consumers, whose food sources were identical in both number and isotope values, and whose physiologies were similarly variable, one could infer that the population whose isotopic variance was greater had a more variable diet than the other. More specifically, one could calculate precisely how much more variable the diet was through the relationship that Var(y_1)/Var$(y_2) =$ Tr$\left(C_{f_1}\right)$/Tr$\left(C_{f_2}\right)$.

Situations will arise where the isotopic values of the food sources are not consistent between locations where, or at times when, the consumers were sampled. Our results confirm those of previous studies (Araújo *et al.*, 2007, Hoeinghaus and Zeug, 2008) that this will confound the results by increasing consumer variation where the sources are more disparate, and vice versa. Clearly, in this scenario, the naive approach illustrated in Figure *2-1*a where one makes the simple assumption that variation in isotopes says something about diet variation is poor. Where possible, the source isotope data should be sampled appropriately (Araújo *et al.*, 2007, Graham *et al.*, 2010, MacKenzie *et al.*, 2014) as, following simple correction of the consumer data by Var(y)/Var(x), the transformed data will become directly correlated to diet variation (Tr$\left(C_f\right)$) (compare Figure *2-1*a with 1b). There may be situations where data on the source variation is not possible to obtain and, in those situations, one must make an implicit assumption that the differences in the variance in sources between consumer populations is negligible.

When considering diet variation as an ecological concept, we have chosen to represent this on the x-axis of Figure 2-1 (a-d) as a combination of both number of sources and the variation in the diet proportions that the consumers draw from. This makes conceptual sense as a larger diet repertoire is synonymous with a larger niche, and so too is larger variation in diet of those sources. With isotope variance scaling with $n/(n-1)$, we can see that differences between lower richness of sources have more of a profound effect on Var(y) than larger values. That is, when $n=3$ the proportional scaling effect is 1.5, whereas when $n=10$ the effect is 1.1 and asymptotes to 1. The empirical consideration here is that, if the numbers of food sources are at the lower end and different between consumer populations, then differences in isotopic variation may be due to constraints of food availability rather than variance in the diet proportions *per se*. At larger source richness, this effect becomes less pronounced except of course when the discrepancies are very large.

In deriving Eq. (11) we made the point that our use of Tr(C_f) is exact when the eigenvalues of C_f are equal which implies that the distribution of proportions is isotropic around the mean. Departures from this assumption, where individuals' diets vary more along one axis of the diet simplex will result in increased deviations from our approximation. The simulated data in Figure 2-1b contain a range of diets of varying anisotropy since they were drawn randomly from a wide range of Dirichlet distributions. There is no single measure for anisotropy, but here we take the difference in the min and max eigenvalues of the covariance matrix of diet proportions and scale it by the dimensionality of the matrix and plot the absolute deviations from the predicted relationship against $n\left(\max_{\alpha}\sigma_{\alpha}^{2}-\min_{\alpha}\sigma_{\alpha}^{2}\right)$. The result is as predicted, with increasing departures from prediction associated with increased anisotropy of the diet distribution (Figure 2-2).

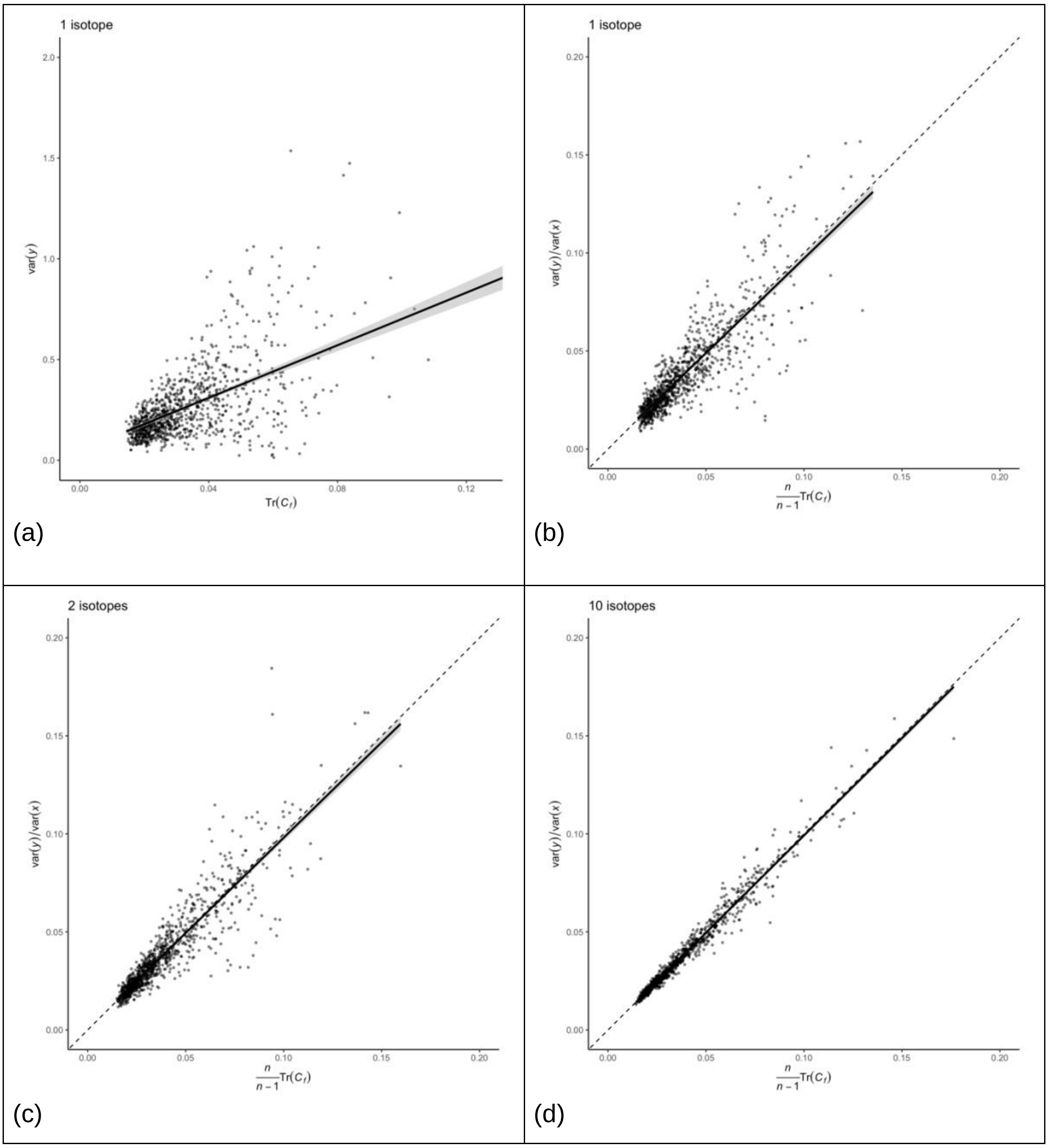
Our analytical models were developed initially for a single isotope, but the predictions generalise to multiple isotope tracers as is commonly employed empirically. The results of the simulations considering 1, 2, and 10 isotopes indicates improved correlations between diet variation and source-corrected isotope variation. Our analytical model can readily be extended to include N multiple isotopes. Returning to Eq. (6), if we compute the mean (over N isotopes) of the population isotopic variance, we would have

$$\frac{1}{N}\sum_{iso=1}^{N}\mathrm{Var}\left(y^{iso}(w)\right)=\frac{1}{N}\sum_{iso=1}^{N}\left\langle P\boldsymbol{x}^{iso}\middle|C_f P\boldsymbol{x}^{iso}\right\rangle \tag{28}$$

As N grows, this expression becomes a mathematical expectation over an ensemble of resource vectors x so, if the distribution over this ensemble is isotropic in the simplex, we get that

$$\begin{aligned}\frac{1}{N}\sum_{iso=1}^{N}\mathrm{Var}\left(y^{iso}(w)\right)&\rightarrow E_{\boldsymbol{x}}\left\langle P\boldsymbol{x}\middle|C_f P\boldsymbol{x}\right\rangle\\ &=\frac{n}{n-1}\mathrm{Tr}\left(C_f\right)E_x\mathrm{Var}(x)\\ &\leftarrow\frac{n}{n-1}\mathrm{Tr}\left(C_f\right)\frac{1}{N}\sum_{iso=1}^{N}\mathrm{Var}\left(\boldsymbol{x}^{iso}\right)\end{aligned}\tag{29}$$

So that



$$\frac{\sum_{iso=1}^{N} \mathrm{Var}\left(y^{iso}\right)}{\sum_{iso=1}^{N} \mathrm{Var}(\boldsymbol{x}^{iso})} \to \frac{n}{n-1} \mathrm{Tr}\left(C_f\right) \tag{30}$$

In other words, if the correlation of isotopic signal between different isotopes and across sources is not too strong, we expect the predicted relationship between isotopic variation and diet variation to become increasingly accurate as the number of isotopes considered (N) grows.

Figure 2-1. The relationship between the observed source-corrected consumer isotope variation(x-axes) and diet variation (y-axes). (a) shows the naive correlation between these two variables. (b),(c) and (d) show correlations for, respectively, 1, 2 and 10 isotopes, after correcting for source variation Var$(\boldsymbol{x})$ and accounting for differences in the number of food sources using the pre-factor $n/(n-1)$ on the trace of the covariance matrix of the diet sources using the pre-factor $n/(n-1)$ on the trace of the covariance matrix of the diet proportions. The black lines in all panels indicated a linear fit with 95% confidence intervals. The dashed lines in b-d represent the $1:1$ analytical prediction from our model. Pearson’s correlation coefficients for a-d are 0.56, 0.87, 0.91 and 0.98.

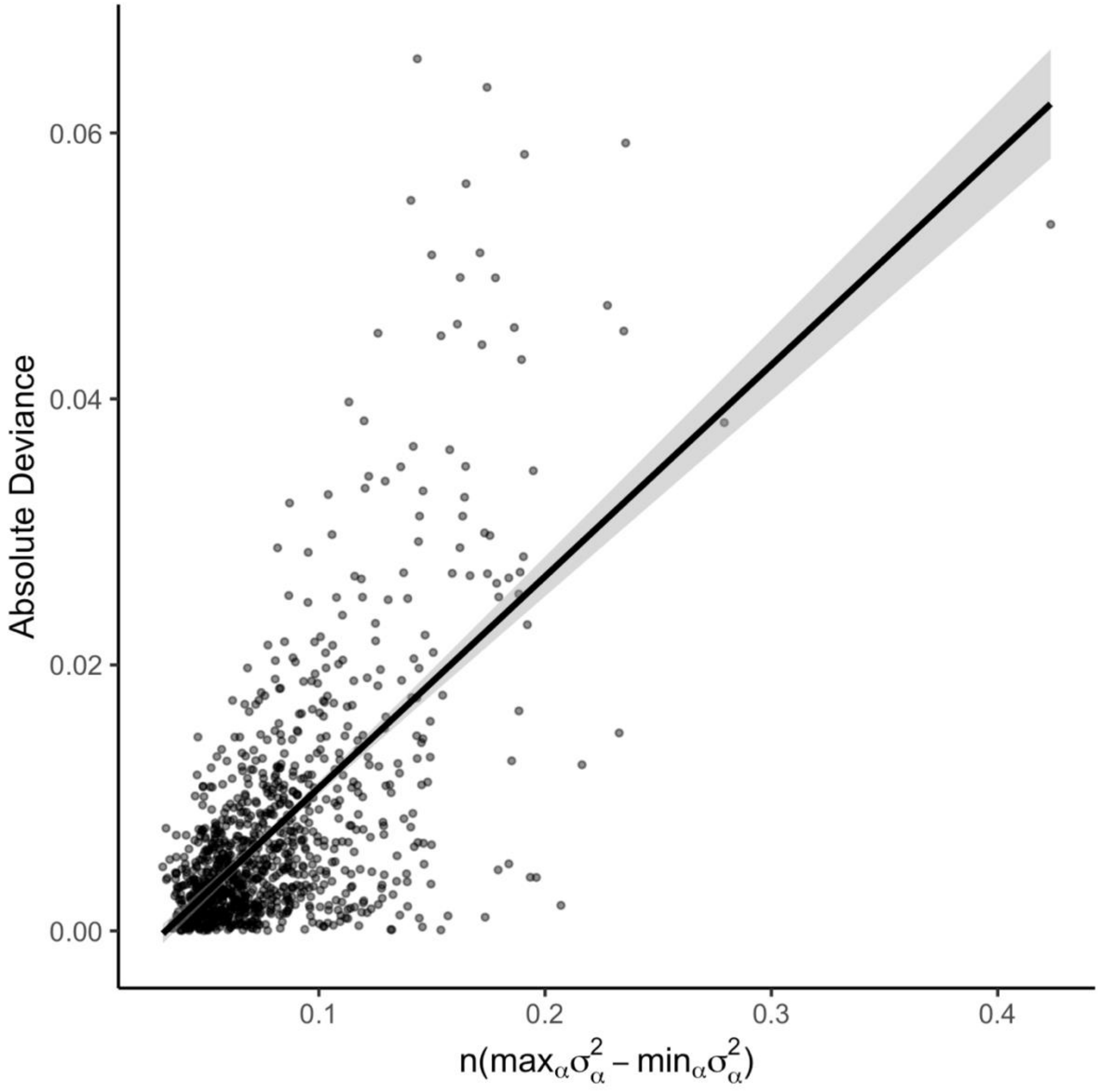


Figure 2-2. The relationship between the absolute deviance of source-corrected consumer variance ("Var" (y)\/"Var" (x)) to the 1:1 line in Figure 1b and our measure of anisotropy of the diet proportions $n\left(\max_{\alpha} \sigma_\alpha^2 - \min_{\alpha} \sigma_\alpha^2\right)$*. The black line indicates a linear fit with 95% confidence region.*

2.4.2 Variation within individuals

The results for the model of within individual variation over time (Eq 23) are similar to the among individual model. Again, we show clearly that variation in stable isotope values is positively correlated on a $1:1$ basis with variation in diet over time and we see the same effect of number of food sources and the same effect of source isotope variation such that the same conceptual considerations and mathematical corrections can be applied. Since we are now considering time explicitly, there arises a question about dynamics in the isotope values of the food sources which are not considered in our model. This makes it necessary to resample baseline isotopes at suitable time points and locations studied or, more riskily, make the implicit assumption that it remains the same. When comparing populations across different geographical locations, the latter assumption cannot be applied, and consistent sampling is necessary (see 3.1 Variation Among Individuals). Alternatively, one may again have to satisfy oneself that, if no data are available for the source isotope values, that their associated variances are similar enough among populations to not introduce bias in the comparison. Equally, with a sufficient number of populations sampled, one may be satisfied to assume that any such bias is independent random noise among populations and that any comparison of consumer niche variance along a gradient can be treated as additional residual error.

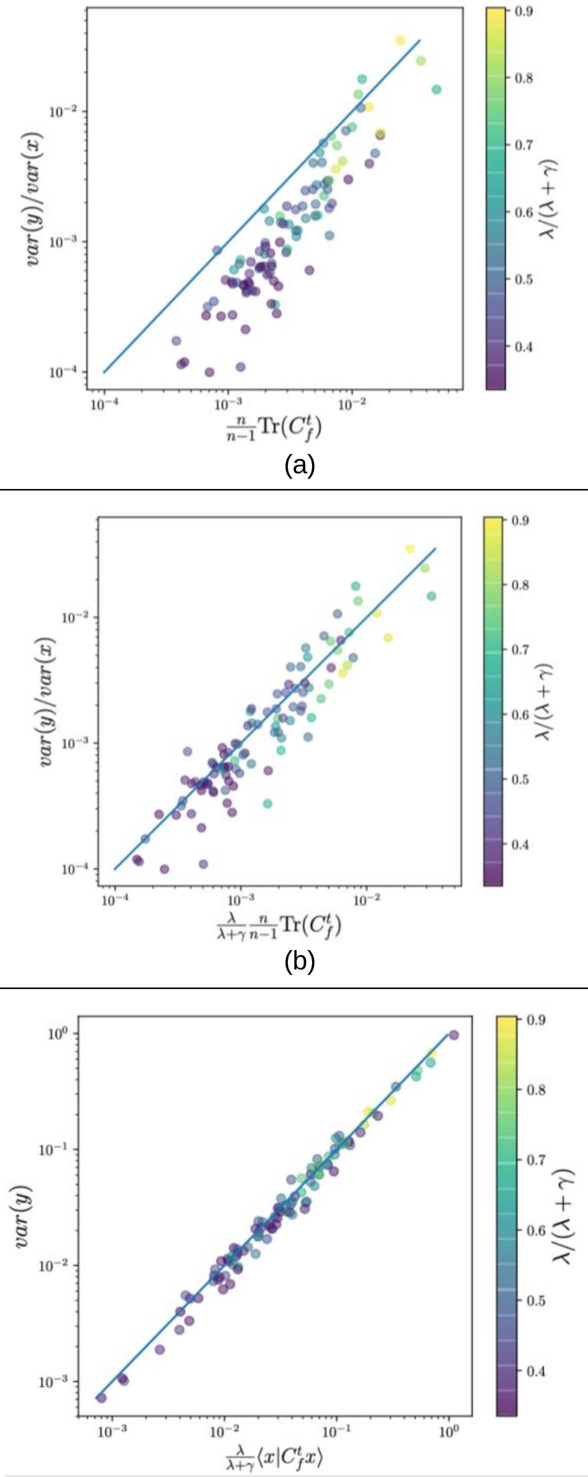
However, this relationship is modified further by the key relationship between the rate at which the diet changes over time (γ) and the rate at which the consumer's tissues incorporate this information (λ). We can see that intuitively, and in general terms, Var(y) grows with tissue turnover rate λ which means that tissues that have a slower turnover rate will have smaller overall variation as they regress towards the mean value of the diet, while tissues with faster turnover rates will more accurately reflect the short timescale fluctuations in diet. In fact, when $\lambda = 0$ and the tissue effectively integrates over infinite time, then Var$(y) = 0$ at one extreme. At the other extreme of rapid tissue turnover, isotopic variance in the consumer is bounded by

$$\mathrm{Var}(y) \leq \frac{n}{n-1}\mathrm{Tr}\left(C_f^t\right)\mathrm{Var}(\boldsymbol{x}) \tag{31}$$

This boundary condition matches the intuition that variation in the isotopes in the consumer cannot be greater than the variation in the underlying food sources and the variation in diet over these sources. The key result from (23) is that tissue turnover (λ) needs to be faster than diet turnover (γ) and matches our conceptual understanding of the process (Phillips *et al.*, 2014). How much faster is a decision that requires subjective consideration, and likely implicit assumptions about the rate of diet turnover which is unlikely to be directly observable empirically.

We illustrate the potential problem by again simulating data over a wide variety of parameter values, including now a range of both diet turnover rates (γ) and tissue turnover rates (λ). Using the same corrections as in Figure *2-1* of dividing consumer isotope variance by resource isotope variance and plotting against a measure of both variance in diet proportions and number of diet sources we again observe a general positive correlation close to $1:1$ (Figure 2-3a). In this situation we have ignored the relative rates of tissue and diet turnover but note that when diet turnover is relatively large compared with tissue turnover the correlation is weaker with points further from the line as $\lambda/(\lambda + \gamma)$ goes to zero. Applying a simple correction to the diet variation axis recovers a more accurate correlation across the range of diet and tissue turnover rates (Figure 2-3b). As a test of the accuracy of the simulation model and with access to all underlying information we note a high level of accuracy and precision of the full analytical model (Figure 2-3c).

Finally, we note from Eq (24) that the assumption made in our model when considering among individual variation (and also typically made in many stable isotope studies in general terms (Phillips *et al.*, 2014)) need not be the case so long as the tissue turnover rate is sufficiently slow so as to integrate over fluctuations in diet. What is useful from our model predictions is that in some cases it is desirable for tissue turnover to be faster than diet turnover, and sometimes the opposite is true. The models allow these nuances to be considered explicitly and will help inform appropriate application to empirical studies. In many situations, and in the desirable case, these insights will guide researchers to choosing appropriate tissues for their specific research question. In situations where such decisions are not possible owing for example to constraints of using non-lethal or non-destructive sampling, then our models allow the constraints and limitations to be identified and provide insights on what is possible to infer from the data, and what explicit and implicit assumptions need to be made.



(a)

(b)

(c)

Figure 2-3. (a) Source-corrected consumer isotope variation correlates positively with diet variation (n/(n-1) "Tr" (C_f)) and points where tissue turnover is fast relative to diet turnover (i.e. λ>γ) are closer to the 1:1 line. (b) Including the pre-factor (λ/(λ+γ)) to diet variation increases considerably the accuracy of the prediction, moving all points closer to the 1:1 line. (c) The full analytical model is accurate and precise when we have full access to all the information available from the simulated data.

2.4.3 Assumptions and limitations

One of the powerful aspects of this modelling approach is that, in making some simplifying assumptions, we can gain clear insights in the main drivers of variation in consumer stable isotope data. The simulations inherently relax some of these assumptions and allow us to observe departures from this expectation; for example, in the case of anisotropic diets, driving deviation from the prediction in Figure 2-2. Clearly, in real systems, there are additional sources of variation and processes that might affect the behaviour of the models. Among these, human and machine errors can be controlled through calibration and standardisation of methods. These will inflate variation at either or both the consumer side or food source side and should do so in an additive sense on Var(y) and Var(x) respectively. We further assume that source isotope values are constant through time, and clearly if longitudinal studies of individuals are conducted over long time periods (e.g. potentially over years using whale baleen (Ryan *et al.*, 2013)), then it is likely that food sources could have shifted and be driving changes in consumer isotopes rather than diet variation itself. The models can be extended to include dynamics on the food source but doing so would require specific decisions to be made on the structure of those dynamics and there is not an obvious approach to be explored that would provide for general insights as there was in our models so far. We would advise that, under such circumstances, specific models be developed and used to explore a priori the expectation of the data prior to analysing the empirical data itself in order to convince oneself that there is a realistic chance of determining a signal on the desired variables from noise and confounding variables. From the beginning, we made the assumption that trophic discrimination factors are constant additive effects and therefore were able to ignore their effect on variance. We know that these effects can vary both within and among individuals and, along with discriminatory metabolic routing of elements, can be an additional source of variation. In ignoring this effect, we are taking the view that the effects are small relative to the other sources of variation in our model. Specific empirical systems could point to situations where this is not true, e.g. in fasting animals or during gestation, and our advice here would follow the general advice of cautioning that, without detailed knowledge of those systems and specific models to describe them, stable isotopes may not be suitable for providing insights on differences in their diet (Phillips *et al.*, 2014, Gorokhova, 2018).

2.5 Conclusion

The distribution of consumers' stable isotope data contains information on the distribution of their diets. Specifically, there is a $1:1$ relationship between the two in terms of their variance, albeit modified and influenced by other factors. A major source of confounding influence is the effect of variation of the isotope values of the consumers' food sources. With these data in hand, a simple correction involving the division of the consumer variance by the source variance yields transformed data that are directly comparable among individuals or populations whose baseline environmental isotope values are different. When considering a time series of isotope values on single individuals or indeed populations, an important constraint on the signal is the relative turnover rates of consumer tissues and diet which will control how the former tracks the latter. Even if the rates are such that they depart from our analytical predictions, we nonetheless expect to see a quantitative relationship between the two, meaning that semi-quantitative comparisons are still possible. Our models give much quantitative confidence to many assumptions made through logically argued theoretical frameworks, make explicit the limitations of the applicability, and provide guidance for empirical studies going forward.

Chapter 3 Technological advances in Stable Isotope Analysis provide ever more insights into animal ecology.

***Authors**:* Jenny Bortoluzzi, Jamie Darby, Andrew L Jackson

***Author contributions**:* I led the writing with strong contributions from JD and supervision by ALJ.

***Status**:* This "In Focus" manuscript has been published at Journal of Animal Ecology

3.1 Abstract

In Focus: Whiteman J.P., Newsome S.D., Bustamante P., Cherel Y., Hobson K.A. (2021) Quantifying capital versus income breeding: New promise with stable isotope measurements of individual amino acids. Journal of Animal Ecology, 00, 1-11. The use of bulk stable isotope analysis (SIA) has become a staple in the field of ecology since the 1980s. This approach has proven its utility, but comes with limitations rooted in assumptions and confounding factors. Compound-specific SIA (CS-SIA) has the potential to address questions out of reach of bulk SIA by providing information on physiological pathways as well as dietary sources of consumer isotopes. Whiteman et al. (2021) provide an excellent example of the power of CS-SIA using amino acid stable isotopes to quantify the extent of capital vs income breeding involved in emperor penguin egg production. By doing so, they reframe an important life history trait as a spectrum, rather than a dichotomy. This showcases the use of CS-SIA as a tool for investigating the resource-allocation strategies employed by this species, and the potential for this technique to untangle the life history strategies of a broad range of species.

Our knowledge of the life histories of far-ranging marine species has advanced more rapidly in recent decades than ever before. This is largely down to developments in telemetry (Weimerskirch *et al.*, 2006) and chemistry-based (Hobson, 1999) techniques, and the innovation of researchers to ask new questions with these tools at hand. Seabirds, whales, sharks and more experience annual and seasonal variation in energetic demands driven by a dynamic environment and species-specific life-history patterns. These traits often relate to reproductive strategies and timings, growth patterns and migrations. The mobility of marine top predators and the variability of their marine ecosystem impedes the study of these systems, though recent developments in ecological research have accelerated our understanding of these processes.

Certain life-history traits are important to understand from a conservation standpoint. For instance, the feather moulting strategies used by different bird species have varying effects on flight ability, depending on intensity and duration (Rohwer *et al.*, 2009). For example, periods of intense moult leave Alcidae seabirds flightless, and as such, are times of increased sensitivity to storm events, surface pollution and food shortages. St John Glew *et al.* (2019) used a combination of stable isotope analysis (SIA) and telemetry data to infer the geographic location of feather development, and therefore habitat use during moulting behaviour of Alcidae seabirds in the North Sea. Another example of SIA being used to study important variations in life-history traits comes from Silva *et al.* (2019), who applied SIA to the skin of Balaenoptera whales to explore their diet and destinations during their winter migration. Taking advantage of the fact that the stable isotopes in the skin are thought to be incorporated over approximately the previous 5 months (Busquets-Vass *et al.*, 2017), the study was able to use skin samples collected in spring to recount the whales' activities over the previous winter. Fish stocks and areas targeted by these whales are identified, and then related back to their population dynamics. These insights may allow for the designation of better directed conservation measures where necessary. Given the mobility of many marine top predators, and the remoteness of their habitats, these features of their ecology would remain a mystery without the development of indirect inference techniques such as SIA.

With SIA, ecologists have worked for years to map food webs and the relationships between organisms in communities. Bulk SIA has long been the basis of such studies: it considers the overall isotopic composition of whole tissues, organisms or soils. The studies described above from St John Glew *et al.* (2019) and Silva *et al.* (2019) used bulk SIA. This requires some prior knowledge or assumptions of stable isotope baselines found in the environment. The isotopic composition of marine primary producers varies spatially, especially in carbon isotopes, and the resulting spatial distribution is known as an isoscape (McMahon *et al.*, 2013). The development of animal tissues can therefore be traced back to the area of an isoscape it was produced in, inferring the broad-scale movements of mobile consumers (Trueman and Glew, 2019). In migratory species such as cetaceans, movements across those isoscapes can be traced in incremental tissue like baleen (Ryan *et al.*, 2013, Trueman *et al.*, 2019). This window into annual variations, beyond that given by short-term tissue samples, provides information on the past ecology of these individuals (sometimes going back decades or centuries by using museum collections, for example). This can be highly significant to understand shifts in ecological behaviours caused by anthropogenic pressures and changing environments; and in turn allows us to make predictions about the future. But the results of these studies using bulk SIA are often hampered by necessary assumptions and uncertainties. Fundamentally incomplete knowledge of environmental baseline isotopes can stymy the construction of the isoscapes in the first place due to the unknown isotopic effects of physiological pathways and temporal averaging between primary producers and prey (Trueman *et al.*, 2019).

Compound specific SIA (CS-SIA), on the other hand, is a molecular-level approach that involves the analysis of individual amino acid or fatty acid stable isotopes, and builds on the principles used in bulk SIA (Boecklen *et al.*, 2011). Advancements in technology mean that it is now easier to study isotopes at a molecular level using gas chromatography or liquid chromatography systems in conjunction with mass spectrometers (Boecklen *et al.*, 2011). This approach potentially provides greater resolution of SIA applicable in trophic ecology, and allows us to study the transfer of nutrients at a physiological level rather than solely at an ecosystem level. One of the key concepts is that it potentially provides a shortcut to accessing indirectly the underlying isotopic baselines by comparing variation between the signatures of key amino acids in the consumer itself.

Whiteman *et al.* (2021) use CS-SIA for this purpose very effectively to examine the underlying physiological mechanisms used by female emperor penguins to create a single large egg. More specifically, they look at the allocation of exogenous and endogenous stores of food into the egg by comparing the stable isotope composition of adult muscle and corresponding egg tissues. Using CS-SIA, the results are much finer than with bulk SIA, as the effect of the penguin’s metabolism on the isotopic composition of essential vs non-essential amino acids is quantifiably different. This allowed the team to trace the source of amino acids in the egg to freshly sourced food, or reallocated stored resources.

In 1980, Drent and Daan introduced two models to describe this resource allocation in egg-forming birds. By looking at the relationship between mass gain in female birds prior to breeding, laying date and clutch size, they proposed two opposing strategies for reproduction: capital and income breeding (Drent and Daan, 1980). Females have to strike a balance between laying at a favourable time, often as early as possible in the breeding season, and amassing and storing enough energy through foraging before laying. The models describe capital breeders as accumulating energy in body reserves while income breeders redirect nutrients and energy straight from the food ingested (Meijer and Drent, 1999). This difference in resource allocation and how it relates to reproduction and growth under different ecological scenarios are key questions in the study of life histories (Stephens *et al.*, 2009).

Clearly, the effective allocation of nutrients for breeding purposes is a huge factor in the reproductive success of a population (e.g. Kitaysky et al., 2000). Many far-ranging seabirds undertake a pre-laying exodus just before breeding, sometimes spending weeks in productive waters far from their breeding colonies. More often than not, females will spend longer away from the colony than males in this time, presumably amassing the nutrients required to create an egg (Brook, 1990). Male emperor penguins need to store energy reserves required to fast for several months while incubating an egg, allowing females to abscond to replenish nutrients spent on egg development (Burns and Kooyman, 2001). CS-SIA has the potential to provide valuable insights into the physiological processes and ecological context involved in this resource management and in a system that is challenging to study. As Whiteman et al. (2021) asked, do egg-forming birds rely on long-term reserves when forming eggs, or does most of the egg-building material come directly from ingested food?

Such information can be linked to the fecundity of a population. What foods are most important during this critical period in the reproductive cycle? Are they amassed over the entire non-breeding period (capital breeding) or are species reliant on seasonal availability of adequate resources (income breeding)? Can reproductive outputs of a population be tied to the availability of these food sources? These questions can be adapted for a range of taxa, especially those that invest heavily in reproduction, as seabirds do. Whiteman et al. (2021), clearly demonstrated how powerful CS-SIA analysis can be to deliver more detailed information on these critical topics.

Chapter 4 A study of the reproductive physiology and feeding ecology of blue sharks, *Prionace glauca*, in the Irish sea (Northeast Atlantic) using a combination of biochemical and biologging methods.

***Authors**:* Jenny R. Bortoluzzi, Nicholas L. Payne, Lauren Meyer, James Gelslechter, Martin J. Kainz, Alison Boyce, Tom Doyle, Lucy Harding, Luke Harman, Haley Dolton, Grace E. McNicholas, Matthew Cobain, David Edwards, Andrew L. Jackson

Author contributions: I conceived the study, collected the data, analysed part of the data, interpreted the results and wrote the manuscript; JG analysed the reproductive hormone samples and analysed data; LM analysed the data on fatty acids; MJK provided supervision laboratory analyses for fatty acids; LH, LH, AB, HD, GEM, MC and DE assisted with data collection and fieldwork. ALJ and NLP provided input on study conception, data analysis and the manuscript.

Special thanks to Save Our Seas Foundation who generously funded the fatty acid and reproductive hormone analysis work carried out for this chapter.

Status: This manuscript is being prepared for publication. Data on bulk and compound specific stable isotope analysis will also be added into the manuscript.

4.1 Abstract

Blue sharks, *Prionace glauca*, are consistently found to be the shark species most at-risk from fishing pressures. They are distributed circumglobally and carry out transoceanic migrations and display spatial sexual segregation. Female blue sharks enter Irish waters in the late Spring and are found there until at least the early autumn but their behaviours and reproductive status while there are largely unknown. Morphological measurements combined with sex steroid hormone analyses were used to identify three classes of maturity in 34 female sharks from June to November (juveniles, subadult and adults). A seasonal shift in resource use was identified from fatty acid analysis combined with biologging and morphological observations. Sexual maturity status was not found to explain this shift but month of sampling was a significant driver. These data suggest a change in diet from small pelagic fish to possible input from gelatinous or planktonic prey, and highlight, once again, the opportunistic behaviour of this pelagic shark.

4.2 Introduction

Despite being one of the most abundant pelagic and the world’s widest-ranging chondrichthyan (Nykänen *et al.*, 2018), much still needs to be learnt about blue shark biology and ecology (da Silva *et al.*, 2021). As the most frequently fished oceanic shark, it is estimated that at least 7 million blue sharks were landed in 2019 with an economic value of up to $441million and that 34% of these catches came from the Atlantic where the population has previously been reported to have declined by up to 70% (Baum *et al.*, 2003, Cappell *et al.*, 2022). With a declining population, they were classed as Near Threatened globally, and in Europe, and as Critically Endangered in the Mediterranean in the latest IUCN Red List assessments (Sims *et al.*, 2016, Rigby, 2019). In 2022, a proposal to add the species into Appendix II of CITES was adopted, regulating its international trade (CITES, 2022, The Shark Trust, 2022). Although the species has been studied in parts of the Pacific and Atlantic, studies specific to Ireland - where blue sharks are seasonal visitors - are sparse. Historically and to this day, they play an important role in local Irish economies as a target for sport angling (Crummey *et al.*, 1991, Fitzmaurice *et al.*, 2005, Inland Fisheries Ireland, 2013, Wogerbauer *et al.*, 2016), yet benefit from no legal protection.

Life history parameters such as fecundity and reproductive strategies are important elements to understanding population growth rates and, along with knowledge of aggregation sites of reproductive females, are key for management and conservation, particularly when the protection of larger areas is not feasible (Wearmouth and Sims, 2008, Yokoi *et al.*, 2017). In Irish waters, the population is predominantly female (Crummey *et al.*, 1991, Fitzmaurice *et al.*, 2005) but information about their reproductive status is lacking and hormonal work is yet to be carried out on this species in the Atlantic. Sex steroid hormone analysis to stage reproductive status in female blue sharks was first shown to be effective by Fujinami and Semba (2020) and work carried out by Rangel *et al.* (2021) on tiger sharks investigated the influence of reproductive status and life stages on diet by combining fatty acid markers with reproductive hormone analysis. Indeed, similarly to stable isotopes, fatty acids are integrated into the tissues of consumers and can be analysed to understand foraging patterns of predators such as fish, marine mammal or sharks (Kirsch *et al.*, 1998). Many fatty acids, such as polyunsaturated fatty acids (PUFAs), cannot be easily synthesised by marine vertebrates and must therefore be obtained from dietary sources and stored in tissues such as the liver, muscle or blood over varying timescales (Beckmann *et al.*, 2013, Beckmann *et al.*, 2014). A variety of abiotic and biotic factors, such as habitat, temperature, or phylogeny, may influence the production and synthesis of different fatty acids (Meyer *et al.*, 2019). This information is retained and transferred within the food web and, as such, understanding the profiles of fatty acids present in the tissues of marine predators can provide insights into their foraging dynamics (Meyer *et al.*, 2019). For example, palmitic acid 16:0 is indicative of pelagic food sources, while high levels of EPA 20:5ω3 may be indicative of cold water and/or demersal feeding (Meyer *et al.*, 2019). As such, variations in fatty acid profiles within and among individuals of a population of consumers can provide insights into variations in their diets and foraging dynamics. Therefore, understanding both reproductive status and diet ecology of a returning migratory population, such as *P. glauca* in Ireland, can provide key information on their use of space and the impacts of activities and environmental conditions on their ecology.

Aside from the very small number of filter-feeding species, elasmobranchs are considered nekton feeders. Blue sharks are seemingly opportunistic consumers that mostly feed on epi- and mesopelagic teleost fish, cephalopods, and, occasionally, mammals and birds throughout most of their range with some ontogenetic, sex and geographical variations (da Silva *et al.*, 2021). Interestingly, crustacea are regularly listed as items found in their stomachs (Kubodera *et al.*, 2006, Vaske Júnior *et al.*, 2009, Markaida and Sosa-Nishizaki, 2010, Cordova-Zavaleta *et al.*, 2018) including euphausiids in California (Harvey, 1989) and free-swimming amphipods in California (Tricas, 1979).

Blue sharks are occasionally described as having a series of knob-like protrusions along the anterior margins of all gill arches which has been suggested to help facilitate handling of invertebrate prey (Compagno, 1984, Rangel *et al.*, 2017). They are also known for having unusually broad pectoral fins, allowing them to swim very slowly (2.5m blue sharks cruise at just 0.3-0.4 $m.s^{-1}$ in Ireland in the Spring, in comparison to a mean of 0.69 $m.s^{-1}$ in scalloped hammerhead sharks and 0.8 $m.s^{-1}$ in white sharks and mako sharks; Iosilevskii et al., 2022, Ahnelt et al., 2020, Ryan et al., 2015) while generating sufficient lift to counteract their negative buoyancy. These slow speeds appear lower than the speed which minimises the cost of transport and so could indicate an energetic strategy of foraging on high densities of very slow moving prey, such as jellyfish (Papastamatiou *et al.*, 2018). Indeed, there is anecdotal evidence from fishers in Ireland that blue sharks do eat gelatinous prey such as jellyfish or ctenophores with reports of captured sharks regurgitating large quantities on deck (pers. comms.).

In this study, we combined analysis of reproductive hormones, fatty acids and biologging to explore the trophic ecology and reproductive status of blue sharks in Irish waters, including the possibility that they derive nutrients from invertebrate prey such as jellyfish. We predict that variation in reproductive hormone levels and maturity status are associated with variation in diet and/or physiological state inferred from fatty acid data. We further predict a change in diet over time coinciding with potential prey presence and specifically investigate whether there is evidence for ingestion and assimilation of lower trophic level organisms such as jellyfish and/or amphipods (free-swimming or parasitic).

4.3 Materials and methods

4.3.1 Study species and sample collection

For this study, 35 blue sharks, *Prionace glauca*, were sampled monthly from West Cork, Ireland (~51.33N, 8.75W) between June and early November 2021 (Figure 4-1). Sharks were captured, through cooperation with charter professionals, using rod and reel angling and brought onboard the boat, onto a mat, after capture. Their gills were ventilated with a seawater hose placed in the mouth, and a wet towel was placed on their heads to reduce stress throughout sampling procedures. Time onboard averaged 11 minutes with a maximum of 16 minutes occurring once.

For each individual captured, a series of measurements and tissue samples were collected. Measurements taken included precaudal length (PCL); fork length (FL); total length (TL); lateral span (LS); frontal span (FS); proximal span (PS); and caudal keel span (CKC) as per Gallagher *et al.* (2014). Any significant markings were noted and photographed (Figure 4-2). Tissue samples taken included a muscle biopsy (~2-

3g) excised from the dorsal musculature using a sterilised scalpel and biopsy punch and 10-20mL of whole blood taken via venipuncture from the caudal artery at the dorsal caudal keel using 18 gauge needles and plastic non-heparinized syringe. Blood samples were then transferred to 10mL sodium (NH) heparinized Vacutainer® collection tubes. Tissue samples were stored on ice while onboard. Once back on land, blood was centrifuged at 2000g for three minutes in 1.5-2mL Eppendorf tubes within a maximum of nine hours post-sampling. Plasma and red blood cells were separated into Eppendorf tubes. Muscle, plasma and red blood cell samples were all temporarily stored at -20ºC while on location and transferred to -80ºC within a maximum of 48 hours until processing.

In addition to shark tissue sampling, samples were taken from a random selection of potential prey items. Fish muscle samples were taken from fish provided by anglers, caught within the previous 24 hours and frozen overnight. Ten jellyfish were caught in July using a handheld fine-mesh net and five were caught onboard the R/V Celtic Explorer in August, all 15 were caught within the same region as shark sampling. An additional 2 jellyfish and ctenophores were caught further offshore in June (50.83N, -7.38W and 51.251N, 9.1325W) onboard the R/V Celtic Explorer. When present, parasitic amphipods were extracted from the bells of jellyfish caught in July and grouped into three samples of 10-30 amphipods each. All potential prey items were frozen at -20ºC before being transferred to -80ºC until analysis.

Finally, a stranded female blue shark individual was found in Kerry (Ireland) and collected in June 2022. A visual assessment of the morphology of its gills was carried out and recorded.

4.3.2 Sex steroid hormones

Reproductive endocrinology, the analysis of levels of sex steroid hormone concentrations, 17β-estradiol (E_2) and progesterone (P_4), in elasmobranchs has been used to provide information regarding the sexual maturity and reproductive cycle of of a population or species, including blue sharks (Awruch, 2013, Fujinami and Semba, 2020). Circulating concentrations of 17β-estradiol (E_2) and progesterone (P_4) were therefore measured in diluted plasma using commercially available immunoassay kits following the manufacturer's instructions. The E_2 AccuLite Chemiluminescence (CLIA) Kit from Monobind, Inc. (Lake Forest, CA) was used to measure E_2 as described for use in finetooth shark (*Carcharhinus isodon*) by Brown et al. (2020) and bonnethead (*Sphyrna tiburo*) by Gonzalez De Acevedo et al. (2021). Validation of the E_2 Acculite CLIA kit for use with blue shark plasma was determined using parallelism to confirm that unknown analytes in serial dilutions of pooled samples (1/1 to 1/128) interacted with antibodies in a manner similar to assay standards. "Cold spikes" were also used to determine percent recovery by adding a known amount of hormone standard to each pooled sample dilution and calculating recovery. Results from E_2 validation tests were used to identify the ideal dilution of plasma to avoid matrix effects, which was determined to be 1/4. Hormone concentrations for each sample were calculated by multiplying by the dilution factor used. Mean intra-assay variability was 12.46% and mean inter-assay variability was 11.54%. Recovery was 55.46% at 1/4.

Initial attempts to validate the use of the P_4 Acculite CLIA Kit (Monobind, Inc.) for measuring P_4 in blue shark plasma were unsuccessful. Therefore, the Lifespan Biosciences Inc. (Seattle, WA) Fish Progesterone (Competitive EIA) ELISA kit was used to measure P_4, as described for blue sharks by Fujinami and Semba (2020). The Lifespan Fish Progesterone kit has been previously validated for use with blue shark plasma; therefore, samples were prepared at a 1/2 dilution following recommendations by Fujinami and Semba (2020). Mean intra-assay variability was 21.10%. Inter-assay variability and recovery were not determined.

4.3.3 Fatty acid analysis

Turnover rates of fatty acids (FA) in tissues of sharks are generally poorly studied. However, blood serum has been suggested as a useful indicator of acute dietary changes and its use is justified to distinguish between diets (Beckmann *et al.*, 2014). Similarly, muscle FA profiles have been found to reflect dietary changes within around three weeks of sampling, though exact times may be species specific (Beckmann *et al.*, 2014, Meyer *et al.*, 2021). Frozen tissue samples (22 shark muscle, 22 shark plasma, 12 fish muscle, 13 jellyfish and 3 amphipod samples) were freeze-dried and homogenised. They were then shipped, processed and analysed at WasserCluster Lunz (Lunz Am See, Austria). Samples were weighed and 2mL of chloroform was added to each one in a glass tube to start the extraction process. Each tube was flushed with N_2 to replace oxygen before being closed and stored at -80ºC overnight. On day two of the extraction, 1mL of methanol, 1mL of chloroform-methanol (2:1), and 0.8mL of NaCl were added to the tubes and closed under N_2 atmosphere. They were then sonicated, vortexed, and centrifuged (3min, 3000g, 4ºC) and a double pipetting method was used to extract the bottom layer of lipids to avoid contact of the extracting pipette with the upper layer. This washing process was repeated twice more by adding 3mL of cold chloroform after extraction of the bottom layer. The extracted layer was transferred into glass vials, placed under N_2 and vaporized until a standardized volume of 1.5mL remained. For each sample, 100μL of this lipid extract was transferred to tin capsules, evaporated and weighed to determine total lipids concentration (mg lipids/g dry weight) in each sample using gravimetric determination. Once the volumes of total lipids were known, calculated volumes of FA methyl esters (FAME) were evaporated to dryness, diluted in 1mL toluene solvent and incubated and 2mL of sulfuric acid H_2SO_4-methanol (1:100M vol/vol) at 50ºC for 16h. The FAME samples were then dissolved in hexane (5mL) and $KHCO_3$ (2%, 2mL), shaken, vortexed and centrifuged at 4ºC for 3 minutes at 1500rpm. The upper organic layer was collected and the process repeated once more. Finally, the pooled organic layer was concentrated by evaporation, dissolved in 1mL hexane and analysed for FA content by gas chromatography with flame ionization detection. FAME concentrations are reported as mass fractions in mg.g^{-1} dry weight.

4.3.4 Biologging

Biologging packages such as that used in the current study have previously been successfully used to study the ecology and physiology of large free-ranging sharks such as tiger sharks, blue sharks, hammerhead sharks, white sharks and more (Payne *et al.*, 2016, Royer *et al.*, 2020, Gallagher *et al.*, 2022, Harding *et al.*, 2022, Papastamatiou *et al.*, 2022). To collect behavioural information, a biologging package was fitted to the pectoral fin of one individual using a plastic cable tie passed through 2 incisions in the fin and connected to a dissolvable time-release link. The package included a camera-logger (Techno-cam, 22-24 grams, resolution 720p, Techno-SmArt Europe); VHF transmitter (Advanced Telemetry Systems, MM100); and a satellite position-only tag (Wildlife Computers SPOT Model 258; ARGOS enabled). Once the time-release link fully dissolved, the package detached from the fin and floated to the surface where the SPOT and VHF tags allowed for retrieval using GPS positioning and a VHF receiver.

4.3.5 Data analysis

We identified change in hormone levels with FL using a cumulative sum change point-analysis (CUSUM CPA) run in Change-Point Analyzer software (Taylor, 2000). The point of change in E2 levels was determined by a bootstrap analysis using a 95% confidence interval. The results from the CPA were used to assign a maturity stage to each shark sampled (“juvenile”, “subadult”, “mature”).

Multivariate statistical analyses were run in PRIMER7 with PERMANOVA to assess differences in FA profiles between blue sharks and prey groups, across tissue types, maturity stages and through time. As squid samples could not be sourced in situ, squid data from Madgett *et al.* (2019) from Southwest Shetland and the Moray Firth (Scotland) were included in this analysis as a proxy, though some FAs are missing from this dataset. Differences among categorical groups were tested by Permutational Multivariate Analysis of Variance (PERMANOVA) with Monte Carlo simulations run on Bray-Curtis similarity matrices calculated from the square-root transformed profile data, following Anderson *et al.* (2008) and Carlisle *et al.* (2021) to determine if *month* of sampling and maturity *stage* influenced the overall FA profiles of sharks. If the number of unique permutations was large (~100 or more), the permutation p-value [p(Perm)] was selected. If the number of unique permutations was low (<100), the Monte Carlo P-value [p(MC)] was selected. Significance was declared at p(MC) or p(perm) <0.05. When differences approach significance (p(perm) < 0.05 but p(MC) ≈ 0.05), both P-values were reported. Differences among groups were then visualised using Principal Coordinates

Analyses (PCO) and Canonical Analyses of Principal Coordinates (CAP). The PCO ordination was used to display differences among monthly samples of shark plasma and shark muscle FA profiles whereas the CAP was used to present differences between these samples and potential prey items. Both were overlaid with vectors of individual FAs.

4.4 Results

A total of 35 live blue sharks were caught during the period of this study (Table 4-1): 34 females (151.72 ± 20.65 cm FL, mean ± SD) and two males (165 and 132 cm FL) (Figure 4-1). Potential bite marks were identified on three female individuals (175 cm FL, 128 cm FL and 182 cm FL; see Figure 4-2). One stranded shark (106 cm PCL) was used for gill morphology observation.

*Table 4-1 Summary data of sampled live blue sharks between June and November 2023. * indicates the individual was tagged with a biologging package. † indicates individuals which were observed with scars.*

ID	Sampling Month	Sex	FL (cm)	TL (cm)	Hormone analysis?	FA Analysis?
BS_01	June	f	127	159	N	N
BS_02	June	f	149	176	Y	Y
BS_03	June	f	160	193	Y	N
BS_04	June	f	158	192	Y	Y
BS_05	July	f	155	191	N	N
BS_06	July	f	135	162	Y	N
BS_07*	July	m	165	199	N	Y
BS_08	July	f	151	185	N	N
BS_09*	July	f	160	195	Y	Y
BS_10	July	f	115	138	Y	N
BS_11	July	f	166	202	Y	Y
BS_12	July	f	143	172	Y	Y
BS_13†	July	f	175	212	Y	Y
BS_14	July	f	136	167	Y	Y
BS_15	July	f	151	184	Y	Y
BS_16	July	f	138	166	Y	N
BS_17†	July	f	128	154	Y	Y
BS_18	July	f	138	168	N	N
BS_19	July	f	121	146	Y	Y
BS_20	July	f	160	196	Y	Y
BS_21	August	f	133	162	Y	Y
BS_22	August	f	161	196	Y	Y
BS_23	August	f	168	211	Y	Y
BS_24	August	f	159	193	Y	N
BS_25	August	f	132	161	N	N
BS_26	August	f	159	193	Y	Y
BS_27	August	f	176	212	Y	N
BS_28	August	m	132	161	N	N
BS_29	September	f	136	165	Y	Y
BS_30	September	f	132	159	Y	Y
BS_31†	November	f	182	230	Y	Y
BS_32	November	f	169	204	Y	Y
BS_33	November	f	191	232	Y	Y
BS_34	November	f	191	230	Y	N
BS_35	November	f	194	237	Y	Y

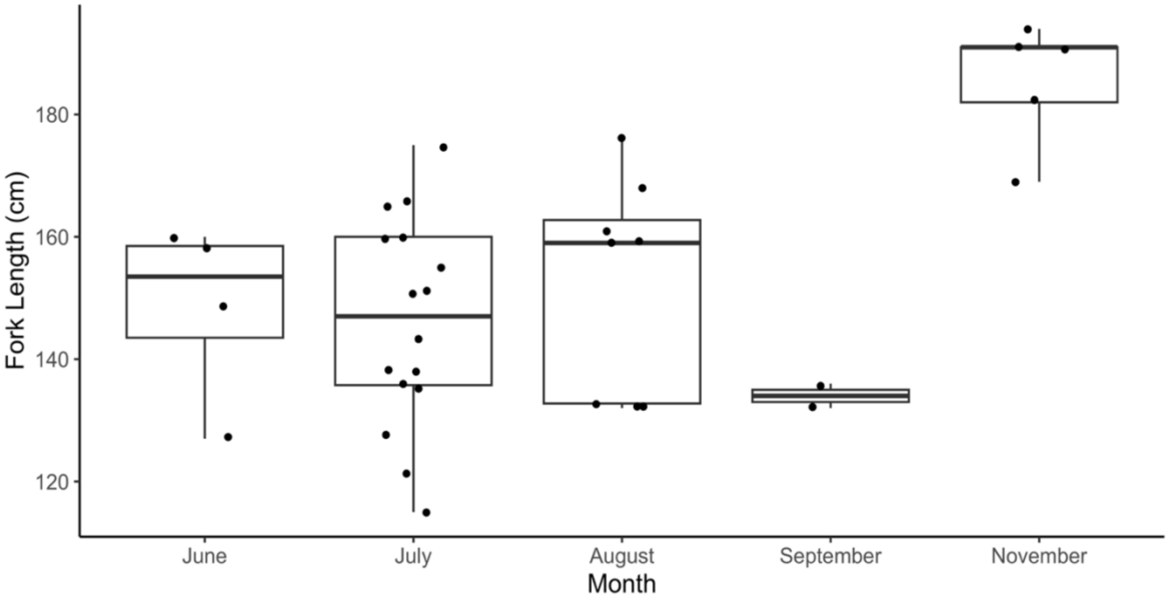


Figure 4-1 Captured blue shark monthly size distribution (Fork length, cm)

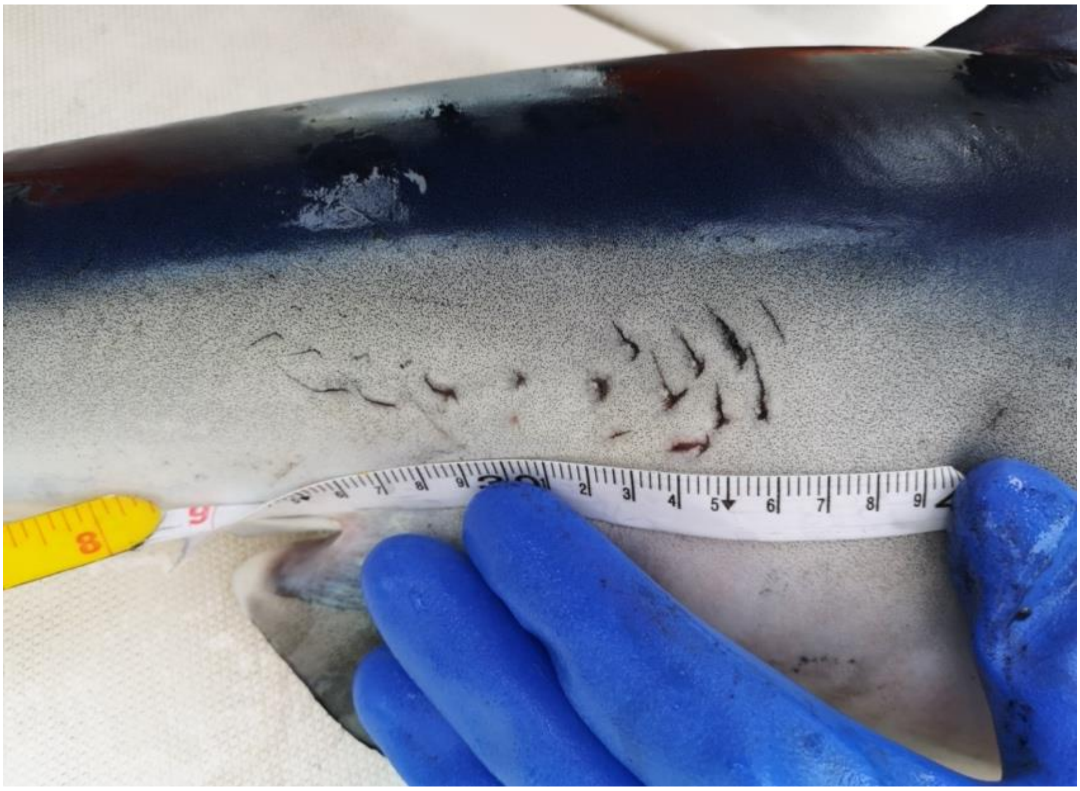


Figure 4-2. An example of potential bite marks observed on the flanks of a female blue shark measuring 128 cm FL.

4.4.2 Female sex steroid hormones

The highest level of E_2 (471.088 pg. mL^{-1}) was measured in a female shark of 160 cm FL while the highest level of P_4 (10.353 ng.mL^{-1}) was measured in a shark measuring 135 cm FL. Concentrations of P_4 were undetectable in two sharks (175 cm FL and 130 cm FL) while the lowest concentration of E_2 was measured in the plasma of an individual of 133 cm FL (Figure 4-3).

CPA results revealed a significant shift in mean E_2 concentrations of female shark blood plasma at 160cm FL with a 95% confidence interval of 159-175 cm FL. We defined the change point (including the 95% CI range) as the size range below which females are likely immature (juvenile), and above which females are likely sexually mature. Female sharks below the CI (<159cm FL) were assigned a maturity stage of juvenile; those within the CI (159 – 175cm FL) were determined to be subadults; and those above the CI (>175cm) were determined to be mature (dashed lines on Figure 4-3). These results are consistent with size ranges found by Pratt (1979) in the north-west Atlantic (subadult range of 145-185 cm FL). In summary, eighteen individuals were assessed as juveniles, ten as subadults, five as mature.

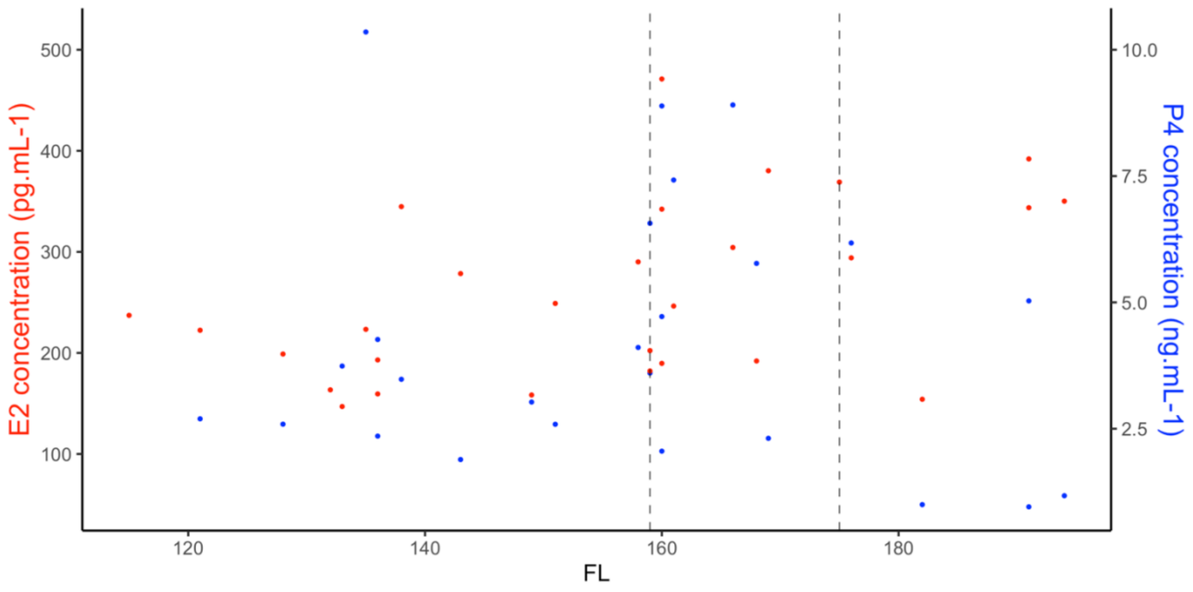


Figure 4-3. Circulating concentrations of 17β-estradiol (E_2, pg. mL^{-1}) and Progesterone (P_4, ng.mL^{-1}) in the blood plasma of female blue sharks. Dashed lines indicate maturity stage classes (juvenile, subadult and mature).

4.4.3 Fatty acid analysis

Overall, variations in shark plasma FA profiles and shark muscle FA profiles could not be explained by stage of maturity (PERMANOVA p(MC) = 0.72 and 0.257 respectively), likely due to a low sample size, particularly of mature individuals.

However, month of sample collection was a significant driver for both plasma and muscle FA profiles (Main test p(MC) = 0.048 and 0.005 respectively).

For plasma, differences between July and August are approaching significance (638 unique permutations, p(Perm) = 0.013 but p(MC) = 0.056), once again likely due to a smaller sample size in August. Differences between July and November were significant (p(Perm) = 0.003). These dissimilarities are illustrated using PCO in Figure 4-4a where November samples are most dissimilar (furthest away) from July samples. Similarly, CAP results (Figure 4-5a) comparing shark plasma to potential prey sources suggests a shift in resource use over time from July to November with decreasing levels of 18:2ω6 (Figure 4-6b) in the latter months for example. Additionally, concentration of 16Me:15 in the plasma of blue sharks increased between July and November, concentrations of which are highest in jellyfish and parasitic amphipods of jellyfish (Figure 4-6c) and partially drove similarities between the two groups (Figure 4-5a).

For muscle, significant differences in the FA profiles of sharks were found between June and November (p(MC) = 0.031); July and August, September and November (p(Perm) = 0.024, p(MC) = 0.03, p(Perm) = 0.001); August and September (p(MC) = 0.036); and September and November (p(MC) = 0.036). PCO analysis (Figure 4-4b) shows clustering of November samples most separate from samples from June, July and September and September from July and August. A CAP presenting FA profiles of shark muscle in relation to potential prey items (Figure 4-5b), once again illustrates a potential shift in resource use between the earlier months (June and July) and the latter (November) potentially driven by decreasing levels of 18:1-9t (Figure 4-6e). Concentrations in muscle of FA 18:1-9t and 16.D9.10 are both seen to decrease over time (Figure 4-6e and f). Both these FAs are lowest or absent in jellyfish and parasitic amphipods, though data for these in squid is missing from our dataset. Concentration of FA 18:0 remains consistently high in shark muscle over time and is highest in jellyfish samples (Figure 4-6a).

While each FA presented in Figure 4-6 are present in a wide breadth of levels in the tissues of sharks and potential prey (<0.25% to >20% of total content), we focused our interpretation on the variation of those concentrations over time as indications of shifting foraging dynamics.

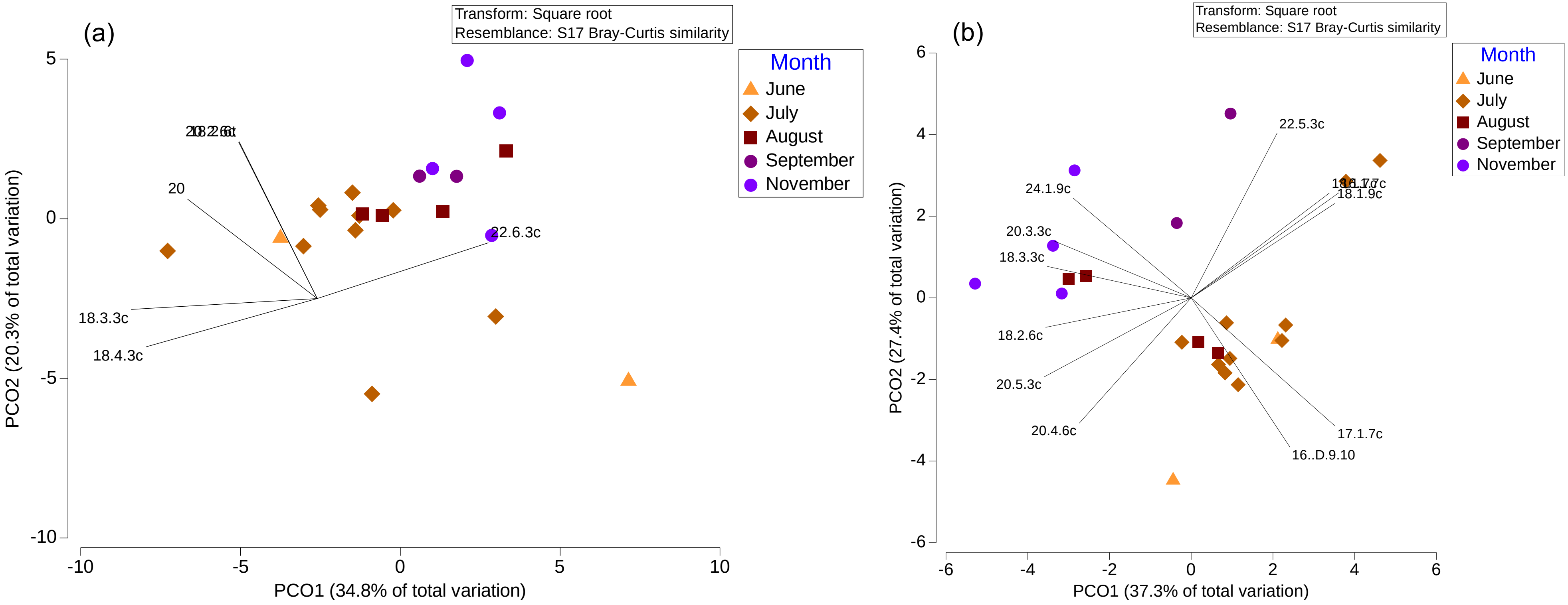


Figure 4-4. PCO ordination provided by PRIMER of (a) shark plasma samples FA profiles by month and (b) shark muscle samples FA profiles by month.

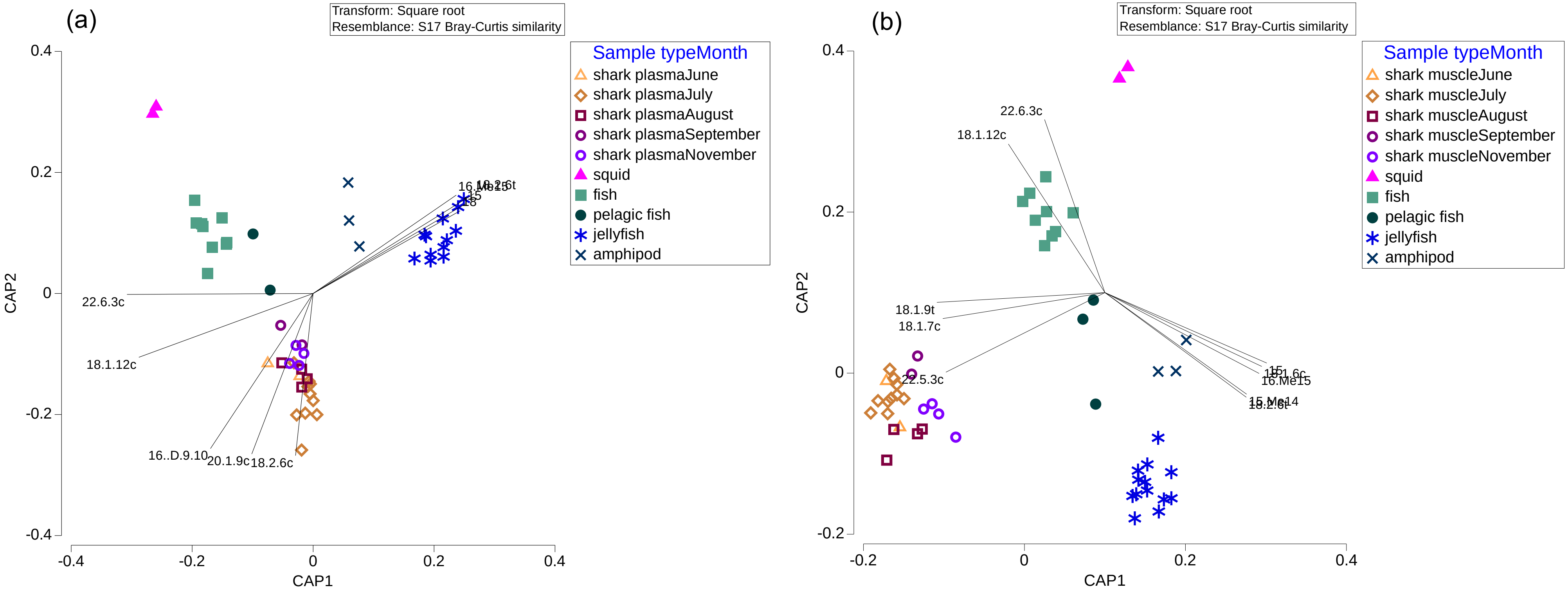


Figure 4-5. Canonical Analysis of Principal Coordinates (CAP) of monthly blue shark, Prionace glauca, (a) plasma and potential prey sources FA profiles and (b) muscle and potential prey sources FA profiles with overlaid vectors of individual fatty acids with CAP correlation values >0.60.

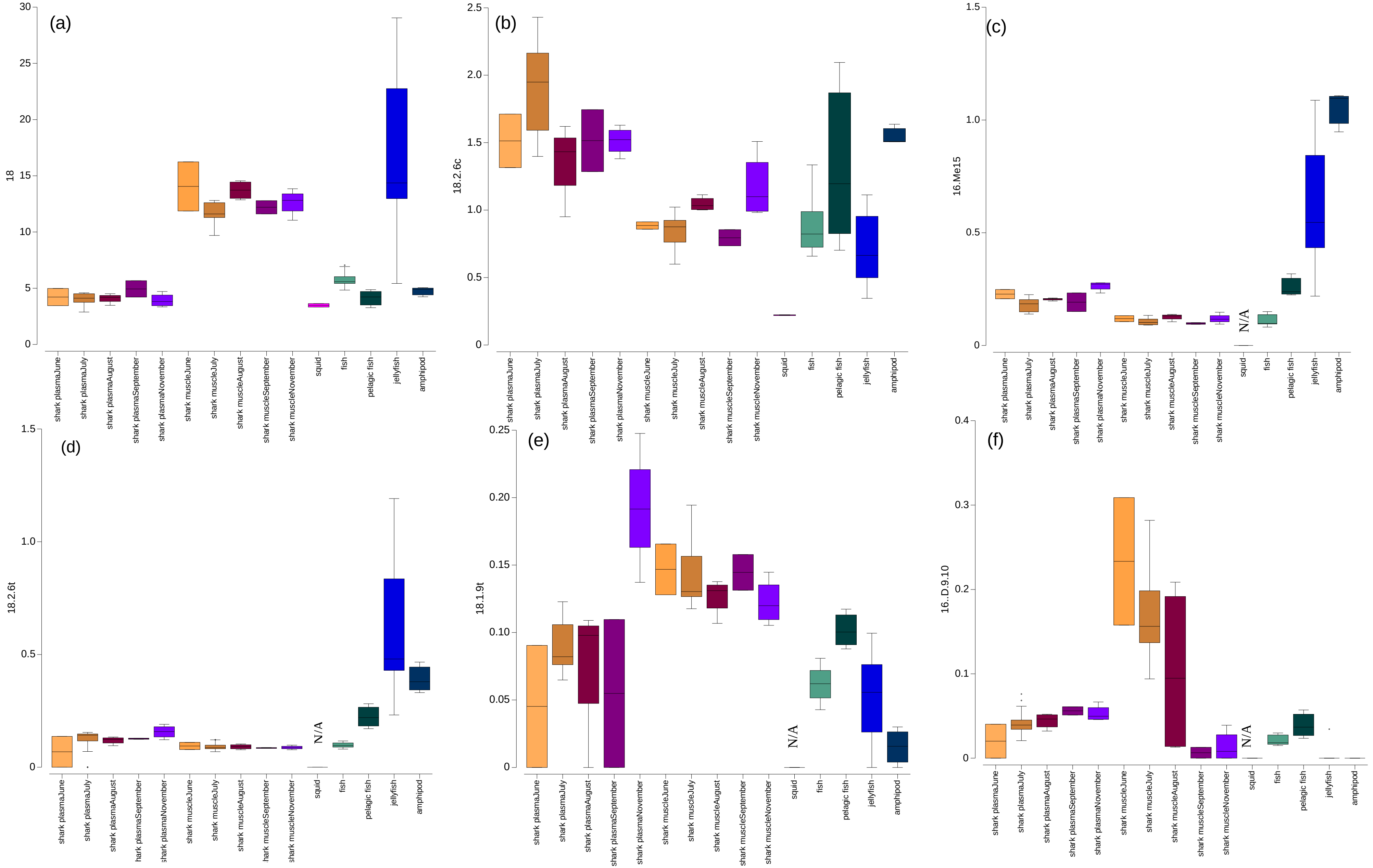


Figure 4-6. Concentration of individual FAs (percentage of total content) in the plasma and muscle of sharks by sampling month and in potential prey sources. Fatty acids (a) 18:0 (b)18:2ω6 (c)16Me:15 (d) 18:2-6t (e) 18:1-9t (f) 16.D9.10. N/A indicates absence of data where the individual FA was not measured in squid samples.

4.4.4 Biologging

An animal-borne camera was used with the aim of capturing novel behaviours and foraging events. The shark was tagged at 51.34337N, 8.75907W and the package surfaced after release at 51.19794N, 8.77378W. In total, 6.5 h of video material was recorded from one shark (male, 199cm TL). No foraging events were captured. We observed one event of possible examination of a jellyfish (Figure 4-7). In a first instance, the shark was seen swimming towards a jellyfish before turning completely on itself and swimming past it again in very close proximity.

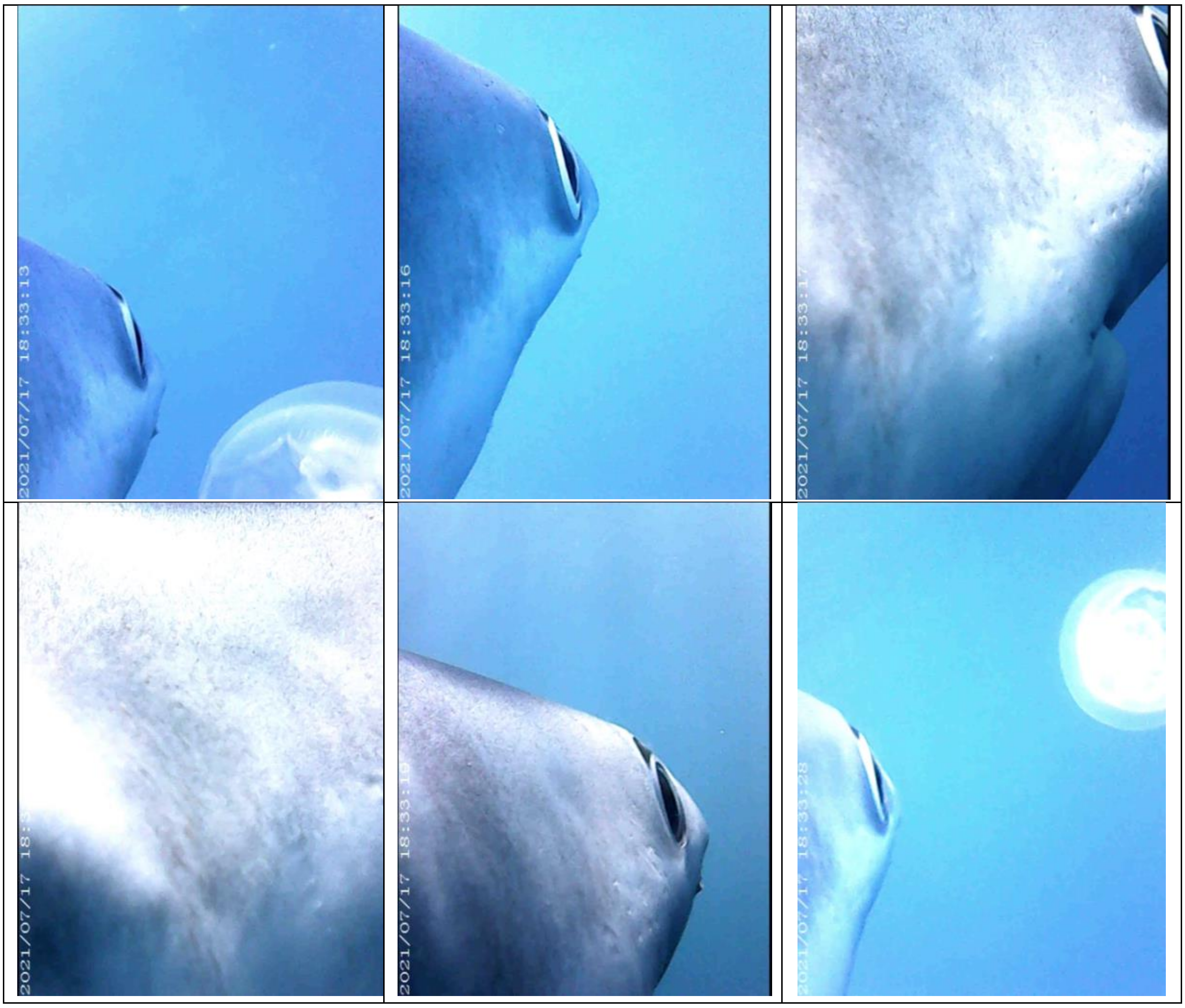


Figure 4-7. Still image series extracted from an animal-borne camera of a male blue shark displaying inquisitive behaviour towards a jellyfish (Techno-Cam, TechnoSmArt Europe)

4.4.5 Gill morphology

One dead stranded shark (female, 106cm TL) was collected from Co. Kerry (Ireland) in June 2022, frozen and dissected in February 2023. A preliminary visual observation of gill arches revealed the presence of node-like soft-tissue structures akin to gill-rakers present on the second and third arches (Figure 4-8) but were not found on the first arch.

Figure 4-8. Node structures observed on the second and third gill arches in situ (left) and extracted (right) from a stranded female blue shark (106cm TL).

4.5 Discussion

By incorporating multiple biochemical tracers and broadscale observations, we gained novel insights into the life history traits of Northeast (NE) Atlantic blue sharks. Through FA analysis, we identified individuals present in Ireland between June and November display a seasonal shift in resource use. Combining these results with circulating sex steroid hormone analysis, we suggest this shift in FA profiles in the tissues of sharks is unlikely to be caused by changes in physiology (reproductive stage) but from variations in diet over time. Our observations and the results from sex steroid hormone revealed that the population of blue sharks caught by anglers in inshore waters (circa 20 nautical miles from the coast) is majority female, with individuals from all three maturity classes captured. We observe indications that some sexually immature females may bear mating scars, similarly to that described by Calich and Campana (2015) and that mature females thought to be largely absent from this region (Nakano and Stevens, 2008) could be present later on in the year when angling effort is much reduced (Crummey *et al.*, 1991). Additionally, consistently with our predictions, our results provide information that blue sharks in Irish summer waters are likely feeding on and assimilating lower trophic prey such as jellyfish or amphipods.

Diet studies of blue sharks in Ireland and the NE Atlantic, though scarce, have identified cephalopods and small pelagic fish as their primary prey items (Stevens, 1973, Henderson *et al.*, 2001, Mendonça, 2009), with evidence of more opportunistic feeding on benthic species, birds, cetaceans and crustacea (Stevens, 1973, Dorman, 1987, Henderson *et al.*, 2001). Relative to other potential food sources, our results did not suggest squid as the primary prey targeted by this population of blue sharks with CAP results suggesting FA profiles in blue sharks are most dissimilar from squid than any other tested prey samples. While the data from squid in this study are not from Ireland, it is unlikely that these values would vastly vary between both locations, and values within the same species or taxa with similar physiologies from different locations (e.g. squid from Scotland and squid from Ireland) would remain closer than values from different species or groups (e.g. squid and pelagic fish) (Meyer *et al.*, 2019). Additionally, we identified a seasonal shift in resource use from their arrival in the early summer (June/July) to the early autumn (November), with clear variations in the concentrations of selected FA such as 16.D9.10 (Figure 4-6f) which declined significantly in the muscle of sharks between June and November. This decrease may be indicative that these sharks primarily feed on prey such as pelagic fish early in the summer but move to a diet less reliant on this prey type later on in the season, possibly feeding on jellyfish where this FA is absent. Other FAs such as stearic acid (18:0; Figure 4-6a) were present in similar concentrations throughout the season in both plasma and muscle of sharks. However, concentrations in both tissue types differed significantly, and those of plasma were low, similarly to those of squid, fish and pelagic fish. In contrast, this acid was present in much higher levels in muscle than in any of these prey items, and in similar concentrations observed in jellyfish.

Many abiotic and biotic factors drive FA profiles of consumers and can confound their use (Meyer *et al.*, 2019). As such, these results must be taken with caution and future work should focus on adding possible missing prey items and, importantly, combining FA analysis with other biochemical and biologging techniques. The combination of this method with stable isotope analysis, for example, could help disentangle which factors are at play here and collection of such data could help elucidate the cause of the observed seasonal variation in the future. In addition to the evidence provided by biochemical tracers, we provide qualitative data in support of the possibility that blue sharks feed on gelatinous or planktonic prey. In their study of the morphology of oral papillae, Rangel *et al.* (2017) described the presence of “gill rakers” in the oropharyngeal cavity of embryonic blue sharks. Our observations confirm that these knob-like soft tissue structures along the gill arches persist at least throughout the early lifetime of the shark (Figure 4-8). They have been hypothesised to be used to improve prey retention by preventing small, perhaps planktonic, prey from slipping out of the gill slits (Compagno, 1984, Rangel *et al.*, 2017) and could be worth comparing to turtle oesophageal papillae or basking shark gill rakers (Matthews and Parker, 1950, Vogt *et al.*, 1998) but appear to be most similar to spiny dogfish gill rakers (Gudo and Homberger, 2002). Gelatinous prey such as jellyfish have been found to make up 30-40% of the stomach volume of the latter (Purcell and Arai, 2001). Additionally, blue sharks swim slowly and opportunistically take advantage of schooling prey such as anchovies (Tricas, 1979) and mating squid (Compagno, 1984). This is further supported by the observation that blue sharks swim at speeds comparable to that of small prey, significantly slower than would be optimal to minimise cost of transport speed (Carey *et al.*, 1990, Papastamatiou *et al.*, 2018). It is possible, therefore, that individuals in Ireland would also employ this strategy when encountering jellyfish blooms in the summer (Kennerley *et al.*, 2021). However, vertebrate energetics involve many competing processes, and movement decisions such as swimming speed can be made to optimise not just foraging success but also reproductive success (Hedenström and Alerstam, 1996), for example, and these decisions may be difficult to isolate without further evidence. While no direct feeding events were recorded, our biologging efforts (Figure 4-7) do suggest that blue sharks display inquisitive behaviour towards jellyfish which may warrant further investigation. By combining our results with other collected observations and anecdotal evidence, we propose that blue sharks, as known opportunistic feeders (Dorman, 1987, McCord and Campana, 2003, Mendonça, 2009, Hernández-Aguilar *et al.*, 2016), may take advantage of the presence of highly abundant gelatinous or planktonic prey in Irish waters in the summer, peaking between July and September (Bastian *et al.*, 2011, Lynam *et al.*, 2011). Recent studies have shown that large marine predators traditionally believed to be exclusively carnivorous may have more diverse diets than originally thought (Leigh *et al.*, 2018, Meekan *et al.*, 2022). Similarly, our results suggest that pelagic sharks thought to feed at mid-to-high trophic levels may, in reality, also be feeding on lower trophic levels or on prey traditionally, yet erroneously, thought of as low-value "trophic dead ends" (Hays *et al.*, 2018). Indeed, while jellyfish are a generally low energy density prey, several large-bodied species, such as the leatherback turtle and the oceanic sunfish, and pelagic predators, such as bluefin tuna and swordfish, have been found to feed in significant amounts on a nutritionally challenging diet of jellyfish (Doyle *et al.*, 2007, Cardona *et al.*, 2012). Additionally, different body components of jellyfish present differences in energy density where gonads and oral arm tissue are generally more nutritious than bell tissue (Doyle *et al.*, 2007). This may present a further hypothesis as to the potential inquisitive behaviour observed on the biologging footage whereby blue sharks could selectively opt to feed on specific jellyfish such as those with developed gonads, or those containing a high number of parasitic amphipods. Indeed, euphausiid and hyperiid amphipods are often found infesting the bells of jellyfish and feeding on their gonads (Doyle *et al.*, 2007). These parasitic crustaceans are known to be comparatively high in energy content (Percy and Fife, 1981) and may present an opportunity for blue sharks to optimise their energy intake from an otherwise low energy density prey. Further studies incorporating additional biochemical tracers such as stable isotope analysis (bulk and compound-specific) or DNA barcoding of gut contents along with additional biologging efforts would be beneficial towards investigating and confirming either of these proposed hypotheses.

While blue sharks are already well-known opportunistic hunters feeding on a variety of prey, the addition of this work to our existing knowledge of blue shark ecology may have implications in our understanding of the pelagic food web and of the role and impact of both blue sharks and jellyfish in their ecosystems. With growing evidence that jellyfish may be making larger nutritional contributions to the diet of marine predators than assumed (Hays *et al.*, 2018), it is possible that changing ocean conditions may encourage opportunistic feeders to take advantage of this increasingly available prey (Licandro *et al.*, 2011, Lynam *et al.*, 2011, Kennerley *et al.*, 2021), particularly when other food sources become scarcer (Essington *et al.*, 2015, Buren *et al.*, 2019, ICES, 2020). Increased reliance on jellyfish as a food source could also lead to increased vulnerability to the threat of plastic pollution (Hays *et al.*, 2016, Schuyler *et al.*, 2016, Macali and Bergami, 2020) by an already vulnerable species (Rigby, 2019, Cappell *et al.*, 2022) with evidence already existing found that blue sharks ingest marine litter, particularly “transparent sheet-like items” (Bernardini *et al.*, 2018).

Chapter 5 Rapid transboundary movements of endangered porbeagle sharks support need for continued cooperative research and management approaches.

Authors Jenny R Bortoluzzi, Andrew L Jackson, Grace E McNicholas, Adam Barnett, Austin J Gallagher, Neil Hammerschlag, William K Roche, Nicholas L Payne

Author Contributions: NLP, GEM and I conceived the study with input from ALJ, AB, AJG, NH and WKR. I carried out the fieldwork, led the data analysis and results interpretation with NLP and GEM. I wrote the manuscript with strong input from GEM and NLP. All authors made contributions to the manuscript.

Status: This manuscript is being prepared for submission to ICES Journal of Marine Science.

5.1 Abstract

Distribution of species across regulatory and physical boundaries poses a recognised challenge to effective management and research, and these transboundary species tend to suffer more-severe population declines. Porbeagle sharks, *Lamna nasus*, are critically endangered in North-eastern Atlantic waters where Exclusive Economic Zones change over small spatial scales. We used high-resolution satellite tags to demonstrate that individual porbeagle sharks in the region display high inter-individual variation in behaviour by undertaking very rapid movements (up to 100 km d^{-1}) through multiple Exclusive Economic Zones (EEZs) and physical habitats, transiting different management zones in a matter of days along different paths. These data highlight the urgent need for regional collaboration and cross-border funding mechanisms to maximise research impact to fill knowledge gaps and improve conservation outcomes for endangered and highly mobile shark species.

5.2 Introduction

Fisheries management tends to rely on the spatial delineation of resources, with territorial boundaries generally reflecting geopolitical criteria. Many commercially important marine species have distributions which cross at least two management boundaries (i.e. Exclusive Economic Zones - EEZs; Palacios-Abrantes et al. 2020), adding complexity to the management of their fisheries. Challenges to this are increasingly compounded by climate change, which can lead to range shifts resulting in increased vulnerability to overharvest (Hammerschlag *et al.*, 2022, Rodriguez-Burgos *et al.*, 2022), necessitating changes in quota allocations and to membership in the management organisations, and shifts from static to dynamic spatial closures (Koubrak and VanderZwaag, 2020, Palacios-Abrantes *et al.*, 2020). These conservation challenges are best exemplified among highly mobile species whereby individuals transit multiple jurisdictional boundaries (Rooker *et al.*, 2019, Ketchum *et al.*, 2020); and the knowledge of these movement patterns and their linkages to important life-history events (e.g., reproduction, parturition) remain key to informed management decisions yet are often incomplete (Wearmouth and Sims, 2008, Yokoi *et al.*, 2017, Daly *et al.*, 2018, Jacoby *et al.*, 2022). Issues like these may, at least partially, explain why species with transboundary distributions seem to experience greater population declines than other species (Palacios-Abrantes *et al.*, 2020), and why wide-ranging sharks and rays have a greater extinction risk (Dulvy *et al.*, 2017). The porbeagle shark, *Lamna nasus,* is globally considered *Vulnerable* by the International Union for Conservation of Nature (IUCN) Red List (Rigby et al. 2019) and was assessed as *Critically Endangered* in 2015 in European waters (Ellis *et al.*, 2015). Their population is now increasing, having previously been estimated to have declined by 50-79% over ~ 60 years in the North East (NE) Atlantic (Rigby *et al.*, 2019, ICES, 2022). Porbeagles are currently split into the following units for assessment and management: (1) the North Atlantic (split into two stocks: the Northwest and Northeast Atlantic), and (2) the Southern Hemisphere (Kitamura and Matsunaga, 2010, Testerman, 2014, Curtis *et al.*, 2016, González *et al.*, 2021). Some data are available on the movements of individual porbeagles in the NE Atlantic, but this information tends to rely on broad scale reconstructions of satellite tracks or low-resolution spatial inference based on conventional mark-recapture tags (Kohler *et al.*, 2002, Pade *et al.*, 2009, Saunders *et al.*, 2010, Bendall *et al.*, 2013, Biais *et al.*, 2017, Cameron *et al.*, 2019, ICCAT-ICES, 2022) that are difficult to relate to precise locations of different EEZs and habitat types. Transjurisdictional movement also should promote international collaboration and data pooling, particularly when extended research timelines are taken into account (Bendall, et al. 2012, ICES-WGEF 2021, ICCAT-ICES 2022). By collecting data on vertical and horizontal movements and environmental conditions, existing knowledge gaps for this species can be addressed, such as (i) the location of critical habitats (Fowler *et al.*, 2004) (ii) their reproductive cycle and fecundity in the North Atlantic (Francis *et al.*, 2008), (iii) general life history knowledge globally (ICCAT Shark Species Group, 2020), (iv) stock structure, including mixing between North West (NW), NE Atlantic and the Mediterranean (ICCAT Shark Species Group, 2020, ICCAT-ICES, 2022), and (v) temporal and spatial distribution of porbeagle sharks in relation to environmental and ecosystem features (ICCAT Shark Species Group, 2020). Thus, allowing for the continued and sustained recovery of the population and its monitoring. Here, we report high-resolution (< 1500m error) tracks of two adult porbeagle sharks tagged off Ireland in 2022, which highlight rapid, transboundary movement patterns in the NE Atlantic, underscoring the need for urgent cross-border, regional and international collaborations to research and ensure continued effective management of this critically endangered population.

5.3 Materials and Methods

The following work was conducted under The Health Products Regulatory Authority (HPRA) Project Authorisation (AE19136/P127). Two female porbeagle sharks ((1) ID229998 244 cm total length; and (2) tag ID222241 280 cm total length) were captured using baited rod and line in collaboration with recreational anglers off the coast of Malin Head, County Donegal, Ireland on the 15th of April 2022. Both individuals were brought onboard, and a seawater hose was placed in the sharks' mouths to ventilate their gills. A wet towel was placed over their eyes to reduce stress. Both sharks were then measured over the curve of the body for total length (TL), fork length (FL), girth (G) and blood sampled. Both were tagged with a Smart Position and Temperature Transmitting tag (SPOT 258**,** Wildlife Computers, Inc., Redmond, WA, USA) and Pop-up Satellite Archival Transmitting tag (miniPAT 348, Wildlife Computers, Inc., Redmond, WA, USA). SPOT tags were attached near the tip of the dorsal fin using nylon bolts, and miniPATs were anchored adjacent to the insertion point of the dorsal fin, using titanium anchor darts with six-inch monofilament tethers.

Table 5-1. Summary data of the two L. nasus double tagged with miniPAT and SPOT tags off north-west Ireland.

Shark	SPOT ID	Tagging date	Sex	FL (cm)	TL (cm)	G (cm)	Tagging location	miniPAT pop-up date	Pop-up latitude	Pop-up longitude
1	21U3296 229998	15-Apr-22	F	210	244	113	Donegal Ireland	13-Oct-22	73.1574	20.1411
2	21U0534 222241	15-Apr-22	F	249	280	166	Donegal Ireland	13-Oct-22	53.3297	0.6332

SPOT tags transmit messages when the free-swimming shark's fin and tag break the surface of the water. Transmitted messages that are received by orbiting ARGOS satellites, when overhead, are used to estimate shark locations based on doppler shift calculations. Each transmission is assigned a quality rating (3, 2, 1, 0, A, B and Z, from best to worst) defining the precision of the location from a radius of a few kilometres down to 250m. We confined our analysis to quality 3-1 locations, representing a <1500m error radius. Tracks were determined by interpolating the minimum straight-line distance between each location. Minimum horizontal swimming speed was calculated as the distance between two successive positions divided by time elapsed. SPOT tags continued to transmit for (1) 266 days until 5th January 2023 and (2) 296 days until 4th February 2023 (Shark 2 is still transmitting daily at this point).

MiniPAT tags remained on the animals until programmed release 6 months (180 days) after tagging. During deployment, the tags measure ambient light (irradiance at 550 nm), depth (0.5 m resolution, ±0.005 m accuracy) and temperature (0.05°C resolution, ±0.1° C accuracy), which are relayed after the detachment from the animal as a 10-minute summary time series. Resulting data were aggregated into depth and temperature bins for each 4-day period to investigate the depth and temperature space exploited by each shark. The analysis was carried out in R studio and hexagonal heatmap of 2D bin counts plots were produced using the package “ggplot2” (Wickham, 2016). The most probable track was calculated with the GPE3 software (Wildlife Computers, Inc., Redmond, WA, USA; Pedersen et al. 2011) which was informed by the cruising speed that provided the best match between the miniPAT and SPOT data for sharks 1 and 2 (1.5 m/s).

5.4 Results

Full tracks derived from the filtered SPOT tag positions of both sharks are presented in Figure 5-1. Over their time at liberty, shark 1 transmitted an average of 9.06 SPOT tag locations per day and shark 2, an average of 3.53 locations per day. Two weeks after tagging, shark 1 (Table 5-1) commenced a complete circumnavigation of Ireland, swimming at least 1500 km in approximately 85 days total. While at liberty, the shark passed through UK and Irish EEZs several times, before continuing northward to come within 40 km of the Danish-Faroese EEZ, and then entering the Norwegian EEZ on August 5th, 2022, where the tag continued to transmit (Figure 5-1). During its circumnavigation of Ireland, the individual travelled parallel to both EEZs in the Irish Sea, frequently crossing borders (min. five times), before residing in a discrete area of the Celtic Sea for two months (Figure 5-1). The latter part of the shark’s journey was rapid, travelling around 1500 km in 18 days at an average speed of at least 85 km d^{-1} (but included periods of ~100 km d^{-1}) and crossing into the Arctic circle on August 12th, 2022. This shark then resided around the Lofoten Archipelago for the next month, before continuing along a north-easterly trajectory, reaching a maximum latitude of 76°N at the end of October, placing the shark within the Svalbard Fisheries Protection Zone (SFPZ). It then began a return southward journey along a similar trajectory and speed (85 km d^{-1}) in early November and entered international waters on November 17th, 2022; Danish-Faroese waters on November 21st; UK waters between the 27th and the 30th November; Irish waters on December 3rd; international waters south of Ireland on December 8th; and continued travelling south in international waters passing between the Azores and Madeira between the 25th and 30th December 2022. On January 5th, 2023, its latest position placed it in the high seas west of the Canary Islands, representing a journey of over 5500 km from the Arctic to West Africa, in two months. In contrast, shark 2 remained within 60km of the tagging site for two months, crossing between Irish and UK EEZs on 4 occasions. In mid-June, this second shark initiated a northward trajectory, travelling 500 km between the Inner and Outer Hebrides, reaching the Orkney Islands in 9 days (~55 km per day) and subsequently travelling down the eastern coast of Britain (Figure 5-1). Here, the shark resided for approximately three months (mid-July – mid-October), before continuing on a northern trajectory, reaching the Moray Firth in mid-November; an average transit speed of 20-30 km d-1. From mid-November 2022 to mid-January 2023, shark 2 remained in Northern Scotland, briefly leaving the Moray Firth on December 3rd, 2022, travelling through the Pentland Firth to the coastal waters north of northern Sutherland, east of Cape Wrath, and returning to the Moray Firth on December 30th.

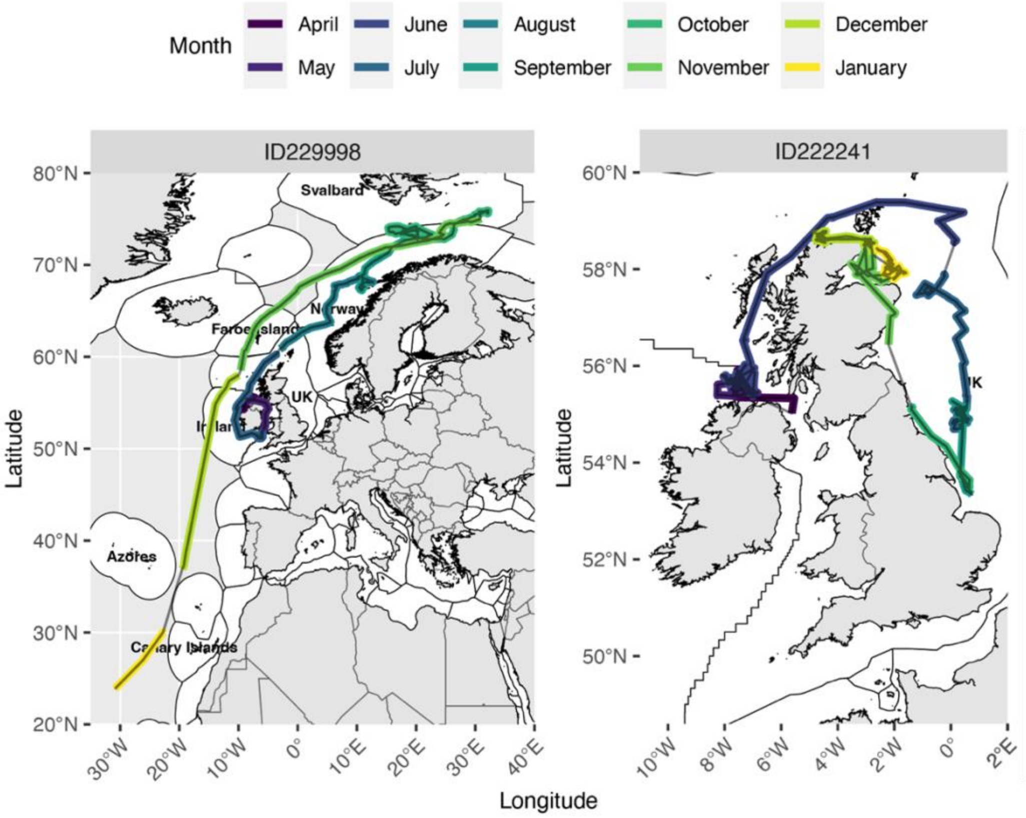


Figure 5-1. Individual tracks for two porbeagle sharks, Shark 1 (left) and Shark 2 (right), tagged off Ireland in April 2022 and tracked until January 2023 using SPOT tag locations. (EEZ: Exclusive Economic Zone, Flanders, 2019).

A comparison of SPOT vs miniPAT tracks (Figure 5-2) reveals differences in track locations sometimes reaching over 200 km (ID229998). SPOT tag tracks of ID229998 showed the shark travelling around Scotland through the inner channel on the east side of the Outer Hebrides whereas miniPAT tracks place it over 100 km west, offshore.

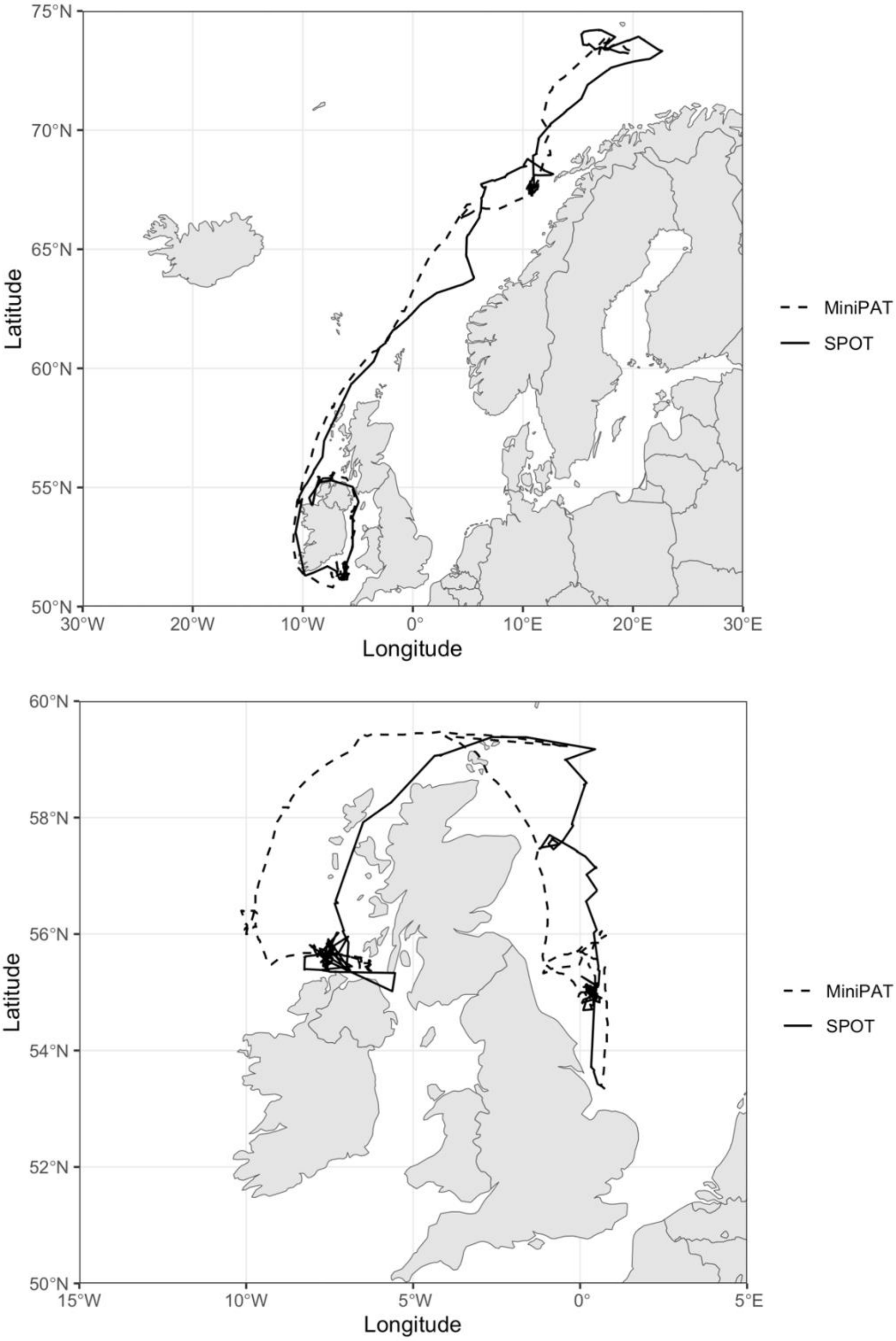


Figure 5-2. Comparison of tracks obtained from miniPAT light-derived geolocations (dashed line) and SPOT GPS and ARGOS satellite locations (solid line) for Shark 1 (top panel; ID 229998) and Shark 2 (bottom panel; ID22241)

The miniPAT data showed both sharks to be generally surface oriented, spending a majority of their time between 0 and 100m depth (Figure 5-3). However, as shark 1 moved northward, it regularly occupied progressively deeper water, and frequently exceeded 400m depth in the northern extent of the Norwegian EEZ and the southern extent of the SFPZ. During the first half of its migration, this individual experienced water temperatures ranging from approximately 10° to 18°C and shifted to cooler temperatures (spending significant time in temperatures below 5°C) in the second half. In contrast, shark 2 (Figure 5-3) remained in shallower waters (< 200m) throughout the deployment period, and, in the latter half (July to October), experienced higher - and a broader range - of temperatures of 7.5° to 20.5°C corresponding to its residency off the eastern coast of Britain.

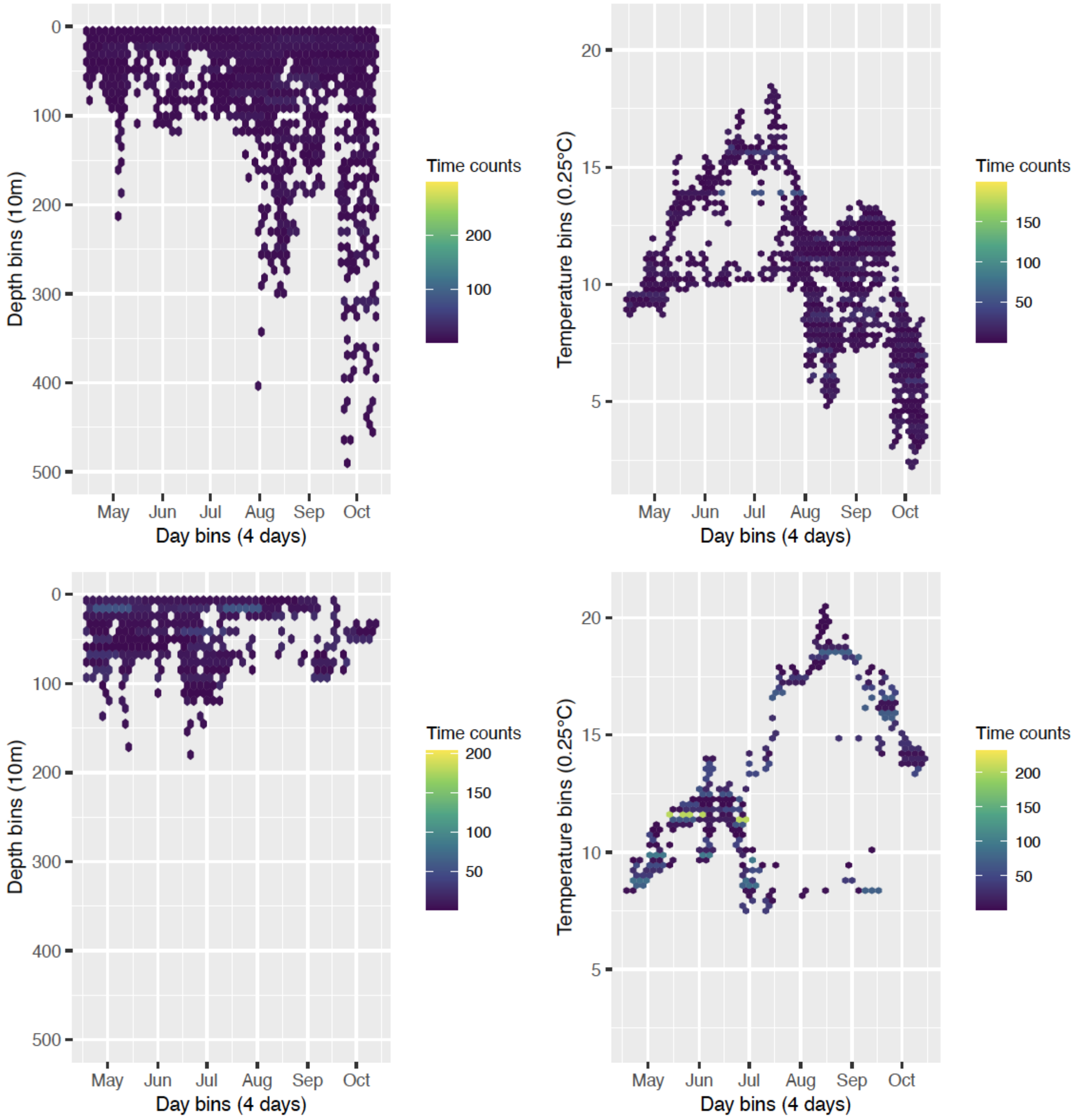


Figure 5-3.Hexagonal heatmap showing the frequency of occurrences in depth and temperature bins for two porbeagle sharks, 1 (top) and 2 (bottom). A bin is defined by time counts within 10m over a 4-day period (left) and 0.25°C within a 4-day period (right).

5.5 Discussion

The tracks from two individuals illustrate that porbeagle in the NE Atlantic show high inter-individual variation in their horizontal and vertical space use. Some individuals seem to undertake rapid, extensive movements across multiple jurisdictional and environmental boundaries in the NE Atlantic over short periods of time. This is in line with previous research which has shown wide-ranging movements and highlighted differences between populations. Southern Hemisphere porbeagles appear to move predominantly latitudinally (Francis *et al.*, 2015) and may travel less extensively than their Atlantic counterparts that travel large distances between summer and winter months (Campana *et al.*, 2015, Biais *et al.*, 2017, Skomal *et al.*, 2021) though data for the Southern hemisphere is insufficient to generalise these conclusions. Within the known North Atlantic distribution of this species, the region encompasses multiple EEZs and Areas Beyond National Jurisdiction (ABNJs) in a relatively small area, which may pose conservation challenges to this species, even among a discrete spatial area - a situation comparable to the Mediterranean region where porbeagle sharks are also assessed as Critically Endangered (Ellis *et al.*, 2016).

However, research into porbeagle movements has largely relied on low resolution tag derived location estimates from PSATs (Pade *et al.*, 2009, Saunders *et al.*, 2010, Campana *et al.*, 2015, Francis *et al.*, 2015, Biais *et al.*, 2017, Skomal *et al.*, 2021). Our study utilises a combination of SPOT and miniPAT tags, providing movement and habitat-use data at a much higher resolution; SPOT tags typically provide location error on the order of <1.5km including above 70 °N and below 70 ° S, whereas light-derived geolocation from PSATs are associated with errors often exceeding 100km and can be even more imprecise north of 70 °N or south of 70 °S (Braun *et al.*, 2018, Wilflife Computers 2022, pers. comm., 8 November). Comparison of the tracks obtained from both tags revealed differences in locations sometimes exceeding 200 km (Figure 5-2). These differences could have consequences for habitat use inferences and could be the difference between management zones. Thanks to the combination of both tags, the data presented here is therefore much more extensive, allowing for individual crossings of jurisdictional boundaries (and their speed) and presence in specific habitats and physical environments to be identified with high accuracy and confidence.

Using these high-resolution data, we contribute to the existing porbeagle tracking dataset in the NE Atlantic (Pade *et al.*, 2009, Saunders *et al.*, 2010, Biais *et al.*, 2017). Although data from these studies range across sexes, ages and tagging periods, sample sizes are low, making it difficult to infer movement trends between classes. Furthermore, by relying on lower resolution PSAT data, identifying specific habitat features of importance has been difficult. Our SPOT/miniPAT data are therefore particularly useful given the ability to identify the precise locations porbeagle sharks used for prolonged periods as well as their variation in use of physical habitat driven by temperature and depth.

These data allow us to build on previous work, generating more-precise confirmation that individual porbeagle sharks transit through multiple EEZs and management zones (e.g. RFMOs) over short periods. The management challenges presented by transboundary marine species are increasingly recognised (Hooker *et al.*, 2011, Campana, 2016, Daly *et al.*, 2018, Rooker *et al.*, 2019, Palacios-Abrantes *et al.*, 2020), and these data allow us to build on previous work, generating more-precise confirmation that individual porbeagle transit through multiple EEZs and management zones (e.g. ICES, ICCAT, OSPAR) over short periods. The population status of NE Atlantic porbeagle, including its moderate intrinsic rate of increase, as well as their low rates of biological productivity (Campana *et al.*, 2015), coupled with data on transboundary movements, highlight the need for continued robust cooperative management and research strategies; particularly considering the apparent likelihood that the population constitutes predominantly mature (possibly pregnant) females (Biais *et al.*, 2017, Cameron *et al.*, 2018). While the EU, Norway, Sweden, and Iceland have all prohibited targeted catches of this species in their waters, and international waters in the case of the EU up until now (Council Regulation 57/2011, ICES, 2021), a new quota has been recommended by ICES in 2023 and 2024 (219 and 231 tonnes respectively; ICES, 2022), and bycatch of porbeagles remains a significant issue (Silva and Ellis, 2019, Gonzalez Triginer, 2020, ICES, 2021). ICCAT, through its Contracting Parties network (CPCs), including the EU and Norway, is tasked with the conservation management of tuna, tuna-like species, and bycatch species, including sharks, in the Atlantic and adjacent seas. Regional fisheries management organisations such as ICCAT generally have jurisdiction for management in high seas but are sometimes regarded as ineffective and have so far failed to halt shark fishing mortality and population decline (Campana, 2016, Juan-Jordá *et al.*, 2022, Pacoureau *et al.*, 2023). Porbeagle-specific recommendations were introduced by ICCAT in 2016, which followed the species being identified as a ‘species of concern’ in 2007 (ICCAT, 2016).

The population status of NE Atlantic porbeagle sharks, including its moderate intrinsic rate of increase, as well as their low rates of biological productivity (Campana *et al.*, 2015), coupled with data on transboundary movements, highlight the need for continued robust cooperative management and research strategies; particularly considering the apparent likelihood that the population constitutes predominantly mature (possibly pregnant) females (e.g. findings from the current study, Biais *et al.*, 2017, Cameron *et al.*, 2018). These data support recent IUCN recommendations that the species be subject to regional and national management measures (e.g. catch limits and bycatch minimisation); and include a follow-through on existing and future international treaties (Rigby *et al.*, 2019). While many existing measures are already in place, leading to the stock being defined as overfished but no overfishing taking place (ICCAT, 2016, ICCAT-ICES, 2022), the robustness of measures could be improved (particularly on the high seas) by a continued effort to fill in knowledge gaps in porbeagle ecology, biology, life-history and physiology. Habitat and movement knowledge is particularly key to the design of area-based management practices such as MPAs (Hyde *et al.*, 2022, Moore and Fowler, 2022).

The movement patterns observed, including the full north to south span of the known range of the Northeast Atlantic population (Rigby *et al.*, 2019). Porbeagles therefore not only transit between EEZs but also beyond international management zones responsible for the management and conservation of this population such as ICES and OSPAR (southern boundaries at 36°N). Our data show a clear preference by these individuals for shallower waters, spending a majority of their time in depths from 0-100m, making them more vulnerable to encountering fishing gear (Cortés *et al.*, 2010, Dulvy *et al.*, 2014). This can be of particular significance in areas of high shipping traffic and fishing effort such as the North Sea, as well as the Iberian, French and Celtic parts of the continental shelf where large pelagic sharks are particularly prone to bycatch (Schroeder and Dannheim, 2006, Queiroz *et al.*, 2019). Despite spending these prolonged periods of time in surface waters, both individuals also experienced regular and rapid changes in physical habitat, characterised by depth and temperature, crossing temperature boundaries as low as 2.5°C and up to 20.5°C. These movements through different physical landscapes not only raises concerns related to their exposure to current anthropogenic threats and activities (Cortés *et al.*, 2010, Queiroz *et al.*, 2016, Andrzejaczek *et al.*, 2022), but also to the effects of changing ocean conditions and habitat degradation on their movements (Lowerre-Barbieri *et al.*, 2019, Vedor *et al.*, 2021). This emphasises the need for governance at appropriate spatial scale and of critical habitats informed by new and continuing research into migration patterns and spatial distribution of the life cycle of species (Lowerre-Barbieri *et al.*, 2019).

Anecdotal evidence suggests, in addition to being an important summer aggregation site for juvenile porbeagles (Cameron *et al.*, 2019), Ireland could be an important reproductive area for the species (Clarke *et al.*, 2016), and therefore worthy of research prioritisation. Results from our study contribute additional clues as to locations worthy of further investigation to identify critical habitats for the species including to the north of the Island of Ireland; the Celtic Deep in the Irish sea (matching tracks from Pade, 2009); the North and East coast of Britain; and the boundary area between the Norwegian and Barents Seas where our individuals spent extensive periods of time. But expanded, long-term multi-year studies are needed to confirm potential inter-annual site fidelity as has been identified in locations such as the Bay of Biscay and the southern Celtic Sea (Biais *et al.*, 2017). To continue addressing uncertainties which remain in our knowledge of porbeagle ecology and biology, ICCAT, ICES and the IUCN have all recommended additional research be carried out on the temporal and spatial distribution, population structure, life-history (e.g. mating, pupping and nursery grounds), and how the species' contributes to livelihoods (Fowler *et al.*, 2004, Rigby *et al.*, 2019, ICCAT Shark Species Group, 2020). In particular, ICCAT CPCs have been encouraged to implement research and monitoring projects at regional level in the Convention area (ICCAT, 2015). However, with the recent departure of the UK from the EU and its collaborative grant schemes, there exists a clear need for new cross-border collaborative mechanisms that will facilitate these multi-jurisdictional research programmes on transboundary species such as porbeagle sharks. This is especially urgent for the island of Ireland given ongoing marine spatial planning discussions there, and the realisation that Marine Protected Areas accrue greatest conservation returns when they overlap with important life history events (Conners *et al.*, 2022) and suitable habitats driven by environmental predictors such as Sea Surface Temperature (Birkmanis *et al.*, 2020). Accordingly, expanded research will reveal the relative importance of Irish waters for porbeagle conservation within the broader region.

Chapter 6 General Discussion

6.1 An ocean under crisis

At the root of the crisis facing our oceans today is severe overexploitation and depletion of resources through overfishing, bycatch and habitat degradation (Duarte *et al.*, 2020). It is currently estimated that over a third of fish populations are currently fished unsustainably, and 60% are fully fished with consequences not just for fish populations, economies and global food security, but for the health of entire ecosystems (Link and Watson, 2019). Moreover, climate change is already accelerating the loss of marine biodiversity (McCauley *et al.*, 2015, Worm and Lotze, 2021), and pollution from a variety of sources is entering the system and accumulating in the water column, sediments and biota, once again with severe impacts on ecosystems and human health (Copat *et al.*, 2013, Naji *et al.*, 2016, Beiras, 2018, Beaumont *et al.*, 2019, Thushari and Senevirathna, 2020).

For chondrichthyans (hereafter referred to as “sharks”), overfishing, often compounded with the aforementioned issues, has led to the decline of over a third of all species to the point of being threatened with extinction and, as a result, the group is now among the most threatened class of vertebrates in the world (Dulvy *et al.*, 2021). Two-thirds of coral-reef associated sharks are currently threatened with extinction (Sherman *et al.*, 2023b); requiem sharks (family Carcharhinidae) make up more than half of the annual reported global shark catches (Sherman *et al.*, 2023a); wide-ranging cosmopolitan species are found to dominate the shark fin trade (Cardeñosa *et al.*, 2022); and since the 1970s, oceanic shark populations have declined by 71% (Pacoureau *et al.*, 2021). By nature, these oceanic migratory species, such as blue sharks and porbeagle sharks discussed in Chapters 4 and 5, are at higher risk of encountering fishers and many of the movements they undertake during their lives lead them to cross jurisdictional boundaries into different Exclusive Economic Zones (EEZs) and Areas Beyond National Jurisdiction (ABNJ) (Campana, 2016). As found in Chapter 5, porbeagle sharks travel great distances in the space of just a few months from areas where they benefit from some protections (e.g. EU waters) to others where they benefit from very little (e.g. the high seas). This mismatch in species distribution and regulatory boundaries presents a significant challenge for management and conservation measures (Palacios-Abrantes *et al.*, 2020) as well as for research efforts (Chapter 5). Additionally, movements are driven by biotic and abiotic factors such as sea surface temperatures, reproduction or food availability, which are themselves not constrained by human-made boundaries but often result in an overlap between shark space use and fishing efforts, especially on the high seas (Queiroz *et al.*, 2016). In order to manage and mitigate the risks these animals face, knowledge of their life-history traits is essential (Chapter 3). In particular, information about habitats and locations of importance as well as the behaviours they display while in these locations (Chapter 4) is critical to adequately and effectively manage them.

6.2 Small but mighty: Ireland’s place in the Northeast Atlantic

“As an island nation with sovereign rights over one of the largest sea areas in Europe, Ireland’s economy, culture and society is inextricably linked to the sea.”

Project Ireland 2040, National Marine Planning Framework (DHLGH, 2021)

The waters around Ireland are rich in biodiversity. The evidence of this is increasingly recognised both nationally and internationally. A prime example of this recognition came in January 2023 when the Greater Skellig (Sceilg Mhichíl) Coast was designated as a Hope Spot by the international marine conservation non-profit Mission Blue, led by Dr Sylvia Earle, which aims to "inspire action to explore and protect the ocean" (Mission Blue, 2023). The Wild Atlantic Way (WAW) developed in 2013 and launched in 2014 has seen huge success in the development of tourism, welcoming 3.7 million overseas visitors in 2017 alone with marine resources and activities playing a core role in this success (Fáilte Ireland, 2013, DHLGH, 2021). For example, for years, Dingle (Co. Kerry), on the WAW, was made famous around the world for its solitary dolphin, Fungi, who drew more than 150,000 people to the town annually (Rogan and Berrow, 1995) until his disappearance in 2020. While current figures do not exist, the whale watching industry is continuously developing and was valued at IR£1.25 million in the early 1990s (Rogan and Berrow, 1995). The industry owes its growing success to the presence of 25 species of cetaceans residing and migrating through Irish waters, including the largest vertebrate on earth, the blue whales; iconic species such as orcas and humpback whales; and lesser known species such as the deep-diving beaked whales (Rogan and Berrow, 1995, Rogan *et al.*, 2018, Fair Seas, 2022). But these trips are also often an opportunity for visitors to experience other wildlife encounters and interest in such tourism in Ireland is high, presenting currently untapped economic opportunities (Gray *et al.*, 2022). The Irish and Celtic Seas are hotspots for seabird populations and are home to around 60 species of both resident and visiting birds, including 38 species of seabirds (Rogan *et al.*, 2018, DHLGH, 2021, Fair Seas, 2022). As for sharks, the island's waters are host to over 70 species of which many are endangered or critically endangered including the porbeagle shark discussed in Chapter 5 and many more (Clarke *et al.*, 2016, Tully *et al.*, 2021).

This wider richness of marine biodiversity was present throughout the work carried out for this thesis. Charter anglers, whose livelihoods depend on these species and whose knowledge of them is invaluable, were collaborators without whom this work would not have been possible. Not a single fieldwork day went by without the sighting of several species of birds, mammals, fish and/or sharks, most often pointed out by our skippers.

Despite all this, only 2.1% of Irish seas currently benefit from any level of protection (Fair Seas, 2022) and basking sharks are the only marine fish that are protected under Section 23 of the Wildlife Act 1976 after a successful campaign in 2021 by the Irish Basking Shark Group and TD Jennifer Whitmore (IBSG, 2022). As Ireland is undergoing a National Marine Framework review (DHLGH, 2021), and with growing public awareness and support for marine issues (in a citizen survey, 92% of the public agreed that more action is needed to improve the health of the ocean; French and McDonough, 2020), now is a time of great opportunity for impactful marine science in Ireland to inform policy and conservation.

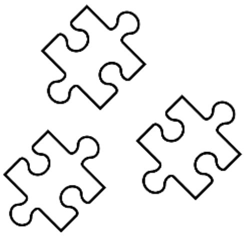
6.3 Science for effective ocean governance

With many of the issues discussed above requiring urgency in our actions, how effective and adequate these measures are depends entirely on the knowledge on which they will be based on. Fundamental knowledge of a species' biology and ecology as well as the ecosystem of which it is part, and humans' cultural and socio-economic reliance on it (Booth *et al.*, 2021), is critical to forming appropriate management and conservation decisions. Regional Fisheries Management Organisations (RFMOs), in charge of managing fisheries on the high seas, rely entirely on research to formulate their policies and lack of adequate data is often cited as a hindrance to effective management (Cronin *et al.*, 2023). In Chapter 5, I encourage an increase in international research collaboration and funding opportunities to fill data gaps in our knowledge of transboundary species, such as porbeagle sharks, to inform their ongoing management. This echoes calls such as that of Cronin *et al.* (2023) who suggest prioritising international and in-country funding to provide a fuller understanding of the status of oceanic shark populations, many of which are currently data poor, and, in turn, develop policy to mitigate their bycatch by tuna-related fisheries. Of course, the benefits of data sharing and collaboration among researchers and policy-makers is not unique to sharks and can be universally applied to species and ecosystems, particularly if data remain available and accessible in the long-term (Hays *et al.*, 2019).

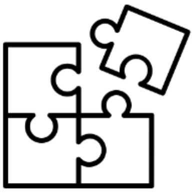
The goal of evidence-based policy is to avoid policy failures resulting from a mismatch between the expectations laid out by decision makers and the actual conditions in the target environment (Jansson, 2016). This mismatch could for example contribute to "paper parks”, when designated protected areas, such as MPAs or Marine Reserves (MR), are ineffective due to poor design, compliance and/or enforcement, for example (Pieraccini *et al.*, 2017). Static or dynamic spatiotemporal management measures such as MPAs or MRs are approaches that can be used to mitigate bycatch or overfishing, by focusing on avoidance of capture or exploitation (Lea *et al.*, 2016, Ortuño Crespo *et al.*, 2020, Cronin *et al.*, 2023). However, their efficacy relies on biological and ecological knowledge of space-use by these species (Lea *et al.*, 2016), knowledge of the ecosystem, and, importantly, must integrate the human dimension (social, economic, cultural and institutional factors) of the issue from conception to enforcement (Charles and Wilson, 2009, Pieraccini *et al.*, 2017). Biologically and ecologically relevant data to inform management, especially Marine Spatial Planning (MSP), can be obtained from tracking studies, such as that explored in Chapter 5. These studies can provide information on fine-scale space use of species in dynamic environments (Lea *et al.*, 2016); how species overlap with threats such as fisheries hotspots (Queiroz *et al.*, 2016, Jacoby *et al.*, 2020, Womersley *et al.*, 2022); locations of aggregations (Crossin *et al.*, 2017, Adams *et al.*, 2021); how species move in, around and beyond existing protected areas and, thus, how these areas can be adjusted, expanded or connected (Lea *et al.*, 2016, Martín *et al.*, 2020, Klimley *et al.*, 2022). But, while tracking studies can provide valuable information for management, their use by policymakers is inconsistent (Pullin *et al.*, 2004, McGowan *et al.*, 2017, Hays *et al.*, 2019). Access to and communication of this and other types of data by policy-makers and stakeholders are often cited as one of the primary barriers to their use (McGowan *et al.*, 2017, Ogburn *et al.*, 2017, Hays *et al.*, 2019). With so much data being published worldwide annually, it can be difficult for stakeholders to keep track. Initiatives to collate and summarise biocentric information to render these data more accessible can be particularly useful to its effective dissemination and use in area-based protections, for example. Such initiatives were first led by the Important and Biodiversity Bird Areas (IBA) approach which identifies sites globally important for the conservation of bird populations (Donald *et al.*, 2019). This approach is entirely data-driven and has contributed to the design of protected areas adapted to the ecology of birds (Donald *et al.*, 2019). Its success has led to the development of many similar approaches such as Key Biodiversity Areas, Important Marine Mammal Areas and, more recently, Important Shark and Ray Areas (ISRAs) (Hyde *et al.*, 2022). Not only are area-based approaches such as ISRAs particularly useful for stakeholders to inform MSP decisions, they can also assist in identifying key knowledge gaps and can be used by scientists to inform research questions (Hyde *et al.*, 2022).

Identifying key gaps in our knowledge at the conception stage of research may seem an obvious step but its relevance to maximise conservation and management can be overestimated or poorly framed by scientists if stakeholders are not included in this step (McNie *et al.*, 2016). For this reason, greater dialogue between policy-makers, management organisations, other stakeholders and scientists at the inception of research questions can ensure ecological data are applied in decision-making processes (McNie *et al.*, 2016, Ogburn *et al.*, 2017, Hays *et al.*, 2019). This can also ensure that attention is paid to the human-dimension of ecological, conservation and management issues throughout the entire scientific process from conception to application (Pieraccini *et al.*, 2017). This inclusion of socio-economic and cultural considerations in science can only be effective when projects incorporate multiple disciplines within their teams.

6.4 From multidisciplinary to interdisciplinary and transdisciplinary research



Multidisciplinary



Interdisciplinary

Transdisciplinary

Throughout this thesis, I have demonstrated the value in approaching research questions using multiple tools. Chapter 1 highlighted the challenges that come with using one method such as stable isotope analysis which is potentially confounded by many factors. However, combining it and others with different biochemical analyses, such as fatty acids or reproductive hormones or different disciplines, such as biologging or telemetry, can help add new pieces to the puzzle (Chapter 4). This is of particular value when the puzzle being studied is in an environment as difficult and opaque as the open ocean and with life histories as dynamic as oceanic sharks. A multidisciplinary approach “draws on knowledge from different disciplines but stays within their boundaries” (Choi and Pak, 2006). In other words, each discipline in such a study may be revealing a new piece of the puzzle but the pieces are not assembled until the end. Often times, like in this thesis, ecological studies are purely biocentric. As discussed previously, this has great value in objectively informing policy and management. However, species, particularly large predators such as sharks, play a number of important roles, not just ecological, but also socio-economic and cultural in their ecosystems (Hammerschlag *et al.*, 2019). These different roles and, more importantly, how they interact with each other need to be understood and integrated into scientific and management processes. This move to integrate interactive disciplines into a coordinated and coherent whole is a step away from a multidisciplinary approach towards an interdisciplinary one (Choi and Pak, 2006). Interdisciplinary research takes the puzzle pieces and looks at how they will fit together from the beginning. Successful interdisciplinary research requires a collaborative approach to problems and questions where biological and ecological knowledge is integrated with social, economic and cultural knowledge (Lovelace *et* *al.*, 2005, MacDonald *et al.*, 2016, Turner *et al.*, 2017). This integration must happen from the beginning and for the collaborating disciplines to be equal in their partnership (Viseu, 2015). Transdisciplinarity takes this collaborative approach one step further by moving beyond academia and engages directly with the production of and use of knowledge by non-academic partners with societal impact at the centre of the research being carried out (Toomey *et al.*, 2015). Transdisciplinarity uses a holistic approach to provide different perspectives and inputs on problems (Choi and Pak, 2006), it adds colour to the puzzle. In Chapter 4, I took steps towards achieving this by letting fishers' knowledge guide my questions and predictions of blue shark behaviours. Much of multiple disciplinary approaches' success is dependent on a relationship rooted in trust between partners (e.g. scientists, stakeholders, policy-makers, managers etc.) and building this trust requires time and good communication (Turner *et al.*, 2017, Pannell *et al.*, 2019, Cvitanovic *et al.*, 2021). In addition to trust, inclusion and respect are critical to the outcome of these collaborations. And while interdisciplinary research is paramount to address the crisis faced by the ocean and sharks today, the scientific community still has a long way to go to address its lack of inclusion and respect. Female leadership is consistently cited as one of the underpinning principles of effective interdisciplinary research, yet women are still under-represented in senior positions in marine science and STEM as a whole (the "leaky pipeline" issue; Howe-Walsh and Turnbull, 2016, Blythe and Cvitanovic, 2020, Shellock *et al.*, 2022). Things are no different in shark science where a majority of women, particularly early career researchers, report experiencing instances of sexism and misogyny from sexual harassment to assault (Macdonald, 2020, St Clair, 2021, Shiffman *et al.*, 2022). Furthermore, while representation of white-presenting women in leadership may be increasing in recent years, the same cannot be said of marginalised races and ethnicities who experience barriers and exclusions at the intersection of their identities (Shiffman *et al.*, 2022). The field of shark conservation (and conservation in general) does not currently reflect the diversity of thought, perspectives, and experiences as the communities it impacts the most. Efforts by groups such as Minorities in Shark Sciences show that the interest by minority groups (including LGBTQIA+ people, gender minorities, people of colour, indigenous people and those from lower socio-economic backgrounds) in the field well and truly exists (Graham *et al.*, 2022). But the barriers to their inclusion are numerous and persistent and both individuals and institutions have their role to play in bringing these barriers down (Smith *et al.*, 2017, de Vos and Schwartz, 2022, Shiffman *et al.*, 2022). Just as in the systems we study, diversity in research, conservation and management presents invaluable benefits that are essential to addressing the issues we have discussed throughout this thesis (Smith *et al.*, 2017, de Vos and Schwartz, 2022). Diversity of thought, experiences and perspectives promotes diversity of solutions, creates safer spaces and builds the confidence of early career researchers, keeping them in the field for the long term. In the context of this work, future efforts on shark ecology and conservation in Ireland should involve interdisciplinary teams including social scientists, environmental economists, fishers and others, right from the conception of projects. If we want to understand how best to protect them, we must also understand how these animals fit within our culture and what role they play in the health and wellbeing of our society. Any early career researcher, such as myself, aiming to achieve this work will need the support of a team of supervisors and collaborators from a diverse background.

Diversity should simply be the norm, not the exception.

Throughout the course of this PhD, I have aimed to foster collaborations within the national and international scientific community as well as with local stakeholders. Capitalising on Ireland's position in the NEA and with Chapter 5 as a starting point, I have been a driving force behind a new international collaborative initiative to holistically fill in knowledge gaps in porbeagle ecology and management in the NEA. In the work carried out for Chapter 4, I initiated and coordinated a team of researchers from multiple disciplines, getting their input from conception to analysis of the study. It has been equal parts challenging and rewarding to exchange knowledge with all these partners and the many more I have interacted with along the way. My hope had been to integrate additional disciplines within this body of work, having planned social science and forensic ecology studies (and even starting some!) over the course of the last four and a half years. This is far easier achieved, though, if the world doesn't lockdown due to a pandemic in the middle of your PhD…

Bibliography

ADAMS, A. J., LEWIS, J. P., KROETZ, A. M. & GRUBBS, R. D. 2021. Bonefish (Albula vulpes) home range to spawning site linkages support a marine protected area designation. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 31**,** 1346-1353.

AHNELT, H., SAUBERER, M., RAMLER, D., KOCH, L. & POGOREUTZ, C. 2020. Negative allometric growth during ontogeny in the large pelagic filter-feeding basking shark. *Zoomorphology,* 139**,** 71-83.

ANDERSON, M., CLARKE, K. & GORLEY, R. 2008. PERMANOVA+ for Primer. Guide to Software and Statistical. *Methods,* 214.

ANDRZEJACZEK, S., LUCAS, T. C. D., GOODMAN, M. C., HUSSEY, N. E., ARMSTRONG, A. J., CARLISLE, A., COFFEY, D. M., GLEISS, A. C., HUVENEERS, C., JACOBY, D. M. P., MEEKAN, M. G., MOURIER, J., PEEL, L. R., ABRANTES, K., AFONSO, A. S., AJEMIAN, M. J., ANDERSON, B. N., ANDERSON, S. D., ARAUJO, G., ARMSTRONG, A. O., BACH, P., BARNETT, A., BENNETT, M. B., BEZERRA, N. A., BONFIL, R., BOUSTANY, A. M., BOWLBY, H. D., BRANCO, I., BRAUN, C. D., BROOKS, E. J., BROWN, J., BURKE, P. J., BUTCHER, P., CASTLETON, M., CHAPPLE, T. K., CHATEAU, O., CLARKE, M., COELHO, R., CORTES, E., COUTURIER, L. I. E., COWLEY, P. D., CROLL, D. A., CUEVAS, J. M., CURTIS, T. H., DAGORN, L., DALE, J. J., DALY, R., DEWAR, H., DOHERTY, P. D., DOMINGO, A., DOVE, A. D. M., DREW, M., DUDGEON, C. L., DUFFY, C. A. J., ELLIOTT, R. G., ELLIS, J. R., ERDMANN, M. V., FARRUGIA, T. J., FERREIRA, L. C., FERRETTI, F., FILMALTER, J. D., FINUCCI, B., FISCHER, C., FITZPATRICK, R., FORGET, F., FORSBERG, K., FRANCIS, M. P., FRANKS, B. R., GALLAGHER, A. J., GALVAN-MAGANA, F., GARCÍA, M. L., GASTON, T. F., GILLANDERS, B. M., GOLLOCK, M. J., GREEN, J. R., GREEN, S., GRIFFITHS, C. A., HAMMERSCHLAG, N., HASAN, A., HAWKES, L. A., HAZIN, F., HEARD, M., HEARN, A., HEDGES, K. J., HENDERSON, S. M., HOLDSWORTH, J., HOLLAND, K. N., HOWEY, L. A., HUETER, R. E., HUMPHRIES, N. E., HUTCHINSON, M., JAINE, F. R. A., JORGENSEN, S. J., KANIVE, P. E., LABAJA, J., LANA, F. O., LASSAUCE, H., LIPSCOMBE, R. S., LLEWELLYN, F., MACENA, B. C. L., et al. 2022. Diving into the vertical dimension of elasmobranch movement ecology. *Science Advances,* 8**,** eabo1754.

ARAÚJO, M. S., BOLNICK, D. I. & LAYMAN, C. A. 2011. The ecological causes of individual specialisation. *Ecology letters,* 14**,** 948-958.

ARAÚJO, M. S., BOLNICK, D. I., MACHADO, G., GIARETTA, A. A. & DOS REIS, S. F. 2007. Using δ13C stable isotopes to quantify individual-level diet variation. *Oecologia,* 152**,** 643-654.

ARNOLD, G. & DEWAR, H. 2001. Electronic Tags in Marine Fisheries Research: A 30-Year Perspective. *In:* SIBERT, J. R. & NIELSEN, J. L. (eds.) *Electronic Tagging and Tracking in Marine Fisheries: Proceedings of the Symposium on Tagging and Tracking Marine Fish with Electronic Devices, February 7–11, 2000, East-West Center, University of Hawaii.* Dordrecht: Springer Netherlands.

AWRUCH, C. A. 2013. Reproductive endocrinology in chondrichthyans: The present and the future. *General and Comparative Endocrinology,* 192**,** 60-70.

AWRUCH, C. A., JONES, S. M., ASOREY, M. G. & BARNETT, A. 2014. Non-lethal assessment of the reproductive status of broadnose sevengill sharks (Notorynchus cepedianus) to determine the significance of habitat use in coastal areas. *Conservation Physiology,* 2.

BAR-ON, Y. M., PHILLIPS, R. & MILO, R. 2018. The biomass distribution on Earth. *Proceedings of the National Academy of Sciences,* 115**,** 6506-6511.

BARNOSKY, A. D., MATZKE, N., TOMIYA, S., WOGAN, G. O. U., SWARTZ, B., QUENTAL, T. B., MARSHALL, C., MCGUIRE, J. L., LINDSEY, E. L., MAGUIRE, K. C., MERSEY, B. & FERRER, E. A. 2011. Has the Earth's sixth mass extinction already arrived? *Nature,* 471**,** 51-57.

BASTIAN, T., HABERLIN, D., PURCELL, J. E., HAYS, G. C., DAVENPORT, J., MCALLEN, R. & DOYLE, T. K. 2011. Large-scale sampling reveals the spatio-temporal distributions of the jellyfish Aurelia aurita and Cyanea capillata in the Irish Sea. *Marine Biology,* 158**,** 2639-2652.

BAUM, J. K., MYERS, R. A., KEHLER, D. G., WORM, B., HARLEY, S. J. & DOHERTY, P. A. 2003. Collapse and conservation of shark populations in the Northwest Atlantic. *science,* 299**,** 389-392.

BEARHOP, S., ADAMS, C. E., WALDRON, S., FULLER, R. A. & MACLEOD, H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of animal ecology,* 73**,** 1007-1012.

BEAUMONT, N. J., AANESEN, M., AUSTEN, M. C., BÖRGER, T., CLARK, J. R., COLE, M., HOOPER, T., LINDEQUE, P. K., PASCOE, C. & WYLES, K. J. 2019. Global ecological, social and economic impacts of marine plastic. *Marine pollution bulletin,* 142**,** 189-195.

BECKMANN, C. L., MITCHELL, J. G., SEURONT, L., STONE, D. A. J. & HUVENEERS, C. 2013. Experimental Evaluation of Fatty Acid Profiles as a Technique to Determine Dietary Composition in Benthic Elasmobranchs. *Physiological and Biochemical Zoology,* 86**,** 266-278.

BECKMANN, C. L., MITCHELL, J. G., STONE, D. A. J. & HUVENEERS, C. 2014. Inter-Tissue Differences in Fatty Acid Incorporation as a Result of Dietary Oil Manipulation in Port Jackson Sharks (Heterodontus portusjacksoni). *Lipids,* 49**,** 577-590.

BEIRAS, R. 2018. *Marine pollution: sources, fate and effects of pollutants in coastal ecosystems*, Elsevier.

BENDALL, V. J., ELLIS, J. R., HETHERINGTON, S. J., MCCULLY, S. R., RIGHTON, D. & SILVA, J. F. 2013. Preliminary observations on the biology and movements of porbeagle Lamna nasus around the British Isles. *Collect. Vol. Sci. Pap. ICCAT,* 69**,** 1702-1722.

BERNARDINI, I., GARIBALDI, F., CANESI, L., FOSSI, M. C. & BAINI, M. 2018. First data on plastic ingestion by blue sharks (Prionace glauca) from the Ligurian Sea (North-Western Mediterranean Sea). *Marine Pollution Bulletin,* 135**,** 303-310.

BIAIS, G., COUPEAU, Y., SÉRET, B., CALMETTES, B., LOPEZ, R., HETHERINGTON, S. & RIGHTON, D. 2017. Return migration patterns of porbeagle shark (Lamna nasus) in the Northeast Atlantic: implications for stock range and structure. *ICES Journal of Marine Science,* 74**,** 1268-1276.

BIRDLIFE INTERNATIONAL 2022. State of the World's Birds 2022: Insights and solutions for the biodiversity crisis. Cambridge, UK: Birdlife International.

BIRKMANIS, C. A., PARTRIDGE, J. C., SIMMONS, L. W., HEUPEL, M. R. & SEQUEIRA, A. M. 2020. Shark conservation hindered by lack of habitat protection. *Global Ecology and Conservation,* 21**,** e00862.

BLOCK, B. A. 2005. Physiological Ecology in the 21st Century: Advancements in Biologging Science1. *Integrative and Comparative Biology,* 45**,** 305-320.

BLYTHE, J. & CVITANOVIC, C. 2020. Five organizational features that enable successful interdisciplinary marine research. *Frontiers in Marine Science***,** 981.

BOECKLEN, W. J., YARNES, C. T., COOK, B. A. & JAMES, A. C. 2011. On the use of stable isotopes in trophic ecology. *Annual review of ecology, evolution, and systematics,* 42**,** 411-440.

BOLNICK, D. I., AMARASEKARE, P., ARAÚJO, M. S., BÜRGER, R., LEVINE, J. M., NOVAK, M., RUDOLF, V. H. W., SCHREIBER, S. J., URBAN, M. C. & VASSEUR, D. A. 2011. Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution,* 26**,** 183-192.

BOLNICK, D. I., YANG, L. H., FORDYCE, J. A., DAVIS, J. M. & SVANBÄCK, R. 2002. Measuring individual-level resource specialization. *Ecology,* 83**,** 2936-2941.

BOOTH, H., SQUIRES, D., YULIANTO, I., SIMEON, B., MUHSIN, ADRIANTO, L. & MILNER-GULLAND, E. J. 2021. Estimating economic losses to small-scale fishers from shark conservation: A hedonic price analysis. *Conservation Science and Practice,* 3**,** e494.

BORTOLUZZI, J., DARBY, J. & JACKSON, A. L. 2021. Technological advances in stable isotope analysis provide ever more insights into animal ecology. *Journal of Animal Ecology,* 90**,** 1394-1397.

BRAUN, C. D., GALUARDI, B. & THORROLD, S. R. 2018. HMMoce: An R package for improved geolocation of archival-tagged fishes using a hidden Markov method. *Methods in Ecology and Evolution,* 9**,** 1212-1220.

BROOK, M. 1990. The Manx Shearwater. . *Polar Record,* 26**,** 248-248.

BUREN, A. D., MURPHY, H. M., ADAMACK, A. T., DAVOREN, G. K., KOEN-ALONSO, M., MONTEVECCHI, W. A., MOWBRAY, F. K., PEPIN, P., REGULAR, P. M. & ROBERT, D. 2019. The collapse and continued low productivity of a keystone forage fish species. *Marine Ecology Progress Series,* 616**,** 155-170.

BURNS, J. M. & KOOYMAN, G. L. 2001. Habitat use by Weddell seals and emperor penguins foraging in the Ross Sea, Antarctica. *American Zoologist,* 41**,** 90-98.

BUSQUETS-VASS, G., NEWSOME, S. D., CALAMBOKIDIS, J., SERRA-VALENTE, G., JACOBSEN, J. K., AGUÍÑIGA-GARCÍA, S. & GENDRON, D. 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: Implications for assessing diet and movement patterns in mysticetes. *PloS one,* 12**,** e0177880.

CABANA, G. & RASMUSSEN, J. B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences,* 93**,** 10844-10847.

CALICH, H. & CAMPANA, S. 2015. Mating scars reveal mate size in immature female blue shark Prionace glauca. *Journal of Fish Biology,* 86**,** 1845-1851.

CAMERON, L. W., ROCHE, W., GREEN, P., HOUGHTON, J. D. & MENSINK, P. J. 2018. Transatlantic movement in porbeagle sharks, Lamna nasus. *Fisheries Research,* 207**,** 25-27.

CAMERON, L. W. J., ROCHE, W. K., HOUGHTON, J. D. R. & MENSINK, P. J. 2019. Population structure and spatial distribution of porbeagles (Lamna nasus) in Irish waters. *ICES Journal of Marine Science,* 76**,** 1581-1590.

CAMPANA, S., FOWLER, M., HOULIHAN, D., JOYCE, W., SHOWELL, M., SIMPSON, M., MIRI, C., EAGLES, M. & HOUSE, M. 2015. Recovery potential assessment for porbeagle (Lamna nasus) in Atlantic Canada. *DFO Can. Sci. Advis. Sec. Res. Doc,* 41**,** 45.

CAMPANA, S. E. 2016. Transboundary movements, unmonitored fishing mortality, and ineffective international fisheries management pose risks for pelagic sharks in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences,* 73**,** 1599-1607.

CAMPANA, S. E., DOREY, A., FOWLER, M., JOYCE, W., WANG, Z., WRIGHT, D. & YASHAYAEV, I. 2011. Migration pathways, behavioural thermoregulation and overwintering grounds of blue sharks in the Northwest Atlantic. *PloS one,* 6**,** e16854.

CAPPELL, R., HOSCH, G., HUNTINGTON, T., PITA, P., VILLASANTE, S., AINSWORTH, G., MALLORY, T., CHEN, H. & PERRY, L. 2022. *Blue Shark: Economic valuation of the global market for blue shark products and interdependent policy analysis for sustainable management and trade*.

CARDEÑOSA, D., SHEA, S. K., ZHANG, H., FISCHER, G. A., SIMPFENDORFER, C. A. & CHAPMAN, D. D. 2022. Two thirds of species in a global shark fin trade hub are threatened with extinction: Conservation potential of international trade regulations for coastal sharks. *Conservation Letters,* 15**,** e12910.

CARDONA, L., ÁLVAREZ DE QUEVEDO, I., BORRELL, A. & AGUILAR, A. 2012. Massive Consumption of Gelatinous Plankton by Mediterranean Apex Predators. *PLOS ONE,* 7**,** e31329.

CAREY, F. G., SCHAROLD, J. V. & KALMIJN, A. J. 1990. Movements of blue sharks (Prionace glauca) in depth and course. *Marine Biology,* 106**,** 329-342.

CARLISLE, A. B., ALLAN, E. A., KIM, S. L., MEYER, L., PORT, J., SCHERRER, S. & O'SULLIVAN, J. 2021. Integrating multiple chemical tracers to elucidate the diet and habitat of Cookiecutter Sharks. *Scientific Reports,* 11**,** 11809.

CEBALLOS, G., EHRLICH, P. R., BARNOSKY, A. D., GARCÍA, A., PRINGLE, R. M. & PALMER, T. M. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances,* 1**,** e1400253.

CHARLES, A. & WILSON, L. 2009. Human dimensions of marine protected areas. *ICES Journal of Marine Science,* 66**,** 6-15.

CHEREL, Y., HOBSON, K. A., BAILLEUL, F. & GROSCOLAS, R. 2005. Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. *Ecology,* 86**,** 2881-2888.

CHOI, B. C. & PAK, A. W. 2006. Multidisciplinarity, interdisciplinarity and transdisciplinarity in health research, services, education and policy: 1. Definitions, objectives, and evidence of effectiveness. *Clin Invest Med,* 29**,** 351-64.

CHOUVELON, T., SPITZ, J., CHEREL, Y., CAURANT, F., SIRMEL, R., MÈNDEZ-FERNANDEZ, P. & BUSTAMANTE, P. 2011. Inter-specific and ontogenic differences in ÃŽÂ´13C and ÃŽÂ´15N values and Hg and Cd concentrations in cephalopods. *Marine Ecology Progress Series,* 433**,** 107-120.

CITES 2022. Consideration of proposals for amendment of Appendices I and II. CoP19 Prop. 37.

CLARKE, M., FARRELL, E., ROCHE, W., MURRAY, T., FOSTER, S. & MARNELL, F. 2016. *Ireland Red List No. 11: Cartilaginous fish [sharks, skates, rays and chimaeras]*.

COMPAGNO, L. J. 1984. FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. *FAO fisheries synopsis,* 125**,** 251-655.

CONNERS, M. G., SISSON, N. B., AGAMBOUE, P. D., ATKINSON, P. W., BAYLIS, A. M. M., BENSON, S. R., BLOCK, B. A., BOGRAD, S. J., BORDINO, P., BOWEN, W. D., BRICKLE, P., BRUNO, I. M., GONZÁLEZ CARMAN, V., CHAMPAGNE, C. D., CROCKER, D. E., COSTA, D. P., DAWSON, T. M., DEGUCHI, T., DEWAR, H., DOHERTY, P. D., EGUCHI, T., FORMIA, A., GODLEY, B. J., GRAHAM, R. T., GREDZENS, C., HART, K. M., HAWKES, L. A., HENDERSON, S., HENRY, R. W., HÜCKSTÄDT, L. A., IRVINE, L. M., KIENLE, S. S., KUHN, C. E., LIDGARD, D., LOREDO, S. A., MATE, B. R., METCALFE, K., NZEGOUE, J., KOUEREY OLIWINA, C. K., ORBEN, R. A., OZAKI, K., PARNELL, R., PIKE, E. P., ROBINSON, P. W., ROSENBAUM, H. C., SATO, F., SHAFFER, S. A., SHAVER, D. J., SIMMONS, S. E., SMITH, B. J., SOUNGUET, G.-P., SURYAN, R. M., THOMPSON, D. R., TIERNEY, M., TILLEY, D., YOUNG, H. S., WARWICK-EVANS, V., WEISE, M. J., WELLS, R. S., WILKINSON, B. P., WITT, M. J. & MAXWELL, S. M. 2022. Mismatches in scale between highly mobile marine megafauna and marine protected areas. *Frontiers in Marine Science,* 9.

COPAT, C., ARENA, G., FIORE, M., LEDDA, C., FALLICO, R., SCIACCA, S. & FERRANTE, M. 2013. Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. *Food and Chemical Toxicology,* 53**,** 33-37.

CORDOVA-ZAVALETA, F., MENDO, J., BRIONES-HERNANDEZ, S. A., ACUNA-PERALES, N., GONZALEZ-PESTANA, A., ALFARO-SHIGUETO, J. & MANGEL, J. C. 2018. Food habits of the blue shark, Prionace glauca (Linnaeus, 1758), in waters off northern Peru. *Fishery Bulletin,* 116**,** 310-324.

CORTÉS, E. 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian journal of fisheries and aquatic sciences,* 54**,** 726-738.

CORTÉS, E., AROCHA, F., BEERKIRCHER, L., CARVALHO, F., DOMINGO, A., HEUPEL, M., HOLTZHAUSEN, H., SANTOS, M. N., RIBERA, M. & SIMPFENDORFER, C. 2010. Ecological risk assessment of pelagic sharks caught in Atlantic pelagic longline fisheries. *Aquatic Living Resources,* 23**,** 25-34.

COSTA, D. P., BREED, G. A. & ROBINSON, P. W. 2012. New Insights into Pelagic Migrations: Implications for Ecology and Conservation. *Annual Review of Ecology, Evolution, and Systematics,* 43**,** 73-96.

COUNCIL REGULATION 57/2011 Council Regulation 57/2011. The fishing opportunities for certain fish stocks and groups of fish stocks, applicable in EU waters and, for EU vessels, in certain non-EU waters. *Official Journal,* L24.

CRONIN, M. R., AMARAL, J. E., JACKSON, A. M., JACQUET, J., SETO, K. L. & CROLL, D. A. 2023. Policy and transparency gaps for oceanic shark and rays in high seas tuna fisheries. *Fish and Fisheries,* 24**,** 56-70.

CROSSIN, G. T., HEUPEL, M. R., HOLBROOK, C. M., HUSSEY, N. E., LOWERRE-BARBIERI, S. K., NGUYEN, V. M., RABY, G. D. & COOKE, S. J.

2017. Acoustic telemetry and fisheries management. *Ecological Applications,* 27**,** 1031-1049.

CRUMMEY, C., RONAN, M. & FAHY, E. 1991. Blue shark Prionace glauca (L.) in Irish waters. *The Irish Naturalists' Journal,* 23**,** 454-456.

CURTIS, T., CORTES, E., DUBECK, G. & MCCANDLESS, C. T. 2016. Statues review report: porbeagle shark (Lamna nasus).

CVITANOVIC, C., SHELLOCK, R. J., MACKAY, M., VAN PUTTEN, E. I., KARCHER, D. B., DICKEY-COLLAS, M. & BALLESTEROS, M. 2021. Strategies for building and managing 'trust' to enable knowledge exchange at the interface of environmental science and policy. *Environmental Science & Policy,* 123**,** 179-189.

DA SILVA, C., KERWATH, S., WILKE, C., MEYER, M. & LAMBERTH, S. 2010. First documented southern transatlantic migration of a blue shark Prionace glauca tagged off South Africa. *African Journal of Marine Science,* 32**,** 639-642.

DA SILVA, T. E. F., LESSA, R. & SANTANA, F. M. 2021. Current knowledge on biology, fishing and conservation of the blue shark (Prionace glauca). *Neotropical Biology and Conservation,* 16**,** 71.

DAHLBERG, A. & MUELLER, G. M. 2011. Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species. *Fungal Ecology,* 4**,** 147-162.

DALSGAARD, J., JOHN, M. S., KATTNER, G., MÜLLER-NAVARRA, D. & HAGEN, W. 2003. Fatty acid trophic markers in the pelagic marine environment.

DALY, R., SMALE, M. J., SINGH, S., ANDERS, D., SHIVJI, M., K. DALY, C. A., LEA, J. S. E., SOUSA, L. L., WETHERBEE, B. M., FITZPATRICK, R., CLARKE, C. R., SHEAVES, M. & BARNETT, A. 2018. Refuges and risks: Evaluating the benefits of an expanded MPA network for mobile apex predators. *Diversity and Distributions,* 24**,** 1217-1230.

DE VOS, A. & SCHWARTZ, M. W. 2022. Confronting parachute science in conservation. *Conservation Science and Practice,* 4.

DENIRO, M. J. & EPSTEIN, S. 1976. You are what you eat (plus a few): the carbon isotope cycle in food chains.

DHLGH 2021. National Marine Planning Framework. *In:* HERITAGE, D. O. H. L. G. A. (ed.). gov.ie/housing.

DONALD, P. F., FISHPOOL, L. D. C., AJAGBE, A., BENNUN, L. A., BUNTING, G., BURFIELD, I. J., BUTCHART, S. H. M., CAPELLAN, S., CROSBY, M. J., DIAS, M. P., DIAZ, D., EVANS, M. I., GRIMMETT, R., HEATH, M., JONES, V. R., LASCELLES, B. G., MERRIMAN, J. C., O'BRIEN, M., RAMÍREZ, I., WALICZKY, Z. & WEGE, D. C. 2019. Important Bird and Biodiversity Areas (IBAs): the development and characteristics of a global inventory of key sites for biodiversity. *Bird Conservation International,* 29**,** 177-198.

DORMAN, J. 1987. Notes on the Stomach Contents of Some Blue Sharks (Prionace glauca, L.), Caught in Courtmacsherry Bay, County Cork. *The Irish Naturalists' Journal,* 22**,** 207-207.

DOYLE, T. K., HOUGHTON, J. D. R., MCDEVITT, R., DAVENPORT, J. & HAYS, G. C. 2007. The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. *Journal of Experimental Marine Biology and Ecology,* 343**,** 239-252.

DRENT, R. & DAAN, S. 1980. The prudent parent: energetic adjustments in avian breeding 1. *Ardea,* 55**,** 225-252.

DUARTE, C. M., AGUSTI, S., BARBIER, E., BRITTEN, G. L., CASTILLA, J. C., GATTUSO, J.-P., FULWEILER, R. W., HUGHES, T. P., KNOWLTON, N., LOVELOCK, C. E., LOTZE, H. K., PREDRAGOVIC, M., POLOCZANSKA, E., ROBERTS, C. & WORM, B. 2020. Rebuilding marine life. *Nature,* 580**,** 39-51.

DULVY, N. K., BAUM, J. K., CLARKE, S., COMPAGNO, L. J., CORTÉS, E., DOMINGO, A., FORDHAM, S., FOWLER, S., FRANCIS, M. P. & GIBSON, C. 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 18**,** 459-482.

DULVY, N. K., FOWLER, S. L., MUSICK, J. A., CAVANAGH, R. D., KYNE, P. M., HARRISON, L. R., CARLSON, J. K., DAVIDSON, L. N. K., FORDHAM, S. V., FRANCIS, M. P., POLLOCK, C. M., SIMPFENDORFER, C. A., BURGESS, G. H., CARPENTER, K. E., COMPAGNO, L. J. V., EBERT, D. A., GIBSON, C., HEUPEL, M. R., LIVINGSTONE, S. R., SANCIANGCO, J. C., STEVENS, J. D., VALENTI, S. & WHITE, W. T. 2014. Extinction risk and conservation of the world's sharks and rays. *eLife,* 3**,** e00590.

DULVY, N. K., PACOUREAU, N., RIGBY, C. L., POLLOM, R. A., JABADO, R. W., EBERT, D. A., FINUCCI, B., POLLOCK, C. M., CHEOK, J., DERRICK, D. H., HERMAN, K. B., SHERMAN, C. S., VANDERWRIGHT, W. J., LAWSON, J. M., WALLS, R. H. L., CARLSON, J. K., CHARVET, P., BINEESH, K. K., FERNANDO, D., RALPH, G. M., MATSUSHIBA, J. H., HILTON-TAYLOR, C., FORDHAM, S. V. & SIMPFENDORFER, C. A. 2021. Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology,* 31**,** 4773-4787.e8.

DULVY, N. K., SIMPFENDORFER, C. A., DAVIDSON, L. N., FORDHAM, S. V., BRÄUTIGAM, A., SANT, G. & WELCH, D. J. 2017. Challenges and priorities in shark and ray conservation. *Current Biology,* 27**,** R565-R572.

ELLIS, J., FARRELL, E., JUNG, A., MCCULLY, S., SIMS, D. & SOLDO, A. 2015. Lamna nasus. The IUCN Red List of Threatened Species.

ELLIS, J., FARRELL, E., JUNG, A., MCCULLY, S., SIMS, D. & SOLDO, A. 2016. Lamna nasus. The IUCN Red List of Threatened Species.

ESSINGTON, T. E., MORIARTY, P. E., FROEHLICH, H. E., HODGSON, E. E., KOEHN, L. E., OKEN, K. L., SIPLE, M. C. & STAWITZ, C. C. 2015. Fishing amplifies forage fish population collapses. *Proceedings of the National Academy of Sciences,* 112**,** 6648-6652.

EVERSHED, R. P., BULL, I. D., CORR, L. T., CROSSMAN, Z. M., VAN DONGEN, B. E., EVANS, C. J., JIM, S., MOTTRAM, H. R., MUKHERJEE, A. J. & PANCOST, R. D. 2007. Compound-specific stable isotope analysis in ecology and paleoecology. *Stable isotopes in ecology and environmental science,* 480.

FÁILTE IRELAND 2013. Wild Atlantic Way. *Route identification report. Available on: http://www.failteireland.ie/FailteIreland/media/WebsiteStructure/Documents/2_Develop_Your_Business/Key%20Projects/WAW_Report_May13_LowRes.pdf (Accessed on 28 March 2020)*.

FAIR SEAS 2022. Revitalising Our Seas: Identifying Areas of Interest for Marine Protected Area Designation in Irish Waters.

FINUCCI, B., CHEOK, J., EBERT, D. A., HERMAN, K., KYNE, P. M. & DULVY, N. K. 2021. Ghosts of the deep – Biodiversity, fisheries, and extinction risk of ghost sharks. *Fish and Fisheries,* 22**,** 391-412.

FITZMAURICE, P., KEIRSE, G., GREEN, P., CLARKE, M. & KENNY, M. 2005. CPUE and associated data for blue shark from the Irish sport fishery. *Collect. Vol. Sci. Pap. ICCAT,* 58**,** 1166-1170.

FOWLER, S., RAYMAKERS, C. & GRIMM, U. 2004. Trade in and conservation of two shark species, porbeagle (Lamna nasus) and spiny dogfish (Squalus acanthias).

FRANCIS, M. P., HOLDSWORTH, J. C. & BLOCK, B. A. 2015. Life in the open ocean: seasonal migration and diel diving behaviour of Southern Hemisphere porbeagle sharks (Lamna nasus). *Marine Biology,* 162**,** 2305-2323.

FRANCIS, M. P., NATANSON, L. J. & CAMPANA, S. E. 2008. The biology and ecology of the porbeagle shark, Lamna nasus. *Sharks of the Open Ocean: Biology, Fisheries and Conservation***,** 105-113.

FRENCH, V. & MCDONOUGH, N. 2020. Ocean Citizen Survey: Perceptions of the Irish public on priorities for the protection and sustainable use of the ocean. Marine Institute.

FRY, B. 2006. *Stable isotope ecology*, Springer.

FUJINAMI, Y. & SEMBA, Y. 2020. Non-lethal assessment of reproductive stage for female blue sharks Prionace glauca using sex steroid hormones. *Journal of fish biology,* 96**,** 1501-1504.

GALLAGHER, A. J., BROWNSCOMBE, J. W., ALSUDAIRY, N. A., CASAGRANDE, A. B., FU, C., HARDING, L., HARRIS, S. D., HAMMERSCHLAG, N., HOWE, W. & HUERTAS, A. D. 2022. Tiger sharks support the characterization of the world's largest seagrass ecosystem. *Nature communications,* 13**,** 6328.

GALLAGHER, A. J., WAGNER, D. N., IRSCHICK, D. J. & HAMMERSCHLAG, N. 2014. Body condition predicts energy stores in apex predatory sharks. *Conservation Physiology,* 2**,** cou022.

GANNES, L. Z., DEL RIO, C. M. N. & KOCH, P. 1998. Natural Abundance Variations in Stable Isotopes and their Potential Uses in Animal Physiological Ecology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 119**,** 725-737.

GARCÍA, V. B., LUCIFORA, L. O. & MYERS, R. A. 2008. The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proceedings of the Royal Society B: Biological Sciences,* 275**,** 83-89.

GONÇALVES-SOUZA, D., VERBURG, P. H. & DOBROVOLSKI, R. 2020. Habitat loss, extinction predictability and conservation efforts in the terrestrial ecoregions. *Biological Conservation,* 246**,** 108579.

GONZÁLEZ, M. T., SEPÚLVEDA, F. A., ZÁRATE, P. M. & BAEZA, J. A. 2021. Regional population genetics and global phylogeography of the endangered highly migratory shark Lamna nasus: Implications for fishery management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 31**,** 620-634.

GONZALEZ TRIGINER, V. 2020. *Distribution range, occurrence and bycatch risk of a large pelagic shark, the porbeagle (Lamna nasus), in Norwegian waters.*

GOROKHOVA, E. 2018. Individual growth as a non-dietary determinant of the isotopic niche metrics. *Methods in Ecology and Evolution,* 9**,** 269-277.

GRAHAM, B. S., KOCH, P. L., NEWSOME, S. D., MCMAHON, K. W. & AURIOLES, D. 2010. Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. *Isoscapes.* Springer.

GRAHAM, J., CACERES, C. & DE AZEVEDO MENNA, D. S. 2022. *Minorities in Shark Sciences: Diverse Voices in Shark Research*, CRC Press.

GRAY, C., PETERS-BURTON, E., SMITH, C. & PARSONS, E. C. M. 2022. Basking shark tourism in Donegal, Ireland – a case-study of public interest and support for shark conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 32**,** 537-550.

GUDO, M. & HOMBERGER, D. G. 2002. The functional morphology of the pectoral fin girdle of the Spiny Dogfish (Squalus acanthias): Implications for the evolutionary history of the pectoral girdle of vertebrates. *Senckenbergiana lethaea,* 82**,** 241-252.

HAMMERSCHLAG, N., MCDONNELL, L. H., RIDER, M. J., STREET, G. M., HAZEN, E. L., NATANSON, L. J., MCCANDLESS, C. T., BOUDREAU, M. R., GALLAGHER, A. J., PINSKY, M. L. & KIRTMAN, B. 2022. Ocean warming alters the distributional range, migratory timing, and spatial protections of an apex predator, the tiger shark (Galeocerdo cuvier). *Global Change Biology,* 28**,** 1990-2005.

HAMMERSCHLAG, N., SCHMITZ, O. J., FLECKER, A. S., LAFFERTY, K. D., SIH, A., ATWOOD, T. B., GALLAGHER, A. J., IRSCHICK, D. J., SKUBEL, R. & COOKE, S. J. 2019. Ecosystem Function and Services of Aquatic Predators in the Anthropocene. *Trends in Ecology & Evolution,* 34**,** 369-383.

HAMMERSCHLAG, N. & SULIKOWSKI, J. 2011. Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. *Endangered Species Research,* 14**,** 135-140.

HARDING, L., GALLAGHER, A., JACKSON, A., BORTOLUZZI, J., DOLTON, H. R., SHEA, B., HARMAN, L., EDWARDS, D. & PAYNE, N. 2022. Capture heats up sharks. *Conservation Physiology,* 10**,** coac065.

HARDING, L., JACKSON, A., BARNETT, A., DONOHUE, I., HALSEY, L., HUVENEERS, C., MEYER, C., PAPASTAMATIOU, Y., SEMMENS, J. M., SPENCER, E., WATANABE, Y. & PAYNE, N. 2021. Endothermy makes fishes faster but does not expand their thermal niche. *Functional Ecology,* 35**,** 1951-1959.

HARVEY, J. T. 1989. Food habits seasonal abundance size and sex of the blue shark prionace glauca in monterey bay california usa. *California Fish and Game,* 75**,** 33-44.

HAYS, G. C., BAILEY, H., BOGRAD, S. J., BOWEN, W. D., CAMPAGNA, C., CARMICHAEL, R. H., CASALE, P., CHIARADIA, A., COSTA, D. P., CUEVAS, E., NICO DE BRUYN, P. J., DIAS, M. P., DUARTE, C. M., DUNN, D. C., DUTTON, P. H., ESTEBAN, N., FRIEDLAENDER, A., GOETZ, K. T., GODLEY, B. J., HALPIN, P. N., HAMANN, M., HAMMERSCHLAG, N., HARCOURT, R., HARRISON, A.-L., HAZEN, E. L., HEUPEL, M. R., HOYT, E., HUMPHRIES, N. E., KOT, C. Y., LEA, J. S. E., MARSH, H., MAXWELL, S. M., MCMAHON, C. R., NOTARBARTOLO DI SCIARA, G., PALACIOS, D. M., PHILLIPS, R. A., RIGHTON, D., SCHOFIELD, G., SEMINOFF, J. A., SIMPFENDORFER, C. A., SIMS, D. W., TAKAHASHI, A., TETLEY, M. J., THUMS, M., TRATHAN, P. N., VILLEGAS-AMTMANN, S., WELLS, R. S., WHITING, S. D., WILDERMANN, N. E. & SEQUEIRA, A. M. M. 2019.

Translating Marine Animal Tracking Data into Conservation Policy and Management. *Trends in Ecology & Evolution,* 34**,** 459-473.

HAYS, G. C., DOYLE, T. K. & HOUGHTON, J. D. R. 2018. A Paradigm Shift in the Trophic Importance of Jellyfish? *Trends in Ecology & Evolution,* 33**,** 874-884.

HAYS, G. C., FERREIRA, L. C., SEQUEIRA, A. M. M., MEEKAN, M. G., DUARTE, C. M., BAILEY, H., BAILLEUL, F., BOWEN, W. D., CALEY, M. J., COSTA, D. P., EGUÍLUZ, V. M., FOSSETTE, S., FRIEDLAENDER, A. S., GALES, N., GLEISS, A. C., GUNN, J., HARCOURT, R., HAZEN, E. L., HEITHAUS, M. R., HEUPEL, M., HOLLAND, K., HORNING, M., JONSEN, I., KOOYMAN, G. L., LOWE, C. G., MADSEN, P. T., MARSH, H., PHILLIPS, R. A., RIGHTON, D., ROPERT-COUDERT, Y., SATO, K., SHAFFER, S. A., SIMPFENDORFER, C. A., SIMS, D. W., SKOMAL, G., TAKAHASHI, A., TRATHAN, P. N., WIKELSKI, M., WOMBLE, J. N. & THUMS, M. 2016. Key Questions in Marine Megafauna Movement Ecology. *Trends in Ecology & Evolution,* 31**,** 463-475.

HAZEN, E. L., MAXWELL, S. M., BAILEY, H., BOGRAD, S. J., HAMANN, M., GASPAR, P., GODLEY, B. J. & SHILLINGER, G. L. 2012. Ontogeny in marine tagging and tracking science: technologies and data gaps. *Marine Ecology Progress Series,* 457**,** 221-240.

HEBERT, C. E., ARTS, M. T. & WESELOH, D. V. C. 2006. Ecological Tracers Can Quantify Food Web Structure and Change. *Environmental Science & Technology,* 40**,** 5618-5623.

HEDENSTRÖM, A. & ALERSTAM, T. 1996. Skylark optimal flight speeds for flying nowhere and somewhere. *Behavioral Ecology,* 7**,** 121-126.

HENDERSON, A., FLANNERY, K. & DUNNE, J. 2001. Observations on the biology and ecology of the blue shark in the North-east Atlantic. *Journal of Fish Biology,* 58**,** 1347-1358.

HERNÁNDEZ-AGUILAR, S. B., ESCOBAR-SÁNCHEZ, O., GALVÁN-MAGAÑA, F. & ABITIA-CÁRDENAS, L. A. 2016. Trophic ecology of the blue shark (Prionace glauca) based on stable isotopes (δ13C and δ15N) and stomach content. *Journal of the Marine Biological Association of the United Kingdom,* 96**,** 1403-1410.

HETTE-TRONQUART, N. 2019. Isotopic niche is not equal to trophic niche. *Ecology letters,* 22**,** 1987-1989.

HEUPEL, M. & SIMPFENDORFER, C. 2010. Science or slaughter: need for lethal sampling of sharks. *Conservation Biology,* 24**,** 1212-1218.

HOBSON, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia,* 120**,** 314-326.

HOEINGHAUS, D. J. & ZEUG, S. C. 2008. Can stable isotope ratios provide for community-wide measures of trophic structure? comment. *Ecology,* 89**,** 2353-2357.

HOOKER, S. K., CAÑADAS, A., HYRENBACH, K. D., CORRIGAN, C., POLOVINA, J. J. & REEVES, R. R. 2011. Making protected area networks effective for marine top predators. *Endangered Species Research,* 13**,** 203-218.

HOWE-WALSH, L. & TURNBULL, S. 2016. Barriers to women leaders in academia: tales from science and technology. *Studies in Higher Education,* 41**,** 415-428.

HUSSEY, N. E., KESSEL, S. T., AARESTRUP, K., COOKE, S. J., COWLEY, P. D., FISK, A. T., HARCOURT, R. G., HOLLAND, K. N., IVERSON, S. J., KOCIK,

J. F., MILLS FLEMMING, J. E. & WHORISKEY, F. G. 2015. Aquatic animal telemetry: A panoramic window into the underwater world. *Science,* 348**,** 1255642.

HUTCHINGS, J. A., MYERS, R. A., GARCÍA, V. B., LUCIFORA, L. O. & KUPARINEN, A. 2012. Life-history correlates of extinction risk and recovery potential. *Ecological Applications,* 22**,** 1061-1067.

HYDE, C. A., NOTARBARTOLO DI SCIARA, G., SORRENTINO, L., BOYD, C., FINUCCI, B., FOWLER, S. L., KYNE, P. M., LEURS, G., SIMPFENDORFER, C. A. & TETLEY, M. J. 2022. Putting sharks on the map: A global standard for improving shark area-based conservation. *Frontiers in Marine Science***,** 1660.

IBSG. 2022. *SHARK SAVED!* [Online]. Available: https://www.baskingshark.ie/post/shark-saved [Accessed February 25th 2023].

ICCAT 2015. 2015-06 Recommendation by ICCAT on Porbeagle Caught in Association with ICCAT Fisheries.

ICCAT 2016. Report of the 2016 intersessional meeting of the shark species group. *Collect. Vol. Sci. Pap. ICCAT.* Madeira, Portugal.

ICCAT SHARK SPECIES GROUP 2020. *Report of the 2020 porbeagle shark stock assessment meeting,* ICCAT.

ICCAT-ICES 2022. Report of the joint ICES-ICCAT benchmark workshop in advance of the North-Eastern Atlantic porbeagle stock assessment. *Collective Volume of Scientific Papers.* ICCAT.

ICES 2020. Herring Assessment Working Group for the Area South of 62° N (HAWG). *ICES Scientific Reports,* 2**,** 1151.

ICES 2021. Working Group on Elasmobranch Fishes (WGEF).

ICES 2022. Porbeagle (Lamna nasus) in subareas 1–10, 12, and 14 (the Northeast Atlantic and adjacent waters). *ICES Advice: Recurrent Advice.*

INLAND FISHERIES IRELAND 2013. Socioeconomic study of recreational angling in Ireland. *Inland Fisheries Ireland, Dublin*.

IOSILEVSKII, G., KONG, J. D., MEYER, C. G., WATANABE, Y. Y., PAPASTAMATIOU, Y. P., ROYER, M. A., NAKAMURA, I., SATO, K., DOYLE, T. K., HARMAN, L., HOUGHTON, J. D. R., BARNETT, A., SEMMENS, J. M., MAOILÉIDIGH, N. Ó., DRUMM, A., O'NEILL, R., COFFEY, D. M. & PAYNE, N. L. 2022. A general swimming response in exhausted obligate swimming fish. *Royal Society Open Science,* 9**,** 211869.

IUCN. 2023. *The IUCN Red List of Threatened Species* [Online]. Available: https://www.iucnredlist.org/ [Accessed 2 Feb 2023].

JACKSON, A. L., INGER, R., PARNELL, A. C. & BEARHOP, S. 2011. Comparing isotopic niche widths among and within communities: SIBER — Stable IsotopeBayesian Ellipses in R. *Journal of Animal Ecology,* 80**,** 595-602.

JACOBY, D. M., WATANABE, Y. Y., PACKARD, T., HEALEY, M., PAPASTAMATIOU, Y. P. & GALLAGHER, A. J. 2022. First descriptions of the seasonal habitat use and residency of scalloped hammerhead (Sphyrna lewini) and Galapagos sharks (Carcharhinus galapagensis) at a coastal seamount off Japan. *Animal Biotelemetry,* 10**,** 1-11.

JACOBY, D. M. P., FERRETTI, F., FREEMAN, R., CARLISLE, A. B., CHAPPLE, T. K., CURNICK, D. J., DALE, J. J., SCHALLERT, R. J., TICKLER, D. & BLOCK, B. A. 2020. Shark movement strategies influence poaching risk and can guide enforcement decisions in a large, remote marine protected area. *Journal of Applied Ecology,* 57**,** 1782-1792.

JANSSON, J. 2016. *Science, information, and policy interface for effective coastal and ocean management*, Taylor & Francis.

JOCHMANN, M. A. & SCHMIDT, T. C. 2015. *Compound-specific stable isotope analysis*, Royal Society of Chemistry.

JORGENSEN, S. J., MICHELI, F., WHITE, T. D., VAN HOUTAN, K. S., ALFARO-SHIGUETO, J., ANDRZEJACZEK, S., ARNOLDI, N. S., BAUM, J. K., BLOCK, B. & BRITTEN, G. L. 2022. Emergent research and priorities for shark and ray conservation. *Endangered species research,* 47**,** 171-203.

JUAN-JORDÁ, M. J., MURUA, H., ARRIZABALAGA, H., MERINO, G., PACOUREAU, N. & DULVY, N. K. 2022. Seventy years of tunas, billfishes, and sharks as sentinels of global ocean health. *Science,* 378**,** eabj0211.

KAY, W. P., NAUMANN, D. S., BOWEN, H. J., WITHERS, S. J., EVANS, B. J., WILSON, R. P., STRINGELL, T. B., BULL, J. C., HOPKINS, P. W. & BÖRGER, L. 2019. Minimizing the impact of biologging devices: Using computational fluid dynamics for optimizing tag design and positioning. *Methods in Ecology and Evolution,* 10**,** 1222-1233.

KENNERLEY, A., LORENZONI, I., LUISETTI, T., WOOD, L. E. & TAYLOR, N. G. H. 2021. Mapping habitats for the suitability of jellyfish blooms around the UK and Ireland. *Hydrobiologia,* 848**,** 1535-1552.

KETCHUM, J. T., HOYOS-PADILLA, M., ALDANA-MORENO, A., AYRES, K., GALVÁN-MAGAÑA, F., HEARN, A. R., LARA-LIZARDI, F., MUNTANER-LÓPEZ, G., GRAU, M., TREJO-RAMÍREZ, A., WHITEHEAD, D. A. & KLIMLEY, A. P. 2020. Shark movement patterns in the Mexican Pacific: A conservation and management perspective. *Advances in marine biology,* 85 1**,** 1-37.

KIRSCH, P. E., IVERSON, S. J., BOWEN, W. D., KERR, S. R. & ACKMAN, R. G. 1998. Dietary effects on the fatty acid signature of whole Atlantic cod (Gadus morhua). *Canadian Journal of Fisheries and Aquatic Sciences,* 55**,** 1378-1386.

KITAMURA, T. & MATSUNAGA, H. 2010. Population structure of porbeagle (Lamna nasus) in the Atlantic Ocean as inferred from mitochondrial DNA control region sequences. *Collect. Vol. Sci. Pap. ICCAT,* 65**,** 2082-2087.

KITAYSKY, A. S., HUNT JR, G. L., FLINT, E. N., RUBEGA, M. A. & DECKER, M. B. 2000. Resource allocation in breeding seabirds: responses to fluctuations in their food supply. *Marine Ecology Progress Series,* 206**,** 283-296.

KLIMLEY, A. P., ARAUZ, R., BESSUDO, S., CHÁVEZ, E. J., CHINACALLE, N., ESPINOZA, E., GREEN, J., HEARN, A. R., HOYOS-PADILLA, M. E., NALESSO, E., KETCHUM, J. T., FISCHER, C., LADINO, F., SHILLINGER, G., SOLER, G., STEINER, T. & PEÑAHERRERA-PALMA, C. 2022. Studies of the movement ecology of sharks justify the existence and expansion of marine protected areas in the Eastern Pacific Ocean. *Environmental Biology of Fishes*.

KOHLER, N. E., TURNER, P. A., HOEY, J. J., NATANSON, L. J. & BRIGGS, R. 2002. Tag and recapture data for three pelagic shark species: blue shark (Prionace glauca), shortfin mako (Isurus oxyrinchus), and porbeagle (Lamna nasus) in the North Atlantic Ocean. *Col. Vol. Sci. Pap. ICCAT,* 54**,** 1231-1260.

KOUBRAK, O. & VANDERZWAAG, D. L. 2020. Are transboundary fisheries management arrangements in the Northwest Atlantic and North Pacific seaworthy in a changing ocean? *Ecology and Society,* 25.

KUBODERA, T., WATANABE, H. & ICHII, T. 2006. Feeding habits of the blue shark, Prionace glauca, and salmon shark, Lamna ditropis, in the transition region of the Western North Pacific. *Reviews in Fish Biology and Fisheries,* 17**,** 111.

LASCELLES, B., NOTARBARTOLO DI SCIARA, G., AGARDY, T., CUTTELOD, A., ECKERT, S., GLOWKA, L., HOYT, E., LLEWELLYN, F., LOUZAO, M., RIDOUX, V. & TETLEY, M. J. 2014. Migratory marine species: their status, threats and conservation management needs. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 24**,** 111-127.

LAYMAN, C. A., ARAUJO, M. S., BOUCEK, R., HAMMERSCHLAG-PEYER, C. M., HARRISON, E., JUD, Z. R., MATICH, P., ROSENBLATT, A. E., VAUDO, J. J. & YEAGER, L. A. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews,* 87**,** 545-562.

LAYMAN, C. A., ARRINGTON, D. A., MONTAÑA, C. G. & POST, D. M. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology,* 88**,** 42-48.

LEA, J. S. E., HUMPHRIES, N. E., VON BRANDIS, R. G., CLARKE, C. R. & SIMS, D. W. 2016. Acoustic telemetry and network analysis reveal the space use of multiple reef predators and enhance marine protected area design. *Proceedings of the Royal Society B-Biological Sciences,* 283**,** 8.

LEIGH, S. C., PAPASTAMATIOU, Y. P. & GERMAN, D. P. J. P. O. T. R. S. B. B. S. 2018. Seagrass digestion by a notorious 'carnivore'. 285**,** 20181583.

LICANDRO, P., HEAD, E., GISLASON, A., BENFIELD, M. C., HARVEY, M., MARGONSKI, P. & SILKE, J. 2011. Overview of trends in plankton communities.

LINK, J. S. & WATSON, R. A. 2019. Global ecosystem overfishing: Clear delineation within real limits to production. *Science Advances,* 5**,** eaav0474.

LOVELACE, S., MCARTHUR, D. & FLORENCE, L. Connecting the oceans and human health through research and education. Proceedings of OCEANS 2005 MTS/IEEE, 17-23 Sept. 2005 2005. 1729-1737 Vol. 2.

LOWERRE-BARBIERI, S. K., KAYS, R., THORSON, J. T. & WIKELSKI, M. 2019. The ocean's movescape: fisheries management in the bio-logging decade (2018–2028). *ICES Journal of Marine Science,* 76**,** 477-488.

LYNAM, C. P., LILLEY, M. K. S., BASTIAN, T., DOYLE, T. K., BEGGS, S. E. & HAYS, G. C. 2011. Have jellyfish in the Irish Sea benefited from climate change and overfishing? *Global Change Biology,* 17**,** 767-782.

LYSTER, S. 1989. The Convention on the Conservation of Migratory Species of Wild Animals (The Bonn Convention) International Law of Migratory Species. *Nat. Resources J.,* 29**,** 979.

MACALI, A. & BERGAMI, E. 2020. Jellyfish as innovative bioindicator for plastic pollution. *Ecological Indicators,* 115**,** 106375.

MACDONALD, B. H., SOOMAI, S. S., DE SANTO, E. M. & WELLS, P. G. 2016. Understanding the science-policy interface in integrated coastal and ocean management. *SCIENCE, INFORMATION, AND POLICY INTERFACE***,** 19.

MACDONALD, C. 2020. The dark side of being a female shark researcher. *Scientific American. Available online at:*

https://www.scientificamerican.com/article/the-dark-side-of-being-a-female-shark-researcher/ *(accessed August 10, 2020)*.

MACKENZIE, K., LONGMORE, C., PREECE, C., LUCAS, C. & TRUEMAN, C. 2014. Testing the long-term stability of marine isoscapes in shelf seas using jellyfish tissues. *Biogeochemistry,* 121**,** 441-454.

MACNEIL, M. A., CHAPMAN, D. D., HEUPEL, M., SIMPFENDORFER, C. A., HEITHAUS, M., MEEKAN, M., HARVEY, E., GOETZE, J., KISZKA, J. & BOND, M. E. 2020. Global status and conservation potential of reef sharks. *Nature,* 583**,** 801-806.

MADGETT, A. S., YATES, K., WEBSTER, L., MCKENZIE, C. & MOFFAT, C. F. 2019. Understanding marine food web dynamics using fatty acid signatures and stable isotope ratios: Improving contaminant impacts assessments across trophic levels. *Estuarine, Coastal and Shelf Science,* 227**,** 106327.

MADIGAN, D. J., BROOKS, E. J., BOND, M. E., GELSLEICHTER, J., HOWEY, L. A., ABERCROMBIE, D. L., BROOKS, A. & CHAPMAN, D. D. 2015. Diet shift and site-fidelity of oceanic whitetip sharks Carcharhinus longimanus along the Great Bahama Bank. *Marine Ecology Progress Series,* 529**,** 185-197.

MANLICK, P. J. & NEWSOME, S. D. 2022. Stable isotope fingerprinting traces essential amino acid assimilation and multichannel feeding in a vertebrate consumer. *Methods in Ecology and Evolution,* 13**,** 1819-1830.

MARKAIDA, U. & SOSA-NISHIZAKI, O. 2010. Food and feeding habits of the blue shark Prionace glauca caught off Ensenada, Baja California, Mexico, with a review on its feeding. *Journal of the Marine Biological Association of the United Kingdom,* 90**,** 977-994.

MARTÍN, G., ESPINOZA, M., HEUPEL, M. & SIMPFENDORFER, C. A. 2020. Estimating marine protected area network benefits for reef sharks. *Journal of Applied Ecology,* 57**,** 1969-1980.

MATTHEWS, B. & MAZUMDER, A. 2004. A Critical Evaluation of Intrapopulation Variation of $\delta^{13}C$ and Isotopic Evidence of Individual Specialization. *Oecologia,* 140**,** 361-371.

MATTHEWS, L. H. & PARKER, H. W. 1950. Notes on the anatomy and biology of the Basking Shark (Cetorhinus maximus (Gunner)). *Proceedings of the Zoological Society of London,* 120**,** 535-576.

MCCAULEY, D. J., PINSKY, M. L., PALUMBI, S. R., ESTES, J. A., JOYCE, F. H. & WARNER, R. R. 2015. Marine defaunation: Animal loss in the global ocean. *Science,* 347**,** 1255641.

MCCORD, M. E. & CAMPANA, S. E. 2003. A quantitative assessment of the diet of the blue shark (Prionace glauca) off Nova Scotia, Canada. *Journal of Northwest Atlantic Fishery Science,* 32.

MCGOWAN, J., BEGER, M., LEWISON, R. L., HARCOURT, R., CAMPBELL, H., PRIEST, M., DWYER, R. G., LIN, H.-Y., LENTINI, P., DUDGEON, C., MCMAHON, C., WATTS, M. & POSSINGHAM, H. P. 2017. Integrating research using animal-borne telemetry with the needs of conservation management. *Journal of Applied Ecology,* 54**,** 423-429.

MCKINNEY, M. L. 1999. High Rates of Extinction and Threat in Poorly Studied Taxa. *Conservation Biology,* 13**,** 1273-1281.

MCMAHON, K. W., HAMADY, L. L. & THORROLD, S. R. 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnology and oceanography,* 58**,** 697-714.

MCNIE, E. C., BEDNAREK, A. T., MEYER, R. & PARRIS, A. S. Designing Usable Environmental Research. 2016.

MEEKAN, M. G., VIRTUE, P., MARCUS, L., CLEMENTS, K. D., NICHOLS, P. D. & REVILL, A. T. 2022. The world's largest omnivore is a fish. *Ecology,* 103**,** e3818.

MEIJER, T. & DRENT, R. 1999. Re-examination of the capital and income dichotomy in breeding birds. *Ibis,* 141**,** 399-414.

MENDONÇA, A. 2009. Diet of the blue shark, Prionace glauca, in the Northeast Atlantic. *Master's Thesis, University of Porto, Portugal*.

MEYER, L., CHAMBERS, S., GERVAIS, C., PETHYBRIDGE, H., BECKMANN, C., BRUCE, B. & HUVENEERS, C. 2021. The use of muscle lipids and fatty acids to assess shark diet and condition. *Journal of Fish Biology,* 98**,** 566-571.

MEYER, L., PETHYBRIDGE, H., NICHOLS, P. D., BECKMANN, C., BRUCE, B. D., WERRY, J. M. & HUVENEERS, C. 2017. Assessing the Functional Limitations of Lipids and Fatty Acids for Diet Determination: The Importance of Tissue Type, Quantity, and Quality. *Frontiers in Marine Science,* 4.

MEYER, L., PETHYBRIDGE, H., NICHOLS, P. D., BECKMANN, C. & HUVENEERS, C. 2019. Abiotic and biotic drivers of fatty acid tracers in ecology: A global analysis of chondrichthyan profiles. *Functional Ecology,* 33**,** 1243-1255.

MICHENER, R. H. & KAUFMAN, L. 2007. Stable Isotope Ratios as Tracers in Marine Food Webs: An Update. *Stable Isotopes in Ecology and Environmental Science.*

MISSION BLUE. 2023. *Greater Skellig Coast Recognized as Ireland's First Hope Spot* [Online]. Available: https://missionblue.org/2023/01/greater-skellig-coast-recognized-as-irelands-first-hope-spot/ [Accessed February 25th 2023].

MOORE, A. B. & FOWLER, S. L. 2022. Important Shark Areas: rationale and need. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 32**,** 710-711.

MORRISON, R. & BULLOCK, C. 2018. A National Biodiversity Expenditure Review for Ireland. *Dublin. University College Dublin*.

NAJI, A., KHAN, F. R. & HASHEMI, S. H. 2016. Potential human health risk assessment of trace metals via the consumption of marine fish in Persian Gulf. *Marine Pollution Bulletin,* 109**,** 667-671.

NAKANO, H. & STEVENS, J. D. 2008. The biology and ecology of the blue shark, Prionace glauca. *Sharks of the open ocean: Biology, fisheries and conservation,* 1**,** 140-151.

NATIONAL BIODIVERSITY DATA CENTRE. 2019. *IPBES & Ireland's biodiversity crisis* [Online]. Available: https://biodiversityireland.ie/ipbes-irelands-biodiversity-crisis/ [Accessed 8th July 2023].

NAVARRO, J., COLL, M., SOMES, C. J. & OLSON, R. J. 2013. Trophic niche of squids: Insights from isotopic data in marine systems worldwide. *Deep Sea Research Part II: Topical Studies in Oceanography,* 95**,** 93-102.

NEWSOME, S. D., MARTINEZ DEL RIO, C., BEARHOP, S., PHILLIPS, D. L. J. F. I. E. & ENVIRONMENT, T. 2007. A niche for isotopic ecology. 5**,** 429-436.

NEWSOME, S. D., YEAKEL, J. D., WHEATLEY, P. V. & TINKER, M. T. 2012. Tools for quantifying isotopic niche space and dietary variation at the individual and population level. *Journal of Mammalogy,* 93**,** 329-341.

NIC LUGHADHA, E., BACHMAN, S. P., LEÃO, T. C., FOREST, F., HALLEY, J. M., MOAT, J., ACEDO, C., BACON, K. L., BREWER, R. F. & GÂTEBLÉ, G. 2020. Extinction risk and threats to plants and fungi. *Plants, People, Planet,* 2**,** 389-408.

NYKÄNEN, M., JESSOPP, M., DOYLE, T. K., HARMAN, L. A., CAÑADAS, A., BREEN, P., HUNT, W., MACKEY, M., CADHLA, O. Ó. & REID, D. 2018. Using tagging data and aerial surveys to incorporate availability bias in the abundance estimation of blue sharks (Prionace glauca). *PloS one,* 13**,** e0203122.

OGBURN, M. B., HARRISON, A.-L., WHORISKEY, F. G., COOKE, S. J., MILLS FLEMMING, J. E. & TORRES, L. G. 2017. Addressing challenges in the application of animal movement ecology to aquatic conservation and management. *Frontiers in Marine Science,* 4**,** 70.

ORTUÑO CRESPO, G., MOSSOP, J., DUNN, D., GJERDE, K., HAZEN, E., REYGONDEAU, G., WARNER, R., TITTENSOR, D. & HALPIN, P. 2020. Beyond static spatial management: Scientific and legal considerations for dynamic management in the high seas. *Marine Policy,* 122**,** 104102.

PACOUREAU, N., CARLSON, J. K., KINDSVATER, H. K., RIGBY, C. L., WINKER, H., SIMPFENDORFER, C. A., CHARVET, P., POLLOM, R. A., BARRETO, R., SHERMAN, C. S., TALWAR, B. S., SKERRITT, D. J., SUMAILA, U. R., MATSUSHIBA, J. H., VANDERWRIGHT, W. J., YAN, H. F. & DULVY, N. K. 2023. Conservation successes and challenges for wide-ranging sharks and rays. *Proceedings of the National Academy of Sciences,* 120**,** e2216891120.

PACOUREAU, N., RIGBY, C. L., KYNE, P. M., SHERLEY, R. B., WINKER, H., CARLSON, J. K., FORDHAM, S. V., BARRETO, R., FERNANDO, D., FRANCIS, M. P., JABADO, R. W., HERMAN, K. B., LIU, K.-M., MARSHALL, A. D., POLLOM, R. A., ROMANOV, E. V., SIMPFENDORFER, C. A., YIN, J. S., KINDSVATER, H. K. & DULVY, N. K. 2021. Half a century of global decline in oceanic sharks and rays. *Nature,* 589**,** 567-571.

PADE, N. G., QUEIROZ, N., HUMPHRIES, N. E., WITT, M. J., JONES, C. S., NOBLE, L. R. & SIMS, D. W. 2009. First results from satellite-linked archival tagging of porbeagle shark, Lamna nasus: Area fidelity, wider-scale movements and plasticity in diel depth changes. *Journal of Experimental Marine Biology and Ecology,* 370**,** 64-74.

PALACIOS-ABRANTES, J., REYGONDEAU, G., WABNITZ, C. C. C. & CHEUNG, W. W. L. 2020. The transboundary nature of the world's exploited marine species. *Scientific Reports,* 10**,** 17668.

PANNELL, J. L., DENCER-BROWN, A. M., GREENING, S. S., HUME, E. A., JARVIS, R. M., MATHIEU, C., MUGFORD, J. & RUNGHEN, R. 2019. An early career perspective on encouraging collaborative and interdisciplinary research in ecology. *Ecosphere,* 10**,** e02899.

PAPASTAMATIOU, Y. P., IOSILEVSKII, G., LEOS-BARAJAS, V., BROOKS, E. J., HOWEY, L. A., CHAPMAN, D. D. & WATANABE, Y. Y. 2018. Optimal swimming strategies and behavioral plasticity of oceanic whitetip sharks. *Scientific Reports,* 8**,** 551.

PAPASTAMATIOU, Y. P., MOURIER, J., TINHAN, T., LUONGO, S., HOSOKI, S., SANTANA-MORALES, O. & HOYOS-PADILLA, M. 2022. Social dynamics and individual hunting tactics of white sharks revealed by biologging. *Biology Letters,* 18**,** 20210599.

PARDO, S. A., KINDSVATER, H. K., REYNOLDS, J. D. & DULVY, N. K. 2016. Maximum intrinsic rate of population increase in sharks, rays, and chimaeras: the importance of survival to maturity. *Canadian Journal of Fisheries and Aquatic Sciences,* 73**,** 1159-1163.

PAYNE, N. L., IOSILEVSKII, G., BARNETT, A., FISCHER, C., GRAHAM, R. T., GLEISS, A. C. & WATANABE, Y. Y. 2016. Great hammerhead sharks swim on their side to reduce transport costs. *Nature communications,* 7**,** 12289.

PAYNE, N. L., TAYLOR, M. D., WATANABE, Y. Y. & SEMMENS, J. M. 2014. From physiology to physics: are we recognizing the flexibility of biologging tools? *Journal of Experimental Biology,* 217**,** 317-322.

PERCY, J. A. & FIFE, F. J. 1981. The Biochemical Composition and Energy Content of Arctic Marine Macrozooplankton. *Arctic,* 34**,** 307-313.

PETHYBRIDGE, H. R., CHOY, C. A., POLOVINA, J. J. & FULTON, E. A. 2018. Improving Marine Ecosystem Models with Biochemical Tracers. *Annual Review of Marine Science,* 10**,** 199-228.

PHILLIPS, D. L., INGER, R., BEARHOP, S., JACKSON, A. L., MOORE, J. W., PARNELL, A. C., SEMMENS, B. X. & WARD, E. J. 2014. Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology,* 92**,** 823-835.

PIERACCINI, M., COPPA, S. & DE LUCIA, G. A. 2017. Beyond marine paper parks? Regulation theory to assess and address environmental non-compliance. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 27**,** 177-196.

POCHEVILLE, A. 2015. The ecological niche: history and recent controversies. *Handbook of evolutionary thinking in the sciences.* Springer.

POST, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology,* 83**,** 703-718.

PRATT, H. L. 1979. Reproduction in the blue shark, Prionace glauca. *Fish. Bull.,* 77**,** 445-470.

PULLIN, A. S., KNIGHT, T. M., STONE, D. A. & CHARMAN, K. 2004. Do conservation managers use scientific evidence to support their decision-making? *Biological Conservation,* 119**,** 245-252.

PURCELL, J. E. & ARAI, M. N. Interactions of pelagic cnidarians and ctenophores with fish: a review. Jellyfish Blooms: Ecological and Societal Importance: Proceedings of the International Conference on Jellyfish Blooms, held in Gulf Shores, Alabama, 12–14 January 2000, 2001. Springer, 27-44.

QUEIROZ, N., HUMPHRIES, N. E., COUTO, A., VEDOR, M., DA COSTA, I., SEQUEIRA, A. M., MUCIENTES, G., SANTOS, A. M., ABASCAL, F. J. & ABERCROMBIE, D. L. 2019. Global spatial risk assessment of sharks under the footprint of fisheries. *Nature,* 572**,** 461-466.

QUEIROZ, N., HUMPHRIES, N. E., MUCIENTES, G., HAMMERSCHLAG, N., LIMA, F. P., SCALES, K. L., MILLER, P. I., SOUSA, L. L., SEABRA, R. & SIMS, D. W. 2016. Ocean-wide tracking of pelagic sharks reveals extent of overlap with longline fishing hotspots. *Proceedings of the National Academy of Sciences of the United States of America,* 113**,** 1582-1587.

QUEIROZ, N., P LIMA, F., MAIA, A., A RIBEIRO, P., P CORREIA, J. & SANTOS, A. M. 2005. Movement of blue shark, Prionace glauca, in the north-east Atlantic based on mark-recapture data. *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom,* 85**,** 1107.

RANGEL, B. D. S., WOSNICK, N., HAMMERSCHLAG, N., CIENA, A. P., KFOURY JUNIOR, J. R. & RICI, R. E. 2017. A preliminary investigation into the morphology of oral papillae and denticles of blue sharks (Prionace glauca) with inferences about its functional significance across life stages. *Journal of anatomy,* 230**,** 389-397.

RANGEL, B. S., HAMMERSCHLAG, N., SULIKOWSKI, J. A. & MOREIRA, R. G. 2021. Dietary and reproductive biomarkers in a generalist apex predator reveal differences in nutritional ecology across life stages. *Marine Ecology Progress Series,* 664**,** 149-163.

RANGEL, B. S., MOREIRA, R. G., RIDER, M. J., SULIKOWSKI, J. A., GALLAGHER, A. J., HEITHAUS, M. R., COOKE, S. J., KAUFMAN, L. & HAMMERSCHLAG, N. 2022. Physiological state predicts space use of sharks at a tourism provisioning site. *Animal Behaviour,* 191**,** 149-163.

RASMUSSEN, K., PALACIOS, D. M., CALAMBOKIDIS, J., SABORÍO, M. T., DALLA ROSA, L., SECCHI, E. R., STEIGER, G. H., ALLEN, J. M. & STONE, G. S. 2007. Southern Hemisphere humpback whales wintering off Central America: insights from water temperature into the longest mammalian migration. *Biology Letters,* 3**,** 302-305.

RIGBY, C., BARRETO, R., CARLSON, J., FERNANDO, D., FORDHAM, S., FRANCIS, M., HERMAN, K., JABADO, R., LIU, K.-M., MARSHALL, A., PACOUREAU, N., ROMANOV, E., SHERLEY, R. & WINKER, H. 2019. *Lamna nasus-Porbeagle. The IUCN Red List of Threatened Species 2019*.

RIGBY, C. L., BARRETO, R., CARLSON, J., FERNANDO, D., FORDHAM, S., FRANCIS, M.P., HERMAN, K., JABADO, R.W., LIU, K.M., MARSHALL, A., PACOUREAU, N., ROMANOV, E., SHERLEY, R.B. & WINKER, H. . 2019. *Prionace glauca. The IUCN Red List of Threatened Species* [Online]. [Accessed 01 May 2021].

RODRIGUEZ, M. A. & GERARDO HERRERA, L. M. 2013. Isotopic niche mirrors trophic niche in a vertebrate island invader. *Oecologia,* 171**,** 537-44.

RODRIGUEZ-BURGOS, A. M., BRICEÑO-ZULUAGA, F. J., ÁVILA JIMÉNEZ, J. L., HEARN, A., PEÑAHERRERA-PALMA, C., ESPINOZA, E., KETCHUM, J., KLIMLEY, P., STEINER, T., ARAUZ, R. & JOAN, E. 2022. The impact of climate change on the distribution of Sphyrna lewini in the tropical eastern Pacific. *Marine Environmental Research,* 180**,** 105696.

ROGAN, E. & BERROW, S. D. 1995. The management of Irish waters as a whale and dolphin sanctuary. *In:* BLIX, A. S., WALLØE, L. & ULLTANG, Ø. (eds.) *Developments in Marine Biology.* Elsevier Science.

ROGAN, E., BREEN, P., MACKEY, M., CAÑADAS, A., SCHEIDAT, M., GEELHOED, S. C. V. & JESSOPP, M. 2018. Aerial surveys of cetaceans and seabirds in Irish waters: occurrence, distribution and abundance in 2015-2017. Department of Communications, Climate Action & Environment and National Parks and Wildlife Service (NPWS)

Department of Culture, Heritage and the Gaeltacht, Dublin, Ireland.

ROHWER, S., RICKLEFS, R. E., ROHWER, V. G. & COPPLE, M. M. 2009. Allometry of the duration of flight feather molt in birds. *PLoS biology,* 7**,** e1000132.

ROOKER, J. R., DANCE, M. A., WELLS, R. J. D., AJEMIAN, M. J., BLOCK, B. A., CASTLETON, M. R., DRYMON, J. M., FALTERMAN, B. J., FRANKS, J. S., HAMMERSCHLAG, N., HENDON, J. M., HOFFMAYER, E. R., KRAUS, R. T., MCKINNEY, J. A., SECOR, D. H., STUNZ, G. W. & WALTER, J. F. 2019.

Population connectivity of pelagic megafauna in the Cuba-Mexico-United States triangle. *Scientific Reports,* 9**,** 1663.

ROYER, M., MALONEY, K., MEYER, C., CARDONA, E., PAYNE, N., WHITTINGHAM, K., SILVA, G., BLANDINO, C. & HOLLAND, K. 2020. Scalloped hammerhead sharks swim on their side with diel shifts in roll magnitude and periodicity. *Animal Biotelemetry,* 8**,** 1-12.

RUTZ, C. & HAYS, G. C. 2009. New frontiers in biologging science. *Biology Letters,* 5**,** 289-292.

RYAN, C., MCHUGH, B., TRUEMAN, C. N., SABIN, R., DEAVILLE, R., HARROD, C., BERROW, S. D. & IAN, O. 2013. Stable isotope analysis of baleen reveals resource partitioning among sympatric rorquals and population structure in fin whales. *Marine Ecology Progress Series,* 479**,** 251-261.

RYAN, L. A., MEEUWIG, J. J., HEMMI, J. M., COLLIN, S. P. & HART, N. S. 2015. It is not just size that matters: shark cruising speeds are species-specific. *Marine Biology,* 162**,** 1307-1318.

SAUNDERS, R. A., ROYER, F. & CLARKE, M. W. 2010. Winter migration and diving behaviour of porbeagle shark, Lamna nasus, in the Northeast Atlantic. *ICES Journal of Marine Science,* 68**,** 166-174.

SCHROEDER, A. & DANNHEIM, J. 2006. Estimation of fishing effort in the ASA of the North Sea. *Final Report of the EU project Q5RS-2002-00787***,** 107-129.

SCHUYLER, Q. A., WILCOX, C., TOWNSEND, K. A., WEDEMEYER-STROMBEL, K. R., BALAZS, G., VAN SEBILLE, E. & HARDESTY, B. D. 2016. Risk analysis reveals global hotspots for marine debris ingestion by sea turtles. *Global Change Biology,* 22**,** 567-576.

SHELLOCK, R. J., CVITANOVIC, C., MACKAY, M., MCKINNON, M. C., BLYTHE, J., KELLY, R., VAN PUTTEN, I. E., TUOHY, P., BAILEY, M., BEGOSSI, A., CRONA, B., FAKOYA, K. A., FERREIRA, B. P., FERRER, A. J. G., FRANGOUDES, K., GOBIN, J., GOH, H. C., HAAPASAARI, P., HARDESTY, B. D., HÄUSSERMANN, V., HOAREAU, K., HORNIDGE, A.-K., ISAACS, M., KRAAN, M., LI, Y., LIU, M., LOPES, P. F. M., MLAKAR, M., MORRISON, T. H., OXENFORD, H. A., PECL, G. T., PENCA, J., ROBINSON, C., SELIM, S., SKERN-MAURITZEN, M., SOEJIMA, K., SOTO, D., SPALDING, A. K., VADROT, A., VAIDIANU, N., WEBBER, M. & WISZ, M. S. 2022. Breaking down barriers: The identification of actions to promote gender equality in interdisciplinary marine research institutions. *One Earth,* 5**,** 687-708.

SHERMAN, C. S., DIGEL, E. D., ZUBICK, P., EGED, J., HAQUE, A. B., MATSUSHIBA, J. H., SIMPFENDORFER, C. A., SANT, G. & DULVY, N. K. 2023a. High overexploitation risk due to management shortfall in highly traded requiem sharks. *Conservation Letters,* n/a**,** e12940.

SHERMAN, C. S., SIMPFENDORFER, C. A., PACOUREAU, N., MATSUSHIBA, J. H., YAN, H. F., WALLS, R. H. L., RIGBY, C. L., VANDERWRIGHT, W. J., JABADO, R. W., POLLOM, R. A., CARLSON, J. K., CHARVET, P., BIN ALI, A., FAHMI, CHEOK, J., DERRICK, D. H., HERMAN, K. B., FINUCCI, B., EDDY, T. D., PALOMARES, M. L. D., AVALOS-CASTILLO, C. G., KINATTUMKARA, B., BLANCO-PARRA, M.-D.-P., DHARMADI, ESPINOZA, M., FERNANDO, D., HAQUE, A. B., MEJÍA-FALLA, P. A., NAVIA, A. F., PÉREZ-JIMÉNEZ, J. C., UTZURRUM, J., YUNENI, R. R. & DULVY, N. K. 2023b. Half a century of rising extinction risk of coral reef sharks and rays. *Nature Communications,* 14**,** 15.

SHIFFMAN, D. S., ARGUEDAS ÁLVAREZ, T., BANGLEY, C. W., BOYT, R., CÔTÉ, I. M., DALY-ENGEL, T. S., DAVIS, A. C. D., GASKINS, L. C., GRAHAM, J., GRAHAM, R. T., JOHRI, S., MACDONALD, C. C., PAIG-TRAN, E. W. M., ROCA, A. I., SCHWIETERMAN, G. D., WHITENACK, L. B., WILEY, T. R. & FERRY, L. A. 2022. What Can Professional Scientific Societies Do to Improve Diversity, Equity, and Inclusion: A Case Study of the American Elasmobranch Society. *Frontiers in Education,* 7.

SHIPLEY, O. N. & MATICH, P. 2020. Studying animal niches using bulk stable isotope ratios: An updated synthesis. *Oecologia,* 193**,** 27-51.

SILVA, J. F. & ELLIS, J. R. 2019. Bycatch and discarding patterns of dogfish and sharks taken in English and Welsh commercial fisheries. *Journal of Fish Biology,* 94**,** 966-980.

SILVA, M. A., BORRELL, A., PRIETO, R., GAUFFIER, P., BÉRUBÉ, M., PALSBØL, P. J. & COLAÇO, A. 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. *Royal Society open science,* 6**,** 181800.

SIMS, D., FOWLER, S., FERRETTI, F. & STEVENS, J. 2016. Prionace glauca (Regional Assessment: Mediterranean): The IUCN Red List of Threatened Species.

SKOMAL, G., MARSHALL, H., GALUARDI, B., NATANSON, L., BRAUN, C. & BERNAL, D. 2021. Horizontal and Vertical Movement Patterns and Habitat Use of Juvenile Porbeagles (Lamna nasus) in the Western North Atlantic. *Frontiers in Marine Science,* 8**,** 624158.

SMITH, N. S., CÔTÉ, I. M., MARTINEZ-ESTEVEZ, L., HIND-OZAN, E. J., QUIROS, A. L., JOHNSON, N., GREEN, S. J., CORNICK, L., SHIFFMAN, D., MALPICA-CRUZ, L., GLEASON BESCH, A. & SHIEL-ROLLE, N. 2017. Diversity and Inclusion in Conservation: A Proposal for a Marine Diversity Network. *Frontiers in Marine Science,* 4.

ST CLAIR, M. 2021. Sexual harassment in marine science. *Women in Ocean Science CIC Available online at: https://www.womeninoceanscience.com/sexual-harassment (accessed May 8, 2022)*.

ST JOHN GLEW, K., WANLESS, S., HARRIS, M. P., DAUNT, F., ERIKSTAD, K. E., STRØM, H., SPEAKMAN, J. R., KÜRTEN, B. & TRUEMAN, C. N. 2019. Sympatric Atlantic puffins and razorbills show contrasting responses to adverse marine conditions during winter foraging within the North Sea. *Movement ecology,* 7**,** 1-14.

STE-MARIE, E., WATANABE, Y. Y., SEMMENS, J. M., MARCOUX, M. & HUSSEY, N. E. 2022. Life in the slow lane: field metabolic rate and prey consumption rate of the Greenland shark (Somniosus microcephalus) modelled using archival biologgers. *Journal of Experimental Biology,* 225.

STEPHENS, P. A., BOYD, I. L., MCNAMARA, J. M. & HOUSTON, A. I. 2009. Capital breeding and income breeding: their meaning, measurement, and worth. *Ecology,* 90**,** 2057-2067.

STEVENS, J. 1973. Stomach contents of the blue shark (Prionace glauca L.) off south-west England. *Journal of the marine biological association of the United Kingdom,* 53**,** 357-361.

SULIKOWSKI, J. A., WHEELER, C. R., GALLAGHER, A. J., PROHASKA, B. K., LANGAN, J. A. & HAMMERSCHLAG, N. 2016. Seasonal and life-stage variation in the reproductive ecology of a marine apex predator, the tiger shark Galeocerdo cuvier, at a protected female-dominated site. *Aquatic Biology,* 24**,** 175-184.

SWEETING, C., JENNINGS, S. & POLUNIN, N. 2005. Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. *Functional Ecology,* 19**,** 777-784.

TAYLOR, W. A. 2000. Change-point analysis: a powerful new tool for detecting changes.

TER STEEGE, H., PITMAN, N. C., KILLEEN, T. J., LAURANCE, W. F., PERES, C. A., GUEVARA, J. E., SALOMÃO, R. P., CASTILHO, C. V., AMARAL, I. L. & DE ALMEIDA MATOS, F. D. 2015. Estimating the global conservation status of more than 15,000 Amazonian tree species. *Science advances,* 1**,** e1500936.

TESTERMAN, C. B. 2014. Molecular ecology of globally distributed sharks.

THE SHARK TRUST. 2022. *Historic Shark & Ray Listings at CITES CoP19* [Online]. Available: https://www.sharktrust.org/news/historic-shark-ray-listings-at-cites-cop19 [Accessed 27 January 2023].

THUSHARI, G. G. N. & SENEVIRATHNA, J. D. M. 2020. Plastic pollution in the marine environment. *Heliyon,* 6**,** e04709.

TILMAN, D., CLARK, M., WILLIAMS, D. R., KIMMEL, K., POLASKY, S. & PACKER, C. 2017. Future threats to biodiversity and pathways to their prevention. *Nature,* 546**,** 73-81.

TOOMEY, A. H., MARKUSSON, N., ADAMS, E. & BROCKETT, B. 2015. Inter-and trans-disciplinary research: a critical perspective. *GSDR Brief***,** 1-3.

TRICAS, T. C. 1979. Relationships of the blue shark, Prionace glauca, and its prey species near Santa Catalina Island, California. *Fish. Bull,* 77**,** 175-182.

TRUEMAN, C. N. & GLEW, K. S. J. 2019. Isotopic tracking of marine animal movement. *Tracking animal migration with stable isotopes.* Elsevier.

TRUEMAN, C. N., JACKSON, A. L., CHADWICK, K. S., COOMBS, E. J., FEYRER, L. J., MAGOZZI, S., SABIN, R. C. & COOPER, N. 2019. Combining simulation modeling and stable isotope analyses to reconstruct the last known movements of one of Nature's giants. *PeerJ,* 7**,** e7912.

TULLY, O., PALMA-PEDRAZA, S., CLARKE, M. & KEANE, J. 2021. The Distribution and Abundance of Elasmobranch Fish in Tralee, Brandon and Dingle Bays in 2018-2019. Marine Institute.

TURNER, L. M., BHATTA, R., ERIANDER, L., GIPPERTH, L., JOHANNESSON, K., KADFAK, A., KARUNASAGAR, I., KARUNASAGAR, I., KNUTSSON, P., LAAS, K., MOKSNES, P.-O. & GODHE, A. 2017. Transporting ideas between marine and social sciences: experiences from interdisciplinary research programs. *Elementa: Science of the Anthropocene,* 5.

UHLENBECK, G. E. & ORNSTEIN, L. S. 1930. On the theory of the Brownian motion. *Physical review,* 36**,** 823.

VASKE JÚNIOR, T., LESSA, R. P. & GADIG, O. B. F. 2009. Feeding habits of the blue shark (Prionace glauca) off the coast of Brazil. *Biota Neotropica,* 9**,** 55-60.

VEDOR, M., QUEIROZ, N., MUCIENTES, G., COUTO, A., COSTA, I. D., SANTOS, A. D., VANDEPERRE, F., FONTES, J., AFONSO, P., ROSA, R., HUMPHRIES, N. E. & SIMS, D. W. 2021. Climate-driven deoxygenation elevates fishing vulnerability for the ocean's widest ranging shark. *eLife,* 10**,** e62508.

VISEU, A. 2015. Integration of social science into research is crucial. *Nature,* 525**,** 291-291.

VOGT, R. C., SEVER, D. M. & MOREIRA, G. 1998. Esophageal Papillae in Pelomedusid Turtles. *Journal of Herpetology,* 32**,** 279-282.

WEARMOUTH, V. J. & SIMS, D. W. 2008. Sexual segregation in marine fish, reptiles, birds and mammals: behaviour patterns, mechanisms and conservation implications. *Advances in marine biology,* 54**,** 107-170.

WEIMERSKIRCH, H., LE CORRE, M., MARSAC, F., BARBRAUD, C., TOSTAIN, O. & CHASTEL, O. 2006. Postbreeding movements of frigatebirds tracked with satellite telemetry. *The Condor,* 108**,** 220-225.

WHITEMAN, J. P., ELLIOTT SMITH, E. A., BESSER, A. C. & NEWSOME, S. D. 2019. A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. *Diversity,* 11**,** 8.

WHITEMAN, J. P., NEWSOME, S. D., BUSTAMANTE, P., CHEREL, Y. & HOBSON, K. A. 2021. Quantifying capital vs. income breeding: new promise with stable isotope measurements of individual amino acids. *Journal of Animal Ecology*.

WHITLOCK, R. E., HAZEN, E. L., WALLI, A., FARWELL, C., BOGRAD, S. J., FOLEY, D. G., CASTLETON, M. & BLOCK, B. A. 2015. Direct quantification of energy intake in an apex marine predator suggests physiology is a key driver of migrations. *Science Advances,* 1**,** e1400270.

WICKHAM, H. 2016. Data analysis. *ggplot2.* Springer.

WOGERBAUER, C., O'REILLY, S., DOODY, C. & ROCHE, W. 2016. Recent data (2007–2013) from the Irish blue shark recreational fishery. *Collect Vol Sci Pap ICCAT,* 72**,** 1150-1166.

WOMERSLEY, F. C., HUMPHRIES, N. E., QUEIROZ, N., VEDOR, M., DA COSTA, I., FURTADO, M., TYMINSKI, J. P., ABRANTES, K., ARAUJO, G., BACH, S. S., BARNETT, A., BERUMEN, M. L., BESSUDO LION, S., BRAUN, C. D., CLINGHAM, E., COCHRAN, J. E. M., DE LA PARRA, R., DIAMANT, S., DOVE, A. D. M., DUDGEON, C. L., ERDMANN, M. V., ESPINOZA, E., FITZPATRICK, R., CANO, J. G., GREEN, J. R., GUZMAN, H. M., HARDENSTINE, R., HASAN, A., HAZIN, F. H. V., HEARN, A. R., HUETER, R. E., JAIDAH, M. Y., LABAJA, J., LADINO, F., MACENA, B. C. L., MORRIS, J. J., NORMAN, B. M., PEÑAHERRERA-PALMA, C., PIERCE, S. J., QUINTERO, L. M., RAMÍREZ-MACÍAS, D., REYNOLDS, S. D., RICHARDSON, A. J., ROBINSON, D. P., ROHNER, C. A., ROWAT, D. R. L., SHEAVES, M., SHIVJI, M. S., SIANIPAR, A. B., SKOMAL, G. B., SOLER, G., SYAKURACHMAN, I., THORROLD, S. R., WEBB, D. H., WETHERBEE, B. M., WHITE, T. D., CLAVELLE, T., KROODSMA, D. A., THUMS, M., FERREIRA, L. C., MEEKAN, M. G., ARROWSMITH, L. M., LESTER, E. K., MEYERS, M. M., PEEL, L. R., SEQUEIRA, A. M. M., EGUÍLUZ, V. M., DUARTE, C. M. & SIMS, D. W. 2022. Global collision-risk hotspots of marine traffic and the world's largest fish, the whale shark. *Proceedings of the National Academy of Sciences,* 119**,** e2117440119.

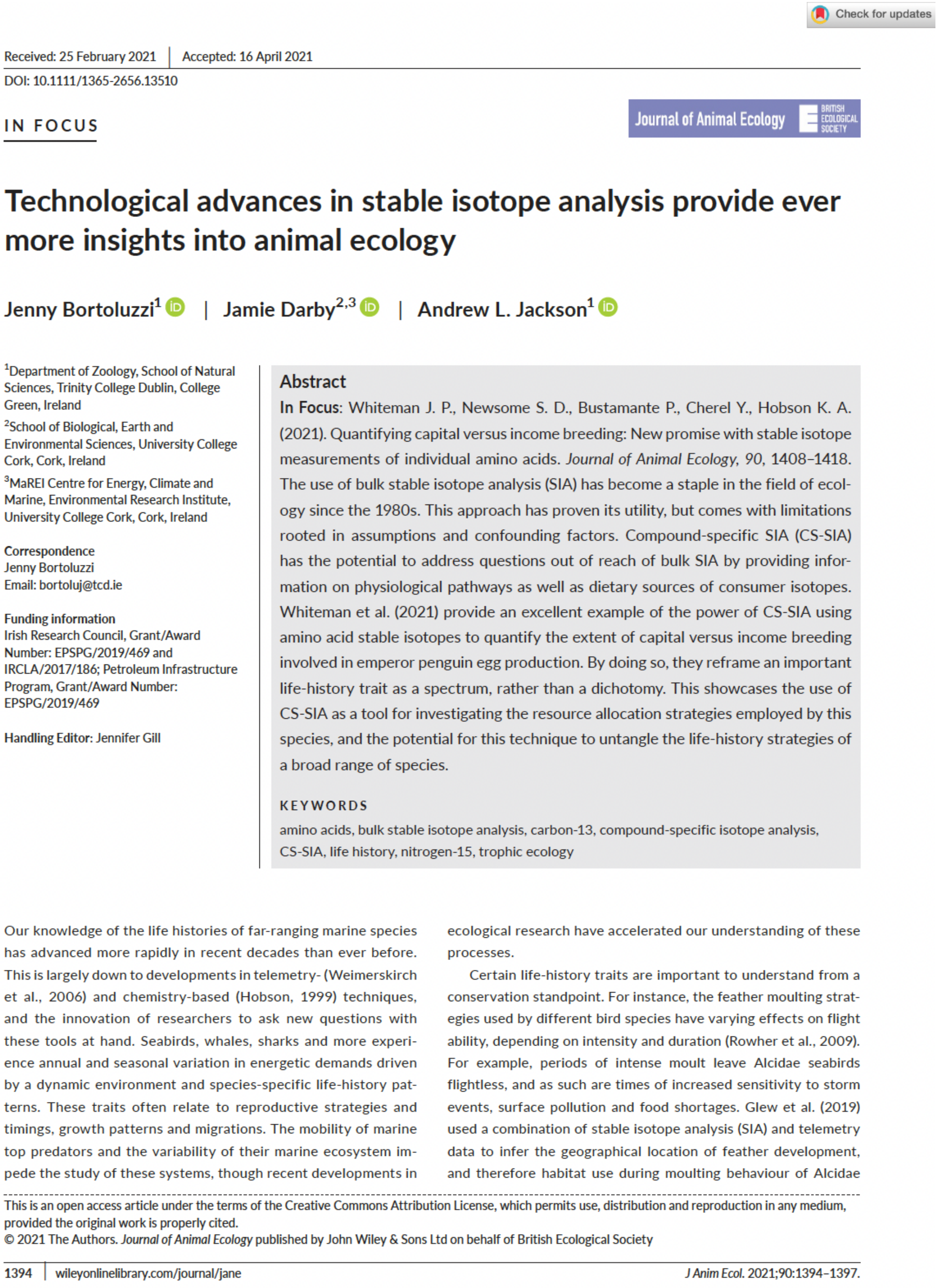
WORM, B. & LOTZE, H. K. 2021. Marine biodiversity and climate change. *Climate change.* Elsevier.

YAN, H. F., KYNE, P. M., JABADO, R. W., LEENEY, R. H., DAVIDSON, L. N. K., DERRICK, D. H., FINUCCI, B., FRECKLETON, R. P., FORDHAM, S. V. & DULVY, N. K. 2021. Overfishing and habitat loss drive range contraction of iconic marine fishes to near extinction. *Science Advances,* 7**,** eabb6026.

YEAKEL, J. D., BHAT, U., ELLIOTT SMITH, E. A. & NEWSOME, S. D. 2016. Exploring the isotopic niche: Isotopic variance, physiological incorporation, and the temporal dynamics of foraging. *Frontiers in Ecology and Evolution,* 4**,** 1.

YOKOI, H., IJIMA, H., OHSHIMO, S. & YOKAWA, K. 2017. Impact of biology knowledge on the conservation and management of large pelagic sharks. *Scientific reports,* 7**,** 1-14.

Publications



Check for updates

Received: 25 February 2021 | Accepted: 16 April 2021

DOI: 10.1111/1365-2656.13510

IN FOCUS

Journal of Animal Ecology BRITISH ECOLOGICAL SOCIETY

Technological advances in stable isotope analysis provide ever more insights into animal ecology

Jenny Bortoluzzi[1] | **Jamie Darby**[2,3] | **Andrew L. Jackson**[1]

[1]Department of Zoology, School of Natural Sciences, Trinity College Dublin, College Green, Ireland

[2]School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland

[3]MaREI Centre for Energy, Climate and Marine, Environmental Research Institute, University College Cork, Cork, Ireland

Correspondence
Jenny Bortoluzzi
Email: bortoluj@tcd.ie

Funding information
Irish Research Council, Grant/Award Number: EPSPG/2019/469 and IRCLA/2017/186; Petroleum Infrastructure Program, Grant/Award Number: EPSPG/2019/469

Handling Editor: Jennifer Gill

Abstract

In Focus: Whiteman J. P., Newsome S. D., Bustamante P., Cherel Y., Hobson K. A. (2021). Quantifying capital versus income breeding: New promise with stable isotope measurements of individual amino acids. *Journal of Animal Ecology*, *90*, 1408–1418. The use of bulk stable isotope analysis (SIA) has become a staple in the field of ecology since the 1980s. This approach has proven its utility, but comes with limitations rooted in assumptions and confounding factors. Compound-specific SIA (CS-SIA) has the potential to address questions out of reach of bulk SIA by providing information on physiological pathways as well as dietary sources of consumer isotopes. Whiteman et al. (2021) provide an excellent example of the power of CS-SIA using amino acid stable isotopes to quantify the extent of capital versus income breeding involved in emperor penguin egg production. By doing so, they reframe an important life-history trait as a spectrum, rather than a dichotomy. This showcases the use of CS-SIA as a tool for investigating the resource allocation strategies employed by this species, and the potential for this technique to untangle the life-history strategies of a broad range of species.

KEYWORDS

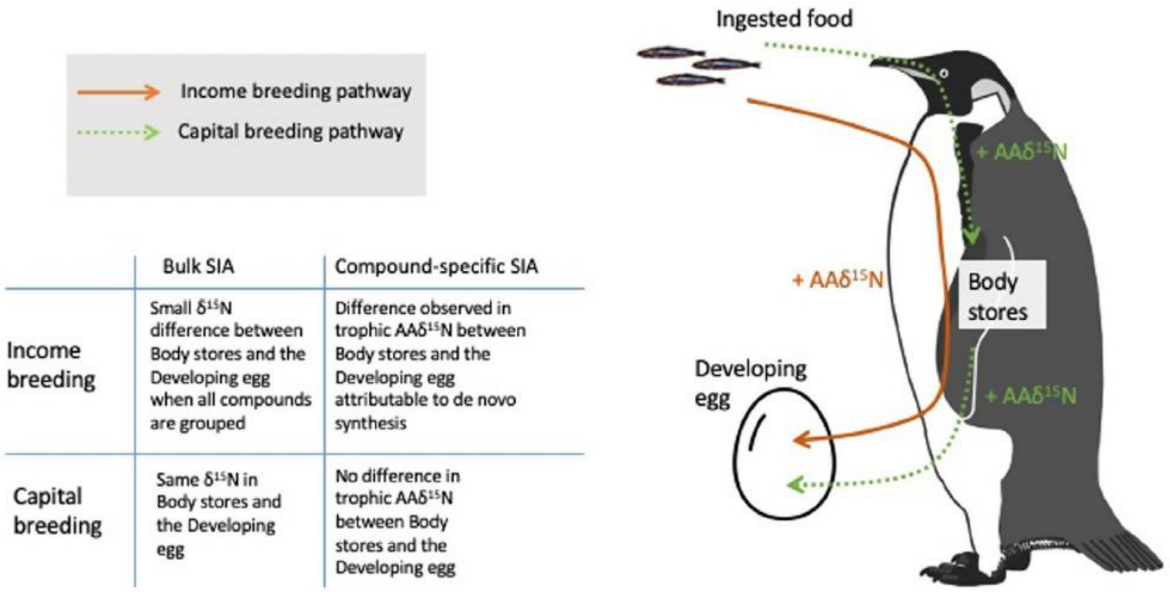
amino acids, bulk stable isotope analysis, carbon-13, compound-specific isotope analysis, CS-SIA, life history, nitrogen-15, trophic ecology

Our knowledge of the life histories of far-ranging marine species has advanced more rapidly in recent decades than ever before. This is largely down to developments in telemetry- (Weimerskirch et al., 2006) and chemistry-based (Hobson, 1999) techniques, and the innovation of researchers to ask new questions with these tools at hand. Seabirds, whales, sharks and more experience annual and seasonal variation in energetic demands driven by a dynamic environment and species-specific life-history patterns. These traits often relate to reproductive strategies and timings, growth patterns and migrations. The mobility of marine top predators and the variability of their marine ecosystem impede the study of these systems, though recent developments in ecological research have accelerated our understanding of these processes.

Certain life-history traits are important to understand from a conservation standpoint. For instance, the feather moulting strategies used by different bird species have varying effects on flight ability, depending on intensity and duration (Rowher et al., 2009). For example, periods of intense moult leave Alcidae seabirds flightless, and as such are times of increased sensitivity to storm events, surface pollution and food shortages. Glew et al. (2019) used a combination of stable isotope analysis (SIA) and telemetry data to infer the geographical location of feather development, and therefore habitat use during moulting behaviour of Alcidae seabirds in the North Sea. Another example of SIA being used to study important variations in life-history traits comes from Silva et al. (2019), who applied SIA to the skin of Balaenoptera whales to explore their diet and destinations during their winter migration. Taking advantage of the fact that the stable isotopes in the skin are thought to be incorporated over approximately the previous 5 months (Busquets-Vass et al., 2017), the study was able to use skin samples collected in spring to recount the whales' activities over the previous winter. Fish stocks and areas targeted by these whales are identified, and then related back to their population dynamics. These insights may allow for the designation of better directed conservation measures where necessary. Given the mobility of many marine top predators, and the remoteness of their habitats, these features of their ecology would remain a mystery without the development of indirect inference techniques such as SIA.

With SIA, ecologists have worked for years to map food webs and the relationships between organisms in communities. Bulk SIA has long been the basis of such studies: it considers the overall isotopic composition of whole tissues, organisms or soils. The studies described above from Glew et al. (2019) and Silva et al. (2019) used bulk SIA. This requires some prior knowledge or assumptions of stable isotope baselines found in the environment. The isotopic composition of marine primary producers varies spatially, especially in carbon isotopes, and the resulting spatial distribution is known as an isoscape (McMahon et al., 2013). The development of animal tissues can therefore be traced back to the area of an isoscape it was produced in, inferring the broad-scale movements of mobile consumers (Trueman & Glew, 2019). In migratory species such as cetaceans, movements across those isoscapes can be traced in incremental tissue like baleen (Ryan et al., 2013; Trueman et al., 2019). This window into annual variations, beyond that given by short-term tissue samples, provides information on the past ecology of these individuals (sometimes going back decades or centuries by using museum collections, for example). This can be highly significant to understand shifts in ecological behaviours caused by anthropogenic pressures and changing environments and, in turn, allows us to make predictions about the future. But the results of these studies using bulk SIA are often hampered by necessary assumptions and uncertainties. Fundamentally incomplete knowledge of environmental baseline isotopes can stymy the construction of the isoscapes in the first place due to the unknown isotopic effects of physiological pathways and temporal averaging between primary producers and prey (Trueman et al., 2019).

Compound-specific SIA (CS-SIA), on the other hand, is a molecular-level approach that involves the analysis of individual amino acid or fatty acid stable isotopes, and builds on the principles used in bulk SIA (Boecklen et al., 2011). Advancements in technology mean that it is now easier to study isotopes at a molecular level using gas chromatography or liquid chromatography systems in conjunction with



	Bulk SIA	Compound-specific SIA
Income breeding	Small δ^{15}N difference between Body stores and the Developing egg when all compounds are grouped	Difference observed in trophic AAδ^{15}N between Body stores and the Developing egg attributable to de novo synthesis
Capital breeding	Same δ^{15}N in Body stores and the Developing egg	No difference in trophic AAδ^{15}N between Body stores and the Developing egg

FIGURE 1 Compound-specific stable-isotopes analysis (CS-SIA) gives a greater degree of accuracy when discussing physiological processes. Here, we explain the basis for the Whiteman et al. (2021) paper that seeks to identify stable-isotope markers that describe capital (pathway 1, green dotted line) and income (pathway 2, orange solid line) breeding. Pathway 1 increases the δ^{15}N of trophic amino acids (AAs) twice before they reach the egg, once through initial metabolism into muscle tissue, and then again through the secondary metabolism of this muscle tissue to mobilise these nutrients for egg formation. Pathway 2 only metabolises these AAs once, on initial digestion, thus the δ^{15}N of trophic AAs in both muscle and egg are the same. CS-SIA can identify the extent to which each pathway is used by targeting these trophic AAs specifically. Bulk SIA does not detect such fine differences, and it is performed on all compounds at once, some of which don't change in δ^{15}N when metabolised, such as essential AAs

mass spectrometers (Boecklen et al., 2011). This approach potentially provides greater resolution of SIA applicable in trophic ecology, and allows us to study the transfer of nutrients at a physiological level rather than solely at an ecosystem level. One of the key concepts is that it potentially provides a shortcut to accessing indirectly the underlying isotopic baselines by comparing variation between the signatures of key amino acids in the consumer itself.

Whiteman et al. (2021) use CS-SIA for this purpose very effectively to examine the underlying physiological mechanisms used by female emperor penguins to create a single large egg. More specifically, they look at the allocation of exogenous and endogenous stores of food into the egg by comparing the stable isotope composition of adult muscle and corresponding egg tissues. Using CS-SIA, the results are much finer than with bulk SIA, as the effect of the penguin's metabolism on the isotopic composition of essential versus non-essential amino acids is quantifiably different. This allowed the team to trace the source of amino acids in the egg to freshly sourced food, or reallocated stored resources (Figure 1).

In 1980, Drent and Daan introduced two models to describe this resource allocation in egg-forming birds. By looking at the relationship between mass gain in female birds prior to breeding, laying date and clutch size, they proposed two opposing strategies for reproduction: capital and income breeding (Drent & Daan, 1980). Females have to strike a balance between laying at a favourable time, often as early as possible in the breeding season, and amassing and storing enough energy through foraging before laying. The models describe capital breeders as accumulating energy in body reserves while income breeders redirect nutrients and energy straight from the food ingested (Meijer & Drent, 1999). This difference in resource allocation and how it relates to reproduction and growth under different ecological scenarios are key questions in the study of life histories (Stephens et al., 2009).

Clearly, the effective allocation of nutrients for breeding purposes is a huge factor in the reproductive success of a population (e.g. Kitaysky et al., 2000). Many far-ranging seabirds undertake a pre-laying exodus just before breeding, sometimes spending weeks in productive waters far from their breeding colonies. More often than not, females will spend longer away from the colony than males in this time, presumably amassing the nutrients required to create an egg (Brook, 1990). Male emperor penguins need to store energy reserves required to fast for several months while incubating an egg, allowing females to abscond to replenish nutrients spent on egg development (Burns & Kooyman, 2001). CS-SIA has the potential to provide valuable insights into the physiological processes and ecological context involved in this resource management and in a system that is challenging to study. As Whiteman et al. (2021) asked, do egg-forming birds rely on long-term reserves when forming eggs, or does most of the egg-building material come directly from ingested food? Such information can be linked to the fecundity of a population. What foods are most important during this critical period in the reproductive cycle? Are they amassed over the entire non-breeding period (capital breeding) or are species reliant on seasonal availability of adequate resources (income breeding)? Can reproductive outputs of a population be tied to the availability of these food sources? These questions can be adapted for a range of taxa, especially those that invest heavily in reproduction, as seabirds do. Whiteman et al. (2021) clearly demonstrated how powerful CS-SIA analysis can be to deliver more detailed information on these critical topics.

ACKNOWLEDGEMENTS

A.L.J. and J.B. were funded by Irish Research Council Laureate Award IRCLA/2017/186 to A.L.J. The position of J.D. is funded by the Irish Research Council and the Petroleum Infrastructure Program, award EPSPG/2019/469. Open access funding provided by IReL.

AUTHORS' CONTRIBUTIONS

J.B. led the writing with strong contributions from J.D. and supervision by A.L.J.

ORCID

Jenny Bortoluzzi https://orcid.org/0000-0002-0496-5358
Jamie Darby https://orcid.org/0000-0002-9757-7150
Andrew L. Jackson https://orcid.org/0000-0001-7334-0434

REFERENCES

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 411–440. https://doi.org/10.1146/annurev-ecolsys-102209-144726

Brook, M. (1990). The manx shearwater poyser. In *Polar record* (Vol. *26*, pp. 246–248). T. A. Poyser and Academic Press Ltd. https://doi.org/10.1017/S0032247400011633

Burns, J. M., & Kooyman, G. L. (2001). Habitat use by Weddell seals and emperor penguins foraging in the Ross Sea, Antarctica. *American Zoologist*, *41*, 90–98.

Busquets-Vass, G., Newsome, S. D., Calambokidis, J., Serra-Valente, G., Jacobsen, J. K., Aguíñiga-García, S., & Gendron, D. (2017). Estimating blue whale skin isotopic incorporation rates and baleen growth rates: Implications for assessing diet and movement patterns in mysticetes. *PLoS One*, *12*, e0177880. https://doi.org/10.1371/journal.pone.0177880

Drent, R., & Daan, S. (1980). The prudent parent: Energetic adjustments in avian breeding 1. *Ardea*, *55*, 225–252.

Glew, K. S. J., Wanless, S., Harris, M. P., Daunt, F., Erikstad, K. E., Strøm, H., Speakman, J. R., Kürten, B., & Trueman, C. N. (2019). Sympatric Atlantic puffins and razorbills show contrasting responses to adverse marine conditions during winter foraging within the North Sea. *Movement Ecology*, *7*, 1–14.

Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: A review. *Oecologia*, *120*, 314–326. https://doi.org/10.1007/s004420050865

Kitaysky, A. S., Hunt, G. L. Jr, Flint, E. N., Rubega, M. A., & Decker, M. B. (2000). Resource allocation in breeding seabirds: Responses to fluctuations in their food supply. *Marine Ecology Progress Series*, *206*, 283–296.

McMahon, K. W., Hamady, L. L., & Thorrold, S. R. (2013). A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnology and Oceanography*, *58*, 697–714. https://doi.org/10.4319/lo.2013.58.2.0697

Meijer, T., & Drent, R. (1999). Re-examination of the capital and income dichotomy in breeding birds. *Ibis*, *141*, 399–414. https://doi.org/10.1111/j.1474-919X.1999.tb04409.x

Rowher, S., Ricklefs, R. E., Rohwer, V. G., & Copple, M. M. (2009). Allometry of the duration of flight feather moult in birds. *PLOS Biology*, *7*, e1000132.

Ryan, C., McHugh, B., Trueman, C. N., Sabin, R., Deaville, R., Harrod, C., Berrow, S. D., & Ian, O. (2013). Stable isotope analysis of baleen reveals resource partitioning among sympatric rorquals and population structure in fin whales. *Marine Ecology Progress Series*, *479*, 251–261. https://doi.org/10.3354/meps10231

Silva, M. A., Borrell, A., Prieto, R., Gauffier, P., Bérubé, M., Palsbøl, P. J., & Colaço, A. (2019). Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. *Royal Society Open Science*, *6*, 181800. https://doi.org/10.1098/rsos.181800

Stephens, P. A., Boyd, I. L., McNamara, J. M., & Houston, A. I. (2009). Capital breeding and income breeding: Their meaning, measurement, and worth. *Ecology*, *90*, 2057–2067.

Trueman, C. N., & Glew, K. S. J. (2019). Isotopic tracking of marine animal movement. In *Tracking animal migration with stable isotopes* (pp. 137–172). Academic Press. https://doi.org/10.1016/B978-0-12-814723-8.00006-4

Trueman, C. N., Jackson, A. L., Chadwick, K. S., Coombs, E. J., Feyrer, L. J., Magozzi, S., Sabin, R. C., & Cooper, N. (2019). Combining simulation modeling and stable isotope analyses to reconstruct the last known movements of one of Nature's giants. *PeerJ*, *7*, e7912. https://doi.org/10.7717/peerj.7912

Weimerskirch, H., Le Corre, M., Marsac, F., Barbraud, C., Tostain, O., & Chastel, O. (2006). Postbreeding movements of frigatebirds tracked with satellite telemetry. *The Condor*, *108*, 220–225.

Whiteman, J. P., Newsome, S. D., Bustamante, P., Cherel, Y., & Hobson, K. A. (2021). Quantifying capital versus income breeding: New promise with stable isotope measurements of individual amino acids. *Journal of Animal Ecology*, *90*, 1408–1418. https://doi.org/10.1111/1365-2656.13402

How to cite this article: Bortoluzzi J, Darby J, Jackson AL. Technological advances in stable isotope analysis provide ever more insights into animal ecology. *J Anim Ecol*. 2021;90:1394–1397. https://doi.org/10.1111/1365-2656.13510

Conservation Physiology
Volume 10 • 2022 10.1093/conphys/coac065
SOCIETY FOR EXPERIMENTAL BIOLOGY
Research article

Capture heats up sharks

Lucy Harding[1,*], Austin Gallagher[2], Andrew Jackson[1], Jenny Bortoluzzi[1], Haley R Dolton[1], Brendan Shea[2], Luke Harman[3], David Edwards[4] and Nicholas Payne[1]

[1]Department of Zoology, Trinity College Dublin, D02 PN40, Ireland
[2]Beneath the Waves, PO BOX 126, Herndon, VA 20172, USA
[3]School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork, T23 N73K, Ireland
[4]West Cork Charters, Shannonvale, Clonakilty, Co. Cork, , P85 FV00, Ireland

***Corresponding author:** Lucy Harding, Department of Zoology, Trinity College Dublin, Ireland. E-mail: hardinl@tcd.ie

Catch-and-release fishing is an important component of ecotourism industries and scientific research worldwide, but its total impact on animal physiology, health and survival is understudied for many species of fishes, particularly sharks. We combined biologging and blood chemistry to explore how this fisheries interaction influenced the physiology of two widely distributed, highly migratory shark species: the blue shark (*Prionace glauca*) and the tiger shark (*Galeocerdo cuvier*). Nineteen sharks were caught by drum line or rod-and-reel angling; subcutaneous body temperature measurements were taken immediately upon capture, with six individuals also providing subsequent subcutaneous body temperature measurements via biologging as they swam freely for several hours post-release. We found that short-term capture caused shark body temperature to increase significantly and rapidly, with increases of 0.6°C–2.7°C for blue sharks (mean, 1.2 ± 0.6°C) and 0.5°C–0.9°C for tiger sharks (mean, 0.7 ± 0.2°C) and with capture-induced heating rates of blue sharks averaging 0.3°C min^{-1} but as high as 0.8°C min^{-1}. Blue shark body temperature was even higher deeper into the white muscle. These heating rates were three to eight times faster than maximum rates encountered by our biologging sharks swimming through thermally stratified waters and faster than most acute heating experiments conducted with ectotherms in laboratory experiments. Biologging data showed that body temperatures underwent gradual decline after release, returning to match water temperatures 10–40 mins post-release. Blood biochemistry showed variable lactate/glucose levels following capture; however, these concentrations were not correlated with the magnitude of body temperature increase, nor with body size or hooking time. These perturbations of the natural state could have immediate and longer-term effects on the welfare and ecology of sharks caught in catch-and-release fisheries and we encourage further study of the broader implications of this reported phenomenon.

Key words: thermal ecology, shark, physiology, catch-and-release, capture, body temperature

Editor: Dr. Steven Cooke

Received 29 April 2022; Revised 26 August 2022; Editorial Decision 1 September 2022; Accepted 9 September 2022

Cite as: Harding L, Gallagher A, Jackson A, Bortoluzzi J, Dolton HR, Shea B, Harman L, Edwards D, Payne N (2022) Capture heats up sharks. *Conserv Physiol* 10(1): coac065; doi:10.1093/conphys/coac065.

Introduction

Catch-and-release fishing is a popular practice in recreational fisheries (Cooke and Schramm, 2007; Danylchuk *et al.*, 2014; Brownscombe *et al.*, 2017) and is often used in scientific research (Wosnick *et al.*, 2018)—in particular, tagging studies (Watanabe *et al.*, 2015; Royer *et al.*, 2020; Harding *et al.*, 2021). Catch-and-release can also occur on commercial fishing vessels where it has been estimated that ~9.1 million tonnes of non-target fish per year globally are caught and released in commercial fisheries (Sepulveda *et al.*, 2019; Gilman *et al.*, 2020). Numerous studies have investigated the physiological and ecological impacts of catch-and-release fishing on teleosts and elasmobranchs (Cooke and Schramm, 2007; Cooke *et al.*, 2013; Gale *et al.*, 2013; Gallagher *et al.*, 2014; Schlenker *et al.*, 2016; Jerome *et al.*, 2017; Gallagher *et al.*, 2019; Sepulveda *et al.*, 2019; Mohan *et al.*, 2020). Catch-and-release fishing is considered a multi-stressor interaction for individuals (Gale *et al.*, 2013), with the negative impacts broadly categorised as lethal or sub-lethal, with potential sub-lethal impacts including altered blood chemistry (Gallagher *et al.*, 2014; Dapp *et al.*, 2016), behavioural impairments (Raoult *et al.*, 2019), reduced growth and reproductive rates and increased disease vulnerability (Gale *et al.*, 2013). Fish can experience oxygen deprivation (Schwieterman *et al.*, 2021), handling-related injuries (Cooke *et al.*, 2013; French *et al.*, 2015) and heat stress (Wosnick *et al.*, 2018, 2019).

Many studies investigating the impacts of catch-and-release fishing have highlighted the potential importance of temperature in these interactions (Meka and McCormick, 2005; Gale *et al.*, 2013; Schlenker *et al.*, 2016; Bouyoucos *et al.*, 2018; Schwieterman *et al.*, 2021), whereby water temperature (T_a) can have a significant effect on post-release survival and a variety of sub-lethal impacts (Gale *et al.*, 2013). Furthermore, body temperature (T_b) is considered an important parameter when assessing an individual's response to stress, their metabolic rate and their energy budgets; all of which may be affected by catch-and-release interactions. However, few studies have measured fish body temperatures during catch-and-release. Measurements of muscle temperature are especially rare, and while two recent studies have inferred surface temperature of sharks' post-capture using thermal imaging of shark skin (Wosnick *et al.*, 2018, 2019), reflectance issues and effects of varying sun and water exposure likely complicate estimates of true operative body temperatures. Sharks are relatively large-bodied fishes with low heat-transfer coefficients (Nakamura *et al.*, 2020) so capture-induced heating could be a particularly relevant issue for them.

When hooked, sharks often exhibit intensive swimming acceleration (Gallagher *et al.*, 2017) and periods of exhaustive exercise (i.e. anaerobic exercise) (Schwieterman *et al.*, 2021), as they attempt to escape. Capture-related exercise and the resulting physiological stress can elevate the metabolic rate (Kieffer, 2000; Mohan *et al.*, 2020) resulting in, among other things, the generation of heat. Furthermore, fish are known to dissipate heat across the gill surface during respiration (Stevens and Fry, 1974; Stevens, 2011; Nakamura *et al.*, 2020) and so during the period of fighting on the hook, these animals may be unable to swim freely, which could provide a mechanism for reduction in heat loss. This excess heat may manifest as an elevation of body temperature in these animals and thus, this study aims to investigate this relationship and determine the extent to which sharks heat up when captured.

In addition to altered thermal dynamics, hooked fish may undergo altered blood biochemistry as a result of the event (Gallagher *et al.*, 2014; Dapp *et al.*, 2016; Mohan *et al.*, 2020; Schwieterman *et al.*, 2021). Capture can often result in hyperkalemia, metabolic and respiratory acidosis (Schwieterman *et al.*, 2021) and declines in blood oxygen concentration, as a result of the often exhaustive exercise and apneic asphyxia associated with capture (Schwieterman *et al.*, 2021). Lactate and glucose are two metabolites widely examined in studies investigating stress in sharks (Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001; Mandelman and Skomal, 2008; Gallagher *et al.*, 2014, 2017). Lactate is a metabolite that is produced anaerobically in the white muscle during exhaustive exercise (Moyes *et al.*, 2006). Glucose is measured as a proxy for the glucocorticoid hormone stress response whereby hepatic glycogen is converted to glucose during gluconeogenesis and released to fuel muscle tissues (Hoffmayer and Parsons, 2001; Prohaska *et al.*, 2021). Body temperature and lactate and glucose are both key elements of metabolic processes. Therefore, by combining our investigations into the body temperature measurements with the blood biochemistry data, we can explore how the two might be related during catch-and-release events.

Materials and Methods

Data collection

To properly evaluate body temperature dynamics in captured marine predatory fishes, we incorporated all phases of the capture and release interaction, from moment of hooking to several hours post-release.

Biologging

We used biologging technology to collect fine-scale, physiological measurements from individuals free-swimming in the wild. Fishing was conducted across three locations: the Bahamas in May 2019, Cape Cod, USA in September 2019 and Co. Cork, Ireland in July–October 2021. We captured tiger sharks by drum lines and blue sharks by rod-and-reel angling. Tiger sharks were secured alongside the boat (remaining submerged during the procedure) and blue sharks were brought on deck with a deck hose placed in the mouth to constantly irrigate the gills with water taken from the ocean surface. Biologging packages were fitted to the first dorsal fin of four tiger sharks (*Galeocerdo cuvier*) and two blue sharks (*Prionace glauca*) (Supplementary Table S1), which were then immediately released. A plastic cable was passed through two 1-cm incisions made in the dorsal fin, which was connected to a dissolvable time-release mechanism that secured the package to the fin. Biologging packages included a mixture of loggers such as accelerometers (recording tri-axial acceleration at 25 Hz and depth at 1 Hz; Techno-Smart AGM-1; 67 × 42 × 19 mm), animal-borne digital cameras (recording at 30 fps; Little Leonardo DVL400M065; 61 × 21 × 15 mm; 29 g in air; 4 x red LED lights) and temperature loggers (recording ambient and body temperature at 1 Hz; Lotek LAT 1810; 11 × 38 mm, 7.6 g in air; Wildlife Computers Mk9; 72 × 17 × 17 mm, 34 g in air). Body temperature was measured via a sensor stalk inserted 4–8 cm into the dorsal musculature of the shark, adjacent to the dorsal fin. To enable retrieval, tag packages also included a VHF transmitter (Advanced Telemetry Systems, MM100) and satellite position-only tag (Wildlife Computers Model 258; ARGOS enabled). Once the time-release mechanism dissolved, the package detached from the fish and floated to the surface, as they were constructed of a positively buoyant material (Diab Syntactic © non-compressible foam). Packages were then located using the ARGOS system and a VHF receiver and retrieved from the ocean surface by boat. A total of four tiger sharks (ranging 155.7–206 kg body mass) and two blue sharks (ranging 25.4–30.7 kg body mass) were tagged.

Additional body temperature measurements of blue sharks

In addition to the biologging work, we collected body temperature measurements from 13 additional blue sharks, from the moment they were landed on deck, until the moment before release (Supplementary Table S1). Temperature probes were fitted to the sharks using the same method as the biologging. The temperature probe was left in place while a work-up was completed on the shark (e.g. recording biometrics, collecting blood samples, etc). Once the work-up was completed, the temperature probe was removed from the muscle and the shark was released. Water temperature was recorded by subsequently placing the temperature logger in the surface water for approx. 2 mins. Additionally, secondary body temperature measurements were taken from 4 of these 12 sharks (i.e. BS32–BS35) to generate a thermal profile of the shark's musculature. To do this, the temperature probe was inserted at a depth of 2 cm into the dorsomusculature, ~20 cm more anterior and closer to the gills than the first probe, left for 1–2 mins, pushed a further 2 cm into the dorsomusculature (to measure body temperature at 4 cm depth) and left for the remaining period of time that the shark was held on deck. The probe was then removed from the shark prior to release.

Caudal peduncle blood sampling

For 14 blue sharks, two blood samples were collected from the caudal peduncle using an 18-gauge needle: one immediately upon capture and one the moment before release (~10 mins later), corresponding with the timing of insertion and removal of the temperature stalk. The time of collection was recorded, and the samples were processed for lactate and glucose immediately after collection on deck, using a HaB direct Lactate Pro™ 2 lactate meter (HaB Direct, 2021) and Accu-Chek Performa blood glucose meter (Accu-Chek, 2021), respectively. A number of lactate/glucose readings could not be recorded due to logistical constraints and/or and user error.

Ethics

All works carried out in Ireland were conducted under The Health Products Regulatory Authority (HPRA) Project Authorisation (AE19136/P127). All works carried outside of Ireland were conducted under local licensing, obtained by Beneath the Waves.

Data analyses

Data handling and statistical analyses were carried out in R Version 4.0.3 (R Core Team, 2020) and IGOR Pro 8 (WaveMetrics, 2020) with Ethographer package (Sakamoto *et al.*, 2009). Time-series analyses were conducted on all biologging data with a focus on the body temperature, water temperature and depth data. A 500-point moving average smoother was applied to the temperature and depth data when plotting the time series.

Upon capture—meaning the moment individuals were landed on deck or secured alongside the boat—the metric 'ΔT' was calculated as the difference between body temperature T_b and water temperature T_a. The total 'hooking time' for each blue shark was calculated (i.e. the time from when the shark was hooked, to when it was brought on deck). Hooking time could not be calculated for the tiger sharks as they were caught using drum lines. Body mass was estimated for each individual using the length–weight relationship $W = aL^b$ where W is weight/body mass (kg), L is fork length (cm) and a and b are published, species-specific coefficients with $a = 3.184 \times 10^{-6}$ and $b = 3.1313$ for blue sharks and $a = 2.528 \times 10^{-6}$ and $b = 3.2603$ for tiger sharks (Kohler *et al.*, 1995).

Multiple Gaussian generalised linear models were conducted to investigate potential relationships between ΔT and hooking time, body mass, lactate concentration and glucose concentration (Supplementary Table S2). Initial and subsequent lactate and glucose concentrations were compared in order to investigate potential lags in stress biomarker elevations following capture.

Results

A total of 19 sharks were caught: 4 tiger sharks and 15 blue sharks. Biologging data were collected from 6 of these individuals (4 tiger sharks and 2 blue sharks), and blood samples were taken from 14 blue sharks and no tiger sharks (Supplementary Table S1). Subsequently, one individual (tiger shark, T2) was excluded from all analyses as it was found to exhibit faulty biologging data.

At the moment of capture, all sharks across both species exhibited elevated T_b relative to T_a (Fig. 1A and B), with elevations ranging from 0.6°C to 2.7°C for blue sharks (mean ± SD = 1.2 ± 0.6°C) and 0.5°C to 0.9°C for tiger sharks (mean ± SD = 0.7 ± 0.2°C) (Supplementary Table S1).

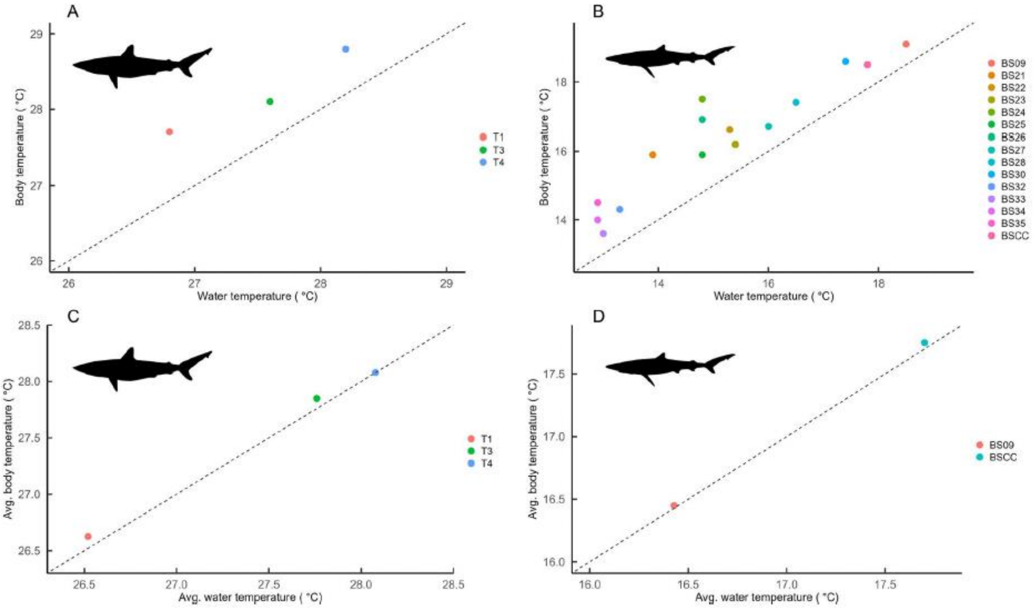


Figure 1: Body temperature elevation of tiger sharks and blue sharks recorded immediately upon capture (panels A and B) and average elevation of body temperature, during a 30-minute period under natural conditions several hours after release (panels C and D). Dashed black lines indicate a 1:1 identity line. (Blue shark image created by Ignacio Contreras and reproduced under the Creative Commons Attribution 3.0 Unported license: https://creativecommons.org/licenses/by/3.0/legalcode).

This elevation in temperature occurred over short periods of time (i.e. hooking time), which ranged from 1.4 to 13 mins for blue sharks ($n = 15$), indicating that warming occurred at an estimated rate of 0.1–0.8°C min^{-1} (mean ± SD = 0.3 ± 0.23°C min^{-1}). These warming rates were unusually high when compared with the post-release data taken from biologged blue sharks BS09 and BSCC (Fig. 2). Free-swimming blue sharks rarely exhibited heating rates greater than 0.1°C min^{-1} (Fig. 2).

The additional body temperature measurements taken from individuals BS32–BS35 revealed a further increase in body temperature, the deeper the temperature probe was inserted, with the difference between readings at 2 cm penetration and 4 cm penetration ranging from 0.20°C to 0.45°C (Fig. 3). The relationship between water temperature and deep muscle temperature was approximately linear despite a somewhat variable relationship between water temperature and shallow muscle temperature (Fig. 3), perhaps representing an uncertainty as to what depth and therefore temperature these sharks were initially hooked at. For all sharks equipped with biologging packages and subsequently released, following the initial elevation of T_b upon capture, T_b underwent a gradual decline, over the first 30 mins for tiger sharks and 15 mins for blue sharks, before returning to match water temperature. During this period of body temperature decline, there was no corresponding decline in water temperature or depth (Fig. 4). Several hours after release, T_b essentially matched T_a during extended periods (Fig. 1C and D).

Regression analyses showed hooking time has no significant influence on ΔT (Fig. 5A; $P = 0.44$); log-transformed body mass has no significant influence on ΔT (Fig. 5B; $P = 0.12$); lactate concentration has no significant influence on ΔT (Fig. 5C; $P = 0.45$); and glucose concentration has no significant influence on ΔT ($P = 0.34$). Multiple linear regressions with ΔT as the response variable and hooking time, body mass, lactate concentration and glucose concentration as predictor variables showed no significant relationships (see Supplementary Table S2, for more details).

Blue shark blood analysis showed lactate levels upon capture ranged from 1.2 to 9.0 mmol l^{-1} (mean = 3.1 mmol l^{-1}, $n = 13$). Following time on deck lactate levels ranged from 2.9 to 9.0 mmol l^{-1} (mean = 5.4 mmol l^{-1}, $n = 11$). Furthermore, 9 out of 11 blue sharks tested twice showed increased lactate levels following time on deck (mean

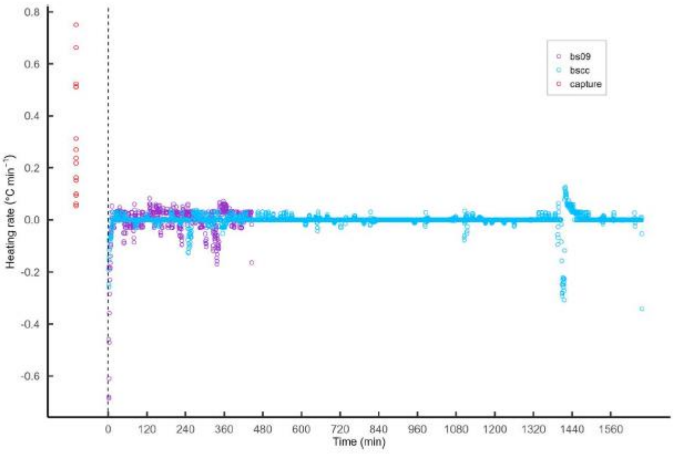


Figure 2: Average heating rate (°C min^{-1}) for each blue shark during capture period (red circles; $n = 15$) and average heating rate every 30 sec for two biologged blue sharks (BSCC and BS09) during post-release phase ($n = 4205$). Capture datapoints (red circles) are placed arbitrarily to left of x-axis to illustrate their collection before the beginning of the time series post-release.

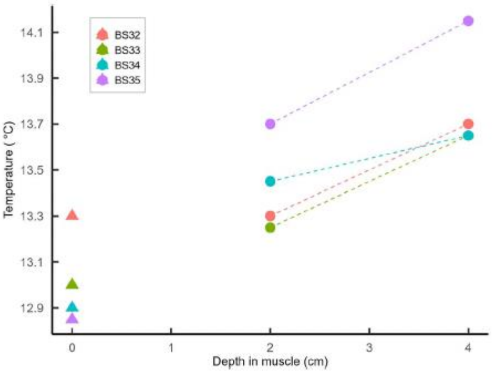


Figure 3: Water temperature measurements (triangles) and body temperature measurements (circles) taken at 2 and 4 cm penetration into the dorsal musculature of four blue sharks.

increase ± SD = 2.5 ± 1.2 mmol l^{-1}), 1 showed no change and 1 showed a decline (Supplementary Table S1). Glucose levels upon capture ranged from 3.1 to 19.7 mmol l^{-1} (mean = 8.6 mmol l^{-1}, $n = 9$). Following time on deck glucose levels ranged from 2.9 to 22.7 mmol l^{-1} (mean = 6.9 mmol l^{-1}, $n = 11$). Of the seven sharks tested twice, only two showed increased glucose levels following time on deck and five showed a decline (mean decrease ± SD = 3.3 ± 6.9 mmol l^{-1}) (Supplementary Table S1).

Discussion

By combining biologging, temperature measurements immediately after capture and blood biochemistry, we show that capture significantly and rapidly (up to 0.8°C min^{-1}) elevates the body temperature of sharks. Subcutaneous muscle temperature was elevated by as much as 2.7°C immediately after capture, and temperature increased deeper into the white muscle, but the magnitude of the temperature elevation above ambient was unrelated to shark size, hooking duration and blood lactate or glucose levels. Following release, T_b rapidly declined toward T_a during the first 30 mins for tiger sharks and 15 mins for blue sharks, and generally approximated T_a within 1 h after release. These results provide new insight into the stress physiology of capture in sharks and highlight the significant influence the fishing process has on shark body temperatures.

Although body temperature elevation has been acknowledged to be of importance when assessing the negative physiological impacts of catch-and-release fishing in the past, it has not been directly recorded under these conditions before. Only through these direct, fine-scale measurements were we able to show definitively that catch-and-release events caused an elevation of the body temperature of sharks. Acute body temperature elevations have been known to cause a number of physiological, ecological and behavioural consequences; in terms of physiology, rapid elevations of body temperature can speed up digestion rates (Carey *et al.*, 1984), increase the expression of heat shock proteins (Renshaw *et al.*, 2012) and affect biological rates, such as metabolism (Morley *et al.*, 2019), potentially contributing to a deficit in the sharks'

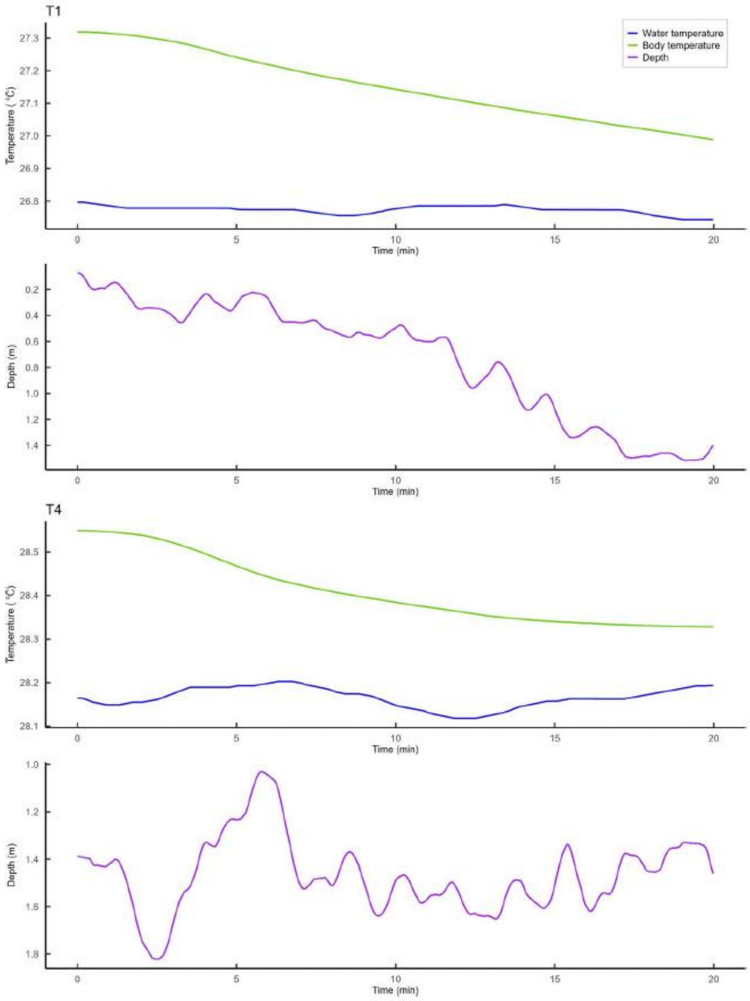


Figure 4: Body temperature, water temperature and depth data for tiger sharks T1 (top panel) and T4 (bottom panel) from moment of release. A 500-point moving average smoother was applied to all data.

daily energy budget, as has been shown before (Bouyoucos *et al.*, 2017, 2018). Regarding behavioural changes, elevations of body temperature can result in distributional changes as sharks are known to adjust their position in the water column as a means to behaviourally thermoregulate (Sims *et al.*, 2006; Nakamura *et al.*, 2020; Watanabe *et al.*, 2021), or may expand their horizontal range if topographically limited (e.g. the tiger sharks in the Bahamas are depth limited and therefore may travel further offshore to seek out colder, deep waters) (Gallagher *et al.*, 2021). While the magnitude of the measured temperature increase deeper into the muscle (0.7°C–1.2°C on average) may not seem dramatic, muscles deeper into the body were higher than subcutaneous tissues for blue sharks, and the subcutaneous temperatures likely underestimate the true temperature elevation of those tissues. This is because we could not measure T_b of sharks prior to capture, so we calculated our estimates of T_b elevation based on sea surface temperatures at the point of capture and our biologging data, which showed shark T_b matches water temperature following sufficient equilibration time after release. Accordingly, our estimates of capture-induced heating of subcutaneous tissues are likely conservative because shark

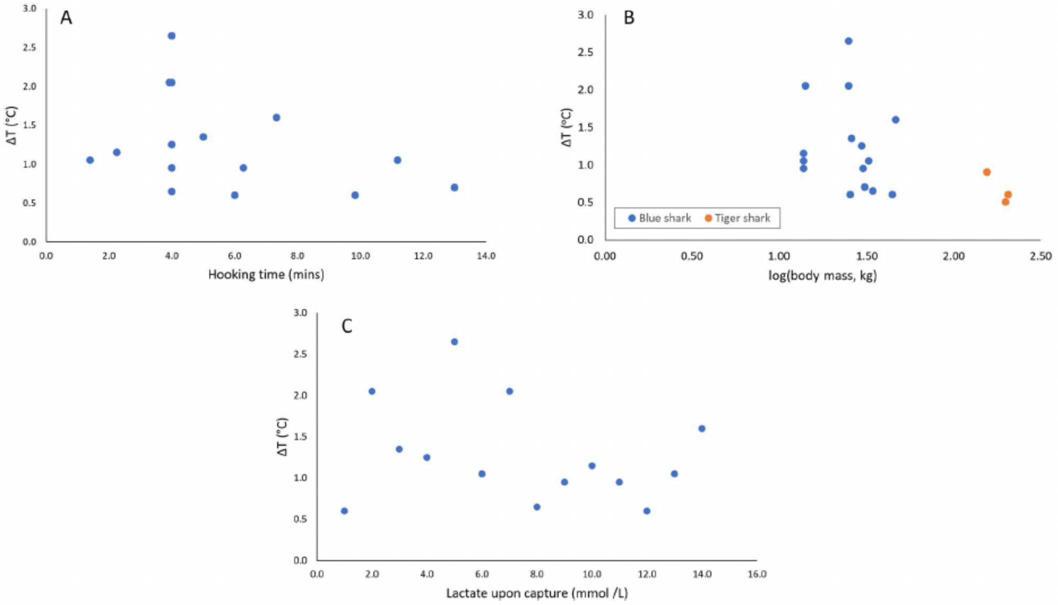


Figure 5: (A–C): (A) Temperature differential (ΔT) against hooking time for blue sharks with no significant relationship. (B) Temperature differential (ΔT) against log (body mass) with no significant relationship. (C) Temperature differential (ΔT) against lactate upon capture for blue sharks with no significant relationship.

T_b may have been lower than surface temperatures if they had been inhabiting cooler (deeper) waters immediately prior to taking the hook, i.e. the true magnitude of heating could be higher than what we report. We do however acknowledge the limited sample size in our study; further collection of measurements of this kind from additional species and individuals could investigate any potential interindividual or interspecies variation. Future work could build on ours and other studies (Wosnick *et al.*, 2018, 2019) that explore how heat is distributed throughout the body of sharks and the associated physiological implications. It is also noteworthy that we report T_b to match T_a for these animals after they have recovered from capture (Fig. 1C and D), because few studies have equivocally shown this to be the case for large ectotherms that have significant thermal inertia (low temperature rate constants; Nakamura *et al.*, 2020).

The simultaneous elevation of body temperature and lactate concentration are undoubtedly due to higher metabolic rates during capture, which is partially through anaerobic pathways. Nevertheless, temperature elevation was not correlated with lactate concentration, and neither were correlated with hooking duration (if anything, all these relationships were slightly negative; Fig. 5). These results were somewhat unexpected, notwithstanding the known lag issues associated with using blood lactate as a direct proxy of the extent of cumulative anaerobic metabolism (blood lactate is chiefly a measure of anaerobic metabolism as it shifts from the white muscle to the blood when an animal switches from aerobic to anaerobic respiration during periods of increased energetic demands; Prohaska *et al.*, 2021), and that other studies have also shown hooking duration as a poor proxy of blood lactate levels (Shea *et al.*, 2022). Further studies with increased sample sizes would be beneficial to investigating this relationship further. Notwithstanding this, our data show that shark body temperature rapidly responds to capture but is not a proxy of blood lactate concentrations over the same time scales. Body temperature should therefore be treated as a new physiological proxy of exhaustion in captured sharks that reflects increased aerobic exercise as well as the mismatch between heat generated in the skeletal muscles and lost at the gills.

Capture-induced heating rates were far more rapid than what our biologged sharks exhibited naturally; some blue sharks heating almost eight times faster than did wild sharks swimming throughout thermally stratified waters. An important next step would be to determine the physiological implications of heating at these rates and magnitudes. There exists a rich literature on physiological responses to acute heating in the laboratory, which provides useful context. For example, Morley *et al.* (2019) compiled data on studies testing the upper temperature limits of marine, freshwater and terrestrial ectotherms under varying rates of warming. The maximum heating rates used in most experimental studies were ~1°C min^{-1}, with most commonly used rates being much lower. These higher rates are often considered to be so fast as to not be ecologically meaningful (Payne *et al.*, 2016, 2021) whereas our sharks heated close to those maximum heating rates manipulated in the laboratory (0.8°C min^{-1}). Heating rate has a well-recognised impact on an ectotherm's thermal limit (Peck *et al.*, 2009; Kingsolver and Umbanhowar, 2018), so it could be instructive to determine physiological implications of this fast heating over the magnitude of temperature increases we documented. This could take the form of laboratory-based studies on physiological indicators of stress in sharks, which are undergoing thermal ramping, such as blood biochemistry, behavioural changes and response to stimuli. Observations from these controlled environments may aid our understanding of how this rapid heating is occurring and affecting the animals physiologically. Another avenue of research could be to explore behaviour post-release through biologging and investigate any relationship with ΔT. Increasing the number of individuals and species of varying lifestyles (e.g. sedentary, active predators) tagged with biologgers would greatly aid these studies.

Future research of this kind, and our own study, have numerous physiological and fisheries-based implications. Firstly, the findings of this study could be incorporated into future ecological models aimed at predicting mortality following catch-and-release angling, perhaps by incorporating a thermal threshold value. Moyes *et al.* (2006) constructed a model to predict the long-term survival of fish released following capture based on several blood markers (including lactate). This study noted that the inclusion of water temperature could have improved their model, with lower water temperatures likely resulting in reduced mortality post-release. We posit that this model could be further improved by the inclusion of body temperature in conjunction with water temperature, as we have shown that body temperature does not directly mimic water temperature in these ectothermic sharks for the first hour(s) after release. We are not aware of any other published studies that documented capture-induced changes in body temperature in large fishes, aside from a report on bluefin tuna captured in nets, which reported a ~2°C increase in body temperature (Addis *et al.*, 2009). Tunas, along with some other species such as lamnid sharks, have specialised physiology, which allows them to retain metabolically derived heat through vascular countercurrent heat exchangers, so could be expected to exhibit even greater temperature elevation during capture than ectothermic species. Many of those species are also of conservation concern, so it could be instructive to explore how catch-and-release fishing might impact the welfare of these animals via T_b elevations.

In summary, we show that catch-and-release angling causes a measurable and rapid increase in the body temperature of sharks. With fast temperature elevations of up to 2.7°C, and possibly greater, it might be important for future research to explore any physiological impacts that this heating might cause, to better manage catch-and-release programs. Welfare outcomes of different handling protocols have been examined in other species (Danylchuk *et al.*, 2007; Brownscombe *et al.*, 2017; Raoult *et al.*, 2019) and exploring implications of factors such as removing sharks from the water (versus leaving them submerged) or catching them near the upper limit of their thermal niche could be helpful for this group of animals.

Funding

This work was supported by Science Foundation Ireland [grant number 18/SIRG/5549 to N. P.]; and the Irish Research Council [grant number IRCLA/2017/186 to A. J.].

Data availability

The data underlying this article are available in the article and in its online supplementary material.

Conflicts of Interest

We know of no conflicts of interest associated with this publication and that any research in the paper not carried out by the authors is fully acknowledged in the manuscript. All sources of funding are acknowledged in the manuscript, and authors have declared any direct financial benefits that could result from publication.

Acknowledgements

The author thanks all those who helped to collect the biologging data and the blood samples, particularly Grace McNicholas, Matthew Cobain, Joe Cooney and Sami Kattan.

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

References

Accu-Chek (2021) Performa. https://www.accu-chek.co.uk/blood-glucose-meters/performa. (date last accessed: 29 August 2022).

Addis P, Locci I, Corriero A, Cau A (2009) Body temperature of the Atlantic Bluefin tuna (*Thunnus thynnus* l.) in the western Mediterranean. In Nielsen JL, Arrizabalaga H, Fragoso N, Hobday A, Lutcavage M, Sibert J, eds, *Tagging and Tracking of Marine Animals with Electronic Devices*. Springer, Dordrecht, Netherlands, pp. 195–207, https://doi.org/10.1007/978-1-4020-9640-2_12.

Bouyoucos IA, Montgomery DW, Brownscombe JW, Cooke SJ, Suski CD, Mandelman JW, Brooks EJ (2017) Swimming speeds and metabolic rates of semi-captive juvenile lemon sharks (*Negaprion brevirostris*, poey) estimated with acceleration biologgers. *J Exp Mar Biol Ecol* 486: 245–254. https://doi.org/10.1016/j.jembe.2016.10.019.

Bouyoucos IA, Weideli OC, Planes S, Simpfendorfer CA, Rummer JL (2018) Dead tired: evaluating the physiological status and survival of neonatal reef sharks under stress. *Conserv Physiol* 6: coy053. https://doi.org/10.1093/conphys/coy053.

Brownscombe JW, Danylchuk AJ, Chapman JM, Gutowsky LFG, Cooke SJ (2017) Best practices for catch-and-release recreational fisheries—angling tools and tactics. *Fish Res* 186: 693–705. https://doi.org/10.1016/j.fishres.2016.04.018.

Carey FG, Kanwisher JW, Stevens ED (1984) Bluefin tuna warm their viscera during digestion. *J Exp Biol* 109: 1–20. https://doi.org/10.1242/jeb.109.1.1.

Cliff G, Thurman GD (1984) Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. *Comp Biochem Physiol* 78: 167–173. https://doi.org/10.1016/0300-9629(84)90111-7.

Cooke SJ, Donaldson MR, Connor CMO, Raby GD, Arlinghaus R, Danylchuk AJ, Hanson KC, Hinch SG, Clark TD, Patterson DA *et al.* (2013) The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. *Fish Manag Ecol* 20: 268–287. https://doi.org/10.1111/j.1365-2400.2012.00867.x.

Cooke SJ, Schramm HL (2007) Catch-and-release science and its application to conservation and management of recreational fisheries. *Fish Manag Ecol* 14: 73–79. https://doi.org/10.1111/j.1365-2400.2007.00527.x.

Danylchuk AJ, Danylchuk SE, Cooke SJ, Goldberg TL, Koppelman JB, Philipp DP (2007) Post-release mortality of bonefish, *Albula vulpes*, exposed to different handling practices during catch-and-release angling in Eleuthera, the Bahamas. *Fish Manag Ecol* 14: 149–154. https://doi.org/10.1111/j.1365-2400.2007.00535.x.

Danylchuk AJ, Suski CD, Mandelman JW, Murchie KJ, Haak CR, Brooks AML, Cooke SJ (2014) Hooking injury, physiological status and short-term mortality of juvenile lemon sharks (*Negaprion bevirostris*) following catch-and-release recreational angling. *Conserv Physiol* 2: cot036. https://doi.org/10.1093/conphys/cot036.

Dapp DR, Huveneers C, Walker TI, Drew M, Reina RD (2016) Moving from measuring to predicting bycatch mortality: predicting the capture condition of a longline-caught pelagic shark. *Front Mar Sci* 2: 126. https://doi.org/10.3389/fmars.2015.00126.

French RP, Lyle J, Tracey S, Currie S, Semmens JM (2015) High survivorship after catch-and-release fishing suggests physiological resilience in the endothermic shortfin mako shark (*Isurus oxyrinchus*). *Conserv Physiol* 3: cov044. https://doi.org/10.1093/conphys/cov044.

Gale MK, Hinch SG, Donaldson MR (2013) The role of temperature in the capture and release of fish. *Fish Fish Ser* 14: 1–33. https://doi.org/10.1111/j.1467-2979.2011.00441.x.

Gallagher AJ, Alsudairy NA, Shea BD, Payne NL, Duarte CM (2021) First application of 360-degree camera technology to marine predator bio-logging. *Front Mar Sci* 8: 707376. https://doi.org/10.3389/fmars.2021.707376.

Gallagher AJ, Meyer L, Pethybridge HR, Huveneers C, Butcher PA (2019) Effects of short-term capture on the physiology of white sharks *Carcharodon carcharias*: amino acids and fatty acids. *Endanger Species Res* 40: 297–308. https://doi.org/10.3354/esr00997.

Gallagher AJ, Serafy JE, Cooke SJ, Hammerschlag N (2014) Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. *Mar Ecol Prog Ser* 496: 207–218. https://doi.org/10.3354/meps10490.

Gallagher AJ, Staaterman ER, Cooke SJ, Hammerschlag N (2017) Behavioural responses to fisheries capture among sharks caught using experimental fishery gear. *Can J Fish Aquat Sci* 74: 1–7. https://doi.org/10.1139/cjfas-2016-0165.

Gilman E, Perez Roda A, Huntington T, Kennelly SJ, Suuronen P, Chaloupka M, Medley PAH (2020) Benchmarking global fisheries discards. *Sci Rep* 10: 14017. https://doi.org/10.1038/s41598-020-71021-x.

HaB Direct (2021) Lactate Pro 2 LT-1730. https://www.habdirect.co.uk/wp-content/uploads/2018/07/b120312-01a-lt-1730.pdf. (date last accessed: 29 August 2022).

Harding L, Jackson A, Barnett A, Donohue I, Halsey L, Huveneers C, Meyer C, Papastamatiou Y, Semmens JM, Spencer E *et al.* (2021) Endothermy makes fishes faster but does not expand their thermal niche. *Funct Ecol* 35: 1951–1959. https://doi.org/10.1111/1365-2435.13869.

Hoffmayer ER, Parsons GR (2001) The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. *Fish Physiol Biochem* 25: 277–285. https://doi.org/10.1023/A:1023210620904.

Jerome JM, Gallagher AJ, Cooke SJ, Hammerschlag N (2017) Integrating reflexes with physiological measures to evaluate coastal shark stress response to capture. *ICES J Mar Sci* 75: 796–804. https://doi.org/10.1093/icesjms/fsx191.

Kieffer JD (2000) Limits to exhaustive exercise in fish. *Comp Biochem Physiol* 126: 161–179. https://doi.org/10.1016/S1095-6433(00)00202-6.

Kingsolver JG, Umbanhowar J (2018) The analysis and interpretation of critical temperatures. *J Exp Biol* 221: 12. https://doi.org/10.1242/jeb.167858.

Kohler NE, Casey JG, Turner PA (1995) Length-weight relationships for 13 species of sharks from the western North Atlantic. *Fish B. NOAA* 93: 412–418.

Mandelman JW, Skomal GB (2008) Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. *J Comp Physiol B* 179: 267–277.

Meka JM, McCormick SD (2005) Physiological response of wild rainbow trout to angling: impact of angling duration, fish size, body condition, and temperature. *Fish Res* 72: 311–322. https://doi.org/10.1016/j.fishres.2004.10.006.

Mohan JA, Jones ER, Hendon JM, Falterman B, Boswell KM, Hoffmayer ER, Wells RJD (2020) Capture stress and post-release mortality of blacktip sharks in recreational charter fisheries of the gulf of Mexico. *Conserv Physiol* 8: coaa041. https://doi.org/10.1093/conphys/coaa041.

Morley SA, Peck LS, Sunday JM, Heiser S, Bates AE (2019) Physiological acclimation and persistence of ectothermic species under extreme heat events. *Glob Ecol Biogeogr* 28: 1018–1037. https://doi.org/10.1111/geb.12911.

Moyes CD, Fragoso N, Musyl MK, Brill RW (2006) Predicting postrelease survival in large pelagic fish. *Trans Am Fish Soc* 135: 1389–1397. https://doi.org/10.1577/T05-224.1.

Nakamura I, Matsumoto R, Sato K (2020) Body temperature stability observed in the whale sharks, the world's largest fish. *J Exp Biol* 223: jeb.210286. https://doi.org/10.1242/jeb.210286.

Payne NL, Morley SA, Halsey LG, Smith JA, Stuart-Smith R, Waldock C, Bates AE (2021) Fish heating tolerance scales similarly across individual physiology and populations. *Commun Biol* 4: 264. https://doi.org/10.1038/s42003-021-01773-3.

Payne NL, Smith JA, van der Meulen DE, Taylor MD, Watanabe YY, Takahashi A, Marzullo TA, Gray CA, Cadiou G, Suthers IM *et al.* (2016) Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Funct Ecol* 30: 903–912. https://doi.org/10.1111/1365-2435.12618.

Peck LS, Clark MS, Morley SA, Massey A, Rossetti H (2009) Animal temperature limits and ecological relevance: effects of size, activity and rates of change. *Funct Ecol* 23: 248–256. https://doi.org/10.1111/j.1365-2435.2008.01537.x.

Prohaska BK, Talwar BS, Grubbs RD (2021) Blood biochemical status of deep-sea sharks following longline capture in the Gulf of Mexico. *Conserv Physiol* 9: coaa113. https://doi.org/10.1093/conphys/coaa113.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raoult V, Williamson JE, Smith TM, Gaston TF (2019) Effects of on-deck holding conditions and air exposure on post-release behaviours of sharks revealed by a remote operated vehicle. *J Exp Mar Biol Ecol* 511: 10–18. https://doi.org/10.1016/j.jembe.2018.11.003.

Renshaw GMC, Kutek AK, Grant GD, Anoopkumar-Dukie S (2012) Forecasting elasmobranch survival following exposure to severe stressors. *Comp Biochem Physiol A* 162: 101–112. https://doi.org/10.1016/j.cbpa.2011.08.001.

Royer M, Maloney K, Meyer C, Cardona E, Payne N, Whittingham K, Silva G, Blandino C, Holland K (2020) Scalloped hammerhead sharks swim on their side with diel shifts in roll magnitude and periodicity. *Anim Biotelemetry* 8: 11. https://doi.org/10.1186/s40317-020-00196-x.

Sakamoto KQ, Sato K, Ishizuka M, Watanuki Y, Takahashi A, Daunt F, Wanless S (2009) Can ethograms be automatically generated using body acceleration data from free-ranging birds? *PLoS One* 4: e5379. https://doi.org/10.1371/journal.pone.0005379.

Schlenker LS, Latour RJ, Brill RW, Graves JE (2016) Physiological stress and post-release mortality of White Marlin (*Kajikia albida*) caught in the United States recreational fishery. *Conserv Physiol* 4: cov066. https://doi.org/10.1093/conphys/cov066.

Schwieterman GD, Winchester MM, Shiels HA, Bushnell PG, Bernal D, Marshall HM, Brill RW (2021) The effects of elevated potassium, acidosis, reduced oxygen levels, and temperature on the functional properties of isolated myocardium from three elasmobranch fishes: Clearnose skate (*Rostroraja eglanteria*), Smooth dogfish (*Mustelus canis*), and Sandbar shark (*Carcharhinus plumbeus*). *J Comp Physiol B* 191: 127–141. https://doi.org/10.1007/s00360-020-01328-8.

Sepulveda CA, Wang M, Aalbers SA (2019) Post-release survivorship and movements of Bigeye Thresher sharks, *Alopias superciliosus*, following capture on deep-set buoy gear. *Fish Res* 219: 105312. https://doi.org/10.1016/j.fishres.2019.105312.

Shea BD, Coulter SK, Dooling KE, Isihara HL, Roth JC, Sudal E, Donovan DJ, Hoopes LA, Dove ADM, Cooke SJ *et al.* (2022) Recreational fishing fight times are not correlated with physiological status of blue sharks (*Prionace glauca*) in the northwestern Atlantic. *Fish Res* 248: 106220. https://doi.org/10.1016/j.fishres.2021.106220.

Sims DW, Wearmouth VJ, Southall EJ, Hill JM, Moore P, Rawlinson K, Hutchinson N, Budd GC, Righton D, Metcalfe JD *et al.* (2006) Hunt warm, rest cool: bioenergetic strategy underlying diel vertical migration of a benthic shark. *J Anim Ecol* 75: 176–190. https://doi.org/10.1111/j.1365-2656.2005.01033.x.

Stevens ED (2011) Design and physiology of arteries and veins | The retia. In Farrell AP ed, *Encyclopedia of Fish Physiology*. Elsevier, San Diego, pp. 1119–1131, https://doi.org/10.1016/B978-0-12-374553-8.00067-8.

Stevens ED, Fry FEJ (1974) Heat transfer and body temperatures in non-thermoregulatory teleosts. *Can J Zool* 52: 1137–1143. https://doi.org/10.1139/z74-152.

Watanabe YY, Goldman KJ, Caselle JE, Chapman DD, Papastamatiou YP (2015) Comparative analyses of animal-tracking data reveal ecological significance of endothermy in fishes. *PNAS* 112: 6104–6109. https://doi.org/10.1073/pnas.1500316112.

Watanabe YY, Nakamura I, Chiang W-C (2021) Behavioural thermoregulation linked to foraging in blue sharks. *Mar Biol* 168: 161. https://doi.org/10.1007/s00227-021-03971-3.

WaveMetrics (2020). Igorpro, WaveMetrics, Lake Oswego, OR, USA.

Wosnick N, Navas CA, Niella YV, Monteiro-Filho ELA, Arruda Freire C, Hammerschlag N (2018) Thermal imaging reveals changes in body surface temperatures of Blacktip sharks (*Carcharhinus limbatus*) during air exposure. *Physiol Biochem Zool* 91: 1005–1012. https://doi.org/10.1086/699484.

Wosnick N, Niella YV, Navas CA, Monteiro-Filho ELA, Freire CA, Hammerschlag N (2019) Multispecies thermal dynamics of air-exposed ectothermic sharks and its implications for fisheries conservation. *J Exp Mar Biol Ecol* 513: 1–9. https://doi.org/10.1016/j.jembe.2019.01.002.