Diego Garcia Yellowfin Tuna Tagging Expedition II

Final Report, September 2019

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Executive Summary

Yellowfin tuna (*Thunnus albacares*) are currently '*Overfished and Subject to Overfishing'* in the Indian Ocean with an urgent need to rebuild the stock. As a global conservation and management strategy, large Marine Protected Areas (MPAs) have been increasing in number and size. The British Indian Ocean Territory (BIOT) Marine Protected Area provides a unique opportunity to investigate the role of MPAs in the protection and management of commercially important pelagic fishes, such as yellowfin tuna. Building on the January 2019 yellowfin tuna tagging expedition that was hindered by unfavourable fishing weather, a team of four Bertarelli Programme in Marine Science researchers, returned to Diego Garcia in September 2019 to continue the work. The primary objectives of this second expedition were to:

1. Deploy mark recapture floy tags on yellowfin tuna within the Diego Garcia recreational fishery.
2. Deploy up to 10 satellite tags on yellowfin tuna, billfish or pelagic sharks (if encountered) to quantify off-shore movement behaviour.
3. Collect tissue samples for isotopic and DNA analyses to provide information on the trophic ecology and habitat use of species within BIOT and on the patterns of connectivity of elasmobranchs and teleosts regionally.
4. Collect environmental DNA samples from around Diego Garcia.
5. Offer training to the Environment Officer and other interested personnel in tagging methods and the taking and storing of DNA and isotope samples.

The expedition again demonstrated that the Morale, Welfare and Recreational (MWR) vessels are appropriate platforms for tagging activities. However, we were again hindered by weather, severely reducing the amount of time we could spend out on the water (44 hours out of a possible 89.5). Mark recapture floy tags were deployed on four yellowfin tuna, two skipjack tuna (*Katsuwonus pelamis*), and one dogtooth tuna (*Gymnosarda unicolor*). No animals were encountered that were suitable candidates for satellite tag deployments. A total of 84 teleost and 14 algal samples were collected for future isotopic analyses (Appendix I). We conducted an extensive environmental DNA bio-blitz around Diego Garcia, with a total of 96 water samples filtered for future analyses. We also provided training to the Senior Fisheries Protection Officers (SFPOs), and Environment Officers (EOs) on appropriate methods used to catch, handle, conventionally tag and sample teleost fishes.

With the Environment Officers and SFPOs now trained and sampling kits provided, we have individuals on-site able to react when the yellowfin tuna are caught. Furthermore, they are also able to undertake tagging activities whilst on the BIOT Patrol Vessel around the outer islands. This should significantly increase the number of tags we can get out and the number of samples we can collect. In addition, yellowfin tuna tagging will be incorporated into the array servicing (Feb/Mar) and recreational fishery expeditions (Darwin+ funding dependent) in 2020. However, in order to get the sample sizes we need, we really need to have access to the offshore deeper schools that exist in BIOT. Understanding the role of the BIOT MPA for pelagic species, such as yellowfin tuna, is a high priority for the BPMS and would contribute significantly to the BIOT draft conservation management plan. As such, a pelagic tagging expedition should be a priority activity for 2020, ideally taking place around November/December to coincide with the traditional peak in industrial fishing activity.

Introduction

Top predators are ecologically significant, yet many are threatened by anthropogenic pressures such as overexploitation (overfishing and illegal fishing). As a global conservation and management strategy, large Marine Protected Areas (MPAs) have been increasing in number and size with the goal of protecting reef and pelagic species and the ocean ecosystems they occupy. However, to date, few empirical studies have addressed their use and effectiveness for protecting pelagic animals. Thus, the British Indian Ocean Territory (BIOT), as an oceanic biodiversity hotspot in the centre of the Indian Ocean where highly migratory pelagic species aggregate at points during their life history, provides a unique opportunity to address this crucial knowledge gap.

Representatives from the Zoological Society of London (ZSL) and Stanford University have been deploying electronic tag technology on sharks and mantas within BIOT since 2013. They have also installed of a significant acoustic receiver array (currently 52 receiver elements) and tagged over 460 fish with four types of tag technology: acoustic, pop-up archival satellite (PAT), smart-positioning or temperature transmitting (SPOT) and biologging camera tags. The data these tags are providing is revealing how the archipelago's different habitats (i.e. lagoons, outer reefs, pelagic environments) shape the behavioural ecology and life history of a diverse range of shark and ray species (Carlisle et al. 2019; Williamson et al. submitted; Andrzejaczek et al. in prep; Jacoby et al. in prep). In addition, they are elucidating the roles that apex predators play in local focal webs (Curnick et al. 2019a). The ability to use these data to estimate spatial, temporal and population dynamics provide vital information that is necessary for protecting the region. Yet, research to date has been focused primarily on elasmobranch species, with only a handful of teleost species tagged so far.

In the Indian Ocean, the Indian Ocean Tuna Commission (IOTC) Working Party on Tropical Tunas (WPTT) has classified yellowfin tuna (*Thunnus albacares*, Near Threatened in the IUCN Red List of Threatened Species) as '*Overfished and Subject to Overfishing'*. Thus, yellowfin tuna are a priority species for the Bertarelli Programme in Marine Science (BPMS). To date, the BPMS team have only tagged a handful of yellowfin tuna within the BIOT MPA (Carlisle et al., 2019) but they are known to be present year-round across the MPA from the historical fisheries record (Curnick et al., in prep). Currently, yellowfin tuna are targeted by the recreational fishery around the island of Diego Garcia. We believe that this fishery could support a unique research opportunity and generate much needed data that would be of interest to science, managers and the IOTC. We undertook an expedition to Diego Garcia in January 2019 to trial a yellowfin tuna tagging project and start to quantify the ecological significance of the BIOT MPA for this commercially important pelagic species within the Indian Ocean. Unfortunately, we were unable to tag any yellowfin tuna on that expedition due to adverse weather conditions (see Curnick et al. 2019b). Thus, we revisited Diego Garcia in September 2019 to target yellowfin tuna around Diego Garcia once more.

Aims and Objectives

After our unsuccessful expedition in January 2019, we analysed the historical fisheries data and sought local advice to find a more suitable time of the year to visit. A team of four BPMS researchers (David Curnick, ZSL; Nick Dunn, ZSL and Imperial College

London; Sammy Andrzejaczek, Stanford University; and Sam Weber, University of Exeter) subsequently visited Diego Garcia between the 3rd September and the 17th September 2019. The primary aim to of the expedition was to investigate the potential for the recreational fishery around Diego Garcia to serve as a source of valuable year-round base-led tuna data collection (tagging, morphometrics, and DNA and stable isotope samples). The primary objectives of this second pilot study were to:

1) Deploy mark recapture floy tags on yellowfin tuna (*Thunnus albacares*) within the Diego Garcia recreational fishery.
2) Deploy up to 10 satellite tags on yellowfin tuna, billfish or pelagic sharks (if encountered) to quantify off-shore movement behaviour.
3) Collect tissue samples for isotopic and DNA analyses to provide information on the trophic ecology and habitat use of species within BIOT and on the patterns of connectivity of elasmobranchs and teleosts across the Indian Ocean.
4) Collect environmental DNA samples from around Diego Garcia.
5) Offer training to the Environment Officer and other interested personnel in tagging methods and the taking and storing of DNA and isotope samples.

This project was undertaken under permit BIOT0003SE19 granted by the UK Foreign and Commonwealth Office and the BIOT Administration.

Methods

Platforms

As in January, we hired a Boston Whaler from the Morale, Welfare and Recreational (MWR) office on Diego Garcia. Although the vessel comes equipped with fishing rods and fishing tackle suitable for yellowfin tuna capture, we brought all of our own lures so we were not dependent on MWR resources. As well as bringing all of our own fishing lures, we also brought all the necessary tagging supplies and sampling equipment into the territory. Any additional resources required were sourced from the science store (vials and ethanol). The boat came with an experienced captain and deckhand and have a maximum capacity of seven people. As the science team consisted of four people, this enabled us to host one additional person onboard each day. The boat maintained constant radio contact with Port Operations and were not allowed beyond three nautical miles offshore from Diego Garcia. MWR boats are usually not permitted to head south beyond Donkey Gate, owing to a previous boating accident in the area. However, we were given special permission to go beyond Donkey Gate for the purposes of scientific research. The MWR boats are not able leave dock before 0730 and have to be back at the marina no later than 1800. Furthermore, the crews were not able to work longer than their contractual eight hours per day. Therefore, each day we planned to go out in two blocks of four hours each day (0730-1130 and 1400-1800), knowing that there was the flexibility to extend to one eight-hour block should the fish be biting.

Fishing methods

Upon arrival at the marina, we sought recommendations by MWR officials, fishers and the boat captains on where and when to fish. The primary fishing method was trolling, targeting depth breaks such as drop-offs or seamounts or along temperature breaks. A spread of five lures (using outriggers) varying in type, colour and size were towed

behind the boat. These lures were trolled at different distances and depths. Cedar plugs, jets, feathers, and Rapalas were used with mono leaders and single hooks as to ensure as little physical damage to the fish as possible. In addition, we targeted areas of concentrated bird activity (booby and terns), a known indicator of tuna activity. However, we occasionally switched baited handlines for sharks when conditions were not conducive for tuna. We had initially planned to spend 12 days out on the water, totalling 89.5 hours of fishing time (Table 1).

Tagging procedures

To date, the team has deployed over 460 electronic tags in BIOT. Most of these tags have reported, indicating a high success with handling, tagging and releasing fish and elasmobranchs in the region. The September 2019 Diego Garcia tagging expedition focused on yellowfin tuna but other species were also caught. The following describes procedures carried out on all species caught, unless otherwise stated.

We used fishing techniques that minimised stress and risk to the animals and ensured that they were rapidly brought to the boat after being hooked. Once at the boat, all fish were carefully lifted onto the MWR vessel, using a supportive sling when necessary. For each animal caught, we collected basic morphometric data (length, sex, body condition) and muscle tissue samples for isotopic analysis (extracted from just below the first dorsal fin). Samples were stored immediately on ice before being transferred to a freezer within our accommodation on Diego Garcia at the end of the fishing session. Fin tissue samples were also collected from yellowfin (taken from a finlet) and sharks (taken from the first dorsal fin) and stored in 70% proof ethanol for subsequent DNA analyses. After all the data were collected, fish were released back into the water with total handling times kept below three minutes to minimise stress.

Any yellowfin tuna caught were tagged with two small mark recapture floy tag (Hallprint type: yellow PDATs (plastic tipped thick dart tags)), one at the base of the first dorsal fin on either side, with a bespoke PDAT stainless steel applicator. These tags are consistent with those used in previous IOTC tagging projects). In addition, larger tunas (>95cm), billfish or pelagic sharks encountered were to be tagged with MiniPAT satellite tags (Wildlife Computers). These satellite tags collect and archive data on depth, temperature, and light levels and detach from the tagged animal after a programmed period of time. Tags will then float to the surface and transmit data to the Argos system, providing information on the movements and habitat use of tagged animals. All tags were to be placed on for programmed durations of 4 (yellowfin tuna) or 12 months (billfish and sharks) to reveal long-term patterns of movements and connectivity. MiniPAT tags were to be attached to two small titanium darts with a reinforced leader – a method developed and refined by the Stanford team during their work on bluefin tuna in the Pacific. Darts are sterilized and then inserted into the dorsal musculature at the base of the dorsal fin. All procedures were approved by the ZSL Ethics committee.

Table 1. The planned body of work between the 3rd and the 17th September 2019 during the second Diego Garcia Yellowfin Tuna Tagging Trial

Time		Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Start	Finish	03/09/2019	04/09/2019	05/09/2019	06/09/2019	07/09/2019	08/09/2019	09/09/2019	10/09/2019	11/09/2019	12/09/2019	13/09/2019	14/09/2019	15/09/2019	16/09/2019	17/09/2019
07:30	08:00		Orientation	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Fly out
08:00	08:30															
08:30	09:00															
09:00	09:30															
09:30	10:00															
10:00	10:30															
10:30	11:00															
11:00	11:30															
11:30	12:00															
12:00	12:30	Fly In		Lunch	Lunch	Lunch	Lunch		Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	
12:30	13:00															
13:00	13:30															
13:30	14:00															
14:00	14:30			Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Lunch	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Pack-up and debrief	
14:30	15:00															
15:00	15:30															
15:30	16:00															
16:00	16:30							Science Talk								
16:30	17:00															
17:00	17:30															
17:30	18:00															

Table 2. The realised body of work between the 3rd and the 17th September 2019 during the second Diego Garcia Yellowfin Tuna Tagging Trial

Time		Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Start	Finish	03/09/2019	04/09/2019	05/09/2019	06/09/2019	07/09/2019	08/09/2019	09/09/2019	10/09/2019	11/09/2019	12/09/2019	13/09/2019	14/09/2019	15/09/2019	16/09/2019	17/09/2019
07:30	08:00		Orientation	Adverse Weather (Brit Rep meeting)	Adverse Weather	Adverse Weather	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging & eDNA sampling	Adverse Weather	Tagging & eDNA sampling		Adverse Weather	Adverse Weather (turtle and shark cove sampling)	Pack-up	Fly out
08:00	08:30											Tagging & eDNA sampling				
08:30	09:00															
09:00	09:30															
09:30	10:00															
10:00	10:30															
10:30	11:00															
11:00	11:30															
11:30	12:00															
12:00	12:30	Fly In		Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	
12:30	13:00															
13:00	13:30															
13:30	14:00															
14:00	14:30			Adverse Weather (turtle cove and banyan cemetry)	Adverse Weather	Adverse Weather (shark cove and plantation)	Tagging & eDNA sampling	Tagging & eDNA sampling	Adverse Weather	Tagging & eDNA sampling	Tagging & eDNA sampling	Tagging (shark)	Tagging (shark)	Adverse Weather	Land based Tagging & eDNA sampling training	
14:30	15:00															
15:00	15:30															
15:30	16:00															
16:00	16:30															
16:30	17:00															
17:00	17:30															
17:30	18:00															

Environmental DNA sampling
At each sampling location, the GPS coordinates were recorded, and two 5-litre water samples were collected. A Niskin water sampling bottle was deployed from the MWR boat to 40m below the surface for the collection of the first water sample. The Niskin bottle was then brought back to the surface and the water sample within poured into a 5L container and stored in a cool box on ice. The Niskin bottle was then washed in surface water before collecting a sample of the surface water, which was also poured into another container and placed in the cool box on ice. Filtration of the water samples took place on land, within three hours of water collection. The container was shaken well to mix the contents and three subsamples of 1L were filtered through sterivex filter capsules using a vacuum pump. Upon completion, the filters were run dry, the outlet end was capped and 2.5mL Longmire's buffer was added to the capsule to preserve any eDNA. The inlet was then capped, and each filter was placed in an individual 100mL WhirlPak bag. A negative control of 500mL filtered fresh water was run every other day.

After filtration, each 5L container was washed thoroughly with a 50% bleach solution, followed by rinsing with filtered fresh water to remove any residue. Individual tubes were used for each filtration and these were also washed with bleach and rinsed before being used again. Nitrile gloves and a lab coat was worn at all times during filtration.

Outcomes

Due to a number of days of adverse weather, namely wind and thunderstorms, we were only able to fish for a total of 44 hours (Table 2). This was about half what we had planned (89.5 hours). This significantly impacted our ability to reach many of our original objectives. Furthermore, as yellowfin tuna, like most pelagic predators, are more likely to be caught when the water is cooler and the sea slightly rougher, being unable to fish during such conditions was prohibitive. Reporting against our original objectives, the outcomes of the expedition are:

Objective 1: Deploy mark recapture floy tags on yellowfin tuna within the Diego Garcia recreational fishery.

Following the same protocol we developed in January, we deployed mark recapture floy tags on four yellowfin tuna, two skipjack tuna (*Katsuwonus pelamis*) and one dogtooth tuna (*Gymnosarda unicolor*) (Figure 1). For yellowfin, two conventional tags were inserted at the base of the first dorsal fin (one on either side) to safeguard against tag loss or shedding. Tags were marked with a unique code identifier (e.g. UK000100), a reference to the location of initial tagging and group responsible (BIOT/BPMS), and a contact email address for reporting its recapture. As skipjack and dogtooth tuna were not the primary focus, just one mark recapture tag was deployed on each.

Objective 2: Deploy up to 10 satellite tags on yellowfin tuna, billfish or pelagic sharks

Unfortunately, no suitable fish were caught during this expedition and therefore no satellite tags were deployed. As a result, these tags are now being stored for a future BIOT tagging expedition. As we were not focussed on sharks on this expedition, we

did not prioritise shark fishing activities. However, we still believe Diego Garcia represents an excellent opportunity to tag sharks, especially reef sharks, given the high densities observed.

Figure 1. Trolling on one of the MWR Boston Whalers around Diego Garcia (left) and attaching a mark recapture tag to a yellowfin tuna *Thunnus albacares* (right).

Objective 3: Collect tissue samples for isotopic and DNA analyses to provide information on the trophic ecology and habitat use of species within BIOT and on the patterns of connectivity of elasmobranchs and teleosts across the Indian Ocean.

A total of 84 fish isotope samples were collected from 11 teleost species including yellowfin tuna, dogtooth tuna, skipjack tuna, kawakawa (*Euthynnus affinis*), great barracuda (*Sphyraena barracuda*), wahoo (*Acanthocybium solandri*), rainbow runner (*Elagatis bipinnulata*), green jobfish (*Aprion virescens*) and red snapper (*Lutjanus sebae*; Appendix I). Additionally, 14 algae samples were taken from inside the lagoon. Samples have been taken back to the Institute of Zoology, Zoological Society of London and stored in -40°C freezers before being sent off for analysis. These data will contribute to our ongoing collaborative research project on trophic ecology in the BIOT MPA (e.g. Curnick et al. 2019a). In addition, we collected heart samples from three kawakawa for later mRNA analyses at Stanford University.

Objective 4: Collect environmental DNA samples from around Diego Garcia.

Water samples were collected from 20 locations around Diego Garcia, 12 of these included samples taken from the surface and at 40m. At eight locations only surface water was collected, six of these were inside the lagoon and two outside the lagoon (Figure 2). Samples were filtered in 1L triplicates, resulting in a total of 96 sample filters.

DNA will be extracted from filters using Qiagen's Blood & Tissue DNA extraction kit, following the methods developed by Spens et al. (2017). After quantification of total DNA using a QuBit fluorometer, metabarcoding will be performed using the MiFish primers (Miya et al. 2015). These primers target a section of the 12S mitochondrial gene of fish and both the universal set of primers and the elasmobranch-specific set

will be used. DNA will be sequenced using the Illumina MiSeq and sequences will be analysed using the MiFish bioinformatics pipeline.

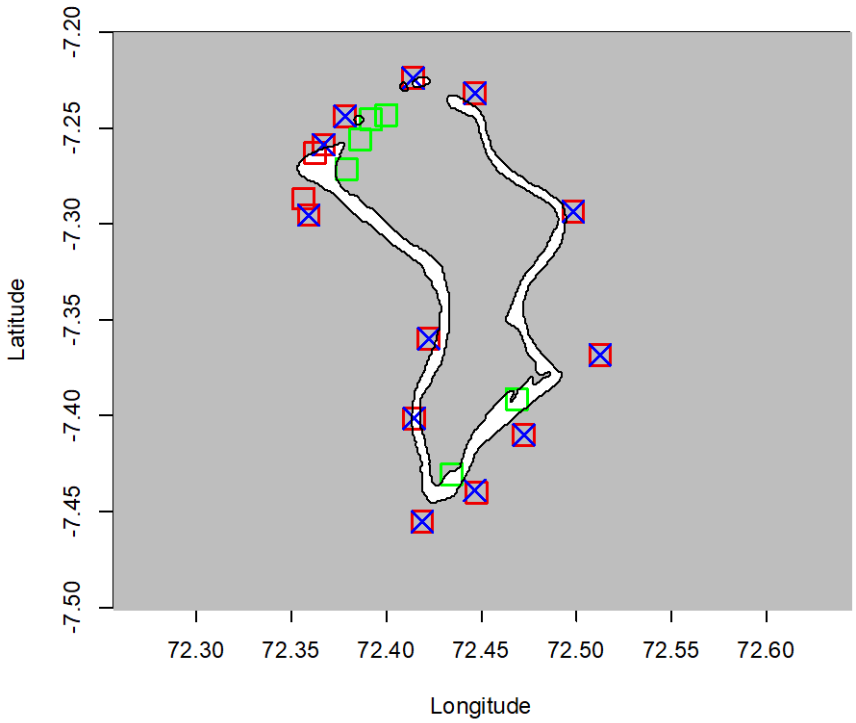

Figure 2. Locations for each eDNA sampling event around Diego Garcia. Surface samples are represented by squares, with lagoon samples in green and outer samples in red. Locations for samples taken at 40m are shown as blue crosses.

Objective 5: Offer training to the Environment Officer and other interested personnel in tagging methods and the taking and storing of DNA and isotope samples.

We provided training to the Senior Fisheries Protection Officers (SFPOs) (John Caddle and Yolanda Barnes), and Environment Officers (EOs) (Harri Morrall and Nadine Atchison-Balmond) on appropriate methods used to catch, handle, conventionally tag and sample teleost fishes. Two tagging and sampling kits were left with the EOs and SFPOs. One to be kept on Diego Garcia and one to be taken on the BIOT Patrol Vessel. Tagging and sampling protocols (Appendix II) and electronic data sheets have also been provided.

Added value

Whilst we were on the island, we were also able to discuss options for reducing the ecological impact of the recreational fishery and provided some guidance on possible minimum landing sizes for focal species to the EO. Furthermore, we were also able to discuss options for sampling seized illegal, unreported and regulated (IUU) catches. This came after a recent seizure where samples had been opportunistically taken by The EO and SFPO at the time (which will be shipped to ZSL for analyses once CITES permits have been acquired). The standardise this process, sampling equipment for IUU catches was provided and IUU sampling protocols developed (Appendix III).

These protocols are now with the SFPOs and EOs to be implemented during the next seizure.

This expedition also provided the opportunity for some valuable knowledge exchange between UK Overseas Territories researchers across the Blue Belt programme. With Dr Sam Weber bringing his experience of running a large tagging programme around Ascension Island, we were able to learn from their project and also develop future projects bringing our two extensive datasets together. We would highly recommend that future expeditions consider bringing researchers from other UK Overseas Territories and reciprocal exchanges be tabled.

Two blogs were also written and are available here

Recommendations and next steps

Unfortunately, our visit again coincided with unfavourable weather conditions and an absence of fish. Indeed, data from the recreational fishery in August (just before we arrived) showed that on the 14 days they went out trolling, they caught yellowfin tuna on 12 occasions, averaging more than four fish a day. Despite using the same captains, boats and methods, we were unfortunate not to encounter more fish. The patchy nature of this fishery and the low bad weather threshold for stopping all small boat activities, makes a solely expedition-based yellowfin tagging approach around Diego Garcia financially and logistically inefficient. With the Environment Officers and SFPOs now trained, we have individuals on-site able to react when the yellowfin tuna are present. Furthermore, they are also able to undertake tagging activities whilst on the BIOT Patrol Vessel around the outer islands. This should significantly increase the number of tags we can get out and the number of samples we can collect. In addition, yellowfin tuna tagging will be incorporated into the array servicing (Feb/Mar) and recreational fishery expeditions (Darwin+ funding dependent) in 2020.

However, in order to get the sample sizes we need, we really need to have access to the offshore deeper schools that exist in BIOT. Understanding the role of the BIOT MPA for pelagic species, such as yellowfin tuna, is a high priority for the BPMS and would contribute significantly to the BIOT draft conservation management plan. As such, a pelagic tagging expedition should be a priority activity for 2020, ideally taking place around November/December to coincide with the traditional peak in industrial fishing activity (Dunn & Curnick, 2019).

Acknowledgements

This work was funded through the Bertarelli Programme in Marine Science. We would like to thank the BIOT Administration for granting us permission to undertake the research, the military personnel on Diego Garcia for their support during fieldwork and the boat captains and deckhands on the Boston Whalers for taking us out each day. We would especially like to thank Commander Kay Burbidge, Major Lee Mildener, Nadine Atchison-Balmond, Harri Morrall, Samuel Bullen, Linsey Billing and Mary Grace Dumlao for all of their assistance.

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Appendices

Appendix I. Isotope samples taken during the September 2019 Diego Garcia yellowfin tuna tagging expedition. FL denotes fork length (cm).

Common name	Latin name	Date sampled	Fork length (cm)	Isotope sample
Kawakawa	*Euthynnus affinis*	08/09/2019	39.5	Muscle
Rainbow runner	*Elagatis bipinnulata*	08/09/2019	46	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	57	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	64	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	47	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	59	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	60	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	63	Muscle
Skipjack tuna	*Katsuwonus pelamis*	08/09/2019	52	Muscle
Baitfish	*Clupidae*	08/09/2019		Muscle
Baitfish	*Clupidae*	08/09/2019		Muscle
Baitfish	*Clupidae*	08/09/2019		Muscle
Green Jobfish	*Aprion virescens*	09/09/2019	58	Muscle
Kawakawa	*Euthynnus affinis*	09/09/2019	52	Muscle
Kawakawa	*Euthynnus affinis*	09/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	09/09/2019	49	Muscle
Kawakawa	*Euthynnus affinis*	09/09/2019	47	Muscle
Rainbow runner	*Elagatis bipinnulata*	09/09/2019	47	Muscle
Rainbow runner	*Elagatis bipinnulata*	09/09/2019	44	Muscle
Rainbow runner	*Elagatis bipinnulata*	09/09/2019	44	Muscle
Wahoo	*Acanthocybium solandri*	09/09/2019	108	Muscle
Dogtooth Tuna	*Gymnosarda unicolor*	10/09/2019	97	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	47	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	50	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	46	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	47	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	36	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	38	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	54	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	52	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	51	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	50	Muscle
Kawakawa	*Euthynnus affinis*	10/09/2019	37	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	52	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	50	Muscle

Rainbow runner	*Elagatis bipinnulata*	10/09/2019	52	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	39	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	46	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	48	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	41	Muscle
Rainbow runner	*Elagatis bipinnulata*	10/09/2019	41	Muscle
Great barracuda	*Sphyraena barracuda*	11/09/2019	108	Muscle
Kawakawa	*Euthynnus affinis*	11/09/2019	45	Muscle
Kawakawa	*Euthynnus affinis*	11/09/2019	44	Muscle
Kawakawa	*Euthynnus affinis*	11/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	11/09/2019	42	Muscle
Rainbow runner	*Elagatis bipinnulata*	11/09/2019	41	Muscle
Rainbow runner	*Elagatis bipinnulata*	11/09/2019	46	Muscle
Red snapper	*Lutjanus Bohar*	11/09/2019	59	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	47	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	50	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	50	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	49	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	47	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	51	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	48.5	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	52	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	49.5	Muscle
Kawakawa	*Euthynnus affinis*	12/09/2019	49	Muscle
Baitfish	*Pseudanthias cooperi*	12/09/2019		Muscle
Baitfish	*Pseudanthias cooperi*	12/09/2019		Muscle
Baitfish	*Clupidae*	12/09/2019		Muscle
Baitfish	*Clupidae*	12/09/2019		Muscle
Rainbow runner	*Elagatis bipinnulata*	12/09/2019	60	Muscle
Rainbow runner	*Elagatis bipinnulata*	12/09/2019	62	Muscle
Yellowfin tuna	*Thunnus albacares*	12/09/2019	73	Muscle
Algae	*Red*	13/09/2019		
Algae	*Red*	13/09/2019		
Algae	*Red*	13/09/2019		
Algae	*Red*	13/09/2019		
Algae	*Red*	13/09/2019		
Algae	*Red*	13/09/2019		
Algae	*Filamentous*	13/09/2019		
Algae	*Filamentous*	13/09/2019		
Algae	*Turf*	13/09/2019		

Algae	*Turf*	13/09/2019		
Algae	*Turf*	13/09/2019		
Algae	*Turf*	13/09/2019		
Algae	*Turf*	13/09/2019		
Algae	*Turf*	13/09/2019		
Algae	*Turf*	13/09/2019		
Algae	*Filamentous*	13/09/2019		
Algae	*Filamentous*	13/09/2019		
Algae	*Filamentous*	13/09/2019		
Kawakawa	*Euthynnus affinis*	13/09/2019	55	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	49	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	53	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	48	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	50.5	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	50	Muscle
Kawakawa	*Euthynnus affinis*	13/09/2019	59	Muscle
Red-bar anthias	*Pseudanthias cooperi*	13/09/2019		Muscle
Baitfish	*Clupidae*	13/09/2019		Muscle
Baitfish	*Clupidae*	13/09/2019		Muscle
Rainbow runner	*Elagatis bipinnulata*	13/09/2019	44	Muscle
Rainbow runner	*Elagatis bipinnulata*	13/09/2019	50	Muscle
Rainbow runner	*Elagatis bipinnulata*	13/09/2019	46	Muscle

Appendix II. BIOT Tuna Tagging Protocol

This project aims to better to quantify the ecological significance of the British Indian Ocean Territory for commercially important tuna species within the Indian Ocean.

Target species

We are predominantly interested in yellowfin tuna (*Thunnus albacares*) as these are classified as *overfished* and *subject to overfishing* by the Indian Ocean Tuna Commission. However, please follow the protocols below if you incidentally catch skipjack tuna (*Katsuwonus pelamis*), bigeye tuna (*Thunnus obesus*), dogtooth tuna (*Gymnosarda unicolor*), as these are also of great interest.

What you will need

All of the technical equipment you need should be in the tuna tagging dry bag. Before starting to fish, please check that the dry bag contains:

1. One tape measure
2. A set of yellow conventional tags
3. A conventional tag applicator
4. Haemostats and scissors
5. A set of labelled vials prefilled with ethanol

In addition, you should have to hand:

1. A clean rag or cloth (soft material)
2. Sling (in science store)
3. Unhooking mat
4. A pair of longnose pliers
5. Clean gloves
6. Digital camera
7. A GPS
8. Notepad

How to tag

Please ensure that all tuna are caught using either rod and line or handlines. Please only use one hook per lure in order to avoid damaging the fish. The use of treble hooks should be avoided where possible. Once a tuna has been hooked, bring it to the boat quickly to ensure that the fish is as healthy as possible (no “playing it” for sport) and to minimise the risk of shark predation. Once alongside the boat, the following steps should be taken:

1. Gently bring the fish onto the boat. For smaller tuna, you should lift by the leader. For larger tuna, please use a sling if available.
2. Wearing gloves, place the tuna down onto a wetted unhooking mat.
3. Immediately place a damp cloth over the eyes to minimise stress.
4. If available, place a water hose into the mouth to irrigate.
5. Remove the hook using the pliers.
6. Using the tape measure, take a fork length measurement. This is from the tip of the snout to the fork in the tail (see Figure 1).
7. Using the haemostats and scissors, take a small amount of tissue (about the size of a large nail clipping) from one of the finlets. It is best to take a leading edge where possible.

8. Place the fin clip in one of the labelled vials, making a note of the vial number.
9. Load a conventional tag into the applicator and insert into the muscle below the dorsal fin, as demonstrated during your training (see Figure 1).
10. Then repeat on the other side so that the animal has two tags, one either side of the dorsal fin. Make a note of the tag numbers.
11. Take a quick photo of the tagged animal.
12. Once both tags are in, take out the hose, remove the eye cover and release the fish back into the water. Where possible drop the fish in headfirst to ensure it gets a rush of water over the gills.
13. Make a note of when and where the fish was caught. A GPS position is preferred although detailed descriptions such as "*in the channel between Ile Poule and Petite Soeur*" are fine.
14. Fill in all of the information on to the tagging data sheet.
15. Store fin clips in a cool, dry location.

This whole process should be very quick and ensure that the animal is out of the water for no longer than two minutes. Remember, every second counts to ensure that the animal has the best chance of survival.

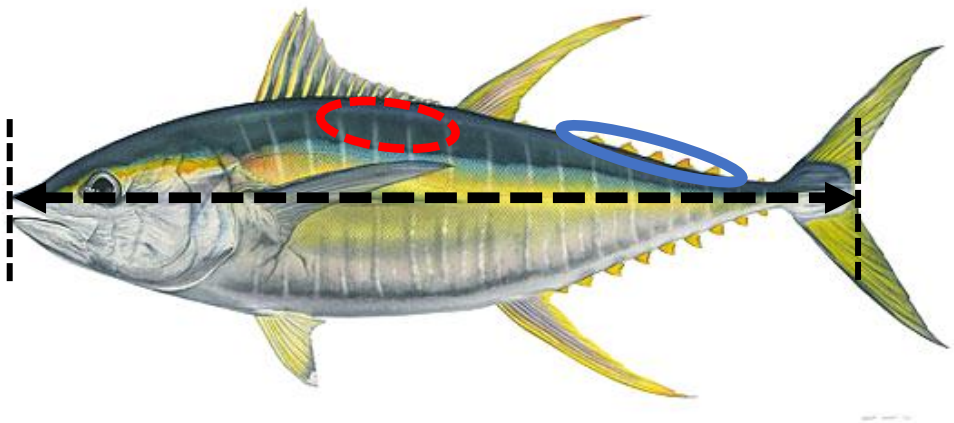

Figure 1. Locations of key sampling areas on a tuna (in this case a yellowfin tuna). The black dashed line represents the fork length measurement. The red circle denotes where the conventional tags should be applied on either side of the dorsal fin. The blue solid circle denotes the location of the finlets where the DNA fin clip sample should be taken.

What to do if the tuna is already dead

If a tuna is already dead, obviously do not tag it. However, please take a fork length measurement, a fin clip and fill in the tagging data sheet as before.

Appendix III. BIOT IUU Sampling Protocol

Seized catches, whilst unfortunate, represent an amazing opportunity to gather important information on rare and endangered species within BIOT. For many of these species, IUU catches represent our only opportunity to gather such data. As such, we ask that the protocols below be followed whenever possible.

What you will need

All of the technical equipment you need should be in the sampling dry bag. Before starting to process the catch, please check that the dry bag contains:

6. One tape measure
7. Haemostats and scissors
8. A set of labelled vials prefilled with ethanol

In addition, you should have to hand:

9. A pair of gloves
10. Digital camera
11. Notepad

How to sample

We are conscious that seized catches may involve hundreds of animals, and time and personnel available for sampling may be limited. As such, the following protocol has been designed to be as streamlined as possible. This is also a suggested workflow, please adjust accordingly on a case-by-case basis.

So, either as the catch is unloaded, or after it has been unloaded:

1. Lay each shark* on its side so that both dorsal fin and upper/ lower sides of pectoral fins, and claspers (if present) are visible.
2. Identify each individual to species level (if possible, see IOTC guide for assistance), sex it (by confirming the presence or absence of claspers between the anal fins) and take a fork length measurement (tip of nose to fork in tail; Figure 1).
3. Place tape measure on or next to the shark.
4. Take a photograph of the shark from directly above, ensuring that the whole of the animal is in view and that the tape measure can be easily read.
5. Using the haemostats and scissors, take a small amount of tissue (about the size of a large nail clipping) from the dorsal fin.
6. Place the fin clip in one of the labelled vials, making a note of the vial number.
7. Repeat for all individuals.
8. Fill in all of the information on to the IUU sampling data sheet.
9. Store fin clips in a cool, dry location.
10. Email/file transfer datasheet and photos to david.curnick@zsl.org for verification.

*For rays, take a photo of the dorsal (top) side for identification purposes. Then, lay them on their backs and follow steps 2-10.

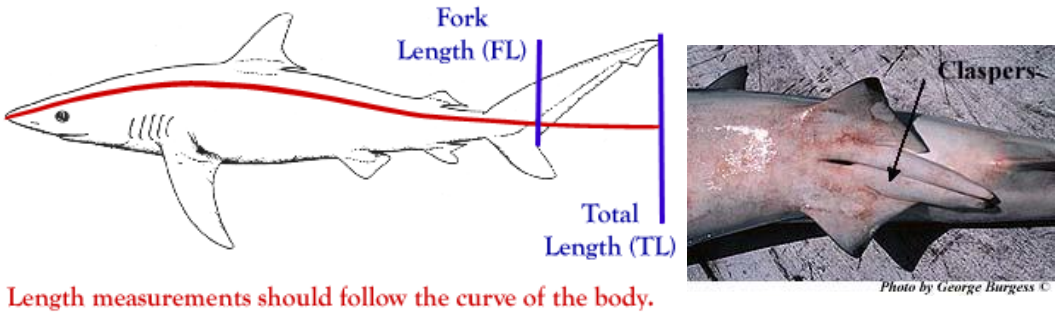

Figure 1. How to take a fork length (FL) measurement of a shark and what male shark claspers look like.

If the catch is large, sharks can be sorted into batches of 3 or 4 with the tape measure placed on the middle shark. In this case, make sure that the photo is taken from directly above and that the tape measure can be easily read.

Please avoid sub-sampling whenever possible. These data are incredibly valuable. If you do need to sub-sample, the please do all of the priority species below and then sub-sample the remaining catch. Particular attention should also be placed on grey reef (*Carcharhinus amblyrhynchos*) and silvertip sharks (*Carcharhinus albimarginatus*) here as these are the focus of much research in BIOT currently.

If time permits, a corresponding weight (in kilograms) of each elasmobranch would be useful.

Priority species for genetic sampling

We are keen to get genetic samples from all of the catch whenever possible. Again, these data are incredibly valuable. However, if time is limited, please prioritise the following IOTC focal, endangered, and CITES listed elasmobranch species:

Pelagic thresher shark (*Alopias pelagicus)*
Bigeye thresher shark (*Alopias superciliosus)*
Silky shark (*Carcharhinus falciformis)*
Oceanic whitetip shark (*Carcharhinus longimanus)*
Bull shark (*Carcharhinus leucas*)
Tiger shark (*Galeocerdo cuvier*)
Shortfin mako shark (*Isurus oxyrinchus)*
Reef manta ray *(Mobula alfredi)*
Oceanic manta ray *(Mobula birostris)*
Blue shark (*Prionace glauca)*
Whale shark *(Rhincodon typus)*
Scalloped hammerhead shark (*Sphyrna lewini)*
Great hammerhead shark *(Sphyrna mokarran)*

Finally, any unusual or uncommon species for the territory such as guitarfishes (Rhinobatidae) or sawfishes (Pristidae) should always be sampled.

What to do if the animal has been mutilated/dressed/processed

If the full body of the animal is no longer intact, please take a fin, muscle or gill raker tissue sample and label it as “unknown species”. We can give you a species ID once we process the genetics.

Please email david.curnick@zsl.org if you have any questions