Population Dynamics Of Marine Turtles Under Harvest

Submitted by

Thomas Brian Stringell

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……  ……

Abstract

Understanding the ecology and life history of marine turtle populations is fundamental for their effective conservation, especially for those that are harvested for food. This thesis presents a collection of six chapters that progress from the applied to the pure; conservation and management in the first chapters through to animal ecology in the latter. A variety of contemporary and multidisciplinary techniques are utilised to explore the structure, populations dynamics and ecology of two marine turtle species, the green turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*), under harvest in the Turks and Caicos Islands (TCI), Caribbean. The work first focuses on the structure of TCI's small-scale fishery and the demographics of turtles landed and incorporates nesting seasonality, adult take, satellite tracking and genetic structure to suggest evidence-based legislative amendments. As part of the study of this fishery, this work reports on how the harvest might increase prevalence of disease in green turtles. As an exploration into the ecology of turtle stocks found in TCI, the work then describes and compares in-water immature and adult sex ratios, genetic differentiation and sex biased dispersal. Finally, stomach content and habitat matching, and stable isotope analyses provide insights into the foraging ecology and suggested keystone roles of sympatric green and hawksbill turtles.

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al. 1992, Glen & Mrosovsky 2004); 26% female at Guadeloupe (GU Kamel & Mrosovsky 2006). Green turtles: 54-68% at Suriname (SUR: Mrosovsky et al. 1984, Godfrey et al. 1996); 70.2% at Poilao Guinea Bissau (GBP Rebelo et al. 2011); 79% at Cyprus (Kaska et al. 1998, Broderick et al. 2000), 92% at Turkey (Broderick et al. 2000, Casale et al. 2000); 75-87% at Ascension Island (ASCI Broderick et al. 2001, Godley et al. 2002, Pintus et al. 2009); 54% average from Tortuguero, Costa Rica (CR Standora & Spotila 1985, Spotila et al. 1987, Horikoshi 1992).

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Table S1. Haplotype frequencies using long sequence lengths (740bp) at hawksbill rookeries and mixed stocks used in the Mixed Stock Analyses. TCI foraging groups that make up the mixed stock are also listed. N denotes the number of samples in each group. Haplotype diversity (*h*) and nucleotide diversity (π) was calculated in Arlequin 3.5 (Excoffier & Lischer 2010), the latter using a Tamura 3-parameter substitution model (Tamura 1992).

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Chapter 5: Taxonomic distinctness in the diet of two species of marine turtle

Table 1. Frequency (proportion of turtles in which present) and average (±SD and range) proportion of biomass of taxonomic diet groups found in stomach content samples of green turtles (n=92) and hawksbill turtles (n=45).

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Figure 1. Biplot of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (‰) for hawksbill turtle (A, n=108) and green turtle (B, n=108) blood plasma samples (circles). Filled circles are turtles for which we also had stomach content samples (n=45 hawksbills and n=92 greens). Diet sources (±SD) are bluegreen algae (bl), red algae (r), green algae (g),

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Figure 7. Mixing model contribution proportions across turtle size classes (cm, CCL). Proportions of sponges to hawksbill turtle diet (A, C), and seagrasses to green turtle diet (B, D) are derived from plasma tissue. Top panel (A, B) shows results from models with uninformative (uniform) priors and bottom panel (C, D) with priors based on relative percentage of diet composition in stomach content samples (taken from Stringell et al. in prep., Chapter 5). Box plots represent the 50%, 75% and 95% credible intervals from dark to light grey.

Chapter 6: Supplementary Information

Table S1. Mean ± SD of stable isotope values (‰) by turtle species, tissue type and size class (CCL). Shaded values indicate average across sizes and total sample size.

Table S2. Taxonomic diet source groups used in the SIAR mixing models and their mean ±SD carbon and nitrogen isotopes (‰). The number of taxa and samples for each source group are given.

Figure S1. Biplot of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (‰) for hawksbill turtles (Ei: A, C) and green turtles (Cm: B, D) and two tissues: red blood cells (squares; A, B) and scute (triangles; C, D). Filled symbols are those turtles that also had stomach content samples (n=45 hawksbills and n=92 greens; see Stringell et al. in prep., Chapter 5). Diet sources (±SD) are bluegreen algae (bl), red algae (r), green algae (g), brown algae (b), seagrasses (sg), sponges (sp), cnidarians (c), and other invertebrates (i).

Figure S2. Convex hulls (joining the extreme most means of turtle size classes:

CCL, cm) of the all-size turtle populations from three tissue types: Top panel, plasma (A, B); middle panel, red blood cells (C, D); bottom panel, scute (E, F). Left panel, hawksbill turtles (Ei: A, C, E); right panel, green turtles (Cm: B, D, F).

Figure S3. Sample-size corrected Standard Ellipse Areas (SEAc) for turtle size classes (CCL, cm) and three tissue types: Top panel, plasma (A, B); middle panel, red blood cells (C, D); bottom panel, scute (E, F). Left panel, hawksbill turtles (Ei: A, C, E); right panel, green turtles (Cm: B, D, F).

Figure S4. Diet source contributions to green turtle blood plasma samples across turtle size classes (CCL, cm). Data are from SIAR mixing models. Each panel represents one of eight sources: bluegreen algae, red algae, green algae, brown algae, seagrasses, sponges, cnidarians, and other invertebrates.

Figure S5. Diet source contributions to hawksbill turtle blood plasma samples across turtle size classes (CCL, cm). Data are from SIAR mixing models. Each panel represents one of eight sources: bluegreen algae, red algae, green algae, brown algae, seagrasses, sponges, cnidarians, and other invertebrates.

Author's Declaration

All chapters presented in this thesis were written by T. B Stringell under the supervision of A.C. Broderick, B.J.Godley, P.B.Richardson.

All molecular analyses, stable isotope sample preparation, and stomach content analysis were conducted at the CEC laboratories. Most DNA sequencing was carried out by Macrogen Europe. Blood hormone enzyme immuno-assays and gonad histology were carried out at the University of Exeter Biosciences laboratories under the supervision of A.Lange and C.Tyler. All stable isotope analysis was carried out at the NERC Life Sciences Mass Spectrometry Facility at the Scottish Universities Environmental Research Centre, East Kilbride, under the supervision of J. Newton and R. McGill. Fieldwork in TCI was carried out as part of the Turks and Caicos Islands Marine Turtle Project under the direction of P.B.Richardon, with the assistance of A. Sanghera, Q.Philips, M.Calosso, J.Claydon, F.Kent, S.Ranger, A.C.Broderick, and in-country support from DEMA and SFS. Numerous SFS volunteers and Staff helped with turtle capture and capture-mark-recapture data recording, particularly at the local South Caicos sites. Numerous fishermen gave boat support and access to landed turtles for sampling. K.Lockhart, L.Seymour and W.Clerveaux provided research permission and CITES permits.

Specific author contributions to chapters are detailed below:

Chapter 1: Marine turtle harvest in a mixed small-scale fishery: Evidence for revised management measures.
Thomas B. Stringell, Marta C. Calosso, John A.B. Claydon, Wesley Clerveaux, Brendan J. Godley, Kathy J. Lockhart, Quinton Phillips, Susan Ranger, Peter B. Richardson, Amdeep Sanghera, Annette C. Broderick.

TBS, AS, QP, PBR, MCC, JABC and ACB conducted fieldwork and sampled turtles. AS, QP, TBS and PBR observed and sampled turtles from the fishery. TBS collated and entered effort, sightings and biological data. KJL provided DEMA fishery landings data. TBS analysed all data, produced all figures and tables and was the

lead author on the manuscript. ACB, BJG and PBR provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 2: Vulnerability of adult marine turtles in a contemporary turtle fishery: Recommendations for legislative change.

Thomas B. Stringell, Wesley Clerveaux, Brendan J. Godley, Quinton Phillips, Susan Ranger, Peter B. Richardson, Amdeep Sanghera, Annette C. Broderick.

TBS, AS, QP, PBR and ACB conducted capture-mark-recapture and nesting surveys and sampled turtles. AS, QP, TBS and PBR sampled turtles from the fishery. TBS carried out all genetic laboratory work and analysed the genetic data. PBR managed the satellite tracking data and helped write the satellite tracking methods and results section of the manuscript. AS, QP, TBS, SR attached the satellite transmitters. TBS collated and entered nesting, effort, sightings and biological data. TBS analysed all data, produced all figures and tables and was the lead author on the manuscript. ACB, BJG and PBR provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 3: Fisher choice may increase prevalence of green turtle fibropapillomatosis disease.

Thomas B. Stringell, Wesley Clerveaux, Brendan J. Godley, Quinton Phillips, Susan Ranger, Peter B. Richardson, Amdeep Sanghera, Annette C. Broderick.

TBS, AS, QP, PBR, and ACB conducted fieldwork and sampled turtles. AS, QP, TBS, PBR observed and sampled the turtle fishery and AS, QP, TBS conducted fisher interviews. TBS collated and analysed all data, produced all figures and tables and was the lead author on the manuscript. ACB, BJG and PBR provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 4: Female biased sex ratios of marine turtles: Insights from life-stages and origin.

Thomas B. Stringell, Carlos Carreras, F Alberto Abreu-Grobois, Brendan J. Godley, Anke Lange, Quinton Phillips, Alan F Rees, Peter B. Richardson, Amdeep Sanghera, Charles R Tyler, Annette C. Broderick.

TBS, AS, QP, PBR and ACB conducted CMR surveys and sampled turtles. AS, QP, TBS and PBR sampled turtles from the fishery. TBS carried out all genetic laboratory work under the guidance of CC with assistance from AFR, and analysed the genetic data. AAG verified unique haplotypes. TBS and AL conducted hormone analysis under the supervision of CRT. TBS collated and analysed data, produced all figures and tables and was the lead author on the manuscript. ACB, BJG, PBR and CC provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 5: Taxonomic distinctness in diet of two species of marine turtle.

Thomas B. Stringell, Brendan J. Godley, Flora Kent, Emma Lewis, Jessica Marsh, Quinton Phillips, Peter B. Richardson, Amdeep Sanghera, Annette C. Broderick.

TBS, AS, QP, PBR, FK and ACB conducted CMR surveys and sampled turtles. AS, QP, TBS, PBR and FK sampled turtles from the fishery. FK and TBS conducted habitat surveys. EL, JM, TBS and FK sorted stomach samples and identified taxa. TBS, FK, EL and JM collated and entered habitat and stomach content data. TBS analysed all data, produced all figures and tables and was the lead author on the manuscript. ACB, BJG and PBR provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 6: Isotopic niche separation, ontogenetic shifts and diet in sympatric marine turtles

Thomas B. Stringell, Brendan J. Godley, Rona McGill, Jason Newton, Quinton Phillips, Peter B. Richardson, Amdeep Sanghera, Annette C. Broderick.

TBS, AS, QP, PBR and ACB conducted CMR surveys and sampled turtles. AS, QP, TBS and PBR sampled turtles from the fishery. TBS sorted stomach samples and identified taxa. TBS prepared all samples and TBS, JN and RMcG conducted stable isotope analysis. TBS analysed all data, produced all figures and tables and was the lead author on the manuscript. ACB, BJG and PBR provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

List of Abbreviations

A	Adult
AIC	Akaikes Information Criterion
AMOVA	Analysis of Molecular Variance
ANOSIM	Analysis of Similarities
ANT	Antigua (Jumby Bay)
ASCI	Ascension Island
AVI	Aves Island, Venezuela
AvTD	Average Taxonomic Distinctness
BIC	Bayesian Information Criterion
BLE	Barbados (Leeward)
BOK	Bioko, Equatorial Guinea
bp	Base Pairs
BRZ	Brazil (Atol das Rocas & Fernando de Noronha, or Bahia)
BVI	British Virgin Islands
BWI	Barbados (Windward)
CCL	Curved Carapace Length
CD	Mean Distance to Centroid
CI	Confidence Interval
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
Cm	*Chelonia mydas*
CMR	Capture-Mark-Recapture
CPUE	Catch Per Unit Effort
CR	Costa Rica (Tortuguero)
CUB	Cuba (Doce Leguas or Southwest)
CYP	Northern and Greek Cyprus
dCR	$\delta^{13}C$ Range
DECR	Department of Environment and Coastal Resources
DEMA	Department of Environment and Maritime Affairs
dNR	$\delta^{15}N$ Range
DRJ	Dominican Republic (Jaragua NP)
DRS	Dominican Republic (Saona Island)
E2	Oestradiol-17ß
Ei	*Eretmochelys imbricata*
EIA	Enzyme Immuno-assay

F	Female
FDR	False Discovery Rate
FLO	Florida, USA
FP	Fibropapillomatosis
F_{ST}	Fixation Index (Haplotype Frequency-based)
GAM	General Additive Model
GBP	Poilao, Guinea Bissau
GLM	Generalised Linear Model
GPS	Global Positioning System
GU	Guadeloupe
h	Haplotype Diversity
Imm	Immature
J	Juvenile
JM	Juvenile Males
M	Male
MCMC	Markov Chain Monte Carlo
MCS	Marine Conservation Society
MDS	(Non-Metric) Multidimensional Scaling
MEX	Yucatan or Quintana Roo, Mexico
MNND	Mean Nearest Neighbour Distance
MON	Montserrat
MSA	Mixed Stock Analysis
mtDNA	Mitochondrial DNA
MTTS	Marine Turtles Time Series Software
nDNA	Nuclear DNA
NERC	Natural Environment Research Council
NIC	Nicaragua (Pearl Cays)
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Analysis of Dispersion (Homogeneity of Variance)
PIT	Passive Integrated Transponder
PRST	Principe & Sao Tome, Gulf of Guinea
PRV	Puerto Rico (Mona Island)
R	Recruit
RBC	Red Blood Cells
SA	Sub-Adult

SC	South Caicos
SCL	Straight Carapace Length
SD	Standard Deviation
SDNND	SD of Nearest Neighbour Distance
SE	Standard Error
SEAb	Bayesian Standard Ellipse Areas
SEAc	Sample Size corrected Standard Ellipse Areas
SFS	The School for Field Studies
SIA	Stable Isotope Analysis
SIAR	Stable Isotope Analysis in R
SIBER	Stable Isotope Bayesian Ellipses in R
SIMPER	Similarity Percentage Routine
SSF	Small-scale fishery
STAT	Satellite Tracking and Analysis Tool
SUR	Suriname (Galibi)
T	Testosterone
TA	Total Area
TAXDTEST	Taxonomic Distinctness Test
TCI	Turks and Caicos Islands
TD	Taxonomic Distinctness
TEF (Δdt)	Trophic Enrichment Factor
TKY	Turkey
TRI	Trinidade, Brazil
TSD	Temperature-Dependent Sex Determination
UKOT	UK Overseas Territory
USV	US Virgin Islands
VarTD	Variation in Taxonomic Distinctness
WCR	Wider Caribbean Region
WIDECAST	Wider Caribbean Sea Turtle Network
WoRMS	World Register of Marine Species
δ13C	^{13}C:^{12}C
δ15N	^{15}N:^{14}N
π	Nucleotide Diversity
Φ_{ST}	Fixation Index (Genetic Distance-based)

Introduction

Fishing affects almost every marine ecosystem. Small-scale fisheries (SSF) contribute to more than half of the world's marine and inland fish catch, nearly all of which is used for human consumption (FAO 2010). With SSF dominating coastal seas (Stewart et al. 2010), the impact on biodiversity can be large (Peckham et al. 2007, Soykan et al. 2008, Mangel et al. 2010, Alfaro-Shigueto et al. 2011). Numerous species are overexploited and greatly reduced (Myers & Worm 2003, Pauly et al. 2005, FAO 2010) and marine turtles are no exception.

For centuries, marine turtles have been a source of meat, eggs, shell, oil and leather and their reduction from historical abundances is widely documented, particularly in the Caribbean region (Jackson et al. 2001, McClenachan et al. 2006). Marine turtles are sensitive to exploitation due to their complex life history traits, including natal philopatry, a broad distribution of life-stages across extensively disbursed habitats, extended period to sexual maturity and multi-decadal generation times (Crouse et al. 1987, Crowder et al. 1994, Heppell & Crowder 1996, FitzSimmons et al. 1997). Moreover, conservation of such wide-ranging species can involve a multitude of stakeholders and nations that have differing regulatory and management frameworks, and cultural, traditional and economic values (Frazier 2002, Blumenthal et al. 2007, Hawkes et al. 2012). Knowledge of critical population parameters, such as in-water sex ratios, size/life-stage and genetic population structure, contributes to the understanding of population dynamics of marine turtles and evidence-based sustainable management. Gaining this knowledge, however, is difficult because of the need to sample turtles at sea. Utilising legal turtle fishery landings to sample turtles, complemented with surveys at sea, provides an ideal opportunity to explore some of these population aspects that would otherwise be difficult to obtain.

One of the few remaining Caribbean nations that permit the harvest of marine turtles for local domestic consumption is the UK Overseas Territory of the Turks and Caicos Islands (TCI). It is thought that TCI has the largest legal and regulated marine turtle fishery in the UK Overseas Territories (Richardson et al. 2009). Despite these regulations the fishery is unmonitored and, until now, there has been no robust quantitative assessment of the size and structure of the fishery. Here, green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles are landed for

consumption. Current TCI turtle fishery legislation is out-dated; it allows the capture of any turtle species at sea with a minimum size of 20inches (51cm) carapace length and 20lbs (9kg) or more in weight (Richardson et al. 2006). Such regulations were inherited from fisheries legislation where minimum landing size is commonly used. But for long-lived species such as marine turtles, protection of large juveniles and reproductive adults is critical to their recovery (Crouse et al. 1987, Crowder et al. 1994, Heppell & Crowder 1996). Of particular conservation concern is the capture of adult hawksbills in TCI, given their critically endangered status (Mortimer & Donnelly 2008).

Exploitation of nesting females from natal rookeries can quickly cause population declines (Bell et al. 2006, McClenachan et al. 2006, McGowan et al. 2008, Kittinger et al. 2013) and protection of nesting rookeries has consequently been one of the tenets of marine turtle conservation. In the Caribbean, there has been a call for a renewed conservation focus on remnant nesting populations, especially for the hawksbill turtle (McClenachan et al. 2006, Mortimer & Donnelly 2008). Genetic characterisation of small and unsampled turtle rookeries in the Wider Caribbean Region is advocated to further elucidate their importance in stock connectivity and their potential to maintain regional genetic diversity (Leroux et al. 2012, Shamblin et al. 2012). The magnitude, seasonality and genetic structure of the green and hawksbill turtle rookeries of TCI are poorly known. They are thought to be remnants of past populations that were subject to regular harvest (Richardson et al. 2009). The legal turtle fishery here may still be a threat to existing populations and affect the recovery of both species.

It is thought that emerging infectious diseases of wildlife are increasing globally with consequences to human, animal and ecosystem health (Cohen 2000, Daszak et al. 2000, Ward & Lafferty 2004, Jones et al. 2008). Harvesting may alter disease prevalence and mortality directly or indirectly (Choisy & Rohani 2006) and when this results in an increase in the disease, may present a serious threat to wildlife or resource conservation, particularly in the management of species of conservation concern. Fibropapillomatosis (FP), a disease characterised by external and internal tumours, has been found in most species of marine turtle, primarily green turtles (Herbst 1994). It is one of the most significant neoplastic diseases in reptiles (Herbst 1994) and has become a global pandemic (Williams et al. 1994). FP

is present in green turtles of TCI, and the influence of the turtle harvest on FP disease dynamics is unknown.

For species with temperature-dependent sex determination (TSD), a better understanding of sex ratios in wild populations and the factors affecting survivorship of the sexes is crucial for supporting effective conservation strategies, especially given predicted scenarios of climate warming. In marine turtles, which exhibit TSD, a greater proportion of female hatchlings are produced at higher nest temperatures and female biased 'primary' sex ratios (pertaining to hatchlings) are common (see Hawkes et al. 2009 for review). Under current climate change scenarios, it is predicted that widespread feminisation of primary sex ratios and a critically reduced proportion of males could hinder population maintenance (Hawkes et al. 2007, Hawkes et al. 2009, Poloczanska et al. 2009, Witt et al. 2010, Fuentes et al. 2011). Studies of juvenile marine turtles captured at sea reveal variation in "secondary" sex ratios (pertaining to post hatchling stages) across sites, but most are also female biased. However, some studies show 1:1 or even male biased adult sex ratios (Chaloupka & Limpus 2001, Stewart & Dutton 2011, Wright et al. 2012), despite female-biased sex ratios in rookeries or immature life stages. This shift from highly skewed female biased primary sex ratios to 1:1 or "Fisherian" ratios (Fisher 1930) in adult stages is an elusive question in marine turtle biology; causes of this marked difference are unknown.

Maternally inherited mitochondrial DNA (mtDNA) has been widely used to assess marine turtle population structure among rookeries and foraging aggregations (see Bowen & Karl 2007, Lee 2008 for reviews). Turtles from different and disbursed rookeries may converge and recruit to mixed origin feeding grounds in shallow coastal waters (Musick & Limpus 1997). Mixed stock analysis (MSA) methodology using mtDNA markers enables groups of individuals in mixed feeding aggregations to be linked to their rookeries of origin (Bolker et al. 2007). Knowledge of mixed stock composition and origin may have profound conservation implications for 'shared' resources under harvest (Moncada et al. 2012).

Green turtles as herbivores and hawksbill turtles as spongivores are thought to have key ecological roles in seagrass and coral reef ecosystems, and their overexploitation has been implicated in detrimental ecosystem changes (Jackson 1997, Bjorndal & Jackson 2003). Understanding the foraging ecology of 'keystone'

species (Paine 1969) is vital to inform effective conservation of the ecosystems in which they function. However, studying trophic role in marine vertebrates is challenging, and for marine turtles, until recently, has been primarily limited to data from stomach content, which represents only a 'snapshot' of feeding and may not adequately reflect diet that is assimilated into bodily tissues over time (Duffy & Jackson 1986, Barrett et al. 2007). Stable isotope analysis (SIA), however, offers insights into foraging ecology because stable isotope ratios of heavy to light isotopes in consumer tissues represent diet integration over short to long time frames (depending on the tissue) and can reliably indicate trophic position (typically from nitrogen isotope ratios, $\delta^{15}N$) and spatial resource use (typically from carbon isotope ratios, $\delta^{13}C$) (Hobson 1999, Post 2002). In marine turtles, stable isotope ratios have been used successfully to identify foraging habitats and diets (for examples see Godley et al. 1998, Hatase et al. 2002, Wallace et al. 2009, McClellan et al. 2010, Dodge et al. 2011, Lemons et al. 2011).

Carr (1986, 1987) proposed that marine turtles undergo habitat shifts at differing life-stages and described how rarely sampled early stage juvenile turtles inhabiting offshore epipelagic habitats (the "lost years") later recruit to coastal waters to feed benthically. Recent SIA research confirmed this likely ontogeny in green turtles and indicated that diet shifted from an omnivorous diet during the first three to five years of their lives to a largely herbivorous diet in later years (Reich et al. 2007, Arthur et al. 2008). Similar SIA studies, however, have not been carried out for hawksbill turtles, although they may also undergo ontogenetic shifts as they recruit to coastal feeding grounds and develop spongivorous diets (Witherington et al. 2012).

The University of Exeter and the Marine Conservation Society were invited by the TCI Government to study the marine turtle fishery as part of a two-year collaborative project. The aims of this study were first to define the magnitude and seasonality of the fishery (Chapter 1) and the nesting population (Chapter 2). The work set out to genetically characterise the TCI rookeries and, using satellite telemetry, establish the degree of residence in adult turtles of TCI waters. These first chapters propose recommendations for fishery management: the introduction of maximum size limits, and a closed season on hawksbill turtle take during the lobster fishing season. An absence of FP in the green turtle fishery but prevalence in turtles surveyed at sea, and how harvest might increase the disease in wild populations are

explored in Chapter 3. Chapter 4 focuses on the genetic structure and sex ratios of the mixed aggregations of foraging turtles in TCI. Sex is determined using circulating blood hormone concentrations (Owens et al. 1978) and calibrated with direct observations and histology of gonads from butchered turtles. Using mtDNA and Bayesian MSA, the probable origin of juveniles is elucidated and sex ratio shifts among life-stages are explored. In the last two companion chapters, the foraging ecology of the two study species is explored using stomach content analysis and SIA: diet in stomach content samples from the turtle fishery is analysed using a technique novel to marine turtle research (taxonomic distinctness: Clarke & Warwick 1998, 2001) (Chapter 5); and niche separation (isotopic niche metrics: Jackson et al. 2011), ontogenetic shifts and diet composition (isotope mixing models: Parnell et al. 2010) are examined using carbon and nitrogen isotope ratios (Chapter 6).

The results of this work provide a baseline for future monitoring by the TCI Government and recommends specific changes to the legislation that may aid the recovery of the turtle species in TCI. The Government is currently incorporating the suggested amendments into the TCI fishing ordinance. As such, it is hoped that this work will contribute to the conservation of the species, not just in TCI but the Wider Caribbean.

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Chapter 1

Marine turtle harvest in a mixed small-scale fishery: Evidence for revised management measures

Thomas B. Stringell[1], Marta C. Calosso[2], John A.B. Claydon[2], Wesley Clerveaux[3], Brendan J. Godley[1], Kathy J. Lockhart[3], Quinton Phillips[3], Susan Ranger[1,4], Peter B. Richardson[1,4], Amdeep Sanghera[4] and Annette C. Broderick[1]

[1] Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ. UK
[2] The School for Field Studies, Center for Marine Resource Studies, South Caicos, Turks and Caicos Islands, BWI
[3] Department of Environment and Maritime Affairs (formerly the Department of Environment & Coastal Resources), South Caicos, Turks and Caicos Islands, BWI.
[4] Marine Conservation Society, Ross on Wye, Herefordshire, HR9 5NB. UK

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Abstract

Small-scale fisheries (SSF) account for around half of the world's marine and inland fisheries, but their impact on the marine environment is usually under-estimated owing to difficulties in monitoring and regulation. Successful management of mixed SSF requires holistic approaches that sustainably exploit target species, consider non-target species and maintain fisher livelihoods. For two years, we studied the marine turtle fishery in the Turks and Caicos Islands (TCI) in the Wider Caribbean Region, where the main export fisheries are queen conch (*Strombus gigas*) and the spiny lobster (*Panulirus argus*); with fin-fish, green turtles (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) taken for domestic consumption. We evaluate the turtle harvest in relation to the other fisheries and recommend legislation and management alternatives. We demonstrate the connectivity between multi-species fisheries and artisanal turtle capture: with increasing lobster catch-per-unit-effort (CPUE), hawksbill turtle catch increased whilst green turtle catch decreased. With increasing conch CPUE, hawksbill catch declined and there was no demonstrable effect on green turtle catch. We estimate 176-324 green and 114-277 hawksbill turtles are harvested annually in TCI: the largest documented extant legal hawksbill fishery in the western Atlantic. Of particular concern is the capture of adult hawksbill turtles. Current legislation focuses take on larger individuals that are key to population maintenance. Considering these data we recommend the introduction of maximum size limits for both species and a closed season on hawksbill take during the lobster fishing season. Our results highlight the need to manage turtles as part of a broader approach to SSF management.

Introduction

Small-scale fisheries (SSF) are estimated to account for more than half of the world's marine and inland fish catch (FAO, 2010). The majority of the world's fishers are located in developing countries and operate using small boats of <12m in length (FAO, 2010). The terms 'small-scale' and 'artisanal' are often used interchangeably. However, SSF are generally commercial fisheries even when they retain traditional aspects (Chuenpagdee et al., 2006). Definitions aside, 'small-scale' does not necessarily mean small impact (McCluskey and Lewison, 2008; Alfaro-Shigueto et al., 2010); catch by individual fishers might not always be substantial, but fleets can be large and have considerable impacts on coastal wildlife (Alfaro-Shigueto et al., 2011; Mangel et al., 2010; Peckham et al., 2007; Soykan et al., 2008). With SSF dominating the global coastal shelf (Stewart et al., 2010), environmental impact is likely to be concentrated in coastal areas that are already likely to be subject to other human pressures (Dunn et al., 2010).

SSF are generally managed by biologically based control measures for single species, e.g. catch quotas, gear restrictions, effort limits, fishing seasons. Most SSF, however, operate as multi-species or mixed fisheries (Salas et al., 2007) and as such single-species based management approaches tend to fail, having indirect effects on other fisheries and fisher behaviours (Béné and Tewfik, 2001). Multi-species or ecosystem-based management approaches that assess multiple biological stocks and their interactions and account for the complexities of fisher behaviours, fleet dynamics, socioeconomic drivers and maintain livelihoods are needed for mixed SSF (Andrew et al., 2007; Béné and Tewfik, 2001; FAO, 2010; Fanning et al., 2011). Knowledge of the dynamics of the whole SSF is key to managing healthy coastal ecosystems and supporting communities that rely on them.

Understanding the impacts of SSF on coastal ecosystems, however, is hindered by a paucity of quantitative information on catches, fishery effort and employment in SSF because of their complexity and the generally poor institutional capacity in developing countries to collect relevant data (Dunn et al., 2010; FAO, 2010; Salas et al., 2007). This, in turn, hinders the formulation of appropriate policies and management in the SSF sector (Andrew et al., 2007; FAO, 2010).

In this paper, we assess a multi-species SSF in the Turks and Caicos Islands (TCI), a UK Overseas Territory (UKOT) in the Wider Caribbean Region (WCR). We examine the artisanal take of two sympatric sea turtle species, the green turtle (*Chelonia mydas*) and hawksbill turtle (*Eretmochelys imbricata*), alongside two of the most important and valuable fisheries in the Caribbean - the Queen Conch (*Strombus gigas*) and the Spiny Lobster (*Panulirus argus*) (FAO, 2007).

A minor artisanal fin-fish fishery also exists in TCI for local consumption, and is likely to develop in the coming years; reliable information on this fishery is absent at present and is therefore unable to be assessed here. Lobster and conch represents almost all of the TCI fishery export, principally to USA markets (Department of Environment and Maritime Affairs - TCI, unpublished data; FAO, 2007). Lobster catch-per-unit-effort (CPUE: kg/fisher/day) has been steadily declining in the Caribbean (Tewfik and Béné, 2004) and despite claims that the TCI conch fishery is at maximum sustainable yield (currently 760 metric tonnes; FAO, 2007), signs of overfishing have been reported since the early 1990s (Medley and Ninnes, 1999; Ninnes, 1994).

The fisheries operate together as a multi-species or mixed SSF, catching lobster, conch, fin-fish and sea turtles during single trips. The mixed SSF is characterised by artisanal free-diving fishers usually operating in crews of two or three from *ca*. 6m fibreglass powerboats. Most catch is landed at various fish processing plants within the TCI, with a relatively small quantity being marketed directly to local restaurants for local consumption. Green and hawksbill sea turtles are also mostly harvested for personal consumption, and although the TCI turtle fishery can be considered artisanal and incidental to the lobster and conch fisheries, it is thought to be the largest regulated and legitimate turtle fishery in the UKOTs (Richardson et al., 2009), and possibly second, in magnitude, only to Nicaragua (Lagueux et al., 2003).

The current TCI sea turtle fishery legislation (Fisheries Protection Ordnance, 1998: see Richardson et al., 2006, 2009 for reviews) permits the harvest of both species >51cm length and >20lbs in weight below the low-water mark (i.e. at sea) There is no closed season and fishers are legally entitled to remove an unlimited number of turtles larger than these minimum size limits at any time of the year. These regulations do not adequately safeguard the survivorship of large juvenile

(sub-adults) and reproductive adults, the vital life stages in population maintenance for long-lived and late-maturing species (Carr et al., 1982; Crouse et al., 1987; Crowder et al., 1994; Heppell and Crowder, 1996, Chaloupka, 2002). Minimum size limits such as these focuses take on large individuals and may impede turtle population recovery, even in small but highly regulated turtle fisheries, e.g. Cayman Islands (Bell et al., 2006). The Cayman Islands recently adopted a maximum size limit of 60cm (Cayman Islands Government, 2008), the first protection measure of its kind in the WCR (Dow et al., 2007). Clearly, in the TCI, a biologically relevant management measure is also needed that discourages the capture of large juveniles and adult turtles in both species.

There is a paucity of up-to-date published information on contemporary small-scale marine turtle fisheries, data from which inform relevant management practices. Current data on the size and structure of this fishery are scarce (Richardson et al., 2009; Rudd, 2003). With recent turtle fishery closures in the neighbouring Bahamas (Fisheries Resources (Jurisdiction and Conservation) Regulations, 2009) and in Trinidad and Tobago (Protection of Turtle and Turtle Eggs (Amendment) Regulations, 2011), and a prevailing protectionist approach to marine turtle conservation within the WCR (see Brautigam and Eckert, 2006; Fleming, 2001; Eckert, 2010), there is a clear need to better contextualise and manage the TCI turtle fishery. At the invitation of the local government, we undertook a two-year study to assess the harvest of marine turtles in TCI. Here we set out to gather data that would inform meaningful suggested changes to current management of the turtle fishery.

Material and methods

Study site

The Turks and Caicos Islands (TCI) is a UK Overseas Territory in the WCR, situated at the southern end of the Bahamas (21° 45N, 71° 35W). Intensive monitoring was carried out at South Caicos, the main fishing centre of the TCI, with regular visits made to the two most populated islands of Grand Turk and Providenciales (Figure 1).

Study species

The green turtle (*Chelonia mydas*) and hawksbill turtle (*Eretmochelys imbricata*) are listed as endangered and critically endangered respectively (IUCN, 2010). Although the TCI turtle fishery is regulated by the Fisheries Protection Ordnance (1998), this legislation only protects turtle eggs and nesting females on the beaches and turtles at sea that are smaller than 20 inches (51cm) carapace length (Richardson et al., 2006).

The spiny lobster (*Panulirus argus*) fishery opens on the 1st August each year and is locally known as “the big grab” when maximum landings are made followed by a gradual decline until closure, usually on 31st March (Tewfik and Béné, 2004). No quota system operates for this fishery.

The queen conch (*Strombus gigas*) fishing season runs from 15 October to 15 July or until the export quota (currently 1.6 million lb. / 0.72 million kg) is reached. The queen conch is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and in order for TCI to engage in international trade, the fishery must be managed sustainably.

Monitoring the artisanal turtle fishery and SSF

Collaboration with fishers facilitated direct counts of hawksbill and green turtles landed for local consumption at key fish landing sites, e.g. fish processing plants and public boat docks or jetties. Several, but not all personal jetties used by one or two fishermen were opportunistically monitored. During a two-year survey period (1 December 2008 - 30 November 2010) dockside observations were made for 544 days at South Caicos, 77 days at Grand Turk and 68 days at Providenciales (Table 1, supplementary Figure S1). A typical dockside observation would last for about 4

hours, usually in the afternoon between 14:00 and sunset or until the last boat had returned to dock. Only counts of turtles that were butchered are included in the analyses; any that were landed and returned to the sea, e.g. perhaps because they were undersize and intercepted by government enforcement officers, were excluded. Associated information about butchered landings, e.g. location, method of and reason for capture, was obtained by informally interviewing fishers. Monthly export fishery records of catch (kg) and effort (boat days) of lobster and conch were collected by government enforcement officers on workday afternoons at the fish processing plants of South Caicos.

Turtle harvest estimation

We surveyed key landing sites in South Caicos (n=4) on 75% of days during the survey period (Table 1, supplementary Figure S1). To compile a complete dataset of turtle harvest for each species in South Caicos and to preserve any structure in harvest seasonality and yearly differences that might exist in the South Caicos data, missing values (days with no dockside coverage) were manually interpolated in a hierarchical manner, as follows: 1) We took the mean number of butchered landings for a particular day of the week for each month in each year. 2) If, however, there were fewer than two days of observations for a particular day of the week in each month, we used the mean number of butchered landings for that day of the week during its quarterly period of that year. 3) If, however, there were fewer than two days (for that particular day of the week) on which data were recorded during its quarterly period of that year (e.g. Sundays during parts of the year), we extended the search and took the mean number of butchered landings for that day of the week during its half-year period of that year. This routine ensured an interpolated mean was obtained for each missing value. Interpolations were carried out in MATLAB v. 2008a. Other interpolation methods were trialled, e.g. linear interpolation and cubic-splines, but these did not preserve the inherent seasonality. The harvest at South Caicos is estimated as the sum of interpolated values and direct counts.

We surveyed the key landing sites on Providenciales (n=3) and Grand Turk (n=1) for 9% and 11% of the survey period respectively (Table 1, supplementary Figure S1), so interpolating missing values for these data was not appropriate. Instead, the data from South Caicos were used to inform the likely harvests at these other islands. Harvest estimates for these two additional sites were calculated by

dividing the sum of turtles landed there by the sum of the proportions of interpolated harvest at South Caicos on the 68 and 77 days of survey at Providenciales or Grand Turk respectively. The estimated TCI harvest is the sum of the three island estimates. All 95% confidence intervals of harvest estimates were taken from the percentiles of the distribution of 10,000 randomised estimates, and calculated using R v 2.13 (R Development Core Team, 2011).

Size classes of the harvest

Carapace length of 765 animals (green turtles n=453; hawksbill turtles n=312) from the fishery and our in-water surveys was measured to the nearest mm using a flexible tape measure along the carapace mid-line from the nuchal notch to the longest caudal tip (Curved Carapace Length – CCL, Bolten, 1999). The size of harvested turtles combined from throughout TCI was compared (Mann-Whitney U test) to those captured during our in-water catch-mark-recapture surveys (see Richardson et al., 2009 for details of in-water survey methods and context). We considered that adult carapace size was 97cm for green turtles, and 78cm for hawksbill turtles, based on mean minimum sizes of nesting females recorded in the region (Hirth, 1997; Witzell, 1983).

Harvested turtles were weighed prior to slaughter (green turtles n=120; hawksbill turtles n=79) using Kern digital scales for turtles under 50kg (± 0.05kg) or Salter analogue scales for those weighing over 50kg (± 0.5kg). Where turtle weight was unknown but size was measured (n=39 green turtles, n=29 hawksbills), CCL was converted to weight using power curve parameters (weight = $8.0x10^{-5}.CCL^{3.07}$, r^2=0.98 for green turtles and $6.0x10^{-5}.CCL^{3.14}$, r^2=0.93 for hawksbills). For each species, total annual landing biomass was estimated using an Horvitz-Thompson-like estimator (Horvitz and Thompson, 1952) by dividing the sum weight of the observed and converted harvest by the proportion of these to the estimated annual TCI harvest (i.e. green turtles: 159 of 239=0.665; hawksbill turtles: 108 of 167=0.647). Confidence limits were calculated by multiplying the average harvested (observed and converted) turtle weight ±1.96.SE by the estimated annual TCI harvest ±95% CI. Edible mass (kg of meat etc.) of a subsample of green turtles (n=7) and hawksbill turtles (n=12) was measured by weighing body parts that were going to be consumed. Edible mass was plotted against total body weight and the parameters from the line of best fit used to estimate edible mass of green (n=159)

and hawksbill turtles (n=108) of known and converted weight. The edible mass of the annual harvest was calculated as above, by scaling up the average and 95% confidence limits of edible mass to the annual harvest estimates.

Seasonality of turtle harvest

Yearly, monthly and daily patterns of interpolated totals of green and hawksbill turtles landed at South Caicos were assessed statistically against the null hypotheses that there are no patterns in turtle catch. Research year, month and day of week were included as fixed factors with their two-way interactions in three-way crossed Permutational Analyses of Variance (PERMANOVAs) using PERMANOVA+ in PRIMER v6 (Anderson et al., 2008). Models were carried out on Euclidean distance with 9999 permutations of residuals under a reduced model and Type III (partial) sums of squares.

Small-scale fishery interactions

We compared mean turtle catch at South Caicos with lobster and conch fishing seasons, survey year and their interactions using two-way PERMANOVAs. Fishing seasons were categorised as: both fisheries open, both fisheries closed, lobster fishery open (conch closed), and conch fishery open (lobster closed). We used generalised linear models (GLMs) with negative binomial errors (using the MASS package in R: Venables and Ripley, 2002). Interpolated monthly totals of hawksbill and green turtle landings were used as response variables (n=24) and related to explanatory variables: survey year, fishing season, conch and lobster fishery CPUE, and catch in the other turtle species. CPUE (kg.boatday^{-1}) was used as an explanatory variable because catch and effort were strongly collinear (Pearson's correlation: Lobster r =0.92; Conch r = 0.96). Minimally adequate GLMs were derived by model simplification and Information Criterion (IC) model selection (Akaikes (AIC) and Bayesian (BIC)) following stepwise deletion and sequential Chi-squared likelihood-ratio tests. Model residuals were checked for autocorrelation and conformity to assumptions.

Results

Turtle harvest estimation

We recorded 194 green turtles and 109 hawksbill turtles landed at the South Caicos docks during 544 days of observation in this 2-year study; turtles were landed year-round on 32% (173 of 544) of the observation days. By interpolating the missing days when data were not gathered (186 days over two years), we estimate that 119 (95% CI: 98 - 140) green and 65 (95% CI: 53 - 77) hawksbill turtles are harvested in South Caicos annually (Table 1). At Providenciales, turtles were landed on 18% (12 of 68) of the days of observation and we estimate the annual harvest to be 38 (95% CI: 0 – 109) green and 72 (95% CI: 26 – 177) hawksbill turtles. For Grand Turk where turtles were landed on 21% (16 of 77) of the days of observation, an estimate of 82 (95% CI: 38 – 128) green and 30 (95% CI: 11 – 61) hawksbill turtles are harvested annually (Table 1; Figure 1). The total annual TCI harvest is estimated at 239 (95% CI: 176 - 324) green turtles, and 167 (95% CI: 114 – 277) hawksbill turtles.

Size classes of the harvest

Harvested turtles were significantly larger (CCL) than those captured during our in-water surveys (Figure 2 a & b) (green turtles: n=453, W=12949, P<0.0001; hawksbills: n=312, W=4194, P<0.0001). Although harvested green turtles during the 2-year study were all below the estimated minimum breeding size recorded at nearby nesting grounds (>98cm Hirth, 1997), 11% (n=12) of harvested hawksbill turtles were within the size of breeding individuals (>78cm Witzell, 1983). Fifty percent (n=77) of harvested green turtles and 33% (n=36) of harvested hawksbill turtles were below the current legal size limit of 51cm CCL; this does not include those released alive by government enforcement officers, as records of these were not always kept, and illegal size-classes may have been underreported.

Harvested turtles that were weighed ranged between 2.4-67.1kg (n=120) and between 5.0-93.0kg (n=79) for green turtles and hawksbills respectively. The mean weight (including those converted from CCL) of harvested green and hawksbill turtles was 18.8kg (SE=1.2, n=159) and 23.8kg (SE=1.9, n=108) respectively and represents 66.5% and 64.7% of the estimated green turtle and hawksbill harvest. Approximately 4.48 (between 2.90-6.82) metric tonnes of green turtles and 3.98 (between 2.30-7.61) metric tonnes of hawksbill turtles were therefore landed

annually. There was a linear relationship between edible mass and total weight (r^2 = 0.96, hawksbills; r^2 =0.85, green turtles: supplementary Figure S2). The mean proportion of edible mass for green turtles and hawksbills was 0.67 and 0.52 respectively and smaller turtles yielded proportionally more edible mass than larger turtles (supplementary Figure S2). This artisanal fishery produced between 1.91-4.29 (mean 2.88) metric tonnes of green turtle edible mass and between 1.14-3.87 (mean 2.00) metric tonnes of hawksbill edible mass.

Seasonality of harvest

Fewer hawksbills were landed in South Caicos in the second year (Pseudo-F_1=5.76, P_{perm}=0.017) and the harvest differed significantly by month (Pseudo-F_{11}=3.68, P_{perm}=0.001) and day of the week (Pseudo-F_6=5.01, P_{perm}=0.001). The structure in hawksbill harvest is driven by low catches on Sundays (see supplementary Figure S3a) and high catches in March, June and August (Figure 3) and contributes to the seasonality consistently between years: 2-way interactions were not significant. Numbers of green turtle captures were not significantly different between years but there was significant structure by month (Pseudo-F_{11}=2.24, P_{perm}=0.015) and day of week (Pseudo-F_6=2.28, P_{perm}=0.04) which were not consistent between years: all 2-way interactions were significant (P_{perm}<0.05) (supplementary Figure S3b).

Small-scale fishery interactions

Hawksbill catch was higher when the lobster fishery was open and the conch fishery closed than in other levels of season (Figure 4: PseudoF_3=4.49, P_{perm}=0.009) and there was no significant effect of year or interaction. Green turtle catch was largely driven by significant differences between seasons in the first year when highest catch occurred with the conch fishery open and lobster fishery closed (season: PseudoF_3=6.82, P_{perm}=0.007). This pattern was not consistent across years (year; PseudoF_1=12.84, P_{perm}=0.003; interaction: PseudoF_3=5.76, P_{perm}=0.007) and in year two no apparent differences occurred between seasons.

In both years, peak lobster CPUE (kg.boatdays^{-1}) occurred at the opening of the lobster fishery (1 August) and declined and stabilised until it closed on 31 March (Figure 3 a & b; see supplementary Figure S4 for separate catch and effort plots). Parsimonious GLM models indicated that as lobster CPUE increased so did hawksbill catch (GLM: χ^2 LR_1=3.73, P=0.05), but green turtle catch declined (GLM:

χ^2 LR_1=3.56, P=0.06) (supplementary Figure S5). In 2009 (Year 1: Figure 3a), the conch export fishery closed on 6 April because the quota was reached. In this year both fisheries therefore closed at around the same time and remained so for four months until August. A large peak in green turtle catch in April 2009 was coincident with this closure. In 2010 (Year 2: Figure 3b) the conch export quota was not reached and the fishery remained open until 15 July creating a period of only two weeks when both fisheries were closed. No corresponding peak in turtle catch of either species was observed during this time. There is a suggestion that with increasing conch CPUE hawksbill catch declines (GLM: χ^2 LR_1=3.09, P=0.08) but no evidence of a relationship with green turtle catch (GLM: χ^2 LR_1=1.53, P=0.22) (supplementary Figure S5).

Discussion

The mixed SSF of TCI is characterised by the targeted fishing of lobster and conch for the export market and the opportunistic catch of several hundred green and hawksbill turtles each year for domestic consumption. Our work in TCI illustrates the connectivity between multi-species fisheries and artisanal turtle capture, and the need to manage turtles as part of a broader approach to SSF management. Seasonality of the turtle harvest appears to be driven primarily by fishery interactions. For example, hawksbill catch is apparently positively dependent on increasing lobster CPUE and inversely related to increasing conch CPUE, and green turtle landings decrease with increasing lobster CPUE. This is almost certainly a result of the different habitats in which these species are found: lobster and hawksbill turtles are most commonly associated with reef habitat, and conch and green turtles with shallow seagrass habitats. Peak hawksbill landings occurred in August and coincided with the opening of the lobster fishing seasons, and in 2009, peak green turtle landings coincided with the closure of both lobster and conch fisheries, demonstrating the potential impact that these fisheries have on marine turtle catch. Our study is the first, of which we are aware, that empirically relates lobster and conch fishing to sea turtle capture. Hawksbill catch, in particular, is significantly dependent on the catch and effort of these fisheries and legislative measures need to embrace this dependency in order to be effective.

Size classes of the harvest: maximum size limits

From our data, the capture of large juveniles and adult turtles is of conservation concern, in particular for the hawksbill turtle given its critically endangered status (IUCN, 2010) and remnant state of nesting populations in the WCR (Blumenthal et al., 2009; Bowen et al., 2007). Eleven percent (n=12) of hawksbills landed in TCI's fishery were of adult size (>78cm Witzell, 1983) (Figure 2b) and foraging adult hawksbills are known to be present in TCI waters year-round since nesting activity has been observed throughout the archipelago in every month of the year (Stringell et al., in prep, Chapter 2). Capture of large hawksbill turtles is likely to be driven by fisher choice and effort allocation, for example, they are easier to catch than green turtles because they are generally less likely to quickly flee from interaction with

humans and are frequently encountered at rest under reef ledges where fishermen dive for lobsters (Authors' pers. obs.).

Despite being the largest green turtle fishery of the UK OTs (Godley et al., 2004b), there were few large juveniles and no adults captured in the two years of our survey period. The paucity of adult green turtles in the harvest is most likely to be a result of a combination of fisher choice and turtle behaviour; fishermen may be unwilling to pursue large, fast swimming adult green turtles because they are difficult to catch and handle, are possibly costly to catch with respect to fuel used, and presumably compete for boat space with more desirable or profitable catches. Additionally, the scarcity of adults in the harvest may be due to low abundance of foraging adults, and the limited time of the year when breeding adults are present in TCI waters: the green turtle nesting season in TCI is seasonal (May-October) (Stringell et al., in prep, Chapter 2). Together with the recovery of major green turtle nesting rookeries in the region (see Broderick et al., 2006, for review), the impact of the TCI fishery on regional green turtle populations is of less concern than that of hawksbills.

Our in-water surveys tended to catch smaller turtles on average than the fishery, probably because our sampling is restricted by safety and logistical constraints to shallower habitats where smaller turtles are typically found (Blumenthal et al., 2010): fishermen often fish on outer reefs and in deeper water habitats. These data probably reflect size-class partitioning in the taxa, where increasing body size is coupled with increasing depth (Musick and Limpus, 1997). Nevertheless, it is clear that fishers most frequently select juvenile turtles of approximately 20kg (or 55cm CCL) and this may be due to several factors: abundance of these size classes and rates of encounter, capture effort, and fisher choices - taste, processing time and optimal yield of edible mass. Our data suggest that turtles of this size yield proportionally more edible mass than larger turtles (supplementary Figure S2), and that proportionally more of the green turtle is consumed than that of the hawksbill. The take of juveniles of this size for both species, however, is likely to be absorbed by the population dynamics without detriment to the populations involved (Heppell and Crowder, 1996; Chaloupka, 2002).

Moncada et al. (1999) reports that 7% of hawksbill turtles captured in Cuba's historic turtle fishery were sexually mature at 61-65cm straight carapace length and

100% at >81cm. We propose an upper size limit of 24 inches (61cm) shell length for both green and hawksbill turtles, similar to that of the Cayman Islands and deliberately precautionary to protect the age classes of most conservation concern: large juveniles and adults of both species (Crouse et al. 1987, Crowder et al. 1994, Heppell and Crowder 1996). The suggested size limit received 88% (n=66) support from the 75 fishers interviewed in September 2011 (Authors, unpublished data). Additionally, because TCI fishers still use imperial measures, it would be relatively practical in terms of compliance and enforcement. Although, approximately 50% of green turtles and 33% of hawksbills landed in the fishery were undersize (Figure 2) - implying either a disregard, a misunderstanding or a sense of biological inappropriateness (e.g. Raakjær Nielsen, 2003) of the present minimum size limits - consultations with fishers to generate understanding of proposed turtle fishery measures indicated almost unanimous support for maintaining a minimum size limit and introducing a maximum size limit (Richardson, unpublished data).

Seasonality of harvest: closed season

The day-to-day structure of turtle harvest likely reflects the general weekly fishing pattern of the mixed fishery and is likely driven by cultural influences e.g. Christianity, such that there are low catches of hawksbill turtles on Sundays. The seasonality results of this study indicate that time-based management controls will affect turtle species differently. The presence of all hawksbill size-classes in TCI waters throughout the year, hawksbill nesting dynamics and the effect of TCI's lobster fishery provide support for a closed season as an appropriate and additional integrated measure that would optimally safeguard threatened hawksbill stocks in the region. Regional peak nesting periods for hawksbill turtles (Beggs et al., 2007; McGowan et al., 2008; Moncada et al., 1999) broadly coincided with peak landings of the species, but not for green turtles (Bell et al., 2006; McGowan et al., 2008; Troeng and Rankin, 2005). Breeding/nesting adult hawksbills are present in TCI waters throughout the year including the peak reproductive season in October, and breeding/nesting green turtles are present seasonally around August (Author's unpublished data). The capture of adult turtles during their reproductive seasons is of conservation concern because their removal reduces the ability for the natal population to be maintained (Heppell and Crowder, 1996; Chaloupka, 2002), and is regulated against in several extant turtle fisheries of the WCR by implementing

harvest restrictions during these periods (e.g. Bell et al., 2006; McGowan et al., 2008; Richardson et al., 2006).

We therefore suggest prohibition on all take of hawksbill turtles during the eight-month lobster open season (August to March inclusive). This would more-or-less align TCI legislation with that of other UKOTs in the WCR (Richardson et al., 2006). Additionally, the greatest market demand is largely for green turtles and thus a reduction in take of hawksbill turtles through a closed season is unlikely to significantly increase the take of green turtles. However, although the nesting season of May to October presents an obvious time period for a potential closed season on green turtles in order to protect breeding/nesting adults, breeding size adults are rarely taken in the harvest (see also Richardson et al., 2009). A closed season on green turtle capture during this period may not be necessary in terms of fishery protection, and is unlikely to be supported by fishers (Campbell et al., 2009). At this time, we do not propose a closed season on green turtle take, and the introduction of, and compliance with the proposed maximum size limit (see later) should protect breeding adults from the fishery.

Turtle harvest estimation

The artisanal marine turtle fishery in TCI is the largest of the UK OTs (Godley et al., 2004b), and our work confirms it as currently the largest documented legal and extant hawksbill turtle fishery in the western Atlantic (Brautigam and Eckert, 2006; Fleming, 2001; Godley et al., 2004b; Richardson et al., 2009). Our harvest estimates are of the few derived by direct observations (Table 2) while most regional estimates are nearly a decade old, and come from fisher interviews, market surveys and logbooks, and as such, may be less accurate (Lunn and Dearden, 2006). For example, previous harvest estimates for TCI that used fisher interviews (Fletemeyer, 1983; Godley et al., 2004a; Richardson et al., 2009) had wider uncertainty (Table 2). These studies also showed much higher median estimates, which may suggest a reduced take since these times, but is more likely simply a result of differing sampling effort and technique. Although we are confident in our harvest estimates, we acknowledge that these are likely to be conservative and minimum estimates because not all fishing docks, especially personal jetties, could be systematically surveyed. For example, fishers at North Caicos, Middle Caicos, and Salt Cay undoubtedly contribute further to the annual harvest, although the fishing

communities here are not nearly as large as those of the three main islands surveyed. Additionally, we know that some fishers butcher turtles at sea (Authors' unpublished data), and there is likely to be an unknown level of foreign poaching in TCI waters, especially from neighbouring Dominican Republic (Fleming, 2001; Richardson et al., 2009); these catches are not included in our estimates because we cannot confidently ascertain the extent of these practices.

Quota management

The fishing community understands the concept of quota because the conch fishery is quota managed via Total Allowable Catch (Béné and Tewfik, 2001). However, implementing, administering, enforcing and monitoring turtle catch compared to quota would require considerable capacity – something that is unlikely to be tenable in a limited fisheries department in TCI (Forster et al., 2011). A licensing system with personal quota, like the Cayman Islands (Bell et al., 2006), may be an option given that all fishermen apply for fishing licences annually, but declaring compliance with personal quota would be unlikely. Supporting biological evidence for turtle quota is not currently available and the impact of such quota on other fisheries is unknown. Therefore, at present we do not advocate quota-based management control measures. Further work is needed to address this possibility.

Closure of the turtle fishery

In many cases where turtle fisheries have been closed, population recovery has resulted (Balazs and Chaloupka, 2004; Beggs et al., 2007; Broderick et al., 2006; McGowan et al., 2008; Troeng and Rankin, 2005). However, in several WCR states, e.g. Anguilla (Godley et al., 2004b), Montserrat (Richardson et al., 2006), BVI (McGowan et al., 2008), monitoring the biological and social consequences of moratoria or fishery closure has been fiscally challenged and not based on detailed study of the turtle fishery itself or as part of a wider multispecies SSF. This is also the case for recent turtle fishery closures in the Bahamas (Fisheries Resources (Jurisdiction and Conservation) Regulations, 2009), and Trinidad and Tobago (Protection of Turtle and Turtle Eggs (Amendment) Regulations, 2011). Our work with the fishing community over the study period found that communities throughout the TCI strongly contest a ban on turtle catch/fishing, expressing particular concern over their removal of artisanal/traditional rights to consume turtles. Compliance with

a fishery closure that was unacceptable to the local community, would present significant enforcement challenges (Raakjær Nielsen, 2003; Campbell et al., 2009; Silver and Campbell, 2005). A fishery closure may also criminalise fishers and drive turtle harvest 'underground' and increase butchering at sea, making future additional monitoring of catch rates impossible. Furthermore, a permanent closure of the turtle fishery may impact other fisheries, for example, by increasing the capture of lobster, conch, and fin-fish for personal consumption. Further work is needed to establish convincing evidence that, in place of other control measures, a closure of the turtle fishery would be biologically relevant and socially acceptable.

Conclusions

In the WCR, the majority of fishers and fisheries are from the SSF sector (Salas et al., 2007). It is therefore important to recognise and mitigate the potential environmental impacts of SSF in this region, consider the complex socio-ecological system associated with SSF (Ostrom, 2009; Liu et al 2007), and to follow the building trend to develop ecosystem-based management strategies that promote sustainability (Belgrano & Fowler 2011). Our results indicate that incorporating the interactions of turtle harvests with mixed SSFs is important to the management of turtle fisheries. We demonstrate that the turtle fishery in TCI is closely tied with the mixed SSF, which is strongly influenced by fisher behaviour, choices and their social environment, an aspect frequently disregarded in fishery management and resource exploitation (Hilborn et al., 1995; Ostrom, 2009). We present empirical biological evidence that supports simple management measures already used by other turtle fisheries in the WCR: the introduction of maximum size limits for both species and a closed season on hawksbill take during the lobster fishing season. These measures are suggested in addition to the existing provisions and are currently being considered by the TCI Government as part of a revision of the Fisheries Protection Ordnance.

Future work could explore a variety of management aspects and tools applicable to this SSF, e.g. Total Allowable Catch quotas for sea turtles and their use in an adaptive management framework, financial management tools such as fines and incentives, multi-species and multi-scale marine management, knowledge use in fisheries management, integrated coastal zone management, spatial management (MPAs for sea turtles), and adaptive governance and participatory strategies. A full

discussion of these is beyond the scope of this paper and outwith the data. However, work is currently underway to facilitate a culture of compliance with the new suggested management measures. Work with fishers and other stakeholders in TCI to explore co-management or community-based management options *sensu* Campbell et al. (2009), has been set up to integrate fishing community concerns and opinion in the design and proposed implementation of recommended turtle fishery management measures, including those mentioned here. It is envisaged that stakeholder participation will be key to effective sustainable management of these resources. If these and other measures are incorporated, TCI will become one of the most highly regulated sea turtle fisheries in the WCR and one that has strongly involved the relevant stakeholders in fishery reform.

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Table 1. Annual harvest estimates of green and hawksbill turtles landed at South Caicos (SC), Providenciales and Grand Turk between 1 December 2008 – 30 November 2010 (Total survey period =730 days). The Turks and Caicos Islands (TCI) estimate is the sum of each island estimate. 95% confidence intervals (CI) are percentiles of the distribution of bootstrapped estimates. Data are from direct dockside observations. 'Interpolated no. turtles captured concurrently at SC' represents the number of turtles (count plus interpolated) captured at South Caicos at the same time as observations were made at Providenciales or Grand Turk. These values are used in calculating the island harvest estimates (see Methods section 2.4 for details).

			Green turtles				**Hawksbill turtles**			
	No. survey days	No. survey days when turtles landed	Observed count from all survey days	Interpolated total (count + interpolated)	Interpolated no. turtles captured concurrently at SC	Annual estimate and 95% CI	Observed count from all survey days	Interpolated total (count + interpolated)	Interpolated no. turtles captured concurrently at SC	Annual estimate and 95% CI
South Caicos	544	173	194	237.02	-	119 (98-140)	109	129.31	-	65 (53-77)
Providenciales	68	12	8	-	25.12	38 (0-109)	13	-	11.62	72 (26-177)
Grand Turk	77	16	16	-	23.14	82 (38-128)	7	-	14.89	30 (11-61)
TCI	-	-	218	-	-	239 (176-324)	129	-	-	167 (114-277)

Table 2. Comparative reported, legal and substantial (>100) annual turtle harvest estimates from several nations in the Wider Caribbean. Harvest estimates for other Caribbean nations can be found in Brautigam and Eckert (2006), Fleming (2001), and Godley et al. (2004b).* denotes a historical quota.

Country	Green turtle	Hawksbill turtle	Year of survey	Method of survey	Source
TCI	176-324	114-277	2008-2010	Direct survey	Present study
TCI	236-1128	184-907	2001-2004	Fisher interview	Godley et al. (2004a), Richardson et al. (2009)
British Virgin Islands	150-450	50-150	2001-2004	Fisher interview	Godley et al. (2004b)
Cuba	280*	500*	1997*	Fishery statistics	Carrillo et al. (1999) Fleming (2001)
St Vincent and the Grenadines	148-214	251-347	1995-1999	Fisher interview	Grazette (2002) in Brautigam and Eckert (2006)
Grenada	488	294	2001	Fisher interview / market survey	Grazette et al. (2007)
Nicaragua	11,000	180-280	1993-2002	Direct survey	Lagueux et al. (2003)

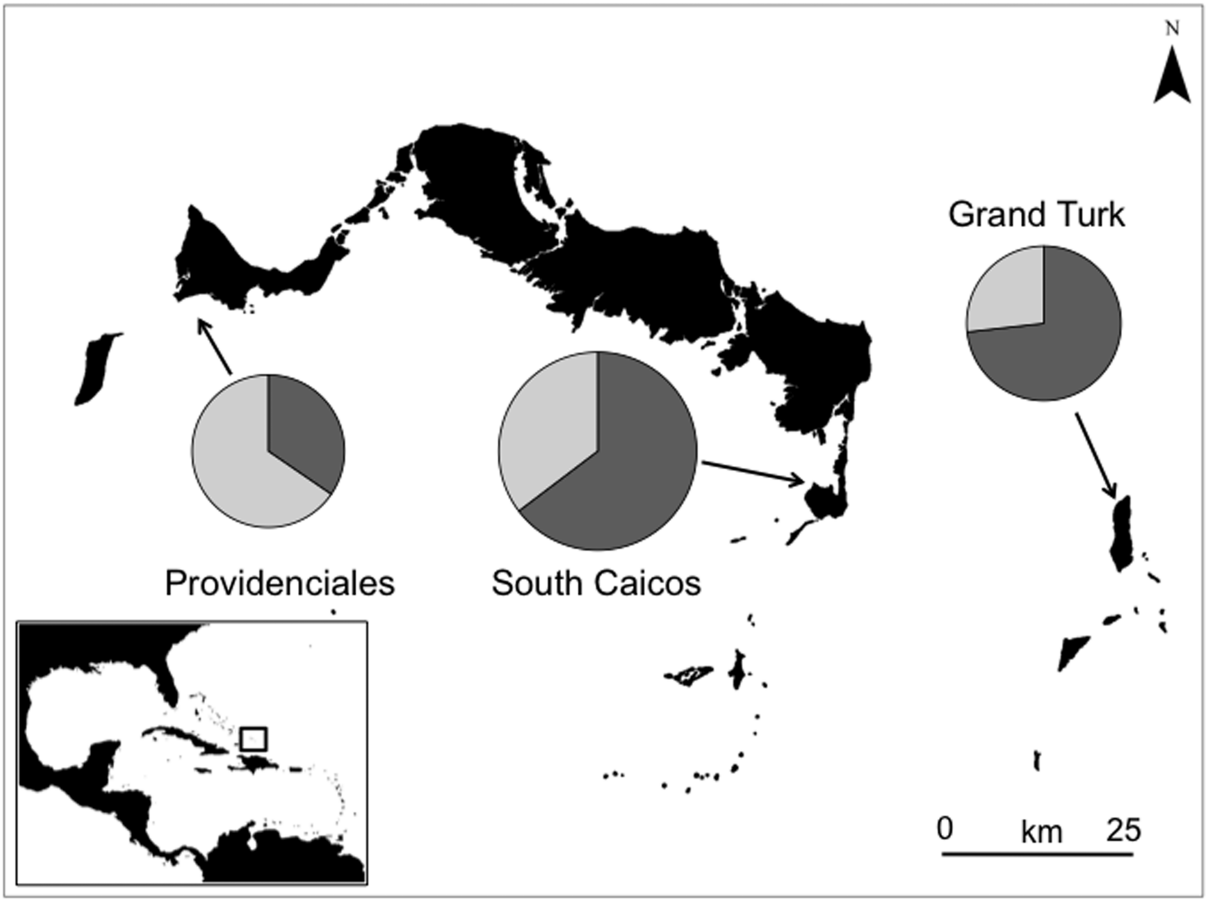

Figure 1. Map and location of the Turks and Caicos Islands. Pie charts show the proportion of the estimated annual harvest of hawksbill turtles (light grey) and green turtles (dark grey) at each surveyed island and are scaled relative to the estimated harvest of both species combined (see Table 1 for values).

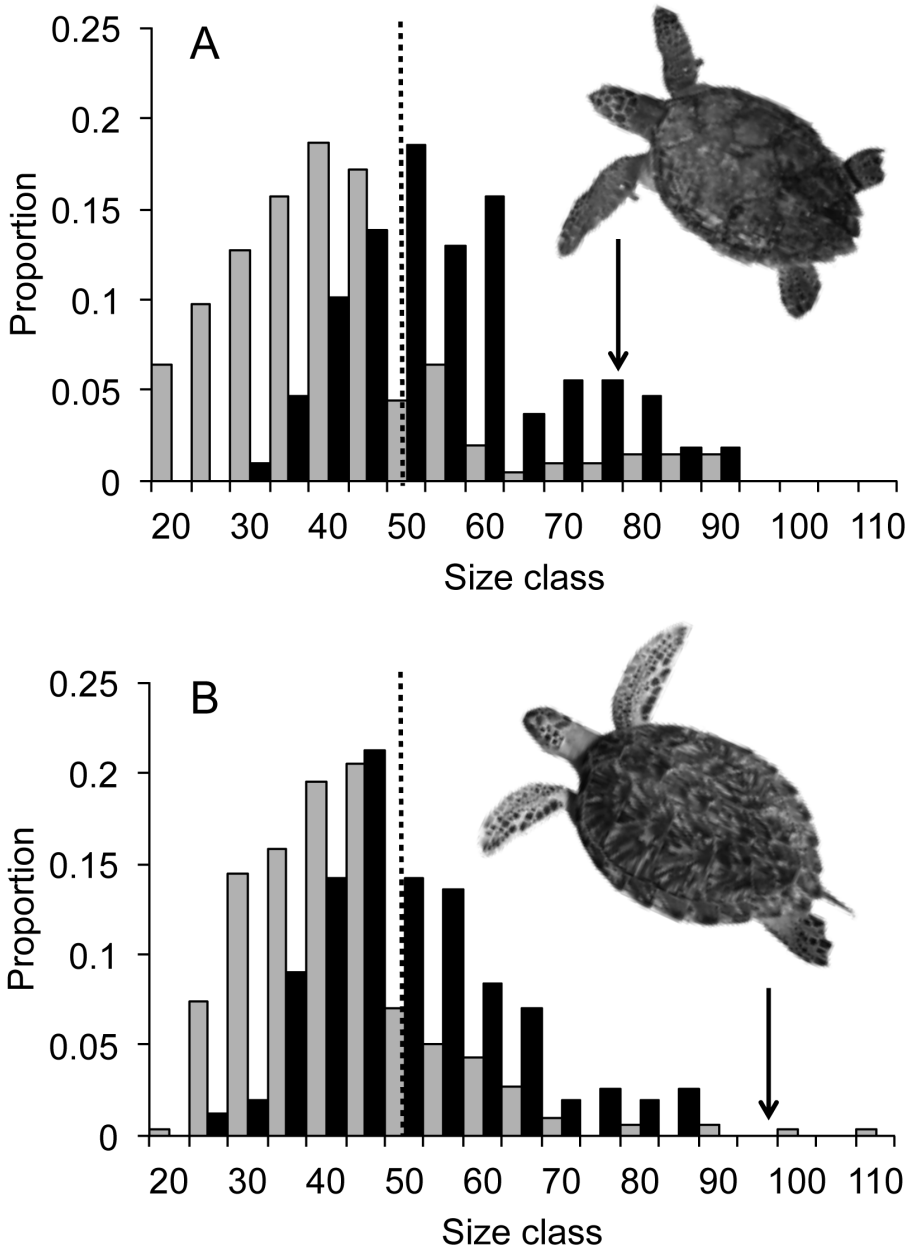

Figure 2. Size-class (CCL, cm) histograms of curved carapace length of A) hawksbill (n= 312) and B) green turtles (n=453) sampled during the 2 year study (December 2008 to November 2010). Turtles sampled from in-water surveys (light grey) and harvested turtles (black) are combined from all islands. Minimum legal size limit (51cm CCL) is shown with a dashed line, and likely minimum breeding sizes (see text) are indicated with arrows. Photos show juvenile hawksbill (A) and green turtles (B) (courtesy of T. Stringell and P. Richardson respectively).

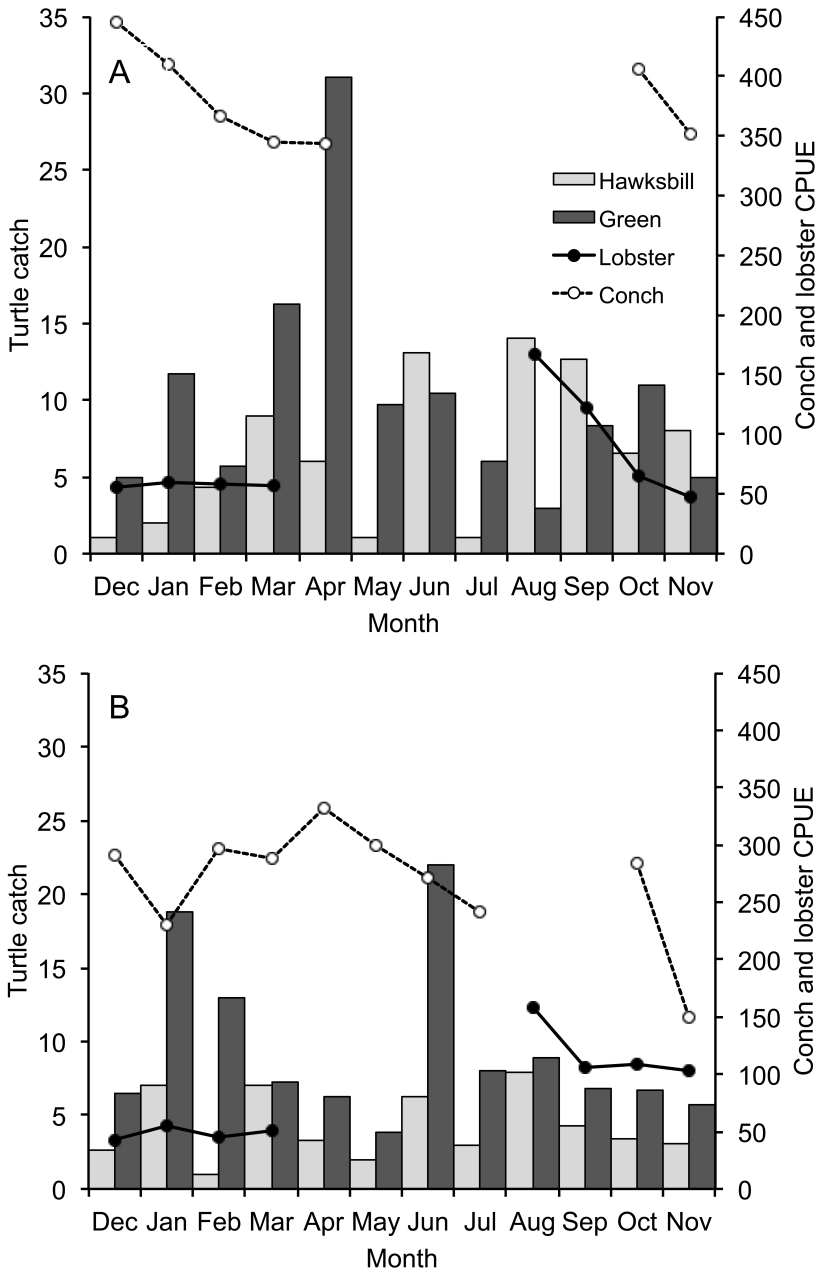

Figure 3. Hawksbill (light grey) and green turtle (dark grey) interpolated monthly landings at South Caicos during A) year 1: 1 December 2008 - 30 November 2009, and B) year 2: 1 December 2009 - 30 November 2010. Fishing CPUE (kg.boat days^{-1}) for lobster (filled circles and solid line) and conch (open circles and dashed line) export fisheries at South Caicos are superimposed.

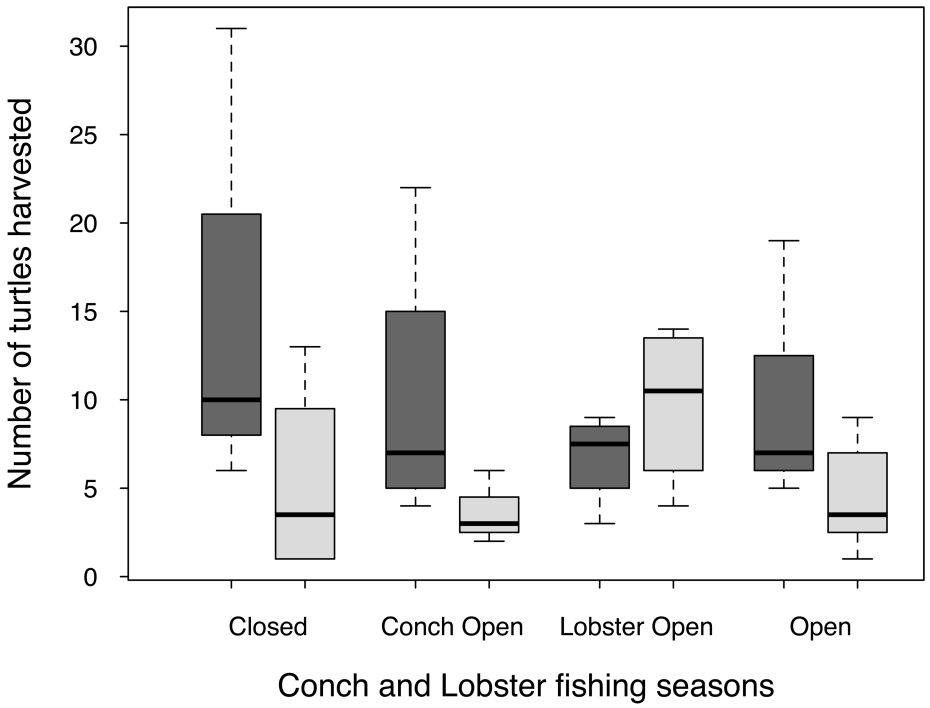

Figure 4. Green turtle (dark grey) and hawksbill turtle (light grey) harvest at each of 4 categories of conch and lobster fishery seasons at South Caicos. Closed and Open categories refer to both fisheries together. 'Conch Open' represents periods when the conch fishery is open and lobster fishery closed, and *vice versa* for 'Lobster Open'. Data from December 2008 to November 2010 (24 months).

Chapter 1: Supplementary Information

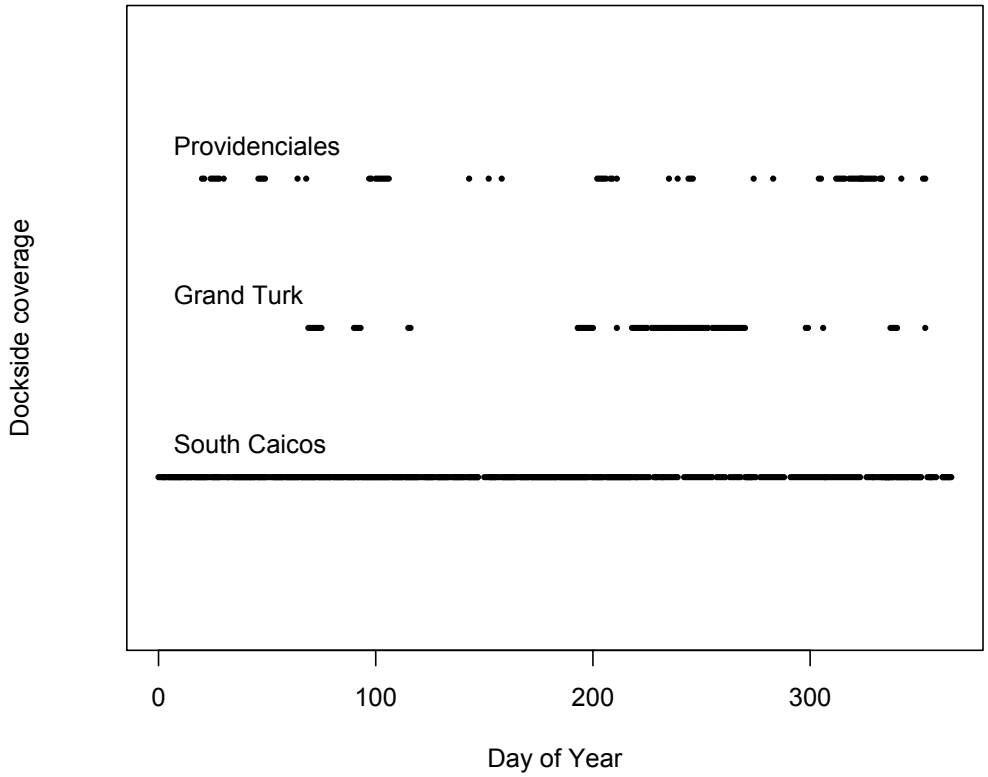

Figure S1. Dockside survey coverage (days) of South Caicos, Grand Turk and Providenciales.

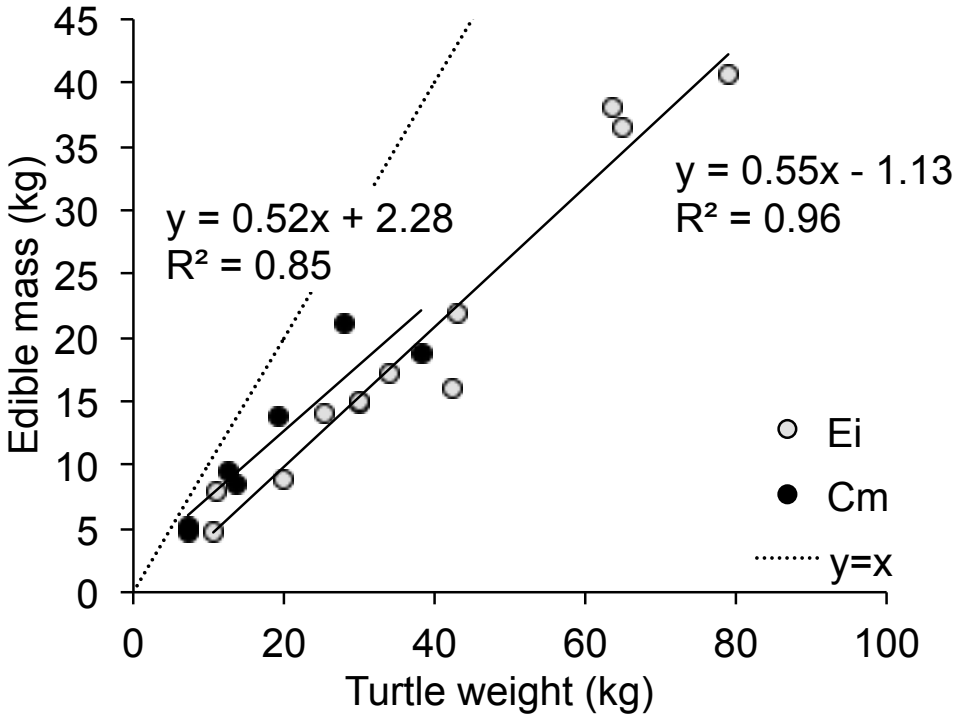

Figure S2. Turtle edible mass and total weight relationships. Equation on left refers to green turtles (black filled circles, n=7) and the equation on right for hawksbill turtles (grey filled circles, n=12). Slope and intercept values were used to calculate the edible mass from the total harvest. The dashed line (*y*=*x*) is shown for comparison.

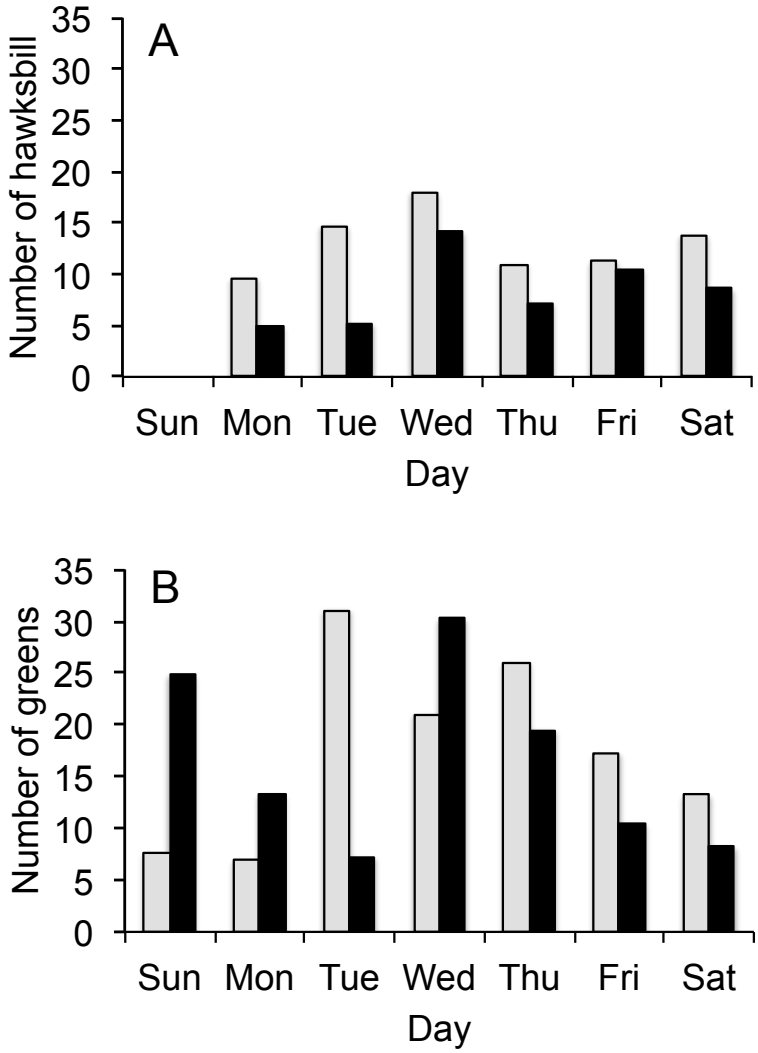

Figure S3. Interpolated sum of hawksbill turtles (A) and green turtles (B) harvested in South Caicos by day of the week. Year 1: 1 December 2008 – 30 November 2009 (light grey); Year 2: 1 December 2009 – 30 November 2010 (black).

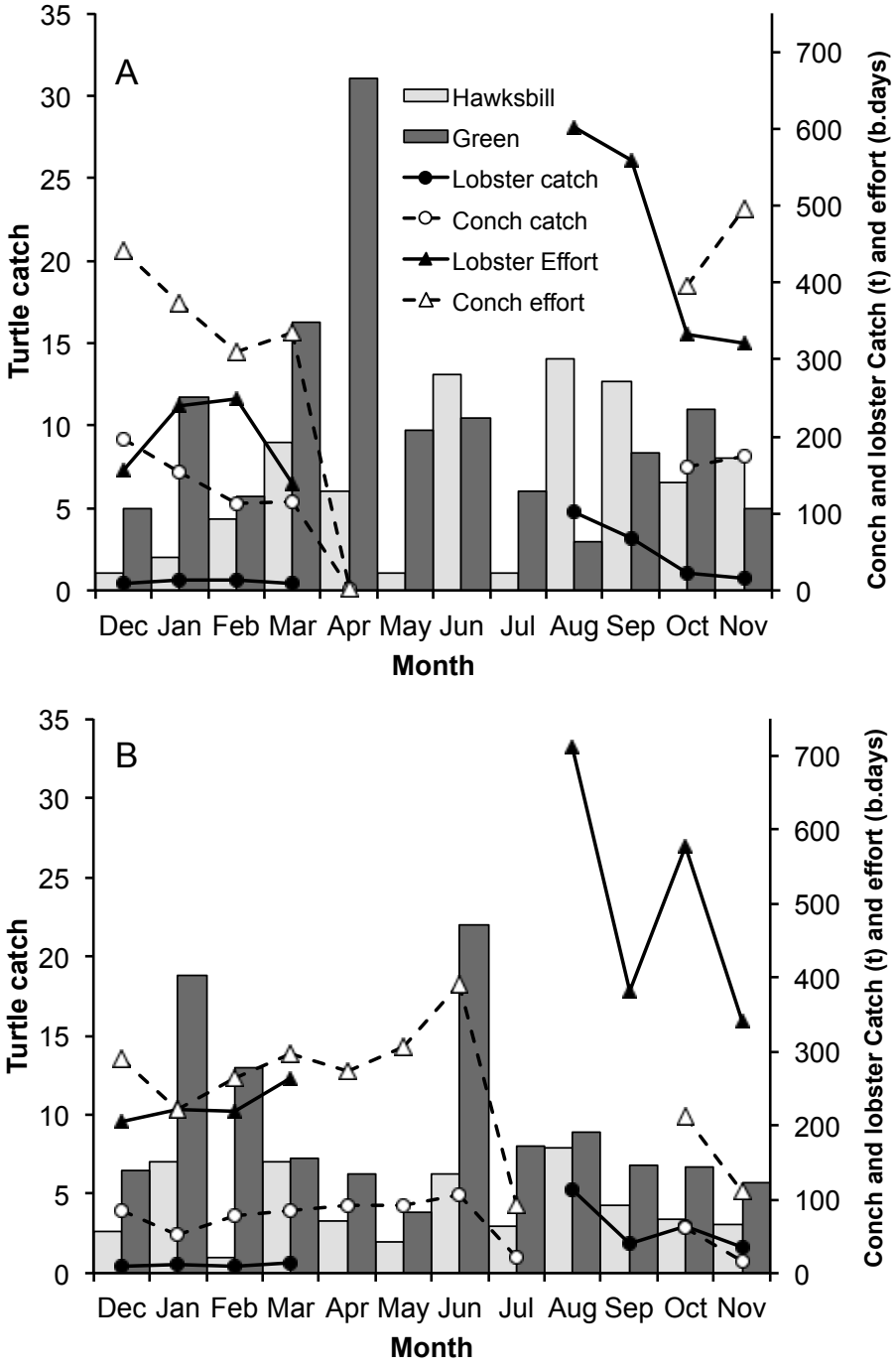

Figure S4. Hawksbill (light grey) and green turtle (dark grey) interpolated monthly landings during A) year 1: 1 December 2008 - 30 November 2009, and B) year 2: 1 December 2009 - 30 November 2010. Fishing catch (metric tonnes; circles) and effort (boat days; triangles) for lobster (filled symbols and solid line) and conch (open symbols and dashed line) export fisheries at South Caicos are superimposed.

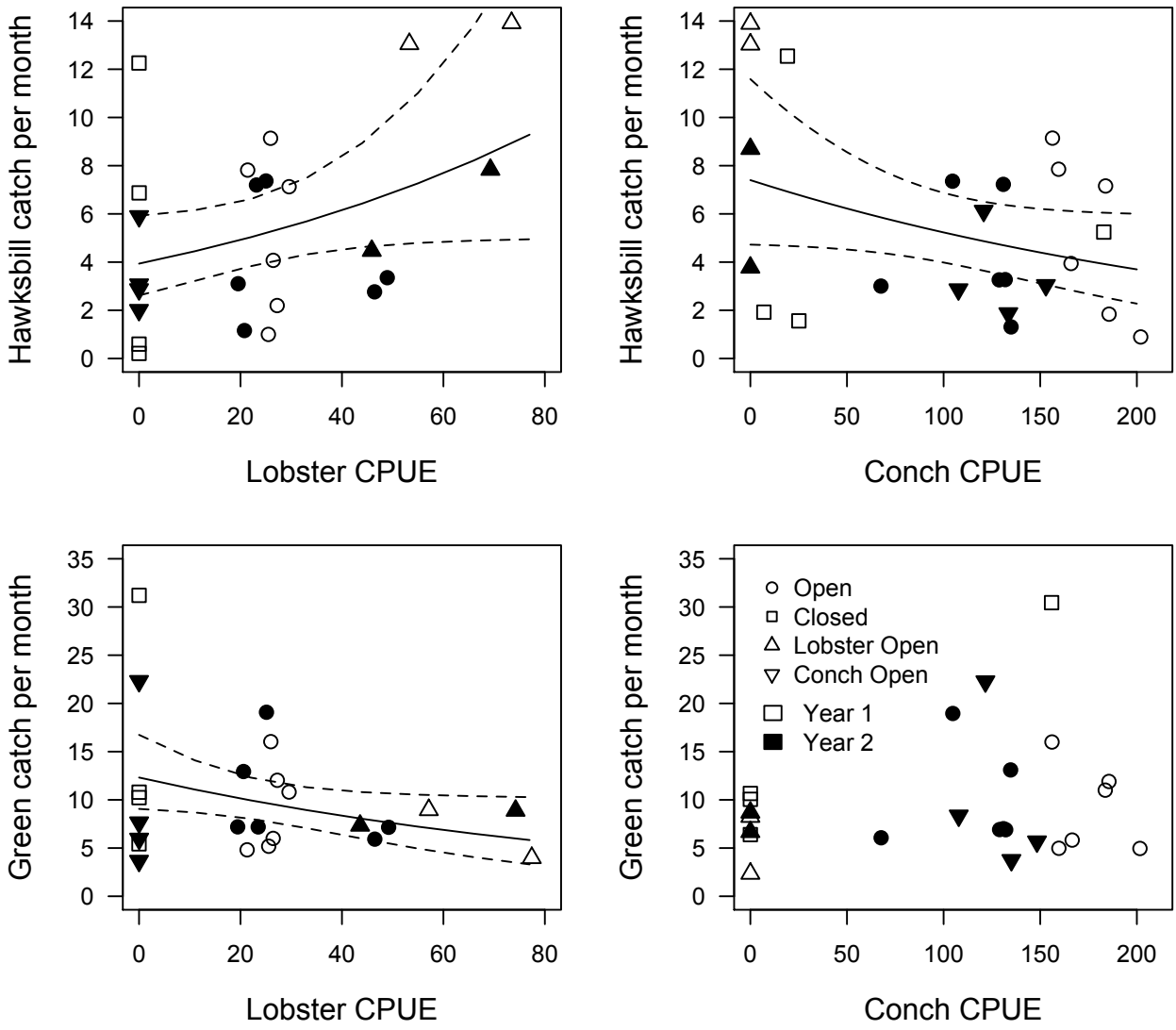

Figure S5. The number of hawksbill (A and B) and green turtles (C and D) harvested per month during the 2-year study period against lobster and conch CPUE (kg.boat days^{-1}) at South Caicos. Lines indicate marginally significant negative binomial GLM fits and 95% confidence intervals (A, P=0.05; B, P=0.08; C, P=0.06; D lines not shown, P=0.22). Point shape and colour represent fishing season and survey year factors.

Chapter 2

Vulnerability of adult marine turtles in a contemporary turtle fishery: Recommendations for legislative change

Thomas B. Stringell[1], Wesley Clerveaux[2], Brendan J. Godley[1], Quinton Phillips[2], Susan Ranger[1,3], Peter B. Richardson[1,3], Amdeep Sanghera[3] and Annette C. Broderick[1]

[1]Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ. UK
[2] Department of Environment and Maritime Affairs, South Caicos, Turks and Caicos Islands.
[3] Marine Conservation Society, Ross on Wye, Herefordshire, HR9 5NB. UK

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Abstract

Fishing has played a dominant role in almost every marine ecosystem, and caused widespread declines in stock abundance. The reduction of sea turtle populations from historical levels is widely known; they are particularly sensitive to exploitation because of life-cycle traits such as longevity and natal philopatry. The take of nesting females is a key conservation concern and globally has led to concentrated efforts to protect this life stage. In the Turks and Caicos Islands (TCI, UK Caribbean Overseas Territory), the legislation provides protection to nesting turtles when they are on the beach but in the water they are subject to regulated take. Here, adults of both green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles are harvested in the TCI, and the hawksbill fishery is estimated to be one of the largest in the western Atlantic. There is a clear need to better contextualise this fishery in order to improve regulation and provide more effective protection for breeding adults. We undertook a two-year study and a combination of nesting beach and in-water surveys, molecular analysis, satellite tracking and collation of fisheries landing data to investigate the seasonality and structure of breeding populations of green and hawksbill turtles in the TCI. We estimate that the current nesting populations in TCI have greatly diminished since the 1980s, perhaps as a result of the legal take of adults. Using these multiple lines of evidence, we highlight the inadequacies of the current regulations in TCI and recommend specific legislative changes employed elsewhere that could improve the management of this traditional turtle fishery and protect breeding adults.

Introduction

Fishing has played a dominant role in almost every marine ecosystem, with numerous species overexploited and greatly reduced (Myers & Worm 2003, Pauly et al. 2005, FAO 2010). Sea turtles are no exception, and their reduction from historical abundances is widely known, particularly in the Caribbean region (Jackson et al. 2001, McClenachan et al. 2006).

Sea turtles are sensitive to exploitation because they are late maturing, exhibit natal philopatry, lay eggs on land and, in most species, their life-cycle involves migrations and distinct life stages where habitats and locations are partitioned (Crouse et al. 1987, Crowder et al. 1994, Heppell & Crowder 1996, Fitzsimmons et al. 1997). This results in genetically differentiated populations that occupy broad marine regions - often entire ocean basins - and cross international boundaries (Bowen & Karl 2007). Conservation of such wide ranging species is, therefore, complex and may often involve a multitude of stakeholders and nations that have differing regulatory and management frameworks, and cultural, traditional and economic values (Frazier 2002, Blumenthal et al. 2007, Hawkes et al. 2012). Despite this complexity, management at the country level rather than multilateral agreements has been suggested as one of the most important steps towards regional conservation (Moncada et al. 2012).

Exploitation of nesting females from natal rookeries can quickly cause population declines (Bell et al. 2006, McClenachan et al. 2006, McGowan et al. 2008, Kittinger et al. 2013) and protection of nesting rookeries has consequently been one of the tenets of sea turtle conservation and has led to recovery in some exploited populations (Bjorndal et al. 1999, Troëng & Rankin 2005, Dutton et al. 2005, Broderick et al. 2006, Richardson et al. 2006, Beggs et al. 2007, Marcovaldi et al. 2007, Chaloupka et al. 2008, Allen et al. 2010). There are, however, many rookeries that have not been entirely extirpated but remain small, particularly in the Caribbean where there is a call for a renewed conservation focus on remnant nesting populations, especially for the hawksbill turtle (*Eretmochelys imbricata*) (McClenachan et al. 2006, Mortimer & Donnelly 2008). Indeed, Leroux et al. (2012) call for the genetic characterisation of smaller and unsampled hawksbill rookeries in the Wider Caribbean Region and suggest that all hawksbill rookeries be treated as distinct management units, because of their potential to maintain regional genetic

diversity. Similarly, Shamblin et al. (2012) call for further investigation of small, remnant green turtle rookeries in the Caribbean to further elucidate their importance in regional stock demographic connectivity.

In some Caribbean nations, legislation regulating the remaining legal turtle fisheries provides minimum size limits that prohibit take of juveniles and permit take of larger size classes (Fleming 2001, Brautigam & Eckert 2006, Richardson et al. 2006). Such regulations were inherited from fisheries legislation where minimum landing size is commonly used, but for long-lived species such as marine turtles, protection of large juveniles and reproductive adults is critical to their recovery (Carr et al. 1982, Crouse et al. 1987, Crowder et al. 1994, Heppell & Crowder 1996). Four of the six UK Overseas Territories in the Wider Caribbean Region still permit the take of turtles (Godley et al. 2004). Of these, the Cayman Islands has recently amended its legislation to protect larger turtles by establishing a maximum size limit and an extended closed season (Cayman Islands Government 2008, Blumenthal et al. 2010). In the British Virgin Islands, Montserrat and the Turks and Caicos Islands, minimum size limits remain in the national legislation regulating these turtle fisheries (Richardson et al. 2006).

The Turks and Caicos Islands (TCI) turtle fishery is regulated by the Fisheries Protection Ordinance (Government of the Turks and Caicos Islands 1998). This legislation prohibits the take of any turtle above the low-water mark (i.e. nesting females) and prohibits the possession, purchase or sale of 'laid' turtle eggs (see Richardson et al. 2006 for review). The minimum size limit at which it is legal to harvest green and hawksbill turtles is *'20 inches in length measured from the neck scale to the tail piece'* or a weight of at least 20 lbs. Turtles of other species can only be taken if they weigh at least 20 lbs. There is no closed season and fishers are legally entitled to remove an unlimited number of turtles larger than these minimum size limits if caught at sea at any time of the year.

Currently, little is known about the magnitude or seasonality of the green turtle (*Chelonia mydas*) and hawksbill turtle rookeries of the TCI, but they are thought to be remnants of past populations that were subject to regular harvest (Richardson et al. 2009). The legal turtle fishery that exists here may still be a threat to existing populations and be impacting recovery of both species. TCI's hawksbill fishery is thought to be one of the largest in the western Atlantic and the take of breeding adults is a conservation concern (Stringell et al. 2013, Chapter 1).

There is a clear need to better understand the dynamics of the nesting populations in order to inform future management of the traditional turtle fishery in the TCI. At the invitation of the local government, we set out to gather data with a view to making empirically based recommendations. We undertook a two-year study to examine seasonality and structure of breeding in green and hawksbill turtles nesting in the TCI and assess these patterns in relation to the fishery. In this multidisciplinary study, we combine observations of the magnitude and spatio-temporal patterns of marine turtle nesting activity, the presence of adults in TCI waters through captures by the fishery, from in-water surveys and satellite tracking and genetically characterise the sea turtle rookeries of TCI using mitochondrial DNA.

Methods

Study Site

The Turks and Caicos Islands (TCI) are located at the southeastern end of the Bahamas chain (21° 45N, 71° 35W). The low-lying archipelago consists of eight main islands and numerous smaller cays, covering approx. 950km^2 at low-tide. The majority of the human population lives on the three islands of Grand Turk, Providenciales and South Caicos. The economy of TCI is driven principally by tourism, offshore finance and fishing. The spiny lobster (*Panulirus argus*) and queen conch (*Strombus gigas*) make up the majority of the fisheries for the export market (Béné & Tewfik 2001, Tewfik & Béné 2004). Sea turtles are captured largely opportunistically and usually for personal consumption, although there is limited directed take for commercial sale and export is prohibited (Richardson et al. 2009). The islands surround a shallow, sandy and productive habitat which is generally fringed by mangroves and creeks, and provides a rich complex of regionally significant foraging habitat for juvenile and adult sea turtles (Richardson et al. 2009), including some from other Caribbean states (Van Dam et al. 2008, Richardson et al. 2010, Hawkes et al. 2012). Most outlying cays and ocean facing beaches appear to be suitable for nesting habitat and they are mostly fringed with coral reefs.

Nesting surveys

During a two-year period from 5 December 2008, 162 surveys were carried out opportunistically at 34 beaches around the islands (Figure 1). Due to the logistical constraints and distances involved in covering the archipelago, frequent and regular nesting surveys of all locations were not possible. Instead, surveys were designed to offer approximate rather than absolute insights into magnitude and spatio-temporal nesting patterns and, therefore, represent minimum counts. Several sites (e.g. Bush Cay, Fish Cay, East Caicos), previously considered key nesting sites (Richardson et al. 2009), were visited relatively frequently (usually every month). Beaches were searched on foot, except for a few occasions when boat-based beach passes were carried out and landings were made when signs of turtle activity were observed. Adult tracks were counted and classified as nesting or non-nesting emergences, and identified to species following standard protocols (Schroeder & Murphy 1999). Hatchling tracks were also recorded and nest contents excavated to confirm species

and obtain tissue vouchers for molecular analysis. Tracks and nest contents were aged as accurately as possible and if hatched nests could not be matched to laying events, we estimated the date the clutch was laid (by subtracting the average incubation period in days: hawksbill: 67 ±8.8days, n=7; green 62 ±2.5 days, n=4; this study). After data were collected, tracks were erased, nests marked and photographed, and locations and GPS positions recorded.

Using Marine Turtles Time Series (MTTS) software version 3.1.9 (Girondot 2010), which fits a negative binomial distribution to temporal data of nest counts, we estimated the annual number of clutches laid by each species. To provide a comparison to historical estimates of the number of females nesting annually (reported in Fletemeyer 1983), we divided the annual nest counts by the regional average number of nests/season/female (=3 nests/female Seminoff 2004, Mortimer & Donnelly 2008) recorded elsewhere.

Adults from the fishery and in-water surveys

Over the two-year study, intensive monitoring of the TCI marine turtle fishery was carried out at South Caicos, Grand Turk and Providenciales. We directly observed hawksbill and green turtles landed for local consumption at fish landing sites, fish processing plants and local boat docks or jetties. For more comprehensive detail regarding the turtle fishery see Stringell et al. (2013) (Chapter 1). An extensive in-water capture-mark-recapture programme was also carried out during the study period where turtles were captured via a combination of “free-diving” and “rodeo-style” methods (Ehrhart & Ogren 1999) from surveys by boat at locations frequented by fishermen (see Richardson et al. 2009 for methods).

Curved carapace length (CCL, notch-tip: Bolten 1999) was measured in turtles captured in the fishery and from in-water surveys. Hawksbill turtles of >78cm CCL and green turtles of >97cm were considered potential adults based on regional average minimum sizes of nesting females (Witzell 1983, Hirth 1997). Here, reported minimum straight carapace lengths (SCL) were converted to CCL using the following corrections, based on measurements of 284 hawksbill turtles and 386 green turtles sampled during the two-year survey: CCL=1.067 x SCL - 0.0074 (hawksbill turtles; R^2=0.99), and CCL=1.0675 x SCL - 0.3636 (green turtles; R^2=0.99). Animals captured in the fishery that were of this potential adult size were examined to determine sex and stage of maturity through gross examination of the gonads (e.g.

by observing presence of follicles, corpora lutea, corpora albicantia and thick walled oviduct in mature females; and presence of cylindrical testes, pendulous epididymi and well developed accessory ducts in mature males: Miller & Limpus 2003) and secondary sexual characteristics (long prehensile tails and strongly curved claws are characteristic of sexually mature males, and a soft, or decornified, plastron develops in sexually active males during the breeding season: Wyneken 2001). We used these fishery-based minimum sizes at maturity to classify adult-sized turtles captured during our in-water research, where no internal examination of gonads was possible. Turtles that were verified as mature or larger than the minimum size at maturity were hereafter classified as adults.

Satellite tracking

As part of a larger study, we attached Sirtrak Kiwisat 101 satellite transmitters to two adult female green turtles and five adult hawksbill turtles (two females, three males) captured in TCI waters (five by fishermen, two by the survey team). The transmitters were attached directly to the highest point of the carapace using two-part epoxy after biometric measurements and samples were taken. The transmitters and attachment were painted with anti-fouling paint. All transmitters used in this study were programmed with a 24 hour-on duty-cycle, and were controlled by a saltwater switch.

Location data were received from Service Argos and the online Satellite Tracking and Analysis Tool (STAT: Coyne & Godley 2005) was used to manage the data. A speed filter was used that removed locations suggestive of minimum travel speeds greater than 5 $km.h^{-1}$. Argos location class data A, B and 1-3 were examined to determine the duration of tracking, nesting and internesting activities, and site residency. Positional data and movements were reconstructed using only location classes 1-3. Nesting emergences were determined from interpretation of the telemetry data, using the location class, distance from shore, depth and temporal criteria described in Tucker (2010) and, where possible, used in combination with ground-truthing via subsequent beach patrols. Migration tracks were discriminated and separated from site residence / foraging ranges by displacement distances from point of release and visual assessment. Foraging site location centroids were determined by calculating the mean latitude and longitude values from resulting residence site location data. To assess internesting and foraging home ranges (areas that accommodate all regular activities of individuals: Hawkes et al 2011),

gaussian kernel density estimates, 90% volume contours and minimum convex polygons (MCP) were calculated from foraging location classes 1-3 using Hawth's Tools in ArcGIS 9.2 (ESRI). Home range sizes (km^2) were calculated from areas of MCPs, which, for each turtle, represents the smallest polygon to encompass all foraging locations.

Genetics

Skin, muscle and blood samples from adults landed in the fishery, captured in-water or on the nesting beach and from dead hatchlings were collected and stored in lysis buffer until DNA extraction at University of Exeter laboratory in UK. Phire Animal Tissue and Phusion Blood Direct PCR kits (Finnzymes ThermoFisher) with LCM15382 and H950g primers (Abreu-Grobois et al. 2006) were used to extract and isolate approximately 830bp fragments of the D-loop control region of mitochondrial DNA (mtDNA). PCR products were analysed on an ABI 3730xl DNA Analyzer (Applied Biosystems) at Macrogen Europe (Netherlands). Sequences were aligned, edited and analysed using Geneious Pro version 5.1 (Biomatters: http://www.geneious.com/) and haplotypes assigned based on reference sequences from GenBank (http://www.ncbi.nlm.nih.gov/), the Archie Carr Center for Sea Turtle Research website for green turtles (http://accstr.ufl.edu/cmmtdna.html), and Abreu-Grobois (pers. comm.) for hawksbill haplotypes (see also Leroux et al. 2012). Haplotype sequences were truncated to 481bp and 740bp for green and hawksbill turtles respectively. Unknown haplotypes were re-extracted, re-sequenced and checked thoroughly against all possible sources.

Haplotype diversity (h) and nucleotide diversity (π) were calculated in the software Arlequin v. 3.5 (Excoffier & Lischer 2010). To test whether male breeding-condition hawksbills and nests were genetically similar, differences in haplotype frequencies were tested with Exact tests of population differentiation (with 100000 permutations and 10000 dememorisation steps), and pairwise F_{ST} statistics (P values from 10000 permutations) using Arlequin. The small sample size of green turtles precluded us from making genetic comparisons between adults and hatchlings. Comparisons of haplotype frequencies between TCI and other regional rookeries are given in Stringell et al (2013; Chapter 4).

Results

Spatio-temporal patterns

Hawksbill nesting distribution was almost entirely restricted to the eastern islands of TCI particularly on uninhabited cays and coasts (Figure 1a), whereas green turtle nesting distribution appeared to be more widespread, with evidence of activity on Atlantic coasts of Providenciales and North Caicos in addition to the more sheltered Isles (Figure 1b).

Of 208 records of turtle activity (non-nesting emergences, nests, hatched nests), the majority (79.8%) were from hawksbill turtles (n=166) (Figure 2). On average across the TCI, adult nesting emergence (proportion of emergences resulting in nests) was 38% for hawksbill turtles and 50% for green turtles.

Hawksbill turtle nesting activity occurred all year round (Figure 2a) whereas green turtle nesting activity was seasonal with nesting only recorded from May-October (Figure 2b). Survey effort (Figure 2c) was minimal in January and May due to logistical constraints, however turtle activity was evident in these months due to inferred lay date from hatched nests or the age of tracks, although no fresh nests were observed.

Magnitude of nesting

We recorded a total of 55 hawksbill turtle and 22 green turtle nests (including hatched nests) during the entire survey period (Figure 2). From 35 and 16 hawksbill turtle nests recorded in 2009 and 2010 respectively, we estimate that 167 (95% CI: 150-185) and 113 (95% CI: 107-120) clutches were actually laid during each of these years in the surveyed beaches of TCI. Averaging these values provides a conservative estimate of 140 (range 107-185) hawksbill turtle nests per year. For green turtles, we recorded five and 17 nests in 2009 and 2010 respectively, from which we estimate 27 (95% CI: 6-47) and 64 (95% CI: 40-87) clutches were actually laid. Averaging these values gives an estimate of 46 (range 6-87) green turtle nests per year. Using the assumption that female turtles lay on average three clutches per year (Seminoff 2004, Mortimer & Donnelly 2008), we estimate the population of nesting turtles in TCI to be 47 (36-62) hawksbill turtles and 15 (2-29) green turtles per year.

Adult captures

Out of 18 captures of potential adult hawksbill turtles (>78cm CCL), 14 were verified as sexually mature (i.e. adults). The average size at maturity for hawksbill turtles was 89.1cm CCL (range 84.7 − 92.2, n=7) for females, and 84.6cm CCL (range 81.6 − 90.5, n=7) for males. All mature males had a tail length of >30cm that extended well beyond the margin of the carapace. Adult hawksbill turtles (n=14) were captured in the fishery (n=5) or during in-water surveys (n=9) throughout the year (1-3 turtles each month except January, April, Jun and August when no landings were observed), with most captures (2-3 per month) around the peak nesting period between September and November (Figure 2A), although two adults were also landed in February. One was landed by fishers in Providenciales (TCI) in October 2010, and was bearing flipper tags that had been attached after the turtle nested in Barbados in October 2008 (J. Horrocks, WIDECAST pers. comm. 2010). In addition to the turtles landed during the study period, fishers in Providenciales landed another flipper tagged adult female hawksbill in February 2012 (not included in this study) that had been tagged whilst nesting in Jumby Bay Antigua in July 2003 (J. Horrocks, WIDECAST pers. comm. 2012).

Green turtles (n=2) were captured and sampled in June and September, during the nesting season for this species in TCI (Figure 2B). Both were mature females, one that was captured foraging in TCI waters and probably nested in Barbuda (Richardson et al. 2010) and one that nested in TCI.

Satellite tracking

All five satellite tracked adult hawksbill turtles remained in TCI waters for the duration of their tracking (between 38 and 1327 days; see Table 1, Figure 3) showing fidelity to specific areas. Residency centroids calculated for one female hawksbill turtle and the three male hawksbill turtles were located between 2.5 km and 4 km from their release sites (Table 1). Hawksbill turtle EiF2 (nest locations shown in Figure 3E), was fitted with a transmitter after it nested on Fish Cay on the 30 June 2009. She was tracked for 38 days before being found stranded dead on Fish Cay on the 08 August 2009. During that time her tracking data suggested she nested once again on nearby Big Ambergris Cay, which was confirmed by beach patrols the following morning. A post-mortem found no determinate cause of death and since she nested again after tag deployment it is unlikely that she was harmed by the attachment. As

we have no idea when or why she died, data was considered uninformative and removed from analysis of tracking data). In contrast EiF1 was tracked for over three years after it was captured on Philips Reef approximately 6.5 km east of East Caicos on 12 October 2009. The turtle was released the next day off the eastern shore of East Caicos, and by the 01 December 2009 the turtle had moved westwards to inshore waters on the north coast of East Caicos (see Figure 3D). Here the tracking data suggests that the turtle laid five clutches of eggs before settling back on Philips Reef on the 24 January 2010. The turtle returned to the north coast of East Caicos the following year (04 October 2011) and laid another five clutches before returning to Philips Reef on the 01 January 2012, where it remained at the time of writing (February 2013). This turtle exhibited A3 post-nesting behaviour (local residence: Godley et al. 2008).

The two satellite tagged green females appeared to be seasonally present in TCI waters (Figure 3A). Turtle CmF1 was captured by fishers on the 30 June 2009 in sea grass habitat in coastal waters north of East Caicos. This turtle was released within 10 km of the capture site the next day and subsequently tracked for 317 days. The turtle travelled back to inshore waters north of East Caicos where it remained for 61 days. It then travelled away from TCI and undertook a migration to Barbuda, where tracking data suggest it may have nested once, before eventually returning to settle back in the same inshore waters of East Caicos, TCI on the 27 January 2010 (see Richardson et al. 2010 for an account of the movements of this female). Turtle CmF2 was tagged after nesting on Gibbs Cay on 12 September 2010 and tracked for 96 days. The turtle exhibited A1 post-nesting behaviour (oceanic and/or coastal movements to neritic foraging grounds) typical of green turtles (Godley et al. 2008). The turtle migrated away from TCI waters on the 16 September 10, travelled through oceanic and coastal waters before settling in coastal waters south of St Croix, USVI by the 01 October 2010, approximately 780 km straight-line distance from Gibbs Cay, where it remained until transmissions ceased on the 17 December 2010.

Genetics

We successfully sequenced the samples from the two satellite tracked adult green turtles, hatchlings from four green turtle nests and 22 hawksbill turtle nests, and 12 adult hawksbills, of which eight had likely bred in TCI (two females, six males, including all satellite tagged animals). Combining these adult hawksbill turtle ‘TCI

breeders' and hatchling samples, taking into account the haplotype frequency adjustments for sequenced mother and hatchlings to avoid the possibility of pseudo-replication, a total of eight hawksbill turtle haplotypes were recorded (Table 2), one of which (EiA81) is so far unique to the TCI nesting population. Haplotypes EiA03 and EiA27 were found only in 'TCI breeding' males and not in any of the 22 nests (Table 2). No significant differences were found between haplotype frequencies of male hawksbill turtles and hatchlings (Pairwise F_{ST}=0.067, P=0.113; Exact test P=0.126), although we note the small sample size. The hawksbill turtle rookery had a haplotype diversity of h=0.407 ±0.128, and nucleotide diversity of π=0.003 ±0.002.

Both green turtle adults and the four nests of hatchlings were found to be of CmA3 and CmA64 haplotypes, the latter from a nest on Providenciales and the only source rookery record to date. Haplotype diversity and nucleotide diversity were calculated as 0.500 ±0.265 and 0.001 ±0.001 respectively.

Discussion

Our data confirm that the Turks and Caicos Islands provide foraging habitat year-round for locally breeding and foraging adult hawksbill turtles and for adult green turtles that likely breed elsewhere. While breeding adult hawksbill turtles are present in the waters of TCI all year and thus are vulnerable to capture by the turtle fishery, the more locally scarce breeding green turtles are probably more seasonal, being recorded only between May – October in the present study and likely move out of TCIs waters during the non-breeding months. The estimated nesting populations are small (47 hawksbill and 15 green turtles/year) and probably represent remnant rookeries (Richardson et al. 2009, McClenachan et al. 2006). Although, the nesting surveys did not include all possible nesting beaches, which may have increased the total estimated number of nesting turtles, our assumed average clutch frequency estimates could have been low in comparison to satellite derived estimates (Rees et al. 2010, Tucker 2010, Weber et al 2013), which would have led to estimates of the nesting populations that would be smaller than estimated here. Additionally, for both species, unique or rare haplotypes have been recorded within the breeding stock of TCI, and are therefore of considerable interest and conservation concern (McClenachan et al. 2006, Leroux et al. 2012).

Although the traditional turtle fishery largely captures juvenile turtles, individuals from breeding populations in TCI, as well as adult turtles from populations breeding elsewhere are legally captured. The current Fisheries Protection Ordinance does not protect breeding-size individuals from TCI or the Wider Caribbean Region. In the following sections we discuss the utility of this study data in revising existing legislation to introduce seasonal closures and maximum size limits for the current fishery.

Rookery genetics

Despite the small size of TCI rookeries, the unique or rare haplotypes recorded in both green and hawksbill turtle populations by this study highlight the importance of protecting such relictual populations in order to maintain regional genetic diversity (Leroux et al. 2012, Shamblin et al. 2012). The hawksbill EiA81 haplotype found in an East Caicos nest is so far unique to the TCI nesting population and undescribed from Caribbean foraging grounds. The EiA13 haplotype from a nest on Gibbs Cay in

the Grand Turk region has previously only been found in nests from Cuban rookeries (Leroux et al. 2012). Haplotype and nucleotide diversity of the hawksbill rookery (see Table 2) is similar to other Caribbean rookeries (see Stringell et al. in prep., Chapter 4), with EIA11 being one of the most prevalent in the region (Leroux et al. 2012). Haplotypes EiA01, EiA13, and EiA81 were found only in the nest samples, thus appear to be representative of breeding females. Two haplotypes (EiA03 and EiA27) were found only in breeding males and may indicate that these individuals, although clearly in breeding condition, may not be originally of TCI stock; EiA03 has been described from rookeries in Antigua, US Virgin Islands and British Virgin Islands (BVI), and EiA27 from rookeries of Montserrat and BVI (Leroux et al. 2012, Formia et al. unpublished). The extended time in which these males were in TCI waters (e.g. >640 days of satellite tracking) however would imply residency. The frequencies of breeding males and nest haplotypes were genetically similar, suggesting that they might be part of the TCI rookery. With small sample sizes, however, it is difficult to draw compelling conclusions; further work is required to establish whether these 'male' haplotypes are represented in TCI nests.

To our knowledge, the TCI represents the only known source rookery for the green turtle haplotype CmA64. Prior to this study, it has been found only in a single foraging green turtle juvenile captured in Indian River Lagoon, Florida (Shamblin pers. comm. 2012). With only four green turtle nests sampled, it is infeasible to compare haplotype and nucleotide diversity to other rookeries in the Greater Caribbean (e.g. Bjorndal et al. 2005); further work is needed to genetically characterise the green turtle rookery of TCI. Better protection of adult turtles in TCI waters than is currently afforded by the regulations is therefore required to facilitate recovery of the small but genetically diverse populations breeding in TCI.

Nesting seasonality and magnitude

This work highlights the challenges involved in monitoring low-magnitude nesting in an extensive archipelago. Considerable investment of both time and money would be required to monitor nesting thoroughly (e.g. SWOT Scientific Advisory Board 2011 gold standard). In the UK Overseas Territories in the Caribbean, where conservation managers are financially constrained (Forster et al. 2011), but have responsibility to manage minor turtle nesting populations, such as TCI, these exacting standards are likely to be untenable. Broad insights can be gained from

simple visual assessments of seasonality, especially in situations with highly irregular and partial nest counts, and are probably sufficient for recommending conservation measures. This study suggests that conservation decisions do not necessarily require massive investment in biological research to provide sufficient insight for sensible and realistic recommendations.

Nesting by hawksbill turtles was recorded year round in TCI, peaking in October, while green turtle nesting peaked in August but occurred between May-October. This is consistent with nesting patterns in neighbouring Caribbean nations (Bjorndal et al. 2005, Velez-Zuazo et al. 2008). Although the magnitude of nesting activity can vary substantially between years (Broderick et al. 2001), the timing of the peak, and duration of the nesting seasons is usually relatively conservative between years (Jackson et al. 2008), characteristics we used to our advantage by steering our survey design on gaining a better understanding of the nesting seasonality in TCI rather than nesting magnitude. Nevertheless, we approximated the relative magnitude of nesting in both green and hawksbill turtles using the MTTS software with the caveat of high expected variation, as reflected, for example, in the wide 95% CI of hawksbill turtle nesting estimate of 2009 as a consequence of the low number of nests that year and the resulting uncertainty in model fit. We assert that the annual estimates should be considered cautiously for reasons given previously and because only two years of data were collected - a small time frame for species with large interannual nesting variation (Broderick et al. 2001). However, the software modelling assumptions are considered well suited for partial survey data such as these (Girondot 2010).

In July 1982, a brief survey of TCI nesting turtle populations was carried out (Fletemeyer 1983) and provides an historical comparison, albeit with high uncertainty, of between 125 and 275 nesting hawksbills and 45 to 105 nesting green turtles (although the estimation technique was not given). This suggests that the current population may be considerably smaller than that in the 1980s. Given the small size of the current breeding populations of marine turtles in TCI, especially in the context of these larger historical numbers, the take of reproductively valuable adults - in particular the higher number of hawksbill turtles taken over this two-year study - is likely to be affecting the recovery potential of these populations.

Adult size and captures

In August 2012, after this study was completed, a female green turtle with shelled eggs in its ovaries and measuring 90.3cm CCL was captured for consumption in Middle Caicos. This adult turtle was considerably smaller than the adult green turtles captured during the study period. However, the smallest nesting green turtle size reported in the region was 73.5cm CCL (converted from 69.2cm SCL) from Tortuguero, Costa Rica (Hirth 1997). In hawksbills, minimum size at maturity has been described in females harvested in Cuba as small as 54-59cm CCL (converted from 51-55cm SCL) and in males at 65-67cm SCL (Moncada et al. 1999) contrary to general sexual dimorphism patterns, where males are typically about 5cm smaller (Witzell 1983, Limpus 1993, Hirth 1997). Meylan et al. (2011) compared green turtle biometric and laparoscopy data across study sites which revealed significant overlaps in size ranges of immature and mature turtles among and within sites and highlighted the difficulties of using size alone to determine maturity. Even though the green turtles we sampled in this study were sexually mature, the use of a size threshold (e.g. 97cm) to classify turtles as adults should be treated with caution.

Management implications: closed seasons

Elsewhere in the Wider Caribbean Region, closed seasons have been introduced into legislation that prohibit take of turtle species during a specified time, usually coinciding with the breeding seasons. For example, take of turtles is prohibited in the Cayman Islands between April and November (Cayman Islands Government 2008), a period which includes the time when breeding adult turtles arrive in Cayman waters and the nesting seasons for loggerhead and green turtles (Bell et al. 2007). A similar closed season is included in the British Virgin Islands legislation, which encompasses the nesting season for green turtles and most of the hawksbill turtle nesting season (Richardson et al. 2006, McGowan et al. 2008). A similar approach could be adopted in the TCI through the introduction of a closed season that covers both the green turtle nesting season and peak nesting of hawksbill turtles. However, the introduction of what may essentially be a ban on turtle capture lasting eight months or more may not be acceptable to the broad collective of stakeholders who currently have year-round use of turtles or be necessary in terms of stock sustainability (Crouse et al. 1987, Campbell et al. 2009, Richardson et al. 2009, Stringell et al. 2013, Chapter 1). The TCI lobster fishery appears to be an important

driver in the number of hawksbill turtles landed, with more hawksbills landed during the lobster open season (Stringell et al. 2013, Chapter 1). Given the relatively low frequency of adult green turtle take by the TCI fishery, an alternative to the composite closed season could be a hawksbill turtle, species-specific closed season coinciding with the lobster open season that traditionally runs from August to March inclusive. Hawksbill turtles are preferred less by consumers in TCI than green turtles (Richardson et al. 2009), and while this measure would still involve a novel eight month restriction on hawksbill take, it would still allow fishers access to green turtles throughout the year, and importantly outside of the lobster season at a time when the green turtle fishery is most valuable (Stringell et al. 2013, Chapter 1).

Management implications: size limits

The Cayman Islands turtle fishery regulations are the only legislation in the Caribbean that provide a maximum size limit for a turtle fishery (Blumenthal et al. 2010). The introduction of maximum size limits for green and hawksbill turtles within the TCI turtle fishery regulations may well be more acceptable measures for local stakeholders compared to a composite closed season for these species. Fishers are already used to the current minimum size limit, and this measure would allow the on-going take of juveniles that make up the majority of animals currently landed anyway (Stringell et al. 2013, Chapter 1). A standard maximum size limit for both species is likely to prove more practical to enforce, but setting such a limit would have to take into account estimated minimum sizes at maturity for the smaller hawksbills to accommodate both species. A conservative maximum size limit of 50cm would therefore take into account the smallest sizes at maturity reported by Moncada et al. (1999) from Cuba. However this is the equivalent of the current minimum size limit of 20 inches (TCI works in imperial measurements) and so is not appropriate if this minimum size limit is maintained (all turtles would be excluded from the fishery: Figure 4). It may therefore be necessary to lower the current minimum size limit, or repeal it altogether, to facilitate fisher access to broader size ranges of juvenile turtles. Even if the minimum size limit is lowered to accommodate a maximum size limit, 50 cm may be deemed too low by stakeholders, as it would significantly impact access to juvenile greens that make up a majority proportion of the current fishery (Stringell et al. 2013, Chapter 1). A maximum size limit of nearer 78cm (e.g. 30 inches) would protect the majority of adult hawksbills and all adult green turtles using

TCI waters, including breeding turtles and those from populations breeding elsewhere. Based on the size distribution of turtles landed in the fishery, such a maximum size limit, in combination with the current minimum size limit, would exclude 44% and 57% of the hawksbill turtle and green turtle populations respectively from the fishery (Figure 4), of which 12% and 6% respectively are >78cm. However, the TCI fishery also lands large juvenile (sub-adult) turtles considered to require protection to facilitate regional population recoveries (Crouse et al. 1987, Crowder et al. 1994, Heppell & Crowder 1996). A precautionary maximum size limit lower than 30 inches set specifically to protect large juveniles would accommodate most uncertainty regarding size at maturity: the current minimum size limit of TCI plus a maximum size limit of 60cm, in line with the Cayman Islands, would protect approximately 72% and 75% of the hawksbill turtle and green turtle populations respectively.

Conclusions

Previous work in the TCI (Richardson et al. 2009) recommended changes to the management of the traditional turtle fishery in TCI, and called for further work to better describe the nature of the fishery and its likely impacts on nesting populations. This study concludes that specific legislative amendments, particularly the introduction of maximum size limits above which animals may not be landed and potential closed seasons, would be beneficial to protection and recovery of the remnant nesting populations in TCI. Management at the country level is also likely to be a key step towards successful regional conservation (Moncada et al. 2012) and of benefit to the populations of turtles in the Wider Caribbean that use TCI as foraging grounds. However, legislative change alone will not facilitate recovery of the turtle populations using TCI waters. Community will and understanding, effective enforcement and stakeholder compliance with turtle fishery legislation will be key factors that decide the future of the turtle populations breeding in TCI and will influence how future generations benefit from this element of TCI's natural heritage.

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Table 1. Deployment statistics of adult turtles satellite tagged in TCI: two female green turtles (CmF) and five adult hawksbills (two females [EiF] and three males [EiM]). Data derived from location classes (LCs) A, B, 1-3. Minimum convex polygons (MCP) calculated from LCs 1-3.

Turtle	CCL (cm)	Date of release	No. days tracked	Distance between release & foraging site centroid (km)	Max Displacement from release (km)	Home range (km^2; MCP)	Migration beyond TCI waters	Foraging ground	Nesting site
EiF1	90.6	13.10.09	>1210*	N: 3.4** IN: 13.4***	63	80.46	No	TCI	East Caicos, TCI
EiF2	85.1	01.07.09	38†	-	58	-	No	Unknown	Fish & Ambergris Cays, TCI
EiM1	81.6	23.09.09	746	2.5	47	38.18	No	TCI	-
EiM2	90.5	02.10.09	640	4	28	29.46	No	TCI	-
EiM3	84.0	01.10.09	1327	3.1	45	18.96	No	TCI	-
CmF1	102.6	25.06.09	317	9.1	1452	216.14	Yes	TCI	Barbuda††
CmF2	112.9	12.09.10	96	778.4	788	27.37	Yes	USVI	Gibbs Cay, TCI

CCL=curved carapace length
N=nesting, IN= internesting
Dates are dd/mm/yy format
* still transmitting at time of writing (04 February 2013)
** two nesting seasons (2009-10 and 2011-12)
*** distance between release site and nesting/internesting centroid
† died
†† a likely single clutch laid in Barbuda (Richardson et al. 2010)

Table 2. Haplotype frequency for hawksbill (Ei) and green turtle (Cm) adults captured in the TCI fishery and from in-water surveys, and hatchlings from individual nests. Frequencies are separated by sex (M=Male, F= Female, U= Undetermined). See Stringell et al. in prep. (Chapter 4) for comparisons with regional haplotype frequencies.

	Breeders (Br)		Foragers (Fg)		Hatchlings (H)	Total (H & Br)	Total (Fg)
Haplotype	F	M	F	M	U		
EiA01					1	1	0
EiA03		1				1	0
EiA11	2	3	2[b]	1	17	21[b]	3
EiA13					1	1	0
EiA27		1				1	0
EiA41					2	2	0
EiA42		1	1			1	1
EiA81[a]					1	1	0
CmA3	1[b]				3	3[b]	0
CmA5			1			0	1
CmA64[a]					1	1	0

[a] source rookery haplotypes unique to TCI (submitted to Genbank).
[b] haplotype frequency adjusted for mother and nest duplicates.

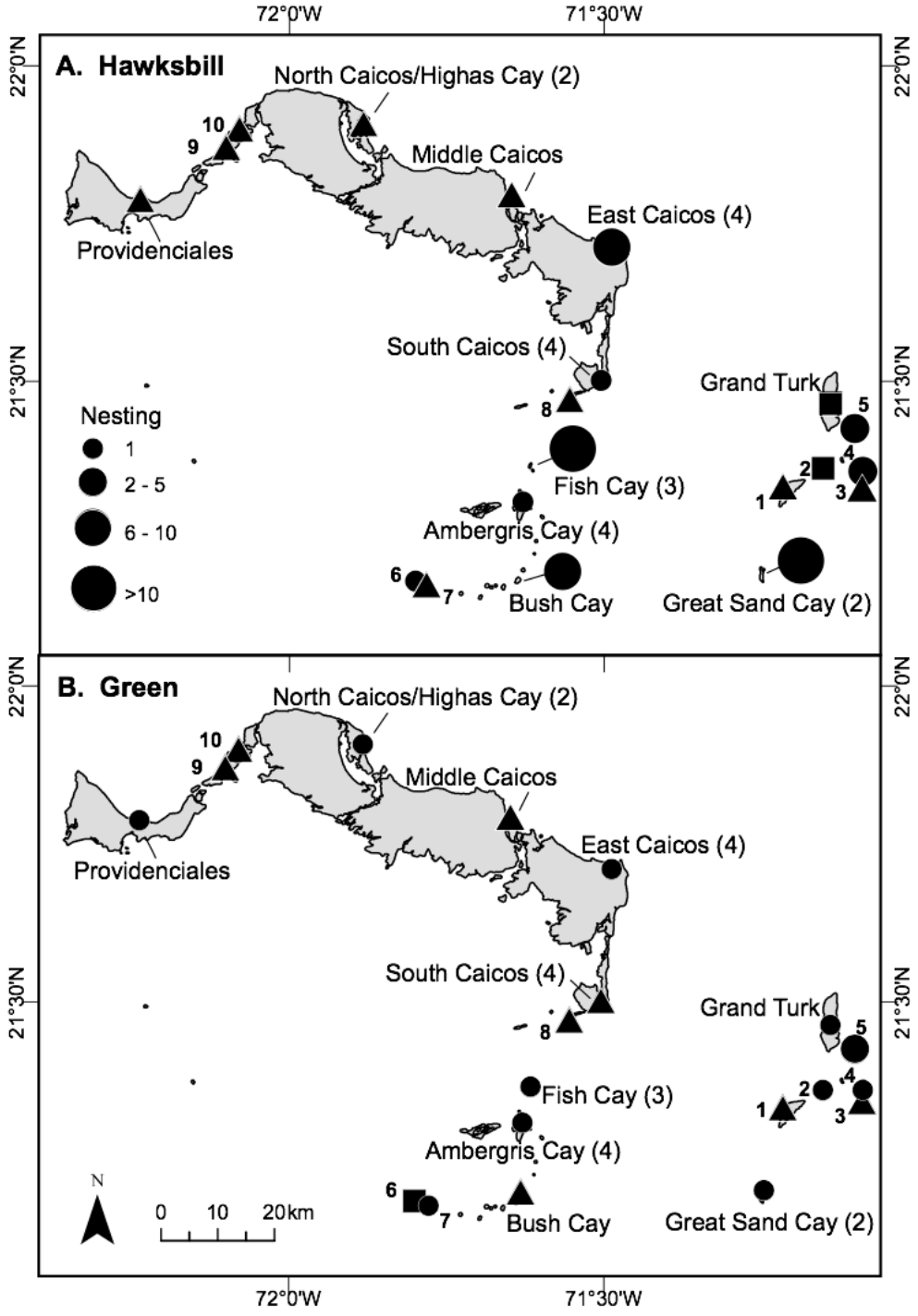

Figure 1. Spatial distribution of hawksbill (A) and green turtle (B) nesting activity. Magnitude of nests recorded is shown by increasing circle size. Locations where only non-nesting emergences were observed are indicated by squares. Survey locations where no turtle activity was observed are shown with triangles. Data are summed over the two-year survey period. Numbers in bold refer to the following locations: 1-Salt Cay, 2-Cotton Cay, 3-Pinzon Cay, 4-Eastern Cay, 5-Gibbs Cay (2 beaches), 6-Weis Cay, 7-Indian Cay, 8-Long Cay, 9-Pine Cay, 10-Dellis Cay. Numbers in parentheses indicate the number of survey beaches at each location, otherwise each label represents a single beach.

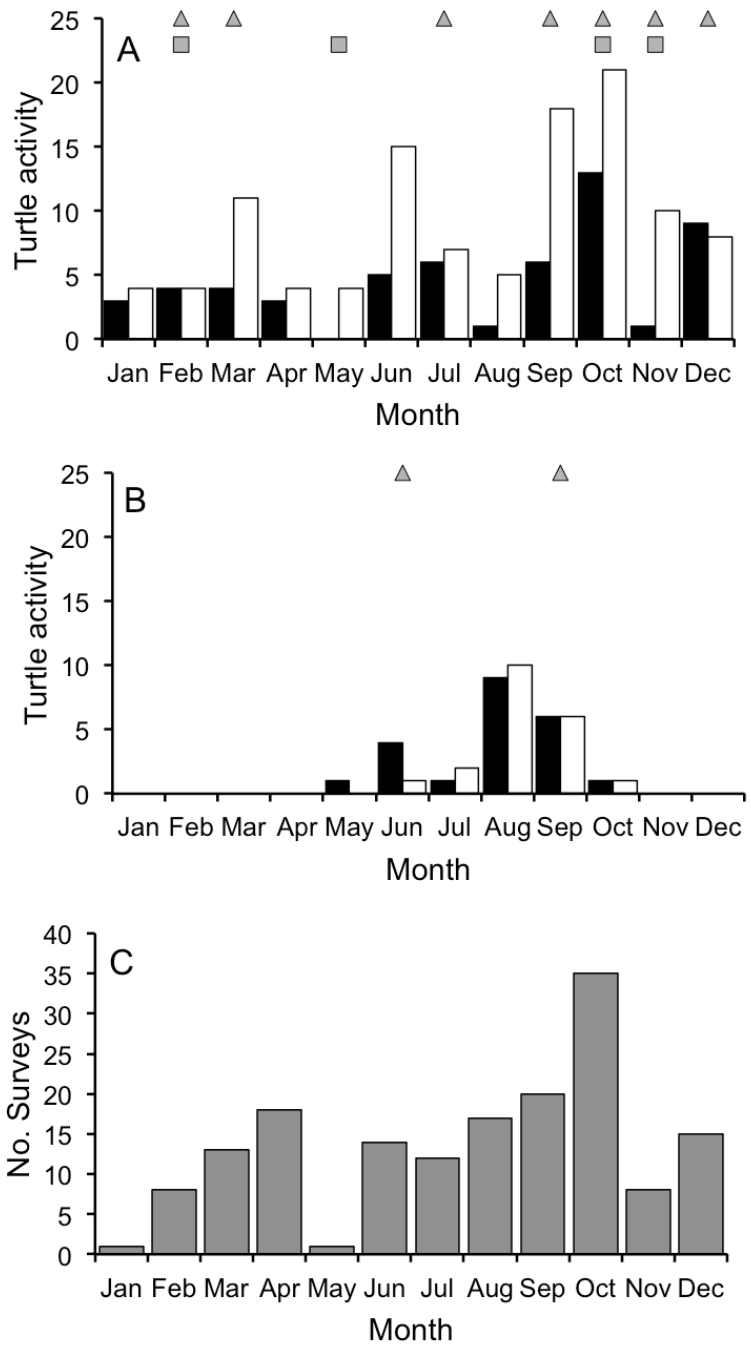

Figure 2. Nesting activity of hawksbill turtles (A) and green turtles (B) in TCI. Black bars indicate number of nests and hatched nests of inferred lay date. Non-nesting emergences are shown as white bars. Survey effort (C) is the number of nesting surveys (n=162) by month. Triangles indicate when one or two adult turtles were captured during CMR (inwater and nesting surveys; two hawksbill turtles were captured in Sep and Oct), and squares indicate turtles captured by fishers (two in November). Data are summed by month and survey locations over the two-year study period.

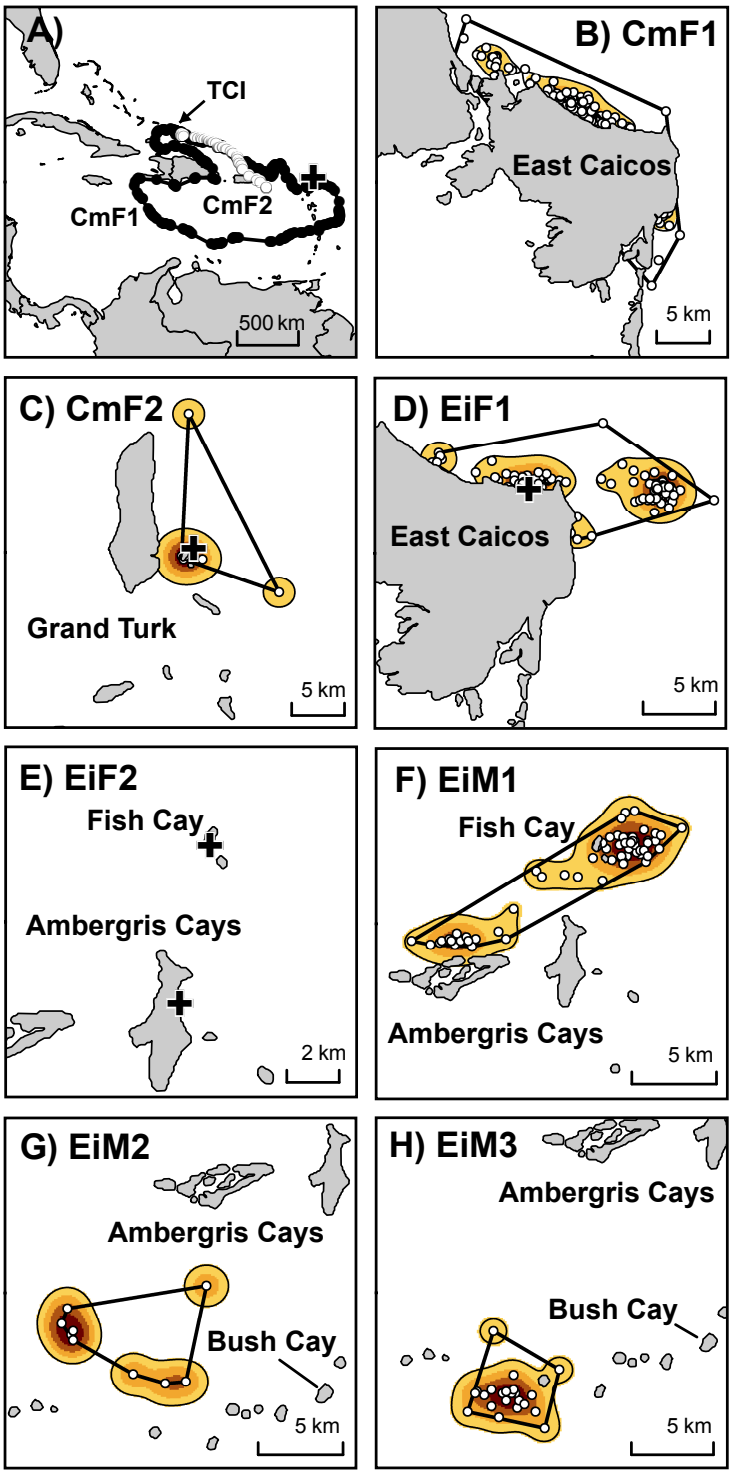

Figure 3. Locations and home ranges - minimum convex polygons (thick straight black lines), kernel density estimates (shading) with 90% volume contours (thin curved black lines) - of seven satellite-tracked turtles: Panel A shows the two green turtle (*Chelonia mydas*, Cm) migration tracks away from TCI territorial waters (see Richardson et al. 2010 for information on CmF1), two female green turtles (B-C: CmF1 and CmF2) and five hawksbill turtle (D-H) (*Eretmochelys imbricata*, Ei: Females EiF1-2 and males EiM1-3). Crosses (+) indicate nesting position for each nesting female (Barbuda: CmF1 (A); TCI: EiF1-2 (D-E), CmF2 (C)). White circles indicate foraging locations - Argos location classes 1, 2, 3 for each turtle up to the time of writing (04 February 2013). Locations are not displayed for the internesting periods of turtles EiF1-2 or CmF2 (see text for further detail).

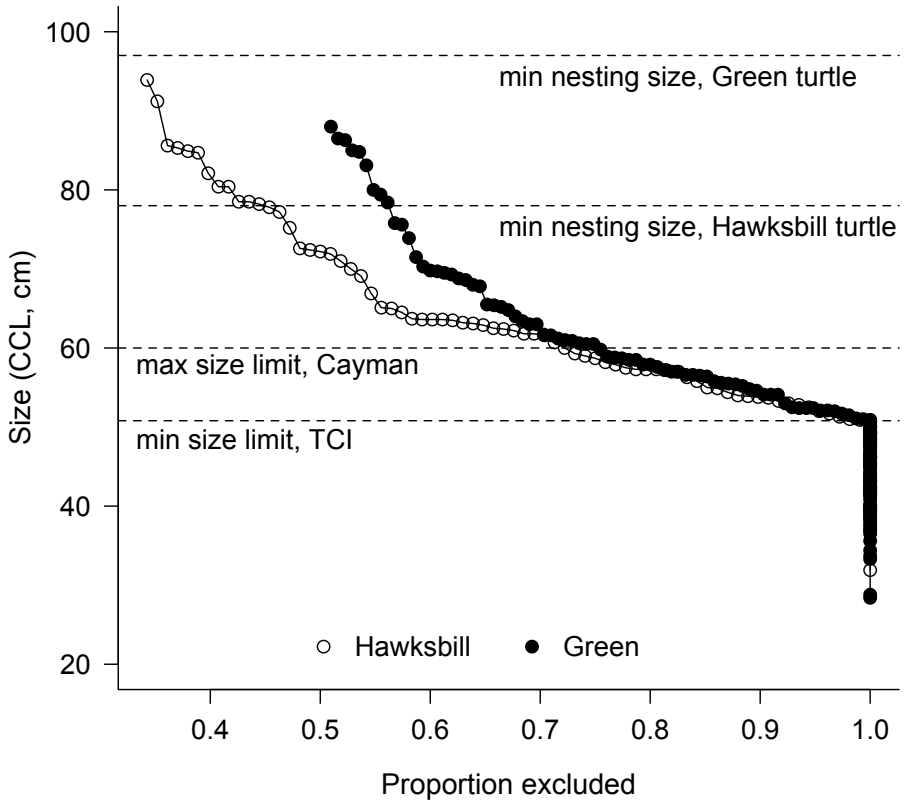

Figure 4. Proportion of hawksbill turtle (open circle, n=108) and green turtle (filled circle, n=155) populations of TCI, as determined from size distribution of harvested turtles (Stringell et al 2013; Chapter 1), potentially excluded from the fishery with various size limits (CCL; curved carapace length, cm). The proportion excluded from the fishery is inclusive of those already excluded by the TCI minimum size limit (51cm). The maximum size limit for Cayman Islands is 60cm. The average minimum nesting size for the region is 78cm for the hawksbill turtle (Witzell 1983) and 97cm for the green turtle (Hirth, 1997).

Chapter 3

Fisher choice may increase prevalence of green turtle fibropapillomatosis disease

Thomas B. Stringell[1], Wesley Clerveaux[2], Brendan J. Godley[1], Quinton Phillips[2], Susan Ranger[1,3], Peter B. Richardson[1,3], Amdeep Sanghera[3] and Annette C. Broderick[1]

[1]Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ. UK
[2] Department of Environment and Maritime Affairs, South Caicos, Turks and Caicos Islands.
[3] Marine Conservation Society, Ross on Wye, Herefordshire, HR9 5NB. UK

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Abstract

Disease in wildlife populations is often controlled through culling. But when healthy individuals are removed and diseased individuals are left in the population, it is anticipated that prevalence of disease increases. Although this scenario is presumably common in exploited populations where infected individuals are less marketable, it is not widely reported in the literature. We describe this scenario in a marine turtle fishery in the Turks and Caicos Islands (TCI), where green turtles are harvested annually for local consumption. During a two-year period, we recorded the occurrence of fibropapillomatosis (FP) disease in green turtles (*Chelonia mydas*) captured during in-water surveys and compared it with those landed in the fishery. Of turtles captured from in-water surveys, 13.4% (n=32) showed externally visible signs of FP. Despite the disease being prevalent in the size classes selected by fishers, FP was not present in any animals landed by the fishery (n=162). FP occurred at specific geographic locations where fishers encounter diseased turtles. The majority (61%) of fishermen interviewed expressed that they had caught turtles with FP and of those that had caught turtles with the disease, 82% chose to return their catch to the sea, selectively harvesting healthy turtles and leaving those with the disease in the population. Our study illustrates that fisher choice may increase the prevalence of FP disease and highlights the importance of this widely neglected driver in the dynamics of exploited wildlife populations.

Introduction

It is thought that emerging infectious diseases of wildlife are increasing globally with consequences to human, animal and ecosystem health (Cohen 2000, Daszak et al. 2000, Ward & Lafferty 2004, Jones et al. 2008). Causes of disease emergence are varied, complex and difficult to study but frequently implicate anthropogenic impacts (McCallum & Dobson 1995, Daszak et al. 2001, Plowright et al. 2008). The consequences of wildlife exploitation on disease dynamics in host species, however, are widely neglected in resource management (Choisy & Rohani 2006).

Sustainable exploitation of wildlife populations that optimises yield while maintaining the population has been a mainstay of conservation biology across taxa for decades (e.g. Gordon 1954, Clark 1976). Resource exploitation is usually driven by complex interactions between nature and humans that can have synergistic effects (Liu et al. 2007) and involve complex and often poorly described socioeconomic systems (Ostrom 2009). It is no surprise, therefore, that there is widespread neglect of the human dimension to exploitation and disease. Harvesting may alter disease prevalence and mortality directly or indirectly (Choisy & Rohani 2006) and when this results in an increase in the disease, may present a serious threat to wildlife or resource conservation, particularly in management of species of conservation concern. For example, overfishing of the food source of harp seals and subsequent seal movement to find alternative prey has been implicated in the spread of phocine distemper virus from infected harp seals to European harbour seals leading to a massively depleting epizootic in the species (Dietz et al. 1989, Heide-Jorgensen et al. 1992, Härkönen et al. 2006).

Disease in animal species is widely controlled by removal of infected hosts (e.g. badgers, (Donnelly et al. 2003); Tasmanian devils, (Lachish et al. 2010); white-tailed deer, (Blanchong et al. 2006). However, culling often not only fails to control the disease but also may increase it. For example, where culling disrupts social structures, a resulting increase in movements of individuals may spread the disease further afield, an effect called perturbation (Donnelly et al. 2003, Woodroffe et al. 2004). But what happens when non-diseased animals in the population are exploited and diseased individuals are left in the population? Such a scenario is presumably common, for example, in fisheries when disease renders fish less marketable (Dobson & May 1987, Kuris & Lafferty 1992, Wood et al. 2010).

However, there are few empirical examples in marine ecosystems. Kuris and Lafferty (1992) report on the effect of returning infected hosts in modelled crustacean fisheries, where the common practice of releasing female crabs, which are preferentially parasitised over male crabs, may result in an increase in the impact of parasitism and affect reproductive output. This scenario may apply in other cases where a fishery takes a disproportionately large percentage of uninfected hosts.

Fibropapillomatosis (FP) is a disease characterised by external and internal tumours and has been found in most species of sea turtle, primarily green turtles (*Chelonia mydas*) (Herbst 1994) (Figure 1). Since its discovery in the 1930s (Smith & Coates 1938) FP has become a global pandemic (Williams et al. 1994) and received much attention in the press, popular media and scientific literature, and is considered one of the most significant neoplastic diseases in reptiles (Herbst 1994). Although fibropapillomas appear to be benign, their location, size and frequency may be debilitating to the host by impeding vision, feeding, swimming, and internal organ function. However, some studies have documented the regression of infection, even in advanced cases (Chaloupka et al. 2009). There is strong evidence that FP is caused by a herpes virus (Lackovich et al. 1999, Lu et al. 2000, Quackenbush et al. 2001, Herbst et al. 2004, Greenblatt et al. 2005a, Patricio et al. 2012) and is associated with various environmental cofactors (Herbst & Klein 1995, Work et al. 2004, Arthur et al. 2008, Van Houtan et al. 2010) but its mode of transmission is yet to be verified, although ectoparasites have been implicated (Greenblatt et al. 2004).

Many green turtle populations have been depleted by exploitation for food, leading to their globally endangered status (IUCN 2010). It is believed that FP might impair recovery of depleted populations (Herbst 1994, Ene et al. 2005) because FP has a high prevalence in immature animals thus impacting the long-term survival of green turtle populations (Greenblatt et al. 2005b). However, in the historically and currently exploited Caribbean metapopulation of green turtles, where multiple nations take turtles for domestic consumption, the dynamics of FP prevalence is unknown. The regionally significant foraging aggregation (Richardson et al. 2009) and the small-scale turtle fishery in the Turks and Caicos Islands (TCI), a UK Overseas Territory in the Caribbean (Stringell et al. 2013, Chapter 1), offered an ideal opportunity to investigate the impact of harvest on FP prevalence in green turtles.

In this study, we ask three broad questions: 1) do we observe different levels of FP prevalence in turtles landed by the fishery and those sampled during

independent in-water surveys; 2) are turtle size and location risk factors for FP; and 3) are fishers selectively harvesting healthy animals?

Methods

Study Site

This study took place over two years between November 2008 and December 2010 in the Turks and Caicos Islands (TCI), a UK Overseas Territory in the Caribbean (21° 45N, 71° 35W). The research team was stationed on South Caicos, the main fishing centre of the TCI, for the duration of this study, with regular visits made to the islands of Grand Turk and Providenciales, the two main population centres, and several visits made to North and Middle Caicos (Figure 2).

Monitoring methods

In-water surveys

We used past information (Godley et al. 2004, Richardson et al. 2009) and local knowledge (sensu Hall & Close 2007) to select survey locations that reflected a range of turtle fishing intensities. We surveyed 14 locations in TCI in an extensive in-water capture-mark-recapture (CMR) programme to sample foraging green turtles. We hand-captured 239 individual turtles via a combination of "free-diving" and "rodeo-style" methods (Ehrhart & Ogren 1999). In general, each location was visited at least 4 times per year to provide a seasonal spread of effort, but logistics and weather occasionally constrained this. We typically used turtle fishermen and their boats for most surveys and tried to emulate the methods they would use to catch turtles; the fishermen themselves made most captures.

Turtle fishery

We observed the turtle fishery at several fish-landing docks throughout the TCI (see Stringell et al. 2013, Chapter 1 for details). A total of 233 green turtles were butchered at the fish-landing docks and 162 visually assessed for presence of FP. We recorded capture location for 89% (n=208) of all butchered turtles and for 92% (n=149) of those assessed for FP. An index of turtle fishing intensity was created from the proportion of turtles harvested at each location during the survey period (Figure 2).

Turtles

Both CMR and harvested turtles were measured by curved carapace length (CCL

cm: Bolten 1999), weighed using Kern digital scales for turtles under 50 kg (±0.05 kg) or Salter analogue scales for those weighing over 50 kg (±0.5 kg), and visually assessed for presence or absence of lesions typical of external FP tumours (Figure 1). Where turtles were recaptured during the 25-month CMR study, only measurements (CCL, weight and location) from first captures were used with the exception of three individuals that developed FP between recaptures (here final capture measurements were used). Capture location was recorded by handheld GPS during surveys, or the location of a butchered turtle's capture approximated after fisher interviews.

Fisher Interviews

We used informal semi-structured interviews with 28 fishers - approximately 10% of licensed fishers in TCI (287 licenses for fishing year 2009/10) - who were asked a series of questions related to FP in turtles and harvesting practice (see supplementary Table S1). Participants at South Caicos (n=13), Providenciales (n=6), Grand Turk (n=5) and North Caicos (n=4), were interviewed by the authors (AS, QP, TBS). These authors were either resident or had spent a significant amount of time with the fishing community over the two-year study period and had established trust, enabling us to gain a unique insight on the turtle fishery.

Interviews were conducted in a casual but guided manner and generally lasted between 5 and 10 minutes but frequently ran over as discussions developed and the subject was revisited over the sampling period to substantiate claims and verify answers. During the interview, an image of a green turtle with FP was shown (Figure 1) and each participant asked whether they had seen green turtles with this disease in TCI waters. Among other questions about FP, fishing locations and opinions on the disease, we asked whether they themselves had caught turtles with FP and what the fate of that turtle was, whether they remember any turtle with FP being landed by others for consumption, and whether they had eaten turtle with or without FP (see supplementary Table S1).

Statistical analyses

To create a null model of FP prevalence in harvested turtles, we took the means of 10,000 randomisations of the number of turtles harvested from locations that had an incidence of FP multiplied by the probability of FP prevalence at these locations as

determined from CMR surveys. Turtle size was restricted to that landed in the fishery (28.8cm-88.0cm CCL). The actual level of FP in the harvest from these locations was compared to this null model. Two-sample *t*-tests were used to test differences in average CCL between groups. These analyses were conducted in R v 2.13.0 (R-Development-Core-Team 2011). We then investigated the risk factors of location, size and their interaction on FP incidence using a two-way crossed mixed-effects permutational ANOVA (PERMANOVA), using PERMANOVA+ (Anderson et al. 2008) and PRIMER v6 (Clarke & Gorley 2006). CCL was the response variable, location a random factor with 13 levels (one location was excluded as an outlier) and FP a binary fixed factor. Where significant differences existed, we investigated pairwise comparisons of CCL of the FP factor at locations where FP occurred.

Results

Fishery vs. CMR

Of the 239 green turtles captured and released during our in-water/CMR surveys, 13.4% (n=32) showed externally visible signs of FP. None of the turtles captured in the fishery that we assessed for FP (n=162) had the disease. The absence of FP in harvested turtles departed significantly (P=0.02) from the null model of prevalence, which was simulated from turtles captured during CMR surveys (n=140) that were of similar sizes to those captured by the fishery (28.8-88.0cm CCL) and only from sites that had an incidence of FP (n=5 sites).

Does FP prevalence change with body size?

Turtles with FP caught in CMR surveys were significantly larger (mean CCL=54.0cm, SD=10.2, n=32) than those without FP (mean CCL=42.7cm, SD=11.4, n=207) ($t_{43.7}$ = 5.71, P<0.0001) (Figure 3). A 3-order polynomial fit with R^2 = 0.84 indicates that FP prevalence peaks at 40% at around the 65-70cm range (Figure 3a). A prevalence value of 50% at 80-85cm size range is probably an artefact of small sample size (n=2). Turtles with FP did not differ in terms of body condition (weight vs. CCL) to those that were FP free (supplementary Figure S1). Turtles captured by the fishery averaged 52.6cm CCL (SD=12.3, n=136; Figure 3b), similar to turtles captured and released with FP ($t_{54.16}$=0.6639, P=0.51). These results indicate that FP is present in the size classes of turtles selected by the fishers and that we would expect to find some harvested turtles to have FP.

Does FP prevalence change with location?

FP occurred only at central island locations where FP prevalence varied from 5%-34% (Figure 2). About 10% (n=23 of 233) of turtles were harvested at these locations (Figure 2). Fishermen therefore exploit turtles from areas where FP occurs and are likely to encounter them.

There was also a strong spatial effect to turtle size, with turtles generally being larger in central locations (Random factor: F_{12}=5.54, P=0.001), and the incidence of FP tended to follow this pattern: four out of the five locations that had turtles with FP had the largest average turtle size (supplementary Figure S2). However, turtles with FP were larger than those without (Fixed factor: F_1=21.31, P=0.002) only at two

locations: Causeway (t_1=2.3, P=0.03, n=22; four turtles with FP) and Jacksonville (t_1=2.2, P=0.024, n=38; two turtles with FP), and not at those locations that had the highest prevalence of FP (Ocean Hole: t_1=1.53, P=0.138, n=32; 11 turtles with FP; Southern Bush: t_1=1.26, P=0.216, n=47; 12 turtles with FP). These results imply that size and location interact and together are risk factors for FP.

Does fisher choice affects FP prevalence?

The absence of turtles with FP that are landed by the fishery is likely due to fisher choice. Of the 28 fishermen interviewed, 21 (75%) were active turtle fishers at the time of the interviews; the remainder were once or had worked closely with turtle fishers (Table 1). Most fishers (61%) had seen or captured green turtles with FP in TCI and 82% of them had returned the turtles to the sea because they did not want to eat diseased meat. Only three fishers reported having harvested turtles with FP and typically cut FP tumours off and sold the meat on to restaurants. The majority of fishers (90%) expressed that they would not harvest turtles with FP in the future; just two of the 21 fishers stated that in the future they would harvest turtles with FP for food, with one stating he would eat meat from turtle with FP, although it was uncertain if he had eaten turtle with FP before.

Discussion

Fishers do encounter turtles with FP, but from our dockside surveys and fisher questionnaires it is clear that fisher choice and harvest practice may explain the absence of the disease in their catch. The fishery selectively harvests healthy turtles and leaves those with the disease in the population, thereby likely increasing the survivorship of turtles with FP through reduced fishing-mortality. This empirical example suggests that harvest and fisher choice may increase the proportion of the population exhibiting FP in green turtle foraging areas in TCI. These results highlight the potential importance of this widely neglected driver in the dynamics of exploited wildlife populations.

Fishery / harvest effects and examples

Culling infected prey theoretically reduces disease prevalence (Holt & Roy 2007). Conversely, predation (or harvest) may increase disease prevalence when the predator or harvester selects only immune or non-diseased individuals (Choisy & Rohani 2006, Holt & Roy 2007). The TCI turtle fishery targets apparently unaffected turtles leading to low fishing mortality of infected individuals and is an empirical example of the latter scenario. It is unknown, however, whether FP transmission is density or frequency dependent. In frequency-dependent transmission there may be an increase in relative abundance of infected hosts and an increase in the prevalence of the disease (Wood et al. 2010), and in density-dependent transmission, removal of uninfected hosts will reduce host density which may lead to a reduction in disease prevalence (Dobson & May 1987, Wood et al. 2010). Either way, harvesting in general reduces the number of hosts, and harvesting uninfected individuals may increase the proportion of infected animals.

In several crustacean fisheries around the world, where diseased products are unpalatable or unmarketable and tend not to be landed in the fishery, the incidence of disease has been correlated with fishing effort (Kuris & Lafferty 1992, Stentiford & Shields 2005, Freeman & MacDiarmid 2009, Bateman et al. 2011). Harvest practice may also have indirect effects on disease (e.g. Dietz et al. 1989, Heide-Jorgensen et al. 1992, Härkönen et al. 2006). Our work also suggests the potential for unintended consequences of harvest practice.

Bias

Differential catchability of infected animals may bias estimates of disease prevalence rates from harvested stocks (Conner et al. 2000). FP may make turtles more susceptible to capture, because tumours can restrict mobility and impair vision (Herbst 1994). If, however, FP infected turtles are more frequently captured by fishers but evidently returned as unsuitable for consumption, estimates from landings would be a stronger indication of the selective harvest. Moreover, if prevalence of FP in harvested turtles is used as an estimate of prevalence in the population, the actual proportion of diseased animals in the population may be much higher. For example, Adnyana et al. (1997) calculated 21.5% prevalence of FP in green turtles from slaughterhouses in Indonesia and extrapolated this prevalence to wild stocks in Indonesian seas. If fisher choice also played a part in the slaughterhouses of Indonesia, then the wild prevalence of FP could have been much greater than reported. Without information on FP prevalence in wild stocks, captured through independent in-water surveys, and knowledge of how fisher choice influence the independent observations of sea turtle fisheries, reliable metrics on disease prevalence would be hard to obtain.

FP risk factors

It is widely reported that turtle size is a risk factor for FP (Chaloupka & Balazs 2005, Foley et al. 2005, Chaloupka et al. 2008, Van Houtan et al. 2010). The absence of FP in small size-classes in the present study (<35cm CCL) may indicate that FP is acquired after turtles recruit to coastal foraging pastures (e.g. Ene et al. 2005), and rarity in large size-classes (>80cm CCL) may suggest either mortality or tumour regression (e.g. Chaloupka et al. 2009). Geographic location is frequently implicated as a risk factor (Van Houtan et al. 2010); FP tends to be more prevalent in near-shore habitats (lagoons, bays) especially those impacted by agricultural industrial urban development, perhaps due to poor water exchange (Herbst 1994, Foley et al. 2005, Santos et al. 2010, Van Houtan et al. 2010). Our in-water survey results further indicate that geographic location and turtle size interact. Fishers exploit turtles from areas where FP occurs and of the size-classes expected to exhibit the disease, yet turtles with FP were not landed as a result of fisher choice. We suggest that fisher choice / harvest may be an important additional risk factor, and one not reported previously in the literature.

Rapid increases in FP have been reported in Hawaii (Chaloupka et al. 2009) and Florida (Foley et al. 2005) since the 1980s and 1930s respectively. Both regions had substantial historical harvests and fisher choice and selective harvest practice may well have played a part in the emergence of disease in these locations.

The long residence times of turtles at sites in TCI (as indicated from recapture histories and satellite tagging data: Stringell et al. in prep., Chapter 2), and the relatively short development times of tumours (three turtles recaptured during the study developed extensive FP in <12 months), could exacerbate the disease. It remains to be seen whether the prevalence of FP in TCI will increase in the coming decade.

Human health

Despite FP being pandemic (Williams et al. 1994) and green turtles being taken for food in artisanal small-scale fisheries (SSF) throughout the world, both historically and at present, comprehensive studies on the human health impact of consuming FP-infected turtles is lacking. While it seems unlikely that there is a human health concern here, it would benefit from further study. SSF and bushmeat hunting have much in common (Milner-Gulland & Mace 1998), and the term “marine bushmeat” has been coined for the artisanal hunting of marine turtles (Alfaro-Shigueto et al. 2011). Most concern with hunting and consumption of bushmeat, however, has been the potential human health issues such as animal-human disease transmission (LeBreton et al. 2006, Harrison et al. 2011) and toxicity (McClenachan et al 2006). McClenachan et al (2006) suggested that hawksbill turtle meat was toxic until the 19th century when it began to be eaten without health consequences as a result of hawksbill turtles consuming more desirable, less toxic sponge species as turtles were overexploited, became less abundant and competition for food was reduced. Few studies consider the effects of hunting on prevalence of disease in wildlife population.

Conclusions and global disease emergence

Disease is a pervasive ecological driver in population dynamics (Plowright et al. 2008) and with suggested global increases (Daszak et al. 2000, Ward & Lafferty 2004, Jones et al. 2008), disease has become pertinent to contemporary wildlife management. As per Plowright et al. (2008), “the goal of this study was not to

conclusively ‘prove’ causation but to amass sufficient evidence to implicate possible ecological and sociological causes of disease emergence”, in order to inform disease prevention and management. Although in many aquatic diseases, causal factors are difficult to isolate due to the complexity of interactions, some anthropogenic impacts have been implicated (Daszak et al. 2001). As such, marine environmental monitoring programmes may utilise diseases as sentinels for ecological status (Stentiford et al. 2009, Lyons et al. 2010, Stentiford et al. 2010). Causal agents of FP in turtles have not been unequivocally isolated. However, incidence of FP in green turtles, that are considered keystone species in tropical seagrass habitats (Bjorndal & Jackson 2003), may prove to be a prime indicator of ecosystem health (Aguirre & Lutz 2004). Our study highlights the possibility that harvesting and fisher choice may increase disease prevalence. Knowledge of such an effect may prove invaluable in informing management decisions for sustainable exploitation and control of epizootics in threatened species.

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Table 1. Results of interviews about fibropapillomatosis (FP) with 28 participants, of which 21 (75%) are currently practicing turtle fishers. See Table S1 for full questionnaire. Questions asked of the future were only to current fishers.

The number of participants who … turtles with FP	% (n)
had seen and captured	61% (17 of 28)
had harvested	18% (3 of 17)
threw back	82% (14 of 17)
(in the future) would not harvest	90% (19 of 21)
(in the future) would harvest	10% (2 of 21)

Figure 1. Green turtle showing externally visible signs of fibropapillomatosis (FP). This image was shown to fishers during interviews.

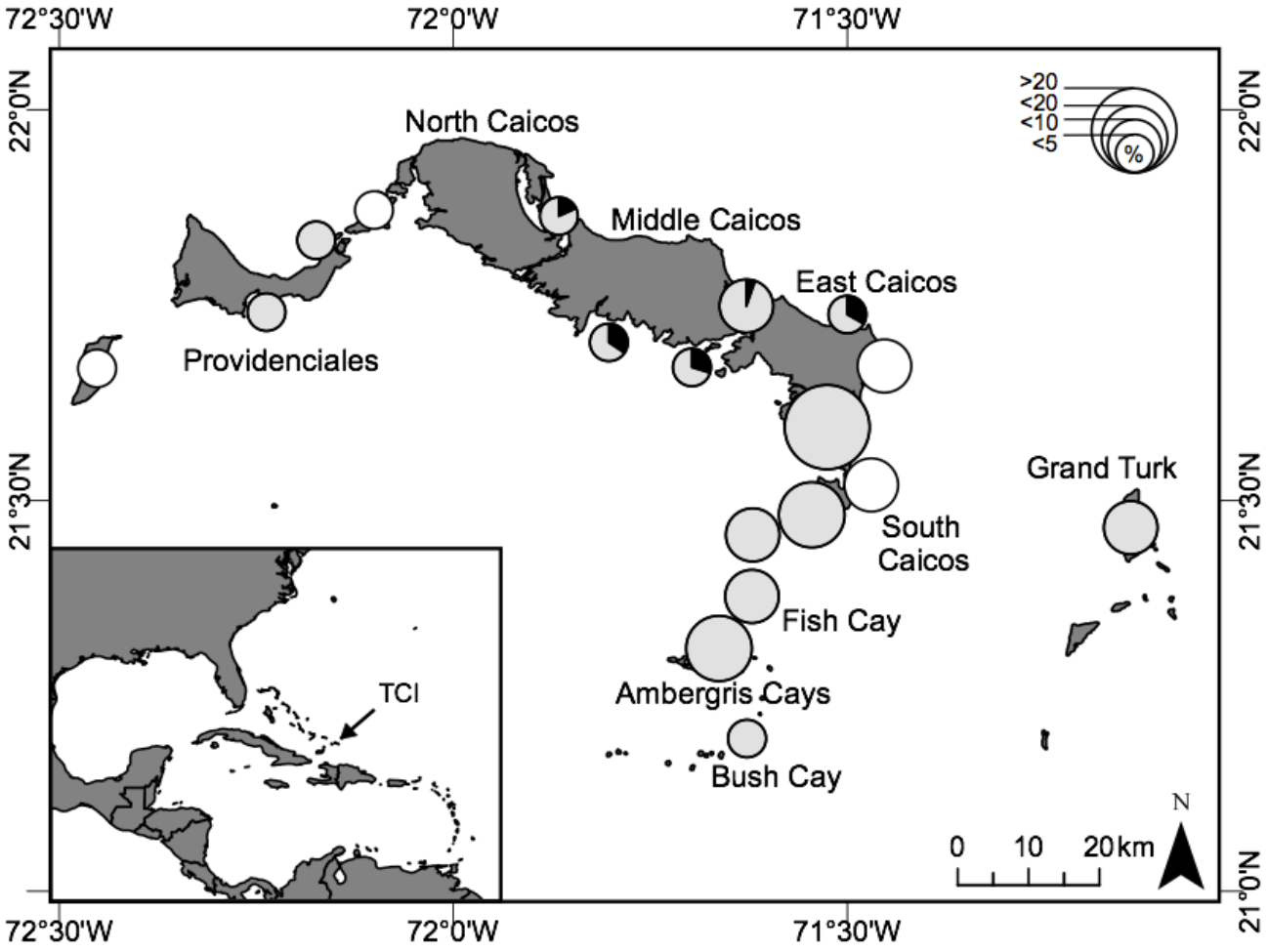

Figure 2. Map of Turks and Caicos Islands (TCI) showing locations (pies) where green turtles were harvested. Size of pies indicates the relative percentage of the total harvest (<5, <10, <20, and >20%; n=233 turtles) during 25 months of survey (Nov 2008 to Dec 2010). Shaded pies indicate areas where we also conducted capture-mark-recapture (CMR) surveys and the prevalence of fibropapillomatosis (black) in turtles caught in these surveys is shown. White circles indicate locations where turtles were harvested but where no CMR surveys were conducted.

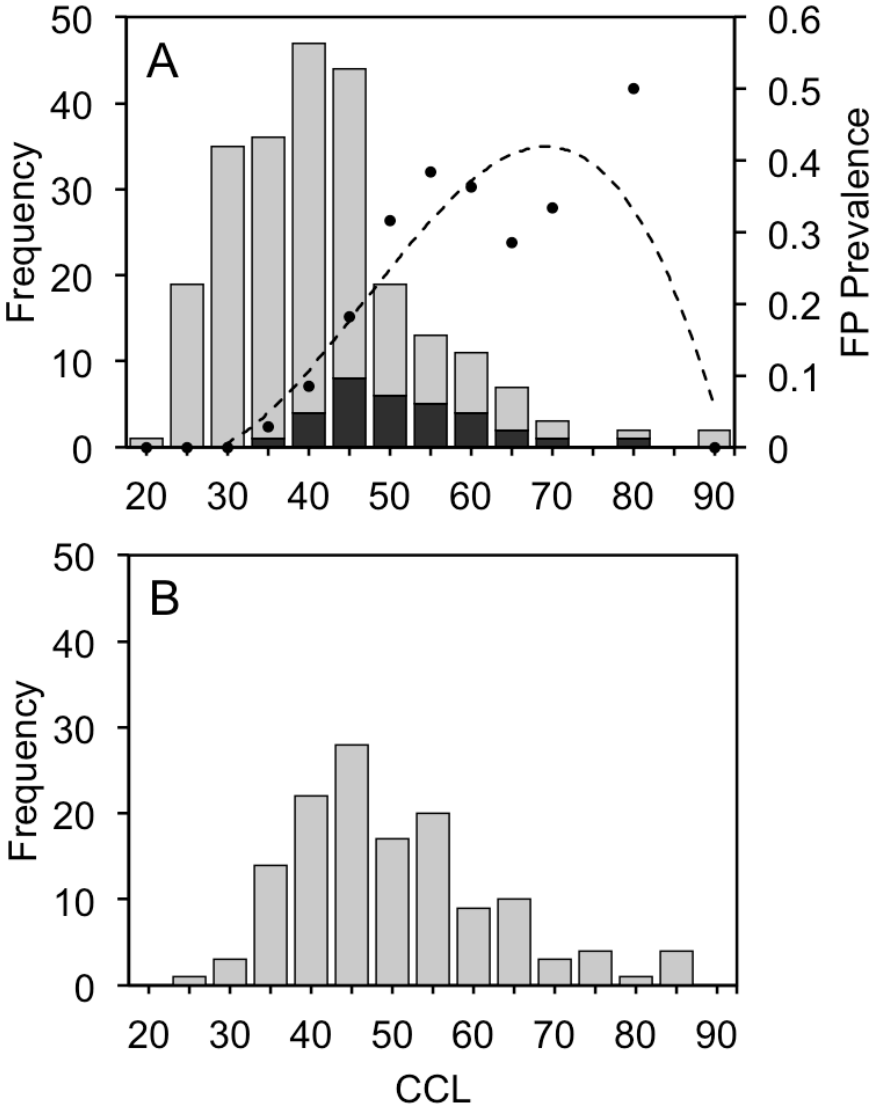

Figure 3. Curved carapace length (CCL, cm) of green turtles captured during capture-mark-recapture surveys (A) and in the fishery (B), showing external signs of fibropapillomatosis (FP) (stacked black bars) or no FP (grey bars). Dots in (A) indicate FP prevalence within each size-class and the dashed line indicates a 3-order polynomial fit (R^2=0.84) of FP prevalence by size.

Chapter 3: Supplementary Information

Table S1. Semi-structured questionnaire used to interview fishers and guide discussions on occurrence of fibropapillomatosis. Figure 1 of main text and a map of TCI was shown to participants during the interview.

Interviewer_____Island/Dock_____
Name of participant_____Date_____Time_____

Fibropapilloma questions

1. Have you seen turtles with diseases/virus infections/growths?_____
   If Yes go to 2. _____
   If No go to 5 _____
2. Can you describe them – what looks unusual about these turtles_____
   _____
3. What species do you see?
   Green_____ Hawksbill_____Loggerhead_____Mullatto_____
4. Where on the turtle do you find this disease/virus infection/growths?
   _____
5. **Show Picture of growths**.
   Have you seen turtles with either large growths or small growths or both?
   If yes go to 6_____
   If no go to 9 _____
6. Where in the TCI do you see turtles with diseases? (point on map & note areas).
   _____
7. Where do you see turtles with diseases most? (map)
   _____
   a. Do you ever go to SouthernBush, Banks, Ocean Hole? (to check if this is hearsay or actual sightings)_____
8. Has the amount of turtles you see with disease changed:
   since you started fishing - Large decrease, small decrease, no change, small increase, large increase
   since 10 years ago- Large decrease, small decrease, no change, small increase, large increase
   since 5 years ago? - Large decrease, small decrease, no change, small increase, large increase
9. Have you ever caught one of these turtles with FP?_____
   a. What did you do with it and why (throw back?)_____
   b. If harvested it, did you chop FP off_____
      i. Did you eat it? Sell it?_____
10. If you were to catch turtles with FP what would you do?_____
    _____
11. Do you remember any turtle being landed with FP?_____
    _____
12. Have you ever eaten any turtle with FP?_____
    a. Have you ever eaten turtle?_____
    b. How would you know if you've eaten a turtle with FP?_____
    _____
13. How do you reckon this affects the turtles that don't have FP?_____
    _____
14. Why do you think turtles have these diseases?_____
    _____

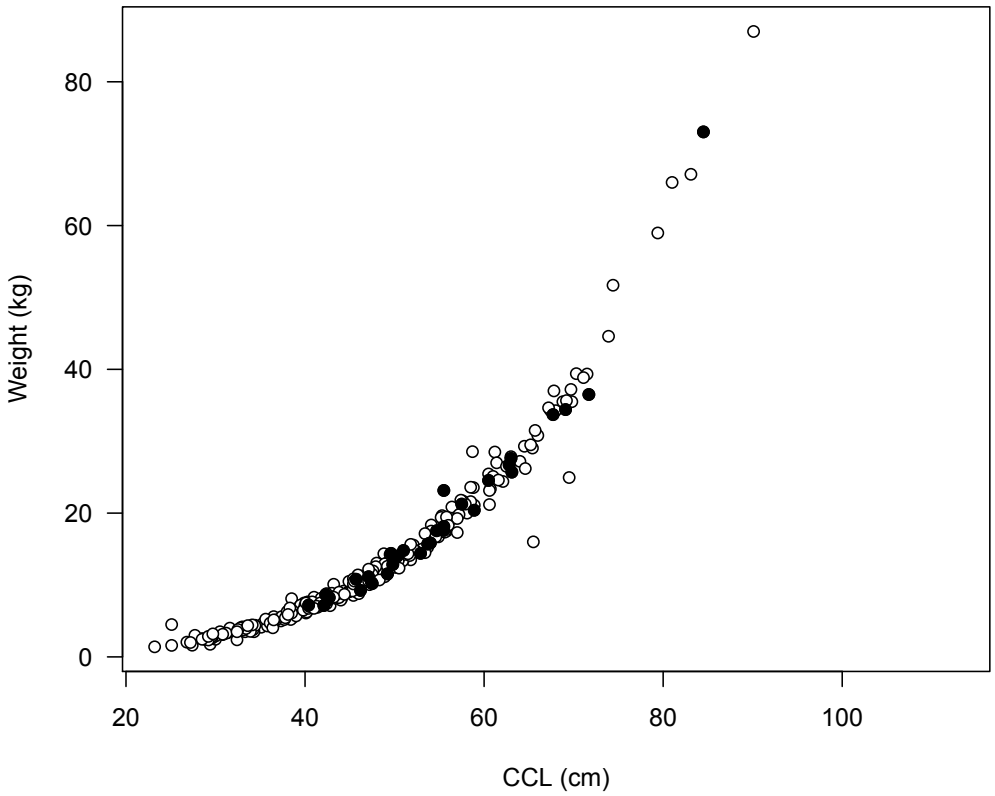

Figure S1. The relationship between curved carapace length (CCL, cm) and weight (kg) of turtles with fibropapillomatosis (FP; black, n=32) and without FP (white, n=207) captured and released during in-water capture-mark-recapture surveys from Nov 2008 to Dec 2010.

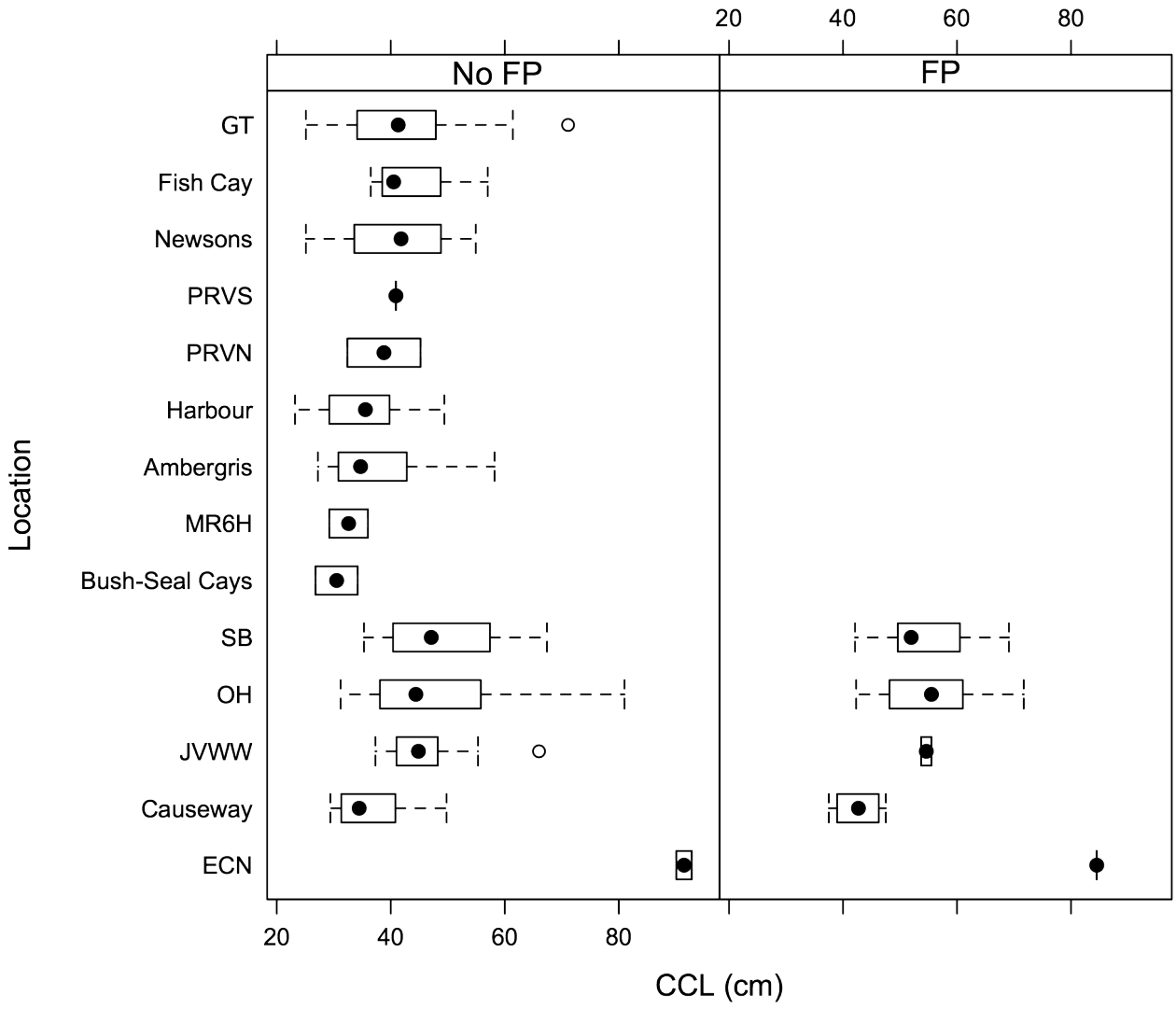

Figure S2. Curved carapace length of turtles captured in capture-mark-recapture surveys without fibropapillomatosis (FP; left panel, n=207) and with FP (right panel, n=32). The top nine locations have no recorded FP prevalence. Box plots indicate median, interquartile ranges and outliers.

Chapter 4

Female biased sex ratios in marine turtles: Insights from life-stages and origin.

Thomas B. Stringell[1], Carlos Carreras[1], F. Alberto Abreu-Grobois[2], Brendan J. Godley[1], Anke Lange[3], Quinton Phillips[4], ALan F Rees[1], Peter B. Richardson[1,5], Amdeep Sanghera[5] , Charles R. Tyler[3] and Annette C. Broderick[1]

[1]Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ. UK
[2]Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Apdo Postal 811, Mazatlán, Sinaloa CP 82000, MX
[3] Biosciences, College of Life and Environmental Sciences, University of Exeter, Stocker Road, Exeter, EX4 4QD UK
[4] Department of Environment and Maritime Affairs, South Caicos, Turks and Caicos Islands.
[5] Marine Conservation Society, Ross on Wye, Herefordshire, HR9 5NB. UK

Manuscript in preparation

Abstract

A better understanding of sex ratios in wild populations and the factors affecting survivorship of the sexes is crucial for supporting effective conservation strategies, in particular for species with temperature-dependent sex determination (TSD) given predicted scenarios of climate warming. In sea turtles, which exhibit TSD, a greater proportion of females are produced at higher temperatures and female biased primary sex ratios are common. Female skewed sex ratios in immature sea turtles captured at sea likely arise from skewed primary sex ratios, sex biased dispersal from rookeries or differential fitness of the two sexes. Immature and adult hawksbill (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) were sampled from the regionally important foraging area and local turtle fishery of the Turks and Caicos Islands (TCI). We identified sex from gonads (via histology and gross morphology) to calibrate sex ratio estimates from blood plasma hormone concentrations for turtles of different size class and haplotype. Sex ratios were highly female biased in recruits in both species, but especially in hawksbills (1M:15F - the highest documented in the Atlantic) but this differed for adult hawksbills where the sex ratio was 1:1. Mixed stock analysis indicated contribution from widespread Atlantic rookeries in both species but a single dominant source for male juvenile green turtles (>80% from Costa Rica). Significant differences in the genetic composition across life-stages were observed with adult hawksbills captured in water most similar to the TCI rookery and differentiated from the immature stock. As no difference in growth rates between the sexes were found, at least in green turtles, the female biases observed in early life-stages (recruits to sub-adults) are unlikely to have resulted from mortality associated with growth rate differences between the sexes. Thus, the extreme female bias in immature life-stages may have resulted from sex biased dispersal and/or skewed primary sex ratios. This work provides insight into the factors that determine in-water sex ratios and origins across life-stages and highlights the need to characterise primary sex ratios at key rookeries in the Atlantic.

Introduction

The genetic theory of natural selection predicts that genotypic sex determination should produce roughly equal numbers of males and females (Fisher 1930). However, sex ratios are often skewed in species with environmental sex determination, of which temperature-dependent sex determination (TSD) is the most common form (Janzen & Paukstis 1991, Janzen & Phillips 2006). Most studies of TSD in reptiles find offspring sex ratios biased towards females (Bull 1980, Janzen & Paukstis 1991), but the evolutionary reasons for this are unknown (Janzen & Phillips 2006). Charnov and Bull (1977) theorised that TSD may have evolved as an adaptive mechanism for maintaining sex ratios to cope with stochastic and unpredictable environmental conditions that differentially influence male versus female fitness (Shine 1999, Warner & Shine 2008). Several differential-fitness models have been proposed to explain the adaptive significance of TSD, some or all of which may apply for any given species where it occurs (Shine 1999). Warner and Shine (2008) provided empirical support for the Charnov-Bull model of differential fitness in a lizard with TSD, and this could apply to sea turtles which exhibit TSD (Yntema & Mrosovsky 1980, Yntema & Mrosovsky 1982, Mrosovsky 1994, Wibbels 2003).

In sea turtles, a greater proportion of females are produced at temperatures above a pivotal value (where a 50:50 sex ratio is produced; typically at temperatures between 28-30°C) with more males produced at temperatures below the pivotal (Yntema & Mrosovsky 1980, Yntema & Mrosovsky 1982). These “primary” sex ratios (pertaining to hatchlings) have been generally found to be female biased (see Figure 1 and Hawkes et al. 2009 for review). Under current climate change scenarios, widespread feminisation of primary sex ratios and a critically reduced proportion of males that could hinder population maintenance are predicted (Hawkes et al. 2007, Hawkes et al. 2009, Poloczanska et al. 2009, Witt et al. 2010, Fuentes et al. 2011). Studies of sex ratios and how they are affected are therefore crucial to determine current sex ratio baselines and for building an understanding of how sea turtles may be impacted by climatic change.

Studies of juveniles sea turtles captured at sea reveal variation in “secondary” sex ratios (pertaining to post hatchling stages) across sites but most are also female biased, likely reflecting the primary sex ratios (e.g. Bolten & Bjorndal 1992, Bolten et

al. 1992, Stabenau et al. 1996, Leon & Diez 1999, Bjorndal et al. 2000, Geis et al. 2003, Blanvillain et al. 2008, Delgado et al. 2010, Hawkes et al. 2013). Some studies, however, show 1:1 or male biased adult sex ratios (Chaloupka & Limpus 2001, Stewart & Dutton 2011, Wright et al. 2012), despite female-biased sex ratios in rookeries or immature life-stages. This shift from highly skewed female biased primary sex ratios to 1:1 or "Fisherian" ratios (Fisher 1930) in adult stages is an elusive question in sea turtle biology. Causes of this marked difference are unknown but may relate to several non-exclusive scenarios: 1) Sex differences in dispersal (e.g. Limpus 1993, FitzSimmons et al. 1997, van Dam et al. 2008, Velez-Zuazo et al. 2008, Hays et al. 2010 in adults; Casale et al. 2002 in juveniles), as a result of differential genetic structuring between the sexes (Casale et al. 2002, but see Maffucci et al. 2013) which may serve to avoid resource competition, intrasexual mate competition and inbreeding (Johnson & Gaines 1990, Perrin & Mazalov 2000, Warner & Shine 2008). 2) Sex biased mortality/fitness, due to differential predation rates between the sexes; hatchlings from cooler nests (i.e. males) have been shown to be larger and have better swimming ability than those from warmer nests (i.e. females) (Gyuris 2000, Booth & Evans 2011) and this may aid more rapid dispersal away from coastal waters where they are vulnerable to predation (Gyuris 1994, Pilcher et al. 2000). Presumably any differences in growth rates between the sexes if present (Chaloupka & Limpus 1997, Limpus & Chaloupka 1997, Bjorndal et al. 2000, Chaloupka et al. 2004) could also have consequences on predation. 3) Sex biased breeding periodicity, for example, male turtles might breed more frequently than female turtles (Limpus 1993, Hays et al. 2010, but see Wright et al. 2012) contributing to a balanced "operational sex ratio" (the ratio of sexually active males to fertilisable females at any given time; Emlen & Oring 1977) even though observed sex ratio might be biased towards females.

Sex determination

Determining sex in marine turtles from external features is thought to be possible only in adults where males have clearly pronounced secondary sexual characteristics including a long prehensile tail, curved claws, and a seasonally softening plastron, when in breeding condition, although complex morphometric methodologies that distinguish sexes in hatchlings have been described (Michel-Morfin et al. 2001, Valenzuela et al. 2004). Sex determination can be achieved by

internal examination of gonads via laparoscopy in juveniles and by dissection and histology in hatchlings (Wood et al. 1983, Wibbels et al. 2000). Laparoscopy, however, is an invasive surgical technique that is logistically demanding and requires trained personnel to perform. An alternative method to sex individuals is to determine concentrations of circulating blood hormones of testosterone (T) and oestradiol-17β (E2) (Owens et al. 1978) and compare these against threshold hormone values for females and males. However, calibration of hormone concentrations with animals of known sex (via observations of gross morphology of gonads by laparoscopy, direct sampling and histology) is desirable, because T values can vary seasonally (in response to temperature), with age and between (and within) individuals, and females produce T as a precursor for E2 (Wibbels et al. 1987, Geis et al. 2003, Braun-Mcneill et al. 2007, Blanvillain et al. 2008, Hawkes et al. 2013). In this study, we accessed a turtle fishery to obtain paired gonad observations/samples and blood samples in order to test and calibrate blood hormone analysis.

Genetics

The generalised life cycle of green and hawksbill turtles suggests that after remaining for a period as pelagic juveniles in oceanic gyres, turtles from different rookeries may converge and recruit to mixed origin feeding grounds in coastal shallow waters (Musick & Limpus 1997). It is thought that turtles may recruit to foraging grounds away from their natal rookery and move between a series of developmental sites as they age (Carr 1968, Meylan et al. 2011), until they return to their natal rookery to reproduce. Maternally inherited mitochondrial DNA (mtDNA) has been widely used to assess sea turtle population structure among rookeries and foraging aggregations (Bowen & Karl 2007 and Lee 2008 for reviews), thus facilitating the conservation and management of genetically distinct units (Moritz 1994, Wallace et al. 2010). With good quality knowledge of mtDNA haplotype frequencies of potential source nesting populations, mixed stock analysis (MSA) methodology enables groups of individuals in mixed feeding aggregations to be linked to their rookeries of origin. MSA can provide powerful insight into the conservation implications of exploiting stocks of mixed origin, e.g. hawksbill harvest and “tortoiseshell” trade in Cuba (Carrillo et al. 1999, Bowen et al. 2007, Godfrey et al. 2007, Mortimer et al. 2007a, Mortimer et al. 2007b, Moncada et al. 2012), and how impacts to nesting populations can affect turtle populations in feeding grounds

(Carreras et al. 2013).

Progressively greater genetic differentiation has been found in later lifestages of turtle populations and results in a spatially “complex population structure” (Bowen et al. 2005, Bowen & Karl 2007). Most research into sea turtle genetic population structure has been based on eggs, hatchlings and nesting females using mtDNA. Recently, interest has turned towards male mediated gene flow in sea turtle populations through nuclear DNA (nDNA) e.g. in microsatellites or SNPs (Roberts et al. 2004, Bowen et al. 2005, Carreras et al. 2011, Wright et al. 2012) and breeding male natal philopatry using nDNA and mtDNA (FitzSimmons et al. 1997, Carreras et al. 2007, Velez-Zuazo et al. 2008, Bagda et al. 2012, Shamblin et al. 2012). Some attention has focussed on haplotype frequencies and genetic differentiation between adult life-stages and sexes (Velez-Zuazo et al. 2008) but few studies (e.g. Casale et al. 2002) have separated sex, different life-stages and residential status when applying mixed stock analyses.

Aims and objectives

Marine turtles are sensitive to exploitation due to their complex life history traits including a broad distribution of life-stages across extensively disbursed habitats, extended period to sexual maturity and multi-decadal generation times. Therefore science-based management of these taxa benefits from knowledge of critical population parameters such as in-water sex ratios, size/life-stage structure and genetic population structure, aspects that aid understanding of their population dynamics and their sustainable management. In this study we present the most thorough and up-to-date sex ratios and genetic profile of the green and hawksbill turtle stocks yet undertaken in the Turks and Caicos Islands to provide a regional perspective for management decisions and to further elucidate relationships with the Wider Caribbean Region. We set out to incorporate life-stage and sex ratio demographics into mtDNA-based MSA to address origin of green and hawksbill turtles and to determine possible genetic origins of any biases in sex ratios of immature life-stages in the Turks and Caicos in-water populations. We explore growth rates between the sexes in the two species, examine known primary sex ratios at rookeries in the Atlantic/Mediterranean, and explore origin of turtles using MSA to describe potential sex biased dispersal.

Methods

Study site

The Turks and Caicos Islands (TCI) is a UK overseas territory in the Caribbean located at the southeastern end of the Bahamas and north of Hispaniola Island (21^{o} 45N, 71^{o} 35W) (Figure 1). The low-lying archipelago consists of several islands and numerous cays that surround a shallow, sandy and productive habitat called the "bank" which is generally fringed by mangroves and creeks. Most outlying cays and ocean facing beaches are surrounded by coral reefs. The hawksbill and green turtle foraging aggregations of TCI, are thought to be regionally significant in abundance (Richardson et al. 2009). However, in the TCI they are subject to a legal harvest. TCI's hawksbill fishery is thought to be one of the largest in the western Atlantic and the take of its breeding adults is a key conservation concern (Stringell et al. 2013, Chapter 1).

Sampling

Over a period of approximately two years (from November 2008 to December 2010), we carried out extensive countrywide in-water capture-mark-recapture (CMR) surveys (via a combination of free-diving and "rodeo-style" methods: Ehrhart & Ogren 1999), nesting surveys (see Stringell et al. in prep., Chapter 2) and directly observed turtles landed in the legal turtle fishery in TCI (see Stringell et al. 2013, Chapter 1). Turtle capture location was recorded using a hand-held GPS or estimated following fisher interviews. Turtles were measured along the midpoint of the carapace (Curved Carapace Length, CCL: Bolten 1999), assessed for secondary sexual characteristics (e.g. males have a long prehensile tail, curved claws, soft plastron), and checked for metal flipper tags and Passive Integrated Transponders (PIT tags) and tagged (if absent) for those released (Balazs 1999). Some individuals had been tagged prior to the present study by authors and collaborators (Richardson et al. 2009).

Tissue samples (skin, muscle and blood) were collected from each turtle for use in genetic and blood hormone analyses. The sampling strategy avoided pseudo-replicates from individuals because harvested turtles were killed and living animals were tagged. Small sections of skin from the trailing edge of the rear flippers of live turtles and/or muscle tissue from butchered animals were sampled with sterile

scalpel blades and transferred immediately to vials of Queens Lysis Buffer (Seutin et al. 1991) and refrigerated until DNA extraction at the University of Exeter laboratory, UK. Blood samples were extracted from the dorsocervical sinus (Owens & Ruiz 1980) of each turtle using sterile 0.8x25mm or 0.8x38mm needles (depending on animal size) and stored in 6ml BD Vacutainer sampling tubes internally coated with Lithium Heparin anticoagulant. Blood samples were stored on ice until they could be transferred to a refrigerator when approximately four drops of whole blood were then transferred to 1.5ml vials of lysis buffer. The vials were then centrifuged for 10mins at 10000 RPM. Blood plasma was pipetted into cryovials and stored at -20 C until transferred to the UK for long-term storage at -80 C and used in blood hormone analyses. Only one sampling occasion per individual was included in the analyses.

Gonad samples were taken from butchered animals, fixed in 5% formaldehyde solution, stored in 90% ethanol and later used in histological examination to confirm sex identified from in-situ gross morphology of the gonads (Wyneken 2001). Gonad tissue was embedded in paraffin, sectioned and stained with hematoxylin and eosin and examined as part of a wider study into gonad ontogeny (e.g. Miller & Limpus 2003).

Laboratory analysis

Blood hormones

Enzyme-immunoassays (EIA) were used to analyse testosterone (T) and oestradiol-17β (E2) concentrations in turtle blood plasma (Owens et al. 1978) following the manufacturer protocols (Cayman Chemical Co. USA: Item 582701 Testosterone EIA Kit; 582251 Estradiol EIA Kit). Steroids from 500µl of blood plasma were extracted with diethyl ether in glass test tubes. Ether was collected in a fresh test tube and evaporated under a gentle stream of nitrogen. Dried samples were resuspended in 0.5ml of EIA buffer (or more if dilution was needed). On 96 well plates, samples were assayed in duplicate along with T and E2 standards (in duplicate) to create standard curves of between 3.9 – 500 pg T/ml and 6.6 – 4,000 pg E2/ml. Plasma hormone concentrations were calculated based on 4-parametric logistic standard curve fits and taking sample dilution factors into account. Each plate was read at 412nm wavelength on a Tecan Infinite m200Pro nanoquant plate reader with multiple reads (2x2) per well and five flashes per read.

To assign sex we used an approach similar to Hawkes et al. (2013) where

threshold values of sex hormones (T and E2) were used to discriminate likely sex, but with our data we also had the known sex from gonad examination. Where gonad sex was known, this took precedence over hormone determination of sex. Blood hormone levels were compared between sexes using t-tests or Mann-Whitney U tests when parametric assumptions were not met. The testosterone: estradiol-17β ratio (T:E2) was used to aid sex-allocation. For each sex, blood hormone concentrations were tested between years using t-tests or Mann-Whitney U tests and against day of year as a continuous variable using general additive models (GAMs) with the mgcv package in R (Wood 2011).

Molecular analyses

For most samples, we extracted and amplified DNA using Phire Animal Tissue and Phusion Blood Direct PCR kits (Finnzymes ThermoFisher), where 0.25mg of tissue or 1ul of 10x diluted blood-buffer solution were added directly to 96-well plates along with kit reagents and primers, and thermally cycled following manufacturer instructions. Forward (H950g: 5'-GTCTCGGATTTAGGGGTTTG-3') and reverse (LCM15382: 5'-GCTTAACCCTAAAGCATTGG-3') primers were used to isolate approximately an 830bp fragment of the D-loop control region of mitochondrial DNA (mtDNA) (Abreu-Grobois et al. 2006). For some samples, DNA was extracted from blood or tissue using QIAamp DNA mini kits (QIAGEN) and amplified by PCR using the same primer pairs and the thermal profile described in Carreras et al. (2006). All PCR products were purified using Exo1 and FastAP enzymes (Fermentas), and sent to Macrogen Europe (Netherlands) where products were cycle sequenced in both directions using ABI BigDye protocols (Applied Biosystems), and analysed on an ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences were aligned, edited and analysed using Geneious Pro version 5.1 and haplotypes assigned manually based on reference sequences from Genbank databases (http://www.ncbi.nlm.nih.gov/), author's database (Abreu-Grobois, see also Leroux et al. 2012) for hawksbill turtles, and the Archie Carr Center for Sea Turtle Research website for green turtles (http://accstr.ufl.edu/cmmtdna.html). Unknown haplotypes were re-extracted, re-sequenced and checked thoroughly against all possible sources. New haplotypes were submitted to the repositories listed above. A multiple alignment of sequences of haplotypes found in TCI were trimmed to 740bp and 481bp in hawksbill and green turtle haplotypes respectively for use in subsequent analyses and regional

comparisons.

Data analyses

Grouping variables

mtDNA sequence data were grouped by various factors to explore the potential influence of temporal sampling, capture location, capture habitat, capture method (harvested or released), life-stage (size) and sex. We first explored temporal structure in terms of survey year, quarterly period, and month. Then, capture location was grouped by distinct sites and sequentially pooled into larger spatial units. Capture habitat was grouped into two categories: reef and seagrass habitats. Capture method of the animal was used as a factor to examine whether there were genetic differences between turtles harvested in the fishery and those captured from CMR surveys. Turtle size (CCL) was used to group turtles into four discrete size classes to represent the following life history stages: new recruits <35cm, juveniles 35-65cm, large juvenile (hereafter sub-adults) green turtles 65-97cm, sub-adult hawksbill turtles 65-78cm, adult green turtles >97cm and adult hawksbills >78cm. Recruit sizes were defined following Velez-Zuazo et al. (2008) for hawksbills and Reich et al. (2007) for green turtles. Sub-adult size was broadly defined following Goshe et al. (2010) and Krueger et al. (2011). Adult turtles were defined by size based on regional average minimum sizes of nesting females (Witzell 1983, Hirth 1997 see Stringell et al. in prep., Chapter 2 for details) and were further split into 'breeders', based on confirmed observations of breeding condition (and assumed resident in TCI, for example, from nesting and satellite tracking; see Stringell et al. in prep., Chapter 2), and 'foragers' where breeding condition was not confirmed and assumed as visitors to TCI. Haplotype frequencies of TCI nests were also used to elucidate patterns in genetic differentiation (see Stringell et al. in prep., Chapter 2).

mtDNA haplotype characterisation

Haplotype diversity (h) and nucleotide diversity (π) for each group (see grouping variables) were calculated in Arlequin v. 3.5 (Excoffier & Lischer 2010). To explore structure in the sequence data, with null hypotheses of no structure, we conducted haplotype frequency based pairwise F_{ST} and genetic distance based Φ_{ST} comparisons, analyses of molecular variance (AMOVAs), and Exact tests of population differentiation (with 100000 permutations and 10000 dememorisation

steps) using Arlequin. P-values for pairwise F_{ST} and Φ_{ST} were calculated from 10000 permutations. The best nucleotide substitution model (Tamura 3-parameter model (Tamura 1992) with gamma correction=0.05) was determined by maximum likelihood in MEGA5 software (Tamura et al. 2011). Principal Coordinate Analysis (PCoA) using GenAlEx v6.5 (Peakall & Smouse 2012) was used to analyse pairwise F_{ST} distances and haplotypes most contributing to the PCoA pattern (Spearman correlation >0.45 for green turtles an >0.7 for hawksbills on the first two PCoA axes) were constructed using PRIMER v6 software (Clarke & Gorley 2006). For multiple comparisons, we employed a modified False Discovery Rate (Narum 2006) instead of Bonferroni corrections.

We incorporated life-stage and sex into the testing procedure to account for possible genetic differentiation between life-stages and sexes and to reflect the genetic makeup of these groups as a result of possible differing sex ratios by life-stage. We sequentially pooled non-significant group pairs and examined each structure in turn from a maximal model consisting of each life-stage-sex combination - due in part to very small sample sizes (<6) associated with some of these groups, particularly the males (see supplementary Tables S1 and S2).

Mixed stock analyses

A Bayesian many-to-many mixed stock analysis (MSA) was carried out with the R "mixstock" package (Bolker et al. 2007) to elucidate potential origins of turtles in TCI. Six chains of 20,000 Markov Chain Monte Carlo (MCMC) iterations were run for each MSA, with a burn-in of 10,000 and thinning by 60. The Gelman-Rubin diagnostic criterion of <1.2 for all variables indicated convergence of MCMC. We used the *foraging ground centric* approach to test the importance of Atlantic and Mediterranean rookeries to the TCI mixed foraging stocks. Due to significant genetic differentiation (see later), we analysed juvenile male green turtles separately from the rest of the immature green turtles (recruit, juvenile and sub-adult females, and sub-adult males) but we combined all immature hawksbills. We tested these groups against potential source rookeries (Figure 1; Table 1; supplementary Tables S1 and S2). We did not test adult hawksbills in an MSA because, due to likely natal philopatry (Carr 1968), we assumed the majority of adults that appeared to be in breeding condition originated in TCI. Furthermore we found no genetic differentiation among the adult groups (of non-nesting adults) and nests. The few that were not in

breeding condition (adult foragers) could not be run in an MSA due to low sample size. Hawksbill sequence data were truncated to 740bp for comparison with other published haplotype frequencies of 16 source rookeries (Table 1a; supplementary Table S1). For compatibility with other published green turtle haplotype frequencies of 16 source rookeries (Table1b; supplementary Table S2), sequences were truncated to 481bp. We incorporated source rookery size (average number of nesting females per year) from published literature (see supplementary Tables S1 and S2) as priors in the MSAs to improve the reliability of the results (Lahanas et al. 1998, Bass et al. 2004, Blumenthal et al. 2009). Where rookery size data were number of nests, we divided by 3 nests/female/year to convert to females/year (Seminoff 2004, Mortimer & Donnelly 2008). Rookery size was tested for Spearman's correlation with arcsine MSA contribution estimates, as were straight-line distances between all source rookeries and TCI measured using the Mapinfo GIS DistanceCalc Tool.

Sex ratios

Sex ratios of the in-water samples were calculated for each life-stage grouping for each species and compared statistically using Chi-squared tests with P-values derived from 10,000 MCMC randomisations. Data from published studies that have estimated primary sex ratios at potential Atlantic/Mediterranean source rookeries were used to inform patterns from the results of in-water sex ratios and Mixed Stock Analyses; Hawksbill turtles: US Virgin Isles (USV, Wibbels et al. 1999), Antigua (ANT, Mrosovsky et al. 1992, Glen & Mrosovsky 2004), Guadeloupe (GU, Kamel & Mrosovsky 2006), Bahia, Brazil (BRZ, Godfrey et al. 1999) (Figure 1a); Green turtles: Cyprus (CYP, Kaska et al. 1998, Broderick et al. 2000), Turkey (TKY, Kaska et al. 1998, Casale et al. 2000), Costa Rica (CR, Standora & Spotila 1985, Spotila et al. 1987, Horikoshi 1992), Suriname (SUR, Mrosovsky et al. 1984, Godfrey et al. 1996), Poilao, Guinea Bissau (GBP, Rebelo et al. 2011), Ascension Island (ASCI, Broderick et al. 2001, Godley et al. 2002, Pintus et al. 2009) (Figure 1b). Where more than one sex ratio reference was available for a rookery, or several locations measured within a study, the percentage female values were averaged.

Growth rates

Flipper tagged turtles recaptured after at least one month were used to determine

growth rates per year. If a turtle was recaptured more than once we took the CCL of the first and last captures. Where sample size allowed, we compared growth rates between sexes using Mann-Whitney U or t-tests and against size (measured from last capture) as a continuous variable using GLMs following initial examination for linearity.

Results

Sex determination

We determined sex of 112 immature hawksbill turtles using histology and morphology of gonads (n=40, 26 of which had paired hormone concentrations) or only using testosterone (T) concentrations (n=72). Sex was determined in 177 immature green turtles from gonads (n=101, of which 55 had paired hormone concentrations) or only using T concentrations (n=76). Twenty-two adult hawksbills (>78cm CCL) were sexed from external or gonad morphology and blood samples were obtained from 14 of these (seven of each sex). No blood samples were collected from the two adult green turtles captured in this study. Testosterone concentrations varied widely in both species (Table 2). For animals whose sex had been confirmed using gonad morphology, concentrations of T differed significantly between sexes in adult hawksbills ($W = 2$, $P = 0.002$, n=14), immature hawksbills ($W = 1$, $P = 0.002$, n=26, although sample size for males was very low, n=3), and immature green turtles ($W=17$, $P<0.001$, n=56). Testosterone was therefore used to develop threshold values for classifying sex in turtles of unknown sex (mostly from CMR surveys) that had concentrations above and below these thresholds.

Our data indicated that hawksbill turtles were likely to be male if they had T concentrations above 518 pg/ml (maximum T value in known females, except in one adult female of breeding size), and immature hawksbills - turtles smaller than 78cm - could be deemed female below 444pg/ml (minimum T value in known males) (Figure 2a). Adult female hawksbills were difficult to sex using blood hormones because of high T concentration (e.g. the single known female at 83cm sampled after egg-laying, Figure 2a). All hawksbills larger than 78cm CCL, however, were sexed through gonads or external morphology (and/or satellite tagged). Sex determination was not considered possible if T concentrations fell between the two threshold values, but no hawksbill samples fell within this range. No hawksbill smaller than 40cm was sexed by both gonad morphology and blood hormones. Only seven turtles this size were landed in the fishery and only one juvenile was sampled for gonads but not for blood and only one recruit (31cm CCL) was captured and killed for consumption in the fishery. Hawksbills recruits (<35cm CCL) were sexed by T concentrations in blood (n=16).

Green turtles with T concentrations above 216 pg/ml were considered male

(maximum T value in females) and below 108 pg/ml were considered female (minimum T value in known males) (Figure 2b). However, between these two threshold values, determining sex was not possible: 15 turtles of unknown sex fell within this range, and were excluded from further sex ratio analysis (Figure 2b). Testosterone did not vary significantly with size between species, sex, life-stage (GLM: P>0.05), or between years (2009 vs. 2010: P>0.05). When tested for temporal effects, female green turtles had higher T levels in the summer (GAM: $F_{1.97}$=3.624, P=0.037) and E2 levels were higher in the summer in samples from unknown sex green turtles (that is, turtles that were not confirmed by histology/gross examination of gonads: GAM, F_1=5.599, P=0.022), although inference from this result is confounded by the effects of both sexes. No significant temporal effect was found with day of year and T for male, female or combined immature or adult hawksbills (GAM: P>0.05). Testosterone concentrations were significantly correlated with increasing E2 concentrations in green turtles (Pearson's R=0.412, t_{101}=4.54, P<0.001), but not in hawksbills (supplementary Figure S1). We found no evidence to suggest that any of our sampled turtles were intersex (that is, both high concentrations of E2 and T: cf Hawkes et al. (2013)) when compared with gonadal histology.

There were no significant differences in E2 concentrations between the sexes (immature hawksbills: W=22.5, P=0.283, n=14; adult hawksbills: W=18, P=0.117, n=10; immature green turtles: W=255.5, P=0.363, n=54) and E2 was therefore not used on its own to sex turtles using threshold values (Table 2, supplementary Figure S2). However, the testosterone: estradiol-17β ratio (T:E2) appeared to discriminate sex in both species, with distinguishable profiles seen in the males (hawksbills: median=788.1, n=9; green turtles: median=80.2, n=15) and females (hawksbills: median=5.2, n=14; green turtles: median=3.9, n=38) (hawksbills: W=122, P<0.001; green turtles: W=548, P<0.001), although some overlap between the sexes was evident and this distinction was more robust in individuals of a curved carapace length (CCL) >50cm (supplementary Figure S3).

Sex ratios

In immature hawksbills there were just 10 males (M) in our sample of 112 turtles (92% female, F) and a shift from a strong female bias in recruits (1M:15F, 94% F) to male bias in breeding condition adults (operational sex ratio, 3:1, 25% F). Juvenile

and sub-adult sizes were also female biased (1:9.6 and 1:10 respectively, 91% F). In non-breeding adults, a 1:1.8 (64% F) ratio was evident (Figure 3). If all hawksbill adults were combined there was an equal (Fisherian) sex ratio of 1:1 (50% F, n=22). There were no significant differences in sex ratios between recruit to sub-adult life-stage pairs, or between adults and earlier stages, but significant differences between breeder/operational sex ratios and all recruit to sub-adult stages (P_{FDR}<0.016). The sex ratio of all adults combined differed significantly from juvenile and recruit stages (P_{FDR}<0.016), but not sub-adults (P=0.051).

In green turtles, there was a female biased sex ratio in all immature stages combined (70% F) and no significant decline in female bias from recruits (1:4.25, 81% F) to later-stage groups (juveniles 1:2.2, 68% F; sub-adults 1:2.25, 69% F) (Figure 3). Estimating the non-breeding adult sex ratio and breeding adult (operational) sex ratio was not possible in green turtles with only a single female sample for each case.

Growth rates

We re-captured 22 hawksbills (mean captures per individual=3 ±1.6 (SD), range 2-7 captures per individual) and 37 green turtles (2.3 ±0.7, range 2-5 captures) over the two-year period, some initially tagged several years prior to our study (Richardson et al. 2009). The time between first capture and the last recapture ranged between 90-1799 days (607.1 ±540.4) for hawksbills and between 28-1374 days (250.2 ±279.9) for green turtles. Other studies used turtles with a time interval of longer than one year between release and recapture in order to reduce biases due to possible differences in growth rates among seasons of the year (Chaloupka & Musick, 1997, Bjorndal et al. 2000). However, a comparison of growth rates between turtles with a recapture time interval of longer than one year (11 hawksbill turtles, 7 green turtles) to those with less than one year (11 hawksbill turtles, 30 green turtles) revealed no significant difference in mean growth rates (hawksbill turtles: $t_{15.6}$=0.736, P=0.473; green turtles: $t_{7.626}$=-0.02, P=0.983). We therefore used growth rate data from turtles recaptured over an interval of at least one month. Only a single male hawksbill (CCL=28.5cm on first capture) was recaptured in this highly female biased population and so a comparison between sexes was not possible in this species. Growth rates for hawksbill females revealed no significant difference between life-stages (ANOVA: F_2=0.43, P=0.657: Figure 4a). In green turtles, growth rates were

not significantly different between sex ($t_{21.9}$=-0.44, P=0.667) or size (CCL) either tested on its own (GLM: $F_{1,35}$=0.87, P=0.357) or in a two-factor model with sex and size (GLM: $F_{2,34}$=0.66, P=0.522: Figure 4b) or sex and life-stage (ANOVA: $F_{3,33}$=0.466, P=0.708). Data were therefore pooled to provide overall average growth rates of 4.2 cm.yr^{-1} (range: 1.05 − 7.44, SD=1.7, n=22) in hawksbills turtles and 6.9 cm.yr^{-1} (range: 1.95 − 11.48, SD=2.3, n=37) in green turtles.

Genetic stocks

For all 118 in-water foraging immature hawksbills there were 20 different haplotypes recorded with 41 polymorphic loci in nucleotide lengths truncated to 740bp (haplotype diversity *h*=0.832 ±0.023; nucleotide diversity π= 0.0087 ±0.0046). When truncated to 384bp for comparison with other foraging ground literature, the TCI Hawksbill haplotype diversity was similar to other regional foraging grounds (Formia et al. unpublished). We discovered one new haplotype in the foraging stock (EiA93: submitted to Genbank). For 16 adult hawksbills (both 'breeders' and 'foragers') there were six haplotypes with 12 polymorphic sites (*h*=0.617±0.135; π=0.0049±0.0029). The TCI hawksbill nesting rookery (n=22 nests) consisted of five haplotypes (*h*=0.407 ±0.128, π=0.0032 ±0.0021; Stringell et al. in prep., Chapter 2), including the unique haplotype EiA81 (submitted to Genbank) and the haplotype EiA13 which was not found among the immature or adult stocks; EiA13 has only previously been found in Cuban nesting rookeries (Leroux et al. 2012).

The foraging green turtles analysed (all non-adult sizes regardless of whether sex was determined) consisted of 134 individuals comprising 15 haplotypes with 19 polymorphic loci in the 481bp nucleotide length sequences. Haplotype and nucleotide diversity of the TCI green turtle foraging population (*h*=0.731 ±0.029; π=0.0071 ±0.0041) is high compared to most other regional foraging stocks (Bass et al. 1998, Lahanas et al. 1998, Bass & Witzell 2000, Bjorndal et al. 2006, Naro-Maciel et al. 2006, Proietti et al. 2009, Proietti et al. 2012, Prosdocimi et al. 2012) and similar to that found in foraging grounds of Almofala, Brazil (Naro-Maciel et al. 2006), Anguilla (Formia et al. unpublished), Barbados (Luke et al. 2004), British Virgin Isles (Formia et al. unpublished) and North Carolina, USA (Bass et al. 2006). A single adult female captured foraging in TCI, and later nesting in Barbuda (see Richardson et al. 2010) had the CmA5 haplotype. The TCI green turtle rookery (n=4 nests) had two haplotypes, CmA3 and CmA64 (*h*=0.500±0.265 and π=0.0010±0.0010), the

latter from a nest on Providenciales and the only source rookery recorded for this haplotype to date (Stringell et al. in prep., Chapter 2). The single green turtle breeder sampled here also had the CmA3 haplotype.

Except for the Exact test between years in hawksbills (P=0.035), there was no significant temporal (year, month) or spatial genetic structure (capture location, habitat, turtle fate - harvested or released) in either species of immature size classes for both distance (Φ_{ST}) and haplotype frequency (F_{ST}) based pairwise comparisons. Since these results are not significant across all testing methods, annual or seasonal genetic differences are thought to be absent or weak at best in both species.

Genetic differentiation: Life-stages and sex

When considering sex or life-stage (excluding adults) as sole grouping factors, there were no significant genetic differences across all testing procedures in either species. The combination of these two factors, however, revealed some differentiation. From a maximal model consisting of each life-stage and sex combination, all non-significant group pairs of recruit, juvenile and sub-adult hawksbills were sequentially pooled into two groups by sex (Male, n=6 vs. Female, n=79: Φ_{ST}=-0.038, P=0.4821, F_{ST}=0.004, P=0.3559, Exact P= 0.2489; see supplementary Table S1 for groups). Due to their similar genetic structure, the sexes were combined into a single 'immature' group (Imm) and the remaining non-sexed but sequenced and sized immature turtle samples were added (n=33) making the global 'immature' group's sample size n=118. Adults (breeding and foraging) of both sexes were genetically similar and were combined (n=16) but were different from Imm (Φ_{ST}=0.199, P=0.0036, F_{ST}=0.125, P=0.0004, Exact P=<0.0001): this differentiation was largely driven by adult breeding males (ABM vs. Imm: Φ_{ST}=0.149, P=0.0270; F_{ST}=0.125, P=0.0025; Exact P=0.0660). Nests (n=22) were genetically similar to adults (Φ_{ST}=-0.002, P=0.3743, F_{ST}=0.009, P=0.3112, Exact P=0.1499) and distinct from Imm (Φ_{ST}=0.305, P<0.0001, F_{ST}=0.209, P<0.0001, Exact P=0.0009). A PCoA indicated that the haplotypes most correlated (Spearman's correlation >0.7 on the first two PCoA axes) to the genetic structure in TCI hawksbill were the rarer haplotypes of EiA66 and 83 and the common EiA01 of immature size classes (see supplementary Table S1 for frequencies).

In green turtles, we compared sub-adults, juveniles and recruits of both sexes (n=85). We had no samples of male recruits and the single adult forager (CmA5) and

breeder (CmA3) were excluded from analyses. The remaining 49 green turtle samples were not sexed and therefore were not used in further analyses. Juvenile males (JM, n=17) were significantly different to sub-adult males (SAM, n=7) and sub-adult females (SAF, n=18) across all testing procedures due to the low frequency of CmA1 haplotype in the JM group (JM vs. SAF: Φ_{ST}=0.484, P=0.0011; F_{ST}=0.194, P=0.0029; Exact P=0.0045. JM vs. SAM: Φ_{ST}=0.568, P=0.0068; F_{ST}=0.301, P=0.0127; Exact P=0.0114. FDR_{10} threshold P=0.0171). Haplotype frequencies were significantly different between JM and juvenile female (JF, n=36) (F_{ST}=0.105, P=0.0166), but not for genetic distance based or exact tests. Female recruits (RF, n=7) were not significantly different to later stage groups of either sex. Incorporating TCI green turtle nest haplotypes into the testing procedure did not reveal any further significant differentiation from other groups, probably due to the small sample size (n=4). As a result of this genetic structure, we kept JM as a distinct grouping and combined all other life-stage and sex groups (except adults) into an 'immature' grouping (Imm, n=68): A PCoA indicated that this genetic structure was largely correlated (Spearman's correlation >0.45 on the first two PCoA axes) with the low frequencies of CmA01 and CmA05 in JM compared to JF and by the single (rare) haplotype (CmA22) only found in JM (see supplementary Table S2 for frequencies).

Mixed stock analysis (MSA)

Only 7% of Imm hawksbill turtles were of TCI origin. The rest were from Barbados Leeward (24%), Cuba and Nicaragua (11% each), Mexico (9%), Guadeloupe (8%), Puerto Rico (7%), USVI (6%), and the remaining rookeries each contributed <5% (Figure 5, supplementary Table S3). Overall, the pattern of contribution is one of mixed origins that is correlated with rookery size (Spearman's R=0.789, P<0.001), but differs from what might be expected if only rookery size was guiding the contribution. For example, the largest hawksbill rookery in the Caribbean is in Mexico, but ranks fourth in terms of contribution to TCI stocks, Nicaragua - the eighth largest rookery - ranks second in contribution and Antigua, the sixth largest rookery, ranks twelfth for contribution (supplementary Table S3; see supplementary Table S1 for rookery sizes).

The MSAs of green turtle haplotypes indicated clear differences in source contributions between JM and Imm stocks: 84% of JM were estimated to have originated from Costa Rica while the majority of Imm came from Florida (>30%),

Costa Rica (>20%) and Mexico (>10%) (Figure 5, supplementary Table S3). Contribution was again correlated to rookery size (Imm: Spearman's R=0.958, P<0.001; JM: Spearman's R=0.958, P<0.001) even when the dominating effect of Costa Rica was removed (Imm: Spearman's R=0.956, P<0.001; JM: Spearman's R=0.949, P<0.001) (supplementary Table S4, see Table S2 for rookery sizes).

There was no correlation between foraging ground-centric contribution estimates and geographic distance of source rookeries for either JM green turtles or Imm of both species (Green turtle: JM: R=0.079, P=0.771; Imm: R=-0.026, P=0.925; Hawksbill: Imm: R=0.181, P=0.502) (see supplementary Tables S1 and S2 for distances). Removal of Costa Rica from the green turtle data, which dominates the JM contribution, gave similar non-significant correlations with distance.

Pairwise haplotype frequency comparisons between Atlantic hawksbill rookeries (Table 2) indicates that the TCI rookery genetic composition is significantly different (high F_{ST} value) to 60% of rookeries and confirmed in most cases by significant Exact tests. There were no significant differences, with low F_{ST} values and therefore genetic similarity, between the TCI rookery and Barbados Windward, Costa Rica, Dominican Republic (Saona Island), Nicaragua, US Virgin Isles and Montserrat, although the latter had few samples and thus comparisons are tentative. The small sample size (n=4) of the TCI green turtle rookery precludes reliable pairwise comparisons of haplotype frequencies with other Atlantic/Mediterranean rookeries, but tentatively suggests similarity with Costa Rica, Mexico, Cuba and Florida.

Discussion

Sex determination

While some studies have use blood hormones solely to sex immature turtles (e.g. Blanvillain et al. 2008, Hawkes et al. 2013), and several have utilised laparoscopy for verification of sex (see Wibbels et al. 2000 for review), our study incorporated paired blood and gonad samples from harvested turtles, providing a definitive sex verification method. With this, we were able to validate our T methodology for sex differentiation across all size-ranges, although it is clear from our results that T concentrations vary greatly between individuals. We found no discernible evidence of seasonal differences, unlike several other studies (Wibbels et al. 1987, Braun-Mcneill et al. 2007, Hawkes et al. 2013). Testosterone concentrations were not related to size within immature life stages/sizes, but in adult hawksbill males, T concentrations were orders of magnitude greater than those of immature turtles. Oestradiol-17β was uninformative on its own in this study, but proved useful when considered in combination with T, and as expected (E2 is an active metabolic product of T), E2 was correlated with increasing levels of T. Despite some turtles having both high T and E2 concentrations, contrary to the suggestion of Hawkes et al. (2013), we found no evidence of intersex in hawksbill turtles from verification of gonads and histology. Rather, it is likely that concentrations of both circulating hormones vary among individuals, with some having high levels, especially in adults (supplementary Figure S1).

Published thresholds of testosterone concentrations in green turtles from the neighbouring Bahamas (Bolten et al. 1992, corrected by an order of magnitude following Braun-McNeill et al. 2007) were remarkably similar to those of our study and provided a similar sex ratio of 68% female (supplementary Table S4). In contrast, sex specific threshold limits in hawksbill turtles from various Caribbean states (Dominican Republic: Leon & Diez 1999, corrected following Braun-McNeill et al. 2007; Puerto Rico: Diez & van Dam 2003, also corrected following Braun-McNeill et al. 2007; US Virgin Islands: Geis et al. 2003; South Florida: Blanvillain et al. 2008) varied more widely (<162 to <261 in females, >182 to >721 in males); yet similar sex ratios were obtained to our results (83% to 93% female), each with low misclassification errors (<3.8%: supplementary Table S4). This suggests that plasma sex hormones can be used in isolation to estimate gonadal sex. Given that immature

turtle stocks, such as those in TCI, are likely to be of mixed genetic origin, it is recommended that, in the absence of site specific blood hormone data (ideally calibrated with known sex samples through observations of gonads), regional average testosterone threshold values would form the basis of relatively accurate sex ratio estimates, provided there was sufficient data to encompass the likely variation in testosterone concentrations due to, for example, temperature, seasonality, breeding and non-breeding periods (Wibbels et al. 1987, Owens 1997, Braun-McNeill et al. 2003, Hawkes et al. 2013).

Sex ratios

Immature stages

Our results reveal strongly female biased sex ratios in immature stages of both species of sea turtle, but especially in hawksbills. A decline in female bias with advancing life-stages to approximately Fisherian ratios in adult stages was found in the hawksbill population of TCI. The 94% female bias in hawksbill recruits (<35cm CCL) and 91% in juveniles (35-65cm CCL) are the highest female biased secondary sex ratios yet to be reported from the Wider Caribbean Region (WCR). Values between 70-80% have been observed in Florida, Dominican Republic and USVI (Leon & Diez 1999, Geis et al. 2003, Blanvillain et al. 2008) and between 69-89% in British Virgin Isles (Hawkes et al. 2013). A slight male bias, however, has been recorded in juveniles at Puerto Rico (44%: Diez & van Dam 2003).

The green turtle foraging population is female biased and our results suggest, albeit without statistical significance, that bias reduces with age from 81% to 69%. This is similar to the in-water secondary sex ratios (of all size life-stages below adult) reported elsewhere in the WCR (Bolten & Bjorndal 1992, Bolten et al. 1992, Stabenau et al. 1996, Bjorndal et al. 2000).

Why hawksbills have such a high female bias in younger stages at TCI foraging grounds is unknown, but it seems reasonable to suppose that the primary sex ratios of at least the rookeries contributing the majority of turtles (Barbados leeward, Nicaragua and Cuba; see Figure 5) are highly female biased. Highly skewed primary sex ratios have been reported in hawksbill nests from Bahia, Brazil (Godfrey et al. 1999), Buck Island USVI (Wibbels et al. 1999) and Antigua (Glen & Mrosovsky 2004, but see Mrosovsky et al. 1992) (Figure 1a). Male biased sex ratios, however, have been reported in Guadeloupe (26% Female: Kamel & Mrosovsky 2006), Florida (7%

female: Dalrymple et al. 1985, although caution is noted as only one hawksbill nest was examined here) and Dominican Republic (Revuelta et al. 2013, estimates from incubated nests) which suggests that female bias cannot be considered a general rule, although rookery sizes here are small and so too their influence on mixed stocks.

In most cases, highly female biased primary sex ratios have been reported in Atlantic and Mediterranean green turtle rookeries (Figure 1b). Tortuguero, Costa Rica, however, had highly varying sex ratio data; in one study sex ratios ranged from 0-100% female depending on where nests were laid (Horikoshi 1992). In another study, Standora and Spotila (1985) found 7% of offspring were female in clutches laid in the vegetation line, 72% female in those near the water and 87% at open beach nest sites. In a further study at Tortuguero, Spotila et al. (1987) found 67% females in 15 nests. Such variation at this key rookery undoubtedly regulates the sex ratios for most of the Caribbean. It is possible that juvenile male green turtles in TCI may have originated from cool nests at the vegetation line of Tortuguero, and other immature turtles of Costa Rica origin from more open sections of this beach.

It is clear from these results that the limited data available on primary sex ratios from the Atlantic and Mediterranean cannot adequately explain the observed sex ratios of in-water stocks, because the primary sex ratios are highly variable between nests, season, years and nest site position (Horikoshi 1992, Kamel 2013) and as a result of other varying influences e.g. cooling rainfall (Godfrey et al. 1996, Miller et al. 2003, Houghton et al. 2007). Such variation in exogenous conditions may be the key to producing enough males for an operational sex ratio (Bell et al. 2010, Hays et al. 2010) capable of sustaining populations in a warming world (Hawkes et al. 2007, Poloczanska et al. 2009, Witt et al. 2010, Fuentes et al. 2011, Hamann et al. 2013). Further primary sex ratio data from other sites around the Caribbean would help elucidate future sex-based MSA.

Adult sex ratios

Why adult sex ratios in TCI are markedly different from those of earlier stages remains unknown, but to our knowledge this is the first study to present such clear evidence of shifting sex ratios between all stages in the same location.

Male adult hawksbills in our study appear to be present at more-or-less equal ratios to females, but there was a male-biased operational sex ratio, although

caution is necessary here as fishermen report that adult males are easier to catch than adult females (T. Stringell pers. obs.). Several studies have suggested that adult males may skew the sex ratio by having higher breeding periodicity than females but maintain a balanced operational sex ratio through multiple mating (Limpus 1993, FitzSimmons et al. 1997, Hays et al. 2010), but others suggest that promiscuity is less prevalent than once thought, with as many fathers as mothers in the green turtle population of Cyprus (Wright et al. 2012). Indeed, in some studies male-biased adult sex ratios have been reported despite female-biased juvenile sex ratios (e.g. green turtles in the southern Great Barrier Reef, Chaloupka & Limpus 2001).

Sample size was limited for adult hawksbills in our study (and very low for adult green turtles due in part to their highly seasonal breeding season and likely small breeding population size; Stringell et al. in prep., Chapter 2), so drawing definitive conclusions from sex ratios in this life-stage is speculative. Clearly, late maturity in marine turtles gives large time differences (decades) between the sex ratios in adult turtle populations and primary sex ratios at nesting beaches. It is possible that historic beach temperatures may have favoured balanced sex ratios in nests, but literature from this time also indicated, in the main, female biased primary ratios (see Hawkes et al. 2009 for review). There are, therefore, several possible balancing mechanisms that appear to make the overall sex ratio of adults similar to 1:1 than that expressed in primary and secondary stages.

Possible sex ratio balancing mechanisms

Sex biased dispersal

Sex biased dispersal or migration could be an important factor in balancing sex ratios. Sex differences in migration/dispersal rates (e.g. Limpus 1993, FitzSimmons et al. 1997, van Dam et al. 2008, Velez-Zuazo et al. 2008, Hays et al. 2010 in adults; Casale et al. 2002 in juveniles), may be a result of differential genetic structuring between the sexes (Casale et al. 2002, but see Maffucci et al. 2013). The results from our mtDNA analysis suggest juvenile male green turtles may be genetically different from most other groups. This would imply possible differences in origin and sex biased dispersal. The MSA indicated that these males were highly likely to have originated from Costa Rica. Alternatively they could be the result of incubation in the early or late parts of the season when temperatures are lowest. Immigration of young

females from several rookeries that return to their origin when they reach adulthood, might explain the shift from female bias to Fisherian ratios with age/size. Incorporating sex, life-stage and genetics has provided some insight in sex biased dispersal in juvenile green turtles (here defined as 35cm-65cm). Given the growth rates of green turtles (Bjorndal et al. 2000), this size cohort implies that hatchlings from Tortuguero beach, Costa Rica, may have hatched a decade or more prior to this study. It is unlikely that it was significantly cooler on average then, than in more recent times, so it is probable that >80% of juvenile male green turtles hatched from cooler times of the season, or from cooler parts of the beach (e.g. from periods of prolonged cooling rainfall (Houghton et al. 2007) or from the vegetation line (Horikoshi 1992)) when and where temperatures promote production of males. But clearly, sand albedo, nest depth and egg position will have confounding influence (Hawkes et al. 2009).

Investigating the likely influence of ocean currents in linking rookeries of origin to the green turtle stocks suggests supply from southwestern Caribbean, Gulf of Mexico and Florida which join the Caribbean current and in turn the Gulf Stream and Antilles current (Figure 1). Conversely, widespread supply to the immature hawksbill stock of TCI is likely from throughout the Wider Caribbean with more probable contribution from the Gulf of Mexico region into the Gulf Stream with return flows to TCI waters via the variable Antilles current; hatchling hawksbills from Barbados may disperse via the Caribbean current, enter the Gulf of Mexico region and recruit to TCI waters as described above (Figure 1). These data perhaps suggest a passive dispersal of hawksbill hatchlings and recruitment to TCI waters. However, a protracted analysis of the influence of currents on the phylogenetics of these Atlantic-wide data is beyond the scope of this paper (but see Blumenthal et al. 2009, Monzón-Argüello et al. 2012, Proietti et al. 2012, and Carreras et al. 2013 for potential approaches).

Lack of genetic differentiation in other life-stage/sex combinations provides no compelling reason to separate groups to examine sex biased dispersal. This highlights some of the issues of relying on MSA to explore such questions: if haplotype frequencies are not differentiated between the life-stage/sex groupings in question then sophisticated tools such as Bayesian MSA will not be able to distinguish origin. Additionally, and perhaps more importantly, annual variations in source contribution to MSA have implications for drawing conclusions (Bjorndal &

Bolten 2008). In their study of green turtles in Bahamas, contributions from Costa Rica varied greatly between years over a period of a decade due to “sweepstake” recruitment pulses (Hedgecock 1994), that is, a small portion of individuals from a few rookeries can produce the majority of recruits by chance - escaping storms, predators, ocean currents (Bjorndal & Bolten 2008). Thus, large variance in annual production of hatchlings that successfully survive to coastal recruitment may drive the composition of mixed stocks and thus the conclusions of the MSA. If such “sweepstakes” recruitment occurred in TCI, then differential recruitment from source stocks also has a sex biased dispersal component. Such insights from our work in TCI will only be gained from long-term data collection, such as those in the Bahamas (Bjorndal & Bolten 2008).

Differential fitness

Another potential sex ratio balancing mechanism might be differential fitness between the sexes that might result from differences in hatchling size, speed of incubation, timing of hatching, growth rates, predation rates etc. Growth rates indicated no significant difference between the sexes implying that this measure of fitness did not explain biased sex ratio. Our hypothesis was that perhaps males grew more slowly in juvenile stages and this might make them more prone to predation for longer. Bjorndal et al. (2000), however, found male green turtles grew faster than females in the Bahamas, but elsewhere females grew faster than males (Limpus & Chaloupka 1997, Chaloupka et al. 2004). In the Great Barrier Reef, hawksbills females grew faster than males (Chaloupka & Limpus 1997, but see Bell & Pike 2012) but no difference between the sexes was found in Barbados (Krueger et al. 2011). These studies illustrate that timing and location have significant influences on growth rates. Predation of juvenile to adult sizes is generally limited to large predators (Heithaus et al. 2008). The highest risk of predation is to earlier stages (eggs and hatchlings) (Gyuris 1994, Heithaus 2013) when growth rate in hatchlings may be more likely to influence fitness. For example male hatchlings from cooler nests tend to incubate for longer and emerge with larger body size (Gyuris 2000), and as such have stronger swimming ability than female hatchlings (Booth & Evans 2011, but see Burgess et al. 2006) to pass through coastal areas before reaching offshore pelagic zones. Transit through the coastal zone at this vulnerable stage is prone to high levels of predation from a wide range of predators of varying sizes

(Gyuris 1994, Gyuris 2000, Pilcher et al. 2000). The sex ratios in later life-stages may therefore be a result of higher production of female hatchlings from rookeries that also produce some male hatchlings with better survival prospects.

Genetic structure and connectivity

Bowen et al. (2005) found progressively greater genetic differentiation among regional foraging sites in older life stage turtles, resulting in “complex population structure” (Bowen & Karl 2007). Similarly, although no structure was found among younger life-stages in our study (except for juvenile male green turtles), significant structure was found between immature stages and adults. Different innate behaviours at different life cycle stages e.g. oceanic hatchling dispersal, juvenile ontogenetic shifts from oceanic to coastal habitats, sequential developmental migrations, and adult breeding migrations (philopatry), are likely to drive this population structure (Bowen & Karl 2007). For example, oceanic-stage juvenile loggerheads in North Atlantic are apparently well mixed with no obvious population structure (Bolten et al. 1998), whilst older life-stages of coastal feeding aggregations are correlated to the haplotype composition of nearest nesting populations (Bass et al. 2004, Roberts et al. 2005). This complex population structure results in a suite of threats differentially affecting each life history stage, which in turn, may differentially affect separate populations (Bowen & Karl 2007). Coastal management strategies may fortuitously provide better protection of nesting colonies, adults and large juveniles - the key life-stages for population maintenance (Crouse et al. 1987). Threats to adults and nests in TCI affect almost exclusively the TCI population, whereas threats to recruits, juveniles and sub-adults also affect other distant rookeries (Carreras et al. 2013). In TCI where nesting rookeries are small, efforts to protect and maintain large and adult size-class turtles in coastal waters are likely of key importance.

Previous studies (Diaz-Fernandez et al. 1999, Bowen et al. 2007, Velez-Zuazo et al. 2008, Blumenthal et al. 2009) indicated the highly mixed nature of the hawksbill turtle aggregations at foraging grounds in the Caribbean. Our MSA results confirmed this for TCI and backs the assertion by Richardson et al. (2009) that this foraging area is regionally significant for both hawksbill and green turtles. Our analysis shows that the immature hawksbill foraging stock is genetically different from the TCI nesting rookery and adults, presenting further evidence of differences between life-

stages and the broad geographic contribution to TCI foraging aggregations.

Due to the small size of the TCI green turtle rookery, contributions to foraging grounds across the Caribbean are likely to be negligible. A clearer indication of connectivity to other mixed stocks may yet be found now that the unique TCI nesting haplotype profile including the endemic Cm-A64 is known and can be identified in future Caribbean foraging stock assays. Prior to this study, this haplotype was found only in a single foraging green turtle juvenile captured in Indian River Lagoon, Florida (Shamblin pers. comm. 2012). Moreover, the unique haplotype discovered in one of four sampled green turtle nests illustrates that, despite being a small and remnant population (Richardson et al. 2009), this green turtle rookery is genetically singular and protection of this unique genetic signature requires urgent action.

In a many-to-many MSA of short hawksbill haplotypes (truncated to 384bp), the TCI hawksbill rookery supplied foraging grounds across the Caribbean, and due to the minor to moderate size of the rookery, contributions were minimal (<10%) (Stringell et al unpublished data). However, once the unique TCI nesting haplotype of EiA81 is characterised in Caribbean foraging stocks, a clearer indication of connectivity to other mixed stocks may be found.

Conservation implications

Knowledge of sex ratios in marine turtle populations is fundamental for their effective conservation and important for determining likely effects of human impacts on population maintenance. In TCI, both hawksbill and green turtles are exploited for food, and the hawksbill fishery is one of the largest documented in the Atlantic (Stringell et al. 2013, Chapter 1). The effects of the fishery in TCI on its sea turtle populations can now be better assessed with a robust understanding of the sex ratios at various life-stages. It is clear that with the highly female biased populations, harvest of juvenile turtles is unlikely to be detrimental to the supply of females.

The mixed composition of the foraging stocks of both species in TCI is an expected result, given the multitude of literature on Caribbean green and hawksbill turtle MSAs indicating similar conclusions for several study sites (see Bowen & Karl 2007 for review). What is interesting about these results, however, is the likely small proportion of turtles in foraging sites that are of TCI origin. TCI fishers have traditionally viewed foraging turtle aggregations as a closed population originating from the TCI and thus a native resource for justifiable exploitation (T. Stringell pers.

obs.). A similar scenario occurred in the 1990s when Cuban authorities wished to exploit hawksbill turtles for shell trade, arguing that the turtles in Cuban waters were of Cuban origin (Carrillo et al. 1999, Moncada et al. 2012 and references therein). MSA played an important part in confirming that their foraging hawksbill stock was mixed, although a high proportion of those foraging turtles also originated from Cuban rookeries (Bowen et al. 2007). Similarly, the TCI turtle fishery is harvesting turtles that originate in other jurisdictions, most of which have protective measures in place (Richardson et al. 2006). For the hawksbill, exploitation of adults is likely to disproportionately affect the native TCI population, although distant sources will still be impacted, perhaps severely in the case of small rookeries. Exploitation of recruits, juveniles and sub-adults, however, is likely to predominantly affect other distant populations e.g. in Barbados, Nicaragua and Cuba (and mainly females). The results of this study have informed the development of new protective measures that facilitate access to foraging juvenile turtles while safeguarding sub-adult and adult turtles in TCI waters (Stringell et al. 2013, Chapter 1, Stringell et al. in prep., Chapter 2).

In a warming world with predicted feminisation of sea turtle populations originating from ‘hot’ beaches (Hawkes et al. 2007, Hawkes et al. 2009, Poloczanska et al. 2009, Witt et al. 2010, Fuentes et al. 2011, Hamann et al. 2013), operational sex ratios may become critical in years to come, although there is some evidence that the Allee effect (a decreased per capita population growth rate at low density, Allee et al. 1949) is less likely to be as critical in turtles as other taxa (Hays 2004, Bell et al. 2010). Climate change, however, may have unintended consequences on sea turtle population maintenance, and it remains to be seen whether turtles will weather the change.

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Table 1. Pairwise F_{ST} values between Atlantic/Mediterranean rookeries and TCI rookeries (TCI.R: hawksbill turtles, n=22; green turtles, n=4) and TCI mixed stocks (green turtle juvenile males (JM, n=17) and other immature turtles (Imm, n=68) and immature hawksbills (Imm, n=118). Data based on hawksbill turtle (A) and green (B) turtle haplotype frequencies. Hawksbill haplotypes are long sequences (740bp), green turtle haplotypes are 481bp. * denotes significant Exact test at P < 0.05. Bold = significant at FDR corrected P values for pairwise comparisons (hawksbill FDR_{31}, P=0.0124; green turtle FDR_{47}, P=0.0113).

A) Hawksbill turtle rookery	Abbrev.	N° samples	N° haps.	F_{ST}		Genetic data source
				TCI.R	TCI.Imm	
Antigua (Jumby Bay)	ANT	72	3	**0.512***	**0.170***	LeRoux *et al* 2012
Barbados (Leeward)	BLE	54	1	**0.868***	**0.317***	Browne *et al* 2010
Brazil (Bahia)	BRZ	66	4	**0.609***	**0.188***	Lara-Ruiz *et al* 2006
British Virgin Islands	BVI	4	4	**0.420***	0.006	Formia *et al* unpubl.
Barbados (Windward)	BWI	30	3	0.028	**0.143**	Browne *et al* 2010
Costa Rica (Tortuguero)	CR	60	7	0.064*	**0.130***	[a] LeRoux et al 2012
Cuba (Doce Leguas)	CUB	70	5	**0.712***	**0.244***	Diaz-Fernandez *et al* 1999
Dominican Republic (Jaragua NP)	DRJ	15	6	**0.274***	**0.063**	Carerras *et al* 2013
Dominican Republic (Saona Island)	DRS	33	4	0.027	**0.144***	Carerras *et al* 2013
Guadeloupe	GU	74	4	**0.788***	**0.429***	LeRoux *et al* 2012
Mexico (Yucatan)	MEX	20	4	**0.612***	**0.317***	LeRoux *et al* 2012
Montserrat	MON	5	3	0.177	-0.050	Formia *et al* unpubl.
Nicaragua (Pearl Cays)	NIC	95	5	0.077*	**0.150***	LeRoux *et al* 2012
Puerto Rico (Mona Island)	PRV	109	7	**0.104***	**0.162***	[b] LeRoux et al 2012
US Virgin Islands (Buck Island)	USV	67	6	-0.002	**0.179***	LeRoux *et al* 2012
Turks and Caicos Islands	TCI	22	5	-	**0.125***	Stringell et al. in prep.; This study
	Total	**796**	**71**			

[a] includes 42 samples from Troeng *et al* 2005

[b] includes 94 samples from Velez-Zuazo *et al* 2008 and 15 resequenced samples from Bass *et al* 1996

Table 1. Cont.

B) Green turtle rookery	Abbrev.	N° samples	N° haps	F_{ST} TCI.R	F_{ST} TCI.JM	F_{ST} TCI.Imm	Genetic data source
Ascension Island	ASCI	245	13	**0.671***	**0.673***	**0.498***	Formia *et al* 2007
Aves Island, Venezuela	AVI	67	2	**0.811***	**0.757***	**0.479***	Shamblin *et al* 2012
Bioko, Equatorial Guinea	BOK	50	2	**0.775***	**0.742***	**0.466***	Formia *et al* 2006
Atol das Rocas & Fernando de Noronha, Brazil	BRZ	69	7	**0.53***	**0.549***	**0.347***	Bjorndal *et al* 2006
Tortuguero, Costa Rica	CR	433	5	0.138	0.06*	**0.446***	Bjorndal *et al* 2005
[a] Southwest Cuba	CUB	28	7	-0.031	0.029	**0.065***	Ruiz-Urquiola *et al* 2010
[b] Northern and Greek Cyprus	CYP	61	3	**0.835***	**0.794***	**0.549***	[d] Bagda *et al* 2012
Florida, USA	FLO	24	3	0.136	0.159*	0.019	Encalada *et al* 1996
Poilao, Guinea Bissau	GBP	51	1	**0.966***	**0.885***	**0.551***	Formia *et al* 2006
Quintana Roo, Mexico	MEX	20	7	0.136	**0.203***	0.005	Encalada *et al* 1996
[c] Gulf of Guinea: Principe & Sao Tome, Gulf of Guinea	PRST	26	7	**0.449***	**0.493***	**0.277***	Formia *et al* 2006
Galibi, Suriname	SUR	58	3	**0.858***	**0.796***	**0.498***	Shamblin *et al* 2012
Turkey	TKY	187	4	**0.953***	**0.925***	**0.733***	Bagda *et al* 2012
Trinidade, Brazil	TRI	99	7	**0.496***	**0.519***	**0.335***	Bjorndal *et al* 2006
Buck Island, US Virgin Islands	USV	49	2	**0.805***	**0.749***	**0.462***	Shamblin *et al* 2012
Turks & Caicos Islands	TCI	4	2	-	-0.072	0.075	Stringell et al. in prep.; This study
	Total	**1471**	**42**				

[a] Two sites combined

[b] Northern Cyprus (Alagadi and Iskele peninsula): Bagda *et al* 2012; Kaska 2000. Greek Cyprus: Lara Bay

[c] Corisco excluded

[d] also Bowen *et al* 1992; Encalada *et al* 1996; Kaska 2000

Table 2. Mean ± standard deviation (SD) and range of testosterone and oestradiol-17β blood hormone concentrations (pg/ml) in hawksbill and green turtles of different life-stages and sexes. Known sex was determined via gonad morphology/histology or external secondary sex features. Total range includes these plus turtles of unknown sex where only blood samples were taken.

Hormone	Life stage	Sex	Hawksbill turtles				Green turtles			
			Mean ± SD	Min	Max	n	Mean ± SD	Min	Max	n
Testosterone	Immature	Total range	184.71 ± 386.03	15.72	3023.95	98	455.25 ± 923.31	8.45	5083.69	131
		Known males	1395.57 ± 1416.90	443.88	3023.95	3	1062.30 ± 957.77	108.06	3123.29	16
		Known females	81.04 ± 101.40	22.46	517.92	23	51.26 ± 48.83	8.45	215.79	39
	Adults	Total range	8535.79 ± 12664.50	13.58	39890.43	14	-	-	-	-
		Known males	16598.31 ± 13965.58	1307.03	39890.43	7	-	-	-	-
		Known females	473.27 ± 892.61	13.58	2473.31	7	-	-	-	-
Oestradiol-17β	Immature	Total range	18.08 ± 26.07	3.18	116.88	32	28.87 ± 57.14	2.33	419.77	102
		Known males	6.39 ± 2.78	3.18	8.00	3	21.43 ± 37.56	3.18	151.96	15
		Known females	11.90 ± 10.73	3.18	40.75	11	28.34 ± 73.36	2.33	419.77	38
	Adults	Total range	62.71 ± 72.17	8.00	191.22	10	-	-	-	-
		Known males	37.52 ± 53.60	8.00	154.60	7	-	-	-	-
		Known females	121.47 ± 86.14	25.18	191.22	3	-	-	-	-

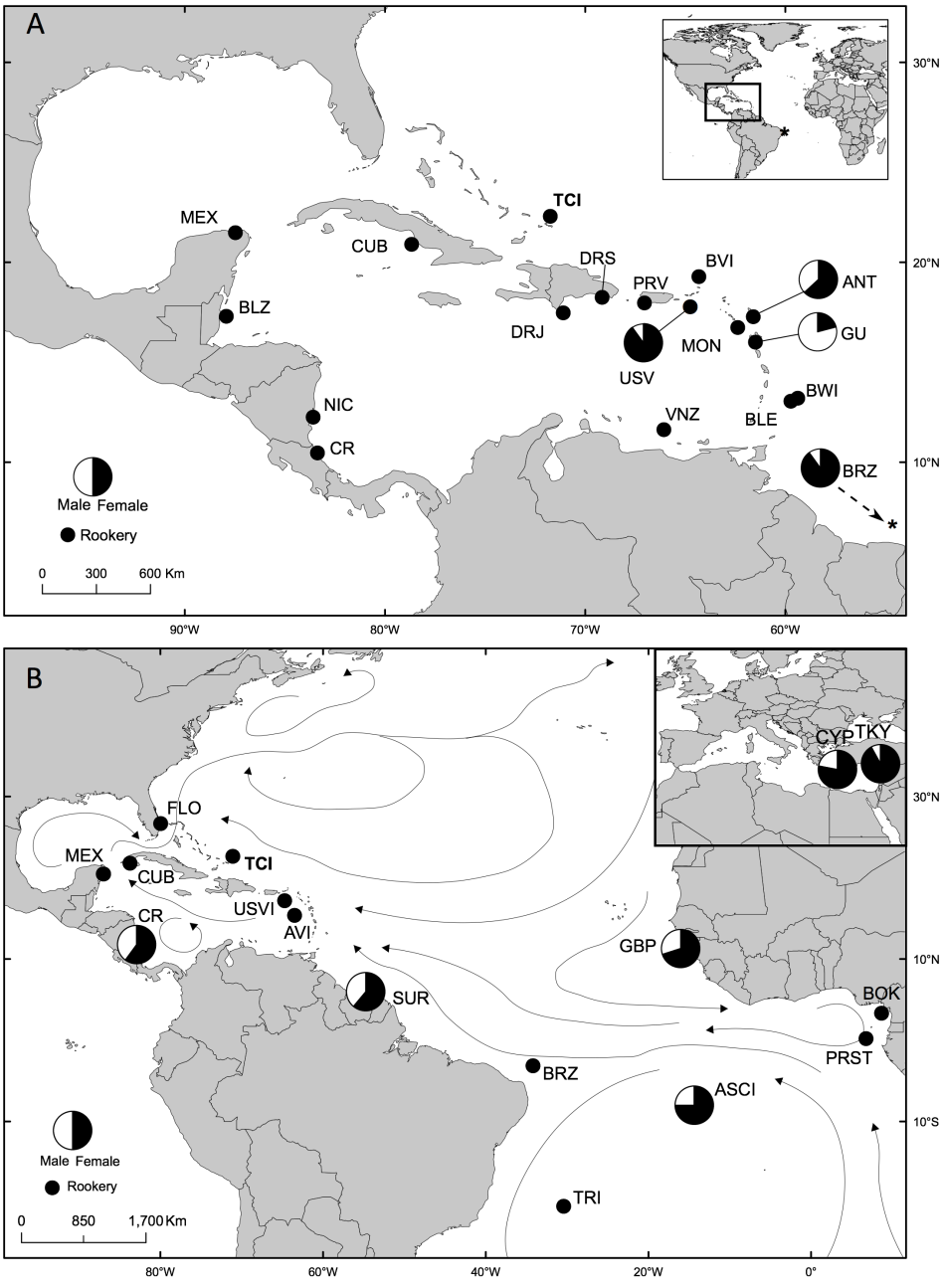

Figure 1. Hawksbill (A) and green turtle (B) rookeries (black circles) used in the MSA. Location labels are listed in Table 1. Arrows in B indicate generalised surface currents applicable for both maps. Brazil (BRZ) rookery indicated on inset of A. Cyprus (CYP) and Turkey (TKY) rookeries indicated on Mediterranean inset of B. Pie charts indicate % female (black) at rookeries where primary sex ratio data exist. Hawksbill turtles: approx. 90% at Bahia, Brazil (Godfrey et al. 1999) and Buck Island US Virgin Islands (USV Wibbels et al. 1999); approx. 63% at Antigua (Mrosovsky et al. 1992, Glen & Mrosovsky 2004); 26% female at Guadeloupe (GU Kamel & Mrosovsky 2006). Green turtles: 54-68% at Suriname (SUR: Mrosovsky et al. 1984, Godfrey et al. 1996); 70.2% at Poilao Guinea Bissau (GBP Rebelo et al. 2011); 79% at Cyprus (Kaska et al. 1998, Broderick et al. 2000), 92% at Turkey (Broderick et al. 2000, Casale et al. 2000); 75-87% at Ascension Island (ASCI Broderick et al. 2001, Godley et al. 2002, Pintus et al. 2009); 54% average from Tortuguero, Costa Rica (CR Standora & Spotila 1985, Spotila et al. 1987, Horikoshi 1992).

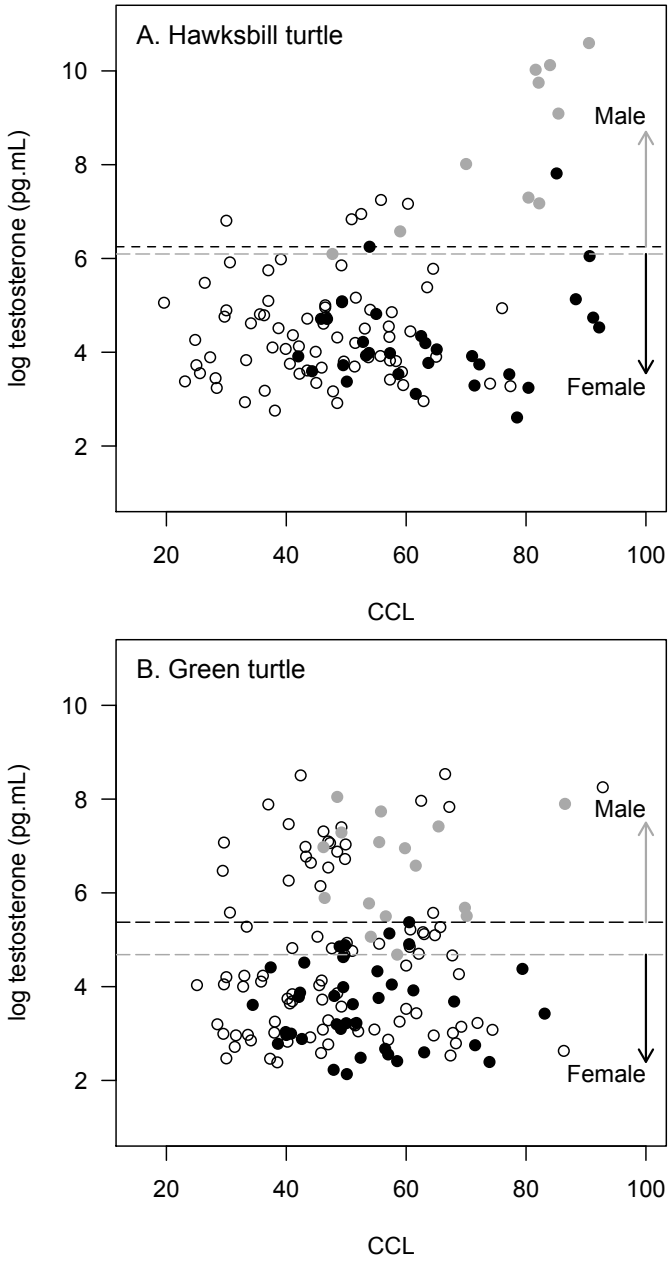

Figure 2. Hawksbill turtle (A) and green turtle (B) testosterone concentration (log pg/ml) plotted against curved carapace length (CCL, cm). Filled circles indicate individuals of known sex derived from gross morphology or histology of gonads: females (black), males (grey). Empty circles are turtles of unknown sex (no observations of gonads). Maximum or minimum testosterone concentrations observed in known sex individuals (dashed lines: colour scheme as before) are used to construct threshold values for determining sex in unknown sex individuals (between the dashed lines, sex determination is infeasible). See Table 2 for ranges of testosterone concentrations for each species, relative to life-stage and sex.

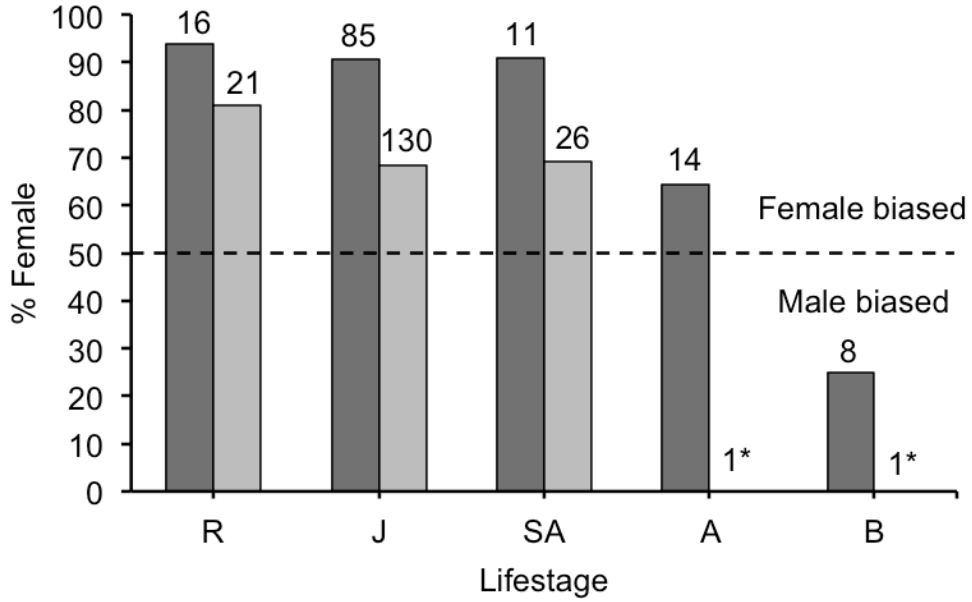

Figure 3. Sex ratios of hawksbill (dark grey) and green turtle (light grey) for the different life-stages. Recruits (R) <35cm curved carapace length (19.6-34.1cm in hawksbill, 25.1-34.4cm in green turtles), Juveniles (J) 35-65cm, sub-adults (SA) 65-97cm in green turtles and 65-78cm in hawksbills, foraging adults (A), breeding adults (B) are here defined as >78cm in hawksbills and >98cm in green turtles (see main text). Numbers above bars indicate sample size. Dashed line indicates equal sex ratio. Sex ratio data in hawksbill turtles: R, 1M:15F; J, 8M:77F; SA,1M:10F; A, 5M:9F; B, 6M:2F; Green turtles: R, 4M:17F; J, 41M:89F; SA, 8M:18F; *No adult green turtle sex ratios are shown because only a single female was captured in each case.

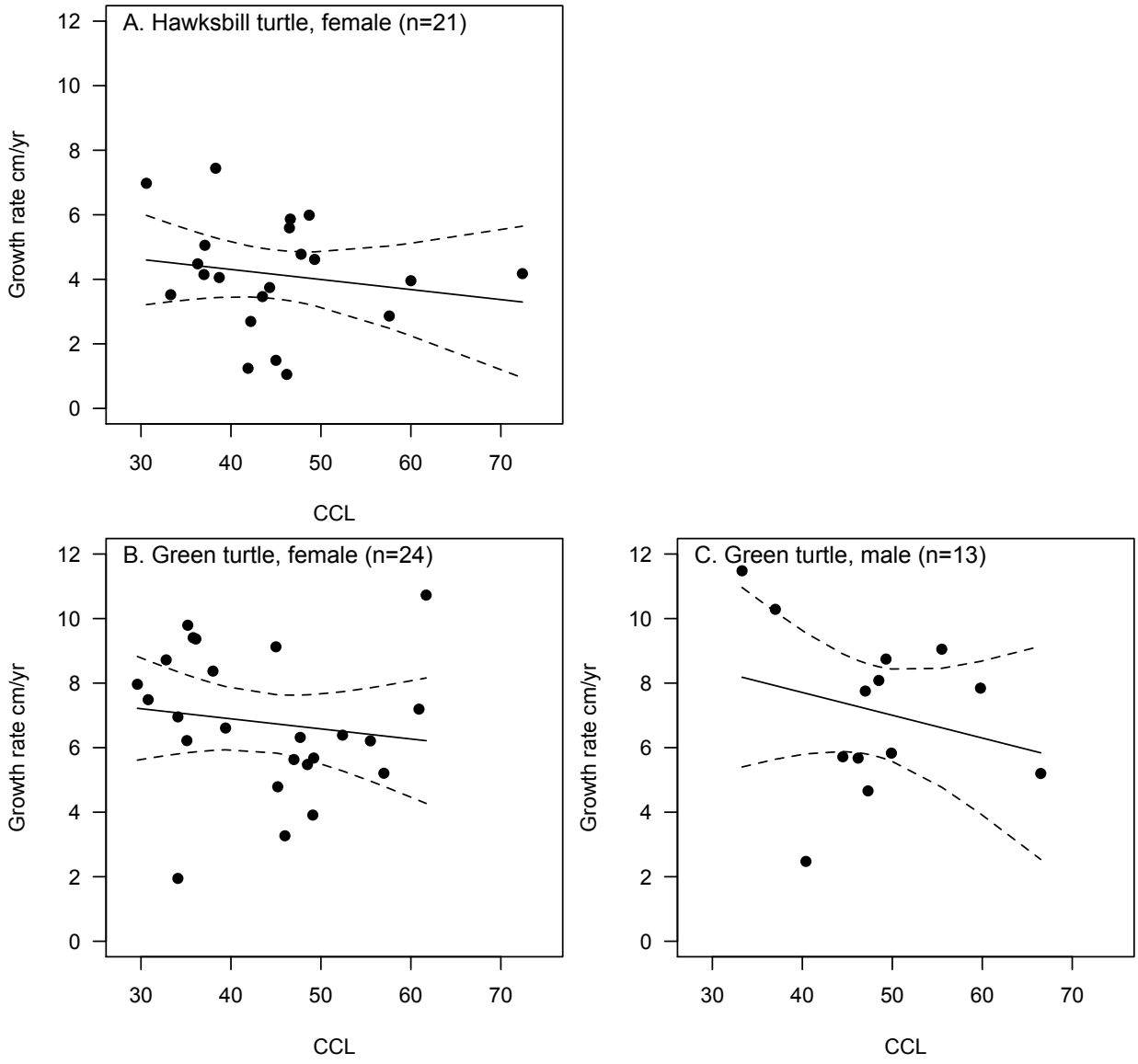

Figure 4. Growth rates in female hawksbill turtles (A) and female and male green turtles (B, C). Data are from known sex individuals, as determined by gross morphology or histology of gonads, or via testosterone concentrations in blood plasma. Lines indicate GLM fit and 95% CI. Curved carapace length (CCL) of final recaptures ranged between 30.6 and 72.4cm in female hawksbill turtles (2 recruits, 18 juveniles, 1 sub-adult; a single male hawksbill turtle recruit [30cm CCL, growth rate 4.18cm/yr] is not shown); between 29.6 and 61.7cm in female green turtles (5 recruits, 19 juveniles), and between 33.3 and 66.5cm in male green turtles (1 recruit, 11 juveniles, 1 sub-adult).

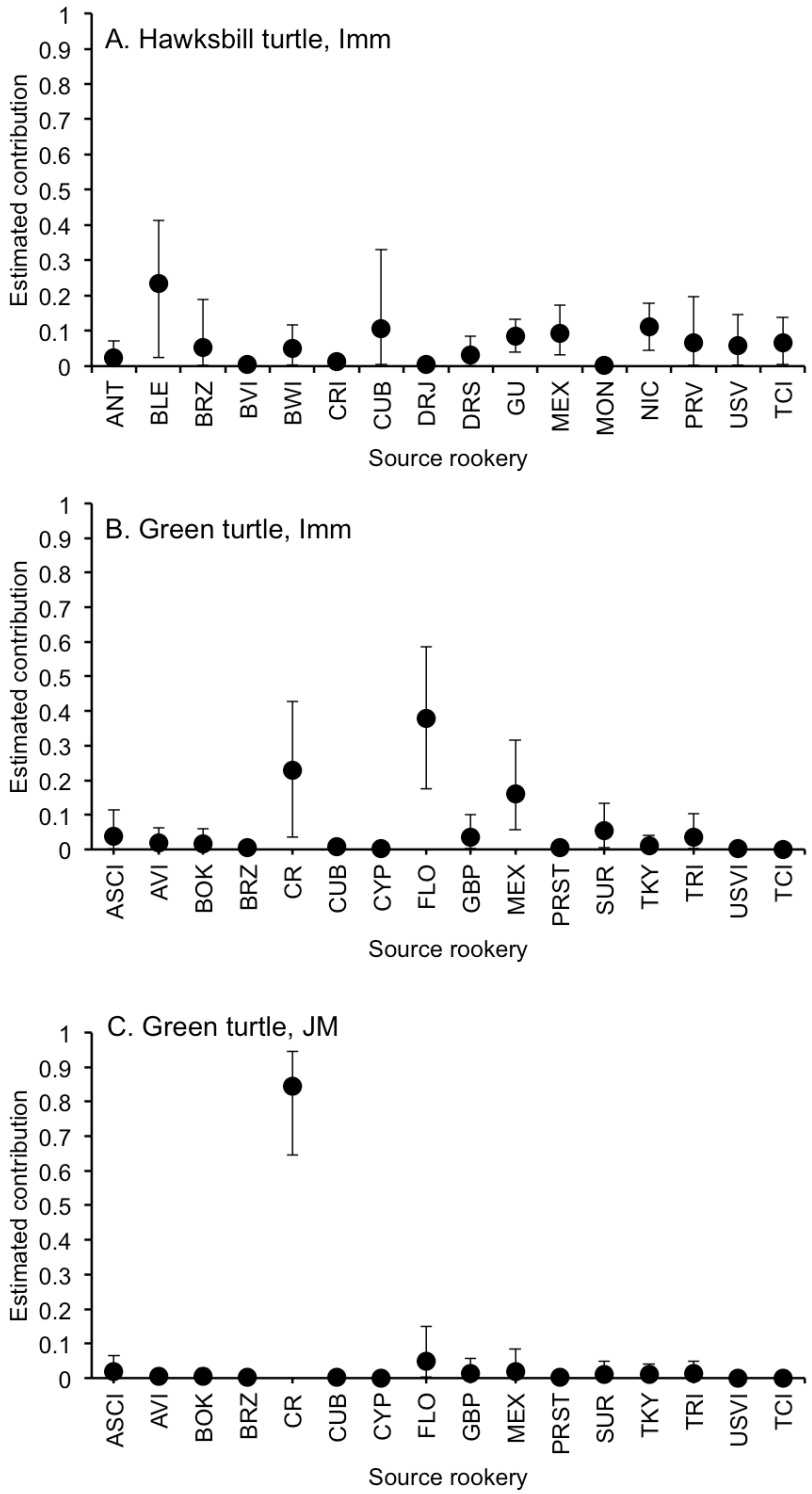

Figure 5. Contribution estimates of Atlantic and Mediterranean source rookeries to TCI foraging aggregations as determined by Bayesian Mixed Stock Analyses using rookery size as weighted priors. Contributions to the immature (Imm) hawksbill stock (n=118) were estimated using 740bp haplotype data. Green turtle mixed stocks consist of Juvenile males (JM, C; n=17) and all other immature turtles (Imm, B; n=68). See supplementary Table S3 for values.

Chapter 4: Supplementary Information

Table S1. Haplotype frequencies using long sequence lengths (740bp) at hawksbill rookeries and mixed stocks used in the Mixed Stock Analyses. TCI foraging groups that make up the mixed stock are also listed. N denotes the number of samples in each group. Haplotype diversity (*h*) and nucleotide diversity (*π*) was calculated in Arlequin 3.5 (Excoffier & Lischer 2010), the latter using a Tamura 3-parameter substitution model (Tamura 1992).

Rookery	Abbrev. (740bp EiA Haplotype)	N	N°. Haps.	1	2	3	9	11	12	13	18	20	21	22	23	24	27	28	29	30	32	36	39	41	42	43	47	51	52	61	62	63	65	66	81n	83	84	90	93[n]	h	h SD	π	π SD	Genetic data Source	Size (females pa)	Distance (km)	Rookery size source
Antigua (Jumby Bay)	ANT	72	3	42	0	29	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.504	0.027	0.0071	0.0038	LeRoux *et al* 2012	100	1168	Mortimer & Donnely 2007
Barbados (Leeward)	BLE	54	1	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	Browne *et al* 2010	367†	1609	Beggs *et al* 2007
Brazil (Bahia)	BRZ	66	4	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0.362	0.069	0.0005	0.0006	Lara-Ruiz *et al* 2006	240	5318	Lara-Ruis *et al* 2006
British Virgin Islands	BVI	4	4	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000	0.177	0.0097	0.0069	Formia *et al* unpubl.	7	837	McGowan *et al* 2008
Barbados (Windward)	BWI	30	3	3	0	0	6	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.476	0.091	0.0033	0.0020	Browne *et al* 2010	37†	1620	LeRoux *et al* 2012
Costa Rica (Tortuguero)	CR	60	7	0	11	0	3	33	5	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0.655	0.057	0.0076	0.0041	[a] LeRoux et al 2012	10	1750	Mortimer & Donnely 2007
Cuba (Doce Leguas)	CUB	70	5	62	0	0	0	1	0	5	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.213	0.064	0.0032	0.0020	Diaz-Fernandez *et al* 1999	400	685	Mortimer & Donnely 2007
Dominican Republic (Jaragua NP)	DRJ	15	6	1	0	0	2	3	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0.848	0.054	0.0043	0.0026	Carerras *et al* 2013	5	464	Revuelta *et al* in press
Dominican Rebublic (Saona Island)	DRS	33	4	3	0	0	0	22	0	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.527	0.089	0.0036	0.0022	Carerras *et al* 2013	33	517	Revuelta *et al* in press
Guadaloupe	GU	74	4	2	0	0	69	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.131	0.053	0.0012	0.0010	LeRoux *et al* 2012	42	1232	Kamel & Delcroix 2009
Mexico (Yucatan)	MEX	20	4	0	0	0	0	0	0	0	0	0	0	2	16	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.363	0.131	0.0011	0.0009	LeRoux *et al* 2012	891	1611	Mortimer & Donnely 2007
Monserrat	MON	5	3	2	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.800	0.164	0.0094	0.0062	Formia *et al* unpubl.	3	1149	Martin *et al* 2005
Nicaragua (Pearl Cays)	NIC	95	5	0	19	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	1	0	0	0	5	0	0	0.612	0.042	0.0058	0.0032	LeRoux *et al* 2012	52	1627	Lagueux *et al* 2003
Peurto Rico (Mona Island)	PRV	109	7	3	0	0	2	60	0	0	1	34	6	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0.600	0.035	0.0033	0.0020	[b] LeRoux et al 2012	247	589	Mortimer & Donnely 2007
US Virgin Islands (Buck Island)	USV	67	6	8	0	2	2	50	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.430	0.072	0.0038	0.0022	LeRoux *et al* 2012	56	861	Mortimer & Donnely 2007
Turks and Caicos Islands	TCI	22	5	1	0	0	0	17	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0.407	0.128	0.0032	0.0020	Stringell *et al* 2013 in prep	47	7	Stringell *et al* 2013 in prep
Total		**796**	**25**	**234**	**30**	**32**	**85**	**266**	**6**	**6**	**3**	**44**	**6**	**2**	**21**	**0**	**2**	**0**	**1**	**2**	**9**	**0**	**0**	**3**	**0**	**24**	**7**	**0**	**1**	**4**	**1**	**0**	**1**	**0**	**1**	**0**	**5**	**0**	**0**								
Mixed stock																																															
TCI immature*	TCI.imm	118*	20	37	3	0	10	28	1	0	0	1	0	0	5	2	0	2	0	0	0	1	1	3	5	6	0	1	0	0	0	1	0	1	0	8	0	1	1	0.832	0.023	0.0087	0.0046	This study			
TCI foraging groups (pooled**)																																															
Young female	YF	79	16	27	3	0	6	18	1	0	0	1	0	0	3	1	0	2	0	0	0	1	1	3	2	5	0	0	0	0	0	1	0	0	0	4	0	0	0	0.823	0.031	0.0082	0.0044	This study			
Young male	YM	6	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0.800	0.172	0.0066	0.0044	This study			
All adult female	ABF	7	4	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.714	0.181	0.0061	0.0039	This study			
All adult male	ABM	9	4	0	0	1	0	6	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.583	0.183	0.0044	0.0029	This study			
Total		**101**	**19**	**31**	**3**	**1**	**6**	**28**	**1**	**0**	**0**	**1**	**0**	**0**	**3**	**1**	**1**	**2**	**0**	**0**	**0**	**1**	**1**	**3**	**5**	**5**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**6**	**0**	**0**	**0**								
TCI foraging groups (separate)																																															
Recruit female	RF	11	9	3	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0.946	0.066	0.0095	0.0055	This study			
Recruit male	RM	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	This study			
Juvenile female	JF	60	13	21	2	0	6	15	1	0	0	0	0	0	3	1	0	1	0	0	0	1	0	2	1	4	0	0	0	0	0	0	0	0	0	2	0	0	0	0.807	0.035	0.0081	0.0044	This study			
Juvenile male	JM	5	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0.900	0.161	0.0074	0.0050	This study			
Subadult female	SAF	8	5	3	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.857	0.108	0.0090	0.0054	This study			
Foraging Adult female	AF	5	4	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.900	0.161	0.0077	0.0052	This study			
Foraging Adult male	AM	3	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	This study			
Breeding female	BF	2	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	This study			
Breeding male	BM	6	4	0	0	1	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.800	0.172	0.0063	0.0042	This study			
Total		**101**	**19**	**31**	**3**	**1**	**6**	**28**	**1**	**0**	**0**	**1**	**0**	**0**	**3**	**1**	**1**	**2**	**0**	**0**	**0**	**1**	**1**	**3**	**5**	**5**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**6**	**0**	**0**	**0**								

* 101 turtles were sexed and sequenced, a further 17 were just sequenced and added.

** groups pooled from separate foraging groups. Young = recruit, juvenile and subadult. All adult is both foraging and breeding adults

[n] new haplotype

† nests pa / 4.1nest per female

[a] includes 42 from Troeng *et al* 2005

[b] includes 94 samples from Velez-zuazo *et al* 2008 and 15 resequenced samples from Bass *et al* 1996

Table S2. Haplotype frequencies using short sequence lengths (481bp) at green turtle rookeries and mixed stocks used in the Mixed Stock Analyses. TCI foraging groups that make up the mixed stock are also listed. N denotes the number of samples in each group. Haplotype diversity (h) and nucleotide diversity (π) was calculated in Arlequin 3.5 (Excoffier & Lischer 2010), the latter using a Tamura 92 3-parameter substitution model. Rookery size (females pa) is calculated from number of nests (Seminoff 2004, Mortimer & Donnelly 2008).

		481 bp CmA Haplotypes		1	2	3	4	5	6	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	29	32	33	35	36	37	38	39	42	44	45	46	48	50	51	52	53	55	56	57	61	62	63	64[n]	h	h SD	π	π SD	Genetic data Source	Size (females pa*)	Distance (km)	Rookery size source
Rookery	Abbrev.	N	No. haps																																																									
Ascension Island	ASCI	245	13	0	0	0	0	0	11	204	9	5	0	0	0	0	0	0	0	0	0	0	0	1	7	1	0	0	0	0	1	0	0	0	0	0	1	0	1	1	2	0	1	0	0	0	0	0	0	0	0	0	0.303	0.038	0.0007	0.0008	Formia *et al* 2007	3800	7090	Broderick *et al* 2006
Aves Island, Venezuela	AVI	67	2	0	0	5	0	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.140	0.055	0.0030	0.0020	Shamblin *et al* 2012	450	1111	Vera 2008
Bioko, Equatorial Guinea	BOK	50	2	0	0	0	0	0	5	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.184	0.068	0.0004	0.0006	Formia *et al* 2006	487	8897	[1] Tomas *et al* 2010
Atol das Rocas & Fernando de Noronha, Brazil	BRZ	69	7	0	0	0	0	0	0	50	7	2	1	5	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.463	0.071	0.0009	0.0009	Bjorndal *et al* 2006	125	4982	[2] Bjorndal *et al* 2006
Tortuguero, Costa Rica	CR	433	5	0	0	395	1	32	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.163	0.023	0.0033	0.0022	Bjorndal *et al* 2005	34804	1754	Troeng & Rankin 2005
[a] Southwest Cuba	CUB	28	7	3	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	1	1	0	0	0	0	0.648	0.089	0.0016	0.0013	Ruiz-Urquiola *et al* 2010	213	1218	[3] Ibarra 2005 cited in Ruiz-Urquiola *et al* 2010
[b] Northern and Greek Cyprus	CYP	61	3	0	0	0	0	0	0	0	0	0	0	0	57	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.126	0.057	0.0003	0.0005	[e] Bagda *et al* 2012	51	9973	[4] Casale & Margaritoulis 2010
Florida, USA	FLO	24	3	11	1	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.562	0.047	0.0013	0.0012	Encalada *et al* 1996	3668	1019	[5] FWCC 2013
Poilao, Guinea Bissau	GBP	51	1	0	0	0	0	0	0	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	Formia *et al* 2006	1500	6037	Formia *et al* 2006
Quintana Roo, Mexico	MEX	20	7	7	0	5	0	1	0	0	0	0	0	0	0	0	1	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.816	0.058	0.0052	0.0033	Encalada *et al* 1996	1547	1568	[6] Seminoff 2004
[c] Gulf of Guinea: Principe & Sao Tome, Gulf of Guinea	PRST	26	7	0	0	0	0	1	1	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.569	0.110	0.0026	0.0019	Formia *et al* 2006	178	8828	[7] Formia *et al* 2006
Galibi, Suriname	SUR	58	3	0	0	1	0	55	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.101	0.054	0.0009	0.0009	Shamblin *et al* 2012	2104	2546	[8] Weijerman *et al* 1998 cited in Seminoff 2004
Turkey	TKY	187	4	0	0	0	0	0	0	0	0	0	0	0	184	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0.032	0.018	0.0001	0.0002	Bagda *et al* 2012	417	10011	[4] Casale & Margaritoulis 2010
Trinidade, Brazil	TRI	99	7	0	0	0	0	0	0	67	19	0	1	0	0	0	0	0	0	0	0	0	0	6	1	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.505	0.052	0.0012	0.0011	Bjorndal *et al* 2006	1200	6501	Almeida *et al* 2011
Buck Island,US Virgin Islands	USVI	49	2	0	0	0	0	45	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.153	0.066	0.0033	0.0022	Shamblin *et al* 2012	33	877	[9] Dow *et al* 2007
Turks & Caicos Islands	TCI	4	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.500	0.265	0.0010	0.0013	Stringell *et al* 2013 in prep	15	0	This study
	Total	**1471**	**42**	**21**	**1**	**437**	**1**	**196**	**19**	**434**	**35**	**7**	**2**	**5**	**241**	**3**	**1**	**5**	**2**	**3**	**2**	**3**	**0**	**7**	**8**	**4**	**0**	**2**	**1**	**0**	**6**	**1**	**1**	**3**	**1**	**2**	**1**	**0**	**1**	**1**	**2**	**5**	**1**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**							
Mixed stocks																																																													
Almofala, Brazil	ALM	117	13	0	0	18	0	28	3	53	3	4	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0.717	0.031	0.0067	0.0038	Naro-Maciel *et al* 2007		4389	
Anguilla	ANG	48	8	3	0	18	0	16	0	7	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0.737	0.038	0.0110	0.0060	Formia *et al* Unpubl.		1004	
Arvoredo Island, Brazil	ARI	115	12	0	0	1	0	25	2	70	5	2	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0.583	0.045	0.0024	0.0017	Proeitti *et al* 2012		6072	
Bahamas	BAH	79	6	2	0	62	0	10	0	1	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.370	0.065	0.0066	0.0038	Lahanas *et al* 1998		429	
Barbados	BAR	60	8	7	0	21	0	13	0	14	1	2	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.773	0.028	0.0105	0.0057	Luke *et al* 2004		1612	
[d] Buenos Aires Province, Argentina	BNA	88	8	0	0	0	0	20	2	59	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.503	0.053	0.0018	0.0014	Prosdocimi *et al* 2012		6749	
Atol das Rocas & Fernando de Noronha, Brazil	BRZ	32	6	0	0	0	0	5	2	20	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.589	0.091	0.0019	0.0015	Bjorndal *et al* 2006		5015	
British Virvin Isles	BVI	66	10	10	0	24	0	22	0	3	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0.741	0.032	0.0107	0.0058	Formia *et al* Unpubl.		849	
Cassino Beach, Brazil	CAB	101	12	0	0	0	0	20	2	62	3	1	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	3	0	0	2	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0.586	0.050	0.0020	0.0015	Proeitti *et al* 2009		6369	
Hutchinson Island, Florida	FLO	62	6	12	1	43	0	3	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.486	0.067	0.0031	0.0021	Bass & Witzell 2000		1002	
North Carolina	NCA	106	12	34	2	43	0	5	0	7	0	0	0	0	0	0	1	2	0	3	0	0	2	0	0	0	2	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.729	0.030	0.0054	0.0032	Bass *et al* 2006		1568	
Sleeping Rocks, Nicaragua	NIC	60	2	0	0	54	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.183	0.062	0.0039	0.0025	Bass *et al* 1998		1476	
Ubatuba, Brazil	UBA	113	10	0	0	2	0	14	0	83	4	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0.446	0.056	0.0021	0.0016	Naro-Maciel *et al* 2007		5842	
TCI juvenile males	TCI.JM	17	5	1	0	13	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.427	0.147	0.0043	0.0028	This study		0	
TCI immature	TCI.Imm	68	11	20	1	25	0	8	0	6	1	1	0	0	0	0	0	1	1	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.758	0.033	0.0077	0.0043	This study		0	
	Total	**1132**	**34**	**89**	**4**	**324**	**0**	**196**	**11**	**385**	**20**	**15**	**0**	**0**	**0**	**0**	**1**	**7**	**2**	**7**	**1**	**5**	**5**	**5**	**9**	**1**	**5**	**2**	**4**	**1**	**10**	**0**	**0**	**2**	**0**	**0**	**2**	**6**	**2**	**4**	**2**	**1**	**0**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**							
TCI foraging groups																																																												
Recruit female	RF	7	4	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.714	0.181	0.0024	0.0020	This study			
Juvenile female	JF	36	6	11	0	15	0	6	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.721	0.046	0.0079	0.0046	This study			
Juvenile male	JM	17	5	1	0	13	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.427	0.147	0.0043	0.0028	This study			
Subadult female	SAF	18	8	4	1	4	0	2	0	4	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.876	0.045	0.0101	0.0058	This study			
Subadult male	SAM	7	3	4	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.667	0.160	0.0046	0.0033	This study			
	Total	**85**	**12**	**21**	**1**	**38**	**0**	**9**	**0**	**6**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**2**	**0**	**0**	**1**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							

[a] Two sites combined

[b] Northern Cyprus (Alagadi and Iskele peninsula): Bagda *et al* 2012; Kaska 2000. Greek Cyprus: Lara Bay

[c] Corisco excluded

[d] Two sites combined: Rio de Plata and El Rincon

[n] new haplotype

[e] also Bowen *et al* 1992; Encalada *et al* 1996; Kaska 2000

*no. nests or mean no. nests divided by 3 (unless stated)

[1] average of 2 seasons

[2] based on Bellini 1995; Bellini & Sanches 1996

[3] Guanahacabibes, Cuba

[4] sum of mean nests per yr / 3

[5] average of 2009 to 2012 / 3.

[6] Yucatan

[7] sum of average of each island estimate

[8] 6313 nests in 1995 / 3

[9] Mean 100-500 tracks * 0.33 nest:false-crawl ratio (Godley *et al* 2001) / 3

Table S3. Hawksbill (A) and green turtle (B) Mixed Stock Analyses foraging ground centric mean contributions ± 95% CIs using models with rookery (source size) weighted priors. Rank contribution and source size shown in parenthesis. Hawksbill long sequence length (740bp) haplotypes were used for the TCI immature mixed stock. Green turtle 481bp sequence length haplotypes were used for the TCI immature and juvenile male mixed stocks. See Table S2 for rookery abbreviations.

A) Hawksbill 740bp		Immature	
Rookery	Relative source size (rank)	Contribution (rank)	95% CI
ANT	0.039 (6)	0.022 (12)	0.000-0.073
BLE	0.145 (3)	0.235 (1)	0.024-0.412
BRZ	0.095 (5)	0.053 (9)	0.001-0.188
BVI	0.003 (14)	0.006 (15)	0.000-0.018
BWI	0.015 (11)	0.049 (10)	0.003-0.118
CRI	0.004 (13)	0.013 (13)	0.001-0.033
CUB	0.158 (2)	0.107 (3)	0.004-0.332
DRJ	0.002 (15)	0.006 (14)	0.000-0.015
DRS	0.013 (12)	0.031 (11)	0.001-0.086
GU	0.017 (10)	0.084 (5)	0.040-0.132
MEX	0.351 (1)	0.093 (4)	0.032-0.173
MON	0.001 (16)	0.003 (16)	0.000-0.009
NIC	0.020 (8)	0.110 (2)	0.044-0.177
PRV	0.097 (4)	0.065 (7)	0.002-0.198
USV	0.022 (7)	0.059 (8)	0.003-0.147
TCI	0.019 (9)	0.065 (6)	0.005-0.139

B) Green turtle 481bp		Immature		Juvenile Male	
Rookery	Relative source size (rank)	Contribution (rank)	95% CI	Contribution (rank)	95% CI
ASCI	0.075 (2)	0.037 (5)	0.036-0.077	0.018 (4)	0.018-0.048
AVI	0.009 (9)	0.019 (8)	0.018-0.044	0.004 (10)	0.004-0.014
BOK	0.010 (8)	0.017 (9)	0.016-0.044	0.006 (9)	0.006-0.020
BRZ	0.002 (13)	0.005 (13)	0.005-0.016	0.001 (13)	0.001-0.005
CR	0.688 (1)	0.229 (2)	0.193-0.198	0.845 (1)	0.200-0.102
CUB	0.004 (11)	0.007 (11)	0.007-0.020	0.002 (11)	0.002-0.007
CYP	0.001 (14)	0.002 (14)	0.002-0.005	0.001 (14)	0.001-0.002
FLO	0.073 (3)	0.378 (1)	0.202-0.208	0.050 (2)	0.046-0.100
GBP	0.030 (6)	0.034 (7)	0.033-0.066	0.015 (5)	0.014-0.043
MEX	0.031 (5)	0.161 (3)	0.104-0.155	0.020 (3)	0.019-0.063
PRST	0.004 (12)	0.006 (12)	0.006-0.017	0.002 (12)	0.002-0.006
SUR	0.042 (4)	0.055 (4)	0.051-0.078	0.012 (7)	0.012-0.036
TKY	0.008 (10)	0.012 (10)	0.011-0.029	0.011 (8)	0.011-0.029
TRI	0.024 (7)	0.037 (6)	0.035-0.068	0.013 (6)	0.013-0.036
USVI	0.001 (15)	0.001 (15)	0.001-0.004	0.000 (15)	0.000-0.001
TCI	0.000 (16)	0.001 (16)	0.001-0.002	0.000 (16)	0.000-0.001

Table S4. Comparison of testosterone thresholds and estimated sex ratios in immature turtles from TCI. For this comparison, sex was assigned solely through testosterone concentrations, even if turtles were of known sex. Turtles of known sex that also had paired testosterone samples (green turtles [Cm], n=55; hawksbill turtle [Ei], n=26) were used to establish the misclassification rate, the difference in numbers of turtles accurately determined and the effect on sex ratio. Unknown sex turtles were those whose testosterone concentrations fell between threshold values.

Source	Species	Testosterone thresholds (pg/mL)		Assigned sex			Mis-classified	% Mis-classified	F : M Ratio	% F	Difference % F	Successfully sexed turtles	Difference from actual
		Male (M)	Female (F)	M	F	Unknown							
This study	Cm	>216	<108	38	87	22	1	1.818	2.3:1	70	1	125	-6
Bolten et al. (1992)	Cm	>200*	<100*	39	84	24	1	1.818	2.2:1	68	-1	123	-7
Average	**Cm**	**>208**	**<104**	**39**	**85**	**23**	**1**	**1.818**	**2.2:1**	**69**	**0**	**124**	**-6**
This study	Ei	>518	<444	7	89	2	0	0	12.7:1	93	1	96	-2
Leon & Diez (1999)	Ei	<200*	<162*	16	80	2	1	3.846	5:1	83	-1	96	0
Diez & van Dam (2003)	Ei	>182*	<170*	16	81	1	1	3.846	5.1:1	84	-1	97	0
Geis et al. (2003)	Ei	>459	<186	8	82	8	1	3.846	10.3:1	91	0	90	-1
Blanvillain et al. (2008)	Ei	>720	<261	6	84	8	0	0	14:1	93	2	90	-3
Average	**Ei**	**>416**	**<245**	**9**	**84**	**5**	**1**	**3.846**	**9.3:1**	**90**	**-1**	**93**	**0**

* Values corrected following Braun-McNeill et al. (2007)

Immature green turtles sexed by testosterone only (n=92; for this study, 76 were successful)

Immature hawksbill turtles sexed by testosterone samples only (n=72; for this study, all successful)

Misclassified samples were of either Male, Female or Unknown determination

% misclassified is calculated from Male or Female Known sex (Unknown category was excluded) i.e. for cm in this study, 1/55*100

Sex ratios calculated from Male and Female determined sex only (Unknown category was excluded)

Difference in % Female is difference to that derived from gonad morphology (known sex samples) plus testosterone only (hormone determined sex) samples

n of successfully sexed turtles is the sum of Male and Female turtles. Difference from actual is in comparison to maximum possible (n=131, cm; n=98, ei)

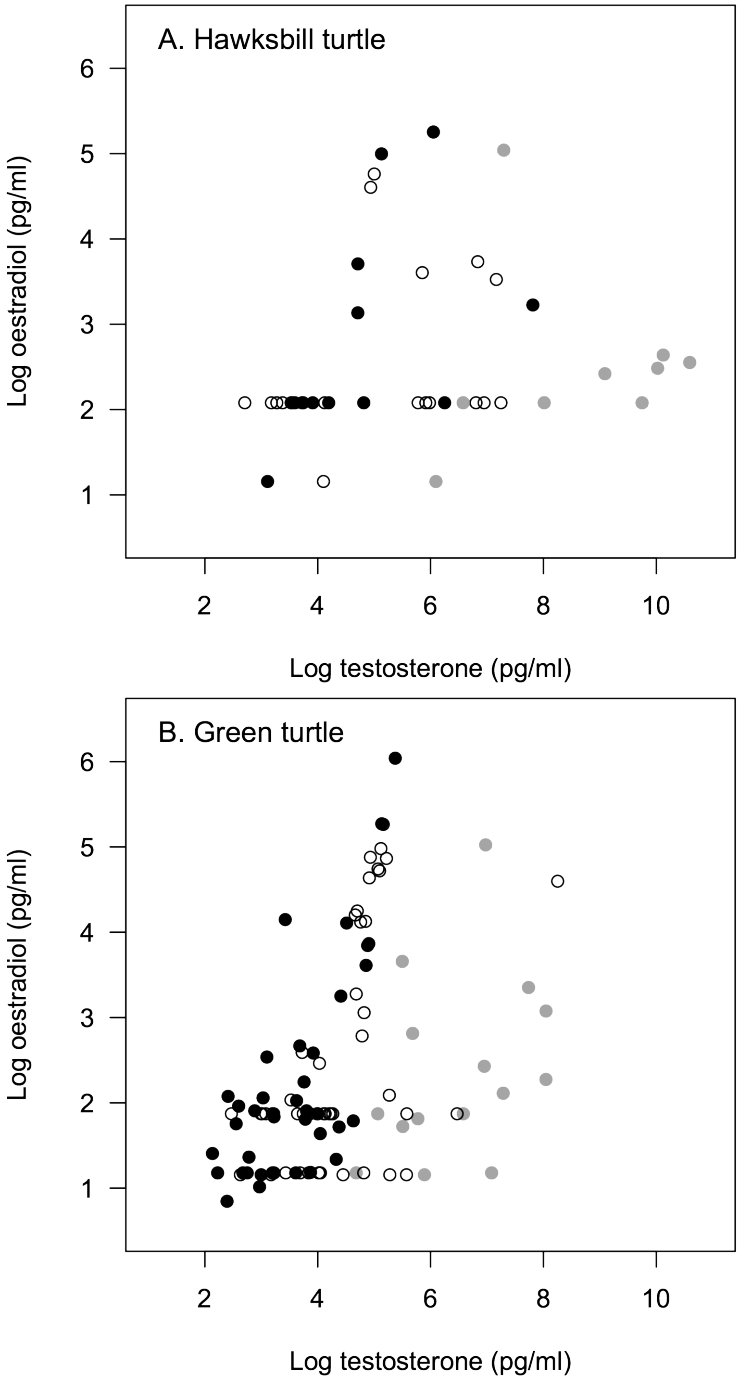

Figure S1. Hawksbill turtle (A) and green turtle (B) log oestradiol-17β agai
testosterone concentrations (pg/ml). Filled circles indicate known sex indiv
from gross morphology or histology of gonads: females (black), males (gre
Unknown sex (no observations of gonads) are empty circles.

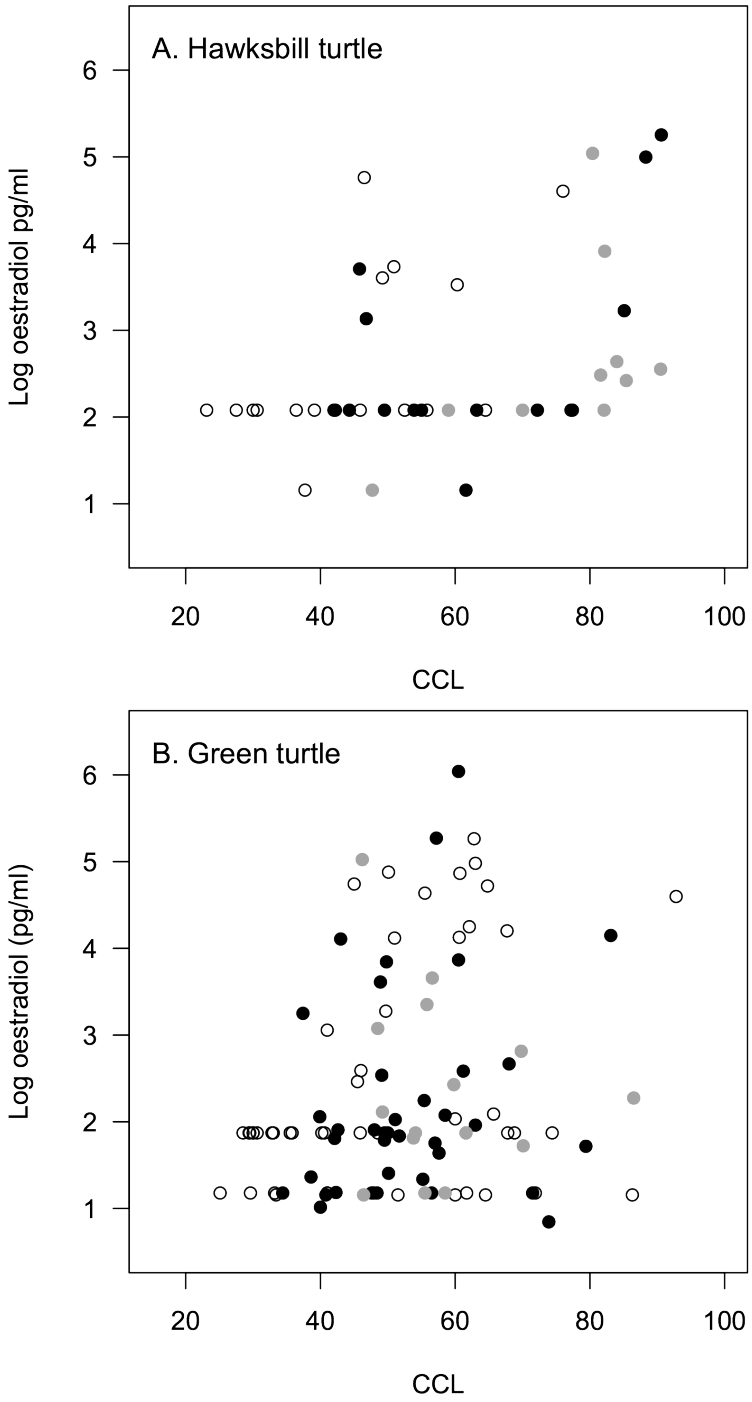

Figure S2. Hawksbill turtle (A) and green turtle (B) log oestradiol-17β (E2) concentration (pg/ml) against curved carapace length (CCL, cm). Filled circles indicate known sex individuals from gross morphology or histology of gonads: females (black), males (grey). Unknown sex (no observations of gonads) are empty circles. Immature green turtle E2 concentrations (pg/ml) ranged from 3.18 to 151.96 in known males (n=15) and 2.33 to 419.77 in known females (n=38). No blood samples were collected from adult green turtles. In immature hawksbills, E2 ranged from 3.18 to 8.00 in known males (n=3) and 3.18 to 40.75 in known females (n=11) and in adult females 25.18 to 191.22 (n=3), and adult males from 8.00 to 154.60 (n=7).

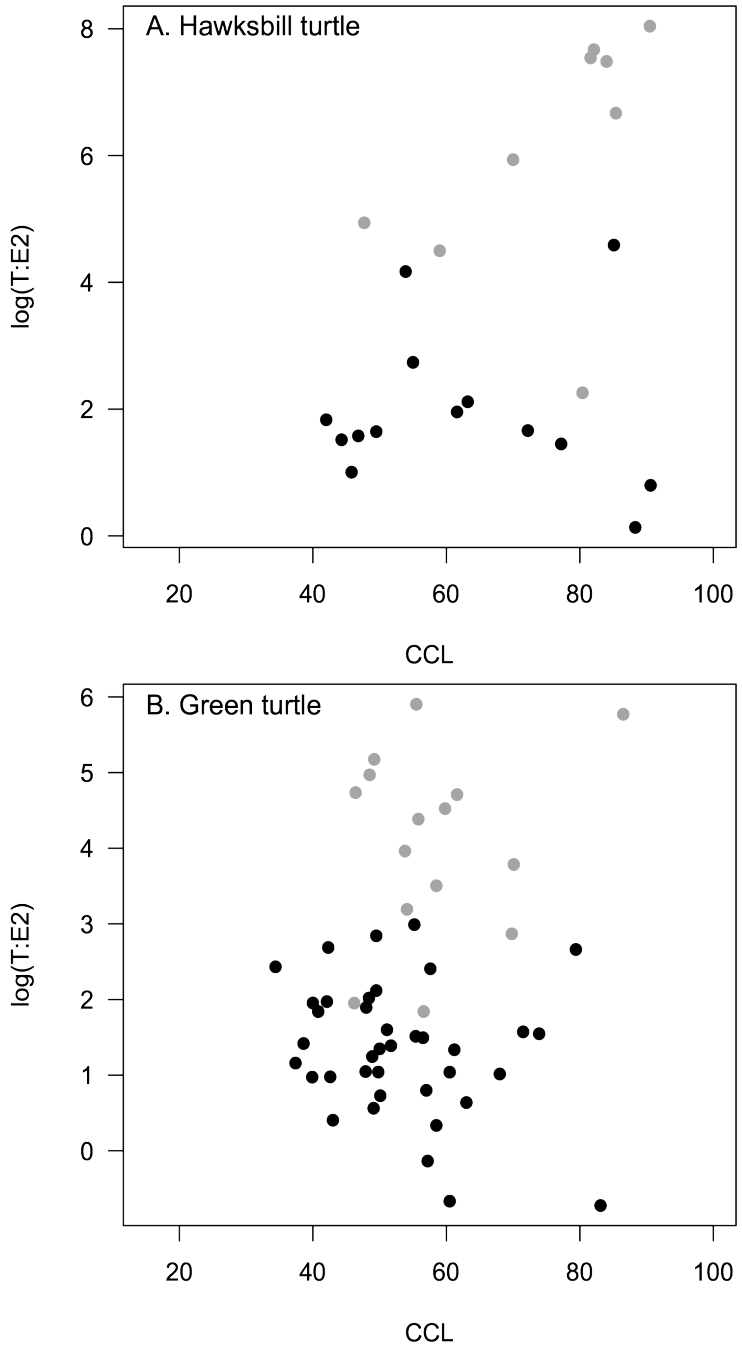

Figure S3. Hawksbill turtle (A) and green turtle (B) log testosterone: oestradiol-17β (T:E2) ratios against curved carapace length (CCL, cm). Filled circles indicate known sex individuals, females (black), males (grey), from gross morphology or histology of gonads. Immature hawksbill turtle T:E2 ratios (unlogged) ranged from 89.9 to 378.0 in known males (n=3) and 2.7 to 64.7 in known females (n=11), and from 9.6 to 3109.2 in adult males (n=7) and 1.1 to 98.2 in adult females (n=3). Immature green turtle ratios ranged from 6.3 to 366.3 in known males (n=15) and 0.5 to 19.9 in known females (n=38). No blood samples were collected from adult green turtles.

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Chapter 5

Taxonomic distinctness in the diet of two species of marine turtle

Thomas B. Stringell[1], Brendan J. Godley[1], Flora Kent[2], Emma Lewis[1], Jessica Marsh[1], Quinton Phillips[3], Peter B. Richardson[1,4], Amdeep Sanghera[4] and Annette C. Broderick[1]

[1]Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ. UK
[2] School of Life Sciences, Heriot-Watt University, Edinburgh, EH14 4AS
[3]Department of Environment and Maritime Affairs, South Caicos, Turks and Caicos Islands.
[4]Marine Conservation Society, Ross on Wye, Herefordshire, HR9 5NB. UK

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Abstract

Understanding the foraging ecology of species is vital to inform effective conservation of the ecosystems in which they function. Marine turtles are considered keystone consumers in coastal ecosystems and their decline through overexploitation has been implicated in the deterioration of reefs and seagrass pastures in the Caribbean. In the present study we analysed stomach contents of green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles harvested in the legal turtle fishery of the Turks and Caicos Islands (Caribbean) to assess the trophic role of these sympatric species. In addition, we carried out habitat surveys. The diet of green turtles (n=92) consisted of a total of 47 taxa: including three species of seagrass (present in 99% of individuals, mostly *Thallasia testudinum*, 95%), 29 species of algae (54%, particularly *Batophora oerstedii*, 18%) and eight species of sponges (28%, particularly *Chondrilla carabensis,* 16%). Hawksbill turtles (n=45) consumed 73 taxa and were largely spongivorous (16 species, sponges present in 100% of individuals, especially *C. carabensis*, 47%) but also foraged on 50 species of algae (present in 73% of individuals, mostly *Padina* spp., 22%) and three species of seagrass (22%, particularly *Syringodium filiforme,* 18%). Plastics were found in trace amounts in 4% and 9% of green and hawksbill turtle stomach samples respectively. There was little overlap in prey items between the sympatric turtle species suggesting niche separation. We used taxonomic distinctness routines to assess the diversity of dietary items found in stomach contents. Green turtles had the most selective diet, whereas hawksbill turtles were less selective than expected compared with relative frequency and biomass of diet items. Results from this study contribute to the fundamental understanding of the foraging ecology of these species and examine their previously suggested keystone roles in maintaining reef and seagrass habitats.

Introduction

The keystone species concept has been central to community ecology since its inception by Paine (1969). The term was originally applied to top predators but has since been relaxed to include species whose presence is crucial in maintaining ecological communities (Mills et al. 1993). Detrimental effects on ecosystems that occur due to the loss of keystone species are widely known; for example, in the northern Pacific, the removal of sea otters during the fur trade, resulted in large increases in their main prey, sea urchins, which caused the collapse of kelp communities through over-grazing (Estes & Palmisano 1974). Marine turtles are large-bodied consumers in coastal ecosystems and are generally considered keystone species; their decline through overexploitation in recent centuries is thought to have contributed to the deterioration of reefs and seagrass pastures in the Caribbean (Jackson 1997, Jackson et al. 2001, Green & Short 2003, Pandolfi et al. 2003, Orth et al. 2006, Waycott et al. 2009). Here, reef-building corals have declined (Bellwood et al. 2004) and in some areas macroalgae and sponges have become dominant (Mumby 2009, McMurray et al. 2010), likely in response to overfishing of their consumers (Mumby et al. 2006, Pawlik et al. 2013).

As the most abundant marine megaherbivore in the Caribbean, green turtles (*Chelonia mydas*) graze principally (but not exclusively) on *Thallasia testudinum* seagrass, and profoundly affect the structure, productivity and nutrient composition of seagrass pastures (Thayer et al. 1982, Thayer et al. 1984, Moran & Bjorndal 2005, 2007, Christianen et al. 2012). It has been suggested that seagrass ecosystems in the Caribbean likely had very different structures and dynamics in times of pre-exploitation of marine turtles, when they existed in huge numbers (Bjorndal & Jackson 2003, McClenachan et al. 2006). Green turtles are thought to maintain grazing plots, and consistent biomass removal increases the nutritional quality of seagrass for the turtle (Thayer et al. 1984) and the speed of nutrient recycling (Thayer et al. 1982). Green turtles are unusual among turtle species in that they are generally herbivorous. However, they have also been recorded as consuming cnidarians, sponges and other invertebrates (Mortimer 1981, Seminoff et al. 2002, Seminoff et al. 2006, López-Mendilaharsu et al. 2008, Arthur et al. 2009; see Bjorndal, 1985 and Bjorndal 1997 for review) and research on Pacific turtle populations suggested immature green turtles are omnivorous (Arthur & Balazs

2008, López-Mendilaharsu et al. 2008). Recent research confirmed the likely ontogenetic shift of green turtles from omnivory in an epipelagic-oceanic habitat (Witherington et al. 2012) during the first three to five years of their lives, to a largely herbivorous diet in coastal-benthic habitats in older turtles (Reich et al. 2007, Arthur et al. 2008). Prey consumed therefore varies within individuals, among populations and through different life stages (Bjorndal 1997). An understanding of diet shifts through the size classes may contribute to our understanding of foraging ecology and the ecosystem roles of green sea turtles.

Hawksbill turtles (*Eretmochelys imbricata*) were originally thought to be indiscriminate omnivores (Carr & Stancyk 1975) but subsequent studies have demonstrated that, although they also consume diverse species of algae (Mortimer 1981, Van Dam & Diez 1997, see Bjorndal, 1997 for review), sponges are probably the primary prey for post-pelagic life stages (Meylan 1988). Post-hatchling hawksbill turtles are thought to have an epipelagic-oceanic stage, similar to green turtles, during which they feed omnivorously on prey in *Sargassum* rafts (see Witherington et al. 2012 for review) before recruiting to coastal areas where they feed benthically on sponges (Bjorndal 1997). In juvenile to adult stages coastal benthic stages, hawksbill diet is thought to be driven by selectivity for certain sponges as well as local abundance of species (León & Bjorndal 2002, Rincon-Diaz et al. 2011).

Sessile sponges rely on toxins, spicules (spike-like skeletal structures) and growth form (e.g. massive form with tough exterior) to deter predators and competitors, and as such there are relatively few sponge predators (Chanas & Pawlik 1995, Pawlik et al. 1995). Hawksbills are the dominant spongivores in reef ecosystems and by removing sponge biomass from reefs, are thought to influence total reef productivity biomass, succession and diversity (Meylan 1988, Bjorndal 1997, Van Dam & Diez 1997); other spongivorous animals, such as nudibranchs, parrotfish and wrasse (Pawlik et al. 1988, Dunlap & Pawlik 1996, Wulff 1997, Dunlap & Pawlik 1998, Hill 1998, Pawlik et al. 2013), do not forage to such an extent (Jackson 1997, Bjorndal & Jackson 2003). Hawksbill turtles reduce sponge overgrowth not only by directly feeding on sponges, but also by exposing the softer inner tissues of sponges, facilitating predation by other species that otherwise would not be able to penetrate the tough exteriors of sponges (Meylan 1988). The precipitous decline of hawksbill turtle populations in the Caribbean, principally from exploitation for their shells (Meylan & Donnelly 1999, McClenachan et al. 2006), has

thus undoubtedly had a profound effect on reef dynamics (Bjorndal & Jackson 2003). Furthermore, predicted effects of climate change on reef and seagrass habitats as a result of rising sea levels and temperatures may make these habitats and associated species vulnerable (Harley et al. 2006, Orth et al. 2006, Hoegh-Guldberg et al. 2007, Hawkes et al. 2009).

Understanding the trophic role and niche width of marine vertebrates is often challenging (Layman et al. 2007). Trophic studies generally require gastric sampling to directly observe what the study species has been eating over a certain time period and location. In comparison to other taxa (Hyslop 1980, Barrett et al. 2007), relatively few studies of sea turtles have utilised stomach sampling (see Mortimer 1981, and Bjorndal 1997 for review, and more recent studies e.g. Brand-Gardner et al. 1999, León & Bjorndal 2002, Seminoff et al. 2002, Arthur & Balazs 2008, López-Mendilaharsu et al. 2008, Arthur et al. 2009, Rincon-Diaz et al. 2011, Santos et al. 2011, Witherington et al. 2012). This is largely due to the logistical difficulties in obtaining samples, which usually involves oesophageal/gastric lavages (see Forbes & Limpus 1993 for technique), or sampling stomachs directly from dead animals through strandings, fishery bycatch or directed take.

In the present study we had the opportunity to collect and analyse stomach contents of green and hawksbill turtles harvested in a legal turtle fishery in the Caribbean (Stringell et al. 2013, Chapter 1). Here, using stomach contents, we set out to assess the trophic role of these sympatric species in the Turks and Caicos Islands. Our aim was to assess dietary preference at an inter-specific level: it was expected that both turtle species would demonstrate clear niche separation, although we were interested in determining the extent of overlap in prey items between species. Secondly we wished to determine whether diets changed with turtle body size (i.e. if an ontogenetic shift existed) and tested the hypothesis of expected specialisation towards herbivory in green turtles and spongivory in hawksbill turtles as they reached maturity. Finally, we discuss this information to elucidate the ecological role of each turtle species and their influence in maintaining Caribbean coastal ecosystems.

Methods

Study Site

The Turks and Caicos Islands (TCI) is a UK Overseas Territory in the Caribbean located at the southeastern end of the Bahamas (21^{o} 45N, 71^{o} 35W) (Figure 1). The low lying limestone islands surrounded by shallow soft sediment areas with mangrove swamps and tidal creeks on the leeward side, contrasting with the fringing reefs and steep drop-offs on the windward side (Doran 1958). The archipelago supports regionally significant foraging stocks of hawksbill and green turtles (Richardson et al. 2009) that are subject to one of the largest legitimate turtle fisheries in the Caribbean (Stringell et al. 2013; Chapter 1). Harvested turtles were sampled for stomach contents, permitting a large number of both species to be studied.

Sampling

Habitat surveys

To characterise the epibenthic macrofaunal communities, shallow (<10m depth) snorkelling surveys were made throughout October 2010. Sixteen survey sites were selected to represent locations where turtle fishing and turtle capture-mark-recapture (CMR) sampling occurred (Figure 1), based on the information acquired during fisher interviews. Reef-based habitats (reef, patch reef, hard bottom and gorgonian plains) and seagrass-based habitats (seagrass, seagrass-algae, algae, coralline algae) were surveyed at these locations, some of which had two or more representative habitats (supplementary Table S1). Approximate survey areas ranged between 0.08 and 1.2 km^2 (see supplementary Table S1). These surveys enabled us to quantitatively describe presence, diversity and abundance of possible prey species at several locations and habitats in order to compare relative proportions of species groups to those found in stomach contents.

The communities at each habitat were described from a total of 1061 photoquadrat images taken at random locations using a housed Canon Powershot G10 digital camera, attached to a 0.25m^2 quadrat framer (the quadrat was divided into 25 cells). Between 14 and 48 photoquadrats were analysed from 15-105 images per habitat (except at Long Cay reef where, due to water depth, only six quadrats were photographed and analysed; supplementary Table S1). At each habitat in each

location, a sample of two to four quadrats were surveyed *in situ* to validate photoquadrat data and species abundance was enumerated by cell frequency counts (see supplementary methods for further details).

Turtles

For two years (from November 2008), we monitored the legal turtle fishery at key landing sites throughout TCI (see Stringell et al. 2013, Chapter 1 for details). Turtle capture location was estimated following fisher interviews. Turtle size was measured along the midpoint of the carapace (Curved Carapace Length cm (CCL): Bolten 1999). The sex of turtles was determined by gross morphology and histology of the gonads of butchered animals or external morphology in adults (see Stringell et al. in prep. for further detail: Chapter 4).

Stomach content samples from 45 hawksbills and 92 green turtles of various sizes were collected directly from butchered animals. Owing to the large volume of digestive material in the gut we chose to collect the contents of the stomach and upper digestive tract. Samples were frozen until sorting.

Turtle stomach contents

Individual stomach contents were sorted and wet mass of each taxon weighed to the nearest 0.01g after blotting dry (Hyslop 1980). If a species weight was <0.01g it was recorded as trace. We also recorded the degree of digestion (categories: fresh, moderate, severe) to aid with further interpretation; most samples were relatively well preserved. Dietary items were identified to the lowest taxonomic level (see supplementary methods for further details).

Data analysis

All multivariate statistical routines were carried out in PRIMER v6 software (Clarke & Gorley 2006) with the PERMANOVA+ add on (Anderson et al. 2008) and univariate tests in R v 2.12 (R Development Core Team 2012).

Habitat

Differences in abundance data (Bray-Curtis similarities of photoquadrat data) between habitats were tested with a one-way permutational multivariate analysis of

variance (PERMANOVA) and for differences in multivariate dispersion by permutation (PERMDISP) (Anderson et al. 2008). Species were also grouped into nine categories: seagrasses, sponges, bluegreen algae, green algae, red algae, brown algae, cnidarians, invertebrates and unknown; and grouped into broad habitat types (reef-based and seagrass-based habitats) with the purpose of comparing turtle stomach content data and tested with a one-way analysis of similarities (ANOSIM, Clarke 1993). (See supplementary methods for further details, including the application of taxonomic distinctness to habitat samples - see below for description of these diversity metrics).

Stomach content

Species biomass was standardised (by total) to account for differences in stomach fullness, and square root transformed. Bray-Curtis similarities were used for subsequent resemblance based tests and visualised in a non-metric multi-dimensional scaling (MDS) with a vector plot overlay of diet species most correlated with the pattern (Clarke 1993). A similarity of percentages (SIMPER) routine (Clarke 1993) was used to examine differences in species composition between turtle species. Differences between turtle species, *a priori* grouping factors (habitat, sex) and turtle size as a covariate were tested using 3-way crossed multivariate permutational analysis of covariance (PERMANCOVA) (Anderson et al. 2008) with permutations under a reduced model and Type 1 (sequential) sums of squares, and non-significant interaction terms sequentially removed. Differences in dispersion among groups were tested using PERMDISP. Diet species were then grouped into nine categories (as above) and visualised for differences in diet groups with size (CCL) between the two turtle species, and tested with a one-way ANOSIM. We compared the relative abundance of these diet groups in hawksbills and green turtles against the relative abundances of the same groups identified in reef and seagrass habitats respectively using a Pearson's Chi-square analysis with Monte Carlo simulated P-values from 10,000 replicates.

To determine how representative stomach content samples were in relation to species available in the habitat, average taxonomic distinctness (AvTD) and variation in taxonomic distinctness (VarTD) were assessed for stomach content samples by turtle species (Clarke & Warwick 1998, Clarke & Warwick 2001a). These diversity measures are based on the relatedness of species drawn at random from a sample,

are independent of the number of species (a better statistical sampling property than richness related estimators), and can be used to compare data from differing sampling effort, spatial and temporal scales (such as stomach samples and habitat species lists) (Clarke & Warwick 1998, Clarke & Warwick 2001a). Here, taxonomic distinctness is defined from a Linnaean tree (taxonomic aggregation file) of macrobenthic species likely in TCI. A regional master list of 565 species was created from species identified in the habitat surveys, stomach content analysis and from searches of databases of sponge, gorgonian, coral, seagrass and algae species previously recorded in TCI and neighbouring Bahamas from the World Register of Marine Species (WoRMS) database (Appeltans et al. 2012).

The two taxonomic distinctness measures were used in a taxonomic distinctness test (TAXDTEST Clarke & Gorley 2006), where stomach content sample data were superimposed on a funnel plot of expected AvTD and VarTD 95% probability limits created from randomised draws of sublists of 2 to 20 species from the regional master list. The weighting of Linnaean tree step lengths was guided by taxon richness of the master file (Clarke & Warwick 1999) and the simulation of random draws was weighted by the frequencies of species found in the habitat surveys (Clarke & Gorley 2006). A Mann-Whitney U test was used to formerly compare the differences in AvTD and VarTD between species.

Stomach sample species richness (S), Simpsons evenness (1-Lambda, calculated on Pi - proportion data: Clarke & Warwick 2001b), AvTD and VarTD were plotted against CCL and tested with GLMs or GAMs after initial exploration of linearity.

Results

Habitat surveys

There were significant differences in species abundance among surveyed habitats and these differences were driven largely by seagrass and algae species (Spearman correlation >0.5) (PERMANOVA: Pseudo-F_7=78.6, P_{perm}=0.001: supplementary Figure S1). Dispersion among habitats was significantly different (PERMDISP: $F_{7,810}$=81.9, P_{perm} =0.001) with patch reefs having the highest mean dispersion (58.9±0.4 SE) and coralline algae habitats having the least (26.0±1.8) (supplementary Figure S1). Grouping species in each photoquadrat into eight categories indicated clear differences between their relative proportions in the broad habitat types, such that algae and cnidarians were more common in reef habitats and seagrass were absent (Reef-based vs. Seagrass-based, ANOSIM: R=0.753, P=0.001: Figure 2).

We identified 108 species of plants and animals from the photoquadrat images. Green algae (Chlorophyta) were the most diverse taxonomic group with 22 species and *Halimeda* the most common genus in this group. Reef habitats were most diverse (had the greatest species richness) but the gorgonian habitat at Harbour (site 10 in Figure 1) was the single most diverse site with 41 species identified (supplementary Table S1). Seagrass density ranged from 15.6 – 148.5 shoots/m^2 (supplementary Table S1). Analyses of taxonomic distinctness of habitat samples (see supplementary methods) indicated that reef based habitats (reef, patch reef, hard bottom and gorgonian plains) were more distinct than seagrass based habitats (seagrass, seagrass-algae, algae, coralline algae); reef photoquadrats mostly fell within the 95% AvTD funnel of the regional expectation, but were generally more variable than expected in terms of VarTD; this pattern was converse with Seagrass based habitats (supplementary Figure S2). These findings indicate that our habitat surveys were likely representative of the species likely to be found in the region.

Stomach content

We identified a total of 93 species in 137 turtle stomach samples (47 species in 92 green turtle stomach samples, and 73 species in 45 hawksbill samples; supplementary Table S2). In green turtles, the diet was mainly herbivorous

(approximately 92% seagrass and algae by biomass) but with varying amounts of sponge (average 7%; Table 1, supplementary Table S2): the seagrass, *Thalassia testudinum* contributed the greatest to biomass (73%). This was followed, in decreasing order, by the seagrass *Syringodinum fiiforme* (16%), the sponge *Chondrilla caribensis* (formerly *C. nucula*) (4%), and the seagrass *Halodule beaudettei* (2%). Remaining species contributed <1% each. *T. testudinum* was found in 95% of all green turtle stomach samples, *S. filiforme* and *H. beaudettei* in 58%, the green algae *Batophora oerstedii* in 18% and *C. caribensis* in 16%. Plastics were found in 4% (n=4) of green turtle stomach contents in trace amounts.

In hawksbill turtles, diet was more varied and omnivorous, with individuals mostly consuming sponges and algae (approximately 99% by biomass) (Table 1, supplementary Table S2): 27% of the biomass comprised of the sponge *C. caribensis*, followed by the sponges *Sidonops neptuni* (17%), *Halichondria melanadocia* (16%), *Scopalina ruetzleri* (8%), *Cinachyrella alloclada* (5%), *Erylus formosus* (4%), and the red algae *Gelidiella acerosa* (3%) and an unidentified red algae (2%). Remaining species contributed <2% each. The most frequently occurring species in the stomach samples were the sponges *C. caribensis*, *H. Melanadocia, S. neptuni* (47%, 29%, 24% respectively) followed by the brown algae *Padina* spp. (22%), the red algae, *Gelidiella acerosa* (18%) and the seagrasses *S. filiforme* and *T. Testudinum* in 18% and 16% of samples respectively. Plastics were found in 9% (n=4) of hawksbill turtle stomach contents in trace amounts.

Green turtles measured between 28.8cm and 88.0 cm (mean=52.8 ± 12.6 SD, n=91) and hawksbills measured between 39.3cm and 91.2 cm (60.4 ± 14.0, n=45). No significant differences were found in diet composition (Bray-Curtis similarities of standardised biomass) with body size, sex and habitat type in which the turtle was found, but there was a clear difference in diet composition between turtle species (PERMANCOVA, turtle species factor: Pseudo-F_1=58.9, P_{perm}<0.001). A SIMPER analysis confirms that *Thalassia* and *Syringodium* seagrasses, *Chondrilla*, *Sidonops* and *Halichondria* sponges together contributed 70% to the dissimilarity between the turtle species: *Thalassia* made the largest contribution and explained 32% of the dissimilarity, and *Chondrilla* explained 13%, with their average abundance being highest in green turtles and hawksbill turtles respectively (Figure 3). There were significant differences in diet variability (multivariate dispersion of Bray-Curtis similarities) between turtle species found at reef and seagrass habitats (PERMDISP:

$F_{3, 123}$ = 18.486, P_{perm}= 0.001). For example, hawksbill turtles captured on reef habitats had the highest mean dispersion of 62.6 ±1.3 (SE) and from seagrass habitats, 53.5 ±6.3, which were not significantly different (PERMDISP: $F_{1, 38}$ = 4.496, P_{perm}= 0.331). Green turtles had significantly lower dispersion than hawksbills from both habitats; green turtles from reefs had a mean dispersion of 35.8±4.0, and from seagrass habitats, 25.7 ±1.7 (PERMDISP: $F_{1, 85}$ = 7.322, P_{perm}= 0.036), suggesting green turtles had the narrowest range of diet of the two species, especially from seagrass habitats.

Grouping stomach content species into diet categories indicated clear differences between the turtle species (ANOSIM: R=0.957, P=0.001: Figure 2) but revealed no discernible pattern with size (supplementary Figure S3), either as a continuous predictor or grouped into 10cm size classes. Turtle size also did not significantly explain the diversity of species in turtle diet expressed as Species richness (S), Species evenness (Simpson's), or VarTD, but there was a weak suggestion of size partitioning in AvTD for green turtles with the taxonomic breadth of diet reducing with larger sizes (GAM: P=0.04) (Figures 4-5).

The analysis of AvTD (on presence-absence stomach content data) indicated that hawksbill turtle stomach samples did not depart significantly from the 'funnel of 95% confidence', indicating that hawksbill turtles fed randomly on what was available in the habitat, that is, their varied diet consisted of species that were as taxonomically related as those chosen at random from a species list of >500 species (Figure 6a). However, 43% (n=40 of 92) of green turtles had significantly lower (P<0.05) AvTD than expected, indicating that they may exhibit strong dietary selectivity, that is, a relatively taxonomically narrow diet in comparison to the habitat, although 57% of individuals had relatively taxonomically wide diets that fell within the habitat probability limits (Figure 6a). There was much less departure from probability limits in the case of VarTD for both species (5%, n=5 of 92 green turtles; no hawksbill turtles), indicating similar variation in distinctness between species in turtle stomachs to those chosen at random (Figure 6b). This result is confirmed by a formal test of these metrics with significantly greater average taxonomic breadth (AvTD) found in hawksbill turtle stomach samples (Wilcoxon: W = 1555, P = 0.01813) but not for VarTD (W = 2193, P = 0.5685).

There was a significant difference between relative percentage biomass of the nine diet groups in average hawksbill turtle stomach content samples and the

relative abundances of these same groups from average reef-based habitat photoquadrats (X^2=164.89, P_{perm}<0.001); that is, many of the same species were present in stomach content samples and the habitat, but not consumed at the same relative proportions. For example, sponges were found in much higher proportions and brown algae at lower proportions in hawksbill turtle stomach content samples than in reef habitats (Figure 2a). Green turtle stomach content samples and seagrass-based habitats also had significantly different relative proportions (X^2=25.67, P_{perm}<0.001), although there was an apparent similarity in seagrass proportions (Figure 2b). These data, which are based on the amounts of each diet item, have differing inferences to the results of the taxonomic distinctness routines, which are based on presence-absence data and Linnaean relatedness.

Discussion

Knowledge of supporting habitats is essential to inform our understanding of the foraging ecology and role of sea turtles in coastal ecosystems. In this study we used an interdisciplinary approach that linked habitat to the diet of two sympatric marine turtle species; to our knowledge, this study is the first to examine taxonomic distinctness of the diet of marine turtles. These taxonomic routines are especially useful for comparison of data sets collected over different temporal or spatial scales and sampling effort (Clarke & Warwick 1998, 2001b). We demonstrated clear niche separation between the two turtle species, but found no evidence of diet shifts with size (ontogenetic shifts) from stomach contents, although AvTD suggested a non-linear change with size in green turtles (see supplementary information, Figure S3).

Apart from seagrasses, the relative proportions of prey species in green turtle stomachs did not statistically match those in seagrass habitats, especially for red algae, green algae and sponge proportions. This suggests a selective feeding strategy and a functional linkage between consumer and habitat that supports the findings of others (Table 1, Bjorndal 1980, Mortimer 1981, Bjorndal 1997, Van Dam & Diez 1997, León & Bjorndal 2002, Seminoff et al. 2002, Rincon-Diaz et al. 2011, Santos et al. 2011). In green turtles, the AvTD routine indicates that for nearly half of the stomach content samples, the relatedness of species in the diet was less taxonomically distinct than that of the species available in the surrounding habitat, also suggesting a degree of selective feeding. The relatively low distinctness in their diet is likely a result of the narrow taxonomic distinctness of the three seagrass species that make up the majority of green turtle diet (in terms of biomass), which are derived from just two families. However, the several algae species (>5% frequency, mainly Chlorophytes: Table 1) found in green turtle stomachs may have elevated the taxonomic distinctness of the stomach samples so that 57% fell within the funnel of taxonomic expectation. Although green turtles can be found in both reef and seagrass habitats, the lower taxonomic distinctness of green turtle diets compared to hawksbill turtles may be largely a result of seagrass-based habitats having lower species diversity than reef-based habitats.

Conversely, since hawksbill turtles are most commonly associated with reef-based habitats (but see Bjorndal & Bolten 2010), we might expect hawksbills to have a diet more diverse than that of green turtles and one that reflects the diversity of

species found in reef systems. In terms of taxonomic breadth (AvTD), we demonstrated that this is the case, with every sample falling within the funnel of taxonomic expectation, suggesting they are generalists/indiscriminate feeders that graze randomly (sensu Carr & Stancyk 1975) and have a diet representative of available species. However, in terms of relative abundance of diet type, hawksbill turtle diet was not representative of reef habitat, a finding that supports selective feeding mostly on sponges and algae (Bjorndal 1997, Van Dam & Diez 1997, León & Bjorndal 2002, Rincon-Diaz et al. 2011). This may be due to several reasons: 1) sponges house many symbiotic, parasitic and commensal animal and plant species (which may have more nutritional value than the sponges themselves) - increasing the apparent taxonomic breadth of diet, 2) sponges may not be easily digestible or nutritious (Bjorndal 1985) and may remain in the stomach longer than other readily digestible taxa, 3) presence-absence data in taxonomic distinctness routines gives equal weighting to rare species; these routines are diversity measures that examine the relatedness of species rather than their abundance, and 4) sponges are from a phylum of especially wide taxonomic breadth; two species of sponge may be as distinct from each other as two unrelated species drawn at random. (This also applies to algae, which encompass several kingdoms and phyla). Caution must therefore be taken when making comparisons with other studies that used abundance or biomass measures because the taxonomic distinctness routines tells us about relatedness not relative proportions. Nevertheless, the breadth of diet as determined by AvTD is a useful companion to relative diet proportions. Furthermore, the findings using this measure are reflected in a stable isotope analyses (SIA) of the same population of hawksbill turtles that show highly mixed diet sources that are not dominated only by sponges, suggesting more of a generalist diet (see Stringell et al. in prep., Chapter 6).

Many of the diet species identified in turtle stomachs are found across the different habitat types and at most locations. For example, the sponge *C. caribensis* occurred in both reef and seagrass habitats. The form of *Chondrilla* (*C. caribensis f. caribensis*) commonly found in hawksbill and green turtle stomachs from our study is more usually associated with seagrass habitats, and over 16% of hawksbill stomachs contained seagrass, suggesting the importance of seagrass habitats to foraging hawksbill turtles (Bjorndal & Bolten 2010). Several sponge species were also found in green turtle diet. While consumption of sponges by green turtles has

been previously reported (Bjorndal 1990), the extent of the finding is surprising. Sixteen percent of samples contained *C. caribensis*, indicating this sponge is likely to be purposefully consumed. Further, Figure 3 illustrates that one green turtle had a diet dominated by sponges, perhaps representing active consumption of these taxa.

One caveat that may have implications on making inferences from our data is that habitat surveys were restricted to shallow depths (<10m), while foraging turtles clearly dive much deeper (e.g. Blumenthal et al. 2009). Diving ability in marine turtles scales with body size (Schreer & Kovacs 1997) and size partitioning by depth is well known (Musick & Limpus 1997). Once turtles recruit from the pelagic zone and settle in TCI they are probably limited to shallow habitats that contain seagrass and patch reefs, while larger turtles are able to forage at greater depths where other food types are found. Consequently, we may have surveyed the habitat of smaller turtles but not larger ones. Therefore the relative abundance of species in our habitat surveys may not fully represent what is found in turtle stomachs. Most published studies that link habitat type to stomach contents are also restricted to shallow survey depths and typically survey only those species that were identified in stomachs (Van Dam & Diez 1997, León & Bjorndal 2002, Rincon-Diaz et al. 2011), biasing the availability of species in random surveys. The taxonomic distinctness routines remove this bias by using species lists (Clarke & Warwick 1998, 1999, 2001b). In our case, a comprehensive list of species recorded primarily from the Bahamas region was used and random draws taken from this list were directed by the relative frequencies of species found in our habitat surveys. This provides a much more robust assessment of habitat linkage than surveys of only those species selected from stomach samples. Nevertheless, taxonomic distinctness assesses diversity rather than abundance, and complements rather than replaces analyses of relative abundance. Further work with this diversity measure would be advantageous for building our knowledge of foraging ecology in marine turtles.

Stomach contents represent only a snapshot of feeding by marine turtles and may not adequately relate to what is assimilated into bodily tissue over time. This is a key disadvantage with stomach content analysis (Duffy & Jackson 1986, Barrett et al. 2007). Diet varies considerably between individuals and locations (Bjorndal 1997) but can also vary in individuals through time, as demonstrated by the different diet components found along the alimentary canal of a green turtle (Arthur et al. 2009). Additionally, some prey species may have been completely digested in

stomach samples, precluding their identification. Videos from animal-borne cameras on green turtles from California (Seminoff et al. 2006) suggest the importance of cnidarians and algae to green turtle diet, so it is possible that soft bodied invertebrates and readily digestible (non-cellulose based) algae are underrepresented in our study, although we note that most stomach samples in our study were surprisingly well preserved, an observation also shared by Mortimer (1981).

Green turtles undergo ontogenetic shifts where small oceanic-pelagic juveniles recruit to coastal-benthic habitats and switch from omnivorous/carnivorous to herbivorous feeding, as demonstrated using SIA (Reich et al. 2007, Arthur et al. 2008, and Stringell et al. in prep., Chapter 6). Stringell et al. in prep. (Chapter 6) suggest that a similar ontogenetic shift also occurs in hawksbills. Part of the present study was to investigate if a similar shift in forage could be observed in stomach content with increasing size of turtles. We might expect to see a shift from omnivory/carnivory to herbivory in green turtles and to omnivory - at a lower trophic level due to intake of sponges rather than higher animal taxa - in hawksbills. Our stomach content analyses results do not show this shift: there were no apparent differences in prey contributions with size in either species. One possible explanation is that small recruits were unlikely to have been well represented in our sample of the fishery - small turtles are less desirable to eat due to low meat yield for processing time and are below legal catch size (Stringell et al. 2013, Chapter 1). Additionally, the size at which hawksbills recruit to coastal habitats is thought to be smaller than that of green turtles (Meylan et al. 2011), therefore the smallest hawksbill in our study may well have been resident for some time. In a SIA by Stringell et al. in prep. (Chapter 6), a possible change from herbivory to omnivory was noted in large juvenile sized green turtles where seagrasses contributed less to the diet. This was not reflected in the stomach content analyses. Larger size green turtles (large juveniles to adults) were also not well represented in the fishery, most likely due to the effort required to catch them and their relative abundance at these sizes (Stringell et al. 2013, Chapter 1). Although the present study had a large sample size, some size classes were not well represented and further sampling of small and large animals would help address this bias.

Given the sponge and algae dominated, yet taxonomically broad, diet of hawksbills, and the selective grazing of green turtles, these sympatric turtle species

are likely to play key grazing roles in Caribbean seagrass and reef systems. Generally there was little overlap in the significant components of diet samples between the sympatric turtle species, suggesting niche separation. Both green and hawksbill turtles are among the largest grazers in the tropics and are thought to have critical roles in regulating the structure and function of reef and seagrass habitats (Bjorndal & Jackson 2003). Some sponge species, notably *C. caribensis,* are superior competitors with corals in reef habitats (Hill 1998, Wulff 2012). Hawksbills, as spongivores, thus undoubtedly play a key role in the ecological interactions between this species and many other sponges, corals and algae. We are gradually building a more complete picture of the ecological dynamics that relate habitat to consumers and predators. Heithaus et al. (2007) suggested that declines in seagrass beds in Bermuda may be linked to increases in green turtle populations (Murdoch et al. 2007), which coincide with declines in tiger sharks in the northwest Atlantic (Baum et al. 2003). This suggests top-down effects of marine predator declines may be profound (Heithaus et al. 2008) not only on regulating the abundance and distribution of grazers (turtles) but on the structure and function of habitats (Thayer et al. 1984). Furthermore, reefs and seagrass beds are vulnerable to climate change, as are the consumers that rely on them (Harley et al. 2006, Orth et al. 2006, Hoegh-Guldberg et al. 2007, Hawkes et al. 2009, Poloczanska et al. 2009, Witt et al. 2010, Fuentes et al. 2011, Hamann et al. 2013).

Along with climate change, removal of large grazers (turtles, manatees) in the Caribbean over an historic timeframe has been implicated in widespread ecosystem changes to reef and seagrass habitats (Bjorndal & Jackson 2003). Present day turtle fishery harvests in the Caribbean are a fraction of what they used to be (McClenachan et al. 2006) but substantial harvests still exist, notably in Nicaragua (Lagueux et al. 2003) and TCI (Stringell et al. 2013). Although the green turtle harvest in TCI is considerably smaller than that of Nicaragua, it is nevertheless substantial and the TCI hawksbill harvest is one of the largest in the western Atlantic (Stringell et al. 2013, Chapter 1). This fishery is therefore likely to have an effect on coastal ecosystem functioning if turtles play their supposed keystone roles.

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Table 1. Frequency (proportion of turtles in which present) and average (±SD and range) proportion of biomass of taxonomic diet groups found in stomach content samples of green turtles (n=92) and hawksbill turtles (n=45).

Diet group	Green turtle				Hawksbill turtle			
	Proportion of turtles	Biomass			Proportion of turtles	Biomass		
		Mean	±SD	Range		Mean	±SD	Range
Seagrasses	0.99	0.91	0.17	(0.00 - 1.00)	0.22	*	0.01	(0.00 - 0.04)
Red algae	0.26	0.01	0.10	(0.00 - 0.97)	0.49	0.10	0.20	(0.00 - 0.70)
Brown algae	0.08	*	0.01	(0.00 - 0.10)	0.49	0.02	0.04	(0.00 - 0.18)
Green algae	0.32	*	0.02	(0.00 - 0.18)	0.49	*	0.01	(0.00 - 0.07)
Unknown algae	0.03	*	*	(0.00 - 0.01)	0.04	*	0.01	(0.00 - 0.07)
Sponges	0.28	0.07	0.14	(0.00 - 0.55)	1.00	0.88	0.21	(0.30 - 1.00)
Cnidarians	0.03	*	*	(0.00 - 0.04)	0.02	*	*	(0.00 - 0.01)
Invertebrates	0.03	*	*	(0.00 - *)	0.09	*	*	(0.00 - *)
Plastic	0.04	*	*	(0.00 - *)	0.09	*	*	(0.00 - *)

* = <0.01 (trace)

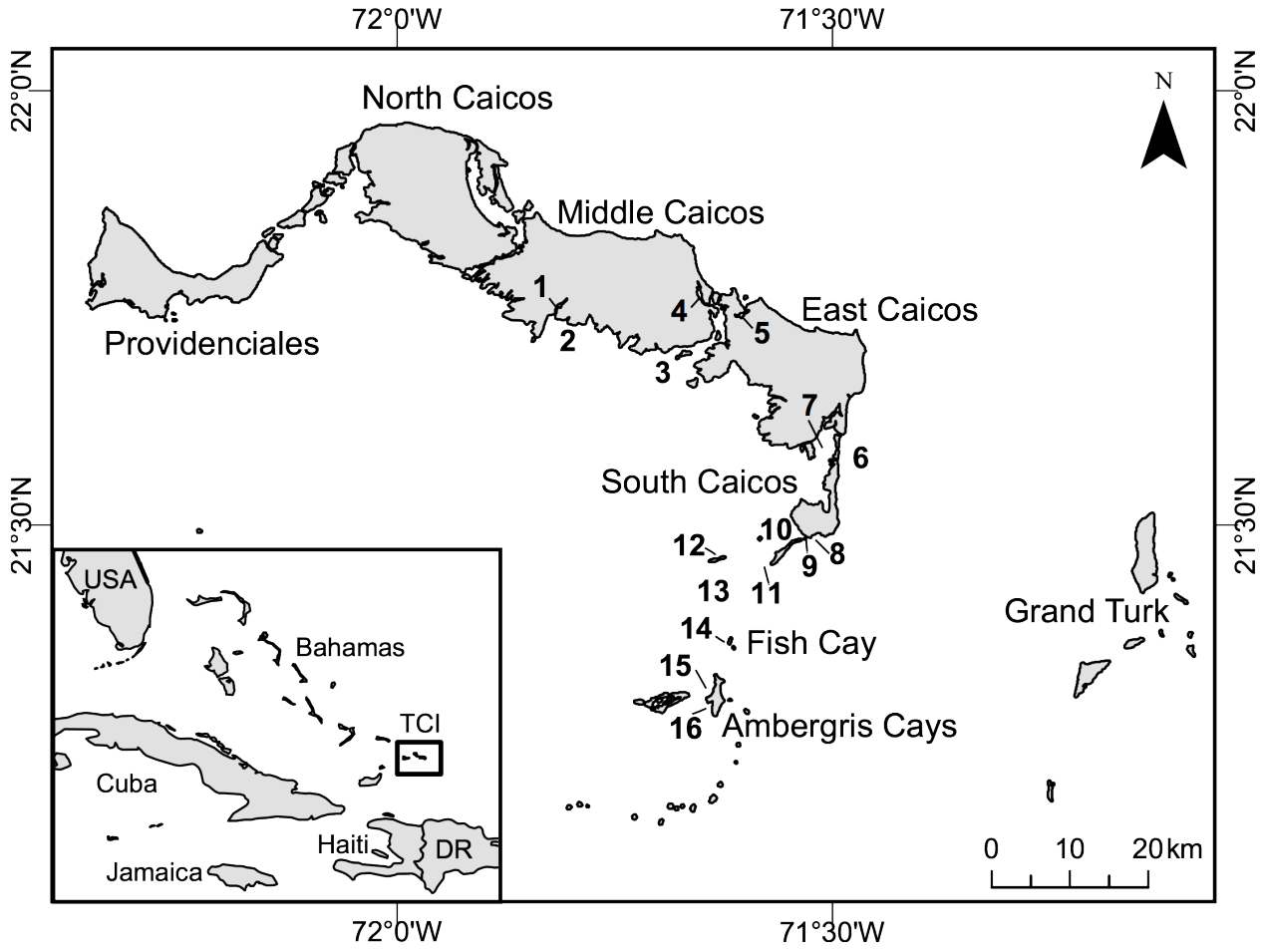

Figure 1. Map of Turks and Caicos Islands (TCI) and location in Wider Caribbean Region (inset, DR=Dominican Republic). Numbers indicate the following survey sites: 1=Man-o-War, 2=Ocean Hole, 3=Southern Bush, 4=Larmer Creek, 5=Jacksonville, 6=Eastside, 7=Nuisance Point, 8=Tuckers Reef, 9=Shark Alley, 10=Harbour, 11=Long Cay, 12=Six Hills, 13=Middle Reefs, 14=Fish Cay, 15=Ambergris, and 16=Ambergris Airport. See supplementary Table S1 for further information on sites, habitats and sampling effort.

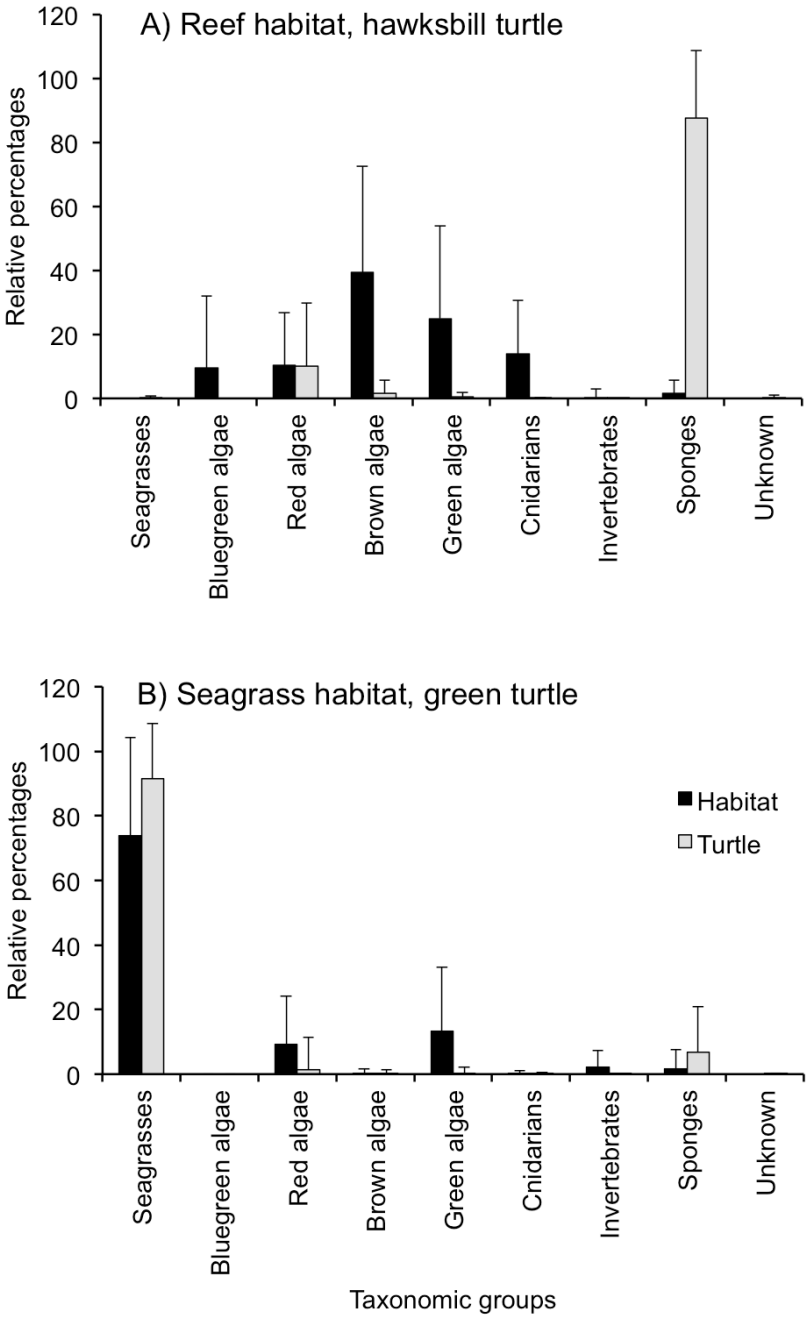

Figure 2. Average relative percentages (± 1 SD, error bars) of taxonomic diet groups found in reef (A) or seagrass (B) habitat photoquadrats (abundance: n=736) and hawksbill (A) and green (B) turtle stomach samples (biomass: n=137). Habitats are represented by black bars and turtle species by pale grey.

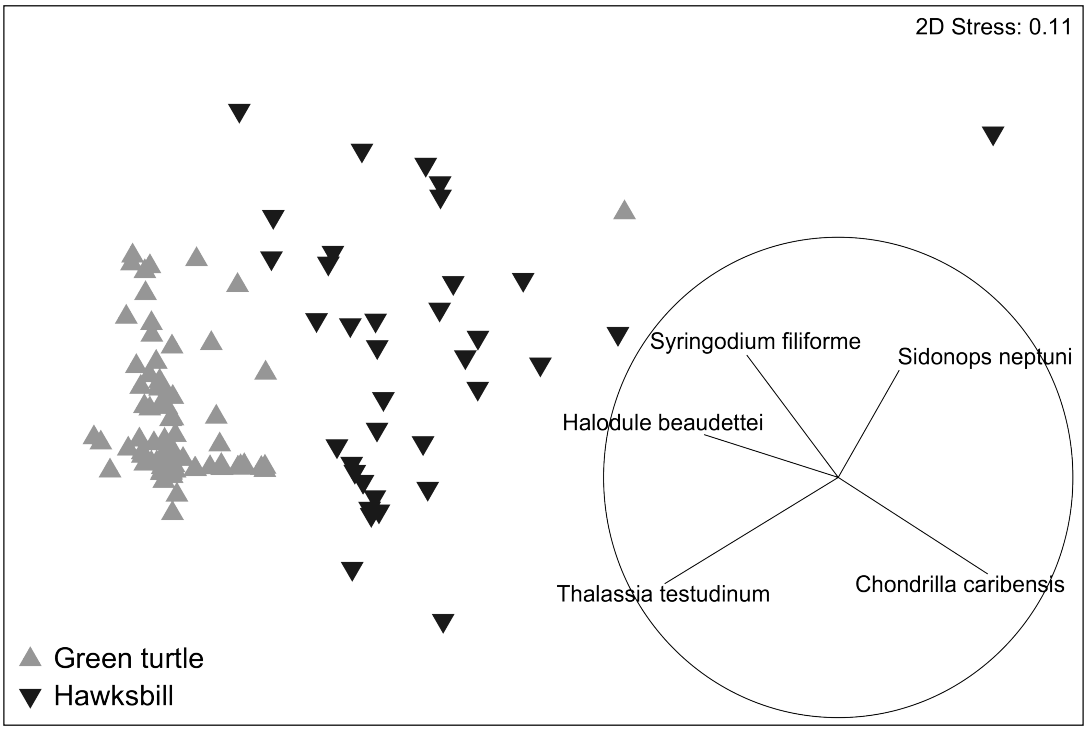

Figure 3. Non-metric multidimensional scaling ordination of stomach content with vector overlay of most contributing species (R>0.5 Spearman's correlation; derived from SIMPER analysis). Stomach content biomass data are standardised, square root transformed Bray-Curtis similarities. Three hawksbill turtle outliers (not shown) lie outside of plot boundary to the northeast and were dominated by *Sidonops neptuni* in their diet.

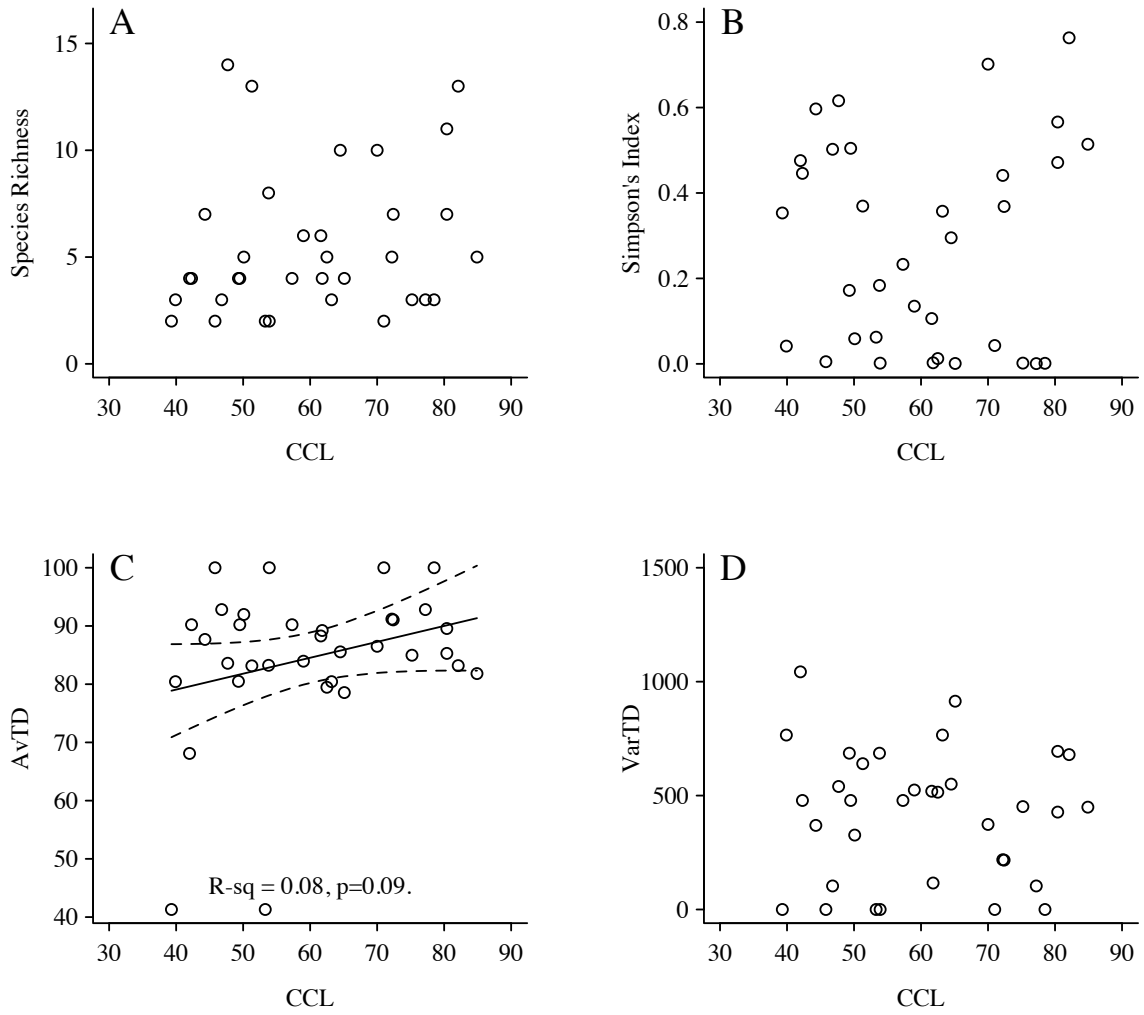

Figure 4. Species diversity measures of stomach content samples against hawksbill turtle size (CCL, cm). (A) species richness, (B) Simpson's index (calculated on biomass), (C) average taxonomic distinctness, (D) variation in taxonomic distinctness.

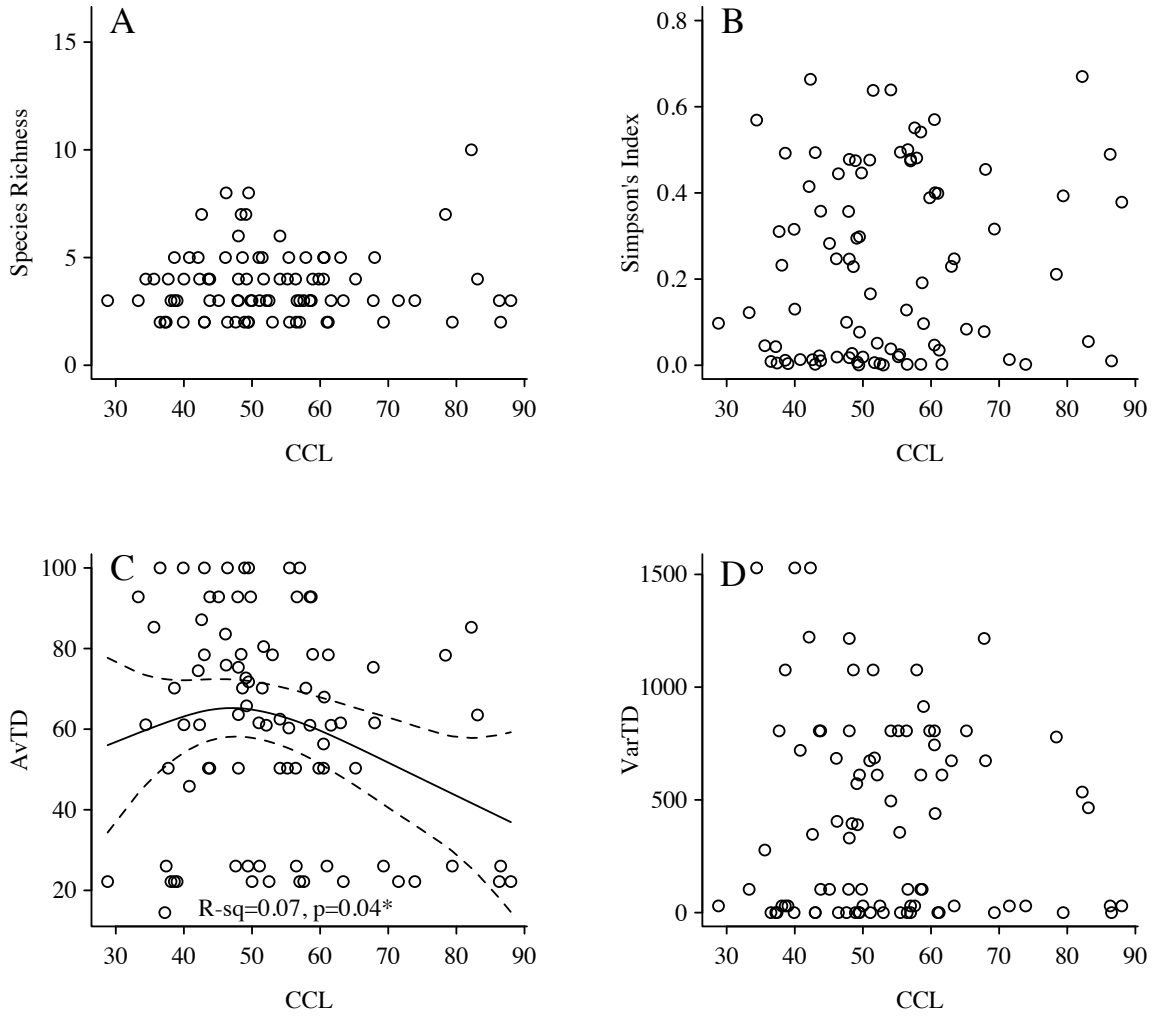

Figure 5. Species diversity measures of stomach content samples against green turtle size (CCL, cm). (A) species richness, (B) Simpson's index (calculated on biomass), (C) average taxonomic distinctness, (D) variation in taxonomic distinctness.

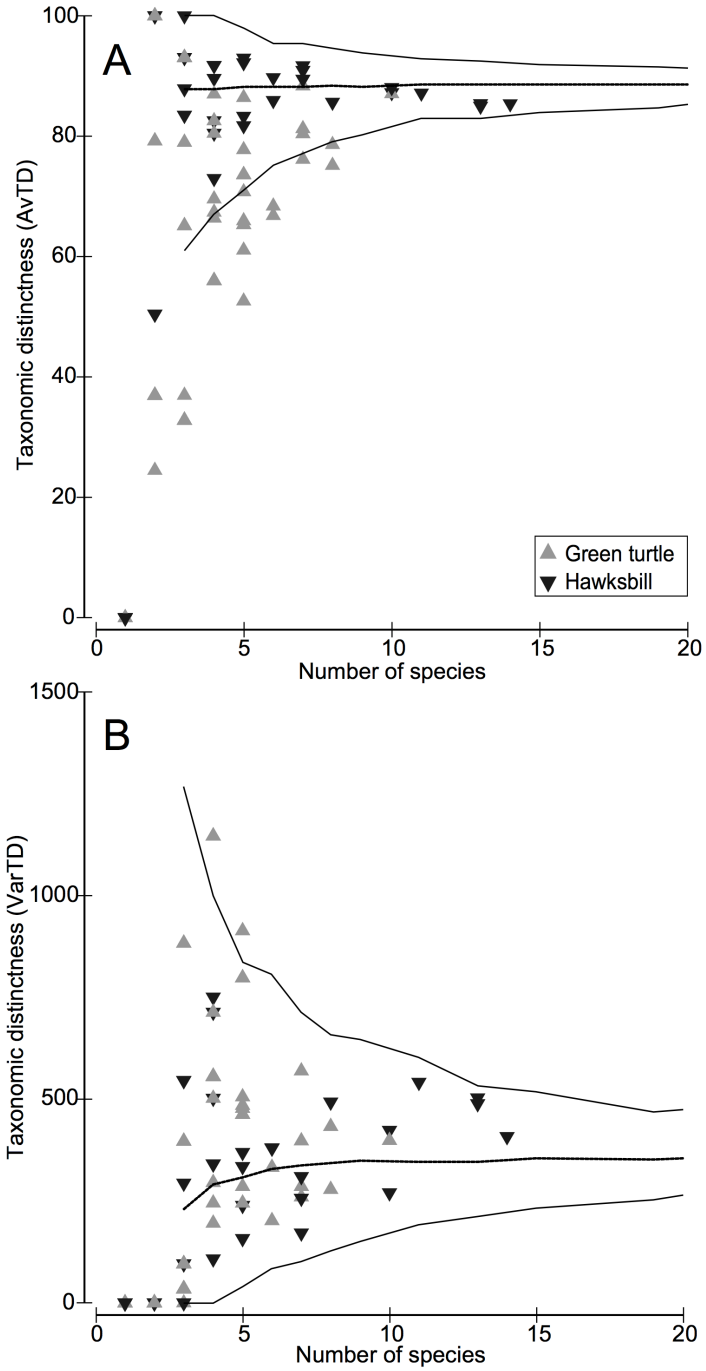

Figure 6. Average (A) and variation (B) in taxonomic distinctness of stomach contents from two turtle species (n=45 hawksbill turtles, n=92 green turtles). Lines indicate the median and upper and lower 95% probability intervals of taxonomic distinctness created from randomised draws of sublists of 2 to 20 species from a regional master list of 565 species. Weighting of Linnaean tree step lengths was guided by taxon richness of the master list and frequencies of species found in the habitat surveys were used to weight the selection of the random species.

Chapter 5: Supplementary Information

Methods

Habitat surveys

At each habitat in each location, a minimum of two (usually four) quadrats were surveyed *in situ* and species enumerated for comparison with photoquadrat data. To test the efficiency of the photographic survey method, photoquadrats were compared to *in situ* quadrats that were assumed to be better resolved than photographs. Square root transformed species abundance data (Bray-Curtis similarities) from photoquadrats were compared with paired *in situ* samples using a mixed effects permutational multivariate analysis of variance (PERMANOVA), with the combined factor of location and habitat as a random effect. There was no significant difference between *in situ* and photoquadrat data (PERMANOVA: $PseudoF_{1df} = 2.48$, $P_{perm} = 0.07$) and thus photoquadrat data were used in further analysis and considered representative of the habitat.

Photoquadrat images were selected for analysis at random. The cumulative number of species per habitat in each site was plotted against the number of quadrats analysed to create species area curves. The number of quadrats analysed in each habitat was determined by the asymptote of these species area curves (see Table S1). A total of 736 images were analysed on screen. Species abundance was enumerated by cell frequency counts (the quadrat was divided into 25 cells). Additionally, broader-scale rapid assessments of habitats at each location were carried out and seagrass shoot densities estimated (see Supplementary Tables S3 and S4).

Species were identified to the lowest taxonomic level and species unidentified in the field from *in-situ* quadrats and rapid assessments were sampled and identified in the laboratory. Most algae, seagrass and coral species were identified to species or genus level. Most turf algae were given a grouping description, e.g. filamentous turf, and gorgonians were mostly identified to family level. Sponges are known to be difficult to identify in the field (Ackers & Moss 2007) and where possible, samples were taken, identified to lowest taxonomic level and each occurrence ascribed a morphotype (growth form) following Bell and Barnes (2001) and Bell et al. (2006).

Species identification from turtle stomach contents

Seagrasses were usually identifiable with the naked eye. Algae were viewed and photographed under a 120X light microscope (Leica M165C, Leica Microsystems Ltd, Heerbrugg), tissue sections taken where necessary and identified using a variety of reference tools (e.g. Taylor 1967, Littler et al. 1989, Littler & Littler 2000, Guiry & Guiry 2012). Sponges were identified by gross morphology, skeletal and spicule slide preparations (Ackers & Moss 2007) by consulting a variety of reference materials (e.g. Weidenmayer 1977, Zea et al. 2009, Kluijver et al. 2012, Van Soest et al. 2012). Other species present in stomachs (e.g. octocorals, gorgonians) were identified using various sources (e.g. Humann & Deloach 1992, Sanchez & Wirshing 2005, Kluijver et al. 2012, Sheppard 2012). Taxa that had two or more unidentified species were numbered to distinguish them, and unidentified sponge species were given a descriptive name of its morphology (see Data analysis: habitat taxonomic distinctness for further details).

Data analysis: habitat

Bray-Curtis similarities from photoquadrat data were visualised between habitats using a non-metric multi-dimensional scaling (MDS) ordination and a vector plot overlaid to indicate species most correlated (Spearman's rank correlation) to the pattern (Clarke 1993) (supplementary Figure S1). Habitats were also described qualitatively following the classification scheme of Mumby and Harborne (1999) (see Table S4; site and habitat locations and descriptions are given in Table S1, see Figure 1 (main text) for map of locations).

Data analysis: habitat taxonomic distinctness

Average Taxonomic Distinctness (AvTD) and variation in Taxonomic Distinctness (VarTD) were assessed by habitat type (Clarke & Warwick 1998, Clarke & Warwick 2001: supplementary Figure S2). These diversity measures are based on the relatedness of species drawn at random from a sample, are independent of the number of species (a better statistical sampling property than richness related estimators), and can be used to compare data from differing sampling effort, spatial and temporal scales (such as stomach samples and habitat species lists) (Clarke & Warwick 1998, Clarke & Warwick 2001). Here, taxonomic distinctness is defined from a Linnaean tree (taxonomic aggregation file) of macrobenthic species likely in

TCI. A master list of 565 species was created from species identified in the habitat surveys, stomach content analysis and from searches of databases of sponge, gorgonian, coral, seagrass and algae species previously recorded in TCI and neighbouring Bahamas from the World Register of Marine Species (WoRMS) database (Appeltans et al. 2012). Taxa that had two or more unidentified species were numbered to distinguish them, for example *Haliclona 1, Haliclona 2*. Unidentified sponge species were initially given a descriptive name of its morphology (*sensu* Bell & Barnes 2001: e.g. orange encrusting sponge) and for more than one unidentified sponge morphology per group, each was numbered (e.g. orange encrusting 1, orange encrusting 2 etc.). Morphological groupings were propagated through the aggregation file in order to preserve its taxonomic structure. To determine how representative the habitat photoquadrat data were in relation to the species composition expected in the region, the two taxonomic distinctness measures were used in a TAXDTEST (Clarke & Gorley 2006), where photoquadrat sample data were superimposed on a funnel plot of expected AvTD and VarTD 95% probability limits created from randomised draws of sublists of 2 to 20 species from the regional master list. The weighting of Linnaean tree step lengths was guided by taxon richness of the master file (Clarke & Warwick 1999) and the simulation of random draws was weighted by the frequencies of species found in the habitat surveys (Clarke & Gorley 2006).

Table S1. Summary of TCI sampling sites, their habitats and descriptions with tidal state and height (m) (tidal phase indicated by S = Springs, N = Neaps) and sampling water depth at time of sampling. Grid references and map code refers to Figure 1. The number of photoquadrat pictures taken (n=1061) and analysed (n=736) after analysis of species area curves and number of species identified from these are given. Seagrass density (m^{-2}) at eight location/habitats estimated by rank (where 1 is sparse and 5 is dense) and quantified using validated photoquadrat shoot counts.

Site	Habitat	Habitat group	Date (time) first surveyed	Time of LW (height, m)	Depth (m)	Location (map code)	Photoquadrats analysed (taken)	Area analysed (m^2)	Approx. survey area (km^2)	N° species	Mean seagrass shoots.m^{-2} (Rank 1-5)	Description (habitat characteristics and dominant species)
Ambergris	Reef	Reef	20/10/2010 (12:52)	13:42 (0.05)	1	21.321566, -71.642364 (15)	48 (49)	12	0.38	38	-	Rocky patch reef (likely to be remains of dead reef) colonised by abundant fleshy brown algae (e.g. *Sargassum, Dictyota).* Soft corals common (e.g. *Briareum asbesinum, Pseudopterogorgia),* occasional sponges and coral species.
Ambergris	Seagrass Algae	Seagrass	20/10/2010 (10:32)	13:42 (0.05)	1	21.319006, -71.637057 (15)	24 (37)	6	0.75	12	55.7 (4)	Soft muddy sand with *Arenicola* mounds. Seagrass with dense patches of algae (*Batophora spp, Penicillus spp* and *Acetabularia spp.)* and occasional sponges.
Ambergris Airport	Seagrass	Seagrass	20/10/2010 (12:02)	13:42 (0.05)	1	21.298467, -71.648736 (16)	30 (56)	7.5	1.19	19	-	Patches of seagrass on sand with occasional hard substrate (usually conch shells). Algae such as *Laurencia spp* and *Penicillus spp* dominant. Occasional soft corals e.g. *Briareum asbesinum* and corals e.g. *Porites porites.*
Eastside	Patch Reef	Reef	17/10/2010 (13:51)	11:21 (0.18)	2	21.559640, -71.485860 (6)	38 (65)	9.5	0.48	32	-	Algal dominated (e.g. *Turbinaria spp)* hard bottom with patches of dead coral structure. Gorgonians, such as *Plexaura spp,* common and occasional coral patches
Fish Cay	Hard Bottom	Reef	14/10/2010 (13:13)	08:14 (0.2)	4	21.574620, -71.520810 (14)	32 (105)	8	0.21	28	-	Hard bottom with fleshy brown algae (mainly *Sargassum spp, Padina spp* and *Lobophora spp)* and some green algae (e.g. *Halimeda spp* and *Dictyoshpaeria cavernosa).* Occasional gorgonians (mainly *Gorgonia spp)* and some corals and sponges.
Fish Cay	Patch Reef	Reef	16/10/2010 (11:25)	10:22 (0.21)	2	21.357757, -71.629195 (14)	33 (35)	8.25	0.43	35	-	Largely dead elkhorn coral with fleshy brown algae and encrusting algae (e.g *Porolithon spp).* Patches of live coral growth (e.g. *Acropora palmata, Porites, Montastrea spp)* and occasional (? m^{-2}) gorgonians.
Harbour	Gorgonian	Reef	08/10/2010 (14:45)	15:46 (-0.03)	3	21.485954, -71.533581 (10)	45 (51)	11.25	0.09	41	-	Hard bottom with turf algae and a fine layer of silt. Large gorgonians common (including *Pseudopterogorgia spp, Plexaurella spp* and *Pterogorgia spp).* Sparse patches of algae (e.g. *Halimeda spp),* some sponges (e.g. *Ircinia spp)* and corals (e.g. *Millepora spp).*
Harbour	Hard Bottom	Reef	07/10/2010 (15:35)	14:55 (-0.04 S)	1.5	21.487173, -71.530855 (10)	28 (32)	7	0.09	21	-	Hard bottom with turf algae and patches of *Halimeda, Dasycladus* and *Sargassum* with a covering of silt. Low relief compared to patch reefs, with occasional corals.
Harbour	Seagrass	Seagrass	07/10/2010 (15:00)	14:55 (-0.04)	2	21.486382, -71.528381 (10)	38 (49)	9.5	0.08	15	78.2 (5)	Dense seagrass patches (*Thalassia testundinum* and *Syringodium filiforme)* with green algae common (mainly *Dasycladus vermicularis, Penicillus spp* and *Halimeda spp)*
Harbour (Shark Alley)	Patch Reef	Reef	22/10/2010 (11:38)	15:01 (0)	2	21.484459, -71.535330 (9)	46 (15)	11.5	0.08	37	-	Patch reef with some areas of dead coral with encrusting algae (e.g. *Porolithon),* and some patches of live coral (e.g. *Millepora spp, Montastrea spp, Dendrogyra cylindricus).* Small patches of algae (e.g. *Halimeda spp)* and occasional gorgonians.
Harbour (Tuckers Reef)	Patch Reef	Reef	11/10/2010 (11:00)	05:30 (0.06)	1	21.484740, -71.528120 (8)	40 (45)	10	0.11	23	-	Patchy reef with *Millepora spp, Acropora palmata, Porites* and *Gorgonia spp.* Surrounded by hard bottom with turf algae. Some patches of dead coral with encrusting algae.
Jacksonville	Seagrass Algae	Seagrass	21/10/2010 (11:49)	15:23 (0.02)	0.6	21.748160, -71.599424 (5)	18 (68)	4.5	0.08	8	15.6 (2)	Sparse seagrass patches (*Thalassia testundinum)* dominated by green algae (e.g. *Halimeda spp).* Sponges such as *Haliclona spp* common. Dead seagrass present.
Larmer	Seagrass	Seagrass	21/10/2010 (10:08)	15:23 (0.02)	2	21.751532, -71.641136 (4)	14 (19)	3.5	0.38	7	65.4 (4)	Seagrass patches on soft muddy sand with large *Arenicola* mounds. Some *Penicillus spp* and *Laurencia* spp.
Long Cay	Patch Reef	Reef	14/10/2010 (14:45)	08:14 (0.2)	6	21.454344, -71.572886 (11)	6 (6)	1.5	0.55	12	-	Dead elkhorn coral colonised by encrusting algae and fleshy brown algae (e.g. *Turbinaria, Sargassum).* Occasional corals (e.g. *Acropora palmata, Porites spp, Montastrea spp),* and gorgonians (e.g. *Gorgonia spp)*
Middle Reefs	Patch Reef	Reef	16/10/2010 (14:26)	10:22 (0.21 N)	2.5	21.439322, -71.590720 (13)	26 (51)	6.5	0.94	18	-	Patches of live and diverse coral (e.g. *Millepora spp, Acropora palmate, Agarcia spp, Montastrea spp)* with some patches of dead coral skeleton with encrusting algae. Occasional small encrusting sponges.
Newsons Point	Algae	Seagrass	17/10/2010 (10:49)	11:21 (0.18)	1	21.572490, -71.522300 (7)	22 (25)	5.5	0.35	9	-	Dense algae patches (mainly *Laurencia spp, Batophora spp,* and *Penicillus spp)* on soft muddy sand.
Newsons Point	Coralline Algae	Seagrass	17/10/2010 (10:37)	11:21 (0.18)	1	21.572380, -71.522430 (7)	34 (61)	8.5	0.39	17	39.7 (3)	Dense algae (mainly coralline algae and *Penicillus spp)* on sand with seagrass dominant. Sponges common (e.g. *Chondrilla nucula)* and occasional corals (*Porites porites).*
Newsons Point	Seagrass	Seagrass	17/10/2010 (12:16)	11:21 (0.18)	0.5	21.590110, -71.506220 (7)	28 (28)	7	0.85	10	62.7 (4)	Dense (4/5) seagrass (*Thalassia testundinum)* patches with green algae (mainly *Penicillus spp* and *Halimeda spp)* and red algae (mainly *Laurencia spp).* Sponges such as *Tedania ignis* and a brown papillate sponge common
Ocean Hole	Seagrass Algae	Seagrass	09/10/2010 (12:41)	04:54 (-0.02)	1	21.726916, -71.811821 (2)	34 (54)	8.5	0.82	8	31.9 (2)	Soft muddy sand with worm holes and mounds. Sparse patches of seagrass with patches of *Batophora spp* and *Halimeda spp*. Papillate sponges present.
Ocean Hole (Man-o-War)	Seagrass Algae	Seagrass	09/10/2010 (10:51)	04:54 (-0.02)	0.5	21.713000, -71.845160 (1)	26 (47)	6.5	0.62	7	148.5 (5)	Dense patches of seagrass (*Thalassia testundinum* and *Halodule beaubettei)* with dense patches of *Laurencia spp* and some *Penicillus spp.*
Six Hills	Gorgonian	Reef	12/10/2010 (14:06)	06:20 (0.11)	3	21.460890, -71.629040 (12)	24 (37)	6	0.18	27	-	Located in an area of strong currents. Hard bottom with fleshy brown algae and large gorgonians

Table S2. Species in diets of green turtles (n=92) and hawksbill turtles (n=45). Frequency (proportion of stomachs in which present), average biomass proportion ± SD, and max. proportion (min. was zero in all cases) of species across stomach samples. Bold species represent those found in >10% of stomach samples. Asterisk denotes trace amount (<0.01 by proportion). Comparison studies that found same top prey species by weight are indicated next to taxon name: *1*-Mortimer (1981); *2*-León and Bjorndal (2002); *3*-Santos et al. (2011); *4*-Seminoff et al. (2002); *5*-Van Dam and Diez (1997); *6*-Bjorndal (1997); *7*-Rincon-Diaz et al. (2011); *8*-Bjorndal (1980). Table is split into three parts for clarity. Parts A and B represent herbivorous diet items.

A

Group	Phylum	Species	Green turtle			Hawksbill turtle		
				Biomass			Biomass	
			Frequency	Mean ± SD	Max	Frequency	Mean ± SD	Max
Seagrass	Magnoliophyta	***Thalassia testudinum 1,6,8***	**0.95**	**0.73 ± 0.32**	**1.00**	**0.16**	*** ± 0.01**	**0.04**
		Syringodium filiforme 1,6	**0.58**	**0.16 ± 0.30**	**1.00**	**0.18**	*****	**0.00**
		Halodule beaudettei 3,6	**0.58**	**0.02 ± 0.04**	**0.20**	0.02	*	**0.00**
		Alismatales	0.03	*	*	-	-	-
Unknown algae	Plantae	Plantae 2	0.03	*	0.01	0.02	* ± 0.01	0.07
		Plantae 1	-	-	-	0.02	*	*
Red algae	Rhodophyta	*Digenea simplex*	0.01	0.01 ± 0.09	0.86	0.09	0.01 ± 0.05	0.34
		Chondria spp	0.05	* ± 0.01	0.09	0.09	*	*
		Corallinaceae	0.04	*	0.05	0.09	0.01 ± 0.03	0.13
		Ceramium spp	0.02	* ± 0.01	0.05	-	-	-
		Rhodophyta 3	0.01	* ± 0.01	0.05	-	-	-
		Spyridia filamentosa	0.02	*	0.02	-	-	-
		Laurencia spp	0.05	*	*	0.07	0.01 ± 0.08	0.54
		Hypnea spp	0.04	*	0.01	-	-	-
		Hypnea musciformis	0.01	*	*	-	-	-
		Rhodophyta	0.01	*	*	0.07	0.02 ± 0.10	0.69
		Gelidium pusillum	0.02	*	*	0.02	*	*
		Rhodophyta 2	0.01	*	*	-	-	-
		Gelidium spp	-	-	-	0.02	*	*
		Gelidiopsis spp	-	-	-	0.04	*	*
		Rhodophyta 1	-	-	-	0.02	*	*
		Rhodophyta 4	-	-	-	0.02	*	0.01
		Rhodophyta 5	-	-	-	0.02	*	*
		Ceramiaceae	-	-	-	0.02	*	*
		Gelidiella acerosa	-	-	-	**0.18**	**0.03 ± 0.10**	**0.50**
		Gracilaria spp	-	-	-	0.02	* ± 0.01	0.05
		Kallymenia spp	-	-	-	0.09	0.02 ± 0.10	0.67
		Halymenia duchassaingii	-	-	-	0.02	0.01 ± 0.08	0.51
		Lejolisia exposita	-	-	-	0.02	*	*
		Gracilaria cervicornis	-	-	-	0.02	*	0.01
		Polysiphonia spp	-	-	-	0.02	*	*

Table S2. Cont.

B

Group	Phylum	Species	Green turtle			Hawksbill turtle		
				Biomass			Biomass	
			Frequency	Mean ± SD	Max	Frequency	Mean ± SD	Max
Brown algae	Ochrophyta	*Dictyota dichotoma var. intricata*	0.01	* ± 0.01	0.10	-	-	0.00
		Padina spp	0.02	*	0.03	**0.22**	**0.01 ± 0.02**	**0.14**
		Rosenvingea intricata	0.02	*	0.01	0.04	*	*
		Dictyota menstrualis	0.01	*	*	0.02	*	*
		Rosenvingea sanctae-crucis	0.01	*	*	0.04	* ± 0.02	0.14
		Canistrocarpus cervicornis	-	-	-	0.04	*	*
		Dictyota spp	-	-	-	**0.13**	*	**0.01**
		Sphacelaria spp	-	-	-	0.04	*	0.01
		Rosenvingea spp	-	-	-	0.02	* ± 0.02	0.16
		Zonaria spp	-	-	-	0.02	*	*
		Dictyopteris spp	-	-	-	0.09	* ± 0.01	0.04
		Sargassum spp	-	-	-	0.09	*	0.02
Green algae	Chlorophyta	*Acetabularia spp*	0.05	* ± 0.02	0.18	0.04	*	*
		Batophora oerstedii	**0.18**	*	**0.01**	**0.13**	*	**0.01**
		Halimeda spp	0.08	*	0.01	0.04	*	0.01
		Acetabularia polyphysoides	0.04	*	0.01	-	-	-
		Chlorophyta A	**0.11**	*	*	0.02	*	0.01
		Dasycladus vermicularis	0.03	*	0.01	0.02	*	*
		Caulerpa verticillata	0.03	*	*	0.04	*	*
		Penicillus capitatus	0.01	*	*	0.02	*	*
		Cladophora spp	0.01	*	*	0.02	* ± 0.01	0.07
		Cladophoraceae	0.01	*	*	-	-	-
		Chaetomorpha spp	0.01	*	*	0.02	*	0.01
		Dictyosphaeria cavernosa	-	-	-	0.09	*	0.02
		Microdictyon marinum	-	-	-	0.07	*	0.01
		Chlorophyta B	-	-	-	0.04	*	0.01
		Chlorophyta C	-	-	-	0.02	*	*
		Chlorophyta D	-	-	-	0.02	*	*
		Avrainvillea spp	-	-	-	0.07	*	0.02
		Halimeda monile	-	-	-	0.02	*	*
		Udotea spp	-	-	-	0.02	*	*
		Bryopsidales	-	-	-	0.02	*	*

C

Group	Phylum	Species	Green turtle			Hawksbill turtle		
				Biomass			Biomass	
			Frequency	Mean ± SD	Max	Frequency	Mean ± SD	Max
Sponge	Porifera	***Chondrilla caribensis f. caribensis 2,5,6,7***	**0.16**	**0.04 ± 0.12**	**0.55**	**0.47**	**0.27 ± 0.40**	**1.00**
		Cinachyrella alloclada	0.02	0.01 ± 0.06	0.44	**0.11**	**0.05 ± 0.19**	**0.98**
		Sidonops neptuni 2,5,6	0.02	0.01 ± 0.04	0.30	**0.24**	**0.17 ± 0.35**	**1.00**
		Niphates erecta	0.03	* ± 0.04	0.32	-	-	-
		Tedania spp	0.01	* ± 0.02	0.19	-	-	-
		Pandaros acanthifolium	0.01	* ± 0.01	0.11	-	-	-
		Spirastrella cunctatrix	0.01	*	*	-	-	-
		Higginsia strigilata	0.01	*	*	-	-	-
		Porifera A	-	-	-	0.02	*	*
		Erylus formosus	-	-	-	0.07	0.04 ± 0.16	0.90
		Porifera B	-	-	-	0.02	0.02 ± 0.15	1.00
		Halichondria (Halichondria) melanadocia	-	-	-	**0.29**	**0.16 ± 0.32**	**1.00**
		Clathria (Thalysias) juniperina	-	-	-	0.04	0.01 ± 0.08	0.53
		Erylus goffrilleri	-	-	-	0.02	*	0.03
		Scopalina ruetzleri	-	-	-	0.09	0.08 ± 0.26	1.00
		Timea mixta	-	-	-	0.07	0.01 ± 0.08	0.55
		Stelletta pudica	-	-	-	0.04	0.02 ± 0.11	0.61
		Desmacella meliorata	-	-	-	0.04	* ± 0.01	0.04
		Lissodendoryx spp	-	-	-	0.02	0.02 ± 0.15	1.00
		Tethya spp	-	-	-	0.02	0.02 ± 0.15	0.98
		Porifera C	-	-	-	0.02	*	*
Cnidaria	Cnidaria	Alcyonacea	0.02	*	0.04	-	-	-
		Hydrozoa	0.01	*	*	-	-	-
		Cnidaria	-	-	-	0.02	*	0.01
Invert	Platyhelminth	*Digenia spp*	-	-	-	0.04	*	*
	Mollusca	Gastropoda	0.02	*	*	0.04	*	*
		Mollusca	0.01	*	*	-	-	-
	Arthropoda	Crustacea	0.01	*	*	-	-	-
		Pycnogonum spp	0.01	*	*	-	-	-
	Echinodermata	Ophiuroidea	-	-	-	0.02	*	*
Other	-	Plastic	0.04	*	*	0.09	*	*
	-	**Sediment**	**0.11**	*	*	**0.43**	**0.04 ± 0.02**	**0.07**
	-	Shell	-	-	-	0.02	*	*
	-	Wood	-	-	-	0.02	*	*

Table S3. Rapid assessment of habitats: biological characteristics measured using the SACFOR abundance scale (see http://jncc.defra.gov.uk/page-2684), substrate type (%), physical characteristics ranked 1-5 (5= high relief, many patches, high density, unstable sediment, many crevices, deep sediment).

Site	Habitat	Seagrass	Algae	Gorgonian	Coral	Sponge	Rock	Boulders	Cobbles	Rubble	Sand	Mud	Relief (1-5)	Patchiness (1-5)	Density (1-5)	Stability (1-5)	Crevices (1-5)	Depth of sediment (1-5)
Harbour	Seagrass	A	C		R		0	0	0	0	100	0	2	3	4	4	1	3
Harbour	Hard bottom		C		O		90	0	0	30	40	0	3	4	2	4	4	1
Harbour	Gorgonian		C	C	F	F	100	0	10	10	10	10	4	2	2	4	3	1
Harbour	Reef		O				90	0	0	0	10	0	5	3	3	5	4	1
Tuckers Reef	Reef		C	F	A	F	50	0	20	40	10	0	5	4	2	4	5	1
Shark Alley	Seagrass		C	C	A	O	0	0	0	0	100	100	1	4	5	2	1	5
Man o War	Algae	A	A			R	0	0	0	0	100	50	1	5	1	1	1	4
Ocean Hole	Reef	S	C			O	30	0	10	10	5	0	5	4	4	4	5	1
Six Hills	Gorgonians		A	O	A	F	100	0	10	10	5	0	4	3	3	4	2	1
Six Hills	Seagrass		A	A	F	F	0	0	0	0	100	40	1	4	4	1	1	5
Southern Bush	Algae	S	A				0	0	0	0	100	40	1	5	2	1	1	5
Southern Bush	Hard bottom	O	S				100	0	20	10	5	0	3	3	4	3	2	1
Fish Cay	Reef		S	F	O	F	40	20	10	20	0	0	5	5	3	5	5	1
Fish Cay	Reef		C	O	S	O	40	0	10	10	0	0	5	5	4	5	4	1
Long Cay	Reef		C	F	A	F	40	0	30	40	0	0	5	5	4	5	5	1
Middle Reefs	Algae		C	O	A	F	20	0	0	0	100	0	2	5	5	3	1	2
Newson's Point	Seagrass	A	A		F	O	0	0	0	0	70	30	2	5	4	3	1	3
Newson's Point	Algae	R	A		R	O	0	0	0	0	70	30	4	5	5	2	1	3
Newson's Point	Reef	A	C			O	40	20	10	50	10	0	4	5	3	4	3	1
Eastside	Algae		C	A	A	O	0	0	5	0	100	10	1	5	4	2	1	1
Ambergris	Seagrass	A	A			R	0	0	0	0	90	30	3	5	4	2	1	3
Ambergris	Reef	A	A		R	O	100	20	20	30	10	0	4	5	4	4	4	1
Ambergris	Algae		A	F	A	R	0	0	0	0	100	30	3	2	3	2	1	2
Jacksonville	Seagrass	A	C			R	0	0	0	0	100	10	1	5	1	2	1	2
Larmer		O	A			C												

Table S4. Classification of habitats surveyed. Dense = >1 individuals m^{-2} for solitary species, or >50% cover for algae/seagrass. Based on the classification scheme outlined by Mumby and Harborne (1999).

First Tier	Second Tier		Third Tier		Code	Examples
	Characteristics	**Dominant Biota**	**Characteristics**	**Secondary Biota**		
Hard Substratum	Patch Reef	Brown turf algae (Phaeophyta, e.g. *Turbinaria spp*)	Dense algae overgrowing coral rubble	Cnidaria Gorgonians, Porifera	PRDPha	Six Hills, Ambergris, Fish Cay, East Side, Shark Alley
		Cnidaria (e.g. *Acropora spp, Millepora spp, Porites spp)*	Dense patchy coral with sparse algae	Encrusitng algae (e.g. *Porolithon spp)*	PRDCni	Middle Reef, Tuckers,
	Hard substratum	Green turf algae (Chlorophyta e.g. *Halimeda spp*)	Continuous sparse turf with occasional corals	Cnidaria, Porifera	HBSChl	Harbour
		Brown turf algae (Phaeophyceae e.g. *Sargassum spp)*	Continuous dense turf with occasional corals	Cnidaria, Porifera	HBDPha	Fish Cay
	Gorgonian	Gorgonians (Alcyonacea) turf algae	Dense	Algae turf (e.g. *Sargassum, Halimeda)* Porifera	GODAlc	Harbour, Six Hills
Soft Substratum	Muddy Sand with mounds	Seagrass e.g. (e.g. *Thalassia spp*)	Dense	Porifera, Chlorophyta	MSDTha	Newson's Point, Harbour, Larmer
		Green algae (e.g. *Halimeda spp, Penicillus spp)*	Sparse, patchy	Seagrass (e.g. *Thalassia spp)*, Porifera	MSSHal	Jacksonville, Ocean Hole
		Green algae (e.g. *Batophora spp*)	Dense	Seagrass (e.g. Thalassia), Porifera	MSDBat	Southern Bush, Ambergris
		Algae dominated (e.g. *Laurencia spp, Penicillus spp)*	Dense, patchy	Porifera	MSDLau	Newson's Point, Man o war
	Hard sand	Seagrass (e.g. *Thalassia spp)*	Sparse, patchy seagrass with algae	*Laurencia spp,* Cnidaria	HSSLau	Ambergris Airport
		Corallinaceae dominated	Dense	*Thalassia* spp, Porifera, Cnidaria	HSDCor	Newson's Point

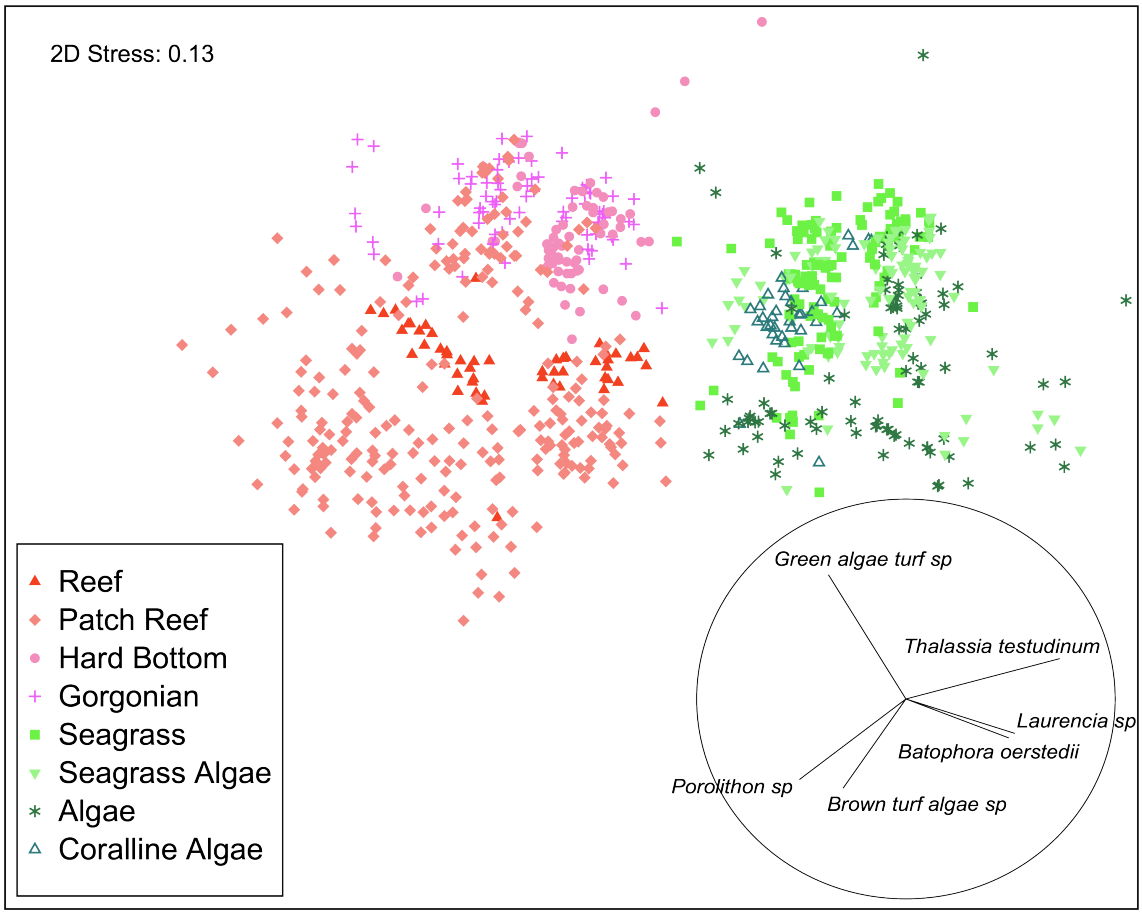

Figure S1. Non-metric multi dimensional scaling ordination of habitat photoquadrats and vector overlay of most contributing species (R>0.5 Spearman's correlation; derived from SIMPER analysis).

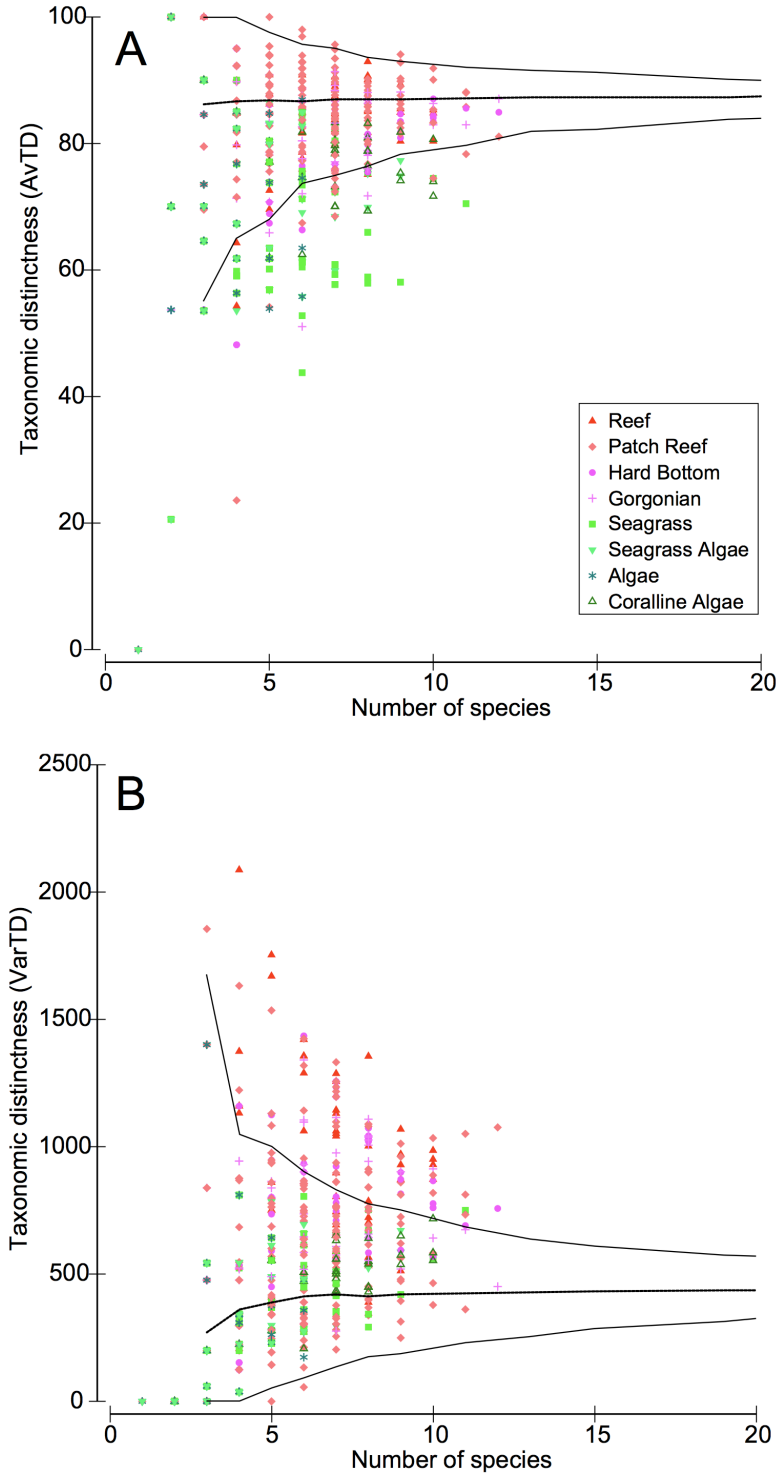

Figure S2. Average (A) and variation (B) in taxonomic distinctness of habitat quadrats. Lines indicate the median and upper and lower 95% probability intervals of taxonomic distinctness created from randomised draws of sublists of 2 to 20 species from a regional master list of 565 species. See supplementary text for details.

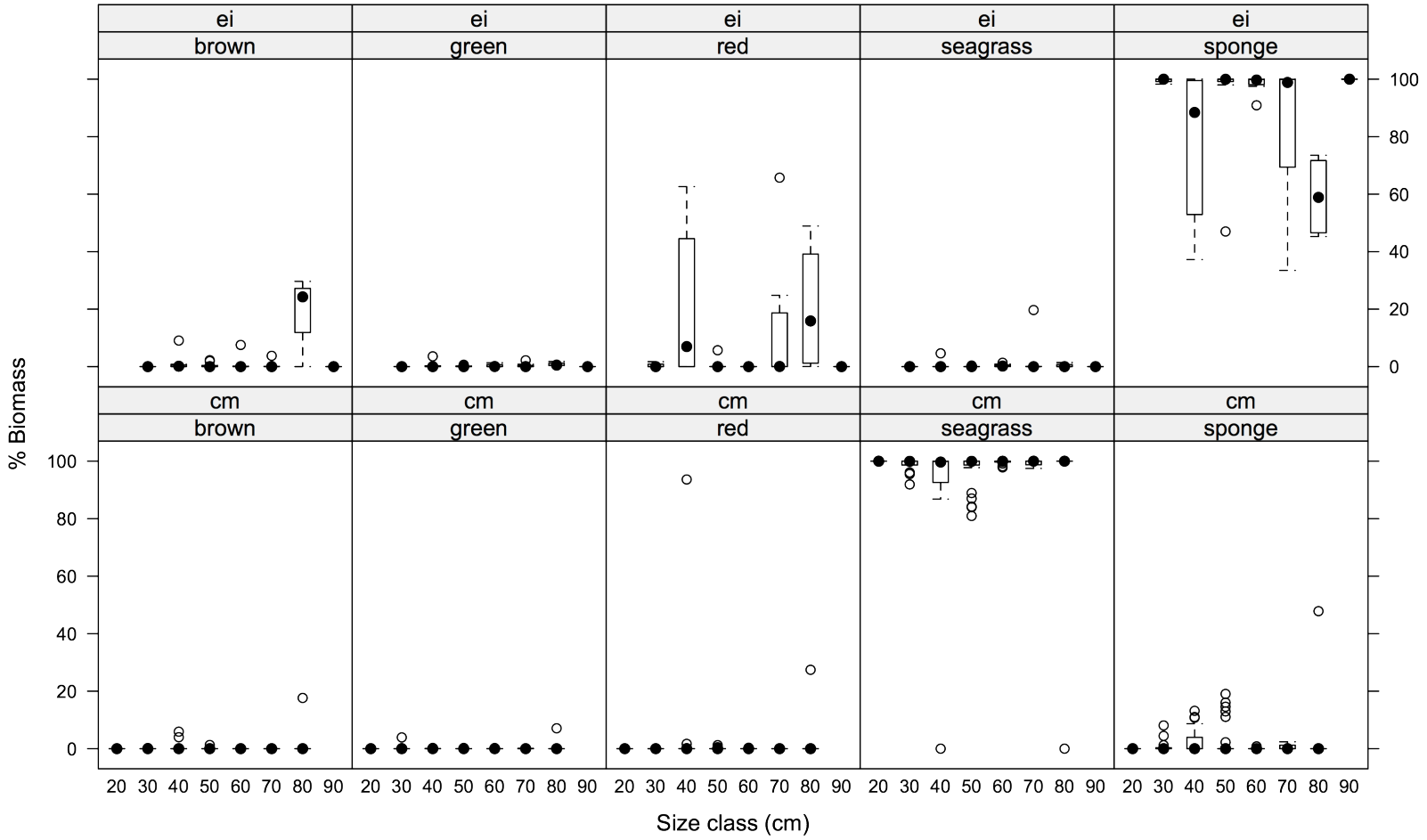

Figure S3. Trellice plot of relative percentage biomass of five main diet groups (brown, green and red algae, seagrasses and sponges) found in stomach content indicates no apparent relationship exists with turtle carapace size class. Hawksbill turtles (*Eretmochelys imbricata*, ei) top panel, green turtles (*Chelonia mydas*, cm) bottom panel.

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Chapter 6

Isotopic niche separation, ontogenetic shifts and diet in sympatric marine turtles

Thomas B. Stringell[1], Brendan J. Godley[1], Rona McGill[2], Jason Newton[2], Quinton Phillips[3], Peter B. Richardson[1,4], Amdeep Sanghera[4] and Annette C. Broderick[1]

[1]Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ. UK
[2] NERC Life Sciences Mass Spectrometry Facility, Scottish Universities Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, G75 0QF, Scotland, UK
[3] Department of Environment and Maritime Affairs, South Caicos, Turks and Caicos Islands, BWI.
[4] Marine Conservation Society, Ross on Wye, Herefordshire, HR9 5NB. UK

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Abstract

Understanding the foraging ecology of marine vertebrates is essential for their effective conservation. Marine turtles are thought to be keystone consumers in tropical coastal ecosystems where green turtles (*Chelonia mydas*) as herbivores and hawksbill turtles (*Eretmochelys imbricata*) as spongivores maintain seagrass and reef habitats respectively. In this study, we use carbon and nitrogen stable isotope ratios in their prey and their tissues to examine the diet, isotopic niche width and trophic position of sympatric marine turtle species in the Turks and Caicos Islands, Caribbean. Isotopic niche metrics were significantly different between the turtle species reflecting inter-specific niche separation. Isotope ratios changed significantly with increasing carapace size in all three tissues (plasma, red blood cells and scute) of green turtles and scute tissue of hawksbills, demonstrating likely ontogenetic shifts. Bayesian mixing models also indicated that diet sources changed with size in green turtles from omnivory at small sizes to herbivory at mid-sizes; seagrass contributions were less dominant at larger sizes, suggesting a potential signal for the onset of developmental migrations. Hawksbill diet was highly mixed but likely similar across size classes. Together, these results suggest that the two turtle species likely play key roles in their respective habitats, with little competition for resources between the species. Conservation of these large grazers may be crucial for maintaining healthy reef and seagrass habitats.

Introduction

Marine turtles are widely believed to be keystone consumers and their decline through overexploitation in recent centuries has been implicated in the deterioration of reef and seagrass systems in the Caribbean (Jackson 1997, Jackson et al. 2001, Pandolfi et al. 2003, Orth et al. 2006, Waycott et al. 2009). It has been suggested that green turtles (*Chelonia mydas*) as herbivores improve seagrass pastures: affecting structure, productivity and nutrient composition of seagrass (Thayer et al. 1982, Thayer et al. 1984, Moran & Bjorndal 2005, 2007, Christianen et al. 2012). Hawksbills (*Eretmochelys imbricata*) as spongivores remove large amounts of sponge biomass, reducing competition with corals for space (Hill 1998) and thus influencing reef succession and diversity (Meylan 1988, Bjorndal 1997, Van Dam & Diez 1997).

Understanding trophic role and niche width of marine vertebrates is often challenging (Layman et al. 2007) and for marine turtles has been primarily limited to data from stomach sampling (Mortimer 1981, Bjorndal 1997, Brand-Gardner et al. 1999, León & Bjorndal 2002, Seminoff et al. 2002, Arthur & Balazs 2008, López-Mendilaharsu et al. 2008, Arthur et al. 2009, Rincon-Diaz et al. 2011, Santos et al. 2011, Witherington et al. 2012). Data obtained from stomach contents has its limitations for diet analyses because it represents a 'snapshot' of feeding rather than diet integration over longer time frames, and under-represents soft-bodied or easily digestible species (Duffy & Jackson 1986, Barrett et al. 2007). A combination of biochemical methods with conventional stomach sampling provides a more detailed interpretation of diet in situations where either method on its own may give less powerful results (Hedd & Montevecchi 2006).

Stable isotope analysis (SIA) offers tremendous potential insights into trophic ecology and spatial resource use (Hobson 1999, Post 2002). The use of nitrogen and carbon stable isotopes in foraging studies depends on different dietary items having different isotopic signatures, which are reflected in the tissues of consumers (Inger & Bearhop 2008). During metabolism, ^{14}N is lost to nitrogenous waste products and the heavy and stable isotope (^{15}N) is preferentially retained in the body (DeNiro & Epstein 1981). Consumer tissues are thus enriched in heavy isotopes of nitrogen relative to their food source (^{15}N:^{14}N, expressed as $\delta^{15}N$), usually by about 3 to 5‰ (Post 2002). This trophic enrichment ensues predictably through the food

chain so that $\delta^{15}N$ is a good indicator of trophic position. Stable carbon isotope ratios (^{13}C:^{12}C, expressed as $\delta^{13}C$) change little through the food chain (generally 0 to 1‰, Caut et al. 2009) but rather tend to trace the importance of different carbon pools to a consumer which can represent different habitats and geographic locations (DeNiro & Epstein 1978). For example, different $\delta^{13}C$ in primary producers (C_3, C_4 and CAM plants, marine algae) result from differing photosynthetic metabolisms (Inger & Bearhop 2008), and $\delta^{13}C$ decreases from marine benthic to pelagic habitats and from marine coastal to oceanic realms (Hobson 1999). SIA therefore provides quantitative information on resource and habitat use, because prey is assimilated and reflected in body tissues (Newsome et al. 2007).

In marine turtles, stable isotope ratios have been used successfully to identify foraging habitats and diets (for examples see Godley et al. 1998, Hatase et al. 2002, McClellan et al. 2010). Preliminary work has been carried out on isotopic turnover rates and discrimination factors in a range of turtle tissues (for captive animal studies see Seminoff et al. 2006, Seminoff et al. 2007, and Reich et al. 2008; for wild animal studies, see Revelles et al. 2007, and McClellan et al. 2010). Stable isotope turnover rates in blood plasma and red blood cells are thought to be in the order of weeks and months respectively (Seminoff et al. 2007, Reich et al. 2008). Turtle carapaces are composed of keratinized “scutes” covering the bony shell of turtles. Scutes are continuously growing tissues that are metabolically inert after synthesis. They “fix” a permanent record of resource use at the time of formation, representing multiple years: the oldest fraction being found at the posterior surface (Vander Zanden et al. 2010). Utilising different tissue types from individual turtles, therefore, allows insights over different temporal scales to assess changes in isotopic signals within and among individuals. Recent research confirmed the likely ontogenetic shift of green turtles from epipelagic-oceanic habitat and omnivory during the first three to five years of their lives to coastal-benthic habitats and a largely herbivorous diet (Reich et al. 2007, Arthur et al. 2008). Similar studies, however, have not been carried out for hawksbill turtles, although they may also undergo ontogenetic shifts as they recruit to coastal feeding grounds and develop spongivorous diets (Witherington et al. 2012).

Stable isotope ratios have been applied to the ecological niche concept (Hutchinson 1957) so that an n-dimensional hypervolume can be constructed from trophic components, $^{15}N/^{14}N$, and environmental components, $^{13}C/^{12}C$, and the

‘isotopic space’ quantified to provide a measure of isotopic niche width (Bearhop et al. 2004, Layman et al. 2007, Newsome et al. 2007, Jackson et al. 2011). Several metrics have been proposed to represent different properties of isotopic niche space (Layman et al. 2007), which have been further developed within a Bayesian framework to provide more robust estimates of these metrics (Jackson et al. 2011). Comparisons of isotopic niche parameters within and among populations of sympatric marine turtle species may reveal insights into their foraging and ecological roles in coastal ecosystems. To elucidate their foraging ecology further, SIA and Bayesian mixing models of key dietary items and consumer tissues offer great promise over conventional diet studies (Moore & Semmens 2008, Parnell et al. 2010). Likely contribution values of putative diet sources can be assessed in order to characterise the consumer’s diet and trophic role.

In this study we sample turtles from two years of capture-mark-recapture surveys and an extant turtle fishery in the Caribbean, to obtain stomach contents and tissue samples of a large number and wide size range of sympatric marine turtle species. In Stringell et al. in prep. (Chapter 5), we undertook conventional dietary sampling from stomach content analysis. In the present companion study, we use stomach content to direct the collection of wild dietary source material, and use these with the tissues of sea turtles to investigate temporal, spatial and size related variation in resource use patterns and isotopic niche width. We address these hypotheses: (i) Are there any size specific changes in isotopic ratios that suggest ontogenetic shifts and inferred trophic status, and are these isotopic signals consistent across different temporal scales, that is, plasma (days to weeks), red blood cells (weeks to months) and scute (months to years)? (ii) Is there isotopic niche separation between the two turtle species and among size classes? From these we hope to achieve the following insights: Do they eat what we think they do? Are the trophic profiles suggestive of these species playing their supposed keystone roles?

Methods

Study Site

The Turks and Caicos Islands (TCI) is a UK overseas territory in the Wider Caribbean Region located at the southeastern end of the Bahamas (21^{o} 45N, 71^{o} 35W). The low lying limestone islands are characterised by shallow soft sediment areas with mangrove swamps and tidal creeks on the leeward side of the islands, contrasting with the fringing reefs and steep drop-offs on the windward side. The archipelago support regionally significant foraging stocks of hawksbill and green turtles (Richardson et al. 2009), which are subject to one of the largest, legitimate turtle fisheries in the Caribbean (Stringell et al. 2013, Chapter 1).

Turtle sampling

Over a period of two years (November 2008 to December 2010), we carried out extensive in-water capture-mark-recapture (CMR) surveys and monitored the legal turtle fishery in TCI at landing sites (see Stringell et al. 2013, Chapter 1 for details of the fishery). Turtle capture location was recorded using a hand-held GPS or estimated following fisher interviews. Turtle size was measured along the midpoint of the carapace (Curved Carapace Length, cm (CCL): Bolten 1999). Turtle size was grouped into seven green turtle and eight hawksbill turtle size classes: group 1, 20-30cm; group 2, 30-40cm; … ; group 8, 90-100cm. Each turtle that was not butchered was released and tagged (metal flipper and passive integrated transponder (PIT) tags: Balazs 1999) unless tags were already present (Richardson et al. 2009). The sex of turtles was determined by gross morphology of the gonads of butchered animals, external morphology in adults, or from circulating testosterone concentrations from blood plasma samples (see Stringell et al. in prep., Chapter 4, for further detail).

Samples were collected from each turtle for use in stable isotope analyses (SIA) and a range of tissues was selected to represent different time-frames of diet integration: blood plasma informing a time scale of days to weeks; red blood cells (RBC), a time scale of weeks to months; and scute (keratin), a time scale of months to years (Seminoff et al. 2007, Reich et al. 2008). Blood was extracted from the dorsocervical sinus of each turtle while alive (Owens & Ruiz 1980) and plasma separated from RBC using a centrifuge (10,000 RPM); both tissue samples were

frozen until processing. Scute samples from green turtles were biopsied from the posterior (oldest) surface of the second lateral/costal carapace scute following Reich et al. (2007). In hawksbill turtles, the scute was too thick for biopsy sampling; instead, scute samples were cut from the trailing edge of the second lateral scute. (See supplementary information for further details). Sampling pseudo-replication was avoided by including only samples taken from marked individuals on one occasion.

Stomach content samples from 45 hawksbills and 92 green turtles of various sizes were collected directly from butchered animals and content identified to lowest taxonomic level (see Stringell et al. in prep., Chapter 5 for details) in order to determine the proportion of diet sources for use in SIA mixing model priors and to direct the collection of diet source samples from the wild. Voucher species of observed and putative turtle prey, for use as source samples in stable isotope analysis, were collected from the wild at several sites during CMR surveys. Samples were frozen until processing.

Laboratory analysis

Sample pre-processing

Diet source samples were defrosted and washed in distilled water and scraped/picked clean of extraneous particles and epiphytes. Some epiphytes, e.g. hydroids on *Sargassum spp.*, were analysed separately for stable isotope ratios where weight allowed. We used some of the stomach contents as sources in SIA because we were unable to collect them from the wild (e.g. because of water depth or low occurrence).

All tissue and habitat voucher samples were dried at 60°C and ground into powder and weighed into tin capsules to the nearest 0.1mg. For animal material, 0.7mg was usually sufficient to obtain carbon and nitrogen peak sizes within the desired range (as defined by laboratory standards), as was 1.2mg for plant material. Occasionally, sample weight had to be reduced or increased depending on carbon and nitrogen content. Lipids were not extracted from turtle tissue samples because carbon:nitrogen ratios were generally less than 3.5 (Post et al. 2007).

Diet source samples containing carbonate - Coralline algae, gorgonians, and sponges - were split into two, and half these samples were acid treated to remove carbonate, which is known to affect the $\delta^{13}C$ values (carbonates result in a higher δ13C than organic carbon, DeNiro & Epstein 1978) (see supplementary methods for

further details). Decarbonated samples were used to determine $\delta^{13}C$ values and untreated samples were used for $\delta^{15}N$. To determine if lipid extraction was required for source samples, several of the more common species were split into two, and lipids were extracted from half using a standard Soxhlet with a 2:1 mixture of methanol and ether solvents. Pre-treated samples were then compared to their paired non-treated samples in SIA. If necessary, a post hoc lipid correction factor was then applied to carbon isotope ratios ($\delta^{13}C$) (Post et al. 2007). However, differences in $\delta^{13}C$ were not significantly different between the lipid extracted and untreated samples (all *t*-tests were P>0.119), and we therefore did not correct for lipids.

Stable Isotopes analysis

SIA was carried out at the Natural Environment Research Council Life Sciences Mass Spectrometry Facility, East Kilbride, Scotland. Carbon and nitrogen isotope measurements were determined simultaneously using an ECS 4010 elemental analyser (Costech, Milan, Italy) interfaced with a ConFlo III and Delta V Plus mass spectrometer (Thermo Fisher, Bremen, Germany). Three internal standards - Sigma Aldrich gelatine, alanine solution and glycine - were run every 10 samples to monitor instrument drift and allow for any corrections to sample values from standard curves. Aliquots of an additional laboratory standard, tryptophan, were also run to determine the carbon and nitrogen contents of the source and consumer samples, and percent and weight of carbon and nitrogen were calculated. Delta notation was used for stable isotope abundances in parts per thousand (‰) relative to international standards: Vienna Peedee belemnite (^{13}C) and atmospheric N_2 (^{15}N):

$$\delta X=(R\text{sample}/R\text{standard} -1) \times 1000 \quad (1)$$

Where *R*sample and *R*standard are ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in the sample and international standard respectively. Measurement precision (SD) was estimated to be 0.08‰ for $\delta^{15}N$ and 0.02‰ for $\delta^{13}C$ (derived from gelatine standards, n=157).

Data analysis

All multivariate statistical routines were carried out in PRIMER v6 software (Clarke & Gorley 2006) with the PERMANOVA+ add on (Anderson et al. 2008) and univariate

and Bayesian tests in R v 2.12 (R Development Core Team 2012).

Turtle size and isotopic changes

Isotope ratios from plasma, RBC and scute tissues were compared separately with turtle size (CCL) as a continuous variable using generalised additive models (GAMs: Wood 2011). Additionally, isotopes were compared together via Euclidean distances in a permutational multivariate analysis of variance (PERMANOVA: Anderson et al. 2008) to compare size-classes for each tissue and species.

Isotopic niche metrics

Using Stable Isotope Bayesian Ellipses in R (SIBER: Jackson et al. 2011) in the Stable Isotope Analysis in R package (SIAR: Parnell et al. 2010), we compared the total isotopic niche width (convex hulls) of the turtle population between species. We also compared various isotopic niche descriptors using Bayesian versions of the Layman isotopic niche metrics (see Layman et al. 2007 for details): $\delta^{15}N$ range - dNR; $\delta^{13}C$ range - dCR; total area - TA; mean distance to centroid - CD; mean nearest neighbour distance - MNND; standard deviation of nearest neighbour distance - SDNND). We then examined the isotopic niche width of turtle size classes within and between turtle species by comparing their Bayesian standard ellipse areas (SEAb) calculated using SIBER. Ellipse areas were compared statistically by calculating the proportion of posterior ellipses of one size class that were smaller or larger than those of another size class; a proportion <0.05 or >0.95 was considered significant (McCarthy 2007). The same approach was used to test for significant differences in ellipse areas and each niche metric between species.

Mixing models

Isotopic discrimination between hawksbill turtles and potential prey was accounted for using Trophic Enrichment Factors (TEF, Δdt) measured in similarly sized juvenile loggerhead turtle (*Caretta caretta*) tissues (Reich et al. 2008); blood plasma (Δdt $\delta^{13}C$: −0.38±0.21‰, Δdt $\delta^{15}N$: 1.50±0.17‰), RBC (Δdt $\delta^{13}C$: 1.53±0.17‰, Δdt $\delta^{15}N$: 0.16±0.08‰), and scute (Δdt $\delta^{13}C$: 1.77±0.58‰, Δdt $\delta^{15}N$: −0.64±0.09‰). For green turtles, blood plasma (Δdt $\delta^{13}C$: −0.12±0.03‰, Δdt $\delta^{15}N$: 2.92±0.03‰) and RBC (Δdt $\delta^{13}C$: −1.11±0.05‰, Δdt $\delta^{15}N$: 0.22±0.03‰) TEFs were taken from green turtles (Seminoff et al. 2006), and scute TEFs from loggerheads (as above, Reich et al.

2008).

Isotope mixing models are widely used to estimate proportional contribution of sources (putative prey items) to a mixture (consumer tissue) to infer diet composition (Phillips & Gregg 2001, 2003, Phillips et al. 2005, Moore & Semmens 2008, Jackson et al. 2009, Parnell et al. 2010). In our study, we had many diet source items with wide variation in isotope ratios, uncertainty in TEFs (e.g. hawksbill tissues and green turtle scute TEFs taken from loggerhead trials, Reich et al. 2008) and several turtle size-class groups we wished to run simultaneously, each with variability in isotope values. Therefore, a Bayesian framework was appropriate to model this natural variation and uncertainty in order to generate robust probability estimates of source proportions (Parnell et al. 2010). We used SIAR with concentration dependence (Phillips & Koch 2002) in the model and uninformed (uniform) priors, with 500,000 MCMC iterations, a 50,000 burn-in and thinning by 45 to reduce sample autocorrelation (Jackson et al. 2009, Parnell et al. 2010). We also ran SIAR with informed priors consisting of proportions of diet items observed in previous stomach content analysis (see Stringell et al. in prep., Chapter 5), and compared model solutions to those using uniform priors. Before running SIAR, sources were grouped into eight taxonomically coherent groups (bluegreen algae, red algae, green algae, brown algae, seagrasses, sponges, cnidarians, and other invertebrates), and consumers classified into size class mixture groups (as before). To test for significant differences in source contributions among turtles sizes, we calculated the proportion of contributions from the MCMC process of one size group that were greater than those of another group (as before). Separate mixing models were run for plasma, RBC and scute tissues.

Results

Isotopic profiles and inter-species niches

As blood plasma tissue is the most rapid turnover of the tissues tested and likely to represent diet incorporation in the order of days to weeks, we chose to report on plasma results to most closely represent the likely time frame of foraging that stomach content samples contain (for coherence with data presented in Stringell et al. in prep, Chapter 5).

Isotopic profiles differed markedly between the turtle species; Hawksbill turtles generally had higher $\delta^{15}N$ nitrogen levels and lower $\delta^{13}C$ carbon isotope levels than green turtles, indicative of their higher trophic position and food sources of a more oceanic/offshore signature (although some overlap in isotopic location is evident, Figure 1). Seagrass sources were isotopically close to most green turtle samples, while other sources, especially sponges, red algae, and cnidarians, were closer to hawksbill turtle samples (Figure 1). Isotope biplots for RBC and scute indicate similar isotopic locational differences as seen with blood plasma (supplementary Figure S1)

The isotopic niche space (convex hulls) of green turtles was much wider than that of hawksbill turtles, with significantly higher Bayesian Layman metrics for green turtles in all cases ($P<0.05$) (Figure 2). This was similarly the case for blood and scute tissues (supplementary Figure S2).

Turtle body size

Ontogenetic shifts

The sizes of turtles sampled ranged from 19.6 to 92.2cm in hawksbill turtles and 25.1 to 102.6cm in green turtles (Figure 3). In hawksbill turtle scute tissue there was a significant linear increase of approximately three units (‰) in $\delta^{13}C$ isotopic values with increasing body size (from −18‰ in smaller sizes [20cm] to −15‰ in larger size turtles [90cm]), and a decline of approximately two units (‰) in $\delta^{15}N$ isotopic values (from +6 to +4‰), although the weak R^2 of the model for $\delta^{15}N$ is noted (Figures 4-5). There were no significant changes with size for either isotope signatures in hawksbill plasma or RBC. Conversely, green turtles show a significant non-linear change with increasing body size in both isotopes and all three tissues, with an increase of approximately seven units (‰) in $\delta^{13}C$ (from −15‰ to −7‰) and a decrease of approximately three units (‰) $\delta^{15}N$ (from +6‰ to +3‰) (Figures 4-5).

PERMANOVAs on the combined stable isotope Euclidean distances for each tissue and species across turtle size classes revealed similar patterns: In hawksbill turtle scute, but not plasma or RBC, there was a significant change (an increase largely driven by $\delta^{13}C$) in isotope ratios with size class which explained 37% of the model (Pseudo-F_7=5.90, P_{perm}<0.001); In all green turtle tissues there were significant differences among size classes (driven by an increase in $\delta^{13}C$ and a decrease in $\delta^{15}N$ with size) and in all pairwise comparisons, isotope distances in smaller size classes were generally significantly different from larger sizes of green turtles.

Intra- and inter-species isotopic niches

As before, we report on blood plasma results for coherence with stomach content samples (Stringell et al. in prep, Chapter 5). Standard ellipse locations shifted from lower $\delta^{13}C$ and higher $\delta^{15}N$ isotopic space in small (young) green turtles to lower $\delta^{15}N$ and higher $\delta^{13}C$ isotopic space in larger (older) turtles, while in hawksbill turtles, isotopic ellipse locations overlapped across size-classes (Figure 6). Bayesian standard ellipse areas (SEAb) in hawksbill turtles, however, generally increased in area and variation with body size (except for the largest size class). But in green turtles, SEAb decreased from the 40-50cm size class with increasing body size (these patterns are more pronounced in RBC and scute tissues; supplementary Figure S3).

For 20-30, 40-50 and 50-60cm size classes, green turtle SEAb from blood plasma tissue (Figure 6) were significantly larger than those of hawksbill turtles (P<0.01), the SEAb area of the 80-90cm hawksbill size class was larger than that of green turtles (P<0.05), and other comparisons were not significant. For blood samples, green turtle SEAb were larger than hawksbill turtles for only the 20-30 and 40-50cm size classes (P<0.01) and for scute samples, green turtle SEAb of 20-30 to 50-60cm size classes were significantly larger than hawksbill turtles (P<0.05) (not shown).

Mixing models: diet changes with size

For the mixing models, over 100 samples of each tissue (only plasma is reported here) were analysed for each turtle species (supplementary Table S1) and over 300 samples of 118 taxa were used as grouped diet sources (supplementary Table S2).

Larger size classes (>70cm) in both turtle species and the smallest size class (20-30cm) in green turtles generally suffered from low sample sizes (minimum of 4; Figure 3, supplementary Table S1).

Green turtles showed a marked statistically significant increase in seagrass consumption with increasing body size until a significant drop in contributions at the two largest size classes (all 10k iterations of source contributions were larger in the 60-70cm size class than 20-30, 70-80 and 80-90cm size classes: $P<0.0001$, Figure 7); the same patterns were observed for RBC and scute (Figures not shown). Models suggest that other dietary items are consumed in those size classes where seagrasses make up less of the diet (in order to sum to one; supplementary Figure S4).

Hawksbill turtles, however, showed a varied diet through all size classes, although there appears to be an increase in sponge and red algae consumption in 40-50 and 50-60cm size classes (although not statistically significant; Figure 7, supplementary Figure S5). While Figure 7 shows the results of plasma tissue, the same patterns were observed for RBC and scute (Figures not shown).

Using priors of the relative proportions from turtle stomach content samples (Stringell et al in prep, Chapter 5), the mixing models on plasma samples gave more variable but elevated contributions for sponges in hawksbill turtles (no significant differences among size classes). In green turtles, the same pronounced increase ($P<0.0001$ between 20-30 and 60-70cm size classes) in seagrass contributions through the sizes and a significant decline ($P<0.001$ between 60-70 and 80-90cm size classes) in the larger sizes, albeit with high uncertainty (the 70-80cm size class was uninformative, most likely due to small sample size) (Figure 7). Proportions of other sources in the diet resulting from mixing models using plasma tissue and uniform priors are shown in supplementary Figure S4 and S5. Diet proportions from weighted priors (not shown) indicated similar patterns but with wider variation.

Discussion

Isotopic profiles and inter-species niches

While it is known that green and hawksbill turtles feed at different trophic levels (Bjorndal 1997), suggesting different ecological niches - green turtles being herbivorous and hawksbill turtles spongivorous - a direct comparison of the feeding ecology of these sympatric species from a single location has so far not been carried out. This study uses SIA to quantify isotopic niche space to infer ecological niche separation between the two species. As expected, the $\delta^{15}N$ isotopic profile of hawksbill turtles suggests they feed at a higher trophic level than green turtles. The interpretation of the differences in $\delta^{13}C$ profiles between the species is likely a reflection of key habitat differences: Hawksbills are typically found on hard-bottom/coral reef habitats and forage on filter-feeding sponges that are influenced by oceanic food sources; Green turtles are typically found on sheltered sediment-based seagrass beds. Our results indicate a high degree of separation in all isotopic niche metrics between these species, with some overlap, perhaps associated with green and hawksbill turtles feeding on sponges in similar habitats, most likely from seagrass pastures (Bjorndal & Bolten 2010). These results imply little competition for resources between the turtle species.

The wider isotopic niche space of green turtles than that of hawksbills, is contrary to that expected by the narrow taxonomic structure of green turtle diet as determined from stomach content samples, but the isotopic niche width is likely to be very much enlarged by the likely ontogenetic shift in diet from small to large sizes. The comparatively narrow isotopic niche width of the hawksbill turtle population, however, supports the findings of the stomach content analysis where the predominant diet was sponges (Stringell et al in prep, Chapter 5).

One pervading issue with isotopic niche metrics is that the breadth of isotopic δ-space may be deceptive because not only may it represent broad taxonomic differences in diet sources but also the magnitude of differences in isotopic signatures of those resources (Newsome et al. 2007).

Ontogenetic shifts

Green turtles

Our results demonstrate an ontogenetic shift in green turtles as also described by

Reich et al. (2007) and Arthur et al. (2008). As they shift from a carnivorous or omnivorous diet to a herbivorous one, a non-linear increase in $\delta^{13}C$ and decrease in $\delta^{15}N$ isotope ratios is seen respectively. This pattern is confirmed in all three sampled tissues that represent different time periods over which diet is incorporated, indicating a gradual change to herbivory and incorporation of coastal signatures. If a rapid ontogenetic and isotopic change were anticipated on recruiting to the coast, we would have expected to see herbivorous (low $\delta^{15}N$ values) and coastal (high $\delta^{13}C$) isotopic signatures in plasma tissue, and perhaps RBCs, across all size classes. It would seem that green turtles may recruit to the coastal feeding grounds of TCI at a wide range of small sizes and develop herbivory gradually, perhaps causing the gradual decline in $\delta^{15}N$ and increase in $\delta^{13}C$ with size in plasma and RBCs rather than abrupt changes expected if diet shifts are immediate.

Both the GAM models, and particularly the mixing models, indicate that as green turtles approach 70cm there appears to be a further shift in isotopic ratios, perhaps indicating another change in dietary components. Although this change may be due to small sample sizes in the larger size classes, this might coincide with the sizes at which green turtles begin developmental migrations to adult foraging grounds, a life stage that may require changes in nutritional demands. If this represents a genuine shift in diet, then it is contrary to consensus (Meylan et al. 2011), but is evident in both GAM plots of $\delta^{13}C$ and $\delta^{15}N$ vs. size (Figures 4-5) and in the SIAR mixing models, where a shift in dietary sources best explains this change in consumer isotopic values (Figures 2, 6, and 7). Large individuals, however, may have been out to sea, incorporating an oceanic influence to their stable isotope ratios. For example, satellite tracking of several of these larger size green turtles captured in TCI, has revealed that some undertook long distance developmental migrations to Cuba and North Carolina (authors' unpublished data); perhaps we sampled sub-adult turtles from other natal origins consisting of differing isotopic signatures. It is possible, therefore, that differences in seagrass signatures from wide geographic areas may influence these inferences (Vander Zanden et al. 2013). Further sampling of these larger sized turtles may elucidate this hypothesis further.

Hawksbill turtles

Our data from scute tissue suggest that as hawksbill turtles increase in size, $\delta^{13}C$ isotopic ratios increase and $\delta^{15}N$ decreases. The linear shift in $\delta^{13}C$ with size is likely

to represent a shift in location. As in other marine turtle species, this may suggest an ontogenetic shift from pelagic/oceanic habitats to benthic/neritic feeding grounds (and an increased intake of lower trophic level diet items (decrease in $\delta^{15}N$)) with increasing size (Musick & Limpus 1997, Bolten 2003, Reich et al. 2007, Arthur et al. 2008, This Study). Hawksbill plasma and RBC, however, did not show any significant differences with size suggesting that recruitment to the coastal feeding grounds might have occurred at sizes smaller than those sampled; ontogenetic shifts were only detectable in the scute tissue which represents a longer history of resource use. This 'missing' size range is likely to have high growth rates in comparison to larger sizes (Chaloupka & Limpus 1997) and therefore may represent a short life stage. This implies that a change in habitat and/or diet could have occurred abruptly, and is coherent with the classical (abrupt) ontogenetic shift model (Bolten 2003, Carr 1986, 1987, Snover et al. 2010). To assess if the observed significant variation in isotope ratios with increasing body size was dependent only on the smallest size classes, we excluded the smallest size classes (in 5cm increments) from the data and re-ran the GAM models. Removing sizes up to 30cm CCL revealed no significant decrease in $\delta^{15}N$ in the remaining sizes ($P=0.227$, $R^2=0.01$), but retained the significant increase in $\delta^{13}C$ ($P=<0.001$, $R^2=0.18$), perhaps suggesting an ontogenetic diet shift that occurs abruptly and a habitat shift that changes gradually. Only by excluding size classes up to 50cm did the remaining sizes show no significant change in carbon isotope ratios ($P=0.07$, $R^2=0.04$), implying that by this body size, any ontogenetic habitat shift is likely to be permanent. The abrupt ontogenetic diet shift differs markedly to that observed in green turtles in this study, which represents a gradual non-linear change across size classes and plastic foraging behaviour, as known for loggerhead turtles, for which the classical ontogenetic model is no longer supported (McClellan & Read 2007).

The change in $\delta^{15}N$ with increasing hawksbill turtle body size (inclusive of all size classes) could represent an increasing intake of lower trophic level diet items, but probably not a change in consumer trophic level, which is typically accompanied by a larger change (fractionation) of approximately 3.4‰ per level (Post 2002). The decline in $\delta^{15}N$ may indicate that, compared to earlier life stages, the diet of larger sized hawksbill turtles might be composed of more algae material that generally has lower $\delta^{15}N$ composition than higher taxa (supplementary Table S2); most (73%) hawksbill turtle stomach samples had algae species in them and contributed

approximately 12% to the average diet in terms of relative abundance (biomass), but there were no significant change in diet with size (Stringell et al in prep, Chapter 5). However, the use of $\delta^{15}N$ as a resource axis may not provide a quantifiable measure of trophic level until we have a better understanding of the environmental and physiological factors that determine the magnitude of changes in $\delta^{15}N$ (Newsome et al. 2007).

Intra-species isotopic niches

Green turtle isotopic niche width (ellipse area) generally decreases from 40cm CCL with increasing body size, indicating that diet may become more specialised (and isotopically narrow) as turtles age (Vander Zanden et al. 2013). This change from a known omnivorous/carnivorous diet in the pelagic small juveniles to a benthic herbivorous diet in juveniles after recruiting to coastal environments is well documented (Bjorndal 1985, Bjorndal 1997, Arthur et al. 2008, Reich et al. 2008).

Hawksbill turtle isotopic niche width increases significantly with body size, up to adult sizes of 80-90cm, indicating diet becomes more isotopically broad. At >90cm there is a decline in mode ellipse area to a level similar to that of small sizes. It is possible that this is an artefact of small sample sizes, but these data could suggest that hawksbill turtles may start off with a more specialised diet and become a more generalist feeder or change to alternative specialised diet with increasing size, although stomach content analysis did not support this hypothesis (Stringell et al in prep, Chapter 5).

Mixing models: diet changes with size

Green turtles

Green turtle diet is composed largely of seagrasses and algae, with an apparent change in diet with size as expressed by an increase to a peak in seagrass consumption at 60-70cm size classes followed by a decrease as other diet items (sponges and algae) contribute to the diet. Larger turtles forage at greater depth (Musick & Limpus 1997) and thus may access a wider variety of food, and as a result have different trophic signatures to smaller turtles. This pattern, however, was not reflected in the stomach content analyses (see Stringell et al in prep, Chapter 5) which indicated no significant differences in diet proportions (biomass) with size. It is clear that SIA probably more realistically reflects what turtles are consuming and

assimilating into their tissues: stomach content analysis is a ‘snapshot’ of feeding which may not be representative of a varied diet of an individual over time (Duffy & Jackson 1986, Barrett et al. 2007) - SIA has the advantage of assessing diet that has been integrated over different time frames depending on the tissue examined (Newsome et al. 2007).

Hawksbill turtles

Mixing models also indicated a highly mixed diet for hawksbill turtles, much more than suggested by the stomach content, which was dominated by sponges (Stringell et al in prep, Chapter 5), and there was little change with size. This was the case even when model priors were used that were heavily weighted towards sponge dominance as determined by stomach content analysis. Sponges house many symbiotic, parasitic and commensal species that increases the apparent taxonomic breadth of diet; it maybe that residents of sponges have more nutritional value than sponges themselves, although invertebrates (a putative proxy for sponge dwellers) did not contribute greatly to the SIAR mix. It is also possible that sponges are digested and incorporated/metabolised far less than we think (e.g. see Bjorndal 1990 for green turtles).

The highly mixed nature of the hawksbill turtle diet indicated by the mixing models might be indicative of source samples that overlap isotopically and may not be as distinct from each other as required for discrimination. Isotopic measurements can only distinguish among resources with contrasting isotopic signatures (Newsome et al. 2007) and the discrimination factors of sources may differ markedly (Post 2002, Vander Zanden et al. 2012). However, picking and choosing source diet items that are distinct seems counterintuitive and biased when diet is known to be taxonomically broad (see Stringell et al in prep, Chapter 5, for review).

Appropriate trophic enrichment factors (TEFs) are critical in getting good mixing models and source partitioning (Seminoff et al. 2006, Newsome et al. 2007, Reich et al. 2008). There are no published TEFs for hawksbill turtles, so their closest relative (loggerhead turtles) was used here instead. Further work is required to determine TEFs for the hawksbill turtle, which may provide clearer signals of diet contributions in the current mixing models.

Concluding remarks

Our study suggests ontogenetic habitat and diet shifts in two sympatric marine turtle species. Green turtles reveal gradual size partitioning and ontogenetic structuring in the foraging patterns of this species; from a pelagic, oceanic and carnivorous phase in early life stages to a neritic, benthic and herbivorous phase in later life stages where they predominantly feed on and incorporate seagrasses. Data on hawksbill turtles suggest they may have a gradual ontogenetic habitat shift and an abrupt ontogenetic diet shift with a change in trophic feeding that represents a much more varied diet than expected, that is, not only dominated by sponges. This mixed diet was confirmed by stomach content analysis (Stringell et al in prep, Chapter 5) and has several implications. 1) Previous studies of hawksbill turtle diet have been somewhat limited by stomach content analyses. Taxa other than sponges that may be incorporated from the diet into bodily tissue may not be well represented in stomach content leading to biased inferences on feeding ecology. 2) The incorporation of diet items other than sponges, especially algae, may be a result of changes in the availability of diet, for example, increases of algal cover in depleted coral reef systems (Mumby 2009, McMurray et al. 2010), likely in response to overfishing (Mumby et al. 2006, Pawlik et al. 2013). Only a longer term analysis of potentially changing isotopic profiles coupled with habitat surveys of known foraging grounds might indicate such ecosystem changes.

Isotope ratios are subject to variation among individuals, locations and seasons, which may mask signals in either $\delta^{13}C$ or $\delta^{15}N$ (McClellan et al. 2010, Vander Zanden et al. 2012). Although our study had a large sample size for analysis, samples were limited from some locations and seasons and in some size classes, particularly small hawksbills and large green turtles. Targeting future sampling to certain locations, seasons and size classes would allow a more complete range of factors to be analysed to further explore the findings of this study. Additionally, incorporating other methods, such as satellite tracking and genetics, may further elucidate life history traits such as migrations between serial foraging grounds, reproductive migrations and foraging strategy dichotomies (Hatase et al. 2002, Hawkes et al. 2006, McClellan et al. 2010).

Analysis of the isotopic niches of sympatric species, such as marine turtles, and monitoring the degree of overlap in normally well separated niches, might provide a valuable indicator of broad ecological changes. Green and hawksbill turtles are

among the largest-bodied grazers in the tropical coastal ecosystems and are likely to have key roles in regulating the structure and function of reef and seagrass habitats (Bjorndal & Jackson 2003). With marine turtles widely considered as keystone species, knowledge of their foraging ecology in coastal ecosystems is essential for effective conservation and SIA has a primary role in delivering these insights.

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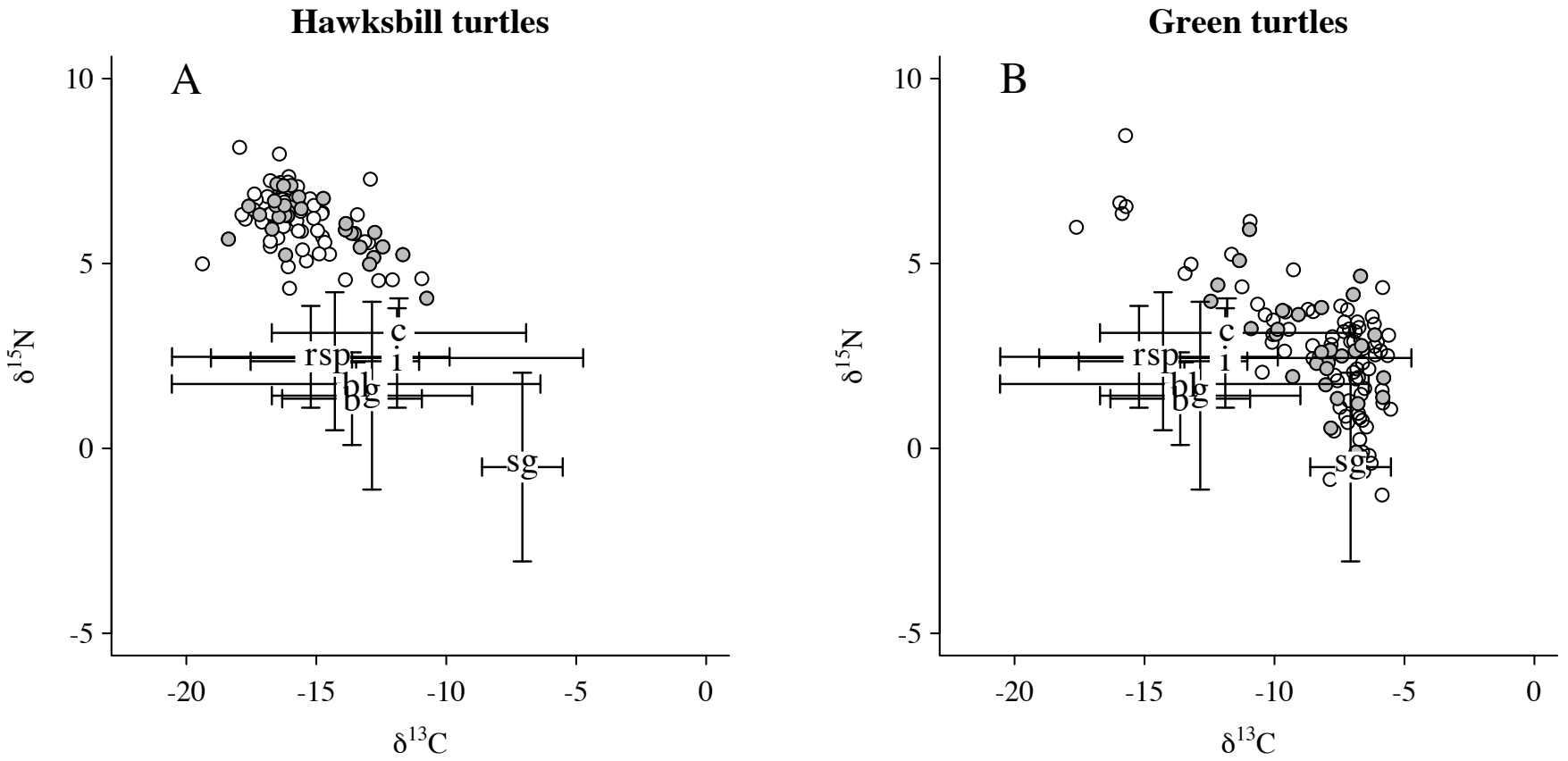

Figure 1. Biplot of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (‰) for hawksbill turtle (A, n=108) and green turtle (B, n=108) blood plasma samples (circles). Filled circles are turtles for which we also had stomach content samples (n=45 hawksbills and n=92 greens). Diet sources (±SD) are bluegreen algae (bl), red algae (r), green algae (g), brown algae (b), seagrasses (sg), sponges (sp), cnidarians (c), and other invertebrates (i).

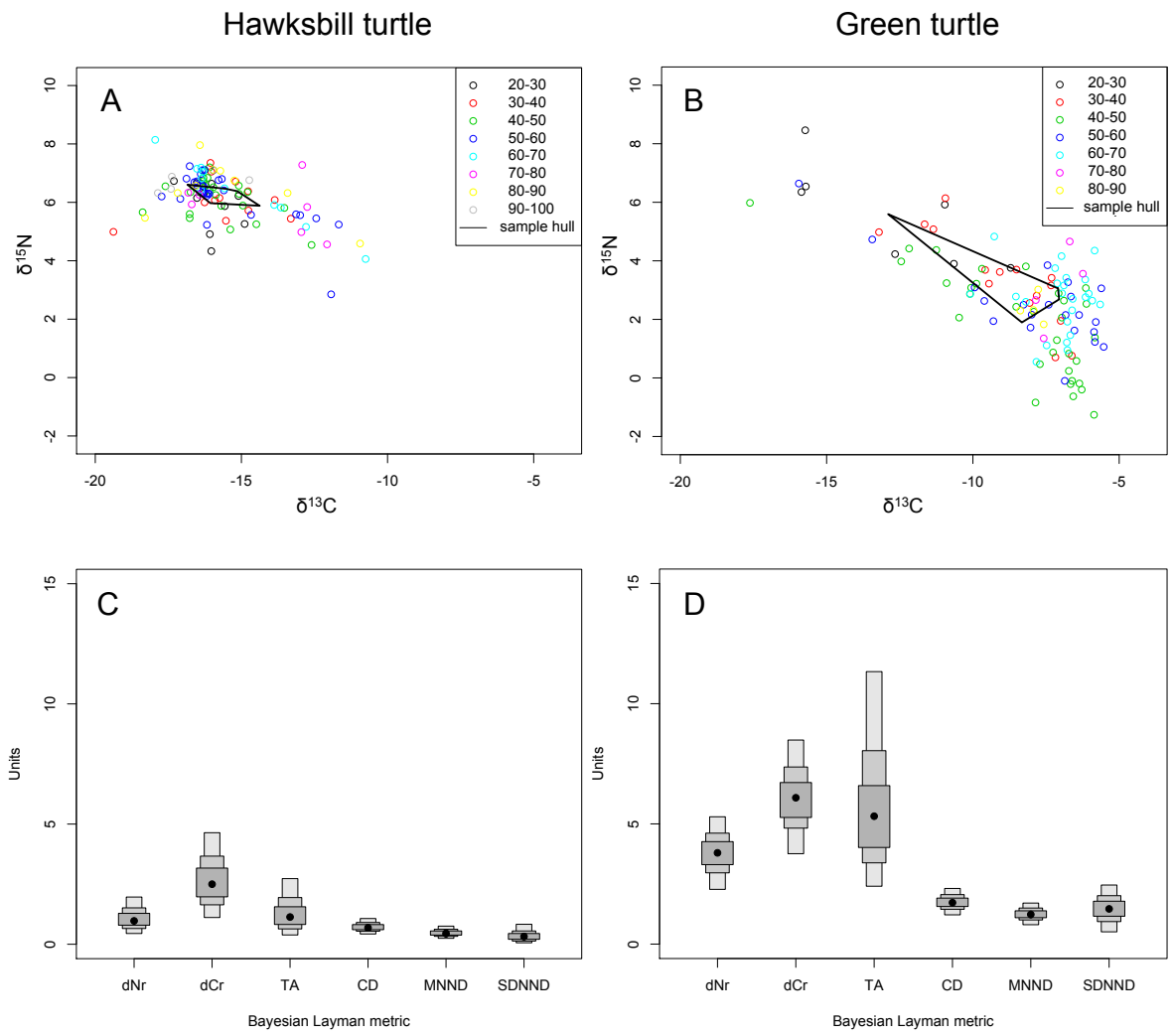

Figure 2. Inter-species isotopic niche metrics for hawksbill turtle (A, C) and green turtle (B, D) blood plasma samples. Standard convex hulls (joining the extreme most means of the turtle size classes: smallest, 20-30cm, … , largest, 90-100cm, CCL) for the all-size population are shown for illustration of one possible iteration of the total niche width (A, B), and various Bayesian Layman niche metrics are given (C, D): $\delta^{15}N$ range - dNR; $\delta^{13}C$ range - dCR; total area - TA; mean distance to centroid - CD; mean nearest neighbour distance - MNND; standard deviation of nearest neighbour distance – SDNND (see Layman et al. 2007 for details on metrics). The Bayesian metrics can be compared between the turtle species.

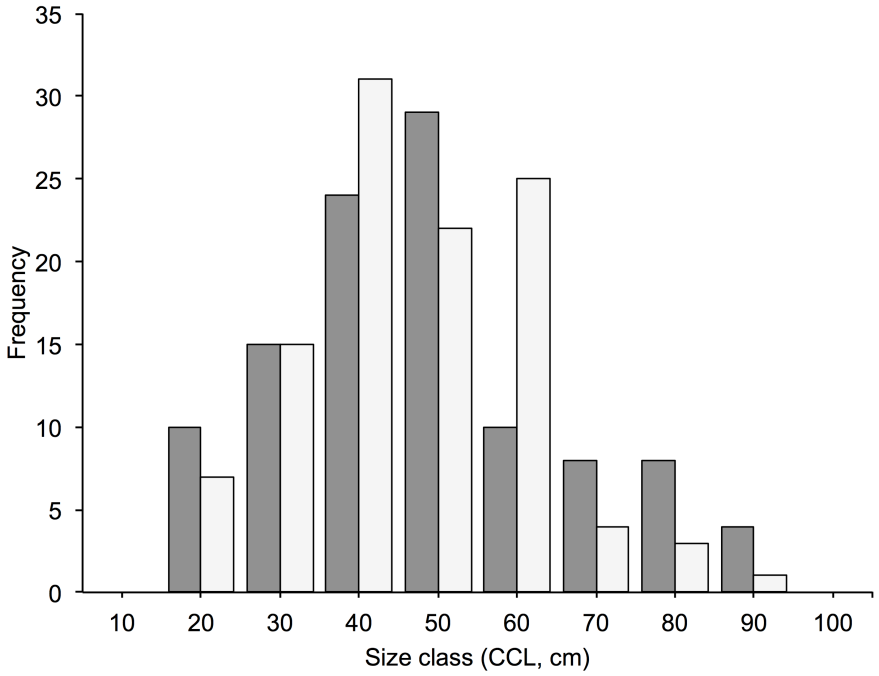

Figure 3. Size frequency histogram of hawksbill (dark grey) and green turtles (light grey) sampled in this study. Sizes are curved carapace length (CCL) taken from turtles that were sampled for blood plasma tissue for use in stable isotope analysis (n=108 for each species).

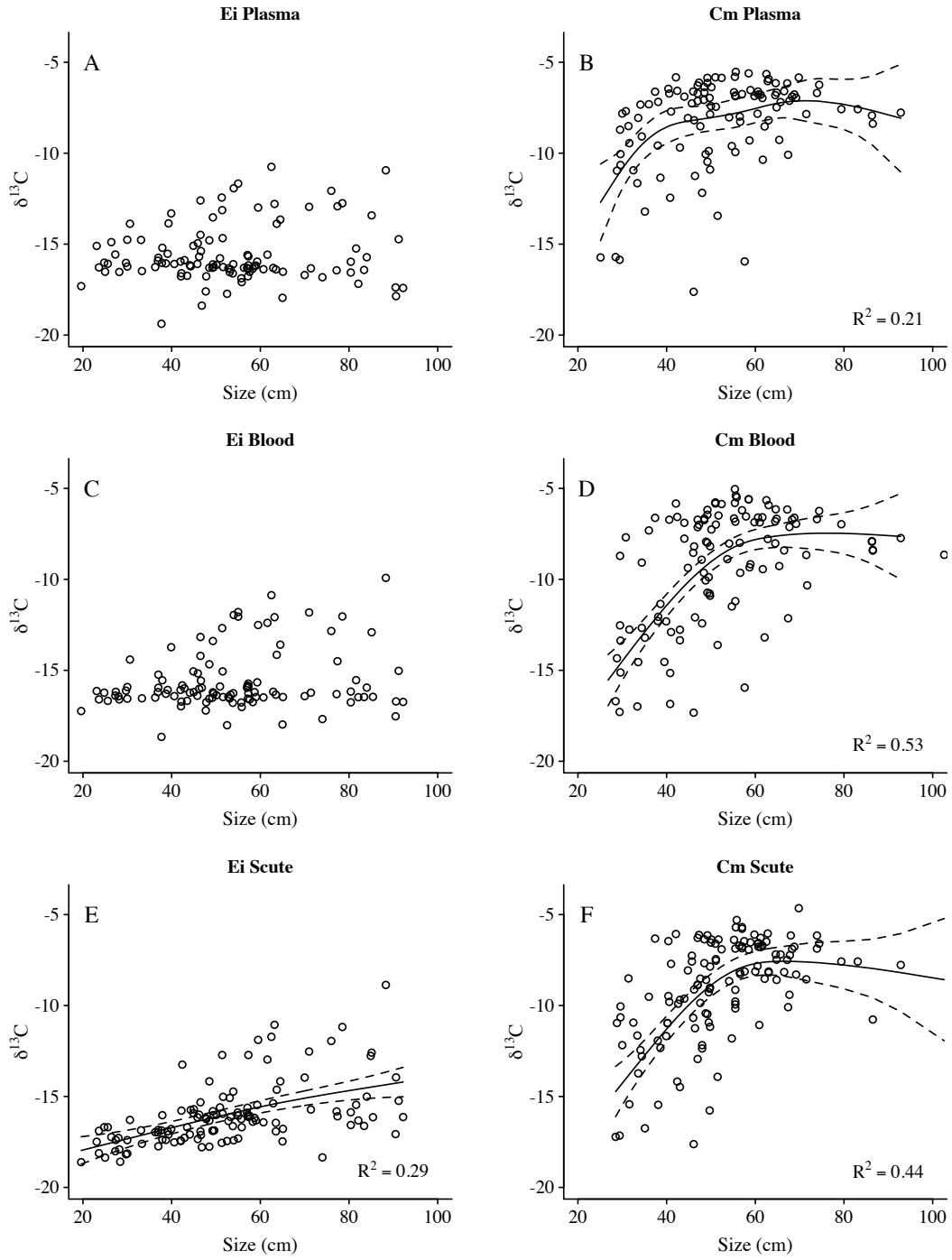

Figure 4. Size (CCL, cm) and δ13C isotope ratios for blood plasma (A, n=108; B, n=108), red blood cells (C, n=107; D, n=123) and scute (E, n=121; F, n=120) tissues from hawksbill turtles (Ei) and green turtles (Cm). Significant GAMs shown with R^2_{adj} values of fit: Green turtle: Plasma, $F_{4.9}$=5.95, P<0.0001, n=108; Blood, $F_{3.6}$=37.58, P<0.0001, n=123; Scute, $F_{3.5}$=25.95, P<0.0001, n=120. Hawksbill scute: $F_{1.9}$=26.14, P<0.0001.

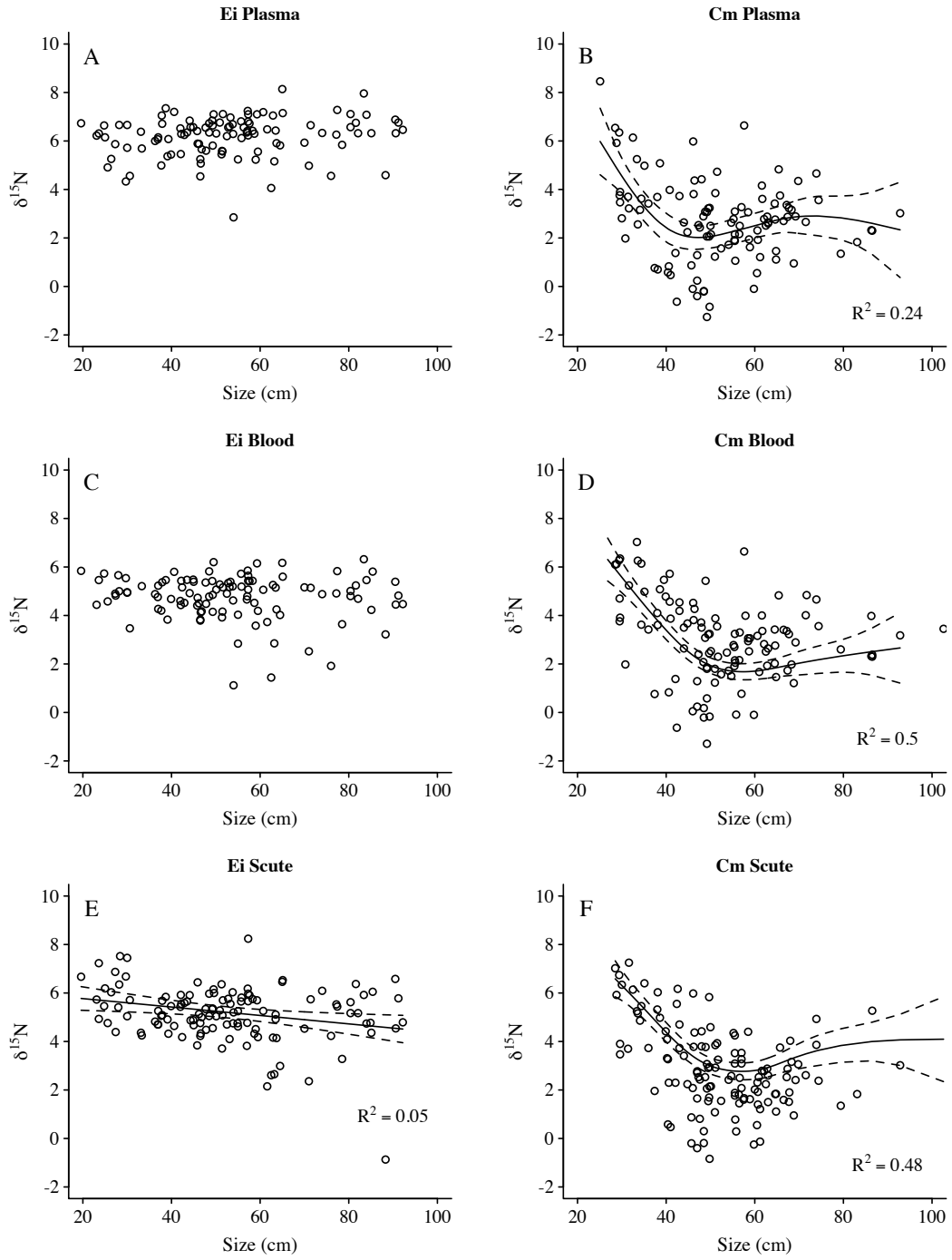

Figure 5. Size (CCL, cm) and δ15N isotope ratios for blood plasma (A, n=108; B, n=108), red blood cells (C, n=107; D, n=123) and scute (E, n=121; F, n=120) tissues from hawksbill turtles (Ei) and green turtles (Cm). Significant GAMs shown with R^2_{adj} values of fit: Green turtle: Plasma, $F_{4.8}$=7.01, P<0.0001; Blood, $F_{4.2}$=29.05, P<0.0001; Scute, $F_{4.6}$=23.33, P<0.0001. Hawksbill scute: GAM d15N, F_1=7.11, P=0.009.

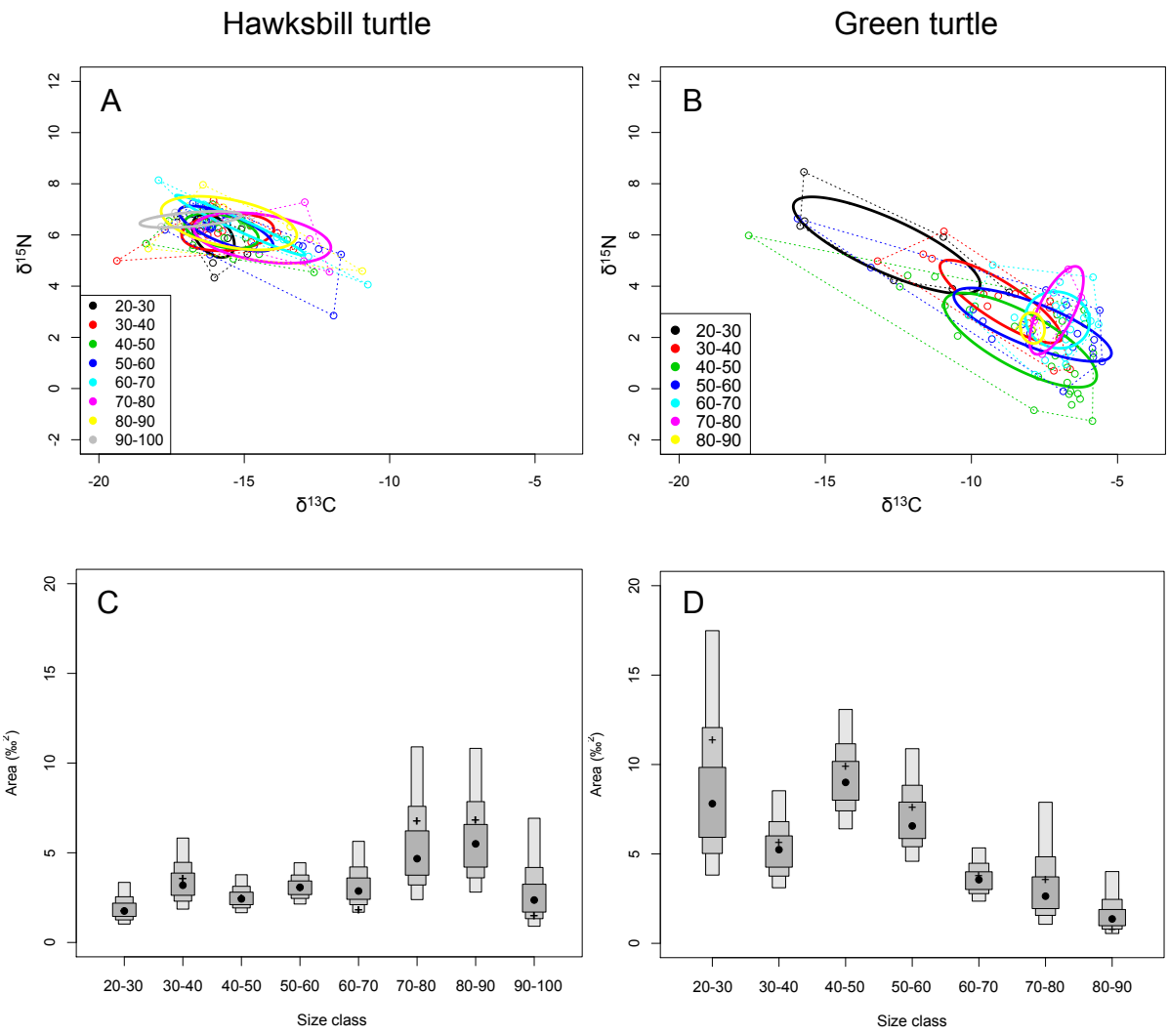

Figure 6. Intra-species isotopic niche space for hawksbill turtle (A, C) and green turtle (B, D) blood plasma tissue across turtle size classes (cm, CCL). Standard ellipse areas are sample-size corrected (SEAc: A, B). Corresponding Bayesian standard ellipse areas (SEAb) are shown for each size class (C, D) and can be compared among sizes and turtle species. Medians of SEAc (cross) and mode of SEAb (dot) are overlaid on the box plots of SEAb, which represent the 50%, 75% and 95% credible intervals from dark to light grey.

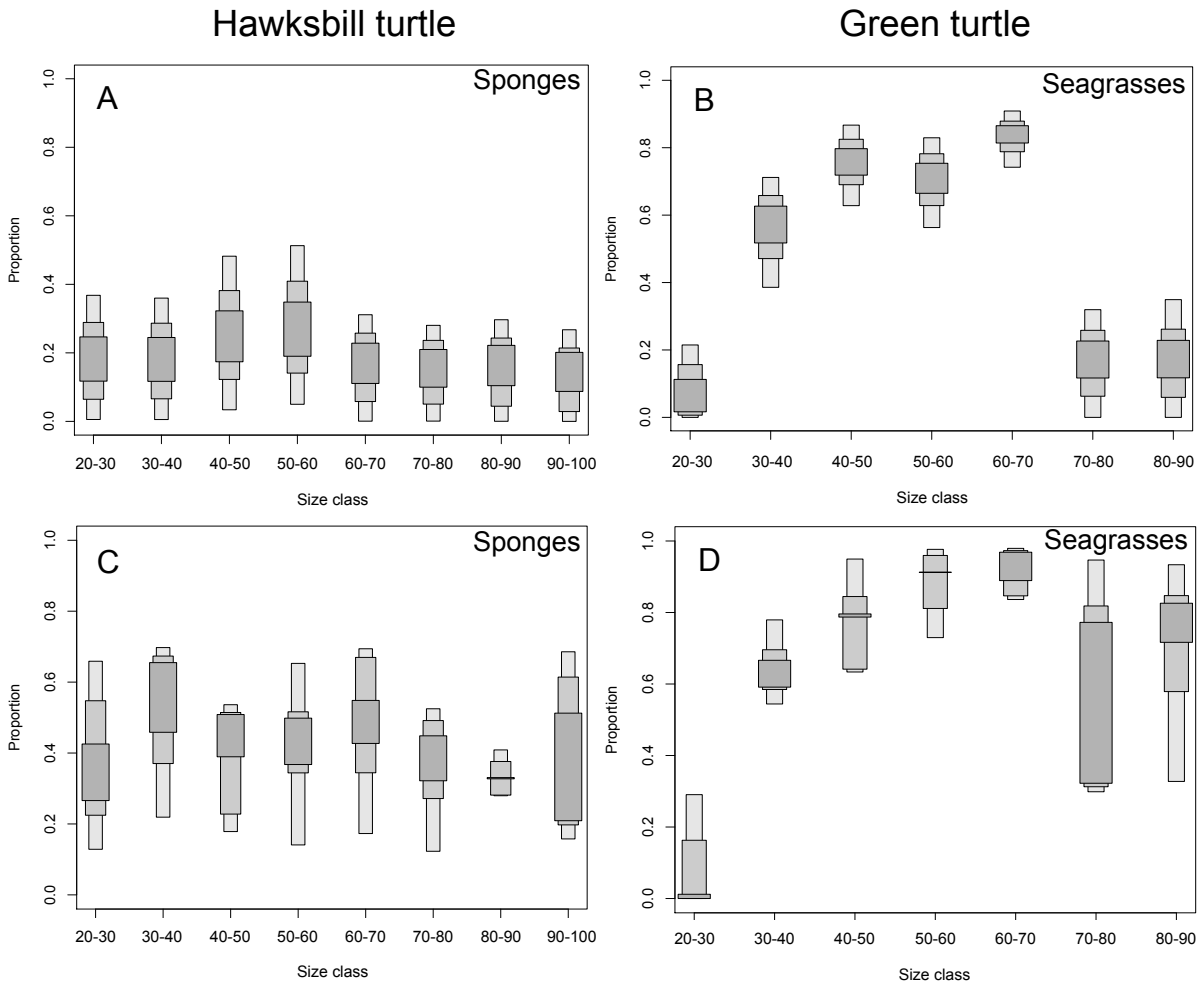

Figure 7. Mixing model contribution proportions across turtle size classes (cm, CCL). Proportions of sponges to hawksbill turtle diet (A, C), and seagrasses to green turtle diet (B, D) are derived from plasma tissue. Top panel (A, B) shows results from models with uninformative (uniform) priors and bottom panel (C, D) with priors based on relative percentage of diet composition in stomach content samples (taken from Stringell et al. in prep., Chapter 5). Box plots represent the 50%, 75% and 95% credible intervals from dark to light grey.

Chapter 6: Supplementary Information

Methods

Turtle sampling

Blood was extracted from the dorsocervical sinus of each live turtle (Owens & Ruiz 1980) using sterile 0.8x25mm or 0.8x38mm needles (depending on animal size) and 6ml BD Vacutainer sampling tubes internally coated with Lithium Heparin anticoagulant, which does not effect SIA (see Lemons et al. 2012). Blood samples were stored on ice in the field and transferred to a refrigerator within hours before being centrifuged for 10mins at 10000 RPM. Blood plasma was pipetted into cryovials and stored at -20°C until transferred to the UK for long-term storage at −80°C. Red blood cells (RBC) were frozen in Vacutainers at -20°C. Scute sampling in green turtles followed Reich et al. (2007) where sterile biopsy punches (2mm or 6mm depending on turtle size) were used to take full scute depth samples from the posterior (oldest) of the second lateral/costal carapace scute.

Collection of diet sources

Throughout October 2010, shallow (<10m depth) snorkelling surveys were carried out at several locations throughout TCI to describe the epibenthic macrofaunal communities of reef-based and seagrass dominated habitats (see Stringell et al. in prep., Chapter 5, for details of methods). Voucher species of observed and putative turtle diet, for use as sources samples in stable isotope analysis, were collected from the wild at several sites during these and earlier capture-mark-recapture surveys during the two year period. Samples were frozen until processing.

Laboratory analysis

Sample pre-processing

All scute samples were thoroughly cleaned with isopropyl alcohol, rinsed in distilled water, dried at 60°C for at least 24 hr. and ground into powder using a Dremmel hand drill with a stainless-steel bit. Plasma, RBC, and habitat voucher samples were dried at 60°C and powdered using a mortar and pestle. Ground samples were

weighed into tin capsules to the nearest 0.1mg. Occasionally sample weight had to be reduced or increased depending on carbon and nitrogen content in SIA - in these cases samples were repeatedly analysed (if sample volume allowed) until sufficient mass was present for accurate detection (sometimes considerable weight was required when nitrogen content was low).

Carbonate containing diet voucher samples were treated with 10% 1M hydrochloric acid for 24 hours to remove carbonate content (method adapted from Topçu et al. 2010). The process was repeated until effervescence stopped. Samples were thoroughly washed with distilled water and left to stand for several hours, the liquid decanted off and the washing process repeated. Finally, the liquid was decanted off and the resultant solids dried at 60°C for at least 24hours.

Table S1. Mean ± SD of stable isotope values (‰) by turtle species, tissue type and size class (CCL). Shaded values indicate average across sizes and total sample size.

Turtle	Tissue	Size (cm)	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	n
Hawksbill	Plasma	20-30	-16.06	0.68	5.97	0.80	11
		30-40	-15.57	1.51	6.11	0.69	12
		40-50	-15.79	1.24	6.16	0.67	23
		50-60	-15.63	1.59	6.25	0.87	29
		60-70	-15.14	2.11	6.38	1.11	11
		70-80	-14.38	2.15	5.88	0.90	7
		80-90	-15.53	2.18	6.46	0.98	9
		90-100	-16.85	1.43	6.61	0.26	4
			-15.61	**1.61**	**6.21**	**0.82**	**106**
	Blood	20-30	-16.39	0.38	5.19	0.50	13
		30-40	-16.01	1.22	4.76	0.53	10
		40-50	-15.85	1.02	4.83	0.69	26
		50-60	-15.60	1.70	4.77	1.02	28
		60-70	-14.81	2.30	4.41	1.36	11
		70-80	-14.52	2.35	4.12	1.46	7
		80-90	-15.18	2.29	4.98	0.91	9
		90-100	-16.50	1.05	4.78	0.44	4
			-15.64	**1.62**	**4.77**	**0.92**	**108**
	Scute	20-30	-17.65	0.61	5.93	0.96	17
		30-40	-17.31	0.63	5.47	1.14	12
		40-50	-16.48	1.03	5.45	1.41	28
		50-60	-15.78	1.32	5.29	0.92	30
		60-70	-14.70	2.21	4.19	1.59	10
		70-80	-14.45	2.46	4.65	1.31	8
		80-90	-14.62	2.49	4.73	2.07	10
		90-100	-15.60	1.33	5.42	0.94	4
			-16.08	**1.76**	**5.26**	**1.33**	**119**
Green	Plasma	20-30	-12.90	2.92	5.59	1.73	7
		30-40	-9.01	1.99	3.40	1.56	15
		40-50	-8.33	2.57	1.90	1.81	32
		50-60	-7.91	2.63	2.50	1.40	21
		60-70	-7.05	1.08	2.68	1.08	24
		70-80	-7.09	0.75	3.06	1.40	4
		80-90	-7.92	0.34	2.37	0.49	4
			-8.29	**2.53**	**2.70**	**1.72**	**107**
	Blood	20-30	-16.04	1.83	6.25	0.97	7
		30-40	-12.76	1.93	4.59	1.00	14
		40-50	-10.04	2.49	2.21	1.68	39
		50-60	-7.58	1.91	1.80	0.97	28
		60-70	-7.97	1.64	1.87	0.73	27
		70-80	-7.76	0.97	2.93	1.11	6
		80-90	-7.83	0.78	2.31	0.82	4
			-9.50	**3.02**	**2.57**	**1.71**	**125**
	Scute	20-30	-15.34	1.69	6.26	0.63	6
		30-40	-12.85	3.48	5.76	1.43	15
		40-50	-9.89	2.56	3.27	1.38	37
		50-60	-7.51	2.23	2.96	1.03	28
		60-70	-7.83	2.06	2.84	0.97	23
		70-80	-7.91	1.52	3.90	1.11	9
		80-90	-8.66	1.50	4.23	0.76	4
			-9.40	**3.21**	**3.65**	**1.58**	**122**

Table S2. Taxonomic diet source groups used in the SIAR mixing models and their mean ±SD carbon and nitrogen isotopes (‰). The number of taxa and samples for each source group are given.

Sources	No. Taxa	No. Samples	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$
Bluegreen algae	1	5	-13.47	7.09	1.74	0.59
Red algae	29	76	-15.21	5.34	2.48	1.38
Green algae	29	77	-12.86	3.85	1.43	2.54
Brown algae	11	37	-13.63	2.69	1.34	1.25
Seagrasses	3	27	-7.07	1.55	-0.50	2.55
Sponges	28	61	-14.29	3.24	2.36	1.87
Cnidarians	10	19	-11.82	4.89	3.12	0.93
Invertebrates	8	12	-11.89	7.16	2.45	1.34

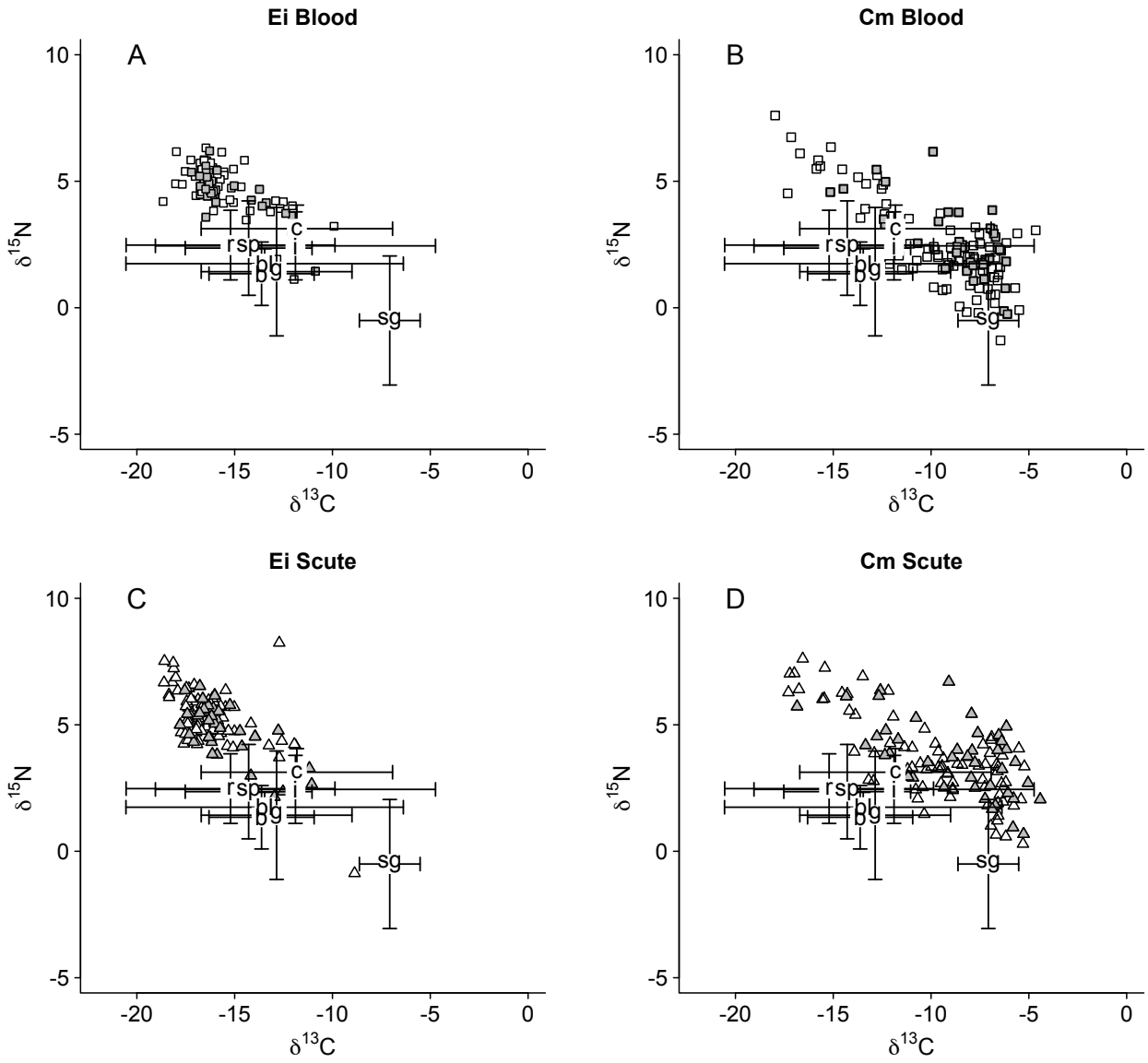

Figure S1. Biplot of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (‰) for hawksbill turtles (Ei: A, C) and green turtles (Cm: B, D) and two tissues: red blood cells (squares; A, B) and scute (triangles; C, D). Filled symbols are those turtles that also had stomach content samples (n=45 hawksbills and n=92 greens; see Stringell et al. in prep., Chapter 5). Diet sources (±SD) are bluegreen algae (bl), red algae (r), green algae (g), brown algae (b), seagrasses (sg), sponges (sp), cnidarians (c), and other invertebrates (i).

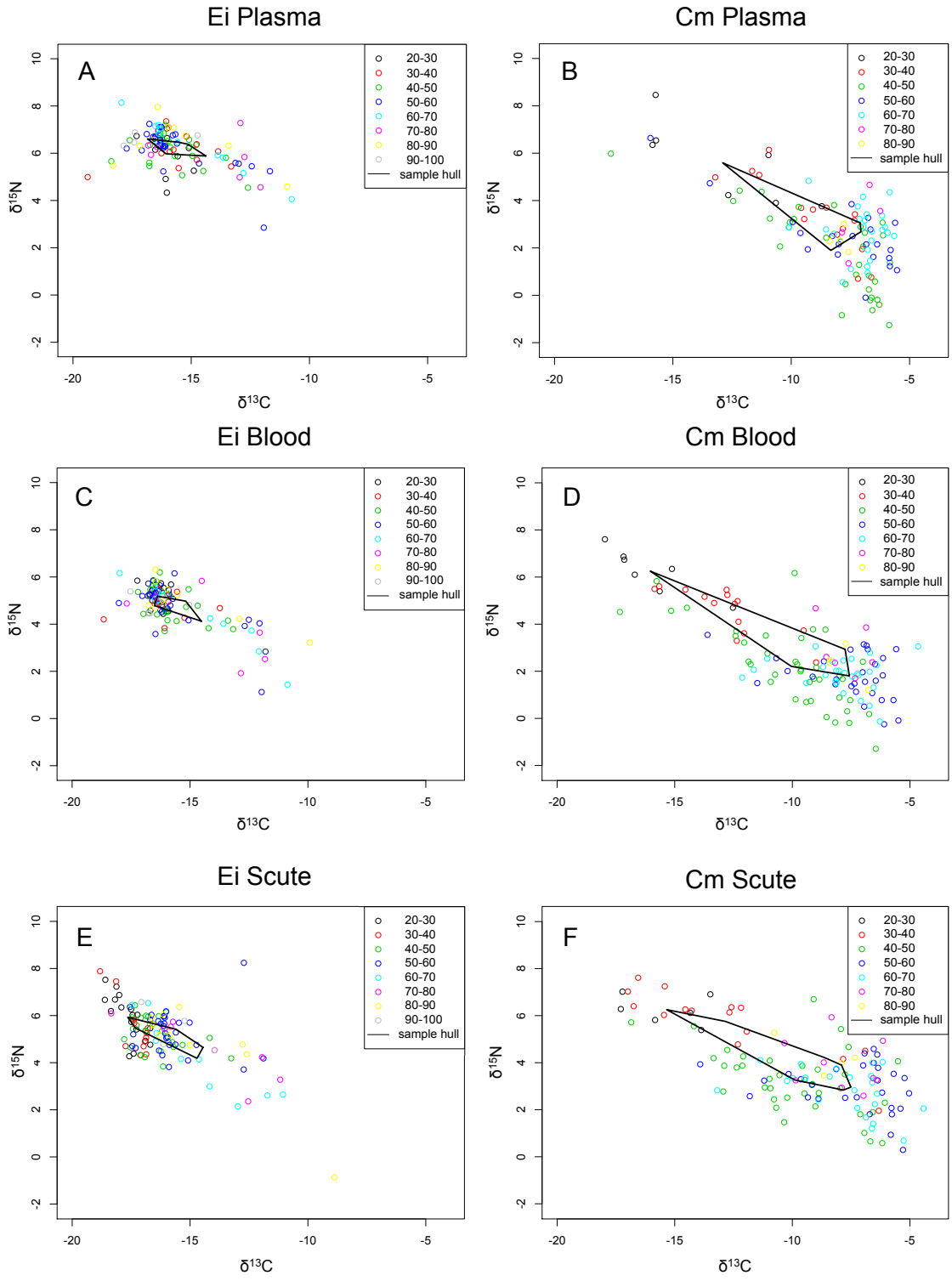

Figure S2. Convex hulls (joining the extreme most means of turtle size classes: CCL, cm) of the all-size turtle populations from three tissue types: Top panel, plasma (A, B); middle panel, red blood cells (C, D); bottom panel, scute (E, F). Left panel, hawksbill turtles (Ei: A, C, E); right panel, green turtles (Cm: B, D, F).

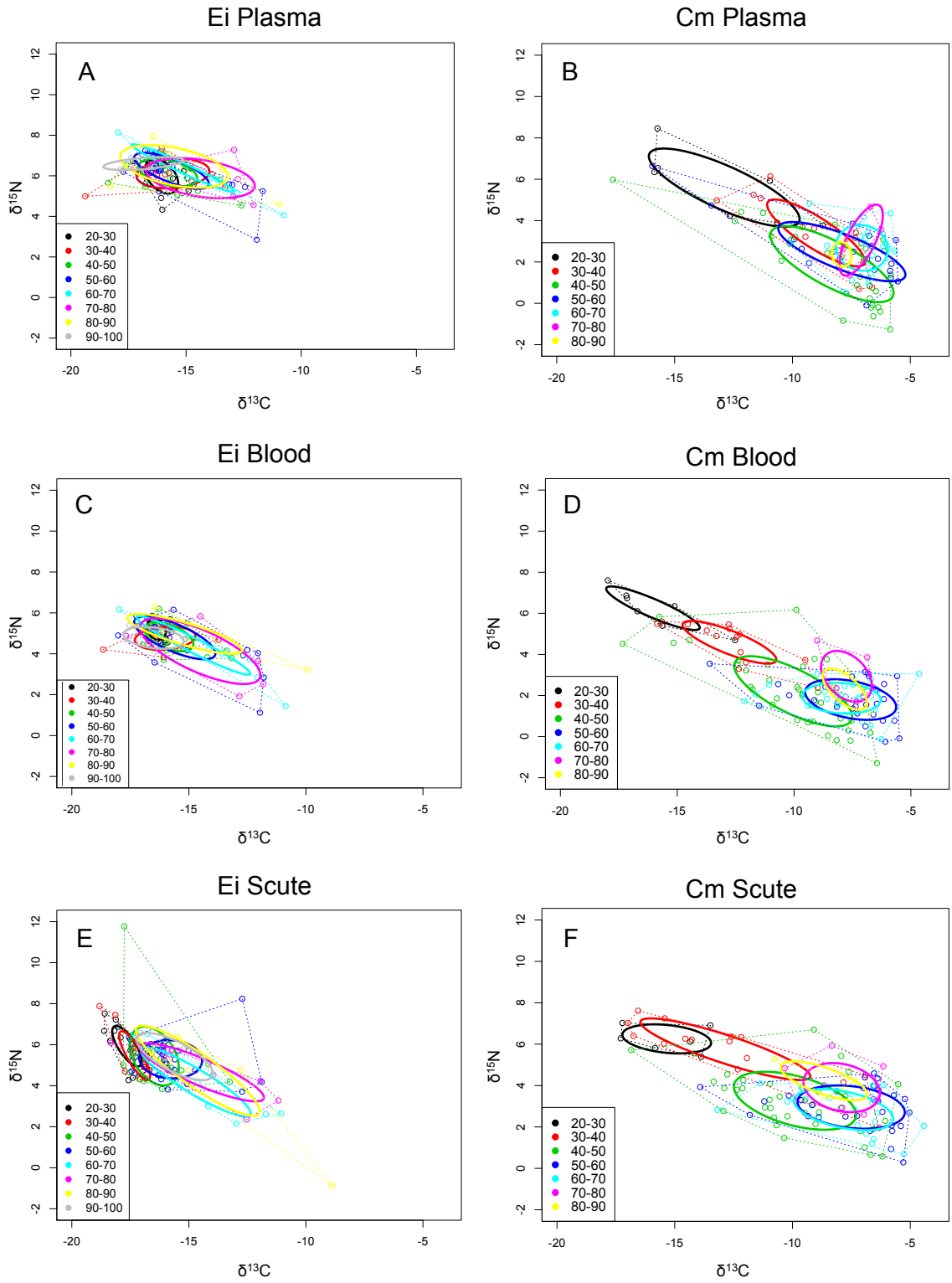

Figure S3. Sample-size corrected Standard Ellipse Areas (SEAc) for turtle size classes (CCL, cm) and three tissue types: Top panel, plasma (A, B); middle panel, red blood cells (C, D); bottom panel, scute (E, F). Left panel, hawksbill turtles (Ei: A, C, E); right panel, green turtles (Cm: B, D, F).

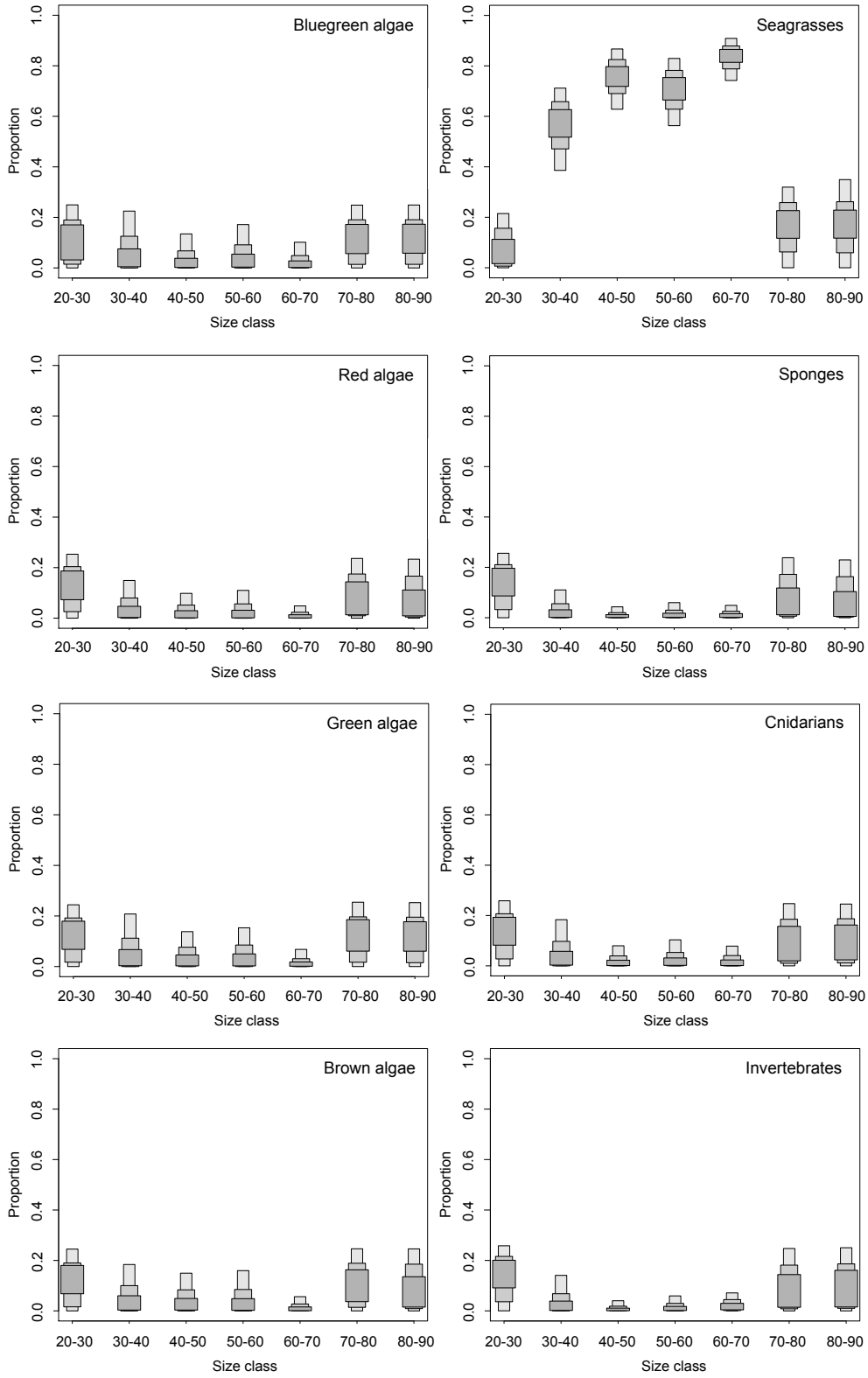

Figure S4. Diet source contributions to green turtle blood plasma samples across turtle size classes (CCL, cm). Data are from SIAR mixing models. Each panel represents one of eight sources: bluegreen algae, red algae, green algae, brown algae, seagrasses, sponges, cnidarians, and other invertebrates.

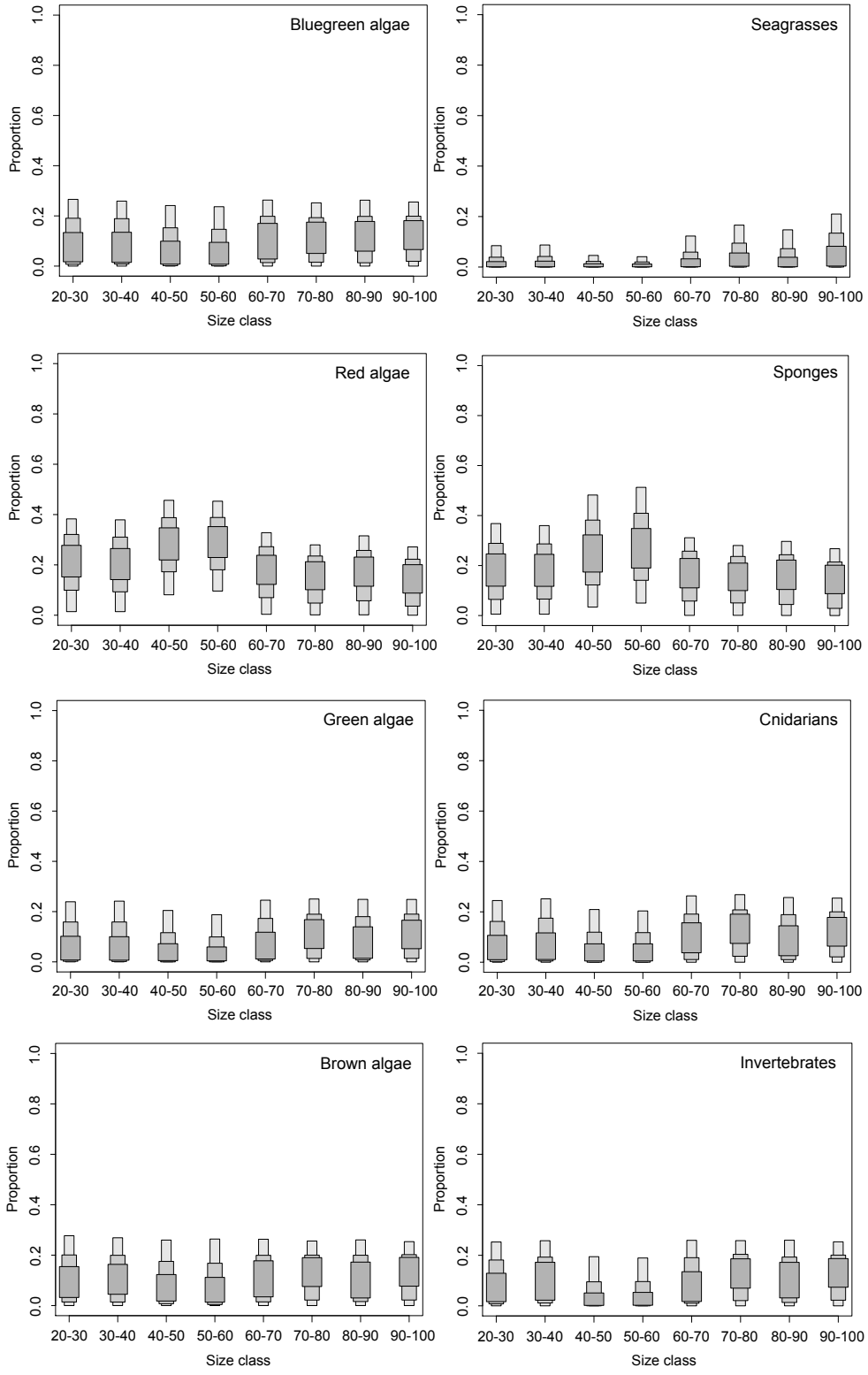

Figure S5. Diet source contributions to hawksbill turtle blood plasma samples across turtle size classes (CCL, cm). Data are from SIAR mixing models. Each panel represents one of eight sources: bluegreen algae, red algae, green algae, brown algae, seagrasses, sponges, cnidarians, and other invertebrates.

Supplementary References

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