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Trophic overlap in mobulid rays: insights from stable isotope analysis

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ABSTRACT: Mobulid rays, a group of closely related filter-feeders, are threatened globally by bycatch and targeted fisheries. Their habitat use and feeding ecology are not well studied, and most efforts have focused on temporally limited stomach content analysis or inferences from tagging data. Previous studies demonstrate a variety of different diving behaviors across species, which researchers have interpreted as evidence of disparate foraging strategies. However, few studies have examined feeding habitats and diets of multiple mobulid species from a single location, and it is unclear if the proposed differences in diving and inferred foraging behavior are examples of variability between species or regional adaptations to food availability. Here, we use stable isotope data from mobulids landed in fisheries to examine the feeding ecology of 5 species at 3 sites in the Indo-Pacific. We use Bayesian mixing models and analyses of isotopic niche areas to demonstrate dietary overlap between sympatric mobulid species at all of our study sites. We show the degree of overlap may be inversely related to productivity, which is contrary to prevailing theories of niche overlap. We use isotope data from 2 tissues to examine diet stability of *Manta birostris* and *Mobula tarapacana* in the Philippines. Finally, we observe a significant but weak relationship between body size and isotope values across species. Our findings highlight challenges to bycatch mitigation measures for mobulid species and may explain the multi-species mobulid bycatch that occurs in a variety of fisheries around the world.

KEY WORDS: Niche overlap · Mixing model · Feeding ecology · Bycatch risk

INTRODUCTION

Mobulid rays are a group of closely related, highly derived filter-feeders that, in many cases, have overlapping habitats and geographic distributions (Couturier et al. 2012). Periods of cladogenesis within the Mobulidae coincide with periods of global warming, and speciation within the family has occurred as recently as within the last million years (Kashiwagi et al. 2012, Poortvliet et al. 2015). Hypotheses for the drivers of these speciation events include fragmentation of productive upwelling environments and reduced food availability during extended periods of global warming, and physical barriers to dispersal and connectivity during ice ages (Poortvliet et al. 2015). The most recently diverged mobulid species

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shows evidence of hybridization (Walter et al. 2014), suggesting that differences in behavior and habitat use, as opposed to geographic isolation, may be the primary factors driving and maintaining speciation (Kashiwagi et al. 2011, 2012).

Over the past decade, demand for mobulid gill plates in Asian medicine has led to targeted fisheries and increased bycatch retention of mobulid rays (Couturier et al. 2012). While these growing targeted fisheries have catalyzed focused conservation and scientific attention for mobulids (Ward-Paige et al. 2013, White et al. 2015, Lewis et al. doi:10.7287/peerj.preprints.1334v1), bycatch of these species likely impacted populations long before large-scale targeted fisheries began. Mobulid rays are vulnerable to incidental capture in gill nets, purse seines, trawls, and even long lines (Croll et al. 2016). Moreover, their low annual reproductive output and conservative demographic characteristics make them highly susceptible to fisheries-induced population declines (Dulvy et al. 2014, Pardo et al. 2016), even when catch rates are low. Understanding horizontal and vertical habitat use, which are likely driven by foraging in many cases, can help determine when mobulid rays are most vulnerable to incidental capture in fisheries and can aid in the development of bycatch mitigation measures (Stewart et al. 2016b).

Several studies have examined the horizontal and vertical movements of mobulid rays using satellite telemetry (Canese et al. 2011, Croll et al. 2012, Braun et al. 2014, Jaine et al. 2014, Thorrold et al. 2014, Stewart et al. 2016b), and others have used direct observations to identify feeding patterns and prey sources (Notarbartolo di Sciara 1988). The results of these studies demonstrate differences in habitat use (e.g. Croll et al. 2012, Thorrold et al. 2014), vertical movements (e.g. Canese et al. 2011, Croll et al. 2012, Braun et al. 2014, Jaine et al. 2014, Thorrold et al. 2014, Stewart et al. 2016b), and in some cases prey sources between mobulid species (Notarbartolo di Sciara 1988). However, few studies have examined the feeding ecology of multiple mobulid species in a given region or within a single species at multiple locations (see Notarbartolo di Sciara 1988, Sampson et al. 2010). Consequently, it remains unclear whether the observed differences in foraging behavior and habitat use are consistent between species or simply a result of regional variations in resource availability. Armstrong et al. (2016) demonstrated that mantas require zooplankton to reach threshold densities before feeding becomes energetically profitable, and therefore regional patterns in prey availability could conceivably have a greater influence on mobulid feeding behavior than morphological and behavioral differences between species.

Researchers are increasingly using stable isotope analysis as a tool for inferring the trophic ecology of animals, providing insight into trophic niche separation (Cherel et al. 2007, Plass-Johnson et al. 2013), trophic overlap (Foley et al. 2014, Jackson et al. 2016), diet composition (Semmens et al. 2009), and diet shifts (Ben-David et al. 1997, MacNeil et al. 2005). Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are 2 of the most commonly used isotopes in ecological studies, as they can provide information on feeding locations and prey types due to predictable changes in $\delta^{13}C$ across habitats (France 1995) and increases in $\delta^{15}N$ at higher trophic levels (Owens 1987). Importantly, the use of stable isotopes to infer trophic dynamics requires a number of experimentally validated parameters, including fractionation and tissue incorporation rates (Gannes et al. 1997), which can be challenging to obtain for species that are not easily studied in a laboratory setting. Further, factors such as diet composition, body condition, organism size, and metabolic rate can influence isotopic incorporation, and few studies have explicitly examined these effects (Gannes et al. 1997, Newsome et al. 2010). While acknowledging these limitations, isotope analysis is particularly useful in marine species, as it can provide a large temporal window into the diet of animals that are otherwise challenging to observe in the wild for long periods (e.g. MacNeil et al. 2005, Cherel et al. 2007). In most regions, mobulid rays are present sporadically and unreliably at sites accessible by researchers, making direct observations challenging (Couturier et al. 2012). With the exception of coastally associated reef manta rays (e.g. Anderson et al. 2011, Jaine et al. 2012) and occasional opportunistic observations (e.g. Stewart et al. 2016b), direct observations of feeding behavior in mobulids are rare and largely restricted to surface feeding and daylight hours, complicating efforts to characterize the trophic ecology of this family. These characteristics make stable isotope analysis a powerful tool for studying the trophic ecology of mobulid rays.

In this study, we examine the trophic ecology and isotopic niche separation of 5 species of mobulid rays using stable isotope analysis. We use a Bayesian mixing model to assess the diet contribution of prey sources identified and sampled from stomach contents at 1 study site, and we compare the isotopic niche areas and isotopic niche separation between species and across regions. We use samples collected from fisheries landings at 3 sites throughout the Indo-

Pacific to examine how resource partitioning may change across regions with varying productivity and resource availability.

MATERIALS AND METHODS

Study sites and sample collection

Our study sites included (1) the coast of northern Peru, (2) Sri Lanka, and (3) the Bohol Sea in the Philippines (Fig. 1). We analyzed muscle samples from 5 species of mobulid rays (4 species per site) from Peru (n = 46), the Philippines (n = 192), and Sri Lanka (n = 102) and liver samples from 2 species in the Philippines (n = 30) (Table 1).

Peru

In Peru, we collected skeletal muscle samples of *Manta birostris* (n = 3), *Mobula japanica* (n = 20), *Mobula munkiana* (n = 18), and *Mobula thurstoni* (n = 5) at landing sites throughout the Tumbes region, in the north of the country, from August 2012 through May 2013. Fishers typically caught mobulid rays within approximately 10 to 30 km of shore (over the continental shelf) as non-discarded bycatch in purse seines and gill nets targeting tuna. Rays were landed either whole, gutted, or in many cases only as pectoral fins. We only included samples in this study from individuals that could be confidently identified from images recorded at the time of tissue collection (Stevens 2014). Where possible, we recorded disc width of landed individuals (Table 1).

Sri Lanka

In Sri Lanka, we collected skeletal muscle samples from *M. birostris* (n = 37), *M. japanica* (n = 27), *Mobula tarapacana* (n=27), and *M. thurstoni* (n = 11) landed at fish markets in Negombo, in the west of the country, and Mirissa, in the south, from November 2010 through August 2013. In most cases, fishers reported catching the mobulids within 20 km of shore on the edge of the continental shelf, while other samples were collected from mobulids caught on long-range expeditions within and outside the exclusive economic zone and off the continental shelf. Rays were landed gutted, and we recorded disc width of individuals where possible (Table 1).

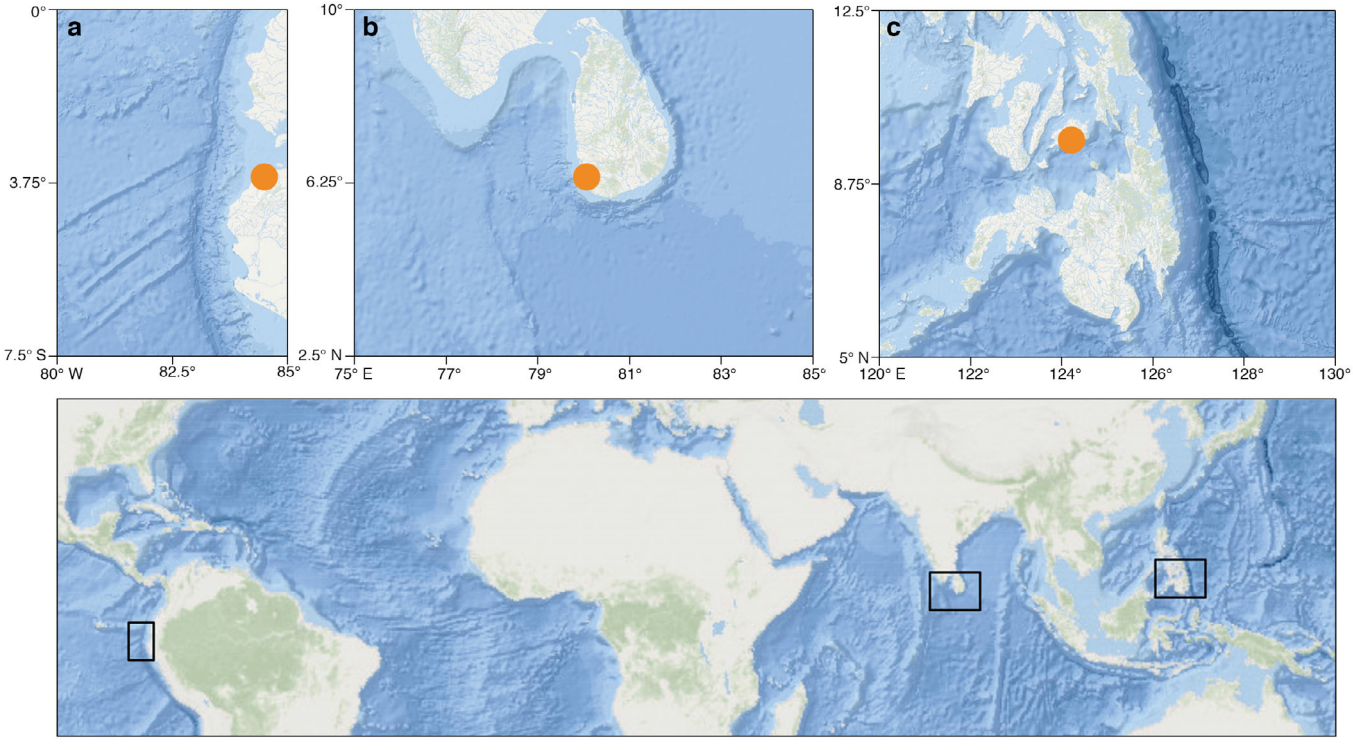

Fig. 1. Study sites. Bounding boxes for chl *a* satellite data are displayed in the lower map, while bathymetric maps of the study sites are displayed within those bounding boxes for (a) Peru, (b) Sri Lanka, and (c) Philippines. Orange circles indicate the locations of primary tissue sample collection sites for each region

Table 1. Summary information for tissues collected from mobulids, including mass conversions and tissue turnover rates, and their prey. All values are means ± SD. All mobulid tissues were skeletal muscle unless otherwise noted. Liver tissues were high in lipids, which leads to lower $\delta^{13}C$ values; therefore, we lipid extracted (LE) the liver tissues and report $\delta^{13}C$ values for intact (bulk) and LE liver tissues (see 'Materials and methods')

Region/species	No. of samples	Disc width (cm)	Converted mass (mean, kg)	Tissue turnover (mean, d)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	LE $\delta^{13}C$ (‰)	Bulk C:N	LE C:N
Peru									
Manta birostris	3	365.3 ± 265.9	601.5	550.8	10.4 ± 1.3	−16.7 ± 1.0	–	3.1 ± 0.1	–
Mobula japanica	20	172.0 ± 61.2	42.9	324.9	10.9 ± 1.0	−17.4 ± 0.4	–	3.1 ± 0.1	–
Mobula munkiana	18	143.4 ± 40.5	25.3	292.4	12.5 ± 0.2	−17.1 ± 0.2	–	3.2 ± 0.1	–
Mobula thurstoni	5	90.4 ± 8.5	8.0	231.9	9.9 ± 0.9	−17.6 ± 0.3	–	3.2 ± 0.1	–
Philippines									
M. birostris	42	438.5 ± 55.4	1024.8	612.8	9.6 ± 0.4	−16.5 ± 0.7	–	3.2 ± 0.2	–
M. birostris liver	15	–	–	245.1	9.1 ± 0.4	−22.9 ± 0.7	−19.8 ± 1.3	17.9 ± 4.8	7.9 ± 1.5
M. japanica	42	192.2 ± 24.3	62.7	350.5	9.9 ± 0.4	−16.2 ± 0.4	–	3.1 ± 0.1	–
Mobula tarapacana	35	237.9 ± 49.6	171.8	428.7	9.8 ± 0.6	−16.1 ± 0.6	–	3.1 ± 0.1	–
M. tarapacana liver	15	–	–	171.5	10.1 ± 0.6	−21.9 ± 0.7	−19.5 ± 0.9	14.5 ± 4.0	7.3 ± 1.2
M. thurstoni	73	147.4 ± 24.1	31.0	304.4	9.5 ± 0.4	−16.2 ± 0.5	–	3.1 ± 0.1	–
Sri Lanka									
M. birostris	37	253.9 ± 54.6	208.0	445.4	10.6 ± 0.8	−17.6 ± 0.4	–	3.2 ± 0.1	–
M. japanica	27	207.0 ± 21.9	80.6	368.5	10.7 ± 0.5	−17.8 ± 0.5	–	3.2 ± 0.1	–
M. tarapacana	27	205.0 ± 44.2	111.4	393.1	10.9 ± 0.8	−17.4 ± 0.6	–	3.2 ± 0.1	–
M. thurstoni	11	126.3 ± 19.9	20.2	279.3	10.4 ± 0.3	−18.0 ± 0.4	–	3.2 ± 0.1	–
Philippines stomach contents									
Euphausiids	40	–	–	–	7.6 ± 0.5	−18.3 ± 0.4	–	3.7 ± 0.2	–
Copepods[a]	3	–	–	–	6.9 ± 0.4	−19.8 ± 0.8	–	3.9 ± 0.5	–
Chaetognaths[a]	3	–	–	–	8.4 ± 0.4	−18.8 ± 0.5	–	3.3 ± 0.2	–
Pteropods	1	–	–	–	5.24	−12.2	–	8.2	–
Pteropods[a]	6	–	–	–	6.2 ± 0.1	−14.6 ± 2.5	–	4.3 ± 0.9	–
Myctophids	11	–	–	–	9.0 ± 0.8	−17.9 ± 0.9	–	3.3 ± 0.4	–
Sardinella spp.	6	–	–	–	9.7 ± 0.4	−17.8 ± 0.8	–	4.1 ± 0.6	–
Cubiceps spp.	3	–	–	–	8.4 ± 0.3	−17.3 ± 0.3	–	3.5 ± 0.1	–

[a]Samples collected in a zooplankton tow

Philippines

In the Philippines, we analyzed samples collected from *M. birostris* (n = 42), *M. japanica* (n = 42), *M. tarapacana* (n = 35), and *M. thurstoni* (n = 73) at a landing site in Jagna, on the island of Bohol, from December 2012 through May 2014. Mobulids were landed primarily during the dry season from mid-November to mid-June. Fishers captured mobulids in the central and eastern Bohol Sea, typically between 5 and 50 km from shore at night and in the top 30 m of the water column over depths greater than 1000 m. We collected skeletal muscle tissue and recorded disc width where possible (Table 1). We collected stomach contents from a subset of individuals across all species, and we collected liver samples from a subset of *M. tarapacana* (n = 15) and *M. birostris* (n = 15). In some cases, prey sources were present in a number of different stomach content samples but in quantities that were too small to prepare for stable isotope analysis (e.g. copepods, chaetognaths, and pteropods; see Rohner et al. 2017 for a detailed analysis of stomach contents). While these prey sources did not make up a substantial portion of the diet during the period when stomach contents were collected, their relative importance may change throughout the year. Because isotope analysis allows for dietary insights over a longer tissue integration period, we chose to include these sources in our analyses. To obtain adequate material for isotope analysis, we performed 1 plankton tow in Pintuyan, Southern Leyte, during February 2016. We only included copepods, chaetognaths, and pteropods from the plankton tow in isotope analyses; no other species or groups that were not present in stomach contents were included.

We visually sorted prey collected in mobulid stomach contents and plankton tows to the species level except for copepods, which we pooled across species to obtain enough material for isotope analysis. We further sorted prey species using microscopy (details reported in Rohner et al. 2017). In 2 cases, fish samples collected from *M. tarapacana* stomachs could not be visually identified. We extracted DNA from these samples at Scripps Institution of Oceanography using a Qiagen DNeasy kit and sequenced 16S genes for genetic identification. We matched these sequences to existing barcodes using the basic local alignment search tool database (Madden 2002), which identified them as *Sardinella* sp. and *Cubiceps* sp. Myctophids were typically found whole and intact in *M. birostris* stomach contents, and we subsampled these to include a portion of skin, connective tissue, and muscle, which we homogenized and included as a single sample per fish. In all cases, only 1 fish was present per stomach content sample (n = 11). The remains of the partially digested *Sardinella* sp. and *Cubiceps* sp. from the *M. tarapacana* stomach contents were homogenized for analysis after removing degraded tissue. We found multiple specimens of *Sardinella* sp. (n = 6) and *Cubiceps* sp. (n = 3) per stomach content sample, and we analyzed each individual fish as a separate prey sample. Euphausiids, copepods, and chaetognaths were each pooled (within species) to obtain sufficient tissue for isotope analysis. We took 1 pooled subsample of euphausiids per stomach content sample (n = 40 samples). We separated partially digested from intact euphausiids within each stomach content sample and only included intact euphausiids in our subsamples for isotope analysis. We took multiple pooled subsamples of copepods (n = 3 samples) and chaetognaths (n = 3 samples) from 1 plankton tow. In the case of pteropods, 1 individual constituted 1 sample, and we obtained specimens from 1 stomach content sample (n = 1 individual) and 1 plankton tow (n = 6 individuals) (Table 1). While additional samples of prey species collected by plankton tows across a longer period of time would have been ideal, logistical constraints restricted our available samples to a single plankton tow to supplement stomach contents in the present study.

Sample storage

As remote tropical sampling locations generally did not allow for preferred preservation methods such as freezing, we stored tissue, stomach content, and plankton tow samples in 95% ethanol. While there is no consensus on the effect of ethanol on $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (Sarakinos et al. 2002, Barrow et al. 2008, Burgess & Bennett 2017), most preservation studies on fish muscle and fin tissue indicate there are negligible effects of ethanol on $\delta^{15}N$ values, and the shift in $\delta^{13}C$ values after long-term storage in ethanol is low (typically a mean increase of 0.5 to 1.5‰) (Kaehler & Pakhomov 2001, Sarakinos et al. 2002, Kelly et al. 2006, Vizza et al. 2013, Stallings et al. 2015). Additionally, Burgess & Bennett (2017) suggest that storage of elasmobranch tissues in ethanol mimics the effects of urea extraction, perhaps by acting as a solvent. In Peru, samples were stored in 96% ethanol + 0.1 mM EDTA, as they were initially intended for genetic analysis. To our knowledge, there are no studies that examine the effects of ethanol and EDTA preservation on isotope values. Sample preservation studies have examined the impacts of DMSO storage on isotope values (Lesage et al. 2010), and in at least 1 case, a DMSO + EDTA solution was used (Hobson et al. 1997). The mean effect of DMSO and DMSO + EDTA storage on isotope values was similar for both $\delta^{13}C$ (−4.74 vs. −5.1) and $\delta^{15}N$ (−0.7 vs −0.9), suggesting that EDTA does not have a substantial additional impact on isotope values. Importantly, where EDTA was added to a DMSO buffer, the variance of isotope values did not change as compared to frozen samples (Hobson et al. 1997), which would impact estimates of isotopic niche area in the present study. The storage and preservation of samples in ethanol (or ethanol + EDTA) makes them challenging or impossible to compare with other isotopic values in the literature for the same species or potential source items that were preserved differently. However, these storage methods should not affect the estimation of isotopic niche area or within-region comparison of sources and consumers given the consistent preservation methodology utilized across samples. We did not compare isotope values among samples that were preserved only in ethanol with those preserved in ethanol + EDTA.

Isotope analysis

For stable isotope sample preparation, we soaked samples in deionized water for 5 min and then rinsed them to remove debris and residual ethanol from storage. We then freeze-dried approximately 10 mg (wet) of tissue from each individual for 24 h using a FreeZone 2.5 freeze drier (Labconco). In the case of

liver samples, we freeze-dried 30 mg (wet) of tissue from each individual for 72 h.

The high lipid content of elasmobranch livers can result in lower $\delta^{13}C$ values (Logan & Lutcavage 2010, Kim & Koch 2012). We therefore lipid-extracted liver samples using petroleum ether (Dobush et al. 1985), following the protocol outlined in Kim & Koch (2012). As lipid extraction with petroleum ether appears to affect $\delta^{15}N$ values (Parng et al. 2014), we subsampled liver prior to lipid extraction and used non-lipid-extracted samples for $\delta^{15}N$ values and lipid-extracted samples for $\delta^{13}C$ values. We did not lipid-extract muscle tissue, as their carbon to nitrogen (C:N) ratios fell below the C:N threshold of 3.5 suggested by Post et al. (2007), indicating these tissues had sufficiently low lipid content that would not affect $\delta^{13}C$ values (see Table 1). We did not lipid-extract prey samples because their C:N values (3.8 ± 0.7, mean ± SD) indicated they were not lipid-rich as defined by Newsome et al. (2010), and the tissue quantity of many prey samples (especially copepods and chaetognaths) was so low that lipid extraction was not practical. However, to assess the possible impacts of the lipid content of prey samples on our mixing models, we did apply mathematical lipid normalization equations (see 'Materials and methods: Statistics and mixing models'). We homogenized pteropods whole without acid-washing or removing carbonate shell components, which may have artificially increased $\delta^{13}C$ values for pteropods in our results (Mateo et al. 2008).

We homogenized dried mobulid muscle tissue and prey fish samples using a Wig-L-Bug dental amalgamator and mobulid liver and zooplankton prey samples manually using a mortar and pestle. We then packaged between 0.5 and 1.0 mg of powdered tissue in 5 × 9 mm tin capsules (Costech). Samples were analyzed at the University of California Santa Cruz Stable Isotope Laboratory using an NC2500 elemental analyzer (CE Instruments) interfaced to a Delta Plus XP isotope ratio mass spectrometer (Thermo-Finnigan) with an acetanilide standard.

We estimated tissue-specific stable isotope turnover times for skeletal muscle by calculating the average mass of each species based on species-specific disc width to mass conversions from Notarbartolo di Sciara (1988) and using the body mass tissue incorporation rates for carbon and nitrogen in teleosts and elasmobranchs described in Kim et al. (2012b). No disc width to mass conversion is available for *M. birostris*, so we used the conversion formula for *M. tarapacana*, which is the second-largest mobulid species in the present study. MacNeil et al. (2006) estimated the incorporation rates of nitrogen in liver tissue to be approximately 40% of the incorporation rates of nitrogen for muscle tissue in a controlled feeding experiment using freshwater stingrays, and we therefore applied this scaling factor to our muscle tissue nitrogen incorporation rates for *M. birostris* and *M. tarapacana* to arrive at approximate nitrogen incorporation rates for liver.

Statistics and mixing models

We performed all statistical analyses using the R software program (R Core Team 2016). We used the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011) to create standard ellipses representing relative isotopic niche widths in bivariate $\delta^{13}C$ and $\delta^{15}N$ space, where the standard ellipse represents bivariate standard deviation. We generated Bayesian credible intervals of the standard ellipse area of each species as well as for the overall mobulid assemblage (all species combined) within each region. We computed the pairwise overlaps between mobulid species within each region and used the mean proportional overlap among species as a metric for community overlap. Bayesian estimation of standard ellipses allows for an unbiased estimate of relative isotopic niche area even at small sample sizes, in contrast with metrics such as convex hulls, which are positively correlated with sample size (Jackson et al. 2011). Therefore, our median estimates of isotopic niche metrics should not be impacted by small sample sizes, although uncertainty and therefore credible interval width should be greater. We excluded *M. birostris* in Peru from isotopic niche area comparisons, as the extremely large credible intervals resulting from small sample size (n = 3) made comparisons uninformative. However, we did include *M. birostris* in the mean proportional isotopic niche overlap calculations for Peru to keep the total number of species constant at each site to allow for comparisons across regions. For all SIBER analyses, we used 2 chains of 10 000 iterations with a burn-in of 1000 and thinning of 10.

To determine the contribution of prey sources to mobulids' diets in the Philippines, we used MixSIAR, a Bayesian stable isotope mixing model (Stock & Semmens 2016). We included 7 prey sources in the model: euphausiids, copepods, chaetognaths, myctophids, *Sardinella*, *Cubiceps*, and pteropods. We subsequently made 2 combinations of 3 groups *a posteriori* by summing their model output posterior distributions based on their functional role, because 7 sources would likely be confounded with only 2 trac-

ers ($\delta^{15}N$ and $\delta^{13}C$) (B. X. Semmens et al. unpubl.). The grouping scenarios were (1) zooplankton (chaetognaths, copepods, euphausiids), fish (myctophids, *Sardinella*, *Cubiceps*), and pteropods; and (2) epipelagic (chaetognaths, copepods, *Sardinella*), mesopelagic (euphausiids, myctophids, *Cubiceps*), and pteropods. We kept pteropods separate from the aggregated groupings due to the dissimilarity of their isotopic signature and possible influences of carbonate shells on the measured isotope values. We did not combine these groups *a priori* (before inclusion in the model) because the combined source groups were not bivariate normally distributed, which is an expectation of the mixing model (B. X. Semmens et al. unpubl.).

Dietary lipids in prey sources may be routed directly to consumer tissues (Newsome et al. 2010, Parng et al. 2014), making it more appropriate to leave prey samples with lipids intact. However, to account for uncertainty in the importance of dietary lipid content, as well as possible influences of carbonate shells in our pteropod samples, we ran 2 versions of our mixing models. The first included bulk prey samples and bulk consumer samples, while the second used correction factors to account for lipid content and carbonate shells. For the second mixing model, we applied muscle tissue-specific fish C:N correction factors for $\delta^{13}C$ (Logan et al. 2008) to our myctophid, *Sardinella*, and *Cubiceps* sources and euphausiid species-specific C:N correction factors for $\delta^{13}C$ to our chaetognath, copepod, euphausiid, and pteropod sources, because euphausiids were the only marine invertebrates for which correction factors were available (Logan et al. 2008). After accounting for lipid content, we applied a correction factor of −6‰ $\delta^{13}C$ to pteropod samples, representing the mean difference in whole versus acid-washed marine gastropods reported in (Mateo et al. 2008). We ran each version of the mixing model (bulk and lipid/carbonate corrected) with both informative and uninformative priors as specified below.

Trophic discrimination factors, or the differences in prey and consumer isotope values that result from metabolic processes, are an important aspect of mixing models, but the determination of appropriate trophic discrimination factors has remained a hotly contested debate in the literature (Gannes et al. 1997, Caut et al. 2008, Hussey et al. 2010b). Determining discrimination factors generally requires laboratory experiments or controlled feeding studies, which are not practical or readily available for many species, including mobulids. Couturier et al. (2013) proposed discrimination factors of 2.4‰ for nitrogen and 1.3‰ for carbon based on stable isotope values of wild reef manta rays and their putative prey sources. These values fall within the range of other published elasmobranch discrimination factors, although laboratory experiments demonstrate considerable variation, even within species (Hussey et al. 2010a, Kim et al. 2012a, Malpica-Cruz et al. 2012). One advantage of Bayesian mixing models is their ability to incorporate uncertainty in parameter estimates and propagate this uncertainty throughout the model (Moore & Semmens 2008). We used mean values of 2.4‰ for nitrogen and 1.3‰ for carbon (Couturier et al. 2013) but used a standard deviation of 1‰ for both isotopes in our mixing models to account for the variability in laboratory-derived trophic discrimination factors, the uncertainty of the discrimination factors proposed for wild reef manta rays, and possible differences between mobulid species. Specifically, we selected a standard deviation of 1‰, as it adequately covers the range of $\delta^{15}N$ and $\delta^{13}C$ trophic discrimination factors reported in laboratory experiments with elasmobranchs (Hussey et al. 2010a, Kim et al. 2012a, Malpica-Cruz et al. 2012).

In addition to trophic discrimination factors, Bayesian mixing models require prior distributions for proportional diet contribution of all prey sources. We ran 2 different iterations of our mixing model. The first model used priors that are uninformative on the simplex, where all possible sets of diet proportions are given equal weight in the prior distributions (B. X. Semmens et al. unpubl.). Priors are specified using a Dirichlet distribution, where an uninformative prior has a probability $\alpha_i = 1/(\text{n sources})$ for each source *i*. The second model used semi-informative priors, which we based on the presence and abundance of the 7 prey sources in stomach content samples from each species. An informative prior could be specified as $\alpha_{i,j}$ = n records of prey species *i* in stomach contents of consumer species *j*. However, in some cases, 1 or 2 prey sources dominated stomach content samples or may have been the only prey sources present within a species' stomach contents, which would have led to extremely informative priors. Using very strong priors essentially eliminates the utility of a mixing model, as the posterior distributions will simply reflect the priors (B. X. Semmens et al. unpubl.). Furthermore, the stomach contents were collected from January to April and therefore are representative of the mobulids' diets during only a third of the year. Muscle tissue represents the integrated diet over a much longer period (Table 1) and is therefore useful in determining longer-term integration of diet contributions. For these reasons, we qualitatively

relaxed the stomach content-informed priors so that the model had a reasonable chance of including any of the possible prey sources, while at the same time providing some weight to the diet preferences implied by stomach content data. Prior distributions for each source are presented in Fig. S1 & Table S1 in the Supplement at www.int-res.com/articles/suppl/m580p131_supp.pdf, and *a posteriori* aggregated priors are presented in 'Results: Mixing models' (see Fig. 5). We ran all mixing models with 3 chains of 300 000 iterations, a burn-in of 200 000, and thinning of 100. We assessed convergence of all Bayesian analyses using visual inspection of chain convergence and autocorrelation plots as well as Gelman-Rubin diagnostics (Gelman & Rubin 1992).

To assess relationships between trophic characteristics and body size, we used Bayesian linear mixed effects models. To do this, we set up models with isotope values ($\delta^{15}N$ and $\delta^{13}C$) as the response (normally distributed), disc width and regions as fixed effects, and species as a random effect. We used a multivariate ANOVA to examine isotopic differences between lipid-extracted liver samples and muscle samples in *M. birostris* and *M. tarapacana*. Because MANOVAs do not provide significance levels for each variable, we used paired *t*-tests to examine within-individual differences in $\delta^{15}N$ and $\delta^{13}C$ values between liver and muscle of individuals from which we collected both tissue types. We applied Bonferroni corrections for repeated measures and report corrected p-values.

Environmental data

We used the xtractomatic package (Mendelssohn 2015) in R to obtain monthly chl *a* values from MODIS satellites for the regions surrounding each of our study sites over the past 10 yr to characterize the long-term patterns and variability in regional productivity. Our bounding boxes were 7.5° S, 85° W to 0° N, 80° W (Peru); 2.5° N, 75° E to 10° N, 85° E (Sri Lanka); and 5° N, 120° E to 12.5° N, 130° E (Philippines) (see Fig. 1). We selected these bounding boxes based on the expected distribution of mobulids in each region, which was supported by bycatch data from the eastern equatorial Pacific (Croll et al. 2016), interviews with fishermen regarding capture locations in Sri Lanka, and records of long-distance movements in several *Mobula* species (Thorrold et al. 2014, Francis & Jones 2017). We averaged chl *a* values over the entire bounding box for a given region and smoothed monthly averages into seasonal (3 mo) averages for plotting purposes.

RESULTS

Isotopic niche areas

The stable isotope values from the 4 mobulid species, and their resulting isotopic niche spaces, largely overlapped among species for those sampled in Sri Lanka and the Philippines, whereas there was a greater separation in isotope values and isotopic niche space among species collected in Peru (Figs. 2 & 3). The mean proportional isotopic niche overlap among species increased from Peru to Sri Lanka to the Philippines (0.10, 0.33, 0.36, respectively), although there was a high degree of overlap between Bayesian credible intervals of these estimates (Fig. 3, Table S2 in the Supplement). Satellite-derived chl *a* values indicate Peru had the greatest mean primary productivity of our 3 study sites, followed by Sri Lanka and the Philippines (1.04, 0.48, 0.19 mg m^{-3}, respectively), while Sri Lanka had the greatest variability (SD) in chl *a* values followed by Peru and the Philippines (SD: 0.38, 0.27, 0.06 mg m^{-3}, respectively) (Fig. 3).

Manta birostris and *Mobula tarapacana*, the largest of the mobulids, had larger isotopic niche areas than *Mobula japanica* and *Mobula thurstoni* in Sri Lanka and the Philippines, while in Peru, *M. japanica* and *M. thurstoni* had much larger isotopic niche areas than *Mobula munkiana* (Fig. 4). We found no pattern between ellipse area and region that was consistent across species. For example, while the median ellipse area of *M. japanica* was largest in Peru and smallest in the Philippines, the median ellipse area of *M. thurstoni* was largest in Peru and smallest in Sri Lanka, and the ellipse area of *M. birostris* was larger than *M. tarapacana* in the Philippines but smaller in Sri Lanka (Fig. 4). We report pairwise comparisons of isotopic niche area credible intervals and median values of isotopic niche areas in Table S3.

Size-based differences in isotopic signatures

Our mixed effects models demonstrated significant but weak relationships between body size (disc width) and isotope values. The median slope across all mobulids demonstrated an increase in the $\delta^{15}N$ value of 0.21‰ per meter increase in disc width and an 89.2% probability of a slope greater than 0. Conversely, mobulids displayed a negative slope in $\delta^{13}C$ values, with a median decrease of 0.23‰ per meter increase in disc width across all species and a 97.47% posterior probability of a slope less than 0. Species-

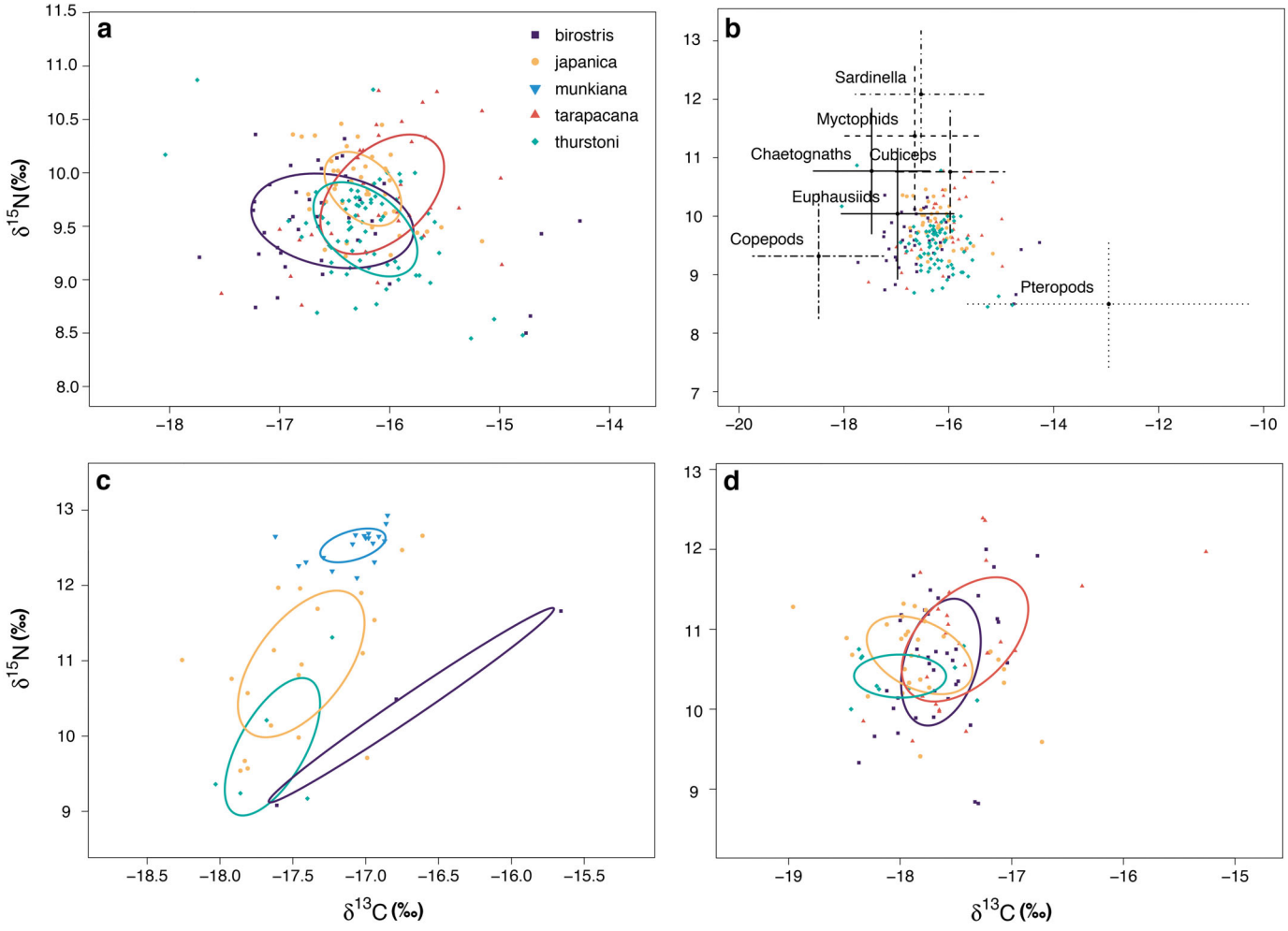

Fig. 2. Isotope data with SIBER ellipses and sources (Philippines). (a) Philippines, (b) Philippines with sources, (c) Peru, (d) Sri Lanka. Ellipses in (a), (c), and (d) represent maximum likelihood standard ellipses for each species, while analyses presented in 'Materials and methods' and 'Results' were performed on Bayesian estimates of standard ellipses (not shown). Bars in (b) represent mean ± SD for each dietary source in the Philippines included in the mixing models. Sources in (b) are corrected for trophic discrimination, including the additional uncertainty in trophic discrimination factors that was incorporated into the mixing models. Legend for species colors and shapes in all regions is listed in (a). See Table 1 for full species names

level relationships and significance values are reported in Figs. S2 & S3 in the Supplement.

Mixing models

The mixing model results from the Philippines support the data from the isotopic niche ellipses indicating that the diets of mobulids in the region are largely overlapping (Fig. 5). Using lipid- and carbonate-corrected prey sources had a minor impact on aggregated posterior distributions, typically reducing the diet contribution from pteropods and slightly increasing the contribution from fish or zooplankton (Table 2, Table S4). All 4 species of *Mobula* appeared to consume similar proportions of the 3 aggregated prey groups (Fig. 5, Table 2): model outputs suggest that diets were dominated by zooplankton, had a substantial input from the pteropod source, and included a smaller but still notable proportion of fish. In many cases, the discrete (non-aggregated) sources were confounded, evidenced by the influence of prior specifications on posterior distributions (e.g. euphausiids in *M. birostris* and *M. tarapacana*, *Cubiceps* in *M. japanica*) (Fig. S1 & Table S5). Aggregated posterior distributions for fish and zooplankton groups, however, were robust to prior specifications (Fig. 5, Table 2). Using a semi-informative prior did not substantially impact median estimates of diet proportions but did substantially reduce credible intervals and the

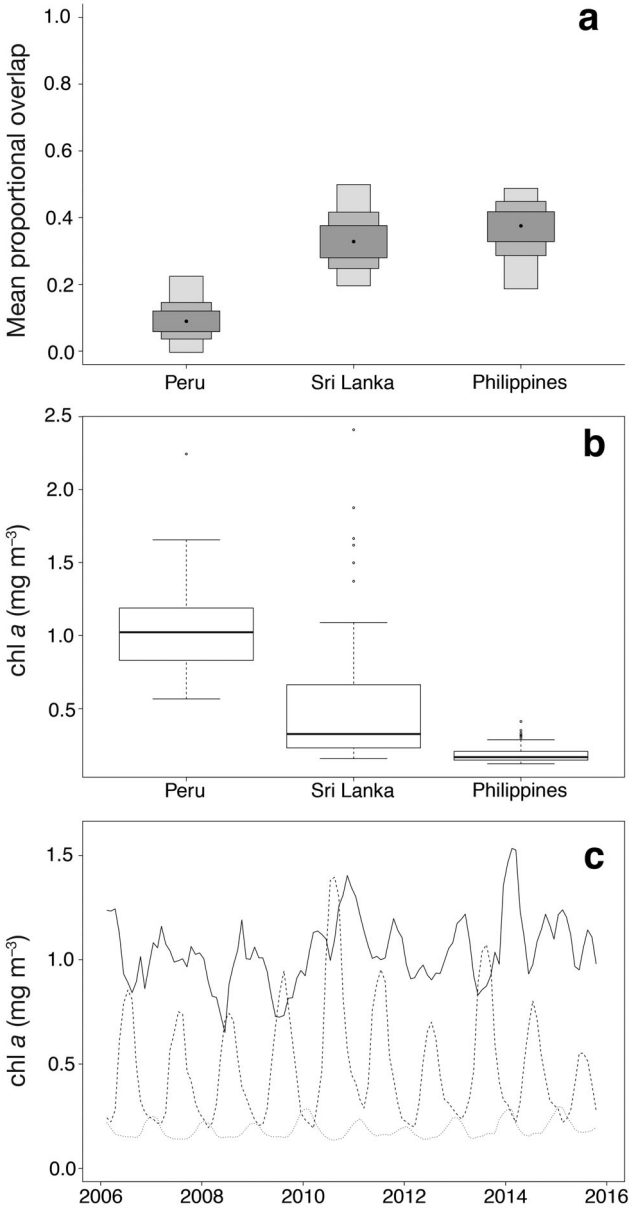

Fig. 3. Niche overlap and environmental data from Peru, Sri Lanka, and the Philippines. (a) Mean proportional overlap between species' isotopic niche areas. Rectangles represent the 50, 75, and 95% credible intervals (dark to light shading, respectively), and black dots represent the mode values. (b) Boxplots of monthly mean chl *a* values for each of the 3 study regions (boundaries defined in 'Materials and methods') across a 10 yr period from 2006 to 2016. (c) Smoothed 3 mo averages of chl *a* values across the same 10 yr period as in (b) for Peru (solid line), Sri Lanka (dashed), and the Philippines (dotted)

skewedness and non-normality of posterior distributions. The 2 exceptions to this were the proportion of fish in the diet of *M. japanica*, which shifted from 0.33 to 0.15 (median) from the uninformative to semi-informative prior, and the normality of the posterior distribution of the proportion of pteropods in the diet of *M. thurstoni*. At the discrete source level, both pteropods and copepods tended to be robust to changes in prior specification across species (Fig. S1). There was more variability in diet contributions from the epipelagic and mesopelagic aggregated sources across species than from the fish and zooplankton groups (Table 2). However, epipelagic and mesopelagic groupings were less robust to prior specifications. There was no seasonal trend in isotope values for euphausiid prey samples, which were collected across multiple months and years. Variability in euphausiid isotope values was similar to the variability in prey samples collected in a single plankton tow (Table 1), suggesting that prey samples from the single plankton tow adequately capture the variability in prey sources for the purpose of diet reconstruction.

Liver samples

The mean effects of lipid extraction on the isotope values from liver samples were an increase of 2.79‰ on $\delta^{13}C$ and a decrease of 0.11‰ on $\delta^{15}N$ (Table 1). In all analyses and discussion of liver samples, we used the $\delta^{13}C$ values from lipid-extracted samples and the $\delta^{15}N$ values from corresponding bulk (non-lipid-extracted) samples unless otherwise specified. Despite repeatedly sonicating liver samples in petroleum ether until the solution was clear instead of a dark orange color (indicating that lipids had been successfully removed), the C:N ratios of liver samples remained high (5.6 to 10.3; Table 1). There was a significant relationship between the bulk C:N ratio and the change in $\delta^{13}C$ between lipid-extracted and bulk liver samples ($p = 0.015$; Fig. S6). The linear regression equation for both species combined was:

$$\delta^{13}C_{\text{lipid-extracted}} - \delta^{13}C_{\text{bulk}} = 0.089 \times C{:}N_{\text{bulk}} + 1.328$$

However, the fit of this relationship was poor ($R^2 = 0.137$). Muscle tissue isotope values from individuals paired with liver samples were no different from the overall muscle tissue isotope values for either *M. birostris* ($\delta^{15}N$: 9.6 ± 0.5; $\delta^{13}C$: −16.6 ± 0.8) or *M. tarapacana* ($\delta^{15}N$: 9.8 ± 0.6; $\delta^{13}C$: −16.2 ± 0.6), indicating that the subsample of individuals with both muscle and liver tissue was representative of the full set of samples.

The multivariate ANOVA indicated that liver samples were significantly different from muscle samples in both *M. birostris* ($p < 0.001$) and *M. tarapacana* ($p < 0.001$). Paired *t*-tests indicated that the $\delta^{15}N$ values were significantly different between muscle and liver in *M. birostris* ($p = 0.049$) but not *M. tarapacana*

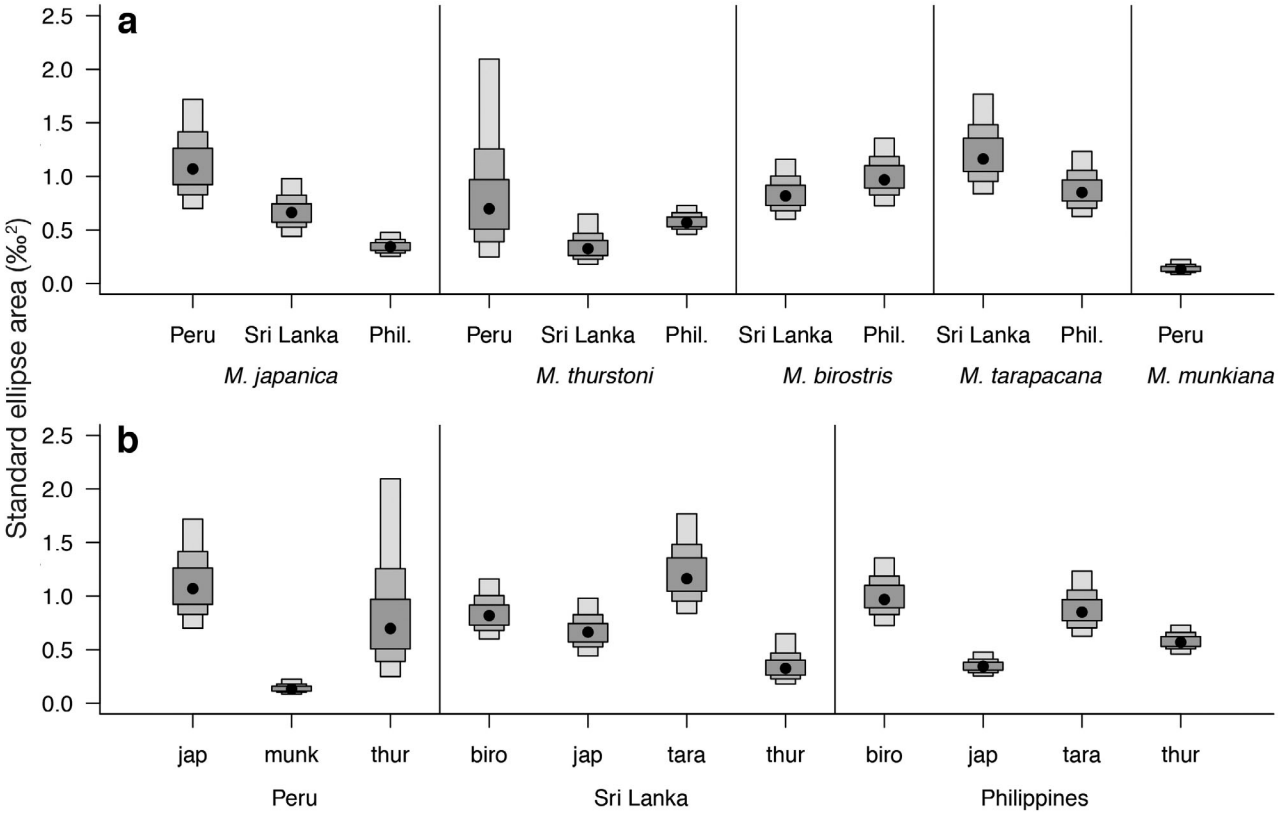

Fig. 4. Niche area by species and region. (a) Between-region comparisons for each species and (b) between-species comparisons for each region. Rectangles represent the 50, 75 and 95% credible intervals (dark to light shading, respectively), and black dots represent the mode values. We excluded *Manta birostris* collected in Peru due to large credible intervals that resulted from small sample size (n = 3). See Table 1 for full species names

($p = 0.53$). The $\delta^{13}C$ values were significantly different between muscle and liver in both species ($p < 0.001$).

DISCUSSION

Niche overlap

Trophic niche partitioning according to body size is commonly observed in nature and is a fundamental theory explaining coexistence of similar species in habitats with limited resources (Hutchinson 1957). For example, in marine fishes, body size frequently correlates with mouth gape, which in turn determines the maximum prey size a consumer can target (Scharf et al. 2000). In filter-feeders, the mechanism that would facilitate trophic niche partitioning is less clearly linked to body size, as prey items are typically orders of magnitude smaller than a filter-feeder's mouth gape. Nevertheless, sympatric rorqual whales demonstrate resource partitioning across a range of body sizes despite morphological similarities, most likely a function of behavioral differences (Santora et al. 2010, Gavrilchuk et al. 2014). Like rorqual whales, mobulid rays are morphologically similar but span a range of body sizes across species. However, despite the wide range of body sizes sampled in our study, we observed a high degree of isotopic overlap for most species within each region. Previous studies found trophic niche overlap between several mobulids in the Gulf of California, Mexico (Notarbartolo di Sciara 1988, Sampson et al. 2010), and our results demonstrate a similar pattern for 5 mobulid species at 3 separate locations across the Indo-Pacific. Additionally, we observed only weak and most likely ecologically irrelevant relationships between body size and $\delta^{13}C$ and $\delta^{15}N$ values in mobulids, further demonstrating inter- and intraspecific trophic similarities regardless of body size.

Niche overlap theory posits that overlap decreases as interspecific competition increases, for example when resources are scarce and species are forced to specialize to outcompete sympatric competitors

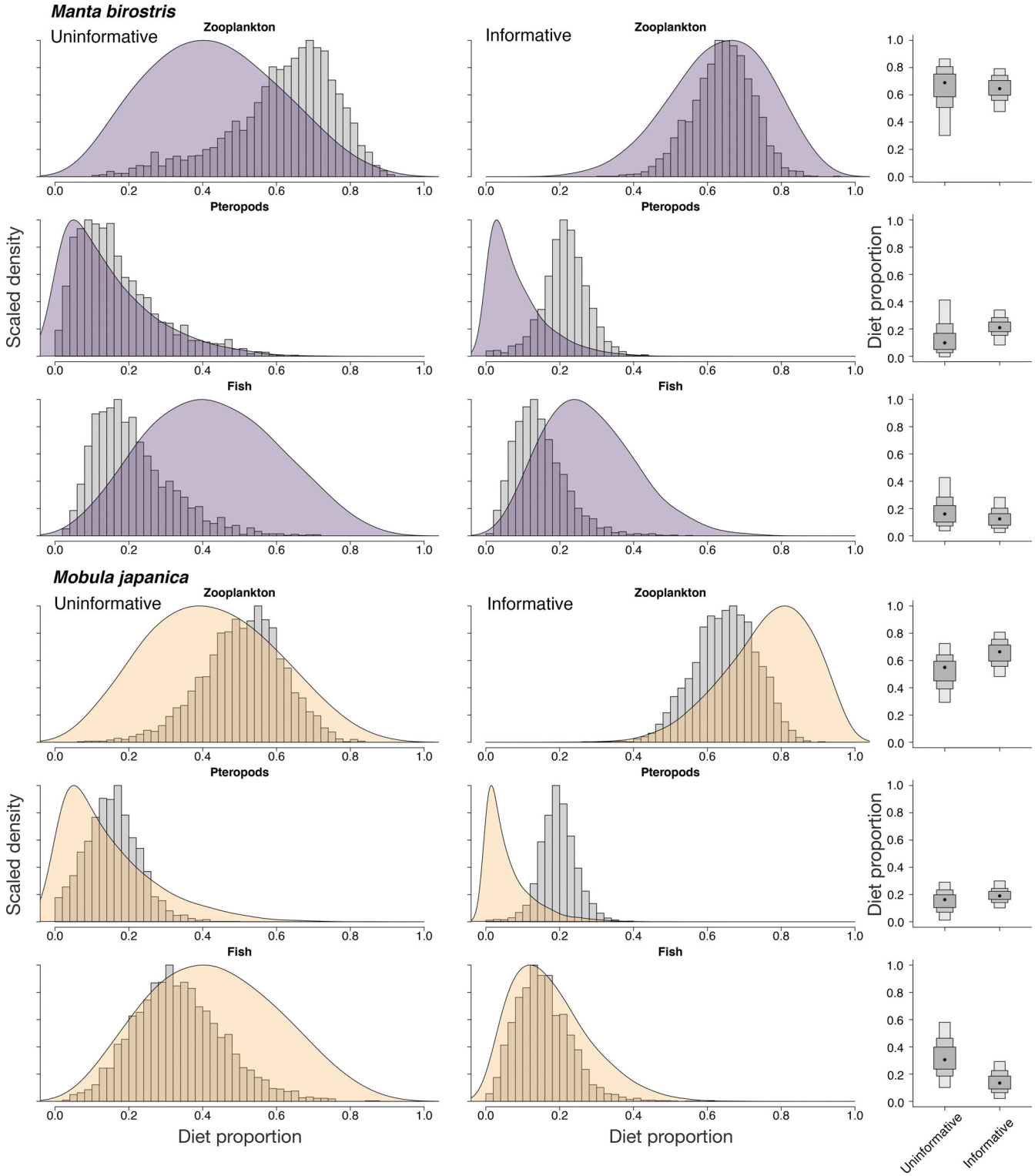

Fig. 5. Mixing model estimates of diet contributions for *a posteriori* aggregated sources. Colored density distributions represent *a posteriori* aggregated prior specifications and grey histograms represent posterior distributions in either the uninformative (left) or informative (right) model runs. The far right panel compares posterior distributions between the uninformative and informative model runs. Rectangles represent the 50, 75 and 95% credible intervals (dark to light shading, respectively), and black dots represent the mode values (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m580p131_supp.pdf shows the prior distributions that were specified for each source in the model as well as source-specific posterior distributions before *a posteriori* aggregation)

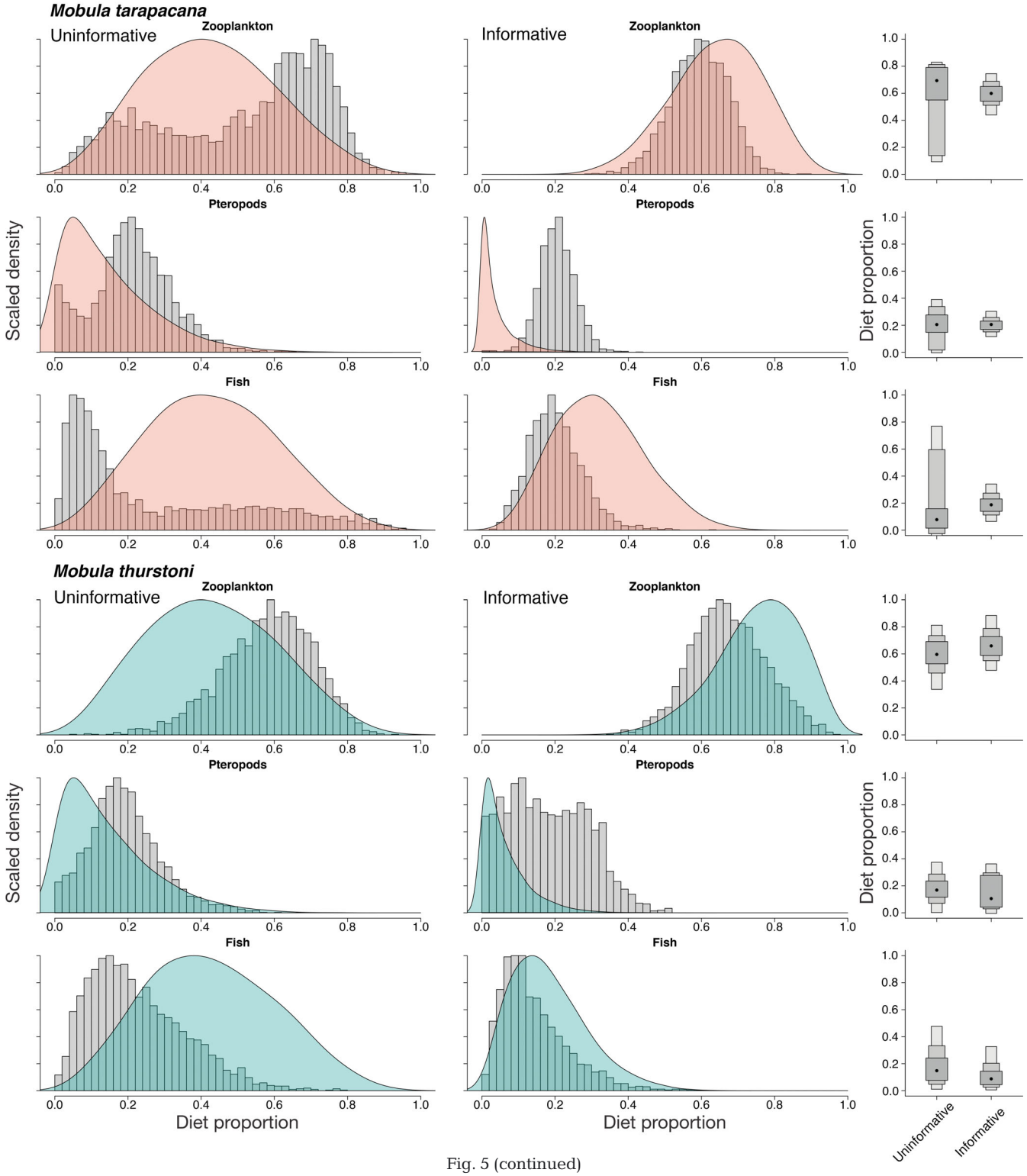

Fig. 5 (continued)

(Pianka 1974, 1981). However, this niche overlap theory (Pianka 1974) has not been definitively supported in observational studies (Porter & Dueser 1982). An alternate explanation for our results is that trophic niche overlap in mobulid rays follows an inverse pattern, where overlap increases as resources become more scarce (e.g. Porter & Dueser 1982). A mechanism for this increasing overlap in nutrient-poor regions may

Table 2. Median diet contributions of *a posteriori* aggregated prey groups from the MixSIAR Bayesian mixing model. Note that epipelagic/mesopelagic groups are different combinations of the same sources in zooplankton/fish groups. Pteropods were kept separate from both grouping scenarios. The groupings and specification of uninformative and semi-informative priors are explained in 'Materials and methods'

Species/source	Uninformative median (95% CI)	Semi-informative median (95% CI)
Manta birostris		
Zooplankton	0.64 (0.26–0.84)	0.64 (0.47–0.79)
Fish	0.19 (0.06–0.48)	0.14 (0.04–0.32)
Pteropods	0.14 (0.03–0.47)	0.21 (0.08–0.33)
Epipelagic	0.55 (0.21–0.78)	0.33 (0.16–0.51)
Mesopelagic	0.28 (0.11–0.56)	0.45 (0.27–0.67)
Mobula japanica		
Zooplankton	0.52 (0.27–0.70)	0.65 (0.48–0.80)
Fish	0.33 (0.12–0.61)	0.15 (0.04–0.32)
Pteropods	0.15 (0.03–0.31)	0.19 (0.10–0.30)
Epipelagic	0.37 (0.15–0.61)	0.21 (0.06–0.41)
Mesopelagic	0.48 (0.21–0.72)	0.60 (0.37–0.78)
Mobula tarapacana		
Zooplankton	0.59 (0.08–0.82)	0.60 (0.43–0.74)
Fish	0.15 (0.02–0.83)	0.19 (0.08–0.36)
Pteropods	0.21 (0.01–0.43)	0.20 (0.12–0.30)
Epipelagic	0.28 (0.04–0.77)	0.19 (0.06–0.40)
Mesopelagic	0.52 (0.04–0.85)	0.60 (0.37–0.78)
Mobula thurstoni		
Zooplankton	0.59 (0.31–0.79)	0.67 (0.48–0.88)
Fish	0.20 (0.04–0.52)	0.12 (0.03–0.37)
Pteropods	0.18 (0.02–0.43)	0.17 (0.01–0.40)
Epipelagic	0.43 (0.19–0.67)	0.32 (0.14– 0.54)
Mesopelagic	0.37 (0.13–0.64)	0.48 (0.28–0.75)

be an increased reliance on high-biomass prey patches that are sparsely distributed and often dominated by 1 or a few prey species (e.g. Rohner et al. 2015, Armstrong et al. 2016). For example, in Peru, where strong equatorial upwelling leads to overall high surface primary productivity, zooplankton prey is likely to be abundant. The density of vertically migrating zooplankton and fish is influenced by surface productivity (Croll et al. 2005, Hazen & Johnston 2010), and therefore an overall increase in zooplankton abundance (surface-associated and deep scattering layers) is expected in a highly productive region such as Peru. Under these conditions, different mobulid species may be able to take advantage of their evolutionarily distinct traits (e.g. size, maximum depth tolerance, thermal inertia) to maximize foraging success by feeding on their preferred zooplankton prey, resulting in greater trophic separation. In contrast, in oligotrophic tropical waters such as our study site in the Philippines, zooplankton should be lower in abundance and more patchily distributed. Numerous studies demonstrate trophic niche separation and resource partitioning in both pelagic and benthic predators in similar oligotrophic regions (e.g. Young et al. 2010, Heithaus et al. 2013, Pardo et al. 2015). However, filter-feeding elasmobranchs such as mobulids appear to require prey densities to exceed a threshold level to make feeding energetically profitable (Armstrong et al. 2016). Consequently, as regional productivity declines, there may be fewer prey patches of adequate density, resulting in multiple sympatric species converging on the same high-density prey sources and therefore greater trophic overlap such as that observed in the present study. This is also supported by observations of mobulid captures in the Philippines. Multiple mobulid species are frequently captured in the same gill nets, which are set at night over deep water in the Bohol Sea when euphausiids are abundant near the surface. Between 2013 and 2014, 25% of 790 recorded fishing trips captured more than 1 species of mobulid in a single net (J. M. Rambahiniarison unpubl.). Whale sharks, another filter-feeding elasmobranch, also rely on dense and often monospecific prey patches to survive in oligotrophic regions (Rohner et al. 2013, 2015), while sympatric rorqual whales exhibit trophic niche separation in highly productive polar foraging grounds (Santora et al. 2010, Gavrilchuk et al. 2014). It is possible that prevalent theories of niche overlap (May & MacArthur 1972, Pianka 1974, 1981) do not adequately describe the trophic dynamics of sympatric marine filter-feeders due to the prey density thresholds they require to meet energetic demands. It may also be the case that large, long-lived, mobile animals are able to escape bottlenecks in resource availability that would otherwise lead to persistent trophic niche differentiation in less productive environments. Importantly, isotopic overlap does not translate directly to trophic overlap, as consumers may feed on mixtures of taxonomically distinct but isotopically similar prey items that would lead to similar consumer isotope signatures. However, analysis of stomach contents in the Philippines verified that the mobulids' diets converge for at least 6 mo of the year (Rohner et al. 2017), effectively ground truthing our inferences from isotope data. This demonstrates the benefits of combining these 2 approaches in dietary studies, and future analysis of mobulid stomach contents in Sri Lanka and Peru would help validate the inferences made here. Our observations of these relationships between trophic dynamics and regional productivity are limited because we have observations from only 3 locations.

Nevertheless, this topic warrants further study in mobulids and perhaps filter-feeders more broadly.

Mobula munkiana appears to be an exception to the pattern of trophic overlap as the only mobulid in our study with an isotopic niche that is almost entirely non-overlapping with other mobulids in the same region. *M. munkiana* had no overlap with *Manta birostris* or *Mobula thurstoni* and only a 6% median isotopic niche overlap with *Mobula japanica* (Table S2 in the Supplement). Notarbartolo di Sciara (1988) found a similar pattern in the Gulf of California, with *M. munkiana* stomach contents dominated by mysids, as compared with the euphausiid prey found in *M. japanica* and *M. thurstoni*. Our results suggest that this trophic niche separation may be consistent for the species throughout its range and that *M. munkiana* may be feeding on an entirely different prey source and/or in an entirely different region from other mobulids in Peru. This is supported in part by differences in landing seasons between *M. munkiana* and other mobulids in Peru (K. Forsberg unpubl.), suggesting that *M. munkiana* may be present along the coast during a different time of year than other mobulids, perhaps due to differences in foraging patterns.

$\delta^{13}C$ and $\delta^{15}N$ values can provide insights into feeding locations and prey types due to predictable changes in $\delta^{13}C$ across marine habitats (France 1995) and increases in $\delta^{15}N$ at higher trophic levels (Owens 1987). In predatory elasmobranchs, changes in isotopic values are common as individuals grow and either change habitats, in the case of an ontogenetic shift, or access larger prey items at higher trophic levels (Estrada et al. 2006, Hussey et al. 2011). Borrell et al. (2011) found a positive relationship between both $\delta^{13}C$ and $\delta^{15}N$ and body size in whale sharks from the northwestern Indian Ocean, suggesting that ontogenetic shifts may also occur in elasmobranch filter-feeders. We found weak relationships between disc width and isotope tracers across all mobulid species. The observed decrease in $\delta^{13}C$ and increase in $\delta^{15}N$ with increasing disc width could indicate a shift to higher trophic level, offshore prey sources in older and larger individuals within species. However, the magnitude of the relationship we found (~0.2‰ per meter disc width) is minimal in comparison to the observed variability in isotope values within a given size class, which often exceeded 2‰ for individuals less than 10 cm apart in size. This suggests that mobulids likely do not experience an ontogenetic shift in feeding behavior and trophic level ($\delta^{15}N$) nor in habitat ($\delta^{13}C$), although there may be some weak overall effect of disc width on trophic dynamics or isotopic fractionation. Notarbartolo di Sciara (1988) found differences in stomach contents between juvenile and adult *M. thurstoni* in the Gulf of California but suggested this was more likely due to the season when either size class was sampled as opposed to a true ontogenetic dietary shift. The individuals sampled in our study spanned a range of disc widths from juveniles to mature adults in all regions and species with the exception of *M. thurstoni* in Peru. This suggests that both juvenile and adult mobulids may occupy the same habitats and target the same prey, as proposed for *M. birostris* by Stewart et al. (2016a). This is further supported by captures of both mature and juvenile mobulids in the same nets in the Philippines (J. M. Rambahiniarison pers. obs.).

The overlap we observed between species' isotopic niches is surprising given the diversity of vertical habitat use and foraging behaviors recorded in mobulids through observational studies and archival tag deployments. *M. japanica* and closely related *Mobula mobular* appear to spend the majority of their time in near-surface habitats, shallower than 50 m depth (Canese et al. 2011, Croll et al. 2012, Francis & Jones 2017). *M. birostris* makes deeper foraging dives and appears to spend substantial amounts of time below 100 m (Stewart et al. 2016b). *Mobula tarapacana* routinely undertakes deep dives below 800 m — in some cases over 1800 m — for periods of 1 to 4 h, presumably to access bathypelagic scattering layers of fishes (Thorrold et al. 2014). These vertical movements are generally linked to observed or inferred foraging behavior and represent a high degree of vertical segregation that could in turn lead to differing trophic niches. However, all of these observations were made in different regions, and our results suggest that the variability in observed behaviors may be a result of regional differences in the location of high-density zooplankton prey as opposed to consistent differences in feeding behavior between species. The few studies that examine multiple mobulid species within a single region support this conclusion. Notarbartolo di Sciara (1988) observed similarities in the euphausiid-dominated stomach contents of adult *M. japanica* and *M. thurstoni* in the Gulf of California, Mexico, which Sampson et al. (2010) later confirmed using isotope analysis. While the majority of stomachs collected from *M. tarapacana* in Notarbartolo di Sciara (1988) were empty, there were traces of euphausiids, among other crustaceans, and 1 stomach contained numerous remains of fishes. Paired with our mixing model results, this suggests that *M. tarapacana* is more piscivorous than the other mobulids. However, the increased proportion of fish

in the diet of *M. tarapacana* is minor in our results, and it is unlikely that the extreme energy expenditure of deep dives to the bathypelagic zone (Thorrold et al. 2014) would justify such a modest dietary contribution. We again posit that such behaviors are likely region-specific, and that *M. tarapacana* from the Philippines may not undertake these types of foraging excursions. Archival tag deployments on *M. tarapacana* in the Philippines could provide insights into vertical habitat use and differences between this region and previous studies in the eastern Atlantic.

Mixing models

Stomach content collections from mobulids landed in the Philippines allowed us to examine dietary overlap in greater detail. Diet contributions from the fish and zooplankton aggregated source groups were similar across species, with the majority of the diet coming from the zooplankton group and lower diet contributions coming from pteropods and the fish group. Our aggregated posterior distributions were robust to our choice of priors, and median values of diet contributions from fish, zooplankton, and pteropods were similar regardless of our use of uninformative or semi-informative priors. The most notable exception to this was *M. japanica*. *M. japanica* muscle tissue had the highest mean $\delta^{15}N$ value of all mobulids in the Philippines, placing them closest to the fish sources we identified from *M. birostris* and *M. tarapacana* stomach contents. In our model using an uninformative prior, *M. japanica* appeared to exceed all other species in their consumption of fish. Even when using semi-informative priors with a lower expected contribution of fish in the diet, our model results still indicated that *M. japanica* consumes a greater proportion of fish than *M. thurstoni* and *M. birostris* and a similar proportion to *M. tarapacana*, despite *M. tarapacana* and *M. birostris* being the only mobulids with stomach content samples that contained fish. There are several possible explanations for the discrepancy between stomach contents and mixing model results for *M. japanica*. The first possibility is that *M. japanica* is more piscivorous during the rainy season, and the landings and stomach content sampling during the dry season did not reflect the overall diet. While telemetry data suggest that *M. japanica* is restricted mainly to near-surface waters (Croll et al. 2012), at least some individuals make regular dives to depths of 200 to 300 m (Francis & Jones 2017). Myctophids and *Cubiceps* sp. such as those found in the stomach contents of *M. birostris* and *M. tarapacana*, migrate vertically to depths that are easily within the recorded diving depths of *M. japanica,* while *Sardinella* sp. typically inhabit near-surface waters. Smaller mobulids have been recorded actively feeding on schools of fish in the western Pacific (Heinrichs 2009), and it is possible that *M. japanica* engages in the same behavior in the Philippines. An alternative explanation is that our trophic discrimination factors are incorrect, given the variability in published discrimination factors for elasmobranchs and the lack of experimentally derived discrimination factors for mobulids. There is some evidence to suggest that trophic discrimination factors may vary with body size and compelling evidence to suggest a relationship between trophic enrichment and dietary protein content (Newsome et al. 2010). However, given the similarity in both the stomach content samples and body sizes of *M. japanica* and *M. thurstoni*, we would expect a similar shift for both species in our model. Given the differences between the 2 species' isotopic signatures, we find it more likely that there is a true dietary difference and a greater contribution from an enriched $\delta^{15}N$ source — fish or possibly an unsampled source — to *M. japanica* in the rainy season. Diet contributions from our epipelagic and mesopelagic source aggregations tended to be more variable across species than fish and zooplankton groups. However, we found epipelagic and mesopelagic groups to be less informative; first, because they tended to be more sensitive to prior specifications than the fish and zooplankton groups (Table 2) and, second, because they provide fewer insights into habitat use, as both the epipelagic prey and the vertically migrating mesopelagic prey can be accessed by mobulids at the surface at night.

The non-aggregated source contributions from our model outputs provide additional information on dietary sources and possible differences between species. However, our mixing model results on specific prey sources should be interpreted with caution, both because the number of sources (7) is far greater than the number of tracers we used (2) and because of the apparent sensitivity of the sources to prior specifications (Fig. S1). Several sources stand out for their consistency across priors and species. In all cases, our mixing models estimated a higher proportion of pteropods in the diets of mobulids than expected based on their presence in stomach contents. Their prevalence in the estimated diet proportions is undoubtedly due to the geometry of the source and consumer isotope values: without pteropods, the consumers would not be contained within the sources in bivariate isotope space, and therefore pteropods must be included in a sufficient proportion

for the mixing model to come to a mathematical solution. Our mixing model runs with carbonate corrections for pteropod shells led to reductions in pteropod diet contributions, illustrating this concept. It is possible that mobulids are feeding heavily on pteropods in the rainy season, when they are not landed by fishers and sampled, explaining the paucity of pteropods in stomach content samples. Alternatively, there may be an additional, unknown prey source that is isotopically similar to pteropods (either bulk or carbonate corrected) but not included in our mixing models. Burgess et al. (2016) hypothesized that *M. birostris* in Ecuador may be relying heavily on mesopelagic prey sources, which are higher in $\delta^{13}C$ and lower in $\delta^{15}N$ as compared with surface zooplankton, similar to the pteropod source in the present study. It is possible that our pteropod source is isotopically similar to unsampled mesopelagic prey sources in the Philippines and that all mobulid species rely heavily on these prey items. However, the pteropod source (with or without carbonate corrections) was isotopically distinct from both mesopelagic fishes and vertically migrating euphausiids that were sampled from stomach contents, suggesting that pteropods may in fact be an important diet item for these species in the Philippines.

Importantly, our aggregated posterior distributions may artificially inflate the proportion of fish included in the diet due to our inclusion of 3 fish sources. In the discrete source posteriors, the proportion of each fish in the diet of *M. japanica* and *M. thurstoni* abuts zero under the semi-informative prior, suggesting that the proportion of the diet coming from each fish species is negligible — very different from the posterior distributions for fish in *M. tarapacana* and *M. birostris* (Fig. S1). However, when these posterior distributions are summed for the *a posteriori* aggregated fish group, the resulting diet proportion is inflated.

Diet-switching

We analyzed multiple tissue types in mobulids from the Philippines to directly examine the possible diet-switching that our mixing model results suggested. Liver is a metabolically more active tissue than muscle and therefore has a faster turnover rate (Tieszen et al. 1983, Hobson & Clark 1992). Previous studies have used liver and muscle tissue to examine dietary stability in elasmobranchs, with isotopic differences between the 2 tissue types interpreted as evidence of seasonal diet switching (MacNeil et al. 2005). The isotopic signatures of the liver samples we collected in the Philippines were significantly different from the paired muscle samples from the same individuals. The greatest difference between tissues was in $\delta^{13}C$ for both *M. birostris* and *M. tarapacana*. However, liver and muscle tissues are expected to fractionate $\delta^{13}C$ differently, with lower $\delta^{13}C$ values in liver samples even after lipid extraction (Pinnegar & Polunin 1999, MacNeil et al. 2005). Controlled experiments and studies of wild populations of teleost and elasmobranch fishes suggest that this difference in $\delta^{13}C$ fractionation between liver and muscle tissue may range from 0.5 to 2‰ (Pinnegar & Polunin 1999, MacNeil et al. 2005, Hussey et al. 2010a), while the shift in $\delta^{13}C$ between our muscle and liver samples was, on average, 2.8‰. It is therefore likely that some portion of the difference in $\delta^{13}C$ is due to variable fractionation rates between the 2 tissues, while some portion is due to true dietary shifts. This may explain the discrepancies between the frequency of some prey sources in stomach contents and the mixing model outputs of diet proportions for *M. birostris* and *M. tarapacana* and possibly the other mobulid species for which liver samples were not collected (Fig. S1). If liver and muscle tissue isotope values were equal, it would suggest a stable diet throughout the tissue integration period of the metabolically slower tissue, while differences suggest diet-switching (MacNeil et al. 2005). However, the C:N ratios of lipid-extracted liver tissues remained higher than corresponding muscle tissues, which may indicate that lipid extraction was not successful, confounding the interpretation of these results.

We found small but significant differences in $\delta^{15}N$ between tissues in *M. birostris* and no significant difference in $\delta^{15}N$ between tissues in *M. tarapacana*. However, $\delta^{15}N$ may also fractionate differently between tissue types, although with a smaller effect than $\delta^{13}C$. Hussey et al. (2010a) found $\delta^{15}N$ values to be 0.37 to 0.89‰ higher in bulk muscle tissue than in bulk livers of 3 captive sharks and 0.11 to 1.18‰ higher in lipid-extracted muscle than in lipid-extracted livers. Similarly, MacNeil et al. (2005) found $\delta^{15}N$ values to be, on average, 0.03 and 0.57‰ higher in lipid-extracted muscle tissue than in lipid-extracted livers of 2 species of non-captive sharks that they concluded had relatively stable diets. While our comparison of $\delta^{15}N$ in bulk muscle and bulk liver tissue seems to suggest that *M. birostris* may switch its diet seasonally in the Philippines, adding a correction factor of 0.5‰ to liver $\delta^{15}N$ values to account for possible fractionation differences between tissues inverts these results, with $\delta^{15}N$ becoming significantly different between tissues in *M. tarapacana* and not signif-

icant in *M. birostris*. These findings illustrate how even small uncertainties in stable isotope ecology, especially in fractionation factors, can have substantial impacts on the interpretation of results, and they highlight the frequently repeated need for species-specific laboratory validation of parameters that are used in isotope analysis.

CONCLUSIONS

Our findings contribute to a limited but growing body of knowledge on the habitat use and ecology of mobulid rays, which are highly vulnerable to exploitation due to their demographic characteristics (Dulvy et al. 2014, Pardo et al. 2016). Both bycatch and targeted fisheries appear to contribute to global declines in mobulid abundance (Ward-Paige et al. 2013, White et al. 2015, Croll et al. 2016). Targeted fisheries can be managed with legislation banning the capture of mobulids, but bycatch remains a more challenging and persistent threat due to the ubiquity of mobulid bycatch in artisanal and commercial fisheries of all types (Croll et al. 2016). The apparent similarity in diets and overlapping isotopic niches between mobulids in this study are consistent with the spatial overlap in bycatch of the various mobulid species (Croll et al. 2016). Identifying the spatial and temporal patterns of mobulids' primary zooplankton prey (for example euphausiids in the Philippines) could aid in predicting their occurrence and relative vulnerability to bycatch-prone fisheries. Our results further indicate that both adults and juveniles are targeting similar prey and are thus overlapping with and susceptible to the same fisheries pressures, which has implications for the catchability of different life stages and consequently the development of age- or size-based fisheries management strategies. Further research is necessary to corroborate many of the patterns observed here over larger spatial and temporal scales and will aid in our understanding of habitat use by mobulids and the development of fisheries bycatch mitigation strategies.

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