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Telemetry-validated nitrogen stable isotope clocks identify ocean-to-estuarine habitat shifts in mobile organisms

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Abstract

1. Throughout their life history, many animals transition among heterogeneous environments to facilitate behaviours such as reproduction, foraging and predator avoidance. The dynamic environmental and biological conditions experienced by mobile species are integrated in the chemical composition of their tissues, providing retrospective insight into movement.
2. Here, we present a unique application of nitrogen stable isotope clocks ('isotopic clocks'), which integrate tissue turnover rates, consumer stable isotope ratios and habitat-specific isotope baselines to predict time-since-immigration and the timing of habitat shifts in a migratory species. Nitrogen stable isotope values of blood plasma collected from juvenile sand tiger sharks *Carcharias taurus*, a species known to undertake seasonal movements between ocean and estuarine environments, were used to derive estimates of time-since-immigration and the timing of seasonal habitat shifts undertaken by this species.
3. Nitrogen isotopic clocks estimated for 65 juvenile sand tiger sharks sampled across 6 years indicated that individual sharks predominantly arrived to estuarine habitats between June and July, with some individuals arriving as early as mid-May. These estimates were validated by comparing isotope-derived estuarine arrival times with those from acoustically tracked individuals. The median estuarine arrival day estimates from our isotopic approach aligned with estimates from acoustic telemetry for each sampling population.
4. Sensitivity analyses indicated that isotopically inferred time-since-immigration and estuarine arrival estimates were robust to variation in isotopic turnover rate and diet tissue discrimination factors under multiple modelling scenarios. This suggests that parameterization of the nitrogen isotopic clock provides reliable estimates of time-since-immigration and day of arrival into new habitats if isotopic variation exists between origin and new locations.
5. Our study presents a unique application of telemetry-validated isotope clocks to derive retrospective estimates of time-since-immigration and timing of habitat shifts for animals that seasonally traverse heterogeneous environments. This

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approach can be readily applied across many temporal and spatial scales, and to other species and ecosystems, to facilitate rapid assessment of changes in animal habitat use and broader ecosystem structure.

KEYWORDS

Carcharias taurus, ecosystem connectivity, habitat use, heterogeneous habitats, movement ecology, shark

1 | INTRODUCTION

The physical, chemical and biological properties of environments through which animals move are often integrated in the biochemical composition of their tissues, either through diet (i.e. catabolized to organic macromolecules) or accretion into hard structures (e.g. bound within otoliths, teeth and bone). The potential of biochemical markers to record migration dynamics is great (e.g. Graham et al., 2010; Trueman et al., 2019), but application lags behind that of conventional tracking approaches (e.g. biotelemetry, Graham et al., 2010; Hobson et al., 2012; Madigan et al., 2014; Moore et al., 2016; Vander Zanden et al., 2018; Trueman et al., 2019). Naturally occurring stable isotope ratios form the methodological basis of most studies because regional variability in the isotope ratios of baseline organisms (defined as low trophic level prey species with high site fidelity), which is largely driven by local environmental (e.g. precipitation, temperature, salinity) and biogeochemical processes (e.g. nitrate vs. nitrite metabolism or C_3 vs. C_4 photosynthesis), is integrated into the tissues of higher organisms that readily migrate (Madigan et al., 2014, in press; Moore et al., 2016). In most cases, the tissues of an animal that has recently migrated from one region to another, assuming both are isotopically distinct, will reflect an admixture of origin and new prey isotope baselines. This is because the individual has not yet reached an isotope 'steady state' with the new system (Madigan et al., 2014), provided that sampling has occurred within the time window of isotope turnover for a given element (Thomas & Crowther, 2015; Vander Zanden et al., 2015). From examination of an individual's 'isotopic clock' (Klaassen et al., 2010), knowledge of these facets can be used to estimate time-since-immigration, and thus the retrospective timing of individual movement into a new system (Madigan et al., 2014, 2017, in press; Moore et al., 2016). Defining the timing of animal migration is important to establish temporal variability in food web dynamics, as well as to encourage cooperation between management boundaries over which individuals may move (Madigan et al., 2021).

Application of an isotopic clock has provided new insight into the migration dynamics of Pacific Bluefin Tuna *Thunnus orientalis* across the Pacific Ocean (Madigan et al., 2014). In Madigan et al. (2014), time-since-immigration and approximate arrival times were estimated for individuals moving from the isotopically distinct, nutrient-poor waters of Eastern Japan, with a lower baseline nitrogen isotope ratio ($\delta^{15}N$), into the highly productive, nitrate-dominated waters of the Eastern Pacific Ocean, with a higher baseline $\delta^{15}N$. The authors were able to define residents from migrants and evaluate whether migration patterns varied across ontogeny. Isotopic clocks have also been applied

across marine-brackish-freshwater gradients; for example, Moore et al. (2016) combined carbon, sulphur and nitrogen ($\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$) isotopic clocks to predict time-since-immigration for juvenile life-history stages of five Pacific salmonids within the Skeena River watershed, British Columbia. This approach was used to confirm estuaries as critical stopover habitats for life-history stages that were too small to track using conventional approaches (Moore et al., 2016). Despite the promise of isotopic clocks to discern important information on animal migrations and habitat use, there have been few applications to other species and study systems, especially at smaller spatial and temporal scales. Similarly, a critical comparison between isotope-derived migration information with that of telemetry approaches (e.g. passive acoustic vs. isotopic clock) has yet to be undertaken.

We present results from both nitrogen isotopic clocks ($\delta^{15}N$) and acoustic telemetry to illustrate how isotopic clocks can be used to estimate time-since-immigration and seasonal movement between heterogeneous marine habitats. Juvenile sand tiger sharks *Carcharias taurus* are used as a case study species as this species performs seasonal ocean-to-estuarine habitat shifts (Kneebone et al., 2012, 2014). Our application to juvenile sharks illustrates that stable isotope clocks are applicable at much finer temporal and spatial scales than documented in previous studies (Madigan et al., 2014; Moore et al., 2016). Our study provides a clear methodological framework that can be applied to other animals that traverse chemically distinct interfaces, to estimate time-since-immigration and infer the timing of habitat shifts, collectively informing the broader ecology of species and ecosystems.

2 | MATERIALS AND METHODS

2.1 | Study location and sampling of juvenile sand tiger sharks

The Great South Bay (GSB), New York, US is a highly productive temperate lagoon that provides critical stopover habitat and food resources for many juvenile life-history stages of marine finfish, especially during the summer and early fall (Olin et al., 2019; Tinoco, 2017). Anecdotal evidence dating back to the early 20th century proposed that GSB has long served as a potential summer nursery ground for juvenile sand tiger sharks *Carcharias taurus* (Thorne, 1928). Juvenile sand tiger sharks are known to migrate considerable distances between southern wintering grounds, extending from Florida to Northern Carolina, and summer nursery grounds in more northern, temperate embayments (Kneebone et al., 2014; Figure 1). In coastal New York,

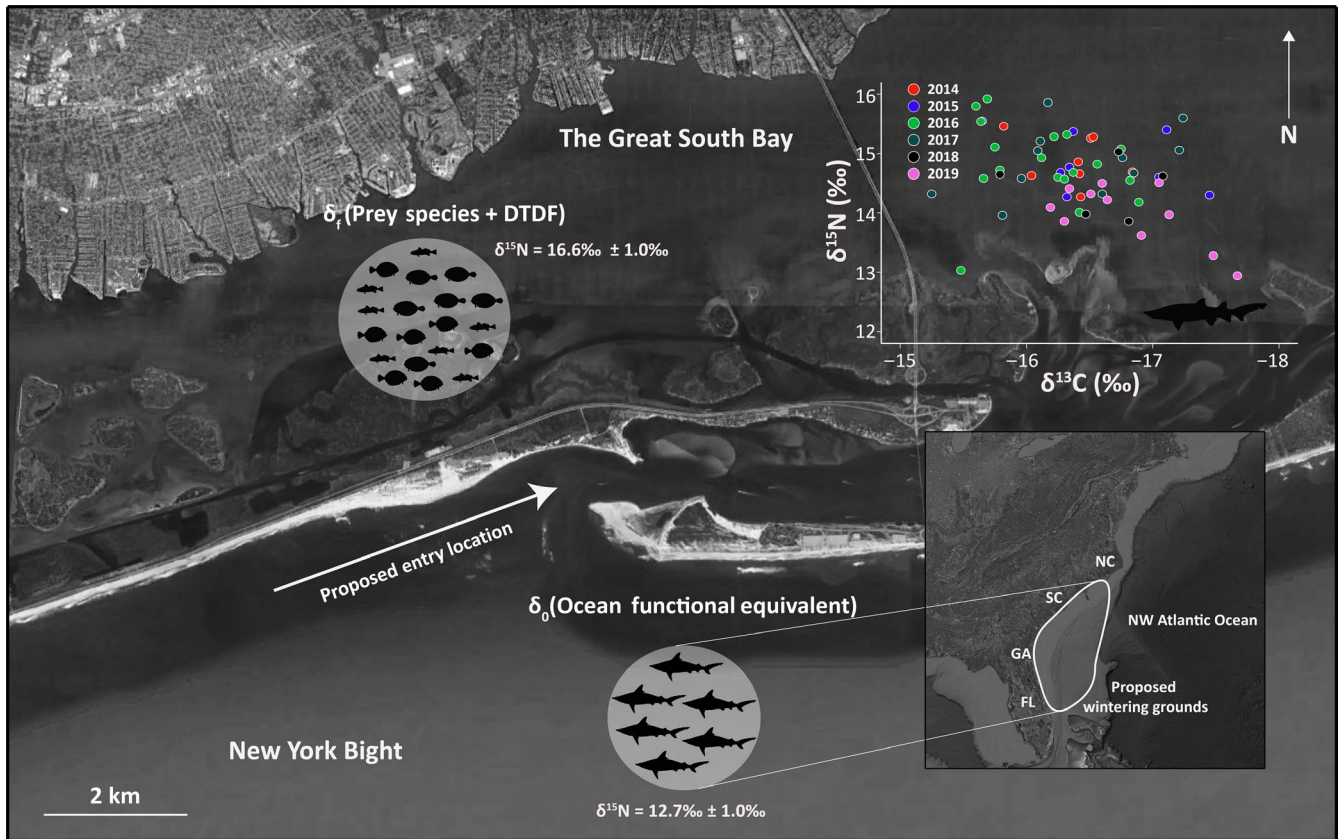


FIGURE 1 Proposed migration route of juvenile sand tiger sharks into Great South Bay, New York from the New York Bight. Top inset depicts the isotopic biplot for juvenile sand tiger sharks captured in GSB by year in relation to δ_0 and δ_f parameters used in the isotopic clock model. Bottom inset represents the proposed seasonal wintering ground of juvenile sand tiger sharks; δ_0 were parameterized based on the isotopic composition of blood plasma sampled from juvenile blacktip sharks from the First Coast, Jacksonville, FL. Source: The Great South Bay and NE US Seaboard, Google Earth, Accessed 9 January 2020

their migration dynamics remain speculative, but offer an opportunity to develop an isotopic clock model to discern the dynamics of ocean–estuarine movements. Juvenile sand tiger sharks were captured between May and September in consecutive years from 2014 to 2019 in GSB (Figure 1) using rod and reel angling and drum lines. Some individuals were recaptured multiple times throughout the six sampling years, and these data were treated as independent fish for the purposes of the isotopic clock analysis. A full description of animal sampling is given in the Supporting Information (Appendix S1).

2.2 | Isotopic clock and statistical resampling procedure

Isotopic clocks are defined by an equation from Klaassen et al. (2010) that describes time as a function of the difference in ecosystem baseline isotope ratios and the rate of turnover for that isotope in an organism:

$$t_i = \frac{\ln\left(\frac{\delta_{0i} - \delta_{fi}}{\delta_t - \delta_{fi}}\right)}{\lambda_i}, \quad (1)$$

where t = time (in days) an individual has been resident in the new system, δ_0 = the assumed isotopic composition of an animal at

isotopic steady state with their origin location, δ_f is the assumed isotopic composition of the animal at isotopic steady state with the new location, δ_t is the isotopic composition of an individual at the time of sampling and λ is the tissue-specific isotopic turnover rate (Figure 2). This equation can be solved by entering a value for each of the input parameters to provide a single estimate. Alternatively, t can be viewed as a distribution of estimates drawn from distributions of input parameters that represents a more realistic characterization of uncertainty (see Heady & Moore, 2013; Klaassen et al., 2010). Thus, t_i is 1:10,000 draws from normal distributions of δ_{0i} , δ_{fi} and λ_i based on the mean (μ) and standard deviation (σ ; Figure 2). The first 1,000 successful estimates of t_i are then used to generate distributions of time-since-immigration, t_i (in days) and day of arrival with the following equation:

$$\text{Arrival Day} = \text{Sampling Day} - t_i. \quad (2)$$

To estimate population-level estimates of t_i and arrival day, the distributions for all individuals can be combined for each year (e.g. Madigan et al., 2014; Madigan et al., 2021). We further explore uncertainty in t_i estimates by conducting sensitivity analyses related to tissue turnover rates and diet tissue discrimination factor/trophic enrichment factor (herein DTF) as described below. Diet tissue

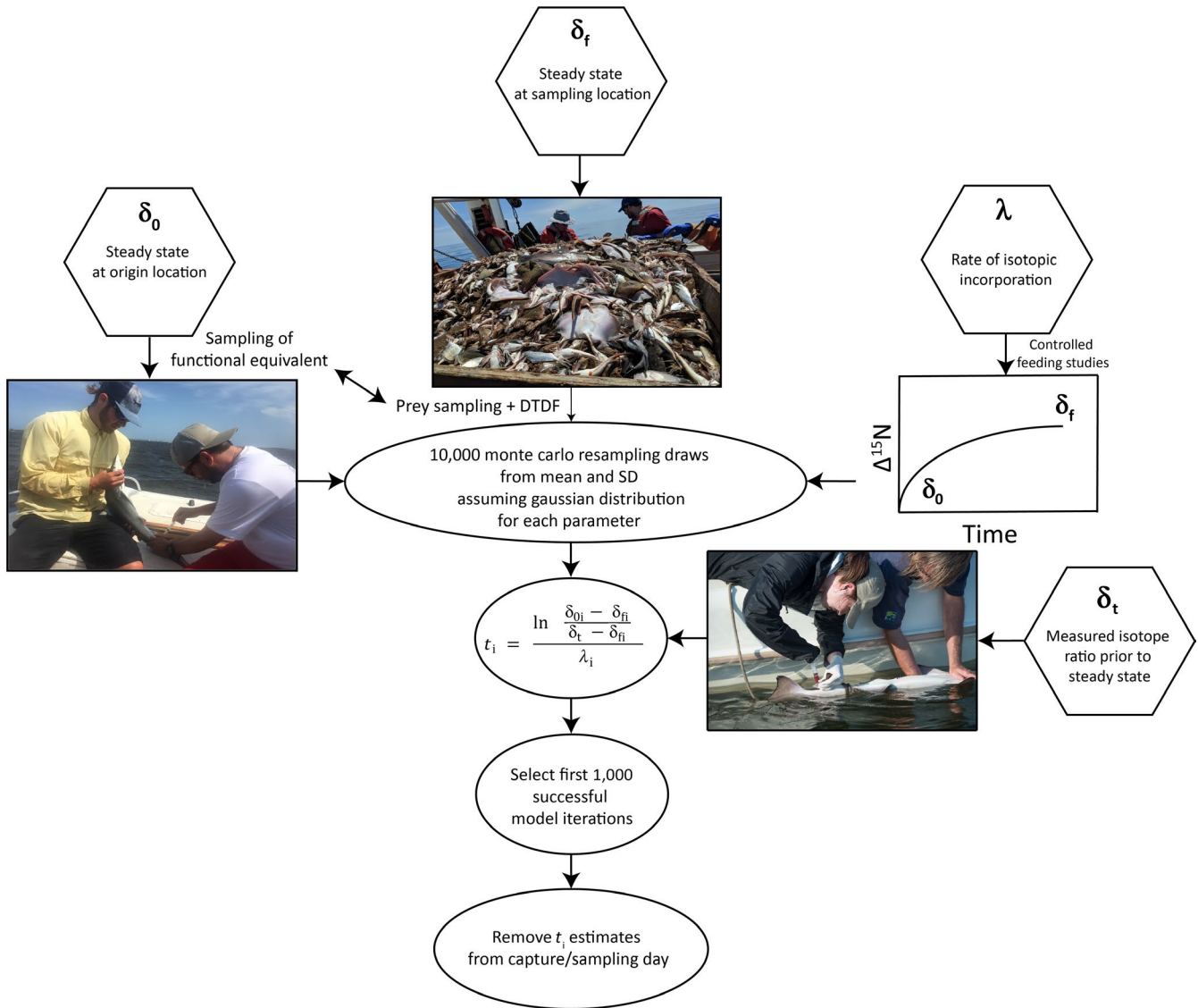


FIGURE 2 Flow diagram illustrating parameterization and implementation of an isotopic clock approach to calculate time-since-immigration and arrival day for highly migratory animals. Note that δ_0 and δ_f parameters can be derived from prey-based sampling + DTFDs or via sampling functionally equivalent species assumed to be at isotopic steady state with the origin or new locations

discrimination factors are defined as the stepwise isotopic enrichment/depletion in a consumer relative to its prey (Caut et al., 2009; Hussey et al., 2010). First, we describe the development of parameter estimates that populate Equation 1.

2.3 | Model parameterization for juvenile sand tiger sharks

We used isotopic clocks to generate time-since-immigration estimates for juvenile sand tiger sharks captured in GSB. Models were parameterized using a single elemental isotope ratio, $\delta^{15}N$, measured in shark blood plasma, for two reasons. First, the high terrestrial nutrient input into estuaries often results in elevated ^{15}N compared to primary producers of continental shelf ecosystems. For GSB specifically, high nitrogen loading from local agriculture (e.g. duck

farms, Gobler et al., 2019) provides sufficient isotopic distinctiveness relative to the coastal ocean, which is required to effectively parameterize isotopic clock models (Madigan et al., 2014, in press). In coastal marine settings, a similar range in $\delta^{13}C$ between baselines is certainly possible, but high variability of planktonic and benthic primary producers that utilize both C_3 and C_4 photosynthetic pathways may be problematic for parameterizing accurate isotopic baselines. Second, the use of $\delta^{13}C$ isotopic clocks in this study system may be further compounded by uncertainty surrounding the lipid dynamics of shark blood plasma (Kim & Koch, 2012). Given the potential for lipid effects, $\delta^{13}C$ was assumed to be less reliable compared to $\delta^{15}N$ for the parameterization of isotopic clocks. Isotopic clocks were implemented for animals captured from 2014 to 2019 (Figure 1; Table 1). The isotopic turnover rate used for ^{15}N of shark blood plasma (expressed in day^{-1}), λ , was $0.0165 \pm 0.003 \text{ day}^{-1}$ based on estimates derived from leopard sharks *Triakis semifasciata*

TABLE 1 Justification of parameterization for primary isoclock model to examine time-since-immigration of juvenile sand tiger sharks into the Great South Bay estuary

Parameter	Mean (± 1 SD)	Species and sampling location	Tissue type	Justification	References
δ_t	—	Juvenile sand tiger sharks, Great South Bay, New York	Blood plasma	Fast turnover rate tissue sampled from juvenile sand tiger sharks to account for time-since-immigration into the GSB (summer and early fall only)	This study
δ_0	$12.7 \pm 1.0\text{‰}$	Juvenile blacktip sharks, First Coast, Jacksonville	Blood plasma	Blacktip sharks are assumed to exhibit similar diets to sand tiger sharks in near-shore environments. The sampling location represents the mid-point of proposed wintering grounds for juvenile sand tiger sharks	Cortés (1999), Kneebone et al. (2014), Morgan et al. (2020)
δ_f	$13.4 + \text{DTDF}$ ($3.2 \pm 1.0\text{‰}$)	Winter Flounder, Summer Flounder, Weakfish, Great South Bay, New York	White muscle	Species represent potential prey items (or functionally equivalent prey) for juvenile sand tiger sharks in GSB given no diet data exist for the New York region. Chosen DTDF is based on a mixed diet of squid and fish (see Kim, Casper, et al., 2012)	This study; Kim, del Rio, et al. (2012), Kim, Casper, et al. (2012)
λ	$0.0165 \pm 0.003 \text{ day}^{-1}$	Leopard shark, captured: San Francisco Bay, maintained: University of California, Santa Cruz	Blood plasma	This represents one of the only isotopic turnover rates published for blood plasma that is derived from controlled feeding experiment. This study is also the source of DTDFs used for the parameterization of δ_f	Kim, del Rio, et al. (2012)

held in experimental conditions (Kim, del Rio, et al., 2012). We used the isotopic turnover rate derived from this study as it represents one of the only published estimates for shark blood plasma that is coupled with estimates of diet tissue discrimination factors that can be used to parameterize δ_f .

Isotopic baselines or functionally equivalent species can be used to define the hypothetical isotopic composition of juvenile sand tiger sharks at relative isotopic 'steady state' with the origin and new ecosystem (Madigan et al., 2021). These 'baselines' reflect the isotopic ratio of species that are assumed to inhabit the same trophic position, thus exhibit similar diet tissue discrimination from dominant source/s of primary production (Moore et al., 2016) or are based on potential prey items corrected for the DTDF (Heady & Moore, 2013; Madigan et al., 2014). In this study, we used existing isotope data from two independent datasets (Table 1; Appendix S1 Table S1) to derive δ_0 and δ_f .

Existing biotelemetry data suggest that the coastal waters of North Carolina and Florida serve as seasonal wintering grounds for juvenile sand tiger sharks (Kneebone et al., 2014). Thus, we assumed δ_0 (i.e. reflecting the animal at isotopic steady state with the origin location) would reflect juvenile sand tiger sharks at isotopic steady state with isotopic prey baselines reflecting southern wintering grounds. Due to a lack of isotopic data from juvenile sand tiger sharks in this region, we parameterized (δ_0) based on literature-derived $\delta^{15}\text{N}$ of blood plasma measured for a functionally equivalent species known to exhibit similar dietary habitats and migration dynamics (based on published diet data, Cortés, 1999), the blacktip shark *Carcharhinus limbatus* ($\delta^{15}\text{N} = 12.7\text{‰}$, Morgan et al., 2020). Owing to the potential relationship between body size and $\delta^{15}\text{N}$ as commonly observed in marine

fishes (Jennings et al., 2002; Galvan et al., 2010), literature-derived values were based on small-bodied individuals that exhibited a similar size range (fork length = 68–155 cm) to juvenile sand tiger sharks sampled in this study (fork length = 89–158 cm). Blacktip sharks were sampled from a central portion of the assumed wintering grounds, the First Coast, Jacksonville, FL (Morgan et al., 2020). We assigned a conservative standard deviation of $\pm 1.0\text{‰}$, which is higher than that reported by Morgan et al. (2020, $SD = 0.83\text{‰}$) to ensure our resampling procedure adequately accounted for potential variation in isotopic baselines.

Estuarine baselines were derived from the isotopic composition of white muscle tissue of small-bodied secondary and tertiary consumers (Appendix S1: Table S1), including weakfish *Cynoscion regalis* ($n = 19$, $TL = 6.5 \text{ cm} - 26.5 \text{ cm}$), summer flounder *Paralichthys dentatus* ($n = 31$, $TL = 11.4 \text{ cm} - 38.5 \text{ cm}$) and winter flounder *Pseudopleuronectes americanus* ($n = 31$, $TL = 7.2 \text{ cm} - 21.0 \text{ cm}$). These species were selected because they likely serve as potential prey items for juvenile sand tigers in estuarine food webs, given the latter's broad dietary preferences noted elsewhere (Cortés, 1999). Even if juvenile sand tiger sharks do not directly prey on these species, their isotopic composition represents that of low to intermediate consumers within the estuarine food web and is assumed synonymous to juvenile sand tiger shark diet. Additionally, our sensitivity analyses (see below) ensured that an extremely broad isotopic range of potential prey could be accounted for in our modelling framework. The mean isotopic composition of all individuals combined ($n = 81$, $\delta^{15}\text{N} = 13.4\text{‰}$) was corrected based on published DTDFs for blood plasma (Kim et al., 2012, see Table 1 for further justification of model parameterization). Our primary model used a DTDF of

$\Delta^{15}\text{N} = 3.2\text{‰}$ because this value represents the mid-point of published estimates for leopard sharks where individuals were fed single diets of squid (DTDF = 2.2‰) and tilapia (DTDF = 4.2‰) in a laboratory setting (Kim et al., 2012). We assume that juvenile sand tiger sharks in natural systems feed across multiple prey types and are therefore more likely to exhibit an intermediate DTDF reflecting a mixed diet. We assigned a conservative standard deviation of $\pm 1.0\text{‰}$ to δ_f , which ensured that variation associated with DTDFs and isotopic baselines was accounted for (Phillips et al., 2014). Furthermore, the effects of uncertainty on estimated time-since-immigration and estuarine arrival were tested across several sensitivity models (see below).

2.4 | Sensitivity analysis

Isotopic turnover rates (λ) and diet tissue discrimination factors (DTDFs) represent some of the most poorly constrained components of stable isotope systems, but are often required to accurately interpret and solve various mixing scenarios (Phillips et al., 2014; Thomas & Crowther, 2015; Vander Zanden et al., 2015). Because these two sources of uncertainty may significantly impact time-since-immigration estimates (hence inferred arrival days) derived using the isotopic clock, we ran several sensitivity analyses to incorporate uncertainty of turnover rates and DTDFs in estimates of time-since-immigration and arrival day. First, we examined the distribution of t_i estimates assuming a constant rate of isotopic turnover, where

$$t_i = \frac{\ln\left(\frac{\delta_{oi} - \delta_f}{\delta_t - \delta_f}\right)}{\lambda} \quad (3)$$

Second, we tested the variability of isotopic clock models to significant variability in DTDFs assigned to prey stable isotope values to derive δ_f . Diet tissue discrimination factors vary considerably across elasmobranchs and because factors such as environmental conditions (e.g. temperature), prey type and ration size (Barnes et al., 2007; Kim, Casper, et al., 2012; Kim, del Rio, et al., 2012; Shipley & Matich, 2020), as well as the amino acid composition of specific tissues (Hussey et al., 2010; McMahon et al., 2010), can influence bulk ^{15}N fractionation during protein synthesis (Bond & Diamond, 2011; Hussey et al., 2012). It is therefore recommended that such models are examined for sensitivity to the use of variable DTDFs (Olin et al., 2013; Phillips et al., 2014). We tested the sensitivity of time-since-immigration estimates to two additional DTDFs that were assigned to δ_f . We used the mean DTDFs reported in Kim, del Rio, et al. (2012) and Kim, Casper, et al. (2012) from leopard sharks fed a constant diet of tilapia ($\Delta^{15}\text{N}_{\text{tilapia}} = 2.2\text{‰}$, $\delta_f = 15.6 \pm 1.0\text{‰}$) and squid ($\Delta^{15}\text{N}_{\text{squid}} = 4.2\text{‰}$, $\delta_f = 17.6 \pm 1.0\text{‰}$).

2.5 | Acoustic telemetry

We examined the accuracy of our primary isotopic clock model by comparing model predictions to validated passive acoustic telemetry data, for each sampling year. Specifically, time-of-first detection was

calculated from an existing passive acoustic telemetry array, which comprised between 19 and 28 VR2W (69 KHz) acoustic hydrophones (Vemco Inc). These were deployed from April to November between 2014 and 2019 (Appendix S1: Figure S1, Table S2).

3 | RESULTS

Blood plasma/serum was collected from 66 juvenile sand tiger sharks between 2014 and 2019 (Table 2); this included recaptures of 14 individuals across multiple sampling years.

3.1 | Primary model

A total of 66 individuals provided 1,000 calculated values of t_i by sampling from distributions of δ_{fi} and δ_{ti} and λ_i . In one individual, the isotopic clock produced time-since-immigration and estuarine arrival day estimates that were implausible based on the known life-history strategy of the species; this individual was subsequently removed from the analyses as per other studies (Moore et al., 2016), leaving 65 individuals for subsequent analyses. A small number of the sampling iterations resulted in negative t_i values, and in some cases, models were unable to resolve t_i because δ_t fell outside of the mixing space (e.g. $\delta_t < \delta_o$, $\delta_t > \delta_p$, $\delta_o > \delta_r$). At the individual level, time-since-immigration periods (t_i) inferred from the primary isotopic clock model varied among sampled individuals. Time-since-immigration periods ranged from 4 to 141 days across all individuals (Figure 3).

All individuals within each sampling year were pooled to provide annual, population-level estimates of estuarine arrival. For most years, at least 50% of the population was assumed to have entered the estuary within mid-to-late June, except for 2014 and 2016, where the most probable estuarine arrival periods were early July and late May, respectively (Figure 3). For years 2014 and 2015, at least 50% of individuals were assumed to have entered estuarine habitat by the first week of July (Figure 3). In 2016, at least 50% of individuals were assumed to have entered by 25 May. For 2017–2019, at least 50% of individuals were assumed to have entered by 20 June (Figure 3). The range of approximate arrival days, as inferred through the passive acoustic telemetry array (time of first detection,

TABLE 2 Size range, sample sizes and blood plasma stable nitrogen isotope and C:N molar ratio data ($M \pm SD$) for juvenile sand tiger sharks sampled in Great South Bay from 2014 to 2019

Year	n	Size range (fork length, cm)	$\delta^{15}\text{N}$	C:N _{molar}
2014	9	103–141	14.9 (0.4)	2.1 (0.7)
2015	10	91–146	15.0 (0.8)	2.0 (0.5)
2016	18	89–151	14.8 (0.7)	2.3 (0.6)
2017	12	89–159	14.7 (0.7)	2.5 (0.6)
2018	6	90–131	14.7 (0.8)	2.8 (1.1)
2019	11	92–143	14.0 (0.5)	3.4 (0.4)

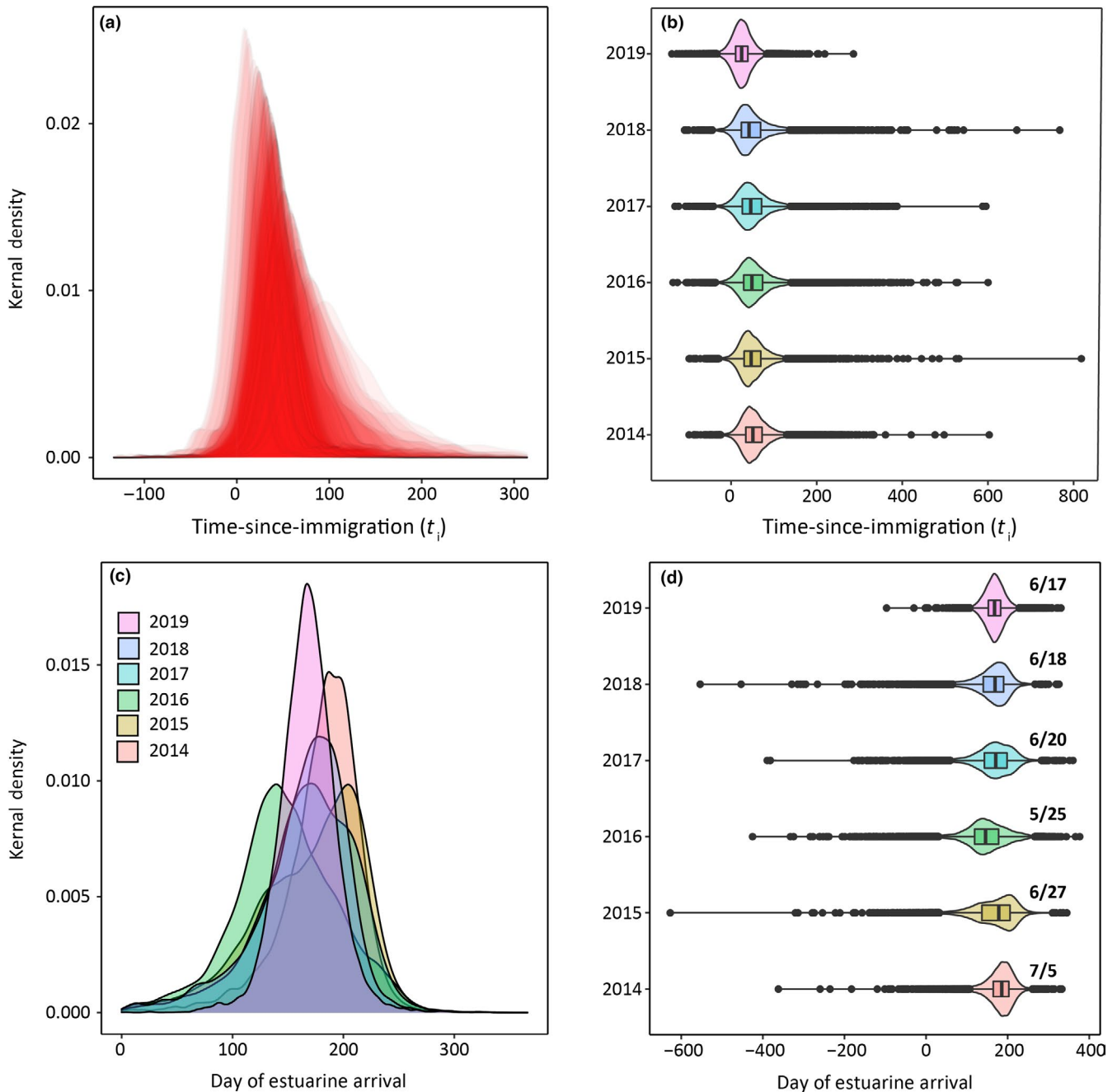


FIGURE 3 (a) Density distributions of time-since-immigration for all individual juvenile sand tiger sharks sampled in this study. (b) Box and violin plots highlighting time-since-immigration estimates across all individuals pooled by year. (c) Density distributions showing arrival days for all individuals pooled by year. (d) Box and violin plots highlighting the full distribution of modelled arrival days; dates represent the median estimates for each sampling population for each year

May to September, Appendix S1: Table S3), overlapped considerably with probable arrival days inferred from the nitrogen isotope clocks. In all cases, the range of entry times captured the median (i.e. 50%) arrival day for the sampled population within each year (Figure 4).

3.2 | Sensitivity analysis

The constant turnover rate isotopic clock model provided median time-since-immigration and estuarine arrival days for

population-level estimates that were within ≤ 2 days of the primary model where isotopic turnover was assigned a SD of 0.003 (Figure 4; Table 3; Appendix S1: Table S4). There were small differences in the range of possible time-since-immigration and arrival day estimates between the primary and constant turnover rate model as inferred through percentiles (Figure 4; Table 3; Appendix S1: Figure S2, Table S4). Across all sampling years, assuming a constant isotopic turnover rate generally reduced the upper 97.5th percentile by ≤ 18 days; there was a negligible effect on the lower 2.5th percentile (Figure 5, Table 3).

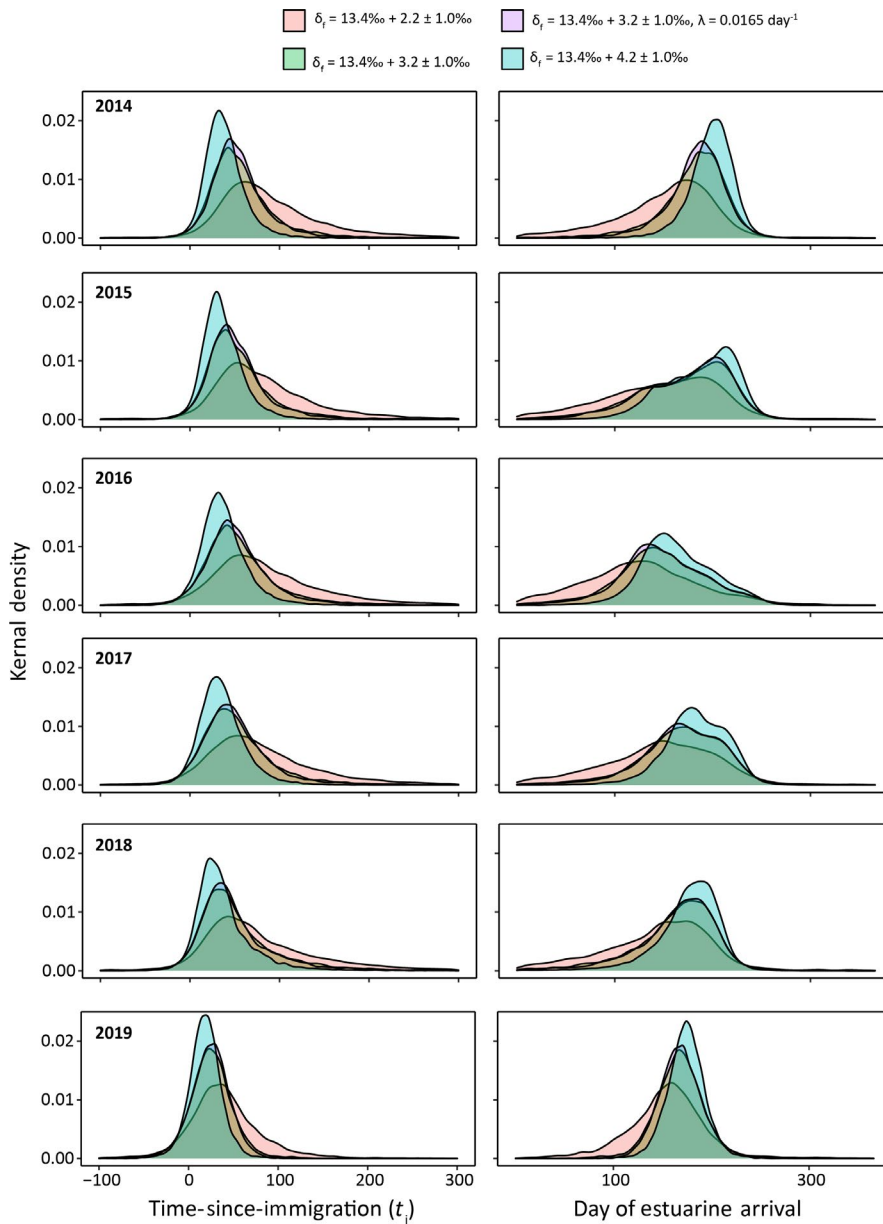


FIGURE 4 Density plots highlighting the sensitivity of time-since-immigration (t_i) and estuarine arrival day across a series of model scenarios, relative to the primary model (green): a constant turnover rate model (pink), and two DTFD sensitivity models where $\delta_f = 15.4 \pm 1.0\%$ (red) and $17.4 \pm 1.0\%$ (blue), respectively. Models were run to provide population-level estimates of time-since-immigration and estuarine arrival for each sampling year (2014–2019)

TABLE 3 Annual population level (2.5th, 25th, 50th, 75th and 97.5th) percentiles of time-since-immigration (t_i) and estuarine arrival date for juvenile sand tiger sharks into Great South Bay from years 2014 to 2019

	2014		2015 ^a		2016		2017		2018		2019	
	t_i	Arrival date	t_i	Arrival date	t_i	Arrival date	t_i	Arrival date	t_i	Arrival date	t_i	Arrival date
0.025	3	3/27/2014	-2	2/11/2015	-8	1/28/2016	-11	2/15/2017	-9	1/23/2018	-25	4/27/2019
0.25	34	6/14/2014	30	5/17/2015	30	4/28/2016	26	5/23/2017	24	5/20/2018	10	6/2/2019
0.5	51	7/5/2014	47	6/27/2015	49	5/25/2016	46	6/20/2017	42	6/18/2018	25	6/17/2019
0.75	72	7/22/2014	69	7/25/2015	74	6/26/2016	71	7/17/2017	69	7/9/2018	39	7/2/2019
0.975	153	8/23/2014	159	8/30/2015	174	8/29/2016	168	8/28/2017	188	8/13/2018	77	8/6/2019

^aOne individual removed from analysis due to erroneous estimates of t_i .

Time-since-immigration (t_i) and estuarine arrival estimates were somewhat sensitive to the use of variable DTFDs assigned to δ_p , especially when the assigned DTFD was lower than the primary model

(Table S5). Assigning a lower DTFD of 2.2% ($\delta_f = 15.6 \pm 1.0\%$) resulted in longer time-since-immigration estimates and earlier estuarine arrival estimates by ≤ 26 days relative to the primary model, which is relatively

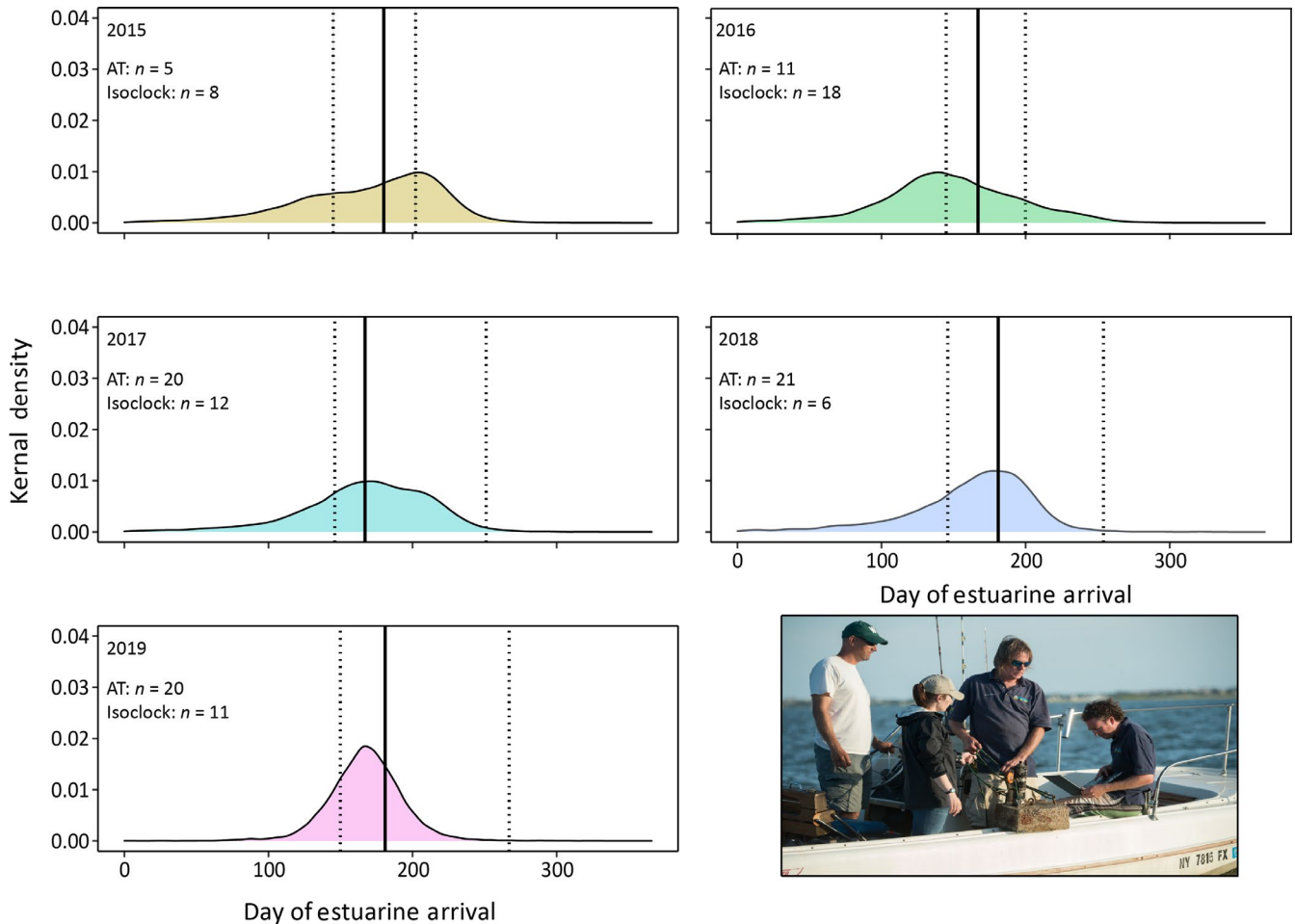


FIGURE 5 Density distributions highlighting annual entry times for juvenile sand tiger sharks modelled using isotopic clocks (density plots) and range of first detection times gathered from individuals detected on a long-term passive acoustic telemetry array. Dashed lines represent the full range of acoustic-derived entry times and solid lines represent the median estimates for each year. The bottom right panel illustrates scientists from the New York Aquarium conducting routine maintenance on their passive acoustic telemetry array. Photo: Julie Larsen Maher © WCS

small compared to the total time-since-immigration time (Figure 3; Appendix S1: Table S5). This model also increased the uncertainty surrounding the median time-since-immigration and arrival times for each year as inferred through percentiles (Table 3; Appendix S1: Figures S2 and S3; Table S5). Assignment of a higher DTDF of $4.2\% \pm 1.0$ ($\delta_f = 17.6 \pm 1.0\%$) resulted in more agreeable median time-since-immigration and estuarine arrival estimates (≤ 15 days) relative to the primary model (Figure 4; Table 3; Appendix S1: Figures S2 and S3, Table S6). Model uncertainty was also lower relative to the primary, constant turnover and lower bound DTDF models (Figure 4; Table 3; Appendix S1: Figure S2 and S3, Tables S4, S5 and S6).

4 | DISCUSSION

Nitrogen stable isotope clocks discerned the timing of ocean–estuarine habitat shifts and provided time-since-immigration estimates for a highly migratory marine species. We demonstrated that the timing of habitat shifts in juvenile sand tiger sharks, as inferred through stable

nitrogen isotopes (i.e. estuarine arrival days), was consistent with passive acoustic telemetry, suggesting that stable isotope clocks can provide complementary information to traditional tracking methods in near-shore settings. This approach has application to a broad range of organisms that seasonally traverse heterogeneous environments in terrestrial, freshwater and marine systems (e.g. crustaceans, fishes, turtles and mammals). Below we discuss the various model considerations and further validation that is required to promote the increased and broad-scale use of this approach in future migratory studies.

4.1 | Model considerations and validation

The ability to rapidly calculate the timing of habitat shifts at relatively low cost has the potential to greatly expand the study of animal migration, and offers an attractive means for studying species that may be too small to tag using traditional approaches (Moore et al., 2016). Here, we provide the first comparison between stable isotope-derived arrival days with those inferred through passive acoustic telemetry.

We observed that the most probable isotope-derived arrival days (at the population level) fell within the range of first detections estimated through acoustic telemetry. This provides strong support that isotopic clock models can accurately predict the arrival periods of juvenile sand tiger sharks into the summer nursery grounds of GSB.

A current limitation of stable isotope-based modelling is the ambiguity surrounding diet tissue discrimination factors and tissue turnover rates for commonly analysed tissues (Gannes et al., 1997; Shipley & Matich, 2020). In many cases, tissue turnover rates and DTDFs are unavailable for the study species, necessitating the use of literature-derived values from taxonomic relatives (Healy et al., 2018). Here, time-since-immigration and arrival day estimates displayed some sensitivity to assuming a constant rate of isotopic turnover as well as variable DTDFs assigned to δ_p . However, the overall ecological interpretation of sand tiger shark migration dynamics remained unchanged regardless of varying some of these parameters (and uncertainty), with arrival days remaining in broad agreement with the entry times inferred from the passive acoustic monitoring array (i.e. estuarine arrival occurring during the late spring/early summer). Combined, these findings suggest that our model design and associated parameterization are robust, providing arrival times that are relatively insensitive to significant changes to model reparameterization (i.e. DTDFs spanning 3‰ plus error). However, we believe that sensitivity analyses are a necessary component of utilizing an isotopic clock approach and should be presented in all future applications. We stress a need for a greater range of empirically derived isotopic turnover rate and DTDF estimates; this would allow the further refining of model estimates when combined with the development of multi-tissue and multi-isotope models now used to measure diet shifts (see Heady & Moore, 2013). Similarly, additional estimates of isotopic turnover rates for shark blood plasma will allow for appropriate sensitivity analyses to be applied; this is important considering that the rate of isotopic turnover may vary with factors such as diet, ambient temperature and growth rate (Vander Zanden et al., 2015), which may change as an animal moves from one habitat to another.

A final consideration of isotope clock models relates to the potential influence of individual-level diet variation on isotopic variability. It is certainly plausible that individual-level diet variation could drive variable isotopic expressions, even if all individuals are at steady state with region-specific baselines. Despite this limitation, if the isotopic distinctiveness of the origin and of new locations are significant (such as those presented here), using the isotopic clock to make population-level inferences, and then employing conservative uncertainty estimates for both baselines, can provide reliable time-since-immigration estimates. This is because the differences in isotopic baseline composition between origin and new locations, in most cases, are greater than the isotopic variation associated with individual-level foraging (Madigan et al., 2021). Therefore, assigning appropriate isotopic baselines in isotopic studies - remains a -challenge owing to the dynamic nature of baseline composition. Future studies applying isotope clocks should consider these challenges and aim to incorporate robust evaluations of animal ecologies (i.e. extent of individual-level variation in diet) and isotopic baselines of varying ecosystems (and

inherent variation in baselines), as well as the application of multiple isotopes or expansion of additional tracers into the modelling framework (see Heady & Moore, 2013).

4.2 | Application to species and ecosystems

Understanding the timing and duration of seasonal habitat use is increasingly needed as researchers seek to understand the effect of climate change on species phenology and the resulting response by ecosystems (Staudinger et al., 2013; Weiskopf et al., 2020). The current research was conducted in the Great South Bay, NY, an ecosystem that is driven by the timing of a suite of predator species that occupy the ecosystem seasonally and include sand tiger sharks, summer flounder, black seas bass *Centropristis striata*, and striped bass *Morone saxatilis*, among others (Nuttall et al., 2011; Olin et al., 2019). Here, the ability to rapidly identify the timing of juvenile shark movements into this system is fundamental to local conservation initiatives; this is because estuarine systems encompass important nursery habitat for juvenile sharks during certain times of the year. Therefore, knowledge of their arrival and time-since-immigration is imperative for providing regional management of their populations and protection of key habitat types.

To fully appreciate the changes to predator-prey relationships and ecosystem dynamics, methods are needed to quantify the timing of movement between ocean and estuarine environments. The case study presented herein provides confirmation that isotopic clocks can be applied to estimate the timing and duration of habitat shifts in a seasonal predator in a coastal ecosystem. Using confirmation through acoustic telemetry, we illustrate the efficacy of isotope clocks to measure shifts between heterogeneous habitats at finer temporal and spatial scales than previous applications of this approach. Overall, this work emphasizes that a broader application across animal taxa and ecosystems is possible.

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AUTHORS' CONTRIBUTIONS

O.N.S., A.L.N., M.G.F., G.A.H. and J.A.O. devised the project; O.N.S., G.A.H., A.L.N., J.S.L., M.D.C., M.W.H. and H.W. conducted the field sampling and contributed to the sample analysis; O.N.S. performed the statistical analysis; O.N.S. wrote the paper with significant contributions from M.G.F., J.A.O. and G.A.H. All authors provided comments and approved the final version of the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data supporting this study are deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.x3ffbg7hv> (Shipley et al., 2021).

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REFERENCES

- Barnes, C., Sweeting, C. J., Jennings, S., Barry, J. T., & Polunin, N. V. (2007). Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. *Functional Ecology*, 21(2), 356–362.
- Bond, A. L., & Diamond, A. W. (2011). Recent Bayesian stable isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, 21, 1017–1023. <https://doi.org/10.1890/09-2409.1>
- Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): The effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46, 443–453.
- Cortés, E. (1999). Standardized diet compositions and trophic levels of sharks. *ICES Journal of Marine Science*, 56, 707–717.
- Galvan, D. E., Sweeting, C. J., & Reid, W. D. K. (2010). Power of stable isotope techniques to detect size-based feeding in marine fishes. *Marine Ecology Progress Series*, 407, 271–278.
- Gannes, L. Z., O'Brien, D. M., & Del Rio, C. M. (1997). Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*, 78(4), 1271–1276.
- Gobler, C. J., Young, C. S., Goleski, J., Stevens, A., Thickman, J., Wallace, R. B., Curran, P., Koch, F., Kang, Y., Lusty, M. W., Hattenrath-Lehmann, T. K., Langlois, K., & Collier, J. L. (2019). Accidental ecosystem restoration? Assessing the estuary-wide impacts of a new ocean inlet created by Hurricane Sandy. *Estuarine, Coastal and Shelf Science*, 221, 132–146. <https://doi.org/10.1016/j.ecss.2019.02.040>
- Graham, B. S., Koch, P. L., Newsome, S. D., McMahon, K. W., & Aurioles, D. (2010). Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In J. West, G. Bowen, T. Dawson & K. Tu (Eds.), *Isoscapes* (pp. 299–318). Springer.
- Heady, W. N., & Moore, J. W. (2013). Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia*, 172, 21–34. <https://doi.org/10.1007/s00442-012-2483-9>
- Healy, K., Guillerme, T., Kelly, S. B., Inger, R., Bearhop, S., & Jackson, A. L. (2018). SIDER: An R package for predicting trophic discrimination factors of consumers based on their ecology and phylogenetic relatedness. *Ecography*, 41, 1393–1400. <https://doi.org/10.1111/ecog.03371>
- Hobson, K. A., Anderson, R. C., Soto, D. X., & Wassenaar, L. I. (2012). Isotopic evidence that dragonflies (*Pantala flavescens*) migrating through the Maldives come from the northern Indian subcontinent. *PLoS ONE*, 7, e52594. <https://doi.org/10.1371/journal.pone.0052594>
- Hussey, N. E., MacNeil, M. A., & Fisk, A. T. (2010). The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. *Hydrobiologia*, 654, 1–5. <https://doi.org/10.1007/s10750-010-0361-1>
- Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: Tissue types, methods, applications and assumptions. *Journal of Fish Biology*, 80, 1449–1484. <https://doi.org/10.1111/j.1095-8649.2012.03251.x>
- Jennings, S., Pinnegar, J. K., Polunin, N. V., & Warr, K. J. (2002). Linking size-based and trophic analyses of benthic community structure. *Marine Ecology Progress Series*, 226, 77–85.
- Kim, S. L., Casper, D. R., Galván-Magaña, F., Ochoa-Díaz, R., Hernández-Aguilar, S. B., & Koch, P. L. (2012). Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. *Environmental Biology of Fishes*, 95, 37–52. <https://doi.org/10.1007/s10641-011-9919-7>
- Kim, S. L., del Rio, C. M., Casper, D., & Koch, P. L. (2012). Isotopic incorporation rates for shark tissues from a long-term captive feeding study. *Journal of Experimental Biology*, 215, 2495–2500. <https://doi.org/10.1242/jeb.070656>
- Kim, S. L., & Koch, P. L. (2012). Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. *Environmental Biology of Fishes*, 95, 53–63.
- Klaassen, M., Piersma, T., Korthals, H., Dekinga, A., & Dietz, M. W. (2010). Single-point isotope measurements in blood cells and plasma to estimate the time since diet switches. *Functional Ecology*, 24, 796–804. <https://doi.org/10.1111/j.1365-2435.2010.01689.x>
- Kneebone, J., Chisholm, J., & Skomal, G. B. (2012). Seasonal residency, habitat use, and site fidelity of juvenile sand tiger sharks *Carcharias taurus* in a Massachusetts estuary. *Marine Ecology Progress Series*, 471, 165–181. <https://doi.org/10.3354/meps09989>
- Kneebone, J., Chisholm, J., & Skomal, G. (2014). Movement patterns of juvenile sand tigers (*Carcharias taurus*) along the east coast of the USA. *Marine Biology*, 161, 1149–1163. <https://doi.org/10.1007/s00227-014-2407-9>
- Madigan, D. J., Baumann, Z., Carlisle, A. B., Hoen, D. K., Popp, B. N., Dewar, H., Snodgrass, O. E., Block, B. A., & Fisher, N. S. (2014). Reconstructing transoceanic migration patterns of Pacific bluefin tuna using a chemical tracer toolbox. *Ecology*, 95, 1674–1683. <https://doi.org/10.1890/13-1467.1>
- Madigan, D. J., Boustany, A., & Collette, B. B. (2017). East not least for Pacific bluefin tuna. *Science*, 357, 356–357. <https://doi.org/10.1126/science.aan3710>
- Madigan, D. J., Shipley, O. N., & Hussey, N. E. (2021). Applying isotopic clocks to identify prior migration patterns and critical habitats in mobile marine predators. In C. L. Madliger, S. J. Cooke, C. E. Franklin, & O. P. Love (Eds.), *Conservation physiology: Integrating physiology into animal conservation and management* (pp. 69–85). Oxford University Press. <https://doi.org/10.1093/oso/9780198843610.003.0005>
- McMahon, K. W., Fogel, M. L., Elsdon, T. S., & Thorrold, S. R. (2010). Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *Journal of Animal Ecology*, 79(5), 1132–1141.
- Moore, J. W., Gordon, J., Carr-Harris, C., Gottesfeld, A. S., Wilson, S. M., & Russell, J. H. (2016). Assessing estuaries as stopover habitats for juvenile Pacific salmon. *Marine Ecology Progress Series*, 559, 201–215. <https://doi.org/10.3354/meps11933>
- Morgan, C., Shipley, O. N., & Gelsleichter, J. (2020). Resource-use dynamics of co-occurring chondrichthyans from the First Coast, North Florida, USA. *Journal of Fish Biology*, 96, 570–579. <https://doi.org/10.1111/jfb.14238>
- Nuttall, M. A., Jordaan, A., Cerrato, R. M., & Frisk, M. G. (2011). Identifying 120 years of decline in ecosystem structure and maturity of Great South Bay, New York using the Ecomath modelling approach. *Ecological Modelling*, 222, 3335–3345. <https://doi.org/10.1016/j.ecolmodel.2011.07.004>
- Olin, J. A., Cerrato, R. M., Nye, J. A., Sagarese, S. R., Sclafani, M., Zacharias, J. P., & Frisk, M. G. (2019). Evidence for ecosystem changes within a temperate lagoon following a hurricane-induced barrier Island Breach. *Estuaries and Coasts*, 43(7), 1625–1639. <https://doi.org/10.1007/s12237-019-00593-3>

- Olin, J. A., Hussey, N. E., Grgicak-Mannion, A., Fritts, M. W., Wintner, S. P., & Fisk, A. T. (2013). Variable $\delta^{15}\text{N}$ diet-tissue discrimination factors among sharks: Implications for trophic position, diet and food web models. *PLoS ONE*, *8*, e77567.
- Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., Semmens, B. X., & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, *92*, 823–835. <https://doi.org/10.1139/cjz-2014-0127>
- Shipley, O. N., & Match, P. (2020). Studying animal niches using bulk stable isotope ratios: An updated synthesis. *Oecologia*, *193*(1), 27–51. <https://doi.org/10.1007/s00442-020-04654-4>
- Shipley, O. N., Newton, A. H., Frisk, M. G., Henkes, G. A., LaBelle, J., Camhi, M., Hyatt, M., Walters, H., & Olin, J. A. (2021). Data from: Telemetry validated nitrogen stable isotope clocks identify ocean-to-estuarine habitat shifts in mobile organisms. *Methods in Ecology and Evolution*, <https://doi.org/10.5061/dryad.x3ffbg7hv>
- Staudinger, M. D., Carter, S. L., Cross, M. S., Dubois, N. S., Duffy, J. E., Enquist, C., Griffis, R., Hellmann, J. J., Lawler, J. J., O'Leary, J., Morrison, S. A., Sneddon, L., Stein, B. A., Thompson, L. M., & Turner, W. (2013). Biodiversity in a changing climate: A synthesis of current and projected trends in the US. *Frontiers in Ecology and the Environment*, *11*, 465–473. <https://doi.org/10.1890/120272>
- Thomas, S. M., & Crowther, T. W. (2015). Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. *Journal of Animal Ecology*, *84*(3), 861–870.
- Thorne, E. (1928). Great South Bay as a shark nursery. *New York Zoological Society Bulletin*, *21*, 114–115.
- Tinoco, A. I. (2017). *Effects of Hurricane Sandy on Great South Bay, Long Island: Assessing water quality, seagrass and associated nekton communities* (MS thesis). Stony Brook University, Stony Brook, New York, pp. 88.
- Trueman, C. N., Jackson, A. L., Chadwick, K. S., Coombs, E. J., Feyrer, L. J., Magozzi, S., Sabin, R. C., & Cooper, N. (2019). Combining simulation modeling and stable isotope analyses to reconstruct the last known movements of one of Nature's giants. *PeerJ*, *7*, e7912. <https://doi.org/10.7717/peerj.7912>
- Vander Zanden, H. B., Chaffee, C. L., González-Rodríguez, A., Flockhart, D. T., Norris, D. R., & Wayne, M. L. (2018). Alternate migration strategies of eastern monarch butterflies revealed by stable isotopes. *Animal Migration*, *5*, 74–83. <https://doi.org/10.1515/ami-2018-0006>
- Vander Zanden, M. J., Clayton, M. K., Moody, E. K., Solomon, C. T., & Weidel, B. C. (2015). Stable isotope turnover and half-life in animal tissues: A literature synthesis. *PLoS ONE*, *10*, e0116182. <https://doi.org/10.1371/journal.pone.0116182>
- Weiskopf, S. R., Rubenstein, M. A., Crozier, L. G. et al. (2020). Climate change effects on biodiversity, ecosystems, ecosystem services, and natural resource management in the United States. *Science of The Total Environment*. <https://doi.org/10.1016/j.scitotenv.2020.137782>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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