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RESEARCH ARTICLE

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Inferring odontocete life history traits in dentine using a multiproxy approach ($\delta^{15}N$, $\delta^{44/42}Ca$ and trace elements)

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Rationale: Understanding the interactions between marine mammals and their environment is critical for ecological and conservation purposes. Odontocetes offer a continuous record of their life history from birth as recorded in annual increments of their tooth dentine. Because dentine is not remodeled and contains collagen, nitrogen stable isotope compositions ($\delta^{15}N$) reflect nursing and weaning events, life history traits that would otherwise be impossible to retrieve in such elusive marine animals. Yet, capturing the magnitude and temporal changes in these events is constrained by tooth size and sampling resolution. Moreover, historical and fossil specimens undergo collagen decay, hence the need to develop the measurements of other proxies.

Methods: Here, we present a multiproxy approach to investigate the use of Ca isotope compositions ($\delta^{44/42}Ca$) in relation to $\delta^{15}N$ and laser ablation profiles for different trace metal (Ba, Mg, Sr, Zn) concentrations across the dentine of a single individual of the common bottlenose dolphin *Tursiops truncatus*.

Results: To help interpret the dentine data, we provide milk elemental compositions and $\delta^{44/42}Ca$ values for two odontocete individuals. We discuss the observed changes in $\delta^{44/42}Ca$ across the dentine as potential markers of birth, weaning interval, incidental ingestion of seawater, trophic level and physiology. Incidental ingestion of seawater during nursing induces a positive offset in $\delta^{44/42}Ca$ values recorded in the early formed dentine.

Conclusions: Life history parameters of individual marine mammals are extremely difficult to retrieve due to limitations in observing specimens in the wild and the methodology presented here offers new ecological and paleoecological perspectives.

1 | INTRODUCTION

Marine mammals are present in all oceanic and coastal and even in some freshwater environments. They occupy a highly diverse range of ecological niches (from herbivorous to top predators), which makes them key actors in the trophic organization of aquatic ecosystems and important sentinels of environmental changes.[1] The advent of modern techniques such as satellite and drone imagery, biologging, telemetry, real-time acoustic monitoring and biomodeling has considerably improved our understanding of marine mammal life history traits over the last few decades.[2] Among these new techniques, the development of new methods of geochemistry has provided new complementary ways to understand and monitor how marine mammals live and lived (e.g. Newsome et al[3]).

While controlled experiments are key to understanding element routing in the body and isotopic fractionation processes,[1–8] a great deal of the information is hampered by the nature, geometry and window of time captured in the tissues of interest.[9–11] Because the community that introduced the use of stable isotope analyses was in large part driven by archeological questions,[12] it is no surprise that tooth enamel was for a long time, and still is, elected as the tissue of choice due to its compactness and durability, preserving information from past contexts.[13–15] Nonetheless, a number of limitations have stimulated discussions such as how the recording of elements and their isotopes during enamel secretion and maturation processes is representative of isotopic compositions[16–18] or trace element contents.[19–24] Another important limitation is that in most mammals, teeth become replaced once (diphyodonty) and the entire enamel crown does not capture a continuous record from birth to death but is limited to a time range encompassing several months to a couple of years.[9] Other tissue types (dentine, cementum) may extend this temporal range, but contrary to enamel, they incorporate diagenetic elements that may blur or erase the original biological signal. On the other hand, enamel contains too little nitrogen (<0.1 wt%[25]) for the purpose of spatially resolved calibration at the submillimeter scale with other geochemical proxies. This is despite recent advances at measuring $\delta^{15}N$ in enamel.[8] Conversely, dentine is not remodeled[26] and comprises about 30 wt% of collagen protein,[15] allowing for spatially resolved measurements of its nitrogen (N) isotopic composition and a direct comparison with other geochemical proxies.

In odontocetes, enamel is deposited prenatally, but dentine (if unworn) covers the prenatal and entire postnatal duration in the form of annual increments known as growth layer groups (GLGs).[27] A single tooth generation (monophyodonty) is maintained during lifetime, and for this reason, odontocetes offer an extensive temporal perspective for life history reconstruction. The initial observation in humans that nursing infants had tissues more enriched in ^{15}N than their mother's[28] opened a vast field of research for the investigation of nursing patterns. In marine mammals, previous studies have investigated the isotopic composition of nitrogen embedded in annually formed dentine GLGs, and the inference of life history traits such as weaning age, feeding preferences or sex-specific ontogenetic differences is now well established.[29–31] Yet, exploration of other geochemical proxies may provide increased spatial resolution and different perspectives to infer life history traits. Moreover, collagen may be altered rapidly during decay and is obliterated in old fossils, so modern organisms represent an opportunity to understand how N isotopes in incremented tissues covary with other proxies.

Here, we investigate the covariation of N isotopes as a reference for life history traits as defined in previous studies[29–31] against a record of trace elements and Ca isotope compositions along a longitudinal transect in the unworn dentine of a female individual of *Tursiops truncatus*. Calcium (Ca) is essential to metabolic function, and its high concentration in bioapatite requires minimal sampling for stable isotope analyses. Despite challenging analytical procedures using multicollector inductively coupled plasma mass spectrometry (MC-ICPMS),[32–34] Ca isotopes are becoming available to study dietary preferences among modern and fossil mammals, although physiological processes behind fractionation need to be constrained.[7,18,35–40] Calcium-normalized strontium (Sr), barium (Ba), zinc (Zn) and magnesium (Mg) ratios are increasingly measured using laser ablation, yet their significance for monitoring metabolism and diet are under discussion.[19–24] Not only do these geochemical proxies offer a fine spatial resolution in incremented tissues, but they would also benefit from a confrontation with N isotope data. We present the first longitudinal study involving a multiproxy approach in the dentine of an odontocete, with $\delta^{15}N$ values serving as an anchor to interpret trace metal concentrations and Ca isotope variations in terms of life history trait records.

2 | MATERIALS AND METHODS

2.1 | Sample

A single tooth was sampled on a stranded bottlenose dolphin (*T. truncatus*) as part of a surveillance work conducted by the research station PELAGIS. Individual M1127 is a female, stranded at Sainte-Marie-de-Ré, France, on 24 March 1979.

2.2 | Tooth sectioning and imaging

The tooth was embedded in epoxy resin and then sectioned longitudinally using a low-speed diamond blade (Buehler Isomet). Each tooth half was hand-polished with silicon carbide grinding papers at 1000 and then 2000 grits. Revealing GLGs was not optimal because we refrained from preparing histological sections following standard decalcification procedures. This decision was reached to preserve the physical and chemical integrity of both halves of the specimen, allowing data replications or additional geochemical samplings, if deemed necessary in the future. Each polished tooth section was digitally photographed under low incidence light to reveal GLGs. Images were treated with the Photoshop filter "solarize" to enhance contrasts of tooth layers. GLGs were not always visible, but for the purpose of this study, we clearly identified the enamel–dentine junction and the dentinal neonatal line, and for postnatal dentine, we were able to propose an approximate GLG count (Figure 1). One tooth half was dedicated to drilling for N isotopes; the other half was first dedicated to laser ablation, followed by micro-drilling for Ca isotopes. All analyses were conducted on the center of the tooth proceeding from the apex to the pulp cavity. Photographs of the sample spots and laser path were digitally assembled to match the timing of mineralization (Figure 1). Recorded peaks and drops in geochemical proxies are discussed in reference to histologically defined zones in the dentine representing landmark moments in the life history of *T. truncatus*, namely the prenatal–birth, nursing, weaning and adulthood periods (Figure 1).

2.3 | Laser ablation ICPMS

The dentine was spatially analyzed for elemental concentration in October 2018 using an Excite 193 nm ArF excimer (Teledyne Photon Machines) laser ablation system under an ultrapure He atmosphere. The laser ablation system was hyphenated to a quadrupole (Thermo Fisher ICAP-Q) ICPMS system. The laser ablation path covered a line transect of less than 3 cm long, and masses of ^{138}Ba, ^{44}Ca, ^{24}Mg, ^{88}Sr and ^{64}Zn were monitored. The laser output was set to 100%, the fluence to 5.06 J/cm^2, the repetition rate to 10 Hz, the spot diameter to 40 µm, the speed to 40 µm/s and the intensity measured on mass ^{44}Ca was approx. 2×10^6 cps. Five reference materials were monitored during the laser ablation session (NIST SRM 1400, MAPS5, MAPS4, NIST610 and NIST612). Laser ablation transects on those standards were analyzed for 60 s under the same acquisition parameters as the tooth sample. A blank measurement was performed for 15 s before and after each sample transect measurement. Results were blank-subtracted, isotopic data were converted into elemental data using natural abundances and data were normalized to calcium. Average ratios for SRM 1400 are as follows: $Sr/Ca = 1.3 \times 10^{-3}$, $Ba/Ca = 2.6 \times 10^{-3}$, $Mg/Ca = 8.2 \times 10^{-3}$, $Zn/Ca = 3.3 \times 10^{-4}$, and agree with those obtained for SRM 1400 from previous studies.[22]

2.4 | Ca isotopes ($\delta^{44/42}$Ca)

As detailed in the literature,[38,41] sampling was performed using a tungsten carbide drill mounted on a precise position drilling MicroMill device. Holes were performed along an apico-basal trajectory on the surface of the sectioned tooth half with each sampling hole representing a single Ca isotope datapoint representing enamel and prenatal and postnatal dentine (Figure 1). This sampling strategy resulted in holes with a maximum diameter of 400 µm and a maximum depth of 300 µm. Following each drilling step, sampling powder accumulated on the rims of the holes and was directly transferred into clean 7 mL Savillex vials for further Ca purification steps in the clean laboratory. All samples were digested in Suprapure concentrated HNO_3, evaporated and then dissolved in 300 µL of Suprapure 1 M HCl, which was purified through a cation exchange resin (AG50X-W12) to separate the matrix from Ca and Sr fractions as described in previous works.[38,41] Calcium fractions were separated from Sr fractions using Sr-specific resin (Eichrom Sr-Spec) in 3 M HNO_3 medium. Blanks for the whole procedure did not exceed 100 ng of Ca.

A MC-ICPMS system (Neptune Plus, Thermo Fisher Scientific) was used for measuring Ca isotopic abundance ratios (^{44}Ca/^{42}Ca, ^{43}Ca/^{42}Ca) following methods in Tacail et al.[32] The purified samples were dried out and retaken in ultrapure 0.05 M HNO_3, and Ca

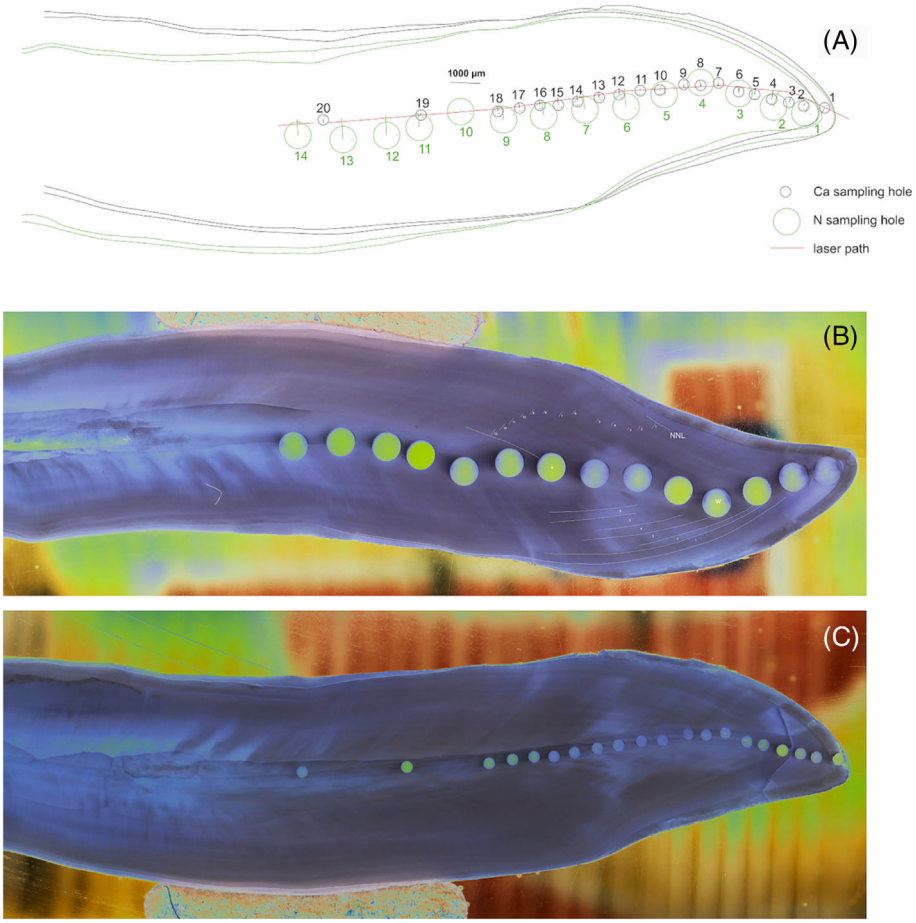

FIGURE 1 Sampling locations and pictures of the sectioned tooth of *Tursiops truncatus*. (A) Superimposed line drawings of the halved tooth sections showing the exact locations of the drilling spots for calcium isotopes (small diameter), nitrogen isotopes (large diameter) and laser ablation profile. (B) Photography of the section used for nitrogen isotope sampling. (C) Photography of the section used for calcium isotope sampling. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Compiled Ca and N isotope values measured on the dentine of *Tursiops truncatus* (M1127).

Date of death	Stranding area	Sex	Genus_species	Pelagis no.	Sample ID	Tissue type	$\delta^{44/42}$Ca (‰)	2SD	$\delta^{43/42}$Ca (‰)	2SD	*n*
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M1	Enamel	−1.21	0.05	−0.61	0.12	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M2	Dentine	−1.14	0.03	−0.56	0.07	4
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M3	Dentine	−0.98	0.04	−0.50	0.06	4
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M4	Dentine	−0.97	0.09	−0.48	0.05	4
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M5	Dentine	−0.94	0.06	−0.46	0.05	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M6	Dentine	−1.07	0.06	−0.55	0.04	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M7	Dentine	−1.19	0.08	−0.63	0.04	4
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M8	Dentine	−1.15	0.02	−0.58	0.08	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M9	Dentine	−1.18	0.07	−0.61	0.09	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M10	Dentine	−1.20	0.07	−0.59	0.08	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M11	Dentine	−1.23	0.05	−0.60	0.10	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M12	Dentine	−1.33	0.05	−0.68	0.05	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M13	Dentine	−1.31	0.07	−0.65	0.07	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M14	Dentine	−1.44	0.03	−0.76	0.04	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M15	Dentine	−1.27	0.08	−0.65	0.02	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M16	Dentine	−1.29	0.09	−0.66	0.10	5
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M17	Dentine	−1.19	0.01	−0.60	0.02	3
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M18	Dentine	−1.18	0.07	−0.62	0.09	4
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M19	Dentine	−0.97	0.08	−0.47	0.08	4
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	M1127-M20	Dentine	−1.01	0.06	−0.50	0.03	3
			STANDARD	-	SRM1486 Cétacé JM	Bone	−1.01	0.06	−0.51	0.08	8
Date of death	Provenance	Sex	Genus_species	Pelagis no.	Sample ID	Tissue type	δ^{15}N (‰)				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-1.raw	Dentine	15.1				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-2.raw	Dentine	14.9				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-3.raw	Dentine	13.8				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-4.raw	Dentine	12.6				

TABLE 1 (Continued)

Date of death	Stranding area	Sex	Genus_species	Pelagis no.	Sample ID	Tissue type	$\delta^{44/42}Ca$ (‰)	2SD	$\delta^{43/42}Ca$ (‰)	2SD	*n*
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-5.raw	Dentine	12.6				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-6.raw	Dentine	12.9				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-7.raw	Dentine	12.9				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-8.raw	Dentine	13.0				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-9.raw	Dentine	13.5				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-10.raw	Dentine	13.8				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-11.raw	Dentine	13.4				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-12.raw	Dentine	13.8				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-13.raw	Dentine	14.0				
23-Mar-75	Sainte-Marie-de-Ré	Female	*Tursiops_truncatus*	M1127	2203_JM-m-14.raw	Dentine	14.1				

concentration was adjusted at 1.5 ppm for all samples and standards. The Ca isotope compositions are expressed using the "delta" notation defined as follows for the $^{44}Ca/^{42}Ca$ ratio:

$$\delta^{44/42}Ca\,(‰) = \left(\left(^{44}Ca/^{42}Ca_{sample}\right)/\left(^{44}Ca/^{42}Ca_{ICP-Ca-Lyon}\right) - 1\right) \times 1000$$

where $(^{44}Ca/^{42}Ca)_{sample}$ and $(^{44}Ca/^{42}Ca)_{ICP\text{-}Ca\text{-}Lyon}$ are the Ca isotope abundance ratios measured in sample and ICP Ca Lyon reference standard, respectively. A single concentrated solution of NIST SRM 1486 was repeatedly purified and measured in the same batches as the samples to control for instrumental accuracy. The calcium isotope values of the tooth samples are available in Table 1.

2.5 | N isotopes ($\delta^{15}N$)

Sampling for N isotopes was performed using a diamond drill bit mounted on a dremel drilling station. Holes were also performed along an apico-basal trajectory on the surface of the sectioned tooth half with each sampling hole representing a single N isotope datapoint corresponding to prenatal and postnatal dentine (Figure 1). Holes form a maximum diameter of 900 µm and a maximum depth of about 500 µm. Sampling powder was weighed immediately following sampling and directly collected in tin capsules. The average sampling weight was about 0.6 mg. The isotopic composition of nitrogen was measured on the bulk sample using an elemental analyzer (vario PYRO Cube, Elementar) coupled in continuous flow to an isotope ratio mass spectrometer (IsoPrime 100, Elementar). Isotope ratios are expressed in the delta notation according to

$$\delta^{15}N\,(‰) = \left(\left(^{15}N/^{14}N_{sample}\right)/\left(^{15}N/^{14}N_{AIR}\right) - 1\right) \times 1000$$

IAEA-N1 and IAEA-N2 reference materials were analyzed with the samples, and the standard deviations of the replicate analyses were lower than 0.20‰. The nitrogen isotope values of the tooth samples are available in Table 1.

2.6 | Milk elemental concentrations and Ca isotopes

In order to help interpret the dentinal record, milk samples were obtained from two stranded lactating odontocetes: *T. truncatus*, found stranded in March 2017 at St-Vaast-la-Hougue, France, and *Delphinus delphis*, found stranded in February 2016 at Bretteville-sur-Ay, France. Each milk sample was kept frozen until sample preparation and analysis, which began in March 2021. Between 200 µL and 1 mL of milk was placed in 30 mL Teflon beakers and dissolved in 4 mL of ultrapure concentrated HNO_3 and 0.5 mL of H_2O_2 for 48 h and evaporated to dryness thereafter. All samples were then taken up in 5 mL of concentrated HNO_3 and 0.5 mL of H_2O_2 with the latter reagent added slowly to limit the exogenous reaction. After monitoring the reaction, the Teflon beakers were closed and put on a hot plate at 90°C for 6 days and were evaporated. The samples were then redissolved in 6 mL of 15 N HNO_3 and transferred into

TABLE 2 Ca isotopes and trace element concentrations for odontocete milk.

Date of death	Stranding area	Genus_species	Pelagis no.	Sample ID	Tissue type	$\delta^{44/42}$Ca (‰)	2SD	$\delta^{43/42}$Ca (‰)	2SD	n	Ca (ppm) AES
17 March 2017	St-Vaast-la-Hougue	*Tursiops truncatus*	**11712457**	**11712457**	Milk	−1.32	0.05	−0.74	0.06	4	111
8 February 2016	Bretteville-sur-Ay	*Delphinus delphis*	**11612226**	**11612226**	Milk	−1.10	0.04	−0.61	0.00	3	101

Abbreviation: LQ, below limit of quantification.

TABLE 2 (Continued)

Date of death	Ca (ppm) quad	Sr (ppm) AES	Sr (ppm) quad	Mg (ppm) AES	Mg (ppm) quad	Zn (ppm) quad	K (ppm) quad	Na (ppm) quad	Ba (ppm) quad
17 March 2017	107.79	LQ	0.18	70	72.34	21.45	1,809.98	3,368.71	0.065
8 February 2016	95.37	LQ	0.14	46	46.07	3.54	927.07	530.80	0.005

Abbreviation: LQ, below limit of quantification.

autoclave Teflon vials specially set up for a microwave digestion system (ultraWAVE, Milestone). The batch of samples was progressively brought to a temperature of 200°C and a pressure of 120 Bar for about 35 min. Samples were then transferred into Teflon beakers and evaporated to dryness on the next day.

All samples were taken up in 1 mL of 0.05 N HNO_3, and one aliquot of 50 µL was taken for Ca, Sr, Ba and Mg concentration analyses on an ICAP (Thermo Fisher) atomic emission spectrometer. Blanks were regularly monitored, and standard NIST SRM 1486 bone meal yielded a value of 247 ppm of Sr, 30% of Ca and 5192 ppm of Mg in agreement with the certified value. The remaining samples were evaporated and retaken in a small volume of 0.4 N HCl before undergoing the Ca purification procedure. The Ca isotope purification and measurements follow the above-described protocol. The concentration and isotope values of the milk samples are available in Table 2.

2.7 | Statistical tests and mixing model

All graphics and statistical tests were made using R version 4.2.1.[42] To enable statistical comparison of $\delta^{44/42}$Ca or δ^{15}N spot analyses with X/Ca laser scan profiles, we resampled X/Ca laser profiles to match the spatial resolution of $\delta^{44/42}$Ca and δ^{15}N datasets. We extracted the X/Ca average and 2 SD values of ratios over the window centered around each $\delta^{44/42}$Ca or δ^{15}N spot, considering a total width corresponding to the sampling point diameter (400 and 900 µm, respectively). Results are shown in Figures 2–4. These resampled X/Ca datasets are compared with $\delta^{44/42}$Ca and δ^{15}N values, respectively, by conducting nonparametric Spearman and parametric Pearson correlation tests. We used the correlation function of the PerformanceAnalytics package.

To estimate the proportion of seawater as a potential dietary source during weaning that is needed to explain the Ca isotope values of early formed dentine, we built a mixing model with seawater and milk as end members. End-member values for seawater and milk are compiled in Table 3.

3 | RESULTS

All data reported from the apex to the cervix account for temporal variations from fetal onto adult stages. N and Ca isotope profiles are compared in Figure 2, and each isotopic system is then compared to laser ablation profiles (Figures 3 and 4). The data used for building the isotopic and elemental profiles are available in the supporting information (Data S1).

3.1 | N isotopes (Figure 3)

The first sampling spot represents prenatal dentine and is the most ^{15}N-enriched of the dataset with a value above 15‰. The second

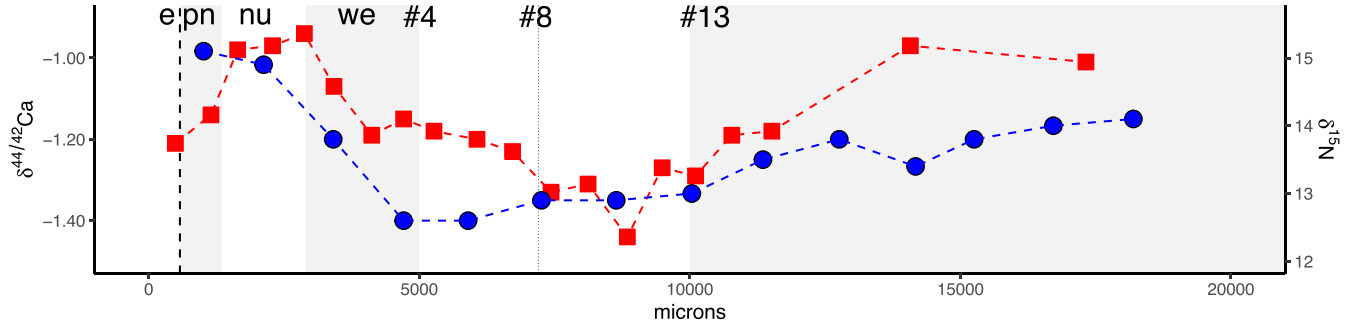

FIGURE 2 Comparison of the incremental profiles of nitrogen isotopes (blue symbols) with calcium isotopes (red symbols) in the dentine of *Tursiops truncatus*. The *x*-axis represents the distance in micrometers from the tooth apex. Gray/white areas represent tissue types and life history events including: e, enamel; pn, prenatal dentine; nu, nursing stage; we, weaning stage. GLGs 4, 8 and 13 are indicated and reflect events during the adult diversified diet as discussed in the main text. [Color figure can be viewed at wileyonlinelibrary.com]

sampling spot has a value just below 15‰ and corresponds to the earliest postnatal dentine (introduction of milk in the diet for a duration of about 1 year). The specimen reaches the lowest value of 12.5‰ from the fourth sampled spot, which corresponds to an estimated age of about 4 years according to GLG count. M1127 would have been fully weaned by the fourth year. The dentinal area from the 8th to the 13th GLG reveals a slight (+0.5‰) increase in $\delta^{15}N$ values, followed at the 13th GLG by a significant increase in $\delta^{15}N$ values reaching a plateau at about 13.8‰.

3.2 | Ca isotopes (Figure 4)

The enamel $\delta^{44/42}Ca$ value of *T. truncatus* is −1.21‰. Dentine $\delta^{44/42}Ca$ values are highly variable with the lowest value at −1.44‰ and the highest at −0.94‰ and an average value of −1.15‰. The pattern of $\delta^{44/42}Ca$ variations shows a rise in values across the neonatal line, then a plateau in the earliest postnatal dentine until the 2nd GLG, followed by an abrupt drop of about 0.25‰ lasting until the 4th GLG, then a short plateau until the 8th GLG, followed by an additional but more gradual drop of the same amplitude until the 13th GLG and a gradual increase for the rest of the postnatal dentine reaching values similar to the earliest postnatal layers.

3.3 | Strontium/calcium

The enamel of *T. truncatus* is nearly devoid of Sr (background level about 0.0010) in comparison to prenatal dentine and the first GLGs. The prenatal dentine shows a rapid increase in Sr/Ca ratios to 0.0050. Immediately at birth (as identified by the neonatal line), Sr/Ca ratios augment and eventually peak at 0.0075. From this peak to the 4th GLG, the Sr/Ca ratios drop to attain the background level for the entire length of the postnatal dentine deposition.

3.4 | Barium/calcium

The enamel of *T. truncatus* is characterized by a bimodal distribution pattern of Ba including low concentration near the outer enamel portion and a high concentration of Ba in the inner portion (peak > 0.00012). The prenatal dentine shows low Ba/Ca ratios (<0.00005). Birth is not characterized by any change in Ba/Ca ratios, and the low background ratios are retained for all the milk-provisioning period. The start of the weaning period, at the start of the second year (according to GLG), witnesses a rise in Ba/Ca ratios, reaching rapidly a level of 0.00007 until weaning is complete. Two transient drops in Ba/Ca ratios are recorded during the weaning period. The rest of the postnatal dentine is stable (0.00007) until the point identified as possible first lactating event (a production of milk by the studied dolphin) where ratios become highly variable.

3.5 | Zinc/calcium

The Zn enamel profile of *T. truncatus* is U-shaped with the enamel outer surface showing high Zn/Ca ratios (>0.002), which immediately drop to a nearly nil value and rebound to high ratios (0.001) just before the enamel–dentine junction. The prenatal dentine is characterized by very low Zn concentrations. As is the case with Ba/Ca ratios, the neonatal line is not characterized by any change in Zn/Ca ratios. A peak in Zn/Ca ratios (0.002) is observed within the milk-provisioning period and seems concomitant with the initiation of the Sr peak, but the Zn peak drops slightly earlier than the Sr peak, that is, just before the start of the weaning period. Here, the Zn/Ca ratios drop below 0.001 during the first GLG and maintain this value until weaning is complete. Thereafter, Zn/Ca ratios increase slightly and remain constant until the point identified as a possible first lactating event, which witnesses a drop in Zn/Ca ratios as well as subsequent waxes/wanes for the rest of the postnatal dentine deposition.

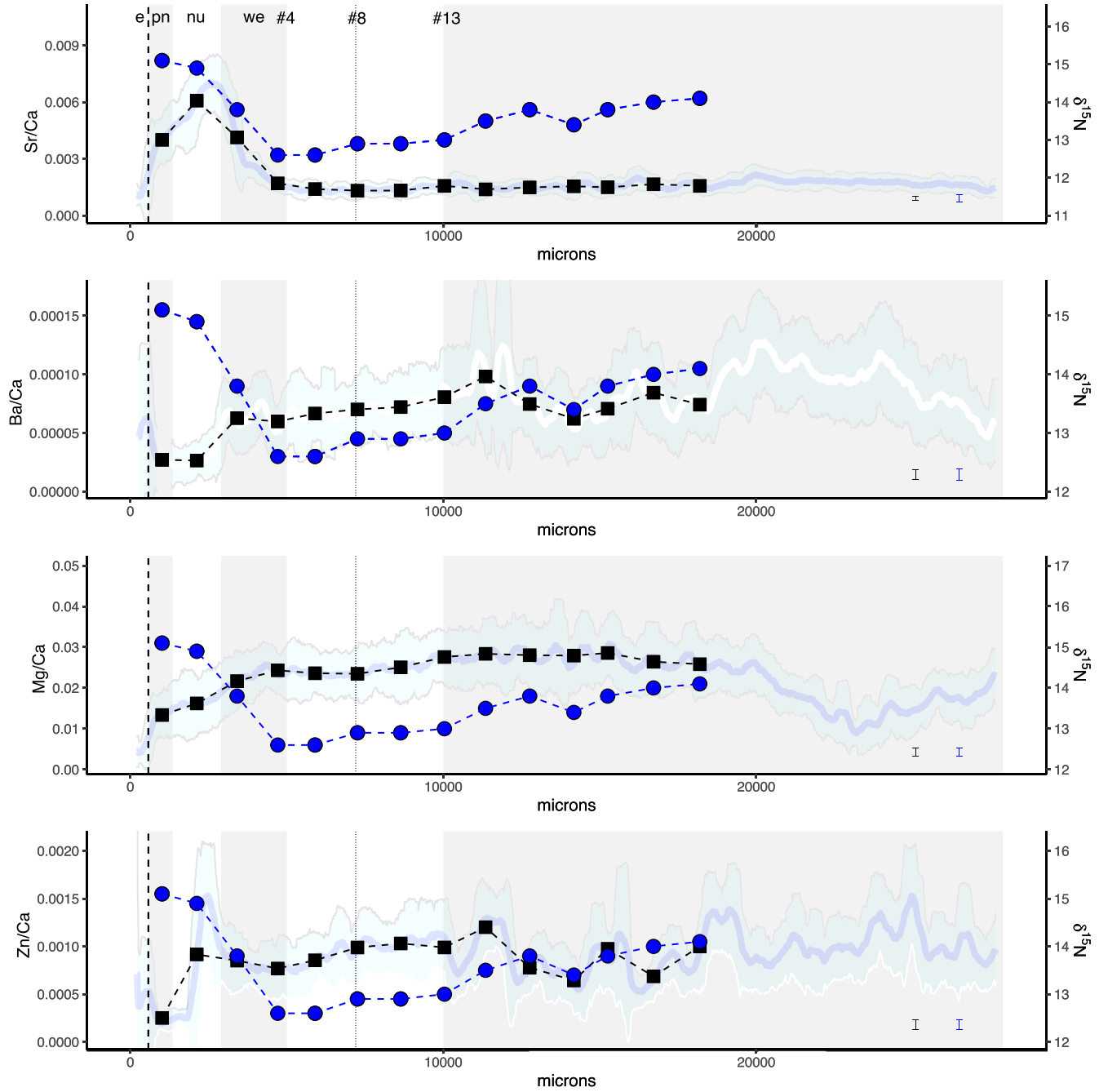

FIGURE 3 Incremental profiles of nitrogen isotopes (blue circles) against trace elements normalized to calcium (black squares) with (A) strontium, (B) barium, (C) magnesium and (D) zinc. The *x*-axis represents the distance in micrometers from the tooth apex. Gray/white areas represent tissue types and life history events including: e, enamel; pn, prenatal dentine; nu, nursing stage; we, weaning stage. GLGs 4, 8 and 13 are indicated and reflect events during the adult diversified diet as discussed in the main text. The light blue line corresponds to the centered moving average for a 420-µm-wide window (31 laser ablation integrations). The light blue shaded area displays the moving 2 SD envelope around the mean, calculated for the same window. Black dots represent the average value of the laser ablation profile with an average 95% confidence interval around the average value of the considered element normalized to calcium; the corresponding nitrogen isotope value has an associated 2 SD error represented by the blue error bar. [Color figure can be viewed at wileyonlinelibrary.com]

3.6 | Magnesium/calcium

In *T. truncatus*, Mg in enamel is poorly concentrated. The enamel–dentine junction is marked by an obvious increase in Mg, attaining a Mg/Ca ratio of about 0.015 and remaining stable during prenatal dentine deposition and the milk-provisioning period. No event is observed at the neonatal line. The weaning period is characterized by a progressive increase from 0.02 to 0.025. When weaning age is

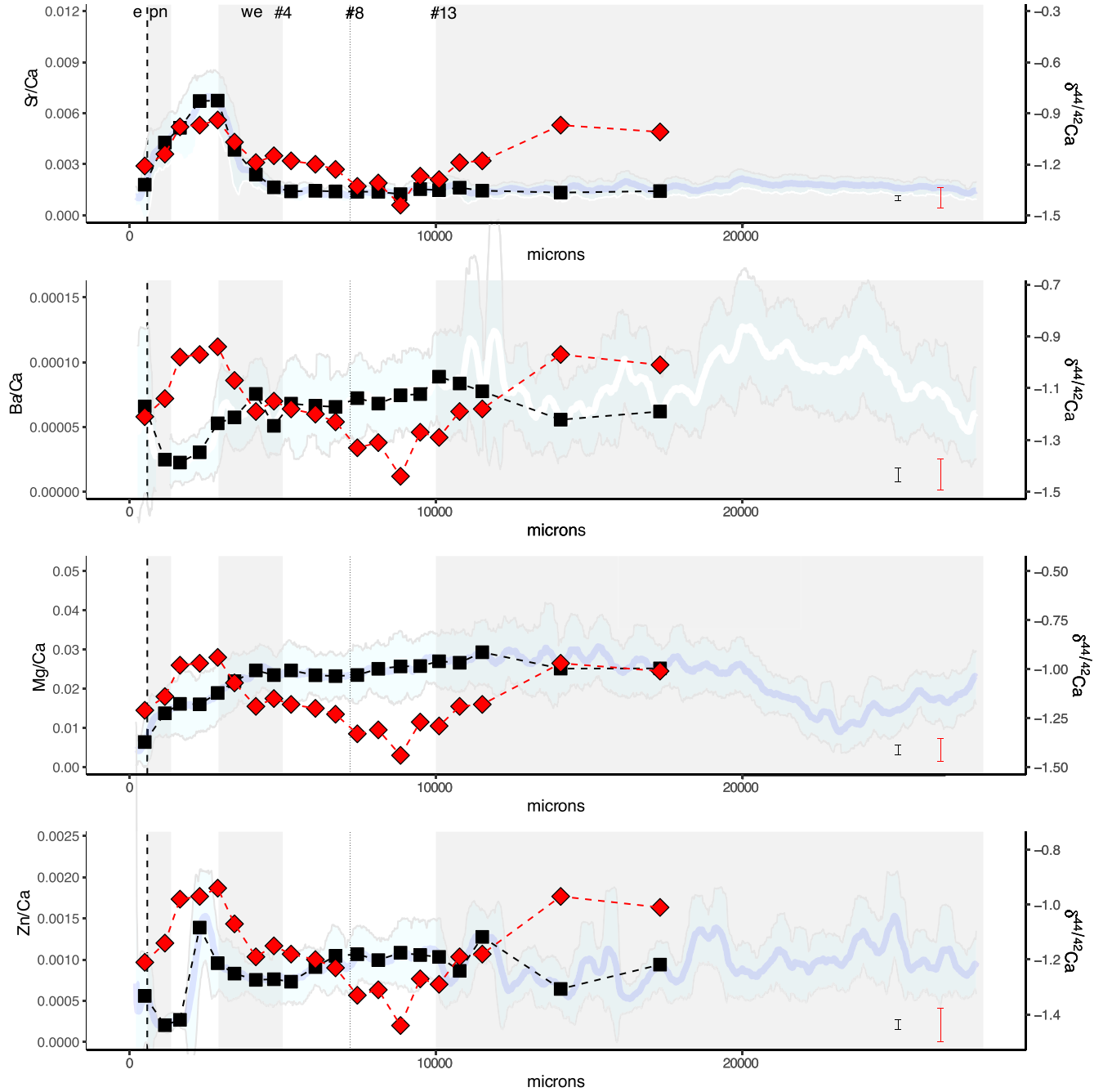

FIGURE 4 Incremental profiles of calcium isotopes (red circles) against trace elements normalized to calcium (black squares) with (A) strontium, (B) barium, (C) magnesium and (D) zinc. The *x*-axis represents the distance in micrometers from the tooth apex. Gray/white areas represent life history events including: e, enamel; pn, prenatal dentine; nu, nursing stage; we, weaning stage. GLGs 4, 8 and 13 are indicated and reflect events during the adult diversified diet as discussed in the main text. The light blue line corresponds to the centered moving average for a 420-µm-wide window (31 laser ablation integrations). The light blue shaded area displays the moving 2 SD envelope around the mean, calculated for the same window. Black dots represent the average value of the laser ablation profile with an average 95% confidence interval around the average value of the considered element normalized to calcium; the corresponding calcium isotope value has an associated 2 SD error represented by the red error bar. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Calcium isotope values and trace metal concentrations (in ppm) of milk and seawater end members used in the mixing model.

End member	$\delta^{44/42}$Ca (‰)	Ca	Ba	Cu	K	Mg	Sr	Zn
Seawater	0.41	418.82	0	0	397.28	1312.71	7.9	0
Milk	−1.21	106.24	0.03	NaN	1368.52	57.63	0.16	12.5

reached, Mg/Ca ratios witness a minor drop (concomitant with a small Ba drop) and eventually remain stable at about 0.025 until the first lactating event where Mg/Ca ratios become dispersed and a long bell pattern is observed for the entire rest of postnatal dentine deposition. The final length of deposition is followed by a drop (tail of the bell) and a slight protracted rebound in Mg/Ca ratios.

3.7 | Statistical comparisons

The calcium isotope profile in the dentine (Figure S1) is positively correlated with Sr/Ca ratios (Spearman $p^{**} = 0.6$, Pearson $p^{**} = 0.66$) and is anti-correlated with both Ba/Ca ratios (Spearman $p^{***} = -0.71$, Pearson $p^{**} = -0.65$) and Mg/Ca ratios (Spearman $p^{*} = -0.48$, Pearson $p^{*} = -0.52$). Other significant correlations involve Ba/Ca versus Mg/Ca ratios (Spearman $p^{***} = 0.84$, Pearson $p^{***} = 0.91$). The nitrogen isotope profile is not significantly correlated to any trace element profile (Figure S2).

3.8 | Milk data

Sampling milk on stranded cetaceans is challenging. Odontocete milk samples are almost devoid of strontium as is the case of human milk.[43] Milk concentrations for K, Na, Mg, Ca, Zn, Sr and Ba are available in Table 2. The two odontocete milks display ^{44}Ca-depleted values of −1.10‰ and −1.32‰, respectively, and close to the values reported for cow milk.[38,44]

4 | DISCUSSION

Early works have suggested that Ca isotopes are a proxy for trophic level inference in terrestrial and marine foodwebs,[45,46] yet investigations on marine mammals have revealed anomalous results.[35] Since these early studies, it now becomes clearer that, as concerns mammals, the nursing effect is driving the Ca isotopic composition of early formed tissues,[38,44] while gestation can modify the isotopic offset between diet and tissues, likely because of changes in the mother's diet and its assimilation.[7] Marine mammals do include a number of outliers in their Ca isotopic composition,[33,35,40] but the fact that those marine vertebrates are not supposed to ingest seawater might partly explain their unique status in the trophic web in comparison to elasmobranchs and fishes.[33,47] Messa et al[40] also discussed potential shifts in trophic sources to explain the observed variability for some odontocetes. In addition, other terrestrial mammals such as cervids or hippopotamids also show anomalous ^{44}Ca-depleted values relative to their expected trophic position,[36,37] but taking into consideration multiple Ca sources, such discrepancies can also be partly explained.[18] Here, we aim to further clarify mechanisms behind Ca isotope variability in odontocetes by using, for the first time, a spatially resolved multiproxy approach on a single individual.

4.1 | Spatially resolved records of the prenatal, nursing and weaning periods

Achieving sampling resolution is a limiting factor and with the exception of the work of Evacitas et al[31] no other δ^{15}N data are available in the literature for prenatal dentine in odontocetes. Here, N isotopes are elevated prenatally, an observation in line with the fact that fetal δ^{15}N values are elevated due to physiological reasons.[48] Also, δ^{15}N values stay elevated during the first year after birth, indicating that the young is nursed on mother milk during its first year, that is, feeding on a trophic level higher than its mother.[28] Such elevated values are consistent with δ^{15}N values reported in the early formed dentine of other odontocetes,[31] suggesting that early deposited dentine records the breastfeeding period.

Previous Ca isotope studies conducted on enamel indicate that an extended breastfeeding period should not induce any change in $\delta^{44/42}$Ca values between the infant prenatal and postnatal/preweaning tissues.[38,39]

During the following recorded periods identified as nursing (= milk provision) and weaning events, $\delta^{44/42}$Ca values follow the patterns of Sr/Ca ratios and δ^{15}N values. The nursing period maintains the highest Ca isotope values of the dataset (at about −0.92‰), the highest Sr/Ca ratios and the highest δ^{15}N values. Our results contrast with those of previous studies on Ca isotopes in mammalian enamel (humans, sheep, deer, reindeer), where milk consumption during nursing implies a trophic isotope effect fractionation toward depleted values in the tissue of the consumer directly after birth followed by a subsequent ^{44}Ca-enrichment toward the end of the weaning process, that is, when the individual starts an adult diversified diet.[18,38,49] Odontocete milk analyzed here for Ca isotopes (−1.32‰ and −1.10‰) is slightly lower or comparable to cow milk values.[38,44] Feeding exclusively on milk during the nursing period would normally induce ^{44}Ca-depleted dentine values in the analyzed *Tursiops* individual. In other words, a trophic offset of −0.54‰ should correspond to dentine values of about −1.7‰.[34] This is not the case, and the discrepancy between the measured dentine value (−0.92‰) and the expected value (−1.7‰) during the nursing period must be explained.

Here, the progressive introduction of non-milk food (weaning) in the diet of *Tursiops* is observed until the fourth GLG as evidenced by a steep decline in δ^{15}N values in agreement with other odontocetes[29–31,50–52] and among seals.[53–55] The weaning interval, as recognized because of the steep decrease in δ^{15}N values, is concomitant with progressive ^{44}Ca-depleted values, eventually reaching $\delta^{44/42}$Ca prenatal values. Again, the Ca isotopic pattern is paradoxical with the N one. What processes are driving the observed changes in the dentine Ca isotope composition?

4.2 | Mixing model and the seawater ingestion hypothesis

Given the sensitivity of Ca isotopes to dietary source variations, the isotopic composition of the early formed dentine could be driven by a

^{44}Ca-enriched source. In the case of ocean-dwelling organisms, seawater is the most obvious and available source of heavy calcium with a concentration of 418 ppm[56] and an isotopic composition of +0.41‰ (see compilation in Martin et al[33]).

According to our mixing model, an incidental ingestion of about 20 vol% of seawater in the infant's diet is sufficient to induce the observed isotopic shift in the early formed dentine values (Figure 5), that is, during the nursing period. During the weaning stage, we interpret the progressive lowering of $\delta^{44/42}$Ca values as a progressive decrease in incidental seawater ingestion, which corresponds to the progressive suppression of milking bouts. Seawater ingestion is therefore a non-negligible component of the Ca isotope composition of early formed mineralized tissues in odontocetes.

Metabolic processes regulate the concentrations of trace metals in the body, and their concentration will be adjusted at the cellular level. Adult dolphins may incidentally ingest seawater during feeding, and they are able to maintain osmotic balance.[57] Yet, as concerns young individuals, concentration surges such as that occurring during incidental ingestion of seawater may not be perfectly regulated in young individuals. Therefore, additional support for the incidental ingestion of seawater during nursing comes from the concentration analysis, modeled, respectively, with elements that are less concentrated (Ca, Mg and Sr) or more concentrated (Ba and Zn) in milk than in seawater (Figure 5; Table 2).

Ba/Ca, Zn/Ca and Mg/Ca ratios do not change across birth, and both Ba/Ca and Zn/Ca stay low. In the presently analyzed tooth dentine of *T. truncatus*, the concomitant increase across the neonatal line of the Sr/Ca ratios and $\delta^{44/42}$Ca values represents the most striking result.

The Sr concentration in human milk is low[58] and cetacean milk is devoid of Sr (this study). Because of its toxicity, Sr is biopurified at the level of the placenta[59] and at the level of the gastrointestinal tract some time after birth around 1 year of age in humans[60] (see also the model in Nava et al[24]). This suggests that pre- and postnatal

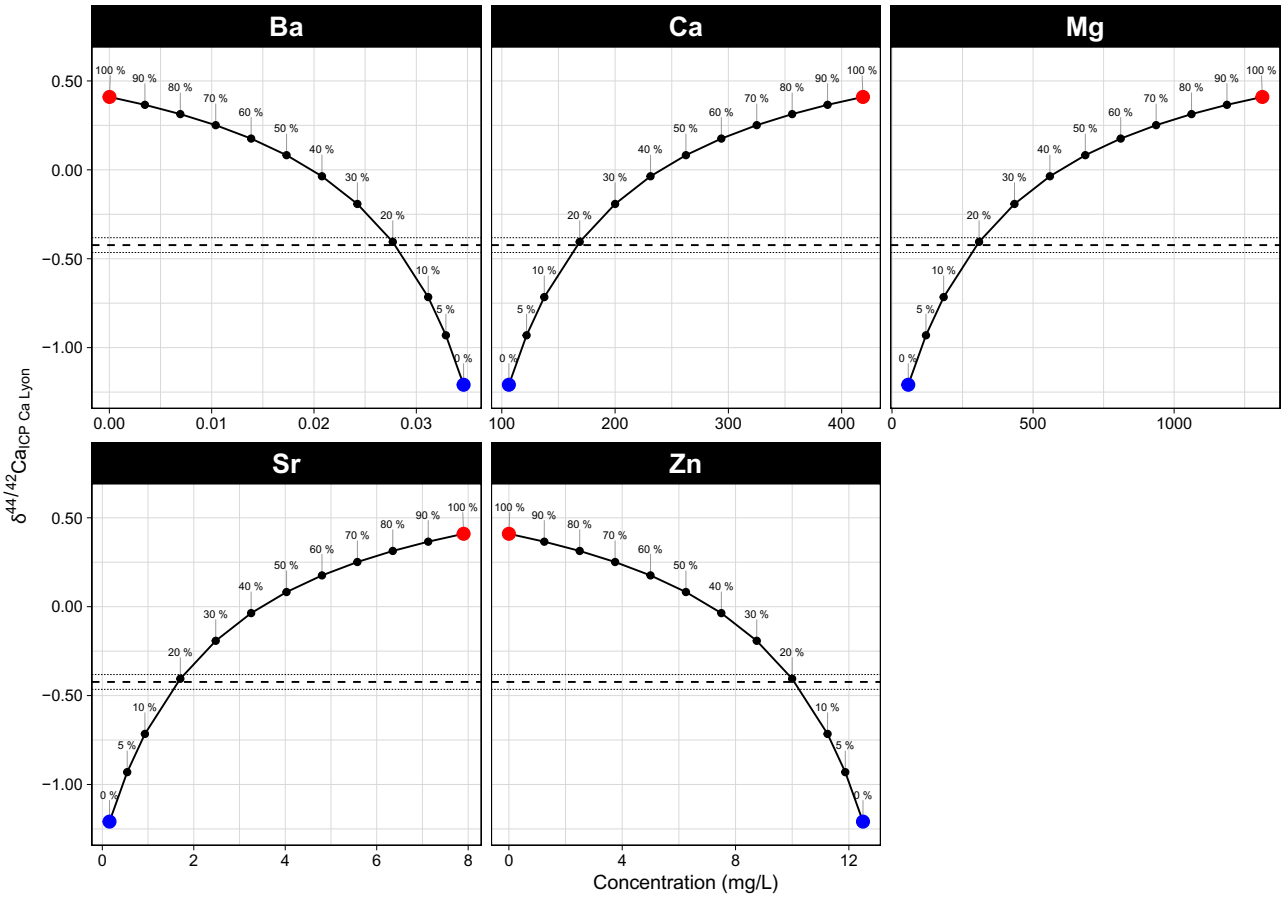

FIGURE 5 Mixing model presenting the expected shifts in the calcium isotope composition of the feeding source with variable input of seawater into dolphin milk. Calcium isotope end members consist of the average dolphin milk value of −1.21‰ (blue circle) and the seawater composition (+0.41‰). The isotopic composition of the source (−0.38‰) ingested by the infant is indicated by the horizontal dashed line and was calculated using the dentine Ca isotope value deposited during nursing (−0.92‰) minus the diet-bone offset of 0.54‰ established in mammals (e.g. Tacail et al[34]). This source mixture corresponds to an input of about 20% of seawater added to the ingested milk consumed by the infant. The same event corresponds to a twofold increase in Sr content in agreement with the observed Sr peak in early formed dentine; some limited contribution of Mg from seawater; and no contribution at all of Ba or Zn from seawater. [Color figure can be viewed at wileyonlinelibrary.com]

mineralized tissues are representative of mother and infant changes in diet, with a negligible contribution by gut biopurification before 1 year of age. Further research on cetacean gastrointestinal development might inform on elemental behavior during that time frame. The interval covering birth, weaning and adulthood records important variations in Sr/Ca ratios,[43] but their interpretations remain challenging. Previous studies conducted on primate enamel have reported contradicting results including a decrease in Sr/Ca ratios across the neonatal line in breastfed infants,[21,61] an increase in Sr/Ca in formula-fed infants[21] or no change at all.[20] Our results on *T. truncatus* highlight a dramatic increase in Sr/Ca ratios across the earliest formed dentine covering the prenatal and nursing periods, attaining a peak at the onset of the weaning period, followed by a dramatic fall during weaning and a subsequent low background value for the rest of the adult period. The earliest increase in Sr/Ca ratios is inconsistent with a breastfeeding hypothesis (e.g. Müller et al[21]) and is instead interpreted here as an incidental ingestion of seawater (Figure 5). The subsequent decrease in Sr/Ca ratios during the weaning period seems explained by the progressive cessation of nursing and therefore the progressive suppression of incidental seawater drinking.

The nursing and weaning interval offers interesting patterns for the other trace metal ratios. During milk provision, Ba/Ca ratios are maintained at their lowest levels while they rapidly increase during the weaning interval, suggesting the incorporation of Ba, during the period of dietary diversification. Barium content in seawater is extremely low,[56] and the observed increase is independent of an environmental interference and may therefore be interpreted in terms of dietary supplementation. This pattern agrees with the model of Nava et al[24] and also contradicts the previous suggestion that Ba records successive milking events as recorded in the enamel of primates[20] and calls for further studies about Ba bioavailability in marine diet, as well as from primate dentine in order to remove any temporal shifts from maturation processes. Zn/Ca ratios show a dramatic "up and down" in the second half of the nursing period. If we consider that Zn is coming from milk, then this variation in dentine is in agreement with previous observations that Zn concentration is highly variable in milk during lactation (see discussion in Müller et al[21]). Zn/Ca then shows not much variation during the weaning and periods thereafter, in agreement with previous data from enamel,[21] and could be an indication that it is maintained relatively constant through homeostatic control. Finally, Mg/Ca ratios are characterized by a slight but well visible and constant increase during the weaning stage, and if confirmed in other individuals, this increase may be a useful indicator of the weaning transition period together with Ba concentration. In fact, Mg/Ca and Ba/Ca show a significant correlation between each other, and both are anti-correlated to Ca isotopes.

In conclusion, the present results indicate that the observed peak in strontium in the early formed dentine is environmental in origin and, together with the change in calcium isotopic composition, corresponds to an incidental ingestion of seawater by the young cetacean. On the other hand, barium and magnesium variations may help identify the onset of the dietary diversification or may reflect physiological controls on their regulation in the body.

4.3 | Adulthood and discerning between diet shifts and physiological changes

The dentine deposited beyond the 4th GLG is considered to represent the adulthood period as evidenced from low $\delta^{15}N$ background values (e.g. Evacitas et al[31]). In the 4th to 13th GLG interval, Sr/Ca ratios vary little, while $\delta^{44/42}Ca$ values and $\delta^{15}N$ values record two events, interpreted here as distinct dietary preferences. The earliest (from 4th to 8th GLGs) is characterized by ^{44}Ca-depleted values, similar to values of the prenatal period, and by the lowest $\delta^{15}N$ values of the dataset. The next event (8th to 13th GLG) shows opposite shifts in Ca isotopes (even more ^{44}Ca-depleted) and N isotopes (toward ^{15}N-enriched values) that meet expectations for a shift in trophic source. The small (0.5‰) N isotopic offset between the two identified time intervals is of comparable magnitude as reported between male and female Risso's dolphins, which were interpreted in terms of dietary differences[31] or between populations of *Orcinus orca* with different diets.[29] The Ca isotopic offset of 0.15‰ between these two adult intervals also agrees with a trophic level effect in the *Tursiops* individual that may have included a greater proportion of ^{44}Ca-enriched prey such as cephalopods early in its adult diet and eventually turned to a more piscivorous diet later in life. This change is not obvious in any of the trace element records.

From the 13th GLG, $\delta^{15}N$ values describe a further progressive enrichment of 1‰. Similar patterns have been reported in odontocetes,[29,31] which was explained either as a change in trophic level or as a change in the diet–tissue discrimination factor associated with a decrease in growth rate.[29] A study involving a population of Risso's dolphins allowed the identification of a similar positive offset from the 9th GLG in females but not in males.[31] A catabolic event involving milk production was suggested for this male–female $\delta^{15}N$ difference.[31] Interestingly, such an interpretation would be compatible with the present Ca isotope record that shows a 0.3‰ increase in $\delta^{44/42}Ca$ values from the 13th GLG as well. Hassler et al,[7] based on experimental data from raised pigs, showed that the gestation and nursing periods did contribute to higher bone $\delta^{44/42}Ca$ values in females as a consequence of milk production and high Ca dietary absorption. Sr/Ca and Mg/Ca ratios do not record any variations, but both Zn/Ca and Ba/Ca ratios record greater amplitudes in their variations, yet we lack the resolution permitting a discussion as to whether this is linked to a perturbation in body metabolism or minor changes in dietary inputs.

5 | CONCLUSIONS

Cetaceans are elusive animals, and several aspects of their life history are easily unnoticed despite surveying efforts. N isotopes are a reliable monitor of trophic levels and used in conjunction with the observed variations in Ca isotopes and trace metals may offer additional insights into life history traits of modern and fossil species. Nitrogen isotopes are rarely available in the fossil record, and together with improving spatial resolution, a better understanding of covariations using multiproxy approaches is required.

The present multiproxy approach provides new insights into Ca isotopic physiology in modern mammals. This strongly suggests that an external input of a source of calcium from seawater plays an important part in determining Ca isotopic values recorded in early deposited mineralized tissues in marine mammals. Youngsters may not have the capability to expel seawater while suckling milk because of the strong suction needed to transfer milk from the nipple into the mouth, hence provoking some incidental ingestion of seawater. Adults, on the other hand, manage to avoid drinking seawater by expelling it while swallowing their solid prey.[62] Cetaceans, as any other mammals, are exposed to heavy dehydration if ingesting seawater. Nevertheless, marine mammals rapidly restore osmotic balance, more rapidly than terrestrial mammals.[57] Incidental ingestion during nursing representing nearly a quarter of seawater input poses physiological questions. Cetaceans possess a higher number of nephrons compared to terrestrial artiodactyls, suggesting a capacity for producing a highly concentrated urine, although it was concluded that such kidneys evolved in response to diving abilities and body size (see review in Ortiz[63]). Then, how is osmotic balance maintained during incidental ingestion of seawater? Are nephrons already developed early in ontogeny? If so, they would be actively solicited during nursing to filtrate the large amount of incidentally ingested seawater. Specific homeostatic regulation of intestinal absorption could also contribute to the adaptation to such stressing osmotic conditions.

Beyond those questions, the spatiotemporal characterization of seawater ingestion as recorded in tooth dentine represents a marker event of the nursing and weaning period. Given the spatial resolution achievable in sampling dentine material for Ca isotopic analysis, future implementations of the methodology should aim at quantifying timing of mineralization against GLGs. Doing so opens perspectives to precisely infer the duration of the nursing and weaning periods in a variety of odontocete species. Such life history parameters may help better understand reproduction strategies and maternal care in modern populations and help conservation efforts. A major limitation to such investigations remains analytical. Yet, new-generation collision cell MC-ICPMS offers perspectives to increase the throughput of Ca isotope measurements on small samples (e.g. Dai et al[64]) and hence could allow the exploration of intra-individual variability at the population level.

Finally, our results also bear perspectives for reconstructing nursing and weaning in long-extinct species. In million-year-old contexts, nitrogen isotopes and trace elements are no longer preserved, whereas Ca isotopes offer a new way to infer life history traits in the face of diagenesis.

AUTHOR CONTRIBUTIONS

Jeremy E. Martin: Conceptualization; investigation; funding acquisition; writing—original draft; methodology; validation; visualization; writing—review and editing; resources. **Théo Tacail:** Investigation; Methodology; validation; writing—review and editing. **Laurent Simon:** Investigation; methodology; validation; writing—review & editing. **Auguste Hassler:** Investigation; methodology; validation; writing—review & editing. **Philippe Télouk:** Methodology; writing—review & editing; visualization; investigation. **Vincent Balter:** Investigation; methodology; visualization; writing—review & editing.

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DATA AVAILABILITY STATEMENT

the dataset used in this work is available as supplementary material.

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