Sequential analyses of bovid tooth enamel and dentine collagen ($\delta^{18}$O, $\delta^{13}$C, $\delta^{15}$N): new insights into animal husbandry between the Late Neolithic and the Early Bronze Age at Tana del Barletta (Ligurian Prealps)

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Received: 26 January 2021 / Accepted: 19 July 2021 / Published online: 13 August 2021 © The Author(s) 2021, corrected publication 2022

Abstract
Tana del Barletta is an upland cave used from the Late Neolithic to the Middle Bronze Age, located in the vicinity of the coast in Liguria (NW Italy). The excavation revealed the presence of a faunal assemblage dominated by caprine and cattle remains. In order to gain new data on late prehistoric farming strategies (e.g. seasonal mobility, coastal grazing, animal diet), intra-tooth series of stable oxygen and carbon isotopes have been obtained from cattle and sheep/goat tooth enamel, along with intra-tooth series of nitrogen and carbon isotopes from cattle dentine collagen. Due to the prevalence of maxillary teeth, a modern calf has also been analysed to assess intra-individual isotopic differences between the maxillary and mandibular dentition. Modern data on oxygen isotope values of meteoric water from different altitudes around the area of the site were used as a reference for interpretation. The results indicate that the water ingested by the herd was mostly characterised by particularly low $\delta^{18}$O values, highlighting the importance of the uplands for the late prehistoric farmers of the region. However, the input of water sourced from lower elevations, especially during the winter months, cannot be dismissed. In addition, the nitrogen isotopic composition of cattle collagen rules out the ingestion of salt-tolerant vegetation or seaweed, suggesting that grazing did not occur directly on the coastal plain.

Keywords Stable isotopes · Cattle · Sheep/goat · Pastoralism · Animal diet · Seasonal mobility

Introduction
Numerous works of environmental archaeology have focused on the rugged territory overlooking the sea on the north-western Italian coast (Liguria). These contributions include geoarchaeological (Macphail et al. 1997), archaeobotanical (Arobbba et al. 2018) and zooarchaeological (Rowley-Conwy 2000; Rowley-Conwy et al. 2020) investigations, as well as studies on archaeometallurgy (Maggi and Pearce 2005), ceramic petrography (Capelli et al. 2008), osteology (Marchi et al. 2006; Sparacello and Marchi 2008; Sparacello et al. 2016, 2017; Dori et al. 2020; Orellana-González et al. 2020; Varalli et al. 2020) and stable isotope analyses of human remains (Pettitt et al. 2003; Goude et al. 2011, 2020; Varalli et al. 2015).

In Liguria, there is abundant evidence supporting the importance of animal husbandry since the beginning of the Neolithic, with a range of zooarchaeological, geoarchaeological and archaeobotanical data suggesting that during the Late Neolithic mobile pastoralism became a paramount subsistence strategy for the communities settled in the region. Most of the archaeological sites consist of caves and rock shelters located in the area of Finale Ligure and in the Pennavaira Valley (Fig. 1). The prevalence of domestic species in the faunal assemblages, the occurrence of deciduous teeth and the presence of dung layers...
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and thick spherulite-rich fumier deposits (Macphail et al. 1997; Rowley-Conwy 1997; Barker et al. 1990; Rowley-Conwy et al. 2020) suggest that these sites were largely used as stables since the Early Neolithic (c. 5800–5200 BC) (Rowley-Conwy et al. 2020) and especially during the Middle Neolithic (Square-Mouthed Pottery: c. 5000–4500 BC), when some of them (i.e., Cave of Arene Candide) were occupied all the year round and animals were fed with fodder collected in the surroundings (Maggi and Nisbet 2000). At the Cave of Arene Candide, during the Impressa phase of the Early Neolithic (c. 5800–5600 BC), animal farming seems to be mostly aimed at milk production (Rowley-Conwy et al. 2020), while with the onset of the Middle Neolithic, the strategy switched towards meat (Rowley-Conwy 1997). The data from the other Ligurian sites are insufficient to draw any firm conclusions but, on a larger geographical scale, the situation in the Liguro-Provençal milieu and Languedoc is rather varied (Rowley-Conwy et al. 2013).

The subsequent onset of the Chassey (c. 4300–3600 BC) and Copper Age (c. 3500–2000 BC) cultures saw a decrease in tree taxa, together with evidence for controlled use of fire (Nisbet 1997) and changes in stratigraphic and sedimentological units (Cruise et al. 2009; Macphail et al. 1997), suggesting that herds were taken to graze in newly created pastures (Maggi and Nisbet 1990; Maggi and Campana 2008; Branch and Marini 2014; Branch et al. 2014; Morandi and Branch 2018). The relevance of pastoral activities for the communities settled in the Liguro-Provençal arc is also shown by the Late Neolithic–Early Bronze Age anthropogenic indicators (Huet 2017a: 21–27) associated with the well-known rock engravings of Mt. Bégo, in the Maritime Alps (De Lumley 1995; Barfield and Chippindale 1997; Huet 2017b).

In this regard, while on the coast there are examples of open-air sites with multiple burials, at higher elevations only multiple burials in small natural caves are known (Maggi and Pearce, in press, with bibliography). Three cases in particular are worth mentioning: the Grotta del Pertuso in the upper Argentina valley (1330 m a.s.l.) (Fig. 1, no. 1), which hosted the remains of a minimum of 18 individuals of all ages and both sexes from the Copper Age to the Early Bronze Age (Del Lucchese and Ricci 1987; Formicola and Balestri 1987; Maggi and Nisbet 1990; Varalli et al. 2015), Tana della Volpe (750 m a.s.l.) (Fig. 1, no. 2), hosting no less than 68 individuals distributed over a thousand years throughout the Copper Age (Del Lucchese and De Pascale 2011) and Arma della Grà di Marmo (1000 m a.s.l.) (Fig. 1, no. 3), with no less than 30 individuals (Ricci 1998). According to Maggi and Nisbet (1990), this and other evidence, including cases of endogamy (Bareschino et al. 2013; Maggi and Pearce, in press), may indicate that whole family groups managed the pastoral activities over a long period of time, rather than individual herders guiding their livestock.

The system of presumed short-distance transhumance towards summer highland pastures, suggested by the occurrence of stable-caves at different altitudes and small burial caves in the uplands, besides archaeological and environmental evidence, continues until the Early Bronze Age (2200–1800 BC).

Later on, an increase in open-air sites occurs, the location of which suggests a more structured arrangement of routes and resources (Maggi and Nisbet 1990; Maggi 1999, 2004). However, even though understanding the nature of animal
husbandry, including seasonal movements, is of paramount importance to better interpret subsistence strategies at the time, isotopic investigations of faunal remains—and oxygen isotope data in particular—are very scant so far (Le Bras-Goude et al. 2006; D’Angela and Longinelli 1993).

In order to address this and assess the potential of stable isotope analyses for contributing to key research questions for the later prehistory of the region, we conducted a pilot study, analysing cattle and caprine tooth enamel and dentine collagen from Tana del Barletta, a small high-altitude cave in the Pennavaira Valley (western Liguria) which was used mainly from the Neolithic to the Bronze Age. The context appears particularly suitable to assess herd mobility in the territory, not only because of the relatively close proximity of mountain pastures and sea shore, which can be expected to produce markedly different oxygen isotope values, but also because of the availability of coastal grazing grounds, which should be traceable by higher δ15N values (Britton et al. 2008).

The Tana del Barletta tooth assemblage consists almost entirely of maxillary teeth. However, nearly all isotopic studies of sequentially formed tissues make use of mandibular molars, whether investigating archaeological material (e.g. Towers et al. 2014; Buchan et al. 2016) or analysing modern samples for reference datasets (e.g. Balasse et al. 2012a). Therefore, due to the scarcity of knowledge regarding the variation of the oxygen isotopic composition in maxillary teeth and their relationship with mandibular teeth (Balasse et al. 2021), we used a modern cattle specimen to assess the reliability of the upper dentition to record intra-tooth variations.

Isotopic background (δ18O, δ13C, δ15N) and current interpretative models for animal mobility

Along with the potential for determining birth seasonality, the application of stable isotopes to detect seasonal mobility in archaeological herds has attracted the attention of the scholars since the early 2000s (Pederzani and Britton 2019) and remains a very frequent approach (Gerling et al. 2017; Chazin et al. 2019; Tomczyk et al. 2019; Bishop et al. 2020; Trentacoste et al. 2020; Ventresca Miller et al. 2020), due to the prominence of the debate revolving around ancient forms of mobile pastoralism (Arnold and Greenfield 2006; Jourdain-Annequin and Duclos 2006).

Isotopic fractionation involves a change in the ratio between light and heavy isotopes during temperature-controlled phase transitions. Condensation preferentially removes heavier 18O from atmospheric water, resulting in a 18O depletion of cloud masses. At high- and mid-latitudes, this creates seasonal temperature fluctuations, which are responsible for cyclic variations in the oxygen isotopic composition of meteoric water (temperature effect) (Dansgaard 1964), and the farther atmospheric water travels inland, away from source water bodies (e.g. oceans), the more it becomes depleted in 18O, with a ~1.8‰ depletion every 1000 km (continental effect) (Rozanski et al. 1993). Furthermore, as clouds rise along an altitudinal gradient, the oxygen isotopic composition of meteoric water in the uplands becomes 18O-depleted relative to warmer lowlands (altitude effect) (Rozanski et al. 1993; Pederzani and Britton 2019). Due to this mechanism, a ~0.15–0.3‰ decrease in δ18O values occurs every 100-m increase in altitude in the Italian peninsula, where the rugged geomorphology particularly accentuates the altitude effect (Longinelli and Selmo 2003; Giustini et al. 2016).

The δ18O value of mammal body water is largely determined by the δ18O of ingested water, and reflected within the δ18O of phosphate and structural carbonate components of skeletal tissues (biogenic apatite and collagen). When the δ18O of precipitation fluctuates throughout the year, incrementally formed tissues, such as tooth enamel, record these seasonal variations, albeit with some attenuation (Fricke and O’Neill 1996).

So far, examinations of archaeological assemblages making use of stable oxygen isotopes have attempted several approaches to infer or rule out herd mobility. These are the amplitude of intra-tooth δ18O values (Mashkour et al. 2005), the relationship between the pattern of variations in the δ18O and δ13C curves (Makarewicz 2017a; Makarewicz et al. 2017; Tejedor-Rodríguez et al. 2021), variations in δ13C intra-tooth series (Balasse and Ambrose 2005) and the combination of δ18O and 87Sr/86Sr ratios (Balasse et al. 2002; Pellegrini et al. 2008; Valenzuela-Lamas et al. 2016). However, especially where stable oxygen isotopes were considered on their own, the multitude of different interpretative strategies used by researchers has highlighted the need for a more rigorous methodological framework to detect past seasonal mobility (see Makarewicz 2017a; Hermes et al. 2018). Analyses of modern animals with known life histories from different environments play thus an important role in the intra- and inter-individual variations that are to be expected (e.g. Britton et al. 2009; Makarewicz and Pederzani 2017; Tornero et al. 2018).

Based on the current knowledge of precipitation/enamel δ18O relationships at mid-latitudes, a general trend valid for cases of vertical mobility seems to be best described by Britton et al. (2009) (see also Henton 2012 and Hermes et al. 2018). A model of wider applicability can be hence extrapolated, where movement to 18O-depleted elevations in summer and to 18O-enriched lowlands in winter leads to a flattening of the pronounced sinusoidal pattern typical of sedentary animals (e.g. Fricke and O’Neill 1996; Fricke et al. 1998), although local variables can play a role in masking this trend (see e.g. Knockaert et al. 2018). Nevertheless, in domesticates, such a pattern may also be produced by certain
herding practices, e.g. the supply of $^{18}$O-enriched leaf water through winter foddering or $^{18}$O-depleted groundwater in summer (Makarewicz and Pederzani 2017; Hermes et al. 2018).

The relative abundance of $^{13}$C and $^{12}$C (usually expressed as $\delta^{13}$C) in vegetation varies according to the photosynthetic pathway of the plants and is further modified by the environment in which plants grow (Lambers et al. 2008). In Mediterranean settings, the great majority of taxa follows the Calvin-Benson cycle ($C_3$ plants) (Deines 1980), which tends to result in lower $\delta^{13}$C values ($\sim -34$ to $-24\%$) (Smith and Epstein 1971; Tauber 1981), although these also moderately fluctuate seasonally and along the altitudinal gradient (Körner et al. 1991; Smedley et al. 1991). A group of native plants following the Hatch-Slack cycle ($C_4$ plants) also occurs in temperate Europe (Pyankov et al. 2010), although they are generally too rare to register a significant impact on consumers’ $\delta^{13}$C values. In the case of Tana del Barletta, it is also relevant to highlight that seaweeds—which obtain their carbon primarily from dissolved CO$_2$ in the oceans—and seaside plants can also exhibit high $\delta^{13}$C values ($\sim -18$ to $-10\%$), falling within a range similar to that of terrestrial $C_4$ plants (Tauber 1981). It is likely that in antiquity the coast of western Liguria was home to a sand-dune habitat favourable to the growth of salt-tolerant $C_4$ Mediterranean halophytes, such as Salsola spp. and Euphorbia peplus (Arobbia et al. 2001; Acosta and Ercole 2015). To enable comparison of modern plant $\delta^{13}$C with archaeological datasets, a $1.5\%$ difference has also to be taken into account, due to the fossil fuel effect (Francay et al. 1999).

When considered jointly, oxygen and carbon stable isotopes can also provide indications of mobility, as in modern specimens’ lowland/highland seasonal migration results in inverted $\delta^{18}$O and $\delta^{13}$C seasonal curves (Tornero et al. 2018). However, the distinction between vertical movement and winter foddering can be difficult to discern (Makarewicz 2017a; Makarewicz et al. 2017).

A number of studies have established a correlation between coastal flora and higher $\delta^{15}$N values (Virginia and Delwiche 1982; Heaton 1987; Page 1995). This is evident in seaweeds, halophytes and even in non-halophytic coastal vegetation, due to nitrogen enrichment of the soil in proximity of the sea. These elevated $\delta^{15}$N values are passed on through the food chain and can be observed in the bone and dentine collagen of the consumers (Britton et al. 2008). Due to the absence of remodelling of primary dentine, this latter allows observation of nitrogen levels during discrete phases of tooth formation. However, instrumental and natural averaging of the signal still occurs to some degree, due to a c. 1.5–2-month equilibration period of the metabolites within the body reservoir (Balasse et al. 2001), as well as to the sampling of growth increments oblique to the axis of the tooth, which causes some mixing of dentine layers deposited over different periods (Balasse et al. 2001; Zazzo et al. 2006). Studies of archaeological bone and dentine collagen from Britain and north-central Europe have found significantly higher $\delta^{15}$N, and sometimes also $\delta^{13}$C values in cattle and caprine specimens from salt-marsh sites, and these isotopic thresholds have been taken as indicative of coastal grazing (Britton et al. 2008; Mühldner et al. 2014).

It is important to note that the carbon and nitrogen isotopic composition of both enamel bioapatite and dentine collagen in cattle also reflects the pre-weaning intake of maternal milk and the post-weaning dietary change along first and second molars. As prior to weaning mother and calf occupy two different trophic levels, a c. 3–3.5‰ offset in collagen $\delta^{15}$N values occurs, the mother’s signal being lower (Balasse et al. 2001; Gillis et al. 2013), whereas M1 enamel $\delta^{13}$C values reflect a progressive enrichment in $^{13}$C, due to rumen development (Towers et al. 2014). In addition, also the oxygen isotopic composition of enamel in cattle and sheep first molars is influenced by the pre-weaning signal, reflecting the composition of mother’s body water, which has already undergone fractionation (Fricke and O’Neil 1996).

Archaeological context

Tana del Barletta is a small cave located at c. 900 m a.s.l. on the steep calcareous slope of Mt. of Gettine, c. 14 km from the coast, in the northern side of the Pennavaira Valley (western Liguria) (N 44°07′57.7″, E 8°03′51.7″) (Figs. 1 and 2). Archaeological investigations during the mid-1980s identified a superimposition of three layers (c. 20 cm apart) allowing radiocarbon dating of the occupations between the Late Neolithic and the Middle Bronze Age, possibly followed by episodic visits in the Iron Age (Del Lucchese et al. 1987; Maggi and Nisbet 1990; Barker et al. 1990). Few artefacts were uncovered by the excavation, including stone tools and potsherds, along with animal bones and very few human remains (Del Lucchese et al. 1987; Barker et al. 1990). Out of a total of 1713 identifiable zoological remains, caprines prevail in the assemblage (822 fragments, from 54 to 74% according to the phase of occupation), followed by cattle (314 fragments, from 21 to 32%) and pigs (127 fragments, from 7 to 20%), while deer and hare only occur in very low amounts (Barker et al. 1990).

The cave of Tana del Barletta is of special interest for its location near the ridge, while most of the contemporary sites in the Pennavaira Valley typically occur in the valley floor (Leale Anfossi 1962; Del Lucchese et al. 1987; Maggi and Nisbet 1990). Animal husbandry is documented in the cave since the Late Neolithic, suggesting the exploitation of the high pastures of Mt. Galero (1708 m a.s.l.), just c. 4.5 km from the site.
It has been proposed that the Pennavaira Valley was probably involved in a system of family-run short-range transhumance, connecting hypothetical main settlements located in the lowlands of the Albenga plain with the mountain pastures above 1000 m a.s.l., c. 20 km inland (Maggi and Nisbet 1990), possibly on a seasonal basis (Barker et al. 1990).

**Materials and methods**

**Archaeological assemblage and chronology**

The faunal assemblage from Tana del Barletta was examined for the presence of bovine and caprine teeth for isotopic analysis. Although the overall number of bovid remains found in the cave amounts to more than a thousand fragments (Barker et al. 1990), teeth are rare and all of them were analysed. In total, ten teeth were recovered: six bovine maxillary teeth (enamel bioapatite and dentine collagen analysis), two caprine mandibular teeth and two caprine maxillary teeth (enamel bioapatite analysis) (Fig. 3). Five of the teeth are dated to phase 3 (representing at least three different Late Neolithic cattle specimens), four to phase 2 (representing two Late Copper Age sheep/goat specimens), one to phase 1 (representing one Early Bronze Age cattle specimen) (Table 1).

As the adjacent teeth TDB-4–5, TDB-7–8 and TDB-9–10 were still inserted in the maxilla/mandible, a fragment from each of the associated jaw bones was also sampled for collagen analysis. Molars have been indicated as M1/3, as no reliable discrimination could be made between isolated cattle upper molars on a purely morphological basis. The difficulty in differentiating loose cattle M1 and M2 is well known to zooarchaeologists (Beasley et al. 1993). When it comes to maxillary teeth, the issue is further complicated by the absence of the typically three-lophed shape of third molars, which makes them readily identifiable in mandibular rows. The small size of the assemblage also impeded any sound assumptions based on tooth size.
Both couples of caprine teeth were still in the jaw and allowed for confident identification of M1, M2 and P4 (fourth premolar) (Table 1).

For cattle teeth, the distinctive distally-pointing curvature of the roots was used to separate left- from right-side dentition. Although Payne’s (1973) and Grant’s (1982) tooth wear stages are not normally reported for maxillary teeth, an approximate indication is here provided (Table 1). Previous zooarchaeological works on Tana del Barletta are limited to a list of taxa and quantification of remains (Barker et al. 1990), without providing data on the minimum number of individuals (MNI) of cattle and caprine specimens. The MNI can at least be estimated as regards the tooth assemblage, on the basis of chronology, tooth laterality, wear and shape (Table 1). In light of these parameters, the assemblage used for this study is likely to represent a minimum of four cattle specimens (TDB-1; TDB-2; TDB-3–5; TDB-6) and two sheep/goat specimens (TDB-7–8; TDB-9–10). In the published report for the site, caprine remains included both sheep and goat and were grouped as *Ovis/Capra* (Barker et al. 1990), while overall, in the late prehistory of the region sheep remains are far more abundant (Rowley-Conwy et al. 2020).

In order to achieve a better determination of the material analysed, specific criteria based on morphological differences in lower dentition were considered, following Halstead et al. (2002). The diagnostic features observable in the assemblage are summarised in Table 2. However, further experimental works on caprine mandibles (Zeder and Pilaar 2010; Gillis et al. 2011) have shown that goat is more...
The chronology of the remains is based on the 14C dates available for the superimposed hearths excavated in the 1980s (Del Lucchese et al. 1987; Barker et al. 1990), produced by the laboratory of the Harwell Atomic Energy Research Establishment, along with three new radiometric dates purposely obtained for this study at the Oxford Radiocarbon Accelerator Unit (Table 3). For the new dating programme, we selected a tooth from the lowest hearth (TDB-5), a tooth from the upper hearth (TDB-6) and a tooth from the caprine jaws originally collected in a reworked deposit on the surface of the cave (TDB-8). TDB-5, still in the jaw with TDB-4, was found in the same stratigraphic unit as TDB-1–2–3 and therefore provides a reworked deposit on the surface of the cave (TDB-8).

The timing of tooth development in goat may not overlap exactly, and delays might occur in maxillary teeth relative to mandibular teeth (Vigal and Machordom 2005). The timing of tooth development in goat may now shown to be Late Copper Age (TDB-7–8–9–10).

The time span of formation of bovid teeth relevant to this study is as follows: cattle M1, from the last c. 4.5 months in utero to 3–2 months of life; cattle M2, from 1 to 12–13 months; cattle M3, from 9–10 to 23–24 months (Brown et al. 1960); sheep/goat M1 starts in utero and reaches completion by 9 months; sheep/goat M2, from 2 to 12 months; sheep/goat P4, from c. 12 to 18–24 months (Weinreb and Sharav 1964; Milhaud and Nézit 1991; Hillson 2005). The timing of tooth development in goat may not overlap exactly, and delays might occur in maxillary teeth relative to mandibular teeth (Vigal and Machordom 1985). However, the rate of tooth growth does not reflect the timing of tooth mineralisation, due to a delay caused by the process of enamel maturation. This phase, where most of the carbonate fraction is deposited, takes c. 6–7 months in cattle (Towers et al. 2014) and c. 5–6 months in sheep/goat (Balasse et al. 2012a), and the apposition of mineralisation fronts makes it virtually impossible to sample discrete growth increments, decreasing the time resolution of sequential sampling (Balasse 2002).

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**Table 2** Summary of morphological features of mandibular caprine teeth which are potentially diagnostic for discriminating *Capra* from *Ovis*

| Sample  | Tooth | Morphological features                                      | Reference                  | Possible indication |
|---------|-------|-------------------------------------------------------------|----------------------------|---------------------|
| TDB-9   | P4    | Absence of vertical rib of mesio-lingual corner             | Halstead et al. (2002), criterion P4.1 | *Capra*             |
| TDB-10  | M1    | Buccal edge of disto-buccal cusps bent towards the distal side | Halstead et al. (2002), criterion M1.2 | *Ovis*              |

**Table 1** Summary of δ18O and δ13C values of bioapatite enamel from cattle and sheep/goat teeth from Tana del Barletta (means refer to mid-range values). LN, Late Neolithic; LCA, Late Copper Age; EBA, Early Bronze Age; TWS, tooth wear stage

| Sample | Taxon | Tooth | Sample Tooth | Number | Jaw side | Grant’s TWS | Phase | Period | No. subsamples | δ18O dist. (‰) | δ18O max. (‰) | δ18O mean (‰) | δ18O range (‰) | δ13C dist. (‰) | δ13C max. (‰) | δ13C mean (‰) | δ13C range (‰) |
|--------|-------|-------|--------------|--------|----------|-------------|-------|--------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| TDB-5  | Bos   | M1    | x left       | 10     | n/a      | Bar. 2      | LN    | 10     | 6.4            | -6.3           | -7.0           | 0.5            | -6.3           | 2.9             | 12             | -12.0          | 8.4             | -11.7          | 2.5             |
| TDB-6  | Bos   | M1    | x right      | 9      | n/a      | Bar. 1      | LN    | 9      | 12.4           | -8.5           | -9.5           | 0.5            | -12.4          | 2.9             | 11.5           | -11.5          | 2.5             |
| TDB-7  | Bos   | M1    | x right      | 12     | n/a      | Bar. 1      | LN    | 12     | 0.0            | -9.0           | -9.0           | 0.0            | 0.0            | 0.0            | 9.0             | -12.2          | 11.7           | 2.8             |
| TDB-8  | Bos   | M1    | x right      | 12     | n/a      | Bar. 2      | LN    | 12     | 0.0            | -9.0           | -9.0           | 0.0            | 0.0            | 0.0            | 9.0             | -12.2          | 11.7           | 2.8             |
| TDB-9  | Bos   | M1    | x right      | 12     | n/a      | Bar. 2      | LN    | 12     | 0.0            | -9.0           | -9.0           | 0.0            | 0.0            | 0.0            | 9.0             | -12.2          | 11.7           | 2.8             |
| TDB-10 | Bos   | M1    | x right      | 12     | n/a      | Bar. 2      | LN    | 12     | 0.0            | -9.0           | -9.0           | 0.0            | 0.0            | 0.0            | 9.0             | -12.2          | 11.7           | 2.8             |

*Possibly slightly broken occlusal surface
**One broken cusp
All dates were recalibrated in OxCal 4.3 (Bronk Ramsey 2009), using the latest available curve (IntCal13; Reimer et al. 2013). The previous chronology of Barletta phases 3 and 2 (Late Neolithic and Copper Age) was confirmed by the new results and supported by the artefacts recovered during the excavation, which included plain and cordoned ware, a flint arrowhead and a serpentinite axe (Del Lucchese et al. 1987).

Modern assemblage

Given the large prevalence of maxillary teeth in the archaeological assemblage, modern remains of cattle were also sampled, in order to investigate whether the isotopic signals measured in mandibular and maxillary teeth are similar and comparable. The animal belonged to a specimen of Piedmontese breed and was always raised in the same location, a calcareous meadow in Paruzzaro (eastern Piedmont, Italy). In the farm, adults were fed with a mixture of hay, maize, beet, soy, bran and sugarcane, while calves were given milk (90%) and a “microfibre” composed of maize, barley, soy, pea and straw (10%). The head was obtained in September, defleshed by burying it in a biologically active topsoil and recovered one year later. The teeth were then extracted using a Dremel cutting wheel. On the mandible and maxilla of the specimen examined, deciduous premolars (p2, p3, p4) appeared to be still in place, while the permanent first molar (M1) had erupted, and the permanent second molar (M2) had become visible.

The precise age at death of the animal was unknown; therefore, it was estimated with reference to the tooth development stage. Based on permanent molars’ eruption timing (M1: between 6 and 12 months; M2: between 12 and 16 months; Brown et al. 1960; Koarai et al. 2016) and wear stage (Jones and Sadler 2012), the animal was likely to be an immature individual about 7–8 months of age, within Jones-Sadler’s early stage C. In order to increase the reliability of the results and compare the maxillary/mandibular signal twice, both the left and right sides of the jaws were sampled. Due to the incomplete stage of formation of second molars, only first molars were sampled for stable carbon and oxygen isotope analysis.

Sampling and analysis of enamel bioapatite ($\delta^{18}O$, $\delta^{13}C$)

Bucco-mesial lobes were selected as sampling points due to enamel thickness and easiness of sampling (Towers et al. 2014). A conventional sampling method drilling the enamel with a diamond-tipped abrasive drill bit perpendicularly to the tooth growth axis was adopted. Depending on the length of the crown, from 7 to 12 (cattle) and from 3 to 7 (sheep/goat) samples per tooth were taken from archaeological material, and from 9 to 11 from modern material. Around c. 8–12 mg (cattle) and c. 4–6 mg (sheep/goat) of enamel powder were collected. Following Balasse et al. (2002), samples were reacted with acetic acid 0.1 M (approximately 0.1 mL solution/0.1 mg powder), at room temperature for 4 h, then rinsed five times and freeze-dried. All samples were reacted with 100% phosphoric acid at 70 °C for 1 h. An amount varying between 0.6 and 0.8 mg of enamel powder was used for measurements. The CO$_2$ produced was analysed on a Thermo Delta V Advantage isotope ratio mass spectrometer coupled to a Thermo gas chromatography-based GasBench II system via a Conflo IV interface at the Chemical Analysis Facility (CAF) of the University of Reading, UK.

Values were calibrated with the international standards NBS 19 (limestone; accepted values: $\delta^{18}O = -2.20‰$, $\delta^{13}C = +1.95‰$) and LSVEC (lithium carbonate; values used in this study: $\delta^{18}O = -26.40‰$, $\delta^{13}C = -46.60‰$) using a
two-point calibration curve with drift correction. All values are reported in per mil relative to the Vienna Pee Dee Belemnite (VPDB) scale. Over the period of analysis, measured values for NBS 19 were $-2.19 \pm 0.14\%$ (δ$^{18}O$) and $1.97 \pm 0.10\%$ (δ$^{13}C$), while for LSVEC these were $-26.44 \pm 0.16\%$ (δ$^{18}O$) and $-46.57 \pm 0.11\%$ (δ$^{13}C$). The accuracy (percent error), based on repeat analysis of an internal carbonate standard (Reading limestone calcite—RLC; accepted values: δ$^{18}O = -1.77\%$, based on repeat analysis of an internal carbonate standard (Reading horse enamel—RHE), was ± 0.20\% for δ$^{18}O$ and ± 0.12\% for δ$^{13}C$. The analytical precision (1σ std. dev.), based on repeat analysis of an internal standard of modern horse enamel prepared at the Reading stable isotope laboratory (Reading horse enamel—RHE), was around 0.20\% for δ$^{18}O$ and 0.12\% for δ$^{13}C$ (see Online Resources 1–2).

**Sampling and analysis of dentine and bone collagen (δ$^{15}N$, δ$^{13}C$)**

Before sampling, all material was cleaned using abrasive dental drill bits. Collagen extraction followed Longin (1971), with modifications according to Collins and Galley (1998). Using a Dremel diamond cutting wheel, a whole tooth loph was cut perpendicularly to the axis into c. 2-mm thick slices (c. 100–500 mg), and when still inserted in the jaw a portion of the bone (c. 250–450 mg) was also sampled. Tooth slices and bone fragments were dissolved for 2–4 days in refrigerated 0.5 HCl, rinsed in acidic (pH 3) deionised water and heated up to 70 °C. Residues were recovered using a 60-90-μm Ezee® filter. The samples were then freeze-dried for 48 h, prior to weighting into tin capsules and duplicate analysis with a Sercon Elemental Analyzer coupled to an Europa 48 h, prior to weighting into tin capsules and duplicate analysis with a Sercon Elemental Analyzer coupled to an Europa Geo 20–20 isotope ratio mass spectrometer at the University of Reading, UK. Values were calibrated using a two-point calibration curve with drift correction, using internal working standards calibrated to internationally certified reference materials including the amino acid methionine (Elemental Microanalysis/MethR), powdered Bovine Liver Standard (NIST1577a-BLS) and a batch of pork gelatine and fish skin prepared at the Reading stable isotope laboratory (Reading pork gelatine—RPG; accepted values: δ$^{15}N = 5.04\%$, δ$^{13}C = -21.54\%$; Reading fish skin—RFS; accepted values: δ$^{15}N = 13.99\%$, δ$^{13}C = -15.64\%$). All δ$^{15}N$ values are reported in per mil relative to the atmospheric N$_2$ (AIR), and δ$^{13}C$ values in per mil relative to the VPDB scale. Analytical precision (1σ std. dev.) over the period of analysis, determined by repeat analysis of internal standards (RPG and RFS), was ± 0.19\% for δ$^{15}N$ and ± 0.06\% for δ$^{13}C$ (see Online Resources 3–5).

**Reference values of environmental water**

In the absence of direct isotope measurements of water sources from around the site, reference data on $^{18}O$ abundance in meteoric water from atmospheric precipitation (henceforth δ$^{18}O_{mw}$) were obtained using the Online Isotopes in Precipitation Calculator (OIPC 3.1; Bowen and Wilkinson 2002; Bowen 2019). Following the algorithms by Bowen and Revenaugh (2003) and Bowen et al. (2005), monthly and mean annual δ$^{18}O_{mw}$ values were calculated for three locations: the cave site, the coastal plain of Albenga (c. 12 km from the site, 10–20 m a.s.l.) and the upland pasture of Mt. Galero (c. 4.5 km from the site, 1700 m a.s.l.) (Table 4). The values interpolated have been checked against direct measurements of environmental water available from the nearest pluviometric stations included in the database of the Global Network for Isotopes in Precipitation (GNIP) (IAEA/WMO 2015): Genoa and Monaco for the coast and Bossea for the upland area (Table 4). Most of these instrumental data were collected before the global rise of temperatures following the first years of the twenty-first century. A slight offset is noticeable, OIPC estimates for the coast being ~3% lower in winter months relative to the average winter values from the IAEA stations of Genoa and Monaco, and ~2% higher in summer months relative to the average summer values from the station of Genoa, while the offset is slightly reduced for winter values at high elevations (~1–2%, Mt. Galero and Bossea IAEA station). Summer values are not available for Bossea, as the warmest month for which data were recorded is March (~8.8%), and among the winter months the lowest values occur in November (but data are lacking for January).

Interpolations from the three locations show a large intra-annual range, spanning from 8.1 (coastal plain) to 9.4% (upland pastures). However, the decade-spanning IAEA records available for Genoa and Monaco indicate a smaller seasonal range (~3% for Genoa, from −6.5 to −3.4% and ~5.5–6% for Monaco, from −6.9 to −1.2%).

**Relationship between oxygen isotopic composition of structural carbonate and environmental water**

Specific formulas for conversion of enamel δ$^{18}O$ values (δ$^{18}O_{en}$) into δ$^{18}O_{mw}$ were applied to the dataset from Tana del Barletta. Although not appropriate for every geographical location (Janzen et al. 2020), these formulas, used before in key studies of herd mobility (Pellegrini et al. 2008; Britton et al. 2009), and routinely applied also in later Quaternary and pre-Quaternary works (e.g. Pushkina et al. 2020; Owocki et al. 2020; Pederzani et al. 2021), have received criticism by Pollard et al. (2011). However, in the Alpine region, a significant correlation between water isotopic composition derived from archaeological cattle bones, altitude and modern water was found by Mayr et al. (2016). Moreover, there is a paucity of comparable δ$^{18}O_{(PO3-4)}$ values from the study region where the site is located (the choice suggested by Pollard et al. 2011); therefore, conversion from
δ18Oen to δ18O(H2O) represents the only applicable approach to tackle issues of ancient mobility. Following such considerations, we decided to use these equations, with the baseline assumption of their rough reliability (Table 5).

After conversion of values from PDB to SMOW scales according to Werner and Brand (2001) (1) and estimations of phosphate bioapatite δ18O values from bioapatite structural carbonate δ18O values according to Iacumin et al. (1996) (2), oxygen isotope ratios in bone phosphate were converted into oxygen isotope ratios in precipitation, as calculated using the inverse form of the equations for cattle (3) and sheep (4) by D’Angela and Longinelli (1990):

$\delta^{18}O_{SMOW} = 1.03091 \times \delta^{18}O_{PDB} + 30.92 \tag{1}$

$\delta^{18}O_{(PO_4^{3-})} = 0.98 \times \delta^{18}O_c - 8.5 \tag{2}$

$\delta^{18}O_{(H_2O)} = \frac{\delta^{18}O_{(PO_4^{3-})} - 24.90}{1.01} \tag{3}$

$\delta^{18}O_{(H_2O)} = \frac{\delta^{18}O_{(PO_4^{3-})} - 27.21}{1.48} \tag{4}$

### Table 4
Geographic data of key locations around the site area and estimates according to the Online Isotopes in Precipitation Calculator (OIPC) for the seasonal amplitude of δ18O values of meteoric water (top); geographic data from pluviometric stations of the International Atomic Energy Agency (IAEA) used to obtain comparable values (bottom). The full instrumentally-measured seasonal amplitude is available only for Genoa and Monaco, along with winter data from Bossea.

| Location                     | Coordinates | Altitude (m a.s.l.) | Min. annual δ18O mw value (OIPC ‰ estimate) | Max. annual δ18O mw value (OIPC ‰ estimate) |
|------------------------------|-------------|--------------------|---------------------------------------------|-----------------------------------------------|
| Upland pastures of Mt. Galero| N 44°09′11.7″ E 8°01′07.3″ | 1700              | −13.4                                       | −4.0                                          |
| Cave of Tana del Barletta    | N 44°07′46.7″ E 8°03′45.1″ | 900               | −11.6                                       | −2.9                                          |
| Coastal plain of Albenga     | N 44°03′52.3″ E 8°12′04.9″ | 18                | −9.6                                        | −1.5                                          |

| Location                     | Coordinates | Altitude (m a.s.l.) | Min. annual δ18O mw value (average ‰ measured) | Max. annual δ18O mw value (average ‰ measured) |
|------------------------------|-------------|--------------------|---------------------------------------------|-----------------------------------------------|
| Bossea                       | N 44°13′60″ E 7°50′00.0″ | 1050              | −11.5 (November)                            | n/a (March = −8.8)                           |
| Genoa                        | N 44°25′12″ E 8°50′00.0″ | 0                 | −6.5 (February)                             | −3.4 (July)                                  |
| Monaco                       | N 43°43′56.6″ E 7°25′24.89″ | 0                 | −6.9 (November)                             | −1.2 (June)                                  |

### Table 5
Maximum and minimum δ18O values of water ingested by cattle and sheep/goat calculated following the equations by Werner and Brand (2001), Iacumin et al. (1996) and D’Angela and Longinelli (1990). Mnd., mandible; Mxl., maxilla.

| Sample | Taxon     | Tooth | Mnd. | Mxl. | Calculated δ18O mw, min. value (%) | Calculated δ18O mw, max. value (%) |
|--------|-----------|-------|------|------|----------------------------------|-----------------------------------|
| TDB-1  | Bos       | M1/3  | x    |      | −8.7                             | −7.0                              |
| TDB-2  | Bos       | M1/3  | x    |      | −8.8                             | −7.4                              |
| TDB-3  | Bos       | M1/3  | x    |      | −9.7                             | −8.8                              |
| TDB-4  | Bos       | M1/2  | x    |      | −9.8                             | −7.7                              |
| TDB-5  | Bos       | M2/3  | x    |      | −10.6                            | −9.5                              |
| TDB-6  | Bos       | M1/3  | x    |      | −11.7                            | −10.3                             |
| TDB-7  | Ovis/Capra| M1    | x    |      | −6.6                             | −4.5                              |
| TDB-8  | Ovis/Capra| M2    | x    |      | −6.1                             | −5.4                              |
| TDB-9  | Ovis/Capra| P4    | x    |      | −7.5                             | −6.9                              |
| TDB-10 | Ovis/Capra| M1    | x    |      | −9.0                             | −7.2                              |
The last step was also performed according to the inverse form of the equation for mammals (5) by Amiot et al. (2004):

$$\delta^{18}O_{(H_2O)} = 1.11 \times \delta^{18}O_{(PO_4)} - 26.44$$  \hspace{1cm} (5)

However, using Eq. (5), the changes in the values appeared to be negligible in the case of cattle (≤ 0.4) but up to 2‰ lower in the case of caprines. Therefore, the data given below will only refer to the values obtained using the genus-specific Eqs. (3) and (4) by D’Angela and Longinelli (1990).

Results

Stable oxygen and carbon isotopes of modern cattle enamel

In the modern cattle, δ^{18}O values range between −5.4 and −11.1‰ and δ^{13}C values between −9.9 and −11.5‰. The intra-tooth variation ranges between 4.4 and 4.9‰ for the δ^{18}O signal and is equivalent to or less than 1‰ for the δ^{13}C signal (Table 6). Overall, the pattern of intra-tooth variations in both mandibular M1 and in the left maxillary M1 is very similar: it follows an increasing slope for the δ^{18}O signal, while the δ^{13}C sequences appear rather flat (Fig. 4). However, the upper right M1, despite its general increasing trend towards the ERJ, shows two drops, one of which is within the most recent part of the sampled enamel.

The maxillary M1 from both sides exhibit δ^{18}O values comprised between −5.4 and −10.7‰/−10.8‰. Mean δ^{18}O values are −8.1‰ and −8.0‰, and the intra-tooth range 5.4 and 5.3‰ for the left and right maxillary M1, respectively. The corresponding mandibular δ^{18}O maxima and minima from both sides are between −6.0‰/−6.3‰ and −10.6‰/−11.1‰. Mean δ^{18}O values are −8.7‰ and −8.3‰, and the intra-tooth range is 4.8 and 4.6‰.

In the maxillary molars, δ^{13}C values are comprised between −11.5 and −10.2‰ (averaging −10.6‰ and −10.8‰). In mandibular molars, δ^{13}C values are comprised between −11.1 and −9.9‰ (averaging −10.5 and −10.4‰).

Stable oxygen and carbon isotopes of archaeological cattle enamel

Bovine teeth display δ^{18}O values comprised between −8.6 and −3.9‰ (Table 1; Fig. 5). Although some reduced intra-tooth variations are visible, the intra-tooth range between maximum and minimum δ^{18}O values is rather narrow, even in teeth representing large time spans such as the M2/3 (from 0.9 to 2.9‰, averaging only 1.6‰). TDB-1 and TDB-6 (M1/3) show a similar, decreasing and finally newly
increasing pattern of δ18O towards the ERJ, while in TDB-4 (M1/2) δ18O values increase towards the ERJ, and in TDB-2–3–5 (M1/3 and M2/3) the δ18O sequences are relatively flat.

δ13C values are relatively homogenous (ranging from −14.6 to −11.3‰), and intra-tooth variations are small (averaging 1.2‰).

**Stable oxygen and carbon isotopes of archaeological caprine enamel**

Caprine δ18O values range from −7.8 to −1.2‰ (Table 1; Fig. 5). Overall, apart from TDB-10, sheep/goat teeth exhibit higher δ18O values than cattle teeth. TDB-7 and TDB-10 (both M1) show a similar, decreasing, pattern of δ18O variations. The intra-tooth range between maximum and minimum δ18O values is quite limited (from 0.8 to 3.2‰, averaging 1.9‰).

δ13C values are relatively homogenous (from −14.6 to −11.9‰), with small intra-tooth variations (averaging 1.4‰).

**Stable nitrogen and carbon isotopes of archaeological dentine and bone collagen**

The C:N ratios (range 3.1–3.5), C content (range 19.6–43.6%) and N content (range 6.7–15.7%) of all dentine samples fall within the range of reliable values (DeNiro 1985; Ambrose 1990). Collagen yield percentages in dentine (between 2.7 and 9.3%, averaging 6.5%) are also sufficient to produce reliable results (Van Klinken 1999) (Online Resource 5). The isotopic composition of cattle collagen appears extremely stable across tooth dentine. All cattle teeth exhibit δ15N values between 3.5 and 5.9‰, with an intra-tooth range often very limited (≤0.3‰). δ13C values are equally homogeneous (from −22.1 to −21.2‰), with most of the teeth having particularly small intra-tooth variations (in five cases ≤0.4‰) (Table 7; Fig. 6).

The C:N ratios (range 3.2–3.3), C content (range 39.1–43.7%) and N content (range 14.1–15.7%) of the archaeological bone fragments (cattle maxilla enclosing TDB-4–5 and upper and lower caprine jaws associated with the contiguous teeth TDB-7–8 and TDB-9–10) fall in the accepted range for well-preserved collagen (DeNiro 1985; Ambrose 1990), except for the jaw related to TDB-9–10, which therefore cannot be taken into account (Online Resource 5). Bone samples exhibit δ15N and δ13C values very close to those recorded in dentine samples (Table 7).

**Discussion**

To shed light on the subsistence strategies of late prehistoric societies settled in Liguria, it will be of paramount importance to assess whether caves containing animal remains were permanently occupied or used as seasonal shelters (Barker et al. 1990; Rowley-Conwy 1997, 2000; Rowley-Conwy et al. 2020). As regards Tana del Barletta, the cave had previously been interpreted as a pastoral shed aimed at the seasonal exploitation of the nearby pastures of Mt. Galero (Fig. 7, triangle), while the plain of Albenga may have been home to one or more permanent villages (Maggi and Nisbet 1990) (Fig. 7, circle).
Stable oxygen isotopes are useful indicators to gain insights into the environment grazed by the livestock penned in the cave, as the $\delta^{18}O$ of tooth enamel broadly reflects the oxygen isotopic composition of ingested water, without undergoing remodelling once mineralisation has occurred (Kennedy 2018). In obligate drinking animals (e.g. Bos sp.), the relationship between the isotopic composition of the water intake and enamel bioapatite is particularly strong, as the composition of the latter largely reflects the $\delta^{18}O$ of the dominant water source (Pederzani and Britton 2019; Pederzani et al. 2021). Therefore, cattle teeth from Tana del Barletta can offer precious information on the isotopic composition of ingested water, which is in turn controlled by environmental and geographical parameters.

### Mandibular and maxillary isotopic composition of cattle first molars

Just a handful of studies have specifically reported the use of maxillary teeth to investigate the isotopic composition of incrementally formed tissues (Balasse et al. 2003, 2017, 2021; Towers et al. 2011; Makarewicz 2017a; Makarewicz and Pederzani 2017), and only very recently archaeological and modern upper molars (from sheep) have been specifically examined (Balasse et al. 2020). In turn, having a majority of studies based on mandibular dentition encourages analysts to select solely archaeological mandibular teeth to obtain comparable data, thus enlarging the gap. Using a modern skull, the isotopic pattern recorded in maxillary and mandibular cattle M1 was compared twice (sampling left-sided and right-sided molars). Although the time span of formation of M1 crowns does not embrace a full annual cycle (Brown et al. 1960), there is a very good match between the range of maximum and minimum $\delta^{18}O$ values recorded in cattle lower molars of both sides and their upper counterparts (less than 1‰ offset). The similarity of the intra-tooth pattern is also evident, despite a slight difference between the right maxillary M1 and the other M1s. The former shows two drops in the values (Fig. 4), which are not seen in the left maxillary M1 and are likely due to an incomplete mineralisation towards the enamel-root junction, noted during sampling, or to the drilling procedure (e.g. deep sampling causing a partial inclusion of dentine).

It is possible that the increasing pattern of variation observed in modern M1s was influenced by the pre-weaning intake of maternal milk. However, the $\delta^{18}O$ values of this latter are strongly related to the same geographical and seasonal parameters determining the $\delta^{18}O$ values of drinking water (Crittenden et al. 2007), which in the teeth analysed appear to equally affect both mandibular and maxillary tooth apatite.

The results suggest that the oxygen isotopic composition of cattle mandibular and maxillary first molars does not differ substantially (cf. Makarewicz and Pederzani (2017) and Balasse et al. (2020) on lower and upper sheep molars). However, potential variations, especially in other molars (Balasse et al. 2021), should be investigated with larger datasets, to assess the degree of inter-individual variability (Robinson et al. 1995; Towers et al. 2014). This would be highly relevant for isotopic studies of cattle dentition from archaeological contexts, in the event of absence of mandibular teeth in the faunal assemblage (see e.g. Balasse et al. 2021) and availability only of maxillary teeth for intra-tooth sequential analysis.

### Low values and narrow amplitude of variation in stable oxygen isotope series

As regards stable oxygen isotopes from the archaeological assemblage, due to the presence of both worn and unworn molars which cannot be precisely identified (M1/3), it is possible that at least in some teeth the results are influenced by the lactation signal, reflecting in M1 the composition of mother’s body water rather than that of meteoric water (Fricke and O’Neil 1996; Franz-Odendaal et al. 2003; Henton et al. 2010). However, the contiguous maxillary molars TDB-4 (M1/2) and TDB-5 (M2/3) are particularly promising to observe the isotopic composition of cattle water intake over a long period, as these teeth represent the longest time span observable for an individual cow in the dataset obtained. According to the known data on cattle tooth development, the series TDB-4–5 is likely to represent approximately one year of life or slightly more (Brown et al. 1960). However, this can only be taken as an approximation, as measured $\delta^{18}O$ values refer to the bioapatite composition after mineralisation, which mostly occurs during the maturation phase (Robinson et al. 1995), lasts at least 6–7 months and may not always progress at a perfectly uniform rate (Towers et al. 2014). The highest and the lowest values in the series, $-4.6‰$ and $-7.5‰$, were selected to calculate the range of $\delta^{18}O$ values of the water ingested by the specimen. By applying conversion equations from $\delta^{18}O_{\text{en}}$ to $\delta^{18}O_{\text{mw}}$, we obtained $-7.7‰$ (warmest period) and $-10.6‰$ (coldest period) (Table 5). If only the c. 1-year time span of TDB-5 is considered, the extremes range from $-9.5$ to $-10.6‰$. Taking into consideration also the other available cattle teeth, among the Late Neolithic molars, the lowest value still occurs within TDB-5, corresponding to $-10.6‰$ of ingested water, and the highest one in TDB-1, corresponding to $-7.0‰$ of ingested water. The minimum value recorded in the only Early Bronze Age molar (TDB-6) is even lower, corresponding to $-11.7‰$ of ingested water. These results allow us to stress two points which appear evident and require an attempt of explanation: markedly low values and unusually narrow range of amplitude of $\delta^{18}O_{\text{en}}$ values, even in teeth formed over 1 year (Fig. 5).
It is apparent that the $\delta^{18}O_{mw}$ values obtained from $\delta^{18}O_{en}$, ranging in obligate drinking cattle from $-11.7$ to $-7.0$‰, are consistently lower than expected, especially as regards the supposedly summer values. The lowest $\delta^{18}O_{mw}$ values ($-11.7$‰ and $-10.6$‰, TDB-6 and TDB-5) are as low as the modelled winter values from the site and winter instrumental data from the weather station of Bossea (Table 4). Considering a certain degree of dampening affecting isotopic intra-tooth records (Passey and Cerling 2002; Hoppe et al. 2004; Blumenthal et al. 2014; Green et al. 2018), largely due to the sampling method (Zazzo et al. 2005; Pederzani et al. 2021), the actual value of the water intake corresponding to the $\delta^{18}O_{en}$ minima may have been even lower, compatible with $\delta^{18}O_{mw}$ modelled values from mountain pastures located above the site.

On the other hand, the highest enamel-derived $\delta^{18}O_{mw}$ values ($-7.0$‰ and $-7.4$‰, TDB-1 and TDB-2) are too low to appear compatible with the modelled ($-1.5$‰) and instrumentally recorded ($-3.1$‰) composition of meteoric water from the coastal plain (especially during warm months), even allowing for dampening effect (Fig. 8). As a confirmation, the series TDB-4/TDB-5, formed over at least one year, never reaches values higher than $-7.7$‰. The potential impact of lactation on the data could not fully explain this anomaly, as it would have probably resulted in slightly higher pre-weaning values (Fricke and O’Neil 1996; Franz-Odendaal et al. 2003), due to the presence of $\delta^{18}O$-enriched plant water in cow’s milk (Camin et al. 2008).

It is difficult to provide a straightforward interpretation for the results of stable oxygen isotope analysis, as direct measurements of water across the altitudinal gradient would be needed, and the number of samples should be increased including more sites. However, at least, as regards Tana del Barletta, a couple of possible scenarios can be advanced.

**Livestock management scenarios inferred from stable oxygen isotope series**

According to the currently available data, the total oxygen isotope amplitude of water ingested by cattle mostly overlaps with the annual range of values expected from relatively high elevations (Fig. 8), suggesting isotopically depleted inputs all-year-round. This fact would have relevant implications in terms of herd management and use of the site, as it would point to a more structured occupation of the highlands, where high-altitude caves do not serve only as seasonal or occasional sheds, but as stable outposts integrated into a pastoral system intensively exploiting upland territories. This view may be contextualised by the presence of human remains pertaining to c. 20 individuals of all ages recovered at the cave of Grotta del Pertuso (1330 m a.s.l.), to explain which Maggi and Nisbet (1990) hypothesised the existence in the area of a blood-tied clan engaged in herding activities.

On the other hand, the isotopic range of the calculated cattle water intake (probably reduced due to signal averaging from amelogenesis) also partly overlaps with winter values from the lowlands (Fig. 8), leaving open the possibility that a certain seasonal mobility occurred, which may have included grazing at low altitudes during cold months.

In addition, more subtle factors may be also responsible for low values of ingested water. For example, particularly negative measurements may be due to local environmental parameters, such as the impact of snow melting on the drinking sources accessed by the animals or the intrusion of isotopically lighter groundwater (Rozanski et al. 1993; Geyh et al. 2000; Hermes et al. 2018). The ingestion of water from buffered sources such as large lakes and rivers, isotopically different from local precipitation (Gonfiantini 1986; Pederzani et al. 2021), can be ruled out, given their absence in the study area.

Also, human behaviour can affect the signal, such as herders provisioning water to the animals from $\delta^{18}O$-depleted sources (Makarewicz 2017b). In this regard, available meteorological records from the station of Mt. Settepani (1375 m a.s.l.) show the presence of c. 50–100 cm of snow nowadays on the area of Mt. Galero during harsh winters (ARPA 2020). Similar environmental variables, as well as frequent grazing at high elevations, may also have affected the unexpectedly low oxygen isotope ratio of meteoric water calculated from cattle and caprine bone at the coastal cave of Arene Candide (D’Angela and Longinelli 1993).

Moreover, the effect of percent error and variation in analytical precision observed in $\delta^{18}O$ measurements is too reduced to determine such low values (see above, and Online Resource 1), and even the use of equations to estimate the oxygen isotopic composition of meteoric water, which according to Pollard et al. (2011) and Pryor et al. (2014) should bear an uncertainty of c. ±1–2‰, may not be enough to account for these results. Finally, the inferred low isotopic values of precipitation cannot be attributed to cooler climatic conditions, as the dates of all samples analysed in this study fall within the end of the Atlantic and the full Subboreal periods, when temperatures were broadly similar to and not cooler than in the current Subatlantic (Marcott et al. 2013). Mean July air temperatures reconstructed for the northern Apennine area also appear rather uniform over the last 5000–6000 years (Samartin et al. 2017), and the Abies forests were already declining, paving the way to the spread of Fagus woodland, which still is the dominant biome in the lower montane belt (Branch and Marini 2014; Branch et al. 2014; Arobba et al. 2018).
Table 7  Summary of δ¹⁵N and δ¹³C values of dentine collagen and jaw bone from cattle and caprine teeth and jaws from Tana del Barletta (means refer to mid-range values). Mnd, mandible; Mxl., maxilla. C:N ratios, C content, N content and collagen yield provided in Online Resource 5.

| Sample | Taxon | Tooth/bone | Mnd. | Mxl. | No. sub-samp. (N) | δ¹⁵N min. (%e) | δ¹⁵N max. (%e) | δ¹⁵N mean (%e) | No. sub-samp. (C) | δ¹³C min. (‰) | δ¹³C max. (‰) | δ¹³C mean (‰) | δ¹³C range (‰) |
|--------|-------|------------|------|------|-------------------|----------------|----------------|----------------|-----------------|---------------|---------------|---------------|----------------|
| TDB-1  | Bos   | M1/3       | x    | 11   | 3.5               | 30.8           | 4.5            | 24.5           | 4.0             | 1.0           | 11            | -21.9         | 33.5          | -21.5         | 2.5            |
| TDB-2  | Bos   | M1/3       | x    | 7    | 3.8               | 16.1           | 4.3            | 2.0            | 4.0             | 0.5           | 7             | -21.6         | 29.15         | -21.4         | 12.1           |
| TDB-3  | Bos   | M1/3       | x    | 7    | 4.8               | 6.0            | 5.9            | 26.0           | 5.1             | 1.1           | 7             | -21.6         | 26.0          | -21.3         | 21.3           |
| TDB-4  | Bos   | M1/2       | x    | 4    | 4.3               | 2.9            | 4.6            | 10             | 4.4             | 0.3           | 5             | -22.1         | 17.3          | -21.3         | 10             |
| TDB-5  | Bos   | M2/3       | x    | 7    | 3.8               | 13.9           | 4.1            | 2.7            | 3.9             | 0.3           | 7             | -21.6         | 6.5           | -21.4         | 21.7           |
| Jaw    | Bos   | bone       | x    | 1    | 3.2               |                |                |                |                 |               |               |               | -21.4         |               |
| TDB-4–5| Bos   | M1/3       | x    | 7    | 5.1               | 16.1           | 5.3            | 22.3           | 5.2             | 0.2           | 7             | -21.4         | 16.1          | -21.2         | 25.8           |
| Jaw    | Ov./  | bone       | x    | 1    | 4.7               |                |                |                |                 |               |               |               | -20.6         |               |
| TDB-7–8| Cap   | x         | 1    | 4.5   |                |                |                |                |                 |               |               |               |               |
| Jaw    | Ov./  | Cap       | x    | 1    | 4.5               |                |                |                |                 |               |               |               | -21.3         |               |

*C:N ratio, C content and N content out of the accepted range
Whichever scenario best describes the situation occurring at Tana del Barletta, the flatness of the $\delta^{18}O_{en}$ trends (Fig. 5) contrasts with the typically sinusoidal pattern usually observed in bovid enamel from high- and mid-latitudes (e.g. Balasse et al. 2012a, b; Towers et al. 2014, 2017; Knockaert et al. 2018), often showing a large ($\geq 3.5\%$) intra-tooth variation due to seasonality (e.g. Buchan et al. 2016; Makarewicz et al. 2017; Tornero et al. 2018), unlike records from low latitudes (Janzen et al. 2020). In this study, four cattle teeth exhibit a maximum intra-tooth range below 1.5\%, and no clear and pronounced S-shaped patterns are identifiable in the dataset, with the exception of TDB-1, along perhaps
with attenuated curves in TDB-4 and TDB-6. The same situation also concerns the only sheep/goat second molar (TDB-8), characterised by a flat curve and a small intra-tooth range (1.0‰). Some seasonal fluctuations may be captured by caprine first molars TDB-7 and TDB-10, but their record does not span a complete annual cycle (Weinreb and Sharav 1964; Milhaud and Nézit 1991; Zazzo et al. 2010). Truncated annual sequences may also be partly responsible for apparently reduced intra-tooth δ¹⁸O ranges in cattle enamel, as, unlike in teeth attached to the jaws, identification as M1, M2 and M3 is not possible for loose two-lished maxillary molars.

However, if flattened δ¹⁸O patterns reflect reality, as it is likely at least in the case of the TDB-4–5 series, this anomaly may be accounted for within the model pushed forward by Britton et al. (2009), according to which migration from cooler summer locations to warmer winter locations results in a buffered pattern lacking the seasonal extremes (cf. Henton 2012). Seasonal movement from the mid-slope zone around the site and the mountain-top pastures may have played a role in the seasonal oxygen isotope signal at the cave site.

**Fig. 7** Map showing the location of Tana del Barletta (star), the mountain pastures of Mt. Galero (triangle), the coastal plain of Albenga (circle) and the IAEA (International Atomic Energy Agency) pluviometric stations considered in this study (1 = Genoa; 2 = Bossea; 3 = Monaco).

**Fig. 8** Amplitude of the calculated δ¹⁸O range of water ingested by cattle (horizontal rectangle) and ranges of each tooth (vertical white rectangles). Grey vertical rectangles show the seasonal amplitude of meteoric water interpolated for the cave site, mountain pastures and coastal plain, and the seasonal amplitude of meteoric water measured at the weather stations of Genoa and Monaco, along with November-March values from Bossea (instrumental data from this location are lacking for the summer period).
in evening out the δ18O gradient observable in the analysed samples, especially reduced in cattle molars. Further intra-tooth sequences spanning long periods from Ligurian upland sites will be crucial to reach a firmer conclusion.

The difference between evaporation-insensitive (cattle) and evaporation-sensitive (caprines) taxa appears clearly in the c. 1-year-spanning sheep/goat contiguous series TDB-7–8 (M1 + M2). The oxygen isotope maxima and minima recorded for this specimen are ~2–2.5‰ higher than the corresponding extremes in cattle samples, probably as a result of 18O-enriched leaf water in the ingested browse caused by evaporative fractionation (Kohn 1996; Kohn et al. 1998). A different picture results from the sheep/goat series TDB-9–10 (P4 + M1), characterised by a particularly low minimum value (~7.8‰), corresponding to ~9.0‰ of atmospheric precipitation, which suggests for this specimen access to the same open water sources ingested by cattle during the cold season. However, in winter, a smaller offset between obligate and semi-obligate drinkers should be expected (Makarewicz 2017a). Further inferences are not possible, as this series does not represent a full seasonal cycle, due to the timing of formation of caprine first molar crowns (c. 6–9 months; Weinreb and Sharav 1964; Milhaud and Nézit 1991; Zazzo et al. 2010).

Assessing coastal grazing through nitrogen stable isotopes

The nitrogen isotopic composition of collagen has been used in previous studies to track the inclusion of 15N-enriched coastal vegetation in the diet, but to date the approach had not been applied to Mediterranean settings. All samples from Tana del Barletta exhibit δ15N values with reduced intra-tooth range (~1‰ or less) consistently comprised between 3.5 and 5.9‰, falling within the expected range for herbivores feeding exclusively on inland plants (Schoeninger et al. 1983) (Table 7; Fig. 6). The limited amplitude of intra-tooth variations may be an effect of sampling through the whole dentine thickness, in addition to further complications due to the formation of secondary dentine later in life (Frank and Nalbandian 1989; Balasse 2003). Interestingly, the M1/2 and M2/3 associated with maxillary bone show decreasing mean values (TDB-4: 4.4 ‰; TDB-5: 3.9 ‰; bone: 3.2 ‰), possibly reflecting the cessation of milk consumption due to the weaning process (Balasse et al. 2012b). This might be indicative of animal grazing within the understory of a densely forested environment, in agreement with the charcoal analysis suggesting a mixed deciduous woodland around the site (Del Lucchese et al. 1987), or point to the intake of 13C-depleted leafy fodder (Balasse et al. 2012b).

Conclusions

Given the lack of knowledge regarding the reliability of maxillary cattle teeth to record oxygen and carbon isotope fluctuations, we assessed intra-tooth variations in four upper and lower M1s from a modern specimen. The measured values...
show similar patterns and very close minima, maxima and range of amplitude. No shifts between lower and upper M1s were observed. However, a c. 5-month discrepancy has been estimated between maxillary and mandibular M3 isotopic records (Balasse et al. 2021). Further research is needed to clarify this issue and better understand the differences in the isotopic signal between upper and lower dentition. This is particularly relevant for archaeological assemblages, where mandibular teeth may not always be available (see e.g. Balasse et al. 2021).

According to the available estimates for the oxygen isotopic composition of environmental water, both maximum and minimum δ18O values of cattle enamel appear particularly low. This may point to the exploitation of high elevations throughout the year, which may have involved a prolonged presence of the herd between the site and upland pastures. In this regard, it should be stressed that, between the 4th and 3rd Millennium BC, palaeobotanical and sedimentological records from the Ligurian mountains suggest anthropogenic disturbance associated with woodland clearance, in order to increase the availability of pastureland (Maggi and Campana 2008; Cruise et al. 2009; Branch and Marini 2014). However, according to the estimates for the isotopic composition of precipitation, for the specimens of Tana del Barletta the input of water from the lowlands in the summer months cannot be ruled out, suggesting the possibility of seasonal animal mobility. Such a scenario would be compatible with a view proposed by previous works, which indicated the transition to the Late Neolithic (marked in Liguria by the Chassey culture) as a possible onset for transhumant pastoralism (Binder et al. 2008; Maggi and Campana 2008).

It is nevertheless unlikely that grazing occurred directly on the coastal plain, as shown by the nitrogen isotopic composition of cattle dentine collagen, the values of which rule out the ingestion of salt-tolerant vegetation or seaweed. This is confirmed by the carbon isotopic composition of cattle dentine and enamel, which falls within the range of inland C3 plants.

The amplitude of the δ18O intra-tooth variation from both cattle and sheep enamel often appears particularly narrow. This pattern contrasts with the characteristic sinusoidal curve observed repeatedly in archaeological and modern domestic herds at temperate latitudes. Such a rather homogeneous trend can be partly explained by assuming some form of seasonal vertical movement of the specimens between the site and a potential lowland settlement (Barker et al. 1990; Maggi and Campana 2008). This would reduce the observable fluctuation, due to the use of drinking sources from higher altitudes in the hot season (18O-depleted), and drinking sources from lower altitudes in the cold season (18O-enriched) (Britton et al. 2009; Henton 2012). However, it should be borne in mind that these considerations are based on the reduced number of samples available from the cave, and larger assemblages from a variety of sites should be analysed in order to reach more secure conclusions which can be generalised on a regional scale.

Finally, the diverse geology characterising the area surrounding the cave in a range of few kilometres appears particularly promising for a future application of strontium isotope analysis to the tooth assemblage from Tana del Barletta. Along this, we aim to obtain direct δ18O measurements from different water sources at various elevations, assessing whether localised environmental variables may have contributed to the markedly low δ18O maximum values observed in the dataset from the site.

Further investigations of contemporary transhumant herds with known feeding histories from different geographical and environmental contexts will also be helpful to resolve certain equifinalities and provide more robust interpretative frameworks. In particular, there appears to be a paucity of isotope studies on southern European and Mediterranean settings, where only in recent years research has been intensified (e.g. Valenzuela-Lamas et al. 2016; Knockaert et al. 2018; Tornero et al. 2018; Trentacoste et al. 2020) and transhumance is a central topic (Maggi et al. 1990a, b; Barker 2005; Arnold and Greenfield 2006; Carrer and Migliavacca 2019).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12520-021-01418-w.

Acknowledgements Dr. Daniele Arobbia provided expertise in the regional flora, Prof. Nick Branch supported the funding application, Dr. Paola Calegari provided references on bovid drinking habits. Thanks are also due to Mariavittoria Parravicini and Fabio Fornoni for providing accommodation during the initial stage of the research. The authors are indebted to two anonymous reviewers for their valuable comments on the manuscript.

Author contribution L.F.M. devised the project, produced the main conceptual ideas and interpretations, wrote the paper with inputs from D.F. and created the figures. L.F.M. and D.F. carried out laboratory work on enamel samples. L.F.M. and G.M. carried out laboratory work on collagen samples. D.F. and G.M. helped supervise the project. R.M. directed the excavation of the site, provided expertise in the archaeology of the region and assistance to obtain faunal samples.

Funding Open Access funding enabled and organized by Projekt DEAL. Radiocarbon dating of teeth from Tana del Barletta was supported by the National Radiocarbon Facility of the Natural Environment Research Council and Arts and Humanities Research Council (UK), grant NF/2017/2/15.

Data availability Not applicable.

Code availability Not applicable.
Declarations

Ethics approval  Not applicable.

Consent to participate  Not applicable.

Consent for publication  Not applicable.

Competing interests  The authors declare no competing interests.

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