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Oxygen and carbon isotopic analysis in terrestrial snail shells: from methodology to interpretations

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ABSTRACT:

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Mollusc shells are among the most common fossil remains in Quaternary sediments. Classical palaeoenvironmental reconstructions are based on mollusc assemblages and the ecological preferences of the species. These approaches can be complemented by stable isotope analysis, which adds information on the environmental conditions under which the organisms lived. Oxygen isotope values in land snail shells are mainly used to estimate the isotopic composition of precipitation and can also provide temperature estimates. The carbon isotope values reflect the diet and the dominant vegetation type (e.g., C3 vs. C4 plants) in their surroundings. Despite decades of study, the processes controlling the incorporation of stable oxygen and carbon isotopes into land snail shells are still not fully understood, especially the relative influence of environmental versus biological factors. This understanding is essential for an accurate interpretation of the isotopic data. This paper reviews research on stable isotope analysis of land snail shells. It summarises methodologies, sampling strategies, and sample preparation techniques for oxygen and carbon isotope measurements. It also discusses applications of isotopic data in palaeoclimatic and palaeoenvironmental reconstructions using modern and fossil shells, highlights interpretative limitations, and examines biological and environmental factors that influence isotopic signatures.

Key words: Stable isotopes; Land snails; Molluscs; Palaeoenvironment reconstructions; Quaternary

INTRODUCTION

Mollusc shells are one of the most abundant animal macrofossils in Quaternary sediments, deposited in marine, freshwater and terrestrial environments (Goodfriend 1992; Berglund and Ralska-Jasiewiczowa 2003; Prendergast *et al.* 2015; Yanes *et al.* 2018; Rech *et al.* 2021). Due to their sensitivity to environmental changes and their widespread distribution, snail shell analysis has emerged as a versatile approach to climate reconstruction, proving to be effective across various regions of the world (Wang *et al.* 2016; Zhai *et al.* 2019).

The vast majority of Quaternary malacological studies have so far focused on the taxonomic composition of mollusc assemblages. Palaeoenvironmental reconstructions were based on the assumed ecological requirements of the identified taxa. This is justified because many of the gastropod remains found in Quaternary sediments belong to extant taxa. Their specific requirements and life preferences, such as moisture content, living habitat, and optimal activity temperature, are simple extrapolations of those of their modern relatives (Goodfriend 1992). It is much more difficult to deal with extinct species, whose ecological preferences remain unknown. In this case, palaeoenvironmental reconstructions based solely on the taxonomic composition of the assemblage may be limited or even impossible.

With the advancement of isotope ratio mass spectrometry, the scope of investigations has expanded to include the analysis of stable oxygen and carbon isotopes in mollusc shells, offering new perspectives for palaeoenvironmental studies. Stable isotope analysis quantifies the ratio between the content of heavy and light isotopes of the same element, reflecting the isotopic composition of the environment in which the shell formed. The main focus of stable isotope biogeochemistry in palaeomalacology is on the shell (Yanes *et al.* 2018). Its main component, calcium carbonate (aragonite), contains the two non-metals: oxygen and carbon, each with at least two easily detectable stable isotopes. The proportions between the heavy and light isotopes, that is $^{18}O/^{16}O$ and $^{13}C/^{12}C$, are variable and depend on several factors.

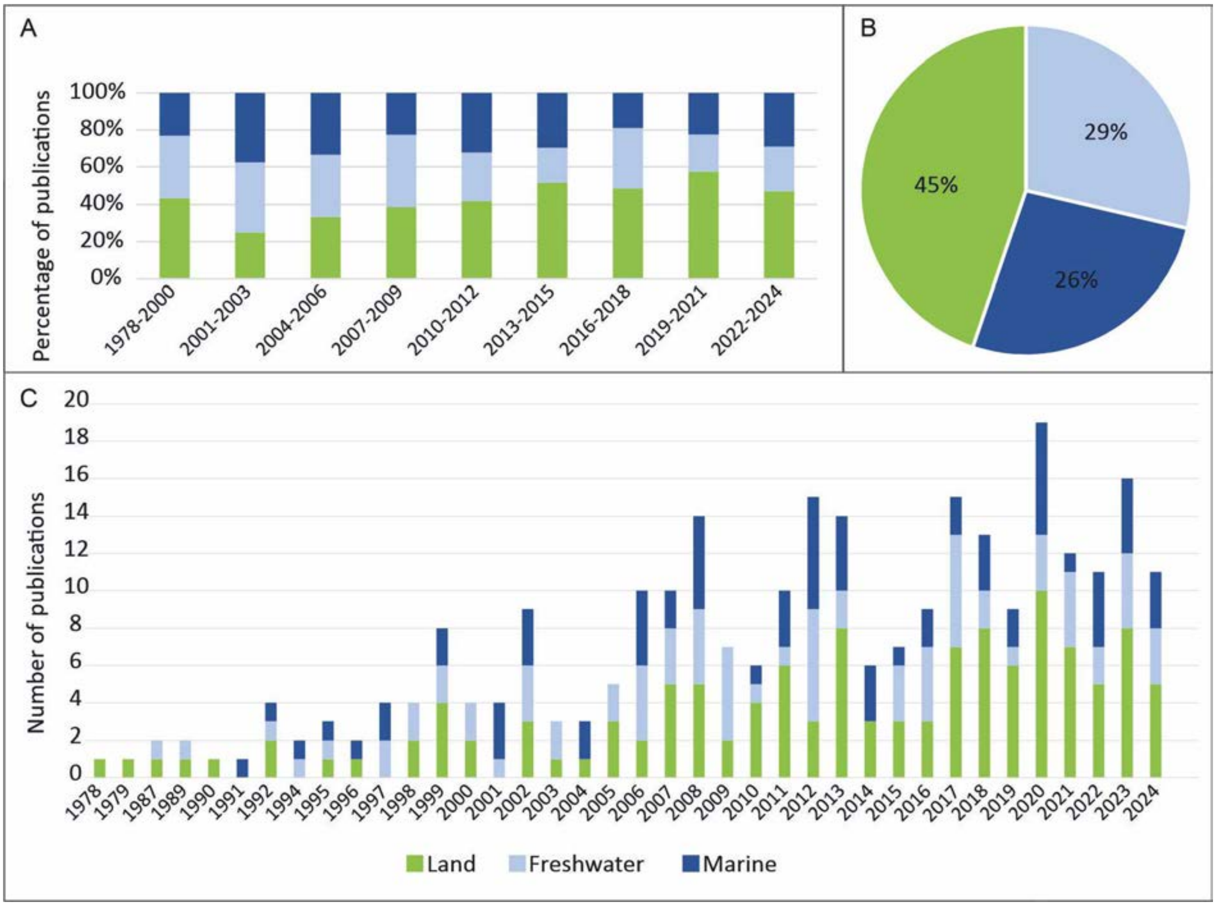
The widespread occurrence of mollusc shells in Quaternary sediments around the world has inspired many studies to examine the relationship between stable shell carbonate composition, environment, climate, and ecology. The results of these studies indicate that the analysis of isotopic values of terrestrial gastropod shells can provide a valuable source of environmental data (Yapp 1979; Goodfriend 1992; Balakrishnan and Yapp 2004; Yanes *et al.* 2011). Studies carried out in different parts of the world have found various relationships between $\delta^{18}O$ and $\delta^{13}C$ values and the environment and the ecology of terrestrial gastropods. The situation is analogous for the shells of aquatic snails.

Shells of marine and freshwater snails are widely used for stable oxygen and carbon isotope studies. They precipitate in isotopic equilibrium with the surrounding water (Fritz and Poplawski 1974; Rahimpour-Bonab *et al.* 1997; Leng *et al.* 1999; Latal *et al.* 2004; Anadón *et al.* 2010; Long *et al.* 2020). The $\delta^{18}O$ values in marine and freshwater snail shells depend primarily on temperature and the isotopic composition of the surrounding water (Fritz and Poplawski 1974; Jones *et al.* 2002). Based on stable oxygen values, it is possible to infer the temperature and isotopic composition of the surrounding water. Additionally, in the case of marine species, the salinity level can be reconstructed (Andreasson and Schmitz 1996; Lécuyer *et al.* 2012; Apolinarska *et al.* 2015; Roy *et al.* 2019; Chen *et al.* 2020). The $\delta^{13}C$ values of snail shells depend on the $\delta^{13}C$ value of dissolved inorganic carbon (DIC) in marine or lake water and, to a lesser extent, on carbon of metabolic origin (Fritz and Poplawski 1974; Goodfriend and Ellis 2002; McConnaughey and Gillikin 2008; Lartaud *et al.* 2010; Apolinarska *et al.* 2015; Wang *et al.* 2019). It is influenced by three main processes: the isotopic composition of inflowing water, the exchange of CO_2 between the atmosphere and water, and water plant photosynthesis (Leng and Marshall 2004; Milano and Szymanek 2019). Changes in the shell $\delta^{13}C$ values can be used to reconstruct relative changes in ancient DIC $\delta^{13}C$ values (Apolinarska and Pełechaty 2017), which in turn, provide insights into shifts in the carbon cycle in both freshwater and marine environments (Hendry and Kalin 1997; Malchus and Steuber 2002; Schöne *et al.* 2004; Lécuyer *et al.* 2012).

Studies on stable oxygen and carbon isotopes in terrestrial snail shells are less developed than those on freshwater and marine snails. However, significant advances have been made in recent years, and the number of publications from the last 10 years (2014–2024) is almost equal to the total number of publications from earlier decades (Text-fig. 1). The study of $\delta^{18}O$ and $\delta^{13}C$ in aqueous taxa is more advanced than in terrestrial taxa, mainly due to the better predictability of the isotopic values of the water in which they lived. Water snails provide precise information on palaeotemperatures and the carbon cycle in freshwater and marine environments, and their application in palaeohydrological reconstructions is well documented (Gasse *et al.* 1987; Kobashi *et al.* 2004; Sessa *et al.* 2012; Apolinarska *et al.* 2016; Szymanek 2017; Asami *et al.* 2021; Mirosław-Grabowska *et al.* 2025). In the case of terrestrial snails, stable oxygen and carbon isotopes show great potential for climatic and environmental research; however, interpreting these results is more complex and requires consideration of additional factors.

Land snails depend on an external heat source, have low migratory capacity, and live relatively short lives (usually 2–5 years) (Kehrwald *et al.* 2010). Furthermore, they are subjected to continuous fluid loss during life processes, resulting in the need for almost constant water intake (Balakrishnan and Yapp 2004; Prendergast *et al.* 2015). During dry and warm periods, some snails enter a state of aestivation (Schweizer *et al.* 2019). All of this, combined with their low metabolic rate, sensitivity to climatic and environmental fluctuations, and the specificity of shell precipitation, makes them ideal candidates for a potential palaeoenvironmental proxy (Yapp 1979; Prendergast *et al.* 2015; Nield *et al.* 2022).

The isotope analyses of fossil and modern shells show that $\delta^{18}O$ in snail shells predominantly reflects precipitation and temperature. In contrast, $\delta^{13}C$ is influenced more significantly by the diet and bal-



Text-fig. 1. Publications on the analysis of oxygen and/or carbon isotopes in snail shells published between 1978 and 2024, categorised by the snails' habitat: A – cumulative percentage of publications across successive time intervals; B – overall percentage distribution of all publications by habitat; C – number of publications per year.

ance of the surrounding vegetation (Yapp 1979; Balakrishnan and Yapp 2004; Colonese *et al.* 2007; Yanes *et al.* 2008; Yanes and Fernández-Lopez-de-Pablo 2017; Padgett *et al.* 2019; Nield *et al.* 2023; Quenu *et al.* 2023). Over the years, numerous significant scientific experiments have been conducted, enhancing our understanding of the influence of environment, diet, and behaviour on the isotopic record in land snail shells (Scott 2002; Metref *et al.* 2003; Balakrishnan and Yapp 2004; Zhang *et al.* 2014, 2018*b*). Due to the remarkable complexity of these interpretations and the extensive array of factors affecting stable oxygen and carbon isotope values, experiments on living organisms have been instrumental in uncovering the intricate mechanisms of isotope incorporation into shells (Balakrishnan and Yapp 2004; Prendergast *et al.* 2015; Zhang *et al.* 2018*a*).

This paper aims to highlight the potential of land snail shells as proxies for palaeoenvironmental and palaeoclimatic reconstructions, focusing on the analysis of stable oxygen and carbon isotopes. The paper also provides an overview and emphasises the importance of modern research methods, which enable more precise palaeoenvironmental studies and complement traditional approaches.

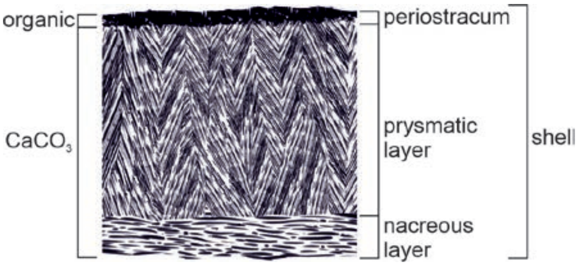
THE SNAIL SHELL AND STABLE ISOTOPES OF OXYGEN AND CARBON

Shell formation and mineralization

The gastropod shell provides an external skeleton and protects against predators and unfavourable environmental conditions (Goodfriend 1986;

Skompski 1991). A snail shell is an organo-mineral compound that typically consists of three distinct layers: the outer periostracum, which remains uncalcified, the prismatic calcareous layer, and the inner nacreous layer, which is calcified (Text-fig. 2) (Suzuki and Nagasawa 2013). The mineral layers usually constitute the dominating part of the shell volume, while the organic layer often constitutes from 1 to 5% of the entire shell (Song *et al.* 2019). The organ responsible for shell formation is the mantle, a thick muscle sheet surrounding the visceral sac that covers the inner surface of the shell. The mantle contains an epithelial gland, a layer of cells that covers the inner surface of the mantle (Marin *et al.* 2012). The epithelium regulates the biomineralization process of shell formation by secreting proteins and minerals and participates in their transport, which later combine and solidify, initiating the formation of the shell (Jones 1935; Wiktor 2004; Addadi *et al.* 2006). The periostracum, an organic membrane, is the first layer to form, followed by the deposition of the calcified layers (Suzuki and Nagasawa 2013). The process begins with the deposition of conchiolin, a natural polymer composed of proteins and chitin, which forms an initial uncalcified layer. After death, conchiolin undergoes a rapid degradation process. Calcified layers include the prismatic layer and the nacreous layer, the latter of which is responsible for the shell's pearly appearance. Aragonite crystals in a nacreous layer diffract light, contributing to the shell's lustre (Marin *et al.* 2012). The mollusc shell develops by either adding calcium carbonate to the edges or growing from the bottom upward. During shell formation, ions and organic matrices are supplied to the interface between the mantle and the shell. The carbonate snail shell is usually composed of two polymorphs of calcium carbonate, namely aragonite and calcite. Aragonite is a metastable form of calcium carbonate. It means that it tends to transform into calcite as a result of diagenesis and post-sedimentary processes. There is also a third variety of calcium carbonate, vaterite. This polymorph is very unstable, and its presence in snail shells, if observed at all, is a small proportion (Hasse *et al.* 2000). Land snail shells are most often composed of aragonite (Tompa 1976, 1979; Fernández *et al.* 2016).

Until now, only one study (Grey *et al.* 2024) of freshwater pearl mussels *Margaritifera margaritifera* has demonstrated a shift in δ^{18}O values of the prismatic layer relative to the isotopic composition of the surrounding water, which is in contrast with δ^{18}O of the prismatic layer, which was consistent with the water isotope signal. The occurrence of this phenomenon in land snail shells remains an open question.



Text-fig. 2. Cross section of snail shell, based on Samek (2004: p. 18).

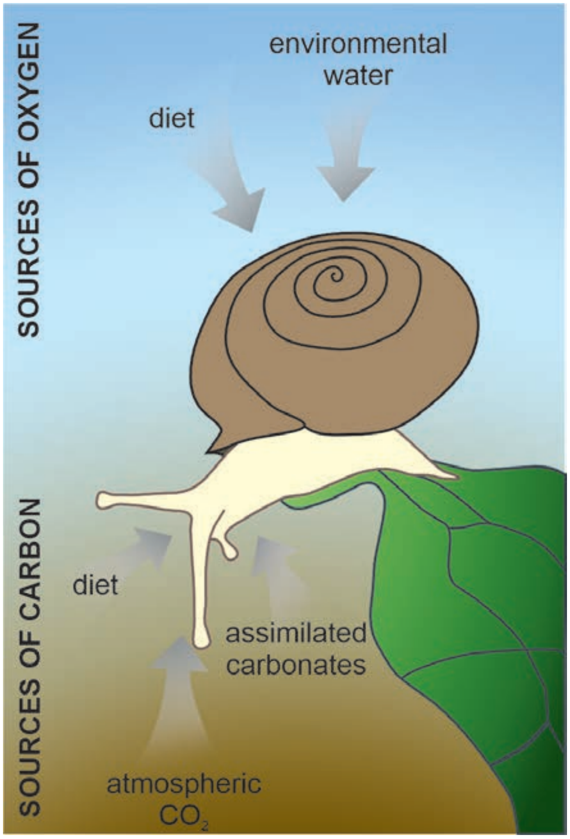
Molluscs enlarge their shells during their life. Growth and development are not continuous processes but periodic ones related to the snail's metabolic activity (Schöne 2008). The presence of growth lines marks individual stages of shell growth. Growth rate is sensitive to environmental conditions, varies between species and individuals, and increases during periods of higher temperatures and humidity. It also depends on food availability. Land snails in temperate regions grow more rapidly during summer and little or not at all during winter and very dry periods, when they aestivate. In the monsoon area, growth usually falls in the summer months, while in the Mediterranean region, snails are most active in winter, autumn, and spring before temperatures become too high and humidity too low (Balakrishnan and Yapp 2004; Zhai *et al.* 2019). Throughout the lifespan, the fastest rate of growth occurs during the early stages of development (Marin *et al.* 2012). Once it reaches its peak, it tends to decline as the animal approaches maturity, until it completely stops. The direction of growth is independent of the growth rate. In many species, spiral growth ceases after reaching sexual maturity, which is usually marked by a well-developed aperture (Goodfriend 1986; Vermeij 2021).

Damaged shells can be repaired using bicarbonate secretions from the mantle. Proteins secreted from the mantle bind to calcium ions, aiding in the formation of calcium carbonate crystals in a specific hierarchical pattern. Despite significant research, the precise mechanisms of this process remain only partially understood (Marin *et al.* 2012).

Sources of shell oxygen and carbon

Land snails take oxygen and carbon from the environment. The oxygen component of the shell comes mainly from: 1) diet and 2) environmental water, which includes, among others, precipitation and ambient water in the form of water vapour and dew. Snails can also absorb liquid water taken up by the surface of the foot (contact hydration) (Text-fig. 3) (Yanes *et al.* 2011; Colonese *et al.* 2013; Colonese 2017; Yanes and Fernández-Lopez-de-Pablo 2017; Padgett *et al.* 2019; Rech *et al.* 2021; Zong *et al.* 2022). Carbon that builds up in the carbonate of the snail shell comes from three main potential sources, differing in isotopic signatures: 1) diet – metabolic CO_2 derived from ingested organic matter, including CO_2 produced during cellular respiration; 2) atmospheric CO_2 – absorbed directly from the surrounding air; 3) ingested inorganic carbonates – CO_2 released through the reaction of dietary $CaCO_3$ with gastric acids (Text-fig. 3) (Yapp 1979; Goodfriend 1992; Scott 2002; Balakrishnan and Yapp 2004; Colonese *et al.* 2007; Yanes *et al.* 2008; Colonese 2017; Yanes and Fernández-Lopez-de-Pablo 2017; Padgett *et al.* 2019; Jenkins *et al.* 2023; Nield *et al.* 2023; Quenu *et al.* 2023).

Terrestrial snails are predominantly herbivorous; however, some species exhibit alternative feeding strategies, functioning as predators, scavengers, or omnivores. Certain species also incorporate detritus into their diet. This dietary diversity reflects their adaptations to various environmental conditions and available food resources. Predatory gastropods usually feed on other gastropods or oligochaetes. Herbivore snails are usually feed on the living parts of plants, leaves, tubers, shoots, or fruits. Snails also eat algae, lichens, and fungi. Each species may be specialised in consuming one type of food that is related to the structure of the mouth apparatus. The least specialised are omnivorous snails, which do not have specific dietary preferences. They feed on various parts of plants, both living and dead, and can eat carrion in the form of dead insects, earthworms, small vertebrates, and vertebrate faeces. The assimilation of water has a significant impact on all kinds of life activities and occurs in different ways in land snails. These organisms can compensate for deficiencies by eating plants or drinking dew or rainwater by absorbing water with the entire surface of the foot (Wiktor 2004).



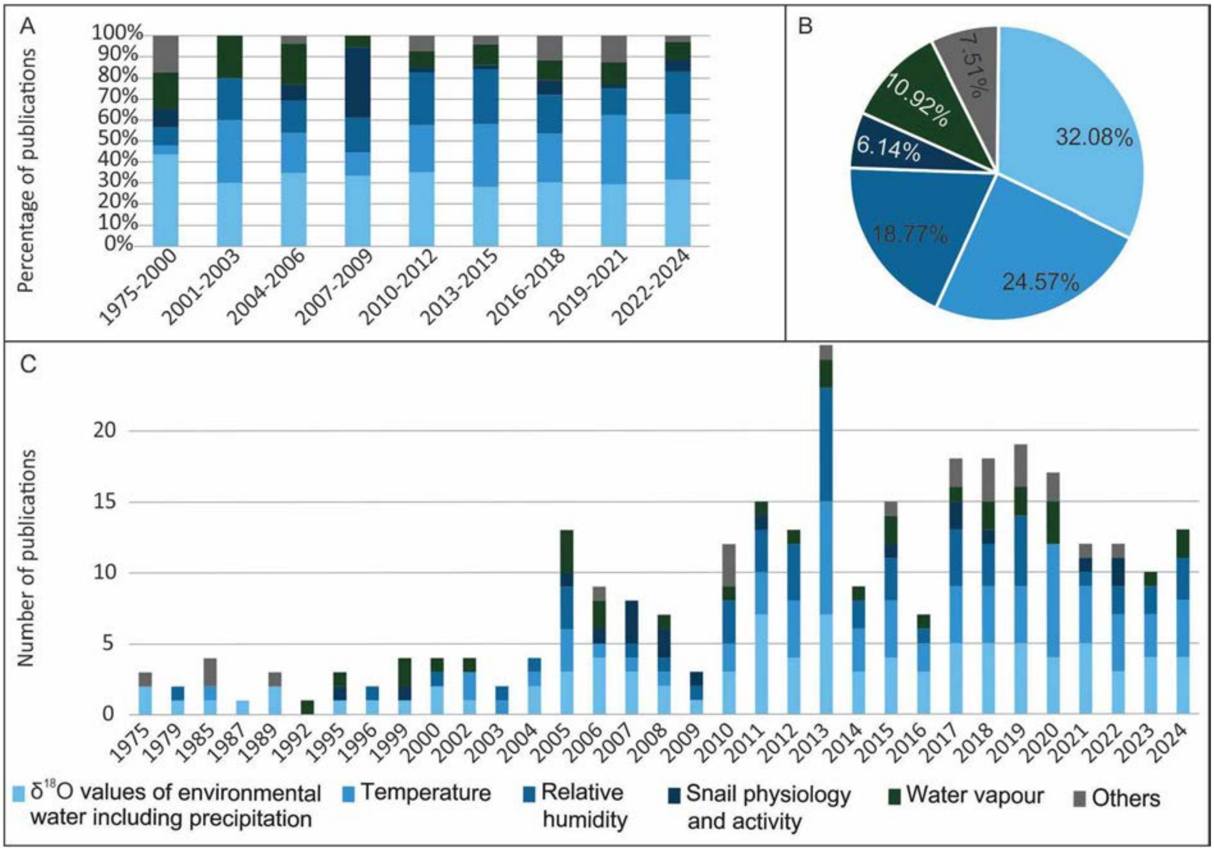
Text-fig. 3. Sources of oxygen and carbon in land snail shells based on literature data cited in the text.

One of the main factors influencing the oxygen isotopic values of the shell is the isotopic composition of the rainfall, which can be classified as part of broadly defined environmental water. Yapp (1979) observed that the differences between $\delta^{18}O$ values in shells and $\delta^{18}O$ values in rainfall are related to the inverse of relative humidity. Lécolle (1985) found a linear correlation between $\delta^{18}O$ in rainfall and altitude, with notable deviations occurring above 1200 metres in oceanic, Alpine, and Mediterranean climates. Goodfriend (1992) indicated that $\delta^{18}O$ values are also influenced by water vapour, suggesting a relationship between atmospheric precipitation and local climatic conditions. In particular, temperature and relative humidity (Balakrishnan and Yapp 2004) impact oxygen isotope fractionation that potentially alters $\delta^{18}O$ values in the shells (Qin *et al.* 2021).

Environmental water is usually referred to as water vapour, dew, and local atmospheric precipitation (Magaritz and Heller 1980; Goodfriend *et al.* 1989; Balakrishnan and Yapp 2004; Dong *et al.* 2020). Hassan (2006) expanded this perspective by including the impact of water loss through the evaporation of snail body fluids and the isotopic values of environmental water during snail activity. Fluid exchange with the environment and metabolic processes (Colonese *et al.* 2007; Prendergast and Stevens 2014), as well as local environmental conditions, also significantly affect $\delta^{18}O$ values in shells

(Balakrishnan *et al.* 2005; Zanchetta *et al.* 2005). The δ^{18}O values may also be related to the seasonality of snail activity, which influences the interpretation of the results according to the time of year (Zong *et al.* 2022). The variability of δ^{18}O in the shells of land snails is due to a complex interaction between local climatic conditions, the properties of environmental water, and the biological processes occurring within the snails. The δ^{18}O values reflect both direct environmental effects and subtle influences related to the behaviour and biology of these organisms. Currently, the main factors considered to influence stable oxygen isotopes in the shells of land snails are precipitation, relative humidity, and temperature. It is also important to consider the influence of other factors, as the understanding of how δ^{18}O values are incorporated into land snail shells has changed over time (Text-fig. 4).

Most studies on stable isotopes in snail shells focus on herbivorous snails, as they represent the dominant group in terms of feeding strategy (Speiser 2001; Le Gall and Tooker 2017; Yanes *et al.* 2018). A model constructed by Goodfriend and Hood in 1983 assumed three main sources of carbon in the shells of land snails: 1) food, 2) respiration, and 3) assimilated mineral carbonates (Goodfriend and Hood 1983; Yanes *et al.* 2008; Wang *et al.* 2019). Carbonate ingestion by terrestrial gastropods appears to be particularly important in areas where carbonate rocks occur on the surface and $CaCO_3$ is readily available. The effect of carbonates ingested can vary between species and locations (Yanes *et al.* 2008; Chiba and Davison 2009; Goodfriend 1992; Goodfriend and Ellis 2002; Wang *et al.* 2016; Padgett *et al.* 2019). Scott's (2002) experimental research under controlled laboratory conditions showed that the carbon in plant food accounts for almost 100% of the carbon that later builds the snail shell. When cultured snails were fed an additional source of $CaCO_3$, the carbonate consumed did not affect the δ^{13}C values (Scott 2002; Metref *et al.* 2003). It is estimated that 60–81% of the carbon in snail shells comes from



Text-fig. 4. The evolution of scientific views on the most important factors affecting oxygen isotope values (δ^{18}O) in land snail shells, based on publications from 1975 to 2024. A – cumulative percentage of views on key factors across successive time intervals; B – overall percentage distribution on the identified factors; C – number of publications reporting major factors influencing δ^{18}O values by year. Figure compiled from literature identified via keyword searches in the Web of Science database.

their plant diet. The proportions of carbon of inorganic origin and atmospheric carbon dioxide vary between 8–27% and 10–17%, respectively (Xu *et al.* 2010; Qin *et al.* 2021).

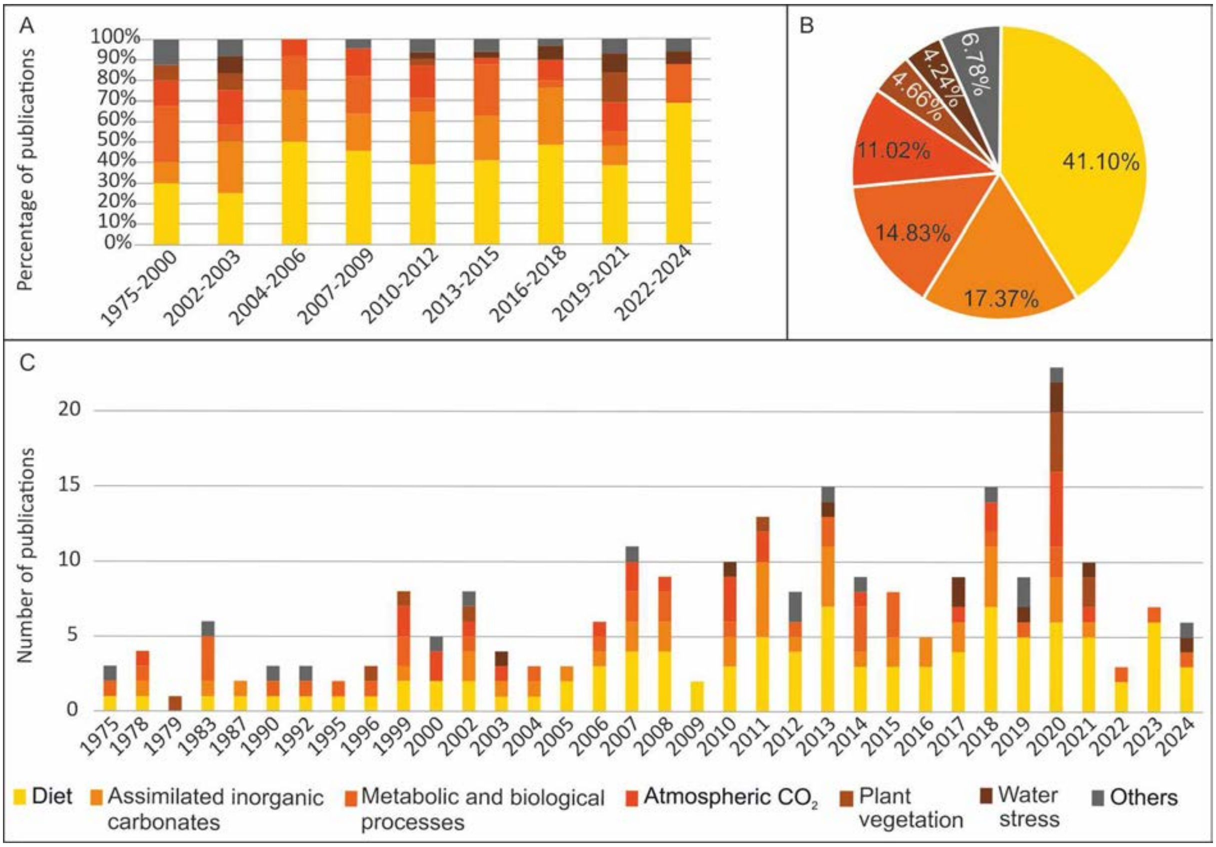
The majority of published studies of fossil and modern land snails assume that they are primarily herbivores that consume vascular plants, which makes their shells indicators of the photosynthetic pathways of the plants they eat and their physiological strategies (Colonese *et al.* 2013; Hassan 2015; Yanes *et al.* 2017, 2018; Zong *et al.* 2022). Local vegetation, specifically the type of photosynthesis (C3 fixation, C4 fixation, Crassulacean Acid Metabolism, also known as CAM photosynthesis), is a critical factor, as highlighted by multiple studies (Balakrishnan *et al.* 2005; Colonese *et al.* 2007; Yanes *et al.* 2011; Stevens *et al.* 2012). Hassan (2006) points out that the isotopic values of organic matter consumed by snails and the climatic conditions, such as aridity, significantly influence δ^{13}C. In more humid environments, where C3 plants dominate, δ^{13}C values tend to be more negative. In contrast, under higher aridity, the scarcity of C3 vegetation, the greater contribution of C4 plants, and increased water stress can result in relatively higher δ^{13}C values, with an additional effect potentially arising from the incorporation of carbon derived from local carbonates (Hassan 2006). Terrestrial plants can perform photosynthesis in three ways: C3 photosynthesis, C4 photosynthesis, and CAM. This adaptation is related to the geographical distribution and the water stress to which plants are exposed. Each of the pathways fractionates atmospheric CO_2 carbon differently, resulting in plant tissues having different δ^{13}C values. Most plants perform C3-type photosynthesis. They do not have special mechanisms to combat wasteful respiration. Furthermore, C3 plants tolerate water stress badly, so they perform well in temperate and humid climates. Their δ^{13}C range is between -33‰ and -23‰ (global average: -27‰ to -26‰) (O'Leary 1981; Balakrishnan *et al.* 2005; Drucker 2022). This group includes all trees, shrubs, forbs, and some grasses (Yanes *et al.* 2018; Padgett *et al.* 2019). C4 plants are subjected to constant water stress and thus forced to reduce photorespiration. They inhabit arid regions. The global δ^{13}C extent of C4 plants ranges from -16‰ to -9‰ (average -13‰ to -12‰) (O'Leary 1988; Balakrishnan *et al.* 2005; Drucker 2022). The C4 photosynthesis pathway is used by approximately 3% of vascular plants. This category includes subtropical and tropical grasses (Hassan 2015). The last major photosynthesis type is CAM. Plants belonging to this group have adapted to dry environments. They are characterised by intermediate fractionation and can show values similar to those for plants of C3 and C4, and intermediate values. A characteristic feature of CAM plants is that they accumulate CO_2 at night and perform photosynthesis during the day. This group includes succulents, some orchids, cacti, and agaves.

Other studies showed the effect of dietary preference on δ^{13}C of the shells (Zhang *et al.* 2014). According to Scott (2002), δ^{13}C values of the diet are reflected in the isotopic composition of body tissues; however, this relationship is less evident in the isotopic record of the shell. In these studies, the δ^{13}C values were, on average, shifted by +12.3‰ relative to the body of the snail in all groups of snails examined. The experiment carried out by Metref *et al.* (2003) revealed a significant influence of diet on the δ^{13}C values of shells, particularly in the case of C3 plants (consistent with Scott's findings). The higher δ^{13}C values observed in snails fed on C4 plants are consistent with the fact that these plants naturally have higher δ^{13}C values than C3 plants, indicating a correlation between the isotopic composition of the diet and the signature recorded in the shell. Similarly, Liu *et al.* (2007) reported the same amplitude of δ^{13}C variation in snail shells and soft tissues, with the shell showing an average enrichment of approximately +14.2‰ relative to body tissues. The consistent difference between the shell and soft tissues suggests that the δ^{13}C values of the shell depend on the isotopic composition of the total carbon pool in the body.

Studies by Colonese (2007), Yanes *et al.* (2011), and Stevens *et al.* (2012) indicate that land snails absorb more CO_2 through respiration than from the surrounding environment and that the isotopic composition of consumed plants and ingested carbonates significantly impact their body carbon isotopes. Equally important environmental factors such as temperature, altitude, rainfall, and local vegetation significantly shape isotopic values in shells (Wang *et al.* 2019; Zhai *et al.* 2019). Quenu *et al.* (2023) conclude that differences in shell δ^{13}C may reflect the efficiency of water use by consumed plants and the influence of seasonal weather conditions. Diet and assimilated inorganic carbonates are most often considered the main factors affecting stable carbon isotopes in land snail shells; however, experimental studies have shown that temperature can also affect δ^{13}C values (Bao *et al.* 2023; Újvári *et al.* 2024) (Text-fig. 5).

Intra- and interindividual isotopic variability

There are quite large differences in oxygen isotopic values within individual shells, up to 4‰ (Prior



Text-fig. 5. The evolution of scientific views on the most important factors affecting carbon isotope values (δ^{18}C) in land snail shells, based on publications from 1975 to 2024. A – cumulative percentage of views on key factors across successive time intervals; B – overall percentage distribution on the identified factors; C – number of publications reporting major factors influencing δ^{13}C values by year. Figure compiled from literature identified via keyword searches in the Web of Science database.

1985; Kehrwald *et al.* 2010; Yanes and Fernández-Lopez-de-Pablo 2017). Intrapopulation variation can also be observed in stable isotope values obtained from shells. Seasonality significantly influences the isotopic record in the shell, while very dry periods of the growing season may not be captured at all (Colonese *et al.* 2007). Snails only build up their shell under favourable temperature and humidity conditions, so only the conditions of some seasons will be recorded in the shell. Hydration stress may be important for some species and types of environment (Moreno-Rueda 2012). This phenomenon involves much faster dehydration of small and young snails compared to mature or larger snails. Due to the nature of their physiology and continuous water metabolism, increased dehydration, and therefore increased water intake, can translate into isotopic values in the shell. The most intense growth of an organism occurs at the beginning of its life (Schöne 2008). Therefore, the early seasons of snail development impact the total isotopic composition of the shell more than the later seasons. Variation in life-history shifts can help explain the substantial differences in isotopic values within a single population.

The behaviour, physiology, and ecology of terrestrial gastropods demonstrate that their ability to survive is directly dependent on their ability to maintain adequate fluid levels in their bodies. Furthermore, due to the narrow specialisation of these molluscs, their locomotive modality, life cycle, activity rhythm, ease of dehydration, and requirements for the environment and climate, they have developed several survival strategies. Consequently, snails show the highest activity at temperatures above freezing. The optimal temperature is estimated to be at least 5–10°C (Skompski 1991). Furthermore, increased snail activity is observed immediately after rainfall or at night when relative humidity is higher than during the day (Balakrishnan and Yapp 2004; Yanes *et al.* 2017). Under stressful conditions, snails enter a state of dormancy. The reason may be temperature (negative or above 27°C) or unfavourable moisture conditions such as drought (Balakrishnan and Yapp 2004). The snail shell only grows when the individual is active, not during hibernation or aestivation (Balakrishnan and Yapp 2004). The shell records information about only the environmental conditions that prevail during the activity of the snail. Therefore, it is important to know which period is represented by the analysed samples. The growing periods vary in length and include different seasons depending on the geographical location (Nield *et al.* 2022). In addition, the rate of carbonate precipitation in the shell can decrease with the age of the snail. However, if samples are taken using the microdrill method along the growth axis of the shells, this may be important for interpretation because we can capture different seasons (Zhang *et al.* 2018*a*).

Previous studies indicate that isotopic variability in snail shells can occur at several levels: between species, between individuals within a single species, and within a single shell as a seasonal record. The magnitude of these differences depends on local environmental conditions, species physiology, food sources, and local climate. This variability is not universal and results from a combination of ecological, microenvironmental and physiological factors, so it should be considered individually for each data set (Goodfriend and Hood 1983; Goodfriend and Magaritz 1987; Goodfriend 1992; Goodfriend and Ellis 2002; Balakrishnan *et al.* 2005; Zanchetta *et al.* 2005; Baldini *et al.* 2007; Colonese *et al.* 2010).

It should be emphasised that the ranges presented are indicative and are based on studies published to date. These values may change with the progress of analyses conducted in different geographic regions and for various species, particularly in the context of local environmental conditions, plant composition, or climate change. Therefore, ongoing studies of isotopic variation in land snail shells might lead to further refinement or expansion of the known ranges of δ^{18}O and δ^{13}C amplitudes.

Species have different adaptations and survival strategies in a changing environment (Qin *et al.* 2021). Depending on the species, CO_2 fractionation and carbon dioxide passing through body fluids can occur differently, resulting in differences in stable isotope values (Yanes *et al.* 2018). Additionally, each genus may retain slightly different environmental data in its shell due to different ecologies. Taxa may have different tendencies to hide among vegetation, even within the same habitat. Furthermore, the diet may vary, which will translate into a different geochemical signal. Some species are more likely to incorporate carbonates into their diet, while others may do so less or not at all (Wang *et al.* 2019; Qin *et al.* 2021). For these reasons, it is useful to study several species from a single site. If possible, the sampling should not be limited to single specimens, but several specimens should be taken for analysis. To avoid errors in the analysis of extreme cases, multi-shell samples should be studied and examined separately, and an average calculated from the results obtained (Kehrwald *et al.* 2010; Apolinarska *et al.* 2015).

Another interesting but not fully studied adaptation of snails is the morphology and colour of the shell. The dark colour has been shown to affect the snail's body temperature, which can be elevated by up to 12°C relative to the ambient temperature. Body temperature is important during shell calcification and can easily obscure the environmental conditions recorded by the δ^{18}O of the shell (Zaarur *et al.* 2011).

Suess effect

An aspect that must also be considered in the study of modern snails is the Suess effect, which is also known as the industrial effect. This results from human activity and leads to a disruption of the carbon cycle. As a result of the burning of fossil fuels, the atmosphere becomes depleted in ^{13}C, resulting in a gradual decrease of δ^{13}C in the atmosphere (Keeling *et al.* 2017) and plants since ca. 1800, with a steep decrease after 1950. This is not only relevant due to the diet of herbivorous snails. The Suess effect translates into δ^{13}C values of the snail shell due to gas exchange that occurs on the surface of the snail's body. The effect of exchange with atmospheric CO_2 on δ^{13}C is believed to be more important for smaller snail species (Prendergast *et al.* 2015). Therefore, the reconstruction of the palaeoenvironment from carbonates from relatively recent terrestrial gastropods must consider the introduction of depleted CO_2 into the atmosphere since the Industrial Revolution (Prendergast *et al.* 2015). The δ^{13}C values of modern (i.e., post-1800) shells should be corrected for the Suess effect by applying an algorithm that would reduce the measured δ^{13}C value to the preindustrial reference level (Köhler 2016; Padgett *et al.* 2019; Dombrosky 2020).

Preservation of isotopic signals in land snail shells

The shells of land snails are predominantly composed of aragonite, which forms prismatic and nacreous layers. Periostracum is typically not preserved in subfossil and fossil shells (see chapter Shell formation and mineralization, and Text-fig. 2). The presence of calcite is generally considered a sign of diagenetic alteration (Hassan 2015; Li *et al.* 2020; Jenkins *et al.* 2023). For fossil shell analysis, it is crucial to know whether a gastropod shell is recrystallised and can be used for isotopic analysis. Shell recrystallisation requires specific conditions. Diagenesis includes not only physical but also chemical processes (Tucker and Wright 1990). The extent of these changes varies depending on factors including time, surface temperature, trace element content, particularly Mg^{2+} ions, and pressure (Sheng *et al.* 2005). For Quaternary land snail shells, which are often buried relatively shallowly beneath the earth's surface, the main factor influencing the diagenetic processes is time (Sheng *et al.* 2005). The thermal transformation of aragonite into calcite needs increased temperature, a condition usually not met in the case of typical Quaternary sediments. The widely accepted concept is that the primary aragonite shell is replaced by an ectypal calcite structure through dissolution and reprecipitation (Sheng *et al.* 2005; Casella *et al.* 2017; Pederson *et al.* 2019; Li *et al.* 2020). Hundreds of thousands of years are required for a phase change in calcite to occur. It is generally assumed that modern gastropod shells have not undergone an aragonite-to-calcite transformation (Zong *et al.* 2022).

Provided that diagenetic alteration took place, the phase transformation of the shell can modify the original isotopic values of carbon and oxygen, affecting the interpretation of the results of palaeoenvironmental and palaeoclimatic reconstructions. To determine the chemical composition and possible signs of recrystallisation of the shell, X-ray diffraction (XRD), Raman spectrometry, Fourier Transform Infrared Spectroscopy (FTIR) and/or electron probe micro-analysis (EPMA) can be applied. Effective approaches are the XRD and Raman spectrometry methods, which allow identification of the presence of calcite. Both $CaCO_3$ polymorphs can be easily identified by analysis of the XRD diffractogram peaks or the position and symmetry of the Raman peaks, which for calcite will be at 712 cm^{-1} and for aragonite at 701–704 cm^{-1} (Lécuyer *et al.* 2020). FTIR also enables the distinction between aragonite and calcite based on the characteristic bending vibration of the carbonate group: the absorption at ~712 cm^{-1} is diagnostic for calcite, whereas aragonite shows split bands near 700–713 cm^{-1} (Vagenas *et al.* 2003; Udomkan and Limsuwan 2008; Chaudhari and Bhongale 2022). However, FTIR spectra may include overlapping bands from organic components, which can interfere with the carbonate signal and complicate interpretation (Balmain *et al.* 1999; Chaudhari and Bhongale 2022). Scanning Electron Microscopy (SEM) allows for the recognition of the traces of dissolution precipitation on the shell surface that can be used as evidence of diagenesis (Hassan 2006). It is recommended that several random samples be checked to verify whether phase transformation changes have occurred in the studied sedimentary context (Li *et al.* 2020).

MICROHABITATS OF TERRESTRIAL SNAILS

To better understand the information recorded in the shells of fossil snails, it is crucial to understand their life requirements, including their microenvironments. A relevant question that arises during research is: can species living within the same ecosystem inhabit (and record) different habitats? Terrestrial snails tend to exhibit more meticulous habitat selection compared to vertebrates. Their distribution is influenced by various factors that constitute their microenvironment, such as moisture, food availability, pH, shelter, type of substrate and microclimate. Studying the microhabitats of terrestrial snails allows one to gain insight into the range of distribution, spatial variability of their biodiversity, species richness, and their associations with occupied habitats. It is important to note that a single microhabitat can represent a wide range of microclimates, which can be reflected in the fauna living in that microhabitat and may impact the dynamics of the population. According to research conducted by Kemencei *et al.* (2014), local conditions can significantly influence the abundance of terrestrial snails, with microhabitats of high abundance characterised by the co-occurrence of a greater number of species. Snails live in an environment, and the environment impacts snails. This interconnected system affects not only the sources of oxygen and carbon for snails and the factors that influence their values, but also many more. It can be said that land snails operate under the principle of "survive where you are" in their lives. This is due to their limited mobility over long distances, which is especially true for small species. For this reason, many of them have specialised in terms of their ecological requirements. This means that some species can only be found in specific habitats, in small areas that meet their vital requirements (Urbański 1952; Dahirel *et al.* 2015). However, this does not exclude the presence of species with broad ecological tolerance that can thrive in various environments. Studies and observations have shown that terrestrial gastropods exhibit a kind of territorialism that is not characterised by defending the inhabited area against other individuals but rather by existing within the limited area. It has been observed that snails moved from their habitat will attempt to return to their homeland if the distance is not too far. Additionally, the relocated snails were restless (Prior 1985). These characteristics greatly facilitate the interpretation of the mollusc assemblage, as the snails found at the site (if not redeposited) should be representative habitat of the immediate area.

METHODS

Field sampling

Initial research on terrestrial gastropods involves the collection of research material in the field. Subsequent stages should focus on laboratory processing and sample preparation for further analysis. The review of currently used techniques will be limited to Quaternary and modern material only.

The sampling method varies depending on the shell material – the modern or the fossil one. The main problem encountered in fossil sites is the uneven accumulation of shell material. Due to possible processes of shell transport in the terrestrial environment, their selective segregation and accumulation, the shell assemblages may contain remains that have not undergone transportation (necrocoenosis) and/or the remains that were redeposited and accumulated outside the original habitat (allocoenosis) (Alexandrowicz and Alexandrowicz 2011). When analysing such accumulations, possible depositional and post-depositional disturbances should be considered. The alteration of the original abundance and species composition may also be influenced by predation or poor preservation of the shells in the sediments. Migration of individuals and transportation by other animals or humans play a secondary role in terrestrial environments; however these processes can be particularly important in archaeological sites (Goodfriend 1992). The studies of a fossil fauna should be supported by analysis of the modern malacofauna in the vicinity of the site, including characteristics of habitats, geological substrate. Information on the vegetation cover, temperature, humidity, degree of shading or habitat insolation, anthropopressure, and degree of environmental pollution is also required (Alexandrowicz and Alexandrowicz 2011).

In geological sites (natural exposures, boreholes, quarries, etc.), quantitative samples are preferred (Goodfriend 1992; Yanes *et al.* 2013). Bulk sediment samples are recommended instead of selecting individual shells from the profile (Goodfriend 1992). Samples of similar volume or weight should be taken (usually 1–3 cm^3) and then wet-sieved through a set of sieves. Shells should be cleaned. Sometimes hydrogen peroxide is necessary for this, but this chemical treatment can affect the properties of the shells. Thus, the use of H_2O_2 is not recommended when isotopic analyses of the outer shell layers or studies on amino acid racemization or epimerization are planned (Goodfriend and Ellis 2002; Colonese *et al.* 2007, 2010; Colonese 2017).

Collecting contextual data

In preparing land snail shells for isotopic analysis, it is advisable to consider examining the microenvironmental elements associated with the studied species, particularly if the focus is on extant species. These analyses are typically conducted along with shell examination, and the resulting data are invaluable for subsequent climatic and environmental interpretations. Data on precipitation, temperature, and relative humidity can be obtained from the meteorological stations closest to the site. There is also the possibility of conducting your own isotopic survey of precipitation. To do this, set up a clean rain gauge outside at the beginning of each rainfall event. To avoid the effect of evaporation, samples should be taken immediately after the rain has stopped, in the glass bottles protected by waterproof seals and stored in the refrigerator (Zong *et al.* 2022).

For each of the sites analysed, climate data should be collected from the nearest weather station. It is useful to collect data on air temperature, precipitation, and oxygen isotopes in meteoric water linked to the Global Network of Isotopes in Precipitation (GNIP). These data can be used to calculate the annual averages and weighted average $\delta^{18}O$ values of precipitation for each site (Bricker *et al.* 2023). Such data are particularly relevant when studying oxygen isotopes, as they directly reflect the isotopic composition of local precipitation. The results obtained are related to the monitoring period. However, by juxtaposing these data with terrestrial gastropods collected during the same period and their isotopic signature, we can build a reference for local relationships between climate and the shell isotope record (Yanes *et al.* 2014; Rech *et al.* 2021). By contrast, for carbon isotope analysis, such climate data have limited significance, as $\delta^{13}C$ values in snail shells primarily reflect the isotopic composition of assimilated vegetation rather than climatic parameters.

An important direction in environmental research involves the analysis of components from the habitat of the studied snails by collecting samples from forest litter, leaves, plant fragments, as well as sediments such as soil or rock fragments from the studied site. Litter and vegetation provide valuable information on ecosystem structure, the dominant vegetation type, and potential sources of organic matter that can influence the carbon isotopic composition in snail shells (Goodfriend and Ellis 2002; Yanes *et al.* 2009). Carbon isotope analysis of plant samples allows for the identification of dominant vegetation types and their contribution to the local ecosystem, which is crucial for interpreting the isotopic signatures of snail shells. To obtain a representative picture of local environmental conditions, samples of litters, plants, lichens, mosses, and fungi should be collected directly from the locations where snails are found. It is also advisable to collect multiple species from each site to account for the diversity of potential food sources. The calculated average isotopic values for each taxonomic group can be considered representative of potential organic carbon sources (Yanes *et al.* 2018).

Collecting soil and sediment samples from the snail's surroundings of the studied sites allows for the assessment of the chemical and mineralogical conditions of the palaeoenvironment that influenced the shell biomineralization process. Particular attention should be paid to the presence of limestone or other carbonate sources in the substrate, as they can provide an additional source of inorganic carbon for land snails (Balakrishnan and Yapp 2004; Yanes *et al.* 2008, 2009, 2011). Field studies have shown that snails can use significant amounts of inorganic carbon from calcareous rocks as a source of calcium for shell construction (Goodfriend and Hood 1983; Goodfriend and Magaritz 1987; Yanes *et al.* 2008, 2018).

Shell sampling

Dry samples can be homogenised (including prismatic and nacreous layers) using an agate mortar and pestle. To avoid contamination, samples should be cleaned and ground separately, and after operation, all equipment should be cleaned with pure deionised water and pure ethanol for analysis (Bricker *et al.* 2023). Depending on the type of planned study, several shells can be combined into one representative sample (Zhai *et al.* 2019; Jenkins *et al.* 2023). However, grouping samples from one site and one layer should be done with caution (Kehrwald *et al.* 2010).

Intraindividual multiple sampling of a shell is also possible (Li *et al.* 2024). One method is to collect powder samples with the use of a microdrill. A sample can be drilled with a carbide drill with a diameter based on the expected resolution of the study. The method involves taking material from all layers of the shell (prismatic and nacreous layers). Different sampling criteria can be adopted, for example, from each whorl or every 1 mm along the growth axis of the shell. The drilled powder should be collected on weighing paper and then transferred to test tubes (Kehrwald *et al.* 2010; Yanes *et al.* 2011; Padgett *et al.* 2019; Qin *et al.* 2021; Quenu *et al.* 2023). After each sample, the drill should be cleaned, for instance, in dilute hydrochloric acid to remove any remaining carbonate powder (Qin *et al.* 2021).

Before isotope analysis, fossil and modern shells should be examined for recrystallisation of aragonite into calcite (see Preservation of isotopic signals in the shells of land snails). This assessment can be performed using three methods. If any signs of recrystallisation are absent, samples can be prepared for further analysis. If signs of recrystallisation are observed, it is still possible to extract aragonite through a flotation process in a heavy liquid that separates calcite from aragonite (Douka *et al.* 2010).

Pre-treatment

The target material for the stable isotope analysis of the shell $\delta^{18}O$ and $\delta^{13}C$ is the mineral part (aragonite) of the shell (Balakrishnan and Yapp 2004; Balakrishnan *et al.* 2005; Yanes *et al.* 2008). Because the analyte of the IRMS measurement is CO_2 released from the aragonite (see subchapter Non-shell samples), any material in a sample that can be a potential source of CO_2 must be regarded as a contaminant and removed before analysis (Zanchetta *et al.* 2005). The most typical contaminants include 1) the outer organic layer of the shell (periostracum); 2) remnants of soft tissues; 3) organic matter from sediment, soil, or litter and 4) carbonates from soil, sediment, or secondary carbonate precipitates.

The first step in preparing shells for isotopic analyses is washing in deionised or distilled water to remove sediments and other contaminants (Balakrishnan and Yapp 2004; Zanchetta *et al.* 2005; Balakrishnan *et al.* 2005). This process, especially when using an ultrasonic bath, is usually sufficient for effective cleaning (Zanchetta *et al.* 2005; Yanes *et al.* 2008, 2009). Alternatively, samples can be ground to a powder using an agate mortar, rinsed with acetone, and dried at 70–80°C (Yapp 1979).

When standard cleaning does not yield satisfactory results, additional procedures are required to remove particles that adhere to the shell surface and interior. To remove the outer organic layer of the shell (periostracum) and other organic impurities, immersion in a solution of 3–5% sodium hypochlorite (NaClO) at room temperature is applied (Scott 2002; Zanchetta *et al.* 2005; Loxton *et al.* 2017). Larger shells can be gently crushed beforehand to accelerate the reaction or a small hole can be drilled in the protoconch to facilitate cleaning the interior of the shell (Zanchetta *et al.* 2005; Wang *et al.* 2016). The reaction takes several hours (up to two days) at room temperature to efficiently remove organic contaminants (Balakrishnan *et al.* 2005; Zanchetta *et al.* 2005; Yanes *et al.* 2008; Quenu *et al.* 2023). Alternatively, a 10% H_2O_2 solution can be used instead of NaClO (Metref *et al.* 2003). Another method involves combustion in a low-temperature oxygen plasma, which effectively removes persistent organic residues; however, this method may alter amino acids and should be used depending on the intended analyses (Zanchetta *et al.* 2005; Colonese *et al.* 2010). Destruction of amino acids can prevent studies such as amino acid geochronology (Penkman *et al.* 2008, 2010, 2013), studies of biomineralization processes and learning about the mechanisms of shell formation (Treccani *et al.* 2006; Mann *et al.* 2007; Yan *et al.* 2008; Pavat *et al.* 2012; Liu *et al.* 2023; Zhang *et al.* 2024), ecotoxicological studies (Soliman and El-Ansary 2007; Holman *et al.* 2011; Schleicherová *et al.* 2024), or comparative studies between taxa (Pavat *et al.* 2012; Liu *et al.* 2023).

Properly prepared samples should be thoroughly rinsed with deionised water and dried. The cleaning process can be aided by using a brush. An alternative method is to soak a shell in a 1% Alconox solution for about 30 minutes, then thoroughly rinse with deionised water and dry at room temperature (Rech *et al.* 2021). This process should be repeated until a satisfactory level of cleanliness is achieved. Some researchers also use a cleaning method that involves briefly immersing the shells in diluted HCl, which accelerates the process, after which the shells should be thoroughly rinsed with deionized water (Engel *et al.* 1994; Goodfriend and Ellis 2002). It is also possible to skip the immersion step and clean the shells in an ultrasonic bath submerged in a 10% H_2O_2 solution at approximately 25°C for 48 hours (Bao *et al.* 2020). After applying reagents, the samples should be rinsed several times with deionized water and dried at room temperature.

If these treatments are unsuccessful and the shells are still coated with hard-to-remove organic particles, a hydrogen peroxide solution can be used (Ubaldini *et al.* 2024). To avoid possible damage, an admixture of an alkalizing substance may be used in the form of a buffer isolated from atmospheric CO_2 (Alexandrowicz and Alexandrowicz 2011; Zhang *et al.* 2020). Shells subjected to this process should later be rinsed several times with deionised water and dried (Colonese *et al.* 2013; Wang *et al.* 2019). According to Zhang *et al.* (2020), this treatment has little effect on the $\delta^{18}O$ values of shells.

Depending on the planned analyses, drying can be carried out at room temperature for several days, preferably without heating and not in the sun (Alexandrowicz and Alexandrowicz 2011). Samples can also be dried in a vacuum at room temperature (Zaarur *et al.* 2011). Some researchers dry samples overnight at a temperature of about 40–60°C (Kehrwald *et al.* 2010; Wang *et al.* 2019; Zhai *et al.* 2019) or at 40–50°C for approximately 12 hours (Scott 2002; Balakrishnan and Yapp 2004; Balakrishnan *et al.* 2005; Zanchetta *et al.* 2005; Yanes *et al.* 2008; Prendergast *et al.* 2015; Yanes *et al.* 2018); however, samples dried this way may not be suitable for some studies such as racemization and epimerization of amino acids. Once organic matter has been removed and the samples are dried, they should be homogenized into a uniform powder for further analyses. The most common and easily accessible method is manual grinding in an agate mortar (Yates *et al.* 2002). Powdering can also be done in a mechanical grinder, which is easier for large samples (Zanchetta *et al.* 2005). If the goal is to analyze isotopic variation along growth lines, the use of dental burrs or a Dremel tool is recommended, as they enable precise sampling from specific growth layers within the shell (Goodfriend and Ellis 2002). Powdered and dried shell samples should be transferred to labelled polypropylene tubes. Samples prepared this way can be subjected to isotopic analyses (Colonese *et al.* 2013; Yanes *et al.* 2018; Rech *et al.* 2021).

Non-shell samples

The collected rainwater samples should be washed with 0.4% CO_2 in helium gas. After adding a small portion of 100% H_3PO_4 acid, the samples should be left to reach equilibrium at 25°C for approximately 48 hours. After this time, when the samples have reached equilibrium, they can be isotopically measured on a mass spectrometer (Yanes *et al.* 2008).

Litter and vegetation samples should be washed with deionised water, then dehydrated in an oven at 40°C and homogenised. The resulting powder can be subjected to isotopic analysis (Colonese *et al.* 2013). However, in snail habitat studies, soil, plants, and shell organic matter samples should be analysed separately. Carbonates should be dissolved in HCl, and the sample should be washed with water and dried in an oven at about 80°C (Hassan 2015). After drying, to determine the percentage of organic carbon content, about 1–4mg of homogeneous powder is weighed in tin capsules. The weighed samples are then transferred to a TOC organic carbon analyser. Carbon dioxide produced by combustion at 1000°C is measured using suitable detectors. The amount of CO_2 released is proportional to the carbon content of the original sample. The carbon isotope results are given in δ notation relative to the standard VPDB (Hassan 2015).

Sediment samples should also be homogenised, preferably by grinding them in a mortar under methanol and treating them with 3% H_2O_2 overnight to eliminate organic impurities. The samples should then be rinsed with deionised water and dried at room temperature. 10–20µg of the sample are treated overnight under vacuum with 100% H_3PO_4 at 25°C, and the resulting carbon dioxide can be sequentially analysed on a mass spectrometer (Yanes *et al.* 2011).

STABLE ISOTOPE ANALYSIS

General information

The carbon and oxygen isotopic ratios are most commonly measured using IRMS equipped with three Faraday cups that collect molecules with three different m/z ratios, typically: m/z = 44 (representing the $^{12}C^{16}O_2$ molecule); m/z = 45 (representing the $^{13}C^{16}O_2$ or $^{12}C^{16}O^{17}O$ molecule); and m/z = 46 (representing mainly the $^{12}C^{16}O^{18}O$ or $^{13}C^{16}O^{17}O$ isotopologues with a minor contribution from $^{12}C^{17}O_2$). The $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios in a sample are calculated from the measured m/z 44/45/46 ratios using dedicated software that utilizes the m/z 44/45/46 ratio in standards of known isotopic composition measured along a sample, for calibration of the measurements.

The isotopic results obtained are reported in δ notation (Hoefs 2021) following the formula:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000\% ,$$

where δ is the $\delta^{18}O$ or $\delta^{13}C$, and R is the ratio of heavy to light isotopes (that is, $^{18}O/^{16}O$ or $^{13}C/^{12}C$, respectively) in the sample or standard. The ^{17}O isotope is usually neglected in most isotopic studies of snail shells. The standard in the equation refers to an international measurement scale and its assigned isotopic ratio (Coplen 2011). For mollusc shells, the commonly used standard is Vienna Pee Dee Belemnite (VPDB) for $\delta^{18}O$ and $\delta^{13}C$ as recommended by the 2005 General Assembly in Beijing. Full analytical data supporting this recommendation can be found in new guidelines for $\delta^{13}C$ measurements in (Coplen *et al.* 2006) (Roberts *et al.* 2018). In isotopic studies, standards rely not only on international measurement scales but also on laboratory reference materials such as NBS-19, which are essential for scale realisation, correction of instrument bias, and ensuring long term measurement accuracy and comparability among laboratories (Coplen *et al.* 2006; Camin *et al.* 2025). A positive δ value indicates enrichment in the heavier isotope of a sample compared to the standard.

Methods of isotope analysis

A stable isotope measurement is conducted with an isotopic ratio mass spectrometer (IRMS). The sample is dissolved in orthophosphoric acid to release gaseous CO_2, which constitutes the analytical fraction. This is usually achieved by reacting powdered aragonite with hot (70°C) orthophosphoric acid (H_3PO_4). Depending on the method, the dissolution occurs in either a vacuum or in a helium atmosphere. The released gas is ionized in the ion source of the spectrometer and then enters the electromagnetic field, where individual mass fractions (44, 45, 46) are separated. To avoid an exchange of oxygen between water and carbonate, the minimum concentration of water in the acid is desirable (McCrea 1950). This is ensured by the use of ">100%" phosphoric acid, which is prepared by dissolution of P_2O_5 in 100% H_3PO_4. To avoid isotopic fractionation during the reaction of carbonate with acid, the acid must be applied in excess. Before the reaction, the sample is flushed with Helium gas to remove atmospheric CO_2 from the reaction vial in continuous flow system such as GasBench II, whereas Kiel IV Carbonate Device and traditional offline methods use vacuum evacuation instead of He flushing. The reaction time depends on the reaction temperature and preparation system. According to McCrea (1950) the reaction of carbonates with phosphoric acids last 6–8 hours at 25°C, 1–2 hours at 50°C, and approximately 20 minutes at 72°C (McCrea 1950; Spötl and Vennemann 2003). In automated system as Kiel, reaction times are determined by the instrument protocol and are typically shorter due to elevated temperatures. The measurement is performed in dedicated collectors (Faraday cups) and reported in delta notation (McCrea 1950).

The resulting CO_2 is purified in a liquid nitrogen U-trap in offline and Kiel system, whereas GasBench II uses additional nitrogen-water trap and can operate without a U-trap. The CO_2 extraction line can be connected to IRMS through a dual-inlet system or a continuous-flow system. The sample dissolution and preparation stages of spectrometric analysis can be conducted offline using a traditional vacuum line or via one of the peripheral devices. Each of these methods has limitations and requirements.

Offline method

Sample preparation is performed offline following the method described by McCrea (1950) using a vacuum line. The reaction vessel contains the sample and, separately, orthophosphoric acid (density 1.92 g/cm^3). After the vessel is filled, the acid is mixed with the sample. The reaction occurs at 25°C for at least two hours. The CO_2 released from the sample is then condensed in a cryogenic trap (liquid nitrogen trap). The final step involves measuring the carbon and oxygen isotopic composition using a mass spectrometer in the Dual Inlet system. The results are reported as δ values relative to the VPDB standard (McCrea 1950).

The offline method is a classical approach to preparing samples for carbon and oxygen isotope analysis in carbonates. It requires minimal specialized equipment, but it requires a relatively large sample, approximately 15 mg. This method is very time-consuming, allowing for the processing of approximately 14 samples per day (McCrea 1950).

Semi-automated continuous flow methods

Stable carbon and oxygen isotope analyses of carbonates are performed using a peripheral attachment (Thermo Scientific GasBench II, Elementar isoFLOW) coupled with a mass spectrometer operating in the Continuous Flow system. The sample is placed in a reaction vessel sealed with a septum-sealed screw cap. Next, the vessel is flushed with helium to replace the ambient atmosphere with pure helium. Subsequently, a few drops of phosphoric acid (density 1.92 g/cm^3) are introduced through the septum. Carbon dioxide is released from the sample through a reaction with the acid. The minimum sample mass is 200 μm, and the reaction time is at least one hour at 72°C. The final step involves measurement using IRMS (Isotope Ratio Mass Spectrometer). The results are in δ notation relative to the VPDB standard. Continuous flow analyses are sensitive to temperature fluctuations. Therefore, the manufacturer recommends applying a linearity correction to mitigate this effect (Grootes *et al.* 1969).

Carbonate isotope analysis in snail shells using GasBench and IRMS setup is a suitable choice for small specimens. The method allows for the measurement of relatively large samples (up to 1 mg), ensuring a representative result for the entire specimen. It is almost fully automated, requiring minimal human involvement, while providing high measurement precision.

Automated Dual Inlet Methods

The third method is a fully automated system for measuring the isotopic composition for carbon and oxygen in carbonates using Dual Inlet system. Available devices include the Kiel IV Carbonate Device (or the Nu Carb Device) coupled with an IRMS. The samples (minimum 20 μg of carbonate) react with orthophosphoric acid (density 1.92 g/cm^3) at 70°C. The released gas is transported and frozen in a trap (liquid nitrogen trap 1) at -160°C. Subsequently, the temperature in trap 1 is increased to -118°C, where the water is retained while the previously released CO_2 is refrozen in trap 2. After this stage, the CO_2 is transferred through a capillary directly to the ion source. A measurement series should include standards. The reaction time ranges from 3 to 10 minutes.

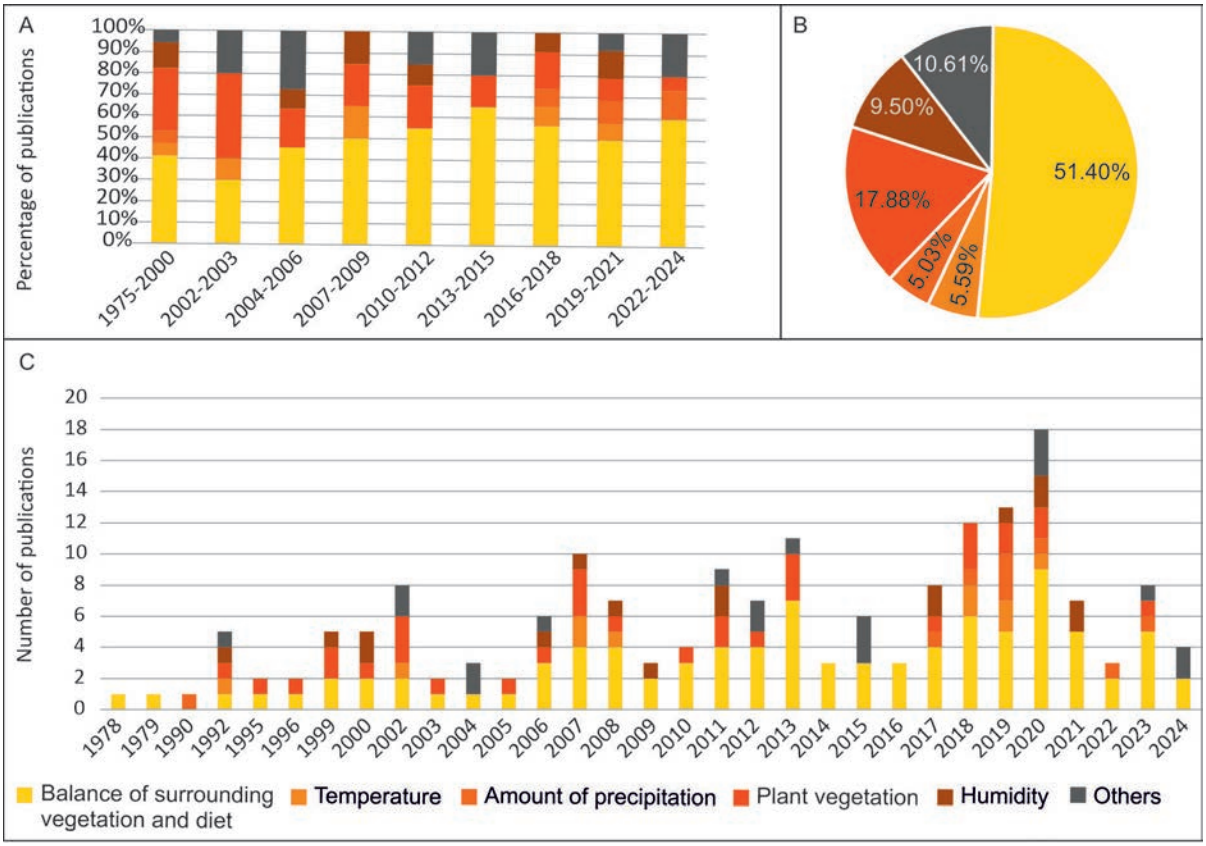
Among the described methods, this requires the smallest sample (20 μm). It allows analysis of growth layers and reconstruction of isotopic changes through an organism's lifespan. It is the only method discussed that enables the automatic removal of water formed during the acid-sample reaction. A measurement series should include internationally recognized standards correlated with VPDB. In practice, several reference materials (either certified standards or internal lab standards of previously measured isotopic composition) are measured along the samples (Berglund and Ralska-Jasiewiczowa 2003; Roberts *et al.* 2018). These materials may have variable isotopic composition; however, their composition must be well known and must refer to the international reference. The use of standards (e.g., NBS 18, IAEA 603, IAEA 610, IAEA CO8) implies that the measurements have been calibrated according to the guidelines of the International Atomic Energy Agency (IAEA) and can be reported versus internationally accepted standards (INTERNATIONAL ATOMIC ENERGY AGENCY 2017).

RECONSTRUCTION OF PALAEOCLIMATE AND PALAEOENVIRONMENT

A thorough understanding of the sources of oxygen and carbon and the mechanisms that determine their incorporation into the shells of land snails makes it possible to reconstruct the palaeoclimate and palaeoenvironment. Due to the low mobility of gastropods, the reconstructed parameters are undoubtedly reflective of local conditions (Yanes *et al.* 2008; Zhai *et al.* 2019). Due to the life cycle of terrestrial snails, we can reconstruct the conditions only for the period when they are active. Information about the dry season or the winter period is not recorded. Additionally, the lifespan of the snail is crucial; depending on its duration, we obtain data from one or multiple seasons (Zaarur *et al.* 2011).

The $\delta^{13}C$ values derived from the shells provide information about the sources of carbon. Currently, most researchers interpret the carbon isotope record as reflecting changes in the distribution and quantity of plants with different photosynthetic pathways (C3 and C4 plants) in response to changes in precipitation (Zaarur *et al.* 2011). This, however, is not reflected in temperate climates, where the key factors appear to be: water stress, humidity, microclimate, and shell growth period (Újvári *et al.* 2021, 2024). Any attempt to use $\delta^{13}C$ values of terrestrial gastropod shells to estimate the distribution patterns of C3 and C4 plants is based on several assumptions: (1) the isotopic values of the shells reflect those of the living organism (Goodfriend and Hood 1983; Goodfriend and Magaritz 1987; Goodfriend 1990); (2) snails consume all types of plants indiscriminately in proportions corresponding to the abundance in the environment (Hassan 2015); (3) the difference in $\delta^{13}C$ between C3 and C4 plants is constant (Hassan 2015); and (4) the fractionation between diet and snail tissue is irrelevant (Hassan 2015). However, this is currently applicable only to environments where C4 plants occur or have occurred in the past (currently mainly in tropical and subtropical regions). In the regions with temperate climates, the dominant vegetation type is C3. Lack of the C4 plants makes the diet narrower in $\delta^{13}C$ compared to subtropical and tropical regions. This makes the minor sources of $\delta^{13}C$ variability (such as inorganic carbonates from soil and rock, atmospheric CO_2, and others) more important in such regions (Goodfriend and Ellis 2002; Zhang *et al.* 2014) (Text-fig. 6) and facilitates their identification. It is also worth considering the influence of the so-called "canopy effect" on $\delta^{13}C$ values in land snail shells. This phenomenon involves a decrease in carbon isotopic values in dense forest vegetation, where a vertical gradient of $\delta^{13}C$ values is observed, from the lowest near the ground surface to higher in the tree crowns (Van Der Merwe and Medina 1991). This effect is due to the low light reaching the undergrowth, leading to more intense isotopic fractionation during photosynthesis (Rodière *et al.* 1996), recirculation of biogenic CO_2 (Creighton and Roney 2009), and limited access to refreshed atmospheric CO_2 (Van Der Merwe and Medina 1991). Reduced $\delta^{13}C$ values in plants are then passed along the food chain to herbivorous organisms (Van Der Merwe and Medina 1991). Consistent with this, animals inhabiting dense forest formations show depletion in ^{13}C of about 1.5–3‰, as confirmed in reindeer, red deer, and roe deer, among others (Rodière *et al.* 1996; Creighton and Roney 2009).

Studies have shown that some snails can be selective in their diet (Zong *et al.* 2022), but many species have a mixed diet. In this context, higher $\delta^{13}C$ values are interpreted as an indicator of the consumption of C4 plants, which is characteristic of arid regions. Lower values of $\delta^{13}C$ are believed to reflect temperate vegetation (Goodfriend 1992; Hassan 2006; Yanes *et al.* 2008; Chiba and Davison 2009; Wang *et al.* 2019; Zhai *et al.* 2019; Jenkins *et al.* 2023). Controlled laboratory conditions showed a positive correlation between the $\delta^{13}C$ values of the shell and the composition of the vegetation cover (Scott 2002; Metref *et al.* 2003; Quenu *et al.* 2023). Therefore, stable carbon isotope values, especially their fluctuations, can be successfully used to document changes in palaeovegetation (Yanes *et al.* 2011). Snails from regions dominated by C3 vegetation and low water stress tend to have more negative $\delta^{13}C$ values than snails from regions with predominantly C4 vegetation and higher water stress (Jenkins *et al.* 2023). Intermediate values are observed in snails from transitional areas. It is important to note that the isotopic values may be modified by the carbon isotopic composition of plants consumed by snails, which depends on plant physiology, their photosynthetic pathway, and environmental conditions such as water stress. Additionally, it is suggested that carbon isotope values in snail shells are more species-dependent than the oxygen isotopes, making it difficult to generalise for carbon (Yapp 1979; Colonese *et al.* 2007). Most researchers reconstruct the balance of surrounding vegetation (in the context of C3 and C4 plants) and the diet of the snails, which are interconnected, based on carbon isotopic values (Metref *et al.* 2003; Zanchetta *et al.* 2005; Wang *et al.* 2019; Qin *et al.* 2021; Zong *et al.* 2022).



Text-fig. 6. Evolution of scientific views on possible reconstructions based on stable carbon isotope values ($\delta^{13}C$) in land snail shells. A – cumulative percentage of major reconstructions approaches across subsequent time intervals; B – overall percentage distribution of main interpretations; C – number of publications presenting specific reconstruction approaches by year. Figure compiled from literature identified via keyword searches in the Web of Science database.

Establishing a relationship between the carbon isotopic values of contemporary gastropods and their habitats is crucial, allowing the acquired data to be applied to fossil records and producing satisfactory results with reduced error.

Understanding the parameters influencing the oxygen isotopic values in the shell enables the reconstruction of local climate and environmental conditions. Enrichment in a heavier isotope is typically interpreted as being due to the climate changing into warmer and drier conditions (Zaarur *et al.* 2011). Several studies have suggested that the isotopic composition of oxygen in the shells of land snails may reflect the isotopic values of precipitation (Goodfriend and Ellis 2002; Zanchetta *et al.* 2005; Kehrwald *et al.* 2010). However, the reconstructed oxygen isotopic composition of water in the snail's body is more positive than that of precipitation, even by several permille and the observed differences were not constant (Prendergast *et al.* 2015). Recent work shows that part of this enrichment may be explained by uptake of dew, which can contribute substantially to the total water intake of land snails – up to 50% under dry conditions, and is characterized by more positive $\delta^{18}O$ values than local precipitation (Da 2025). These discrepancies and the flux balance model (Balakrishnan and Yapp 2004; Zhang *et al.* 2018*a*) suggest that important factors responsible for shell $\delta^{18}O$ value include $\delta^{18}O$ in environmental water vapour, relative humidity (RH), and precipitation temperature (Balakrishnan and Yapp 2004). The values of environmental water vapour and ingested water are difficult to monitor and vary significantly between species and their microenvironments. This complicates laboratory studies and increases interpretive uncertainty in the $\delta^{18}O$ results obtained from the shells (Zong *et al.* 2022).

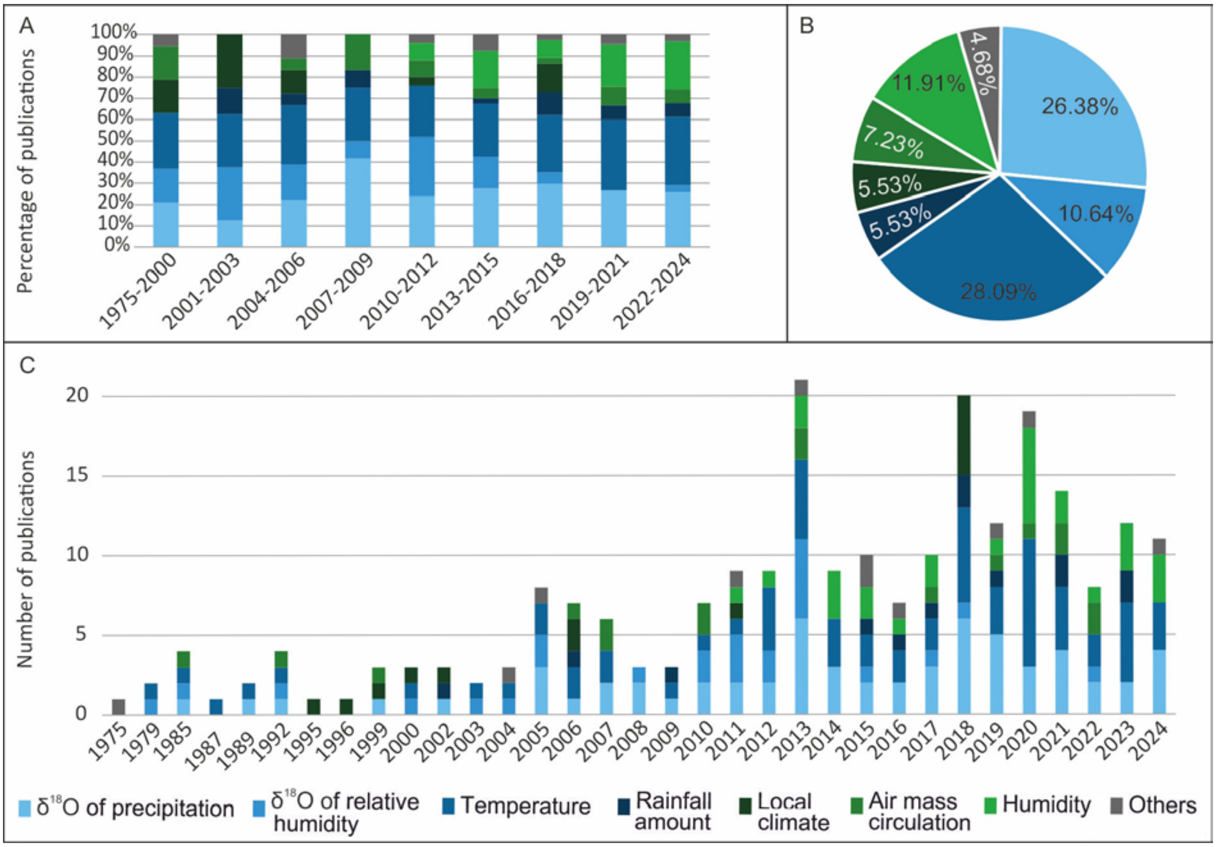
Despite these challenges, $\delta^{18}O$ values in land snail shells have been widely used to infer palaeoclimate parameters, including precipitation $\delta^{18}O$, temperature and humidity (Zanchetta *et al.* 2005; Yanes *et al.* 2009; Kehrwald *et al.* 2010; Prendergast *et al.* 2015). According to the Balakrishnan and Yapp model, $\delta^{18}O$ of precipitation, $\delta^{18}O$ of water vapour, relative humidity (RH) and air temperature are the factors that most control the $\delta^{18}O$ values of the land snail shell (Balakrishnan and Yapp 2004; Colonese 2017; Nield *et al.* 2022). Stable isotope estimates from land snail shells should ideally be interpreted in the context of comparable isotopic data obtained from the shells of their closest living relatives collected contemporaneously from well-studied habitats (Yanes *et al.* 2008). Such attempts have been made; however, these results carry some degree of uncertainty (Wang *et al.* 2019). The reconstructed temperature may reflect not only the ambient temperature, but also the physical or body temperature at which the snail precipitated its shell, which does not necessarily match environmental conditions (Colonese *et al.* 2007; Zaarur *et al.* 2011; Wang *et al.* 2016; Zhai *et al.* 2019; Lécuyer *et al.* 2020; Qin *et al.* 2021) (Text-fig. 7).

Clumped Isotopes Δ_{47}

The palaeothermometer based on clumped isotopes Δ_{47} is an alternative approach to measuring the formation temperature of carbonate minerals. It is based on the clumping of the rare isotopes of oxygen and carbon, i.e., ^{18}O and ^{13}C, in CO_3^{2-} groups in the carbonate mineral. This means that we study not only the bulk isotopic composition ($^{18}O/^{16}O$ and $^{13}C/^{12}C$) but also the fraction of ^{18}O and ^{13}C atoms bonded together in the same carbonate ion group ($^{13}C^{18}O^{16}O_2^{2-}$) (Ghosh *et al.* 2006; Bricker *et al.* 2023). The homogeneous isotope exchange reaction can be written using the formula:

$$Ca^{13}C^{16}O_3 + Ca^{12}C^{18}O^{16}O_2 \leftrightarrow Ca^{13}C^{18}O^{16}O_2 + Ca^{12}C^{16}O_3$$

The formation of ^{13}C and ^{18}O bonds in carbonates is only limited by the temperature at which its precip-



Text-fig. 7. Evolution of scientific views on possible reconstructions based on stable oxygen isotope values ($\delta^{18}O$) in land snail shells. A – cumulative percentage of major reconstructions approaches across subsequent time intervals; B – overall percentage distribution of main interpretations; C – number of publications presenting specific reconstruction approaches by year. Figure compiled from literature identified via keyword searches in the Web of Science database.

itation occurs, with a greater proportion of clumped ^{13}C-^{18}O bonds forming at lower temperatures (Zhang *et al.* 2018*a*).

Research on the relationship between Δ_{47} in land snail shells and the temperature of shell calcification was initiated by Zaarur *et al.* (2011) and further developed by Zhang *et al.* (2018*b*). More recent climate-chamber experiments and studies of natural populations show that in many species, aragonite shells precipitate close to clumper isotope equilibrium (Guo *et al.* 2019; Dong *et al.* 2020; Bao *et al.* 2023; Újvári *et al.* 2024). However, species specific deviation can occur. As a result, Δ_{47} values in terrestrial snail shells are considered a promising tool for reconstruction of the mean temperature of the snail activity season. Δ_{47} primarily reflects temperature and is less sensitive than bulk isotopes ($\delta^{18}O$ and $\delta^{13}C$) to variability in the isotopic composition of water, food, or ingested carbonates. Deviations of Δ_{47} from equilibrium values are usually attributed to kinetic isotope effects. These effects are related to dehydration or dihydroxylation and CO_2 degassing during shell precipitation from body fluids, rather than changes in growth rate, metabolism, or pH (Zhang *et al.* 2018*b*; Guo *et al.* 2019; Dong *et al.* 2020; Bao *et al.* 2023; Újvári *et al.* 2024).

Several studies have reported that the temperatures calculated from Δ_{47} terrestrial snail shells differ from the mean annual ambient temperatures by several degrees (Zaarur *et al.* 2011; Wang *et al.* 2016). This suggests that the aragonite in shells forms close to isotopic equilibrium reflecting the body temperature of the snail, not the ambient temperature (Zaarur *et al.* 2011). Zhang *et al.* (2018) found that there were no significant differences in Δ_{47} values between snails fed differently and drinking water with different isotopic signatures. This demonstrates the lack of effects of influence of the life history on Δ_{47}. Moreover, subsequent laboratory and field studies confirmed that shell Δ_{47} values are generally not affected by mineralogy or diet but that minor, species-specific kinetic deviations can occur (e.g., Guo *et al.* 2019; Dong *et al.* 2020; Bao *et al.* 2023; Újvári *et al.* 2024).

To correctly interpret the results of the Clumped Isotope analysis on modern shells, preliminary studies are necessary. These consist of determining the activity periods of the gastropods, taxon-specific ecophysiological traits defining the living environment including vegetation, and additional variables, such as internal physiology or shell characteristics (morphology, colouration) (Bricker *et al.* 2023). For extinct species, the use of Clumped Isotopes to reconstruct palaeotemperatures is not straightforward. Given that snail shells should record the average temperatures prevailing during shell precipitation, they may provide valuable information about the environmental conditions experienced during snail activity. However, the temperature recorded by Δ_{47} does not necessarily correspond to the mean annual or seasonal ambient temperature, because the shell calcification may occur only during specific periods of activity (Zaarur *et al.* 2011; Wang *et al.* 2016). Clumped Isotopes are more reliable when considering the flux balance model developed by Balakrishnan and Yapp (2004) (Zhang *et al.* 2018*b*). However, limited information is available on the relationship between modern shell geochemistry and the palaeoenvironmental conditions in the regions with contrasting climates. Such studies are needed to further validate proxies for temperature reconstruction from snail shells Δ_{47} (Wang *et al.* 2016; Zhang *et al.* 2018*b*; Zhai *et al.* 2019; Dong *et al.* 2020; Bricker *et al.* 2023).

DISCUSSION AND CONCLUSIONS

Stable isotope research is a rapidly growing field of science that shows significant potential, particularly within the expanding realms of interactions between the biological, archaeological, and geological sciences. It has been used successfully in malacology as a proxy to investigate past climates and environments (e.g., (Yanes *et al.* 2011; Colonese *et al.* 2013; Yanes and Fernández-Lopez-de-Pablo 2017; Padgett *et al.* 2019; Rech *et al.* 2021; Zong *et al.* 2022). Many variables should be considered for any interpretation to be correct. Discovering all the factors that must be considered is not easy, and many of the mechanisms that control gastropod behaviour are still unknown. Fortunately, our state of knowledge is constantly improving. The isotopic composition of precipitation can now be reconstructed with a high degree of probability based on the available data. Today, we can, with a high degree of probability, model reconstruction, based on the data obtained, of, among other things, the isotopic composition of precipitation. Carbon isotope ($\delta^{13}C$) data allow for the reconstruction of diet and the composition of the surrounding vegetation (Text-fig. 6), while stable oxygen isotope values ($\delta^{18}O$) provide information on the isotopic composition of environmental water (precipitation, relative humidity) and ambient temperature (Text-fig. 7).

Terrestrial snail shells from Quaternary sediments hold a large potential for reconstructions due to their widespread occurrence and the ability to decode information about past environmental conditions recorded in their shells. Initially, these reconstructions were based on an analysis of the taxonomic composition of the malacofauna. With the advancement of new technologies and research methods, isotopic analysis has gained prominence, allowing for more precise reconstructions of climatic and environmental conditions. Due to their unique characteristics, terrestrial snails serve as excellent recorders of climatic and environmental changes, encapsulating these changes in their carbonate shells. Deciphering this signal is a complex process that requires advanced research methods. Over the past few decades, numerous field studies and experimental laboratory rearing have contributed significantly to understanding the sources of oxygen and carbon in snail shells and the factors influencing the isotopic values of these elements (e.g., Yapp 1979; Goodfriend 1992; Scott 2002; Metref *et al.* 2003; Zhang *et al.* 2014, 2018*a*). The analysis of stable oxygen and carbon isotopes from subfossil land snail shells is best interpreted in the context of analogous data from modern snails. Parallel analyses would best address the following questions: 1) What factors influence the stable oxygen and carbon isotopes recorded in the shell? 2) What can be reconstructed from isotope records in land snail shells? Given the many factors influencing both issues, each case should be analysed individually, considering local conditions. Ideally, both modern reference snails and subfossil snails should belong to the same species. This allows us to assume that the environmental, climatic, and behavioural requirements of the species have not changed significantly and that the mechanisms that affect the species in the past and present are identical. If the species under study is no longer found in the study area today, the closest living relative should be analysed as a reference instead (Yanes *et al.* 2008).

The primary sources of carbon for terrestrial snails are diet, assimilated inorganic carbonates and atmospheric CO_2 (e.g., Yapp 1979; Goodfriend 1992; Scott 2002; Balakrishnan and Yapp 2004; Colonese *et al.* 2007; Yanes *et al.* 2008; Jenkins *et al.* 2023; Yanes and Fernández-Lopez-de-Pablo 2017; Padgett *et al.* 2019; Nield *et al.* 2023; Quenu *et al.* 2023) (Text-fig. 3). Oxygen sources include diet and environmental water, especially precipitation (e.g., Yanes *et al.* 2011; Colonese *et al.* 2013; Yanes and Fernández-Lopez-de-Pablo 2017; Padgett *et al.* 2019; Rech *et al.* 2021; Zong *et al.* 2022) (Text-fig. 3). The $\delta^{18}O$ and $\delta^{13}C$ values in the shells are influenced by factors such as the isotopic signal of the ingested carbonates, metabolic processes, relative humidity, temperature and other environmental parameters (Balakrishnan *et al.* 2005; Colonese *et al.* 2007; Wang *et al.* 2019; Bricker *et al.* 2023). These factors complicate unequivocal interpretation, necessitating further research and methodological development.

The analysis of stable oxygen and carbon isotopes in terrestrial snail shells is a crucial tool in palaeoclimatic reconstructions, especially for inferring the composition of vegetation (e.g., C3/C4 plant proportion) and the environmental conditions such as humidity and temperature. However, there are still many issues that can be addressed in future work. A topic worth exploring further is the integration of data from multiple locations and taxa with modern climate data from the same periods. This effort would facilitate a comprehensive analysis of the parameters that control the $\delta^{18}O$ and $\delta^{13}C$ values of the shells (Quenu *et al.* 2023). Research on the ecology of terrestrial gastropods remains valuable. Based on this, research into the $\delta^{13}C$ and $\delta^{15}N$ of food sources could be expanded to a broader spectrum. Studies could include isotopic analysis of decaying animal tissues, sediments, wood ash, and other materials. Furthermore, it would be valuable to analyse and compare syntopic small snails with larger ones, considering their different ecological habits. Field observations and laboratory experiments on snail diets are still needed, as the results would expand current knowledge and fill existing gaps (Yanes *et al.* 2018). Some researchers anticipate that isotopic studies of land snail shells will become a proxy to complement maps of the oxygen isotope distribution in precipitation (Zanchetta *et al.* 2005; Kehrwald *et al.* 2010; Prendergast *et al.* 2015; Yanes *et al.* 2018). According to these plans, land snails could be used as an independent data set capable of constraining general atmospheric circulation model simulations extending back to the Late Pleistocene (Prendergast *et al.* 2015). Furthermore, $\delta^{18}O$ isotopic values in some species may track environmental changes, such as cave infiltration, but with potentially much greater spatial coverage. Such species may serve as alternatives for studying palaeoclimate (Rech *et al.* 2021).

Future work should consider interspecific differences in ecophysiological traits, including changes in food preferences throughout the year. Variable behaviour in seasonal contexts, differing thermoregulatory capacities, and variability in longevity of individuals within and between genera also merit observation. This is particularly important when taxa at different sites do not belong to the same genus. To correctly interpret results in the context of palaeoclimate and palaeoecology, a thorough understanding of the ecophysiology of existing taxa is necessary. Furthermore, it is useful to verify whether the shells of land snails precipitate in isotopic equilibrium by measuring Clumped Isotopes Δ_{47} in the shells (e.g., Bricker *et al.* 2023). Studies of changes in the ecophysiological habits of terrestrial gastropods in the process of adaptation to a changing environment could also be carried out, which would significantly contribute to the development of fossil site studies and palaeoclimatic reconstructions (Wang *et al.* 2016).

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