RESEARCH ARTICLE

Use of amino acid isotope analysis to investigate capital versus income breeding strategies in migratory avian species

Nico Lübcker[1] | John P. Whiteman[2] | Oliver N. Shipley[1,3] | Keith A. Hobson[4,5] | Seth D. Newsome[1]

[1]Department of Biology, University of New Mexico, Albuquerque, New Mexico, USA

[2]Department of Biological Sciences, Old Dominion University, Norfolk, Virginia, USA

[3]Beneath The Waves, Herndon, Virginia, USA

[4]Environment and Climate Change Canada, Saskatoon, Saskatchewan, Canada

[5]Department of Biology, University of Western Ontario, London, Ontario, Canada

Correspondence
Nico Lübcker
Email: nlubcker2021@unm.edu

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Abstract

1. Income and capital breeding represent opposing ends of a continuum of reproductive strategies. Quantifying nutrient allocation to reproduction is challenging, but recent advances in compound-specific stable isotope analysis hold promise for tracing the source of individual compounds allocated to reproduction.
2. Here, we describe a novel approach of using measured carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values of individual amino acids (AAs) in pectoral muscle of egg-laying females and egg yolk as a useful tool to quantify the reliance on income versus capital breeding in migrating species. We used white-fronted (*Anser albifrons frontalis*), lesser snow (*A. caerulescens caerulescens*) and black brant (*Branta bernicla nigricans*) geese breeding in tundra ecosystems of northern Alaska as model organisms.
3. All three species relied on mixed capital–income breeding strategies, but models based on AA isotope data estimated higher proportional contributions of endogenous resources to yolk synthesis compared to results based on bulk tissue isotope analyses.
4. Tracing income versus capital nutrient allocation in migratory species at the compound level is a major advance from the current 'elemental' perspective obtained from bulk tissue stable isotope analyses. Our framework is applicable to all taxonomic groups, as long as there is a sufficient spatial or temporal isotopic gradient between resources obtained during the breeding and non-breeding periods.

KEYWORDS
biomonitoring, breeding strategy, compound-specific stable isotope analysis, egg production, resource allocation

1 | INTRODUCTION

Resource allocation trade-offs between survival and reproduction are a fundamental aspect of individual fitness and affect the long-term persistence of populations (Lameris et al., 2019). Income and capital breeders represent opposing ends of an exogenous-to-endogenous resource allocation continuum, with migratory avian and mammalian species exhibiting variable reliance on each (Hupp et al., 2018; Meijer & Drent, 1999; Ruthrauff et al., 2021). This may be attributed to variability in environmental conditions, resource phenology, individual physiology, body size and genotype (Williams et al., 2017). Identifying where individuals fall

along the capital–income continuum, and the plasticity of this important life-history trait in increasingly stochastic environments, is pertinent to understanding how species may respond to environmental change (e.g. Jaatinen et al., 2016; Lameris et al., 2019). For instance, changes in the contribution of endogenous versus exogenous resources to reproduction in migratory species can serve as a proxy response to such changes at different foraging locations. Such proxies are especially relevant to species' persistence in increasingly stochastic environments (Williams et al., 2017), such as those at high latitudes (Zuckerberg et al., 2020).

Conventional methods for studying capital versus income breeding relied on correlating clutch mass gain with the macronutrient loss in laying females (reviewed in Hobson, 2006). More recently, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis of bulk tissues (e.g. muscle, yolk) has proven useful (Hobson, 1995; Hobson & Jehl, 2010). Bulk tissue isotopes provide a quantitative means of directly tracing the relative inputs of endogenous versus exogenous nutrient sources to reproduction (Hobson et al., 2000; O'Brien et al., 2000). This approach hinges upon differences in the isotopic baseline reflecting primary production in foraging locations used during different periods of an organism's annual life cycle and is applicable to those species that migrate to breed (Hobson, 2006). Bayesian stable isotope mixing models (Moore & Semmens, 2008) have been used to quantify respective contributions between local and endogenous sources of carbon to eggs when they differ isotopically (e.g. Hobson, 1995; Hobson & Jehl, 2010; Klaassen et al., 2017; Sharp et al., 2013). Such approaches, however, can be heavily influenced by assuming the magnitude of isotopic discrimination between endogenous reserves and egg nutrients, and cannot distinguish among sources and routing of specific compounds (e.g. amino acids and fatty acids) that are important for animal nutrition and reproduction, especially for herbivores that consume diets with low protein contents (e.g. Twining et al., 2018; Whiteman et al., 2021).

Isotopic analysis of individual compounds is a useful tool for assessing the physiological status of consumers (e.g. Lübcker, Whiteman, Millar, et al., 2020; Shipley et al., 2022; Whiteman et al., 2021). Essential and non-essential amino acids (AAs) show different isotope discrimination between a consumer and its diet (Whiteman et al., 2019). The carbon backbone of essential AAs cannot be synthesized by animals and must therefore be routed directly from dietary protein originating from primary producers and fungi, with minimal ^{13}C fractionation (McMahon et al., 2010) or synthesized by gut microbiota (Newsome et al., 2020). In contrast, non-essential AAs can be synthesized de novo as well as routed directly from diet, resulting in more variable patterns of isotopic discrimination (McMahon & McCarthy, 2016). Nitrogen isotope dynamics of AAs follow a different categorization based on the degree of trans- and deamination they experience during metabolism. 'Source' AAs undergo minimal trans- and/or deamination, which results in minimal ^{15}N fractionation during trophic transfer; thus, these AAs typically reflect the nitrogen isotope composition of primary producers at the base of the food web. 'Trophic' AAs are tightly coupled to a dynamic nitrogen pool (generally in the form of glutamate) and undergo

considerable trans- and deamination resulting in greater ^{15}N fractionation during trophic transfer (McMahon & McCarthy, 2016; O'Connell, 2017). Finally, the glycolytic AAs, glycine and serine, have recently emerged as potential indicators of anabolic versus catabolic states of protein balance as related to the Cahill and/or Cori metabolic cycles (e.g. Lübcker, Whiteman, Millar, et al., 2020). These patterns render isotopic values of AAs particularly useful for discerning a suite of physiological processes.

Recent work has illustrated how AA $\delta^{13}C$ and $\delta^{15}N$ can trace protein allocation to reproduction in mammals (Lübcker, Whiteman, Newsome, et al., 2020) and birds (Whiteman et al., 2021). This method exploits inter-tissue differences in isotopic offsets between non-essential or essential AAs or groups of AAs (e.g. trophic vs. source) as a proxy for tissue catabolism (Whiteman et al., 2021). Remobilized endogenous AAs are ^{15}N-enriched, and if used for egg synthesis, result in larger $\delta^{15}N$ offsets between trophic and source AA pairs of yolk compared to $\delta^{15}N$ offsets in muscle tissue. Whiteman et al. (2021) showed that these offsets can reflect a spectrum of income or capital breeding strategies, without the need to characterize the isotopic composition of consumer diets or apply discrimination factors—the latter remaining a contentious source of error (Stephens et al., 2022). That study focused on a marine obligate capital breeder, the emperor penguin (*Aptenodytes forsteri*; hereafter EMPE), whose AA isotopic patterns contrasted with non-migratory and income breeding herring gulls (*Larus argentatus smithsonianus*; hereafter HEGU; Hebert et al., 2016). Importantly, those species did not recently travel among isotopically distinct biomes to breed. It remains a question whether and how one can apply the compound-specific isotope approach to cases where birds may use a mixed capital–income breeding strategy and migrate between isotopically distinct habitats immediately before the breeding season. Here, we aimed to assess the performance of the AA-based approach in examining mixed breeding strategies in migratory species, and whether this method can be applied without the need to characterize the isotopic composition of dietary sources available in the different foraging habitats utilized during their annual life cycle.

For migratory income species moving between isotopically distinct wintering and summer breeding sites, and whose body protein pool (e.g. skeletal muscle) has a relatively slow turnover rate compared to the duration of migration, $\delta^{13}C$ and $\delta^{15}N$ values of maternal tissues are expected to differ from local resources (Hobson & Clark, 1992), providing the necessary isotopic contrast to identify capital versus income sources of nutrients. For income breeders, we expect that essential AA $\delta^{13}C$ and/or source AA $\delta^{15}N$ values will differ between yolk and muscle, reflecting the baseline isotopic composition of the food webs utilized on the breeding and non-breeding grounds respectively (Figure 1). For capital breeders, we expect that essential AA $\delta^{13}C$ and/or source AA $\delta^{15}N$ in muscle and yolk to be indistinguishable, with both reflecting the baseline $\delta^{13}C$ and $\delta^{15}N$ of their wintering grounds. Predictions for non-essential AA $\delta^{13}C$ and/or trophic AA $\delta^{15}N$ values in both capital and income breeders are determined by underlying variability in the isotope composition of their prey in breeding and non-breeding grounds, as well as metabolic reactions associated
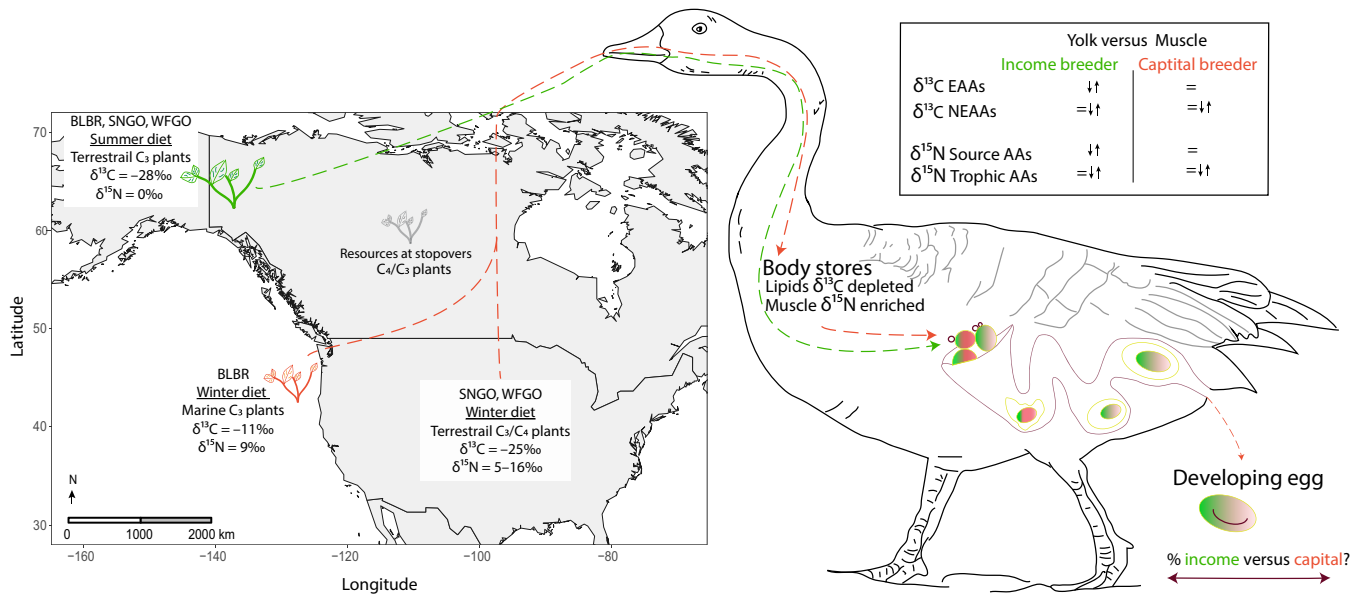
FIGURE 1 Expected outcome of income versus capital breeding strategies on yolk and muscle amino acid (AA) δ^{13}C and δ^{15}N values in geese breeding in the Colville River Delta region of Alaska. δ^{13}C values of essential AAs (EAAs) measured in the yolk and muscle of capital breeders should be identical (=) due to routing from endogenous pools. δ^{13}C values of non-essential AAs (NEAAs) can be similar, higher (↑) or lower (↓) than muscle δ^{13}C, depending on the δ^{13}C at the base of the food web and the macromolecular origin of endogenous carbon used to synthesize AAs de novo in the case of capital breeders. Muscle and yolk source AA δ^{15}N should similarly be equal in capital breeders, but trophic AA δ^{15}N values of yolk should be higher than muscle if newly synthesized AAs are used to produce yolk. For income breeders, trophic AAs δ^{15}N values can be similar, higher or lower than muscle δ^{15}N, depending on the δ^{15}N at the base of the food web.

with amino acid catabolism in capital breeders. In general, for non-essential AAs, we expect greater δ^{13}C variation between income and capital breeders because AAs used to synthesize yolk can be mobilized directly from muscle with little fractionation or synthesized de novo from carbon derived from relatively ^{13}C-enriched protein or ^{13}C-depleted lipid sourced from either exogenous (diet) or endogenous (skeletal muscle or adipose fat) resources. For income breeders, δ^{15}N values of trophic AAs in yolk may be lower or higher than muscle, depending on the baseline δ^{15}N values of the wintering/stopover versus breeding sites. Trophic AA δ^{15}N values of yolk should be higher than those of muscle if AAs in yolk were synthesized de novo from remobilized muscle proteins, but this pattern can be obscured by the amount of endogenous or exogenous resources that are used for egg synthesis, as well as underlying baseline δ^{15}N variability between non-breeding and breeding grounds (Figure 1).

Arctic-breeding geese present a useful model to explore the reproductive capital versus income paradigm using an AA isotope-based approach because they have been well studied with bulk tissue isotope analysis (e.g. Hupp et al., 2018). It was long believed that large-bodied migratory geese breeding at high latitudes adopt a strictly capital breeding strategy to combat adverse foraging conditions upon arrival in spring (reviewed by Hobson, 2006; Klaassen et al., 2006). However, bulk tissue isotopes provided strong evidence to suggest that these species adopt a mixed capital–income strategy for the allocation of proteins and lipids to eggs (Gauthier et al., 2003; Hobson et al., 2011; Klaassen et al., 2017; Sharp et al., 2013). The long-term research program highlighted in Hupp et al. (2018) focused on three sympatric breeding geese on the Beaufort coast of

northern Alaska, white-fronted goose (WFGO; *Anser albifrons frontalis*), lesser snow goose (SNGO; *Anser caerulescens caerulescens*) and black brant goose (BLBR; *Branta bernicla nigricans*), providing a unique opportunity to compare estimates of endogenous contributions of proteins to eggs based on both bulk tissue and AA isotope analysis. We also explore whether δ^{15}N offsets between trophic and source AAs (Δ^{15}N$_{\text{Trophic-source}}$) within tissues similarly reflect capital versus income breeding strategies. Whiteman et al. (2021) suggested that yolk Δ^{15}N$_{\text{Trophic-source}}$ should exceed that of muscle if remobilized ^{15}N-enriched AAs are used for egg synthesis. This approach assumes that differences between trophic and source AA δ^{15}N values in primary producers at the base of the food web, known as beta (β) values, are consistent between different biomes. We investigate how variation in β could affect the interpretation of Δ^{15}N$_{\text{Trophic-source}}$ values as an indicator of muscle catabolism (e.g. Whiteman et al., 2021). Our study elevates this AA-specific approach to trace income versus capital nutrient allocation in migratory species, applicable to a wide range of avian, reptile and mammal species.

2 | MATERIALS AND METHODS

2.1 | Study species, study area and sample collection

Our study was based on tissues provided by a long-term study of geese breeding at the Colville River Delta in northern Alaska (70.4°N, 150.6°W; see Hupp et al., 2018; U. S. Fish and Wildlife Service

migratory bird permit MB789758-1). In spring and summer 2012, pectoralis muscle was collected from early arriving female WFGO, SNGO and BLBR. Unpaired egg tissues collected from co-occurring individuals of each species were obtained following clutch completion. We included muscle and yolk samples from 24 and 25 individuals respectively (Table 1). Geolocator tracking data of all three species are presented in Hupp et al. (2018). Black brant goose wintered along coastal waters from Alaska to the south-western United States where they are known to forage primarily on common eelgrass (*Zostera marina*) which has high $\delta^{13}C$ (–11 to –13‰) and $\delta^{15}N$ (~9‰) values, as expected for a marine species (Hupp et al., 2018; Jaschinski et al., 2008; Thayer et al., 1978; Figure 1). White-fronted goose primarily used inland agricultural habitats in winter and spring staging areas (Hupp et al., 2018) ranging from the Mississippi River valley through to the Canadian prairies. Lesser snow goose winter origins were broader and likely included upland agricultural and coastal sites in the southern United States and northeast Mexico. Low bulk $\delta^{13}C$ values of muscle tissues (–25.1 to –19.0‰) of arriving SNGO and WFGO in the Arctic indicate they primarily used C_3-based habitats during winter and staging (Table 1); however, minor use of C_4-based agricultural plants (corn or sorghum) or freshwater habitats is also possible (e.g. Alisauskas & Hobson, 1992). Muscle $\delta^{15}N$ values (6.0–7.7‰) indicate these species largely consumed terrestrial resources (Alisauskas & Hobson, 1992). Upon arrival on the breeding grounds, all three species switched to consuming a local, terrestrial, C_3-based diet with low $\delta^{13}C$ (–28‰) and $\delta^{15}N$ (0‰) characteristic of Arctic food webs (Hupp et al., 2018), which provided the necessary isotopic difference between endogenous and exogenous nutrients.

All three species are highly synchronous breeders (Hupp et al., 2018). The WFGO are the first to arrive at the breeding site in early May and spend the most time there before initiating clutches (Table 1), whereas BLBR are the last to arrive in late May. All three species tend to initiate clutches around the same dates (Hupp et al., 2018). Muscle tissue of geese has an isotopic incorporation rate that depends on body mass, but in general, the samples collected upon arrival reflected dietary resources integrated from late winter through to arrival (Carleton & Martinez del Rio, 2005; Hobson & Clark, 1992). In contrast, yolk is synthesized during a 2- to 3-week period of rapid follicular growth preceding egg laying (Jaatinen

et al., 2016). The isotopic composition of yolk could reflect AAs acquired or synthesized while on or close to the breeding ground or alternatively allocated from muscle stores that largely reflect resources consumed during the non-breeding season (Alisauskas & Ankney, 1992).

2.2 | Bulk tissue $\delta^{13}C$ and $\delta^{15}N$ analysis

Muscle and egg yolk were freeze-dried and ground to a fine powder. Lipids are depleted in ^{13}C and were extracted using a 2:1 chloroform: methanol solution (Folch et al., 1957) during three sequential 24-h soaks (~72h total). Samples were then triple rinsed with deionized water and oven-dried at 60°C for 48h. For samples that had enough material for both bulk tissue and AA stable isotope analyses (Table 1), we weighed 0.5–0.6mg of dried lipid-extracted tissue into tin capsules and measured $\delta^{13}C$ and $\delta^{15}N$ values with a Costech ECS 4010 Elemental Analyser coupled to a Thermo Scientific Delta V Plus Isotope Ratio Mass Spectrometer (EA-IRMS) at the University of New Mexico Center for Stable Isotopes (UNM–CSI). Lipid removal can increase bulk tissue $\delta^{15}N$ by 0.9 ± 0.5‰ (Elliott & Elliott, 2016; Elliott et al., 2014). We applied no $\delta^{15}N$ lipid correction and used models with lipid-extracted bulk tissue $\delta^{15}N$ values to be consistent with the data used in Hupp et al. (2018) to which we compare our findings. Analytical precision was estimated at ± 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ using three interspersed internal reference standards (casein, tuna muscle and serine) which were calibrated against IAEA N1, IAEA N2 and USGS 43 for $\delta^{15}N$ and NBS 21, NBS 22 and USGS 24 for $\delta^{13}C$.

2.3 | Amino acid $\delta^{13}C$ and $\delta^{15}N$ analysis

An 8–12mg aliquot of lipid-extracted muscle and yolk was hydrolysed in 1mL of 6N HCL at 110°C for 20h and then dried at 110°C under a gentle stream of N_2. Amino acid films were then subjected to a two-step derivatization (Silfer et al., 1991) with 4:1 2-isopropanol: acetyl chloride (1h at 110°C) and 1:1 dichloromethane: trifluoroacetic anhydride (10min at 110°C). During hydrolysis, asparagine and aspartate are converted to aspartic acid (Asx) and glutamate and glutamine are

TABLE 1 Summary of data from pectoralis muscle and yolk samples collected from three goose species that exhibit sympatric breeding in the Colville River Delta region of the Alaska Arctic. *N* denotes the muscle and yolk sample sizes used for bulk tissue carbon ($\delta^{13}C$ in per mil (‰)) and nitrogen ($\delta^{15}N$) stable isotopes and for amino acid $\delta^{13}C$ and $\delta^{15}N$ analysis in brackets. The approximate arrival dates and interval between arrival and the initiation of nest incubation were obtained from Hupp et al. (2018). We used a Wilcoxon rank-sum test to assess differences between the muscle and yolk $\delta^{13}C$ and $\delta^{15}N$ values.

| Species | *N* | | Arrival date on breeding site | Days after arriving and clutch initiation | Bulk $\delta^{13}C$ (‰) | | Bulk $\delta^{15}N$ (‰) | |
	Muscle	Yolk			Muscle	Yolk	Muscle	Yolk
Black brant geese (BLBR)	10 (10)	10 (8)	23–28 May	~6–13 day	−13.2 ± 1.3	−20.6 ± 1.6***	11.5 ± 1.4	8.9 ± 1.8**
Lesser snow geese (SNGO)	6 (6)	8 (7)	12–16 May	~16 day	−23.9 ± 2.4	−24.3 ± 0.9	6.3 ± 0.2	6.6 ± 0.3
White-fronted geese (WFGO)	5 (6)	7 (5)	~9th May	~25 day	−20.1 ± 0.8	−24.7 ± 0.3***	6.9 ± 0.8	5.5 ± 0.6*

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

converted to glutamic acid (Glx). We measured the $\delta^{13}C$ and $\delta^{15}N$ values of seven essential AAs (phenylalanine (Phe), lysine (Lys), valine (Val), threonine (Thr), leucine (Leu), tyrosine (Tyr) and isoleucine (Ile)) and six non-essential AAs (alanine (Ala), glycine (Gly), proline (Pro), serine (Ser), Asx and Glx). Amino acids were separated using a Thermo Scientific Trace 1310 Gas chromatography containing a BPx5 60m column (ID 0.32mm, film thickness 1.0μm), reduced to N_2 at 1000°C in a Thermo Scientific IsoLink II combustion interface and Thermo Scientific Delta V Plus IRMS. Samples were analysed as duplicate injections, bracketed by either a single or duplicate injection of an in-house reference standard comprising a mixture of powdered AAs (Sigma Aldrich) of known isotopic composition measured via EA-IRMS (Table S3).

The derivatization technique adds carbon atoms to the original compound, and the $\delta^{13}C$ of the underivatized samples ($\delta^{13}C_{USA}$) was determined for each AA as follows:

$$\delta^{13}C_{USA} = \left(\delta^{13}C_{DSA} - \delta^{13}C_{DST} + \left(\delta^{13}C_{UST} \times P\right)\right) \times P^{-1}, \quad (1)$$

where DSA is the derivatized sample, DST is the derivatized standard, UST is the underivatized standard for each AA and P is the proportion of carbon atoms added.

The underivatized sample $\delta^{15}N$ values are determined ($\delta^{15}N_{USA}$) for each AA as follows:

$$\delta^{15}N_{USA} = \left(\delta^{15}N_{DSA} + \left(\delta^{15}N_{DST} - \delta^{15}N_{UST}\right)\right). \quad (2)$$

Both bulk tissue and AA isotope ratios are reported in standard delta (δ) notation as parts per mill (‰) relative to the international standard Vienna Peedee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$. Analytical precision for 13 AAs was assessed by calculating the within-run standard deviation (SD) of $\delta^{13}C$ and $\delta^{15}N$ of each AA of the sample duplicates, averaging 0.2‰ for $\delta^{13}C$ (range 0.1‰ (Glx) to 0.3‰ (Lys)). The within-run SD of $\delta^{15}N$ for the sample duplicates averaged 0.3‰ (range 0.2‰ (Lys) to 0.4‰ (Phe)). The within-run SD of $\delta^{13}C$ of the AAs in the reference material averaged 0.3‰ while the within-run SD of the $\delta^{15}N$ of AAs in the reference material averaged 0.4‰ (Table S4).

We also compared our geese data to previously published AA $\delta^{13}C$ and $\delta^{15}N$ values of muscle and yolk of EMPE (Whiteman et al., 2021) and HEGU (Hebert et al., 2016). The EMPE muscle ($n=5$) and yolk ($n=5$) samples were not from the same individual (unpaired), while Hebert et al. (2016) reported the AA $\delta^{15}N$ for paired muscle and egg homogenate (yolk and albumin) of HEGU ($n=4$).

2.4 | Statistical analyses

All analyses were performed using R (R Development Core Team 21, version 4.1.0) in the RStudio interface (version 1.4.1717). Normality was assessed using a Shapiro–Wilk test, and visual inspection of residual plots confirmed homoscedasticity. Wilcoxon rank-sum tests (R package *Coin*; Hothorn et al., 2008) were used to test for differences between the median muscle and yolk AA $\delta^{13}C$ and $\delta^{15}N$ values.

For analyses of bulk tissues, we used Bayesian stable isotope mixing models including both $\delta^{13}C$ and $\delta^{15}N$ values (R Package

'*MixSIAR*'; Stock et al., 2018) to quantify the proportional contribution of income versus capital nutrients to egg yolk protein. Source data included the mean ($\pm$SD) $\delta^{13}C$ and $\delta^{15}N$ values of lipid-free pectoral muscle and plant leaves (Hupp et al., 2018), while lipid-free yolk $\delta^{13}C$ and $\delta^{15}N$ values were used as consumer data. We used two sets of trophic discrimination factors in mixing models. For the first set (Model 1), we assumed that the bulk diet-yolk and muscle-yolk trophic discrimination factors were identical to those used in previous studies: $\Delta^{13}C$ of 0.0 ± 1.0‰ and $\Delta^{15}N$ of $+3.4\pm1.0$‰ (Hobson, 1995; Hupp et al., 2018).

For the second set (Model 2), we used muscle-yolk $\Delta^{13}C = 1.0\pm1.0$‰ and $\Delta^{15}N = 1.0\pm0.5$‰ (Cherel et al., 2005; Lübcker, Whiteman, Millar, et al., 2020). These values were chosen based on two assumptions. First, that catabolism of protein into free AAs do not result in isotopic enrichment of residual protein (muscle), provided that deamination of the AA does not occur; as a result, if catabolized AAs are directly routed to yolk synthesis without being deaminated, the $\delta^{15}N$ of muscle and yolk would be more similar (Lübcker, Whiteman, Millar, et al., 2020). Second, that discrimination factors between muscle and plasma (i.e. reported for fasting birds and mammals in Cherel et al., 2005 and Lübcker, Whiteman, Millar, et al., 2020) are a reasonable proxy for those between muscle and yolk, as free AAs in the plasma are used to synthesize the precursor to egg yolk in the liver (vitellogenin; Deeley et al., 1975).

We also constructed a mixing model in which the mean $\delta^{13}C$ value of essential AAs and the mean $\delta^{15}N$ value of source AAs from muscle and diet items were used as potential inputs to predict the mean values of yolk (Model 3). No discrimination factors were used for this model because consumers cannot synthesize these AAs de novo, and thus, their tissue isotopic composition directly reflects that of the input (although symbiotic microbes may alter this dynamic; see Newsome et al., 2020). We used AA isotope data for muscle tissue to represent dietary sources during the non-breeding season. We lacked AA isotope data of potential dietary sources on the breeding grounds; thus, we assumed that the available pool of essential AAs in local terrestrial C_3 plants had comparable $\delta^{13}C$ and $\delta^{15}N$ values as the bulk plant tissue. This assumption has been validated in a recent study by Besser et al. (2022), where the difference in mean $\delta^{13}C$ values between the bulk tissue and essential AAs in a large dataset of C_3 plants ($n=60$ samples) was only −0.4‰, which is within the analytical error and standard deviation of the bulk plant tissue $\delta^{13}C$ collected from the breeding grounds (Table S1). For goose muscle, the mean of the essential $\delta^{13}C$ AAs (Phe, Lys, Val, Thr, Leu, Tyr, Ile) and mean $\delta^{15}N$ of source AAs (Lys, Phe, Tyr) of each individual were calculated as input, and the analogous process was applied to yolk isotope values.

Finally, we constructed a fourth model to assess if the endogenous supply of essential/source AAs (Model 3) was higher than for non-essential/trophic AAs (Model 4) during rapid yolk formation. Non-essential/trophic AAs can be obtained from the diet or synthesized de novo, while essential/source AAs need to be obtained from the diet or from catabolized endogenous (muscle) stores. If the demand for essential/source AAs outstrips the supply

for exogenous resources during rapid yolk deposition, while sufficient non-essential/trophic AAs are synthesized de novo or routed directly from the diet towards yolk synthesis, it could result in differential estimates of capital versus income breeding for different AA groups. This model included the mean $\delta^{13}C$ value of the non-essential $\delta^{13}C$ AAs (Pro, Asx, Ser, Gly, Ala, Arg, Glx) and the mean $\delta^{15}N$ value of trophic AAs (Val, Thr, Leu, Ile, Ala, Asx, Glx, Pro, Arg) of each individual, using the same $\Delta^{13}C$ and $\Delta^{15}N$ as Model 2 (Table 2; Table S2).

Mixing models were run for 100,000 iterations with a burn-in period of 50,000 and thinning by a factor of 50 over three Markov chains. A Gelman–Rubin diagnostic test was used to assess model convergence, and all models had a scale reduction factor <1.1, indicating model convergence (Gelman & Rubin, 1992). We report the median and 95% credibility intervals (CI) of the predicted proportional contribution of endogenous or exogenous nutrients to the yolk.

TABLE 2 Predicted median proportional contribution of income versus capital amino acids (AAs) allocated to yolk synthesis of three Artic-breeding goose species; black brant goose (BLRB), snow goose (SNGO) and white-fronted goose (WFGO), with 95% credibility intervals (CI). We constructed four models (1–4) based on bulk tissue carbon (C) and nitrogen (N) isotope values (measured in per mill (‰)) with two different muscle–yolk isotopic discrimination factors (1 and 2), essential (for C) and source (for N) AAs (3) and non-essential (for C) and trophic AAs (for N) only (4). Essential AAs included Phe, Lys, Val, Thr, Leu, Tyr and Ile; source AAs included Lys, Phe, Tyr; non-essential AAs included Pro, Asx, Ser, Gly, Ala, Arg and Glx; trophic AAs included Val, Thr, Leu, Ile, Ala, Asx, Glx, Pro and Arg. Amino acid abbreviations are defined in Section 2.

Species	Component of yolk and muscle tissue in model	Model	Muscle–yolk discrimination (‰)		Median % contribution of endogenous AAs to yolk synthesis (95% CI)
			^{13}C	^{15}N	
BLBR	Bulk C & N	1	1.0	1.0	49 (41–58)
		2	0.0	3.4	50 (43–57)
	AA$_{essential}$ C & AA$_{source}$ N	3	0.0	0.0	71 (66–76)
	AA$_{non-essential}$ C & AA$_{trophic}$ N	4	0.0	3.4	69 (64–74)
SNGO	Bulk C & N	1	1.0	1.0	77 (64–87)
		2	0.0	3.4	49 (39–58)
	AA$_{essential}$ C & AA$_{source}$ N	3	0.0	0.0	96 (89–100)
	AA$_{non-essential}$ C & AA$_{trophic}$ N	4	0.0	3.4	91 (85–97)
WFGO	Bulk C & N	1	1.0	1.0	27 (19–37)
		2	0.0	3.4	36 (29–43)
	AA$_{essential}$ C & AA$_{source}$ N	3	0.0	0.0	79 (69–92)
	AA$_{non-essential}$ C & AA$_{trophic}$ N	4	0.0	3.4	74 (68–80)

We calculated differences in $\delta^{15}N$ between trophic and source AA pairs ($\Delta^{15}N_{T-S}$) for muscle and yolk for $\Delta^{15}N_{Glx-Phe}$, $\Delta^{15}N_{Pro-Phe}$, $\Delta^{15}N_{Glx-Lys}$ and $\Delta^{15}N_{Pro-Lys}$ for each species of goose, and compared results to previously published data for EMPE (Whiteman et al., 2021) and HEGU (Hebert et al., 2016) to evaluate if these AA pairings can be used as an index of the proportional contribution of income versus capital resources used in yolk production. For HEGU, we averaged the egg homogenate AA $\delta^{15}N$ of the two eggs collected per individual as reported in Hebert et al. (2016) and recalculated the difference between the egg and muscle AA $\delta^{15}N$ (Figure S2).

3 | RESULTS

3.1 | Bulk muscle–yolk $\delta^{13}C$ and $\delta^{15}N$

Bulk $\delta^{13}C$ and $\delta^{15}N$ differed between yolk and muscle for both BLBR (Table 1; Wilcoxon rank-sum test $\delta^{13}C$: $W=80$, $p<0.001$; $\delta^{15}N$: $W=69$, $p<0.01$) and WFGO ($\delta^{13}C$: $W=25$, $p<0.01$; $\delta^{15}N$: $W=23$, $p<0.05$). Yolk and muscle for SNGO did not differ in bulk $\delta^{13}C$ and $\delta^{15}N$.

3.2 | Muscle–yolk amino acid $\delta^{13}C$

Many essential $\delta^{13}C$ AAs differed between yolk and muscle, but patterns differed across the three species (Figure 2a). Black brant goose exhibited the most obvious trend, with all seven essential AA $\delta^{13}C$ averaging 5.9 ± 2.4‰ lower in yolk than in muscle (Wilcoxon rank-sum test of median values; Lys: $W=97$, $p<0.001$; Thr: $W=88$, $p<0.01$; Ile: $W=100$, $p<0.001$; Val: $W=97$, $p<0.001$; Leu: $W=100$, $p<0.001$; Phe: $W=92$, $p<0.001$; Tyr: $W=90$ $p<0.001$). In contrast, for SNGO, yolk was higher than muscle for four essential AAs (Lys: $W=7$, $p<0.05$; Val: $W=3$, $p<0.01$; Ile: $W=8$, $p<0.05$; Phe: $W=7$, $p<0.05$), while yolk and muscle did not differ for three essential AAs (Thr, Leu, Tyr). The WFGO was more similar to BLBR than SNGO, as yolk was lower than muscle for four essential AA (Thr, $W=32$, $p<0.05$; Ile: $W=34$, $p<0.01$; Leu: $W=8$, $p<0.05$; Tyr: $W=32$, $p<0.05$), although there was no difference for three AAs (Lys, Val, Phe).

For non-essential AAs, BLBR again had the most striking results, with all six non-essential AA $\delta^{13}C$ values being lower in yolk than in muscle (Wilcoxon Signed-Ranks Test for Pro: $W=91$, $p<0.01$; Glx: $W=97$, $p<0.001$; Asx: $W=98$, $p<0.001$; Ser: $W=100$, $p<0.001$; Gly: $W=100$, $p<0.001$; Ala: $W=100$, $p<0.001$; Figure 2b). For SNGO, yolk and muscle non-essential $\delta^{13}C$ were similar with only two exceptions: yolk Pro $\delta^{13}C$ was higher, and yolk Gly $\delta^{13}C$ was lower, than muscle (respectively: $W=7$, $p<0.05$; $W=47$, $p<0.01$). White-fronted goose was again more similar to BLBR, with almost all non-essential yolk $\delta^{13}C$ significantly lower in yolk than in muscle (Glx: $W=35$, $p<0.01$; Asx: $W=34$, $p<0.01$; Gly: $W=35$, $p<0.01$; Ser: $W=36$, $p>0.05$; Ala: $W=35$, $p<0.01$). The only exception was Pro, which did not differ between yolk and muscle ($W=13$, $p=0.530$).
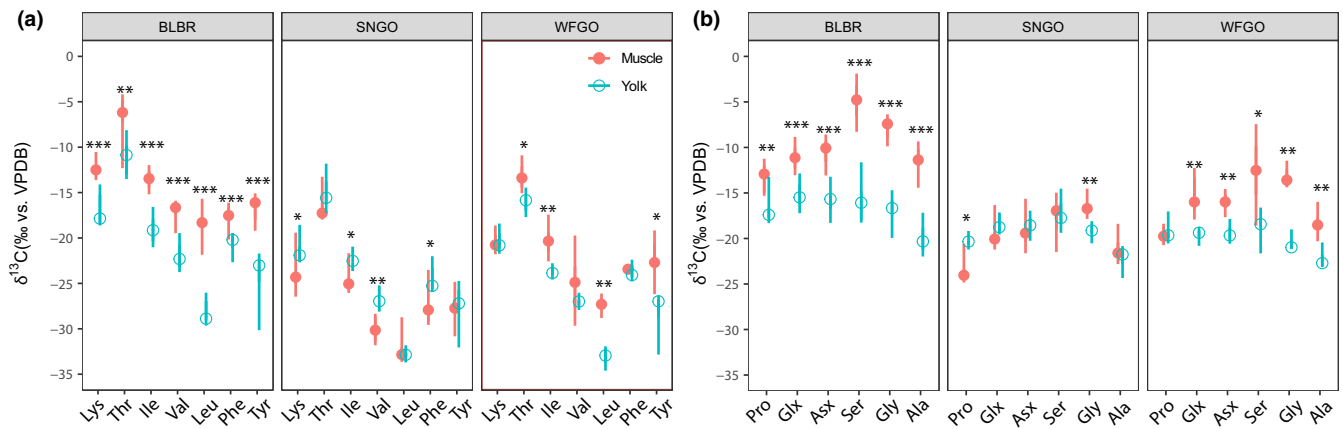
FIGURE 2 Median (a) essential and (b) non-essential amino acid (AA) $\delta^{13}C$ values of yolk (blue circles) and pectoralis muscle (red circles) of three Artic-breeding goose species; black brant goose (BLRB), snow goose (SNGO) and white-fronted goose (WFGO). Solid error bars reflect the 5% and 95% confidence intervals around the median $\delta^{13}C$. Amino acid abbreviations are defined in Section 2. Significance in AA $\delta^{13}C$ between tissues was based on a Wilcoxon rank-sum test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3.3 | Muscle–yolk amino acid $\delta^{15}N$

For BLBR, source AA $\delta^{15}N$ was slightly lower in yolk than in muscle for Tyr and Lys (Wilcoxon rank-sum test Tyr: 1.6‰ lower; $W=42$, $p < 0.05$; Lys 2.2‰ lower; $W=93$, $p < 0.001$) but did not differ for Phe ($W=47$, $p=0.880$; Figure 3a). For SNGO, yolk $\delta^{15}N$ was higher than muscle for Phe ($W=0$, $p < 0.001$) while the other AAs did not differ. For WFGO, yolk and muscle source AA $\delta^{15}N$ were similar (Figure 3a).

For Gly and Ser, $\delta^{15}N$ values were similar between yolk and muscle in BLBR and WFGO. For SNGO, Gly and Ser values were ~2‰ higher in yolk compared to muscle ($W=0$, $p < 0.001$: Ser $W=1$, $p < 0.01$; Figure 3a). For Thr, $\delta^{15}N$ of yolk were higher than muscle in SNGO ($W=1$, $p < 0.01$) and WFGO ($W=0$, $p < 0.01$; Figure 3c).

For BLBR, values of $\delta^{15}N$ for six of seven trophic AAs were lower in yolk than in muscle (Ala ($W=77$, $p < 0.5$), Glx ($W=85$, $p < 0.01$), Asx ($W=93$, $p < 0.001$), Val ($W=91$, $p < 0.01$), Leu ($W=95$, $p < 0.001$) and Ile ($W=93$, $p < 0.01$); Figure 3b). Almost all of these differences were larger than in other species, particularly for Ile, which was 5.9‰ lower in the yolk. For SNGO, yolk Pro and Ala $\delta^{15}N$ were higher than in muscle (Pro: $W=2$, $p < 0.01$; Ala: $W=2$, $p < 0.01$), whereas the remaining AAs exhibited the opposite trend (Val: $W=48$, $p < 0.001$, Leu ($W=48$, $p < 0.001$) and Ile ($W=48$, $p < 0.001$). For WFGO, yolk trophic AA $\delta^{15}N$ were lower than muscle for four AAs (Pro: $W=35$, $p=0.051$), Val ($W=38$, $p < 0.05$), Leu ($W=42$, $p < 0.01$) and Ile ($W=37$, $p < 0.05$). The AA data of each individual are presented in Tables S5 and S6.

3.4 | Bayesian mixing models: Income or capital breeders?

Models 1 and 2 contrasted capital and income strategies using bulk tissue data and different sets of discrimination factors for each model, as described in the Methods The results suggested that both SNGO and BLBR relied predominantly on capital resources (Figure S1). For SNGO, muscle was estimated to contribute 77% or 49% of the yolk

nitrogen, depending on whether the muscle–yolk $\Delta^{15}N$ was assumed to be 1.0 ± 0.5‰ or 3.4 ± 1.0‰ (Table 2). For BLBR, these contributions from muscle were estimated to be 49% or 50%. White-fronted goose relied more on income breeding than the other two species, with endogenous stores contributing 27% or 36% of the yolk nitrogen.

Models 3 and 4 contrasted breeding strategies using mean stable isotope values of essential/source AAs and non-essential/trophic AAs, respectively. These results suggested that all three species relied predominantly on capital resources. Model 3, which was based on source and essential AAs, indicated that capital, endogenous AAs contributed 71% of yolk AAs for BLBR, 96% of yolk AAs for SNGO and 79% of yolk AAs for WFGO (Figure 4; Table 2). These estimates of capital contribution decreased marginally in Model 4 when based on the contribution of trophic and non-essential AAs: 69% of yolk AAs for BLBR, 91% of yolk AAs for SNGO and 74% of yolk AAs for WFGO (Figure 5).

3.5 | Trophic and source amino acid offsets: Income or capital breeders?

For capital breeders, such as EMPE (Figure S2a), $\Delta^{15}N_{Pro-Phe}$ (Wilcoxon rank-sum test $W=0$, $p < 0.01$) and $\Delta^{15}N_{Pro-Lys}$ ($W=0$, $p < 0.01$) were higher in yolk compared to muscle. For a previously published dataset of income breeding HEGUs, no differences in the $\Delta^{15}N_{T-S}$ were observed between egg tissue and muscle ($\Delta^{15}N_{Glx-Phe}$: $W=3$, $p=0.147$; $\Delta^{15}N_{Pro-Phe}$: $W=7$, $p=0.886$; $\Delta^{15}N_{Glx-Lys}$: $W=4$, $p=0.343$; $\Delta^{15}N_{Pro-Lys}$ $W=5$, $p=0.486$; Figure S2c). In our study, all four pairings of $\Delta^{15}N_{T-S}$ were generally smaller for yolk than for muscle across the three goose species; this stands in contrast to the patterns observed for both a marine capital breeder (EMPE) and a freshwater/terrestrial income breeder (HEGU). Yolk $\Delta^{15}N_{Glx-Phe}$ was smaller than muscle for all three goose species (BLBR −2.3‰; $W=85$, $p < 0.01$; SNGO −2.2‰; $W=47$, $p < 0.01$; WFGO −1.7‰; $W=37$, $p < 0.05$; Figure S2b). The $\Delta^{15}N_{Pro-Phe}$ was smaller in yolk than in muscle for BLBR and WFGO but not in
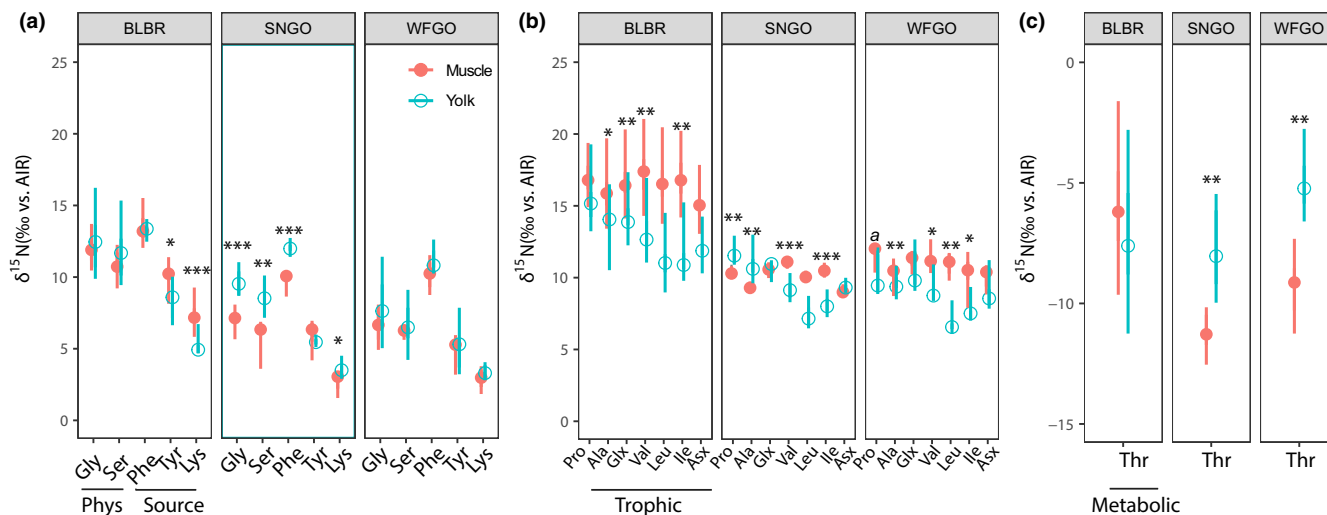
FIGURE 3 Median (a) source and (b) trophic amino acid (AA) $\delta^{15}N$ values of yolk (blue circles) and muscle (red circles) of three Artic-breeding goose species; black brant goose (BLRB), snow goose (SNGO) and white-fronted goose (WFGO). Threonine (Thr) is plotted separately (c) due to its characteristically low $\delta^{15}N$ values. Solid error bars reflect the 5% and 95% confidence intervals around the median $\delta^{13}C$. Amino acid abbreviations are defined in Section 2. Glycine (Gly) and Serine (Ser) are often considered physiological AAs (Phys), while Thr is considered a metabolic AA. Significance in AA $\delta^{15}N$ between tissues was based on a Wilcoxon rank-sum test (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

SNGO (BLRB: $\Delta^{15}N_{Pro-Phe}$ 1.2‰ lower; $W=75.5$, $p=0.058$; WFGO: $\Delta^{15}N_{Pro-Phe}$ −2.5‰; $W=41$, $p<0.01$). The values of $\Delta^{15}N_{Glx-Lys}$ and $\Delta^{15}N_{Pro-Lys}$ were smaller in yolk than in muscle for WFGO only (respectively, $W=31$, $p<0.05$; $W=34$, $p<0.01$, Figure 6).

4 | DISCUSSION

Building on AA isotope studies of capital (Whiteman et al., 2021) versus non-migratory income (Hebert et al., 2016) species, we applied this approach to three closely related migratory geese that move between isotopically different biomes and in which mixed capital–income reproduction strategies were assumed. As expected, all three species utilized mixed capital–income breeding consistent with previous findings (Hupp et al., 2018), but higher proportional contributions of capital resources to yolk synthesis were observed when assessed using individual AAs. Complementary data for potential dietary resources at the breeding site are required to quantify the proportional contribution of exogenous versus endogenous resources allocated to yolk synthesis. Below, we outline considerations required to elevate the promising application of AA isotopes to trace nutrient allocation during reproduction.

4.1 | Amino acid $\delta^{13}C$ and $\delta^{15}N$: Income versus capital breeding

Patterns in AA $\delta^{13}C$ and $\delta^{15}N$ values among yolk and muscle of all three species were difficult to interpret without considering the isotopic composition of prey available on the breeding and non-breeding grounds. For example, yolk AA $\delta^{13}C$ values of BLRB were ~6–7‰ lower on average than that of muscle (Figure 2),

suggesting that their carbon skeletons were synthesized from sources with distinctly lower $\delta^{13}C$ baselines than those used to synthesize muscle prior to arrival on the breeding grounds. While this pattern is suggestive of income breeding, black brant forage almost exclusively on ^{13}C-enriched eelgrass *Zostera marina* ($\delta^{13}C = \sim-11$‰) during the non-breeding season and then switch to an Arctic terrestrial C_3 plant diet ($\delta^{13}C = \sim-28$‰) during the summer breeding season (Figure 1; Hupp et al., 2018), a large shift in baseline $\delta^{13}C$ values of ~17‰. Considering that the difference between yolk and endogenous (muscle) $\delta^{13}C$ is only ~6–7‰ and the yolk has a mean $\delta^{13}C$ value of −17.5‰, mixing models based on bulk tissue data showed that ~50% of the yolk was sourced from endogenous reserves indicating use of a mixed capital–income breeding strategy.

Isotope mixing models based on AA isotope data suggest that all three species relied on mixed capital–income breeding strategies but were more reliant on capital than estimated by bulk tissue isotopes (Figures 4 and 5; Table 2). For example, most essential/source AAs (~71%; Model 3) and non-essential/trophic AAs (69%; Model 4) in black brant yolk were sourced from endogenous essential AAs catabolized from muscle (Figure 4). These estimates are significantly higher than those based on bulk tissue (95% CI: 41–58; Models 1 and 2). Likewise, endogenous sources accounted for 96% of essential/source AAs in the yolk of lesser snow goose, which is also higher than bulk tissue estimates (~71%, Hupp et al., 2018). Models show that white-fronted goose also used a mixed breeding strategy but were more reliant on capital with 79% and 74% of yolk essential/source and non-essential/trophic AAs, respectively, originating from endogenous stores. The AA-based predictions for white-fronted goose differed from previous studies (Hupp et al., 2018) and models based on bulk tissue data reported here, showed that only ~36% of yolk was derived from endogenous stores (Table 2).

FIGURE 4 Isotope biplots (left panels) and Bayesian mixing model (MixSIAR) results (right panels) depicting the proportional contribution of endogenous (muscle) or exogenous (plant) amino acids to yolk synthesis in black brant goose (BLBR) (a, b), lesser snow goose (SNGO) (c, d) and white-fronted goose (WFGO) (e, f). The mean essential δ^{13}C and source AA δ^{15}N values of the yolk and muscle were used as consumer and dietary source values respectively (Model 3). Error bars indicate uncertainty (±1 SD) source AA and bulk muscle tissue isotope values. Posterior distribution plots of exogenous and endogenous contributions to yolk formation in the right panels indicate the predicted contributions of essential and source AAs to yolk synthesis.
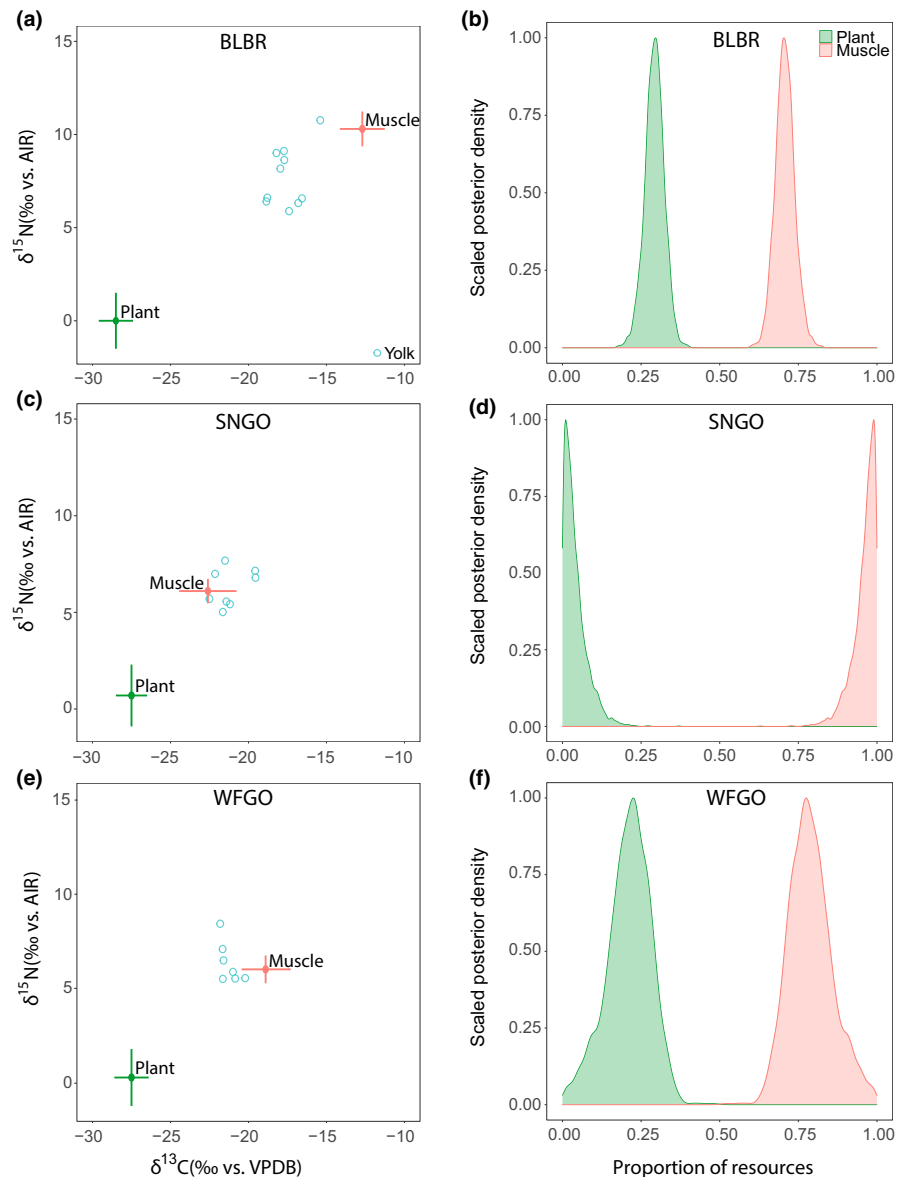
Of the three species, white-fronted goose spends the most time between arrival at the breeding site and clutch initiation and thus were expected to rely more on income breeding compared to black brant (Table 1). Values of δ^{15}N for several trophic AAs were lower in white-fronted goose yolk than in muscle, which is contrary to expectations when extensive catabolism of muscle and/or de novo synthesis of AAs occurs (Lübcker, Whiteman, Millar, et al., 2020; Whiteman et al., 2021). These patterns could reflect differences in β values among the primary producers that fuel the food webs used by geese in the non-breeding versus the breeding season (see below). Alternatively, geese have been observed to feed on staging grounds close spatially and temporally to their breeding grounds and clutch initiation, a strategy known as proximate capital (Klaassen et al., 2006; Schmutz et al., 2006), which could provide an additional source of AAs that would not be reflected in muscle tissue due to its slower rate of isotopic incorporation (Hobson & Clark, 1992; Hupp et al., 2018). Mixing models are sensitive to potentially missing prey, assumptions regarding trophic discrimination factors and β values,

which could have contributed to differences in predictions based on bulk tissue and the AA-based approach, warranting further investigation. Our derivatization protocol (Silfer et al., 1991) measured ca. 97% of all amino acids present in yolk (Mori et al., 2020), but the bulk stable isotopes could also reflect other nitrogen and carbon-containing molecules not removed by the lipid extraction, such as, for example, phosphoproteins (Mecham & Olcott, 1949). Other nitrogen-containing molecules, however, represents less than ca. 0.2% of the yolk crude protein (Attia et al., 2020), but could further explain discrepancies between bulk tissue and AA-based predictions.

4.2 | Consideration of AA δ^{15}N β values

Careful consideration of how β values vary among primary producers consumed by migratory consumers is needed to interpret patterns in consumer AA δ^{15}N values (Ramirez et al., 2021). In
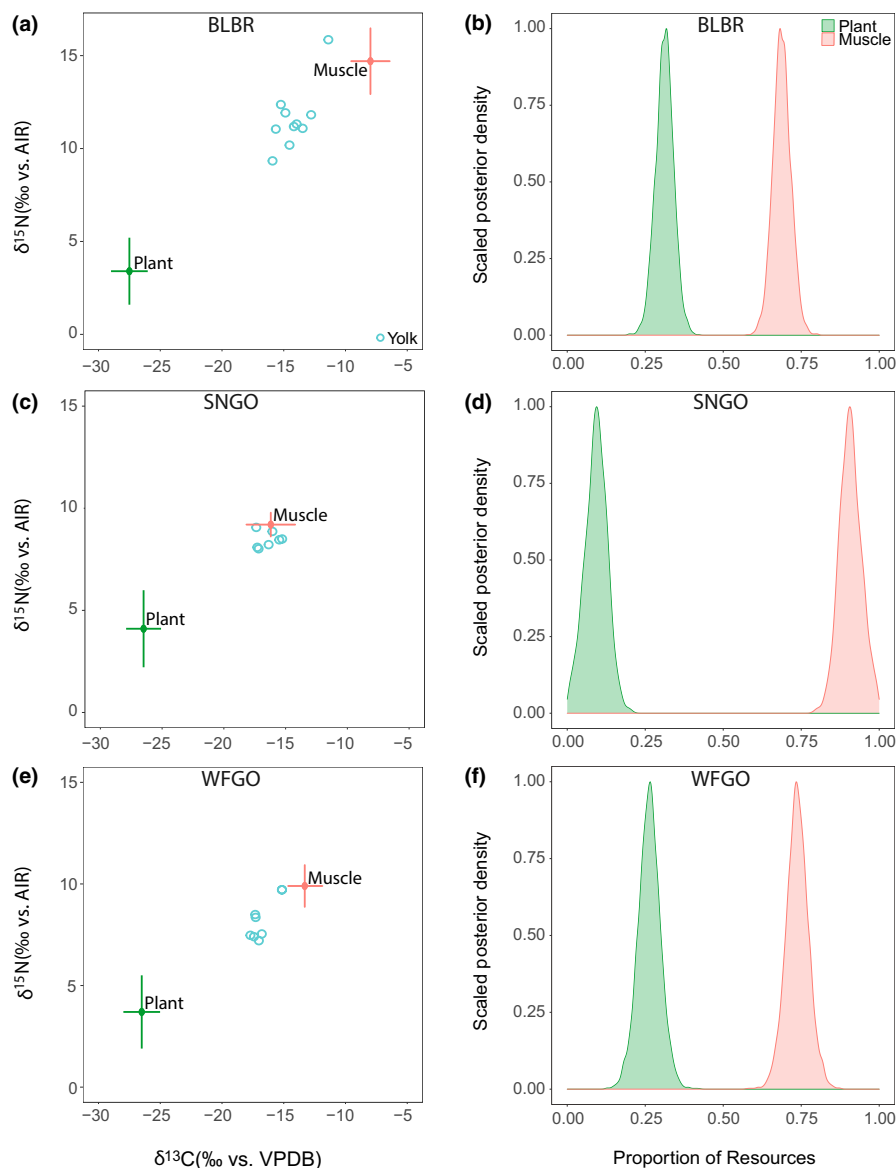
FIGURE 5 Isotope biplots (left panels) and Bayesian mixing model (MixSIAR) results (right panels) depicting the proportional contribution of endogenous (muscle) or exogenous (plant) amino acids to yolk synthesis in black brant goose (BLBR) (a, b), lesser snow goose (SNGO) (c, d) and white-fronted goose (WFGO) (e, f). The mean non-essential $\delta^{13}C$ and trophic AA $\delta^{15}N$ values of the yolk and muscle were used as consumer and dietary source values respectively (Model 4). Source AA data are corrected for discrimination and error bars indicate combined source AA and discrimination uncertainty ($\pm 1\,SD$). Posterior distribution plots of exogenous and endogenous contributions to yolk formation in the right panels indicate the predicted contributions based on a muscle–yolk nitrogen discrimination factor of $1.0 \pm 0.5‰$ (solid colours) and $+3.4 \pm 1.0‰$ (opaque colours) following Hupp et al. (2018).

contrast to previous work on capital breeding emperor penguin (Whiteman et al., 2021) that forage in pelagic marine ecosystems characterized by relatively invariant β values, the migratory geese examined here consume aquatic and terrestrial autotrophs throughout the year that have varying β values. For example, all three geese species forage on a mixture of aquatic ($\beta_{Glx-Phe}$ values: +3–5‰) and terrestrial primary producers ($\beta_{Glx-Phe}$ values: −5–6‰) during migration and the non-breeding season (winter), but then switch to forage solely on terrestrial plants during the summer breeding season (Figure 6; Ramirez et al., 2021). Underlying differences in the primary producer β values determine the $\delta^{15}N$ offsets between trophic and source AAs ($\Delta^{15}N_{Glx-Phe}$) in muscle (capital). Specifically, the $\delta^{15}N$ of trophic AAs of muscle is influenced by the proportion of terrestrial and aquatic resources consumed during the non-breeding period (Figure 6a). Yolk represents the mixture of both income and capital resources, and the $\Delta^{15}N_{Glx-Phe}$ of yolk reflects the proportion of muscle (capital) and terrestrial plants (income) used to synthesize this egg component.

For all three geese species, the measured $\Delta^{15}N_{Glx-Phe}$ of yolk was ca. −2‰ lower than that of muscle (Figure 6b) despite the expectation that yolk $\Delta^{15}N_{Glx-Phe}$ should be higher because it is catabolized from muscle. This suggests that the β values of potential prey in non-breeding and breeding grounds need to be better quantified before patterns in $\Delta^{15}N_{Glx-Phe}$ can be used as a quantitative index for how much muscle catabolism contributes to yolk synthesis (Whiteman et al., 2021).

4.3 | Supply and demand: Amino acid deposition in yolk

The allocation of resources to reproduction can be viewed as a trade-off between the physiological costs of carrying excess reserves while migrating and the supply versus demand of AAs required for yolk synthesis, which occurs within a narrow 2- to 3-week window before egg laying (Jaatinen et al., 2016). Essential
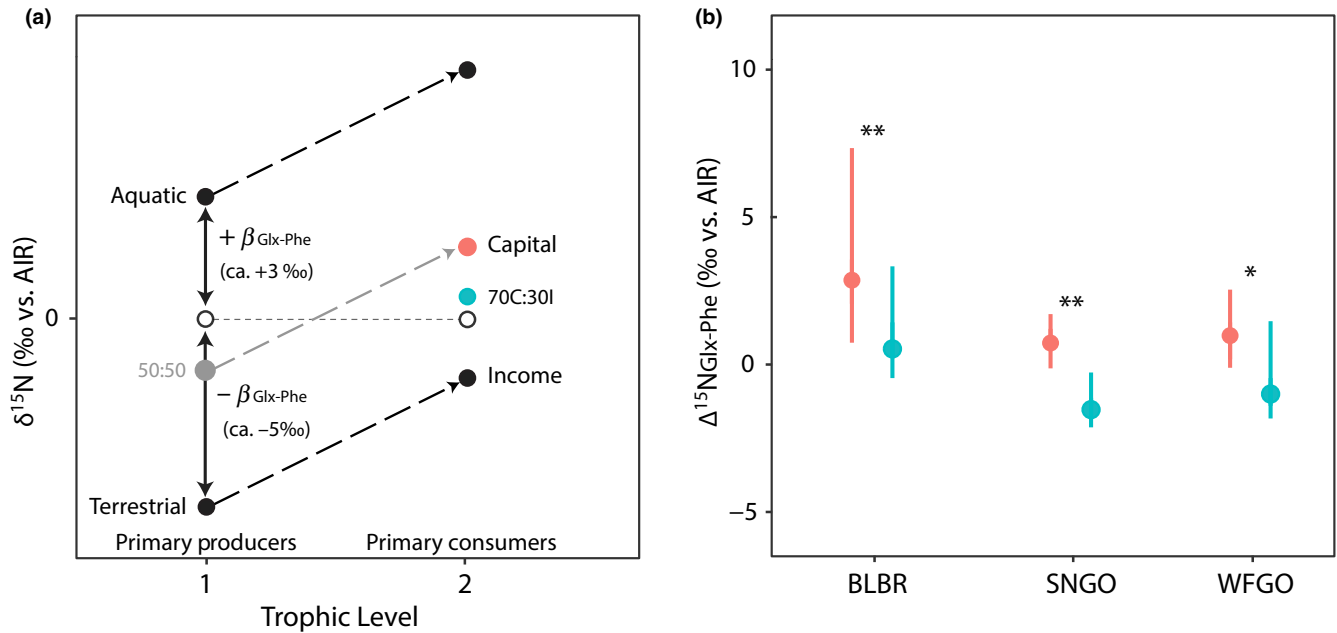
FIGURE 6 Schematic illustration of how underlying differences in β values affect the offset in $\delta^{15}N$ values ($\Delta^{15}N_{Glx\text{-}Phe}$) between Glx (closed circles) and Phe (open circles) measured in goose muscle (red) and yolk (blue). This framework is similar to that proposed by Chikaraishi et al. (2009) to estimate trophic position, with $\beta_{Glx/Phe}$ representing the $\delta^{15}N$ difference between Glx and Phe synthesized by primary producers. Panel (a) demonstrates the expected positive muscle $\Delta^{15}N_{Glx\text{-}Phe}$ of geese (primary consumers) foraging on aquatic primary producers (e.g. phytoplankton and macroalgae) with positive $\beta_{x/y}$ values ($\beta_{Glx\text{-}Phe} + 3‰$) during the non-breeding season. If we assume an income breeding scenario in which yolk was completely synthesized from terrestrial plants at the breeding site with negative $\beta_{Glx\text{-}Phe}$ of $-5‰$, the yolk $\Delta^{15}N_{Glx\text{-}Phe}$ would be lower than muscle $\Delta^{15}N_{Glx\text{-}Phe}$ assuming the diet to muscle and diet to yolk trophic enrichment of Glx is comparable (dashed lines are parallel) and there is minimal discrimination in Phe across trophic levels. Because these species eat a mixture of aquatic and terrestrial resources during the non-breeding season, the muscle trophic AA $\delta^{15}N$ would be a mixture of the two sources as shown by the grey circle representing a 50:50 mixture of aquatic and terrestrial resources. The proportion of muscle (capital) and terrestrial plants at the breeding site (income) uses to synthesize yolk would ultimately determine the pattern in $\Delta^{15}N_{Glx\text{-}Phe}$ among tissues (Panel b). If we assume 70% of yolk AAs come from capital (C) sources and 30% from income (I), then the yolk would have $\Delta^{15}N_{Glx\text{-}Phe}$ that is lower than the muscle, despite evidence for muscle catabolism as observed in all three geese species. This highlights the need for additional captive feeding experiments to characterize trophic enrichment of AAs and the need to quantify $\beta_{Glx/Phe}$ of potential prey in non-breeding and breeding grounds before using $\Delta^{15}N_{Glx\text{-}Phe}$ as a quantitative index of how much muscle catabolism contributed to yolk synthesis (Whiteman et al., 2021). Significance in AA $\Delta^{15}N_{Glx\text{-}Phe}$ values between tissues was based on a Wilcoxon rank-sum test (*$p < 0.05$; **$p < 0.01$). Black brant goose (BLRB), snow goose (SNGO) and white-fronted goose (WFGO). Solid error bars reflect the 5% and 95% confidence intervals.

AA requirements for egg production can outweigh the supply through normal foraging by up to twofold (Meijer & Drent, 1999; Robbins, 1981), and this deficit can be met by compensatory feeding and/or the catabolism of endogenous stores. One of the potential advantages of using AA isotope analysis is that the proportional contribution of exogenous versus endogenous sources of individual AAs can be modelled separately (Figures 4 and 5). The predicted endogenous supply of essential/source AAs (Model 3) was similar to the endogenous supply of non-essential/trophic AAs (Model 4) during rapid yolk formation in all three species (Table 2). Future studies that include prey AA isotope values from the breeding grounds as end members can evaluate the contribution of capital versus income resources of individual AAs (e.g. García-Seoane et al., 2023). Finally, additional information on the timing of when specific AAs are deposited during yolk synthesis is required to better understand how physiological processes associated with catabolism and reproduction impact the isotopic patterns observed here.

This information could be obtained in controlled captive feeding experiments where isotopically distinct diets are switched at the onset of rapid follicular growth.

5 | CONCLUSIONS

We tested the prediction that AA $\delta^{13}C$ and $\delta^{15}N$ values of yolk and muscle can reflect a spectrum of income or capital breeding strategies at the compound level in three migratory goose species that utilize different habitats over their annual life cycle. Estimated proportions of endogenous reserves to reproduction based on both bulk tissue and AA isotope analysis suggested that all three species relied predominantly on capital resources when assessed at the compound level. Patterns in AA $\delta^{13}C$ and $\delta^{15}N$ suggested that all three geese species included some exogenous resources in yolk synthesis, but we could not quantify their

proportional contribution without complementary data for potential dietary resources (plants) at the breeding site. Specifically, consideration of habitat-specific β values needed to be included when using $\Delta^{15}N_{T-S}$ offsets as a measure of income versus capital allocation of AAs to reproduction. Overall, our study expands the promising application of AA isotopes as an eco-physiological tool to trace nutrient allocation during reproduction and establishes a framework that could be applied to a diverse array of taxonomic groups. Importantly, we reveal that the capital versus income paradigm should be restructured in an ecophysiological context to consider more precisely the nature of AAs involved (i.e. source vs. trophic, essential vs. non-essential). A similar approach could presumably also consider fatty acid contributions to capital versus income breeding.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14150.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.xsj3tx9mg (Lübcker et al., 2023).

ORCID

Nico Lübcker https://orcid.org/0000-0001-7141-6669
John P. Whiteman https://orcid.org/0000-0002-3348-9274
Oliver N. Shipley https://orcid.org/0000-0001-5163-3471
Keith A. Hobson https://orcid.org/0000-0002-2525-1178
Seth D. Newsome https://orcid.org/0000-0002-4534-1242

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Description of amino acid standards and data used to investigate capital versus income breeding strategies in migratory avian species.