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The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*)†

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The difference in isotopic composition between a consumer's tissues and that of its diet is a critical aspect of the use of stable isotope analyses in ecological and palaeoecological studies. In a controlled feeding experiment with the Atlantic salmon, *Salmo salar*, we demonstrate for the first time that the value of tissue-diet isotope spacing in nitrogen in a growing animal is not constant, but varies inversely with growth rate. The value of tissue-diet isotopic spacing in N reflects N use efficiency. Thus, in salmon, growth rate is accompanied by, or requires, increased N use efficiency. The total range in tissue-diet isotopic spacing in N seen in the experimental population of 25 fish was 1‰, approximately 50% of the total trophic shift. Mean equilibrium tissue-diet isotopic spacing (± standard deviation) in salmon averaged 2.3‰ (±0.3‰) and 0.0‰ (±0.3‰) for N in muscle and liver, respectively, and 2.1‰ (±0.1‰) and 1.6‰ (±0.3‰) for C in muscle and liver, respectively. Feeding with a mixed dietary source (wheat and fish-meal origin) resulted in tissue-diet isotopic fractionation in both C and N due to the differential digestibility of food components with distinct isotopic composition. The rate of change in isotopic composition of *S. salar* tissues was dominated by growth, but the estimated contribution of metabolic turnover to change in tissue N was relatively high for an ectothermic animal at ca. 20–40%. The estimated half-life for metabolic turnover of the tissue N pool was ca. 4 months in both muscle and liver tissue. This is the first study to demonstrate a direct relationship between tissue-diet isotopic spacing in N and growth rate and adds to the growing list of factors known to influence the level of isotopic separation between a consumer's tissue and that of its diet.

Stable isotope analysis (SIA) is commonly used to infer diet and trophic level in ecosystem studies. SIA offers advantages over gut content analysis as a method to study ecosystem structure because the isotopic composition of animal tissue reflects the average diet assimilated over a length of time, usually of the order of weeks to months. SIA may also be performed retrospectively using archived, historic or archaeological materials,[1,2] allowing reconstruction of ecosystem or ecological change over long time periods.

The use of tissue isotope composition as a proxy for diet requires that the relationship between the isotopic composition of a consumer's tissues and that of its diet (termed tissue-diet spacing, or $\Delta_{t\text{-}d}$ where $\Delta =$ (for instance) δ^{15}N consumer – δ^{15}N diet) is well known and, ideally, is constant.[3] The magnitude of $\Delta_{t\text{-}d}$ is an average of all isotopic fractionation reactions occurring within the body, and is assumed to be dominated by fractionation in favour of light isotopes during the production of waste products (e.g. urea, ammonia). Recent studies have suggested that $\Delta_{t\text{-}d}$ in both N and C is rather more complex than first assumed, with a variety of metabolic and dietary factors influencing the magnitude of the trophic shift.[4–9] In fish, tissue-diet isotopic spacing appears to be a function of the quantity and quality of food ingested with the magnitude of $\Delta_{t\text{-}d}$ falling with increased feeding rate, reflecting changes in the metabolism of compounds such as essential amino acids, lipids and carbohydrates.[7,8,10]

Rapid growth may be triggered by increased access to food involving changes in the proportion of non-essential amino acids sourced directly from dietary protein (i.e. changes in nitrogen use efficiency). If so, tissue-diet isotopic spacing within individual growing animals of the same species will be a function of growth rate. The aim of this study is first to determine values of $\Delta_{t\text{-}d}$ in muscle and liver tissues of Atlantic salmon (*Salmo salar*) as a first step for field applications of SIA techniques, and secondly to examine the relationship between growth rate and $\Delta_{t\text{-}d}$ in C and N in a

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rapidly growing fish with a simple controlled feeding experiment.

EXPERIMENTAL

Growth of salmon

Ninety MOWI Norwegian hybrid salmon (1 + age), raised to smolt stage (transition from fresh water to sea water) on a common diet, were obtained from Yorkshire Salmon Ltd. (Skipton, UK) in May 2004. The masses of a further 196 fish drawn from the same population were determined to estimate mean initial mass.

All fish were kept communally in a single constant-flow ambient seawater tank (capacity 1500 L) at the University of Portsmouth Marine Station, Eastney, UK. Salmon were fed from a single batch of EWOS booster feed diet (Table 1) for 315 days to ensure equilibration with the new diet. Feed was added to the tank once or twice daily until uneaten pellets remained floating on the surface. Daily feeding levels were between approximately 100 and 300 g of pellet feed. During the course of the experiment, any mortalities were removed and frozen.

At the start of the experiment, six fish were randomly selected, sacrificed by schedule 1 methods, and muscle and liver samples were taken to determine the initial isotopic composition. In all the sampled fish, white muscle samples were taken from a region below the dorsal fin and above the lateral line. Portions of liver were dissected and the hind gut contents were removed following excision of the lower colon immediately anterior of the anus. Muscle, liver and gut content samples were immediately frozen at −20°C until preparation and analysis.

Tissue preparation

Dissected tissues were oven dried at 60°C overnight then ground to a homogeneous powder using a ceramic pestle and mortar. Samples were split and one subsample was treated to remove lipids using a 10:5:4 methanol/chloroform/water extraction.[11] Extractions were performed at least three times until a clear supernatant was obtained. Following lipid extraction, samples were oven dried at 60°C. Samples of pellet feed were taken repeatedly throughout the duration of the experiment, oven-dried and lipid-extracted using the same procedure as applied to liver and white muscle.

Analytical methods

Carbon and nitrogen isotope ratios for dried whole and extracted fish tissues and feed were measured by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Costech (model ECS 4010) elemental analyser (EA) combined with a ThermoFinnigan Delta Plus XP mass spectrometer. Approximately 0.8 mg of each sample was combusted in a tin cup for the simultaneous determination of C and N isotope ratios. Three laboratory standards were analysed for every 8–10 unknown samples in each analytical sequence, allowing instrument drift to be corrected if required. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from the international standards V-Pee dee belemnite (carbon) and AIR (nitrogen), according to the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The measurement precision of both $\delta^{15}N$ and $\delta^{13}C$ was estimated to be $\leq$0.3‰.

Table 1. Composition of experimental feed (EWOS booster feed 3 mm pellet size). Pellets are composed of fish meal, fish oil, wheat and shrimp meal

EWOS booster feed components	Wt %
Oil	23
Protein	48
Fibre	1.5
Ash	13

RESULTS

Full results for fish growth and isotopic composition of tissues and feed are provided in the Appendix.

Salmon growth

Following smolting, six individuals became feeble and died within 12 weeks. A further four individuals jumped into an outflow pipe and died. Four individuals were randomly chosen and sacrificed at week 19 to monitor general health. Following failure of an air pump during the night of 30 September 2004, after 26 weeks, 30 salmon died, presumably due to asphyxiation. These salmon were removed, weighed, measured and immediately frozen at −20°C prior to dissection. No further mortalities occurred. The remaining salmon were removed on 5 February 2005, sacrificed by schedule 1 methods, weighed, measured and dissected immediately after sacrifice.

Individual mass increase was estimated as the difference between the mass of the fish at sampling and the mean initial mass. Calculating mass increase relative to the mean initial mass introduces error into our estimates of individual growth rate. The 95% confidence interval around the mean initial mass is ±1 g. Taking the upper and lower confidence intervals on the mean initial mass as likely maximum and minimum initial weights for any individual fish, we estimate errors on individual growth rate values as ±2.5%.

Average growth rates (G, month^{-1}) were estimated as:

$$G = (w_t/w_i)^{1/t} \quad (2)$$

where w_t = weight of the fish at time t, and w_i = the mean initial weight (48.5 ± 1 g).

The mean growth rate (±standard deviation (SD), n = 25) for fish sampled at week 45 was 1.19 ± 0.02 month^{-1}. Individual mean growth rates ranged from 1.14 to 1.22 month^{-1}. Thus all fish sampled at week 45 were increasing in mass, with significant differences in the rate of addition of new tissue (Table 2).

Isotopic data

The mean isotopic compositions of the pre-experiment hatchery feed, muscle and liver tissues at the start of the experiment, experimental feed, muscle and liver tissues at weeks

Table 2. Mean weights of fish at the start of the experiment, and 45 weeks after the start of the experiment

	Initial mass (g)	Mass week 45 (g)
Mean	48.5	341.7
N	196	25
SD	6.7	68.7

N = number of fish weighed, SD = standard deviation.

26 and 45 and hind gut contents at week 45 are provided in Table 3. All tissues were isotopically enriched in both C and N relative to diet. Hind gut contents were isotopically depleted in N compared with diet.

Effects of lipid extraction

Lipid extraction resulted in significant ($P < 0.01$) increases in $\delta^{13}C$ ratios in muscle, liver and feed reflecting the loss of lipids depleted in ^{13}C. Lipid extraction resulted in a greater positive increase in $\delta^{13}C$ ratios in muscle and liver at week 45 than at week 26. The differences in the effect of lipid extraction ($\delta^{13}C_{extracted} - \delta^{13}C_{whole}$) between the sample dates were small (0.1‰ difference in muscle, 0.3‰ difference in liver) but significant ($P < 0.01$), suggesting either that growth was accompanied by an increase in lipid content of muscle and liver, or that individuals succumbing to oxygen starvation at week 26 had systematically lower lipid contents.

Lipid extraction also resulted in large positive increases in $\delta^{15}N$ in muscle (week 45; mean increase in $\delta^{15}N$ values following lipid extraction = 0.9‰), and liver (week 45; mean increase in $\delta^{15}N$ values following lipid extraction = 0.5‰). These values are similar to those seen in white muscle (0.8‰) and liver (0.6‰) in rainbow trout, *Oncorhyncus mykiss*.[11] $\delta^{15}N$ values in pellet feed increased by 1.3‰ following lipid extraction, similar to the marked effect of lipid extraction on $\delta^{15}N$ values in whole minnows (2.8‰).[12]

Increases in $\delta^{15}N$ values after lipid extraction may be caused by loss of unspecified ^{15}N-depleted water-soluble, polar low molecular weight nitrogenous molecules. In mammalian skeletal muscle tissue, extractable N may amount to 10% of total N, and would be expected to contain ^{15}N-depleted molecules that will be excreted, such as ammonia. Carnivorous fish such as salmonids experience large fluxes of ammonia into the plasma a few hours after feeding.[13,14] Plasma ammonia concentrations in rainbow trout approach levels responsible for acute ammonia toxicity in fasting fish, and fish muscle may also contain high levels of ammonia produced during anaerobic protein metabolism.[15] During smolting in salmon, ammonia production increases.[16] A significant proportion of total N in fish (particularly salmonid) skeletal muscle and liver tissue is thus likely to be in the form of extractable N. As ammonia is likely to be ^{15}N-depleted compared with tissue protein, loss of ammonia from muscle during the open vessel extraction procedure will tend to increase $\delta^{15}N$ values during lipid extraction. In our study, variance in $\delta^{15}N$ values was significantly reduced following lipid extraction, presumably suggesting removal of varying amounts of ^{15}N-depleted ammonia from tissues. Lipid-extracted $\delta^{15}N$ values were, therefore, used to calculate tissue-diet spacing and to investigate relationships between tissue isotopic composition and growth rate.

Table 3. Mean isotopic composition ($\delta^{13}C$, $\delta^{15}N$) of pellet feed and *S. salar* tissues

	Initial (Hatchery)				Experimental											
					Week 26						Week 45					
	Feed		Muscle		Feed		Muscle		Liver		Muscle		Liver		Hind Gut	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Whole																
Mean	−21.4	9.6	−19.9	14.2	−22.2	7.5	−19.6	10.4	−20.6	9.9	−19.5	10.2	−20.8	8.7	−22.0	6.5
SD	0.1	0.5	0.3	0.3	0.3	0.9	0.4	0.3	0.5	0.8	0.2	0.3	0.1	0.3	0.4	0.4
n	2	2	6	6	11	11	29	29	26	26	25	25	24	24	9	9
Extracted																
Mean	−20.2	9.4	−18.9	14.7	−21.0	8.8	−19.0	11.4	−19.1	10.3	−18.9	11.1	−19.4	9.2	−22.0	6.9
SD	0.2	0.3	0.2	0.5	0.5	0.6	0.3	0.4	0.2	0.7	0.1	0.3	0.4	0.3	0.8	0.8
n	2	2	6	6	15	15	31	31	28	28	25	25	24	24	15	15

SD = standard deviation, n = number of measurements.

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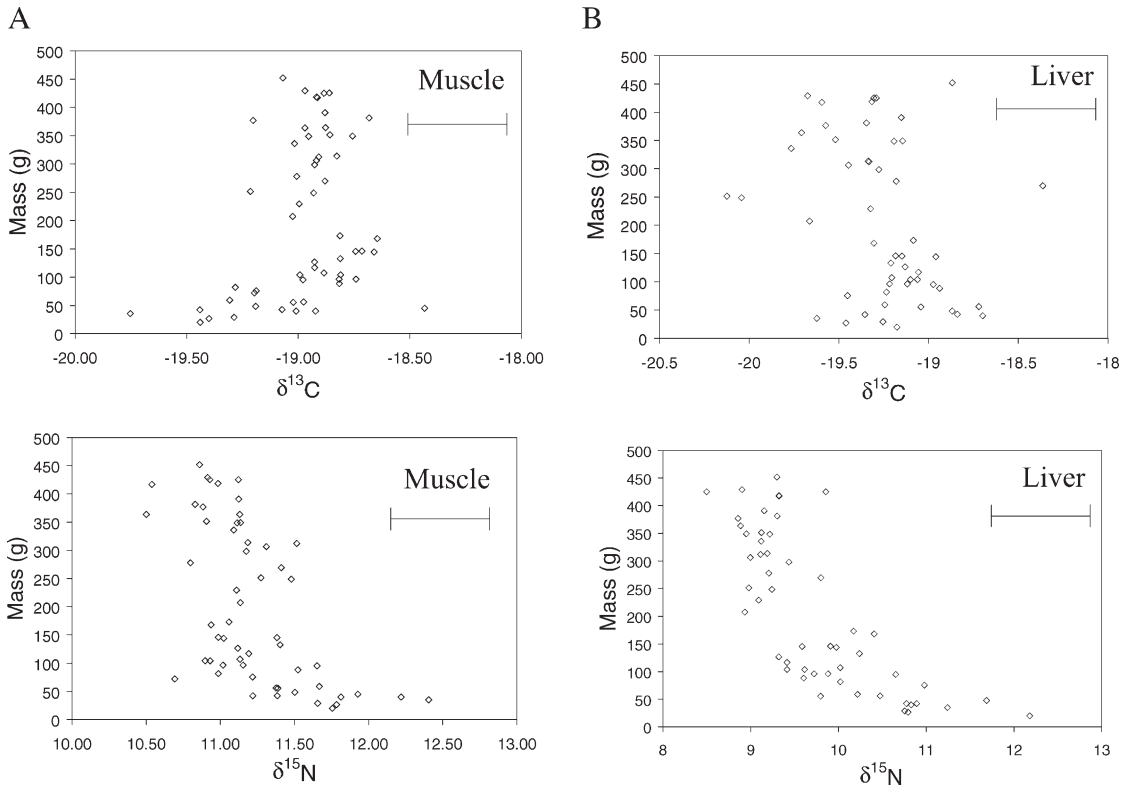

Figure 1. Evolution of δ^{13}C and δ^{15}N values in lipid-extracted muscle (A) and liver tissues (B) of growing *S. salar* (bar indicates 0.3‰ maximum estimated error in isotopic analyses).

Evolution of tissue isotopic composition

Muscle δ^{13}C values increased and δ^{15}N values decreased throughout the duration of the experiment (Fig. 1). With growth, muscle tissues approach equilibrium values after an approximately 3-fold increase in body mass (Fig. 2(A)). Liver δ^{13}C and δ^{15}N values show similar relationships (Fig. 2(B)), but liver δ^{13}C values show higher variance in samples taken at week 45 (F-test: $P \ll 0.01$), possibly indicating inconsistent removal of lipids within the extraction process.

The relative contribution of growth and metabolic turnover to the rate of change in isotopic composition in fish was estimated by comparing measured data with that predicted from growth models.[17–23]

The rate of isotopic change in response to growth alone is described by simple mass balance models:

$$\delta_t = \delta_e + (\delta_i - \delta_e) \times (w_i/w_t) \quad (3)$$

Taking δ_e as the mean tissue composition in fish at week 45, growth-only mass balance models explain a significant

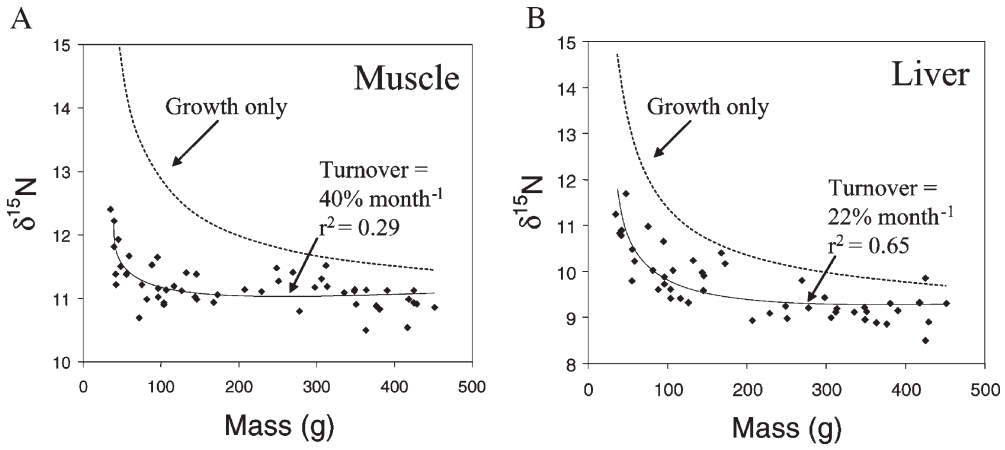

Figure 2. Evolution of δ^{15}N values in lipid-extracted muscle (A) and liver (B) tissues of growing *S. salar*. Dashed curves indicate δ^{15}N values predicted from growth-only models ($\delta_t = \delta_e + (\delta_i - \delta_e) \times (w_i/w_t)$). Solid curves indicate δ^{15}N values predicted from a combined growth-metabolism model ($\delta_t = \delta_e + (\delta_i - \delta_e) \times (w_i/w_t) \times C^t$) where values of C (the proportion of initial N pool remaining after time period t) were estimated from the measured data by non-linear fitting.

amount of stable C and N variation in muscle (δ^{15}N: $r^2 = 0.46$, $P < 0.01$; δ^{13}C: $r^2 = 0.30$, $P < 0.01$) and stable N variation in liver ($r^2 = 0.68$, $P < 0.01$). Growth-only models of isotope change predict equilibration with the new diet after an approximately 6-fold increase in body mass. In fact the isotopic composition of C and N in both muscle and liver tissue approached equilibrium conditions more rapidly than predicted by growth-only models (Fig. 2), suggesting that metabolic turnover or amino acid recycling contributed significantly to the isotopic evolution of salmon tissues.

To estimate the contribution of metabolic turnover to change in the isotopic composition of muscle and liver tissue, a combined growth-metabolism model[19] was used:

$$\delta_t = \delta_e + (\delta_i - \delta_e) \times (w_i/w_t) * C^t \quad (4)$$

where C is the proportion of the initial N pool remaining after 1 t-period (calculated in months). The value of C was solved by iteration improving prediction of δ_t and 95% confidence intervals were estimated by bootstrapping.[24] The combined growth-metabolism model explained approximately 30% of the variation in the isotopic composition of N in muscle ($r^2 = 0.29$, $P \ll 0.01$) and approximately 70% of the isotopic variation in N in liver tissue ($r^2 = 0.69$, $P \ll 0.01$). Estimates of the rates of metabolic turnover of tissue N in *S. salar* (1–C)[19] are $40 \pm 5\%$ month^{-1} in muscle and $22 \pm 3\%$ month^{-1} in liver, similar to the 20% month^{-1} turnover rates estimated for N in the muscle tissue of the migrating goby (*Rhinogobius* sp.).[19]

The time required to replace 50% of the initial tissue N by turnover may be estimated from:[19]

$$t^* = \log 2/(\log G - \log C) \quad (5)$$

where G is the average growth rate (Eqn. (2)).

The time required to replace 50% of the original N in both muscle and tissue in *S. salar* was estimated as ca. 1 and 2.25 months, respectively. It is important to note that the simple models used above do not consider recycling of amino acids from tissues other than liver or muscle into the free amino acid pool.[25–27] Estimates of metabolic turnover derived from the above models are thus likely to be overestimates.

Tissue-diet spacing

All tissues approach a constant 'equilibrium' once body weight exceeds ca. 200 g (i.e. ca. 300% increase in mass from an initial mean mass of 48.5 g) (Fig. 2). We therefore use the isotopic composition of tissues of fish with >300% increase in mass to calculate mean tissue-diet spacing (Table 4).

Values of the tissue-diet isotopic spacing are consistently lower in liver than in muscle in both lipid-extracted and whole tissues. Tissue-diet spacings in both C and N calculated from lipid-extracted muscle and liver tissues are significantly lower than those calculated from whole data (t-test: $P < 0.01$).

Influence of growth rate on tissue-diet spacing

δ^{15}N values of lipid-extracted muscle tissues for fish that had equilibrated to the new diet (>300% increase in mass) were significantly influenced by growth rate (Fig. 3; $r^2 = 0.28$, $P < 0.01$). Fish with faster growth rates exhibited lower muscle δ^{15}N values and therefore lower tissue-diet isotopic spacing in N. Whole muscle tissue values are more scattered and show no significant relationship with growth rates. No significant relationship was found between growth rate and liver δ^{15}N values or between growth rate and δ^{13}C values for liver or muscle tissue.

DISCUSSION

Dynamics of isotopic change

As in other diet-switching experiments with juvenile fish,[17,19,20,22,23] isotopic change in the tissues of sub-adult Atlantic salmon is dominated by the addition of new tissue. Assuming no contribution of recycled amino acids, metabolic turnover of existing muscle and liver nitrogen occurred at rates of approximately 20–40% month^{-1}, consistent with previously reported estimates of rates of muscle C turnover of 24–66% month^{-1}.[20] Following a diet switch, muscle and liver tissues in post-smolt *S. salar* reach equilibrium after a ca. 300% increase in body mass, equivalent to approximately 8 months growth under the experimental conditions used. Marked differences in growth rate between individuals were seen in the current experiment, and, in natural populations, variations in growth rate and/or metabolic rate between individuals following a change in diet will lead to differences in isotopic composition and contribute to within-population variation.[17]

Value of tissue-diet spacing

Nitrogen

Values of the tissue-diet isotope spacing in N in both muscle (2.3‰, SD = 0.3‰) and liver (0.0‰, SD = 0.3‰) are relatively low compared with often quoted average values of ca. 3.4‰.[28,29] Several compilations of experimental and field observations of trophic level shift in the isotopic

Table 4. Mean δ^{13}C and δ^{15}N values (±SD) in whole and lipid-extracted feed, muscle and liver tissues and mean values (±SD) of tissue-diet isotopic spacing in C and N in muscle and liver tissue of Atlantic salmon

	Diet (SD)	Muscle (SD)	Liver (SD)	$\Delta_{t\text{-}d}$ Muscle (SD)	$\Delta_{t\text{-}d}$ Liver (SD)
Whole data					
N	11	25	24		
δ^{13}C	−22.2 (0.3)	−19.5 (0.2)	−20.8 (0.1)	2.7 (0.1)	1.4 (0.1)
δ^{15}N	7.5 (0.9)	10.2 (0.3)	8.7 (0.3)	2.7 (0.3)	1.2 (0.3)
Extracted data					
N	15	25	24		
δ^{13}C	−21.0 (0.4)	−19.0 (0.1)	−19.4 (0.4)	2.1 (0.1)	1.6 (0.3)
δ^{15}N	8.8 (0.6)	11.1 (0.2)	9.2 (0.3)	2.3 (0.3)	0.0 (0.3)

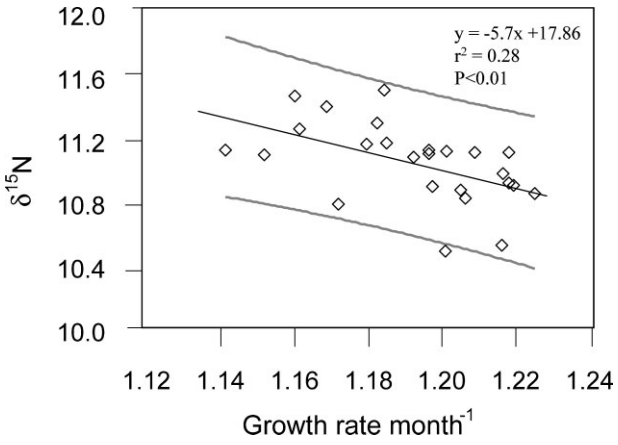

Figure 3. Relationship (regression ±95% confidence interval) between growth rate ($G = (w_t/w_i)^{1/t}$) and lipid-extracted muscle $\delta^{15}N$ values for fish experiencing >300% increase in body mass.

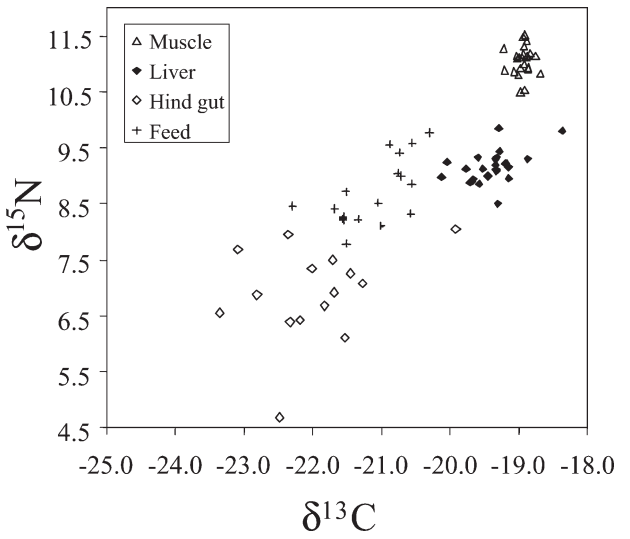

Figure 4. Stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$ values) of lipid-extracted muscle and liver tissues, hind gut contents and experimental feed from *S. salar* in isotopic equilibrium with the experimental diet (i.e. >300% increase in mass).

composition of C and N have been provided recently.[6,30,31] Ammoniotelic fish typically display relatively low values of $\Delta^{15}N_{t\text{-}d}$ (mean = 2.3‰, n = 32, standard error = 0.2‰).[6] Increasing N use efficiency results in lowered tissue-diet isotopic spacing, suggesting that ammoniotelic fish have relatively high N use efficiency. This may be linked to the fact that protein is the principal energy source for carnivorous fish in general and salmonids in particular. Our experimental values are consistent with the low values of $\Delta^{15}N_{t\text{-}d}$ typical for marine fish and add to the growing body of evidence concluding that values of trophic level isotopic shift in N are highly variable within and between taxa.[5–8] The lower $\delta^{15}N$ values and thus lower values of $\Delta^{15}N_{t\text{-}d}$ seen in liver than in white muscle are consistent with the greater proportion of essential amino acids in liver protein.[11]

Carbon

The values of the isotopic shift in C in both muscle and liver (2.1‰, SD = 0.1‰ and 1.6‰, SD = 0.3‰, respectively) are at the high end of published values for fish (mean values of $\Delta^{13}C$ muscle-diet in ammoniotelic animals = 0.4‰, n = 49, standard error = 0.2‰),[6] but the values are similar to those found in previous experimental studies feeding fish on pellet diets derived from mixed animal and vegetable products.[17]

Carbon and nitrogen isotope ratios in animal tissues are affected by diet quality—specifically the relative efficiency with which different dietary components are assimilated.[7,8] Synthetic feeds such as that used in the current study are commonly of both plant and animal origins (Table 1) with differing digestibility. In the current study, hind gut contents of fish sacrificed after 45 weeks were found to have lower δ values for both C and N than diet (F.ig. 4) indicating that components of the pellet with low δ values (e.g. C-3 derived plant products, lipids, and/or microbially produced ammonia) were less efficiently digested. The values for isotopic spacing between *bulk* diet and tissue calculated above are explained partially by differential digestibility of diet components and are overestimates of the equilibrium isotopic spacing between *assimilated* diet and muscle and liver tissue. We suggest that hind gut contents or faecal materials should be sampled and analysed in any controlled feeding isotope experiment to ensure that the isotopic composition of assimilated diet is the same as that of the bulk diet.

Influence of growth rate on tissue-diet isotope spacing

The maximum range in $\delta^{15}N$ values of muscle tissue in fish equilibrated to diet (>300% increase in body mass) is 1‰ (10.5–11.5‰). The $\delta^{15}N$ value of muscle tissue is significantly correlated with growth rate (Fig. 3, $r^2 = 0.28$, $P < 0.01$), suggesting that the metabolic requirements or consequences of growth affect the value of diet-tissue spacing in N. As tissue-diet spacing in N reflects N use efficiency, the observed relationship between growth rate and tissue-diet isotopic spacing indicates a direct link between growth rate and N use efficiency.

Liver tissue contains a greater proportion of essential amino acids than muscle and therefore liver $\delta^{15}N$ values are lower than those in muscle[11] (Fig. 4). Excluding a single outlier, the difference in $\delta^{15}N$ values between muscle and liver tissues in experimental *S. salar* shows a significant negative relationship with growth rate (Fig. 5; $r^2 = 0.3$, $P < 0.01$). The proportion of non-essential amino acids synthesised *de novo* therefore decreases with increasing growth rate, confirming that growth rate is accompanied by increasing N use efficiency.

The observed relationship between growth rate and N use efficiency may arise in two ways: (1) Faster growing individuals are those with higher food intake rates, leading to greater N use efficiency, as seen in experimental studies with carp[7,8] (however, it should be noted that excess protein ingestion would be expected to reduce N use efficiency and increase tissue-diet spacing); and (2) genetic variation in N use efficiency leads to variation in individual growth rates.

In the first case, growth rate is a consequence of increased feeding level, whereas in the second, growth rate is encouraged by increased metabolic efficiency regardless of

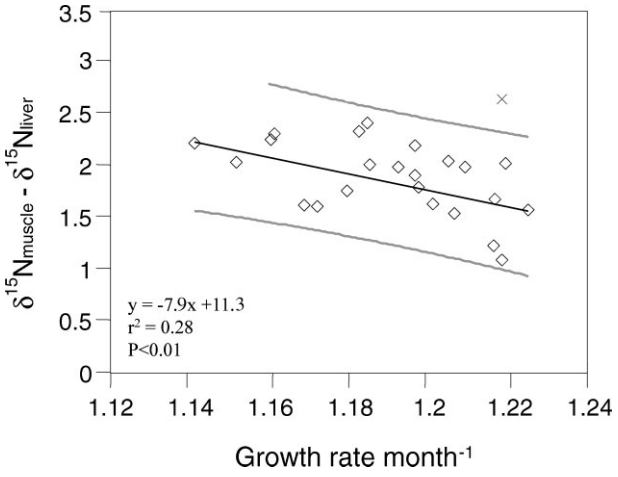

Figure 5. Relationship (regression ± 95% confidence interval) between growth rate ($G = (w_t/w_i)^{1/t}$) and N isotopic separation between muscle and liver for fish experiencing >300% increase in body mass (x = outlier not included in regression calculation).

feeding level. Simple experiments could be designed to distinguish between these mechanisms. The critical point is that in our experimental population of *S. salar* growth rate is directly related to increased N use efficiency, and thus growth rate influences diet-tissue isotopic spacing in N.

CONCLUSIONS

In *S. salar*, and probably other ammoniotelic fish, growth rate is a function of N use efficiency and therefore affects tissue-diet isotopic spacing in N. Stable isotope analyses may therefore be used to assess the requirements for and consequences of growth. In ecosystem studies, variation in growth rates will contribute to within-population variation in tissue $\delta^{15}N$ values, and may be particularly significant when comparing individuals at different ontogenetic stages.

Tissue-diet isotope spacing in N in white muscle tissues of experimentally reared *S. salar* averages 2.3‰, significantly lower than the value of ca. 3.4‰ commonly quoted in marine ecological studies, but consistent with recent computations of the value of tissue-diet isotope spacing in N in marine fish.

The values of tissue-diet isotope spacing measured in experimental studies may be significantly influenced by differential assimilation of dietary components with differing isotopic composition. Analyses of hind gut contents or faeces should be routinely conducted when assessing tissue-diet isotope fractionation to monitor potential differential assimilation of mixed diet components.

The change in C and N isotope values for salmon over time is dominated by growth and addition of new tissue, but metabolic turnover rates are estimated at 20–40% turnover month^{-1}. Rapidly growing young fish with high metabolic levels such as salmon will equilibrate to a new diet relatively quickly, of the order of a few months.

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APPENDIX

				Whole data						Lipid-extracted data					
				$\delta^{13}C$			$\delta^{15}N$			$\delta^{13}C$			$\delta^{15}N$		
Date sampled	Week no	Length (mm)	Weight (g)	Muscle	Liver	Hind gut	Muscle	Liver	Hind gut	Muscle	Liver	Hind gut	Muscle	Liver	Hind gut
11.06.04	11	161	41.9	−20.1	−20.8		10.8	10.5		−19.4	−19.4		11.4	10.8	
08.06.04	11	135	19.8		−21.8			11.3		−19.4	−19.2		11.8	12.2	
06.06.04	11	154	35.1	−20.5	−21.6		10.9	10.8		−19.8	−19.6		12.4	11.2	
11.06.04	11	132	26.7	−20.5	−21.2		10.7	11.2		−19.4	−19.5		11.8	10.8	
17.06.04	12	144	29.0	−20.2	−20.6		10.2	10.6		−19.3	−19.3		11.7	10.8	
2.07.04	14	184	75.6	−19.7			10.2			−19.2	−19.5		11.2	11.0	
18.07.04	17	168	59.0	−19.7			10.6			−19.3	−19.2		11.7	10.2	
29.07.04	18	175	48.0	−19.9	−20.7		10.6	10.8		−19.2	−18.9		11.5	11.7	
5.08.04	19	191	81.7	−19.7	−20.5		9.8	9.4		−19.3	−19.2		11.0	10.0	
5.08.04	19	177	71.8	−19.6			9.9			−19.2			10.7		
6.08.04	19	164	42.4	−19.7	−20.6		10.4	9.9		−19.1	−18.8		11.2	10.9	
13.08.04	20	175	55.5	−19.6	−20.3		9.9	9.7		−19.0	−19.0		11.4	9.8	
09.08.04	20	168	55.8	−19.4	−21.8		10.3	9.7		−19.0	−18.7		11.4	10.5	
31.08.04	23	176	45.0	−19.6	−20.5		10.2	10.5		−18.4			11.9		
01.10.04	26	216	145.5	−19.3	−20.6		10.9	9.2		−18.7	−19.1		11.4	9.6	
01.10.04	26	209	104.0	−19.3	−20.0		10.5	9.9		−18.8	−19.1		10.9	9.6	
01.10.04	26	206	107.0	−19.3	−20.4		10.2	9.5		−18.9	−19.2		11.1	10.0	
01.10.04	26	193	104.0	−19.4	−20.7		10.3	9.1		−19.0	−19.1		10.9	9.4	
01.10.04	26	175	96.2	−19.4	−20.6		10.0	9.2		−18.7	−19.1		11.2	9.9	
01.10.04	26	210	144.0	−19.3			10.4			−18.7	−19.0		11.0	10.0	
01.10.04	26	203	95.1	−19.4	−20.4		10.5	10.2		−19.0	−19.0		11.7	10.7	
01.10.04	26	214	132.7	−19.5			10.4			−18.8	−19.2		11.4	10.2	
01.10.04	26	186	96.2	−19.2	−20.5		10.3	9.3		−18.8	−19.2		11.0	9.7	
01.10.04	26	214	126.5	−19.3	−20.7		10.8	8.9		−18.9	−19.1		11.1	9.3	
01.10.04	26	157	39.9	−19.5	−20.4		10.7	10.2		−19.0	−18.7		11.8	10.8	
01.10.04	26	214	145.9	−19.3	−20.7		10.3	8.9		−18.7	−19.2		11.0	9.9	
01.10.04	26	228	173.0	−19.4	−20.8		10.5	9.2		−18.8	−19.1		11.1	10.2	
01.10.04	26	179	88.4	−19.3	−20.3		10.4	9.4		−18.8	−18.9		11.5	9.6	
01.10.04	26	164	39.7		−19.3			11.4		−18.9			12.2		
01.10.04	26	204	116.7	−19.4	−20.5		10.3	9.6		−18.9	−19.1		11.2	9.4	
01.10.04	26	217	168.0	−19.3	−20.6		10.8	9.1		−18.6	−19.3		10.9	10.4	
04.02.05	44	290	298.1	−19.5	−20.5		9.6	9.2		−18.9	−19.3	−19.9	11.2	9.4	8.0
04.02.05	44	287	277.9	−19.6	−20.7		10.0	8.5		−19.0	−19.2	−22.4	10.8	9.2	7.9
04.02.05	44	327	418.5	−19.4	−20.7		10.2	8.9		−18.9	−19.3	−21.3	11.0	9.3	7.1
04.02.05	44	304	351.2	−19.3	−21.0		10.2	8.6		−18.9	−19.5	−21.7	10.9	9.1	6.9
04.02.05	44	262	251.2	−19.5			10.3			−19.2	−20.1	−22.2	11.3	9.0	6.4
04.02.05	44	255	207.4	−19.5	−20.7	−21.3	10.4	8.9	6.2	−19.0	−19.7		11.1	8.9	
04.02.05	44	314	363.9	−19.3	−20.6	−22.2	10.3	8.8	6.4	−18.9		−21.5	11.1		7.3
04.02.05	44	332	451.8	−19.8	−20.6	−21.8	9.8	9.0	6.7	−19.1	−18.9	−21.5	10.9	9.3	6.1
04.02.05	44	313	348.4	−19.6	−20.8	−22.0	10.2	8.7	6.6	−19.0	−19.2	−23.1	11.1	9.2	7.7

RCM

04.02.05	44	300	306.3	−19.3	−20.8	−22.3	10.4	8.3	6.3	−18.9	−19.4	−22.5	11.3	9.0	4.7
04.02.05	44	319	376.7	−19.4	−20.7		9.7	8.4		−19.2	−19.6		10.9	8.9	
04.02.05	44	313	429.2	−19.5	−20.8	−22.0	9.7	8.1	6.0	−19.0	−19.7		10.9	8.9	
04.02.05	44	304	313.6	−19.3	−20.6		10.1	8.7		−18.8	−19.3		11.2	9.2	
04.02.05	44	307	349.0	−19.4	−20.7		10.2	8.7		−18.8	−19.1	−23.3	11.1	9.0	6.6
04.02.05	44	320	381.2	−19.3	−20.8		9.8	8.1		−18.7	−19.3		10.8	9.3	
04.02.05	44	334	425.1	−19.3	−20.9		10.6	8.8		−18.9	−19.3		11.1	8.5	
04.02.05	44	324	425.2	−19.6	−20.7		10.7	9.2		−18.9	−19.3		10.9	9.9	
04.02.05	44	302	312.1	−19.4	−20.9	−21.8	10.5	8.6	6.6	−18.9	−19.3	−22.0	11.5	9.1	7.4
04.02.05	44	290	269.6	−19.5	−20.7		10.3	8.4		−18.9	−18.4		11.4	9.8	
04.02.05	44	309	390.6	−19.5	−20.9		10.2	8.9		−18.9	−19.2	−21.8	11.1	9.2	6.7
04.02.05	44	315	417.1	−19.5	−20.9	−22.5	10.0	8.6	7.3	−18.9	−19.6	−22.8	10.5	9.3	6.9
04.02.05	44	305	335.7	−19.9	−21.0		9.9	8.7		−19.0	−19.8		11.1	9.1	
04.02.05	44	309	363.6	−19.4	−20.9	−22.2	10.4	8.7	6.1	−19.0	−19.7		10.5	8.9	
04.02.05	44	262	248.8	−19.4	−20.9		10.6	9.0		−18.9	−20.0	−21.7	11.5	9.2	7.5
04.02.05	44	269	229.3	−19.4	−20.5		10.3	8.7		−19.0	−19.3	−22.3	11.1	9.1	6.4

Feed values

Whole data		Lipid-extracted data	
$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
−22.8	8.8	−20.3	9.8
−22.0	8.3	−20.5	9.6
−21.8	7.0	−20.7	9.0
−22.7	5.4	−20.9	9.6
−22.5	8.2	−20.6	8.9
−22.0	8.1	−20.7	9.4
−22.3	7.8	−20.6	8.3
−22.0	6.9	−20.8	9.0
−22.0	8.3	−21.0	8.5
−22.6	7.1	−21.7	8.4
−22.2	8.0	−21.3	8.2
−22.0	7.2	−21.5	7.8
		−21.6	8.3
		−21.6	8.2
		−22.3	8.5
		−21.6	8.2
		−21.5	8.7
		−21.0	8.1