Stable isotope turnover rates and fractionation in captive California yellowtail (Seriola dorsalis): insights for application to field studies

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Stable isotope analysis (SIA) measurements from long-term captivity studies provide required parameters for interpretation of consumer SIA data. We raised young-of-the-year (14–19 cm) California yellowtail (Seriola dorsalis) on a low δ15N and δ13C diet (pellet aquaculture feed) for 525 days, then switched to a high δ15N and δ13C diet (mackerel and squid) for 753 days. Yellowtail muscle was sequentially sampled from each individual after the diet switch (0 to 753 days) and analyzed for δ15N and δ13C, allowing for calculation of diet-tissue discrimination factors (DTDFs) from two isotopically different diets (low δ15N and δ13C: pellets; high δ15N and δ13C: fish/squid) and turnover rates of 15N and 13C. DTDFs were diet dependent: Δ15N = 5.1‰, Δ13C = 3.6‰ for pellets and Δ15N = 2.6‰, Δ13C = 1.3‰ for fish/squid. Half-life estimates from 15N and 13C turnover rates for pooled yellowtail were 181 days and 341 days, respectively, but varied considerably by individual (15N: 99–239 d; 13C: 158–899 d). Quantifying DTDFs supports isotopic approaches to field data that assume isotopic steady-state conditions (e.g., mixing models for diet reconstruction). Characterizing and quantifying turnover rates allow for estimates of diet/habitat shifts and “isotopic clock” approaches, and observed inter-individual variability suggests the need for large datasets in field studies. We provide diet-dependent DTDFs and growth effects on turnover rates, and associated error around these parameters, for application to field-collected SIA data from other large teleosts.

Fundamental understanding of fish biology, feeding behavior, population dynamics, and movement patterns has expanded in recent years due to technological advances in tools such as electronic tags for animal tracking1,2, chemical tracer analyses3, and ‘big data’ analysis4,5. However, knowledge gaps remain for all fish species6,7, which can be addressed with evolving tools for more comprehensive, ecosystem-based understanding and management of fish species. Stable isotope analysis (SIA) is one tool that has rapidly expanded in marine ecosystem studies. Ecological studies using SIA rely on the fact that marine consumers reflect the isotopic composition of the prey they consume, which varies with prey ecology, region, and environmental conditions8,9. This premise has allowed researchers to investigate diet, trophic dynamics, and movements of marine predators8,10,11 with SIA, most commonly of nitrogen (δ15N) and carbon (δ13C). More recently, the quantity of SIA data for marine predators has grown to allow for global analyses of marine predator ecology and habitat use. For example, the synthesis of thousands of SIA values across ocean basins has allowed for characterization of global trophic geography in sharks12 and tunas13.

Underlying consumer SIA data are complex physiological and ecological isotopic dynamics, which ultimately determine consumer SIA values. Isotopic compositions of consumer tissues reflect those of diet, which guided early applications of SIA to feeding ecology. Consumer tissue δ15N and δ13C increase systematically during trophic transfer due to fractionation and re-routing dynamics of amino acids and other organic compounds during digestion, assimilation, and tissue turnover14. The resultant differences between consumer and diet SIA values,
represented by several equivalent terms (diet-tissue discrimination factors [DTDFs], trophic discrimination factors [TDFs], or trophic enrichment factors [TEFs]), have been used for trophic level estimates and food web reconstruction. DTDF estimates for δ15N (Δ15N) have generally been higher than those for δ13C (Δ13C), and an early synthesis of DTDF values reported a widely used, multi-taxon mean of 3.4 (± 1.0 SD) for Δ15N and 0.4 (± 1.3 SD) for Δ13C30. Many early ecological studies applied these first multi-taxon DTDF estimates to estimate diet and trophic position, but subsequent laboratory studies have provided many more taxon- or species-specific DTDFs, demonstrating the importance of applying specific DTDF values due to substantial variability across taxa, tissue types, and diet31,32. While statistical frameworks and software packages that reconstruct predator diets using SIA (e.g. MixSIR, SIAR)16,17 are able to incorporate error around DTDF values18, mixing models are better parameterized by laboratory studies that provide more accurate DTDF estimates19.

Understanding isotopic turnover rates in predator tissues is required to quantify timing of diet shifts or animal movements. Following a shift to an isotopically different diet, predator tissue δ15N and δ13C values change over time until reaching consistent values that reflect the new diet (i.e., steady-state conditions). These rates are largely dictated by the physiological processes of new tissue synthesis (growth) and anabolic and catabolic ‘tissue turnover’ processes (broadly referred to here as ‘metabolism’). Captive studies in fish have shown muscle isotopic turnover rates as low as several days (young-of-the-year [YOY] winter flounder Pseudopleuronectes americanus25) to months (summer flounder Paralichthys dentatus, arctic sculpin Myoxocephalus scorpioides26,27) to > 1 year (mado Atypichthys latus, Pacific bluefin tuna Thunnus orientalis25,26). These differences are largely driven by fish size and life-stage; since relative growth (proportion of new mass to initial mass over time) is a primary driver of isotopic turnover dynamics, turnover rates will vary with relative growth rates ontogenetically (faster turnover in younger fish) and inter-specifically (faster turnover in fish with high growth rates). Isotopic turnover rates have most often been described with exponential fit models, allowing for calculation of half-life (t1/2) values for comparison across studies26–27. Recent reviews and meta-analyses of laboratory-derived isotopic turnover rates have reported predictable allometric relationships of slower turnover rates (higher t1/2) with increasing fish body size26–28. Turnover rates are also tissue-dependent; more metabolically active ‘fast-turnover’ tissues (e.g. blood plasma, liver) and ‘slow-turnover’ tissues (e.g. muscle, bone collagen) can reflect a shift in diet of large predatory fish in timeframes of days/weeks or months/years, respectively29.

Laboratory-based experiments, under controlled or semi-controlled conditions, quantifying species- and lifestage-specific isotopic turnover improve the understanding of stable isotope dynamics and have direct applications to wild SIA studies. Turnover rates and the time to steady-state allow researchers to put diet estimates in temporal context by roughly demonstrating the dietary timeframes represented by SIA composition of various tissues. Quantified turnover rates also allow applied ‘isotopic clock’ techniques30–32, which use isotopic endmembers (of predator, diet, and/or region) and measured predator SIA values to estimate the timing since a shift in diet and/or habitat. Measured SIA values in fish that recently migrated thus allow retrospective reconstructions of large-scale movements, such as entire river migrations by lake sturgeon (Acipenser fulvescens)33 and trans-Pacific migrations by Pacific bluefin tuna34. However, accuracy of these migratory timeframes is maximized with accurate species-specific isotopic turnover rates, which are best calculated from laboratory experiments.

The California yellowtail (Seriola dorsalis) is an ecologically and economically important teleost predator in the California Current Ecosystem. At typical capture sizes (40 cm to > 100 cm), yellowtail are a relatively large-sized, highly mobile, high trophic level predator in pelagic and coastal ecosystems35. The lack of experimentally-derived DTDFs and turnover parameters for yellowtail limits the interpretation of SIA data for (1) temporal and spatial patterns in trophic dynamics and (2) timing of movement patterns, as yellowtail are known to shift from pelagic to coastal habitats with increasing size36,37. To calculate these isotopic parameters for this species, we raised YOY yellowtail to steady-state on a low δ15N and δ13C diet for 525 days, then switched larger (~ 40 cm) yellowtail to a high δ15N and δ13C diet and sequentially sampled muscle from individuals over 753 days (see Fig. 1). This allowed for quantification of DTDFs in yellowtail, as well as isotopic turnover in individuals and

Figure 1. Schematic showing experimental design to quantify dynamics of δ15N and δ13C in captive California yellowtail Seriola dorsalis. Sizes (cm) below x-axis show approximate changes in yellowtail fork length during the phases of the experiment. Lines showing changes in yellowtail muscle δ15N (solid line) and δ13C (dashed line) are theoretical changes based on typical isotopic turnover dynamics in teleosts.
across the study population. These isotopic parameters can be applied to field-collected SIA data for active, high trophic level teleosts that currently lack laboratory-derived SIA data.

Results

Yellowtail diet δ15N and δ13C values. The δ15N and δ13C values of pellet feed (n = 10) were 5.8 ± 0.1‰ SD and −22.6 ± 0.4‰, respectively. The δ15N and δ13C values of Pacific mackerel (n = 6) and market squid (n = 6) were (mackerel) 13.5 ± 0.1‰ and −19.7 ± 0.1‰ and (squid) 14.7 ± 0.3‰ and −17.9 ± 0.5‰, resulting in weighted mean fish/squid (weighted by % fish and % squid presented to yellowtail; 68% and 32% respectively) diet δ15N and δ13C values of 14.0 ± 0.8‰ and −19.0 ± 0.9‰ (Table 1).

Diet-tissue discrimination factors. We calculated two DTDF values for δ15N and δ13C (Δ15N and Δ13C) from captive yellowtail due to the demonstrated correlation between fish DTDF values and diet δ15N and δ13C values (Caut et al. 2009). The two diets used here differed highly in δ15N and δ13C values (Table 1). For YOY yellowtail fed pellet feed (low δ15N and δ13C diet) for 525 d, Δ15Nlow and Δ13Clow were 5.1 ± 0.3‰ and 3.6 ± 0.1‰, respectively (n = 21) (Table 1). Individual yellowtail Δ15Nlow values ranged from 3.4–3.9‰ and Δ13Clow values from 4.6–5.7‰. For larger yellowtail fed fish/squid (high δ15N and δ13C diet) for 595–753 d, Δ15Nhigh and Δ13Chigh were 2.6 ± 0.2‰ and 1.3 ± 0.4‰, respectively (Table 1). Individual yellowtail Δ15Nlow values ranged from 2.3–2.9‰ and Δ13Chigh values from 0.7–2.0‰.

We compared the observed relationship between DTDF and diet δ15N and δ13C values with relationships quantified previously in fishes by Caut et al. (2009) and Hussey et al. (2014). We used experimental diet δ15N and δ13C values to calculate estimates of Δ15Nlow and Δ13Clow from Caut et al. (2009) (Eqs. 6, 7), and of Δ15Nlow and Δ13Nhigh from Hussey et al. (2014) which only quantified the relationship for Δ15N (Eq. 8). Calculated estimates were: Δ15Nlow = 4.2‰; Δ13Clow = 2.0‰; Δ15Nhigh = 2.1‰; Δ13Chigh = 1.2‰ (Caut et al. 2009); 15Nlow = 4.4‰; Δ15Nhigh = 2.2‰ (Hussey et al. 2014). By modifying the constants in Eqs. 6 and 7 (Caut et al. 2009) to fit results here, we provide predictive equations for DTDFs of yellowtail based on diet δ15N and δ13C values:

\[
\Delta^{15}N_{\text{yellowtail}} = -0.317(\delta^{15}N_{\text{diet}}) + 6.939
\]

\[
\Delta^{13}C_{\text{yellowtail}} = -0.639(\delta^{13}C_{\text{diet}}) - 10.839
\]

Time-based δ15N and δ13C turnover. Pooled yellowtail δ15N values increased from 0–378 d, increasing from 10.9 ± 0.3‰ to 15.8 ± 0.6‰ over this period (Fig. 2, Table 2). After 378 d, time-based increases in δ15N were lower, reaching apparent steady-state at 595 d at 16.4 ± 0.2‰ (Fig. 2). δ15N values then remained consistent to 753 d (16.4 ± 0.2‰). The exponential model fit grouped yellowtail data well \(r^2 = 0.94\); (Fig. 2) and provided a time-based estimate of \(\delta^{15}N_{t=5}\) of 181 d (Fig. 2b) (see Table 3 for model parameters and 95% confidence intervals).

Individual yellowtail δ15N turnover rates were variable, with a range of individual \(t_{0.5}\) values of 99–239 d (Fig. 2a). All individuals reached consistent, steady-state values by 595 d, and high individual \(t_{0.5}\) values were driven by relatively low δ15N values at intermediate timesteps, resulting in exponential model fits that reached asymptotic steady-state values late in the experiment for some individual fish (Fig. 2a). However, intra-individual

| Group | Isotope | Tissue | n  | Mean (SD) | C:N (SD) (mass) | DTDF | Mean | SD | Time in capt |
|-------|---------|--------|----|-----------|----------------|------|------|----|--------------|
| Feed  | \(\delta^{13}C\) | -      | 10 | -22.6 (0.4) | 7.0 (0.5) |       |      |    |              |
|       | \(\delta^{15}N\) | -      | 10 | 5.8 (0.1) | 7.0 (0.5) |       |      |    |              |
| Mackerel | \(\delta^{13}C\) | WM     | 6  | -19.7 (0.1) | 3.2 (0) |       |      |    |              |
|        | \(\delta^{15}N\) | WM     | 6  | 13.5 (0.1) | 3.2 (0) |       |      |    |              |
| Squid  | \(\delta^{13}C\) | WM     | 6  | -17.9 (0.5) | 3.4 (0) |       |      |    |              |
|        | \(\delta^{15}N\) | WM     | 6  | 14.7 (0.3) | 3.4 (0) |       |      |    |              |
| Fish/squid mean | \(\delta^{13}C\) | WM     | 12 | -19.0 (0.9) | -      |       |      |    |              |
| Weighted by mass | \(\delta^{15}N\) | WM     | 12 | 14.0 (0.8) | -      |       |      |    |              |

Table 1. Mean δ15N, δ13C, and C:N ratio values of California yellowtail (Seriola dorsalis) muscle tissue, captive feed, and calculated diet-specific DTDF values for pellet and fish/squid diet. δ13C values are arithmetically lipid-corrected according to Logan et al. 55. *The δ13C values of Dietlow (pellet diet) could not be corrected for carbonate and or lipid due to unknown pellet composition; this DTDF is based on bulk pellet δ13C values.
turnover dynamics were consistent with first-order exponential model predictions, with high exponential model fits across individual yellowtail ($r^2$ range 0.97–0.99, mean 0.98 ± 0.1; Fig. 2a) and higher $r^2$ values for all individuals than for the single model fit across all yellowtail ($r^2 = 0.94$; Fig. 2b).

Pooled yellowtail muscle $\delta^{13}C$ values also increased consistently throughout the experiment (Fig. 3 and Table 2). $\delta^{13}C$ values increased from 0–532 days, increasing from -19.0 ± 0.1‰ to −17.6 ± 0.2‰ over this period (Fig. 3). Subsequent $\delta^{13}C$ values were generally consistent, reaching −17.5 ± 0.1‰ at 753 d. Model fits showed an asymptote at longer time periods than for $\delta^{15}N$ both for individual yellowtail (Fig. 3a) and grouped data.
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(Fig. 3b). The exponential model fit grouped yellowtail data well ($r^2 = 0.91$; Fig. 3b and Table 3) and provided a time-based estimate of $^{13}$C $t_{1/2}$ of 341 d (Fig. 3b; see Table 3 for model parameters and 95% confidence intervals).

Across individual yellowtail, turnover rates of $\delta^{13}$C were more variable than those of $\delta^{15}$N, with a range of individual $\delta^{13}$C $t_{1/2}$ values of 158–899 d (Fig. 3a). All individuals reached steady-state values by the end of the experiment, though the dynamics of $\delta^{13}$C change at earlier timesteps resulted in exponential model fits that reached asymptotic steady-state values late in the experiment, or not at all, in individual fish (Fig. 3a). As for $\delta^{15}$N, intra-individual turnover of $\delta^{13}$C was fairly consistent with the first-order exponential model ($r^2$ range 0.89–0.97, mean 0.93 ± 0.3; Fig. 3a); most individual model fits were better than the single model fit across all yellowtail ($r^2 = 0.91$; Fig. 3b).

Growth-based $\delta^{13}$C and $\delta^{15}$N turnover. Yellowtail that remained in captive conditions for adequate time periods for individual turnover estimates ($n = 10$) showed substantial but variable growth in captivity. Relative growth in mass $W_R$ (see Eq. 13) by the end of the experimental period (753 d) ranged from 1.7–3.5 (mean 2.7 ± 0.5 SD). Growth rate constants for individual fish ranged from 0.0008 to 0.0023 d$^{-1}$ (0.0017 ± 0.0005 d$^{-1}$).

The model-estimated growth rate constant ($k'$; see Eq. 15) for the grouped population of yellowtail (0.0017 d$^{-1}$) was the same as the mean across individuals (0.0017 ± 0.0005 d$^{-1}$).

Change in yellowtail $\delta^{15}$N and $\delta^{13}$C values were well described by exponential model fits to $W_R$ (Fig. 4, Table 4). Model fit of $W_R$ to change in $\delta^{15}$N was slightly better ($r^2 = 0.96$) than that for $\delta^{13}$C ($r^2 = 0.91$) (Fig. 4, Table 4). For $\delta^{15}$N, the relative growth in mass required for 50% turnover and 95% turnover was 1.14 and 1.77, respectively (Fig. 4a, Table 4). For $\delta^{13}$C, the $W_R$ required for 50% turnover and 95% turnover was 1.15 and 1.82, respectively (Fig. 4b, Table 4).

Relative proportion of isotopic turnover due to growth and metabolic processes. Sequential sampling of individual yellowtail allowed for calculation of the proportion of isotopic turnover due to growth ($P_g$) and proportion of turnover due to metabolic processes ($P_m$) (see Eqs. 19, 20) across individual fish and the grouped population of captive fish. Overall, growth contributed more to turnover of $^{15}$N ($P_g = 84\%$, $P_m = 16\%$) than $^{13}$C ($P_g = 44\%$, $P_m = 56\%$) for the grouped population of captive yellowtail. These proportional estimates varied in individual yellowtail. $P_g$ for turnover of $^{15}$N ranged from 26 to 45% (36 ± 8%), and $P_g$ for turnover of $^{13}$C ranged from 40 to 100% (65 ± 26%). Metabolic processes $P_m$ contributed to the majority of $^{15}$N turnover in all fish (55–74%, 64 ± 8%). For $^{13}$C turnover, $P_m$ was more variable, contributing 0% to several fish, but to the slight majority (51–60%) of $^{13}$C turnover in 4 of 10 fish (0–60%, 35 ± 26%). Growth rate $k'$ of individual fish was significantly correlated with $P_g$ for turnover of both isotopes, with faster growth rates resulting in higher proportion of turnover due to growth for $^{15}$N (linear regression, $P = 0.02$) and $^{13}$C (linear regression, $P = 0.03$) (Fig. 5).

Applicability to field data. Based on three metrics (variance, range, standard deviation), yellowtail muscle $\delta^{15}$N values showed the lowest variability at the beginning ($t = 0$ d) and end ($t = 753$ d) of the diet switch experiment (Fig. 6). Variability rapidly increased after the diet switch and was highest between 100–400 d (Fig. 6). These temporal patterns of variability were less evident for $\delta^{13}$C, but $\delta^{13}$C values also were generally most consistent at the beginning and end of the experimental period (Fig. 6).
Figure 4. Isotopic change with fish growth for (a) $\delta^{15}$N and (b) $\delta^{13}$C in captive California yellowtail *Seriola dorsalis*. Points represent individual measurements and lines show associated model fits (solid lines) and 95% confidence intervals (dashed lines). Model equation and associated parameters (95% confidence intervals) shown for both isotopes. $G_{0.5}$ and $G_{0.95}$ show the amount of relative growth by mass (mass_{final}/mass_{initial}) required for 50% turnover and 95% turnover, respectively, of each isotope in yellowtail muscle.

Table 4. Parameter estimates and 95% confidence intervals for relative growth-based ($W_g$) exponential fit models for $\delta^{15}$N and $\delta^{13}$C values of muscle in California yellowtail (*Seriola dorsalis*). Estimated growth-based half-life ($G_{0.5}$) and growth required for 95% isotopic turnover ($G_{0.95}$) is shown for each isotope.

| Isotope | Parameter (95% CI) | $r^2$ | $G_{0.5}$ | $G_{0.95}$ |
|---------|---------------------|--------|-----------|-----------|
| $\delta^{15}$N | $a = -10.2$ (−29.1, 9.1), $l = 5.242$ (3.327, 7.156), $c = 16.4$ (16.2, 16.6) | 0.96 | 1.14 | 1.77 |
| $\delta^{13}$C | $a = -20.0$ (−68, 28), $l = 5.0$ (2.590, 7.407), $c = -17.6$ (−17.6, −17.5) | 0.91 | 1.15 | 1.82 |

Figure 5. Proportion of isotopic turnover in muscle due to growth ($P_g$) increases with growth rate in yellowtail *Seriola dorsalis*. Points represent individual measurements of $\delta^{15}$N (filled circles) and $\delta^{13}$C (open circles) and lines show associated linear fits (dark dashed lines) and 95% confidence intervals (light dashed lines). Positive relationships were significant between $P_g$ and $k'$ for both $\delta^{13}$C (linear regression, $P=0.03$) and $\delta^{15}$N (linear regression, $P=0.02$).
At the start of the diet switch, when yellowtail began feeding on 100% fish/squid, Bayesian mixing model estimates showed 100% pellet diet at $t = 0$ (Fig. 7). After ~160 days of fish/squid diet, yellowtail muscle $\delta^{15}N$ and $\delta^{13}C$ values resulted in mixing model estimates of 50% pellets, 50% fish/squid (Fig. 7). Approximately 520 days were required for mixing model estimates to reflect 100% fish/squid diet (Fig. 7), representing ~3 $\delta^{15}N$ half-life periods and ~1.5 $\delta^{13}C$ half-life periods.

**Discussion**

Species-specific turnover experiments provide crucial parameters for application to stable isotope ecology studies. We reared a large teleost in semi-controlled conditions (here, controlled diet with fluctuating temperature and salinity) to provide DTDFs and isotopic turnover estimates, which are relatively rare due to the difficulty of large fish husbandry and the timeframes required for such experiments. We demonstrate that DTDFs are...
diet-dependent in California yellowtail, similar to other fish reared on similar diets, and provide DTDF values and predictive algorithms that allow for diet-dependent discrimination factors to be estimated for active large-bodied teleosts. The relatively long turnover rates reported here (steady-state conditions after > 1 yr) demonstrate the long timeframes required for wild fish to reach steady-state conditions with local diet. Finally, turnover rates provide parameters for field-based estimates of diet shifts and movements, and growth-based models allow for scaling of reported turnover rates with animal size and associated changes in relative growth rates.

Yellowtail Δ 15N and Δ 13C estimates were strongly influenced by diet. Δ 15N and Δ 13C were 5.1‰ and 3.6‰ for low δ 15N and δ 13C diet (pellets) and 2.5‰ and 1.3‰ for high δ 15N and δ 13C diet (fish/squid). Our Δ 15N and Δ 13C values are different from means that have been reported in DTDF reviews: (Δ 15N) 3.4‰10,38, 3.2‰39, 2.9‰40, 2.5‰41, and 2.0‰42 and (Δ 13C) 0.4‰10,42 and 0.5‰40. However, taxon- and species specific DTDFs have proven to be broad, and our values fit within the wide ranges of specific DTDFs reported across reviewed studies (Δ 15N = 3.2–9.2‰ and Δ 13C = 8.8–61.1‰14). Δ 13C values typically have been described as low, citing minimal fractionation of 13C between trophic levels and thus being useful as tracking the source of productivity in the diet of upper trophic level consumers. However, our Δ 13C values of 3.6‰ and 1.3‰ suggest a much higher degree of fractionation of 13C. However, our calculated Δ 13C, from pellet diet, should be interpreted with caution as pellet composition was unknown, pellet C:N was high (7.0 ± 0.5), and unknown pellet composition limited assessment of the effects of carbonates and/or lipids and precluded appropriate arithmetic correction of pellet δ 13C values.

Despite the wide ranges around reported DTDF means above, those means often have been (and continue to be) applied to field data in analyses as similar mixing models, particularly when species-specific laboratory-derived DTDFs are lacking, with application of Δ 15N = 3.4‰ and Δ 13C = 0.4‰10 being particularly common14. However, inappropriate application of DTDFs can substantially affect results and interpretations of field studies. DTDFs are most commonly used in Bayesian mixing models (e.g. MixSIR, SIAR)16,17 which are highly sensitive to inaccurate DTDFs, producing highly erroneous estimates when DTDFs vary by > 1‰ and potentially less25,43. While many factors can influence DTDFs, including consumer physiological state, diet composition and quality, consumer size, age, and tissue composition14, recent studies have demonstrated a strong negative relationship between DTDFs and diet δ 15N and δ 13C values26,27. Our calculated DTDFs, while different from commonly applied mean values, were similar to those estimated from predictive equations (based on diet δ 15N and δ 13C values) in both Caut et al. (2009) and Hussey et al. (2014) (see Results). Yellowtail Δ 15N high and Δ 13C high estimates were also similar to those of Pacific bluefin tuna (Thunnus orientalis) (Δ 15N = 1.9 ± 0.4‰; Δ 13C = 1.8 ± 0.3) fed a similar diet of California Current-sourced squid and sardine44. This both corroborates the importance of diet δ 15N and δ 13C in determining DTDF and provides a slightly modified predictive equation (Results, Eqs. 1, 2), calculated from our two DTDFs, that can be applied to field-collected yellowtail data and potentially to other similar fish (e.g., large-bodied ectotherms) when species-specific estimates are not available.

Bayesian mixing models allow for multiple DTDFs to be entered as inputs. However, Caut et al. (2009) found that >70% of studies used a single DTDF even when diet resources had substantially different δ 15N and δ 13C values. Our different DTDFs in the same yellowtail, when fed isotopically distinct diets, supports the assertion that a single DTDF is inappropriate in such cases. Based on our results, we recommend using our yellowtail-specific algorithm for field SIA data from yellowtail and potentially from similar fish. Since our algorithm is only calculated from the two DTDFs determined here, the equations from Caut et al. (2009) and Hussey et al. (2014) may also provide more robust and useful alternative and/or complementary DTDFs than static, mean DTDFs when applying Bayesian mixing model approaches to test data.

Yellowtail muscle turnover rates of 15N (t 0.5 = 181 d) and 13C (t 0.5 = 341 d) were higher (i.e., isotopic turnover in muscle took longer) than many values reported for teleosts, which have shown half-lives on the order of days to weeks (see literature syntheses26–27). While the incorporation of prey into consumer tissue and associated iso- topic fractionation is a complex process, influenced by growth rate, thermoregulation, environment, diet quality, and nutrient routing, synthesis studies have shown a strong relationship between turnover rate and body size in fish25–27. However, most fish in captive turnover experiments are relatively small (< 1 kg, and often less than 100 g24–26). Our turnover rates were similar to larger-bodied (> 1 kg) teleosts and chondrichthians (e.g. leopard shark Triakis semifasciata44, sandbar shark Carcharhinus plumbeus45, Pacific bluefin tuna46; gage grouper Mycteroperca microlepis46). In addition, our turnover rates were comparable to those that would be estimated from body size alone for vertebrate ectotherm muscle (~ 100–200 d)25,27, suggesting that captive yellowtail turnover dynamics were generally typical for ectothermic fish of their size, as well as the endothermic Pacific bluefin tuna.

Our results differed from the relatively consistent turnover rates of 13C and 15N in muscle that have been found across several taxa, and are predicted by metabolic scaling theory26,27. In contrast, we found slower turnover rates of yellowtail muscle 13C that were roughly 2 × those of 15N; similar dynamics were previously described in large-bodied (> 5 kg) Pacific bluefin tuna and sandbar sharks24,45. It is possible that this was partially driven by relative differences in yellowtail δ 13C and δ 15N endmember values, as the higher change of δ 15N (~ 6‰) than δ 13C (~ 15‰) (Fig. 2, 3) would likely drive more rapid change in 15N turnover in early stages of the experiment. Yellowtail were also fed a mixed fish/squid diet, and the possibility of intra-individual feeding variability (different proportions of fish and squid) and the different δ 13C values of fish and squid (~ 19.7 and ~ 17.9‰, respectively) could have driven different individual turnover rates and overall variability in turnover rates of δ 13C. While several other mechanisms exist that potentially drive differences between 13C and 15N turnover rates in muscle (e.g., contributions of red blood cells, respiratory exchanges of C; Thomas and Crowther 2015), it is most likely that the relative contributions of growth and metabolic processes drive these differences in active, large-bodied fish. In larval and juvenile fish, muscle isotopic turnover is generally dominated by rapid growth20,22,47. This is well demonstrated in bluefin tuna; larval (< 1 g) Atlantic bluefin Thunnus thynnus showed a 15N half-life of 2.5 days with 100% turnover attributed to growth48, while sub-adult (> 4.5 kg) Pacific bluefin showed a 15N half-life of 167 days with 38% turnover attributed to growth49. In yellowtail here, growth contributed more to 15N turnover (84%) than to 13C turnover (44%), and growth was also found to contribute more to turnover of 13C (59%) than
15N (38%) in large-bodied Pacific bluefin. Turnover rates of individual yellowtail provide additional insight to the relative importance of variable growth rates, as intra-specific differences in growth rate significantly affected turnover rates of both 15N and 15C (Fig. 5).

It is reasonable that as fish size increases and relative growth decreases, metabolic processes will play a greater role in isotopic turnover. However, why this would differentially affect 15N and 15C is unclear, and likely includes metabolic dynamics of tissue protein turnover, routing of amino acids and lipids into muscle tissue proteins, and fractionation processes during catabolism. The isotopic dynamics of these processes are not well enough described or understood to assess their relative influence on 15N and 15C turnover. While the processes underlying this result require further investigation, the implication is that δ15N and δ13C values of wild large-bodied fish should be considered independently, as δ15N values will represent longer timeframes of past resource use. This will influence both Bayesian mixing model diet reconstructions (as δ15C values will represent less current diet inputs than will δ13N and isotopic clock approaches (that apply specific turnover rates of 15N and 15C to isotopic data to estimate time since diet shifts and/or migrations).

Though individual yellowtail growth rates in identical captive conditions varied (Wt = 2.7 ± 0.5; k = 0.0017 ± 0.0005 d−1), models of relative growth Wt versus change in δ15N and δ13C fit well (r2 = 0.96 and 0.91, respectively). The similarity of the growth required for 50% isotopic turnover (G0.5) for 15N and 15C (14% and 15%, respectively) suggests that turnover dynamics of both isotopes are similarly influenced by growth. Since time-based estimates of 15N turnover were substantially shorter than 13C, this reinforces the likelihood that metabolic processes play a more influential role in turnover of muscle 15N in yellowtail of this size. Model fits suggest that the relative growth in mass necessary for 95% turnover (G0.95) of 15N and 15C will occur with 77% and 82% increase in growth by mass, respectively (Table 4). Since relationships of age and growth, and length versus mass, have been described for California yellowtail15,22, these estimates can be scaled to larger or smaller yellowtail to estimate turnover rates. In general, turnover rates will decrease with increasing yellowtail size due to the decrease in relative growth rates with size. For example, based on relationships in Baxter et al. (1960), the mass doubling times (Wt = 2) for a 1 kg, 2 kg, 4 kg, and 8 kg yellowtail are ~ 350, 550, 1000, and > 2000 d, respectively. These size-specific differences in relative growth will in turn change the time necessary for muscle isotopic turnover.

Substantially less growth was required for turnover of 15N and 15C than in similarly sized Pacific bluefin tuna with only slightly lower specific growth rates (0.0016 d−1) than yellowtail (0.0017 d−1), as 72% and 209% increases in growth were required for 50% turnover of 15N and 15C, respectively, in Pacific bluefin tuna muscle24. This again indicates the relative importance of metabolic processes in turnover of muscle 15N and 15C in active, large-bodied fish. As regional endotherms, Pacific bluefin tuna have higher internal temperatures and consequently higher metabolic demands than ectotherms, presumably leading to increased rates of the metabolic functions that influence turnover of 15C and 15N. As such, growth-based turnover estimates here may be more appropriately applied to other ectothermic teleosts, while those from Pacific bluefin may be more appropriate for other endothermic fish (e.g., other Thunnus species, lamnid sharks, opah Lampris spp).

Our results lend insight into the application of various laboratory-derived isotopic parameters to wild fish. First, our different DTDFs from diets of different isotopic composition strongly support other studies that call for diet-based DTDFs14,15. Gaut et al. (2009) found that > 33% of studies used Δ15N and Δ13C values that were > 2‰ different than estimated diet-dependent discrimination factors (DTDFs), which would render mixing model results highly misleading. In the absence of specific DTDFs for the study species, estimated DTDFs from algorithms provided in Gaut et al. (2009) and Hussey et al. (2014) may provide a useful starting point.

Second, the variability of δ15N and δ13C observed at the start, middle, and end of the diet switch experiment suggests that intra-specific variability may be lower for end-member isotopic parameters (DTDFs) than middle-member parameters (turnover rates). Accordingly, applied DTDFs will be more reliable than turnover rates in isotopic clock approaches. Isotopic clocks have been applied to large active teleosts with some success31; in one study, an isotopic clock applied to Pacific bluefin tuna suggested two ‘pulses’ of arrival in a new isotopic environment34, and these results were later corroborated by an extensive electronic tagging study of juvenile bluefin2. However, in that isotopic clock used a bootstrapping technique incorporating the error around arrival rates, was applied to a large sample size (n = 428 individuals), and showed wide variability of arrival time estimates around peak arrival times34. Consequently, application of an isotopic clock to one or several individuals may yield highly inaccurate results depending on prior isotopic turnover rates in those specific individuals (which cannot be measured). As such, we suggest that the turnover rates here be used in analytical frameworks that incorporate uncertainty and, if possible, be applied to large datasets.

Third, an ongoing question with Bayesian mixing model diet reconstructions is the time required for consumers, and mixing model estimates, to reflect current feeding conditions. Our application of the Bayesian mixing model MixSIR to yellowtail feeding on fish/squid showed that ~ 520 days were required to reflect this diet. However, contrary to expectations, this timeframe did not require 3–4 half-life periods for both isotopes and was not limited by the isotope with slower turnover (13C), which would have required > 1000 days (≥ 3 half-lives of 13C) to reflect current diet. The limiting isotope was that with more rapid turnover (15N), and accurate mixing model estimates required ~ 3 half-life periods of that isotope. Thus while mixing model applications must continue to consider the prior timeframes represented by diet estimate outputs, estimates may require less time to reflect current feeding than predictions from turnover rates alone. Further analyses using simulated or lab-derived data will further clarify these dynamics to constrain the timeframes represented by mixing model outputs.

The challenges of rearing active, fast-growing teleosts in captivity while tracking diet and isotopic turnover make fully controlled conditions difficult to maintain. In this study, diet type, quantity, and ratios were maintained, but seawater temperature and salinity were dependent on local conditions (though temperature was monitored, and relatively constant at ~ 18 °C). Individual fish grew at different rates despite uniform rearing conditions; while variable fish size may have influenced turnover rates and DTDFs (due to physiological changes

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during growth), it also allowed for investigation of the relationship of growth and turnover rates (Fig. 4). DTDF estimates are also complicated by the inability to fully characterize the nutritional and isotopic composition of ingested prey. In particular, the uncertainty of biological sources of commercial pellet feed, which were unavailable, and the high C:N of pellet feed suggests that DTDFs from this diet should be interpreted with caution. Analysis of fish and squid muscle excludes other ingested parts (e.g., bones containing calcium carbonate). However, field studies with omnivorous animals are subject to the same uncertainty due to mixed diet, making the uncertainty around these reported estimates appropriate for field-collected data. Tracking turnover in individual fish provided some quantification of expected uncertainty from a mixed diet, but the parameters provided here should be interpreted and applied with the same considerations of uncertainty and variability that are inherent to all isotopic ecology studies.

Laboratory studies using diet switches with captive animals provide species-specific isotopic parameters that aid interpretation of wild data. Our calculated yellowtail DTDFs corroborate diet-dependence of DTDFs and the importance of applying multiple DTDFs in mixing model approaches. Turnover rates demonstrated that intra-specific variability of isotopic turnover can be substantial, contributing to the error reported around turnover rate estimates. While quantitative framework now exists for predicting DTDFs based on diet and turnover rates based on body size, the variability in those published relationships and the variation we observed here show that species- and lifestyle-specific studies still provide the most precise isotopic parameters to ecologists for targeted species of study, especially for large-bodied animals for which fewer data exist. Future studies will both expand the breadth of species for which important isotopic parameters are necessary, refining models that allow for estimates of DTDFs and isotopic turnover based on animal type, diet, body size, and lifestyle. This will continue to improve interpretations of field data using stable isotope approaches.

Materials and methods

Ethics statement. All procedures used in these experiments were in accordance with protocol SW1401 of the SWFSC Animal Care and Use Committee. YOY California yellowtail were collected under California Department of Fish and Wildlife Scientific Collection Permit #SC-11522. Experimental protocols were approved by the National Oceanic & Atmospheric Administration Southwest Fisheries Science Center (NOAA SWFSC) Animal Care & Use Committee. Reporting of methods and results were in compliance with ARRIVE guidelines for animal research.53

Collection and captive husbandry of yellowtail. YOY California yellowtail (14–19 cm) were collected from offshore floating kelp mats near San Diego, CA, USA on September 12, 2012. Fish were caught using unbaited sabiki bait rigs, then immediately transferred to an onboard flow-through holding tank. On land, fish were transported in the holding tank on the trailered vessel to NOAA’s SWFSC Experimental Aquaria Facility in La Jolla, CA, where they were transferred via dipnet to holding tanks. Fish were first held in 300 × 150 × 90 cm oval tanks (~ 3200 L) with flow-through, filtered local seawater at local ambient seawater temperature (~ 18 °C) and reared on a diet of Bio-Oregon BioTrout feed pellets. Pellets were presented immediately to newly transferred fish and fish began feeding 0–7 days after capture. Yellowtail were fed pellets 6 days/week to apparent satiation. After 525 days, the now larger yellowtail (42–50 cm) were transferred to a larger circular tank (diameter 3.7 m, capacity 9600 L) with the same filtered seawater at the same ambient temperatures (~ 18 °C) and tagged in the dorsal musculature with uniquely numbered and colored Floy plastic spaghetti tags. Yellowtail were switched to a diet of Pacific mackerel (Scomber japonicus) and market squid (Doryteuthis opalescens), both sourced off the coast of southern California (McRoberts Sales Co.). Yellowtail were fed mackerel and squid (by mass: 62% mackerel, 38% squid) 6 days/week to apparent satiation. Yellowtail muscle was sampled at t = 0 (the day of diet switch), followed by sampling intervals ranging from 27 to 119 days (mean interval 60 ± 27 days) depending on perceived condition of yellowtail and conditions for sampling. Samples were collected by lowering tank water levels, capturing yellowtail in a rubber knotless net, then transferring fish to vinyl cradles. Biopsy punches (Cook Quick-Core G07821) were used to remove 0.1–0.2 g of white muscle from the dorsal musculature. When possible, fork length (FL; cm) was measured at the time of sampling. Muscle tissue was also collected from the diet (dorsal musculature from mackerel and mantle tissue, with the outer membrane removed, from squid) throughout the study for SIA. Sampling continued until yellowtail were removed due to poor condition, suffered natural mortality, or became too large to remain in the holding tank (t = 753 days after diet switch, the endpoint of the study).

YOY yellowtail (13.7–18.8 cm, 15.7 cm ± 1.7; 0.02–0.08 kg; 0.04 kg ± 0.02) fed well on pellet diet, increasing ~ threefold in length and ~ 40-fold in mass over 525 days. After 525 days, 21 similar-sized (42–50 cm FL, 46.0 cm ± 2.5; 1.0–2.2 kg, 1.7 kg ± 0.3) individual yellowtail were selected for the diet switch experiment to fish/squid (Fig. 1). During the course of the diet switch experiment, 11 individual fish were removed from captive conditions before apparent isotopic steady-state due to poor physical condition, lack of feeding, or natural mortality. A total of 10 yellowtail fed consistently in captivity for a long enough period to reach apparent steady-state with new diet (595–753 days) allowing calculation of individual turnover rates in these fish. This allowed for estimates of Δ15Nlow and Δ15Clow and population-wide isotopic turnover estimates from 21 fish subjected to the diet switch, and Δ15Nhigh and Δ15C high and individual yellowtail turnover estimates from 10 individuals in captive conditions for 595–753 days that reached steady-state with new diet.

SIA of yellowtail and diet. Yellowtail dorsal muscle tissue and prey muscle tissue samples were immediately stored in cryovials at −20 °C. Feed pellets were analyzed whole. All samples were then frozen at −80 °C and subsequently lyophilized and ground to a homogenous powder for isotope analysis. The δ13C and δ15N values of all samples were determined at the University of Hawaii using an on-line C–N analyzer coupled with a Delta XP
isotope ratio mass spectrometer. Replicate reference materials of atmospheric nitrogen and V-PDB were analyzed every 10 samples, and analytical precision was < 0.2‰ for δ13C and δ15N. Isotope ratios are described by:

$$\delta X = \left( \frac{R_A}{R_{\text{standard}}} - 1 \right) \times 1000,$$

where $q$ is the isotope of interest, $X$ is an element of interest, $R_A$ is the ratio of the rare to the common isotope, and $R_{\text{standard}}$ is the isotope standard Air or V-PDB. Isotope values are reported as per mille (%).

**Arithmetic corrections of δ13C values.** While both chemical and arithmetic lipid extractions have been shown to be effective methods to correct bias in δ13C values due to lipid-content, arithmetic corrections preserve sample integrity and simplify sample preparation. Studies have noted effects of chemical lipid extraction on δ13C values and suggested separate treatment for δ13C and δ15N analyses, and biopsy samples did not always provide adequate material for such treatment (especially from smaller yellowtail), we chose to arithmetically correct for lipid content. δ13C values of yellowtail muscle and diet items (mackerel and squid) were arithmetically lipid-normalized based on mass C:N ratios using muscle- and organism type-specific lipid normalization algorithms. For yellowtail and mackerel, we used a muscle-specific lipid correction algorithm derived from a suite of fish species:

$$\delta_{13C} = \frac{P - (P \times F / C \times N)}{\delta_{13C_{\text{tissue}}}}$$

where $\delta_{13C}$ is the arithmetically-corrected δ13C value, C:N is the C/N ratio by mass of the specific sample, and $P$ and $F$ are parameter constants based on measurements by Logan et al. Relatively low C:N ratios in squid (3.4 ± 0) led to minimal differences in δ13C and δ15C in squid. Since pellet composition was unknown and no appropriate arithmetic δ13C correction was available, bulk δ13C values are reported for pellets.

**Calculating DTDF.** We calculated two DTDFs (Δ15N and Δ13C), one for pellet diet and one for fish/squid diet. For both DTDFs, Δ15N and Δ13C were calculated from the mean difference between yellowtail muscle and respective diet δ15N and δ13C values when yellowtail were at isotopic steady-state. $\Delta_{15N_{\text{low}}}$ and $\Delta_{13C_{\text{low}}}$ were calculated before the diet switch, after yellowtail fed on pellets for 525 days. $\Delta_{15N_{\text{high}}}$ and $\Delta_{13C_{\text{high}}}$ were calculated after yellowtail that had reached steady-state with the fish/squid diet using the weighted (by proportion mass in diet) mean δ15N and δ13C values of fish/squid diet. DTDF values were calculated according to the equation:

$$\Delta_{\text{diet}} = \text{mean}(\delta_{\text{yellowtail}} - \delta_{\text{diet}})$$

where Δdiet represents the diet- ('low' or 'high') and isotope-specific DTDF, $\delta_{\text{yellowtail}}$ is the δ15N or δ13C value of yellowtail that reached steady-state with diet, and $\delta_{\text{diet}}$ is the mean δ15N or δ13C value of the food (pellet or fish/squid). For the fish/squid diet, δ13C values were arithmetically lipid-corrected and weighted by the proportional mass of each item in the diet (62% and 38%, respectively). For $\Delta_{15N_{\text{low}}}$ and $\Delta_{13C_{\text{low}}}$, we assumed YOY yellowtail had reached steady-state with pellet diet after 525 days. This was supported by the length of time (525 days) and relative growth (massfinal/massinitial = ~2 kg/0.05 kg = ~40) of yellowtail on pellet feed, both of which are substantially higher than what is demonstrably necessary for small fish to reach steady-state with diet.

Since we calculated two different DTDFs that were diet-dependent, as has been previously demonstrated, we compared our experimentally-derived DTDF values to the diet-dependent DTDF algorithms reported by Caut et al. 2009 (Δ15N and Δ13C) and Hussey et al. 2014 (Δ15N only), both of which used DTDFs for fish to derive linear equations for diet-based estimates of DTDFs. We used the fish white muscle equation from Caut et al. 2009 for Δ15N and Δ13C:

$$\Delta_{15N} = -0.281(\delta_{15N_{\text{diet}}}) + 5.879$$

$$\Delta_{13C} = -0.248(\delta_{13C_{\text{diet}}}) - 3.477$$

and the fish (muscle and/or whole) equation from Hussey et al. 2014 for Δ15N:

$$\Delta_{15N} = -0.27(\delta_{15N_{\text{diet}}}) + 5.92$$

and compared those estimated DTDFs to our experimentally-derived values.

**Time-based isotopic turnover.** Sequential sampling of individual yellowtail allowed for quantification of turnover rate in (1) all yellowtail that reached steady-state with new diet and (2) the pooled population of captive yellowtail. We used exponential fit models and to quantify yellowtail muscle tissue turnover rate of δ15C and δ15N, as used previously:

$$\delta_t = ae^{-\lambda t} + c,$$

where $\delta_t$ is the stable isotope value at time $t$, $a$ and $c$ are parameters derived from the best fit, and $\lambda$ is a data-derived first-order rate constant. Parameters $a$ and $c$ represent specific parameters: $a$ = difference (%) between initial and final steady-state values and $c$ is the model-estimated final isotope steady-state value. The isotope-specific half-life ($t_{1/2}$) was then calculated:
for different λ values derived for δ^{13}N and δ^{13}C, for both individual yellowtail and the grouped population. We used a modified equation from Buchheister and Latour\textsuperscript{21} to calculate the time needed to obtain a given percentage (α) of complete turnover:

\[ t_{\alpha/100} = \ln (1 - \alpha/100)/\lambda \]  

(11)

where \( t_{\alpha/100} \) is the time needed to attain α% turnover and λ is the data-derived first-order rate constant.

**Growth-based isotopic turnover.** Fish length (fork length or FL; cm) was recorded at \( t_0 \), various time steps throughout the experiment concurrent with tissue sampling, and \( t_c \). Direct measurements of yellowtail mass (kg) were taken at \( t_c \). Some mass measurements were taken with FL during the experiment; however direct measurements of mass during sampling were not always possible due to the difficulty of weighing large, active fish and the priority to minimize stress on captive fish during sampling events. When only length was available for individual yellowtail at specific time points, mass at time \( t \) (\( W_t \)) was estimated using the length–weight equation from Baxter 1960:

\[ W_t = 7.747 \times 10^{-8} (\text{FL}_t \text{mm})^{2.84} \]  

(12)

Relative gain in mass (\( W_g \), hereafter referred to as 'relative growth') was then calculated:

\[ W_g = W_f/W_i \]  

(13)

where \( W_f \) is the measured final mass and \( W_i \) is the initial mass estimate from SL. Using the equation from Ricker\textsuperscript{61} for \( W_f \):

\[ W_f = W_i e^{k't} \]  

(14)

where \( k' \) is the group specific growth-rate constant, we derive \( k' \):

\[ k' = \ln (W_R)/t \]  

(15)

and can obtain the growth rate constant \( k' \) for individual fish using relative growth (\( W_g \)) and time in captivity \( t \). Hesslein et al.\textsuperscript{52} describes the isotope value of a fish at time \( t \) (\( \delta_i \)) as:

\[ \delta_i = \delta_f + (\delta_i - \delta_f)e^{-(k' + m)t} \]  

(16)

where \( \delta_i \) is the final isotope value at steady-state with diet, \( \delta_f \) is the initial isotope value before the diet switch, \( m \) is the metabolic turnover constant. This is a modification of Eq. (3), where \( \delta_i = c, (\delta_i - \delta_f) = a \), and \( (k' + m) = \lambda \). Thus we calculate \( \lambda \) from Eq. (3), \( k' \) from Eq. (13), and use Eq. (14) to calculate the metabolic constant \( m \) based on turnover rates of δ^{15}N and δ^{13}C\textsuperscript{24}. We can also calculate the amount of relative growth needed to achieve a percent turnover of δ^{13}C and δ^{15}N\textsuperscript{21,24}:

\[ G_{\alpha/100} = \exp (\ln (1 - \alpha/100)/\lambda) \]  

(17)

and growth-based turnover can be calculated:

\[ G_{0.5} = \exp (\ln (0.5)/\lambda) \]  

(18)

where \( G_{0.5} \) is the growth-based half-life and λ is the data-derived rate constant from growth-based model fits to yellowtail muscle δ^{13}C and δ^{15}N. We estimated the proportion of isotopic turnover due to growth (\( P_g \)) and the proportion of turnover due to metabolic turnover (\( P_m \)) as the proportion of \( k' \) and \( m \), respectively, of the overall isotopic turnover constant \( \lambda \textsuperscript{21,24} \):

\[ P_g = k'/\lambda \]  

(19)

\[ P_m = m/\lambda \]  

(20)

We applied Eqs. 13–20 to δ^{13}C and δ^{15}N values in yellowtail muscle tissue and report growth turnover rate constants and overall estimated contribution of growth and turnover to observed δ^{15}N and δ^{13}C turnover in captive yellowtail.

**Applicability to field data.** Typical applications of δ^{13}C and δ^{15}N data from field-collected animals include approaches that assume isotopic steady-state with diet (e.g. Bayesian mixing models) and approaches that utilize isotopic values after an assumed or inferred shift in diet and/or habitat (e.g. isotopic clock approaches). Sequential sampling of individual yellowtail allowed for investigation of variability in δ^{13}C and δ^{15}N values at steady-state with pellet feed, at various time intervals after a diet switch, and at steady-state with fish/squid diet. Sequential sampling reduced the influence of individual variability in assessment of isotopic parameters. We compared time after diet switch to three metrics of variability (variance, range, standard deviation) of δ^{13}C and δ^{15}N throughout the experimental period to assess the robustness of approaches assuming steady-state and/or changing δ^{13}C and δ^{15}N values in wild predators.
Since tissue δ¹³C and δ¹⁵N values represent a time-integrated signature of prior feeding, Bayesian mixing model approaches represent both prior and current diet until consumers reach steady-state. To allow comparison of isotopic turnover rates and the time required for Bayesian modeling to adequately represent current diet, after diet switch we applied the Bayesian mixing model MixSIR" to all timesteps of the diet switch experiment. For inputs, we used δ¹³C and δ¹⁵N from sampled yellowtail, two different DTDFs calculated here (one for pellet feed, one for fish/squid; see Results), and mean δ¹³C and δ¹⁵N values of pellets and fish/squid as diet inputs. For each timestep we ran 10⁴ iterations and uninformative priors. Mixing model outputs allowed for estimation of the time required for the model to represent the actual current diet of yellowtail following the diet switch (100% fish/squid).

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Author contributions
D.J.M., O.E.S., J.R.H., H.D. conceived study. O.E.S. and J.R.H. performed fieldwork. O.E.S. performed feeding, husbandry, and animal sampling. D.J.M. performed sample analysis and statistical analyses. D.J.M. wrote the manuscript, with contributions from all authors.

Competing interests
The authors declare no competing interests.

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