A giant's appetite: how body size drives the diet and trophic position of the Japanese giant salamander

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Abstract

In predators, growth often drives ontogenetic dietary shifts (ODSs), leading to increasing trophic position (TP) with body size as growing individuals gradually incorporate larger prey in their diet. In species exhibiting extreme size variation, particularly those with gigantism, TP may increase markedly with body size, as large individuals might gain access to prey considerably higher in the food chain and inaccessible to smaller conspecifics. This can ultimately lead to apex predator status in the largest individuals. In this study, we investigated for the first time ODSs in one of the world's largest amphibians, the Japanese giant salamander (*Andrias japonicus*). We combined stomach content and stable isotope analysis ($\delta^{15}N$, $\delta^{13}C$) from 160 individuals across a broad size range to quantify dietary patterns and TP changes. We found a non-linear increase in TP with body size, from approximately 3.0 to 5.1, with a marked inflection point at a snout–vent length of 39 cm. This threshold corresponded to a clear dietary transition: from primarily consuming aquatic insects, to feeding predominantly on fish, anurans, and freshwater crabs. This transition likely reflects morphological and physiological adaptations associated with gigantism, enabling the exploitation of large prey. Our findings suggest that gigantism may be adaptive in predators such as giant salamanders by promoting ecological opportunities, allowing individuals to access high trophic levels through extensive growth and ultimately function as apex predators. These results contribute to a broader understanding of the ecological consequences of body size evolution in predatory vertebrates, highlighting how extreme growth can reshape species' ecological roles.

Keywords

Introduction

Body size is a key life-history trait shaping ecological dynamics in animals (Elton 1927, Leaper and Huxham 2002, Cohen et al. 2003, Takatsu and Kishida 2015), influencing how organisms interact with their environments (Hildrew et al. 2007). In trophic ecology, increasing body size generally allows animals, especially predators, to access different food resources, often including larger prey at higher trophic levels (Cohen et al. 1993, Reuman and Cohen 2004, Brose 2010), although some large predators, such as many whale species, primarily feed on small prey items (Roman et al. 2014). As individuals grow, dietary changes—known as ontogenetic dietary shifts (ODSs)—are widespread across the animal kingdom and play a crucial role in population dynamics and food web stability (Miller and Rudolf 2011, Rudolf and Lafferty 2011, Roos and Persson 2013, Nakazawa 2015, Nilsson et al. 2018, Sánchez-Hernández et al. 2019). The relationship between body size and ODSs can be quantified by tracking changes in trophic position (TP) across growth stages (Werner and Gilliam 1984, Nosaka et al. 2015), which constitutes an integrative metric, providing key insights into the ecological role of organisms within their ecosystem.

The relationship between body size and TP is particularly important in species expressing gigantism. Gigantism refers broadly to the evolution of exceptionally large body size relative to closely related species or ancestral forms, often conferring ecological advantages such as access to larger prey, reduced predation risk, and increased competitive dominance (Peters 1986, Vermeij 2016). Today, many taxa exhibit gigantism, including the African elephant (*Loxodonta africana*), the blue whale (*Balaenoptera musculus*), the saltwater crocodile (*Crocodylus porosus*) and the colossal squid (*Mesonychoteuthis hamiltoni*). Gigantism often results in a particularly wide size range between juvenile and adult stages, potentially leading to substantial differences in ecological roles throughout ontogeny. Species exhibiting gigantism may undergo broad shifts in trophic position (Woodward et al. 2005), reflecting changes in their dietary habits and predatory strategies. However, this is not universal—baleen whales, for instance, grow to enormous sizes but maintain a consistent diet of small prey, resulting in little change in TP as they mature (Roman et al. 2014). Studying gigantism may provide key insights into the physical, ecological and evolutionary principles that govern the evolution of body size (Moran and Woods 2012). While many studies of gigantism have focused on homeothermic species, large ectotherms remain comparatively understudied. Ectothermy can have profound ecological consequences for predators, affecting metabolic rates, energetic requirements, and activity patterns (Clarke and Johnston 1999, Gillooly et al. 2001), which in turn influence trophic position, prey selection, and the timing of ontogenetic dietary shifts. In some low-energy environments, such as caves, these physiological constraints can result in markedly different trophic trajectories compared to large homeotherms (Lunghi et al. 2022).

Changes in TP are linked to ODSs, which are of particular interest when studying large predators, which often occupy the highest trophic levels, i.e. apex predators (Estes et al. 2011). TP of large predators is generally expected to increase along ontogeny, reflecting dietary shifts as body size grows. In some species, these shifts can lead to a non-linear relationship between body size and TP (Cohen et al. 1993, Woodward and Hildrew 2002, Riede et al. 2011), where feeding strategies change abruptly once a critical body size threshold is reached, allowing the exploitation of previously inaccessible resources such as larger prey (Werner and Gilliam 1984, Nosaka et al. 2015). However, the body size-TP relationship varies across ecosystems and taxonomic groups, with studies reporting positive, hump-shaped, or even negative relationships (Layman et al. 2005, Segura et al. 2015, Burress et al. 2016, Romero-Romero et al. 2016, Ou et al. 2017, Potapov et al. 2019).

The role of gigantism in large predators remains globally poorly understood (Hammerschlag et al. 2019), especially in freshwater ecosystems. The largest apex predators in many freshwater food webs include crocodilians (Delany and Abercrombie 1986, Lang 1987), large predatory fish like the wels catfish (*Silurus glanis*), and mammals such as freshwater dolphins (Vejřík et al. 2017, Echeverria et al. 2022). Large-bodied apex predators also exist among amphibians, particularly giant salamanders (Cryptobranchidae), a lineage which dates back to the Middle Jurassic (Gao and Shubin 2003). These fully aquatic salamanders persist in North America, China, and Japan. They are considered ambush predators using suction feeding (Matsumoto et al. 2024). The Japanese giant salamander (*Andrias japonicus*), which is endemic to Japan (Duret et al. 2025a), is among the largest amphibian species, reaching lengths of up to 1.5 meters. However, its trophic ecology and pivotal role in lotic freshwater ecosystems remains poorly understood. Existing studies have mainly focused on its stomach content composition (Tago 1931, Tochimoto 2002, Okada et al. 2008), leaving significant gaps in our understanding of its trophic ecology and ODSs. Given its status as a unique apex predator, the Japanese giant salamander provides an excellent opportunity to investigate how body size influences dietary shifts and explore the broader ecological implications of gigantism in freshwater ecosystems.

Our study aims to investigate the relationship between body size and trophic position (TP) in the Japanese giant salamander, and to explore the ecological implications of ontogenetic dietary shifts (ODSs) associated with gigantism—particularly the access to previously inaccessible resources in this apex predator. To this end, we used a combination of stomach content analysis (SCA) and stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Fry 1988, Post 2002, McCutchan et al. 2003, Parnell et al. 2010). SCA provides direct insights into diet composition but is limited by prey digestion rates and short-term dietary snapshots (Hyslop 1980, Denoël and Joly 2001a, Costa et al. 2014, Lejeune et al. 2018, Lunghi et al. 2024). By contrast, SIA of nitrogen isotopes ($\delta^{15}N$) allows for a time-integrated assessment of TP, as $\delta^{15}N$ increases predictably along trophic levels (Fry 1988, Post 2002, McCutchan et al. 2003, Thomas and Crowther 2015, Lejeune et al. 2022), providing indirect but quantitative insights into assimilated diet and TP. We specifically addressed the following questions: (i) Is there a positive relationship between body size and TP? (ii) To what extent does TP vary across the size range of individuals, and what does this variation reveal about their ecological role in the context of gigantism? (iii) What is the maximum TP reached by

giant salamanders? (iv) Can we identify a threshold in the body size–trophic position relationship that reflects an ODS, such as access to larger prey (e.g. fish) beyond a certain size?

We hypothesize that the relationship between body size and TP follows a non-linear trajectory (Fig. 1), with an abrupt shift in prey composition upon reaching a critical body size threshold, and resulting in a large TP range between smaller and larger individuals. Under a linear ontogenetic dietary shift (ODS), we would expect a gradual transition where both small and large prey contribute to the diet across a wide size range. However, in *A. japonicus*, we anticipate a sharp decline in the consumption of small prey such as aquatic insects once individuals reach a size that enables them to efficiently capture larger, more energy-rich prey such as fish and anurans. This sudden dietary shift likely arises from morphological and functional changes linked to gigantism (e.g., increased gape size and feeding efficiency), which open new ecological opportunities by allowing individuals to prey on larger, more energy-rich organisms that are inaccessible to smaller conspecifics (Werner and Gilliam 1984). By identifying the presence and timing of this dietary threshold, our study provides key insights into the trophic implications of gigantism in apex predators. Understanding these ontogenetic shifts is essential for assessing the role of giant salamanders in freshwater food webs and informing conservation strategies to protect both the species and its prey communities.
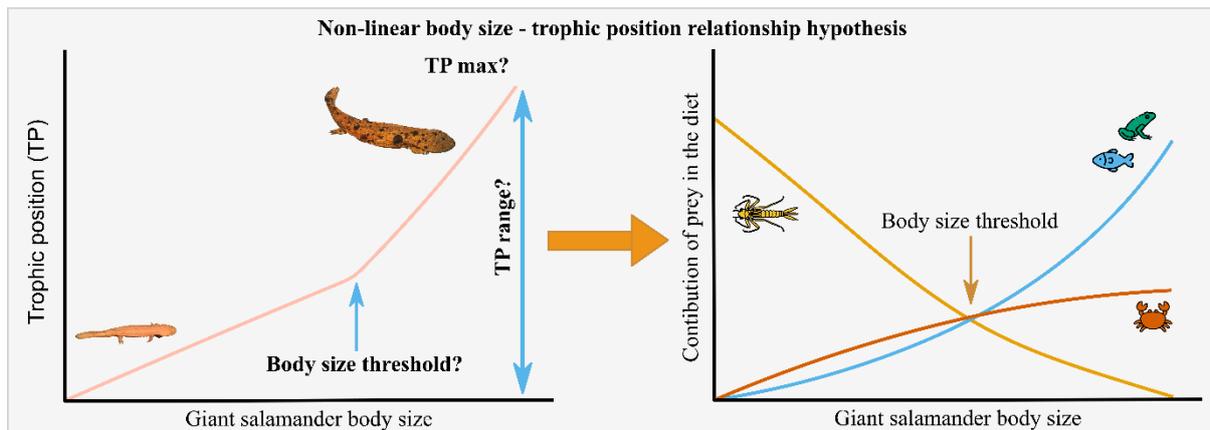

Figure 1. Hypothesis of non-linearity in ontogenetic dietary shift in Japanese giant salamanders through body size-dependent changes in $\delta^{15}N$ and trophic position (TP). A critical body size threshold marks a sudden increase in $\delta^{15}N$ and TP, indicating an abrupt dietary shift where larger individuals begin consuming more anurans and fish, altering both diet composition and trophic position. Photo of the larval and adult giant salamanders: Mathieu Denoël.

Material and methods

Study area

This study was conducted in the upper Ichi River watershed (Ikunocho Kurokawa, Hyogo Prefecture, Japan), focusing on an 8 km stretch, including a 6.5 km section of the Ichi River (35°12'N – 134°52'E; Fig. 2a) and a 1.5 km section of its tributary, the Nagano River (35°13'N

– 134°53'E) (map of the study area in the Supporting information). Elevation ranges from 390 m to 510 m above sea level. The river is oligotrophic with clear water, minimal aquatic vegetation and banks lined with mixed forests (deciduous trees and commercially planted conifers). Surrounding agricultural lands primarily consist of rice paddies. The region has a humid subtropical climate with hot, humid summers and mild winters.

Sampling

We captured Japanese giant salamanders of all body sizes using dip nets during 32 nocturnal surveys (April–June 2023). In total, 160 different individuals were captured and identified using 12-mm microchips (Biolog-ID, 134.2 kHz) and a handled reader (Agrident APR 500 RFID reader). Among them, 25 individuals were already tagged, 130 received a new microchip and 5 individuals were not tagged due to their small size and were identified using visual patterns. We measured each individual to the nearest 0.1 cm (snout-vent length (SVL), from the tip of the snout to the end of the cloaca, and total length (TL); Fig. 2b). We collected stomach contents via stomach flushing (Joly 1987), using a handheld aquarium pump to flush river water into the stomach, inducing regurgitation in a dip net (mesh size: 1 mm). Stomach contents were analysed directly in the field. The prey items were identified to the most precise taxonomic level possible (Kawai and Tanida 2018), and each prey category was weighed to the nearest 0.01 g (wet mass) (Supporting information). Prey items were also photographed (Nikon Z6II; macro-objective 105 mm) for further analysis in the laboratory if needed.

To investigate the potential influence of habitat use on stomach content prey composition of Japanese giant salamanders, we recorded local habitat characteristics, including water velocity, water depth, river width, relative channel position, and the percentage of agricultural land cover within a 100 m radius. The relative channel position was calculated as the ratio of the distance to the riverbank to the total river width (see details in Supporting information).

We collected a small sample of muscle from the caudal tail (5 by 2 mm) from each individual for stable isotope analysis (SIA). We collected potential food sources for SIA from six river sections (five sections in Ichi river, one in Nagano river; minimum six samples per taxa). Within each river section, we used fishing nets (50 x 25 x 25 cm; mesh size: 5 mm) to capture fish and dip nets (50 x 25 x 25 cm; mesh size: 1 mm) to collect aquatic insects and freshwater crabs, which were also hand-collected by overturning rocks. We collected muscle samples of fish and freshwater crabs and whole-body aquatic insects for SIA.

We sampled baselines of the aquatic food web, focusing on two key sources: terrestrial baseline, represented by leaf litter in the river, and aquatic baseline, represented by periphyton. We also hand-collected aquatic gastropods (*Semisulcospira* spp.) as an additional proxy for the aquatic baseline (periphyton grazers). The slight discrepancy in sample size between stomach content analysis ($n = 151$) and stable isotope analysis ($n = 160$) was due to the decision not to stomach flush the very small salamanders or those in low body condition. Specifically, the small larval individuals ($n = 4$) were captured but excluded from stomach content analyses due to methodological constraints.
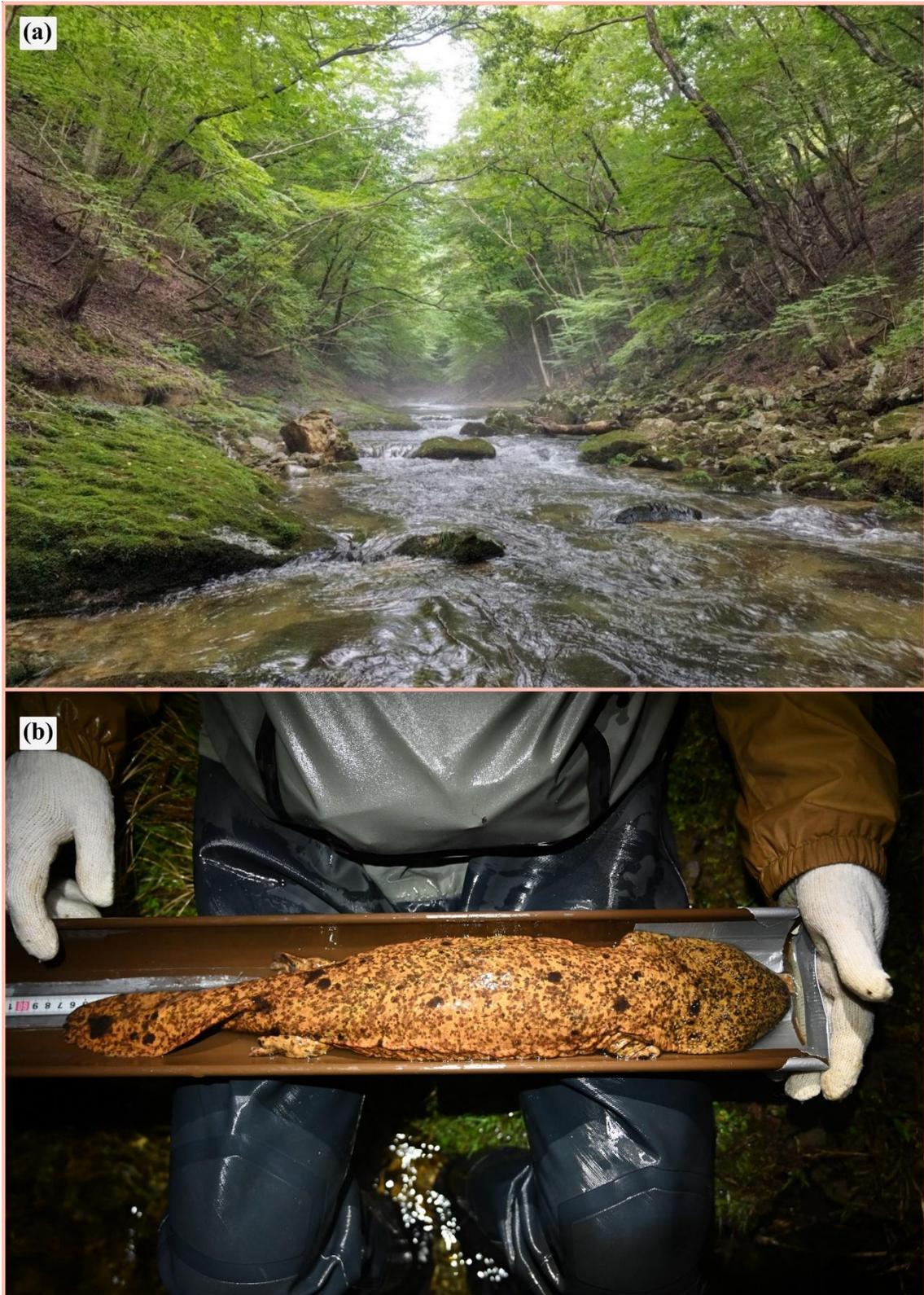
Figure 2. Study site: Ichi river (a) and length measurement of a Japanese giant salamander (b). Photo of the river by Clément Duret and photo of the salamander by Tiphanie Bartet.

Stable isotope data processing

All samples for stable isotope measurements were oven-dried at 60°C for 72 hours (Mktrshy incubator) and ground into a homogeneous powder. Since giant salamander samples could not be exported out of Japan, we measured their carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in Japan (Faculty of Food and Agricultural Sciences, Fukushima University) using an isotope ratio mass spectrometer (Delta-V Advantage with Flash Elemental Analyser, Thermo Fisher Scientific). We analysed food sources and baselines samples in Belgium (Laboratory of Trophic and Isotope Ecology (LETIS), University of Liège) using an isotope ratio mass spectrometer (isoprime PrecisION) with an elemental analyser (Vario MICRO cube, Elementar, Germany).

Isotope ratios were conventionally expressed as δ values in ‰ (Coplen 2011). Reference materials for stable isotope analysis included certified standards from the International Atomic Energy Agency (IAEA, Vienna, Austria) in Belgium and from the Center for Ecological Research (CER), Kyoto University, and the Institute of Biogeosciences (BioGeos), Japan Agency for Marine-Earth Science and Technology (JAMSTEC) in Japan (Tayasu et al. 2011). Details of the reference materials and internal standards are provided in Supporting information. Additionally, samples from a single fish specimen were analysed in both laboratories and used as a control sample to ensure consistency across analyses.

Stable isotope mixing model analysis

We developed stable isotope Bayesian mixing models using the R package 'simmr' (Govan et al. 2023) for each giant salamander with uninformative priors to estimate the contribution of four prey categories (fish, freshwater crabs, frogs, aquatic insects) to the isotopic composition of giant salamanders, based on their occurrence in stomach contents. Before running the models, we ensured that these categories had distinct isotopic compositions to enhance the models' discriminatory power. We ran mixing models using a Markov Chain Monte Carlo (MCMC) algorithm with the following parameters: three chains, length = 100,000, burn-in = 50,000, and thin = 50. We assessed convergence of the Markov chains for each mixing model using Brooks-Gelman-Rubin diagnostics and we visually checked model fit by plotting the posterior predictive distribution of each model. We applied trophic discrimination factors (TDFs) for each prey category following McCutchan et al., 2003: 1.3 ± 0.3 (‰) for $\delta^{13}C$ and 1.4 ± 0.21 (‰) for $\delta^{15}N$ for freshwater crabs and aquatic insects, 1.3 ± 0.3 (‰) for $\delta^{13}C$ and 3.3 ± 0.26 (‰) for $\delta^{15}N$ for fish and frogs.

Statistical analysis

Diet and habitat

For each prey category identified in stomach contents, we calculated several metrics. The occurrence (O) refers to the number of stomachs in which the prey category was found. The frequency of occurrence (FO, %) was calculated as $FO = O/N_s * 100$, where N_s is the total number of stomach contents analysed. We also determined the total prey count (N), which represents the total number of prey items found for each prey category across all stomach contents. The proportion of the total prey count (%N) was calculated as $\%N = N/N_p *100$,

where N_p is the total number of prey items (all categories combined) found across all stomach contents. In addition, the total mass (M) of each prey category was calculated as the sum of the total wet mass (g) of all prey items across all stomach contents. Finally, the proportion of the total prey mass (%M) was calculated as %M = M/M_t * 100, where M_t is the total wet mass of all prey items (all categories combined) found in all stomach contents.

To assess the potential influence of the salamanders' habitat on variations in prey proportions in their stomachs, we performed a distance-based redundancy analysis (dbRDA) using PRIMER version 7 software (Clarke and Gorley 2015) and PERMANOVA + add-in (Anderson et al. 2008). We constructed a resemblance matrix based on Bray-Curtis distances, calculated from the proportions of prey in each stomach after applying a square-root transformation to the data. This transformation was used to down-weight the influence of highly dominant prey categories while preserving ecological relevance. The predictor variables included in the analysis were the five environmental variables mentioned earlier. The dbRDA was computed with 9,999 permutations with a forward selection of the variables to assess significance of the results.

Relationship between $\delta^{15}N$, trophic position, and body size

We estimated the $\delta^{15}N$-derived trophic position of Japanese giant salamanders (TP_{SIA}) using the R package tRophicPosition (Quezada-Romegialli et al. 2018). For each individual, we applied a Two Baselines Bayesian model, incorporating both terrestrial and aquatic baselines (Supporting information), with $\lambda = 1$ for the trophic level of the baseline organisms. A trophic enrichment factor (TEF) of 2.4 was used, based on the tissue type analysed, dietary sources, and trophic level of giant salamanders (Stephens et al. 2023). In some sections, $\delta^{15}N$ values of periphyton were unavailable. Because the Bayesian model requires both baselines to be at the same TP (λ), we used snail isotope values as proxies. In sections where both snail and periphyton $\delta^{15}N$ values were available, we calculated an isotopic difference of 1.7 ‰, which was subtracted from snail $\delta^{15}N$ values in sections lacking periphyton to estimate their aquatic baseline.

We examined the relationships $\delta^{15}N$–SVL and TP–SVL using generalized additive models (GAMs), which allow for non-linear relationships (Wood 2017). These models were compared to linear models using the Akaike Information Criterion (AIC) to evaluate potential non-linear effects. After developing the GAMs, we applied breakpoint models to test the hypothesis that there is a drastic shift in the rate of increase in $\delta^{15}N$ isotope ratio and TP at a specific SVL. Specifically, we used a piecewise linear model (segmented regression) to detect the breakpoint SVL. Piecewise linear models are effective for identifying such thresholds (Muggeo 2003, Ficetola and Denoël 2009) and were implemented using the R package '*segmented*' version 2.1-2 (Muggeo 2008). The breakpoint ranges were preliminarily identified using GAM plots of the $\delta^{15}N$–SVL and TP–SVL relationships.

Ontogenetic variation of the diet

To investigate body-size-dependent variation in the diet of Japanese giant salamanders, we developed GAMs using prey proportions obtained from both stomach content and stable isotope data. For the stomach content analysis, the mass proportions of the four prey categories

(fish, freshwater crabs, frogs, and aquatic insects) were used as response variables, with snout-vent length (SVL) included as a smooth explanatory term to capture potential non-linear relationships. For the stable isotope analysis, the proportional contributions of prey categories estimated by the mixing models were used as response variables, with SVL included as a smooth term. We fitted all GAMs using thin plate regression splines implemented in the 'mgcv' package in R (Wood 2017). We assessed model adequacy and robustness through diagnostic checks, including residual analysis and evaluation of model assumptions. All analyses were conducted in R (R-4.3.2).

Results

Stomach content analysis

From the 151 salamanders that were flushed, we recorded a total of 1,316 prey items (total wet mass: 5,392.07 g). The mean ± SD number of prey items per stomach was 8.83 ± 5.66, with a mean ± SD mass of 37.43 ± 44.00 g. A wide variety of prey were found in the stomachs of giant salamanders (table 1), with almost exclusively aquatic prey (99.39% of all identified prey items). The four main prey categories (>94% of total number of prey items, >97% of total prey mass) were Japanese freshwater crabs (*Geothelphusa dehaani*), fish, anurans, and aquatic insects. Identified fish species included the Dark chub (*Nipponocypris temminckii*), Chinese minnow (*Rhynchocypris oxycephala*), amago or red-spotted masu salmon (*Oncorhynchus masou macrostomus*), lizard goby (*Rhinogobius flumineus*) and Japanese torrent catfish (*Liobagrus reinii*). Anurans were primarily represented by the Kajika frog (*Buergeria buergeri*), with occasional occurrences of Japanese wrinkled frogs (*Glandirana rugosa*) and black-spotted frogs (*Pelophylax nigromaculatus*). Aquatic insects included larvae of Plecoptera, Megaloptera, Hemiptera, Trichoptera, Ephemeroptera, and Odonata. Other prey items included a freshwater prawn (*Palaemon paucidens*), aquatic gastropods (*Semisulcospira spp.*), a snake (*Lycodon orientalis*) and mammal remains, likely from a species of Soricidae. Additionally, one case of cannibalism was identified.

Habitat use

Overall, salamanders were found in shallow parts of the rivers (mean ± SD depth = 19.7 ± 13.8 cm), with low water velocity (mean ± SD = 0.14 ± 0.20 m/s), near riverbanks (mean ± SD relative channel position = 0.17 ± 0.13) and in areas with minimal agriculture (mean ± SD = 3.9 ± 9.9 % land cover). The dbRDA analysis (Supporting information) selected four out of the five habitat variables and indicated a weak influence of habitat parameters on stomach contents composition. Water velocity (*Pseudo-F$_{148}$* = 3.1993, *p* = 0.0358, explained variance = 0.020) and the proportion of agricultural areas (*Pseudo-F$_{149}$* = 7.1468, *p* = 0.0018, explained variance = 0.046) showed significant effects, while water depth (*Pseudo-F$_{146}$* = 1.363, *p* = 0.2626, explained variance = 0.008) and river width (*Pseudo-F$_{147}$* = 2.7495, *p* = 0.0676, explained variance = 0.017) were not statistically significant.

Table 1. Stomach content of the Japanese giant salamander ($n = 151$). Metrics include the occurrence (O), the frequency of occurrence (FO, in %), the total number of prey items found across all stomachs (N), the proportion of the total number of prey items (%N), the total wet mass of prey items in all stomachs (M, in g), and the proportion of total wet mass (%M). The four bolded rows represent the four main prey categories used in subsequent analyses of prey proportions.

Prey items	O	FO	N	%N	M	%M
Aquatic prey						
Decapod crustaceans						
Freshwater crab	**144**	**95.36**	**640**	**48.63**	**2087.22**	**38.71**
Freshwater shrimp	8	5.29	41	3.12	29.83	0.55
Teleost fish	**81**	**53.64**	**125**	**9.50**	**314.21**	**5.83**
Aquatic insect (larvae)	**93**	**61.59**	**226**	**17.17**	**95.22**	**1.77**
Plecoptera	31	20.53	43	3.27	27.23	0.51
Megalopetera	37	24.50	78	5.93	39.21	0.73
Hemiptera	28	18.54	44	3.34	3.73	0.07
Trichoptera	4	2.65	4	0.30	0.22	0.00
Ephemeroptera	3	1.99	3	0.23	0.11	0.00
Odonata	26	17.22	31	2.36	22.19	0.41
Undetermined	17	11.26	23	1.75	2.53	0.05
Aquatic Gastropod	24	15.89	31	2.36	10.90	0.20
Anurans	**95**	**62.90**	**244**	**18.54**	**2757.25**	**51.14**
Japanese giant salamander	1	0.66	1	0.08	27.7	0.51
Terrestrial prey						
Scolopendridae	1	0.66	1	0.08	1.62	0.03
Calopterygidae (adult)	1	0.66	1	0.08	1.33	0.02
Mammals	2	1.32	2	0.15	41.36	0.77
Arachnida	1	0.66	1	0.08	0.06	0.00
Snake	1	0.66	1	0.08	25.01	0.46
Coleoptera	2	1.32	2	0.15	0.36	0.01

Stable isotopes

Food web structure and stable isotope analysis

SIA allowed us to reconstruct food web structure and identify the Japanese giant salamander as apex predator (Fig. 3). $\delta^{15}N$ values of giant salamanders ($n = 160$) ranged from 4.3 ‰ (minimum SVL = 4 cm, TL = 5.5 cm) to 11.6 ‰ (maximum SVL = 67.3 cm, TL = 106 cm). $\delta^{13}C$ values (-21.6 to -15.2 ‰) indicated a predominantly aquatic carbon source. Compared to other prey groups (i.e. fish, freshwater crabs, anurans and aquatic insects), giant salamanders typically occupy the top of the food web (Fig. 3).
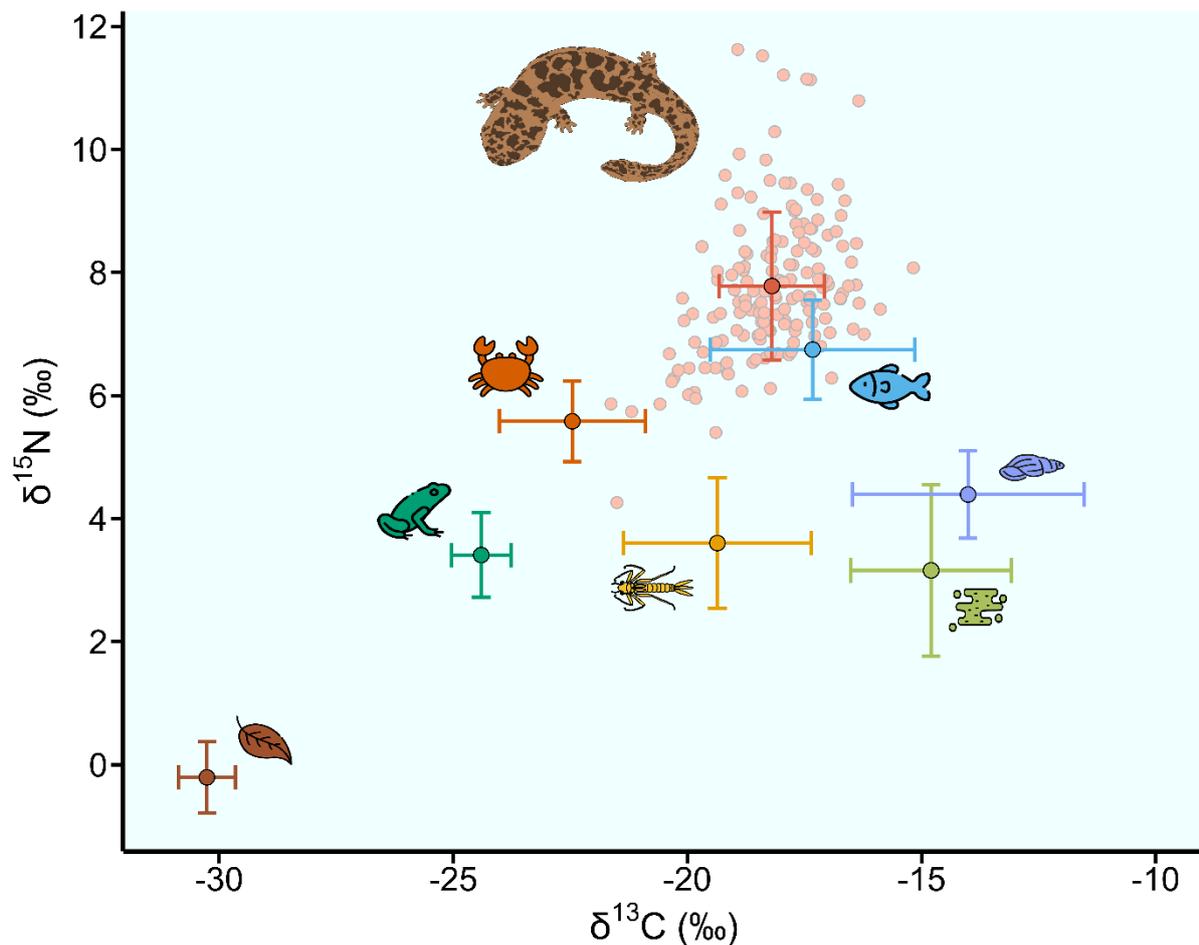
Figure 3. Trophic structure of the community dominated by the Japanese giant salamander, based on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios. The isotopic compositions of each group are presented as mean ± SD. Giant salamanders = orange, teleost fish = blue, freshwater crabs = light brown, anurans = dark green, aquatic insects = yellow, gastropods = light purple, terrestrial basal source (leaf litter) = dark brown, aquatic basal source (periphyton) = light green. Individual data are also presented for *A. japonicus* (light pink dots).

Ontogenetic shift of $\delta^{15}N$ values and trophic position of Japanese giant salamanders

The results from the GAM highlight a significant nonlinear relationship between $\delta^{15}N$ ratios of *A. japonicus* and their SVL ($F_{4.529} = 76.09$, $p < 0.001$; Supporting information). The GAM outperformed the linear model, as indicated by the AIC values (GAM: AIC = 313.4; Linear model: AIC = 368.1).

Similarly, there was a significant nonlinear relationship between the $\delta^{15}N$-derived trophic position of *A. japonicus* (TP$_{SIA}$) and SVL ($F_{3.326} = 42.83$, $p < 0.001$; Fig. 4). The GAM outperformed the linear model, as indicated by the AIC values (GAM: AIC = 154.9; Linear model: AIC = 176.7). The piecewise linear model performed based on this observation identified the breakpoint at SVL = 39.40 ± 2.00 cm (estimate $\pm$ SE), corresponding to a total length (TL) of approximately 62 cm. Using $\delta^{15}N$ values, the breakpoint was identified at a SVL of 42.57 ± 1.13 cm (Supporting information). The increase rate of trophic position is higher in salamanders with an SVL above 39.40 cm (Supporting information). The smaller individual captured had the lowest TP (SVL = 4 cm, TL = 5.5 cm, TP$_{SIA}$ = 1.92) and the larger individual had the highest TP (SVL = 67.3 cm, TL = 106 cm, TP$_{SIA}$ = 5.04). All giant salamanders, except for the five smallest individuals (SVL between 4 and 12.4 cm), had undergone metamorphosis (i.e. closure of the gill slits).
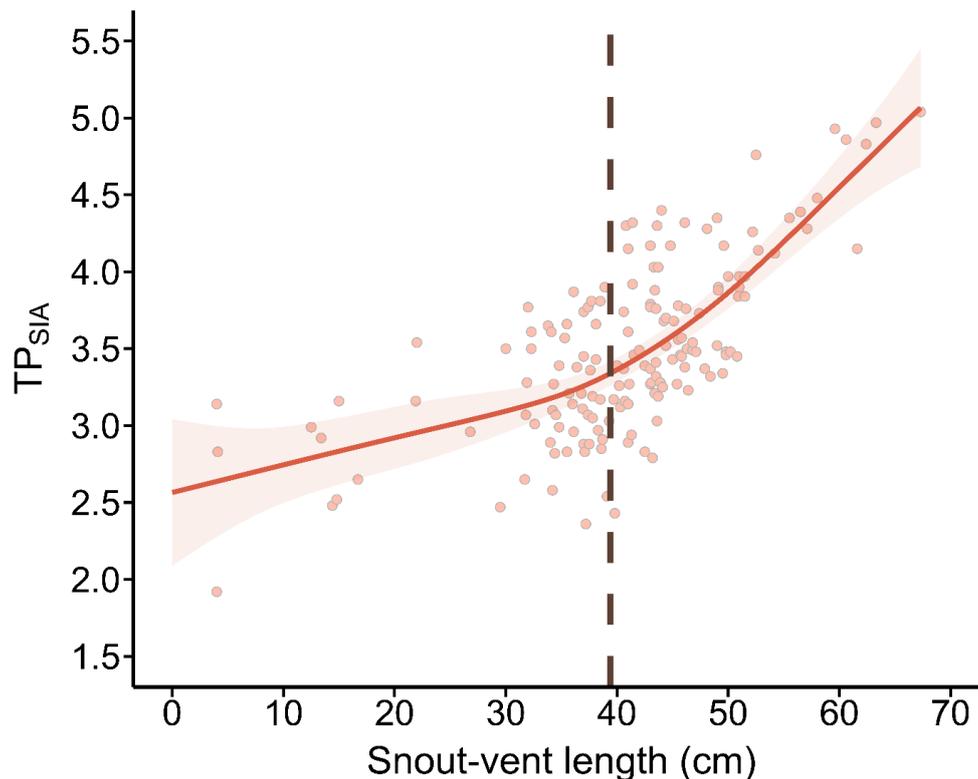

Figure 4. Increase in trophic position calculated from stable isotope data (TP$_{SIA}$) as a function of SVL in Japanese giant salamanders. The trend line represents GAM with 95% confidence interval (shaded areas). The dashed vertical line represents the threshold at a SVL of 39.40 cm. Each dot corresponds to an individual value.

Ontogenetic dietary shift

GAMs results revealed an ontogenetic shift in prey composition of stomach contents for each prey category separately (freshwater crabs: $F_{1.871} = 3.635$, $p = 0.021$; fish: $F_{2.563} = 3.079$, $p = 0.023$; anurans: $F_{1.116} = 5.346$, $p = 0.022$; aquatic insects: $F_{2.644} = 6.117$, $p < 0.001$; Fig. 5a). A continuous decline in the proportion of aquatic insects is observed with increasing salamander size, while for crabs, an increase is observed with a plateau reached at larger sizes. An almost linear increase is seen in the proportion of anurans from 0 to 0.3. Finally, the proportion of fish in the diet increases up to a SVL of about 30 cm (proportion ~0.17) and then decreases in larger salamanders.

Ontogenetic shift in food source contribution through stable isotope mixing models

GAMs developed with stable isotope mixing models results (Fig. 5b; Supporting information) confirmed an ontogenetic shift for freshwater crabs ($F_{4.715} = 13.42$, $p < 0.001$), fish ($F_{3.783} = 50.18$, $p < 0.001$), anurans ($F_{3.613} = 4.095$, $p = 0.003$) and aquatic insects ($F_{3.918} = 41.25$, $p < 0.001$). The contribution of aquatic insects is highest in smaller salamanders but declined with increasing body size. Freshwater crabs and anurans contribution remains stable until SVL ~43 cm but decreases beyond this threshold (Fig. 5). Fish contribution increased continuously with salamander SVL, followed by a shift in the GAM curve after reaching an SVL threshold of 39 cm.

Discussion

Ontogenetic dietary shifts (ODSs) are widespread among vertebrate predators and have important ecological consequences (Nakazawa 2015). Using stomach content and stable isotope analysis, we found clear evidence of a significant ODS in the Japanese giant salamander, along with a positive non-linear relationship between body size and trophic position (TP). Specifically, juveniles occupy a TP around level 3 (i.e. secondary consumer), increasing slowly with body size until a snout-vent length (SVL) of 39 cm. Beyond this threshold, TP rises sharply, bringing the species to the top of the food web (TP ~ 5) at around 60 cm in SVL. Our results emphasize the crucial role of body size in shaping the trophic ecology of giant salamanders and reinforce their apex predator status. Further, the non-linear TP–SVL relationship suggests that the ability to capture larger prey at a certain developmental stage may constitute a key adaptive feature of the evolution towards gigantism in Cryptobranchidae. More broadly, gigantism in vertebrates is often associated with ecological advantages, including reduced predation risk, enhanced competitive dominance, and access to a wider range of prey resources (LaBarbera 1989). In the case of giant salamanders, their exceptional size likely provides both trophic and evolutionary advantages.
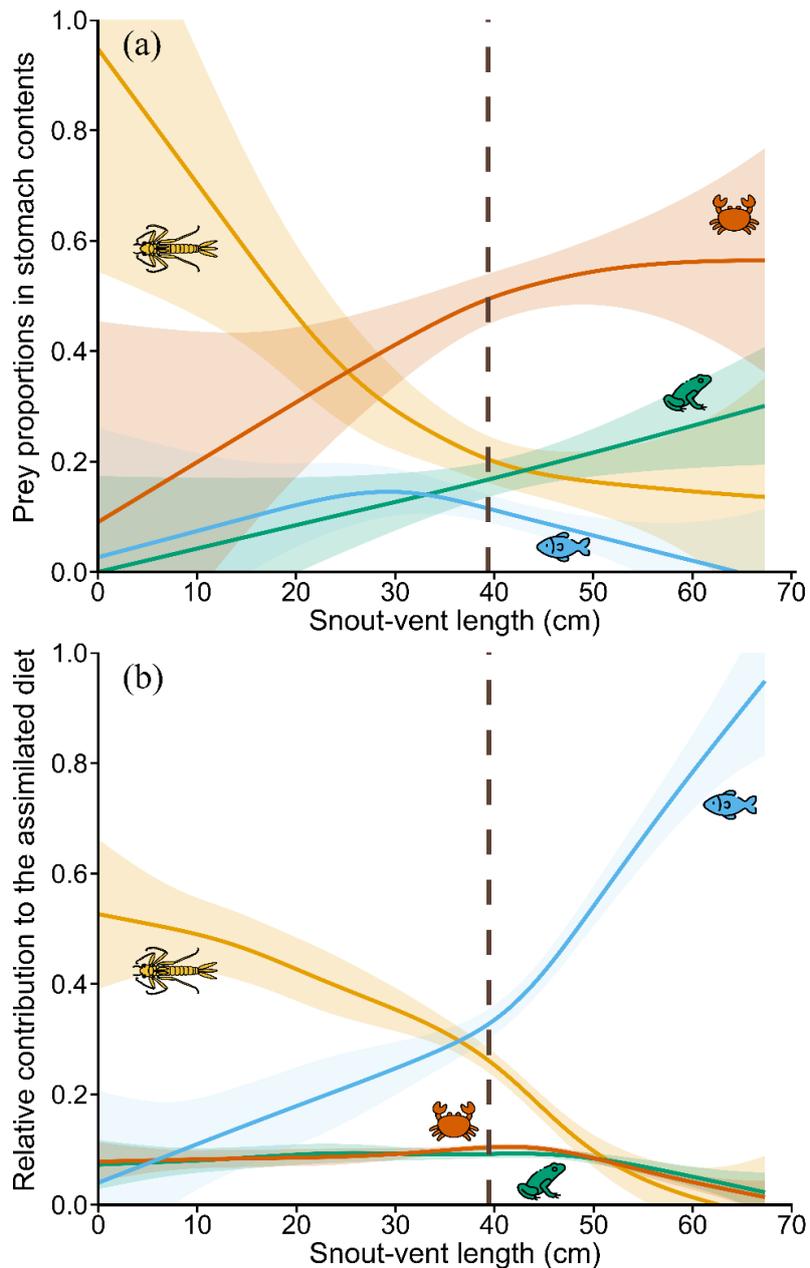
Figure 5. Ontogenetic variation in the proportion of ingested prey (based on stomach content analysis) (a) and in the contribution of prey to the assimilated diet (based on isotope mixing models) (b) of the Japanese giant salamanders. Trends lines represent GAMs with 95% confidence interval (shaded areas): yellow = aquatic insects, dark green = anurans, light brown = freshwater crabs and blue = fish. The dashed vertical line represents the threshold at a SVL of 39.40 cm.

Diet of Japanese giant salamanders

Stomach content analysis (SCA) confirmed the generalist predatory behavior and broad dietary spectrum of *A. japonicus* (table 1), primarily including four prey types: freshwater crabs, fish, anurans and aquatic insects. Occasional consumption of snakes and mammals suggests opportunistic predation on drowning or amphibious prey. This aligns with previous findings that crabs and fish are giant salamanders' dominant prey (Okada et al. 2008). Other giant

salamanders exhibit similar generalist feeding behaviours, such as North American hellbenders (*Cryptobranchus alleganiensis*) which primarily consume crayfish and fish (Peterson et al. 1989) or Chinese giant salamanders (*Andrias davidianus*), identified as apex predators preying on various animals, including conspecifics (Song 1994).

SCA first reveals a continuous decline in aquatic insect consumption with increasing body size (Fig. 5a). Small-sized giant salamanders are likely constrained by their mouth width, limiting access to larger prey like adult anurans or large fish. Based on the proportions of different prey in stomachs, a clear dietary shift is evident at the intersection of the aquatic insects and anuran proportion curves (Fig. 5a). While the presence of freshwater crabs in the diet also increases with size, this change appears more gradual (Fig. 4). As in other apex predators such as the Gharial (*Gavialis gangeticus*), the Alpine newt (*Ichthyosaura alpestris*) and the tiger salamander (*Ambystoma mavortium*), large individuals continue consuming some small prey items despite capabilities of preying on larger prey (Thorbjarnarson 1990, Denoël and Joly 2001b, Denoël et al. 2006). Future studies should specifically investigate the diet of small larval giant salamanders. However, their small size and cryptic behavior (e.g., hiding in leaf litter) make them difficult to sample extensively, and cannot be all stomach-flushed. Nevertheless, our stable isotope analysis suggests that larvae primarily feed on aquatic insects.

Trophic position

The ontogenetic shift in the trophic position (TP) of the Japanese giant salamander is particularly pronounced (Fig. 4). TP calculated for each individual using $\delta^{15}N$ values (TP_{SIA}) revealed a continuous, yet nonlinear, increase with body size, aligning with previous studies on other aquatic apex predators like crocodilians (Radloff et al. 2012, Hanson et al. 2015, Villamarín et al. 2018) and freshwater fish (Romanuk et al. 2011, Ou et al. 2017). However, while many of these studies identified linear or hump-shaped relationships, often with showing a slight declines in $\delta^{15}N$ or TP at larger body sizes, our results clearly indicate a continuous increase, with a significant shift in this increase at an SVL of 39.40 ± 2.00 cm (Fig. 4).

Discrepancy between prey consumption and assimilation

It is important to acknowledge the significant limitations of stomach content analysis (SCA). The most notable is that SCA provides only a snapshot of what the organism has most recently consumed (Fry 2006). This major limitation commonly leads to discrepancies with stable isotope analysis (SIA), as observed in our study and common in aquatic predators (Lejeune et al. 2023). Isotope mixing models estimate the proportion of assimilated food sources and their contribution to tissue synthesis in the studied consumer (Govan et al. 2023). Therefore, while each method provides different perspectives, they offer complementary insights into diet composition. This emphasizes the necessity of integrating both stomach content and stable isotope approaches to accurately assess the trophic ecology of long-lived predators like Japanese giant salamanders. In SCA, we estimated prey proportions using wet mass of prey collected from stomach flushing, but digestion and assimilation rates vary among prey. This is particularly relevant for freshwater crabs, whose exoskeleton constitutes the majority of their total mass while it is only minimally assimilated by the consumer. Although bacteria with chitinolytic activity have been identified in the gut microbiota of giant salamanders (Zhai et al.

2024), their ability to digest and assimilate crab exoskeletons appears limited. Moreover, since giant salamanders swallow their prey whole without chewing, most ingested crabs were found intact following flushing, suggesting that digestion and assimilation may be further hampered. Conversely, fish are a highly assimilable food type. This can directly explain that, while freshwater crabs constituted a dominant prey according to SCA (table 1; Fig. 5a), they accounted for only a small proportion of assimilated prey based on SIA (Fig. 5b). Another discrepancy between the two methods is observed in the estimated proportion of amphibians in the diet. SCA suggests that the proportion of amphibians increases with body size, as some stomachs contained multiple frogs, with up to 16 individuals found in a single stomach. However, stable isotope mixing models indicate that the amphibian contribution to the diet remains relatively stable across body sizes. One possible explanation is that small salamanders may consume tadpoles, which may be quickly digested beyond recognition (Lejeune et al. 2023) and could have been undetectable in SCA. Additionally, frog consumption by giant salamanders is likely underestimated in mixing models for larger individuals due to seasonal availability. The Kajika frog, the most consumed amphibian, is present in rivers from April to August (Matsui and Maeda 2018). Since isotope data reflect past diet, and salamanders were sampled from April to June, anurans contribution may be underrepresented, emphasizing the influence of prey phenology on diet estimates.

Morphological implications and link to gigantism

Ontogenetic changes or evolutionary trends affecting body size, such as gigantism, can act as intrinsic factors of ecological opportunity, promoting niche differentiation and adaptation by providing access to underused or previously inaccessible resources (Lejeune et al. 2021). In Japanese giant salamanders, cranial skeletogenesis suggests potential ontogenetic adaptations for feeding (Ishikawa et al. 2022), aligning with our findings. The significant and non-linear ODS identified in our study may result from underlying morphological changes in the skull which might enable larger individuals to capture prey that would be too large or too fast for smaller salamanders. Skull ossification, combined with the ability to open the mouth widely —a unique feature of giant salamanders compared to other salamanders—likely facilitate the capture of larger prey and may at some point, during ontogeny, create a breaking point generating the ODS pattern observed in our study. Notably, this dietary shift is not linked to metamorphosis, as the size of salamanders at the time of metamorphosis is three times smaller than the threshold body size at which we observed the dietary shift. Additionally, their suction-feeding mechanism and retention of some larval traits (paedomorphosis; Fabre et al. 2020, Bonett et al. 2022), driven by hyobranchial skeleton depression to increase water flow (Heiss et al. 2013), further enhances their predatory efficiency. Selection for gigantic body size throughout evolution is often favoured in contexts of increased resource abundance or ecological release from predators or competitors, while miniaturization typically arises from resource or habitat constraints (Bonett et al. 2009), although the latter can also provide trophic advantage in resource-rich, highly diverse communities (Lejeune et al. 2018). Paedomorphosis itself can mediate the evolution towards either small or large body sizes (Lejeune et al. 2018, Bonett et al. 2022). Therefore, further studies on functional morphology and diet are needed to better understand the evolutionary pathways leading to variation in body size and gigantism, particularly in relation to paedomorphic traits (Bonett et al. 2022). Our results show that, in

Japanese giant salamanders, gigantism allows to access a broader prey spectrum, including prey that are not typically accessible to other salamander species such as large fish, crabs and metamorphosed anurans (Fig. 5) (Zambrano et al. 2010). This dietary shift, combined with key morphological changes, may have played a key adaptive role shaping the evolutionary trajectory towards gigantism in the Cryptobranchidae family. By extending their trophic niche, larger salamanders reduce competition with conspecifics, while simultaneously decreasing their own predation risk. Thus, gigantism in these salamanders is not merely a byproduct of growth but a fundamental adaptation that has likely contributed to their evolutionary success and persistence over millions of years.

Conclusions

Our study highlights the critical role of body size in shaping ontogenetic diet shifts (ODS) and how, by extending the range of body size increase throughout ontogeny, gigantism may promote ecological opportunity in large predators. Through stomach content and stable isotope analyses, we demonstrated a clear ODS in the Japanese giant salamander, with larger individuals consuming disproportionately larger prey. This shift, occurring at an SVL of 39 cm, might result from morphological adaptations linked to cranial skeletogenesis likely enhancing predatory efficiency. Understanding the relationship between diet and body size in apex predators is essential for uncovering the ecological opportunities offered by gigantism. Given the role of a large body size in enabling access to larger prey, these insights can also inform conservation and management strategies, particularly for preserving key prey species such as fish in rivers inhabited by giant salamanders. Furthermore, our findings provide a broader perspective on the evolutionary and adaptative implications of gigantism in giant salamanders and how their large size influences their ecology and trophic behaviour.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Clément Duret: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Methodology (equal); Resources (equal); Software (lead); Validation (equal); Writing – original draft (lead). **Benjamin Lejeune**: Conceptualization (equal); Investigation (equal); Methodology (equal); Software (supporting); Validation (equal); Writing – review and editing (supporting). **Gilles Lepoint**: Formal analysis (supporting); Methodology (supporting); Validation (equal); Writing – review and editing (supporting). **Tiphanie Bartet**: Data curation (equal); Methodology (supporting); Writing – review and editing (supporting). **Sumio Okada**: Methodology (equal); Resources (equal); Writing – review and editing (supporting). **Keitaro Fukushima**: Formal analysis (supporting); Methodology (supporting); Resources (equal); Writing – review and editing (supporting). **Osamu Kishida**: Funding acquisition (equal); Project administration (equal); Resources (equal); Validation (equal); Writing – review and editing (supporting). **Mathieu Denoël**: Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Validation (equal); Writing – review and editing (lead).

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Supporting information

The Supporting information associated with this article is available with the online version.

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