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RESEARCH ARTICLE

Behavioural tactics across thermal gradients align with partial morphological divergence in brook charr

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Abstract

1. Understanding how animals balance environmental constraints is essential for predicting species persistence under climate change. In thermally stratified lakes, cold water fishes such as brook charr (*Salvelinus fontinalis*) must navigate vertical gradients in temperature and oxygen to optimize foraging while avoiding physiological stress. We hypothesized that individuals would exhibit behavioural tactics that reflect a trade-off between accessing warm surface waters to exploit profitable prey and avoiding thermal stress, with greater constraints expected on epilimnetic use as surface temperatures rise.
2. Using high-resolution acoustic telemetry, we quantified fine-scale patterns of thermal habitat use, vertical foray behaviour and diel timing in a wild brook charr population across the summer stratification period. We also assessed whether behavioural thermoregulation aligned with morphological differences, testing whether divergent behaviours reflect partial ecotypic divergence.
3. As surface temperatures rose, brook charr reduced their use of the warm epilimnion, making fewer and shorter vertical forays. Hypolimnion use increased concurrently but was unrelated to limiting oxygen concentrations, indicating that deep water use was not physiologically constrained and may reflect an alternative foraging behaviour. Epilimnetic forays peaked at dusk and varied with moon phases, consistent with crepuscular visual foraging. Hypolimnetic use peaked at dawn and dusk but showed no response to moonlight.
4. Diel patterns shifted seasonally: in warmer months, epilimnetic access was restricted to twilight hours, while cooler months saw broader surface use throughout the day. Principal component analysis of vertical movement and temperature exposure traits revealed two behavioural tactics: a 'warm' tactic, characterized by frequent epilimnetic forays and warmer average thermal exposure, and a 'cool' tactic, associated with greater hypolimnetic use and cooler average temperatures. These tactics corresponded with partial morphological divergence within the two sexes.
5. Linear discriminant analysis showed that males following the warm tactic were morphologically distinct from cool ones, differing in traits related to

feeding and swimming performance, while females showed weaker morphological differentiation.

6. Together our findings reveal repeatable habitat use and consistent thermal tactics that reflect trade-offs between foraging and thermal stress. Vertical gradients in temperature and resource distribution may thus promote fine-scale individual specialization and phenotypic divergence in cold water species facing lake warming.

KEYWORDS

acoustic telemetry, behavioural thermoregulation, diel movement patterns, morphological divergence, *Salvelinus fontinalis*, thermal stratification, vertical forays

1 | INTRODUCTION

Fish, as ectotherms, are strongly constrained by the thermal conditions of their environment. Ambient water temperature regulates their distribution, activity, and performance, with direct consequences for survival, growth and reproduction (Kieffer et al., 1998; Magnuson et al., 1979). When temperatures deviate from optimal ranges, fish adjust their behaviour—primarily through active habitat selection and behavioural thermoregulation—to maintain physiological performance (Amat-Trigo et al., 2023; Haesemeyer, 2020). However, their capacity to adapt to thermal stress is asymmetrical. According to Brett's heat-invariant hypothesis, lower thermal limits may shift with local adaptation, whereas upper limits remain relatively fixed—potentially constraining persistence in warming environments (Brett, 1971; Buckley et al., 2022; Ern et al., 2023; Martin & Huey, 2008).

Seasonal thermal stratification in lakes creates steep vertical temperature gradients that divide the water column into distinct layers (i.e. epilimnion, metalimnion and hypolimnion) which strongly influence fish distribution and activity (Flood et al., 2021). Many species remain within preferred temperature zones to optimize metabolic efficiency (Guzzo et al., 2019), yet may periodically enter suboptimal thermal environments to access resources or avoid predators (Thomas et al., 2023). This trade-off is especially pronounced in cold-water species such as salmonids, which often ascend into warmer epilimnetic waters to forage despite the associated physiological costs (Guzzo et al., 2017). These excursions elevate metabolic demands by increasing oxygen consumption and energy expenditure through heat transfer from the surrounding water (Clarke & Johnston, 1999). To mitigate these costs, salmonids often employ behavioural tactics such as brief foraging bouts followed by rapid returns to cooler refugia, a pattern documented across multiple species in stratified systems (Armstrong et al., 2016; Bertolo et al., 2011). Although the general relationship between fish behaviour and thermal gradients is well established, fine-scale patterns of vertical forays across thermal layers remain poorly understood in natural settings. Most studies have focused on broad habitat use or diel vertical shifts (Cote et al., 2020; Goyer et al., 2014), while few have quantified behavioural metrics such as foray frequency, duration and timing across thermal strata. In particular, the role of the hypolimnion remains understudied (but see e.g. Roberts et al., 2012; Rodrigues et al., 2022), despite its increasing importance in stratified systems experiencing surface warming and deep water hypoxia (Bartosiewicz et al., 2019).

The physiological constraints of different thermal layers differ markedly. In the epilimnion, temperature can reach lethal levels, strictly limiting how long fish can remain in surface waters before incurring thermal stress (Pépino et al., 2015). In contrast, the hypolimnion is often characterized by reduced thermal stress but also low oxygen availability (hypolimnetic hypoxia), which can constrain fish movement and influence habitat selection (Fang & Stefan, 2009; Roberts et al., 2012; Rodrigues et al., 2022). Unlike acute thermal stress, short-term exposure to hypoxia exerts more gradual effects, impairing aerobic performance without causing immediate physiological failure (Chapman & Mckenzie, 2009). Climate change is intensifying these constraints by warming surface water and worsening hypolimnetic hypoxia through stronger thermal stratification and diminished oxygen replenishment. This combination of stressors is of particular concern for salmonid species, which already occupy a narrow thermal window for optimal growth and survival (McCullough et al., 2009). As warming and hypoxia intensify, understanding how salmonid species navigate thermal gradients to balance physiological stress and foraging needs is critical for predicting their future distribution and persistence under climate change.

Brook charr (*Salvelinus fontinalis*) provide an ideal model for studying how fish navigate thermal constraints due to their reliance on both warm and cold water habitats (Pépino et al., 2024). First, the species colonized many postglacial lakes of the Canadian Shield which exhibit pronounced vertical temperature gradients creating distinct thermal zones that brook charr must navigate to balance thermal stress, oxygen availability and foraging needs (Smith et al., 2020). Second, brook charr display resource polymorphism in many of these lakes, where a littoral ecotype feeds mainly on zoobenthos and a pelagic ecotype feeds mostly on zooplankton implying habitat-specific foraging tactics (Bourke et al., 1997; Pépino et al., 2018; Proulx & Magnan, 2004; Rainville, Filion, et al., 2021). However, no direct relationship between morphology and behaviour has yet been demonstrated. Similar patterns of habitat-related resource polymorphism are well documented in other *Salvelinus* species, particularly Arctic charr (*S. alpinus*), where morphs specialized for deep, pelagic and littoral zones show distinct morphological and behavioural adaptations to their thermal and foraging environments (Hawley et al., 2016; Jonsson, 2001). Behavioural studies similarly show that brook charr use the epilimnion, metalimnion and hypolimnion at different times, demonstrating flexible habitat use (Pépino et al., 2024). This flexibility makes them an excellent model for studying behavioural thermoregulation in response to temperature gradients and resource availability.

This study focuses on fish behavioural thermoregulation by addressing key gaps in our understanding of fine-scale vertical movement in stratified lakes, pinpointing how fish foraging behaviour varies across thermal zones and shifts with changing summer temperatures. Specifically, we quantify the temporal patterns of vertical forays in relation to temperature and oxygen availability. Beyond individual metrics, we classified fish into distinct thermoregulation behavioural tactics based on their use of thermal strata, potentially shaped by asymmetrical physiological constraints. We then tested whether these thermoregulation behaviours were associated with morphological differences, exploring whether thermal niches align with potential foraging morphotypes. We hypothesize the following: (1) as surface temperatures rise, brook charr will reduce forays into the epilimnion due to thermal stress; (2) like Arctic charr (Jonsson, 2001), some individuals may increasingly use deeper zones such as the hypolimnion to access cooler temperatures, but remain constrained by low oxygen availability; (3) epilimnetic forays will peak at dusk, coinciding with brook charr foraging activity patterns (Bourke et al., 1996), while forays into the hypolimnion will show less predictable patterns due to competing influences of temperature, food availability and oxygen constraints; and (4) thermoregulation behaviours will correspond to distinct morphotypes, suggesting integrated thermal and trophic tactics. By combining fine-scale behavioural metrics, thermal profiles and morphological data, this study provides new insight into how a cold water species manages competing environmental constraints and whether alternative tactics are linked to phenotypic divergence.

2 | MATERIAL AND METHODS

2.1 | Lake characteristics

The study was conducted in Lake Ledoux (46°38′ N, 73°15′ W), located in the Mastigouche Wildlife Reserve, Québec, Canada, from 2017 to 2018. Lake Ledoux is a small, oligotrophic lake typical of temperate regions, with a surface area of 11.9 ha, an average depth of 5.5 m, and a maximum depth of 18.0 m. Its physicochemical properties are consistent with those reported for similar lakes in the region (Brassard et al., 2023; Magnan, 1988). Aquatic vegetation is primarily concentrated in shallow waters (0–2 m depth; P. Magnan, unpubl.), corresponding to the littoral zone, which serves as a key foraging habitat for brook charr due to the abundance of preferred prey species. Brook charr is the sole fish species present in Lake Ledoux, where sport fishing is strictly regulated by the Québec Government (Bourke et al., 1996; Pépino et al., 2024).

To monitor thermal conditions, temperature profiles were recorded from late June 2017 to early September 2018 using thermographs (iButtons DS1922L in 2017 and iBCod DS1921G-F5 in 2018; Alpha Mac Inc.). Sensors were deployed at 0.5 m intervals from the surface to depths exceeding 10 m, with thermograph lines anchored to a floating raft positioned at the lake's deepest point. While the iBCod sensors are fully submersible, iButtons were encapsulated in aquarium-grade silicone (SCS1200 clear sealant) to protect them from prolonged water exposure. Temperature readings were collected at 4-h intervals throughout the study. Lake Ledoux exhibited thermal stratification from mid-May to the end of October. Stratification characteristics were analysed using the ‘rLakeAnalyzer’ package (Winslow et al., 2013) in R (R Core Team, 2017) and, considering the small size of the lake as well as the stable dynamics of the thermocline, linearly interpolated per hour. To test sensitivity to thermocline position, we also applied a conservative classification: the hypolimnion was defined as starting 1 m below its thermocline, and the epilimnion as ending 1 m above it. This approach allowed us to evaluate whether behavioural patterns were robust to small shifts in thermal structure and to avoid the fine-scale gradients within the metalimnion that can complicate the detection of behavioural thermoregulation.

Dissolved oxygen concentrations were monitored in the epilimnion, metalimnion and hypolimnion with four probes (miniDOT® Loggers, PME, USA) placed at 1,5, 3, 5 and 8 m depth. Oxygen was monitored every minute between July 4 and October 23 in 2018. The probes were retrieved weekly for cleaning and to check their status. As a dense cover of water mites was observed for the two shallower probes after the first week of deployment, the probes were subsequently equipped with automatic whippers to avoid clogging.

2.2 | Acoustic telemetry system

We employed an acoustic telemetry positioning system to continuously monitor brook charr movements and habitat use with high spatiotemporal resolution (<1 min, <5 m) using a VEMCO Positioning System (VPS). Specifically, we deployed the HR2 VPS technology, which uses a rapid pulse rate and high-power setup (no Pulse Position Modulation, HR delay of 10–14 s) to minimize signal ‘collisions’—a common issue when multiple tagged individuals are in close proximity (e.g. Guzzo et al., 2018). The system comprised 23 hydroacoustic receivers (HR2-180 k-100; VEMCO Inc.) and four reference tags (V9TP-2x-180k-34m; VEMCO Inc.), ensuring full lake coverage. Each fish was equipped with an acoustic transmitter recording both depth and temperature, allowing reconstruction of three-dimensional positions (X, Y calculated within the array based on time difference of arrival via the receiver network, Z and temperature broadcast alternatively by the tag sensors). The HR2 VPS was operational from 21 June 2017 to 6 November 2018. Additional details on the HR2 VPS systems, as well as performance assessments, are provided in the supplementary information of Pépino et al. (2024).

2.3 | Fish capture and tagging

Brook charr were captured using Alaska traps (1.0 × 1.8 m mouth opening, two 1 × 15 m wings, 1.27 cm mesh size; Fipec Industries) over two consecutive years (12–20 June 2017 and 5–7 June 2018). Following capture, fish were temporarily held in an enclosure (3 × 4 × 6 m depth) and surgically tagged on the same day. Tagging followed standard protocols (Adams & Maitland, 1998; Bridger & Booth, 2003), previously validated for this species and study system (Goyer et al., 2014; Pépino et al., 2015, 2024). In 2017 ($n = 30$) and 2018 ($n = 15$), brook charr (mass: 226–600 g; total length TL: 291–391 mm) were equipped with 4.0 g acoustic transmitters (V9TP-2x-180k-34m; VEMCO Inc., no Pulse Position Modulation, HR delay of 10–14 s with a high power set-up), which alternately recorded internal temperature and fish depth. Transmitter weight never exceeded 2% of fish body mass, complying with best practice guidelines to minimize behavioural and physiological impacts (Bridger & Booth, 2003; Mellas & Haynes, 1985). All individuals were released in apparent good condition and exhibited normal post-release behaviour. Full details are provided in the supporting information of Pépino et al. (2024). The research study was approved by the Animal Care Committee of the Université du Québec à Trois-Rivières (Comité de Bons Soins aux Animaux—CBSA; permit numbers: 2016--P.M.42) and by the Ministère des Forêts, Faunes et Parcs (MFFP; SEG permit numbers: 2016-06-21-080-04-S-P; 2017-04-27-051-04-S-P; 2018-05-21-050-04-S-P).

2.4 | Data preparation and filtering

Detection data were filtered to retain only periods of thermal stratification: 24 June to 31 October in 2017 and 19 May to 8 September in 2018 (Figure S1). This ensured that analyses targeted behaviour relevant to stratified lake conditions, when distinct thermal habitats (epi-, meta- and hypolimnion) were clearly defined. Detections outside the lake boundaries and those from individuals inferred to be dead (i.e. stationary for >24 h, $n = 4$) were excluded. To reduce artefacts from post-tagging recovery, we excluded the first 10 days following tagging, as fish activity was observed to stabilize thereafter.

Missing fish depth or temperature values were linearly interpolated within a 1-min window, independently for each variable. This preserved the temporal resolution necessary for accurate derivation of vertical movements and foray events for testing our hypothesis that individuals engage in short, thermally constrained excursions across depth layers.

2.5 | Thermal habitat use and vertical forays

To characterize brook charr thermal behaviour and habitat use across depth strata, we derived several variables from the telemetry data. These included the following: (1) fish depth, combining sensor and interpolated values; (2) distance from the lake bottom, calculated as the difference between lake bathymetric depth and fish depth at each detection point; (3) distance to the nearest shoreline, derived from a shapefile based on bathymetric mapping; and (4) swimming speed in body lengths per second (TL/s), using consecutive positional data and each fish's total length (TL) recorded at tagging. These metrics provided a foundation for describing individual movement and habitat use across thermal and spatial gradients.

To assess how fish used distinct thermal habitats, we focused on vertical movements into and out of the epilimnion and hypolimnion (thermal layers with different temperature and oxygen characteristics, and thus likely to impose different ecological trade-offs). We defined a 'foray' as a movement into one of these layers followed by a return to the metalimnion. Specifically, a foray began at the first detection within the target layer (e.g. epilimnion) and ended at the first subsequent detection in a different layer. Thermal boundaries were determined using hourly thermocline depths (see Lake characteristics section), ensuring that foray detection accounted for shifting thermal structure.

For each individual, we quantified multiple aspects of foraying behaviour: (1) total number of forays, (2) duration of each foray (from entry to exit) and daily summary statistics including the following: (3) mean foray duration, (4) total daily time spent in the target layer and (5) maximum duration of a single foray per day. These metrics allowed us to investigate fine-scale behavioural tactics and potential ecological roles of vertical forays, particularly under thermal constraints.

2.6 | Classifying thermal behaviour and habitat use tactics

To identify individual-level behavioural thermoregulation and assess whether brook charr segregated into consistent thermal ecotypes (as previously reported in Lake Ledoux by Bertolo et al., 2011 and Goyer et al., 2014), we calculated summary behavioural metrics across the entire study period. These variables included the following: (1) mean body temperature; (2) mean foray duration into the epilimnion and hypolimnion; (3) proportion of time spent in the epilimnion and hypolimnion; (4) mean swimming speed, both overall and within each layer; (5) mean distance to shore; and (6) mean distance to the lake bottom. These metrics were selected to reflect habitat affinity, foraging behaviours and thermal exposure.

To assess whether brook charr exhibited distinct thermal tactics based on behaviour alone, we performed a principal component analysis (PCA, *prcomp* function in R) on metrics of vertical movement, temperature exposure and space use to identify dominant axes of behavioural variation and reduce dimensionality. All variables were standardized prior to PCA. The first principal component (PC1) was used to classify individuals into two groups based on thermal habitat use: individuals with negative PC1 scores were classified as adopting a 'warm' tactic, while those with positive scores followed a 'cool' tactic (see details in the Results section). To assess the consistency of this classification under peak thermal stress, we repeated the PCA using data from July and August—the warmest period of the summer. This seasonal comparison allowed us to evaluate whether individuals consistently expressed divergent thermal tactics, as would be expected if behavioural divergence is shaped by trade-offs between foraging opportunities and thermal or oxygen stress. These classifications served as the basis for subsequent analyses of diel activity patterns and morphology. As the fish studied were adults of similar sizes (total length of 341 ± 25 mm), and the total length was not correlated with the first axis of the PCA ($R^2 = 0.17$), we did not include it in the analysis.

To further contextualize habitat use, we assigned each detection to a vertical habitat category: benthic (within 0–2 m from the lake bottom) or pelagic (beyond this threshold). These were combined with vertical layers (epilimnion, metalimnion and hypolimnion) to define composite spatial–thermal habitat types (e.g. epilimnetic–pelagic). For each fish, we calculated the proportion of detections in each composite category. This analysis was designed to test whether brook charr occupied distinct three-dimensional niches, potentially consistent with ecotypic specialization in foraging tactic and habitat preference. It also allowed us to assess whether habitat use patterns aligned with the thermal tactics identified earlier. Having classified these tactics, we next evaluated whether these behavioural types differed in the timing and structure of vertical movements.

2.7 | Diel patterns in foray timing

To test whether vertical forays were temporally structured, particularly with respect to diel cycles, we modelled the likelihood of foray initiation throughout the 24-h period. This analysis was motivated by previous studies suggesting crepuscular peaks in brook charr foraging activity (Bourke et al., 1996). We hypothesized that forays into the epilimnion, potentially exposing fish to warmer temperatures, would be concentrated around dusk to optimize feeding benefits due to access to emerging insects.

We used hierarchical generalized additive models (HGAMs) with cyclic cubic regression splines to model diel variation in foray probability and with a random effect for individual fish. Following Iannarilli et al. (2024), we split each day into 1-h intervals and defined binary indicators ($\text{StartForay}_{i,t,j}$) for whether fish i initiated at least one foray into the target thermal layer during time interval t on day j. This was done separately for forays into the epilimnion and hypolimnion. To reduce model complexity, we aggregated these indicators across all days per fish and time interval, modelling the counts as binomial outcomes (successes vs. failures).

Covariates included sunrise and sunset times, calculated using the *bioRad* R package (Dokter et al., 2019) and lunar illumination, derived from the *lunar* R package (Lazaridis, 2022). Each model featured the following: (1) a fish-specific cyclic smoother for time of day, allowing for individualized diel activity curves, (2) a global cyclic smoother for time of day, which acted as a prior to constrain individual-specific smoothers and capture the overall diel trend across fish and (3) a random effect for individual fish to account for repeated measures. Models were fitted using the *bam* function from the *mgcv* package (Wood, 2017), and predictions with 95% confidence intervals were obtained using *predict.bam*.

We first constructed global models over the full study period for forays into the epilimnion and hypolimnion separately. To assess how diel patterns shifted with seasonal thermal regimes, we then fit monthly models from June through October. These models excluded covariates to focus solely on diel variation, minimizing overfitting and enabling cleaner comparisons of temporal activity shifts across months.

We also analysed hourly variation in time allocation across thermal layers per month. For each hour, we calculated the proportion of time each fish spent in a given layer ($\text{TimeForay}_{i,t,j}$), defined as minutes in the layer divided by 60. These values were modelled using Gaussian HGAMs with a similar smoothing structure (with a random effect for individual fish). Together, these analyses allowed us to evaluate whether diel behaviour aligned with predictions of thermally constrained foraging.

2.8 | Morphological differentiation of thermal ecotypes

To investigate whether thermal ecotypes (classified as 'warm' or 'cool' tactics based on PCA-derived depth-use patterns; see *Classifying thermal behaviour and habitat use tactics* for more details) exhibited corresponding morphological divergence, we conducted a morphometric analysis using both geometric landmarks and measured traits. Standardized photographs were taken of each fish, and 25 landmarks were placed using tpsDig2 (Rohlf, 2012a; Figure S2). Following established procedures (Arbour et al., 2011; Dermond et al., 2019), curvature correction was applied using four lateral line landmarks. Curvature was minimal and showed no systematic differences between ecotypes. Landmark files were generated with tpsUtil (Rohlf, 2012b).

To evaluate morphological differentiation, we performed a linear discriminant analysis (LDA, using the *MASS* R package (Venables & Ripley, 2002)) and used a forward selection process to identify the best model (i.e. highest overall classification accuracy). We retained 11 traits: body height, dorsal fin base length, eye width, head height, head length, mouth width, pectoral fin length, pelvic fin height, peduncle height, snout-to-eye distance and lower jaw length. The LDA grouping factor was a composite of thermal tactic and sex, allowing us to assess interactions between thermal habitat use and sexual dimorphism. Assumptions of homogeneity of within-group covariances were verified with permutation tests. Classification accuracy was assessed via leave-one-out cross-validation. Finally, we computed canonical correlation coefficients to assess the contribution of each trait to group separation, following Zuur et al. (2009). This helped identify which morphological traits best explained ecotype divergence and informed potential links between form and ecological function.

3 | RESULTS

3.1 | Thermal habitat use and vertical forays

As surface temperatures increased over the summer months, brook charr showed shifts in vertical foraying behaviour across thermal layers (Figure 1). Consistent with expectations, individuals reduced their use of the epilimnion under warmer surface conditions. Specifically, the number of epilimnetic daily forays ($Z = -26.35$, $p < 0.001$), the total daily duration spent in the epilimnion ($F_{1,3037} = 1286$, $R^2 = 0.29$, $p < 0.001$) and the maximum daily foray duration ($F_{1,3037} = 378.7$, $R^2 = 0.11$, $p < 0.001$) into this layer all declined significantly with increasing epilimnion temperature (Figure 1a). In 2017, the average number of daily forays dropped from over 50 in June to 21 in August; the decline was even steeper in 2018, with a drop from 67 in June to just 10 in August. Similarly, total time spent in the epilimnion decreased from more than 2 h and 30 min in June 2017 to under 1 h and 30 min in August. In 2018, this decline was even more pronounced, from over 3 h and 30 min in June to less than 1 h in August. In contrast, the mean duration of individual forays showed a weaker relationship with temperature ($F_{1,3037} = 112.9$, $R^2 = 0.03$, $p < 0.001$), varying by less than 2 min across months within a year and by approximately 3 min across years.

In the hypolimnion, the number of forays ($Z = 24.44$, $p < 0.001$) and total time spent ($F_{1,2143} = 28.67$, $R^2 = 0.01$, $p < 0.001$) increased as epilimnion temperature rose (Figure 1b). In 2017, the number of hypolimnetic forays increased from 28 in June to 46 in August, and total time increased from just under 2 h in June to over 3 h and 15 min in July. In 2018, forays rose from 11 in June to 46 in August, with total time increasing from just over 1 h to around 2 h. However, both maximum daily foray duration ($F_{1,2143} = 5.621$, $R^2 = 0.002$, $p = 0.017$) and mean foray duration ($F_{1,2143} = 0.6635$, $p = 0.41$) showed weak or no clear trends with temperature, varying by about 6 min across months and by less than 1 min on average. These patterns were consistent when using both the default and conservative definitions of thermal layer boundaries (Figure S3). Oxygen availability in the hypolimnion (mean = 4.9 mg/L, SD = 1.4) was not significantly related to the number ($F_{1,1026} = 0.4172$, $p = 0.5185$) or duration ($F_{1,644} = 4.895$, $p = 0.02$) of forays. For example, the number of hypolimnetic forays remained around 35 in both July and September, despite oxygen concentrations being highest in July and lowest in September. Similarly, total daily foray time declined from 23 min in July to 15 min in September (Figure S4).

Use of habitat categories (benthic vs. pelagic) showed some seasonal structure, though not a consistent relationship with epilimnion temperature across years (Figure 2). In early summer 2018, for instance, individuals transitioned from using epilimnetic benthic habitats to greater use of benthic areas in the metalimnion, which remained the most frequently used habitat across the season. In contrast, increased use of epilimnetic benthic zones in fall 2017 may reflect reproductive activity rather than foraging, given the known timing of brook charr spawning. This interpretation is supported by patterns in Figure 1, where forays into the epilimnion become longer during the same period—suggesting that reproductive forays may leave a distinct behavioural signature compared to shorter, more frequent foraging events. Across all foray metrics, we observed substantial inter-individual variability, with wide spreads in both the median and upper quantiles (Figure 1; Figures S3–S5). This suggests that, although there were consistent population-level responses to temperature, individuals differed markedly in the extent and timing of their foray behaviour, as well as in their use of habitat categories.

3.2 | Classifying thermal behaviour and habitat use tactics

The first two PCA axes captured the major variations (i.e. 51.6%) of individual behaviour (Figure 3). PC1, which explained the most variance (30.6%), represented a gradient in vertical thermal habitat use: individuals with negative scores spent more time in warmer surface waters, made prolonged forays into the epilimnion and exhibited higher swimming speeds, while those with positive scores used cooler, deeper waters and the hypolimnion more extensively. PC2 captured variation in habitat use at intermediate depths, with individuals differing in benthic versus pelagic use within the metalimnion. No clear separation in benthic/pelagic use emerged within the epilimnion or hypolimnion (Figure S6).

These patterns remained consistent when the analysis was restricted to the warmer summer months (July–August; Figure S7). We used PC1 to assign individuals to two behavioural groups: a ‘warm’ tactic (negative PC1 scores), characterized by warmer thermal exposure (mean fish temperature: 12.8 ± 1.5°C), more epilimnetic forays and higher swimming speeds; and a ‘cool’ tactic (positive PC1 scores), characterized by cooler ambient temperatures (mean: 10.2 ± 1.7°C), greater hypolimnetic use and a greater average distance from shore.

3.3 | Diel patterns in foray timing

In the general model spanning the full study period (Figure 4), epilimnetic use peaked at dusk, as predicted. ‘Warm’ individuals were nearly twice as likely to access the epilimnion compared to ‘cool’ individuals, and the probability of epilimnetic forays increased with lunar illumination, suggesting a role for visual foraging. In contrast, hypolimnetic use showed no response to lunar illumination intensity. Unexpectedly, activity in the hypolimnion clearly peaked at both dawn and dusk, with ‘cool’ individuals exhibiting higher overall probabilities of use than ‘warm’ individuals. Notably, ‘warm’ fish were more likely to access the hypolimnion at dawn than at dusk.

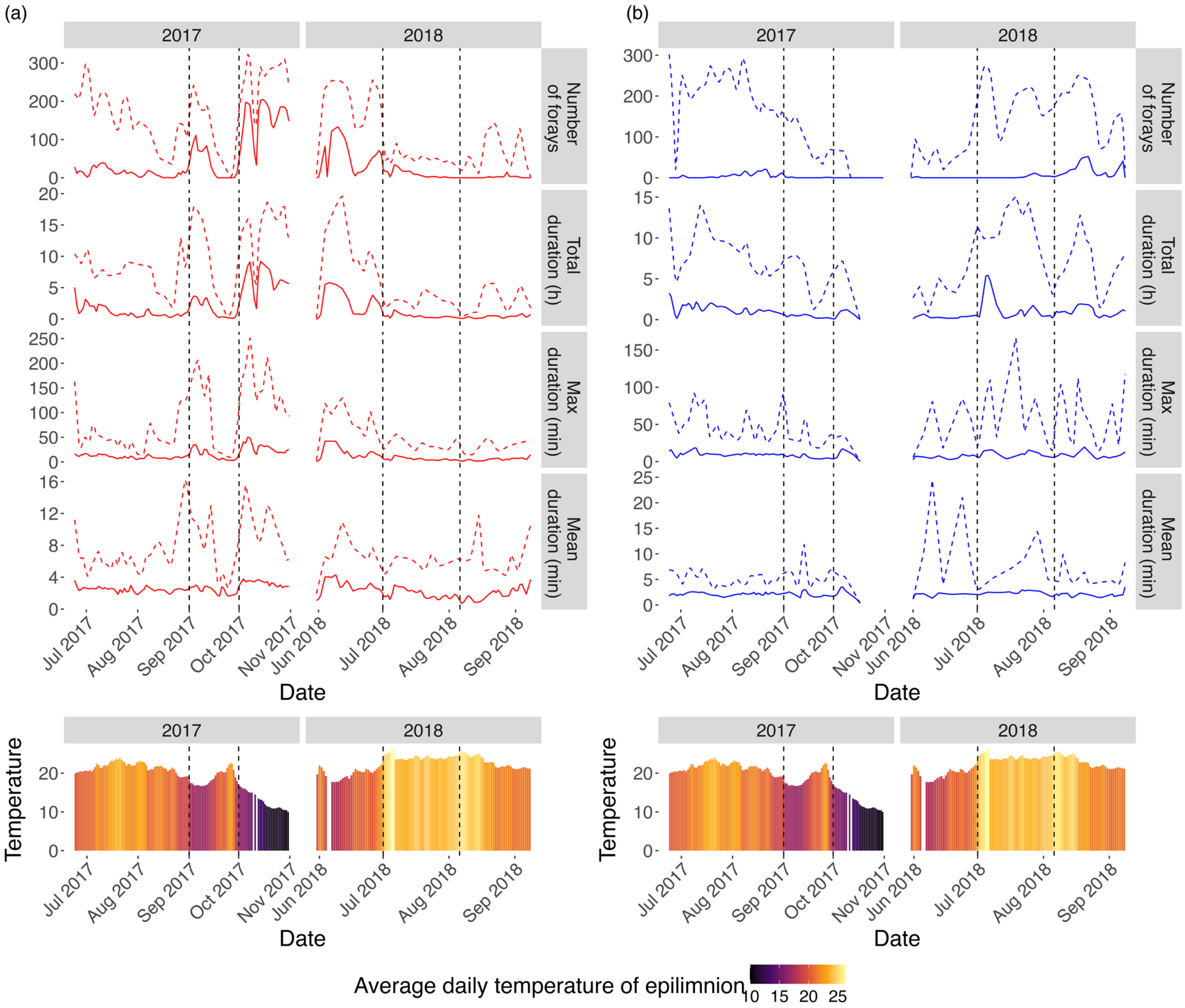

FIGURE 1 Metrics describing brook charr vertical habitat use as a function of day of year and average daily epilimnion temperature. Panels show summary statistics for forays into the (a) epilimnion and (b) hypolimnion, including the following: number of forays per day (defined as a transition into the target layer following a detection in a different layer), total daily duration of forays (in hours), maximum foray duration (in minutes) and mean foray duration (in minutes). Data are shown separately for the summers of 2017 and 2018. Solid lines represent the 50th percentile (median), and dotted lines the 95th percentile for each metric. Line colour corresponds to the thermal layer: red for epilimnion and blue for hypolimnion. Finally, the replicated panel at the bottom shows the average daily temperature of the epilimnion. The dotted vertical lines represent dates of interest, where temperature either decreases or increases, leading to change in the metrics. See Appendix for (i) daily depth estimates of thermal layers (Figure S1); (ii) an alternative version using a more conservative threshold of ≥1 m from the metalimnion boundary (Figure S3); (iii) a version of panel (a) for hypolimnion forays in 2018 coloured by average daily dissolved oxygen (Figure S4); and (iv) per-fish histograms of epilimnion foray durations (Figure S5).

Individual variation in diel foray timing and intensity was substantial (Figure S8), with patterns varying among behavioural tactics. 'Cool' individuals tended to concentrate their forays into deeper layers and exhibited broad diel distributions, while 'warm' individuals showed more consistent crepuscular activity. This suggests that diel behaviour is not uniform across the population but reflects underlying differences in thermal tactics or ecotypes.

Although monthly models revealed some seasonal plasticity, individual-level patterns were more striking. During the warmest months (July–August), epilimnetic forays occurred primarily around dusk, likely reflecting convergence of favourable conditions for visual foraging—sufficient light to locate prey but cooler temperatures that reduce thermal stress—rather than strictly a thermoregulatory response (Figures S9). In cooler months, when surface temperatures declined seasonally and oxygen remained sufficient, these temporal constraints relaxed. Fish accessed surface waters more continuously throughout the day, possibly reflecting reduced thermal stress or engagement in non-foraging behaviour such as reproductive activity. However, it is also plausible that dusk peaks in warmer months primarily reflect

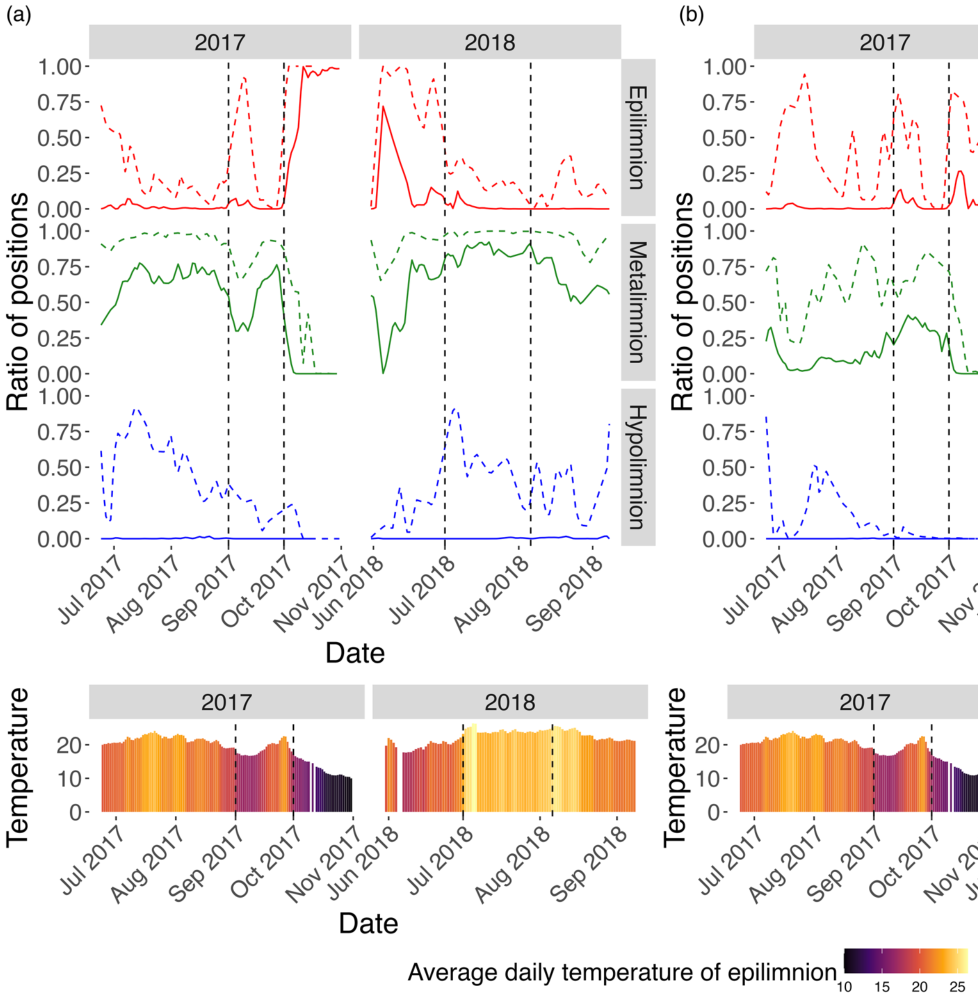

FIGURE 2 Proportion of brook charr detections in (a) benthic and (b) pelagic zones within each thermal layer (epilimnion, metalimnion, hypolimnion), across day of year and average daily epilimnion temperature. The benthic zone is defined as detections occurring within 2 m of the lake bottom. Summers 2017 and 2018 are shown in separate facets. Solid lines show the 50th percentile (median), and dotted lines the 95th percentile for each metric. Line colours indicate thermal layer: red (epilimnion), green (metalimnion) and blue (hypolimnion). Finally, the replicated panel at the bottom shows the average daily temperature of the epilimnion. The dotted vertical lines represent dates of interest, where temperature either decreases or increases, leading to change in the metrics. See Appendix for daily depth estimates of thermal layers, Figure S1.

increased prey availability, such as insect emergence, rather than temperature-driven constraints alone.

3.4 | Morphological differentiation of thermal ecotypes

The assumption of multivariate homogeneity of within-group covariance was met (permutation test; $n=999$, $F_{3,37}=1.838$, $p=0.16$), supporting the validity of the LDA. Wilks' λ statistic indicated a significant overall group effect ($\lambda=0.07$, $p<0.001$), and the first two linear discriminant (LD) axes accounted for over 90% of the total morphological variation (Figure 5a).

LD1 explained 58.8% of the variation and primarily discriminated individuals by sex (Figure 5a). This axis was strongly and positively associated with traits related to overall body and head morphology, including body height (BH), dorsal fin base (DB), pectoral fin length (PC), eye width (EW), head length (HL), head height (HH) and snout-to-eye distance (SE; Figure 5b). LD2 accounted for 33.6% of the variation and partially discriminated individuals by thermal tactics. It was most strongly associated with eye width (EW), pelvic fin length (PH), jaw length (SJ), peduncle height (PS), body height (BH) and mouth width (MW).

Males following the 'warm' tactic were morphologically distinct from 'cool' ones, with no overlap along LD2 (Figure 5a; Table 1). In contrast, females showed substantial overlap between tactics and overlap also occurred between males and females of the same tactic. Overall, 'warm' tactic individuals tended to have larger mouths, longer pelvic fins and larger eyes, while 'cool' tactic individuals had smaller mouths and pelvic fins, and lower body height (Figure 6).

Cross-validation using a jackknife (leave-one-out) procedure yielded an overall classification accuracy of 70%, compared to 25%

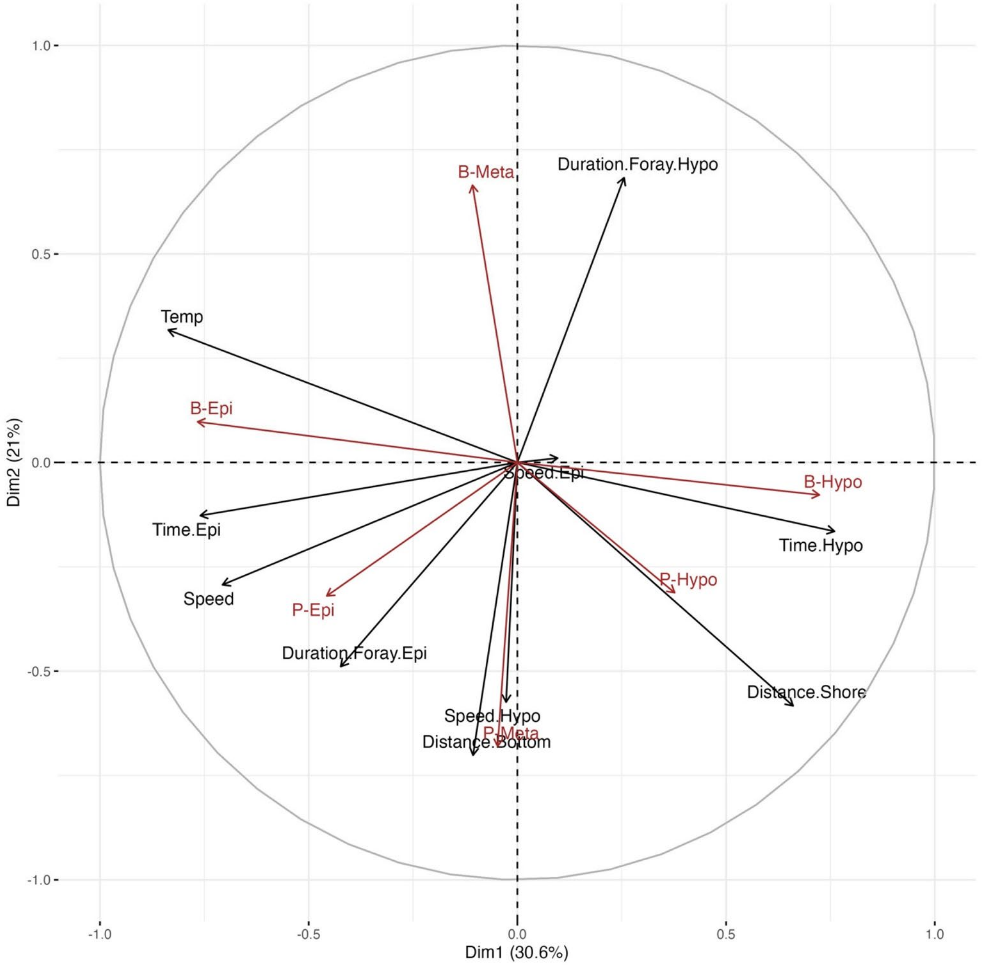

FIGURE 3 Variable contributions to the first two axes of a principal component analysis (PCA) describing brook charr vertical behaviour and habitat use. The PCA was based on standardized, per-fish values for: mean body temperature (°C; Temp), proportion of time spent in the epilimnion and hypolimnion (0–1; Time.Epi and Time.Hypo), average swimming speed across all layers (TL/s; Speed), average speed in the epilimnion and hypolimnion (Speed.Epi, Speed.Hypo), mean distance from the lake bottom (m; Distance.Bottom), mean distance to shore (m; Distance.Shore) and mean foray durations into the epilimnion and hypolimnion (minutes; Duration.Foray.Epi, Duration.Foray.Hypo). Supplementary variables (not used in the PCA) represent the proportion of detections in combinations of habitat type and thermal layer (dark red). Habitat is classified as benthic (within 2 m of the lake bottom; B) or pelagic (P), combined with depth zone: Epi = epilimnion, Meta = metalimnion, Hypo = hypolimnion (e.g. B-Epi = benthic epilimnion, P-Hypo = pelagic hypolimnion). These values sum to 1 for each fish. See Appendix for a version of this figure restricted to July–August to assess seasonal variation in PCA structure (Figure S7).

expected by chance (Table 1). Classification accuracy varied across groups, ranging from 62.5% in cold-tactic males to 77.7% in warm-tactic males. Misclassifications were more frequent in females, particularly in distinguishing between warm and cold tactics, while males were more likely to be misclassified as females of the same tactic.

4 | DISCUSSION

We investigated how brook charr navigate fine-scale trade-offs among temperature and foraging opportunities in a thermally stratified lake. We hypothesized that individuals would exhibit distinct vertical movement patterns shaped by asymmetrical thermal constraints (e.g. Pépino et al., 2015)—with surface warming more strongly limiting access to the epilimnion than low oxygen constrains use of the hypolimnion, consistent with Brett's heat-invariant hypothesis—as well as by spatial or temporal variation in prey availability (Leroux et al., 2023, 2025). We also predicted that these behaviours would be associated with morphological traits. Using high-resolution telemetry, we identified two consistent thermal behavioural tactics (i.e. 'warm' and 'cool') characterized by contrasting habitat use, movement timing and morphology. These findings are consistent with our initial hypothesis that thermal constraints are asymmetrical along the thermal gradient. Notably, they also reveal persistent and substantial use of the hypolimnion throughout the stratified season, a pattern rarely documented in brook charr. However, across both tactics, benthic zones—particularly in the metalimnion—remained the most frequently used habitat type (Figure S6), highlighting the central role of benthic prey resources in structuring behaviour. This novel result suggests that cold, deep habitats may

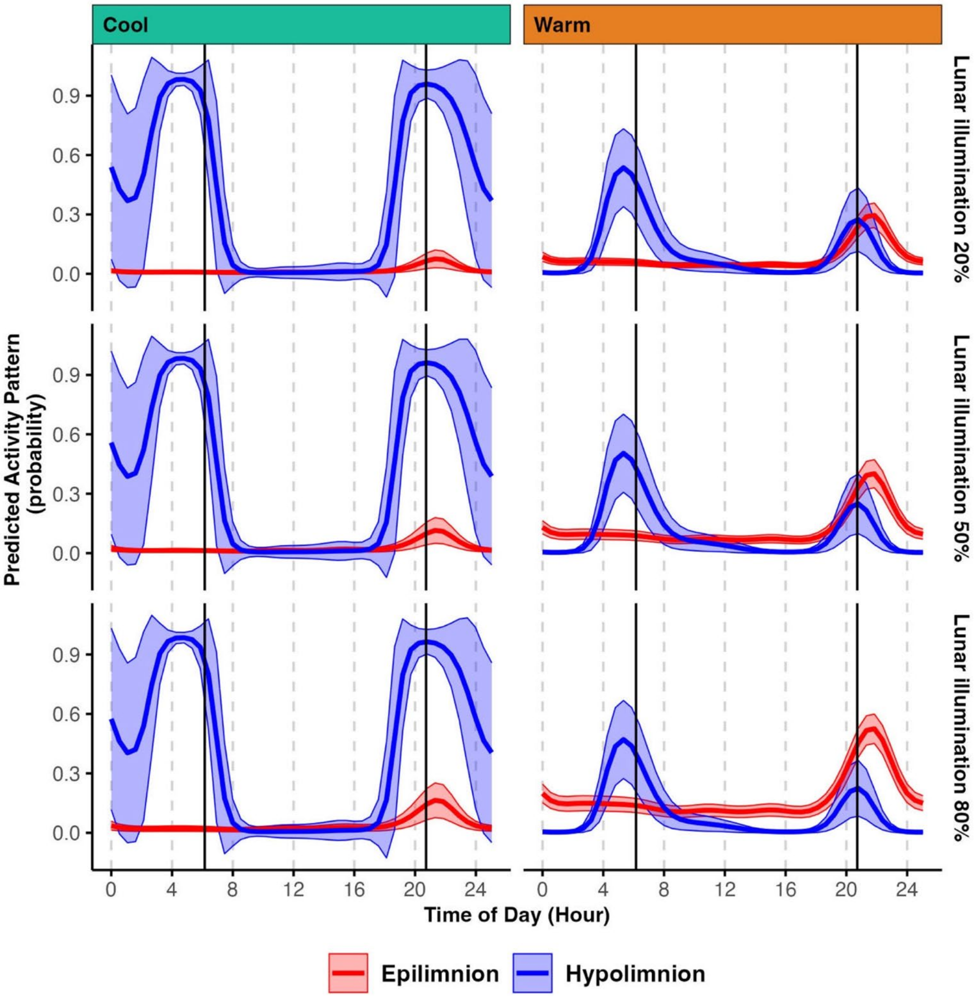

FIGURE 4 Predicted diel vertical movement patterns of brook charr grouped into 'warm' and 'cool' behavioural categories based on their position along the first principal component axis (PC1) of the PCA on thermal behaviour. Panels show the predicted probability (range: 0–1) of transitioning into the epilimnion (red) or hypolimnion (blue) over a 24-h cycle, estimated from separate hierarchical generalized additive models (HGAMs) fitted to each behavioural group. Black vertical lines indicate sunrise and sunset times, which were included as covariates in the models; lunar illumination (percentage of moonlight) was also included. Predictions are shown for an 'average' day, defined by median sunrise and sunset times across the study period. See Appendix for the following: (i) predictions from an HGAM fitted to a representative individual (Figure S8); and (ii) monthly models fitted separately for each behavioural category (Figure S9).

support overlooked foraging opportunities and could play a broader ecological role in the thermal behaviour of cold water species facing lake warming. Such fine-scale ecological specialization within a single population underscores how intraspecific variation facilitates niche partitioning and coexistence in heterogeneous freshwater environments, and may enhance population resilience under environmental change.

4.1 | Thermal habitat use and vertical forays

Brook charr adjusted their vertical movements in response to seasonal and diel changes in thermal structure. During warmer months (July–August), the use of the epilimnion was largely confined to crepuscular periods, particularly dusk. This pattern is likely driven not by short-term thermal fluctuations within the epilimnion but by prey availability, as dusk corresponds to peak activity of benthic invertebrates such as chironomid pupae (Bourke et al., 1996; Grossman et al., 1980) and gammarids (Grossman et al., 1980), as well as zooplankton vertical migration in this system (Naud & Magnan, 1988). As surface waters cooled in fall, diel epilimnetic use broadened and hypolimnion occupancy increased, highlighting the importance of stable thermal refuges at depth. It is important to note that these shifts may also reflect reproductive activity, particularly in October (Bourke et al., 1997).

These patterns are consistent with studies showing that salmonids often trade off thermal exposure and foraging opportunity under heterogeneous thermal environments (Armstrong et al., 2016; Brewitt & Danner, 2014; Pépino et al., 2024; Rainville et al., 2022). They also echo findings from acoustic telemetry studies in other cold-water species such as lake trout, where vertical movements reflect fine-scale behavioural thermoregulation in response to both prey and habitat constraints (Guzzo et al., 2017).

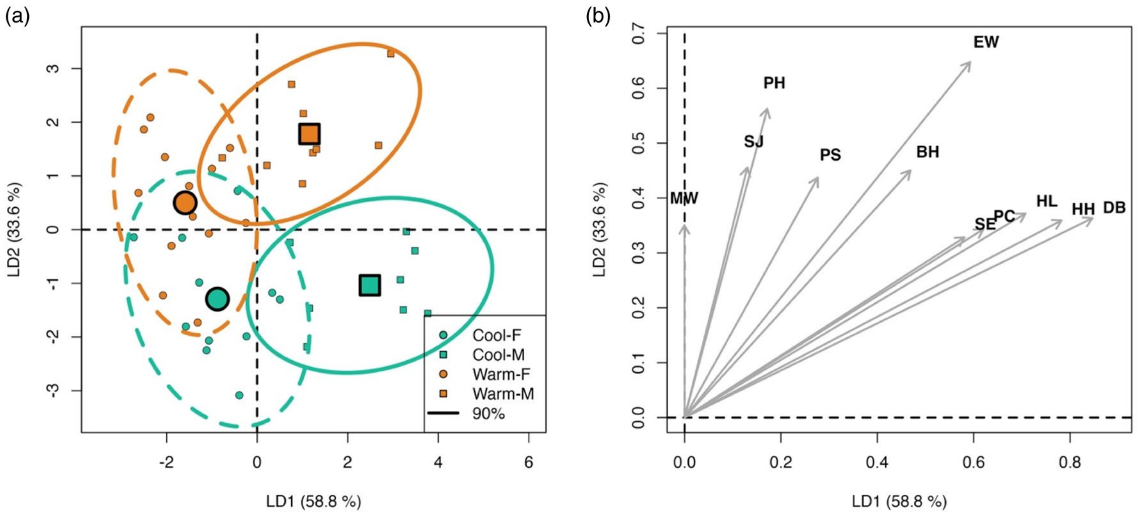

FIGURE 5 Morphological differentiation in brook charr on linear discriminant analysis (LDA). (a) Individual discriminant scores (small symbols) and group centroids (large symbols) on the first two LDA axes. Fish are grouped by Classification × Sex, where classification refers to thermal type based on PCA results. Orange = 'warm' fish; teal = 'cool' fish; circles = females; squares = males. Ellipses encompass 90% of individuals per group. (b) Canonical correlation coefficients showing the contribution of each morphological trait to the LDA axes. Traits include the following: BH, body height; DB, dorsal fin base length; EW, eye width; HH, head height; HL, head length; MW, mouth width; PC, pectoral fin length; PH, pelvic fin height; PS, peduncle height; SN, snout-to-eye distance; SJ, lower jaw length (see Figure S2 for a schematic of trait locations).

TABLE 1 Jackknife-based classification accuracy (%) of brook charr assigned to four groups based on thermal tactic ('cold' or 'warm') and sex (female or male), using linear discriminant analysis of morphological traits.

	Predicted				
Observed	Cool-F	Cool-M	Warm-F	Warm-M	Proportion (%)
Cool-F	8	0	2	1	72.7
Cool-M	3	5	0	0	62.5
Warm-F	3	0	9	1	69.2
Warm-M	0	0	2	7	77.7

Note: Classification accuracy is reported for each group separately (Cold-F, Cold-M, Warm-F, Warm-M) as well as the overall classification rate. Expected accuracy from random classification is 25%.

Hypolimnion use remained stable across oxygen conditions, with values occasionally falling below 5 mg/L, a commonly cited threshold for salmonid performance (Magnuson et al., 1979). This suggests that either oxygen levels remained within tolerable bounds or that prey availability at depth outweighed potential physiological costs—a possibility supported by studies showing that fish may tolerate mild hypoxia to exploit profitable resources (Rahel & Nutzman, 1994; Railsback & Harvey, 2002). Overall, these results highlight brook charr's dynamic balancing of thermal and trophic constraints, as reflected in the fine-scale timing and depth of forays.

4.2 | Classifying thermal behaviour and habitat use tactics

Multivariate classification of movement and thermal exposure revealed two major behavioural tactics: 'warm' individuals, which made more frequent and sustained excursions into warm surface waters, and 'cool' individuals, which remained predominantly in cooler depths and used the hypolimnion more consistently. These tactics were evident both across the full summer period and when analyses were restricted to the warmest months (July–August), suggesting stable behavioural specialization rather than transient flexibility. Importantly, while reproductive activity likely influenced behaviour in the fall—particularly increased use of epilimnetic benthic habitats—this did not alter the broader classification of individuals into warm and cool thermal tactics, which remained consistent across the stratified period.

This behavioural classification aligns well with previous work in Lake Ledoux, which identified a gradient of foraging tactics based on diel activity and space use (Bertolo et al., 2011; Goyer et al., 2014) and where individuals were similarly categorized into 'warm' and 'cool' types. Our findings thus reinforce and extend this classification by linking it directly to thermal habitat use and individual morphology. Although we did not assign individuals to

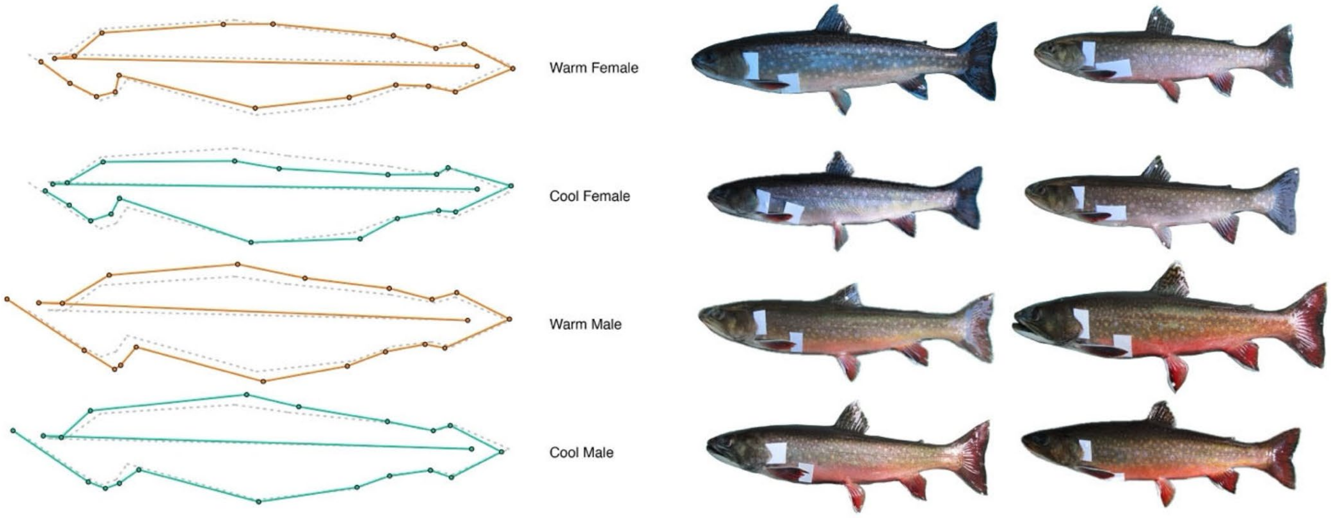

FIGURE 6 Mean body shapes of brook charr grouped by thermal tactic and sex. Average body shapes are shown for each classification group defined by thermal tactic (warm = orange, cool = teal) and sex. Solid coloured lines represent the mean shape of each group, while the dashed grey line indicates the grand mean shape across all individuals. Shape differences have been exaggerated by a factor of 5 to enhance visual interpretability. Representative photographs of individual fish are included alongside the outlines to illustrate morphological differences more intuitively.

morphotypes based on capture zones (e.g. littoral vs. pelagic), the thermal tactics we observed map onto similar ecological gradients described in other polymorphic salmonids (Chavarie et al., 2013; Zimmerman et al., 2007). While two dominant tactics emerged, we also observed considerable individual variation in metalimnion use and foray behaviour. This continuum of thermal responses supports the idea that behavioural variation in this system is not strictly categorical but instead spans a gradient of individual specialization.

The 'cool' individuals' use of deeper, pelagic-associated zones suggests a foraging tactic targeting prey in or near the hypolimnion. Stable isotope evidence supports this interpretation, showing that brook charr feeding in deeper waters of similar systems have distinct dietary signatures (Glaz et al., 2012) These findings point to niche partitioning along thermal, spatial and dietary axes that appear continuous rather than discrete. This also aligns with observations by Rainville, Pépino, and Magnan (2021), who found that while some individuals specialize in either littoral or pelagic resources, many exploit both across longer time frames—highlighting dietary flexibility and opportunism. Such plasticity may be driven by temporal fluctuations in resource availability, such as seasonal mayfly emergence or the late-summer decline in benthic invertebrates, and suggests that individual specialization may not map cleanly onto morphotypes (Amat-Trigo et al., 2023; Power et al., 2005).

We also observed a gradient in the use of the metalimnion, with some individuals favouring benthic habitats and others using pelagic areas. This pattern could explain previously observed muscle carotenoid signals (Rainville, Filion, et al., 2021) in presumed 'cool' individuals, which suggests a non-negligible contribution of zooplankton to their diet. This horizontal structuring indicates that brook charr may partition the intermediate depth zone not only vertically but also horizontally, expanding the range of accessible resources and potentially reducing intraspecific competition. Benthic areas in the metalimnion likely support prey such as insect larvae or emerging aquatic insects, while pelagic zones may provide access to zooplankton and midwater invertebrates (Eloranta et al., 2011; Knudsen et al., 2006). Similar fine-scale horizontal partitioning has been documented in polymorphic lake trout and Arctic charr, where individual foraging tactics are associated with differential use of benthic versus pelagic microhabitats (Chavarie et al., 2016; Knudsen et al., 2010). This multi-dimensional niche use—across depth, temperature and horizontal structure—highlights the potential for fine-scale individual specialization throughout the water column.

While our primary focus was on thermal and vertical habitat use, spatial variation was implicitly captured through depth-layer usage as well as fish positions relative to shoreline and lake bottom. Qualitative patterns in space use suggest that spatial habitat partitioning may reinforce thermal niche differentiation. For example, 'cool' individuals tended to avoid the shallowest areas of the lake, especially during the warmer months (June to September), whereas 'warm' individuals used these areas more frequently. Besides this seasonal pattern, horizontal distributions appeared broadly similar across tactics. To illustrate these trends, we include two supplementary figures (Figures S10 and S11) showing the spatial distribution of detections by month, layer, and thermal tactic. These maps reveal subtle spatial segregation but also considerable overlap—patterns that may be shaped by underlying individual variation (i.e. such as body size), as maps were not stratified at the individual level. A more detailed spatial analysis incorporating bathymetry, habitat structure and individual movement trajectories was beyond the scope of this study, but represents a valuable direction for future work.

4.3 | Diel patterns in foray timing

Both 'warm' and 'cool' individuals increased use of the hypolimnion during crepuscular periods, at both dawn and dusk. These timing patterns suggest targeted foraging behaviour, though not necessarily linked to pelagic prey. Unlike epilimnetic forays, hypolimnetic excursions did not vary with lunar phase and did not coincide with known vertical movements of zooplankton in Lake Ledoux, which are primarily concentrated in the upper layers (Leroux et al., 2023, 2025). Instead, the timing and depth of these forays suggest a focus on benthic prey targets. Chironomid larvae, which ascend towards emergence, are likely candidates due to their predictable vertical movements at dawn and dusk and their accessibility near the sediment surface (Learner et al., 1990), and may confer a competitive advantage to fish capable of intercepting them early in this process. Another likely prey item is the large-bodied burrowing mayfly *Ephemera* spp., observed in abundance at the surface of Lake Ledoux during summer (P. Magnan and M. Pépino, pers. obs.), which emerge from the sediment during crepuscular periods and may be captured prior to surfacing (Bourke et al., 1996; Whelan, 1980). These prey are abundant in low light, low oxygen habitats and exhibit diel movement patterns that align with the timing of charr forays, though uncertainty remains about the precise prey targets and sensory cues involved. Whereas direct feeding observations would further clarify these associations, the more frequent hypolimnetic forays of 'cool' individuals suggest a degree of specialization for exploiting these resources. This supports the interpretation that hypolimnion use reflects not just thermal avoidance, but a foraging tactic that balances energetic gain against the potential costs associated with low oxygen availability (Eloranta et al., 2011; Railsback & Harvey, 2002). However, given that, observed oxygen levels in the hypolimnion occasionally fell below 5 mg/L, a commonly cited threshold for salmonid performance (e.g. Magnuson et al., 1979); this interpretation should be made cautiously. It is also important to note that oxygen was only measured at a single depth (8 m) and at a single location in the lake, limiting our ability to fully characterize oxygen conditions encountered by fish during these forays.

4.4 | Morphological differentiation of thermal ecotypes

The behavioural tactics we observed were associated with subtle but consistent differences in body shape. 'Cool' individuals were more streamlined, with narrower heads, lower body height and thinner caudal peduncles, while 'warm' individuals exhibited deeper bodies and broader heads. These differences align with ecomorphological expectations: fusiform shapes minimize drag and support sustained swimming in open water (Gatz, 1979; Webb, 1984), while deeper bodies and larger heads enhance manoeuvrability and burst performance, aiding benthic foraging in complex habitats (Malmquist et al., 1992; Walker, 1997). This pattern is broadly consistent with previous work on other salmonids (e.g. Chavarie et al., 2013).

Interestingly, in our system, 'warm' individuals, despite having body shapes more typically associated with manoeuvrability, exhibited higher swimming speeds than the more streamlined 'cool' individuals. This is unexpected, as more fusiform shapes are generally associated with enhanced sustained swimming (e.g. Peres-Neto & Magnan, 2004). One possibility is that observed swimming speed reflects motivation or behavioural state rather than morphological capacity. The higher speeds of 'warm' individuals may indicate a more active foraging mode, characterized by frequent pursuit bouts or stop-and-start movements typical of littoral or nearshore habitats. Conversely, the slower, more continuous movements of 'cool' individuals align with a cruising strategy suited to pelagic or deeper benthic zones, where prey are encountered more diffusely. This apparent mismatch underscores the need for integrative approaches that combine behavioural, morphological and physiological data to better understand performance in natural settings.

Our findings are consistent with those reported in other polymorphic salmonids such as lake trout and Arctic charr (e.g. Chavarie et al., 2013; Hawley et al., 2016), where morphological divergence corresponds to differences in depth use and foraging strategy. However, this may be one of the first studies to document comparable patterns in brook charr—a species not typically associated with sustained hypolimnion use. This highlights the ecological relevance of deep water habitats for cold water fishes more broadly, and supports the emerging view that such habitats may harbour overlooked axes of specialization.

Variation in head morphology may also affect feeding efficiency. Longer lower jaws of warm tactic individuals, for instance, can increase mouth gape and speed of closure, improving capture of mobile prey (Kotrschal, 1989; Norton, 1991; Wainwright & Richard, 1995). These traits also resemble secondary sexual characteristics common in male salmonids and may involve trade-offs between reproductive investment and feeding efficiency (Kazyak et al., 2013). Indeed, such trade-offs have been documented in other salmonids, where sexually selected traits can reduce swimming performance or increase energetic costs (Makiguchi et al., 2017). Although we did not assess reproductive status, sex was included in the morphological analysis and emerged as a dominant axis of variation, highlighting the potential for sex-linked traits to influence morphology–function relationships.

Eye size presented another intriguing pattern: individuals following the 'cool' tactic had smaller eyes, contrary to our expectation that larger eyes would enhance visual sensitivity in the dimly lit hypolimnion (e.g. Andersson et al., 2024). This result also contrasts with findings from Rainville, Pépino, and Magnan (2021), where pelagic ecotypes had larger eyes across multiple lakes. However, the relatively larger eyes of 'warm' individuals in Lake Ledoux may reflect their frequent use of the epilimnion, which is thought to involve visual cues, while foraging in a more complex habitat of the littoral zone (e.g. Lisney et al., 2020). Smaller eyes in 'cool' individuals may indicate reliance on non-visual senses (e.g. chemoreception; Keefe, 1992), or reflect trade-offs favouring hydrodynamic efficiency or metabolic savings (Schmitz & Wainwright, 2011). It is also possible that eye size is constrained by developmental or genetic factors, reflecting local adaptation to deeper, less visually demanding environments. Clarifying the functional and evolutionary drivers of these differences will require further investigation into sensory ecology and developmental plasticity.

Together, these results are consistent with findings in other polymorphic salmonids, where body shape is linked to habitat depth and diet (Chavarie et al., 2013; Rainville, Pépino, & Magnan, 2021; Robinson & Parsons, 2002). In systems like Lake Ledoux, where morphotype distinctions may be subtle or continuous, morphological variation may not fully predict tactic but still contributes to reinforcing behavioural divergence and ecological partitioning.

5 | CONCLUSIONS

Our results highlight intraspecific behavioural specialization in brook charr facing lake warming, characterized by consistent individual differences in habitat use, thermoregulation behaviour and morphology. This fine-scale specialization facilitates coexistence in a shared, heterogeneous environment and underscores the ecological importance of intraspecific diversity (Araújo et al., 2011; Bolnick et al., 2003). By integrating fine-resolution telemetry, environmental data and morphology, our study provides novel evidence for individual specialization in thermal and foraging niches within a single fish population. Such diversity may buffer populations against environmental change, but lake warming and deoxygenation could reduce the availability of critical habitats for the sustainability of cold water species. Understanding how individuals partition thermal and trophic resources is critical for predicting species' responses to climate change and conserving behavioural and morphological diversity in freshwater systems, especially as behavioural plasticity may offer one of the few rapid pathways for adaptation (Sinclair et al., 2016; Sunday et al., 2014).

AUTHOR CONTRIBUTIONS

Data acquisition: MP, AB and PM. *Conceptualization*: AS, MP, AB and PM. *Data curation*: AS and PM. *Formal analysis*: AS. Coding: AS and MP. *Methodology*: AS, MP, AB and PM. *Visualization*: AS. *Writing—original draft*: AS. *Writing—review and editing*: AS, MP, AB and PM. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare there are no competing interests.

DATA AVAILABILITY STATEMENT

Data and code available from the Borealis digital repository: https://doi.org/10.5683/SP3/MZ0DIM (Stahl et al., 2025).

STATEMENT ON INCLUSION

Our study brings together authors from a number of different countries, including scientists based in the country where the study was carried out. Whenever relevant, literature published by scientists from the region was cited; efforts were made to consider relevant work published in the local language.

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SUPPORTING INFORMATION

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

Figure S1: Seasonal variation in stratification metrics in Lake Ledoux: (A) Water temperature (°C) and (B) depth (m) of thermal layer boundaries as a function of day of the year. In panel (A), solid lines indicate the mean daily temperature within each thermal layer: red for the epilimnion, green for the metalimnion and blue for the hypolimnion. In panel (B), solid lines represent the lower boundary of the epilimnion (red), the upper boundary of the hypolimnion (blue) and the thermocline (green). Vertical dashed lines mark 31 October 2017 and 19 May 2018—cut-off dates used to define the analysed stratified period. Outside this window, thermal stratification was weak or inconsistent, and layer boundaries could not be reliably identified.

Figure S2: Schematic representation of morphological traits measured in brook charr. Blue dashed lines indicate the 11 linear traits recorded at the beginning of the study. Measurements include: BH, body height; DB, dorsal fin base length; EW, eye width; HH, head height; HL, head length; MW, mouth width (measured dorsally); PC, pectoral fin length; PH, pelvic fin height; PS, peduncle height; SN, snout-to-eye distance; and SJ, lower jaw length. Green dots denote curve points used to 'unbend' fish before conducting geometric morphometric analyses.

Figure S3: Metrics describing brook charr vertical habitat use as a function of day of year and average daily epilimnion temperature. Panels show summary statistics for forays into the (A) epilimnion and (B) hypolimnion, including number of forays per day (defined as a transition into the target layer following a detection in a different layer), total daily duration of forays (in hours), maximum foray duration (in minutes) and mean foray duration (in minutes). A fish is considered to be in the epilimnion or hypolimnion when located at least 1 meter from the adjacent metalimnion boundary. Solid lines represent the 50th percentile (median), and dotted lines the 95th percentile for each metric. Line colour corresponds to the thermal layer: red for epilimnion, blue for hypolimnion. Finally, the replicated panel at the bottom shows the average daily temperature of the epilimnion. The dotted vertical lines represent dates of interest, where temperature either decreases or increases, leading to change in the metrics. See Figure S1 for (i) daily depth estimates of thermal layers; (ii) a version of panel (A) for hypolimnion forays in 2018 coloured by average daily dissolved oxygen (Figure S4); and (iii) per-fish histograms of epilimnion foray durations (Figure S5).

Figure S4: Metrics describing brook charr use of the hypolimnion as a function of day of the year and average daily dissolved oxygen (mg/L) at 8 meters depth. The figure summarizes hypolimnion forays, including the following: (i) number of forays per day, defined as transitions into the hypolimnion following a detection in a different layer; (ii) total daily foray duration (in hours); (iii) maximum foray duration (in minutes); and (iv) mean foray duration (in minutes). Only days with available dissolved oxygen data are included. Finally, the panel at the bottom shows the average daily dissolved oxygen at 8 m depth (light blue = oxygenated, dark blue = hypoxic). See Figure S1 for (i) daily depth estimates of thermal layers; (ii) an alternative version using a more conservative threshold of ≥1 m from the metalimnion boundary (Figure S3); and (iii) per-fish histograms of epilimnion foray durations (Figure S5).

Figure S5: Individual-level histograms of epilimnion foray durations in brook charr. Histogram of foray durations into the epilimnion (in minutes) for each tagged individual. Each bar represents the number of forays of a given duration. Fish are ordered by their position along the first principal component (PC1) from the PCA of vertical habitat use. Facet title colours indicate thermal classification based on the PCA: teal for 'cool' individuals and orange for 'warm' individuals. Mean body temperature (°C) is shown below each fish ID for reference.

Figure S6: Proportional habitat and depth layer use per fish. Each facet represents an individual brook charr, showing the proportion of detections in different combinations of habitat (benthic = within 2 m of the lake bottom; pelagic = elsewhere) and thermal layer: epilimnion (red), metalimnion (green), and hypolimnion (blue). For each fish, the stacked bar totals 1, representing the full distribution of detections across habitat–layer categories. Fish are ordered based on their PC1 score from the PCA of vertical behaviour. Facet title colour indicates thermal classification from the PCA: 'cool' fish are labelled in teal and 'warm' fish in orange. Mean body temperature (°C) for each fish is displayed beneath the fish ID.

Figure S7: Variable contributions to the first two axes of a principal component analysis (PCA) describing brook charr vertical behaviour and habitat use, based on data from July and August only. The PCA was based on standardized, per-fish values for: mean body temperature (°C; Temp), proportion of time spent in the epilimnion and hypolimnion (0–1; Time.Epi and Time.Hypo), average swimming speed across all layers (TL/s; Speed), average speed in the epilimnion and hypolimnion (Speed.Epi, Speed. Hypo), mean distance from the lake bottom (m; Distance. Bottom), mean distance to shore (m; Distance. Shore), and mean foray durations into the epilimnion and hypolimnion (minutes; Duration.Foray.Epi, Duration.Foray. Hypo). Supplementary variables (not used in the PCA) represent the proportion of detections in combinations of habitat type and thermal layer. Habitat is classified as benthic (within 2 m of the lake bottom; B) or pelagic (P), combined with depth zone: Epi = epilimnion, Meta = metalimnion, Hypo = hypolimnion (e.g. B-Epi = benthic epilimnion, P-Hypo = pelagic hypolimnion). These values sum to 1 for each fish. This figure allows assessment of whether PCA structure is sensitive to seasonal environmental variation by focusing only on the peak summer months.

Figure S8: Individual-level diel vertical movement patterns. Predicted probability (range: 0–1) of transitioning into the epilimnion (red) or hypolimnion (blue) as a function of time of day (hour), based on HGAMs fitted separately for each fish and each layer. Models include sunrise and sunset times as fixed effects. Facet titles are coloured by thermal classification based on PCA results: 'cool' fish are labelled in teal, and 'warm' fish in orange. Fish are ordered based on their PC1 score from the PCA of vertical behaviour. Mean body temperature (°C) for each fish is displayed beneath the fish ID.

Figure S9: Monthly diel vertical movement patterns of brook charr by thermal behavioural category. Predicted diel probability of transitioning into the epilimnion (red) or hypolimnion (blue) for brook charr grouped into 'warm' and 'cool' categories based on their position along the first principal component axis (PC1) from the PCA of vertical thermal behaviour. Separate hierarchical generalized additive models (HGAMs) were fitted for each behavioural group within each month (June–October), allowing assessment of temporal variation in vertical movement patterns. Predictions are shown over a 24-hour cycle. Black vertical lines indicate sunrise and sunset times, which were included as covariates. An 'average' day is defined by the median sunrise and sunset times for each month.

Figure S10: Monthly spatial maps of depth-layer usage by brook charr thermal tactics. Each panel shows the geographic distribution of individual fish detections within the lake, faceted by month (June–October), thermal tactic ('warm' or 'cool') and vertical layer (epilimnion, metalimnion, hypolimnion). Points represent density of individual detections, allowing visualization of how habitat use varies spatially and temporally across tactics.

Figure S11: Spatial maps of depth-layer usage by brook charr thermal tactics. Each panel shows the geographic distribution of individual fish detections within the lake, faceted by thermal tactic ('warm' or 'cool') and vertical layer (epilimnion, metalimnion, hypolimnion). Points represent density of individual detections, allowing visualization of how habitat use varies spatially across tactics.

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