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Correlates of Post-Introduction Displacement in a Conservation Translocation of Threespine Stickleback

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ABSTRACT

1. Predicting the distance between an individual's release point into a new environment and their subsequent location ('displacement') could be useful during biological 'introduction' events (e.g., invasions/translocations) because variation in displacement could lead to spatially-structured ecological and evolutionary effects, as well as implications for efforts to control introduction outcomes (e.g., establishment success).
2. Two challenges limit our ability to predict post-introduction displacement. First, displacement is shaped by individual movement, and many factors affect movement. As such, considerable recent effort has focused on finding particularly influential factors, including bold and exploratory behaviour. Second, movement patterns often differ between environments. Predicting post-introduction displacement therefore benefits from assessments in the most relevant environment—that in which the introduction is occurring. Conservation translocations provide a rare opportunity to overcome both challenges by allowing assessment of how behaviour might be associated with post-introduction displacement in the new environment.
3. Our study leveraged a conservation translocation where threespine stickleback (*Gasterosteus aculeatus*) from seven source populations were introduced into one 'recipient' lake. Our study focused on comparisons of the genetics (source population ancestry) and behaviour (bold and exploratory) of individuals subsequently showing lower displacement (captured at the release site) versus greater displacement (captured farthest from the release site). Bold behaviour was assessed as latency to emerge from shelter, whereas exploratory behaviour was the number of unique sections crossed in an open field.
4. Fish from the different source populations showed different bold and exploratory behaviours; yet those population-level differences were not associated with post-introduction displacement. At the individual level in the recipient lake, however, fish showing greater displacement 1 month after release were less exploratory than those showing lower displacement. This association between capture location and exploratory behaviour was no longer evident a year later, implying that the original association was related to initial post-release displacement.
5. Our work highlights how exploratory behaviour might be associated with displacement following introduction into a new environment. More broadly, our study provides rare insights into the extent to which post-introduction displacement is predictable in a real-world setting, and it also underscores the value of conducting experimental manipulations in nature.

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1 | Introduction

Phenotypic variation within a species can have a diverse set of ecological effects that shape populations, communities, and ecosystems (Bolnick et al. 2011; Hendry 2017; Des Roches et al. 2018; Rota et al. Accepted). As a concrete example, Trinidadian guppies (*Poecilia reticulata*) adapted to high- versus low-predation environments have differing effects on community structure (e.g., chironomid biomass) and ecosystem function (e.g., leaf decomposition rates; Bassar et al., 2010). Similarly, threespine stickleback (*Gasterosteus aculeatus*) adapted to lake versus stream environments can have differing effects on factors ranging from prey abundance to dissolved phosphorus concentrations (Matthews et al. 2016). Phenotypic variation also shapes evolutionary trajectories because natural and sexual selection act directly on this variation, the extent and nature of which then can shape evolutionary responses (Hendry 2017; Arnold 2023). An important applied context for considering these effects of variation among individuals occurs in the case of biological 'introductions', such as invasions or conservation translocations. Given that introduced organisms can have dramatic effects on their environments (Simberloff 2011) and can undergo rapid evolutionary changes (Whitney and Gabler 2008), the nature and influence of phenotypic variation likely alters introduction outcomes (e.g., Forsman 2014; González-Suárez et al. 2015).

One important consideration in biological introductions is the spatial spread of organisms from their point of release or invasion (Hastings et al. 2005). This spread is unlikely to be phenotypically homogeneous through novel environments, thus leading to spatially-structured phenotypes (Shine et al. 2011) and, hence, spatially-structured ecological and evolutionary effects. Such spatial variation can be generated by several movement-associated processes, such as dispersal or habitat selection, which then result in variable patterns of net 'displacement' – that is, distances between an introduction point (e.g., release location) and subsequent locations. For example, cane toads (*Rhinella marina*) with longer limbs have a greater dispersal ability, leading to toads at invasion fronts (i.e., greater displacement) having longer limbs than toads behind the invasion fronts (i.e., lower displacement; Phillips et al. 2006). As another example, when threespine stickleback adapted to different environments (lake vs. stream) are displaced to intermediate locations, 90% return to their original habitat type (Bolnick et al. 2009). When phenotypic variation shapes initial displacement, an important question then becomes whether the initial pattern is maintained (as might be expected via habitat selection, local adaptation, or plasticity) or subsequently disappears (as might be expected if it was specifically associated with processes shaping the rate of spreading). In short, variation in displacement can have long-lasting implications for the ecology and evolution of introduced organisms.

In several applied contexts it can be valuable to *predict* patterns of displacement through an understanding of the phenotypic variation. For example, invasive gypsy moths (*Lymantria dispar*) occur as either a flying morph that can spread quickly throughout environments or a flightless morph that only spreads slowly (Robinet and Liebhold 2009). In simulated invasions, the flightless morph generates larger populations that are more likely to become established and that could be more challenging to eradicate (Robinet and Liebhold 2009). Predicting displacement could also benefit conservation translocations or habitat restorations by designing releases of phenotypes most likely to spread throughout the desirable area but not into undesirable areas. Indeed, "hyperdispersal"—where organisms move out of the translocation site and no longer contribute to population establishment—is a likely contributing factor in many translocation failures (and is likely underestimated and underreported; reviewed in: Bilby and Moseby 2024).

Predicting patterns of displacement, however, can be difficult because many factors could be important. As examples, male brook trout (*Salvelinus fontinalis*) are estimated to disperse over twice the distances of females (Hutchings and Gerber 2002), and mosquitofish (*Gambusia affinis*) that are less social disperse farther than mosquitofish that are more social (Cote et al. 2010). Further complicating prediction, movement often differs dramatically between introduced and resident organisms (e.g., Reinert and Rupert Jr 1999; Bauder et al. 2014; Doden et al. 2022). For example, over a two-year study with American beavers (*Castor canadensis*), translocated adults moved up to 19.69 river kilometres whereas residents moved only 0.86 km (Doden et al. 2022). In attempts to explain variation in displacement, behaviour has received special attention (Phillips and Suarez 2012). Two potentially relevant behaviours are bold (risk taking) and exploratory behaviour (movement in an unfamiliar environment). Bolder and more exploratory individuals might initially move farther when placed in new locations. For instance, in Hart's rivulus (*Aneblepsoides hartii*), bolder individuals moved farther when introduced into an artificial stream (Fraser et al. 2001). In bullheads (*Cottus perifretum*), exploratory behaviour was similarly predictive of distance moved following release into a stream (Kobler et al. 2009).

A potential practical route to predicting displacement comes from the realization that much of the phenotypic variation in introductions, whether intentional or not, can arise from the use of multiple source populations (e.g., Facon et al. 2008). For instance, many behaviours differ dramatically among populations (e.g., Michelangeli et al. 2019). In mosquitofish, for example, individuals from bolder or more asocial populations dispersed farther than individuals from shyer or more social populations—regardless of their individual behavioural phenotype (Cote et al. 2011). Individual and population-level traits—especially bold and exploratory behaviours—thus could be useful in understanding, and ultimately predicting, patterns of post-introduction displacement. Exploring this possibility requires studying how displacement patterns emerge within new environments—ideally the specific environments into which introductions are likely to occur. However, experimentally introducing organisms into nature is often impossible, whether for logistical, ethical, or legal reasons, and leveraging 'natural' introductions is often not feasible, given that most introductions are cryptic (reviewed in: Morais and Reichard 2018). Conservation translocations provide exceptional opportunities to circumvent many of these challenges and test predictions about post-introduction displacement in real-world settings.

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1.1 | Our Study

We investigated how source population identity and behaviour might predict patterns of post-introduction displacement in a conservation translocation of threespine stickleback (*Gasterosteus aculeatus*—henceforth just "stickleback"). Specifically, stickleback from seven natural source lake populations were introduced at a common release point in a recipient lake for the purposes of restoration (Hendry et al. 2024). Stickleback are highly relevant to the question at hand because they are often introduced into novel environments, both for conservation translocations in their native range (e.g., Bell et al. 2016; Swan et al. 2018; Hendry et al. 2024) and because they are invasive in many freshwater systems (reviewed in: Makhrov et al. 2024). Moreover, even within lakes, stickleback populations can show microgeographic spatial structuring (e.g., Maciejewski et al. 2020), suggesting the possibility that initial patterns of displacement could be associated with longer-term spatial patterns. Our study provides a rare opportunity to investigate how several factors might be associated with post-introduction displacement in a natural setting.

We first used controlled behavioural assays to measure bold and exploratory behaviours on individuals collected from the seven source populations. Bold and exploratory behaviours were assessed as latency to emerge from shelter and the number of unique sections crossed in an unfamiliar arena, respectively. Although bold and exploratory behaviours are often studied as repeatable "personalities", we here instead use single point-in-time measurements of behaviour, which presumably include a combination of consistent differences among individuals and individual-level plasticity (Réale and Montiglio 2021), both of which could shape movement patterns following translocation. We next collected individuals from the recipient lake 1 month following introduction—specifically capturing individuals near the point of release and at the far end of the lake. Capture location thus serves as a proxy for net displacement from the release point. These post-release individuals were genotyped at diagnostic markers to determine if source populations showed differences in displacement. Finally, we repeated the collections and behavioural assays 1 year later to determine whether any associations between behaviour and capture location persisted a year after the introduction. This second year likely no longer reflects variation in initial movements underlying displacement but could provide insight into longer term settlement patterns.

Data analyses were designed to answer three questions. Question 1: To what extent does the "source population" of origin (i.e., fish from one specific lake versus another lake) predict capture location? Here we quantify the extent to which source population, and the *average* behaviour of fish from each source population, can predict capture location (near vs. far) when individuals are introduced into a new environment. Question 2: To what extent is *individual* behaviour associated with capture location? Here we quantify the association between individual behaviour and capture location in the recipient lake. Question 3: Are initial patterns transitory—or do they persist through time? For this final question, we assess the extent to which behavioural patterns change from 1 month after release into the recipient lake to 1 year after the release.

2 | Methods

2.1 | Lake Descriptions and Stickleback Introductions

The translocation was conducted in G Lake (hereafter 'the recipient lake') on the Kenai Peninsula in Alaska (60°25′47.6″, 151°10′37.7″). The recipient lake has a surface area of 7 ha and a maximum depth of 9.4 m (Hendry et al. 2024). In 2018, the Alaska Department of Fish and Game treated the lake with the chemical rotenone to eradicate invasive Northern Pike (*Esox lucius*), thus rendering the lake subsequently fishless (Couture et al. 2022). As part of an effort to re-establish the native fish fauna, members of our team were given the opportunity to reintroduce stickleback to G Lake. A first attempt at reintroduction was made in June of 2019—but that introduction was unsuccessful, as confirmed by subsequent sampling over the next 3 years (Hendry et al. 2024).

Our team therefore reintroduced stickleback again into the recipient lake in May 2022 (Figure 1—point 2). The introduced fish originated from seven source populations in Alaska (Table 1; for a map see Figure 3 in Hendry et al. 2024). Three of the source populations (South Rolly, Spirit, and Wik lakes) contain stickleback that correspond to the "limnetic" ecotype and four of the source populations (Finger, Tern, Walby, and Watson lakes) contain stickleback that correspond to the benthic ecotype (Haines et al. 2023; Hendry et al. 2024). These ecotypes are not relevant to the present study because our goal here was to assess variation in behaviour and displacement regardless of their ecotype. Moreover, exploratory linear models confirmed that benthic and limnetic ecotypes did not differ in bold or exploratory behaviour, and so ecotype effects are unlikely to be present in the recipient lake (bold: $F_1 = 0.06$, SS = 3682, $p = 0.81$, $n = 58$; exploratory: $F_1 = 3.32$, SS = 51.95, $p = 0.07$, $n = 58$). In total, 3495 stickleback (495–500 from each of the seven source populations) were introduced into the recipient lake between May 19 and May 25, 2022. The releases took place over 6 days in a series of "waves," with each wave consisting of approximately 50 fish from each of the source populations. Additional details on the introductions and source populations can be found in Hendry et al. (2024).

2.2 | Behavioural Trials

Gee's (G40 − ¼″ mesh) minnow traps were used to collect stickleback from each of the source populations and from the recipient lake. For the source populations, traps were set in easy-to-access locations near the road or at public access points. Thirty fish were collected from all source populations, except Watson ($n = 28$), South Rolly ($n = 25$), and Wik ($n = 0$). Because we did not capture any stickleback over several hours of effort in Wik, that lake had to be excluded from some analyses corresponding to Question 1—specifically, analyses investigating the extent to which behaviour varies among the source populations, and how mean source population behaviour is associated with displacement in the recipient lake. In the recipient lake, minnow traps were set along the shoreline near to the introduction point ("near" traps; ~0–50 m from the introduction point) and on the opposite shoreline farthest from the introduction point ("far" traps; 250–300 m from the introduction point 'as the crow

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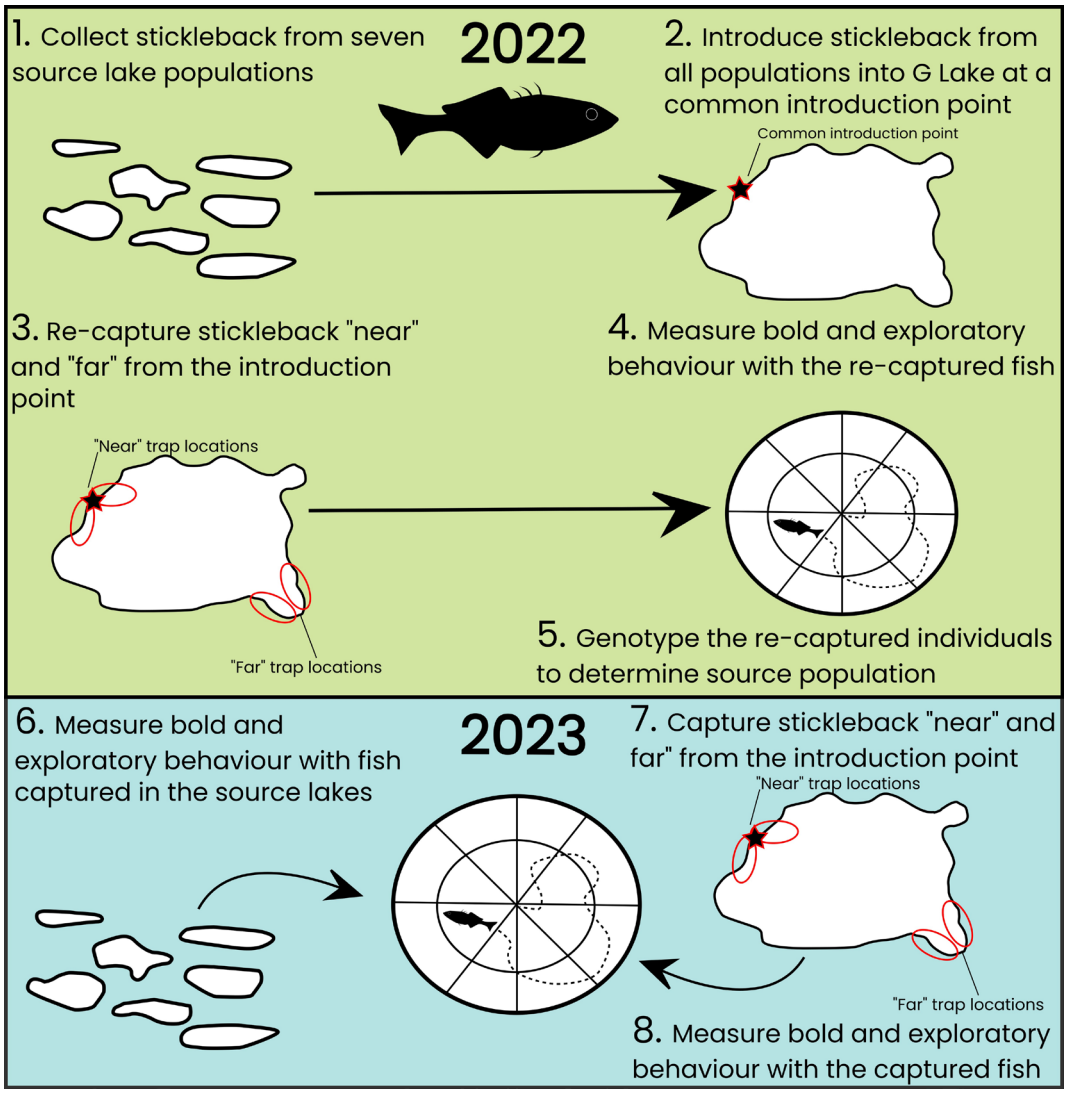

FIGURE 1 | Experimental timeline. The green panel shows work that was conducted in 2022, and the blue panel shows work conducted in 2023.

flies' or 435–500 m along the shoreline; Figure 2a). We specifically chose to trap at contrasting distances from the introduction point to try and maximize variation in traits that might contribute to variation in displacement. This decision was largely motivated by the fact that conducting experimental work in nature is challenging, and especially so for detecting fine-scale differences in noisy environments. We therefore chose to prioritize higher power (only two contrasts) vs. finer-scale resolution, and future studies could consider alternative approaches, such as trapping along outward gradients (e.g., Heckley et al. 2024).

Source population collections took place in 2023, which assumes that behavioural differences among those lakes in 2023 will reflect any differences in the previous year (2022) when fish were collected for the translocations. This assumption is reasonable because stickleback behaviour can be highly consistent over long time periods (King et al. 2013). Recipient lake collections took place in both 2022 and 2023 (Figure 1—points 3 and 7) to assess behavioural differences shortly after introduction (June 22–26, 2022; 31–37 days after the first fish were introduced) versus a full year after introduction (June 14–17, 2023). In 2022, intensive trapping in the recipient lake allowed us to collect 60 individuals, which might seem low but was to be expected given the low density of introduced fish: 3495 fish into a 7 ha lake followed by some expected mortality. In 2023, reproduction by the introduced fish had increased stickleback density, and we were able to easily capture 100 individuals.

Captured fish were held in trap-specific holding buckets equipped with air bubblers before being used in the trials, which were conducted at the side of the lake. The experimental arena was a 1.8 m–diameter plastic "kiddie pool" filled with about 200 L of lake water, similar to those used in other studies of stickleback behaviour (e.g., Laskowski and Bell 2014; Bensky et al. 2017). The bottom of the experimental arena was partitioned into sixteen sections based on eight equal wedges (like a pie) that were also divided mid-way between the edge of the arena and the centre (Figure 2b,c). A holding container made from a red plastic cup with a magnetic door was placed in the centre of the arena. To standardize conditions and also avoid direct sunlight and other external influences that might scare the fish or influence their movement, the arena was placed inside a gazebo that had its sides covered with blackout curtains (Figure 2d). Note that the gazebo itself allowed

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TABLE 1 | Information on the location and ecotype of the source populations, as well as the number of fish from each source population that were released into G lake.

Source population	Coordinates	Ecotype	Number released
Finger	61°36′34.3″ N, 149°15′52.2″ W	Benthic	500
South Rolly	61°40′00.3″ N, 150°08′12.7″ W	Limnetic	500
Spirit	60°36′01.1″ N, 151°00′45.2″ W	Limnetic	500
Tern	60°31′49.7″ N, 149°33′12.6″ W	Benthic	500
Wik	60°43′02.8″ N, 151°14′30.3″ W	Limnetic	495
Walby	61°04′23.3″ N, 149°46′18.1″ W	Benthic	500
Watson	60°32′09.1″ N, 150°27′42.7″ W	Benthic	500

diffuse light that approximately mimicked conditions an hour or so before dusk. After every trial, we removed one bucket of water from the kiddie pool and added one new bucket of lake water.

For each trial, we haphazardly selected one fish from the holding buckets. The fish was placed in the central holding container and left there for a three-minute acclimation period, after which a magnetic door was removed by hand, granting the fish access to the rest of the arena. We first recorded the amount of time, in seconds, that it took for the entire length of the fish to emerge from the holding container (latency to emerge, our measure of bold behaviour). If the fish did not emerge after 10 min, it was assigned a latency of 600 s, and the container was gently tilted from behind by the observer until the fish emerged. After emergence, we recorded—over a five-minute period—the number of unique sections of the grid drawn along the arena bottom that were entered by the fish, as well as the total number of sections crossed. These last two behavioural measures are sometimes used to represent separate behaviours (e.g., exploration vs. activity). However, the two measures were highly correlated in our dataset: the weakest correlation was $r = 0.74$ (South Rolly Lake) and the strongest was $r = 0.88$ (Watson Lake). Hence, subsequent analyses used

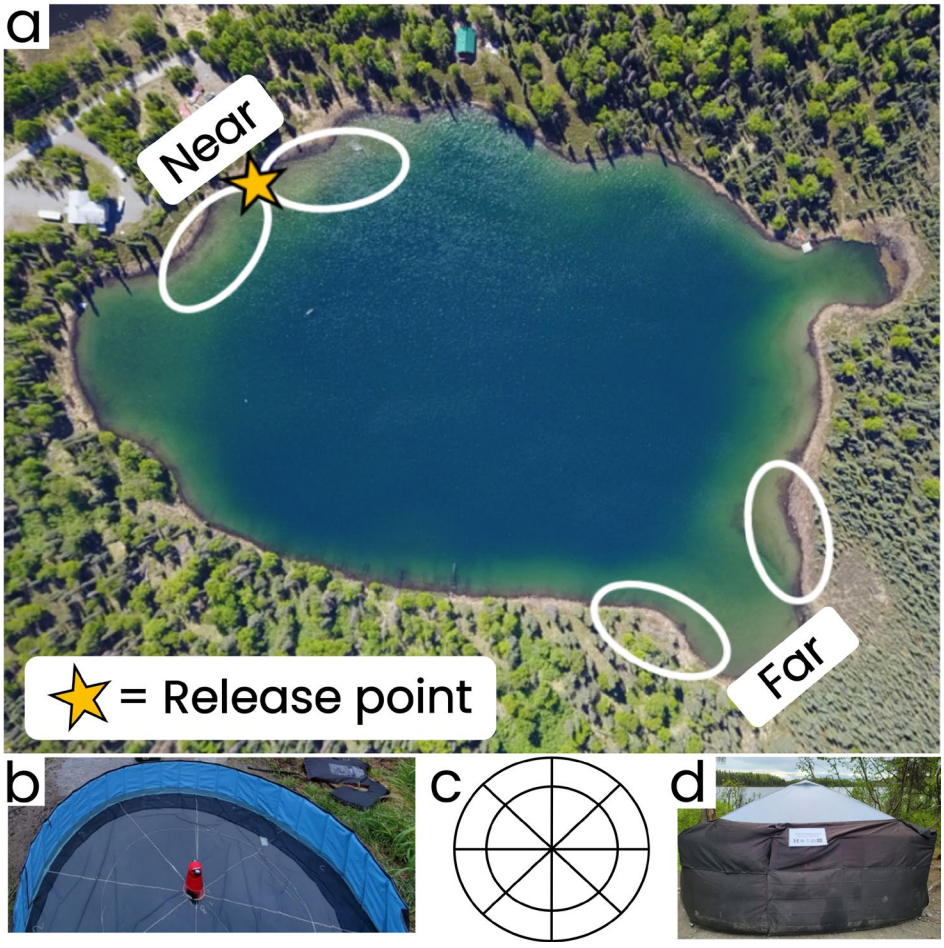

FIGURE 2 | Aerial photograph of the recipient lake (Panel a) and photographs/illustrations of the experimental arena used for behavioural trials (Panels b–d). On the recipient lake photograph (Panel a), the release point and the general areas (ellipses) where traps were set "near" to or "far" from to the release point to capture variation in post-introduction displacement are indicated. For the experimental arena, the water-filled kiddie pool (Panel b) had a grid on the bottom (Panel c) and was covered by a gazebo and surrounded by black-out curtains (Panel d).

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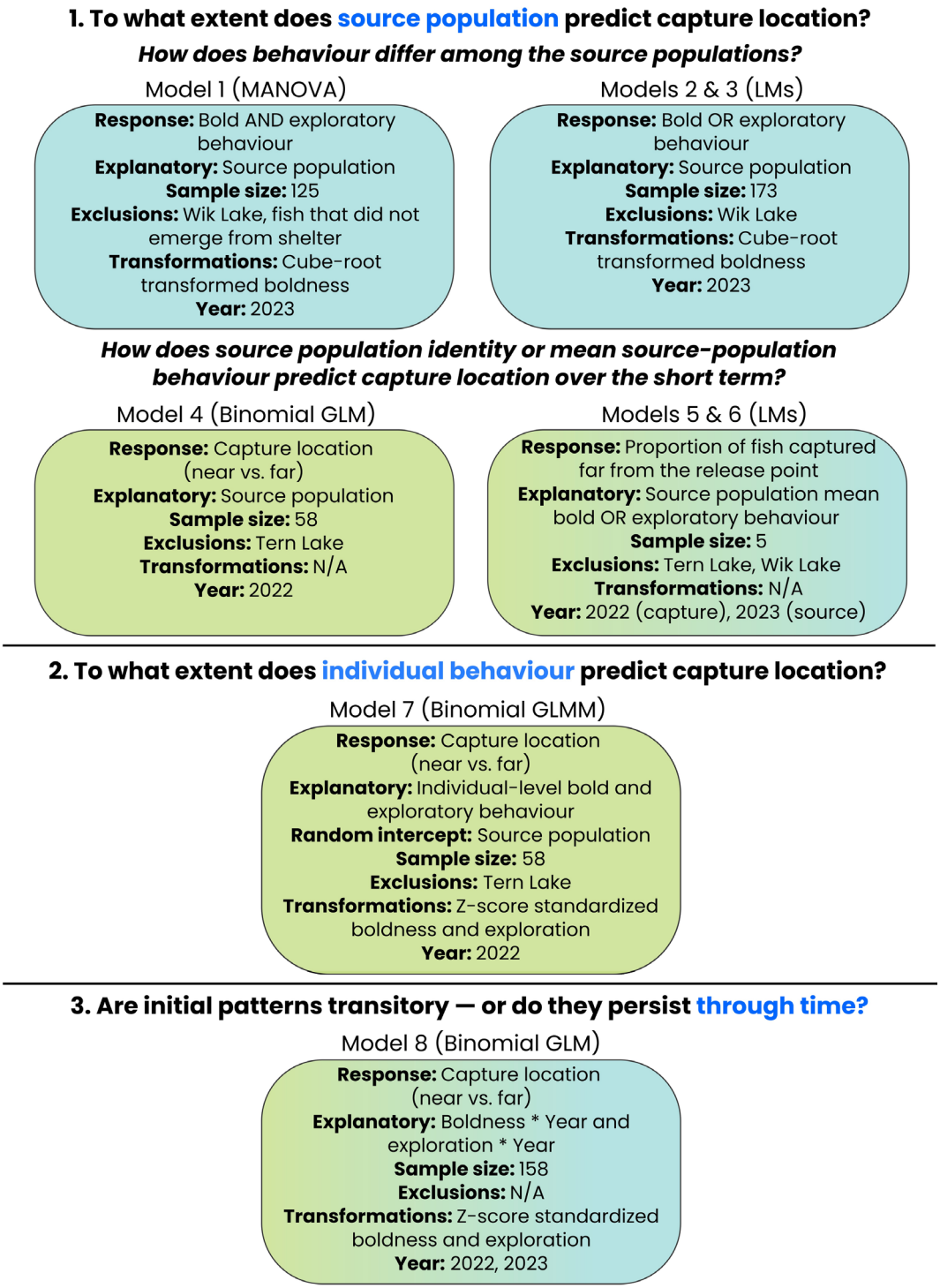

FIGURE 3 | Details about each specific model included in our analyses.

only the number of unique sections crossed, which we consider "exploratory behaviour" for consistency with the common definition that exploratory behaviour represents movement in an unfamiliar environment (Réale et al. 2007). Measuring two behaviours in succession is a standard approach (e.g., Laskowski et al. 2015; Fox et al. 2025), but therefore also means the behaviours are not independent. Still, the average correlation between bold and exploratory behaviours was only −0.37 in the source lakes (min = −0.06, max = −0.71), and −0.26 across both years in the recipient lake (year 1 = −0.23, year 2 = −0.30). After each fish's five-minute trial, it was euthanized with an overdose of buffered MS-222. Fin-clips were taken for each individual and preserved in 95% ethanol, after which the fish was preserved in formalin.

2.3 | DNA Genotyping

Assessing source population variation was straightforward for fish captured directly from the source populations, but

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it required genotyping from fish captured from the recipient lake in 2022. To isolate DNA for the 60 experimental fish from that year, we first placed fin clips into a solution of tissue digestion buffer and proteinase K, which was kept overnight at 55°C, and then subjected to a series of phenol-chloroform and ethanol washes. The extracted DNA was then used to screen each fish for Single Nucleotide Polymorphisms (SNP) suitable for identifying source populations. Specifically, poolseq data were used to identify SNPs on multiple chromosomes that were unique and fixed (or nearly so) for each source population (Hendry et al. 2024). Details of the development of the genotyping arrays and downstream analysis can be found in Bolnick et al. (2024) and Eckert et al. (2026). Using this method, we can accurately assign individual stickleback to the source population from which they were collected 100% of the time (Bolnick et al. 2024). Genotyping failed for two fin clips, and so our final dataset includes $n = 58$ samples for the recipient lake collected one month after introduction. Note that we did not genotype fish from the recipient lake in 2023 because interbreeding among populations meant that most fish would no longer be assignable to a single source population—which was the required inference for questions posed in the present paper.

2.4 | Data Analysis

Analyses were conducted in the R environment v 4.3.1 (R Core Team 2024). For effect sizes, we extracted eta^2 for each term in the linear models (LM), including the multivariate analysis of variance (MANOVA; Ben-Shachar et al. 2020). We similarly extracted R^2 for each term in generalized linear models (GLM) and mixed effects models (GLMM). Test statistics and p-values for all models were extracted using the car package (Fox and Weisberg 2019) with a type 2 sum of squares for models without interactions between predictor variables and a type 3 sum of squares for the model with interactions. Model diagnostics were assessed manually and with the DHARMa (Hartig 2022), rstatix (Kassambara 2023), and heplots (Friendly 2007) R packages (model diagnostics are provided in the Supporting Information).

2.5 | Question 1: To What Extent Does Source Population (i.e., Average Behaviour) Predict Capture Location?

To investigate how source population can predict capture location in the recipient lake, we began by examining how behaviour (measured in the assays) differed among the source populations—for fish captured from those recipient lakes (Model 1, Figure 3). The motivation for these initial analyses was that, if behaviour differs among source populations and is linked to displacement, then population-level differences in behaviour could be associated with capture location following release of individuals from those populations into a novel environment. These analyses excluded Wik Lake as we did not catch any fish in Wik Lake (see above). For this objective, we used a MANOVA to assess the possible effect of source population jointly on the response variables latency to emerge (bold behaviour) and the number of unique sections crossed (exploratory behaviour) in source lake stickleback. MANOVA is the appropriate test for investigating the effect of a single independent variable on the means of more than one dependent variable (Bray and Maxwell 1985). Source population was considered a fixed effect because the conceptual motivation for our study was that the particular source populations might have properties best suited for specific translocation objectives. We were therefore ultimately interested in the behaviours of specific source populations. We then also modelled univariate associations between source population and each behaviour as a separate response variable in two LMs (Models 2 and 3, Figure 3). To better meet model assumptions, bold behaviour was also cube-root transformed and the MANOVA was conducted with a subset of the data where we excluded the fish that did not emerge from shelter. The univariate LMs, however, could be analysed with those fish assigned a latency of 600 (see above).

We next assessed whether source population identity or the average behaviour of fish from a given source population might predict capture location in the recipient lake over the short-term (i.e., the 2022 data; Models 4–6, Figure 3). Both sets of analyses excluded Tern Lake because no fish of Tern Lake ancestry were collected in the recipient lake. Wik Lake was included in the source population identity analyses because fish of Wik ancestry were captured in the recipient lake, but Wik was excluded from the average behaviour analyses because we did not capture any fish in Wik Lake itself. This variation in capture success in Wik Lake was owing to variation between the years. We were able to capture fish in 2022 for the initial introduction, but not when we re-sampled in 2023. For source population identity, we started with a GLM where capture location (near vs. far) was the response variable, source population (inferred by genotyping) was the fixed effect, and errors were binomial because there are only two possible outcomes (Dobson and Barnett 2018; Model 4, Figure 3). Because source population is the only effect here, this model conceptually integrates the effects of all biological properties that might differ among source populations. Next, to consider if any source population effects could be specifically related to the typical behaviours of those populations, we replaced the "source population" fixed effect with the average bold or exploratory behaviour score of fish from each source population as the fixed effect (Models 5 and 6, Figure 3). The response variable here was the proportion of fish from each source population captured in far traps (i.e., far from the release point) in the recipient lake. Note that these LMs could not use individual fish and so have very low power ($N = 5$ populations) and should therefore be considered exploratory.

2.6 | Question 2: To What Extent Does Individual Behaviour Predict Capture Location?

To next examine how individual behaviour was associated with capture location on the short term (i.e., 2022) within the recipient lake, we used a binomial GLMM with capture location (near vs. far) as the response variable in lme4 (Model 7, Figure 3; Bates et al. 2015). The predictor variables in this case were individual-level data on bold and exploratory behaviour, each standardized by converting to a z-score. For this model, source population was

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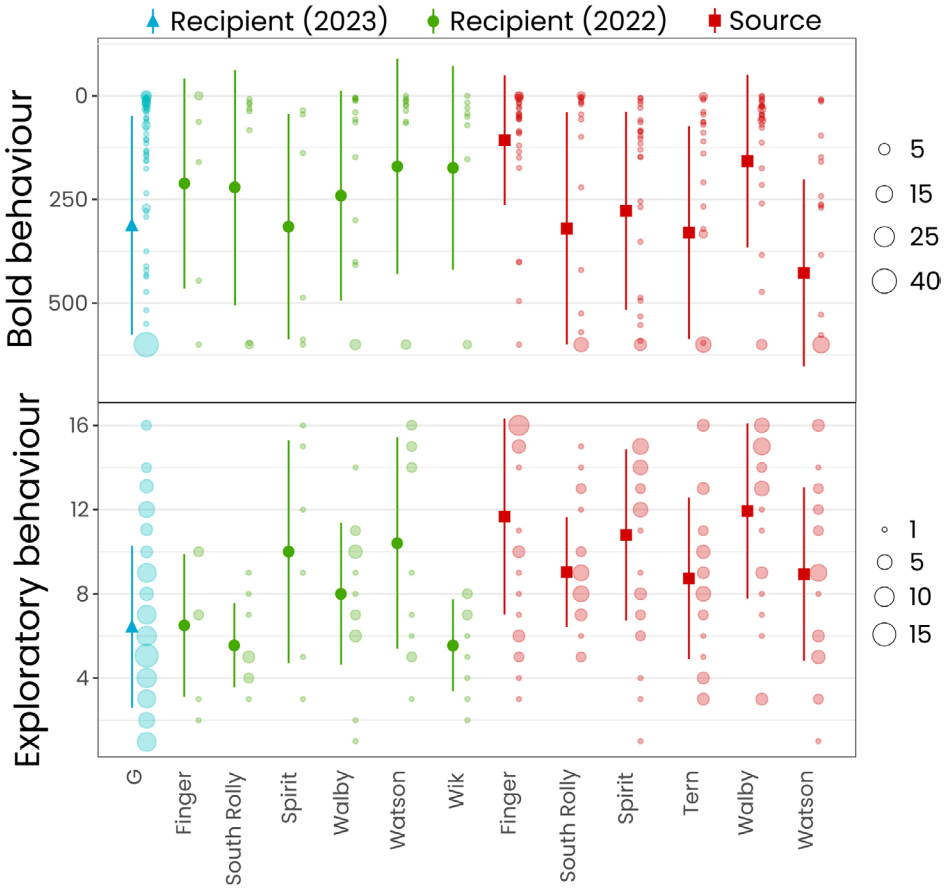

FIGURE 4 | Differences in bold (top panel) and exploratory behaviour scores (bottom panel) among all sampling events. The shapes with error bars represent fish captured in the recipient lake in 2022 (green circles; with the specific source population assessed through genotyping) and 2023 ('G', blue triangles), as well as fish captured directly from the source lakes in 2023 (red squares). Raw behaviour scores are scaled by sample size (the number of individuals with the same behavioural score). The shapes with error bars denote the mean and standard deviation for each population and are shifted to the left of the data points for clarity. The y-axis for bold behaviour is reversed for more intuitive interpretation (i.e., bold behaviour increases approaching the top of the figure). Wik Lake fish were introduced into the recipient lake but do not have a corresponding data point for behavioural assays conducted on fish captured directly from the source population as we were unable to conduct those assays (see text).

a random (rather than fixed) effect because we are no longer interested in the direct effects of specific source populations, which was tested in Question 1. Instead, we are here interested in the effects of individual behaviour on displacement, and so we need to control for source population identity via a random effect.

2.7 | Question 3: Are Initial Patterns Transitory—Or Do They Persist Through Time?

Finally, we assessed the extent to which behaviours associated with displacement changed over time by comparing data for fish collected from the recipient lake in 2022 versus 2023 (Model 8, Figure 3). Here we constructed a binomial GLM with capture location as the response variable, individual bold and exploratory behaviours (standardized) as predictor variables, and interactions between those behavioural variables and year (as a fixed effect as we were specifically interested in the order of the 2 years). Source population was not included in this model because, in 2023, fish had been interbreeding and so were not genetically assigned to a specific sole-origin source population.

3 | Results

3.1 | Question 1: To What Extent Does Source Population Predict Capture Location?

The source populations differed in bold and exploratory behaviours (MANOVA: Pillai's trace = 0.20, $F_{(10,238)} = 2.69$, $p < 0.01$, $n = 125$, Table S1; LMs: bold: $F_{(5,167)} = 7.15$, $p < 0.001$, $n = 173$, Table S2; exploratory: $F_{(5,167)} = 3.88$, $p < 0.01$, $n = 173$, Table S3). The differences were not trivial: source population explained a tenth of the variance in both behaviours combined and, when considered separately, almost a fifth of the variance in bold behaviour and a tenth of the variance in exploratory behaviour (MANOVA: $\text{eta}^2 = 0.10$; LMs: bold: $\text{eta}^2 = 0.18$; exploratory: $\text{eta}^2 = 0.10$). Source populations tended to fall into two groups: those with higher bold (averaging 106.9–277.4 s) and exploratory (averaging 10.8–11.9 squares) behavioural scores (Finger, Spirit, and Walby), and those with lower bold (averaging 320–427 s) and exploratory (averaging 8.7–9.0 squares) behavioural scores (South Rolly, Tern, and Watson; Figure 4) (For bold behaviour, a lower value means that the fish emerged faster and were therefore bolder).

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Despite this variation in behaviour among fish from the source populations, capture location in the recipient lake was not associated with source population ($\chi^2_5 = 4.00$, $p = 0.55$, $n = 58$; $R^2 = 0.07$; Table 2; Table S4). Unsurprisingly, then, the average bold and exploratory behaviours of stickleback collected from each source population were not associated with the proportion of individuals captured near versus far in the recipient lake (bold: $F_{(1,3)} = 0.84$, $p = 0.43$, $n = 5$, Table S5; exploratory: $F_{(1,3)} = 2.74$, $p = 0.20$, $n = 5$, Table S6). However, despite the lack of statistical significance in the latter population-level analysis (i.e., average values for $n = 5$ populations), it is worth noting that variation in capture location seemed to be qualitatively associated with mean bold behaviour ($eta^2 = 0.22$) and mean exploratory behaviour ($eta^2 = 0.48$). We provide this qualitative interpretation only to suggest that studies examining more populations might well reveal effects of some population-level behavioural differences on movement following release into new locations.

3.2 | Question 2: To What Extent Does Individual Behaviour Predict Capture Location?

A GLMM analysing bold behaviour and exploratory behaviour explained about a fifth of the variance in the capture location of individuals in the recipient lake ($R^2 = 0.18$). Practically all this explanatory power could be attributed to exploratory behaviour ($R^2 = 0.18$). Specifically, individuals that were captured near (rather than far from) the introduction point crossed more unique sections in the arena ($\chi^2_1 = 9.68$, $p \leq 0.01$, $n = 58$; Figure 5; Figure S1; Table S7). Stated another way, individuals that were displaced farther in the recipient lake explored less in the behavioural assays. By contrast, bold behaviour was not associated with capture location (bold: $\chi^2_1 = 1.50$, $p = 0.22$, $n = 58$; $R^2 = 0.02$; Figure 5; Figure S1; Table S7).

3.3 | Question 3: Are Initial Patterns Transitory—Or Do They Persist Through Time?

Analysing individual-level data from the 2 years revealed an interesting interaction between exploratory behaviour and year ($\chi^2_1 = 8.11$, $p < 0.01$, $n = 158$; $R^2 = 0.07$). As shown in the above analysis, fish captured in 2022 far from the release location explored less than those captured near the release location. In 2023, however, no association between capture location and exploratory behaviour was evident (Figure 5). On the other hand, bold behaviour was not associated with capture location in either year (bold: $R^2 = 0.02$; $R^2 =$ year: 0.02; $R^2 =$ bold-year interaction: $R^2 = 0.03$; Table 3; Figure 5).

TABLE 2 | Number of individuals captured in 'near' and 'far' traps in 2022.

Source population	Near	Far
Finger	3	3
South Rolly	4	5
Spirit	3	3
Tern	0	0
Walby	10	6
Watson	6	6
Wik	2	7

4 | Discussion

Predicting displacement can help to understand ecological and evolutionary processes, and such predictions could be especially useful during introduction events, such as biological invasions or conservation translocations. We approached this topic by asking to what extent the location where fish were captured following introduction (near an introduction point vs. farther from that introduction point) was related to their source population of origin and/or individual behaviour, as well as how any spatial patterns might change from 1 year to the next. To implement this work, we leveraged a large-scale conservation translocation in Alaska, where stickleback from seven source populations were introduced into a single lake. We found that the different source populations did show differences in behaviour. However, source population and behaviour were not strongly associated—at the population level—with capture location following introduction into the new lake. Instead, capture location was associated with exploratory behaviour at the individual level, such that stickleback captured farther from the introduction point (i.e., those with greater displacement) explored less than did stickleback captured near the introduction point (lower displacement). This last effect disappeared by the following year, implying it was associated with the initial displacement. We now discuss these findings in more detail, highlighting opportunities for future research and some possible applied implications.

4.1 | Question 1: To What Extent Does Source Population Predict Capture Location?

Fish from the different source populations differed in bold and exploratory behaviours, a finding that matches previous work showing how these behaviours can differ dramatically among populations of stickleback (Lacasse and Aubin-Horth 2012; De Winter et al. 2016) and other fishes (e.g., Brown and Braithwaite 2004; Brown et al. 2005; Archard and Braithwaite 2011). These results also confirm that (1) the methods used in our assays were suitable for discerning behavioural differences among populations, and (2) considerable potential existed for population-level differences to shape displacement in the recipient lake. Despite source population differences in behaviours hypothesized to influence displacement, source population and the average behaviour of fish from those populations were not associated with capture location following release into the recipient lake.

Several potential explanations can be advanced for the apparent lack of population-level effects on displacement. First, bold and exploratory behaviours in the assays might not reflect bold and exploratory behaviours after release into the recipient lake. However, such assays are common when assessing how translocated individuals might behave when released into nature (e.g., Sinn et al. 2014; Bamber et al. 2020; Germano et al. 2017). Second, the recipient lake might be too small (7 ha) to observe

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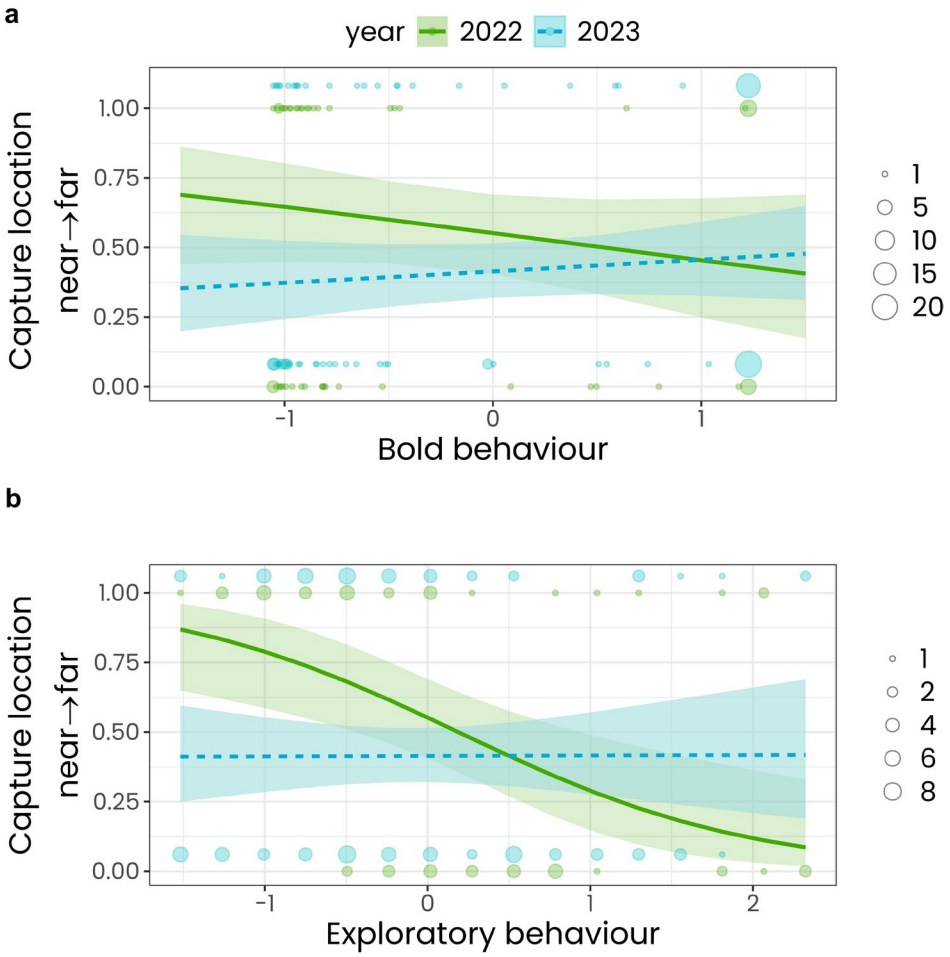

FIGURE 5 | The association between exploration and displacement that was evident in 2022 was no longer evident by 2023. The trend lines with confidence intervals (for bold and exploratory behaviours) are predicted by the model, and the data points are the raw data scaled by sample size (the number of individuals with the same behavioural score). The 2023 data points have been shifted vertically for clarity. Given that the results were qualitatively consistent between Models 7 and 8 (see Figure 3), this figure is showing model-predicted results from Model 8 only (i.e., including data from 2022 and 2023). See Figure S1 for predictions from Model 7 (i.e., 2022 data only).

TABLE 3 | ANOVA output from generalized linear model assessing the effects of behaviour and year on capture location (near vs. far).

Coefficient	χ^2	df	p
Bold	1.55	1	0.21
Year	2.32	1	0.13
Exploratory	**13.03**	**1**	**<0.001**
Bold: Year	2.20	1	0.14
Exploratory: Year	**8.11**	**1**	**<0.01**

Note: Significant factors ($p < 0.05$) are bolded.

population-level differences in displacement, which might instead require studies on larger spatial scales or on shorter time frames after introduction. Third, the populations might have had differential survival following release into the lake, eliminating spatial patterns because only individuals with certain phenotypes survived. Fourth, behaviours other than those we studied might be better predictors of displacement—for example, stickleback movement in rivers has been associated with variation in the social environment (Laskowski et al. 2015). Still, the "source population" effect would be expected to integrate all such behaviours. Fifth, some source population effects might be present, and we simply needed more power (i.e., more individuals or populations) to statistically confirm those effects (see suggestive trends noted in the Results). Finally, variation in displacement might be more dependent on individual-level characteristics than population-level differences. For example, an unmeasured factor that might shape post-introduction movement is sex, as sex has been associated with stickleback movement in some environments (e.g., Cano et al. 2008)—although, in a previous study, we did not find evidence of sex-biased movement with experimentally displaced lake stickleback (Heckley et al. 2024). Future studies could consider other individual-level characteristics, as our focus on individual-level characteristics in this study emphasized behaviour (see Question 2).

A key take-away for future work with stickleback is that fish from all source populations were quickly distributed throughout the entire recipient lake. Thus, the specific source population of stickleback in new environments is unlikely to strongly impact ecological and evolutionary outcomes associated with displacement—at least in small and isolated lakes. Therefore, rather

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than focusing on variation in displacement, researchers investigating intraspecific variation in a translocation context should perhaps direct their attention to other ecologically and evolutionarily important traits, such as body size, reproductive rate, or local adaptation. Stickleback populations differ dramatically in these and many other traits (Bell and Foster 1994), at least some of which are surely important for mitigating or facilitating the impacts of introduced stickleback, as has been reported for other introduced fishes (e.g., Yonekura et al. 2007).

4.2 | Question 2: To What Extent Does Individual Behaviour Predict Capture Location?

Despite an absence of clear population-level influences (see Question 1), individual behaviour was associated with capture location. Specifically, individuals captured nearest the introduction point (i.e., those with lower displacement) explored more in the experimental arenas than did individuals captured farthest from the introduction point (i.e., greater displacement). The direction of this difference is perhaps surprising because it might seem intuitive for individuals that explore more (i.e., move more on a small scale) to have displaced farther (i.e., move more on a large scale). We suggest two potential reasons for why—in our study—more exploratory individuals were captured closer to the release point.

First, although exploratory behaviour on a small scale and displacement on a large scale are both generally associated with "movement," movement has different functions at those two scales. For instance, exploratory behaviour is primarily related to information acquisition (Rojas-Ferrer et al. 2020), whereas many other movement processes can generate displacement. These processes can be shaped by many factors, such as avoiding predators, parasites, or inbreeding, as well as seeking out breeding sites or resource opportunities (Bowler and Benton 2005). Further, acquiring information about the environment (exploration) could trade off with the movements generating displacement if more exploratory individuals spend more time investigating their local environments, thus moving more slowly away from those environments (displacement). At least one other study found a similar pattern: juvenile flying squirrels (*Pteromys volans*) that disperse longer distances also explore less (Selonen and Hanski 2006). With stickleback, one mark-recapture study did not find that exploratory behaviour was associated with movement in a river (Laskowski et al. 2015). Although flying squirrels are not stickleback, and river stickleback are not lake stickleback, our point is that multiple studies have found outcomes contradicting the seemingly straightforward expectation that more exploratory individuals should exhibit greater displacement.

Second, the direction of causality between behaviour and displacement is not clear in many studies (e.g., Myles-Gonzalez et al. 2015). In our case, we assayed behaviour *after* capturing individuals that displaced different distances, rather than measuring the behaviour of individuals first and then monitoring their subsequent displacement. The reason for this order of assessment in our study was that the behavioural assays take time (three fish per hour) and so could be done for only a small subset of the 3495 fish that were released (The fish needed to be released over a short time period to ensure a balanced design while avoiding high summer temperatures; Hendry et al. 2024). If we had instead directed our effort to assaying the same number of fish ($N = 60$) *before* the release, the chances of catching these assayed fish near and far a month later would have been very low, as the 60 total post-release individuals that we captured required 5 days of effort and represented only 1.7% of the fish released. Regardless, the order of our assessment (displacement first and then the behavioural assays) means that lower exploratory behaviour could have caused greater displacement, greater displacement could have caused lower exploratory behaviour, or both variables could have been associated with some other causal variable. Regardless of the direction of causality, the strong—and surprising—association between exploratory behaviour in an experimental arena and displacement distance in a lake suggests the value of further work to explore this association in other contexts.

4.3 | Question 3: Are Initial Patterns Transitory—Or Do They Persist Through Time?

Finally, we assessed how spatial patterns of behavioural variation might shift over time in the recipient lake by repeating our sampling and assays a year later. In the second sampling period (1 year after introduction), we found no notable spatial patterns of behavioural variation, in contrast to the initial (1 month after introduction) individual-level association between exploratory behaviour and displacement. It is always possible that environmental factors that differ between the shorelines (near versus far from the release site) changed between the 2 years and are underlying this change. Although we did not attempt to measure such variables, we can at least note that no differences were visually evident to us while sampling.

Alternatively, the inter-annual change in pattern might provide additional (albeit indirect) evidence that the initial near versus far behavioural difference reported above is indeed associated with initial movements underlying displacement. Although stickleback can live for 2–3 years in the wild, and so it is theoretically possible that we captured stickleback in 2023 that had been released in 2022, those individuals would be *at least* 2 years old in 2023, putting them at the higher end of a normal stickleback lifespan for this region (e.g., in Walby lake, one of the source populations, stickleback live for a maximum of about 2 years; Heins et al. 2000). It is therefore highly likely that all fish (or at least the vast majority) captured in 2023 were the result of within-lake recruitment—as also inferred from overall abundances (much higher in 2023 than 2022) and individual fish sizes (consistent with year-old fish). That is, the fish captured and assayed in 2023 were most likely the offspring of fish that had been introduced and bred in 2022. The near versus far (relative to the introduction site) distinction was therefore not relevant to the 2023 fish, which had lived their entire lives in the recipient lake. Stated another way, the "far" fish captured in 2022 had presumably moved farther than the "near" fish, whereas that would not be case in 2023. These considerations suggest that the behaviour-distance association observed in 2022 was specifically associated with displacement—as it disappeared in the next generation (when displacement from the introduction point was irrelevant).

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Temporal change in the behaviour-distance association also has practical implications. For instance, it suggests that associations between phenotypes and displacement can be ephemeral—and therefore should be tested on multiple time scales. This idea supports previous assertions of interesting behavioural (and other trait) differences on the "wave front" of invasions or range expansions (e.g., Liebl and Martin 2012; Myles-Gonzalez et al. 2015; Gruber et al. 2017). Such wave-front effects are expected to be transitory and can disappear after the trailing edge of phenotypes "catches up" to the wave front. In the case of stickleback in our 7 ha lake, this shift appears to have taken only a single generation—and perhaps much less as we only assayed 1 month after introduction (a near vs. far behavioural difference was evident) and 12 months after introduction (no behavioural difference was evident).

5 | Conclusions

Phenotypic variation can have strong ecological and evolutionary effects (Bolnick et al. 2011; Hendry 2017; Des Roches et al. 2018). Thus, predicting how individual phenotypes are associated with patterns of post-introduction displacement could provide important insight into how eco-evolutionary processes unfold in novel environments. Motivated by these possibilities, we investigated the extent to which source population and behaviour could predict post-introduction displacement of stickleback from seven populations translocated into a single lake in Alaska. In doing so, our study leveraged a rare opportunity to investigate the correlates of post-introduction displacement in nature.

Although the populations differed in behaviour (bold and exploratory), neither source population nor their average behaviour predicted whether individuals were found near or far from the release point a month after introduction. Our study thus complements previous findings that individual stickleback can move long distances (Ward et al. 2013), and that invasive stickleback spread rapidly to new locations (Makhrov et al. 2025). When our study and these past findings are considered together, a practical interpretation might be that pretty much any source population will yield similar displacement outcomes when released into lakes.

We did find a strong—and initially surprising—association between exploratory behaviour and short-term displacement at the individual level: fish captured *farther* from the release site explored *less* in experimental arenas. This pattern had disappeared a year later, which suggests that it could be specifically associated with initial movement-associated processes following introduction, although additional work will be needed to disentangle cause and effect. Regardless, our study illustrates that exploratory behaviour could serve as a possible tool to predict displacement—although its utility will require deeper scrutiny. It also underscores the value of conducting experimental manipulations in nature to investigate how complex ecological processes, such as displacement, play out in natural systems. That is, behaviours assayed in controlled arenas might not predict—at least not as one might expect—displacement in nature.

Author Contributions

Conceptualisation: A.M.H., A.P.H. and K.M.G. Developing methods: A.M.H., C.L.P., D.I.B., L.E., A.M.B., R.D.H.B., G.E.H., A.P.H. and K.M.G. Conducting the research: A.M.H. and F.D. Data analysis: A.M.H. Preparation of figures and tables: A.M.H. Writing: A.M.H., R.D.H.B., A.M.B., D.I.B., F.D., L.E., G.E.H., C.L.P., A.P.H. and K.M.G.

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Ethics Statement

This work was conducted in accordance with approved IACUC protocols at the University of Illinois Urbana-Champaign (Protocol #: 21031), with permission of the State of Alaska Department of Fish and Game (Permit #: SF2023-110 and SF2022-106).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Individuals captured near the introduction point ("weaker" dispersers) crossed more sections in an artificial arena (more exploratory) 1 month after introduction (Panel B) but did not differ in bold behaviour (Panel A). The trend lines with confidence intervals are predicted by the model, and the data points are the raw data scaled by sample size (the number of individuals with the same behavioural score). **Figure S2:** Raw bold and exploratory scores for fish sampled in the recipient lake in 2022 (G22) and 2023 (G23) and in each of the source lakes. The data points are scaled by sample size (the number of individuals with the same behavioural score for each population). **Table S1:** Results for "model 1"—multivariate analysis of variance (MANOVA) assessing the effect of source population on bold and exploratory behaviour together. Approx. *F*, approximate *F* statistic; Den, denominator; df, degrees of freedom; Num, numerator; *p*, *p*-value. **Table S2:** Results for "model 2"—linear model assessing the effect of source population on bold behaviour. See Table S1 for a list of abbreviations. **Table S3:** Results for "model 3"—linear model assessing the effect of source population on exploratory behaviour. See Table S1 for a list of abbreviations. **Table S4:** Results for "model 4"—binomial generalized linear model assessing the effect of source population on capture

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location (near vs. far). See Table S1 for a list of abbreviations. **Table S5:** Results for "model 5"—linear model assessing the effect of mean source population bold behaviour on the proportion of fish captured far from the release point. See Table S1 for a list of abbreviations. **Table S6:** Results for "model 6"—linear model assessing the effect of mean source population exploratory behaviour on the proportion of fish captured far from the release point. See Table S1 for a list of abbreviations. **Table S7:** Results for "model 7"—binomial generalized linear mixed model assessing the effects of individual-level bold and exploratory behaviour on capture location. Source population was included as a random intercept. Bold and exploratory behaviour scores were z-score standardized. See Table S1 for a list of abbreviations. **Figure S3:** DHARMa R package model diagnostics for models 2 and 3. **Figure S4:** DHARMa R package model diagnostics for models 4–6. **Figure S5:** DHARMa R package model diagnostics for models 7, 8.

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