

Research



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Long-term environmental stability does not erode plasticity in nest building responses to changing ambient conditions

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The primary function of animal nests is to protect developing offspring from hostile and fluctuating environments. Animal builders have been shown to adjust nest construction in response to changes in their environment. However, the extent of this plasticity, and its dependence on an evolutionary history of environmental variability, is not well understood. To test whether an evolutionary history with flowing water impacts male ability to adjust nests in response to flow regime, we collected three-spined sticklebacks (*Gasterosteus aculeatus*) from three lakes and three rivers, and brought them into reproductive condition in controlled laboratory aquaria. Males were then allowed to nest under both flowing and static conditions. Nest building behaviour, nest structure and nest composition were all recorded. In comparison to males building nests under static conditions, males building in flowing water took longer to construct their nests and invested more in nesting behaviour. Moreover, nests built in flowing water contained less material, were smaller, more compact, neater and more elongated than nests built under static conditions. Whether males came from rivers or lakes had little impact on nesting activities, or male capacity to adjust behaviours in response to flow treatment. Our findings suggest that aquatic animals which have experienced a stable environment over a long period of time retain plasticity in nest-building behaviours that allow them to adjust nests to ambient flow conditions. This ability may prove crucial in coping with the increasingly unpredictable flow regimes found in anthropogenically altered waterways and those resulting from global climate change.

This article is part of the theme issue ‘The evolutionary ecology of nests: a cross-taxon approach’.

1. Introduction

Parents provide care for developing young to optimize their development (e.g. thermoregulation; [1]) and/or to minimize external threats (e.g. predation; [2]). Although intensive parental behaviours usually promote reproductive success, such activities are also energy-demanding, requiring investment in foraging to provision young [3,4] and other specialized activities, such as alarm calling [5] or fanning eggs [6]. Additionally, parental care may increase conspicuousness to predators [7,8]. Since the costs and benefits of parenting can differ depending on the environment in which care takes place, selection should favour individuals that can adjust parenting activities to best balance the costs and benefits in the prevailing environment [9]. Despite the extensive literature on parental care [10–12], the plasticity of pre-copulatory parenting effort (i.e. nesting-site selection, nest construction) has received comparatively little attention.

In animals that build nests, nest-building typically precedes fertilization and hence is often the first stage of parental care that faces environmental pressures that may affect offspring production [13,14]. For example, flycatchers (*Ficedula hypoleuca*) are capable of selecting nest sites with lower predation risk by

gathering information from heterospecific nesting attempts [15]. Failure to exhibit plasticity in nesting behaviour can lead to the selection of suboptimal nest locations or microhabitats, which can directly impact breeding success [16] and have cascading consequences for population viability. In anthropogenically disturbed environments, this also has the potential to lead to ecological traps, whereby traits that are normally adaptive become disadvantageous in the changed environments [17]. Plastic nesting activity allows parents to optimize nest microhabitats to improve offspring viability in changing environments. To date, studies investigating nest-building activity, mostly focus on birds and reptiles [18], with fewer studies considering nest-building (and especially its plasticity) in aquatic animals (but see [19–21]). This is surprising given the severe impacts of anthropogenic activities and climate change on aquatic ecosystems [22–24].

Water velocity is a key component of aquatic environments that influences physical (e.g. turbidity, water conductivity, substrate type) and biological factors (e.g. vegetation density, prey availability, predation risk, population density) [24–28]. On one hand habitats with flowing water tend to have higher dissolved oxygen levels [29], a lower risk of predation (e.g. [30,31]) and a reduced likelihood of algal blooms [32], which may reduce the costs of nest building. On the other hand, flowing water can come with greater fluctuations in water temperature [33], reduced vegetation cover, increased costs of locomotion [34,35], and a higher chance of nest destruction (washout) owing to shear stress action [36–38]. These natural disturbances are likely to generate selection for particular nest characteristics in flowing water, which is worth investigating, especially when considering the dramatic alteration of water flow regimes caused by climate change and human intervention [39,40].

Indeed, previous research has shown that fish building nests in flowing water select nest locations (e.g. at the edges of rivers; [41]) and adjust the orientation of their constructions [42] to optimize flow rates through the nest as well as building structurally different nests to those constructed under static conditions [43,44]. Parents in static water typically express weaker preferences for nest sites [44] presumably owing to the more uniform and less challenging hydrodynamic landscape. Instead, variations in oxygen levels [6,45], increased risk of egg cannibalism [46] and the risk of stolen fertilization [47] in static water may necessitate changes in nest design (e.g. size of nest entrance) or behaviour (e.g. fanning frequency) to ensure incubation success. Intra-specific lake-stream divergence therefore offers a valuable opportunity to examine phenotypic plasticity and local adaptation in nesting behaviour, which can be under both natural (e.g. defence against predators) and sexual selection (mate attraction; [48]). Individuals with greater nesting plasticity are expected to be favoured by the greater temporal and spatial variation of water flow in rivers, which provides a more challenging and dynamic habitat compared to lakes, where plastic responses to variation in water flow may be less advantageous. Although habitat-related differences in nest ecotypes are apparent (e.g. [49,50]), it remains unclear whether parents from river and lake populations are equally capable of adjusting nesting behaviour in response to alterations in water current.

The capacity to adjust nest structure according to prevailing conditions should be favoured in aquatic animals where nest microhabitats play a decisive role in embryo

development and survival (e.g. [51–53]). Fish living in flowing water, for example, might be selected to construct nests with a uniformly streamlined and compact shape that enhances their resistance to strong currents; by contrast, inhabiting still water habitats, which are expected to pose fewer constraints on nest design, may allow for greater variation in nest characteristics, thus facilitating their development as extended sexual traits that signal the builder's quality [48,54]. On the other hand, flowing water environments typically show considerable temporal and spatial variation in water flow. Thus, fish that inhabit river environments may be selected for greater plasticity in nest building behaviour and their nest structure. Understanding how nest-building fish respond to variation in flow is important in the context of changes in flow regimes resulting from climate change (e.g. extreme flooding events; [55]) and other anthropogenic disturbances such as dam operations [56,57], and water abstraction [58,59].

Here, we aimed to determine whether the ability of male three-spined sticklebacks (*Gasterosteus aculeatus*) to adjust their nest building behaviour in response to variation in water flow depends on their evolutionary history with still or flowing water environments. In sticklebacks, males are the sole contributor to nest-building, and their nests serve both as incubators for eggs as well as a signal to attract females [48,54,60]. After establishing a territory, male sticklebacks construct nests by transporting nest material (e.g. filamentous algae and other plant material) and sticking this to the substratum (e.g. sand, gravel) using a glue-like protein – ‘spiggin’ – produced by the kidney [61,62]. In natural populations, whether male sticklebacks build nests at all can vary between populations [63] and depends on the level of predation risk [64]. When they do build nests, sticklebacks show a strong preference for particular nesting sites [53,65–67], and such preferences can vary according to habitat of origin [68]. When choosing among flow regimes, sticklebacks from lakes and streams typically favour habitats that resemble their home conditions [69]. Different populations also exhibit variation in nest characteristics; for example, in the use of decorative nest ornaments [70,71], and the size of nests [72]. Further, individual males in some river populations are capable of adjusting nesting behaviour in response to changing flow conditions; for example, males incorporate more nesting glue and build more compact nests [44], and upregulate the expression of spiggin genes [62] when constructing nests in flowing water. However, it is not known how widespread nesting plasticity is, or whether such plasticity exists in populations of fish that have no recent evolutionary history of exposure to flowing water.

To address this question, we collected three-spine sticklebacks from three river and three lake populations and allowed individual males to build nests under both flowing and static conditions sequentially in the laboratory, where the order of flow regimes was randomized. We explicitly tested: (i) the effect of ambient water flow; (ii) the effect of habitat of origin and; (iii) the interactive effect of ambient water flow and habitat of origin on aspects of nest-building behaviour (including nest site choice and construction speed), nest design (including materials used to construct nests as well as the size and shape of nests) and nest orientation. We predicted that if evolutionary history affects the ability of males to adjust their nest building behaviour in response to flow regime then males from river environments

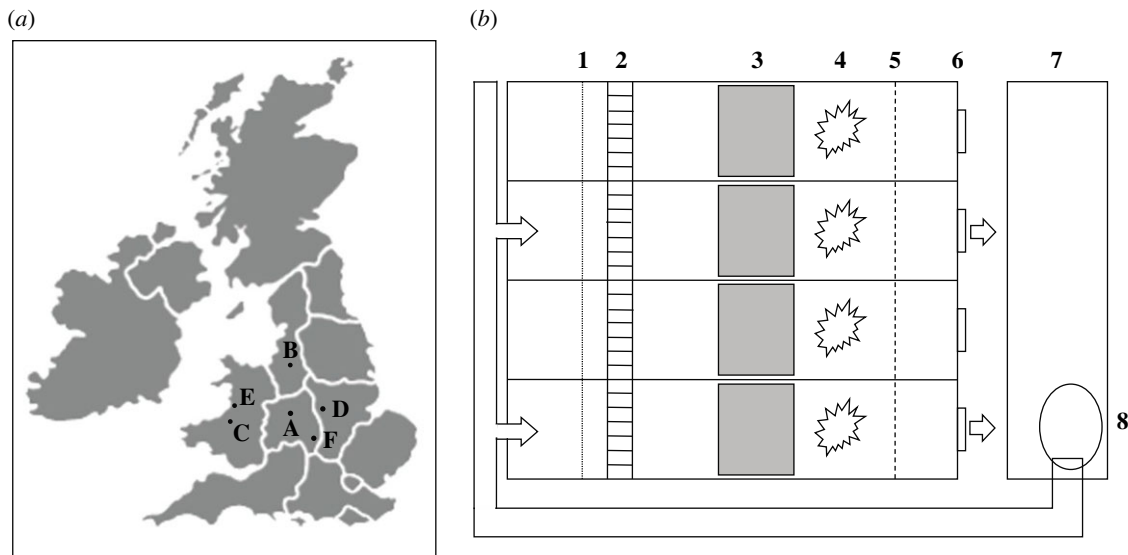


Figure 1. (a) Location of the six populations. Fish were collected from three lakes, all located in different hydrological catchments: A, Aqualate Mere (52.781° N, 2.339° W); B, Tatton Mere (53.331° N, 2.384° W); and C, Llyn Eiddwen (52.283° N, 4.044° W); and from three natural rivers: D, River Eye (52.763° N, 0.905° W); E, River Ystwyth (52.331° N, 3.896° W); F, River Welland (52.650° N, 3.255° W). (b) Schematic diagram of nesting channels. Channels were created in large plastic trays and separated by solid plastic barriers. Flow was created in two of the four channels using an 8000 l h⁻¹ capacity water pump (8) which pumped water from a sump tank (7) positioned below the outflow of the channels. Pumped water entered the channels via a 32 mm corrugated hose and passed through a sponge baffle (1) and 50 mm collimator made of plastic straws (2) to create a laminar flow in the 45 cm × 13 cm nesting channel. Water then passed through a mesh barrier (5) before exiting via the outflow (6). Channels contained a gravel substrate, a 10 cm Petri dish containing washed sand (3) and nesting materials (see text for details) (4). Water depth was maintained at 10 cm.

would be better able to adjust their nests than males from lake environments, because these males naturally experience greater variation in water velocity.

2. Methods

(a) Fish collection and husbandry

Adult male and female three-spined sticklebacks were collected using wire minnow traps from six populations (three natural, postglacial lakes and three rivers) across England and Wales (UK) in April and May 2009 (figure 1a). To ensure phylogenetic independence of the populations used, each river and lake population was located in a different catchment system that drained independently into either the North Sea or Irish Sea (see the electronic supplementary material, table S1 and figure S1). Three-spined sticklebacks colonized northern European freshwaters from ancestral marine populations following the retreat of glaciers during the last deglaciation period, *ca* 30 000–10 000 YBP. Thus, the six studied populations are expected to have been isolated from one another for at least the past 10 000 years [73]. Following capture, fish were transported to aquarium facilities at the University of Leicester and housed in single-sex 54 l stock tanks at densities up to 0.5 fish l⁻¹ for one to four months until showing signs of reaching breeding condition (i.e. red nuptial coloration on the throat) when they were then used in experiments (between May and July 2009). Fish were kept at 17 ± 1°C under a light : dark cycle of 16 : 8 and fed a mixture of brine shrimp (*Artemia* spp.) and bloodworms (*Chironomus* spp.) ad libitum and to excess daily.

(b) Nesting trials

To test the effects of water flow and habitat type of origin on male nest building behaviour and nest structure, we placed males from the three lakes (Aqualate Mere: *n* = 12; Tatton Mere: *n* = 25; Llyn Eiddwen: *n* = 19; total *n* = 56) and three rivers (Eye: *n* = 15;

Ystwyth: *n* = 16; Welland: *n* = 22; total *n* = 53) individually into nesting channels where they were exposed to either flowing or static water conditions (figure 1). Once a male completed a nest he was removed, placed in a different nesting channel offering the alternate flow treatment and allowed to build a second nest. As males came into breeding condition, they were allocated alternately to each flow treatment. This ensured that males from each population were evenly represented in each treatment over the duration of the experiment. Trial durations (i.e. the overall time the male spent completing a nest) varied from 2 to 36 days. A male was removed from the assigned channel if he did not initiate nest-building after 29 days (38 out of 193 trials). Several of the nests did not last until the completion of the trial period (9 out of 155 nests). Nest-building success in response to the treatment effect was assessed based on the probability of nest building, time spent constructing a nest and the likelihood of the nest remaining intact until the trial's end. Nesting behaviour and nest characteristics under both flow conditions were also recorded for each male (see below).

Nesting channels were constructed in large plastic trays (figure 1b). The flow rate in each channel was measured at four positions using an electromagnetic flow meter (Model 130 801, Valeport Ltd, Totnes, UK) prior to the nesting trials. Mean water velocity measured across all positions in the channels under the 'flow' treatment ranged from 3.88 to 8.95 (mean ± s.e. = 6.04 ± 0.09 cm s⁻¹; *n* = 100), corresponding approximately to the mean flow rate chosen by nesting males [44]. Water velocity was negligible under the static treatment. Once placed in their nesting channels, males were fed bloodworms twice daily and experienced temperatures of 19 ± 0.1°C.

To encourage nesting, males were provided with nesting materials including natural coloured gravel, 0.2 g of white filter wool, two hundred 5 cm-long, dark green polyester threads, 0.5 g of brown coarse bristles (cut to 3 cm) and a 10 cm square Petri dish containing sand. These materials were chosen to reflect the functional diversity of natural nesting materials that male sticklebacks use to construct nests in the wild. To stimulate nesting behaviour, males were presented with a gravid female daily

for 20 min. Prior to nest building, gravid females were released into each male's channel to allow full interaction, but once males began nest building the gravid females were constrained within a glass jar to ensure that they did not interfere with the nest [44]. The males were checked for signs of nest building, and the stage of nest construction was recorded each day (according to [74]). We measured male body mass before and after each nesting cycle to examine the effects of water flow and habitat of origin on change in body condition.

(c) Behavioural observations

From the day that males began gluing threads into the nest pit (i.e. stage 2 of construction; [74]), the nesting behaviour of males was recorded daily following the removal of stimulus females. A 1.3 MP webcam (Trust International BV, Dordrecht, the Netherlands) was mounted 70 cm above each nesting channel, allowing all parts of the channel to be viewed simultaneously, and software ('ActiveWebcam' www.pysoft.com) was used to allow multiple cameras to record at the same time onto a PC. Nesting behaviour was transcribed from these videos watched in real-time. Behaviours recorded included the amount of time each male spent at his nest, the amount of time a male spent tending his nest (i.e. adjusting and shaping nest materials), as well as the number of times a male glued his nest (i.e. dragging his cloaca across the nest), collected materials and crept through the nest. These durations and frequencies were converted to proportions of total trial duration and rates (per min), to account for small differences in video duration (ranging from 1765 to 1820 s). Each male had two video recordings (one in each flow treatment), and behavioural data were extracted from a total of 143 recordings (static water: $n = 77$; flowing water: $n = 66$) deemed suitable for analysis.

(d) Nest location and orientation

The effects of habitat of origin and water flow on the position and orientation of the nest within each channel was determined from 110 (static water: $n = 55$; flowing water: $n = 55$) and 109 recordings (static water: $n = 55$; flowing water: $n = 54$), respectively, owing to our inability to measure these parameters from some videos. To record the nest position, the channel was divided into three zones along the channel length, the sections were classified by distance from the water inflow ('closest', 'middle', 'furthest'; figure 3).

Nest orientation was estimated by watching males creep through the nest (i.e. wriggling through the nest to form a distinct tunnel with an exit; [75]) and his orientation during fanning bouts. Stickleback nest tunnels are unidirectional, with fanning always directed at the entrance to the nest [21,76]. To measure nest orientation, a 360° protractor was used to measure the angle of the tunnel, relative to the water current (figure 4). Nests classified as 180° were oriented with the entrance of the tunnel directly downstream of the tunnel's exit, so that all fanning behaviour was directed into the current. An orientation of 360°/0° meant that the entrance of the nest was directly upstream of the exit and fanning behaviour was directed in the same direction as the ambient flow.

(e) Nest removal and analysis

When completed (i.e. once an entrance was evident, or a male was observed to creep through the nest in the presence of a female; [48]), nests were removed from the channels using laminated card (following the methods of [48]). Nests were then dried (at 60°C for 24 h) and digitally photographed from above for later analysis. Several components of nest shape were quantified, including nest compactness (= bulk area of nest/total area of nest), nest neatness (= $1 - [\text{number of loose thread ends}/$

$(2 \times \text{number of total threads})$), nest elongation (= length of nest major axis/length of nest minor axis), nest roundness (= $(4\pi \times \text{total area of nest})/\text{nest perimeter}^2$) (see [44,48,77] for details). The composition of nests was also quantified. Intact nests were first weighed (total dry weight, to 0.001 g) before being separated into their constituent parts (sand, gravel, filter wool, bristles and threads), which were each weighed (to 0.001 g) separately.

A total of 23 males died during the course of the study (see Results). At the end of the experiment, we euthanized all surviving males, photographed the left-hand side of their body for later measurement of body length and the area of red nuptial throat coloration using IMAGEJ (following the procedure in [78]). Since the production of spiggin by the kidney affects nest neatness and gluing frequency [61,62], we dissected and weighed the kidneys of males to examine potential differences between lake and river populations. Although there were population differences in body length ($F_{5,70} = 13.282$, $p < 0.001$), males from the two habitat types (river versus lake) did not differ in their mean length ($F_{1,3,996} = 0.537$, $p = 0.505$), coloration ($F_{1,3,762} = 1.645$, $p = 0.273$) or kidney weight ($F_{1,3,556} = 1.063$, $p = 0.368$) (see the electronic supplementary material for details).

(f) Statistical analyses

To analyse each trait, we used mixed models with population identity (ID; $n = 6$) and male ID ($n = 109$) specified as random factors, to account for non-independence of males from the same population and repeated measurement of the same males. Male habitat of origin (river or lake), water flow treatment (static or flowing) and body length (standardized to a mean of 0 and standard deviation of 1) as well as all two-way interactions were included as fixed factors in initial models. Given the known influence of male body size on nesting activities [71,79], we included it in all of our models unless otherwise stated. After running initial models, non-significant interactions were removed to allow interpretation of the main effects [80]. When there was a significant interaction between water flow treatment and habitat of origin, we ran separate models for the static and flowing water treatments to clarify how lake and river populations responded to changes in flow. We also included nest order in all models as a fixed factor, to account for potential variation between first and second nests. For transparency, summaries of all model outputs (both initial and final) are provided in the electronic supplementary material. Results are presented as mean \pm s.e., with a significance level set at $\alpha = 0.05$ (two tailed). p -values were calculated using the *Anova* function in the *car* package of RSTUDIO v. 1.3.1093 with R v. 4.0.5 [81]; F -tests with Kenward-Roger d.f. were performed for linear mixed models (LMMs) fitted with the *lme4* package, while type III Wald chi-square tests were conducted for generalized linear mixed models (GLMMs) fitted with the *glmmTMB* package. For GLMMs, dispersion tests (conducted using the *DHARMA* package) were used to ensure that the data variance was not greater than the model assumption. Details on model type and error distribution for each trait are given below, along with any deviations from this general approach.

(i) Male survival and condition

To determine whether habitat of origin or water flow treatment affected the survival of nesting males, we ran a GLMM with a binomial error. We did not include body length in this model because we did not measure length for males that died during the study. To determine whether habitat of origin or water flow affected the change in body weight of surviving males during a nesting cycle, we used an LMM. Because males were in nesting channels for differing amounts of time depending on how long it took them to build a nest (see next section) we also ran this model including the time taken to complete a nest

(total time to reach stage 3) as an additional covariate. If the treatment effect changed after controlling for the construction time, it would suggest that differences in change in body weight arise owing to variation in time spent building a nest.

(ii) Nest-building success

To test how habitat of origin and water flow treatment affect the ability of males to build and maintain a nest, we ran separate GLMMs on a series of nest-building steps. These steps included: the likelihood of building a nest (binomial); the time taken to initiate nest construction (i.e. time taken to reach stage 2 of nest construction) (negative binomial error); the time taken to complete a nest after building had commenced (i.e. time taken from stage 2 to stage 3) (binomial); and whether the nest remained intact until the end of the trial period (binomial). We analysed the time to complete a nest using a binomial error because in the majority of trials (129 out of 150) it took males less than one day to compete the entrance of his nest. Body length data was not collected for non-nesting males during the experiment, so body length was not included in our analysis of likelihood of building a nest.

(iii) Nesting behaviour

Prior to testing the effects of habitat of origin and water flow on nesting behaviour we conducted a principal components analysis (PCA) to reduce some of our interrelated behavioural measures (proportion of time spent at the nest, rate of gluing and proportion of time spent tending the nest) into a composite trait (nesting behaviour). Prior to running this PCA we scaled the component variables, checked their distribution and transformed them when necessary to meet assumptions of normality, ensuring that skewed data, or data measured on different scales, did not bias the results of the PCA. The likelihood of 'creeping-through' and the likelihood of collecting nesting material were not included in this PCA because their binomial distribution did not permit transformation. This PCA produced one eigenvector with an eigenvalue greater than one. This eigenvector explained 78.6% of the variation in the data. All component traits loaded positively on this eigenvector, so high values of nesting behaviour represent a general increase in investment in nesting (see the electronic supplementary material for a full summary of PCA results). We then used an LMM to test the effects of habitat of origin and water flow treatment on nesting behaviour, and separate GLMMs with binomial errors to test the effects on the likelihood of creeping-through and the likelihood of collecting nest material.

(iv) Nest location and orientation

To test whether water flow treatment or habitat of origin affected where males built their nests, we conducted a two-step analysis. First, we assessed whether males from different habitats (lakes or rivers) adjusted their nesting location in response to flow treatment by comparing the probability of building a nest in one of three locations (closest, middle, furthest; figure 3) under static and flowing water conditions using χ^2 goodness-of-fit tests. We used Monte Carlo simulation with 2000 replicates to calculate *p*-values and set the significance level at $\alpha=0.05$ (two-tailed). Since there was no significant effect of water flow treatment on nest location among males within each habitat type (see Results), we pooled the data from flowing and static water trials for the second step of our analysis, which aimed to test for an effect of habitat of origin on nest location. In the second step, we determined whether the distribution of nests across the three zones differed between males from lakes and rivers, as well as whether each differed from a random distribution (i.e. 33.34% for each location). Body length was not included in this analysis.

For nest orientation, we used a Bayesian mixed-effects model with circular data, implemented in the *bpnreg* package (see [82]

for details). We included habitat of origin, water flow treatment, and body size (standardized) as separate fixed factors, and population ID as a random factor. We estimated the mean orientation angle and its 95% highest posterior density (HPD) interval for each group. If the HPD intervals for each group overlapped, it indicated that the circular means did not differ significantly between groups, and there was no strong effect of the treatments on nest orientation.

(v) Nest characteristics

Before testing the effects of habitat of origin and water flow treatment on nest characteristics we used three PCAs to reduce the various nest measurements into composite traits that best explained the variation in our data. Prior to running each PCA we scaled each variable, checked their distribution and transformed them to meet assumptions of normality when necessary. The first PCA incorporated measures that quantified the different nesting materials used to build nests (sand mass, gravel mass, filter wool mass, number of threads, number of bristles). This PCA produced two eigenvectors with eigenvalues greater than one that we used in subsequent analyses. The first (PC1), explained 38% of the variance in our data and was negatively correlated with all the component traits included in the analysis; thus, PC1 can be considered an inverse measure of overall 'nest mass'. The second (PC2), explained 24% of the variance in our data and was negatively correlated with the number of threads and number of bristles incorporated into the nest, but positively correlated with sand weight, gravel weight and filter wool weight. Hence, PC2 represents the trade-off between bulky materials that weigh the nest down and more buoyant materials that knit the nest together. We call this eigenvector 'nest composition'. The second PCA we ran incorporated different measures of nest size (total nest area, bulk area and nest weight). This PCA produced a single eigenvector (PC1) with an eigenvalue greater than one. PC1 explained 72% of the variance in our data and was negatively correlated with all measures of nest size included in the analysis. Therefore, this PC can be considered to represent an overall (inverse) measure of 'nest size'. The third PCA incorporated measures of nest shape (nest neatness, nest compactness, total elongation and total roundness). This PCA produced two eigenvectors with an eigenvalue greater than one. PC1 explained 48% of the variance in our data and was negatively correlated with nest neatness, nest compactness and total elongation but positively correlated with total roundness. High values of 'nest shape PC1' indicate rounder nests with lower neatness and compactness, while PC2 explained 39% of the variance in our measures of nest shape and was positively correlated with all component variables except total elongation. High values of 'nest shape PC2' indicate rounder nests with high neatness and compactness.

Following PCA we used LMMs to test the effects of habitat of origin and water flow treatment on the resulting composite nest characteristics—'nest mass', 'nest composition', 'nest size', 'nest shape PC1' and 'nest shape PC2'.

3. Results

(a) Male survival and change in body condition

The survival of males in the study was not affected by habitat of origin, either as a main effect (binomial error, $\chi^2_1 = 0.059$, $p=0.808$) or through its interaction with flow treatment ($\chi^2_1 = 0.046$, $p=0.831$), but it was consistently lower in the flowing water treatment ($\chi^2_1 = 8.213$, $p=0.004$). Of 109 males in the study, 20 (18.3%) died while experiencing the flowing water treatment, while three (2.7%) died in the static treatment.

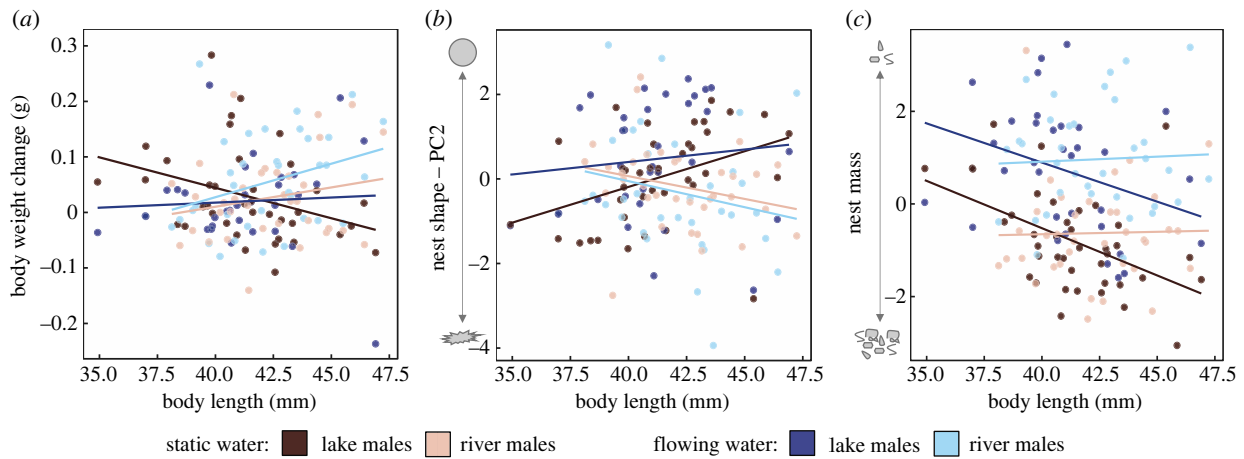


Figure 2. Effect of male body size on nesting activity. (a) Change in body weight, (b) PC2 of nest shape (see text for details), and (c) nest mass (i.e. PC1 of nesting materials). Positive values in (b) represent neater, more compact and rounder nests, while negative values represent the opposite. Positive values in (c) represent less material contained in the nest, while negative value indicates more material. Colours represent the water flow treatment (brown = static water, blue = flowing water). Darkness indicates the habitat of origin (dark = lake males, light = river males). The sample distribution and regression line in each combination of habitat of origin and flow treatment are shown.

By contrast, habitat of origin interacted with male body length to produce a marginally significant effect on the change in body weight over each nesting cycle (LMM: $F_{1,15.010} = 4.765$, $p = 0.045$), with larger river males gaining more weight than larger lake males (figure 2a). This effect persisted (LMM: $F_{1,14.924} = 5.079$, $p = 0.040$) after controlling for the difference in construction time between treatments (LMM: $F_{1,128.753} = 69.696$, $p < 0.001$; electronic supplementary material, figure S5), indicating that it was not a result of being in the treatment for differing amounts of time. Flow treatment, on the other hand, did not affect the change in body weight among surviving males (LMM: $F_{1,69.116} = 0.582$, $p = 0.448$).

(b) Nest-building success

Male habitat of origin had no effect—either as a main effect or through its interaction with flow treatment—on any of our measures of nest-building success, including the likelihood of nest building (binomial error, interaction: $\chi^2_1 = 0.064$, $p = 0.800$, main effect: $\chi^2_1 = 0.105$, $p = 0.746$), time spent initiating nest construction (negative binomial error, interaction: $\chi^2_1 = 2.039$, $p = 0.153$, main effect: $\chi^2_1 = 0.039$, $p = 0.844$), time spent completing the nest (binomial error, interaction: $\chi^2_1 = 0.007$, $p = 0.935$, main effect: $\chi^2_1 = 0.710$, $p = 0.399$), or the likelihood of the nest persisting until the end of treatment (binomial error, interaction: $\chi^2_1 = 0.001$, $p = 0.999$, main effect: $\chi^2_1 = 0.008$, $p = 0.931$). Water flow treatment, however, had pronounced effects on nest-building success. Among the 109 males, the likelihood of building a nest was much lower in flowing water compared to static water (i.e. 72% of males built nests in flowing water versus 89% in static water: binomial error, $\chi^2_1 = 7.965$, $p = 0.005$). Further, males took longer to initiate nest construction in flowing water than in static water (i.e. males took 8 days to reach stage 2 in flowing water and 6 days under static conditions: negative binomial error, $\chi^2_1 = 8.068$, $p = 0.005$). Once males began nest building, however, most completed their nests within 1 day, irrespective of water flow treatment (binomial error, $\chi^2_1 = 1.245$, $p = 0.265$). Finally, nests initiated under flowing conditions were less likely to persist until the end of the treatment period than those built under static conditions, although this effect was only marginally significant (binomial error, $\chi^2_1 = 3.855$, $p = 0.050$).

The only measure of nest-building success to be affected by nest order was the time to begin nest construction. Males took 10 days to begin building their first nest, but only 4 days to begin their second (negative binomial error, $\chi^2_1 = 59.654$, $p < 0.001$). For other non-significant effects, see the electronic supplementary material.

(c) Nesting behaviour

There was no interactive effect between habitat of origin and flow treatment on male nesting behaviour (LMM: $F_{1,70.573} = 0.164$, $p = 0.687$) or the likelihood of being observed to collect materials during the observation period (binomial error, $\chi^2_1 = 2.038$, $p = 0.153$). Similarly, no significant main effect of habitat of origin, or water flow treatment, was detected for either behavioural trait (electronic supplementary material). However, males were more likely to be seen creeping through their nest in the flowing water treatment compared to the static treatment, although the difference was only marginally significant (binomial error, $\chi^2_1 = 4.153$, $p = 0.042$).

Neither nest order nor body size had any effect on any of the nesting behaviours measured (electronic supplementary material).

(d) Nest location and orientation

The distribution of nests across the three zones did not differ between the flowing and static water treatments for either lake males ($\chi^2 = 2.477$, $p = 0.330$) or river males ($\chi^2 = 3.971$, $p = 0.151$). However, after pooling data from flowing and static treatments, habitat of origin had a strong effect on the distribution of nests throughout the nesting channel ($\chi^2 = 12.200$, $p = 0.002$). The distribution of nest locations selected by males from lakes differed from that expected by chance, with males building more often in the zone furthest from the inflow ($\chi^2 = 30.464$, $p < 0.001$; figure 3). By contrast, the distribution of nests built by males from rivers did not differ from random ($\chi^2 = 4.778$, $p = 0.099$).

Water flow treatment had a strong and uniform effect on nest orientation. When building under flowing water conditions, males built nests with entrance holes facing towards the outflow, at an approximate angle of 180° to the inflow (figure 4), which required the males to swim against the

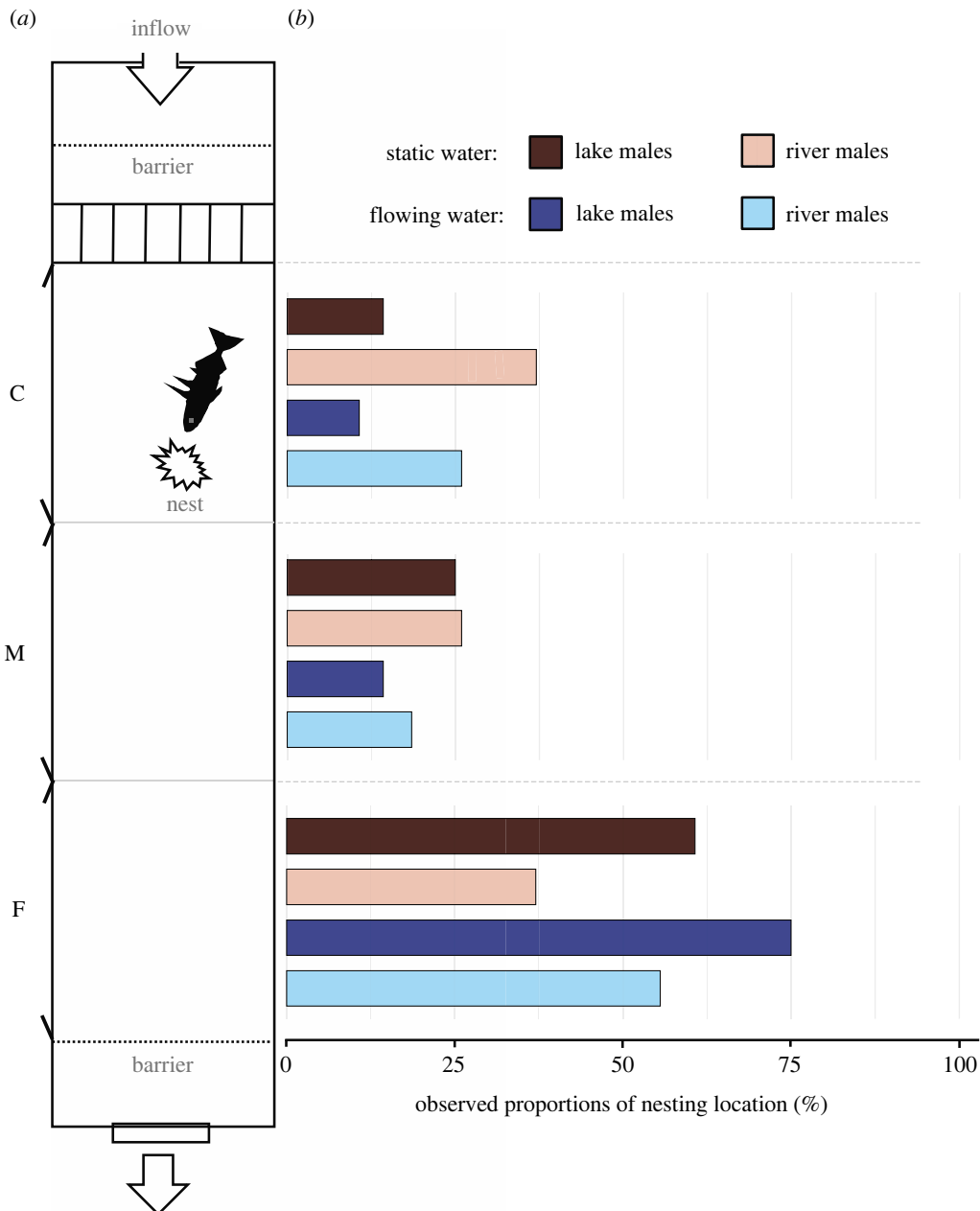


Figure 3. The effects of habitat of origin and water flow treatment on nest site location. (a) A diagram of the nesting channel divided into three zones: furthest (F; with a gravel substrate), middle (M; including the Petri dish with sand), and closest (C; with a gravel substrate) relative to the location where the water flowed into the channel (i.e. inflow). (b) Histogram showing distribution of nest sites chosen by males from lake and river populations building under flowing and static water treatments. Colours represent the water flow treatment (brown = static water, blue = flowing water). Darkness indicates the habitat of origin (dark = lake males, light = river males).

flow to enter their nests. There was no effect of habitat of origin on nest orientation. The mean angle of nest entrances made by lake-dwelling males was estimated at 178° (95% HPD intervals: 170° to 195°) in flowing water, which did not differ from that of the river males in flowing water (mean = 175° ; 171° to 199°). Under static conditions, nests were far less uniformly oriented, and again the mean values of nests built by males from lakes (100° to 356°) and rivers (119° to 46°) overlapped. Body size did not have an effect on nest orientation (electronic supplementary material, figure S7).

(e) Nest characteristics

There was no interaction between male habitat of origin and water flow treatment on nest mass ($F_{1,69.395} = 0.304$, $p = 0.583$), nest composition ($F_{1,68.683} = 0.752$, $p = 0.389$), nest size

($F_{1,69.030} = 2.146$, $p = 0.148$) or nest shape (PC1: $F_{1,68.449} = 0.001$, $p = 0.978$; PC2: $F_{1,67.938} = 2.366$, $p = 0.129$). Male habitat of origin also had no main effect on any of our composite nest characteristics (electronic supplementary material). However, there was an interactive effect of habitat of origin and male body size on 'nest shape PC2'. This interaction shows that nests built by larger males from lakes were neater, more compact and rounder than those constructed by larger males from rivers (body length*habitat interaction on nest shape PC2: $F_{1,15.782} = 5.044$, $p = 0.039$; figure 2b).

Water flow treatment, on the other hand, had strong effects on all composite nest characteristics except 'nest composition' ($F_{1,70.020} = 0.949$, $p = 0.333$) and 'nest shape PC2' ($F_{1,69.126} = 0.796$, $p = 0.375$). Nests built in flowing water contained less material ('nest mass': $F_{1,70.479} = 89.573$, $p < 0.001$; figure 5a), and were smaller than those built under static conditions

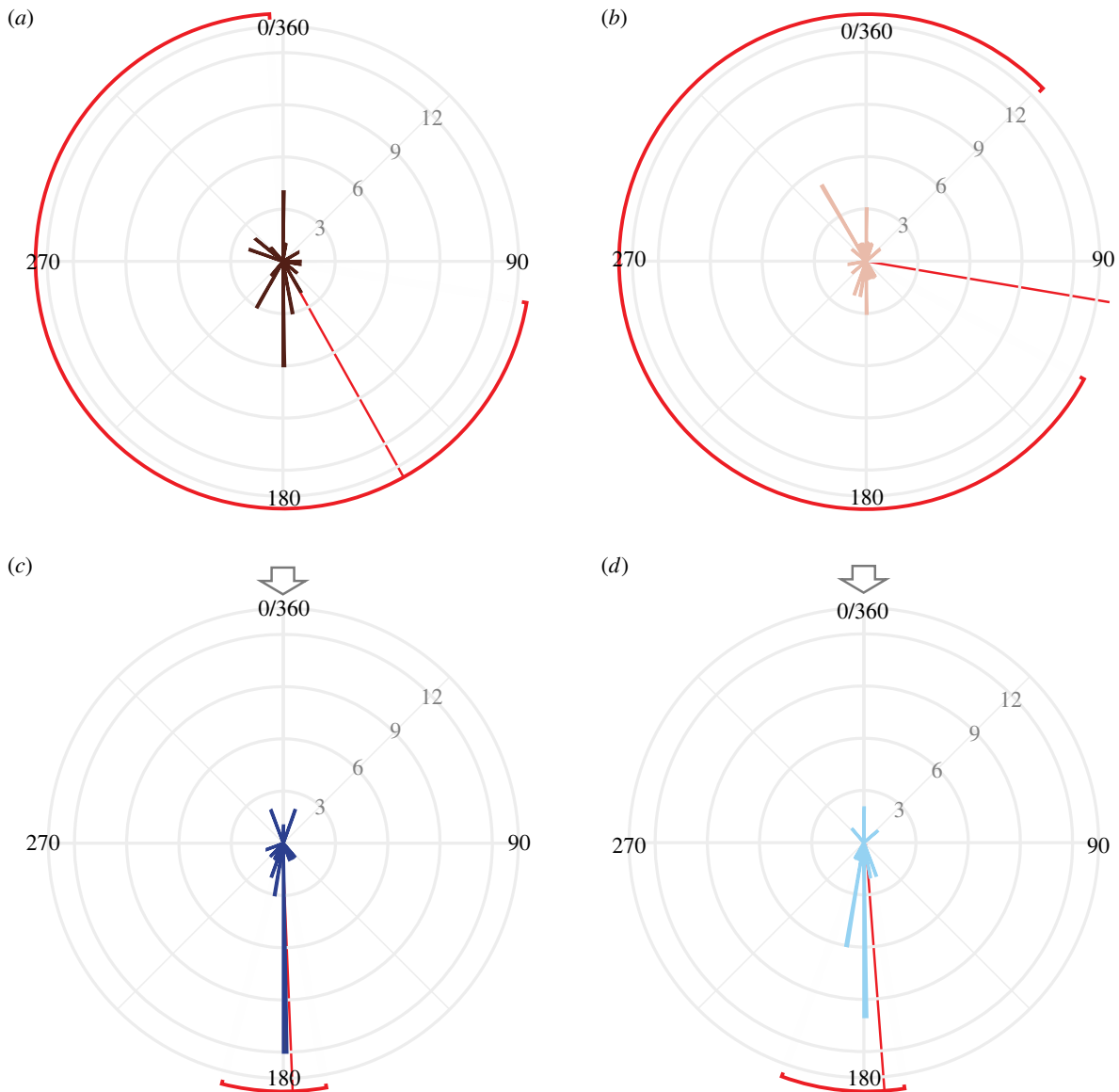


Figure 4. Circular plots illustrating the effects of habitat of origin and water flow treatment on orientation of nests built by male sticklebacks. (a) Lake males building in static water; (b) river males building in static water; (c) lake males building in flowing water; and (d) river males building in flowing water. Colours represent the water flow treatment (brown = static water, blue = flowing water). Darkness indicates the habitat of origin (dark = lake males, light = river males). Lines represent orientation angles (0–360°), and the length of each line indicates the number of observed nests (ranging from 0 to 12). Arrows indicate the direction of water flow (in flowing water treatments). Nest orientation was determined by observing males creeping through the nest. A 180° orientation indicates that the nest entrance faced the outflow; an orientation of 360° or 0° indicates that the nest entrance faced the inflow. Mean and 95% highest posterior density generated by Bayesian mixed-effects model are shown in a red colour.

(‘nest size’: $F_{1,70.227} = 72.248$, $p < 0.001$; figure 5b). Nests built in flowing water were also more compact, neater and more elongated than those built under static conditions (‘nest shape PC1’: $F_{1,69.491} = 72.721$, $p < 0.001$; figure 5c).

Larger males built nests containing more material than smaller males (‘nest mass’: $F_{1,51.600} = 4.467$, $p = 0.039$; figure 2c). Finally, there were no effects of nest order on any of the nest characteristics (see the electronic supplementary material).

4. Discussion

Since water flow can determine the functionality of nest structure and the effectiveness of nest-building behaviours (e.g. [83,84]), it is plausible that stickleback males plastically adjust their nest structure according to ambient water flow

regimes. Such plasticity might be expected to be adaptive in situations where flow regimes exhibit temporal and/or spatial variation during the breeding season; conversely, less variable habitat types might select for greater specialization and lower levels of plasticity. Previous studies have shown genetic divergence among stickleback populations in a wide range of traits in relation to environmental variation (e.g. [85–88]) and there is evidence of population divergence in mating behaviour [89], raising the possibility that the habitat of origin might also influence a male’s nest-building plasticity under varying conditions of water flow. To test this, we experimentally manipulated water flow experienced by the same males from three river and lake populations to examine their nesting plasticity. We predicted that nest-building success (i.e. the likelihood of successfully building and maintaining a nest, and the speed of constructing a nest) would generally be lower in flowing water [34,37,44], and

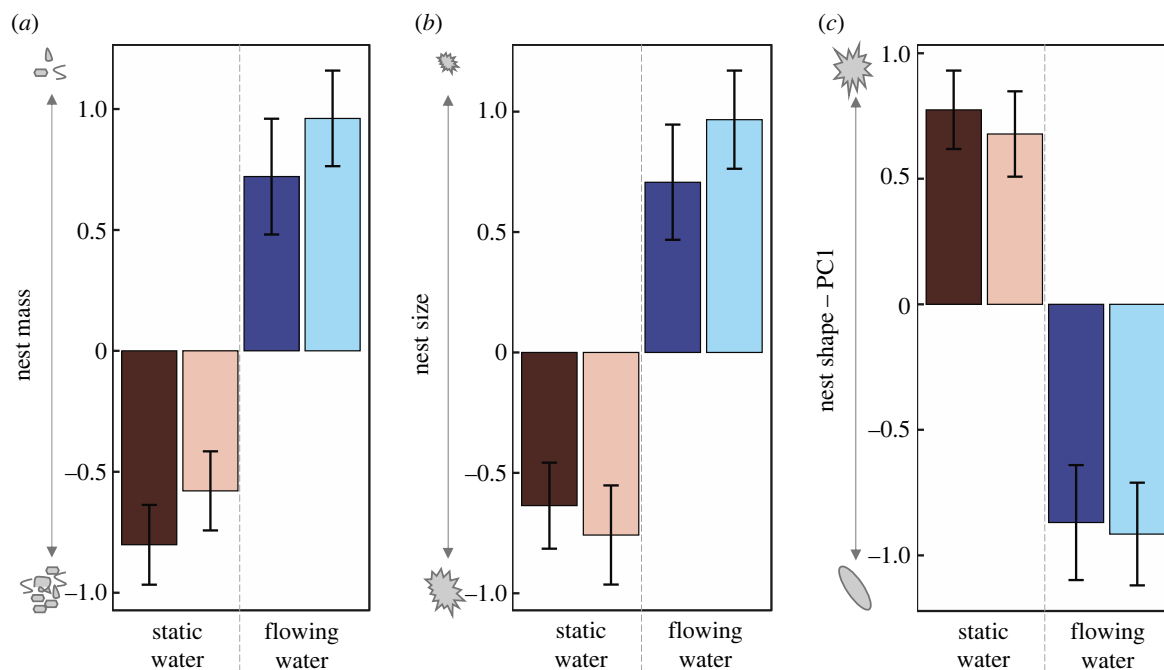


Figure 5. Effects of habitat of origin and water flow treatment on (a) nest mass (i.e. PC1 of nesting material): positive values represent less material, while negative values represent more material, (b) nest size (i.e. PC1 of nest size): positive values indicate smaller nests, while negative values represent bigger nests, and (c) PC1 of nest shape: positive values represent a rounder, messier and less compact nest, while negative values indicate a more elongated, neater and more compact nest. Dark brown = lake males in static water, light brown = river males in static water; dark blue = lake males in flowing water, light blue = river males in flowing water. Error bars indicate mean \pm s.e. Detailed statistical analyses are provided in the electronic supplementary material.

that males would adjust their nest building behaviour and nest characteristics in flowing water to minimize energy expenditure and avoid nest destruction. Given that water velocity varies more in rivers than in lakes, we additionally predicted that males from rivers would exhibit greater plasticity in nesting behaviour to suit ambient flowing water conditions than males from lakes, which we predicted would build more uniform nests.

Our results showed that under flowing water conditions, male sticklebacks experienced higher mortality, were less likely to build a nest and when they did, the males took longer to initiate the construction process, which is likely to reduce their overall reproductive output in the restricted breeding season [90,91]. This increased nest-building effort, however, did not result in greater structural stability; nests built in flowing water were less likely to persist until the end of the treatment period, most likely owing to 'washout' (e.g. [92]). These results suggest that building a nest under flowing water conditions is more challenging than under static water conditions. The relatively high mortality rate in the flowing water treatment might be attributed to the exposure to constant flow during the energetically demanding nest building phase. By contrast to previous studies [44] that used artificial rocks to generate low-flow areas, we aimed to minimize heterogeneity in water current under flowing water. Despite the strong effects of ambient water flow on nest building and nest structure, however, we found very little evidence for divergence in these traits between river and lake populations. The only nesting variable measured that differed between males from rivers and lakes was where they built their nest; lake males were more likely to nest away from the inflow area, whereas the nests of river males were randomly distributed throughout the nesting channel. Habitat of origin was also associated with

male change in body weight while nest building, but only in interaction with initial body size; larger males from rivers gained more weight than larger males from lakes during nesting trials. Further, we found no evidence that river males were better able to adjust their nest building to flowing water conditions than lake males (i.e. we found no interactive effects). Males from both habitat types exhibited equivalent adjustments in nest building behaviour, both qualitatively and quantitatively, under the two flow regimes imposed. In summary, while ambient flow conditions clearly have a significant impact on nest-building activities, there is little evidence to suggest divergence between river and lake populations in their capacity to adapt nesting behaviour and nest structure to changes in flow regime.

In their natural flowing water habitats, fish from rivers tend to avoid nesting in strong currents [66,83] and prefer nest sites close to the river's edge [41], because it is likely to reduce the energy required for locomotion and it allows fish to evade sudden changes in ambient velocity. On the other hand, male sticklebacks under static water conditions display weaker preferences for specific nesting locations [44]. Here, we expected that males nesting in flowing water would prefer to nest away from the water inflow (where the water current may be more turbulent) and on gravel substrates which may be less likely to wash away. Further, if males from rivers are better able to adjust their behaviour in response to water flow variation, we predicted to see this response for river males but not lake males. By contrast to our expectation, we found no effect of water flow treatments on the distribution of nests within the nesting channel. We did however, find an unexpected effect of habitat of origin, with males from lakes being more likely to build nests in the zone furthest from the inflow. The fact that lake males were more likely to build nests farthest from the inflow in

both still and flowing water makes this result difficult to explain. One potential explanation that is unrelated to the water flow treatment is that males were choosing nesting locations based on substrate type. However, this also seems unlikely given that lake males have previously been shown to prefer sandy substrates for nesting [68] and the lake males here, built their nests predominantly in only one of the two available zones that had a gravel substrate.

As in previous studies (e.g. [44]), our study revealed pronounced changes in nest characteristics built by the same males between flowing and static water conditions, that align with the functional requirements of nest design. For instance, the positioning of nests built in flowing water meant that the nest entrances were oriented so that males faced the current when fanning. Although this nest orientation might make fanning less efficient, it could be an adaptation to protect the nest from washout (if the nest opening faced upstream into the inflow current, then it is possible that the flowing water would 'catch' in the entrance and tear the nest from the substrate) [42] and may provide males with better positional control while fanning and tending the entrance of their nest. Another possibility is that olfactory signals (which are considered long-distance signals in flowing water; [93]) released from the nest and flowing downstream might serve as a form of sexual communication, guiding the female directly to the nest entrance (e.g. [94,95]). In flowing water, males also increased the neatness of their nests, which is consistent with previous research that has highlighted the role of spiggin in nest construction under strong currents [44,62]. Additionally, the finding of increased nest compactness in flowing water may provide better insulation against outside environmental stressors, while nests in static water tended to be looser and perforated to increase dissolved oxygen levels [96]. Finally, we found that males built smaller and more streamlined nests in flowing water, which has previously been suggested to reduce drag and increase resistance to water flow [44], in line with principles of fluid dynamics [97]. An alternative explanation for the observed changes in nest design is that the physical strength of water flow reshaped the nest or washed parts of the material away. However, this explanation is unlikely to account for all the differences that we see. For instance, increased compactness and neatness, require males to adjust the amount of spiggin used to incorporate loose threads into the nest. This suggests that males exhibit adaptive plasticity in response to flow environments. Moreover, we observed that males performed more nesting behaviours and were more likely to creep through their nests in flowing water, which may help to maintain a proper nest structure for spawning under strong water current conditions.

Our results provide further support for the hypothesis that male sticklebacks can respond to changes in flow conditions by adjusting their nest characteristics and relevant behaviours. However, in contrast to our predictions, males from both habitats appeared to be equally capable of adjusting their nest-building behaviours to prevailing water flow conditions. This is surprising given the expectation that river-dwelling males should be better adapted to cope with flowing water conditions owing to the energetically demanding nature of nest construction and the physiological demands of maintaining performance [61,62,91,98,99]. This capacity could arise through genetic adaptation, transgenerational plasticity and/or early-life environmental factors

[100–102], but we were unable to differentiate between these mechanisms using wild-caught individuals in this study. Conducting common garden experiments across generations exposed to similar flow environments could help to disentangle the potential roles of evolutionary divergence and phenotypic plasticity (e.g. [103]). Nevertheless, we found no interaction between habitat type and flow treatment, suggesting that these mechanisms were unlikely to play a major role in driving the plasticity. Alternatively, gene flow, arising through migration from an adjacent but divergent habitat types or the transfer of fish by wild birds between water bodies, might prevent local adaptation [104] and result in similar nesting responses between lake- and river-dwelling individuals. This could be verified through genetic data. It should be noted, however, that the lake and river populations used in our study have been separated for at least 10 000 years [73]; therefore, for the plastic nest-building response to flowing water to be conserved through gene flow, a modest rate of migration would need to occur in all three lake populations, along with little 'maintenance' cost of the plasticity in lake habitats [105].

On the other hand, the conservation of plasticity in lake populations could reflect the importance of being able to manipulate nest structure to optimize fitness in response to variation in a range of environmental variables, such as optimizing oxygen availability [45,106] or predation risk [107,108]; or it could be that local currents generated by wave action or wind still generate selection for plasticity in response to flow even in otherwise static lake habitats. Taken together, our results show that stickleback fish from both river and lake habitats are capable of detecting and adjusting their nesting behaviour to changes in flow regimes. An interesting next step would be to investigate whether the adjusted nest designs correlate with improved breeding success, through increased female attraction [109] and/or better incubation of eggs and embryos [18] within their respective environments.

Plasticity of reproductive traits is often influenced by a male's characteristics and/or available resources. For instance, smaller males are often more sensitive to environmental cues and exhibit greater plasticity of reproductive traits, such as nest defence [110] and sneaky mating [111], because of fewer opportunities to mate [112], lower dominance [113] and higher risk of depleting energy reserves [45,114]. In sticklebacks, larger males are often preferred by females [54,60], are better at protecting young [115], and tend to build larger nests [71,77]. Consistent with this, our experiment revealed that larger males, regardless of the water flow conditions they built their nest in, or the habitat they originated from, incorporated more material into their nests than smaller males, probably improving the stability and protective value of the nest (e.g. [116]). However, we did not find body size-dependent adjustments to nest structure in response to water flow. Instead, larger males from lakes consistently built rounder, neater and more compact nests across flow treatments than larger males from rivers, but such a difference was not evident among smaller males. In other words, body size differences between populations may be key in determining the ability to mount effective plastic responses to nest building under different flow conditions. Notably, larger males from rivers appeared to gain more body weight during a nesting cycle, indicating that they may have innate advantages that enable them to thrive

under the experimental conditions tested. This suggests that they may be better equipped in general to adapt to variable flow patterns associated with a changing climate.

To conclude, freshwater ecosystems are among the most heavily impacted environments affected by anthropogenic activities, which frequently manifest as changes to natural patterns of water flow. This study sheds light on the implications of environmental change for the ability of nest-building fishes to adapt to these altered flow regimes resulting from a variety of human activities [117]. Our results reveal that sticklebacks from post-glacial lakes—which have not experienced directional flow for thousands of generations—are equally capable of detecting and adjusting their nesting behaviour in response to changes in flow regimes as stickleback from rivers. This suggests significant residual capacity to exhibit plastic nest-building responses. Overall, our study underscores the potential for organisms to respond and adapt to changes in their environment, even in the face of prolonged periods of environmental stability.

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