Behav Ecol Sociobiol (2010) 64:1927–1935
DOI 10.1007/s00265-010-1003-3

ORIGINAL PAPER

Flow regime affects building behaviour and nest structure in sticklebacks

Ben J. Rushbrook · Megan L. Head ·
Ioanna Katsiadaki · Iain Barber

Received: 6 January 2010 / Revised: 25 May 2010 / Accepted: 16 June 2010 / Published online: 20 July 2010

Abstract Animals usually build nests to provide protection, for themselves or their offspring, from adverse environmental conditions. However, different nest structures may be better at providing protection in different environments. The ability to adjust building behaviour and design of nests in response to variation in environmental conditions is therefore likely to be important in determining individual fitness. Here, we look at how the nests of three-spined stickleback (*Gasterosteus aculeatus*) males differ between still and flowing water conditions. Within flowing water treatments, we find that males select nesting sites with lower than average flow. We also find that nests built in flowing water are smaller and more streamlined than those built in still water. Nests built in flowing water contain more spiggin—an endogenous glycoprotein secreted by nesting males—per gramme of nest material than still water nests, though the absolute spiggin content of nests did not vary with flow regime. These results suggest that male sticklebacks may adjust nest-site selection and nest structure to suit environmental conditions in which they are building. Because flow regime is a factor that is often altered by anthropogenic activities, such as impoundment, channelization and abstraction, this study may also have implications for the conservation of freshwater fish populations.

Keywords Nest building · Stickleback · Animal construction · Spiggin · Phenotypic plasticity

Introduction

Nest building is a taxonomically widespread and important reproductive behaviour that has direct fitness consequences for both nest builders and their offspring. Nests function to protect developing embryos and young from adverse environmental conditions (Hansell 2000), thus providing a safe and suitable environment for offspring development. The specific functions that nests perform to increase offspring fitness are many and varied. For instance, nests may prevent desiccation (Biju 2009), aid in thermoregulation (Bult and Lynch 1997; McGowan et al. 2004), or provide protection from predators (Spencer 2002; Peluc et al. 2008) and pathogens (Little et al. 2008; Fleming et al. 2009).

The structure and design of nests are inextricably linked to the environment in which they are found. Thus, to maximise reproductive success, nest building behaviour, like other reproductive behaviours, must be adjusted to suit local environmental conditions (Candolin and Salesto 2006). There is increasing evidence that nest builders can influence the functional capacity of their nests by altering building behaviour in response to varying environmental conditions. As a consequence, nests may exhibit plasticity or variation in structure, design, and location in response to both biological and abiological environmental variables. For example, in tree pipits *Anthus trivialis*, the orientation

Communicated by K. Lindström

B. J. Rushbrook · I. Barber
Institute of Biological, Environmental and Rural Sciences,
Aberystwyth University,
Aberystwyth, UK

M. L. Head · I. Barber (✉)
Department of Biology, University of Leicester,
Adrian Building, University Road,
Leicester LE1 7RH, UK
e-mail: ib50@le.ac.uk

I. Katsiadaki
Cefas Weymouth Laboratory,
Weymouth, UK

of nests with respect to the sun influences thermoregulation and hatching success of offspring. As a consequence, nest builders adjust the orientation of nests throughout the season to optimise solar radiation (Burton 2006). Likewise, nest builders can alter the location of nests, and many animals adjust their nest-site selection behaviour in response to environmental factors, such as increased predation risk (Spencer 2002; Eggers et al. 2006).

In aquatic environments, one of the primary environmental factors that may influence the functional capacity of nests is water velocity (Mori 1995; Dauwalter 2007; Grabowski and Isely 2007). Living in flowing water habitats such as rivers and streams can impose high costs on organisms inhabiting them, and these organisms have evolved many behavioural (Asaeda et al. 2005; Park and Park 2005) and morphological adaptations (reviewed in Langerhans 2008) to overcome these costs. For fish that build nests in flowing water, one of the principal risks they face is the loss of nests as a result of washout during periods of high flow (Lukas and Orth 1995; Mori 1995). Additionally, building in areas of high flow may incur increased energetic costs associated with collecting and manipulating nesting material in strong currents. Therefore, decisions about nest location and nest design may reflect an attempt by nest builders to trade off the benefits (i.e. increased nest ventilation (Järvi-Laturi et al. 2008) and silt-free substrate (Johnston 2001)) and the costs of building in flowing water. For example, Vinyoles et al. (2002) demonstrated that male river blennies (*Salaria fluviatilis*) altered the orientation of their nest entrance in relation to water velocity, so that water velocity at the nest entrance was similar across nests, regardless of prevalent current speed. Fish may also adjust the design or structure of the nest to make them more streamlined or resilient to flow (Noltie and Keenleyside 1987; Bruton and Gophen 1992), or preferentially select areas of low flow to avoid the costs of nesting in flowing water (Dauwalter 2007; Castello 2008).

Three-spined sticklebacks (*Gasterosteus aculeatus*) are small, nest-building fishes that have colonised a wide range of flowing and still water habitats across the northern hemisphere (Wootton 1976). Male sticklebacks build nests that function as a focus for courtship and a receptacle for eggs. Nest building in this species involves digging a pit in the sediment, followed by the collection and gluing together of sediment and plant material to form a mat within the pit (van Iersel 1953). The male then creates a tunnel in this mass through which the female and male pass during spawning (Wootton 1976). The nesting glue, named spiggin, is a 203-kDa cysteine-rich glycoprotein (Jakobsson et al. 1999) that is synthesised in the kidney of male sticklebacks and secreted via the cloaca.

The nests of three-spined sticklebacks show considerable variation in structure, design and location between geographically separate populations (reviewed by Rowland 1994; Rushbrook and Barber 2008), between sympatric populations occupying different ecological niches (Olafsdottir et al. 2006; Rushbrook and Barber 2008; Raeymaekers et al. 2009), and between individuals within populations (Kraak et al. 2000; Barber et al. 2001). Population-level differences in nest building may potentially arise from adaptive divergence in behaviour (Foster 1999), from plastic responses to variable environments (Bruton and Gophen 1992), or from environmental constraints such as the availability of nesting materials or substrate types (Ochi et al. 2001).

Here, we investigate the effects of water flow on the structure, shape and nest-site selection of male sticklebacks. Previous work on second-generation lab-reared fish from the Misty Lake/River system in British Columbia has suggested that differences in nest structure and nest-site selection of stickleback from lake and river populations have a genetic component (Raeymaekers et al. 2009). However, little is known about environmental effects on stickleback nest-building behaviour. We predict that, if male sticklebacks are capable of adjusting nest-building behaviour in response to altered flow regimes, nests built in flowing water should be smaller and more streamlined than those built in still water. Likewise, if nest building in flowing water is costly, we predict that males will build their nests in areas of low flow.

Methods

Fish collection and husbandry

Adult male and female three-spined sticklebacks were collected from the Afon Ystwyth, mid-Wales, UK (N52°23′55″ W4°05′08″) during June–July 2006. This is a broad coastal river with water velocities during the time the fishes were collected, ranging between 1.6 and 20.7 $\mathrm{cm\,s^{-1}}$ (measured at four distances to the bank across eight sites on two occasions, separated by 1 week in June 2006). Fishes were held in mixed sex groups in laboratory stock tanks (750 mm×200 mm×380 mm) at 18±1°C, under a 16-h light:8-h dark photoperiod, for 5 days prior to transfer to nesting pools. During this period, males were fed ad libitum a mixed diet of frozen bloodworm (chironomid larvae), live whiteworm (*Enchytraeus* sp.) and *Daphnia* sp.

Nesting arena tanks

Two circular nesting arenas constructed from rigid-walled wading pools (see Fig. 1) were filled to a depth of 20 cm. Flow conditions were generated by directing the output of a

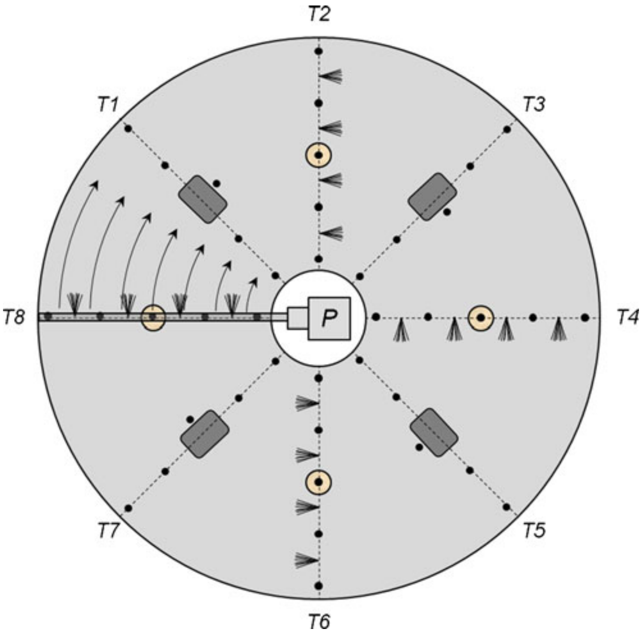

Fig. 1 Diagram of the arena tanks used in the study. Wading pools (1.5 m diameter) were fitted with a central island that housed an 8,000-Lh^{-1} water pump (*P*), the outlet of which could be directed into a spray bar to produce a circular current. By turning a valve, pools could be switched between the 'static' treatment (with the pump running, but output directed into the island) and the 'flow' treatment. Flow rates were measured at five equally spaced points along each of eight notional radial transect lines (*T1–8*). To generate heterogeneous flow regimes when the pools are operated under flow conditions, artificial 'rocks' (gravel-filled plastic containers, 140×100×100 mm) were added to both pools at the centre of *T1*, *T3*, *T5* and *T7*. In addition, sand was provided in plastic dishes (Ø=90 mm, 15-mm deep) sunk into the gravel between the artificial rocks. Two clumps of 50 nesting threads were placed on either side of each dish to provide material for nest building

high-capacity water pump, housed within the central island of the tank, into the nesting arena via a perpendicular spray bar. Still conditions were generated within the arena by recirculating the pump output back into the island, ensuring that temperature (19.5±1°C) and background noise levels were similar in the two treatments. Artificial rocks and an excess of nesting materials (sand and polyester threads) were made available throughout the arena.

Current velocity for the 'flow' treatment was quantified at five evenly spaced sample points along eight radial transect lines using an electromagnetic flow meter (Model 801, Valeport Ltd, Totnes, UK). The mean of three 30-s timeframe readings at each point was calculated. Flow rates at equivalent positions in the two pools did not differ significantly (paired *t* test: $t_{40}=-0.03$, $p=0.973$), and the pools offered the same distribution of current velocities (Kolmogorov–Smirnov two-sample test: $p=0.905$). Current velocity was lowest close to the central island and increased across the channel, with artificial rocks generating areas of reduced flow immediately in their wake.

Nest-building trials

We tested the effects of flow environment on nest construction by encouraging individual males to build nests under both flowing and still regimes (in random order), each experienced for a period of 7 days. At the start of each trial, four males expressing nuptial coloration were weighed (initial mass, M_I: to 0.001 g), measured (initial standard length, L_I: to 1 mm) and dorsal spine-tagged (Barber and Ruxton 2000) prior to being introduced as a 'quartet' into one of the nesting pools. To encourage nest building, four gravid females were presented, for 10-min periods twice a day, in glass jars spaced evenly throughout the pool. Males were observed during female presentations to determine nest ownership. The current velocity at any new nest site was then recorded, and the distance of new nest sites to the centre of the downstream edge of the closest upstream rock, and to the centre of the nesting tank, was measured.

Each day, after recording new nest details, the pumps were switched off briefly to allow feeding and visual inspection of the nests. Nests with a visible entrance, or where the building male had been observed 'creeping through' during female presentations (van Iersel 1953), were considered complete. These nests were photographed in situ from above, carefully removed on an acetate sheet and stored at −80°C in a ziplock bag for subsequent quantitative analysis of spiggin content (see below). To prevent nesting material from becoming a limiting resource, extra threads were added to the pools when nests were removed, and on day 3 of each 7-day period.

After female presentations and nest inspections were completed on day 7, all nests were removed, and nesting threads were replaced. The current regimes in each pool were then switched so that fish previously experiencing flow conditions were subjected to still conditions, and vice versa. Female presentations in the switched treatment continued on day 8. The daily protocol detailed above was then followed for 7 days further. On completion of the final set of female presentations and nest inspections, all males were removed, and pools were drained, cleaned and had conditions reset. In total, eight quartets (i.e. 32 males) were exposed to both flowing and still water treatments in the study.

Analysis of nest structure

Individual males sometimes completed more than one nest under one flow regime, and all completed nests were removed from pools and analysed. However, as nest structure in sticklebacks is highly repeatable under constant conditions (Rushbrook et al. 2008), results for nest structure presented here are based only on each male's first completed nest in each flow treatment, to avoid pseudoreplication. Nest measurements and shape indices were obtained from in situ nest images using ImageTool 3.0 (available at http://ddsdx.uthscsa.edu/dig). Nest compactness was calculated as the bulk area of the nest divided by its total area (Barber et al. 2001). Nest area and elongation (i.e. length of the major axis divided by the minor axis) were calculated from the area of the concave polygon that enclosed threads that were either fully intertwined within the nest, or where both ends had been actively incorporated into the nest (Rushbrook et al. 2008).

Spiggin content of nests

Nests were thawed and blotted before being weighed (nest wet mass, M_N, to 0.001 g). Each was then placed in a 10-ml plastic tube with 2 ml of spiggin buffer (Katsiadaki et al. 2002), heated to 80°C for 30 min to denature the spiggin, and centrifuged at 3,000×*g* for 15 min. One millilitre of spiggin extract from each sample was then processed using the spiggin-specific enzyme-linked immunosorbent assay protocol (Katsiadaki et al. 2002; Rushbrook et al. 2007). Spiggin content is expressed as units of extracted spiggin per gramme M_N.

Statistical analysis

Wilcoxon matched pair, signed-ranks tests were used to test the effect of flow regime on the number of nesting attempts made, the number of nests completed and the number of males within each quartet that completed nests. For males that completed nests under both still and flowing regimes, the number of nests completed under each treatment was also compared using Wilcoxon matched pair, signed-ranks tests.

The length and residual mass of males that completed and did not complete nests under flow conditions were compared using Kruskal–Wallis analyses of variance (ANOVA). To test whether males nesting under flow conditions were selective in their choice of nest site with respect to current velocity, one-tailed Kolmogorov–Smirnov two-sample tests compared the distribution of current velocity at the sites of all nesting attempts with the distribution of available current velocities.

Males from four of the eight quartets completed nests under flow conditions. Data from these replicates were used to examine aspects of nest-site choice in relation to nest completion. For nests built under flowing water conditions, mixed model ANOVA was used to examine variation in flow rate at the sites of completed and noncompleted nests. Mixed model ANOVAs were also used to compare the proximity of completed and noncompleted nests to the nearest upstream rock under flowing conditions, and to determine whether flow regime had an effect on the proximity of nests to upstream boulders or to the centre of the pool. In all cases, male quartet was fitted as a random factor, and nonsignificant interaction terms were dropped from the model before retesting the main effects.

To determine the effects of flow on nest mass, spiggin content and shape, we used paired *t* tests. Prior to analysis, measures of nest mass and spiggin content were $\log_{10}$-transformed to normalise variances, and proportional nest shape characteristics (compactness, elongation and roundness) were arcsine square root-transformed.

Analyses were performed using the Minitab (version 16) statistical software package.

Results

Flow regime, nesting attempts and nest completion

A total of 97 nesting attempts were observed under still conditions; of which, 45 (46%) resulted in completed nests. Under flow conditions, 39 nesting attempts were made; of which, 16 (41%) were completed. Although more than twice as many nesting attempts were made under still conditions, nest completion rates were unaffected by flow regime ($\chi^2=0.32$, $df=1$, $p=0.57$). Within quartets, males made significantly more nesting attempts, and completed more nests, under the still than the flow treatment (Wilcoxon signed-ranks tests: attempts: $W_7=28.0$, $p=0.022$, Fig. 2a; completions: $W_7=28.0$, $p=0.022$, Fig. 2b). There was also a nonsignificant tendency for more of the individual males in each quartet to complete nests under still conditions ($W_7=25.5$, $p=0.063$; Fig. 2c).

Nine males completed nests under both treatments. Among these males, there was a nonsignificant tendency to complete more nests under still than flow conditions (Wilcoxon signed-ranks tests: $W_6=20.0$, $p=0.059$; Fig. 2d). Males that completed at least one nest under flow conditions did not differ in body length from those that only completed nests under still conditions (Kruskal–Wallis ANOVA, $H=0.66$, $p=0.42$). However, there was an almost significant tendency for flow-nesting males to have higher residual mass (i.e. be in better condition; $H=3.77$, $p=0.052$).

Nest-site selection under flow conditions

Under flow conditions, the distribution of current velocities recorded at the sites of nesting attempts did not match the distribution of available current velocities (Kolmogorov–Smirnov two-sample test, one-tailed: $p<0.001$), with males avoiding areas of highest flow (Fig. 3). The average current velocity at the sites of completed and noncompleted nests did not differ (mean±SD, completed: 6.1 ± 3.2 cms^{-1}, $n=16$; noncompleted: 6.9 ± 2.4 cms^{-1}, $n=11$; $F_{1,26}=0.52$, $p=0.48$), nor was it affected by quartet ($F_{3,26}=2.39$, $p=0.25$), and there was no significant interaction term. There was no effect

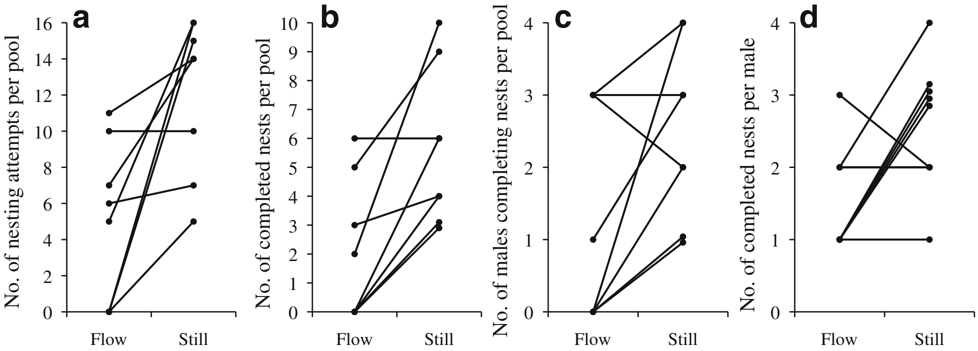

Fig. 2 Four 'reaction norm' plots showing the (**a**) number of nesting attempts, (**b**) number of completed nests, (**c**) number of males nesting and (**d**) number of completed nests per male, under the two treatments

of flow regime on the proximity of completed nests to upstream boulders (mean±SD, still: 31.4±13.3 cm, *n*=29; flow: 32.6±20.3 cm, *n*=16; $F_{1,44}$=0.37, *p*=0.55), though quartet had a significant effect after dropping the nonsignificant interaction term from the model ($F_{3,44}$=3.96, *p*=0.015). However, nests completed under the flowing regime were situated closer to the centre of the flow pool than those completed under the still regime (mean±SD, still: 51.6±11.2 cm, *n*=29, flow: 42.9±8.6 cm, *n*=16; $F_{1,44}$=6.40, *p*=0.015). For nest sites selected under flow conditions, proximity of the nest to the nearest boulder did not differ between completed and noncompleted nests (mean±SD, completed: 32.6±20.3 cms^{-1}, *n*=16; noncompleted: 23.8±19.5 cms^{-1}, *n*=11; $F_{1,26}$=2.63, *p*=0.12) or quartet ($F_{3,26}$=1.54, *p*=0.23), and there was no significant interaction term.

Effect of flow regime on nest size, shape and spiggin content

Among males that built under both regimes, the first nests built under flow conditions were smaller and had lower mass than those built under still conditions (area, t_9=2.96, *p*=0.018; mass, t_9=4.83, *p*=0.001; Fig. 4a, b). The first nests built under flow conditions were also significantly more elongated (t_9=2.76, *p*=0.024; Fig. 4c), and tended to be more compact (t_9=2.14, *p*=0.065).

Although the absolute amount of spiggin extracted per nest did not vary significantly between flow regimes (t_9=−1.54, *p*=0.161), when corrected for nest mass, the relative spiggin content of first nests built under flow conditions significantly exceeded that of nests built under still conditions (t_9=2.68, *p*=0.028; Fig. 4d).

Discussion

Environmental conditions may affect nest-building behaviour and nest design at a number of levels. First, participation in nest-building activities may be more costly in some environments than others, and this may alter how individuals invest resources in nest building. Second, different environments may select for different nesting behaviour or nest structures,

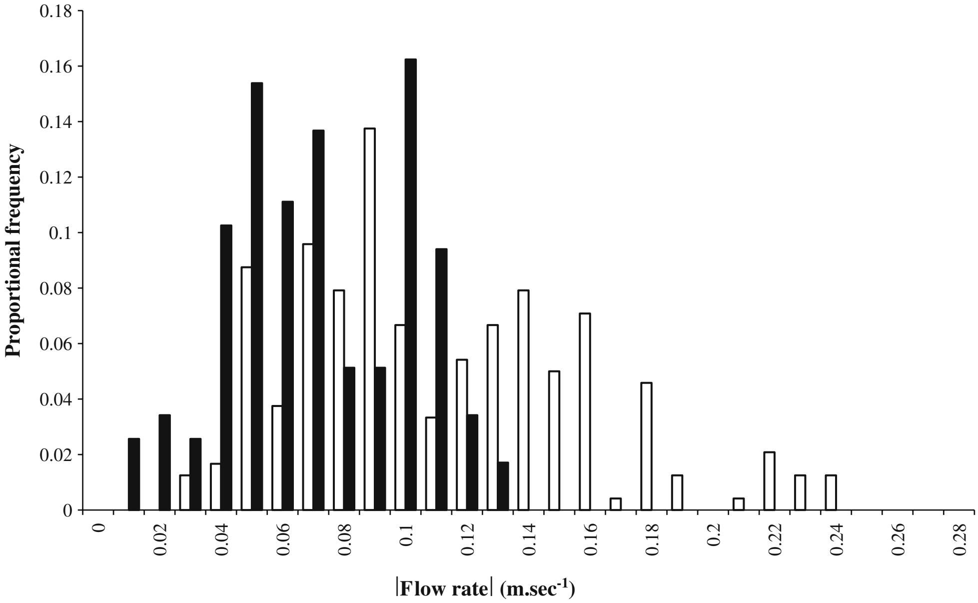

Fig. 3 Single panel, *black* and *white* histogram showing the proportional frequency of flow rates

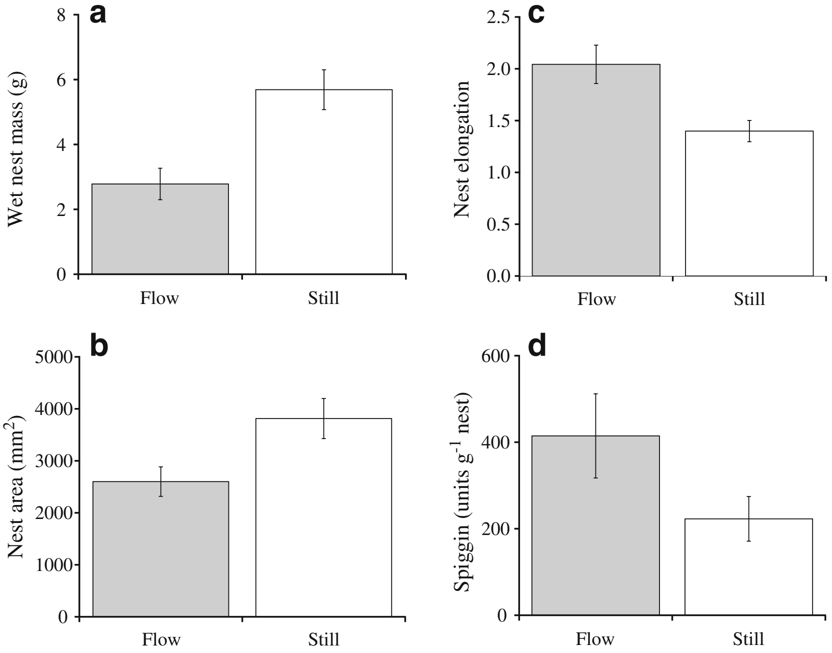

Fig. 4 Four histograms with *grey* and *white* bars showing various nest characters

so that individuals build nests to suit the conditions in which they are found. Here, we show that flow regime affects the nest-building behaviour of three-spined sticklebacks at both of these levels.

Nest building in sticklebacks is energetically costly. This is demonstrated by the fact that nesting behaviour is condition-dependent, with males kept on low food rations (Wootton 1984), and those harbouring energetically demanding parasites (Candolin and Voigt 2001; Rushbrook and Barber 2006; Macnab et al. 2009) showing reduced or absent nesting behaviour. Our results show that fewer males completed nests, and individual males completed fewer nests under flowing compared to static conditions, suggesting that building in flow exacerbates the costs of nest building. Building nests in flowing water is likely to be more costly for a number of reasons. First, swimming against a current is energetically demanding (Standen et al. 2002), so individuals inhabiting flowing water may have less energy available to invest in reproductive behaviours including nest building (Milinski and Bakker 1992). Second, nest building itself requires the collection, transport and manipulation of nesting material, and building in flowing water may increase the costs of these activities. Altering the costs of material collection can alter or reduce nest-building behaviour. For example, when the costs of transporting nest-building material increase, golden hamsters (*Mesocricetus auratus*) alter their nesting behaviour by making fewer trips and carrying more nesting material per trip (Guerra and Ades 2002).

Our results also show that males nesting in flowing water are, on average, in better condition than those nesting in still water, suggesting that flowing water prevents poor-condition males from nesting. This suggests that flow regime could affect which individuals in a population reproduce and may thus have important implications for how selection operates on traits and the evolution of populations (Candolin and Salesto 2006). This possibility warrants further investigation.

Nest-site selection may ameliorate some of the costs of nesting in flowing water. Water flow in natural river systems is likely to be highly variable, both temporally and spatially. In many fish species, nest builders base nest-site selection on water current, often choosing locations with lower than average flow, e.g. three-spined stickleback (Hagen 1967; Mori 1994), relict darter (Piller and Burr 1999), giant pirarucu (Castello 2008) and small mouth bass (Dauwalter and Fisher 2008). Under the flowing-water-regime-nesting males experienced in our study—which provided an equivalent range of flow rates to those in the natal river (Rushbrook 2007)—males preferred to nest in areas with lower than average flow, and no male completed a nest at a site with a flow exceeding 13 cmsec^{-1} (see also Mori 1994). Furthermore, males nested closer to the island under flow conditions than they did under still conditions; the nature of the circular current created under the flow treatment meant that water velocities were highest close to the outer rim of the pool. However, males building under flow conditions did not preferentially select nests in sheltered areas behind artificial boulders. This contrasts with a previous study which showed that anadromous three-spined sticklebacks nesting in streams prefer to nest downstream of vegetation stands (Hagen 1967). The fact that males did not choose to nest behind boulders, despite these areas having low directional flow, suggests that directional water current is not the only factor that males consider when choosing a nest site. Areas behind boulders may be more turbulent, and unpredictable water flow created by eddies may make nest building difficult (Vogel 1996). Similarly, areas of low flow in the lee of boulders may be prone to siltation (Hynes 1970), which may increase the costs of parental care (Potts et al. 1988; Jones and Reynolds 1999).

Increased costs of nest building in flowing water could also alter the structure of nests. The observed reduction in nest size under flow conditions could reflect depleted energy reserves as a result of coping with flowing water, or the fact that collection, transport and manipulation of nesting material have higher energetic costs in high water velocities. However, the fact that the total amount of spiggin incorporated into nests did not depend on flow regime suggests that differences in structure are unlikely to be due to differential costs of building. Spiggin production is arguably the most costly aspect of nest building in sticklebacks, with kidney spiggin levels being responsive to both parasitic infection and food availability (Rushbrook 2007; Rushbrook et al. 2007). Also, it has previously been shown that sticklebacks from streams build smaller nests than those from lakes when reared under common conditions, suggesting that building small nests under flow may be adaptive, and that this aspect of nest structure may have a genetic component (Raeymaekers et al. 2009).

As well as being smaller, nests built in flowing water were more elongated and tended to be more compact than nests built in still water. These differences in nest structure could demonstrate the ability of males to adjust nest structure in response to prevailing environmental conditions. There is little research considering how selection should act on fish nests in different environments, so it is difficult to assess the adaptive significance of differences in nest form that we see here. However, we can gain insight into the possible selective forces acting on nests in flowing water by considering hydrodynamic studies. In flowing water, organisms or structures that attach to the substrate are predominantly affected by two forces—lift and drag (Vogel 1996)—that are important in the evolution of size and form (Langerhans 2008). For instance, substrate-dwelling species of water mites living in flowing water environments are smaller, flatter and have fewer protruding hairs than still water counterparts (Pennak 1978). Similarly, shell shape and size of freshwater limpets may be adapted to prevailing water currents (e.g. Denny et al. 1985). For fish nests, the characteristics of nest size and shape in this and previous studies (Noltie and Keenleyside 1987; Bruton and Gophen 1992) potentially increase nest success in flowing water. Due to the action of lift and drag, nests that are well anchored, small, elongated and more compact are less likely to be washed away in flowing water environments.

In this study, we found flow-built nests to contain more spiggin per gramme of nest material than those built under still conditions, but this appears to be driven by the reduced size of flow-built nests, as the absolute spiggin content of nests remained constant between treatments. One interpretation of this result is that the synthesis and/or secretion of spiggin are unresponsive to changes in flow regime, at least over the timescales examined here. Alternatively, the fact that males did not reduce the amount of spiggin incorporated into smaller flow-built nests may suggest that the relative frequency of gluing behaviour, or the amount of glue released per gluing movement, is increased during nest building in flow. Detailed behavioural investigations of males building nests under divergent flow regimes, in concert with expression studies of the genes that code for spiggin synthesis in sticklebacks (Kawahara and Nishida 2006; Kawahara and Nishida 2007), would provide significant insight into the mechanisms underlying this result.

Building nests to suit local conditions is not only likely to increase nesting success but may also be important for subsequent reproductive behaviour such as courtship and parental care. Nest location and structure may play an important role in mate attraction. Females are expected to prefer males that build nests that are well adapted to local conditions (Collias and Victoria 1978; Johnson and Searcy 1993; Hoi et al. 1994; Hoi et al. 1996), and aspects of nest structure may also provide valuable information to females about male quality (Kvarnemo et al. 1998; Barber et al. 2001). Female choice in sticklebacks has previously been shown to depend on nest location (e.g. height above the substrate (Östlund-Nilsson 2000) and distance from the shore (Blais et al. 2004)). Likewise, females may base mate-choice decisions on nest structure. For instance, female 15-spined sticklebacks have been found to prefer nests with more tångspiggin (a protein similar in function and chemistry to spiggin) (Östlund-Nilsson 2001), and female three-spined sticklebacks have been found to prefer more ornamented nests (Östlund-Nilsson and Holmlund 2003). Nest structure and location may also be important in determining parental care. For example, in sand gobies, *Pomatoschistus minutus*, males nest in either sandy or rocky bottom substrates. Nests built on rocky substrates are more open than those built on sandy bottoms and thus require less ventilation via male fanning behaviour (Järvi-Laturi et al. 2008).

In our study, differences in nest structure between the flow treatments and the choice of areas with lower than average flow for nest building may reflect male consideration of future costs associated with mate attraction and parental care, as well as nesting success. However, further research is required to test this possibility.

While the differences that we see in nest structure between flowing and still water conditions are consistent with an adaptive explanation, we are unable to rule out the possibility that physical constraints of building in a flowing water environment may also influence nest form. For instance, directional water flow could remove nesting material from the nest, making it smaller and pull loose threads to make the nest more elongated. Further studies manipulating nest structure in flowing water and looking at the fitness consequences of such manipulations are needed before any adaptive significance of such differences can be firmly concluded.

In summary, the results we present here are consistent with the hypothesis that nest-building behaviour in sticklebacks is under both genetic (Rushbrook et al. 2008; Raeymaekers et al. 2009) and environmental controls (Rushbrook and Barber 2008). Our results also suggest that male sticklebacks may be plastic in their nest-building behaviour in response to flow regime. Changes in flow regimes alter the signalling environment under which males and females interact, and such changes have the potential to disrupt adaptive mate-choice decisions (Wong et al. 2007; van der Sluijs et al. 2010). Because flow regimes are regularly altered by anthropogenic activities, including river impoundment, channelization and abstraction, our results may also have relevance for the conservation of nest building species, and hence, for aquatic biodiversity.

Acknowledgements We thank Rory Geoghegan and Gareth Owen for technical assistance. We acknowledge the support of the Natural Environment Research Council via a studentship to BJR (NER/S/A/2003/11389) and a research grant to IB (NE/F019440/1). We are grateful to Kai Lindström and two anonymous referees for constructive comments on our manuscript.

References

Asaeda T, Fujino T, Manatunge J (2005) Morphological adaptations of emergent plants to water flow: a case study with *Typha angustifolia*, *Zizania latifolia* and *Phragmites australis*. Freshw Biol 50:1991–2001

Barber I, Ruxton GD (2000) The importance of stable schooling: do familiar sticklebacks stick together? Proc R Soc Lond B 267:151–155

Barber I, Nairn D, Huntingford FA (2001) Nests as ornaments: revealing construction by male sticklebacks. Behav Ecol 12:390–396

Biju SD (2009) A novel nesting behaviour of a treefrog, *Rhacophorus lateralis* in the Western Ghats, India. Curr Sci 97:433–437

Blais J, Rico C, Bernatchez L (2004) Nonlinear effects of female mate choice in wild threespine sticklebacks. Evolution 58:2498–2510

Bruton MN, Gophen M (1992) The effect of environmental factors on the nesting and courtship behaviour of *Tilapia zillii* in Lake Kinneret (Israel). Hydrobiologia 239:171–178

Bult A, Lynch CB (1997) Nesting and fitness: lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. Behav Genet 27:231–240

Burton NHK (2006) Nest orientation and hatching success in the tree pipit *Anthus trivialis*. J Avian Biol 37:312–317

Candolin U, Voigt HR (2001) No effect of a parasite on reproduction in stickleback males: a laboratory artefact? Parasitology 122:457–464

Candolin U, Salesto T (2006) Effects of increased vegetation cover on nesting behavior of sticklebacks (*Gasterosteus aculeatus*). Behav Ecol Sociobiol 59:689–693

Castello L (2008) Nesting habitat of *Arapaima gigas* (Schinz) in Amazonian floodplains. J Fish Biol 72:1520–1528

Collias NE, Victoria JK (1978) Nest and mate selection in village weaverbird (*Poceus cucullatus*). Anim Behav 26:470–479

Dauwalter DC (2007) Spawning chronology, nest site selection and nest success of smallmouth bass during benign streamflow conditions. Am Midl Nat 158:60–78

Dauwalter DC, Fisher WL (2008) Spatial and temporal patterns in stream habitat and smallmouth bass populations in eastern Oklahoma. Trans Am Fish Soc 137:1072–1088

Denny MW, Daniel TL, Koehl MAR (1985) Mechanical limits in wave-swept organisms. Ecol Monogr 55:69–102

Eggers S, Griesser M, Nystrand M, Ekman J (2006) Predation risk induces changes in nest-site selection and clutch size in the Siberian jay. Proc R Soc Lond B 273:701–706

Fleming RI, Mackenzie CD, Cooper A, Kennedy MW (2009) Foam nest components of the tungara frog: a cocktail of proteins conferring physical and biological resilience. Proc R Soc Lond B 276:1787–1795

Foster SA (1999) The geography of behaviour: an evolutionary perspective. Trends Ecol Evol 14:190–195

Grabowski TB, Isely JJ (2007) Spatial and temporal segregation of spawning habitat by catostomids in the Savannah River, Georgia and South Carolina, USA. J Fish Biol 70:782–798

Guerra RF, Ades C (2002) An analysis of travel costs on transport of load and nest building in golden hamster. Behav Processes 57:7–28

Hagen DW (1967) Isolating mechanisms in three-spined sticklebacks (*Gasterosteus aculeatus*). J Fish Res Board Can 24:1637–1692

Hansell M (2000) Bird nests and construction behaviour. Cambridge University Press, Cambridge

Hoi H, Schleicher B, Valera F (1994) Female mate choice and nest desertion in penduline tits, *Remiz pendulinus*—the importance of nest quality. Anim Behav 48:743–746

Hoi H, Schleicher B, Valera F (1996) Nest size variation and its importance for mate choice in penduline tits, *Remiz pendulinus*. Anim Behav 51:464–466

Hynes H (1970) The ecology of running waters. Liverpool University Press, Liverpool

Jakobsson S, Borg B, Haux C, Hyllner SJ (1999) An 11-ketotestosterone induced kidney-secreted protein: the nest building glue from male three-spined stickleback, *Gasterosteus aculeatus*. Fish Physiol Biochem 20:79–85

Järvi-Laturi M, Lehtonen TK, Pampoulie C, Lindstrom K (2008) Paternal care behaviour of sand gobies is determined by habitat related nest structure. Behaviour 145:39–50

Johnston CE (2001) Nest site selection and aspects of the reproductive biology of the pygmy sculpin (*Cottus paulus*) in Coldwater Spring, Calhoun County, Alabama. Ecol Freshw Fish 10:118–121

Johnson LS, Searcy WA (1993) Nest site quality, female mate choice and polygyny in the house wren *Troglodytes aedon*. Ethology 95:265–277

Jones JC, Reynolds JD (1999) Costs of egg ventilation for male common gobies breeding in conditions of low dissolved oxygen. Anim Behav 57:181–188

Katsiadaki I, Scott AP, Hurst MR, Matthiessen P, Mayer I (2002) Detection of environmental androgens: a novel method based on enzyme-linked immunosorbent assay of spiggin, the stickleback (*Gasterosteus aculeatus*) glue protein. Environ Toxicol Chem 21:1946–1954

Kawahara R, Nishida M (2006) Multiple occuffences of spiggin genes in sticklebacks. Gene 373:58–66

Kawahara R, Nishida M (2007) Extensive lineage-specific gene duplication and evolution of the spiggin multi-gene family in stickleback. BMC Evol Biol 7:209

Kraak SBM, Bakker TCM, Hocevar S (2000) Stickleback males, especially large and red ones, are more likely to nest concealed in macrophytes. Behaviour 137:907–919

Kvarnemo C, Svensson O, Forsgren E (1998) Parental behaviour in relation to food availability in the common goby. Anim Behav 56:1285–1290

Langerhans RB (2008) Predictability of phenotypic differentiation across flow regimes in fishes. Integr Comp Biol 48:750–768

Little TJ, Perutz M, Palmer M, Crossan C, Braithwaite VA (2008) Male three-spined sticklebacks *Gasterosteus aculeatus* make antibiotic nests: a novel form of parental protection? J Fish Biol 73:2380–2389

Lukas JA, Orth DJ (1995) Factors affecting nesting success of smallmouth bass in a regulated Virginia stream. Trans Am Fish Soc 124:726–735

Macnab VL, Katsiadaki I, Barber I (2009) Reproductive potential of *Schistocephalus solidus* infected male three-spined stickleback *Gasterosteus aculeatus* from two UK populations. J Fish Biol 75:2095–2107

McGowan A, Sharp SP, Hatchwell BJ (2004) The structure and function of nests of long-tailed tits *Aegithalos caudatus*. Funct Ecol 18:578–583

Milinski M, Bakker TCM (1992) Costs influence sequential mate choice in sticlebacks, *Gasterosteus aculeatus*. Proc R Soc Lond B 250:229–233

Mori S (1994) Nest site choice by the three-spined stickleback, *Gasterosteus aculeatus* (form Leiurus), in spring-fed waters. J Fish Biol 45:279–289

Mori S (1995) Spatial and temporal variations in nesting success and the causes of nest losses of the fresh-water 3-spined stickleback, *Gasterosteus aculeatus*. Environ Biol Fishes 43:323–328

Noltie DB, Keenleyside MHA (1987) Breeding ecology, nest characteristics, and nest-site selection of stream- and lake-dwelling rock bass, *Ambloplites rupestris* (Rafinesque). Can J Zool 65:379–390

Ochi H, Onchi T, Yanagisawa Y (2001) Alloparental care between catfishes in Lake Tanganyika. J Fish Biol 59:1279–1286

Olafsdottir GA, Ritchie MG, Snorrason SS (2006) Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks. Biol Lett 2:250–252

Östlund-Nilsson S (2000) Are nest characters of importance when choosing a male in the fifteen-spined stickleback (*Spinachia spinachia*)? Behav Ecol Sociobiol 48:229–235

Östlund-Nilsson S (2001) Fifteen-spined stickleback (*Spinachia spinachia*) females prefer males with more secretional threads in their nests: an honest-condition display by males. Behav Ecol Sociobiol 50:263–269

Östlund-Nilsson S, Holmlund M (2003) The artistic three-spined stickleback (*Gasterosteus aculeatus*). Behav Ecol Sociobiol 53:214–220

Park BK, Park SS (2005) Effects of stream hydraulic conditions on foraging strategies of false dace, *Pseudorasbora parva*, in the lentic ecosystem. J Environ Biol 26:635–643

Peluc SI, Sillett TS, Rotenberry JT, Ghalambor CK (2008) Adaptive phenotypic plasticity in an island songbird exposed to a novel predation risk. Behav Ecol 19:830–835

Pennak RW (1978) Fresh-water invertebrates of the United States, 2nd edn. Wiley, New York

Piller KR, Burr BM (1999) Reproductive biology and spawning habitat supplementation of the relict darter, *Etheostoma chienense*, a federally endangered species. Environ Biol Fishes 55:145–155

Potts GW, Keenleyside MHA, Edwards JM (1988) The effect of silt on the parental behavior of the sea stickleback, *Spinachia spinachia*. J Mar Biol Assoc UK 68:277–286

Raeymaekers JAM, Delaire L, Hendry AP (2009) Genetically based differences in nest characteristics between lake, inlet, and hybrid threespine stickleback from the Misty system, British Columbia, Canada. Evol Ecol Res 11:905–919

Rowland WJ (1994) Proximate determinants of stickleback behaviour: an evolutionary perspective. In: Bell MA, Foster SA (eds) The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford, pp 297–344

Rushbrook BJ (2007) Individual and population level variation in nest construction in the three-spined stickleback (*Gasterosteus aculeatus*). PhD thesis. Aberystwyth University

Rushbrook BJ, Barber I (2006) Nesting, courtship and kidney hypertrophy in *Schistocephalus*-infected male three-spined stickleback from an upland lake. J Fish Biol 69:870–882

Rushbrook BJ, Barber I (2008) A comparison of nest building by three-spined sticklebacks *Gasterosteus aculeatus* from still and flowing waters. J Fish Biol 73:746–752

Rushbrook BJ, Katsiadaki I, Barber I (2007) Spiggin levels are reduced in male sticklebacks infected with *Schistocephalus solidus*. J Fish Biol 71:298–303

Rushbrook BJ, Dingemanse NJ, Barber I (2008) Repeatability in nest construction by male three-spined sticklebacks. Anim Behav 75:547–553

Spencer RJ (2002) Experimentally testing nest site selection: fitness trade-offs and predation risk in turtles. Ecology 83:2136–2144

Standen EM, Hinch SG, Healey MC, Farrell AP (2002) Energetic costs of migration through the Fraser River canyon, British Columbia, in adult pink (Oncorhynchus gorbuscha) and sockeye (O. nerka) salmon as assessed by EMG telemetry. Can J Fish Aq Sci 59: 1809-1818

van der Sluijs I, Gray SM, Amorim MCP, Barber I, Candolin U, Hendry AP, Krahe R, Maan ME, Utne-Palm AC, Wagner H-J, Wong BBM (2010) Communication in troubled waters: responses of fish communication systems to changing environments. J Evol Biol

van Iersel JJA (1953) An analysis of the parental behaviour of the three-spined stickleback (*Gasterosteus aculeatus* L.). Behaviour (Suppl) 3:1–159

Vinyoles D, Cote IM, De Sostoa A (2002) Nest orientation patterns in *Salaria fluviatilis*. J Fish Biol 61:405–416

Vogel S (1996) Life in moving fluids: the physical biology of flow, 2nd edn. Princeton University Press, Princeton

Wong BBM, Candolin U, Lindstrom K (2007) Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. Am Nat 170:184–189

Wootton RJ (1976) The biology of the sticklebacks. Academic Press, London

Wootton RJ (1984) A functional biology of sticklebacks. University of California Press, Berkeley