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Nest construction and presence do not alter territorial aggression in male threespine stickleback

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Abstract

Constructing a nest within a mating territory provides a clear benefit to the resident, particularly by improving the opportunity to mate. It is unclear whether animals who use nests exclusively for reproductive purposes account for either the effort invested or the resulting increase in mating potential when valuing their territories. We sought to explicitly reveal a nest's added subjective resource value through within-group comparison of aggressive behaviour before and after nest construction. An increase in aggression following construction would indicate that the resident perceives greater subjective value in his territory, and thus values the nest. Threespine stickleback, *Gasterosteus aculeatus*, fish demonstrate stereotypical aggressive behaviours during an easily induced territorial defence. The male's nest is used exclusively for reproductive purposes, avoiding any confound of shelter. Contrary to our hypothesis, neither nest presence, timing of construction, nor nesting outcome was associated with differences in behavioural measures of territorial aggression. Assessed behaviours were robust, repeatable and inter-correlated. We conclude that territorial aggression is neither predictive of nor altered by nesting in threespine stickleback fish. Our results suggest that nests used transiently for a portion of the mating season add negligible subjective resource value to a territory. We additionally demonstrate that examinations of territorial aggression in sticklebacks do not need to control for nest building, improving statistical power by decreasing dropout rates. These results dovetail with recent work in other fish species to suggest that assessment of territorial aggression absent a nest may be practicable for fish in general.

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Author contributions

N.J. conceived and designed the experiment; M.F. performed the experiments; N.J. analysed the data and wrote the paper.

Conflicts of Interest

None.

Keywords

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Territoriality is widespread in the animal kingdom, drastically influencing fitness by allowing animals to gain sole access to resources such as food and mating opportunities. Although holding a territory provides clear benefits, it also carries time and energy costs, as well as a risk of injury in territorial conflicts (Hinsch & Komdeur, 2017). The choice to engage in territorial conflict, either as a defending resident or as an intruder, is a complex decision composed of many subprocesses (Arnott & Elwood, 2008; Injaian & Tibbetts, 2015; Reichert & Quinn, 2017). The amount of effort invested into defending a territory can be used to measure an animal's subjective appraisal of the territory's value (Hinsch & Komdeur, 2017; Hollander, Titeux, & van Dyck, 2012; O'Connor et al., 2015).

Resource value can be divided into two factors: subjective and objective value (Arnott & Elwood, 2008). Objective value reflects intrinsic or fundamental qualities of the territory, such as size (in m²), calories of food provided or number of potential mates. Because these measures are empirical, the objective value of a territory is the same for all contestants. However, not all contestants will assign identical importance to each quality. For example, a starving animal may value food above all else, while a healthy animal may consider it alongside shelter and mating opportunity. These subjective valuations are specific to the contestant, reflecting differences in perceived value, typically based on prior experience or investment in exploiting a resource (Stockermans & Hardy, 2013). Different internal states further shift individual subjective valuations (Enquist & Leimar, 1987). Knowledge of the territory differs between competitors, typically to the resident's distinct advantage. Subjective valuations can even differ over time for a given individual, reflecting fluctuating motivations or other inconstant facets of internal state.

Reproduction is key to fitness, and becoming a parent clearly shifts physiological state. Parenthood has repeatedly been shown to increase animal perception of risk and value, reviewed in Arnott and Elwood (2008). The concern of mitigating risk to offspring also extends to maintaining a safe living space. In crayfish (*Procambarus clarkia*), maternal females are more likely to initiate shelter-related aggression and to win compared to nonmaternal females or males (Arnott & Elwood, 2008). Similarly, in American lobsters, *Homarus americanus*, maternal females outcompete non-maternal females for shelters, whether the maternal females are residents or intruders (Arnott & Elwood, 2008). Furthermore, gaining access to mating opportunities can influence the perceived or subjective value of a resource. In house crickets, *Acheta domestica*, males who are isolated from females are more likely to initiate aggression and win more fights (Brown, Smith, Moskalik, & Gabriel, 2006). Current monopolization of females causes escalation in male cottonwood borers, *Plectrodera scalator*, although in these nonterritorial insects, prior pairing does not influence aggression (Goldsmith, Stewart, Adams, & Trimble, 1996). In blackbirds, as competition for nesting sites increases, so does the frequency of conspecific aggression (Diniz, Oliveira, Marini, & Duca, 2019).

Holding a mating or breeding territory has clear objective value, as it implicitly affords the chance to reproduce. A nest, often required for mating, represents a substantial investment into improving a territory. It is a self-initiated change to the territory with inherent, objective value to the resident. The literature is mixed, however, as to whether animals' individual subjective valuations account for the likelihood of reproducing within a nesting territory and correspondingly increase with the presence of a nest. Nest proximity and stage was positively correlated with winning aggressive encounters in house finches, *Haemorrhous mexicanus* (Jonart, Hill, & Badyaev, 2007), and with increased aggression in stickleback fish (Bakker, 1994).

Nest use varies widely across taxa, but this has not been considered in reference to subjective territorial value. In house finches, who reuse nests (Evenden, 1957), nesting stage was correlated with winning (Jonart et al., 2007). However, for the many species that do not reuse nests between reproductive attempts, a nest's value is transient - more analogous to a pitched tent than to a furnished house. If the major limiting factor is gaining the territory, then the addition of a reproduction-exclusive nest might be subjectively inconsequential despite the objective value the nest provides. Blackbirds, which typically do not reuse their nests (Ellison, 2008), significantly decrease levels of female-female aggression during advanced stages of nesting (i.e. having eggs or nestlings), without altering male-male aggression (Diniz et al., 2019).

To examine how subjective value of a mating territory is influenced by nests exclusively used for breeding purposes, we used threespine stickleback, *Gasterosteus aculeatus*, fish a classic system for the study of behaviour. Males defend nesting territories during the summer breeding season and do so exclusively for reproductive purposes. However, nests themselves are not reused between nesting cycles - up to five of which can occur within a single breeding season in the wild (Rushbrook, Dingemanse, & Barber, 2008; Wootton, 1976). Territorial establishment precedes nesting (Rowland, 1994; Van Iersel, 1953), allowing us to assess the subjective value of the territory in the absence of a nest. Stickleback males initiate mating-specific behaviours upon completion of a nest (Wilz, 1975), revealing a corresponding internal or physiological state change. Male territorial defence presents stereotypically, is easily induced in the laboratory and is representative of field studies (Bell, 2005). Accordingly, stickleback fish are an ideal system in which to investigate how nests alter subjective value of a breeding territory. Specifically, our goal was to determine whether individual rates of aggression towards a territorial intruder differ depending on the presence of a nest. Resident male sticklebacks' aggression scales with the proximity of an intruder to the resident's nest (reviewed in Rowland and Bolyard, 2000), suggesting that nests are subjectively valuable. Additionally, previous work by Wootton (1970) indicated that sticklebacks who do nest are marginally more aggressive after nesting than those that do not nest. However, why some fish do not nest remains unknown, so differences in territorial aggression could be due to some other difference between groups - for example, those that nest may at all times be more aggressive than non-nesters, or the nesters' territories may have been more objectively valuable to begin with, etc. Changes in aggression following nesting within an individual stickleback have not been investigated, leaving open the question of how much subjective value, if any, a completed nest adds to a male's territory. We hypothesized that residents' aggression during territorial defence would

increase with completion of a nest, driven by an increased subjective value of the territory. The objective value increases with the investment on construction and with the immediate mating opportunity afforded by the nest; a subsequent increase in aggression would indicate a corresponding increase in the perceived, subjective, value. While most resource value studies use a between-group design, we sought to directly examine subjective value by comparing aggression within the same individuals before and after completing their nests.

METHODS

Animals

Freshwater adult fish were collected from Putah Creek, California, U.S.A. in summer 2017 and housed in the laboratory in 83-litre ($107 \times 33 \times 24$ cm) group tanks containing ~20 individuals with recirculated freshwater (5 ppm salt). The room was maintained at 18 °C on a 16:8 h light:dark 'summer' photoperiod. Males showing signs of nuptial coloration were weighed and measured (standard length from the tip of the mouth to caudal peduncle), and then moved to individual, visually isolated 9.5 litre ($32 \times 21 \times 19$ cm) tanks lined with gravel and containing a synthetic plant. Each was allowed to acclimate for 24 h prior to any behavioural measurements.

Response to a Territorial Intrusion (Flask Assay)

We measured aggression in male sticklebacks by recording the response of a resident fish to a simulated territorial intrusion on three occasions, with 1 day between trials (see Fig. 1). In each trial, an intruder, confined to a glass flask, was placed in the resident's tank. The interactions between the resident and intruder were scored for 5 min starting after the resident oriented towards the intruder. Fish were given up to 5 min to orient to the intruder, making 10 min the maximum possible duration of a trial. We recorded the times to orient towards and first bite at the intruder, as well as the number of approaches, bites and charges (lunges). Alternatively, the trial was terminated if the resident fish did not orient to the intruder within 5 min. Fish were not dropped from the study for failure to orient; all fish received all three trials. Individual intruders were not used more than four times per day. All behavioural assays were carried out during 1530–1800 h in September and October 2017. After the third trial, males were removed from the experiment and their nesting materials and nests were removed from the tank. Water cycled for ~36 h before tanks were reused.

Nesting Phase

The first trial occurred in the absence of nesting material to serve as a prenesting control for all fish. Immediately following the first trial, nesting materials in the form of algae and a sand-filled nesting box were added to the tank. The second and third trials took place 3 and 5 days after the first intrusion, in consideration of previous work (N. James, personal observation), suggesting a median time of 3–4 days for males to complete a nest. Nesting progress was recorded prior to each trial, noting the presence or absence of a completed nest or whether the resident had exhibited nesting behaviour, i.e. digging in sand or interacting with algae. A nest is considered complete if it features a visible tunnel (Van Iersel, 1953).

Ethical Note

All animal work was done in compliance with the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign (IACUC protocol 15077). During all simulated territorial intrusions, the resident and intruder were separated to prevent any injuries from occurring. Group housing was used to provide a normal social environment prior to and following the experiment. All capture of fish was done to the highest standards (IACUC and U.S. law) by trained researchers with prior approval. Collections were kept to a minimum to avoid disrupting the native population. Fish received daily maintenance of feeding and illness checks by laboratory members. Additionally, daily room and water checks were done by university Division of Animal Research technicians.

Data Availability

The complete data set and the final R scripts used for analysis are publicly available on the Open Science Framework (<https://osf.io/y47ur>).

Data Analysis

Data analysis was performed in R (v.3.5.1). For complete details on the analysis, please see the R scripts. Specific sample sizes are given for all analyses. Descriptive statistics (mean \pm SD) were generated by the ‘psych’ (v.1.8.4) and ‘FSA’ (v.0.8.21) packages. Behavioural repeatability was calculated using ‘rptR’ (v.0.9.22) as a Poisson distribution with a simple model of trial as a fixed variable and individual as a random variable. These results agreed with the model-free, nonparametric intraclass correlation (ICC) with the ‘nopaco’ package (v.1.0.6). Correlations between all the measured behaviours, nonparametric analyses of homogeneity of variances, and Wilcoxon rank-sum tests were conducted with the R ‘stats’ package (v.3.5.1). As the behaviour data were non-normal, we analysed the data for time and nesting effects using a general linear mixed model with Poisson distribution (multivariate: MCMCglmm v.0.2.0; univariate: ‘lme4’ v.1.1–121) and separately in a model-free nonparametric rank-based method with correction for repeated measures (‘nparLD’ v.2.1) similar to a MANOVA that is robust to outliers and small sample sizes (Bathke et al., 2018; Forstmeier; Schielzeth, 2011; Harrison et al., 2018; Noguchi, Gel, Brunner, & Konietzschke, 2012; Schuster & Lubbe, 2015; Zuur; Ieno, & Elphick, 2010). Both analyses were in agreement. To compensate for multiple comparisons, the ANOVA-type statistic, Wilcoxon test and *t* test *P* values were minimally adjusted using the Holm method of the statistical package’s ‘p.adjust’ function. Post hoc Dunn tests through FSA similarly used its default Holm correction.

To test whether males that built nests differed in their aggression from non-nesters, we initially used a multivariate multiple regression general linear mixed model (MCMCglmm) with bites and charges as the response variables and trial and outcome as fixed predictors and individual as a random variable. This showed no main effect of nesting phase but a strong main effect of trial (Appendix, Table A1). To follow up on nesting phase, we ran between-group Wilcoxon tests with either bites or charges as the response variable and nest presence as the predictor of interest. To ensure there was no effect of nest presence, we used a within-subject Wilcoxon test limited to either early or late nesters comparing bites or charges in the trial immediately pre- and postnesting. Given the correlation between bites

and charges, which can bias multivariate GLMMs, we further examined the effect of trial using univariate GLMs (lme4's 'glmer') for each aggression measure independently as the response variable with trial as a fixed variable and individual as a random variable. Effect sizes for between trial comparisons were calculated using Vargha and Delaney's *A* (VDA).

RESULTS

In all, 90 males started and completed the experiment, totalling 270 trials. Ten trials involving nine individual males were halted after the resident failed to orient within the 5 min period. In total, 35 completed nests: 16 'early nesters' between trials 1–2 and 19 'late nesters' between trials 2–3. The 'nesters' collectively comprised these two nesting outcomes. A few males ($N=6$) showed 'nesting behaviours' (i.e. digging in the nesting box or moving algae around) but did not complete a nest before trial 3 and so were not considered 'nesters'. The final 47 males fell into the last nesting outcome, 'non-nesters'.

Intruders Provoked Aggressive Behaviour

Resident males were responsive to the intrusion, quickly orienting to the intruder at a mean of 40 ± 59 s and first biting at 92 ± 71 s after orienting. Even without a nest, male sticklebacks aggressively defended their territories, biting an average of 11.0 ± 13.6 times per trial and charging 6.0 ± 7.3 times per trial. Across all fish and outcomes, bites and charges were significantly positively correlated (Fig. 2). Time to bite was significantly negatively correlated with number of bites (Fig. 2), as expected: fish that started biting sooner bit more times overall.

Nest Presence Does Not Alter Aggression

The presence or absence of a nest was not associated with any differences in aggression during any trial (Fig. 3). Within trial 2, males who completed their nests ($N=16$) compared to those who had not nested ($N=64$) were not more aggressive (Wilcoxon signed-rank test: bites: $Z=-0.45$, $P_{\text{adj}}=1$; charges: $Z=-1.95$, $P_{\text{adj}}=0.20$). Within trial 3, males who completed their nests ($N=35$) compared to those who had not nested ($N=47$) were not more aggressive (Wilcoxon signed-rank test: bites: $Z=-0.01$, $P_{\text{adj}}=1$; charges: $Z=-1.03$, $P_{\text{adj}}=0.91$).

Aggressive Behaviour Did Not Change after Completing a Nest

Completing a nest did not alter a fish's aggressive response to an intruder when comparing within individuals. Early nesters ($N=16$) had no increase in aggression between trials 1 and 2 (Wilcoxon signed-rank test: bites: $Z=-0.63$, $P_{\text{adj}}=1$; Fig. 4a; charges: $Z=-0.03$, $P_{\text{adj}}=1$; Fig. 4c). Late nesters ($N=14$) had no increase in aggression between trials 2 and 3 (Wilcoxon signed-rank test: bites: $Z=-1.38$, $P_{\text{adj}}=0.34$; Fig. 4a; charges: $Z=-0.40$, $P_{\text{adj}}=1$; Fig. 4c). When considering all nesters ($N=33$), there was no increase in aggression between the trial prior to starting a nest and the trial after finishing a nest (Wilcoxon signed-rank test: bites: $Z=-0.23$, $P_{\text{adj}}=1$; Fig. 4b; charges: $Z=-0.39$, $P_{\text{adj}}=1$; Fig. 4d).

No Difference in Territorial Aggression Between Early and Late Nesters

The timing of nest building was not associated with any differences in aggression. Early nesters ($N = 16$) and late nesters ($N = 14$) did not differ in aggression (Wilcoxon signed-rank test: bites: $Z = -0.23$, $P_{\text{adj}} = 0.82$; charges: $Z = -1.25$, $P_{\text{adj}} = 0.42$) during the trial immediately preceding nest completion (i.e. comparing early nesters in trial 1 versus late nesters in trial 2). Similarly, no difference in aggression was observed in the trial after finishing their nest (Wilcoxon signed-rank test: bites: $Z = -0.75$, $P_{\text{adj}} = 0.45$; charges: $Z = -1.25$, $P_{\text{adj}} = 0.42$). In other words, we found no difference in aggression between the early nester and late nester groups.

No Difference in Resource-Holding Potential Between Nesters and Non-nesters

There was no difference in size (Wilcoxon rank-sum test with continuity correction: standard length: $Z = -0.36$, $P_{\text{adj}} = 0.94$; Welch's t test: mass: $t_{82} = 1.02$, $P_{\text{adj}} = 0.94$) between fish who eventually completed a nest (standard length: 43 ± 3 mm; mass: 1.13 ± 0.20 g; $N = 36$) and those who did not (standard length: 43 ± 5 mm; mass: 1.11 ± 0.26 g; $N = 48$). Resident males ranged from 10% smaller to 24% larger than intruders. Size differences between the resident and intruder fish were similar ($t_{82} = -0.89$, $P_{\text{adj}} = 0.93$) between nesters ($7 \pm 5\%$) and non-nesters ($6 \pm 5\%$). There was no correlation between size (length, mass or size difference to intruder) and any measured behaviour.

Trial Effect

There was a main effect of trial on overall aggression (GLM: posterior mean = 0.28, effective sample size = 6264, $P_{\text{MCMC}} = 0.0008$; Fig. 5). Bites increased a small but significant amount after trial 1 (all fish: $VDA_{1-3} = 0.34$; non-nesters: $VDA_{1-3} = 0.34$; Appendix, Table A2), peaking at trial 3, but did not change significantly between trial 2 and 3. Charges also increased a small but significant amount after trial 1 (all fish: $VDA_{1-2} = 0.35$; non-nesters: $VDA_{1-2} = 0.34$; Appendix, Table A2), peaking at trial 2, with no significant change between trial 2 and 3. Due to the correlation between the various measures of aggression (see Fig. 2), we analysed each behaviour for change across trials separately (Table 1, post hoc analysis Appendix, Fig. A1).

Significant Repeatability of Behaviour within Individuals

Regardless of nesting outcome, all measured behaviours exhibited moderate to high and significant repeatability (see Table 2). The main measures of aggression, biting and charging, had the highest repeatability ($R_{\text{Bites}} = 0.65$, $R_{\text{Charges}} = 0.65$) of all measured behaviours.

DISCUSSION

Overall, aggression (bites and charges) was not significantly predicted by nesting phase (multivariate GLM: $P_{\text{Nesting Behaviour}} = 0.68$, $P_{\text{Nest}} = 0.35$). Furthermore, the presence of a nest did not increase any metric of aggression in territorial male sticklebacks. Given the robustness and repeatability of the various behaviours (Table 2), especially bites and charges, we can be confident in our measurement of aggression. Biting and charging behaviours were consistent under all comparisons, both within individuals (pre-nest versus

postnest: Fig. 4b, d) and between groups (early versus late timing: Fig. 4a, c; nester versus non-nester outcome: Fig. 3). Neither nest presence, timing of construction, nor outcome were associated with differences in territorial aggression. We therefore conclude that aggression is neither predictive of nor altered by nesting in threespine stickleback fish.

Here we measured aggression using the traditional stickleback ‘flask assay’ as a simulated territorial intrusion. Visual cues are sufficient to elicit an aggressive response in sticklebacks; no water exchange or physical contact is necessary (Huntingford, 1976; Peeke, 1969; Tinbergen, 1951). While this assay is representative of field studies (Bell, 2005), it is worth noting that there is a major difference in that neither the intruder nor resident can vacate the territory during the 5 min trial, potentially increasing the level of hostility and the total number of interactions. Additionally, use of a confined intruder produces a higher mean number of bites compared to other simulated intrusion methods, but in all methods the overall trend is consistent (Wootton, 1971). Thus, while our measures of aggression were highly repeatable (Table 2), they are expected to exceed those of a wild territorial intrusion.

The increase in bites and charges across trials observed in this study may be explained by several potential factors. The most immediate of these is that the increase may represent a sensitization to intrusion. However, habituation leading to reduced aggression towards a specific territorial intruder is typical in the short term (Peeke, 1982, 1983; Rowland, 1982, 1988) and persists for 3–5 days (Peeke, Blankenship, & Figler, 1979). This ‘dear enemy’ effect is common in territorial animals across taxa and important for minimizing risky aggressive interactions. Thus, we find this explanation unlikely.

Alternatively, the increase in aggression across trials may suggest that the 24 h acclimation period following transfer to an empty tank was insufficient for males to become fully territorial. Residency time is well known to influence fight investment across taxa (dos Santos & Peixoto, 2017). The addition of nesting materials may have contributed to the increase in aggression observed during the second trial in particular. However, the significant repeatability in aggressive behaviours suggests that any effect of nesting materials was equivalent in both nesters and non-nesters. In any case, early nesters were not distinct from late nesters (Fig. 4b, d), indicating that regardless of the increase in territorial aggression over time, there was no change in aggression from the nest’s presence.

In consideration of these factors, we recommend a longer acclimation period in future designs. Typical acclimation times for juvenile fish have previously been reported at 2 days (Bell, Backström, Huntingford, Pottinger, & Winberg, 2007; Dingemanse et al., 2007) to three (Lacasse & Aubin-Horth, 2013). This timing is corroborated by in situ observations in which wild males reach sexual coloration within 1–3 days (Rowland, 2000).

Practically, this experiment shows that researchers examining territorial aggression in sticklebacks using the traditional flask assay do not need to control for nest building. Accordingly, future studies can now disregard the nesting cycle to employ faster, simpler experimental designs compared to current stickleback methods (Bakker, Bruijn, & Sevenster, 1989; Bell, Bukhari, & Sanogo, 2016; Huntingford, 1976; Peeke & Bell, 2012; Peeke et al., 1979; Rittschof et al., 2014; van den Assem & van der Molen, 1969). With the

constraint of nest construction removed, future experiments will enjoy substantially reduced dropout rates, directly providing increased sample sizes and thus statistical power. Happily, animal welfare is also advanced by the use of such a 'reduction alternative strategy' (Fenwick, Griffin, & Gauthier, 2009), as sufficient data to answer research questions in territorial aggression may now be obtained using fewer animals.

There is no doubt that nests are subjectively valuable, as previous studies (Bakker, 1994; Rowland, 1994) show more vigorous conflict with closer proximity to the nest. There was no ceiling on aggression in the course of this experiment, as bites and charges increased across trials (Fig. 5). Resource holding potential was the same for both nesters and non-nesters and was not correlated with any measure of aggression. In combination, these results suggest that the plot of land itself is the largest determining factor in territorial defence and that the addition of a mating-exclusive nest is inconsequential despite its objective value. These results are not only useful for understanding the ecological process of territory conflict, but also have important implications for game theory modelling (Hinsch & Komdeur, 2017).

While parenthood clearly represents a major state change (Arnott & Elwood, 2008), the subjective value of intermediate steps towards reproduction are still under investigation. Gaining access to mating opportunities has been shown to increase subjective value of a territory, although the amount is variable depending on the species examined. It has been suggested that the subjective value of mating opportunity is influenced by mating system (polygamy, seasonal monogamy, etc.) - monogamists have reason to be more selective about mate choice, while polygamists can afford to hold relaxed criteria (Clutton-Brock, 1989; Tiddi; Heistermann; Fahy, & Wheeler, 2018).

Similarly, the subjective value of nests has been seen at both extremes (Bakker, 1994; Diniz et al., 2019; Jonart et al., 2007). In that vein, we are proposing that nest use may underlie variation in subjective value between species. Here we examine a subset of nesting contexts - nests used solely for reproductive purposes on an already claimed mating territory. By limiting our focus to a single context, we were able to reduce the factors contributing to subjective value of the territory. If the major limiting factor is gaining the territory, then the addition of a reproduction-exclusive nest might be subjectively inconsequential despite the objective value of the nest. Our results show that territory holders did not attack more intensely after nesting, indicating no change in the subjective resource value of their territory. Thus, we find that the core value of a nest may derive from its use. A nest's value may lie in its use as a shelter either for the residents or their offspring, rather than as a mating platform. This supports the idea that territorial value is informed both by the immediate needs and circumstances of the individual but also by the species' ecology.

These results dovetail with recent work showing the relative importance of subjective resource valuation to resource holding potential in other species (cichlids devalue multiple territories: O'Connor et al., 2015; gobies discount mating opportunities: Svensson, Lehtonen, & Wong, 2012) and the current methods in trout (Johnsson & Näslund, 2018; Sundström; Löhmus, & Johnsson, 2003), suggesting assessment of territorial aggression in the absence of a nest may be practicable for fish in general. To our knowledge, we

additionally provide the first quantification of behavioural repeatability for the common flask-style intruder assay in sticklebacks using the established measures of aggression.

Finally, we were able to directly examine how the subjective value of a mating territory is influenced by nests used exclusively used for breeding purposes. Since fitness is determined by successful reproduction, not merely the opportunity to mate, the expectation is that the subjective value of a territory would account for the progress towards mating contributed by the nest. However, since many species across taxa do not reuse nests even between reproductive attempts, a nest represents at most a transient increase in value. By using sticklebacks, we were able to examine the subjective value of the nest without the confound of shelter. Although building a nest is an investment into improving a territory as well as a necessary precondition to mating, it does not alter how aggressively the territory is defended.

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Appendix

Table A1

Multivariate multiple regression multiple regression (MCMCglmm) of (bites, charges) ~ trial*outcome and individual fish as a fixed effect

	Posterior mean	Effective sample	pMCMC
(Intercept)	0.46	6928	0.07
Trial	0.28	6068	0.002
Outcome nesting behaviour	-0.12	7750	0.87
Outcome nesters	0.66	7633	0.08
Trial: Nesting behaviour	0.19	7219	0.44
Trial: Nesters	-0.15	6838	0.24

Iterations = 3001:99991; thinning interval = 10; sample size = 9700; deviance information criterion (DIC): 2458.

Table A2

Post hoc comparison across trials for significant measures of territorial aggression

Comparison	All fish, P_{adj}		Non-nesters, P_{adj}		
	Bites	Charges	Bites	Charges	Time to 1st bite
Trials 1, 2	0.01	0.02	0.14	0.06	0.02
Trials 1, 3	0.003	0.13	0.07	0.16	0.01
Trials 2, 3	0.65	0.41	0.62	0.55	0.88

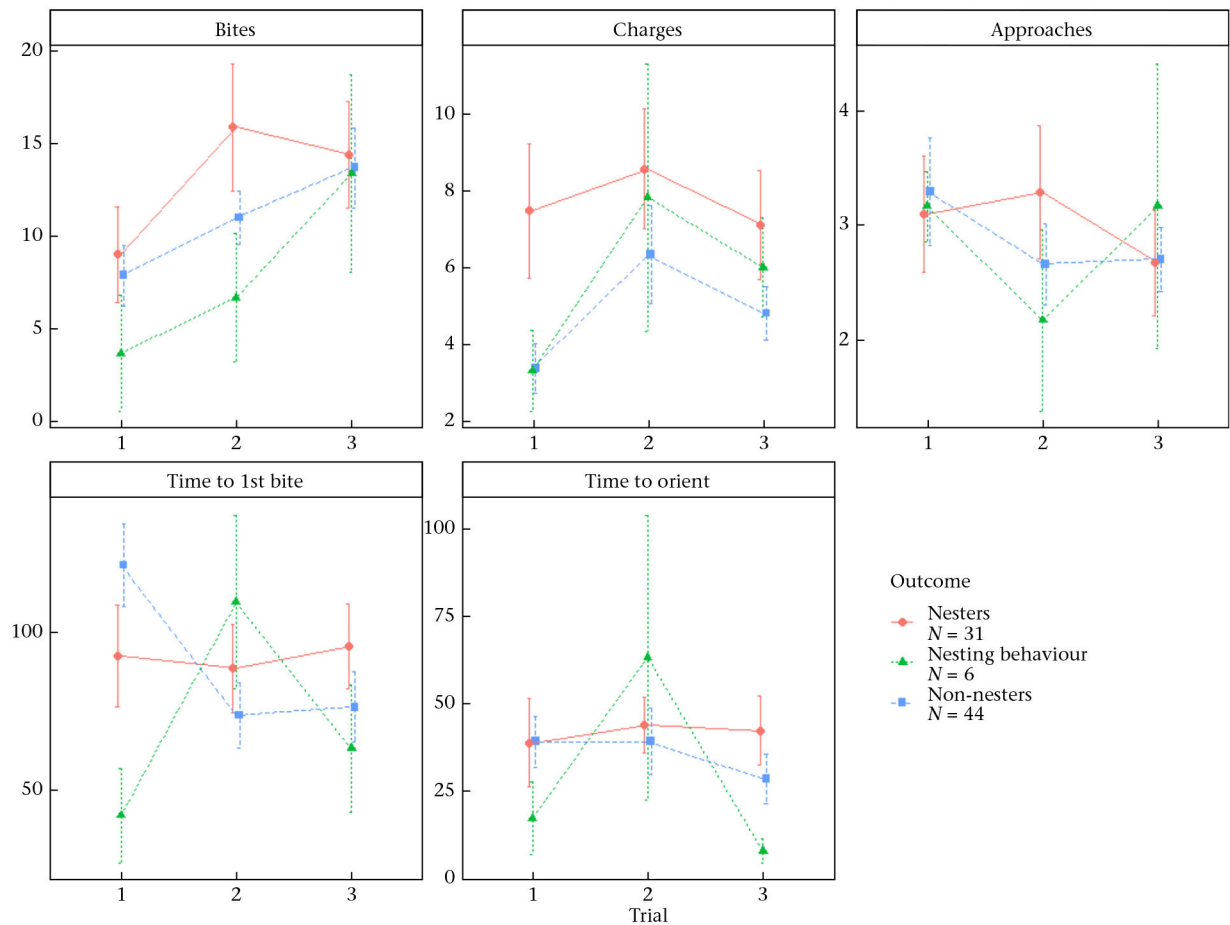


Figure A1.

Counts of each behavioural measure of territorial aggression (mean \pm SE) across all trials for each nesting outcome.

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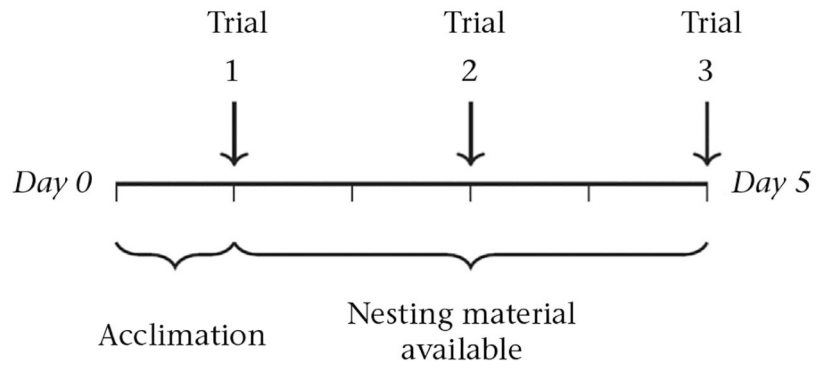


Figure 1. Experimental timeline. Fish were given nesting materials following trial 1.

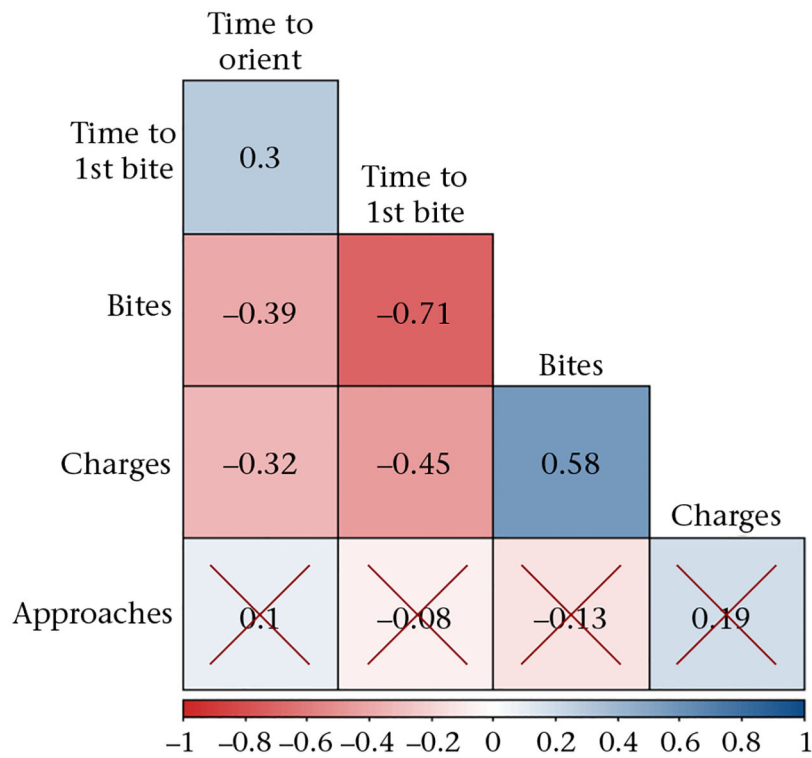


Figure 2. Correlations between behavioural measures of territorial aggression ($N = 81$). Numerical values and colour both represent the strength of the correlation, with crossed out boxes indicating nonsignificance ($P > 0.05$).

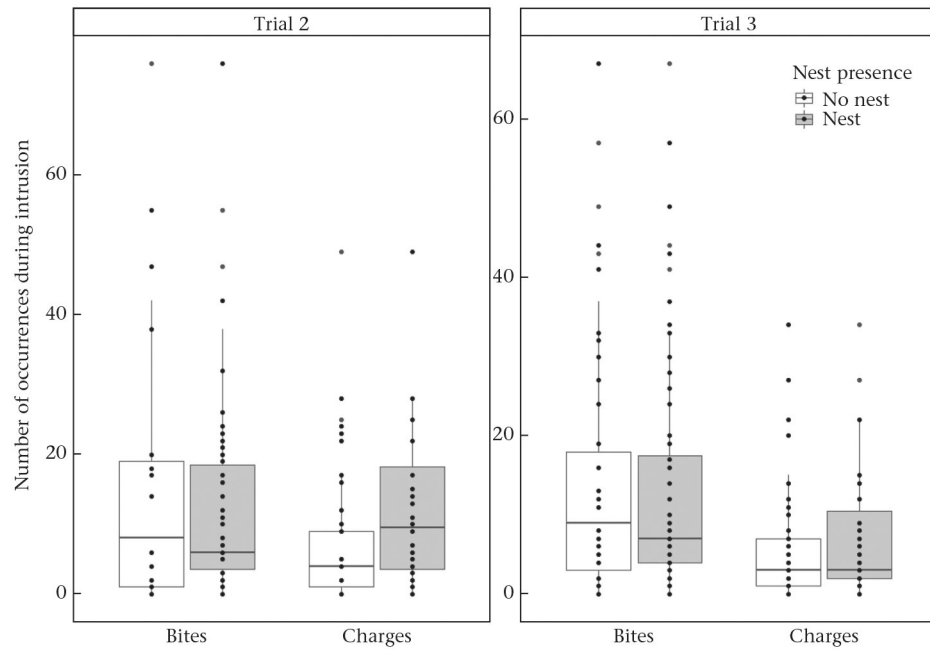


Figure 3.
) Behavioural measures of territorial aggression by males with and without nests during trials in which nesting materials were available. Box plots show 25% and 75% quartiles (boxes), medians (lines in the boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles).

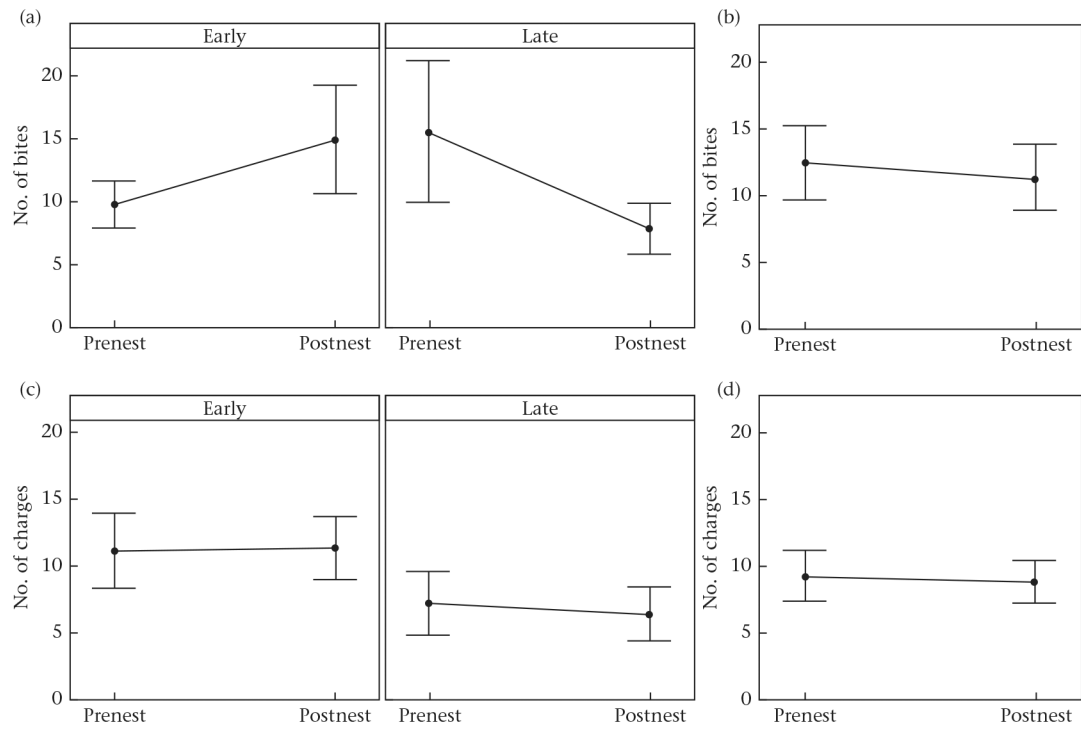


Figure 4. Mean (\pm SE) number of bites and charges by (a, c) early and late nesting resident males and (b, d) all nesting resident males ($N=35$), respectively, immediately before and after constructing a nest.

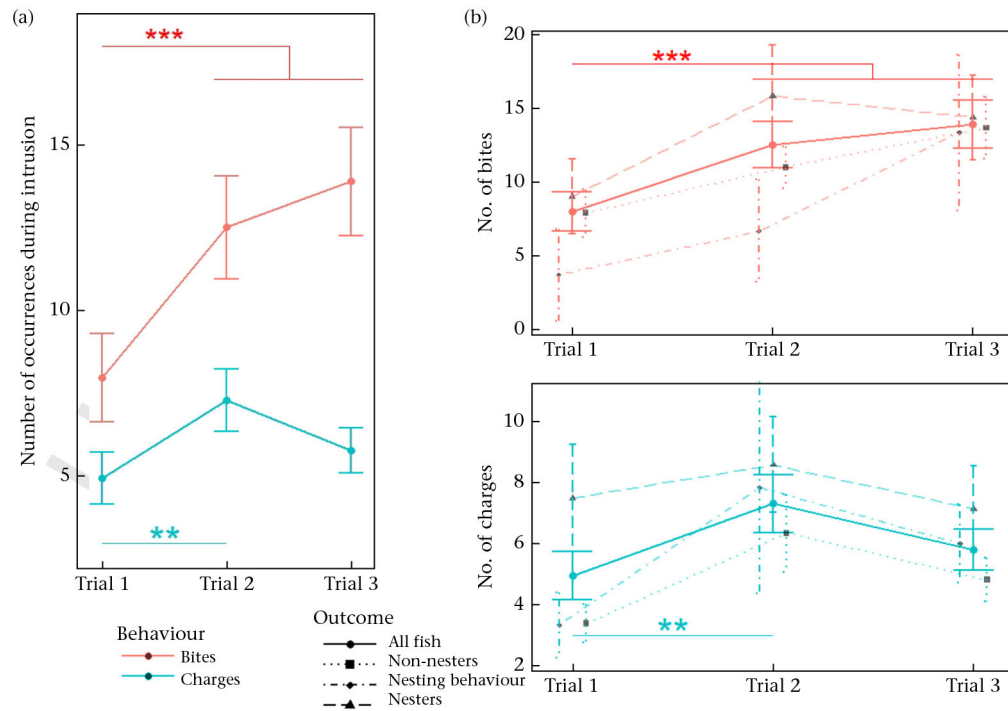


Figure 5. Aggressive behaviour counts (a) across trials for all fish (mean \pm SE) and (b) for each nesting outcome (mean), with bites and charges shown separately. ** $P < 0.01$; *** $P < 0.001$.

Table 1

Adjusted *P* value of trial effect for each behaviour analysed univariately (by 'glmer') along with the remaining degrees of freedom

Behaviour	GLM		
	Z	<i>P</i> _{adj}	df
All fish			
Bites	10.47	4.51E-25	257
Charges	1.94	0.05	202
Approaches	-2.42	0.03	258
Time to orient	-6.38	5.38E-10	258
Time to 1st bite	-18.35	1.73E-74	258
Non-nesters			
Bites	8.02	3.08E-15	137
Charges	2.80	0.01	109
Approaches	-2.33	0.02	138
Time to orient	-9.92	1.37E-22	138
Time to 1st bite	-26.61	2.48E-155	138
Nesting behaviour			
Bites	5.81	3.15E-08	15
Charges	1.94	0.12	10
Approaches	6.34E-05	1	15
Time to orient	-2.98	0.01	15
Time to 1st bite	2.04	0.12	15
Nesters			
Bites	5.17	1.15E-06	99
Charges	-0.57	0.83	77
Approaches	-1.13	0.77	99
Time to orient	1.90	0.23	99
Time to 1st bite	0.81	0.83	99

Response to a territorial intruder was significantly repeatable across all trials, demonstrating the reliability of the flask-confined intruder paradigm

Table 2

Behaviour	All fish			Non-nesters			Nesters		
	R	SE	P _{adj}	R	SE	P _{adj}	R	SE	P _{adj}
Bites	0.65	0.06	1.05E-17	0.71	0.04	2.90E-13	0.63	0.10	3.26E-07
Charges	0.65	0.06	1.67E-18	0.66	0.11	1.26E-10	0.67	0.11	1.11E-08
Approaches	0.41	0.08	4.04E-09	0.40	0.08	2.39E-05	0.47	0.15	2.97E-05
Time to orient	0.36	0.08	2.75E-07	0.45	0.08	2.70E-06	0.31	0.15	0.003
Time to 1st bite	0.16	0.08	0.03	0.28	0.09	0.01	0.16	0.13	0.14

Table contains original scale approximations of *R*, which were similar to nonparametric intraclass correlation (ICC) estimates of repeatability.