READ ME: IMPORTANT INFORMATION FOR DATA ANALYSIS

HOW DATA WAS COLLECTED

All data collected for this experiment was collected on male Threespine stickleback (*Gasterosteus aculeatus*) collected from Little Campbell River- Anadromous Site at the start of the reproductive season (May 2017 and May 2018) Please see publication for exact information on fish collection and husbandry.
All data was uploaded into JMP versions 14.1.0 and 15.0.0 for statistical analyses.

Comprehensive behavioral data as a complete data set can be found in the "BehavioralDATA_BORIS_WithPerMinCalculations_DRYAD" data sheet. Behavioral data was collected and analyzed in four steps:

Step 1: fish were videotaped using an HD Sony camcorder positioned in front of the tanks during each of the unique behavioral interactions (including experimental contexts of a nesting male under various presentations, i.e. (i) presentation of a stimulus female within a UV transparent plexiglass container, (ii) presentation of a gravid female for mating, (iii) presentation of a rival male, and (iv) presentation of a model predator. Further, videos were recorded of control males within control tanks being presented with an empty UV transparent plexiglass container).

Step 2: All videos were given random video identifiers by behavioral interaction type so as to prevent observer bias. No videos were analyzed until all videos were completed. Video analysis occurred during 2020 without direct knowledge of the color scores from each fish to again prevent observer bias. All videos were analyzed using BORIS behavioral software, which allowed for ethograms to be keyed in the system and behaviors quantified withing given videos. Not all behaviors could occur for each interaction type, so only videos that could be quantified were (see Supplemental Tables S1 and S2). After all videos were analyzed, the data from each fish across all interaction types was transferred to a comprehensive data sheet.

Step 3: A master spreadsheet was created from all fish and videos, with data of behaviors scored imported from BORIS output and n/a denoting behaviors that could not occur (see Supplemental Tables S1 and S2). These behaviors were keyed in the data sheet as "_total", which includes the total number of times that a behavior occurred within the video timeline scored. From there, "behavior per minute" calculations were employed to standardize values used in analysis. Each "total" value was divided by the total minutes in which the video was scored and recorded in the data sheet as "_perminute" to indicate the standardized values.

Step 4: All statistical analysis of behaviors occurred within JMP versions 14.1.0 and 15.0.0. To analyze behavioral data by specific interaction type, the master spreadsheet was further broken down into the specific behavioral contexts. For example, if analyzing specific behavioral associations within the "female stimulus context", only the behavioral values from the Stimulus Female interaction were included in analysis through "include/exclude and hide/unhide" row functions in JMP. Further, all Log1P values were generated within JMP from the "_permin" behavioral values, as well as all Principal Component Analysis (PCA) values for behavioral contexts noted within the publication.

Color data was collected for three different areas of the body, including the ventral throat, lateral body, and pelvic spine. The comprehensive data with complete datasets can be found in the "ColorData_Stickleback_DRYAD" data sheet. It was collected as follows:

1. **Ventral Throat Chroma**: Following methods outlined within the publication; spectrophotometry was employed to collect reflectance values for two spots at 1nm intervals from 350-700nm along the ventral throat. The stimulation of the cones in the stickleback eye was then calculated for the 350-700nm range in order to provide quantam photon catches from each cone type used in matrix calculations of hue (h.theta, h.phi), and saturation/chroma (r.vec, i.e. the distance from the achromatic center). R.vec values were of particular interest here so were recorded for each fish at each stage, with the r.vec values from each fish averaged to create the final r.vec value used in analysis here (i.e. "MeanThroatColor_r.vec_ByStage")

2. **Lateral Body Coloration:** Following methods outlined within the publication, photographs of the lateral body of each fish at each stage were taken. From there, photographs were uploaded into Adobe Photoshop, and RGB values were recorded for each of 9 spots across the body within 5 areas commonly known to express nuptial coloration, including the Lower jaw, operculum, pectoral plate, ventral area between the lower jaw and pelvic spines (i.e. "VPS"), and lateral plates. Color measurements were corrected relative to a gay card in order to standardize the RGB values by dividing the R, G, and B values from each point by the RGB values generated from four points along the grey card, creating R_{stand}, G_{stand}, and B_{stand}. From here, Red Intensity (IR) was calculated by dividing the R_{stand} by the sum of R_{stand}, G_{stand}, and B_{stand}. The final Red intensity (IR) values were recorded for each spot along the male at each stage and uploaded into the color spreadsheet Please see Fig. 2 of the main text for geographic location along the body for each spot analyzed here.

Principal Component Analysis (PCAs) was used to create an overall value for lateral body coloration. All 9 spots were included and PCAs were generated for experimental males and control males separately, as outlined in the publication.

3. **Pelvic Spine Coloration:** Following methods outlined within the publication, photos of the erected pelvic spines were taken and uploaded into Adobe Photoshop. Using photoshop, R, G, and B values from each of 14 spots across 8 sections of the pelvic spine were recorded. Final Red Intensity (IR) values were recorded for each spot and averaged to create the value analyzed here denoted as "SpineColor_Iaverage".

REQUIRED INFORMATION FOR EACH SPECIFIC DATA SHEET

DATA SHEETS CONTAING BEHAVIORAL DATA

"BehavioralDATA_BORIS_WithPerMinCalculations_DRYAD"

All raw and per minute behavioral data can be found in this spreadsheet. Data includes behaviors performed by each fish within each behavioral context across all three stages of the reproductive cycle. In total there are 7 possible videos for experimental fish (Stimulus female interaction occurring in stages 2, 3, and 4; Male-Male interaction occurring in stages 3 and 4; Mating interaction occurring in stage 2 only; Predator interaction occurring in stage 4 only) and 3 possible videos for control fish (Control Interaction occurring in stages 2, 3, and 4). As previously stated, the total number of behaviors were recorded with "_total" denoted in the spreadsheet and the behaviors per minute were calculated and recorded with "_perminute" denoted in the spreadsheet. The "_perminute" calculations were the only values used in further analyses within this experiment. Log1P transformations of the "_perminute" behaviors were generated within JMP and used for statistical analyses. Please see publication for additional details of statistical analysis. N/a values are used to indicate behaviors that could not be performed and are highlighted in grey.

"PSEnearFleeLead_FleeLeadnearPSE_Data_PercentageCalculations_DRYAD"

This data file, including four different data sheets (one for each applicable behavioral interaction) addresses the number of "flees or leads performed within 5 seconds of a pelvic spine erection" and "pelvic spine erections performed within 5 seconds of a flee or lead" within the Stimulus Female Interaction, Male-Male interaction, Mating interaction, and Predator interaction, which were the only interactions in which a flee or lead could be performed. These numbers were reported via the BORIS behavioral output and recorded in a distinct sheet from the large data set by behavioral context.

For calculations of the percentages of "flees or leads performed within 5 seconds of a pelvic spine erection" and "pelvic spine erections performed within 5 seconds of a flee or lead", only videos in which the focal behavior occurred, i.e. a 'flee' or 'lead' for the question of "flees or leads performed within 5 seconds of a pelvic spine erection" or 'pelvic spine erection' for the question of "pelvic spine erections performed within 5 seconds of a flee or lead". These values are denoted in the "subset" columns. Any value marked as 'excluded' means that the focal behavior was not performed and therefore it was not included in percentage calculations. The mean percentage values were calculated from the subset values, followed by both the standard error of the mean and standard deviation, as noted in each sheet.

"PelvicSpineErections_Means_TTestData_DRYAD"

This data file was created for calculation of un-pooled T-tests assessing differences between the mean number of pelvic spine erections performed by control fish within the control context to experimental fish within each of the unique experimental contexts (i.e. Stimulus

Female interaction, Mating interaction, Male-Male interaction, and Predator interaction). The values used are the mean Log1P transformed number of pelvic spine erections performed per minute by each fish within each of the behavioral contexts. N/a values denote fish that could not be calculated due to inability to measure the behaviors within a particular interaction.

DATA SHEETS CONTAINING COLOR DATA

"ColorData_Stickleback_DRYAD"

This spreadsheet contains all the reported data values for each of the three types of color patches explored in this study across each fish from each of the four stages color was measured. The values reported include: (i) "spinecolor_Iaverage", i.e. mean red intensity (IR) value of the pelvic spine, (ii) "MeanThroatColor_r.vec_ByStage", i.e. the mean r.vec value generated from the two throat color spots assessed, and (iii) Red intensity values generated for each of the 9 individual color spots located along the lateral body. Each of these data points was generated using methodology outlined above. "Null" values indicate values that could have been recorded, but which we were unable to gather data for due to either premature fish death or technical failures.

      Because color was only recorded once per reproductive stage, stages with fish that go through multiple behavioral contexts (e.g. experimental fish within stages 2, 3, and 4) will show repeated values for each fish. To best highlight this, we have blocked each set of color data values with <u>borders</u> (indicating the complete set of fish within each behavioral context and stage) and <u>colors</u> (to indicate the values from each unique stage, which may include repeated data values if multiple behavioral contexts occurred within that stage). Details are as follows:

      All control male color values are not highlighted, appearing white in the data sheet. Because control male interactions occurred only once within stages 2, 3, and 4, there are no repeated values for these data sets within each stage. Because no behavioral interactions occurred in stage 1 (i.e. initial measurements), there are no repeated values for this stage either.

      All experimental male values within stage 1 (i.e. initial measurements) are highlighted in **RED**, with no values repeated because no behavioral interactions occurred within stage 1. All experimental male values within stage 2 are highlighted in **YELLOW** (with values repeated twice for "stimulus female" context and "mating context"). All experimental male values within stage 3 are highlighted in **ORANGE** (with values repeated two times, including "stimulus female" context and "male-male context"). All experimental male values within stage 4 are highlighted in **PURPLE** (with values repeated three times, including "stimulus female" context, "male-male" context, and "predator" context).

"AllColorSpots__Stages1-4_ExperimentalANDControl_withlength_DRYAD"

This data file includes three separate sheets, one for each of the unique color patch types explored here. Within each sheet, data for each fish across each of the four reproductive stages is recorded. Pelvic Spine Color sheet contains "spinecolor_Iaverage" values for each experimental and control fish at each stage, the Throat Color sheet contains

""MeanThroatColor_r.vec_ByStage" for each experimental and control fish at each stage, and the Lateral Color sheet contains the "PC1_LateralColor" values generated in JMP from the Red Intensity values of each of the 9 lateral body spots within experimental and control fish. "Null" values indicate values that could have been recorded, but which we were unable to gather data for due to either premature fish death or technical failures

Further included is a column on fish length, recorded in mm. Fish length was only recorded one time, at the start of the experiment, and so the values for each fish remain the same across each of the reproductive stages.

EMPHASIS OF IMPORTANT NOTES FOR EACH DATA SPREADSHEET

Within the **"ColorData_Stickleback_DRYAD"**:

-Color was only measured once per reproductive stage.
-Null values in cells indicate values that could have been recorded, but which no values exist due to either premature fish death or technical failures.
-All null values were excluded from analysis.
-Borders around data indicate the data set for each type of fish (either control or experimental males) within each stage by behavioral interaction.
-Highlighted cells indicate experimental males.
-Stage 1 has no repeated values for either control or experimental fish.
-Control fish have no repeated values for stages 2-4.
-Control fish values all appear as white cells, while each of the experimental fish values are highlighted based upon the stage in which the measurement occurred.
-Values from experimental fish in stage 1 are highlighted in **RED**. Values from experimental fish in stage 2 are highlighted in YELLOW and repeat twice (i.e. "stimulus female" & "mating" interactions). Values from experimental males in stage 3 are highlighted in **ORANGE** and repeat twice (i.e. "stimulus female" & "male-male" interactions). Values from experimental fish in stage 4 are highlighted in **PURPLE** and repeat three (i.e. "stimulus female", "male-male", and "predator" interactions).

Within the **"BehavioralDATA_BORIS_WithPerMinCalculations_DRYAD"** sheet:

-The columns with the blue heading are the "per minute" values calculated as the number of behaviors performed per minute of video. These values were then used for further analyses as noted in the publication.
-All Log1P calculations of the "per minute" values were generated within JMP as needed for statistical analysis.
-Cells highlighted in GREY and containing the value n/a indicate behaviors that could not be performed within each the specific behavioral interaction noted. Because the values could not be performed, they were not scored and *should not be considered* within any analysis.

-N/A values that extend across an entire row (i.e in categorical and nominal columns) indicate the video could not be scored due to either premature fish death or technical failures.
-All n/a values were excluded from analysis

Within the "PSEnearFleeLead_FleeLeadnearPSE_Data_PercentageCalculations_DRYAD" sheet:

-Any cells highlighted in **RED** were excluded from percentage calculations because the behavior of interest was not performed and therefore would lead to inaccurate assumptions if included. If the behavior was not performed, there can be no calculations to determine what percent of that behavior occurred near another. For example, if examining the percentage of Flees/Leads performed within 5 seconds of a pelvic spine erection by a fish... if no flees/leads were performed, that data point was excluded from further evaluation. Note- the "subset" columns show exactly which fish were included in percentage calculations, with any ineligible fish being noted. The two questions, "Pelvic Spine Erections performed within 5 sec of a Flee/Lead" and "Flees/Leads performed within 5 sec of a Pelvic Spine Erection" are both represented in this data sheet.
-This data file contains four sheets, one for each of the behavioral contexts in which a Flee or Lead could occur

Within the "PelvicSpineErections_Means_TTestData_DRYAD" sheet:

-Mean values were calculated from the LOG1P transformed pelvic spine erections per min values.
-Null values indicate values that could have been obtained but weren't due to either premature fish death or technical failures.

Within the "AllColorSpots_Stages1-4_ExperimentalANDControl_withlength_DRYAD" sheet:

-This data file contains three different sheets, one for each of the different color patches.
-This sheet contains standard length measurements for each fish. Standard lengths were taken just once, at the start of the experiment, so each fish will have repeated length values across all reproductive stages.
-Cells containing n/a denote values that could have been recorded, but which no value exists for due to either premature fish death or technical failures.
-All cells containing n/a were excluded from analysis.