

Pair-bonding influences affective states in a monogamous fish species

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Appendix S1. Details on study animals and housing conditions

Individuals were from our laboratory breeding stock, which originally came from local commercial distributors. At the beginning of the experiments, the fish were approximately one year old and were sexually mature. They were housed in tanks (450 L, 150×50×60 cm) equipped with artificial plants, artificial shelter, rocks and gravel at $25 \pm 1^\circ\text{C}$ under a 12:12 light:dark cycle until the beginning of the training. The fish were housed in same-sex tanks (40-50 fish per tank) to ensure sexual receptivity and to avoid reproduction before the experiments. The fish were fed daily with Cichlid XL flakes (Tetra®, Germany). All of the experiments were performed between March and November 2017. The experiment was carried out with females for two main reasons. First, the cues used by the females to choose a partner (body length) have been described in previous studies (Dechaume-Moncharmont et al. 2011, 2013), which allowed for the formation of dyads of contrasting males (i.e., differing in body length) for the binary choice test. Second, the protocol for quantifying female preference for a male (time spent in front of the preferred male) and her investment in reproduction (spawning latency, time spent attending the eggs) has been validated (Dechaume-Moncharmont et al. 2011, 2013; Laubu et al. 2016). Similar information is scarce for males.

Appendix S2. Details on the training protocol for the judgement bias test (Experiment 2)

Before Experiment 2, each female was housed with two females, one in each adjacent compartment (Fig. 1), to limit social isolation. She was trained first to find a reward (one chironomid larva) in an open box (see main text for a description of the box), then in a half-open box, and finally in a box completely covered by a lid. These boxes were always located in the same place (either on the left or right side of the compartment) and were associated with the same colour lid (either black or white), which both characterized the positive signal in the remainder of the experiment. Once a female had learned to open the box (Fig. 1b-c), she was trained to discriminate between the rewarded box (positive signal) and an unrewarded box (negative signal). These two boxes differed according to a random combination of spatial (box on the left or right side of the tank with a distance of 20 cm between these two positions, Fig. 1a) and visual (black or white lid) cues. They were presented sequentially.

The training consisted of successive sessions, with only one session per day (between 2:00 and 4:00 p.m.) to avoid overfeeding and maintain motivation for the test. Each session consisted of the presentation of three positive boxes and three negative boxes, in a random order, with one box at a time, and with 5 minutes between two consecutive presentations. After training, the females promptly opened the positive box and refrained from opening the negative box (electronic supplementary material, Fig. S2). A female was considered to have successfully learned the signals when (i) her average latency to opening the positive box was shorter than that to opening the negative one and (ii) the latencies to opening the three positive boxes

were all shorter than the latencies to opening the three negative boxes the day prior to the first judgement bias test. On average, 3.04 ± 2.21 sessions (mean \pm sd) were sufficient for a female to learn the task.

In preliminary tests, we also verified that the fish did not respond to potential chemical cues from the chironomid larva. Naive fish (which had not learned to associate the signal with the reward) did not spontaneously inspect a covered box containing a larva. Similarly, experienced females, which had learned to avoid the negative box, were not attracted by this box when a larva was added. We also verified the absence of innate bias for side or colour in the training sessions; the latency was not affected by either the spatial position (positive signal: $\chi^2_1 = 0.289$, $p = 0.591$; negative signal: $\chi^2_1 = 0.381$, $p = 0.537$) or the colour (positive signal: $\chi^2_1 = 0.871$, $p = 0.351$; negative signal: $\chi^2_1 = 0.630$, $p = 0.427$) of the signal.

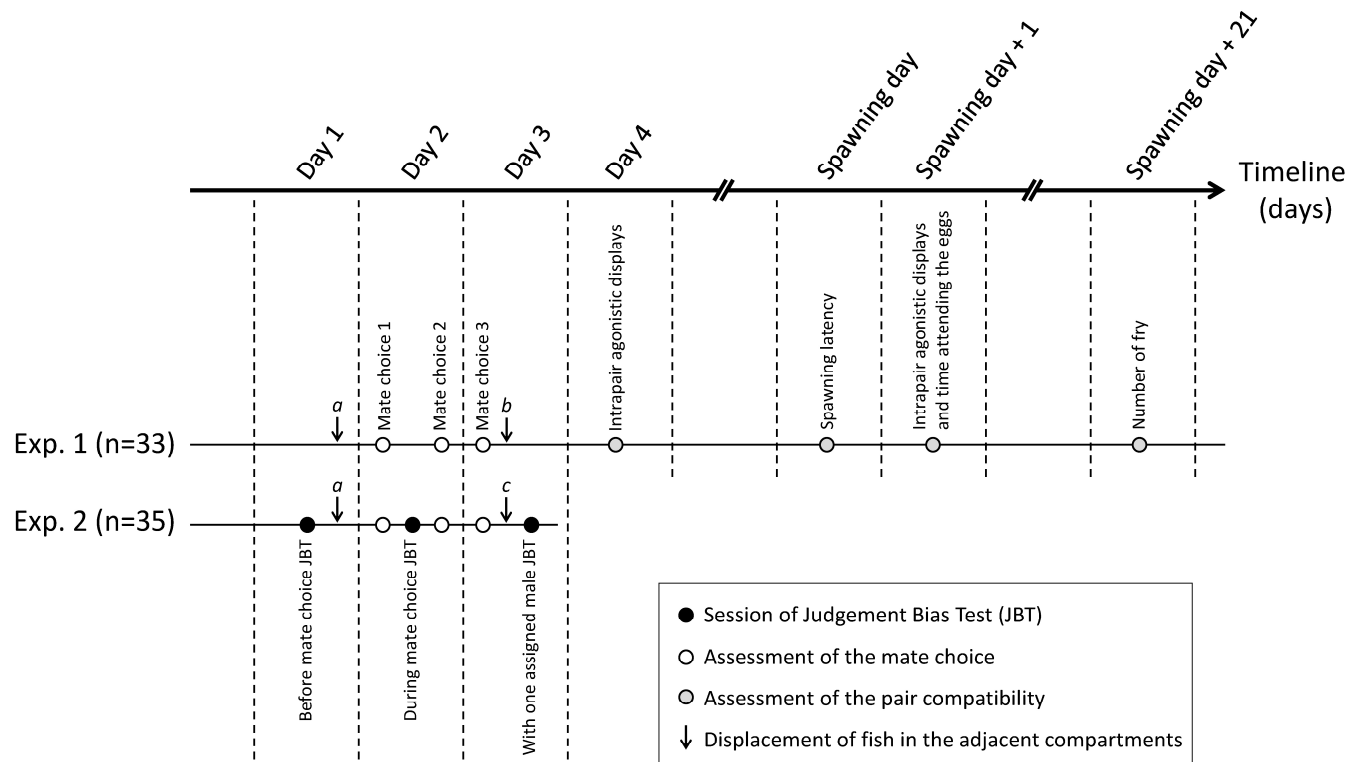


Figure S1. Timeline representing the sequence of events for each experiment. The 68 females were randomly divided into two experimental groups. Experiment 1 ($n = 33$ females) was designed to assess the ability of the binary choice test to accurately identify the preferred male for each female and predict the long-term reproductive success of the pair. At least one week prior to the experiment, the females were housed with two females, one in each adjacent compartment (Fig. 1). At the end of the first day (event ‘a’), the adjacent females were removed and replaced by two males. The observations of female preference for a male in binary a choice test (open circle) were then performed three times to assess the repeatability of this measure. Immediately after the third observation period of mate choice, the female was randomly assigned either her preferred partner ($n = 16$) or her non-preferred partner ($n = 17$). The assigned male was introduced into the female’s compartment, while the other one was removed from his compartment (event ‘b’) for several measures of pair compatibility until 21 days after spawning (grey circle).

Prior to Experiment 2 ($n = 35$ females), each female was trained to learn the positive and the negative signals during daily sessions (see electronic supplementary material, Appendix S2, for details about the training protocol). During Experiment 2, the female preference for a male (open circle) was assessed following the same protocol as in Experiment 1. In addition, we assessed the effect of the assigned male on her affective state using three successive daily sessions of the judgement bias test (JBT, solid circle). The training sessions and the first JBT session (‘before mate choice’) were performed in the presence of two females, one in each adjacent compartment. Then, as in Experiment 1, the adjacent females were removed and replaced by two males at the end of the first day (event ‘a’). The second JBT session (‘during mate choice’) was performed in the presence of these two males. On the third day, the females were randomly assigned either their preferred ($n = 17$) or non-preferred ($n = 18$) male; this male remained in his compartment, while the other male was removed (event ‘c’). Then, the third JBT session (‘with one assigned male’) was performed.

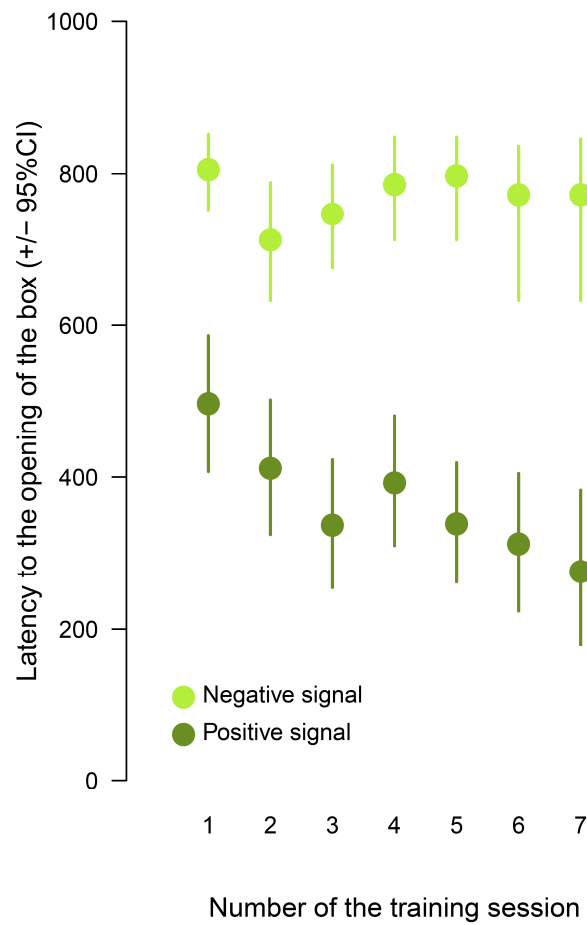


Figure S2. Learning curves for the positive and negative signals used in the judgment bias test. Prior to Experiment 2, the fish were individually trained to discriminate between the positive and negative signals. The latency time (in seconds) to the opening of the box was significantly affected by the interaction between the signal (positive or negative) and the number of sessions (mixed-effects Cox model for repeated measures with the individual as a random factor: $\chi^2_1 = 10.02$, $p = 0.0015$). The latency significantly decreased over time for the positive signal ($\chi^2_1 = 20.00$, $p < 10^{-5}$) and remained constant for the negative signal ($\chi^2_1 = 0.223$, $p = 0.64$).

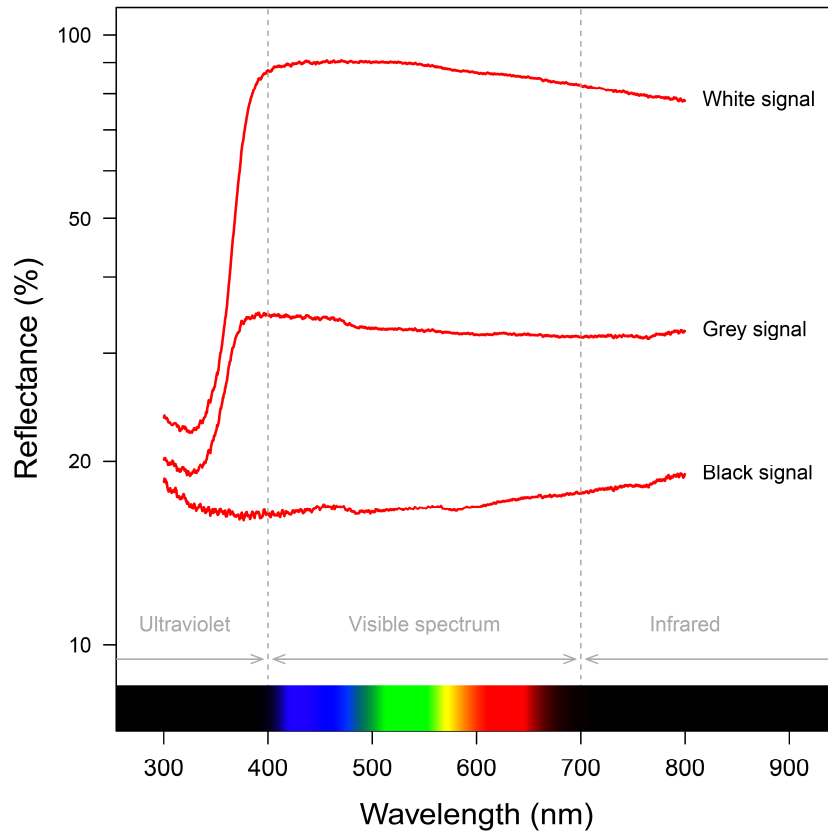


Figure S3. Spectral reflectance curves of each of the three types of lids used in the judgement bias tests. The ambiguous signal was positioned halfway between the position of the positive and negative signals. The ambiguity of the signal also came from the grey colour of the lid, which was a shade between the black and white lids used for the positive and negative signals. Cichlid fish have the rhodopsine Rh1 gene expressing rod photoreceptor cells, allowing perception of shades of grey (Trezise & Collin 2005). The Weber-Fechner law (Nieder & Miller 2003) states that the perceived brightness P is proportional to the logarithm of the measured intensity I for the stimulus: $P = k \times \log(I)$. The regular brightness gradation (black, grey, and white) was thus calibrated along a logarithmic scaling resulting in visually equidistant steps such that the ratio of perceived brightness between black and grey signals $P_{Black}/P_{Grey} = 0.81 \pm 0.05$ (mean \pm sd) was similar to that between grey and white signals $P_{Grey}/P_{White} = 0.83 \pm 0.04$. Brightness was measured using a spectrophotometer (USB2000+, Ocean Optics, IDIL Fibres Optiques, Lannion, France). Following the methods by Biard et al. (2017), the lid samples were illuminated at an angle of 90° with a deuterium-tungsten lamp (DH2000), and the reflected light was measured at the same angle with a 200 μm fibre optic reflection probe (QR200-7-SR-BX). An opaque black tube was fitted at the end of the measuring probe to exclude ambient light and standardize the measuring distance (3 mm). The percentage of reflectance was measured every 0.333 nm in the 300-800 nm spectral range. For each value of the wavelength λ , the reflectance $R(\lambda)$ was calculated relative to pure white (Spectralon Diffuse Reflectance Standard, WS-1-SL) and pure dark (generated by closing the shutter of the spectrometer) references, as $R(\lambda) = 100 \times [(\text{sample-white})/(\text{white-dark})]$. Two randomly chosen lids were analysed for each colour with three independent measures per lid (the probe was lifted and then placed back on the sample again). When these values for the reflectance across the spectrum range were consistent over the repeated measures (Repeatability = 0.98, 95%CI = [0.94; 1], $p < 10^{-5}$), the reflectance curves were averaged for each colour and plotted on a logarithmic scale according to the Weber-Fechner law. The brightness was calculated as the average reflectance over the spectral range.

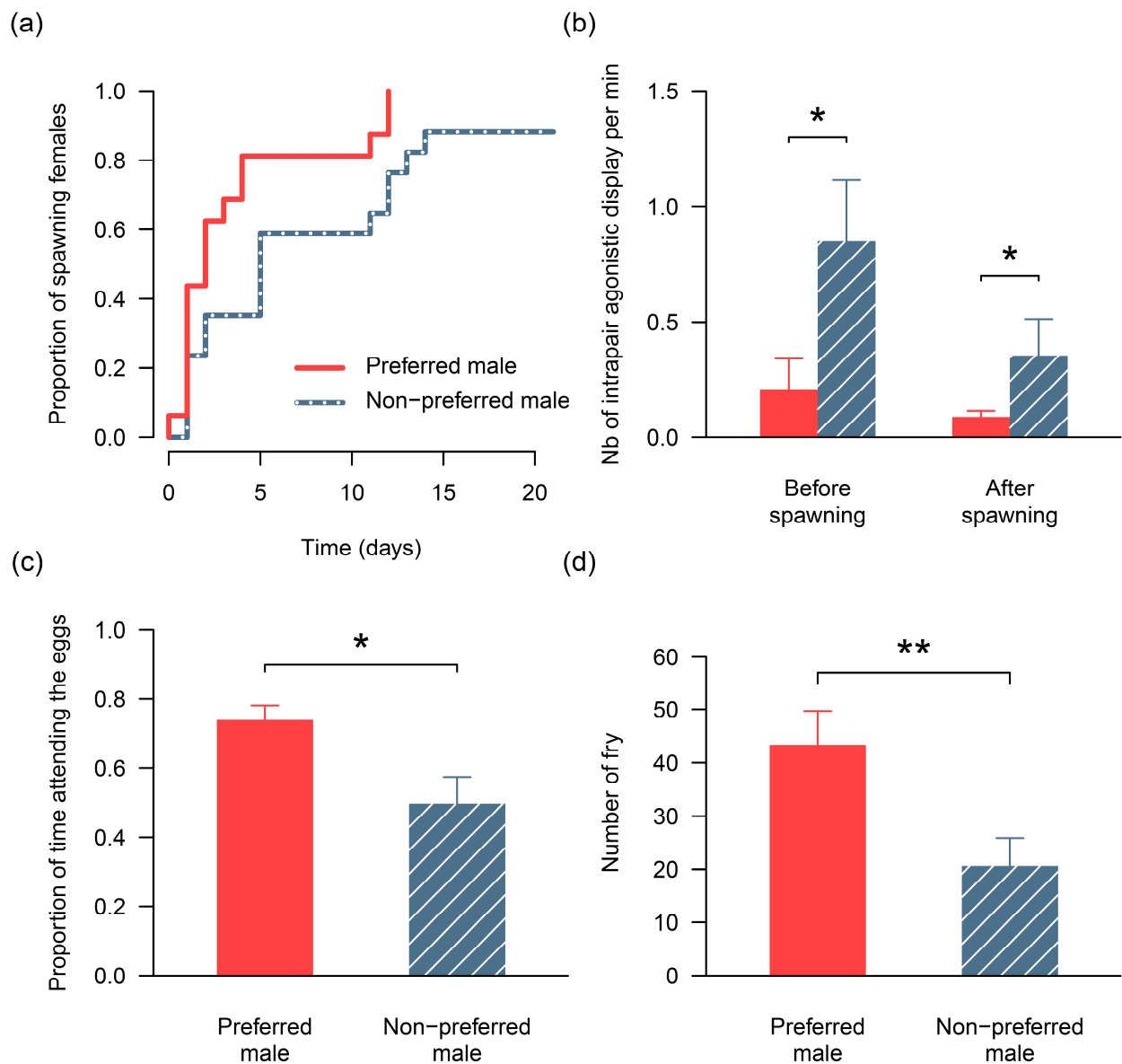


Figure S4: Pair compatibility and reproductive success (Experiment 1) as a function of the assigned male (preferred male in red, non-preferred male in striped blue): (a) spawning latency, (b) proportion of time spent attending the eggs by the female, (c) frequency (number of displays per minute) of aggressive interactions between partners before and after spawning, (d) number of fry three weeks after spawning. The error bars denote the standard error.

Table S1. Experiment 2: effect of the box position and lid colour on the latency to the opening of the box.

| Dependent variable | Fixed effects | χ^2 | df | p |
|---------------------------|--------------------------|----------------------------|-----------|----------|
| Positive signal: | | | | |
| Latency | Colour \times Position | 0.82 | 1 | 0.37 |
| | Position | 1.04 | 1 | 0.31 |
| | Colour | 0.38 | 1 | 0.54 |
| Ambiguous signal: | | | | |
| Latency | Colour \times Position | 1.20 | 1 | 0.27 |
| | Position | 0.17 | 1 | 0.68 |
| | Colour | 2.19 | 1 | 0.14 |
| Negative signal: | | | | |
| Latency | Colour \times Position | 0.18 | 1 | 0.67 |
| | Position | 1.05 | 1 | 0.30 |
| | Colour | 0.58 | 1 | 0.44 |

Each female was trained to discriminate between a positive and negative signal characterized by a randomly attributed combination of position (on the left or right side of the tank) and colour (white or black lid). The effect of the learned signals on the latency to the opening of the box was assessed in the control contexts using a mixed effects Cox regression model with female identity as a random variable. We followed a stepwise selection model procedure to identify the best model. As the interaction terms (Colour \times Position) were non-significant, we then assessed the effect of each variable separately.

Table S2. Experiment 2: latency to the opening of the box as a function of the context ('before mate choice', 'during mate choice', and 'with one male') and the treatment (preferred vs. non-preferred male).

| Dependent variable | Fixed effects | χ^2 | df | p |
|--------------------------|---------------------|----------|----|------------------|
| Positive signal: | | | | |
| Latency | Context × Treatment | 1.62 | 2 | 0.44 |
| | Context | 2.06 | 2 | 0.36 |
| | Treatment | 0.50 | 1 | 0.48 |
| Ambiguous signal: | | | | |
| Latency | Context × Treatment | 9.29 | 2 | 0.0096 ** |
| Negative signal: | | | | |
| Latency | Context × Treatment | 2.12 | 2 | 0.35 |
| | Context | 0.91 | 2 | 0.63 |
| | Treatment | 1.13 | 1 | 0.29 |

The females' affective state was repeatedly estimated in three different contexts: 'before mate choice', 'during mate choice', and 'with one randomly assigned male' (either the preferred male or non-preferred male). The first two contexts were used as control contexts to estimate the repeatability of the female's affective state and to verify that the two treatment groups (preferred vs. non-preferred male) did not differ before the third context ('with one assigned male'). The latency to the opening of the box as a function of the signal (positive, ambiguous or negative) was analysed using Cox mixed-effect models with female identity as a random variable to take the repeated measures design into account. For each signal, we followed a stepwise selection model procedure to identify the best model. When the interaction term was non-significant, the effect of each variable was assessed separately. For the two trained signals, the response latencies did not change as a function of the context or the treatment, which was a central assumption in the judgement bias paradigm. Conversely, females changed their response to the ambiguous signal differently across contexts depending on the treatment. As the interaction term was significant, we then analysed the female response separately by context (Table S3) or by treatment (Table S4). Here and elsewhere, the significant terms are highlighted in bold.

Table S3. Experiment 2: latency to the opening of the box as a function of the treatment (preferred male vs. non-preferred male) calculated separately for each context.

a) Context: before mate choice

| Dependent variable | Fixed effects | χ^2 | df | p |
|---------------------------|----------------------|----------------------------|-----------|----------|
| Positive signal: | | | | |
| Latency | Treatment | 2.71 | 1 | 0.10 |
| Ambiguous signal: | | | | |
| Latency | Treatment | 0.09 | 1 | 0.76 |
| Negative signal: | | | | |
| Latency | Treatment | 0.25 | 1 | 0.62 |

b) Context: during mate choice

| Dependent variable | Fixed effects | χ^2 | df | p |
|---------------------------|----------------------|----------------------------|-----------|----------|
| Positive signal: | | | | |
| Latency | Treatment | 0.092 | 1 | 0.76 |
| Ambiguous signal: | | | | |
| Latency | Treatment | 0.76 | 1 | 0.38 |
| Negative signal: | | | | |
| Latency | Treatment | 0.035 | 1 | 0.85 |

c) Context: one assigned male

| Dependent variable | Fixed effects | χ^2 | df | p |
|---------------------------|----------------------|----------------------------|-----------|----------------|
| Positive signal: | | | | |
| Latency | Treatment | 0.99 | 1 | 0.32 |
| Ambiguous signal: | | | | |
| Latency | Treatment | 4.25 | 1 | 0.039 * |
| Negative signal: | | | | |
| Latency | Treatment | 2.06 | 1 | 0.15 |

As there was a significant interaction between the context and treatment (Table S2), the latencies to the opening of the box were analysed separately for each context: before mate-choice (a), during mate-choice (b) and with one assigned male (c). The latency was analysed using Cox models. We verified that the two treatment groups (preferred male and non-preferred male) did not differ within the first two contexts. Conversely, there was a significant effect of the treatment in the third context and solely for the ambiguous signal: the response latency was significantly longer for the females assigned their non-preferred male than for those assigned their preferred male (HR = 2.52, 95%CI = [1.02; 6.17]).

Table S4. Experiment 2: latency to the opening of the box as a function of the context ('before mate choice', 'during mate choice', and 'with the assigned male') and separately for each treatment (preferred or non-preferred male).

a) Treatment: preferred male

| Dependent variable | Fixed effects | χ^2 | df | p |
|--------------------------|---------------|----------|----|------|
| Positive signal: | | | | |
| Latency | Context | 1.98 | 2 | 0.37 |
| Ambiguous signal: | | | | |
| Latency | Context | 1.62 | 2 | 0.45 |
| Negative signal: | | | | |
| Latency | Context | 0.10 | 2 | 0.95 |

b) Treatment: non-preferred male

| Dependent variable | Fixed effects | χ^2 | df | p |
|--------------------------|---------------|----------|----|------------------|
| Positive signal: | | | | |
| Latency | Context | 2.12 | 2 | 0.35 |
| Ambiguous signal: | | | | |
| Latency | Context | 9.66 | 2 | 0.0080 ** |
| Negative signal: | | | | |
| Latency | Context | 3.60 | 2 | 0.17 |

As there was a significant interaction between context and treatment (Table S2), the latencies to the opening of the box were analysed separately for each treatment. The latency was analysed using mixed effects Cox models with female identity as a random variable in order to take the repeated measures design into account. When the female was assigned her non-preferred male, the context had a significant effect on the latency to the opening of the ambiguous box: her response latency was higher in the third context than in the first two contexts (Tukey *post-hoc* pairwise comparisons "before mate choice" vs. "during mate choice": $p = 1.00$, "before mate choice" vs. "with one assigned male": $p = 0.027$, "during mate choice" vs. "with one assigned male": $p = 0.019$).

Table S5. Experiment 2: latency to the opening of the box as a function of the preference score and the treatment (preferred vs. non-preferred male) in the third context (with one assigned male) and separately for each signal (positive, ambiguous, or negative).

| Dependent variable | Fixed effects | χ^2 | df | p |
|--------------------------|-------------------------------------|----------|----|----------------|
| Positive signal: | | | | |
| Latency | Preference score \times Treatment | 0.24 | 1 | 0.62 |
| | Preference score | 0.62 | 1 | 0.43 |
| | Treatment | 0.99 | 1 | 0.32 |
| Ambiguous signal: | | | | |
| Latency | Preference score \times Treatment | 4.66 | 1 | 0.031 * |
| Negative signal: | | | | |
| Latency | Preference score \times Treatment | 0.0017 | 1 | 0.97 |
| | Preference score | 0.11 | 1 | 0.74 |
| | Treatment | 2.06 | 1 | 0.15 |

The latency to the opening of the box as a function of the signal was analysed using Cox models. For each signal, we assessed how the female response latency was affected by the interaction between her preference score (measuring the strength of her preference for one male during mate choice test) and the treatment (preferred male vs. non-preferred male). We followed a stepwise selection model procedure to identify the best model. When the interaction term was non-significant, we then assessed the effect of each variable separately. For the positive and negative signal, the response latencies did not change as a function of the preference score and the treatment. Conversely, the interaction term was significant for the response to the ambiguous signal. The response latencies to this signal were thus analysed separately by treatment (Table S6).

Table S6. Experiment 2: latency to the opening of the ambiguous box in the third context ('with one assigned male') as a function of the preference score and separately for each treatment.

| Dependent variable | Fixed effects | χ^2 | df | p |
|--------------------------------------|----------------------|----------------------------|-----------|----------------|
| Treatment: preferred male | | | | |
| Latency | Preference score | 0.54 | 1 | 0.46 |
| Treatment: non-preferred male | | | | |
| Latency | Preference score | 4.33 | 1 | 0.037 * |

There was a significant interaction between the preference score and the treatment on the response latency to the ambiguous signal in the full model (Table S5). We thus assessed the effect of the preference score on the response latency to the ambiguous signal separately for each treatment (either preferred or non-preferred male). The latency was analysed using Cox models.

Table S7. Experiment 1: frequency of intra-pair agonistic displays (Fig. S3c) as a function of the treatment (preferred *vs.* non-preferred male) and spawning status (before *vs.* after spawning).

| Dependent variable | Fixed effects | χ^2 | df | p |
|-----------------------|------------------------------------|----------|----|------------------|
| Frequency of displays | Treatment \times Spawning status | 0.058 | 1 | 0.810 |
| | Treatment | 7.73 | 1 | 0.0054 ** |
| | Spawning status | 4.12 | 1 | 0.0425 * |

The frequency of intra-pair agonistic displays (number of displays between partners per minute, regardless the identity of the initiator of the interaction) was recorded either before spawning (24h after pairing) or 24h after spawning. These repeated-measure data were analysed using generalized linear mixed models with female identity as a random variable. We followed a stepwise selection model procedure to identify the minimal model. As the interaction term (Treatment \times Spawning status) was non-significant, it was dropped from the model in order to assess the effect of the two variables separately. The frequencies of the displays were significantly smaller when the female was paired with her preferred male than with her non-preferred male (treatment: estimate $\beta = -1.39$, 95%CI = [-2.28; -0.49]), and in presence of the spawn (Spawning status: $\beta = -0.87$, 95%CI = [-1.69; -0.051]).



Movie S1: Illustration of the judgment bias test (Experiment 2). Each female was trained to open boxes covered by a movable lid, and to discriminate between a positive (rewarded with one chironomid larva) and a negative (unrewarded) signal characterized by a randomly attributed combination of box position (on the left or right side of the tank) and lid colour (white or black). For instance in this video, the female has learned that the positive signal was the box on the left side and covered with a black lid, and the negative signal was on the right side and covered with a white lid. Her affective state was assessed as the response to an ambiguous signal placed in an intermediate position between the two learned signals and covered with grey lid. Given the response latency of the female, the speed of the video was increased (see the information on the video).

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