OXFORD UNIVERSITY PRESS | Behavioral Ecology

Mate choice plasticity in a coral reef fish

Journal:	*Behavioral Ecology*
Manuscript ID	BEHECO-2015-0663.R1
Manuscript Type:	Original article
Keywords:	coloration, mating competition, sexual selection, aggression, damselfish, Chrysiptera cyanea

Mate choice plasticity in a coral reef fish

SEBASTIAN WACKER[1], SARA ÖSTLUND-NILSSON[1,2], ELISABET FORSGREN [1,3], CAIT NEWPORT[1,4], TROND AMUNDSEN[1]

[1] Department of Biology, Norwegian University of Science and Technology, Norway

[2] National Library of Norway

[3] Norwegian Institute for Nature Research, Norway

[4] Department of Zoology, University of Oxford, UK

Short title: Mate choice plasticity

Correspondence: Sebastian Wacker, Department of Biology, Norwegian University of Science and Technology, NO-7491, Norway, e-mail: sebastian.wacker@ntnu.no; tel: +47-73596257; fax: +47-73596100

ABSTRACT

Mate choice and intrasexual mating competition are the processes that drive sexual selection and can be interrelated in multiple ways. Female preference may be altered by exposure to male-male interactions. This could happen because of a reduced ability of females to evaluate males in the presence of male-male competition or because females use information from male interactions to form mating preferences. In this study, female preference for a male tail ornament was measured in the presence and absence of male-male competition in a coral reef fish, the damselfish *Chrysiptera cyanea*. Despite the striking colors of coral reef fishes, few studies have investigated the role of their coloration in the context of sexual selection. When male-male interactions were prevented, females showed a temporal change in preference with respect to male tail coloration, preferring brightly colored males early in the study period and dull males later on. Thus, females did not become less selective, but reversed their preference function within the breeding season. When males were allowed to interact, male-male agonistic interactions were extremely frequent and male courtship activity was strongly reduced. In this situation, females still displayed a sexual interest in males, but were indiscriminate with regard to male tail coloration. Taken together, these results provide compelling evidence for plasticity of female mating preference, related to the social context and time in the breeding season.

Key words: coloration, mating competition, sexual selection, aggression, damselfish, *Chrysiptera cyanea*

INTRODUCTION

Sexual selection is driven by the processes of mate choice and intrasexual mating competition (Darwin 1871; Andersson 1994). The two processes are often tightly interrelated rather than acting independently (Bradbury and Davies 1987; Qvarnström and Forsgren 1998; Wong and Candolin 2005). For example, females may choose mates on the basis of their performance in intrasexual competition, either following observation of competition (Valone and Templeton 2002; Doutrelant and McGregor 2000) or based on resources males have acquired by means of competition (Candolin and Voigt 2001; Creighton 2001; Wacker and Amundsen 2014). This has stimulated theoretical (Kokko 2005) and empirical (Qvarnström and Forsgren 1998; Wong and Candolin 2005) work on whether females should prefer dominant males. Females may benefit from observing male-male competition because it allows them to evaluate male traits related to competition. On the other hand, male-male competition may hamper the evaluation of male traits expressed during courtship. This may occur when male agonistic interactions lead to a reduction of courtship and in particular when males interfere with the courtship of competitors (Howard et al. 1997; Östlund-Nilsson and Nilsson 2000). Traits expressed in intrasexual agonistic encounters and during courtship may not be the same, and females may or may not use information from both processes in their choice of mates. Male-male competition may thus weaken or strengthen female mate preference for a certain trait. It is important to note that this question is different from the relative importance of male-male competition and female choice in determining male mating success (Qvarnström and Forsgren 1998; Wong and Candolin 2005; Hunt et al. 2009). Male-male competition may for example override female choice by precluding females to mate with males of the preferred phenotype. In such a case, the preference is retained but not realized in actual mating. This is fundamentally different from a change in female preference when exposed to male-male competition.

While the above scenarios suggest that female choice should often be affected by male-male interactions, behavioral studies typically focus on one of the two processes, aiming to preclude the other (Wong and Candolin 2005). Mate choice is most commonly studied by 'choice tests'. In those tests, individuals are most often given the choice between two opposite sex individuals that are prevented from interacting with each other by a visual barrier (Wagner 1998; Dougherty and Shuker 2015). This approach allows one to attribute the observed biases in female behavior to preference alone. However, female preference in the presence of male-male competition may be different and may in many species be a closer representation of preference expressed in the wild (Wong and Candolin 2005). The potential effect of male-male competition should therefore be taken into account when studying female preference in species where females may observe male competition.

In this study, we analyze female preference for male tail coloration in the absence and presence of male-male competition in a coral reef fish. *Chrysiptera cyanea* is a territorial damselfish inhabiting coral reefs in the Central Indo-Pacific (Froese and Pauly 2015). Despite their extravagant coloration, sexual selection on coloration in coral reef fishes has generally been little studied (Amundsen 2003; Petersen and Warner 2002; but see: damselfishes: Gronell 1989; Haley et al. 2004; wrasses: Kuwamura et al. 2000; Warner and Schultz 1992). This is surprising in light of the considerable interest in sexual selection on body size and other morphological characters in reef fishes (e.g. cardinalfishes: Okuda et al. 2003; damselfishes: Schmale 1981; Hoelzer 1990; Knapp and Warner 1991; Goulet 1998; Manica 2009; Oliver and Lobel 2013; gobies: Kitamura et al. 2002; Sekiya and Karino 2004; wrasses: Kohda et al. 2005). *C. cyanea* is particularly suitable for studying sexual selection on coloration because male mating success is highly variable and males differ widely in tail coloration (Thresher and Moyer 1983; Gronell 1989). Female mating preferences therefore potentially translate into strong sexual selection on males. A previous field study on the species documented that both male tail ornament and body size were related to mating

success in the wild (Gronell 1989). However, as is generally the case for correlative field studies, it cannot be stated with certainty whether selection on males was a result of female mate choice, male-male competition or their interaction. In a related laboratory study that did not allow male-male interactions, females preferred large colorful males over small dull ones (Gronell 1989). Based on a multiple regression including body size and coloration, Gronell (1989) reported a preference for coloration as such (albeit weak). However, the inherent correlation between male total length and (absolute) ornament length may render the reported preference for coloration rather than size inconclusive. In the present study, we test whether females prefer males with more colorful tails when given the choice between two similarly sized males that differ in tail coloration (Figure 1).

Our experiment consisted of two steps. In the first step, females were given the choice between two males that differed in tail coloration and that were prevented from interacting by an opaque barrier. In the second step, the same two males were allowed visual but not physical contact, and the response of the female as well as male-male interactions were recorded. The tail fin is actively displayed towards both females (this study) and males (Thresher and Moyer 1983; Gronell 1989) and may therefore be of importance in both male-male competition and female choice. Male-male aggressive interactions in the presence of females are not uncommon in the species (Thresher and Moyer 1983) and may thus affect a potential female preference for male tail coloration. If females primarily choose males on the basis of male tail coloration, we would expect the preference to be largely unaffected by male interactions, because the orange tail is displayed in both courtship and male-male competition. If, instead, female mate choice is affected by traits that can best be evaluated when males compete, we would expect female preference to change. The aim of this study was to test whether female *C. cyanea* show a preference for male coloration and whether this preference changes when males are allowed to interact.

METHODS

The study was carried out during 30 October to 17 December 2010 at Lizard Island Research Station (LIRS), Great Barrier Reef, Australia (14° 40' S, 145° 27' E).

Study species

Chrysiptera cyanea males and females are bright blue, with males having conspicuous orange coloration on their tail fins (Figure 1). Males are larger than females and size distributions are only slightly overlapping (female TL (total length): 38-54 mm; male TL: 49-73 mm; Gronell 1989). Together with a female biased adult sex ratio, almost discrete size distributions suggest that the species is a protogynous hermaphrodite (Thresher and Moyer 1983). The peak reproductive season appears to be October to January at the study site (own observations). Both males and females are territorial (Thresher and Moyer 1983; Gronell 1989). Males defend nests and guard eggs until hatching (Thresher and Moyer 1983; Gronell 1989). Nests typically consist of dead clams (*Tridacna* spp.) embedded in coral, or holes in coral rubble, located on shallow reef flats, often in areas with much coral rubble (Gronell 1989; own observations). Aggressive male-male interactions involve mutual or one-sided fin displays (male erects dorsal, anal and pelvic fins and fans tail fin) and chases (Thresher and Moyer 1983; Gronell 1989). Those interactions can be swift or long-lasting (> 30 minutes), and may result in nest take-overs (Thresher and Moyer 1983; Gronell 1989). Agonistic interactions between adult males and females have not been observed despite extensive observations in the field (authors' personal observations). Spawning takes place in the early morning and females sample males the day prior to spawning (Gronell 1989). During mate sampling, both sexes may court and females may enter the nests of several males, sometimes re-entering the same nest repeatedly (Gronell 1989). Female courtship can involve fin displays (own observations) and a solicitation posture ("body inclined head-up and fins still"; Gronell 1989).

Male courtship consists of typically swift approaches to the female and directed lead swims to the nest (Thresher and Moyer 1983; Gronell 1989). Several females can spawn with the same male and females can spawn in intervals of four days (Gronell 1989).

Collection and husbandry

Fish were caught at reef flats near the research station (ca. 0.5 to 3 km) by SCUBA diving, using clove oil to immobilize the fish (Munday and Wilson 1997) and transported by boat to the station. In the lab, fish were measured for total length (TL) on a measuring board (to the nearest 0.5 mm), weighed (wet weight, to the nearest 0.01 g) and photographed (for measurement of size and coloration, see below), within two days after capture. Males were marked with visible implant elastomers (VIE, Northwest Marine Technology, Shaw Island, Washington) for individual recognition. Both males of a trial were marked yellow, on the left or right side respectively, at the underside of the body. The VIE tags were inconspicuous and we did not notice any adverse effects on behavior or welfare. Males and females were housed individually in 5-10 L aquaria, males for 3-16 days (median: 7 days) and females for 3-17 days (median: 8 days) from capture to the start of the trial. Variation in housing time resulted from challenges of catching fish of suitable size and coloration. Fish were caught during a total of 13 trips (26 October to 19 December), over the entire study period. Housing time did not differ significantly between the bright and the dull male (see below for color classification) used in a trial (paired t-test: $t_{24} = -1.50$, $P = 0.15$). Female housing time did not affect female choosiness (i.e. preference for either male, disregarding coloration; data not shown). There was a statistical trend for female housing time to increase over the course of the study, but female housing time did not affect female preference for male tail coloration (data not shown). During individual housing, fish were fed *ad lib* twice a day with live brine shrimp (*Artemia* sp.), marine fish flakes, and a mixture of (previously frozen) brine shrimp, bloodworms and mysids. Aquaria were placed outdoors under a semi-transparent roof,

providing natural daylight and shelter. All aquaria were provided with air stones and a flow of surface seawater at ambient temperature. Air and water flow were paused in experimental aquaria during observations, to avoid negative effects on visibility.

Experimental setup

Female preference for male coloration was studied in a classic dyadic mate choice set-up (Wagner 1998; Dougherty and Shuker 2015). Two males were presented to a female and female preference was quantified as bias in position and courtship behavior. Study aquaria had a water volume of approx. 40L and were divided into a female compartment on one side and two adjacent male compartments on the other side (Figure 2). PVC tubes (4.2 cm diameter, 6-10 cm long) were placed in each of the three compartments, acting as potential nest substrate and shelter. Aquaria were covered with grey, matte PVC on three sides to prevent mirror effects and visual contact among fish in neighboring aquaria. The female of a trial was physically but not visually separated from the males by a transparent plexiglass divider (Figure 2). In the first step of the study (hereafter referred to as 'Step 1'), the two males were separated by an opaque plexiglass divider, preventing visual and physical interaction. In the second step of the study ('Step 2'), males were separated by a transparent plexiglass divider only, allowing visual but not physical contact between the males.

Each set of individuals, one female and two males, was used only once in the experiment, but was tested consecutively in Steps 1 and 2 in order to compare responses of individual females between the two choice situations (steps). Thus, female preference was always first quantified in the absence of male-male competition. This was done to prevent females experiencing male-male competition already in the first step, which might have affected their preference in the second step when male-male interaction would have been precluded.

To allow acclimation to the test tanks, males were transferred to their compartments of the study tank 2-10 days (median: 4 days) prior to the trial and females one to three days (median: 1 day; in one case nine days) prior to the trial. Until the start of a trial, opaque dividers prevented visual contact between any fish in the aquarium. Fish were fed the evening before the trial, but not on the day of the trial. Step 1 of a trial was started between 7:30 and 9:30 am by lifting the opaque (but not the transparent) divider between the female and the males. Females were then allowed to visually interact with males for ca. 30 min, during which the aquarium was filmed from the front (female's side) with a digital HD camcorder (Step 1). After 30 min, the opaque divider between the female and the males was reinstalled. The fish were then given a recovery time of approx. 30-90 min, before Step 2 was started (between 8:30 and 11:00 am). At the start of Step 2, the opaque (but not the transparent) dividers between the female and the males, and between the two males, were removed. All fish were then allowed to interact visually and were filmed for 30 min.

Pairs of males were selected to be similar in body size but to differ in orange tail coloration. Matching was based on TL as measured on a board and subjective judgment of tail coloration on a 7-graded ordinal scale. Quantitative measures of tail coloration and a more exact measure of TL were later obtained from digital images (see below) and used for analyses. Pairs of similarly sized males were selected to differ maximally in coloration, given the available individuals at any given time. This procedure resulted in variation in tail color contrast among trials, which was taken into account in the analysis. Bright and dull males did not differ significantly in standard length (SL; paired t-test: t_{24}=1.4, P=0.16) or body mass (t_{22}=-1.1, P=0.28), but there was a trend for bright males being in worse condition at capture (t_{24}=-1.8, P=0.09) and bright males had significantly longer tails (t_{24}=2.6, P=0.017) (Table S1). Notably, the differences in condition and tail length were small in biological terms, despite being statistically significant. Condition was calculated as residuals from a regression of body mass (mg) on male SL (mm), and the average difference between bright and dull males was

only ca. 0.1g or 3% of average male body mass (Table S1). In order to avoid stress that could have affected the experimental outcomes, the fish were not weighed and measured at, or shortly before, the day of the experimental trial. It is therefore unknown whether males differed in condition at the time of the trial, which typically took place a week after capture and measurement, during which fish were fed ad lib. The average difference in tail length amounted to only ca. 3% of the average tail length (Table S1). Whenever possible, the two males and females within a trial were collected from different reefs (or different parts of a large reef flat), to minimize the likelihood of prior familiarity. This was achieved in 25 out of 30 replicates. The position of the bright and dull males in the aquaria was balanced among trials.

Behavioral recordings

From the 30 min video films for Steps 1 and 2 respectively, we recorded female position relative to the males, and female courtship directed at the males, in order to quantify female preference. We also recorded position, courtship and agonistic behavior of the two males, to analyze whether female preference was related to male behavior. Behavioral recordings were carried out blind to male tail color measurements.

Female and male positions were recorded by scan sampling at 20 s intervals. We recorded whether the female was (i) in front of the left male, (ii) in front of the right male or (iii) in a central neutral zone (Figure 2). At the same time, we recorded whether the female was (i) in proximity to the male-female divider (within a fish length), (ii) not in proximity to the divider or (iii) inside the tube (Figure 2). Finally, we recorded whether the female was oriented towards the male compartments or away from them, including female positioning in parallel to the divider as 'towards the male compartments'. In the analyses of female preference, a female was considered to associate with a particular male when she was in front of him and at

the same time (i) in immediate proximity to the divider, and/or (ii) oriented towards the male compartment. These criteria conservatively limited the data analyzed for preference to situations that represent a clear female interest in one or the other male, excluding ambiguous positions.

We recorded male position as whether a male was (i) in proximity to the male-female divider (within a fish length), (ii) in proximity to the male-male divider, (iii) not in proximity to any divider or (iv) inside the tube (Figure 2).

Courtship by both sexes and male agonistic behavior were recorded by continuous focal observations of the video recordings. Female courtship included fin displays (female erects dorsal fins) and sigmoid displays (female bends body in a sigmoid shape). Male courtship included approaches (male swims towards female in a fast and directed movement of at least half a fish length), fin displays (male erects dorsal fin and fans tail fin in proximity to the female), and lead swims (male swims towards nest and enters it in a directed movement). Male lead swims were only included when they followed an approach or fin display within 2 sec (as was usually the case in the context of courtship) in order to conservatively exclude cases in which the male might have sought shelter. Courtship frequency may be highly affected by female position. We therefore also quantified male courtship propensity, recording whether a male showed any courtship within 3 sec from when the female entered his side of the aquarium (excluding all cases in which the female stayed <3 s). Propensity measures are often more precise expressions of motivation and effort, as they are not affected by frequencies of encounters (Forsgren et al. 2004, de Jong et al. 2012). Recorded male intrasexual agonistic behaviors included approaches (male swims in a directed manner towards the other male) and fin displays (male erects dorsal fin and fans tail fin in proximity to the other male).

Male courtship behaviors were entered into a principal component analysis (PCA) to reduce the number of variables for further analysis. In addition to fin displays, approaches and lead swims, we included male courtship propensity and the proportion of scans in which the male stayed in proximity to the male-female divider in the PCA. All variables were standardized to have a mean of zero and a standard deviation of one. The extracted PCA components were varimax rotated (Tabachnick and Fidell 2001), facilitating the interpretation by more clear loadings of variables into PCA components (Table 1). Separate PCAs were carried out for the two steps of the study. Correlation matrices for the two PCAs are presented in Table S2 and eigenvalues and percentages of variance explained are given in Table 1. For both steps, we extracted the first two components, which had eigenvalues above one, for further analysis. In both steps, fin displays, approaches and lead swims loaded positively on the first component (PC1) and courtship propensity loaded positively on the second component (PC2; Table 1). Proximity to the female compartment loaded positively on the second component for Step 1, but negatively for Step 2 (Table 1). A high PC1 score therefore reflects high courtship frequencies, whereas a high PC2 score reflects a high propensity to court the female in case of an encounter. PC2 also reflects a high or low proportion of time spent in proximity to the female compartment, respectively, for Step 1 and Step 2. The two female courtship behaviors were not correlated ($r = 0.027$) and we therefore analyzed female fin and sigmoid displays as frequencies of behavior.

The start of a trial was defined as the time at which the female had entered both males' sides of the aquarium (i.e., had seen both males). Lifting the opaque divider at the start of the trial and occasional movements of a person near the aquarium sometimes disturbed the fish, which typically sought shelter in the PVC tubes in such events. In most trials, however, all fish left the tubes after a few minutes (but see below) and started to interact. In order to minimize disturbance effects, we limited data collection from the videos to the second half of the trials (15-30 min after the start). For Step 1, we recorded female and male position (by scan

sampling) for the entire 30 min period of the trial in order to analyze whether the first and second halves of the trials differed in the proportion of time the females spent with the more colorful male. This was not the case and we therefore conservatively excluded the first half of the trials for all measures, for reasons given above. In Step 2, male agonistic behavior was recorded for only 5 min (15-20 minutes after the female had entered both males' sides of the aquarium), because frequencies of agonistic behaviors were very high. Other parameters were recorded for 15 min, as in Step 1.

Color quantification

We quantified the orange tail ornament and body size of all males from digital images. Males were placed individually in a miniature aquarium and photographed laterally, keeping the fish in a stable position. Images were taken with a digital single-lens reflex camera (Canon EOS 50D) with a 105 mm lens. Images were taken in natural light in a shaded position, normally around noon, with a fixed aperture opening (f/6) and sensor sensitivity (ISO 800), adjusting exposure time to light conditions. We included a 5 cm size standard and a color standard in the image, the latter to control for variation in color attributable to variation in ambient light.

Images were analyzed in Photoshop CS5 (Adobe, Inc., San Jose, CA). TL was measured from the snout to the distal end of the tail fin ('ruler' tool). SL was measured from the snout to the caudal peduncle. These measures were used for analyses instead of the measuring board recordings taken just after capture, because lengths taken from standardized photos are likely more precise. Tail fin area was measured by marking the outline of the tail fin with the 'magnetic lasso tool', including the area up to caudal peduncle. The area of the orange tail ornament was marked and measured using the 'magic wand' tool, adjusting tolerance and reference pixel until the selection matched the orange area.

Before color quality was measured, images were first adjusted for variation in lightness, using the black and white reference on the color standards ('levels' tool in Photoshop). Colors were then measured in the CIE L*a*b* color space, which quantifies color in the three channels L* (lightness), a* (the balance between green and magenta) and b* (the balance between blue and yellow) (Chen et al. 2004; Svensson et al. 2006). Prior to analysis, L*, a* and b* measurements of the orange tail ornament were corrected for image variation by extracting the residuals from log-log regressions of the tail color measurements on L*, a* and b* of the orange color standard.

The three color channels (L*, a*, b*) and the size of the orange ornament relative to total tail fin area (%) were entered into a PCA to reduce the number of variables for further analysis. All variables were standardized to have a mean of zero and a standard deviation of one. The extracted PCA components were varimax rotated, which facilitated the interpretation as each variable loaded clearly into a single PCA component (Table 2). We extracted the first two components (see Table 2 for eigenvalues and percent variance explained). The three variables of color quality loaded into the first component (PC1), with L* loading negatively and a* and b* loading positively. Relative size of the orange area loaded positively into the second component (PC2; Table 2). A high PC1 score therefore reflects a more orange (composed of magenta (a*) and yellow (b*)) and darker (i.e. lower L*) ornament (termed 'ornament color' hereafter). A high PC2 score reflects a large orange ornament relative to the size of the tail fin (termed 'ornament size' hereafter). A correlation matrix of the variables entered into the PCA is presented in Table S3.

Ethical note

All procedures were carried out with permission from Queensland Fisheries Department, the University of Queensland Animal Ethics Committee, and the Great Barrier Reef Marine Parks

Authority. Individuals were not allowed to interact physically, precluding physical injury. Individual compartments were equipped with PVC tubes, which were actively used as shelter in cases of disturbance (see below).

Statistical analysis

Males within a trial were classified as 'bright' or 'dull' on the basis of ornament color (PC1) and ornament size (PC2). Within each trial, we first calculated the contrast between the two males for each of the measures. For each trial, we then used the measure with the stronger contrast (i.e., the higher absolute value) to decide which male was brighter and which was duller. However, the male that scored higher on ornament color did typically also score higher on ornament size (18 out of 25 trials). Both ornament color and ornament size were PCA components and thus had similar means and standard deviations. See Table S1 for details on color and body size of bright and dull males.

Female preference for male coloration

We analyzed female preference for male coloration with generalized linear models (GLM). Female preference was quantified by three different variables: (i) the proportion of time females spent near the bright male, and the proportions of (ii) fin displays and (iii) sigmoid displays by the females that were directed at the bright male. Those proportions were calculated per trial, dividing the number of events (scans or behaviors) directed at the bright male by the total number of events (bright plus dull male). A separate GLM was fitted for each of the three measures of preference (response variables) and for each step of the experiment (Step 1 and Step 2).

Trials varied considerably in how much the two males differed in tail coloration (Table S1), which may have affected female preference. We therefore included the within trial contrasts in male tail coloration as explanatory variables into the GLMs on female preference. The contrasts were calculated as within trial differences (bright male minus dull male) in PC1 (ornament color) and PC2 (ornament size). We centered the two explanatory variables to have a mean of zero. By centering the male color contrast variables, the model intercepts provide estimates of preference under average male color contrast. Each of the GLMs thus combines testing for overall female preference for male tail coloration (represented by the intercept) with testing whether female preference was affected by how much the two males differed in tail coloration (represented by the slopes).

Female preference may have been affected by other male phenotypic traits than tail coloration. As reported above, we found that bright and dull males within a trial differed somewhat in condition and tail length. We present versions of the GLMs testing for female preference that include centered within trial differences (bright minus dull male) in SL, condition and tail length in the supplementary material (Table S4). None of these covariates had a significant effect on female preference. In the Results section we therefore present GLMs limited to the focal trait, male tail coloration.

We fitted the GLMs for female preference with a quasi-binomial error structure, as the response variables were proportions and the residuals were over-dispersed. Because the numbers of scan samples, and especially female courtship behaviors, strongly varied among trials, we fitted the response variables with the 'cbind' function in R (R Development Core Team, 2011), which weighs proportions based on the number of observations within a trial.

Female preference may have been affected by male courtship. We therefore tested whether (i) bright and dull males differed in courtship activity (paired t-tests) and (ii) whether male

courtship was related to female preference (Pearson correlations). We tested the latter by correlation rather than regression models, as female preference (position and courtship) and male courtship are likely to interact rather than being a one-way process.

Temporal change in female preference

Graphical inspection of the data suggested that female preference changed over the study period, i.e. females tested early seemed to differ in their preference from females that were tested late in the study period. We therefore performed post-hoc tests for a temporal change in female preference. We used GLM regressions to test for an effect of time, fitting a separate model for each of the three female preference variables, for each step of the experiment (Step 1 and Step 2). The three preference variables (position, fin displays, sigmoid displays) were entered as proportions towards the large male, as described above. Time, i.e. the date a trial was carried out, was entered as the number of days after the date of the first trial. Thus, the first trial (30 October) was assigned 0 days, and the last trial (23 December) was assigned 54 days.

Effects of male-male competition on male and female behavior

Female preference might have been affected by male phenotypic traits that were not measured, and possibly differently so in the two steps of the experiment. We therefore analyzed whether overall female preference, independent of specific male traits, changed from Step 1 to Step 2. For each of the two steps of each trial, we calculated overall female preference as the proportion of scan samples and fin displays by females related to the male that received more of the respective behavior. We then used paired t-tests to test for a change in overall preference from Step 1 to Step 2. We also used paired t-tests to analyze whether the

frequencies of female courtship and male courtship and agonistic behavior changed from Step 1 to Step 2.

Excluded trials

In some trials, males remained in their PVC tubes for substantial parts of the trials, probably for reasons of disturbance as described above. For Step 1, we excluded four trials in which one or both males stayed inside the tube for more than 60% of the time. For comparison, males of the included trials stayed inside the tube for a median of 7% of the time (range 0-51%). We also excluded one trial in which the female stayed inside the nest tube for more than 60% of the time. For Step 2, we excluded all five trials that were excluded in Step 1, conservatively assuming that the effects of disturbance in Step 1 could have been carried over to Step 2. In Step 2, we also excluded one trial where a male stayed inside the tube >60% of the time and two trials in which the female did not leave the tube or never entered both males' sides of the tank. The exclusion of trials resulted in the reduction of sample size from 30 to 25 for Step 1 and to 22 for Step 2.

All statistical analyses were carried out in R, version 2.13.1 (R Development Core Team, 2011). Model assumptions for statistical tests were validated by graphical inspection of residual distributions. GLMs with appropriate link functions were used whenever residual distributions were unsuitable for linear models, and paired t-tests were used to account for non-independence of data collected in Step 1 and Step 2 of a trial.

RESULTS

Female preference for male coloration

We tested for female preference for male tail coloration and whether the preference was affected by how much males differed in tail coloration. Female preference was quantified as bias in position, fin displays or sigmoid displays, with separate GLMs fitted for each of the variables (Table 3). We found no overall female preference with regard to male tail coloration. The proportion of time the female spent near the bright male and the proportion of courtship behaviors directed towards the bright male did not differ significantly from the no-preference expectation of 50% (intercepts in Table 3). Neither was female preference affected by how much males differed in coloration. The within trial contrasts in male ornament size and ornament color did not significantly affect female preference when preference was quantified as biases in female position or sigmoid displays (slopes in Table 3). When preference was quantified as bias in female fin displays, the within trial contrast in male ornament color had a significant positive effect on female preference in Step 1 (slope in Table 3). Thus, with an increasing difference in male ornament color, females performed an increasing proportion of fin displays towards the bright male. However, this effect was not robust to the removal of two highly influential data points (slopes after removal: ornament size: 0.17 ± 0.14, $P = 0.24$; ornament color: 0.01 ± 0.15, $P = 0.95$). While those two data points were correct recordings of actual behaviors, they were outliers due to, in one case, an extreme contrast in ornament color, and in the other case an exceptionally high total number of fin displays. Finally, there was a borderline significant negative effect of ornament size on female preference quantified as bias in fin displays in Step 2 (slope in Table 3).

Male behavior may have affected female preference. We therefore tested whether bright and dull males differed in behavior and whether female preference was related to male behavior. Bright and dull males did not differ significantly in courtship behavior PC1 (courtship frequency) (Step 1: paired t-test: $t_{24} = 0.64$, $P = 0.53$; Step 2: $t_{21} = 0.63$, $P = 0.54$) or PC2 (courtship propensity) (Step 1: paired t-test: $t_{24} = 0.19$, $P = 0.85$; Step 2: $t_{21} = 0.06$, $P = 0.95$). Neither did bright and dull males differ in the frequency of intrasexual agonistic fin displays

(Step 2: paired t-test: t_{21}= -0.80, P = 0.43). There was no clear relationship between male behavior (courtship or agonistic) and female preference (Table 4). The only significant correlation was between preference measured as position and male courtship PC1 (courtship frequency), and only in the absence of male-male interaction (Step 1; Table 4).

Temporal change in female preference

When male-male interactions were precluded, female preference changed over the course of the study period. Females tested early in the study period expressed a preference for bright males, whereas females tested late preferred dull males. This effect was evident whether female preference was quantified from time spent in association with either male or from courtship directed at the males (Figure 3). The proportion of time spent near the bright male decreased significantly over the course of the study (GLM: t = -3.9, P < 0.001; Figure 3a). Similarly, there was a significant decrease in the proportion of female fin displays (GLM: t = -4.2, P < 0.001; Figure 3b) and sigmoid displays (GLM: t = -3.6, P = 0.003; Figure 3c) directed at the colorful male over the course of the study. The 95% confidence intervals of the slopes revealed that this change was from a significant preference for bright males early to a significant preference for dull males late (Figure 3a-c). The reversal from preference for bright to preference for dull males occurred in late November, ca. 30 days after the first trial was carried out (Figure 3a-c). In contrast, when male-male interaction was permitted (Step 2), we found no significant change in female preference (position or courtship) over the course of the study (all P > 0.6; Figure 3d-e).

The temporal change in female preference may have been related to temporal patterns in confounding male or female traits. There was a significant temporal effect on within trial differences (bright minus dull male) in male condition (linear regression: r^2 = 0.23, P = 0.014; FigureS1a), with bright males being in increasingly worse condition relative to dull ones over

the course of the study period. There also was a significant temporal effect on within trial differences in male SL (linear regression: $r^2 = 0.16$, $P = 0.046$; Figure S1b). However, the estimated effect on SL amounted to less than 1.5 mm (<4% of mean male SL) over the study period (54 days). There was no significant temporal change in within trial differences in male body mass, ornament color or ornament size or female TL or condition (all $r^2 < 0.08$, all $P > 0.17$).

Effects of male-male interaction on male and female behavior

While female preference was only related to male tail coloration in Step 1, there was no decrease from Step 1 to Step 2 in female preference independent of male traits. The proportion of time spent with the preferred male did not differ significantly between Step 1 and Step 2 (medians: Step 1: 0.69, Step 2: 0.71; paired t-test: $t_{21} = 0.15$, $P = 0.88$). Neither did the proportion of fin displays towards the preferred male differ significantly between Step 1 and Step 2 (Step 1: 0.61, Step 2: 0.64; paired t-test: $t_{19} = -0.54$, $P = 0.59$).

Male courtship decreased strongly when male-male competition was allowed. Male-male interactions (Step 2) were very frequent (Figure 4) and males spent much time in proximity to the male-male divider (median = 60%, 51-70%). There was a significant decrease in the number of courtship behaviors (sum of behaviors; paired t-test: $t_{21} = -11.49$, $P < 0.0001$; Figure 4) and the courtship propensity (Table 5) from Step 1 to Step 2. In females, there was a significant decrease in sigmoid displays, but not in fin displays (Table 5). The proportion of time females spent in proximity to the male-female divider increased significantly from Step 1 to Step 2 (Table 5).

DISCUSSION

We tested whether female *C. cyanea* prefer more colorful (bright) males, as expressed by time spent near such males and courtship directed at bright males. The results showed no overall female preference for males with more colorful tails. Instead, female preference for male coloration changed over the course of the studied part of the breeding season, from preference for males with more colorful tails early to preference for males with less colorful tails late. We further explored whether female preference was affected by seeing males compete, as suggested by previous theoretical (Kokko 2005) and empirical work (reviewed in Wong and Candolin 2005). While female preference was related to male coloration in the absence of male interaction (changing over time), females were indiscriminate with regard to male coloration when males were allowed to interact.

Temporal variation in mate choice

Within a period of less than two months, female preference changed from positive to negative with regard to male tail coloration. While the studied period did not cover the species' entire breeding season, it covered a large part of the peak breeding season (authors' observations; see Methods). The observed dynamics in female preference are therefore potentially of central importance for sexual selection in the species. Our findings are in line with a growing body of evidence for within-season variation in the strength and direction of sexual selection (e.g. Downhower et al. 1987; McLain 1992; Blanckenhorn et al. 1999; Forsgren et al. 2004; Kasumovic et al. 2008; Wacker et al. 2014). More specifically, our results are in line with the small number of studies that have found that within-season variation in mate choice leads to temporal variation in sexual selection (e.g. Qvarnström et al. 2000; Reaney and Backwell 2007; reviewed in Jennions and Petrie 1997; Widemo and Sæther 1999). However, within-season variation in mate choice is typically manifested in a change in choosiness, i.e., the choosing sex becoming more or less selective (e.g. Forsgren 1997; Borg et al. 2006), rather than a change in the preferred mate phenotype. Within-season variation in the preferred

phenotype, including a reversal as found in this study, is expected only when the optimal mate phenotype changes over the course of the breeding season. Such a reversal has previously been found in the fiddler crab *Uca mjoebergi*, where females prefer males with large claws early, but males with small claws late in the breeding cycle (Kahn et al. 2013; Clark and Backwell 2015). Larval release in this species is highly synchronized, which requires eggs to develop slower or faster, depending on when they were spawned (Reaney and Backwell 2007). Females prefer males with smaller claws late in the breeding cycle because these defend smaller, warmer burrows that allow faster larval development. Early in the breeding cycle, when optimal larval development time is longer, they prefer males with larger claws and larger (colder) burrows (Kahn et al. 2013).

We suggest that the preference reversal in *C. cyanea* may be related to seasonal variation in mating competition and to the costs of paternal care. In many animal species, including fishes, the most competitive males are expected to breed early in the season. Breeding early is often advantageous because offspring fitness is expected to increase with a longer growth period (Cargnelli and Gross 1996). Provided that more colorful males are competitively superior, they may start breeding earlier than dull ones. Such an early start may lead to accumulated costs of paternal care (Clutton-Brock 1991; Smith and Wootton 1995) and mating competition (Bonduriansky et al. 2008; Lukas and Clutton-Brock 2014) as the season progresses. Later on in the season, less colorful males may then have more resources to invest into paternal care than more colorful ones, because they have cared for fewer previous broods (Berglund 1991; Wacker et al. 2014). In our study, there was some evidence for bright males to be generally in poorer condition, and the difference between bright and dull males was stronger among the males caught later in the study period. However, the effect was small and the results far from conclusive. More studies are required to reveal the potential relationships between tail coloration, life history, and condition in the species.

Our results highlight the importance of considering temporal variation when studying mate choice, especially in species that breed repeatedly within a breeding season. If temporal variation in mate choice is not explicitly addressed and analyzed for, results like those of the present study can easily be misinterpreted as an absence of any mating preference. It is currently unknown how widespread within-season variation in mate choice, and especially in mate preferences, is. This reflects a generally limited consideration of life history aspects in sexual selection studies, despite its likely fundamental importance (Kokko 1997; Griffith 2000; Cornwallis and Uller 2010).

Effect of male-male interaction on female choice

When males were allowed to interact visually, male-male agonistic behaviors were extremely frequent. This resulted in a strong decrease, but not an absence, of male courtship. Despite intense male-male aggression and decreased male courtship, females continued to express a sexual interest in the males, reducing their courtship rate only slightly and in fact spending more time in proximity to the males. While females retained a sexual interest, this was no longer related to male tail coloration. It appears unlikely that this was because male-male competition hampered the assessment of male traits (Wong and Candolin 2005). First, *C. cyanea* females of our study had previous knowledge of male tail coloration as displayed in the absence of male-male competition. Second, male tail coloration is even more prominently displayed in male-male than in male-female interactions (Gronell 1989, own observations). It thus appears more likely that female preference was affected by one or more other (unmeasured) traits when males were allowed to interact, overriding the previously expressed preference with regard to male tail coloration. However, we cannot exclude the possibility that females can assess male tail coloration only, or more precisely, during male courtship undisturbed by other males. Mate preference for traits that can better be evaluated during intrasexual competition has been found in, for instance, the pipefish *Syngnathus typhle*

(Berglund and Rosenqvist 2001), three-spined sticklebacks (*Gasterosteus aculeatus*; Candolin 1999) and Siamese fighting fish (*Betta splendens*; Doutrelant and McGregor 2000).

The results of the present study illustrate that preference quantified in the absence of male-male competition, as in classical mate choice set-ups, may not always reflect female preference as expressed in more complex situations, involving male-male interaction (see Kangas and Lindström 2001; Lehtonen and Lindström 2009; Casalini et al. 2009). In species where females frequently observe males compete before making mating decisions, preferences expressed in the absence of competition may have little effect on realized mate choice. *Chrysiptera cyanea* males and females are territorial (Thresher and Moyer 1983) and behavioral interactions between neighboring individuals, including male-male competition, are frequent throughout the studied part of the breeding season (authors' observation). Intersexual interactions occur both in the absence and presence of male-male competition (S. Wacker et al., unpublished data). Thresher and Moyer (1983) reported that during spawning periods, several males are often present near a male's nest, frequently involving male-male aggression and occasionally courtship interruption. In the present study, males were simultaneously faced with a rival male that could not retreat and a potential female mate. In this situation, males clearly prioritized intrasexual aggression over courtship. The combined findings of Thresher and Moyer (1983) and this study suggest that females frequently observe male-male interactions prior to mating, and that it may affect their mate choice. Further studies are needed to reveal to what extent male-male competition affects female mating preference in *C. cyanea* in the wild.

Sexual selection on coloration in coral reef fishes

This study confirms that female *C. cyanea* can be choosy with regard to male coloration (Gronell 1989), but also that they show a preference that is dynamic rather than static. The

temporal effects and effects of social environment complicate inference on how female preference affects sexual selection in the wild. Our results suggest that the total effect of female preference on sexual selection across the breeding season depends on how reproduction (i.e. population reproductive output) and the degree of male-male competition are distributed over the breeding season. Those parameters are currently not known in sufficient detail to infer the resulting total effects on sexual selection. Gronell's (1989) large-scale field study of *C. cyanea* found male mating success to be highly variable and positively related to the relative size (length) of the orange tail ornament (Gronell 1989). The study did not report selection in relation to time of season. However, it appears that the majority of data were collected during the present study's early part. Our finding of a female preference for colorful males early in the study period thus appears in line with the positive sexual selection for more colorful males in the field (Gronell 1989). Taken together, studies of *C. cyanea* have solidly documented that male mating success is related to orange tail coloration in the wild (Gronell 1989) and are suggestive for a contribution of female choice to this relationship (Gronell 1989, lab study part; this study). Complementary to female choice, a greater mating success of more 'orange' males could be caused by coloration playing a role in male-male competition. This hypothesis is so far unexplored, despite the fact that males very clearly display their tails in intraspecific interactions (Gronell 1989).

Sexual selection on male coloration by female choice has generally been little explored in reef fishes, but has been suggested to explain interspecific variation in coloration of pomacentrid (Thresher and Moyer 1983) and labrid (Robertson and Hoffman 1977) fishes. Within individual reef fish species, female choice for male coloration has been found in the damselfish *Stegastes leuconstictus* (Haley et al. 2002, 2004) and the wrasse *Thalassoma bifasciatum* (Warner and Schultz 1992). The reef fish species for which a color preference has been documented are, like very many reef fishes, highly territorial (Ehrlich 1975). Territoriality may facilitate mate sampling and thereby mate choice because individuals are

bound to defined locations. Potential mates can thus be sampled repeatedly and re-visited for spawning, as reported for *C. cyanea* (Gronell 1989). For *C. cyanea*, further studies are needed to test whether the temporal dynamics in mate choice translate into seasonal dynamics in sexual selection in the wild, and how this affects sexual selection on male tail coloration across the entire breeding season. At a general level, increased efforts are needed to understand the role of sexual selection in the evolution of the highly diverse and conspicuous coloration of coral reef fishes and its potential importance for the process of speciation (Amundsen 2003; Ritchie 2007; Kraaijeveld et al. 2011). The latter question is highly relevant given the exceptional diversity of fish in this environment.

Conclusions

Our results strongly emphasize the plasticity of female mate choice. Female preference in *C. cyanea* was not only affected by the social context (male-male interactions), but also by time in the breeding season. While this study is among the first to document a reversal of female mate preferences over the course of a breeding season, temporal variation in mate choice may be more common than currently acknowledged. Knowledge about plasticity in mating preference and the underlying mechanisms is essential for understanding life histories, including investment in current reproduction and the development of sexual characters. Further empirical and theoretical work is needed to understand the determinants of variation in mate choice across time and social context.

FUNDING

This work was supported by a grant from the FRIFORSK program of the Research Council of Norway (grant number 191271) to TA.

ACKNOWLEDGEMENTS

We are grateful to the managers and staff of the Lizard Island Research station for generous support, and to Lexa Grutter and N. Justin Marshall for invaluable advice on reef fish ecology and reef fish work. We thank Anne Hoggett, Marianne Pearce, Eva McClure, Yolanda Bosiger, Yarema Reshitnyk and Morten Fyhn Amundsen for invaluable help with field and lab work.

REFERENCES

Amundsen T. 2003. Fishes as models in studies of sexual selection and parental care. J Fish Biol. 63: 17-52.

Andersson M. 1994. Sexual Selection. Princeton, NJ: Princeton University Press.

Berglund A. 1991. Egg competition in a sex-role reversed pipefish: subdominant females trade reproduction for growth. Evolution. 45: 770-774.

Berglund A. 1994. The operational sex ratio influences choosiness in a pipefish. Behav Ecol. 5: 254-258.

Berglund A, Rosenqvist G. 2001. Male pipefish prefer dominant over attractive females. Behav Ecol. 12: 402-406.

Blanckenhorn WU, Morf C, Mühlhäuser C, Reusch T. 1999. Spatiotemporal variation in selection on body size in the dung fly *Sepsis cynipsea*. J Evolution Biol. 12: 563-576.

Bonduriansky R, Maklakov A, Zajitschek F, Brooks R. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. Funct Ecol. 22: 443-453.

Borg ÅA, Forsgren E, Amundsen T. 2006. Seasonal change in female choice for male size in the two-spotted goby. Anim Behav. 72: 763-771.

Bradbury JW, Davies NB. 1987. Relative roles of intra- and inter-sexual selection. In: Bradbury JW, Andersson MB, editors. Sexual Selection: Testing the Alternatives. New York: John Wiley and Sons. p. 143–163.

Candolin U. 1999. Male–male competition facilitates female choice in sticklebacks. P R Soc B. 266: 785-789.

Candolin U, Voigt HR. 2001. Correlation between male size and territory quality: consequence of male competition or predation susceptibility? Oikos. 95: 225-230.

Cargnelli LM, Gross MR. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). Can J Fish Aquat Sci. 53: 360-367.

Casalini M, Agbali M, Reichard M, Konečná M, Bryjová A, Smith C. 2009. Male dominance, female mate choice, and intersexual conflict in the rose bitterling (*Rhodeus ocellatus*). Evolution. 63: 366-376.

Chen Y, Hao PW, Dang AR. 2004. Optimal transform in perceptually uniform color space and its application in image coding. Lect Notes Comp Sci. 3211: 269-276.

Clark HL, Backwell PRY. 2015. Temporal and spatial variation in female mating preferences in a fiddler crab. Behav Ecol Sociobiol. 69: 1779-1784.

Clutton-Brock TH. 1991. The Evolution of Parental Care. Princeton, NJ: Princeton University Press.

Cornwallis C, Uller T. 2010. Towards an evolutionary ecology of sexual traits. Trends Ecol Evol. 25: 145-152.

Creighton E. 2001. Mate acquisition in the European blackbird and its implications for sexual strategies. Ethol Ecol Evol. 13: 247-260.

Darwin C. 1871. The descent of man, and selection in relation to sex. London: John Murray.

de Jong K, Forsgren E, Sandvik H, Amundsen T. 2012. Measuring mating competition correctly: available evidence supports operational sex ratio theory. Behav Ecol. 23: 1170-1177.

Doutrelant C, McGregor PK. 2000. Eavesdropping and mate choice in female fighting fish. Behaviour. 137: 1655-1668.

Dougherty L, Shuker D. 2015. The effect of experimental design on the measurement of mate choice: a meta-analysis. Behav Ecol. 26: 311-319.

Downhower JF, Blumer LS, Brown L. 1987. Seasonal variation in sexual selection in the mottled sculpin. Evolution. 41: 1386-1394.

Ehrlich P. 1975. The population biology of coral reef fishes. Annu Rev Ecol Syst. 6: 211-247.

Forsgren E. 1997. Mate sampling in a population of sand gobies. Anim Behav. 53: 267-276.

Forsgren E, Amundsen T, Borg ÅA, Bjelvenmark J. 2004. Unusually dynamic sex roles in a fish. Nature. 429: 551-554.

Froese R, Pauly D, editors. 2015. FishBase. www.fishbase.org

Goulet D. 1998. Spawning success in the damselfish *Amblyglyphidodon leucogaster*: the influence of eggs in the nest. Anim Behav. 55: 651-664.

Griffith S. 2000. A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. P R Soc B. 276: 1115-1119.

Gronell A. 1989. Visiting behaviour by females of the sexually dichromatic damselfish, *Chrysiptera cyanea* (Teleostei: Pomacentridae): a probable method of assessing male quality. Ethology. 81: 89-122.

Haley MP, Smith JK, Itzkowitz M. 2002. Female choice for males in the beaugregory damselfish. Caribb J Sci. 141: 391-406.

Haley MP, Smith JK, Itzkowitz M. 2004. Female choice and male courtship behaviour in the beaugregory damselfish. Behaviour. 141: 391-406.

Hoelzer G. 1990. Male-male competition and female choice in the Cortez damselfish, *Stegastes rectifraenum*. Anim Behav. 40: 339-349.

Howard RD, Martens RS, Innis A, Drnevich JM, Hale J. 1997. Differential effects of mate competition and mate choice on eastern tiger salamanders. Anim Behav. 55: 1151-1163.

Hunt J, Breuker CJ, Sadowski A, Moore AJ. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. J Evol Biol. 22: 13-26.

Jennions M, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol Rev. 72: 283-327.

Kahn AT, Dolstra T, Jennions MD, Backwell PRY. 2013. Strategic male courtship effort varies in concert with adaptive shifts in female mating preferences. Behav Ecol. 24: 906-913.

Kangas N, Lindström K. 2001. Male interactions and female mate choice in the sand goby, *Pomatoschistus minutus*. Anim Behav. 61: 425-430.

Kasumovic MM, Bruce MJ, Andrade MCB, Herberstein ME. 2008. Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. Evolution. 62: 2316-2325.

Kitamura J, Taru M, Sunobe T. 2002. Female preference of the gobiid fish *Eviota abax* in relation to elongation of the first dorsal spine in males. J Ethol. 20: 133-135.

Kohda M, Sasaki Y, Ohnishi N, Matsumoto K. 2005. Preliminary study on female choice of mates versus sites in a wrasse, *Cirrhilabrus temminckii*. Ichthyol Res. 52: 406-409.

Kokko H. 1997. Evolutionarily stable strategies of age-dependent sexual advertisement. Behav Ecol Sociobiol. 41: 99-107.

Kokko H. 2005. Treat'em mean, keep'em (sometimes) keen: evolution of female preferences for dominant and coercive males. Evol Ecol. 19: 123-125.

Knapp R, Warner R. 1991. Male parental care and female choice in the bicolor damselfish, *Stegastes partitus*: bigger is not always better. Anim Behav. 41: 747-756.

Kraaijeveld K, Kraaijeveld-Smit FJL, Maan ME. 2011. Sexual selection and speciation: the comparative evidence revisited. Biol Rev. 86: 367-377.

Kuwamura T, Karino K, Nakashima Y. 2000. Male morphological characteristics and mating success in a protogynous coral reef fish, *Halichoeres melanurus*. J Ethol. 18: 17-23.

Kvarnemo C, Simmons LW. 1999. Variance in female quality, operational sex ratio and male mate choice in a bushcricket. Behav Ecol Sociobiol. 45: 245-252.

Lehtonen TK, Lindström K. 2009. Females decide whether size matters: plastic mate preferences tuned to the intensity of male–male competition. Behav Ecol. 20: 195-199.

Lukas D, Clutton-Brock T. 2014. Costs of mating competition limit male lifetime breeding success in polygynous mammals. P R Soc B. 281: 20140418.

Manica A. 2009. Female scissortail sergeants (Pisces: Pomacentridae) use test eggs to choose good fathers. Anim Behav. 79: 237-242.

McLain D. 1992. Population density and the intensity of sexual selection on body length in spatially or temporally restricted natural populations of a seed bug. Behav Ecol Sociobiol. 30: 347-356.

Munday P, Wilson S. 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. J Fish Biol. 51: 931-938.

Myhre L, de Jong K, Forsgren E, Amundsen T. 2012. Sex roles and mutual mate choice matter during mate sampling. Am Nat. 179: 741-755.

Östlund-Nilsson S, Nilsson GE. 2000. Free choice by female sticklebacks: lack of preference for male dominance traits. Can J Zool. 78: 1251-1258.

Okuda N, Fukumori K, Yanagisawa Y. 2003. Male ornamentation and its condition-dependence in a paternal mouthbrooding cardinalfish with extraordinary sex roles. J Ethol. 21: 153-159.

Oliver S, Lobel P. 2013. Direct mate choice for simultaneous acoustic and visual courtship displays in the damselfish, *Dascyllus albisella* (Pomacentridae). Env Biol Fish. 96: 447-457.

Passos C, Tassino B, Reyes F, Rosenthal GG. 2014. Seasonal variation in female mate choice and operational sex ratio in wild populations of an annual fish, *Austrolebias reicherti*. PLOSone. e101649.

Petersen CW, Warner RR. 2002. The ecological context of reproductive behavior. In: Sale PF, editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. San Diego, CA: Academic Press. p. 103-118.

Qvarnström A, Forsgren E. 1998. Should females prefer dominant males? Trends Ecol Evol. 13: 498-501.

R Development Core Team 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reaney LT, Backwell PRY. 2007. Temporal constraints and female preference for burrow width in the fiddler crab, *Uca mjoebergi*. Behav Ecol Sociobiol. 61: 1515-1521.

Ritchie M. 2007. Sexual selection and speciation. Annu Rev Ecol Evol Syst. 38: 79-102.

Robertson D, Hoffman S. 1977. The roles of female mate choice and predation in the mating systems of some tropical labroid fishes. Zeitschrift für Tierpsychologie. 45: 298-320.

Schmale MC. 1981. Sexual selection and reproductive success in males of the bicolor damselfish, *Eupomacentrus partitus* (Pisces: Pomacentridae). Anim Behav. 29: 1172-1184.

Sekiya Y, Karino K. 2004. Female mate preference in goby *Eviota prasina*: do secondary sexual traits influence female choice. Zool Sci. 21: 859-863.

Smith C, Wootton RJ. 1995. The costs of parental care in teleost fishes. Rev Fish Biol Fisher. 5: 7-22.

Svensson PA, Pélabon C, Blount JD, Surai PF, Amundsen T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens*, Gobiidae)? Funct Ecol. 20: 689-698.

Tabachnick BG, Fidell LS. 2001. Using Multivariate Statistics. Boston, MA: Allyn & Bacon.

Thresher R, Moyer J. 1983. Male success, courtship complexity and patterns of sexual selection in three congeneric species of sexually monochromatic and dichromatic damselfishes (Pisces: Pomacentridae). Anim Behav. 31: 113-127.

Valone TJ, Templeton JJ. 2002. Public information for the assessment of quality: a widespread social phenomenon. Philos T R Soc B. 357: 1549-1557.

Wacker S, Amundsen T. 2014. Mate competition and resource competition are inter-related in sexual selection. J Evol Biol. 27: 466-477.

Wacker S, Amundsen T, Forsgren E, Mobley K. 2014. Within-season variation in sexual selection in a fish with dynamic sex roles. Mol Ecol. 23: 3587-3599.

Wagner W. 1998. Measuring female mating preferences. Anim Behav. 55: 1029-1042.

Warner R, Schultz E. 1992. Sexual selection and male characteristics in the bluehead wrasse, *Thalassoma bifasciatum*: mating site acquisition, mating site defense, and female choice. Evolution. 46: 1421-1442.

Widemo F, Sæther S. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. Trends Ecol Evol. 14: 26-31.

Wong B, Candolin U. 2005. How is female mate choice affected by male competition? Biol Rev. 80: 559-571.

Figure 1. (a) Male *Chrysiptera cyanea* photographed under standardized conditions, (b) orange tail quantification, and (c-f) examples of variation in male tail coloration. The area outlined in (b) indicates the orange part of the tail, which was measured in size (relative to the whole fin) and color quality. The shown tails are examples of bright (c,e) and dull (d,f) males used in the study. Males (c) and (d) differ mainly in the relative size of the orange area, whereas males (e) and (f) differ mainly in the quality of the orange color.

Figure 2. Aquarium set-up, as seen from above, in a test for female mate choice in *Chrysiptera cyanea*. Dashed lines indicate dividers, separating the aquarium into two male compartments and one female compartment. The divider between the two males was opaque in Step 1 of the study and transparent in Step 2. The divider between the female and the two males was transparent. Each compartment was equipped with a PVC tube (shaded boxes), which served as a shelter and mimicked a potential nest for the males. Grey solid lines indicate zones used to record the position of fish during scan sampling. These zones indicate where an individual would be considered to be in proximity (< one fish length) to another individual's compartment (horizontal: male-female; vertical: male-male) and, for the female compartment, a neutral zone in which the female was considered not to associate with either male.

Figure 3. Female preference for male tail coloration in *Chrysiptera cyanea* in relation to the date the trial took place, measured as days after the first trial. Female preference was tested in (a-c) the presence (Step 1) and (d-e) the absence (Step 2) of male-male competition. Female preference is quantified as the bias in (a,d) position, (b,e) female fin displays and (c) female sigmoid displays, calculated as the proportion of scans or behaviors associated with the more colorful male. A score above 0.5 thus indicates a preference for the bright male, a score below 0.5 a preference for the dull male. The size of points indicates the number of behaviors or scan-sampled positions underlying the calculated proportion for a particular female (sizes not

comparable among panels), reflecting the weighting of data points in the models. Solid lines represent relationships estimated by generalized linear models and dashed lines their 95% confidence intervals. Female sigmoid displays were not analyzed for Step 2 because they occurred in too few trials.

Figure 4. Frequencies of male *Chrysiptera cyanea* courtship (sum of approaches, fin displays and lead swims) and agonistic behaviors (fin displays) in a female choice test. Males were either prevented from interacting by an opaque divider (Step 1) or allowed to interact visually (Step 2). Agonistic behaviors were recorded over 5 min only, but multiplied by three to match 15 min observation time for courtship behavior.

Table 1. Principal components analysis (PCA) on measures of male sexual interest in a female mate choice study in *Chrysiptera cyanea*. Loadings and variance explained are given for two separate PCAs, on the two parts of the study (Step 1 and Step 2). Courtship behaviors (fin display, lead swim, approach) were entered as frequencies. Courtship propensity is the proportion of cases in which a male responded with courtship to encounters with the female. Male position, i.e. proximity to the female compartment, was measured as a proportion of scan samples. Strong loadings (>0.4) are highlighted in bold.

	Eigenvalue	% variance	Fin display	Lead swim	Approach	Courtship propensity	Association with female
Step 1							
PC1	2.0	37	**0.80**	**0.75**	**0.79**	0.15	-0.05
PC2	1.4	31	0.15	-0.32	0.39	**0.68**	**0.90**
Step 2							
PC1	2.0	40	**0.81**	**0.67**	**0.87**	0.22	0.29
PC2	1.2	24	-0.08	-0.09	0.13	**0.83**	**-0.69**

Table 2. Principal component analysis (PCA) on measures of CIE color quality (L*, a*, b*) and orange area (proportion orange in tail fin) in *Chrysiptera cyanea* males. Loadings after varimax rotation and variation explained by each component. Strong loadings (>0.4) are highlighted bold.

	Eigenvalue	% variance	L*	a*	b*	orange area
PC1	2.8	60	**-0.88**	**0.92**	**0.85**	0.26
PC2	0.7	28	-0.21	0.18	0.30	**0.96**

Table 3. Generalized linear multiple regressions on female preference for male coloration in *Chrysiptera cyanea*. Female preference is quantified as either association with the more colorful male (bias in female position) or bias in courtship (fin displays and sigmoid displays). Male coloration (size and color of the orange tail ornament) is quantified as the contrast between the bright and dull males of a trial. A separate GLM was fitted to each of the three variables of female preference and for each step of the experiment, respectively. All estimates are given on logit scale. Predictor variables were centered prior to analysis. The model intercepts therefore represent female preference given average color contrast between males, with an intercept of zero expected in the absence of female preference for male coloration.

		Intercept		Ornament size		Ornament color	
Female preference	*N*	Estimate ± *SE*	*P*	Estimate ± *SE*	*P*	Estimate ± *SE*	*P*
Step 1							
Female position	25	-0.08 ± 0.21	0.73	0.06 ± 0.26	0.84	0.15 ± 0.30	0.63
Fin displays	23	0.11 ± 0.16	0.50	0.14 ± 0.16	0.38	**0.53 ± 0.22**	**0.026**
Sigmoid displays	16	0.40 ± 0.35	0.27	0.34 ± 0.46	0.47	0.60 ± 0.57	0.31
Step 2							
Female position	22	0.09 ± 0.22	0.70	-0.55 ± 0.28	0.067	-0.01 ± 0.31	0.97
Fin displays	22	-0.03 ± 0.18	0.89	**-0.41 ± 0.20**	**0.050**	-0.02 ± 0.27	0.96

Table 4. Pearson correlation coefficients (95% CI) for correlations among variables quantifying female preference (position, fin displays and sigmoid displays) and variables quantifying male courtship (PC1, PC2) in *Chrysiptera cyanea*. Variables were entered as contrast between males within trials (bright male - dull male) and as bias in female preference (proportion of time or behaviors associated to bright male). Sample sizes vary due to the exclusion of trials in which no female fin or sigmoid displays occurred.

Female preference	*N*	Courtship frequency (PC1)	Courtship propensity (PC2)	Agonistic behavior
Step 1				
Position	25	**0.42 (0.03,0.70)**	-0.30 (-0.62,0.11)	
Fin displays	23	0.29 (-0.14,0.63)	-0.32 (-0.65,0.10)	
Sigmoid displays	16	0.39 (-0.14,0.74)	-0.27 (-0.68,0.26)	
Step 2				
Position	22	0.15 (-0.29,0.54)	-0.26 (-0.62,0.18)	0.02 (-0.40,0.44)
Fin displays	22	0.36 (-0.07,0.68)	-0.31 (-0.65,0.13)	-0.14 (-0.53,0.30)

Table 5. Male and female courtship in a female choice study in *Chrysiptera cyanea*. Behavior was quantified in the absence (Step 1) and presence (Step 2) of male-male interactions. Paired t-tests are used to test for a change in male and female behavior from Step 1 to Step 2.

	Step 1		Step 2		Step 1 - Step 2				
	median	range	median	range	mean	SD	t	df	P
Male behavior									
Courtship propensity	0.57	0.33-0.75	0.17	0.03-0.5	0.36	0.18	9.3	21	<0.001
Female behavior									
Fin display	18	0-140	19	3-56	7.4	24.8	1.4	21	0.18
Sigmoid	3	0-34	1	0-4	3.9	7.6	2.4	21	0.027
Association with males	0.20	0.04-0.78	0.33	0.16-0.67	-0.10	0.21	-2.34	21	0.029

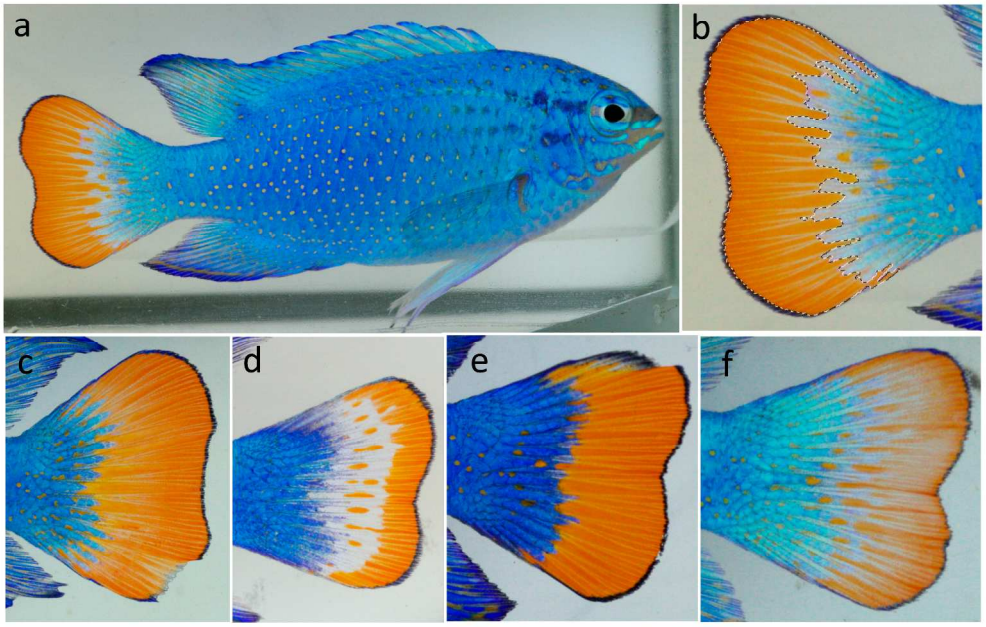

Figure 1. Male tail coloration.
264x197mm (300 x 300 DPI)

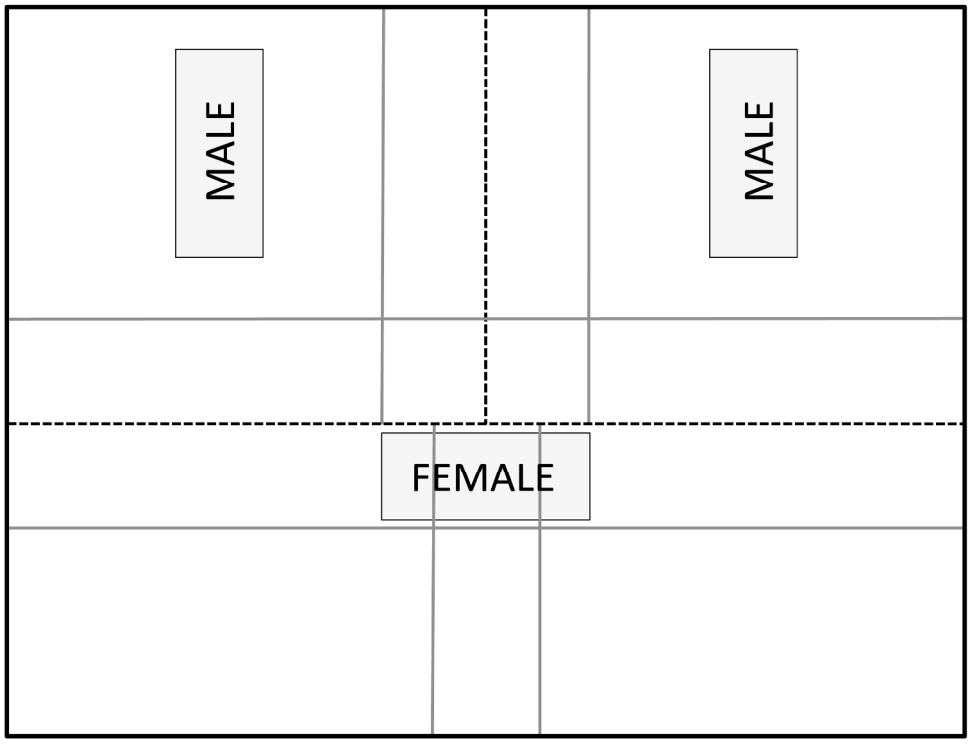

Figure 2. Experimental set-up.
255x197mm (300 x 300 DPI)

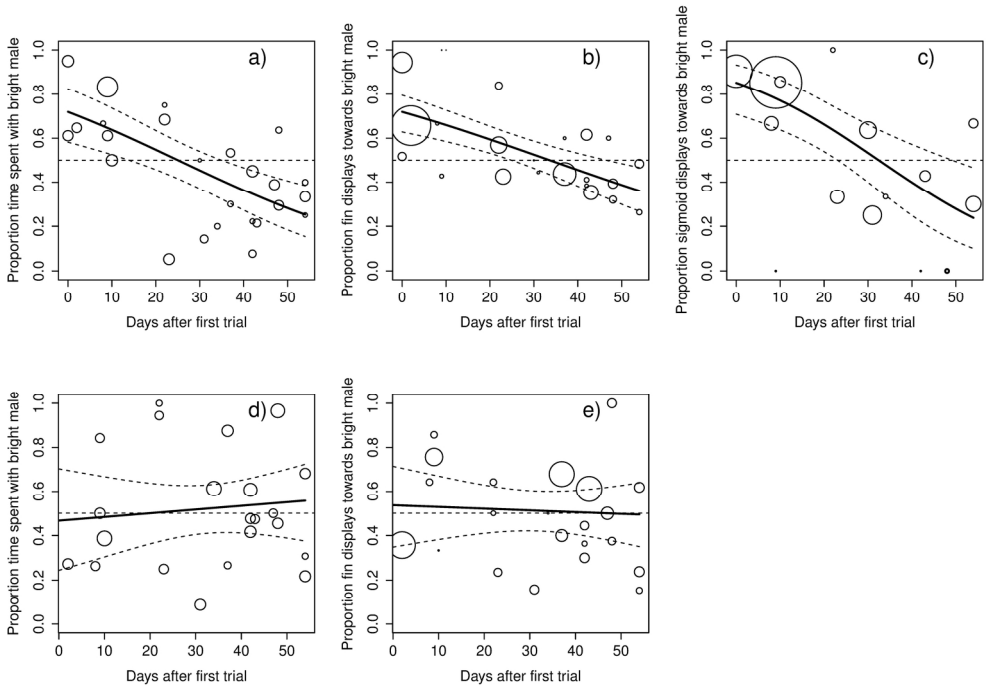

Figure 3. Female preference in relation to season.
189x133mm (300 x 300 DPI)

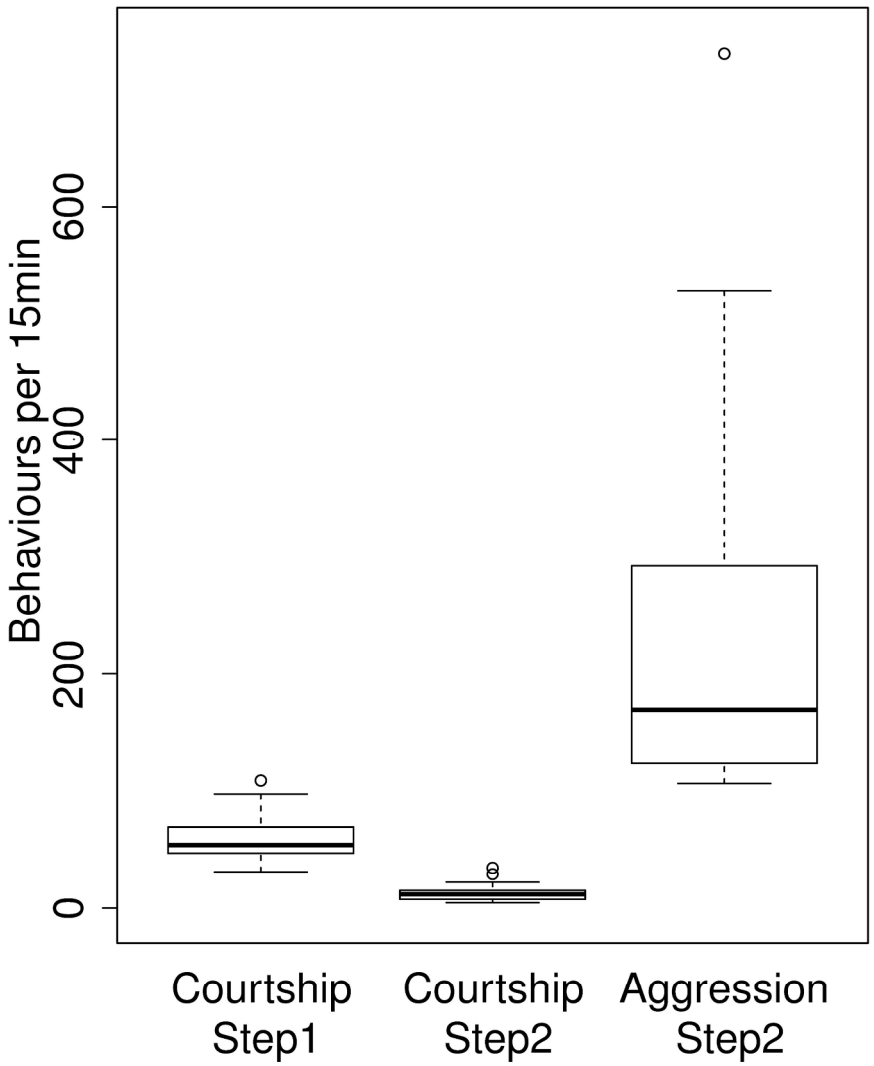

Figure 4. Male courtship and agonistic behaviour.
211x273mm (300 x 300 DPI)