Temporal variability in a multicomponent trait: nuptial coloration of female two-spotted gobies

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Animals that breed more than once may face different environmental and physiological conditions at each reproductive event. Costs and benefits of sexual ornaments could therefore vary both within and between breeding seasons. Despite this, the ornaments are often assumed to be fixed, and temporal changes in ornamentation have rarely been investigated. Female two-spotted gobies (*Gobiusculus flavescens*) have colorful orange bellies when sexually mature and nest-holding males prefer females with more colorful bellies. This nuptial coloration is caused both by the carotenoids-rich gonads being directly visible through the skin and by the chromatophore pigmentation of the abdominal skin. Toward the end of the breeding season, males become rare and females become the more competitive sex. We show that female ornamentation of *G. flavescens* is a complex multicomponent trait and that the separate components, as well as their interactions, are variable. As gonads matured, they became more colorful while the abdominal skin became more transparent, causing more intense belly coloration in sexually mature females. However, coloration varied greatly also among fully mature females, suggesting that it may not only be a signal of readiness to spawn. Indeed, belly coloration predicted gonad carotenoid concentration, but there were several important seasonal differences in color expression. Females sampled toward the end of the breeding season were more colorful. This was due to seasonal increases in both gonad carotenoid concentration and skin coloration. Thus, at a time when competition over males is stronger and the terminal reproductive event approaches, females appear to invest more in signaling. *Key words:* mate choice, nuptial signal, primary sexual character, seasonal variation, secondary sexual character. *[Behav Ecol 20:346–353 (2009)]*

The expression of sexual ornaments is known to vary greatly between individuals (Darwin 1871), but ornaments also vary within individuals, for example, between (Griffith and Pryke 2006) and within (Griffith and Sheldon 2001) breeding seasons. Although temporal changes in ornament expression may merely reflect senescence or wear (e.g., in bird plumage), they can also be adaptive. One such example is the need to attract mates only during periods of sexual receptivity (Baird 2004; Massironi et al. 2005; Heath and Frederick 2006) or to deflect courtship during stages of nonreceptivity (Hager 2001). Because signals that indicate reproductive state will benefit both signalers and receivers, they do not have to be costly to remain honest (Maynard Smith and Harper 2003). However, if ornaments are used as signals of quality in mating competition, this should raise the question of how signal honesty is maintained. One such mechanism is the handicap principle, which states that only high-quality individuals can handle the cost of extravagant ornaments (Zahavi 1975). In species where the benefits of ornaments vary over time, it would be adaptive for individuals to adjust their investment into the ornament. Although temporal variation in ornaments can be expected, few studies take the stage of the breeding season or the phase of the breeding cycle into account when quantifying ornaments (but see Candolin 2000a).

Sexual traits based on carotenoid pigmentation attract particular interest because carotenoids have important functions apart from color signaling (e.g., Olson and Owens 1998). For example, certain carotenoids are precursors of vitamin A and retinoids (Schiedt et al. 1985), may stimulate immune defenses (Amar et al. 2001), act as antioxidants (reviewed in Edge et al. 1997), and can be deposited by females into eggs (Steven 1949). Carotenoids cannot be synthesized *de novo* by animals but have to be ingested and may thus be in short supply. Therefore, there may be trade-offs in the allocation of carotenoids between signaling and other functions, such as maintaining the immune system (Lozano 1994) or producing high-quality eggs (Blount et al. 2004).

In the two-spotted goby (*Gobiusculus flavescens*), females develop colorful orange bellies when sexually mature, and males exhibit a strong mating preference for the most colorful females (Amundsen and Forsgren 2001). This nuptial belly coloration derives both from the carotenoid-rich eggs that are directly visible through the semitransparent abdominal skin and from red and yellow chromatophores in the abdominal skin (Svensson et al. 2005, 2006, Figure 1). It therefore combines 2 previously described phenomena, a partially transparent abdominal skin in gravid females, as in brook sticklebacks (*Culaea inconstans*, McLennan 1995) and straight-tailed razorfish (*Xyrichtys martinicensis*, Baird 1988), and colorful patches on the skin covering the gonads, as in convict cichlids (*Cichlasoma nigrofasciatum*, Beeching et al. 1998) and lagoon gobies (*Knipowitschia panizzae*, Massironi et al. 2005). Female *G. flavescens* with intense belly coloration lay eggs with a higher
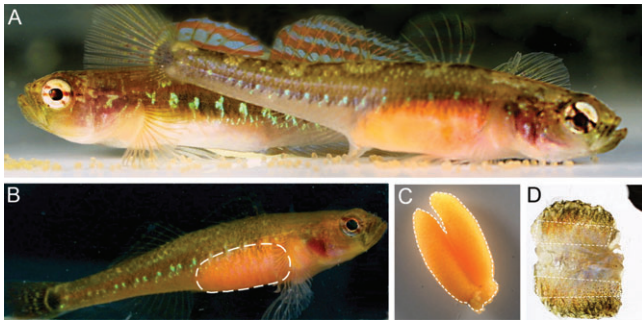

Figure 1
(A) A spawning pair of *Gobiusculus flavescens* demonstrating male and female ornamentation. The female is in front depositing eggs.
(B) Examples of photographs used to quantify belly coloration,
(C) gonad coloration, and (D) abdominal skin coloration and transparency, in female *G. flavescens*. The dashed lines indicate the areas analyzed.

carotenoid concentration than do drab females (Svensson et al. 2006). Carotenoid-rich eggs have been shown to be of higher quality in terms of increased fertilization rate, hatching success, and larval survival (e.g., Verakunpiriya et al. 1997; Ahmadi et al. 2006). As *G. flavescens* males invest heavily in offspring during egg incubation, the male preference for colorful females may be explained as a way to increase their reproductive success. Interestingly, sex roles in *G. flavescens* change over the course of the breeding season, and this may strongly affect the costs and benefits associated with the female nuptial coloration. Early in the breeding season, there is a shortage of mature females (male-biased operational sex ratio [OSR]; Emlen and Oring 1977), male–male competition is intense and males actively court females (Forsgren et al. 2004). Later in the season, however, the ratio of mature individuals, OSR, becomes female-biased, leading to a drastic increase in female courtship, as well as female–female agonism (Forsgren et al. 2004).

Although ornaments are often treated as single traits, most displays are actually made up of multiple components (e.g., Candolin 2003). The different components of the female *G. flavescens* nuptial signal and the seasonal shift in sex roles, make this species ideal for addressing questions about multi-component ornaments and seasonal variation in ornament expression. Moreover, relatively few studies of female ornaments exist in animals with conventional sex roles, particularly ornaments that are only expressed in females (Amundsen and Pärn 2006; Chenoweth et al. 2006). We therefore investigated temporal variation in the nuptial coloration of *G. flavescens* females. We expected female belly coloration to increase as gonads matured. Among mature females, we expected coloration to be more intense late in the breeding season, when the OSR is female biased (Forsgren et al. 2004). We also tested

whether belly coloration signals gonad carotenoid concentration. Finally, we quantified how gonad color, skin color, and skin transparency interact to produce nuptial belly coloration, and if these components vary within, and between, breeding seasons (Table 1).

METHODS

The coloration of female bellies, gonads, and abdominal skin was quantified using digital image analysis, and the total carotenoid concentration of the gonads was determined spectrophotometrically. All fish were collected within a 3 km² area at the mouth of the Gullmarsfjord on the Swedish west coast (58°14.9′N, 11°26.9′E), in May–July of 2002, 2004, and 2005.

Study species

The two-spotted goby (*G. flavescens*) is a marine fish that typically lives for only 1 year, with both females and males reproducing repeatedly during the single reproductive season (Johnsen 1944). Whereas males have brightly colored fins and iridescent blue lateral spots, females develop strikingly orange bellies during the breeding season, and males prefer females with the most colorful bellies (Amundsen and Forsgren 2001, Figure 1). Males typically mate with 2–6 females (Mobley KB, Amundsen T, Forsgren E, Svensson PA, Jones AG, unpublished data) and provide exclusive paternal care of the eggs (Skolbekken and Utne-Palm 2001). Female courtship involves approaching the male and bending the body (sigmoid display), a behavior that seems to emphasize the rotund and colorful belly (Amundsen and Forsgren 2001).

The major carotenoids present in the gonads of female *G. flavescens* are three 4(4′)-Ketocarotenoids, namely astaxanthin, adonixanthin (3,3′-dihydroxy-β,β-carotene-4-dione), and idoxanthin (3,3′,4′-trihydroxy-β,β-carotene-4-dione) (Svensson 2006). 4(4′)-Ketocarotenoids are known to act as precursors of vitamin A (Schiedt et al. 1985) and to positively affect embryonic development in fishes (Verakunpiriya et al. 1997; Ahmadi et al. 2006).

Female coloration and gonad maturity

In 2005, a total of 105 two-spotted goby females were collected between 23 and 30 June. Snorkellers caught females regardless of maturity to obtain a representative sample of the population. Live females were photographed with a digital camera under standardized conditions, as described previously (Svensson et al. 2005, 2006; Figure 1). The females were killed with an overdose of MS 222. Total length was measured to the nearest 0.5 mm using a measuring board. Wet weight was measured to the nearest mg using a Mettler balance, after carefully blotting the fish with tissue paper. Females were then dissected, the abdominal skin excised, and the gonads removed and weighed to the closest mg. The gonadosomatic index (GSI)

Table 1

Summary of the different aspects of female nuptial coloration in *Gobiusculus flavescens* quantified in this paper

Term	Explanation	Quantified with	Quantified as
Belly coloration	The overall nuptial signal of a live female	Photography	Redness (CIE a*)
Gonad carotenoids	The concentration of carotenoids in gonads (i.e., eggs)	Spectrophotometry	Total carotenoids ($\mu g \cdot g^{-1}$)
Gonad coloration	Coloration of excised gonads (i.e., eggs)	Photography	Redness (CIE a*)
Skin coloration	Coloration of the abdominal skin biopsy	Photography	Redness (CIE a*)
Skin transparency	Transparency of the abdominal skin biopsy	Photography	Transparency (%)

See also Figure 1.

was calculated as GSI = gonad mass/(total fish mass–gonad mass). Gonads were photographed on a white background, whereas abdominal skin was photographed on a light table (Svensson et al. 2005, Figure 1). Chromatophore pigment disperses fully after death, so photographs of abdominal skin depict the largest possible contribution of skin pigmentation (Svensson et al. 2005). All digital images were analyzed in Adobe Photoshop 4.0, after being converted to the CIE L*a*b* color space, which is standardized, perceptually uniform and device independent (Chen et al. 2004). CIE L*a*b* has been frequently used in fish color quantifications, especially in connection with carotenoid-based colorations (e.g., Skrede and Storebakken 1986). The a*-channel is commonly referred to as "redness" and expresses the balance between green and magenta (Margulis 2006). In the version of Lab used by Photoshop 4.0, a* = 0 is pure green, a* = 128 is a neutral gray, and a* = 255 is pure magenta. In the present study, "more colorful" indicates a higher a*-value. Belly, gonad, and skin coloration was quantified by measuring the a*-channel in the selected areas (Figure 1). The transparency of abdominal skin was calculated using the amount of light (L*-channel) permeating the skin, relative to the background light level in the image. Color systems based on the human vision, such as CIE L*a*b*, may be inappropriate when studying color signals in animals. However, *G. flavescens* have tristimulus vision similar to humans and lack UV receptors (Utne-Palm and Bowmaker 2006); therefore, the CIE L*a*b* system is likely to capture essential aspects of coloration as perceived by the fish. For more details on the photographic procedures and the image analyses, see Svensson et al. (2005).

Variation in coloration within and between seasons

Gravid females were collected during 2 periods (early and late in the breeding season) in 2 years (2002: May 29–June 1 and July 8–11; 2004: June 1–4 and July 12–16). Late May to early June is hereafter termed "early breeding season" and early to mid July "late breeding season" (Forsgren et al. 2004). Snorkellers caught 14 (in 2002) and 15 (in 2004) gravid females at each of 4 sampling sites, both early and late in the season. Thus, a total of 112 females were collected in 2002 and 120 in 2004. Photography, image analyses, and measurements of length, mass, and GSI were performed as described above. In 2004, gonads were immediately frozen at −20 °C for later carotenoid analysis. Extraction of gonad carotenoids was done using acetone and *n*-hexane as described in Svensson et al. (2006). After extraction and evaporation, the carotenoids were redissolved in 80:20 *n*-hexane:acetone. The total carotenoid concentration was determined using a Perkin Elmer Lambda 40 spectrophotometer (Perkin Elmer Instruments, Norwalk, CT). Concentrations were calculated from the absorbance maximum (λ_{max} = 472 nm) and the specific extinction coefficient for all-*E*-astaxanthin in *n*-hexane ($E_{1\%,\ 1cm}$ = 2100). Compensation for background absorption was performed as described by Bjerkeng (1992).

Statistics

Data analyses were performed using R 2.2.1 (R Development Core Team 2007). Results are presented as mean values ± 1 standard error (SE). Proportions were arcsine square root transformed before analyses whenever this improved the normality of the residual errors (Crawley 2002). In the analysis of gravid females collected in 2002 and 2004, period (early or late breeding season) and year (2002 or 2004) were entered as fixed factors, whereas site was entered as a random factor in mixed-effects models. Models were fitted using restricted maximum likelihood, and likelihood ratios were used to calculate

P values (Crawley 2002). In all regressions, we tested whether a curvilinear model significantly improved the fit compared with the linear model. This was done by comparing the Akaike information criterion (AIC) of models with and without log transformation of the *x* axis. If the fit was not significantly improved (ΔAIC < 2) by transformation, the untransformed linear regression was used. Estimates of slopes and effect sizes are presented as β ± 1 SE. Path analyses of the relationships between belly, gonad, and skin coloration were carried out in concordance with Shipley (2000) after standardizing all variables.

RESULTS

Female coloration and gonad maturity

As expected, these females varied greatly in gonad maturity (GSI range: 0.06–0.44), representing the full range from immature to ready-to-spawn females. Gonad maturity was strongly related to both overall nuptial belly coloration and its separate components (Figure 2). First, belly coloration increased with gonad maturity (GSI), implying that the female ornament is intensified as gonads mature (Figure 2A). This relationship had a significant curvilinear component, that is, it was significantly improved by log transforming the *x* axis (linear model: AIC = 479.1, R^2 = 0.46; log-transformed model: AIC = 474.6, R^2 = 0.49, F = 96.7, degrees of freedom [df] = 102, P < 0.0001). Individual variation in belly coloration was high at all stages of gonad maturity, including the most mature (i.e., ready-to-spawn) females. Gonad coloration also increased nonlinearly with GSI, that is, more mature gonads were more colorful (linear model: AIC = 487.3, R^2 = 0.21; log-transformed model: AIC = 476.4; R^2 = 0.29, F = 41.4, df = 102, P < 0.0001; Figure 2B). Abdominal skin transparency also increased nonlinearly with GSI (linear model: AIC = 573.0, R^2 = 0.61; log-transformed model: AIC = 568.8, R^2 = 0.62, F = 168.3, df = 102, P < 0.0001; Figure 2C). Finally, abdominal skin coloration decreased linearly with GSI (R^2 = 0.21, F = 27.0, df = 102, P < 0.0001, Figure 2D). The observed increase in skin transparency and decrease in skin coloration is likely due to the skin being stretched as the gonads grow.

Variation in coloration within and between seasons

In the following investigations, only gravid females were used. Females from 2004 were, on average, slightly larger than fish caught in 2002 (total length 2002: 44.0 ± 0.2 mm, total length 2004: 45.2 ± 0.2 mm, $F_{1,226}$ = 16.6, P = 0.0001; mass 2002: 0.74 ± 0.01 g, mass 2004: 0.84 ± 0.01 g, $F_{1,226}$ = 53.89, P < 0.0001). However, there were no significant differences in length or mass between early and late breeding season in either year (all $F_{1,226}$ < 2.55, P > 0.11). Although only visibly gravid females were collected, gonad size (GSI) was still variable and was related to both time of season and year (interaction period × year: $F_{1,224}$ = 9.02, P < 0.003). When analyzing the years separately, GSI increased over the season in 2002 ($F_{1,107}$ = 54.78, P < 0.0001) but not in 2004 ($F_{1,115}$ = 1.28, P = 0.26).

Gravid females had more colorful bellies in 2002 compared with 2004 ($F_{1,226}$ = 69.9, P < 0.0001). In both years, belly coloration was highest late in the season (2002: β = 4.86 ± 0.58; 2004 β = 3.33 ± 0.61, $F_{1,226}$ = 93.5, P < 0.0001; Figure 3A). Females had significantly more colorful gonads in 2002 compared with 2004 ($F_{1,226}$ = 4.56, P < 0.03; Figure 3B). Notably, in both years, gonads were more colorful late in the season (2002: β = 2.90 ± 0.77; 2004: β = 3.66 ± 0.73, $F_{1,226}$ = 37.5, P < 0.0001; Figure 3B).
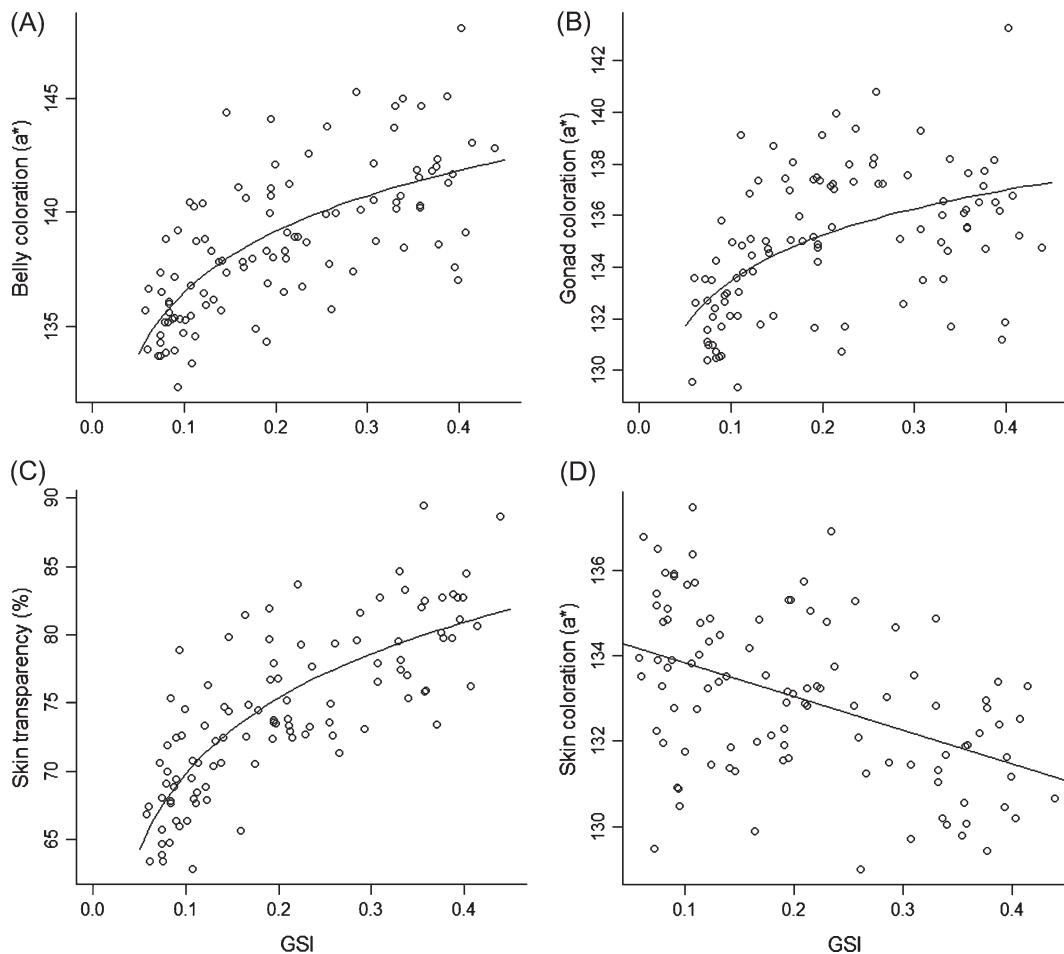
Figure 2
The effects of gonad maturation (GSI) on different components of nuptial coloration in female *G. flavescens*: (A) belly coloration, (B) gonad coloration, (C) skin transparency, and (D) skin coloration.

In 2004, we also quantified gonad carotenoids and the coloration and transparency of the abdominal skin. The total gonad carotenoid concentration was higher late in the breeding season ($\beta = 2.42 \pm 0.40$, $F_{1,106} = 37.7$, $P < 0.0001$; Figure 3C). Abdominal skin biopsies were also significantly more colorful ($\beta = 3.34 \pm 0.52$, df = 115, $t = 6.45$, $P < 0.0001$; Figure 3D) and less transparent ($\beta = -2.09 \pm 0.51$, df = 115, $t = 4.11$, $P = 0.0001$; Figure 3E) late in the season. Pigmentation of both gonads and abdominal skin therefore increased over the course of the breeding season, whereas skin transparency decreased. This led to a seasonal increase in female nuptial belly coloration.

Sources of female belly coloration

We used path analysis on the data collected in 2004 to summarize the relative contributions of gonad and skin pigmentation to overall belly coloration, and how these differ between early and late breeding season (Figure 4). In this analysis, we pooled data from the 4 sites. Early in the breeding season, the relationship between gonad coloration and the gonad carotenoids was very strong (Figure 4A). However, neither variation in gonad nor skin color had statistically significant effects on variation in belly coloration. The only factor having a significant effect was skin transparency. The overall relationship between gonad carotenoids and belly coloration was relatively weak ($r = 0.12$).

Late in the breeding season, the effect of gonad carotenoid concentration on gonad coloration remained strong, albeit slightly weaker than in the early season (Figure 4B). In contrast to the early season, not only skin transparency but also gonad coloration and skin coloration had significant direct effects on belly coloration. The overall relationship between gonad carotenoids and belly coloration was now stronger ($r = 0.25$). This suggests that both abdominal skin and gonad coloration had a larger effect on nuptial coloration late in the season. There was no significant correlation between gonad coloration and skin coloration, in either early or late season, indicating that a female with colorful gonads did not necessarily have colorful abdominal skin.

Analyzing the relationship between gonad carotenoid concentration and belly coloration directly, we found no significant relationship early in the season ($R^2 = 0.01$, $F_{1,53} = 0.76$, $P = 0.39$). However, there was a significant positive relationship late in the season ($R^2 = 0.07$, $F_{1,54} = 4.29$, $P = 0.04$; Figure 5). Notably, these R^2 values correspond well to the correlation coefficients obtained through the path analyses ($r = 0.12$ and 0.25, Figure 4). The finding that skin coloration was higher later in the season (Figure 3D) was corroborated by the offset between the 2 linear relationships presented in Figure 5. That is, irrespective of gonad carotenoids, the extra skin pigments present late in the season caused bellies to be more colorful at this time ($\beta = 2.53 \pm 0.71$, $t = 3.57$, $P < 0.001$; Figure 5).
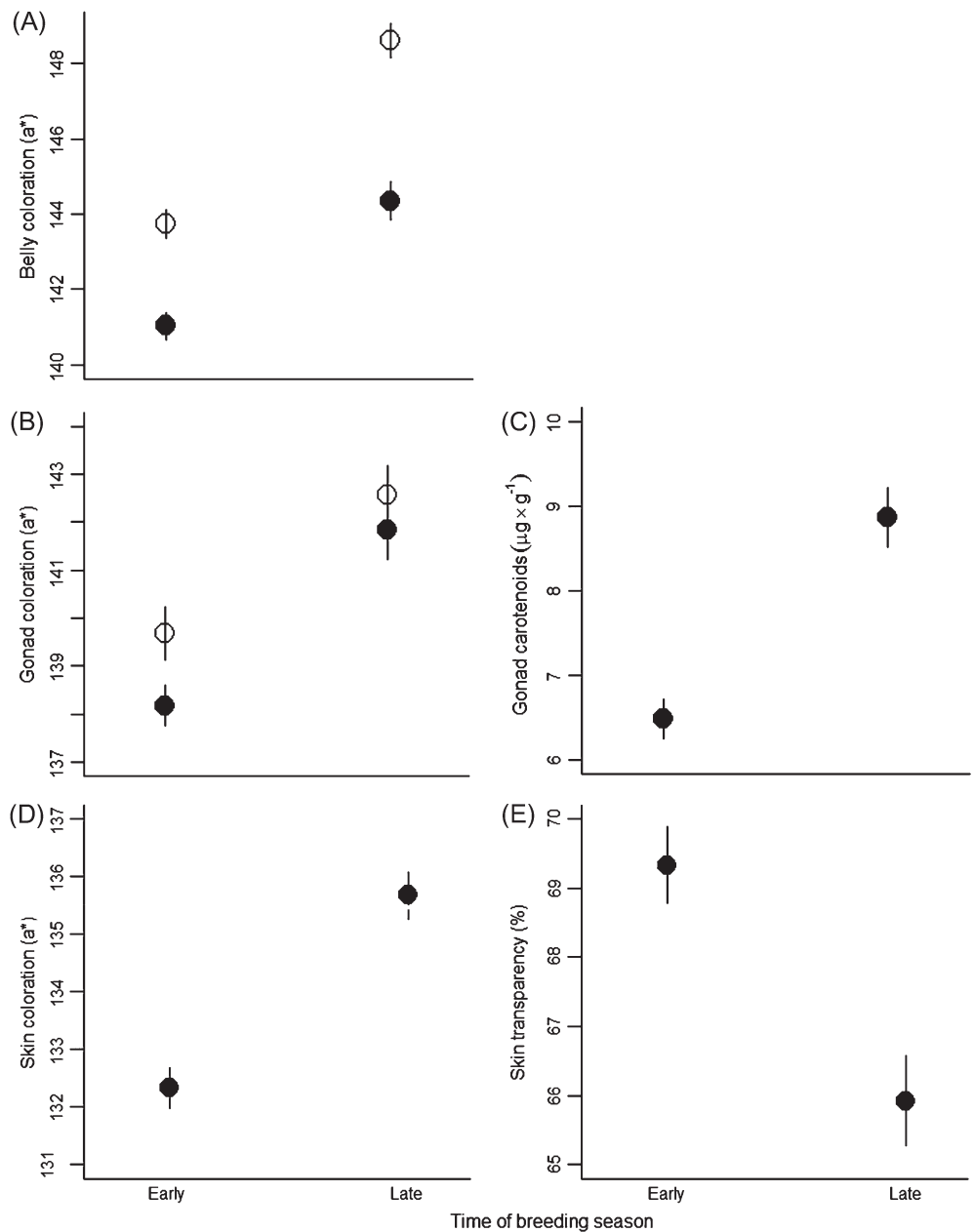
Figure 3
Different components of nuptial coloration of gravid female *G. flavescens* sampled early and late in the breeding season of 2002 (open circles) and 2004 (solid circles). (A) Belly coloration, (B) gonad coloration, (C) total gonad carotenoid concentration, (D) abdominal skin coloration, and (E) abdominal skin transparency. Coloration is expressed as CIE a*, redness. Mean ± 1 SE.

DISCUSSION

This study shows that all components of the nuptial signal of female *G. flavescens*, and their interactions, vary within and between breeding seasons. Our study did not aim to test seasonal changes within individuals but sampled a wild population of females at different times. Future work should investigate whether the seasonal dynamics of *G. flavescens* coloration reflect individual changes, different color strategies among early and late breeders, differential mortality, or some other process. Whichever of these scenarios apply, the seasonal differences are interesting and may be adaptive. Our study is the first to show, in fishes, that the expression of a female color signal used in mating competition varies over the course of the breeding season in a potentially adaptive manner.

Female coloration and gonad maturity

Belly coloration showed a positive asymptotic relationship with GSI. Males could therefore use belly coloration to distinguish between receptive and unreceptive females, and the colorful female belly could have evolved as a ready-to-spawn signal (McLennan 1995; Heath and Frederick 2006). However, belly coloration was highly variable also among fully mature females (Svensson et al. 2006, Figure 2A), suggesting that it may also convey other information, for example, indicating female quality.

It is possible that *G. flavescens* females exploit an existing male preference for sexually mature (i.e., more colorful) females, that is, more colorful, females, to gain mating advantages late in the season when nest-holding males are in short supply (Forsgren et al. 2004). The observed increase in skin pigmentation late in the season can be interpreted as females adaptively augmenting their attractiveness at a time when competition over males is strong. Alternatively, it can be interpreted as an adaptive terminal investment in ornamentation, that is, that females intensify skin pigmentation as the last reproductive cycle approaches (Candolin 2000a). Finally, the increase in skin coloration could also be nonadaptive, for example, if dietary
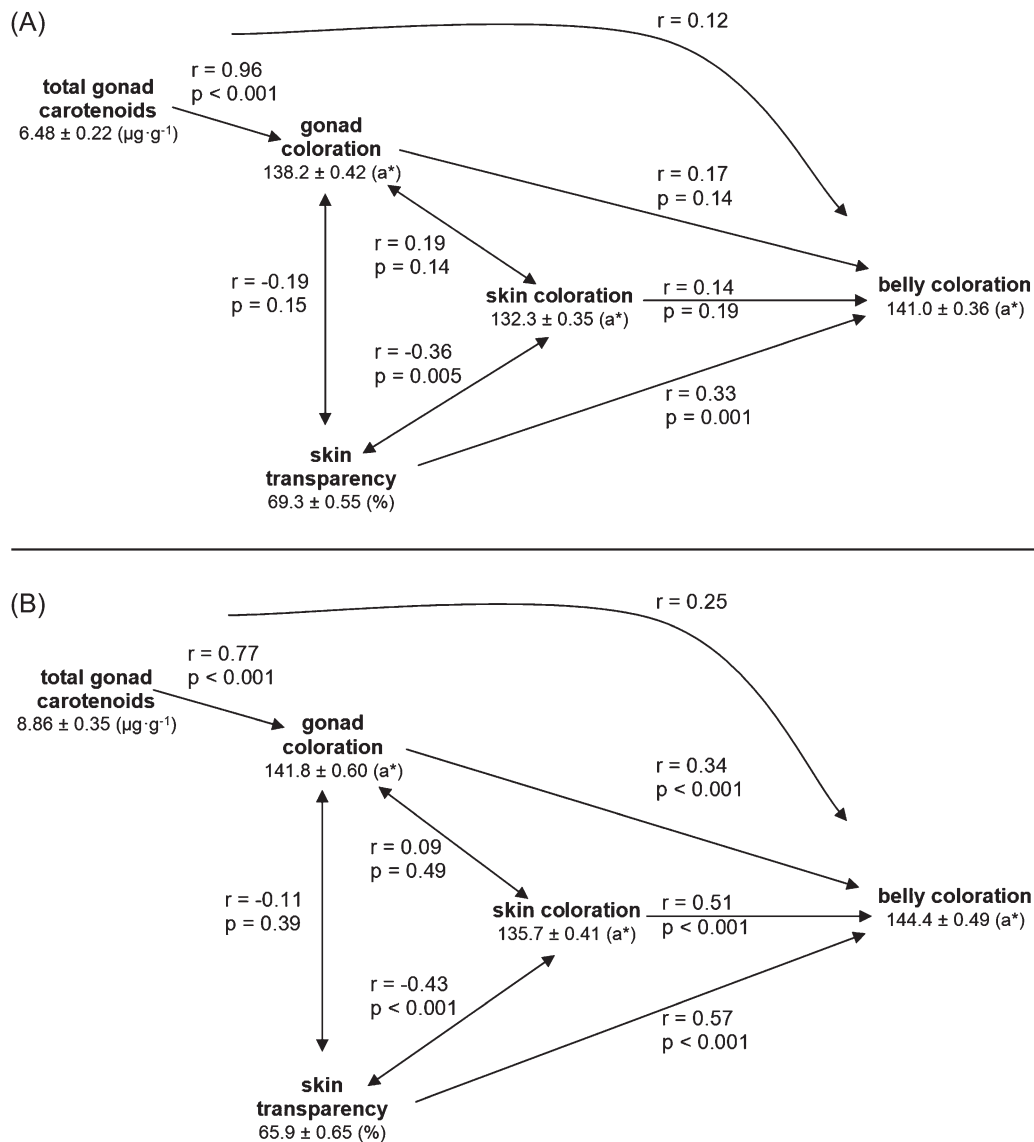
Figure 4
Path analyses of the components contributing to nuptial coloration in gravid female *Gobiusculus flavescens* (A) early and (B) late in the breeding season of 2004. Single-headed arrows are causal effects, *r* = path coefficients from linear models. Double-headed arrows have unresolved causality, *r* = Pearson's correlation coefficients. Quantities are means ± 1 SE.

pigments become more accessible later in the summer, or if drabber females have a higher mortality rate.

Sources of female belly coloration

The analyses showed that both the carotenoid-based gonad coloration and the abdominal skin coloration contribute to belly coloration in gravid females. The path diagrams demonstrate complex interactions between gonads, abdominal skin, and belly coloration. For example, the relationship between gonad coloration and belly coloration describes 2 separate phenomena. First, the transparency of the skin allows a direct view of the gonads. If transparency is high, this means that belly coloration and gonad coloration have a causal relationship as they are largely the same trait. Second, there could be positive covariation in pigment concentration of the 2 types of tissue. However, we found no indication of this as gonad and skin coloration appeared uncorrelated. The seasonal patterns were very similar in 2002 and 2004, but belly coloration and gonad

coloration were generally more intense in 2002. We have no evidence to explain this difference. However, water temperature was somewhat higher in 2002 (4 °C early and 2 °C late in the season), but it is unknown if and how this might have affected, for example, prey abundance or composition.

Signal content in gravid females

At first glance, the colorful belly of *G. flavescens* females appears to be a direct consequence of gonad carotenoid concentration as gonads are directly visible through the transparent skin (i.e., an "abdominal window" *sensu* Baird 1988, Figure 1). Belly coloration may therefore be considered more as a primary, than a secondary, sexual character (Darwin 1871). However, skin chromatophores also contributed significantly to the belly coloration (Svensson et al. 2005, Figure 4). Therefore, belly coloration also has the properties of a traditional secondary sexual character. As the importance of skin pigmentation increased over the breeding season, belly
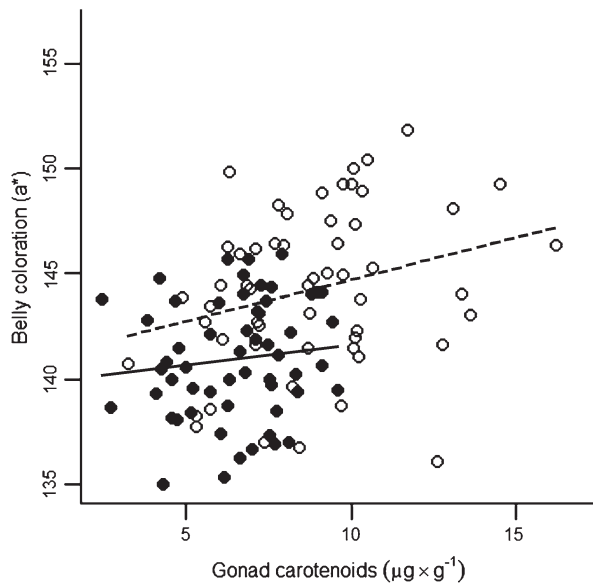
Figure 5
Relationships between gonad carotenoids and belly coloration in gravid female *Gobiusculus flavescens*, early (solid circles) and late (open circles) in the breeding season of 2004.

coloration was more of a primary sexual character early, and more of a secondary character late, in the season. This blurring of the classic distinction between primary and secondary sexual characters might not be unique to *G. flavescens* as partially transparent female bellies exist in several other teleosts (e.g., Baird 1988; McLennan 1995). If we view the belly coloration of *G. flavescens* females as a secondary sexual character, we should also ask about the signal content and signal honesty. In female lagoon gobies (*K. panizzae*), yellow pigment on the belly skin indicates fecundity (Massironi et al. 2005). This seems unlikely in *G. flavescens* as ready-to-spawn females only show a weak relationship between belly coloration and clutch size (Svensson et al. 2006). Furthermore, *G. flavescens* males show only a weak preference for more fecund females (Pélabon et al. 2003), in sharp contrast with their strong preference for colorful females (Amundsen and Forsgren 2001). There was a close link between the carotenoid concentration and the coloration of gonads. Because carotenoids are known to improve egg development (Verakunpiriya et al. 1997; Ahmadi et al. 2006), one could argue that belly coloration actually signals egg quality rather than quantity. However, at best, only 7% of the variation in belly coloration was explained by variation in gonad carotenoids. This suggests that female belly coloration is an imprecise indicator of egg carotenoid content. Nonetheless, a male that chooses a very colorful female (a* >145) over a very drab one (a* <142) late in the season would earn a 2-fold gain in egg carotenoids. Late in the season, males may express such choosiness without suffering costly delays as gravid females are abundant and the OSR is heavily female biased. At this time of the season, males are often surrounded by shoals of 5–20 courting females (Forsgren et al. 2004).

Potential costs of female belly coloration

Signal honesty can be maintained through different processes. A signal may be unfakeable, that is, an index that is causally linked to the quality being signaled, or it may be costly to express (Maynard Smith and Harper 2003). The direct view of the gonads through the abdominal window suggests that belly

coloration of female *G. flavescens* is, at least partly, an unfakeable index of gonad carotenoids. However, skin chromatophores also affect the signal, and their ability to regulate belly coloration over short or long terms may obscure this direct link with egg carotenoids (Svensson et al. 2005; Nilsson Sköld et al. 2008).

As females could "cheat" by disproportionately increasing their skin coloration, we speculate here about possible costs that could help maintain the honesty of the signal. First, intensely orange bellies may lead to increased conspicuousness to predators (Endler 1980). Second, *G. flavescens* females may have to trade-off carotenoids between skin coloration and other uses like immune defense and egg production. One proposed constraint on the evolution of female ornamentation is that incorporating resources into the eggs will be more beneficial than "wasting" them on ornaments (Fitzpatrick et al. 1995; Chenoweth et al. 2006). This seems unlikely in *G. flavescens* because gonad and skin coloration were uncorrelated, but both increased over the reproductive season. Moreover, the trade-off between eggs and ornament is relaxed because carotenoids deposited in the gonads will also increase belly coloration. Third, being colorful could have socially imposed costs that help maintain signal honesty. In sticklebacks, low-quality males can invest disproportionately into ornamentation, but this is prevented by male–male competition (Candolin 2000a, 2000b). Because female *G. flavescens* live in shoals (Svensson et al. 2000), there may be similar social costs of exaggerated belly coloration, especially late in the breeding season when female–female agonism is common (Forsgren et al. 2004). One should also consider that signals can be dishonest during certain circumstances (Maynard Smith and Harper 2003), for example, when signaling increases during terminal efforts (Candolin 2000a). This does not appear to be the case in female *G. flavescens* because belly coloration was in fact a better predictor of gonad carotenoids toward the end of the season. Therefore, the raised signaling intensity late in the season did not add noise to the signal.

In conclusion, the colorful belly of female *G. flavescens* can, at least in part, be regarded as a signal of readiness to spawn. However, by choosing a more colorful female, a male is also more likely to receive eggs with a higher carotenoid concentration. Females display more intense belly coloration toward the end of the breeding season, as a result of changes in both skin and gonad pigmentation and their interaction. A potential for dishonest signaling in *G. flavescens* females calls for studies focusing on if, and how, honesty is maintained in this system.

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