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Chromatic interaction between egg pigmentation and skin chromatophores in the nuptial coloration of female two-spotted gobies

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Summary

In two-spotted gobies (*Gobiusculus flavescens* Fabricius 1779), females develop an orange belly as they approach sexual maturity. Bright belly coloration is preferred by males and has been suggested to act as a female ornament. This coloration is unusual in that it originates partly from pigmentation of the abdominal skin but also from strongly pigmented gonads directly visible through the skin. In addition, females have been observed to temporarily become more colourful during courtship and competition. To understand how gonad and skin pigmentation interact in this nuptial coloration, the potential for colour modification *via* regulation of skin chromatophores was investigated. Noradrenaline caused aggregation of chromatophore pigment and was used to experimentally reduce the contribution of skin chromatophores to the nuptial coloration. Chromatophore pigment aggregation caused bellies to become less colourful and abdominal skin biopsies to become less colourful and more transparent. There was a strong positive relationship between belly coloration and the coloration of the underlying gonads. This shows that belly coloration honestly reflects egg pigmentation, mainly because the transparency of the abdominal skin allows other fish to see the gonads directly. Interestingly, when noradrenaline caused pigment to aggregate and thereby increased the transparency of the skin, the relationship between belly and gonad coloration weakened. We conclude that female *G. flavescens* have a potential to use skin chromatophores to rapidly alter their nuptial coloration, thereby affecting the efficacy with which information about gonad coloration is conveyed.

Key words: sexual selection, nuptial signal, female ornament, courtship, *Gobiusculus flavescens.*

Introduction

In several teleost fishes, females develop nuptial colour patterns as they become sexually receptive (McLennan, 1995; Beeching et al., 1998; Takahashi, 2000). These can come about through pigment cell differentiation, proliferation and migration and normally take several days to develop (Kelsh, 2004; Sugimoto, 2002). In addition to such seasonal colour changes, fish colour patterns may be modified nearly instantaneously, either by reflective changes in active iridophores (Mähtiger et al., 2003) or through aggregation or dispersion of skin chromatophore pigments (Kodric-Brown, 1998; Burton, 2002). Ephemeral changes in chromatophore pigment dispersion are partly under neurohumoral regulation (Fujii and Oshima, 1994; Aspengren et al., 2003) and may provide camouflage (e.g. Healey, 1999) or, in a social context, signal aggression, submission or sexual interest (DeMartini, 1985; Kodric-Brown, 1998; Höglund et al., 2002). Female nuptial colour patterns may therefore become more conspicuous during courtship, not only by behaviours that generally emphasize the coloured areas, e.g. sigmoid display (Baird, 1988; Swenson, 1997; Takahashi, 2000; Kuwamura et al., 2002), but also by actual colour change using chromatophores (Rowland et al., 1991; Kodric-Brown, 1998; Berglund, 2000). The latter has been described, for instance, in the sex-role reversed pipefish, *Syngnathus typhle*, where females display a barred pattern during courtship and intrasexual competition, the intensity of which can be changed within minutes (Berglund and Rosenqvist, 2001). Despite other, often anecdotal, descriptions of teleosts where females exhibit temporary changes in nuptial coloration, few studies have shed light on function and mechanisms, possibly because ephemeral coloration is difficult to quantify.

The two-spotted goby, *Gobiusculus flavescens*, is a small, semi-pelagic marine fish with paternal care of the eggs (Gordon, 1983; Skolbekken and Utne-Palm, 2001; Bjelvenmark and Forsgren, 2003). It is sexually dimorphic with both males and females exhibiting ornamentation during the reproductive season. While males have brightly coloured fins and iridescent blue spots, females develop increasingly orange bellies as gonads mature. Amundsen and Forsgren (2001) hypothesized that the female belly coloration is caused mainly by the pigmented eggs being visible through the semi-transparent abdominal skin but also by pigment in the skin

itself. The development of nuptial coloration over the course of the reproductive season suggests that steroids are involved in its regulation (Burton, 1981; Fujii and Oshima, 1994). Notably, the degree of belly coloration varies even among fully mature females (T. Amundsen, E. Forsgren, C. Pélabon and P. A. Svensson, unpublished), and males show preference for females with the most colourful bellies (Amundsen and Forsgren, 2001). Female courtship involves approaching the male and bending the body (sigmoid display), a behaviour that seems to emphasize the round and colourful belly (Amundsen and Forsgren, 2001). Both during courtship and in agonistic interactions, females can temporarily attain strikingly orange bellies, often while performing repeated sigmoid displays [U. Berger (2002), Diplomarbeit im Fach Biologie, Westfälische-Willhelms-Universität, Münster, Germany].

Early in the breeding season there is a shortage of mature females, and males court females actively (Forsgren et al., 2004). However, the sex roles are dynamic, and later in the season mature females increase in proportion, leading to a drastic increase in female courtship as well as female–female agonism (Forsgren et al., 2004). The belly coloration of female *G. flavescens* may therefore function as a ready-to-spawn signal or an ornament, or both. Little is known, however, about the relative contributions and possible interaction of gonad and skin pigmentation to the female nuptial coloration. Likewise, no studies have been performed on chromatophore regulation in this species.

In the present study, we tested the hypothesis that skin chromatophores interact with egg coloration in determining the externally visible belly coloration of female *G. flavescens*. Specifically, we investigated the potential of chromatophores to alter female belly coloration through pigment dispersion and aggregation. By doing so, we reveal whether live females have the potential to modify their belly colour, used in sexual signalling during courtship and competition.

Materials and methods

Capture and selection of fish

G. flavescens were collected by snorkellers in June 2004 in the Gullmar fjord, Sweden (58°15′ N, 11°27′ E). From a holding tank of >100 females, 10 mature females were selected for each of two experiments described below (total length = 45.2±1.7 mm, N=20). To cover the variation of the natural population with such a small sample, only the most drab and most colourful females (as judged by P.A.S.) were selected.

Chromatophore assays

Preliminary light microscopy observations showed that the chromatophore pigment dispersed after death. Therefore, rather than using potential dispersing factors such as MSH (melanocyte stimulating hormone), ACTH (adrenocorticotropic hormone) or prolactin, killing the fish was used as a dispersing treatment. Although many factors may have aggregating effects on the *G. flavescens* chromatophore pigments, noradrenaline is known to be one of the most general regulators of melanophores and erythrophores in fish, by binding to α_2-adrenoceptors (Fujii and Oshima, 1994). Noradrenaline was therefore used to aggregate the chromatophore pigment. These two treatments were chosen to approach maximal and minimal contributions of skin chromatophores to coloration and thus estimate the potential for colour modification by chromatophores. For details regarding chromatophore regulation, e.g. at the second messenger and the cytoskeleton level, see Fuji and Oshima (1994), Nilsson Sköld et al. (2002) and Aspengren et al. (2003).

Experiment 1: whole fish

To investigate belly coloration, 10 *G. flavescens* females were photographed three times: (1) when the fish were alive, (2) 60 min after the fish were killed by decapitation, when the chromatophore pigment had fully dispersed, and (3) after exposing the dead fish to 10 μmol l^{-1} noradrenaline for another 60 min to allow for pigment aggregation.

Fish were placed in a 7×4×2 cm aquarium containing either seawater (live and unexposed dead treatments) or 10 μmol l^{-1} noradrenaline (noradrenaline-exposed treatment). Considerable attention was given to the standardization of

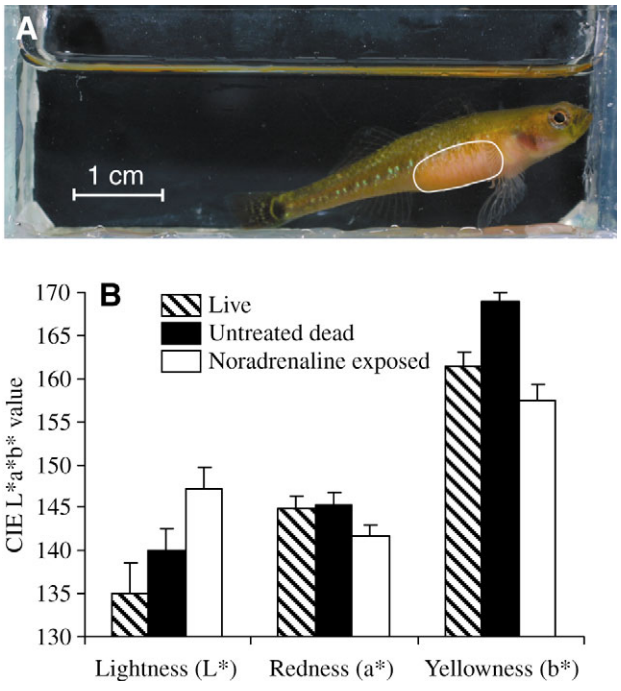

Fig. 1. The role of skin chromatophore pigmentation on belly coloration in whole *G. flavescens*. (A) An example of a photograph used to quantify the belly coloration of *G. flavescens* females, where the oval shape describes the area selected for the colour quantification. (B) Effects of death and noradrenaline exposure on belly coloration of female *G. flavescens*, measured as CIE L*a*b*. The coloration was quantified from digital images taken when the fish were alive (hatched bars), 60 min after being killed (solid bars) and after 60 min exposure to noradrenaline (open bars). Means ± S.E.M., N=10.

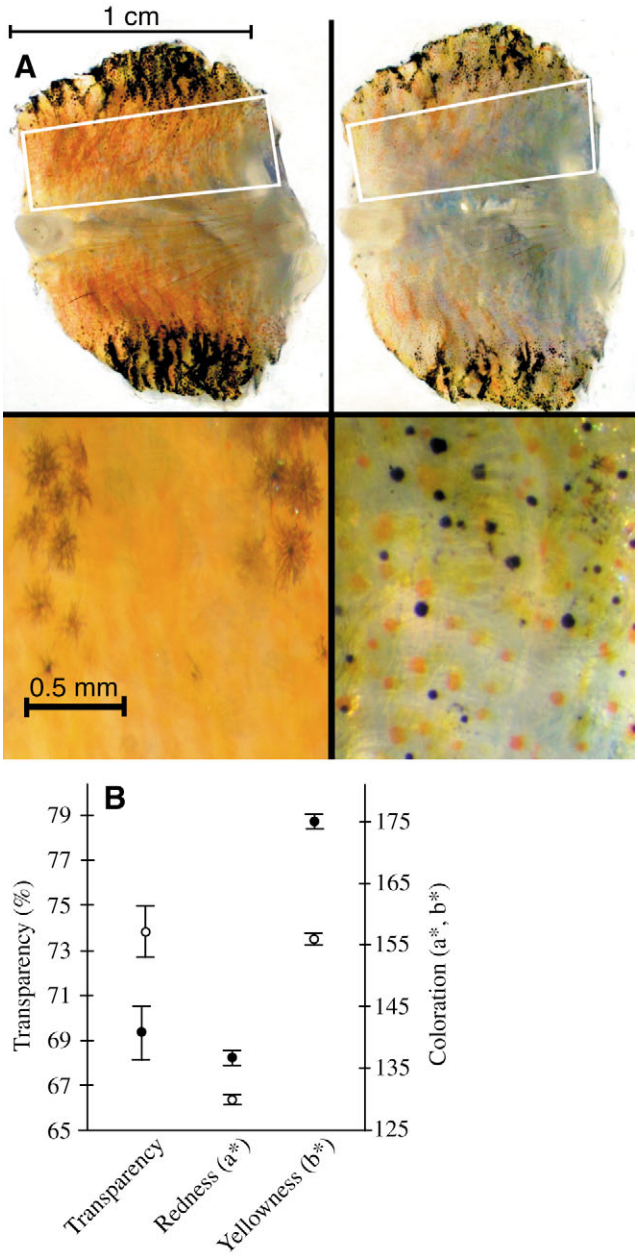

Fig. 2. The role of skin chromatophores on abdominal skin coloration and transparency. (A) An example of photographs of abdominal skin biopsies from *G. flavescens* before (left) and after (right) 60 min exposure to noradrenaline. These photographs were taken on a light table with all light coming through the biopsies. In the upper half, the boxes in the photographs describe the area selected for colour quantification. In the lower half, magnified sections of abdominal tissue show the presence of chromatophores, with either dispersed (left) or aggregated (right) pigment. (B) Effects of chromatophore pigment aggregation caused by noradrenaline exposure on the transparency and coloration of abdominal skin biopsies from *G. flavescens* females. The transparency and coloration (CIE a* and b*) were quantified before (filled circles) and after exposure to noradrenaline (open circles). Means ± S.E.M., *N*=10.

photographic conditions. In a dark room, a Canon D30 digital camera with a Canon 50 mm f/2.5 EF Compact Macro lens (Canon Norge AS, Oslo, Norway) and a Senz stereo macro flash (Photax AB, Nybro, Sweden) were used to take an image of each side of every fish (Fig. 1A). Exposure time (1/60), aperture (f8) and flash power settings were kept constant for all photographs.

Experiment 2: skin biopsies and gonads

To specifically examine the role of chromatophores in the skin overlying the gonads, abdominal skin biopsies were further investigated. A second group of 10 females was killed by decapitation, their abdominal skin was excised, and the gonads were removed. The skin biopsies were placed in saltwater for ~60 min to allow time for full chromatophore pigment dispersion. Then they were placed on a light table and photographed with the light shining through the skin (Fig. 2A). After exposure to 10 μmol l^{-1} noradrenaline for another 60 min, the skin biopsies were photographed a second time. The camera (same as above) and light table were cloaked in black fabric to avoid any ambient light affecting the photograph. Exposure time (1/60) and aperture (f8) were kept constant for all photographs. A black, opaque object placed adjacent to the skin biopsy was used as a zero-light reference in the image analysis.

Since no chromatophores were observed on the gonads or on the gonad epithelium, noradrenaline was not expected to affect the coloration of gonads themselves. To test this assumption, a control experiment was performed. All gonads were placed in Petri dishes containing 9 psu water, and a photograph was taken. The gonads were then placed in Petri dishes containing 10 μmol l^{-1} noradrenaline for 60 min, and a second photograph was taken. The Petri dishes were photographed placed on a white background, and one image was taken of each gonad from directly above using the same camera, lens and flash. Exposure time (1/60), aperture (f32) and flash power settings were kept constant for all photographs.

The close-up photographs of skin biopsies shown in Fig. 2A were taken with a Canon D30 digital camera mounted on a Wild M3Z stereo dissecting microscope (Leica, Stockholm, Sweden).

Image analysis

All image analyses were performed using Adobe Photoshop 4.0 (Adobe Systems Inc., Mountain View, CA, USA). The digital images were converted to CIE L*a*b*, a colour space recommended by the Commission International de l'Eclairage (CIE). CIE L*a*b* consists of three parameters: the L* value (lightness) gives the relative lightness ranging from total black to total white, the a* value ('redness') represents the balance between red and green, and the b* value ('yellowness') represents the balance between yellow and blue. In contrast to, for example, RGB colour space, CIE L*a*b* is a standardized, perceptually uniform and device-independent colour space (Chen et al., 2004). It has been frequently used in fish colour quantifications, especially in connection with carotenoid-based colorations (e.g. Skrede and Storebakken,

1986; Smith et al., 1992; Hatlen et al., 1998; Craig and Foote, 2001).

Colour systems based on human vision might be inappropriate when studying colour signals in animals with unknown visual pigments, for instance if animals are sensitive to UV light (Bleiweiss, 2004). The results of Amundsen and Forsgren (2001) show that *G. flavescens* females scored as 'colourful' by human observers were preferred by males over females scored as 'drab'. Thus, there seems to be an overall agreement between human vision and fish vision in this species, at least in the yellow–red part of the spectrum. In addition, retinal absorbance data suggest that *G. flavescens* has tristimulus vision similar to humans and lacks UV receptors (A. C. Utne-Palm, unpublished data). Therefore, using CIE L*a*b* values from digital photographs is likely to be an appropriate method for colour quantification in our case.

For the photographs of whole fish, the belly area was selected with the lasso tool of Photoshop. This was defined as the roughly elliptic area between the anal pore, the pectoral fin base and the blue spots below the lateral line (Fig. 1A). The mean values of lightness, redness and yellowness of the selected area were measured using the histogram tool. The average values from the two photographs of each fish were used in the analyses.

For the photographs of abdominal skin biopsies, only skin located directly lateral of the gonads was measured, i.e. the area below the lateral line and above an imagined line between the pelvic girdle and the anal pore (Fig. 2A). Lines were drawn on the images using landmarks to ensure that the same area was selected in both photographs, and the selection was done using the lasso tool. To remove any differences in light intensity between photographs, the L* channel was normalized by setting the opaque object as black (L*=0) and the background as white (L*=255), an operation that does not affect the values of redness and yellowness. The average values of lightness, redness and yellowness in the selected area were measured using the histogram tool. Since these photographs were taken on a light table where all light permeated through the skin, the L* of the skin biopsy was directly related to its transparency. As the concept of skin transparency is relevant to the understanding of this colour signal, we converted L* to a measurement of transparency. This was done by calculating the percentage of L* in the selected area relative to the L* of the background (transparency=100×L*/255).

From the pictures of gonads, the gonads were selected using the lasso tool and the average values for lightness, redness and yellowness in the selected area were measured using the histogram tool.

Reagents

Stock solutions of noradrenaline (Sigma Aldrich, St Louis, MO, USA) were stored at –20°C and diluted to the experimental concentrations in phosphate-buffered saline (PBS; 136.9 mmol l^{-1} NaCl, 2.7 mmol l^{-1} KCl, 1.5 mmol l^{-1} KH_2PO_4 and 8.0 mmol l^{-1} Na_2HPO_4 at pH 7.4) just before use.

Results

Belly coloration of whole fish

Belly coloration was quantified from photographs on three occasions (live, untreated dead and noradrenaline exposed). Since the same 10 females were used in the three photographs, the data were analysed as a block design without interaction, using a general linear model with 'individual' and 'treatment' as factors. Tukey's honest significant difference (HSD) was used as a *post-hoc* test on the factor 'treatment'.

There was a considerable range in belly coloration among individual females (live fish: L*=120–155, a*=138–151, b*=155–171). Noradrenaline exposure significantly increased the lightness (L*) compared with live fish (Tukey HSD, N=10, P=0.031) but not compared with untreated dead fish (Tukey HSD, N=10, P=0.26) (Fig. 1B). There was no significant difference in L* between live and untreated dead fish (Tukey HSD, N=10, P=0.49).

Redness (a*) did not differ between live and untreated dead fish (Tukey HSD, N=10, P=0.82), but was significantly reduced after noradrenaline exposure compared with live fish (Tukey HSD, N=10, P=0.003) and untreated dead fish (Tukey HSD, N=10, P=0.001) (Fig. 1B).

Yellowness (b*) increased significantly after death (Tukey HSD, N=10, P=0.001) and decreased significantly after noradrenaline exposure compared with untreated dead fish (Tukey HSD, N=10, P<0.0001) (Fig. 1B). However, there was no significant difference between live and noradrenaline-exposed fish (Tukey HSD, N=10, P=0.081). In conclusion, the results show that noradrenaline induced pigment aggregation, which caused increased lightness and reduced coloration of the bellies. This confirms a role of chromatophores in female belly coloration.

Abdominal skin and gonad coloration

Observations of the skin indicated the presence of melanophores (black-brownish pigment), erythrophores (red pigment) and xanthophores (yellow pigment) on a whitish background colour. All observed females had highly transparent abdominal skin and largely lacked the silvery peritoneum that in many fishes surrounds the abdominal cavity. On the part of the skin directly overlying the gonads, there were mainly erythrophores and xanthophores present (Fig. 2A). Gonads and skin biopsies were subject to two treatments only: untreated and noradrenaline exposed. Therefore, gonad coloration and abdominal skin coloration and transparency were analysed with pairwise t-tests. Percentages were arcsine square root transformed prior to analyses.

Aggregation of chromatophore pigments following noradrenaline exposure caused the transparency to increase from 69% to 74% (transparency, paired t-test N=10, t=–5.02, P<0.001) while decreasing redness (a*, paired t-test N=10, t=5.74, P<0.001) and yellowness (b*, paired t-test N=10, t=15.62, P<0.0001) (Fig. 2B). Compared with erythrophores and xanthophores, melanophores appeared to respond slower to noradrenaline, but this was not further investigated.

In an experiment aimed to verify methodology, it was

confirmed that the observed changes in coloration and transparency were indeed an effect of noradrenaline, rather than postmortem paling (data not shown). Even after several hours, unexposed tissue was much darker and more colourful compared with tissue exposed to noradrenaline.

Exposing excised gonads to noradrenaline did not affect any of the three CIE L*a*b* colour parameters (pairwise *t*-tests, N=10, all P>0.34). No coloration or chromatophores were observed on the gonad epithelium, which indicates that the gonad coloration originates solely from pigments deposited in the eggs.

Relationships between belly colour, abdominal skin colour and gonad colour

Regression analyses were performed to analyse the relationship between gonad coloration and belly coloration at different levels of chromatophore pigment aggregation. Since gonads were not affected by noradrenaline exposure, only data from unexposed gonads were used in the regressions. Redness (a*) was chosen for these analyses, since this parameter produced the best correlation between belly and gonad colour. Redness also produces the best correlations between gonad colour and egg carotenoid concentration (T. Amundsen, J. D. Blount, C. Pélabon and P. A. Svensson, unpublished). There was a significant relationship between gonad redness and belly redness, regardless of whether the fish were alive, dead or treated with noradrenaline (live fish, N=10, r^2=0.80, F=31.45, P<0.001; untreated dead fish, N=10, r^2=0.75, F=23.98, P=0.001; noradrenaline exposed fish, N=10, r^2=0.49, F=7.67, P=0.024) (Fig. 3). However, after noradrenaline exposure (when chromatophore pigment was aggregated), gonad redness explained less of the variation in belly redness (49%) compared with untreated dead fish and live fish where pigment was more dispersed (75% and 80%, respectively). To investigate whether the estimated slopes differed between regressions, Δa* was defined as the difference in belly redness between two treatments. If the slope of Δa* plotted against gonad redness was significantly different from zero, this would correspond to a difference in slope between those two treatments. The slope from the regression using fish exposed to noradrenaline was significantly lower than the slope in the regression using live fish (N=10, r^2=0.64, F=14.24, P=0.005). This result shows that the belly coloration is formed by the additive effects of skin chromatophore pigment and gonad coloration. The regression slope using untreated dead fish did not differ from the slope using live fish (N=10, r^2=0.08, F=0.71, P=0.42) or using fish exposed to noradrenaline (N=10, r^2=0.26, F=2.39, P=0.13). Thus, chromatophore pigment aggregation caused belly colour to reveal gonad colour less efficiently, both by reducing the r^2 (increasing the variation) and by significantly reducing the slope of the relationship (Fig. 3).

There was no significant correlation between gonad coloration and abdominal skin coloration, regardless of whether the skin had been exposed to noradrenaline (unexposed, N=10, Pearson r=–0.12, P=0.73; exposed, N=10, Pearson r=0.29, P=0.41). Thus, the observed positive relationship between gonad and belly coloration could not be explained by a correlation between gonad and skin coloration. This, together with the observed high transparency of the abdominal skin, suggests that the observed relationship between gonad and belly coloration is indeed largely due to the gonads being directly visible through the abdominal skin.

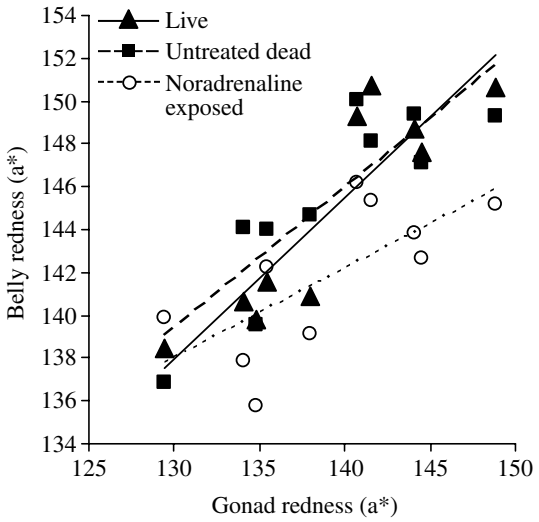

Fig. 3. The relationship between gonad and belly coloration (CIE a*) in the presence and absence of noradrenaline in *G. flavescens* females. Live and untreated dead fish had reasonably dispersed chromatophore pigment, while noradrenaline caused pigment aggregation. The coloration was quantified from digital photographs.

Discussion

Belly coloration

Exposure to noradrenaline caused increased lightness and reduced red and yellow coloration of the belly of *G. flavescens* females. These results were expected, given the whitish background coloration of abdominal skin and the observed presence of melanophores, erythrophores and xanthophores. The results from the noradrenaline treatment show that the presence of chromatophores increases belly coloration and that pigment aggregation/dispersion has the potential to rapidly regulate female belly coloration. It is likely that chromatophores play a role in the changes in female belly coloration observed during female courtship and competition.

Although death caused pigment to disperse compared with live fish, the effects of death on coloration were equivocal in that yellowness but not lightness nor redness increased significantly. This weak response may be due to black/brown and, in particular, red pigment being almost fully dispersed also in live fish.

Observations of courtship suggest that female *G. flavescens* regulate chromatophores in specific parts of the body to modify the coloration of, for example, the belly area [U. Berger (2002), Diplomarbeit im Fach Biologie, Westfälische-Willhelms-Universität, Münster, Germany]. It is inherently difficult to study such ephemeral patterns in a quantifiable manner, since constraining the fish is necessary for accurate colour

measurements, while females confined in too small volumes will not court. Instead, death was used as a treatment to obtain full pigment dispersion. Because death seemed to affect all types of chromatophores in all parts of the fish, it is unlikely that the 'death treatment' mimics the colour increase observed during courtship [U. Berger (2002), Diplomarbeit im Fach Biologie, Westfälische-Willhelms-Universität, Münster, Germany], and the effects of death should be interpreted cautiously.

Colouration of abdominal skin biopsies and gonads

There are two interesting characteristics of the abdominal skin in female *G. flavescens*. The first is its transparency, which seems to function as an 'abdominal window' (cf. Baird, 1988) that allows direct assessment of gonad coloration. The second characteristic is the presence of chromatophore pigments (mainly red and yellow; Fig. 2A), which may be viewed more as a traditional secondary sexual character.

The transparency of the abdominal skin biopsies was high and increased after noradrenaline had caused chromatophore pigment to aggregate. This corresponds to previous studies where aggregation of all chromatophores made the skin more transparent (Fujii and Oshima, 1994). Darwin (1871) distinguished between primary sexual characters, which are the reproductive organs themselves, and secondary characters such as ornaments. The high degree of transparency of abdominal skin in our study is interesting in its own right, since a direct display of the gonad coloration blurs the distinction between primary and secondary sexual characters. Partially transparent abdominal skin in mature females has previously been described in some teleost species, for instance brook stickleback (*Culaea inconstans*; McLennan, 1995) and straight-tailed razorfish (*Xyrichthys matinicensis*; Baird, 1988). One could argue that the observed transparency in these relatively small fishes is a mere side effect of the skin being stretched by large gonads. This seems unlikely for *G. flavescens*, however, since all mature females investigated (even with small gonads) lacked the opaque silvery peritoneum common to most fishes. In comparison, females of closely related and similarly sized *Pomatoschistus* species retain the opaque peritoneum as gonads mature (P. A. Svensson, personal observation). It is therefore possible that the transparency of abdominal skin in female *G. flavescens* has evolved as a signal or a component of a signal, aiming to attract males by displaying the colourful gonads (Amundsen and Forsgren, 2001).

The red and yellow coloration of abdominal skin biopsies decreased after noradrenaline exposure, confirming that abdominal skin coloration is caused by chromatophore pigments. Several studies have described teleost species where females have colourful patches on the skin covering the gonads, e.g. in the convict cichlid (*Cichlasoma nigrofasciatum*; Beeching et al., 1998), the stream goby (*Rhinogobius brunneus*; Takahashi and Kohda, 2004), the lagoon goby (*Knipowitschia panizzae*; Massironi et al., 2005) and the pink belly wrasse (*Halichoeres margaritaceus*; L. H. LaPlante, personal communication). Interestingly, *G. flavescens* females thus seem to use a combination of two previously described phenomena: a nuptial signal that involves a transparent, yet highly pigmented, abdomen. Furthermore, because the skin pigmentation is chromatophore based, it is adjustable and may be used to temporarily modify the belly colour intensity, for instance during courtship and agonistic behaviours.

Relationship between belly and gonad coloration

Amundsen and Forsgren (2001) hypothesized that the male preference for colourful females can be adaptive if females with colourful bellies have more carotenoids in the eggs and therefore provide the males with higher quality offspring. Our results show a strong relationship between belly coloration and gonad coloration. This could not be explained as a correlation between skin and gonad pigmentation but instead suggests that belly colour reflects gonad pigmentation directly. If highly pigmented eggs are of higher quality (Pettersson and Lignell, 1999; Blount et al., 2002), male *G. flavescens* may therefore be able to assess the quality of their potential offspring directly by observing the female belly coloration.

There was a rather small difference in redness between live and untreated dead fish (Fig. 3), possibly because live fish already had almost fully dispersed red pigment (cf. a* in Fig. 1B). The aggregation effect of noradrenaline was, on the other hand, considerable. Although most of the 10 females produced the weakest belly colour when pigment was aggregated, the effect of aggregation varied. Interestingly, the strongest effect of pigment aggregation occurred on the most colourful females (Fig. 3). This implies that colourful females have a larger potential of modifying their appearance, and possibly attractiveness, through the use of chromatophores.

Surprisingly, the positive relationship between belly colour and gonad colour was weakened when noradrenaline caused skin pigment to aggregate. So, despite the fact that less pigment was 'obstructing the view' of the gonads and despite increased transparency, pigment aggregation caused belly coloration to be a poorer predictor of gonad coloration. This pattern could be interpreted as abdominal skin chromatophores acting as amplifiers of gonad coloration (*sensu* Hasson, 1989), i.e. the skin pigment allows males to more accurately estimate gonad coloration (which may be a more costly and therefore more honest trait). In comparison, Berglund (2000) suggested that in female pipefish, an ephemeral barred pattern functions as an amplifier by aiding the males in correctly assessing female size (which is a female trait preferred by males). On the other hand, skin and gonad coloration of female *G. flavescens* may be viewed as two components of the same signal, which together determine a female's attractiveness (Candolin, 2003). A third alternative is that skin and gonad pigmentation convey different types of information. For example, the gradual increase in belly coloration as gonads mature could signal a general readiness to spawn, while the quick change caused by chromatophores could signal interest in a specific mate.

When interpreting the somewhat counterintuitive effect of pigment aggregation, it is important to recall that the treatments affected pigment in the entire fish and not only the quantified belly area. Furthermore, females may differ in the

degree of pigment dispersion when photographed alive and may respond to noradrenaline to different extents. The methodological limitations inherent in this study thus call for future more-detailed investigations of the role and the regulation of abdominal skin chromatophores in the female nuptial signal of *G. flavescens*, especially during natural behaviours. An interesting question is whether females with colourful eggs use chromatophores differently in, for instance, courtship compared with females with paler eggs. Kodric-Brown (1998) proposed that ephemeral colour changes can be used in both mate attraction and intrasexual conflict and should therefore be subjected to sexual selection. She also suggested that such colour changes can be combined with behavioural displays to increase the efficacy of a given signal. Since all of these aspects seem to be present in *G. flavescens*, it is an excellent model system for further investigations, both in the field of pigment cell physiology and in behavioural ecology. There is clearly a need for studies illuminating the relationships between female coloration, carotenoid based egg pigmentation and female quality.

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