

Mouth Color Signals Thermal State of Nestling Dark-Eyed Juncos (*Junco hyemalis*)

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Abstract

In many species of birds, nestlings have brightly colored mouths. Some studies have found that mouth color is related to hunger, and may serve to solicit feedings from parents. We devised two experiments to test the hypothesis that mouth color is an indicator of hunger in nestling dark-eyed juncos (*Junco hyemalis*), and neither experiment produced results to support the hypothesis. We did find, however, that mouth redness saturation increased for the duration of our experiments (60 min). We devised a third experiment to investigate the effect of a different stressor, temperature. In the third experiment, mouth redness decreased in saturation when microenvironment temperature increased following a period of cooling. These findings suggest that mouth color indicates thermal state of nestling dark-eyed juncos and may function as a signal to the female to brood them.

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Introduction

In species with altricial development, offspring must compete with their siblings for access to limited parental investment while at the same time minimizing energy costs and exposure to potential predators. Nestling birds have been widely used to study the evolution of signals designed to solicit parental investment. Much of this research has focused on the costs of signal production (Bachman & Chappell 1998; Kilner 2001) or whether these signals are honest indicators of need (reviewed by Kilner & Johnstone 1997; Royle et al. 2002).

Begging vocalizations, which are relatively easy to measure and manipulate, have been the subject of most studies. Recent years have seen an increased interest in the use of visual signals such as color patterns.

One of the first of these studies focused on canaries (*Serinus canaria*) and found that nestlings' mouths 'flushed' brightly red when they begged for food (Kilner 1997). The red flush increased in saturation (dominance or purity of hue) with longer periods of food deprivation. Studies on great tits (*Parus major*) and barn swallows (*Hirundo rustica*) have shown that artificial reddening of nestling mouths stimulates greater feeding rates by parents (Götmark & Ahlström 1997; Saino et al. 2000). Collectively these studies suggest that nestling mouth color is a signal to solicit food from parents, although mouth color changes in species lacking the 'flush' shown by canaries have been examined to a limited extent (Kilner & Davies 1998).

Other hypotheses have been proposed to explain conspicuous mouth coloration of nestling birds. The contrast between the brightly colored mouth and the light-colored, outer flanges may provide a conspicuous feeding target for parents, especially in cavity-nesting species (Pycraft 1907; Ficken 1965). In agreement with this hypothesis, Kilner & Davies (1998) found that cavity-nesting birds showed greater contrast between the colors of the gape and the surrounding flanges than did bird species that nest in open cups. A different, although not mutually exclusive, hypothesis is that mouth color varies with respect to offspring quality. Saino et al. (2000) showed that immune system challenges in nestling barn swallows decreased mouth color, suggesting that color is an honest signal of immunocompetence. Thus there appears to be no single explanation for conspicuous mouth color in altricial nestling birds.

The current study examines the signal function of mouth color in nestling dark-eyed juncos (*Junco hyemalis*). Juncos are open-cup nesting passerine birds with altricial young (Nolan et al. 2002). We studied a population of juncos that has been the subject of ongoing research on adaptive variation in male testosterone levels (Ketterson & Nolan 1992; Ketterson et al. 1992, 1996, 2001). Our initial interest in nestling mouth color arose from our attempts to explain the suppressive effect testosterone had on male parental behavior (Ketterson et al. 1992; Schoech et al. 1998). We were interested in solicitation displays given by nestling juncos and the way in which hormone levels might affect adult responses to these displays. Such a relationship presupposes that displays (e.g. mouth color) are signals of nestling hunger. Thus, our initial prediction was that nestling junco mouth color saturation would increase in response to food deprivation and decrease following satiation, similar to the pattern observed in other species (Kilner 1997; Kilner & Davies 1998). When these predictions were not supported by our first two experiments, we considered other explanations for changes in nestling mouth color and tested the possibility that it varied with thermal state and might signal a need to be brooded.

Methods

Study Area and Study Species

We conducted this research on a color-banded population of dark-eyed juncos near the Mountain Lake Biological Station (MLBS) in Giles County, VA (see Chandler et al. [1994] for a description of the study area). The demographics of this population have been monitored since 1983. A detailed description of the phenology and life history of dark-eyed juncos can be found elsewhere (Nolan et al. 2002), but we summarize important points below.

Male juncos begin establishing and defending territories at MLBS in early April. Females arrive several weeks later and start to build nests. Nests are typically constructed on the ground beneath overhanging roots or vegetation. The first nestlings appear in early to mid-May. Broods usually contain three to five nestlings (occasionally two), which remain in the nest for 12 d. Junco nestlings, which are fed a variety of larval and adult arthropods, have red mouths surrounded by fleshy, yellow flanges, both of which decrease in prominence and color saturation after 6 d of age. Males and females contribute approximately equal effort to feeding nestlings, but only females incubate eggs and brood young.

Experiment 1: Effects of Nestling Body Size and Satiation

This experiment was conducted May 20–Jul. 26, 1999. We measured mouth color of pairs of nestlings from 23 different broods at 2–3 d of age (d 0 = hatching). In the field, we weighed nestlings to the nearest 0.1 g with a Pesola® scale and measured their tarsometatarsi (hereafter tarsus) to the nearest 0.1 mm with dial calipers. We then selected the largest ($\bar{x} \pm \text{SE}$; mass = 7.6 ± 0.2 g, tarsus = 12.3 ± 0.2 mm) and smallest (mass = 5.8 ± 0.3 g, tarsus = 10.8 ± 0.3 mm) nestling from each brood, placed them in a cotton-lined glass bowl, and transported the bowl to a light-sealed darkroom at MLBS. We recorded the time when nestlings were removed from the nest (\bar{x} = 13:40 hours, range = 07:50–16:55 hours EST), the time of transport from the nest to the darkroom (21.2 ± 1.1 min), and the outdoor temperature at the time of removal ($17.87 \pm 0.57^\circ\text{C}$). A multiple linear regression showed that none of these variables significantly affected initial mouth color (t_1 , see below; time of removal: partial- t = -0.12 , p = 0.90; time of transport: t = -0.75 , p = 0.46; outdoor temperature: t = -1.33 , p = 0.19). This and other experiments were conducted under both clear and overcast conditions.

We used the still image mode on a Sony DCR-TCV 103 Digital 8 Camcorder to photograph nestlings in an arena enclosed on three sides by black paperboard to reduce reflected light. The arena was lit with two 65-W incandescent bulbs mounted 50 cm overhead. During each photographic session we placed the subject nestling inside a small, raised ring (o.d. = 70 mm, i.d. = 35 mm, ht = 20 mm) to position it beneath the camera, which was placed on a tripod 50 cm above the ring. While it was impossible to standardize perfectly the height at which nestlings begged, differences among individuals were within ± 1 cm.

We stimulated nestlings to open their mouths (i.e. beg) by touching their bills with forceps or by tapping on the raised ring. We took a series of three or four digital photographs of each nestling at four different times (t_1 – t_4). The first series of photographs was taken upon arrival in the darkroom (t_1). The second and third series of photographs were taken 20 and 40 min later (t_2 and t_3 , respectively). Our decision to monitor mouth color changes for 40 min before feeding was based on similar methodology used by Kilner (1997). Immediately after the third series of photographs, the nestling was fed to satiation with canned kitten food (Friskies Pet Care Inc., Glendale, CA) from a 1.0 cm³ syringe (E. Kennedy et al. unpublished data). Satiation was determined when the nestling would no longer beg for food, usually after it had eaten 0.4–0.6 cm³ of food. We took a fourth series of photographs 20 min later (t_4). From each series of photographs at each time interval (t_1 , t_2 , t_3 , and t_4) we selected and analyzed the image with the greatest exposed mouth area. We kept nestlings in separate cotton-lined bowls in the dark at a temperature of $24 \pm 2^\circ\text{C}$ when they were not being photographed. The two nestlings were photographed separately, one immediately after the other.

Nestling mouth images were exported to a Macintosh platform computer for analysis with Adobe® Photoshop® version 5.0 (Adobe Systems, Inc., San José, CA) (see Kilner 1997). We determined the degree of red light saturation (RGB color system) of the mouth by calculating the median saturation level (0–255, where 255 is completely saturated) of all pixels within the exposed area of the gape. We used median saturation level because mean values were often skewed by a few unusually bright pixels. To reduce possible observer bias, we analyzed mouth color while blind to the identity of the nestling.

To standardize the mouth color score among images, A color standard was included in each image. The standard was a 2.5×1.5 cm paperboard panel from a box of 35-mm film (Fujichrome, Fujifilm, Inc.). A 20-pixel area of the standard was used to obtain the red saturation level as described above. For example, Adobe® Photoshop® might provide a median redness saturation value of 160 for a nestling's mouth. The redness of the color standard in the same image might be 65, yielding a standardized redness value of 2.46. Higher standardized values correspond to increased redness saturation. Different color standards were used in each experiment to guard against possible discoloration over time.

Experiment 2: Effect of Food Deprivation

This experiment was conducted on pairs of nestlings from 12 different broods of 2–3 d of age from May 17–June 12, 2000. We controlled for differences in body size by selecting the two nestlings from each brood that were most similar in body mass. If there was no pair of nestlings with similar masses, we used tarsus length as a secondary criterion. Measurements of body mass and tarsus length were taken as described in experiment 1.

We transported nestlings to the light-sealed darkroom as described above. Upon arrival (t_1), we took photographs of the mouths of both nestlings, one immediately following the other. Then we randomly assigned the nestlings to one

of two treatments: fed or food-deprived. Using forceps, fed nestlings were immediately provided two fly larvae (approximate mass = 0.1 g each, Grubco, Inc., Hamilton, OH), a process that was repeated every 10 min for the next 60 min. The food-deprived nestling was handled in a similar way as the fed nestling but was given no food. We took photographs of both nestlings at 20-min intervals and analyzed these images as described above in experiment 1.

On a few occasions the fed nestling did not beg for food; in these cases, we assumed the nestling was satiated and did not force it to eat. All nestlings in the fed treatment consumed at least 1.0 g of food in the 60-min period. This rate of food consumption is similar to that observed in the field for much larger (6 d old) nestlings (E. D. Clotfelter et al. unpublished data). We are confident that nestlings in the fed treatment were satiated throughout the experimental period.

Experiment 3: Effect of Temperature

This experiment was conducted on 15 nestlings from five broods at 3 d of age from July 3–27, 2001. We followed a protocol similar to those of the previous experiments. We removed nestlings from their nests, transported them to the same light-sealed darkroom, and took photographs of mouth color four times (t_1 – t_4) during a 60-min period. Unlike the previous experiments, however, the cotton lined bowls were mounted on ring stands 8 cm above a laboratory warming plate. For the first two series of photographs (t_1 and t_2), the artificial ‘nest’ microenvironment was at room temperature ($22 \pm 1^\circ\text{C}$ during this experiment). After the second series of photographs at t_2 , we turned the warming plate on to its lowest setting where it remained for the duration of the experiment. Temperature, hereafter referred to as microenvironment temperature, was monitored with a soil thermometer placed on the cotton lining immediately adjacent to the nestling’s body. The warming plate increased microenvironment temperature to $36.6 \pm 0.8^\circ\text{C}$ by t_3 and to $39.1 \pm 0.5^\circ\text{C}$ by t_4 . Nestlings were returned to their true nests at the conclusion of the experiment. We analyzed mouth color from these photographs using the methods described above for experiment 1.

Ethical Note

The period of food deprivation we used in experiments 1 and 2 was based on published mouth color studies (Kilner 1997) and field observations of junco feeding behavior (Ketterson et al. 1992; Schoech et al. 1998; E. D. Clotfelter et al. unpublished data). We monitored nestlings throughout these experimental periods to ensure their welfare. The microenvironment temperature settings we used for experiment 3 were based on typical avian brooding temperatures (e.g. Choi & Bakken 1990; Evans 1992; Visser 1998). We conducted several pilot studies to determine the efficacy and safety of the warming protocol, with no harm done to any birds. Our experimental manipulations did not negatively affect the survival of nestling juncos. The proportion of nests used in our study (3 years

pooled) that succeeded in producing young (35%) was not significantly different from the success rate of the total population (25%; $\chi_1^2 = 1.69$, $p = 0.19$). Our work was conducted with the required federal permits, the permission of local landowners and under the supervision of the Institutional Animal Care and Use Committees of Indiana University (protocols 99-048 and 00-012) and Providence College (protocol 010423).

Statistical Analyses

We used SPSS 11.01 (SPSS, Inc., Chicago, IL) for our statistical analyses. Figures display $\bar{x} \pm SE$ and differences were considered significant at $p < 0.05$. Data were tested for normality and equality of variances. In experiment 1, nestlings from the same nest (large, small) were analyzed separately to avoid problems of non-independence. For experiments 1–3, we used repeated measures ANOVA to test for temporal changes in mouth color. In experiment 3, we used a nested ANOVA with ‘nest’ as the nesting factor to account for non-independence of birds from the same brood.

Results

Experiment 1: Effects of Nestling Body Size and Satiation

We found no relationship between body size and mouth color at time t_1 for either large (multiple linear regression; mass: partial- $t = 0.40$, $p = 0.69$; tarsus: $t = -0.42$, $p = 0.68$, $n = 20$) or small nestlings (mass: $t = -0.90$, $p = 0.38$; tarsus: $t = 1.1$, $p = 0.29$, $n = 20$). This analysis does not include six nestlings (three large, three small) that would not beg for the first photograph.

During the 60-min observation period, we brought nestlings into the darkroom (t_1 – t_4), the redness of their mouths became increasingly saturated (repeated measures ANOVA, large nestlings: $F_{3,42} = 5.11$, $p = 0.004$; small nestlings: $F_{3,48} = 4.42$, $p = 0.008$; Fig. 1). Several nestlings ($n = 6, 4, 4$, and 9 in t_1 – t_4 , respectively) could not be induced to beg at each interval, resulting in total sample sizes (large and small combined) of 40, 42, 42 and 35 nestlings at each interval. Note that mean redness saturation did not decrease after t_3 , despite the fact that we fed nestlings to satiation at this time.

Experiment 2: Effect of Food Deprivation

As in the first experiment, nestling mouth redness changed significantly between t_1 – t_4 ($F_{3,63} = 3.21$, $p = 0.029$; Fig. 2). The increase in saturation occurred regardless of whether the nestlings were fed, as indicated by the absence of a significant interaction between nestling feeding treatment and time ($F_{3,63} = 0.15$, $p = 0.93$). The observed power of the mouth color change over time was 71.4%, but was only 7.7% for the interaction between time and nestling feeding treatment.

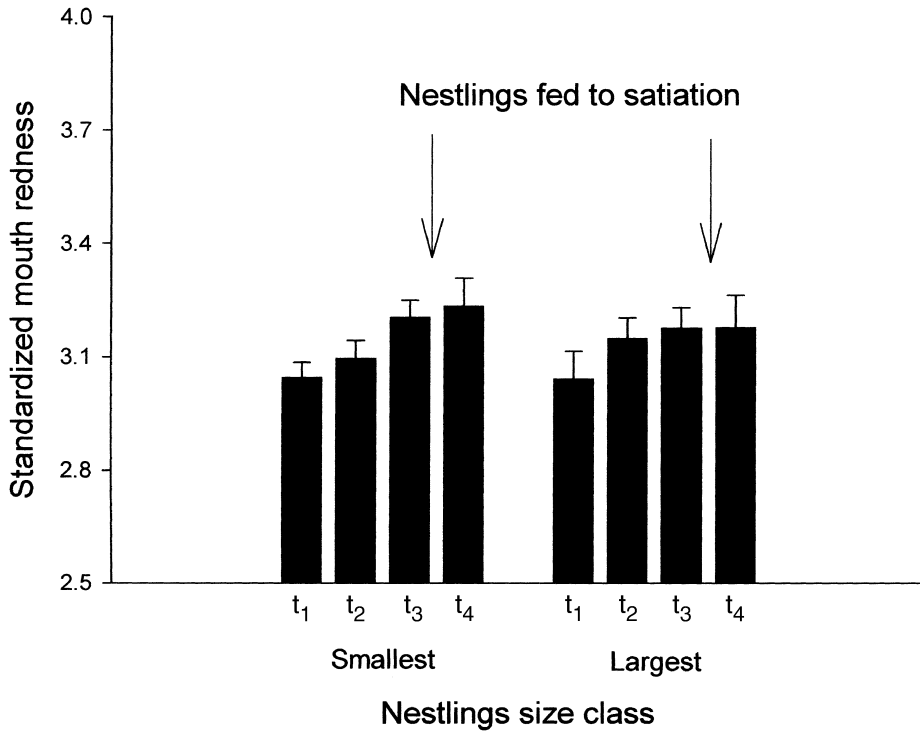


Fig. 1: Mouth redness of dark-eyed junco nestlings photographed at 20-min intervals over a 60-min period following removal from the nest ($\bar{x} \pm \text{SE}$). The smallest and largest nestlings, determined by mass and tarsus length relative to brood mates, are shown on the left and right side, respectively. The change in color over time was statistically significant for both size classes (small nestlings: $F_{3,48} = 4.42$, $p = 0.008$; large nestlings: $F_{3,42} = 5.11$, $p = 0.004$). Nestlings were deprived of food for the first 40 min and then fed to satiation with canned cat food (see arrow). Data are from 46 nestlings selected (see text) from 23 broods

Experiment 3: Effect of Temperature

The redness saturation of nestling mouths decreased significantly when microenvironment temperature increased following 40 min of exposure to room temperature (22°C; repeated measures nested ANOVA, $F_{3,21} = 4.09$, $p = 0.02$; Fig. 3). The nesting variable 'nest number' had no effect on changes in mouth redness as indicated by the non-significant interaction between nest number and mouth color at each interval ($F_{12,21} = 1.60$, $p = 0.17$, observed power = 62.3%). When we regressed mouth redness on microenvironment temperature, we found a significant negative relationship (regression coefficient = -0.0028 , $t = -2.06$, $p = 0.044$).

Discussion

Our first two experiments demonstrated that neither satiation (experiment 1) nor deprivation (experiment 2) significantly affected mouth color of dark-eyed

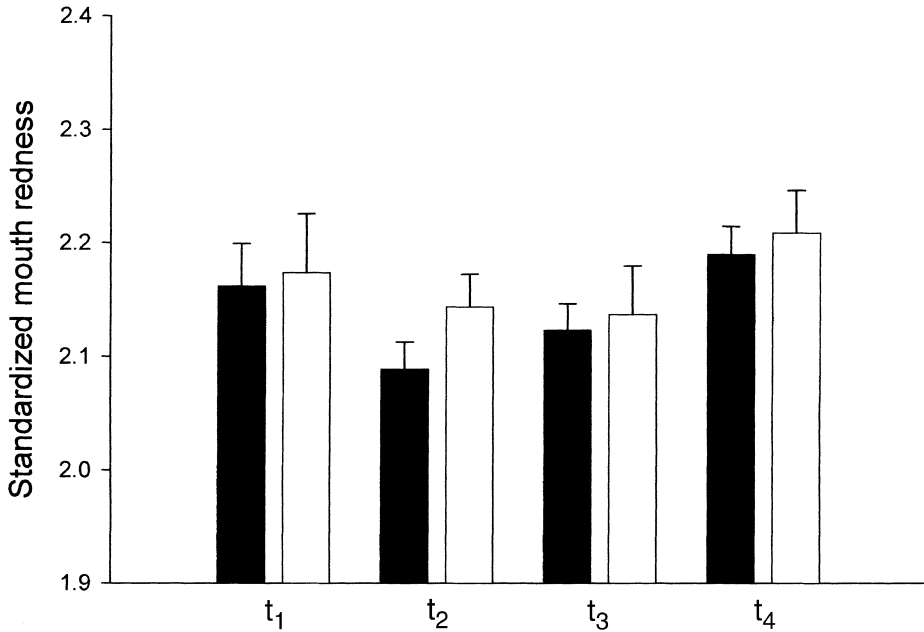


Fig. 2: Mouth redness of dark-eyed junco nestlings photographed at 20-min intervals over a 60-min period following removal from the nest ($\bar{x} \pm \text{SE}$). Half of these nestlings were deprived of food throughout the 60-min period (■), while the other half were fed (see text) during this period (□). Change in mouth color over time was significant ($F_{3,63} = 3.21$, $p = 0.029$), but there was no significant effect of food deprivation ($F_{3,63} = 0.15$, $p = 0.93$). These data are from 24 nestlings from 12 broods ($n = 12$ for each bar except fed nestlings at t_4 , where $n = 11$)

junco nestlings. In both experiments mouth color was a dynamic trait that increased in saturation over the course of our 60-min observation periods. This temporal variation prompted us to examine the importance of temperature, which led to experiment 3. In this experiment we found that mouth redness saturation decreased when microenvironment temperature increased.

Mouth Color and Nestling Hunger

Although there was no visible ‘flush’ as Kilner (1997) described in canaries, we found that mouth redness in nestling juncos increased significantly over a time period similar to that used in her studies. Also unlike canaries, changes in junco mouth color were unrelated to hunger. Discrepancies between the two studies may be due to differences in feeding ecology and mode of parent provisioning between canaries and juncos. Kilner & Davies (1998) suggested that mouth color is a signal of nestling hunger only in species that feed their young by regurgitation. In regurgitation feeding, parents fill their esophageal crops with food (e.g. seeds or carrion) and return to the nest to divide it among one or more nestlings. Regurgitation takes more time than delivering individual food items, which gives

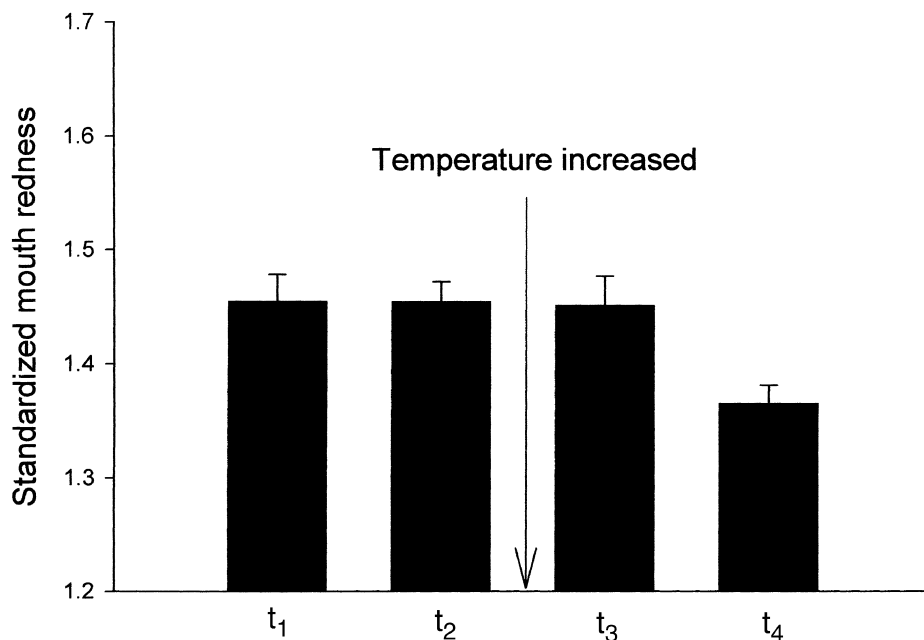


Fig. 3: Mouth redness of dark-eyed junco nestlings photographed at 20-min intervals over a 60-min period following their removal from the nest ($\bar{x} \pm \text{SE}$). Nestlings were allowed to cool toward the room temperature of 22°C in t₁ and t₂, then warmed to 36.6 ± 0.8°C by t₃ and to 39.1 ± 0.5°C by t₄ (see arrow). Change in mouth color over time was significant (nested ANOVA, $F_{3,21} = 4.09$, $p = 0.02$). Data are from 15 nestlings from five broods (nested ANOVA used to account for non-independence of nestlings)

parents an opportunity to identify the neediest (or most persistent) nestling. Therefore, selection may have favored the use of mouth color to signal need in nestlings of these species. Dark-eyed juncos do not feed their young by regurgitation, which makes our findings consistent with Kilner & Davies' (1998) predictions.

Mouth Color and Temperature

In experiments 1 and 2 we found that the longer nestlings were out of the nest the greater the redness saturation of their mouths. In experiment 3 we found that mouth redness decreased when we increased the temperature within artificial nests. Collectively these findings suggest that mouth color varies with nestling thermal state, and may function as a signal to solicit brooding by the female. Numerous studies of non-passerine birds and some of mammals have found evidence of vocal signals to induce brooding or warming (Allin & Banks 1970; Okon 1970; Conover & Miller 1981; Evans 1992, 1994). To our knowledge, our study is the first to demonstrate the possible use of a visual signal such as mouth color for this purpose. It is worth noting that mouth color did not change

significantly until microenvironment temperature reached 39°C, which raises the intriguing possibility that mouth color changes may signal heat stress instead of cold stress, and therefore function to solicit parental shading rather than brooding. Future studies should test this hypothesis by examining nestling mouth color over a wider range of temperatures.

Changing mouth color by increased perfusion of the mouth vasculature may be a less-costly way for nestlings to signal thermal stress than raising the head and vocalizing [see Bachman & Chappell (1998) and references therein for discussion of energy costs of nestling begging]. Altricial nestlings have been shown to beg less frequently under cold conditions, due in part to decreased muscular performance (Choi & Bakken 1990; Leonard & Horn 2001). For example, Choi & Bakken (1990) found that nestling red-winged blackbirds (*Agelaius phoeniceus*) maintained at low body temperatures suffered a 30–60% decrease in their ability to raise their heads. At the same temperatures, however, their ability to open their mouths was largely unaffected, meaning that they might still be able to signal thermal stress to their parents with changes in mouth color.

When homeotherms are cold they usually constrict their blood vessels to slow the rate of cooling, therefore it may appear paradoxical that nestling juncos increase blood flow to their mouths when temperature decreases. At this age, however, nestling juncos are poikilothermic (Dunn 1975). Poikilothermic, altricial nestlings are capable of minimal metabolic thermogenesis, and must huddle together to slow the rate of heat loss (O'Connor 1984; Visser 1998). When poikilotherms are cold and have access to an external heat source such as the sun or a brooding parent, they dilate their blood vessels and increase blood flow to carry heat from the periphery to the core. Just as nestling birds open their mouths in anticipation of being fed, they may also dilate their blood vessels in anticipation of being warmed.

The ontogeny of homeothermy and mouth color in dark-eyed junco nestlings provides indirect support for our hypothesis that mouth color indicates thermal state. Approximately 6–7 d after hatching, nestlings begin to regulate their body temperature, which coincides with a decrease in mouth color saturation. The developmental relationship between mouth color and homeothermy remains largely unexplored in other species, although many passerines achieve homeothermy at approximately the same developmental stage (Dunn 1975; O'Connor 1984).

Do changes in microenvironment temperature reflect changes in nestling body temperature (T_b)? Numerous studies of altricial neonates show that T_b of single nestlings in laboratory environments (similar design as used in the current study) are only slightly above ambient temperature (reviewed in Visser 1998). Simulation studies based on empirically measured thermal conductance values show that nestlings less than 10 g (average from our three experiments = 7.07 ± 0.16 g) cool from 40°C (brooding temperature) to 30°C (lower range of physiological tolerance) in as little as 10 min (Visser 1998). Therefore, manipulations of microenvironment temperature such as we performed in experiment 3 should cause rapid, correlated changes in nestling T_b .

In summary, we found that mouth color of nestling dark-eyed juncos changed over time and in response to changes in their thermal environment. We found no evidence that mouth color was related to hunger in this species. The proximate and ultimate causes of nestling mouth color are far from resolved, however, in this or any other species. Future studies should examine the relationship between mode of parental provisioning and mouth color, as well as the role of the spectral environment of the nest (Endler 1993). Additional experiments that monitor parental responses to mouth color manipulation (Götmark & Ahlström 1997; Saino et al. 2000) would also be informative. Finally, mouth color dynamics should be examined across a greater range of temperatures to better understand the function of this visual signal.

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