Handling-stress initially inhibits, but then potentiates yawning in budgerigars (*Melopsittacus undulatus*)[†]

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Abstract

In mammals, yawning is associated with social and physiological stress, as well as thermoregulation, but little is known about why yawning occurs in stressful contexts or how it is integrated with natural stressors. To investigate the stress-sensitivity of yawning in birds, budgerigars (*Melopsittacus undulatus*) were exposed to a handling stressor that simulated a predatory encounter. Each bird was captured, gently held for 4 minutes, and then released and videotaped for 1 hour (experimental). On a separate day (± 24 hours), the undisturbed animal was videotaped for 1 hour (control). The relationship between handling-induced yawning and body temperature was assessed in a separate experiment, in which the under-wing temperatures of the same birds were measured at 1-minute intervals during a 4-minute holding period. After handling-stress, yawning frequency was initially suppressed, then sharply increased within 20-minutes. Under-wing temperature increased during handling, and individuals' final temperatures at minute 4 were negatively correlated with their latencies to yawn after handling. Thus, stress-induced hyperthermia may be responsible for associations between yawns and stress. These results indicate that yawning may offer a sensitive, non-invasive measure of stress in birds.

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1   Yawning is phylogenetically old and ubiquitous among vertebrates (Baenninger 1987),

2   but little is known about its physiological function. It is commonly thought to equilibrate

3   oxygen and carbon dioxide imbalances in the blood, but there is no experimental support for

4   this hypothesis (Provine et al. 1987b). On the other hand, there is strong evidence to suggest

5   that yawning is associated with social (Baenninger 1997) and physiological stress (Gallup and

6   Gallup 2008), including thermal challenges in birds (Gallup et al. 2009). In line with this,

7   yawning may increase brain arousal, and this arousing function may explain the behaviour's

8   association with stress (Baenninger 1997, but see Guggisberg et al. 2007).

9   Yawning, or behavior resembling yawning, is associated with stressful events in several

10   non-human primates, as well as other mammals. In crested black macaques (*Macaca nigra*),

11   yawning occurs during intense agonistic interactions and other hostile social situations

12   (Hadidian 1980). Troisi et al. (1990) interpret yawning by subordinates in this context as a

13   response to stress, and those by dominants as a threat display. In these macaques, yawning

14   also follows abrupt, startling disturbances, such as thunder, that may induce low-level, acute

15   stress (Hadidian 1980). Likewise, in grey-cheeked mangabeys (*Cercocebus albigena*), yawning

16   occurs in close temporal proximity to alarm calling in the presence of predators (Deputte 1994).

17   Among primates, similarities between threat displays and yawning confounds interpretations of

18   yawning (Vick & Paukner 2010), making it informative to study yawning in species without

19   open-mouth threat displays. Yawning is also associated with physical stress in laboratory rats

20   (*Rattus norvegicus*) in that foot-shock strongly increases yawning (Moyaho & Valencia 2002).

21   Taken together, there appears to be a close relationship between stress and yawning in a range

22   of mammals.

23   In budgerigars (*Melopsittacus undulatus*), the only birds in which yawning has been

24   experimentally studied, yawning occurs more frequently as ambient temperature increases (22

25   – 34°C) towards body temperature (Gallup et al. 2009). In addition, yawning in budgerigars is

26   significantly correlated with other avian thermoregulatory behaviours (e.g., panting, wing

27   venting) (Gallup et al., in press), suggesting that yawning is triggered by the need to decrease

28   elevated body and/or brain temperatures. If temperature regulation is one general function of

29   yawning in homeotherms, stress-associated yawning may be a response to increases in body

30 temperature induced by external stressors (i.e., stress-induced hyperthermia, see Olivier et al.

31 2003 for review). For instance, when common eider ducks (*Somateria mollisima*) are handled,

32 their body and skin temperatures increase within four minutes following the start of the trial

33 (Cabanac & Guillemette 2001). Stress-induced hyperthermia may produce the intimate

34 relationship between stress, yawning and thermoregulation.

35   Although yawning is linked with stress across diverse contexts in mammalian species, no

36 experimental studies in birds have evaluated the effect of stress on yawning. Because yawning

37 is an overt, distinguishable behaviour, characterizing its relation to stress may make it a suitable

38 behavioural measure of stress. In this study, the stress-yawn relationship was investigated in

39 budgerigars by inducing acute stress through handling. The degree of handling—i.e., gentle

40 restraint in a gloved hand following a quick capture—was comparable to that experienced by

41 laboratory animals during routine measurements, and was similar to that used in wild birds

42 (e.g., Cabanac & Guillemette 2001; Carere & van Oers 2004; Fucikova et al. 2009). Each bird's

43 yawning and stretching frequencies were recorded both following brief handling (experimental)

44 and during a similar period with no preceding disturbance (control).   It was hypothesized that

45 yawning would increase in frequency after the handling session relative to the control period,

46 and also show no temporal pattern during the control period. Because of the temporal

47 association between yawning and stretching among humans and rodents (reviewed by

48 Baenninger 1997), stretching was also recorded. Yawning and stretching in budgerigars are

49 temporally associated in non-experimental settings (unpublished data). Interestingly, this

50 temporal relationship is de-coupled as temperatures increase, presumably when yawns serve a

51 thermoregulatory function that stretching does not (Gallup et al., in press). Thus, if stress-

52 induced yawning is specifically related to body temperature and brain arousal, stretching

53 should remain unaffected after a simulated predatory encounter.

54   **Methods**

55 *Study Animals*

56   Budgerigars in this study included six males and four females (*n* = 10) from a research

57 population bred and housed at Binghamton University (Binghamton, NY, U.S.A.). During these

58 experiments, birds were housed as a flock in an indoor aviary (1.8 × 1.8 × 1.8 m), with a

3

59 constant room temperature of 23°C and light:dark cycle of 12:12 hr (time on: 0700 hr, time off:

60 1900 hr). The flock has been continuously housed there for over 1 year and was fully acclimated

61 to their living conditions.

62 *Experimental procedure*

63   Each bird was caught with a small net from the aviary 24 hours before experimentation.

64 The same capture method was used whenever birds were inspected, weighed or moved, and

65 although they responded with flight, they were accustomed to this procedure. Following

66 capture, birds were placed alone in a wire-mesh testing cage (0.4 × 0.3 × 0.3 m) in the same

67 room, but visually occluded from the home aviary. Food and water were available *ad libitum* as

68 in the aviary. The next day, a trained researcher entered the testing room, turned on the

69 camera, and then left for one hour, allowing the bird to acclimate to the researcher's entrance

70 (acclimation period). Following acclimation, birds were video-recorded for 60 minutes in one of

71 the two conditions: (a) an experimental condition that included brief handling and (b) a control

72 condition that included no disturbance. Since yawning is affected by time of day (unpublished

73 data), all recordings took place between 1300 and 1500 hours, using a Panasonic digital

74 camcorder (PV-GS150) with remote control. The experimental condition began when a trained

75 researcher used a small net to capture the bird (~30 seconds), and then restrained it gently in a

76 gloved hand for four minutes. The bird was then immediately returned to the testing cage and

77 the recording session began. For the control condition, no one entered the room, the bird was

78 not handled prior to taping and the camera was remotely activated. Each bird experienced

79 both conditions within 48 hours and trials were counterbalanced with respect to condition

80 order (1 trial per bird per day). This experimental protocol was approved by the Institutional

81 Animal Care and Use Committee at Binghamton University (Protocol #656-09), and in accord

82 with the ASAB/ABS Guidelines for the Use of Animals in Research, unnecessary stress was

83 minimized.

84 *Measuring body temperature during stressor*

85   To assess the stress-response associated with handling, under-wing skin temperatures

86 were measured during a separate handling session. Temperature data were collected

87 approximately two months after the initial handling experiment, at the same time of day, to

88   reduce the number of metrics during each experiment. Temperature data were collected on

89   the same birds as used in the behavioural experiment, except that two had died of natural

90   causes between experiments (final n = 8).

91   The procedure was identical to the earlier experiment, except that temperature was

92   recorded and birds were not videotaped following the handling session. Birds were caught and

93   then isolated for 24 hours. The next day, birds were handled for four minutes using the same

94   methods described above. While in hand, an Oakton Acorn® Temp 5 Thermistor (Oakton

95   Instruments, Vernon Hills, IL; accuracy ± 0.2°C) was placed against the bird's body, underneath

96   the wing. Skin temperatures were recorded at 1-minute intervals to the nearest 0.1°C. In order

97   to reduce the time needed for the thermistor to reach budgerigar resting temperature for the

98   first reading, the thermistor probe was bathed in warm water (~104°F) prior to the start of the

99   experiment.

100   *Analyses*

101   A researcher who was blind to the trial condition scored the video recordings. The

102   frequencies and times of both yawns and stretches were recorded for each bird. Yawning was

103   recognized as a wide opening of the beak and slight closing of the eyes, followed by a brief

104   pause (the acme state) with stretching of the neck. Stretching consisted of an initial posterior-

105   dorsal or posterior-lateral extension of at least one leg, followed by further posterior extension

106   of one or both wings. Since pilot data indicated that yawning frequency varied with time after

107   handling (unpublished data), the temporal distribution of each behaviour was described as the

108   number of total yawns or stretches occurring in each of three successive 20-minute time

109   intervals.

110   The differences between yawning and stretching frequencies across both trial

111   conditions were initially tested using a three-way analysis of variance (ANOVA) including as

112   within-subject factors, trial condition and time interval, and as between-subject factors, trial

113   order. All interactions were included in the initial model, but factors were removed from

114   subsequent models when their main effects and interactions were not significant ($p > 0.05$).

115   Differences in the latency to the first yawn or first stretch were assessed with a two-way

116   ANOVA (within subjects: trial condition and between subjects: trial order). Bonferroni

117   corrections were used to compare main effects. Where there was a significant interaction

118   between time interval and trial condition, only comparisons (paired *t*-test) between two

119   conditions at a single time interval were reported. A Friedman's Test was used to compare

120   body temperatures across the four 1-minute intervals and Wilcoxon Signed-Ranks Tests were

121   performed for pair-wise comparisons.   Correlations between yawning, or stretching, and body

122   temperature during the experimental condition were analyzed using Kendall's tau correlations.

123   Parameters were reported as means ± SEM, statistical tests were performed with α = 0.05, and

124   for ANOVAs, partial $ή^2$ were also reported.

125   **Results**

126   *Yawning and stretching*

127   In total, 63 yawns (42 by the six males, 21 by the four females) were observed during 20

128   hours of observation (10 trials, 2 hours/bird). To answer the question whether there were

129   differences in the latency to yawn between control and experimental conditions, the model

130   included trial condition, trial order and the interaction between these two factors. For this full

131   model, there was no difference in yawning latency between trials categorized by order ($F_{1,8}$ =

132   0.62, *p* = 0.45, partial $ή^2$ = 0.07) and no interaction between trial condition and trial order ($F_{1,8}$ =

133   1.50, *p* = 0.26, partial $ή^2$ = 0.16). After removing trial order from the model, the latency to the

134   first yawn was significantly later in the experimental condition than in the control condition

135   (1322 ± 144 versus 787 ± 189 seconds; $F_{1,9}$ = 5.96, *p* = 0.04, partial $ή^2$ = 0.40, Figure 1A).

136   Of the 63 total yawns, 34 yawns (3.4 ± 0.54 per bird) occurred during the experimental

137   condition and 29 yawns (2.9 ± 0.31 per bird) occurred during the control condition. To

138   investigate whether there were differences in yawning frequencies between trial conditions

139   and across time intervals, the model included trial condition, time interval, trial order and all

140   interactions between these factors. In the full model, there was no difference in total yawning

141   frequencies between the two trial conditions ($F_{1,8}$ = 1.02, *p* = 0.34, partial $ή^2$ = 0.11) or between

142   the three 20-minute time intervals ($F_{2,16}$ = 0.65, *p* = 0.54, partial $ή^2$ = 0.08). There was also no

143   difference in yawning frequencies between trial orders ($F_{1,8}$ = 0.02, *p* = 0.90, partial $ή^2$ < 0.01).

144   There was also no interaction between trial order and condition ($F_{1,8}$ = 2.00, *p* = 0.20, partial $ή^2$

145   = 0.20), between trial order and time interval ($F_{2,16}$ = 0.42, *p* = 0.67, partial $ή^2$ = 0.05), or

6

146   between trial order, time interval and trial condition ($F_{2,16} = 0.64$, $p = 0.54$, partial $\acute{\eta}^2 = 0.07$).

147   After removing trial order from the model, there was a significant interaction between time

148   interval and trial condition ($F_{2,18} = 3.88$, $p = 0.040$, partial $\acute{\eta}^2 = 0.30$, Figure 1B). Paired

149   comparisons between the trial conditions within each time interval indicated that yawning

150   frequency was (1) lower in the experimental than control during the first 20-minutes ($t_9 = 2.23$,

151    $p = 0.05$), (2) greater in the experimental than control during the second 20-minute interval ($t_9 =$

152   -2.45, $p = 0.04$) and (3) not different during the final 20-minute interval ($t_9 = -1.05$, $p = 0.32$).

153   A total of 69 stretches (41 by males, 28 by females) were observed during the 20 hours

154   of observation. The same set of analyses was run to investigate whether the latency to stretch

155   differed between control and experimental conditions. Unlike yawning, there was no

156   difference in latencies to the first stretch between the experimental and control conditions

157   (1913 ± 307 versus 1931 ± 403 seconds; $F_{1,8} = 0.00$, $p = 0.97$, partial $\acute{\eta}^2 = 0.00$). In addition,

158   there was no difference in stretching latency between trial orders ($F_{1,8} = 0.31$, $p = 0.59$, partial

159    $\acute{\eta}^2 = 0.04$), and there was no interaction between trial condition and trial order ($F_{1,8} = 1.34$, $p =$

160   0.28, partial $\acute{\eta}^2 = 0.14$).

161   To investigate whether there were was a difference in stretching frequencies between

162   conditions and across intervals, the full model included trial condition, time interval, trial order

163   and interactions between these factors. For this model, stretching frequencies did not differ

164   between trial conditions ($F_{1,8} = 0.03$, $p = 0.86$, partial $\acute{\eta}^2 < 0.01$) or trial orders ($F_{1,8} = 0.79$, p =

165   0.40, partial $\acute{\eta}^2 = .09$). There was no interaction between trial order and condition ($F_{1,8} = 0.17$, p

166   = 0.69, partial $\acute{\eta}^2 = 0.02$), or between trial order and time interval ($F_{2,16} = 0.45$, $p = 0.64$, partial

167    $\acute{\eta}^2 = 0.05$). Unlike yawning, there was no significant interaction between time interval and trial

168   condition ($F_{2,16} = 2.05$, $p = 0.16$, partial $\acute{\eta}^2 = 0.20$). There was also no interaction between trial

169   condition, time interval and trial order ($F_{2,16} = 0.35$, $p = 0.71$, partial $\acute{\eta}^2 = 0.04$). When removing

170   trial order from the model, stretching differed significantly across the three time-intervals ($F_{2,18}$

171   = 4.31, $p = 0.030$, partial $\acute{\eta}^2 = 0.32$). Post-hoc corrections showed no significant pair-wise

172   comparisons (all p's > 0.05).

173   *Temperature changes with handling*

174   During the temperature assessment session, budgerigar body temperature steadily

175 increased during the handling session (Figure 2A). Average temperature differed across the

176 four-minute intervals (Friedman's test: χ_3^2 = 18.04, p < 0.01). All pair-wise comparisons

177 between time intervals showed a significant increase from one interval to the next (p's < 0.05),

178 except between the second and third minute, which is nearly significant (p = 0.06). A

179 budgerigar's previously recorded behaviour (yawns or stretches) was then correlated with this

180 individual's under-wing temperature. Under-wing temperatures at the end of the handling

181 sessions were strongly and negatively correlated with the latency to first yawn (Kendall's tau

182 correlation: b = - 0.62, p = 0.03, Figure 2B). This indicates that birds with higher body

183 temperatures following handling yawned sooner during the experimental condition. Under-

184 wing temperatures at the fourth minute were not correlated with an individual's total yawn

185 frequency (b = 0.15, p = 0.61) or with the number of yawns during any one of the three 20-

186 minute intervals (all p's > 0.05). Increases in temperature—i.e., difference between the final

187 and first minute—were not correlated with (1) yawn latency (b = -0.18, p = 0.53), (2) total

188 yawning frequencies (b = 0.42, p = 0.16) or (3) yawning frequencies across each 20-minute

189 interval (all p's > 0.05). Unlike yawning, stretching by individuals was not correlated with either

190 temperature at the fourth minute or change in temperature (p's > 0.05).

191   **Discussion**

192 These results illustrate that yawning in budgerigars is affected by handling stress. Yawns were

193 initially suppressed, but then increased in frequency after 20 minutes. As handling may

194 simulate escape from a predator, initially suppressing yawns may adaptively reduce attention-

195 getting movements and/or reduce conflict with other anti-predatory behaviours. Because

196 acute stress increases body temperature (e.g., Cabanac & Guillemette 2001), a spike in yawning

197 after 20 minutes is adaptive, since research suggests yawning is a thermal stabilizing

198 mechanism that decreases brain and/or body temperature (e.g., Gallup & Gallup 2007, in press;

199 Gallup et al. 2009). This interpretation is supported by the strong negative correlation between

200 the individuals' body temperature after handling and their latencies to first yawn (see figure

201 2B), indicating that higher body temperatures may trigger birds to yawn sooner. In contrast to

202 yawning, stretching did not change in frequency after the stressor. Stretching frequencies were

203   also unrelated to the individuals' body temperatures, suggesting that stretching lacks a

204   thermoregulatory role (Gallup et al. 2009).

205   These results are consistent with previous findings in other species that demonstrate a

206   temporal association between yawning and stress. For instance, in South African ostriches

207   *(Struthio camelus australis)*, yawning did not occur during intense activity, but did occur when

208   startling stimuli were recognized as innocuous, presumably sometime after the stressor (Sauer

209   & Sauer 1967).   When rats were exposed to a novel environment, yawning gradually increased,

210   peaking after 30 minutes (Moyaho & Valencia 2002).   Similarly, when foot shocked at fixed, 10-

211   minute intervals, yawning was initially low, but then gradually increased and peaked by 40

212   minutes. On the other hand, increases in yawning were less pronounced when rats were foot-

213   shocked at random intervals (Moyaho & Valencia 2002). This is consistent with the budgerigar

214   data, because it shows that yawning occurs during a recovery period following a stressor: when

215   foot-shocked at known intervals, rat yawning dramatically increases, but when randomly foot-

216   shocked, yawning does not increase as dramatically, presumably because the stress-state

217   persists. These data suggest that yawning is related to the recovery period following a stressor

218   and may be an adaptive response that increases vigilance as the environment becomes more

219   predictable (refer to Greco et al. 1993).

220   The appearance of yawns during the second 20-minute interval is in accord with the

221   view that yawning is a thermoregulatory behaviour in budgerigars (Gallup et al. 2009, in press).

222   The increased yawns observed during the second 20-minute interval may be explained by

223   temperature increases that follow handling stress (Olivier et al. 2003). Similar to the effect of

224   handling on eider ducks (Cabanac & Guillemette 2001), handling increased budgerigar under-

225   wing temperature. This increase in temperature was substantial and rapid, approximating 2°C

226   within three to four minutes. Cabanac and Guillemette (2001) demonstrated that duck

227   temperature peaked by 10 minutes of handling, and hyperthermia was maintained for at least

228   30 minutes. Therefore, if the time course of body temperature is similar in budgerigars, the

229   spike in yawning during the second interval may have been a compensatory mechanism to

230   reduce brain and/or body temperatures following the simulated capture and escape.

231   Moreover, latency to yawn was negatively correlated with skin temperature measured at the

232   fourth minute. This indicates that birds who responded to stress with greater temperature

233   increases needed to yawn sooner, but not at higher frequencies. In short, increases in

234   metabolic activity following stress inevitably cause increases in body temperature. Whether or

235   not the temperature increase is adaptive or a metabolic byproduct is unclear, however yawning

236   may provide a means to regain thermal homeostasis after a stressful event.

237   Since yawning is an easily distinguishable behaviour, these results suggest that

238   measuring yawns may provide a suitable method to detect and qualitatively measure stress

239   non-invasively. It is difficult to measure stress without disturbing an animal, making accurate

240   assessment of stress difficult. For instance, in laboratory settings, collecting blood to measure

241   corticosterone (CORT) levels inherently produces an emotional response, thereby affecting

242   plasma concentrations of stress hormones, such as CORT (Thanos et al. 2008). To appreciate

243   the application of yawning as a technique to measure stress, it is important to note the

244   sensitivity of this relationship. Although the birds used in this experiment were accustomed to

245   daily human contact over a period of many years, the flock continues to respond to human

246   entry with increased movement and vocalization (personal observation). In a pilot study,

247   entering the room to turn on a camera was sufficient to inhibit yawns during the first 20

248   minutes of the control condition (unpublished data), which is why recordings were remotely

249   started in the control trials. This is not unreasonable, as the heart rates of laboratory mice

250   increase when a technician enters the colony and this effect persists for at least two weeks

251   after the first exposure (Kramer et al. 2004). Monitoring yawns may provide a sensitive

252   measure of individual responsiveness to acute stressors.

253   In summary, these results illustrate a relationship between yawning, stress and

254   thermoregulation in birds. This report provides critical insight into the association between

255   yawning and arousal. It is the first to show that yawning is delayed after a simulated predator's

256   attack and also replicates previous studies, showing that yawns are strongly associated with

257   changing body temperature. These findings also suggest that yawning may provide a non-

258   invasive measurement of stress in field and laboratory settings. Follow up studies should

259   measure other physiological parameters related to stress (e.g., plasma-CORT), and then

260   correlate these with yawning.

Acknowledgments

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Figures and figure captions

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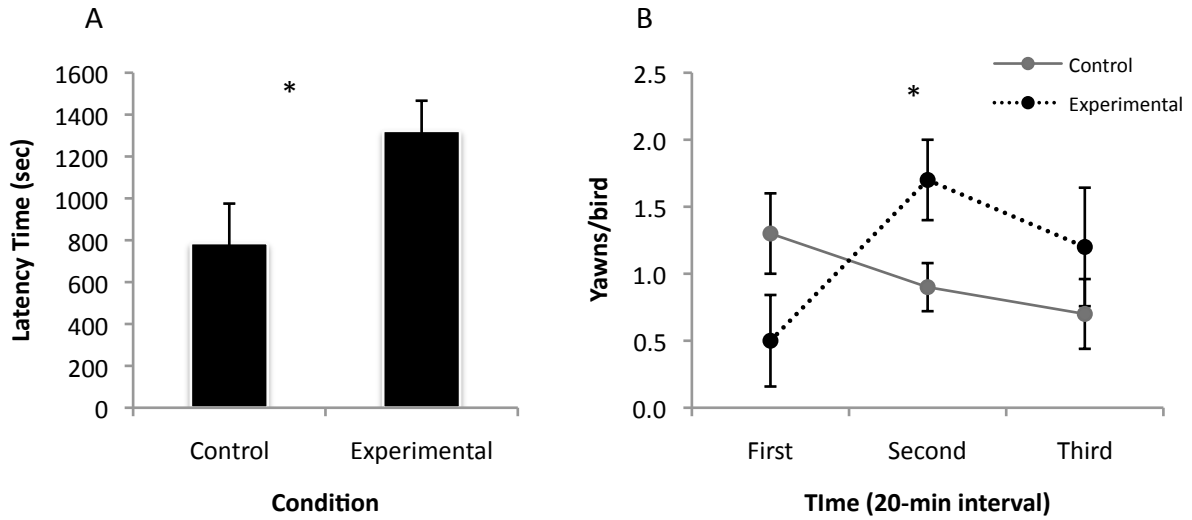
Figure 1. Mean (± SEM) effects of brief handling on yawning frequency. **(A)** The latency period
was the time between the first yawn and the experiment's start (measured in sec). **(B)** This
graph shows the time course of yawning across the two conditions. The yawning frequencies
for each bird were binned into 20-minute intervals, and at each time-interval, yawns were
compared between conditions. **(A and B)** * $p < 0.05$ for control *versus* experimental.

336

337
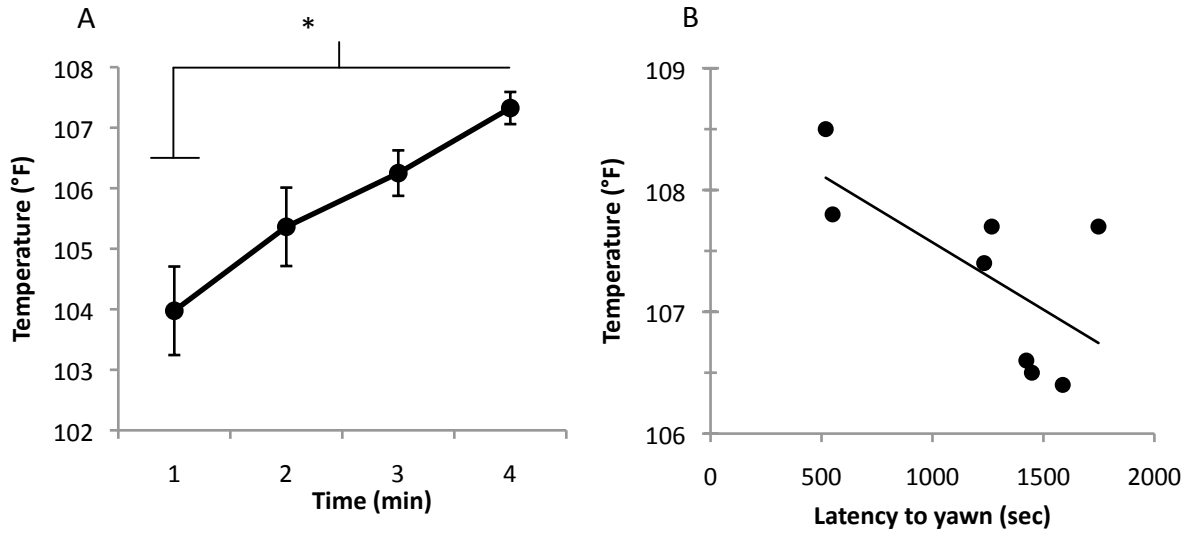
Figure 2. Mean (± SEM) physiological effects of brief handling on under wing temperature. **(A)**
Shown here are the birds' under wing temperatures during the 4-minute handling session. To
reduce the number of simultaneous metrics, measurements were taken during a separate
handling session (* $p < 0.05$ compared to temperature at the first minute). **(B)** Each bird's
latency to yawn during the experimental condition was correlated with its final temperature.