CIRCULATING PROLACTIN OF INCUBATING MALE WILSON'S PHALAROPES CORRESPONDS TO CLUTCH SIZE AND ENVIRONMENTAL STRESS[1]

DAVID J. DELEHANTY,[2,3] LEWIS W. ORING[2] AND ALBERT J. FIVIZZANI
Department of Biology, University of North Dakota, Grand Forks, ND 58202

MOHAMED E. EL HALAWANI
Department of Animal Science, University of Minnesota, St. Paul, MN 55108

Abstract. We measured the effect of clutch-size changes on levels of circulating prolactin (prl) among incubating male Wilson's Phalaropes in order to identify the influence of clutch-size as an exogenous determinant of circulating prl, and to better understand the endocrinology of clutch abandonment. One year of the study was characterized by unusually high temperatures and drought, and incubating males appeared to be heavily stressed based on ambient temperatures and rates of weight loss. We used the contrasting environmental conditions between years to compare levels of circulating prl among males experiencing different levels of environmental stress. We found a strong inter-year effect, with significantly lower prl during the high stress year. We also found a clear effect of clutch-size on level of circulating prl during the low stress year of the study. Males with increased or decreased clutch-size had lower levels of circulating prl than did control males. Because prl levels were so variable, we sought alternative explanations to year and clutch-size effects. We examined nine environmental and physiological factors *a posteriori* for a relationship with circulating prl. Although we identified weak correlates, none of the additional factors could account for the variation in prl explained by year and clutch-size. Incubating male Wilson's Phalaropes appear to experience an adaptive endocrine change when the potential fitness of their clutch is compromised.

Key words: Wilson's Phalarope, prolactin, stress, clutch-size, incubation, nest abandonment.

INTRODUCTION

Wilson's Phalarope (*Phalaropus tricolor*) is a small, sex-role reversed shorebird that breeds on the northern prairies of North America. Females lay a determinate clutch of four eggs. Males provide all incubation and parental care (Höhn 1969, Colwell and Oring 1988). Incubating male Wilson's Phalaropes exhibit a strong behavioral response to clutch-size changes (Delehanty and Oring 1993). Males abandon incubation with increasing frequency as clutch-size is reduced; they allow remaining eggs to perish. Males are especially sensitive to clutch reductions that occur early during the incubation cycle. If clutches are experimentally supplemented, males also abandon incubation, often very quickly. One can readily envision how ceasing to incubate is adaptive when clutch-size declines or nest parasitism occurs. For example, by engaging in incubation males likely incur costs such as increased risk of predation, physiological stress, and reduced opportunity to pursue alternative reproductive strategies. If so, then incubation becomes relatively more costly as the potential fitness benefit of engaging in incubation declines with declining clutch-size. There is likely a lower limit to clutch-size below which the costs of incubation exceed the potential fitness benefits. Below this threshold, males would benefit by abandoning incubation. Similarly, if incremental increases in clutch-size substantially increase the cost of incubation relative to potential fitness benefits, then a sufficiently large clutch will lead to incubation costs that exceed potential benefits. Again, males would benefit by abandoning incubation. But, because incubation is facilitated by strong endocrine cues (Goldsmith 1983), a rapid behavioral shift from incubating eggs to abandoning them must mean either that males are able to override hormonal mediators, or that they experience an adaptive endocrine change.

Prolactin (prl) has been widely implicated in the initiation and maintenance of avian incubation behavior (Goldsmith 1983, 1990, Buntin 1986, Lea 1987). Among sex-role reversed shorebirds, prl levels and male incubation appear to be tightly linked. Elevated levels of circulating prl are correlated with persistent incubation in male Wilson's (Oring et al. 1988) and male Red-necked (*P. lobatus*) Phalaropes (Gratto-Trevor et al. 1990), both species with exclusive male incubation. In Wilson's Phalarope, prl rises with the onset of incubation, remains elevated through the incubation period, and declines during brooding (Oring et al. 1988). In Spotted Sandpipers (*Actitis macularia*), where males provide most incubation, both sexes experience elevated prl during incubation but levels in males exceed those of females (Oring et al. 1986a). Males and females share incubation in the monogamous Semipalmated Sandpiper (*Calidris pusilla*). Here, both sexes have elevated prl during incubation, and levels are similar between sexes (Gratto-Trevor et al. 1990).

Many internal and external factors might affect prl levels of incubating male phalaropes. For example, prevailing environmental conditions, body condition, circadian or annual rhythms (Meier et al. 1969, 1971), steroid profiles, and handling stress (El Halawani et al. 1985) could directly or indirectly affect prl levels. Gratto-Trevor et al. (1990) observed annual differences in the relative level of circulating prl during early-incubation in Red-necked Phalaropes and suggested that low prl levels in one year might have been due to unusually cold and wet weather, causing the phalaropes to forage more and, thus, incubate less. Prolactin among incubating male Wilson's Phalaropes is at least partially influenced by external stimuli (Oring et al. 1988). Elevated prl levels persist in males with experimentally lengthened incubation periods and terminate early in males with experimentally shortened incubation periods. Oring et al. (1988) hypothesized that eggs in the nest cup provide important stimuli for the maintenance of elevated prl among incubating males.

Our primary objective was to test the effect of clutch-size on circulating prl by comparing prl levels in male Wilson's Phalaropes incubating normal, four-egg clutches to levels in males incubating clutches with altered sizes. Because clutch-size changes are associated with clutch abandonment, this test also yields insight into the endocrinology of clutch abandonment. Reduced prl levels among males with altered clutch-sizes would suggest that males undergo an adaptive endocrine response to clutch-size changes.

Fortuitously, one breeding season during our study was characterized by drought and exceptionally high temperatures. This allowed us to make an inter-year comparison of circulating prl among males experiencing very different environmental conditions. Finally, because circulating levels of prl in incubating males proved to be quite variable, we tested a suite of additional factors *a posteriori* for any association with prl among incubating males. Our purpose was to use alternate explanations to rigorously challenge any association of prl with clutch-size or year. We examined nine factors intended to estimate body mass and size, physiological condition, environmental stress, handling stress, and daily and seasonal rhythms experienced by incubating males.

METHODS

We studied phalaropes during 1988 and 1989 at two sites in the northern portion of Wilson's Phalarope breeding range, the Shields site (51° 27′ N, 105° 10′ W) and the Wreford site (51° 24′ N, 105° 02′ W). The sites, 14 km apart, are near the headwaters of Last Mountain Lake in the mixed-grass prairie of southcentral Saskatchewan, Canada. Both sites consisted of secluded, grazed pasture interspersed with shallow wetlands. We performed fieldwork from early May through early July, spanning the period that phalaropes were initiating nests and incubating eggs. We worked exclusively on the Shields site during 1988. Several wetlands of the Shield's site were dry in the spring of 1989, thus, in late May of 1989, most work was shifted to the Wreford site. We located phalarope nests by watching breeding birds from 3 m and 4.5 m towers and by walking through nesting cover and flushing phalaropes from nests.

CLUTCH-SIZE MANIPULATIONS

Delehanty and Oring (1993) provide a detailed description of clutch-size manipulations. Briefly, we reduced normal, four-egg clutches by one or two eggs, usually within two days of clutch completion. We also observed natural egg attrition in clutches, something that we suspect was caused by mice removing eggs. If the nest and

clutch were free of any shell, albumin, or yolk, and the male persisted to incubate, then we included the male in our sample of males with reduced clutches. Of the 17 males incubating clutches of reduced size, 5 males were incubating two-egg clutches and 12 were incubating three-egg clutches. We supplemented four-egg clutches by adding two more eggs. Six-egg clutches simulated an extreme level of nest parasitism for Wilson's phalaropes, and males tended to abandon these clutches quickly. We confirmed that abandonment of reduced and supplemented clutches was due to clutch-size per se, rather than nest disturbance or presence of foreign eggs. We did this by switching two eggs from some four-egg clutches with two eggs from other four-egg clutches. The behavior and nest success of males incubating these four-egg "cross-clutches" did not differ from that of males incubating unmanipulated four-egg clutches (Delehanty and Oring 1993). Also, prl levels of cross-clutch males were indistinguishable from those of males incubating unmanipulated four-egg clutches. Males with reduced or supplemented clutches were not included in our analysis if they abandoned incubation within one day of incurring the clutch-size change.

During both years, we located many nests after clutch completion. We estimated stage of incubation for those clutches that eventually produced chicks using the mean incubation period of nests monitored from egg-laying to hatch during a given year. Males with four-egg clutches from nests for which incubation stage was estimated were included in analyses as full-clutch controls.

To examine abandonment of supplemented clutches more closely, during the second half of the 1989 breeding season, nests found with their clutches already complete were randomly assigned to a second clutch-supplementing experiment, the "mid-incubation" experiment. Ten clutches were increased to six eggs in mid-incubation by adding two eggs for at least two days, until the incubating male was captured. Nine nests were designated as controls and these clutches were left unaltered.

MEASURING PROLACTIN AND NINE POTENTIAL CORRELATES

We captured each male at the nest one to three times during incubation using walk-in traps or mist nets. When possible, we captured each

male once during early-incubation (day 4 through day 7), once during mid-incubation (day 8 through day 17), and once during late-incubation (day 18 through hatch).

Approximately 0.7 ml of peripheral blood was collected into heparinized microhematocrit tubes. To minimize the effect of handling on circulating prl (El Halawani et al. 1985), blood from all but one phalarope was collected within 10 min of capture ($\bar{x} \pm$ SD = 7.5 $\pm$ 2.8 min, minimum = 2 min, max. = 19, n = 165). Blood was immediately placed on ice and centrifuged within several hours. Plasma was drawn off and frozen until assayed. We took several morphometric measures of each male after collecting the blood sample. Males were then immediately released within 100 m of their nest.

Prolactin was measured using the turkey radioimmunoassay described by Burke and Dennison (1980) and Burke and Papkoff (1980). This assay, utilizing ^{125}I-labeled Turkey (*Meleagris gallopavo*) prl, was validated for use with Wilson's Phalarope by comparing dose-response relationships of Wilson's Phalarope prl with prl from broody hen turkeys, and by comparing the immunoreactivity of prl from respective pituitary homogenates (Oring et al. 1988). All samples were measured in a single assay to avoid inter-assay variation. Intra-assay variation was 6.80% (corrected coefficient of variation, V* [Sokal and Rohlf 1981] for 3 prl controls interspersed in the assay). The minimum detectable dose was .099 ng per assay tube. The anti-turkey-prl antibody was from the same stock as used by Oring et al. (1988) in their analyses of prl in Wilson's Phalarope.

We measured nine additional factors: (1) body mass, by weighing males at each capture using a hand-held Pesola scale, (2) wing length, using length of flattened wing chord, (3) circulating testosterone (T), assayed following the methods of Fivizzani et al. (1986), (4) hematocrit, as the percentage of packed red blood cells to total blood volume in hematocrit tubes, (5) handling stress, measured as minutes elapsed between capture and completing blood collection, (6) days incubated, as the number of days a male had incubated his current clutch, (7) time of day of capture, (8) date of capture, and (9) ambient temperature °C, as temperature at time of capture at the capture site. We also obtained records of daily high and low temperatures from an Envi-

ronment Canada weather station located approximately 15 km NNE of the study sites.

STATISTICAL ANALYSIS

Preliminary analysis of males with four-egg clutches indicated that during early-incubation males had not yet attained maximal prl levels, a pattern previously observed in Wilson's Phalaropes (Oring et al. 1988). Because most supplemented-clutch males abandoned clutches during early-incubation, we compared their prl levels only to those of early-incubation males with four-egg clutches. Thus, for the first clutch-supplementing experiment, our comparisons were among males with submaximal prl. Because the mid-incubation experiment was a separate clutch-supplementing experiment, we analyzed it separately. Typically, males with supplemented clutches abandoned incubation very quickly (Delehanty and Oring 1993). Because of this strong behavioral response, we thought it unlikely that supplementing clutches led to elevated prl. Thus, tests involving prl levels of supplemented-clutch males were one-tailed and assumed supplemented-clutch males exhibited lower levels of prl than control males. All other tests of prl levels were two-tailed as were other comparisons unless otherwise specified.

Our primary analysis consisted of reduced-clutch and full-clutch males in both years, together with nine potentially explanatory additional factors. We used analysis of covariance (ANCOVA; SAS 1988) and interactive model building (Henderson and Velleman 1981) to test for clutch-size and year effects on prl and to challenge those findings using the nine alternative explanations. We compared reduced- and full-clutch males captured during mid- and late-incubation when prl levels were maximal. Mean values were used for those males captured more than once. For males that experienced a clutch-size change between captures, we randomly selected one capture; data from captures with different clutch-size were excluded.

We concatenated year and treatment into a single class variable with four levels (year-by-treatment). This procedure allowed us to maximize the statistical power of our preplanned comparisons for year and clutch-size effects. It did this by yielding an experiment-wide estimate of mean square error that could be used to compare prl levels of full-clutch males between years and to compare prl levels of full-clutch

TABLE 1. ANCOVA probability values based on type-III sums of squares for preplanned (year-by-treatment) and *a posteriori* variables. The year-by-treatment effect remains significant even after removing variation explained by *a posteriori* variables that were systematically selected and included in the model based on their significant P-values.

Source	df	MS	F-ratio	P-value
Year-by-treatment	3	1,830.27	14.86	< 0.001
Date	1	717.92	5.83	0.021
Temperature	1	1,558.33	12.65	0.001
Time of day	1	644.21	5.23	0.028
Log(T)	1	691.91	5.62	0.023
Time by Log(T) interaction	1	666.69	5.41	0.025
Error	38	123.16		

versus reduced clutch males within each year. We tested each of the nine additional variables for heterogeneity of slopes with the year-by-treatment variable. Using type-III sums of squares and an alpha level of 0.05, we dropped nonsignificant interactions one at a time. We did not observe any significant interactions between the year-by-treatment variable and any of the nine factors. Again using type-III sums of squares, we tested for, and dropped one at a time, all nonsignificant pairwise interactions among the nine factors. Finally, we dropped, one at a time, all nonsignificant single factors. Because the year-by-treatment variable remained highly significant in the final ANCOVA model, we used contrasts (SAS 1988) to make specific preplanned pairwise comparisons among levels within the year-by-treatment variable. To look for a year effect, we compared prl of full-clutch males between years. To look for a treatment effect, we compared full-clutch males to reduced-clutch males within each year.

RESULTS

We observed a strong year effect and clutch-size effect on prl among incubating male Wilson's Phalaropes. The year-by-treatment variable was highly significant in our final ANCOVA model (type-III SS; $F_{3, 38} = 14.9$; $P < 0.001$; Table 1). Year-by-treatment explained 36.1% of total variation in prl. Full-clutch males had significantly lower levels of circulating prl in 1988, the year of heat and drought, than did full-clutch males in 1989 (contrast; $F_{1, 38} = 39.8$; $P < 0.001$; Fig. 1). Reduced-clutch males did not differ from full-clutch males in 1988 (contrast; $F_{1, 38} = 0.01$;
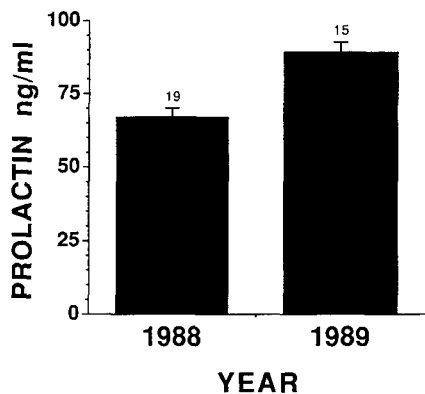
FIGURE 1. Levels of circulating prolactin for males incubating full, four-egg clutches during 1988 and 1989. Bars indicate one standard error. Numbers above bars indicate sample size. Prolactin was significantly lower in 1988.

$P = 0.91$), but reduced-clutch males had significantly lower prl than full-clutch males in 1989 (contrast; $F_{1,38} = 8.1$; $P = 0.007$; Fig. 2).

Five of nine factors examined *a posteriori* were unrelated to prl levels in our ANCOVA analysis: mass, wing chord, hematocrit, handling stress, and days incubated. Four single factors and one interaction were significant in the final ANCOVA model (Table 1). Ambient temperature and date of capture were significant as single factors. Time of day and circulating T were significant single factors and also were retained in the model as a weak, but significant, interaction. Because gonadal hormones and prl may interact to promote incubation (Buntin 1986), we looked further for a pattern between T and prl. However, plotting and regression of prl and log(T) did not suggest a linear relationship between the two hormones (Fig. 3). No combination of factors provided an alternate explanation to the variation explained by year and clutch-size (Table 1).

Supplemented-clutch males in early incubation did not exhibit lower prl than full clutch males in early incubation during the 1988 breeding season ($t_{19} = 0.14$, $P = 0.45$; Fig. 2). During early incubation in the 1989 breeding season, however, there was a strong trend towards lower prl among supplemented-clutch males versus full-clutch males ($t_{11} = 1.70$, $P = 0.06$; Fig. 2). Supplementing clutches during the mid-incubation period in 1989 did result in lower circulating prl ($t_7 = 1.9$, $P = 0.05$; Fig. 4).
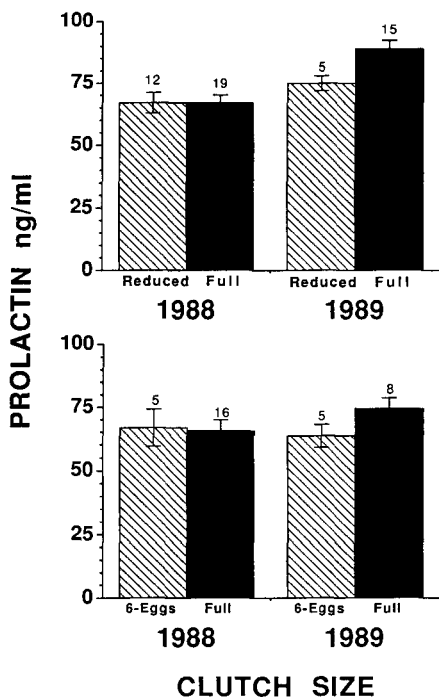

FIGURE 2. Levels of circulating prolactin for males incubating reduced, full (four-egg), and supplemented clutches during 1988 and 1989. Bars indicate one standard error. Numbers above bars indicate sample size. Incubating males with altered clutch-sizes exhibited significantly lower circulating prolactin than males incubating full, four-egg clutches in 1989, but not in 1988 when prolactin levels were suppressed in all incubating males. Comparisons for supplemented clutches versus full clutches were made during early incubation when circulating prolactin was submaximal.
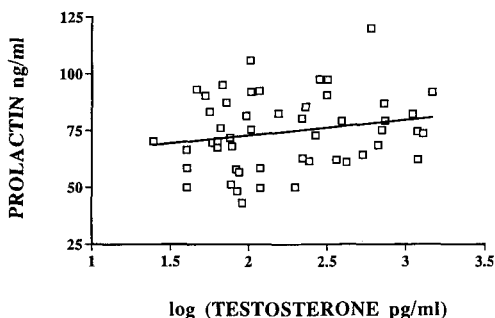

FIGURE 3. Linear regression of circulating prl and T among male Wilson's phalaropes incubating after day 7 of the incubation cycle. Prolactin and log(T) were not linearly related among males (prl $= 6.88$ log(T) $+ 59.10$; $R^2 = 0.040$, $F_{1,49} = 2.03$, $P = 0.16$).
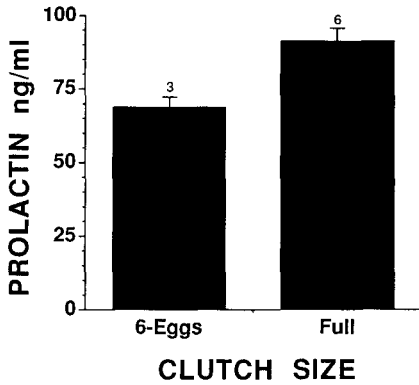
FIGURE 4. Levels of circulating prolactin among male Wilson's phalaropes incubating full, four-egg clutches and males incubating clutches that were supplemented to six eggs during the middle of the incubation cycle. Bars indicate one standard error. Numbers above bars indicate sample size. Supplementing clutches to six eggs resulted in significantly lower levels of circulating prl among supplemented-clutch males.

DIFFERENCES BETWEEN YEARS

The 1988 breeding season was characterized by extreme heat and prolonged drought. Daily high temperatures in May and June averaged 4.9°C warmer in 1988 than 1989 (paired t-test; t_{60} = 4.4, $P < 0.001$), and daily lows in 1988 averaged 2.8°C warmer than 1989 (t_{60} = 3.4, P = 0.001). These differences were particularly pronounced during the latter half of May through June when phalaropes were initiating nests and incubating eggs. The mean ($\pm$ SD) high in the 24 hours preceding each 1988 capture was 31.0 $\pm$ 5.8°C (n = 50 days), significantly higher than the 1989 mean high of 22.4 $\pm$ 5.0°C (n = 43; t = 7.6, $P < 0.001$).

During early incubation, mass of full-clutch males did not differ between years (mean $\pm$ SE 1988: 49.6 $\pm$ 1.0 g, n = 11; 1989: 48.2 $\pm$ 0.6 g, n = 14) (t-test; t_{23} = 1.3, P = 0.20). However, in 1988, incubating males experienced a greater rate of weight loss per gram of body weight than did males in 1989 (mean $\pm$ SE 1988: 5.7 $\pm$ 1.3 $\times$ 10^{-3} g g^{-1} day^{-1}, n = 11; 1989: 1.2 $\pm$ 1.2 $\times$ 10^{-3}, n = 14) (t_{23} = 2.5, P = 0.02). Overall, incubating males lost more weight per day of incubation in 1988 than in 1989 (t_{23} = 2.6, P = 0.02). Full-clutch males in 1988 lost an average of 0.3 $\pm$ 0.1 g day^{-1} (n = 11) between first and second capture, a rate significantly greater than zero (one-tailed t_{10} = 4.4, P = 0.001). In 1989,

full-clutch males lost an average of 0.1 $\pm$ 0.1 g day^{-1} of incubation (n = 14) between first and second capture, not significantly greater than zero (one-tailed t_{13} = 1.0, P = 0.13).

Because of the significant weight loss and low level of circulating prl in full-clutch males during 1988 relative to 1989, we compared *a posteriori* the rate of weight change to rate of prl change between captures among full-clutch males in mid- or late-incubation. During 1988, we found no relationship between weight change (g day^{-1}) and prl change (ng ml^{-1} day^{-1}) (linear regression: R^2 = 0.004; $F_{1,6}$= 0.02, P = 0.88). However, during 1989 males that lost weight during incubation also tended to experience a decline in circulating prl (linear regression: R^2 = 0.31; $F_{1,9}$ = 4.06, P = 0.07).

DISCUSSION

We observed a strong year effect and clutch-size effect on prl among incubating male Wilson's Phalaropes. The year effect is intriguing because low levels of circulating prl coincided with a year of extreme heat in which males lost weight rapidly, and many males looked and felt emaciated when captured and handled late in the incubation period. The heat of 1988 was accompanied by drought, and ephemeral wetlands used by males evaporated quickly. In June of 1988, we observed many males foraging in remaining wetlands with rapid, intense movements and with little preening, resting, or scanning. The intensity of their movements and lack of caution over our presence made their foraging appear frantic to us, and distinctly different from the foraging behavior that we normally observed. We felt that males were experiencing considerable environmental stress in 1988.

Males with reduced or supplemented clutches abandoned incubation with greater frequency than full-clutch males (Delehanty and Oring 1993). Each of three clutch-size manipulations in 1989 resulted in lower levels of circulating prl among males with altered clutch-sizes. These results indicate that level of circulating prl is influenced by clutch-size. These results also suggest that Wilson's Phalarope males experience an adaptive adjustment in circulating prl in response to partial clutch loss or nest parasitism. Males with compromised potential fitness had lower prl; sufficiently low prl presumably accompanies cessation of incubation.

Despite systematically searching for alterna-

tive explanations for year and clutch-size effects, none of the nine environmental or physiological factors examined provided an alternative explanation. Because our systematic search for alternative explanations used probability values as the basis for being retained in the ANCOVA model, we cannot say with certainty whether the *a posteriori* relationships identified are spurious or biologically real. We suspect that three factors, date, temperature, and time of day, were retained as weak but significant factors because of idiosyncratic differences between years. The breeding season ended earlier in 1988, the year of drought, than in 1989, meaning that 1988 included fewer late capture dates than did 1989. Similarly, because 1988 was much hotter than 1989, ambient temperature at time of capture tended to be much warmer. We also trapped birds over a broader daily time period during 1988. Thus, time of day and year tended to be correlated. The retention of T in the model is interesting because steroidal hormones and prl may interact in the onset of incubation behavior (Buntin 1986). However, subsequent analysis did not show a clear relationship between T and prl among incubating males. Although we cannot fully explain these *a posteriori* relationships, our point is that these relationships clearly are not alternative explanations to the annual and experimentally induced differences in prl that we observed.

Males typically left the study area after abandoning incubation, and we did not attempt to capture them. Our sample consisted of males that persisted to incubate despite our experimental clutch-size manipulations. Thus, our measures are conservative in the sense that if some minimum level of circulating prl is necessary for incubation behavior to continue, then males with levels below the minimum were not sampled.

We suspect that we did not observe a clutch-size effect during 1988 because males already had relatively low prl levels. Greater weight loss among incubating males in 1988 versus 1989 indicated greater environmental stress during 1988. The positive relationship between rate of weight loss and rate of prl decline among 1989 males, and the high rate of weight loss among 1988 males, suggest that prl levels among 1988 full-clutch males might already have been dampened by mid-incubation.

These results extend our current understanding of the role of prl in incubation. Riddle et al.

(1935) first proposed that prl induced incubation behavior in chickens (*Gallus gallus*), and persistent incubation behavior has been induced in ovariectomized female domestic turkeys through administration of ovine prl after a steroid "primer," a sequence that imitates the steroid and prl pattern of egg-laying (El Halawani et al. 1986). Youngren et al. (1991) were able to induce incubation behavior in domestic turkeys by intracranial perfusion of ovine prl.

Elevated prl in individuals providing primary incubation has been demonstrated in several wild species including: Ruffed Grouse (*Bonasa umbellus*) (Etches et al. 1979), three albatross species, Wandering (*Diomedea exulans*), Grey-headed (*D. chryostoma*), and Black-browed (*D. melanophris*) (Hector and Goldsmith 1985), the Pied Flycatcher (*Ficedula hypoleuca*) (Silverin and Goldsmith 1983), the Spotted Sandpiper (Oring et al. 1986a, 1986b), the Semipalmated Sandpiper and Red-necked Phalarope (Gratto-Trevor et al. 1990), and Wilson's Phalarope (Nicoll et al. 1967, Oring et al. 1988). Together, the shorebird studies provide broad evidence for an association of elevated prl and male incubation behavior in sex-role reversed shorebirds (Fivizzani et al. 1990, Oring and Fivizzani 1990).

A small number of studies have sought to change prl levels in female birds through clutch-size or incubation length manipulations or to change incubation behavior by manipulating the endocrine or sensory environment of females (El Halawani et al. 1980, 1986, Hall and Goldsmith 1983, Oring et al. 1988, Youngren et al. 1991). We were able to induce changes in prl among incubating male phalaropes by altering clutch-sizes, and thus the fitness potential of the clutch.

Lehrman (1959) proposed that the egg in the nest could act as a stimulus to the endocrine system in birds. One way that clutch-size could affect prl levels is through tactile stimulation by eggs on the incubation patch (Jones 1969). There have been few direct tests of the nest or eggs as stimuli promoting the release of prl. Removing nest boxes from incubating female turkeys led to a decline in prl after 8 hours (El Halawani et al. 1980). Prolactin declined to pre-incubation levels in Ruffed Grouse when eggs were removed from the nest after 23 days of incubation (Etches et al. 1979). Anesthetizing the incubation patch for 9 hours in incubating female domestic ducks led to a significant decline in prl; removing half of the eggs of the

clutch, however, did not (Hall and Goldsmith 1983). No differences in prl levels were observed in female Pied Flycatchers incubating experimentally altered clutch-sizes of 3 to 11 eggs (Silverin and Goldsmith 1983). However, the Pied Flycatcher and the domestic duck have a naturally variable clutch-size, unlike phalaropes. Further, the elevated prl level of female Pied Flycatchers appears to be under strong endogenous control prior to hatch (Silverin and Goldsmith 1984, 1990). Thus, female Pied Flycatchers may be insensitive to altered clutch stimuli prior to hatch. This differs from phalaropes, which appear to be very sensitive to the stimulus provided by the clutch. Prolactin levels of male phalaropes remain elevated only as long as eggs are present during experimentally lengthened or shortened incubation periods (Oring et al. 1988). This study demonstrates lower circulating prl in males with altered clutch-sizes.

The ability of male Wilson's Phalaropes to recognize and abandon clutches with reduced fitness potential suggests an underlying physiological basis for making the behavioral shift from persistent incubation to allowing eggs to perish. Adaptive changes in circulating prl may help to mediate this behavioral shift.

ACKNOWLEDGMENTS

LITERATURE CITED

BUNTIN J. D. 1986. Role of prolactin in avian incubation behavior and care of young: is there a causal relationship? Ann. New York Acad. Sci. 474:252–267.

BURKE, W. H., AND P. T. DENNISON. 1980. Prolactin and luteinizing hormone levels in female Turkeys (Meleagris gallopavo) during a photoinduced reproductive cycle and broodiness. Gen. Comp. Endocrinol. 41:92–100.

BURKE, W. H., AND H. PAPKOFF. 1980. Purification of Turkey prolactin and the development of a ho-

mologous radioimmunoassay for its measurement. Gen. Comp. Endocrinol. 40:297–307.

COLWELL, M. A., AND L. W. ORING. 1988. Breeding biology of Wilson's Phalarope in southcentral Saskatchewan. Wilson Bull. 100:567–582.

DELEHANTY, D. J, AND L. W. ORING. 1993. Effect of clutch size on incubation persistence in male Wilson's Phalaropes (Phalaropus tricolor). Auk 110:521–528.

EL HALAWANI, M. E., W. H. BURKE, AND P. T. DENNISON. 1980. Effect of nest-deprivation on serum prolactin level in nesting female Turkeys. Biol. Reprod. 23:118–123.

EL HALAWANI, M. E., J. L. SILSBY, E. J. BEHNKE, AND S. C. FEHER. 1986. Hormonal induction of incubation behavior in ovariectomized female Turkeys (Meleagris gallopavo). Biol. Reprod. 35:59–67.

EL HALAWANI, M. E., J. L. SILSBY, S. C. FEHER, AND E. J. BEHNKE. 1985. The influence of acute or repeated immobilization on plasma prolactin levels in the Turkey (Meleagris gallopavo). Gen. Comp. Endocrinol. 59:410–415.

ETCHES, R. J., A. GARBUTT, AND A. L. MIDDLETON. 1979. Plasma concentrations of prolactin during egg laying and incubation in the Ruffed Grouse (Bonasa umbellus). Can. J. Zool. 57:1624–1627.

FIVIZZANI, A. J., M. A. COLWELL, AND L. W. ORING. 1986. Plasma steroid hormone levels in free-living Wilson's Phalaropes, Phalaropus tricolor. Gen. Comp. Endocrinol. 62:137–144.

FIVIZZANI, A. J., L. W. ORING, M. E. EL HALAWANI, AND B. A. SCHLINGER. 1990. Hormonal basis of male parental care and female intrasexual competition in sex-role reversed shorebirds, p. 273–286. In M. Wada, S. Ishii, and C. Scanes [eds.], Endocrinology of birds: molecular to behavioral. Springer-Verlag, Berlin.

GOLDSMITH, A. R. 1983. Prolactin in avian reproductive cycles, p. 375–387. In J. Balthazart, E. Prove, and R. Gilles [eds.], Hormones and behaviour in higher vertebrates. Springer-Verlag, New York.

GOLDSMITH, A. R. 1990. Prolactin and avian reproductive strategies. Proc. Int. Ornithol. Congr. 20:2063–2071.

GRATTO-TREVOR, C. L., L. W. ORING, A. J. FIVIZZANI, M. E. EL HALAWANI, AND F. COOKE. 1990. The role of prolactin in parental care in a monogamous and a polyandrous shorebird. Auk 107:718–729.

HALL, M. R., AND A. R. GOLDSMITH. 1983. Factors affecting prolactin secretion during breeding and incubation in the domestic duck (Anas platyrhynchos). Gen. Comp. Endocrinol. 49:270–276.

HECTOR, J. A. L., AND A. R. GOLDSMITH. 1985. The role of prolactin during incubation: comparative studies of three Diomedea albatrosses. Gen. Comp. Endocrinol. 60:236–243.

HENDERSON, H. V., AND P. F. VELLEMAN. 1981. Building multiple regression models interactively. Biometrics 37:391–411.

HÖHN, E. O. 1969. The phalarope. Sci. Am. 220 (6):105–111.

JONES, R. E. 1969. Hormonal control of incubation patch development in the California Quail Lo-

phortyx californicus. Gen. Comp. Endocrinol. 13: 1–13.

LEA, R. W. 1987. Prolactin and avian incubation: a comparison between Galliformes and Columbiformes. Sitta 1:117–141.

LEHRMAN, D. S. 1959. Hormonal responses to external stimuli in birds. Ibis 101:478–496.

MEIER, A. H., J. T. BURNS, AND J. W. DUSSEAU. 1969. Seasonal variations in the diurnal rhythm of pituitary prolactin content in the White-throated Sparrow, *Zonotrichia albicollis*. Gen. Comp. Endocrinol. 12:282–289.

MEIER, A. H., D. D. MARTIN, AND R. MACGREGOR, III. 1971. Temporal synergism of corticosterone and prolactin controlling gonadal growth in sparrows. Science 173:1240–1242.

NICOLL, C. S., E. W. PFEIFFER, AND H. R. FEVOLD. 1967. Prolactin and nesting behavior in phalaropes. Gen. Comp. Endocrinol. 8:61–65.

ORING, L. W., AND A. J. FIVIZZANI. 1990. Reproductive endocrinology of sex-role reversal. Proc. Int. Ornithol. Congr. 20:2072–2080.

ORING, L. W., A. J. FIVIZZANI, M. A. COLWELL, AND M. E. EL HALAWANI. 1988. Hormonal changes associated with natural and manipulated incubation in the sex-role reversed Wilson's Phalarope. Gen. Comp. Endocrinol. 72:247–256.

ORING, L. W., A. J. FIVIZZANI, AND M. E. EL HALAWANI. 1986a. Changes in plasma prolactin associated with laying and hatch in the Spotted Sandpiper. Auk 103:820–822.

ORING, L. W., A. J. FIVIZZANI, M. E. EL HALAWANI, AND A. GOLDSMITH. 1986b. Seasonal changes in prolactin and luteinizing hormone in the polyandrous Spotted Sandpiper, *Actitis macularia*. Gen. Comp. Endocrinol. 62:394–403.

RIDDLE, O., R. W. BATES, AND E. L. LAHR. 1935. Prolactin induces broodiness in fowl. Am. J. Physiol. 111:352–360.

SAS INSTITUTE INC. 1988. SAS/STAT user's guide, version 6.03. SAS Institute Inc., Cary, NC.

SILVERIN, B., AND A. R. GOLDSMITH. 1983. Reproductive endocrinology of free living Pied Flycatchers (*Ficedula hypoleuca*): prolactin and FSH secretion in relation to incubation and clutch size. J. Zool. 200:119–130.

SILVERIN, B., AND A. R. GOLDSMITH. 1984. The effects of modifying incubation on prolactin secretion in free-living Pied Flycatchers. Gen. Comp. Endocrinol. 55:239–244.

SILVERIN, B., AND A. R. GOLDSMITH. 1990. Plasma prolactin concentrations in breeding Pied Flycatchers (*Ficedula hypoleuca*) with an experimentally prolonged brooding period. Horm. Behav. 24:104–113.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman, San Francisco.

YOUNGREN, O. M., M. E. EL HALAWANI, J. L. SILSBY, AND R. E. PHILLIPS. 1991. Intracranial perfusion induces incubation behavior in turkey hens. Biol. Reprod. 44:425–431.