THE ROLE OF PROLACTIN IN PARENTAL CARE IN A MONOGAMOUS AND A POLYANDROUS SHOREBIRD

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ABSTRACT.—We compared circulating prolactin levels in two species of shorebirds which have very different social systems, and which breed sympatrically at La Pérouse Bay, 40 km east of Churchill, Manitoba. Semipalmated Sandpipers (*Calidris pusilla*) are monogamous and share incubation equally, although females normally desert broods earlier than males. Red-necked Phalaropes (*Phalaropus lobatus*) are facultatively polyandrous, and only males care for eggs and young.

High prolactin values were correlated with persistent incubation behavior in male Red-necked Phalaropes, and male and female Semipalmated Sandpipers. Prolactin levels in Semipalmated Sandpipers increased dramatically at the onset of incubation, and were not different between the sexes. Incubating male phalaropes had greater prolactin values than the non-incubating males and females. Changes in prolactin levels, however, did not explain the early brood desertion of female Semipalmated Sandpipers. Prolactin levels did not decline with age of brood in either sex of this species. *Received 16 October 1989, accepted 29 April 1990.*

PROLACTIN is involved in a wide variety of physiological and behavioral events in vertebrates (Bentley 1982). However, controversy continues regarding prolactin's role in initiation and maintenance of incubation in birds. Early studies by Riddle and others (e.g. Riddle et al. 1935, Riddle and Lahr 1944) showed that injections of mammalian prolactin induced incubation behavior in female domestic fowl (*Gallus gallus*) and Ringed Turtle-Doves (*Streptopelia risoria*). Subsequent studies (Saeki and Tanabe 1955, Lehrman and Brody 1961, Opel and Proudman 1980, Höhn 1981) did not yield the same result, although incubation behavior was induced by prolactin in ovariectomized female Wild Turkeys (*Meleagris gallopavo*), after the birds were "primed" with injections of estradiol and progesterone (El Halawani et al. 1986).

Recent development of radioimmunoassays to measure avian prolactin has allowed researchers to follow changes in plasma levels throughout the breeding season, and relate these changes to behavior. The fact that plasma prolactin levels often are elevated before persistent incubation has led some authors to suggest that prolactin induces incubation behavior (e.g. Lea et al. 1981). Others have concluded (from egg-removal studies and studies that involve anesthetization or denervation of brood patches) that either tactile or visual presence of the nest and eggs stimulates incubation and results in elevated plasma prolactin, which in turn maintains incubation behavior (e.g. Hall and Goldsmith 1983). Still others propose a combination of these processes (El Halawani et al. 1986). Other studies of turkeys, bantam hens, and Pied Flycatchers (*Ficedula hypoleuca*) have shown that incubation can persist for at least a short time in the absence of high circulating levels of prolactin (Lea et al. 1981, El Halawani et al. 1980, Silverin and Goldsmith 1984).

The issue is further complicated by the fact that some researchers have found seasonal increases in prolactin levels in the absence of incubation or nestling-feeding behavior. In some instances such increases have been linked to onset of photorefractoriness (e.g. European Starlings [*Sturnus vulgaris*], Dawson and Goldsmith 1983, Goldsmith and Williams 1984; White-crowned Sparrows [*Zonotrichia leucophrys*], Hiatt et al. 1987).

Our primary objective was to examine the pattern of circulating levels of prolactin at dif-

ferent stages of the reproductive cycle in two species of shorebirds with very different breeding systems. In the Red-necked Phalarope (*Phalaropus lobatus*), only males incubate (Hildén and Vuolanto 1972). Females desert their males as soon as the clutch is completed, and then attempt to obtain subsequent mates (Reynolds 1987). Semipalmated Sandpipers (*Calidris pusilla*) are monogamous, with incubation shared equally by both members of the pair (Ashkenazie and Safriel 1979a, b). If changes in circulating levels of prolactin are primarily related to incubation behavior, prolactin levels should be similar in both sexes of the Semipalmated Sandpiper, and greater in incubating male phalaropes than in females and nonincubating males. In both species plasma prolactin should increase substantially at the onset of incubation behavior. However, if prolactin levels increase seasonally—without a change in behavior—in males and females of both species, its function may primarily relate to photorefractoriness.

The gradual decline of plasma prolactin levels of attending parents of altricial and semialtricial young after hatch has been related to a decrease in feeding or brooding with increased age of nestlings (e.g. Silverin and Goldsmith 1984, Hector and Goldsmith 1985). In contrast to species with altricial young, prolactin levels decline dramatically at hatch (or within two days afterwards) in virtually all species examined with precocial young (Dittami 1981, Goldsmith 1982a, Goldsmith and Williams 1980, Hall and Goldsmith 1983, Wentworth et al. 1983). Spotted Sandpipers (*Actitis macularia*) appear to be an exception, because prolactin levels did not decline in the first two days after hatch (Oring et al. 1986a). In addition, prolactin levels in Wilson's Phalaropes (*Phalaropus tricolor*) declined gradually and reached basal levels by nine days posthatch (Oring et al. 1988). These authors suggested that the gradual decrease is related to the decline in brooding behavior with age of chicks, as found in other shorebirds.

In view of the proposed relationship between prolactin levels and brood-care behavior, particularly in shorebirds, a second objective of this study was to examine hormonal control of brood care in the Red-necked Phalarope and Semipalmated Sandpiper, both of which have precocial young. In phalaropes, however, only males participate in brood care (Hildén and Vuolanto 1972, Reynolds 1987). Almost all fe-

male Semipalmated Sandpipers gradually desert their broods to the care of the male soon after hatch (Ashkenazie and Safriel 1979a, b; Gratto and Cooke 1987). Therefore we predicted that prolactin levels of females would decline at rates faster than those of males.

METHODS

Field.—Individuals of both species were observed at La Pérouse Bay (58°24′N, 94°24′W), 40 km east of Churchill, Manitoba, on the Hudson Bay coast, in the summers of 1985–1987. The 3-km² study area is situated in the Mast River delta, and consists primarily of low islets of *Salix brachycarpa* or *Betula glandulosa* and mixed sedges and grasses, in fresh water.

Populations of both Semipalmated Sandpipers and Red-necked Phalaropes were studied in the area from 1980 to 1987, and most birds were already individually color-banded. The breeding system of each species was well known. Nonincubating birds were captured in mist nets, and incubating birds in walk-in nest traps. Adults were marked with individual color-band combinations, and observed throughout the breeding season to determine their breeding status and behavior. For Semipalmated Sandpipers, the largest member of a pair (as determined by bill length) was assumed normally to be the female (Prater et al. 1977). Sex determination often was verified by behavior (flight displays, copulation), which indicated that sexing by size was very accurate (Gratto and Cooke 1987). Sexes of autumn migrants were determined by examination of gonads after the birds were collected. Red-necked Phalaropes were sexed by plumage and behavior. Most birds were scored for body molt on the head, back, and breast.

Blood samples were collected during the following stages of the breeding cycle:

1. *Spring transient* (spr)—birds captured in early June that did not remain to nest in the study area (collected for Semipalmated Sandpipers only).
2. *Prelay* (prel)—paired or unpaired birds captured before laying a clutch, that later bred in the study area.
3. *Lay* (lay)—birds captured during the laying period, before the clutch was complete.
4. *Early incubation* (e inc)—birds captured early in the incubation period, normally day 1 (day last egg was laid) to day 4. For female phalaropes, this refers to the time most male phalaropes in the population were in early incubation. Female Red-necked Phalaropes immediately deserted their mates after egg laying and attempted to obtain subsequent mates. Therefore, behavior of "early incubation" females was identical to that of prelay females. These data were collected to determine if changes in steroid levels of female phalaropes were
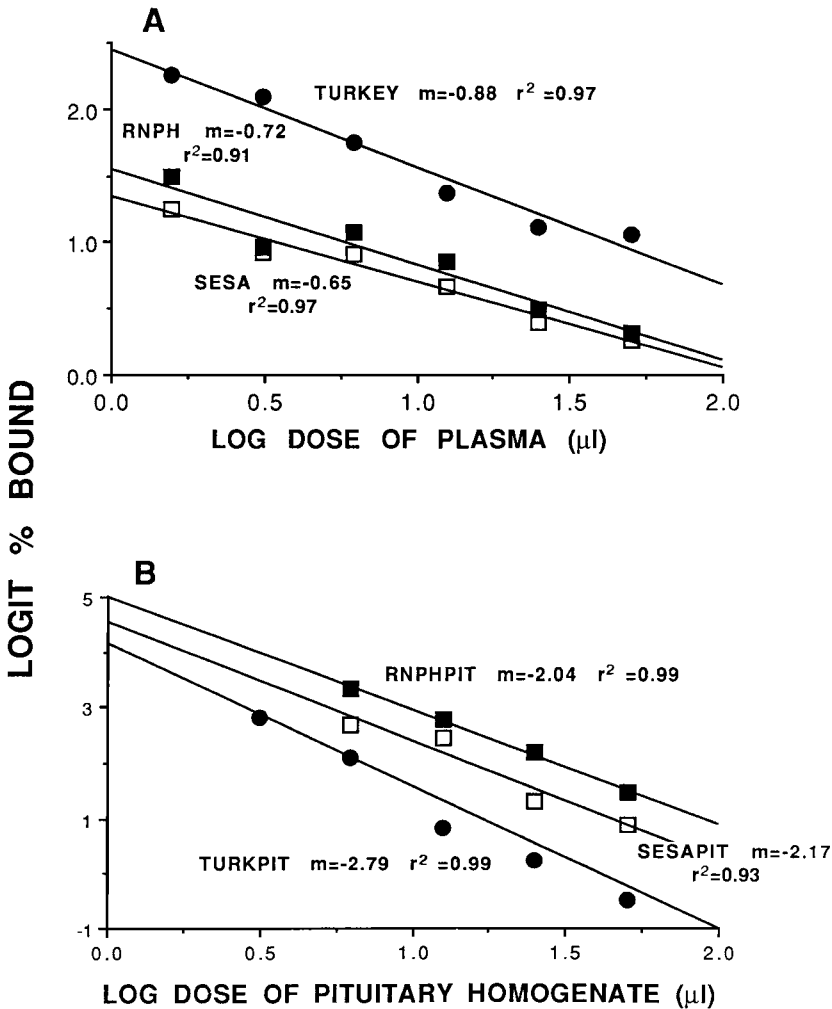
Fig. 1. Radioimmunoassay dose-response series of (A) plasma from broody turkey hens (TURKEY), incubating Semipalmated Sandpipers (SESA), and incubating Red-necked Phalaropes (RNPH); (B) pituitary homogenate of turkey (TURKPIT), Semipalmated Sandpipers (SESAPIT), and Red-necked Phalaropes (RNPHPIT). The slope of each line is indicated by "m."

dependent on season or behavior. Virtually all female phalaropes had left the study area by the time male phalaropes were in late incubation.

5. *Late incubation* (l inc)—birds captured late in the incubation period, before the eggs started to pip, normally day 14-16.
6. *Pip* (pip)—birds captured when at least one egg was pipped and before any hatched, normally day 17-19 (collected only in 1985).
7. *Brood* (brd)—birds captured while they attended the brood, after at least one young had hatched.
8. *Fall transient* (fall)—unbanded adults captured from migratory flocks in late July (collected only from Semipalmated Sandpipers).

Blood was sampled by puncturing the brachial vein with a small (25 G) needle, and collected in heparinized microhematocrit tubes. Most samples (88%, $n = 472$) were collected between 1100 and 1900. Using the largest consistent subsample of the data (incubating and brooding Semipalmated Sandpipers), we found no significant relationship between sampling time and plasma prolactin level in any year (Pearson correlations: 1985, $n = 93$, $r = 0.03$, $P = 0.79$; 1986, $n = 46$, $r = -0.14$; 1987, $n = 62$, $r = -0.20$, $P = 0.11$).

In the field, samples were kept on ice and transported to camp later in the day. At camp, samples were immediately centrifuged. Plasma was drawn off with a microsyringe, placed into labeled plastic tubes,

and stored for transport in liquid nitrogen. At the University of North Dakota, samples were frozen at −20°C until analysis.

Laboratory.—Prolactin values were assayed with the turkey prolactin radioimmunoassay of Burke and Dennison (1980) and Burke and Papkoff (1980). Only two prolactin assays were performed. The first assay (initiated on 24 April 1986) of all 1985 prolactin samples of both species used duplicate 50 μl samples. The second assay (initiated on 2 February 1988) of all 1986 and 1987 prolactin samples of both species used duplicates of 25 μl each. Intra-assay variation was similar in each, with an average of 8.83% based upon differences among multiple-pool sample potency estimates (controls) in the mid-range of the curve, and a minimum detectable dose of 0.307 ng. Statistical comparison of multiple common serum pool estimates in each assay yielded no significant difference between means (ANOVA, $P > 0.05$).

The turkey prolactin radioimmunoassay was previously validated for two other shorebird species, Spotted Sandpipers (Oring et al. 1986a, b) and Wilson's Phalaropes (Oring et al. 1988). This radioimmunoassay was validated for use with Semipalmated Sandpiper and Red-necked Phalarope plasma by comparing the dose-response relationship of plasma from broody turkey hens against plasma from incubating Semipalmated Sandpipers and Red-necked Phalaropes. The dilution series for all three were linear and very similar (Fig. 1A). There was no significant difference between the slopes for turkey and Red-necked Phalaropes (ANCOVA, $P = 0.26$). The difference in slopes between turkey and Semipalmated Sandpipers was marginally significant (ANCOVA, $P = 0.04$). The dose-response relationship of young turkey pituitary homogenate also was compared with pituitary homogenate from both species of sandpipers. Again the dilution series for all three were linear and very similar (Fig. 1B). The difference between slopes for turkey and Semipalmated Sandpipers was not significant (ANCOVA, $P = 0.19$). The difference between slopes for Red-necked Phalarope and turkey pituitary homogenate was marginally significant, because of the strong linearity of the dilution series in these two species (ANCOVA, $P = 0.04$).

Further evidence to support the validity of the assay for the shorebird species was generated by comparing molecular size of the immunologically reactive component of Semipalmated Sandpiper, Red-necked Phalarope, and young turkey pituitary homogenates. Each pituitary homogenate was subjected to molecular sieve column chromatography. The supernatant from each was applied to a 1.5 × 50 cm Sephadex G100 column and eluted with 0.1 M phosphate-buffered saline containing 0.1% BSA. One-milliliter fractions were collected from the column and 50-μl aliquots of each were assayed for immunoreactive prolactin. Using the turkey prolactin assay, we compared the elution patterns of each species for im-
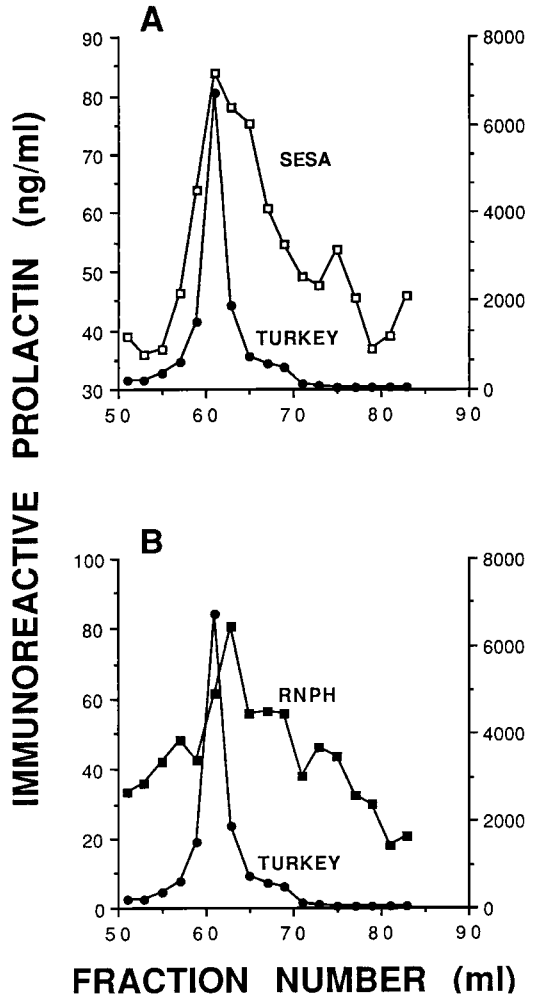

Fig. 2. Elution profiles of pituitary homogenates from incubating Semipalmated Sandpipers (SESA) and Red-necked Phalaropes (RNPH), and young turkeys (TURKEY) after chromatography on a 1.5 × 50 cm Sephadex G100 column. Scales on the left refer to sandpipers; all turkey values should be read from the scale on the right.

munological reactivity. These patterns were very similar with primary peaks in the same molecular size range (Fig. 2).

Effects of blood sampling on behavior of the birds, and on hormone levels, was minimal (Colwell et al. 1988, Gratto-Trevor et al. 1991). Virtually all samples were collected in <15 min.

An ANOVA was used to compare all status groups for each species and sex each year (e.g. female phalaropes in 1985). Status groups with a sample size of one were deleted from this analysis (e.g. laying female phalaropes in 1985). If the ANOVA revealed
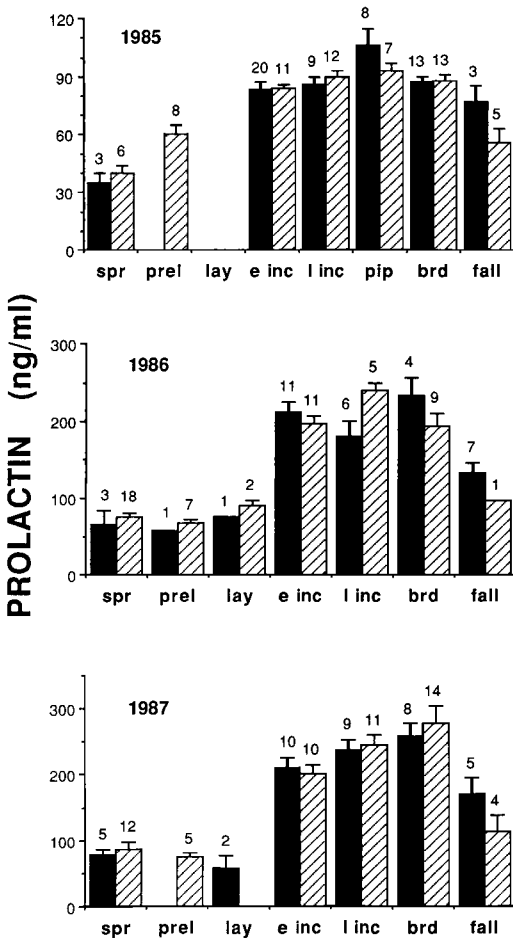
PROLACTIN (ng/ml)

Fig. 3. Mean circulating prolactin levels in male (hatched bars) and female (solid bars) Semipalmated Sandpipers during the 1985 to 1987 breeding seasons at La Pérouse Bay. Abbreviations of status groups are explained in text. Error bars denote SE; numbers above bars are sample sizes.

significant differences ($P < 0.05$), we used the GT2 test for multiple comparisons and unequal sample sizes to determine specific significant differences between status groups: for example, prelay vs. early incubation (SAS Institute Inc. 1982). Unless otherwise stated, any differences noted were significant at $P < 0.05$. Because samples from 1985 and those from 1986–1987 were assayed separately, it is not possible to compare actual hormone levels between years, just patterns of change. We combined data from different years only when we examined changes in individuals during a single season (or differences between members of a pair) when there were no significant differences among years (ANOVA, $P > 0.05$).

RESULTS

PROLACTIN AND INCUBATION

Semipalmated Sandpipers.—Prolactin levels were low in both sexes during spring, prelay, and lay. Levels increased significantly during early incubation, and did not change significantly until they declined at autumn migration (Fig. 3, Table 1).

When all preincubation values (spring, prelay, and lay) were combined within a year, there was never a significant difference between prolactin values of males and females (Table 2). There also were no significant differences between the sexes during incubation or brooding.

The difference in prolactin levels between members of a pair was compared when both birds were captured during incubation, no more than four days apart. Again, there was no significant difference between the sexes (paired t-test, all years combined, $n = 26$, mean ($\pm$SE) female–male $= 11 \pm 8$ ng/ml, $P = 0.17$).

We captured a number of birds several times during a single breeding season. Prolactin levels increased in all seven instances where birds were caught first during prelay and later during incubation or brooding. This increase was significant (paired t-test, all years and sexes combined, $n = 7$, $\bar{x} = 95 \pm 28$ ng/ml, $P = 0.02$). In the 12 instances where birds were caught first in early incubation and subsequently in late incubation, 6 increased, 5 decreased, and 1 remained the same. The difference was not significant (paired t-test, all years and sexes combined, $\bar{x} = 19 \pm 11$ ng/ml, $P = 0.10$). Of the 19 Semipalmated Sandpipers captured first during incubation and later at brooding, 15 prolactin values increased and 4 decreased. The difference was not significant (paired t-test, all years and sexes combined, $\bar{x} = 28 \pm 16$ ng/ml, $P = 0.10$).

Red-necked Phalaropes.—We found no significant differences between prelay and "early incubation" prolactin levels (Fig. 4, Table 3) for female Red-necked Phalaropes. In phalarope males, few differences between status groups were significant (Fig. 4, Table 3). Levels appeared to increase between prelay and early incubation in 1985 and 1987 (only significant in 1987), but not in 1986. Prolactin levels at prelay were always less than those at late incubation or brooding, although the differences were not always significant.

TABLE 1.   Semipalmated Sandpiper: statistical comparison of prolactin levels across breeding stages; results of ANOVA and GT2 multiple comparison tests (Type I family error rate = 5% across each year-sex comparison). Probability and F-values are for ANOVA, while only significant differences are shown for GT2 tests. Abbreviations of status groups are in text.

Year	ANOVA F	ANOVA P	Significant GT2 tests
Female			
1985	8.8	0.0001	spr < e inc, l inc, pip, brd, fall e inc < pip
1986	10.7	0.0001	spr < e inc, l inc, brd fall < e inc, brd
1987	15.0	0.0001	spr < e inc, l inc, brd, fall lay < e inc, l inc, brd fall < brd
Male			
1985	23.9	0.0001	spr < prel, e inc, l inc, pip, brd prel < e inc, l inc, pip, brd fall < e inc, l inc, pip, brd
1986	49.5	0.0001	spr < e inc, l inc, brd prel < e inc, l inc, brd lay < e inc, l inc, brd
1987	19.1	0.0001	spr < e inc, l inc, brd prel < e inc, l inc, brd e inc < brd fall < l inc, brd

In 1985 and 1987, there were no significant differences in prolactin levels between females and preincubation males (Table 4). In 1986, preincubation males had significantly higher values than females. Incubating males in all three years had significantly higher prolactin values than those of females (when the one extremely high value for 1985 "lay" females was excluded). Incubating males also had significantly higher values than preincubating males in all years except 1986, when prolactin levels did not increase in males until late incubation.

Two female phalaropes were captured during prelaying and "early incubation" in one season, and prolactin levels in each changed only -2 ng/ml. Prolactin levels in 7 of 8 male phalaropes increased between preincubation and incubation or brooding, but the difference was not significant (paired t-test, all years combined, $\bar{x} = 39 \pm 19$ ng/ml, $P = 0.08$). The mean difference for males between early incubation and late incubation was 79 ± 44 ng/ml, which also was not significant (paired t-test, all years combined, $n = 7$, $P = 0.12$). In these 7 birds, levels increased in 6 and decreased in 1. There was no significant change in males captured

first during incubation and later during brooding (paired t-test, all years combined, $n = 7$, $\bar{x} = -3 \pm 23$ ng/ml, $P = 0.89$). Levels increased in 3 birds and decreased in 4.

TABLE 2.   Comparison of circulating prolactin levels between sexes in the Semipalmated Sandpiper at different times in the breeding season. "Preincubation" refers to all spring, prelay, and lay birds.

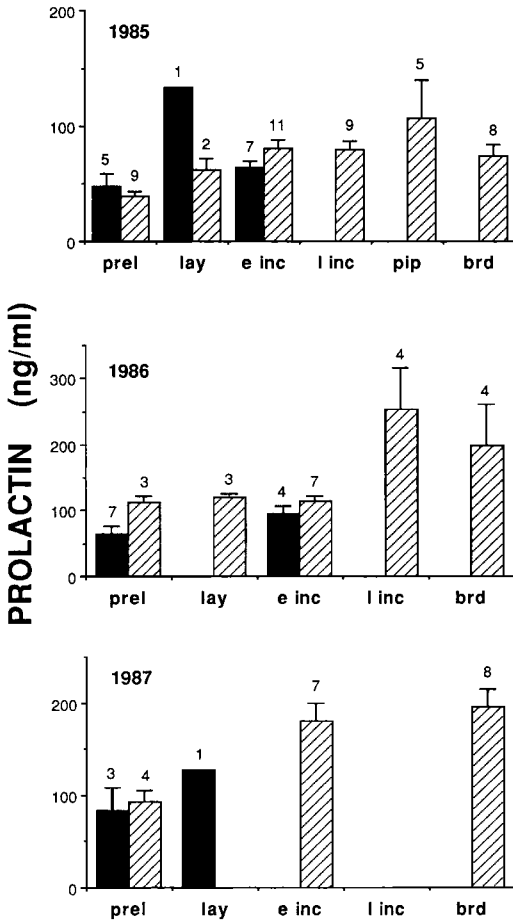|	Prolactin (ng/ml)				
	Females			Males	
Stage/yr	n	$\bar{x} \pm$ SE	P^a	n	$\bar{x} \pm$ SE
Preincubation					
1985	3	35 ± 5	0.10	14	51 ± 4
1986	5	66 ± 10	0.35	27	75 ± 4
1987	7	72 ± 8	0.45	17	83 ± 8
Incubation					
1985	37	89 ± 3	0.73	30	88 ± 2
1986	17	201 ± 11	0.55	15	210 ± 10
1987	19	224 ± 11	0.96	21	223 ± 11
Brooding					
1985	13	87 ± 3	0.88	13	88 ± 3
1986	4	232 ± 23	0.21	9	194 ± 16
1987	8	259 ± 18	0.61	14	278 ± 26

^{a} t-test.

Fig. 4. Mean circulating prolactin levels in male (hatched bars) and female (solid bars) Red-necked Phalaropes during the 1985 to 1987 breeding seasons at La Pérouse Bay. Abbreviations of status groups are explained in text. Error bars denote SE; numbers above bars are sample sizes.
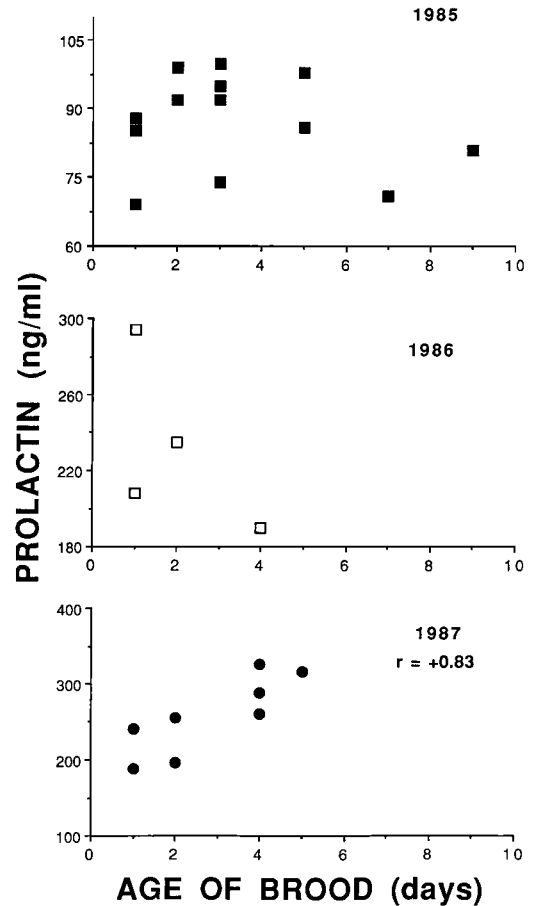
Fig. 5. Relationship between circulating prolactin levels and age of brood for female Semipalmated Sandpipers in each year at La Pérouse Bay. Only 1987 was significant ($r = +0.83$, $P < 0.05$).

PROLACTIN AND BROODING

There were no negative correlations between circulating prolactin levels of female Semipalmated Sandpipers and age of brood (Fig. 5) in any year. There was a significant positive correlation in 1987 ($r = +0.83$, $P < 0.05$).

There were no significant correlations between prolactin and brood age for male Semipalmated Sandpipers (Fig. 6). As noted previously, there was no significant difference between male and female prolactin levels during brooding in any year (Table 2). There also was no significant difference between members of the same pair caught with the brood on the

same day (paired t-test, all years combined, $n = 17$, mean female-male = -23 ± 23 ng/ml, $P = 0.32$).

In male Red-necked Phalaropes, there was a significant negative correlation between prolactin and age of brood in 1985, one of the two years (1985, 1987) with sufficient brood data (Fig. 7, $r = -0.70$, $P < 0.05$). Results from 1987 were not significant, although there was a suggestion of a decline.

DISCUSSION

PROLACTIN AND INCUBATION

High levels of circulating prolactin were correlated with incubation behavior in the Semi-

TABLE 3. Red-necked Phalarope: statistical comparison of prolactin levels across breeding stages; results of ANOVA and GT2 multiple comparison tests (Type I family error rate = 5% across each year-sex comparison). Probability and F-values are given for ANOVA, while only significant differences are shown for GT2 tests. Abbreviations of status groups are explained in text.

Year	ANOVA		Significant GT2 tests
	F	P	
Female			
1985	1.9	0.49	
1986	3.4	0.10	
Male			
1985	3.5	0.01	prel < pip
1986	2.8	0.06	
1987	5.8	0.01	prel < e inc, brd

palmated Sandpiper. Prolactin levels increased significantly at the onset of persistent incubation. Sexes incubate equally, and prolactin values were not significantly different between the sexes at any time during the breeding season.

Only two previous studies have examined avian species in which both members of the pair participate equally in incubation. No differences in prolactin levels between the sexes were found during incubation and brood care in male and female Ringed Turtle-Doves (Cheng and Burke 1983). The sexes incubate equally, and both produce crop sac "milk." In the Black-browed (*Diomedea melanophris*) and the Gray-headed (*D. chrysostoma*) albatross, in which species the sexes incubate equally in bouts lasting a number of days, prolactin values also were not different between the sexes. By contrast, in the Wandering Albatross (*D. exulans*), in which males and females also incubate equally, pro-
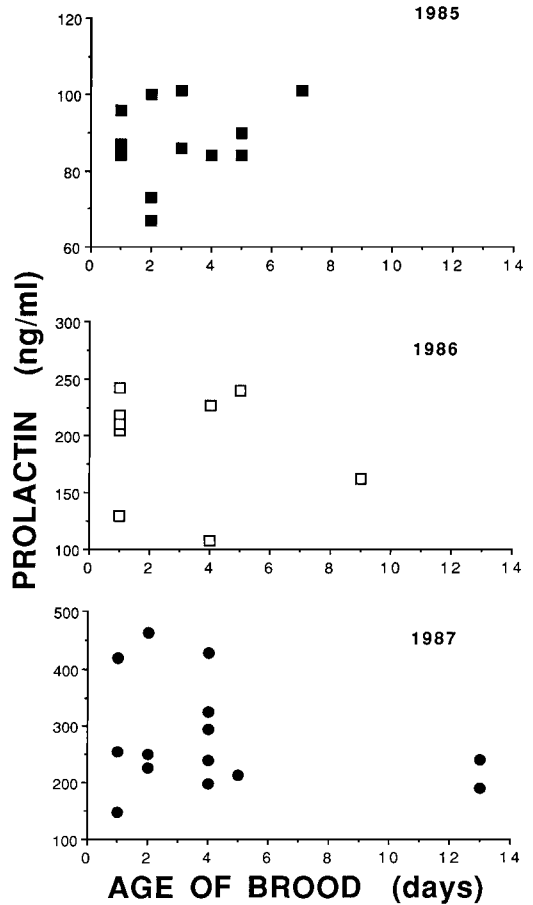

Fig. 6. Relationship between circulating prolactin levels and age of brood for male Semipalmated Sandpipers in each year at La Pérouse Bay. None was significant (Pearson correlations, $P > 0.05$).

TABLE 4. Comparison of circulating prolactin levels among incubating male, and nonincubating male and female Red-necked Phalaropes. The highly elevated value for the only laying female in 1985 was excluded. Comparisons are *t*-tests.

	Prolactin (ng/ml)								
	Preincubation males			Females			Incubating males		
Year	n	$\bar{x} \pm$ SE	P^a	n	$\bar{x} \pm$ SE	P	n	$\bar{x} \pm$ SE	P^b
1985	11	43 ± 17	0.08	12	57 ± 6	0.005	25	86 ± 7	0.0001
1986	6	116 ± 5	0.002	11	75 ± 9	0.01	11	165 ± 30	0.14
1987	4	92 ± 12	0.94	4	95 ± 20	0.02	7	181 ± 19	0.01

[a] All comparisons are *t*-tests.
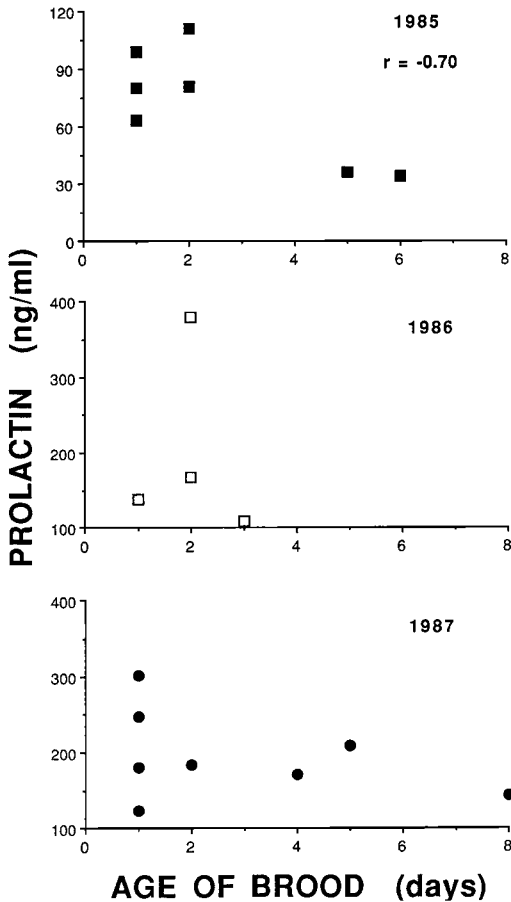[b] Incubating vs. preincubation males.

Fig. 7. Relationship between circulating prolactin levels and age of brood for male Red-necked Phalaropes in each year at La Pérouse Bay. Only 1985 was significant ($r = -0.70$, $P < 0.05$).

lactin levels were significantly higher in females than in males. Possible reasons for this difference were not discussed (Hector and Goldsmith 1985).

In species where only one sex incubates, that sex has higher levels of prolactin than the other during incubation (e.g. Mallard [*Anas platyrhynchos*], Goldsmith and Williams 1980; Bar-headed Goose [*Anser indicus*], Dittami 1981; Common Canary [*Serinus canaria*], Goldsmith 1982b; Pied Flycatcher, Silverin and Goldsmith 1983; White-crowned Sparrow, Hiatt et al. 1987; Wilson's Phalarope, Oring et al. 1988). In the Black Swan (*Cygnus atratus*), females participated in incubation more often than males, and also had slightly higher plasma prolactin levels

(Goldsmith 1982a). Female Spotted Sandpipers normally incubated much less than their mates did late in incubation, and females had lower prolactin levels at that time (Oring et al. 1986b).

It is not surprising that we found incubating male Red-necked Phalaropes had levels of plasma prolactin higher than those of females. Nevertheless, the fact that early-incubating males in 1986 did not have elevated prolactin levels seems to contradict the hypothesis that high values of circulating prolactin coincide with incubation behavior. However, in this case the exception seems to support the rule. Red-necked Phalaropes often desert their nests for extended periods (sometimes days) in cold or wet weather, when food is presumably difficult to obtain. At the time that most phalaropes began their nests in 1986, minimum temperatures were lower than normal, and precipitation much higher (average June minimum temperature: 1985 = 2.0°C, 1986 = 1.0°C; total June precipitation: 1985 = 30 mm, 1986 = 117 mm [Churchill Weather Office Reports unpubl.]). Extensive observations on two nests at this time indicated that incubation was greatly reduced (dropped from the normal 50–70% of daytime 1-h observation period to 0% on particularly poor-weather days; Mallory 1987). This finding further emphasizes the relationship between high prolactin values and persistent incubation. Perhaps periods of poor weather and low insect availability result in decreased prolactin release from the pituitary, and produce low circulating prolactin levels. Certainly a decrease in incubation behavior when foraging conditions are poor has been noted in the Pectoral Sandpiper (*Calidris melanotos*), with single-sex incubation (Norton 1972). Because both sexes incubate in the Semi-palmated Sandpiper, incubation constancy is near 100% (Norton 1972), and no decline in incubation is evident under poor weather conditions. As expected, the poor weather conditions early in incubation in 1986 did not affect prolactin levels in sandpipers.

Even though the 1986 "early incubation" phalaropes had low prolactin levels and reduced incubation, they were sufficiently tenacious to enter a nest trap. This supports the idea that once a bird is "primed" by high gonadal steroids and eggs are present, high plasma levels of prolactin are not necessary for at least short periods of incubation. The presence of eggs, and the act of incubation itself, may result

eventually in elevated prolactin levels (e.g. Goldsmith 1983, Lea 1987).

The extent of male Red-necked Phalarope incubation is variable and depends upon weather conditions. Therefore, it may be expected that their overall patterns of circulating prolactin levels changed from year to year. Even within a year, the period of incubation varied from 18 to 24 days in this species (e.g. 1985, $n = 14$, $\bar{x} \pm$ SD = 20.3 $\pm$ 1.9 days). Semipalmated Sandpipers with biparental care, on the other hand, incubate constantly, and the length of the incubation period varied little (e.g. 1985, $n = 16$, $\bar{x} \pm$ SD = 19.6 $\pm$ 0.73). The patterns of circulating prolactin were much more consistent in this species.

PROLACTIN AND BROODING

Our results for Red-necked Phalaropes agree with those from Wilson's Phalaropes (Oring et al. 1988): prolactin levels declined gradually with increasing age of the brood. Because diurnal brooding behavior generally decreases during the first week posthatch in male Red-necked Phalaropes that attend young, it is possible that circulating prolactin levels in this species are directly related to both incubation and chick brooding. However, this pattern of decline was significant only in one year. Cold weather is not uncommon after hatch in the subarctic. By affecting the amount of brooding, ambient temperature might produce a more inconsistent pattern of prolactin decline after hatch, compared with patterns in more southern study areas.

Although diurnal brooding behavior also generally decreased during the first week posthatch in Semipalmated Sandpipers, we found no evidence in either sex of a decline in plasma prolactin with increasing brood age. This should have been particularly evident in females, because they gradually separate themselves from the chicks much earlier than males (Ashkenazie and Safriel 1979a, b; Gratto and Cooke 1987). In fact, prolactin levels increased significantly in one year. We cannot explain this result, and reasons for the overall lack of decline in prolactin after hatch are not obvious. All previous studies on birds that attend young report either an immediate precipitous decline or a gradual decrease in prolactin (for review, see Goldsmith 1983). Perhaps these high prolactin levels post-

hatch are related to molt and migratory fattening (e.g. Dawson and Goldsmith 1983, 1984). Both sexes of Semipalmated Sandpipers initiated body molt during incubation, and began premigratory fattening soon after leaving the brood. Red-necked Phalaropes, on the other hand, did not initiate molt during incubation or brooding.

Meier proposed that the timing of seasonal conditions in birds is based upon changing temporal relations among hormones, including prolactin (for review, see Meier and Russo 1985). Although we found no evidence for circadian rhythm of prolactin secretion, our sampling regime was not designed to maximize detection of daily changes. Therefore, it is possible that a phase shift in the timing of prolactin release is the basis for prolactin's role in both incubation and photorefractoriness.

The patterns of circulating prolactin levels in Semipalmated Sandpipers and Red-necked Phalaropes breeding in the subarctic indicate that high prolactin levels are correlated with persistent incubation behavior. This is made evident by the fact that there was no difference in prolactin levels between the equal incubating sexes of the Semipalmated Sandpiper, and by the fact that incubating male Red-necked Phalaropes had greater prolactin values than the nonincubating females. The apparent effect of poor weather conditions on plasma prolactin levels in male phalaropes emphasizes the difference in incubation constancy between these two species. However, changes in prolactin levels did not explain the early brood desertion of female Semipalmated Sandpipers. Although we cannot explain why plasma prolactin levels do not decline during brooding in Semipalmated Sandpipers (and is not pronounced in Red-necked Phalaropes), this phenomenon appears unique among avian species examined.

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