Changes in Circulating LH, Sex Steroid Hormones, Thyroid Hormones and Corticosterone in Relation to Breeding and Molting in Captive Humboldt Penguins (Spheniscus humboldti) Kept in an Outdoor Open Display

Authors: Otsuka, Ryoko, Aoki, Kiyoshi, Hori, Hidemasa, and Wada, Masaru

Source: Zoological Science, 15(1) : 103-109

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.103

ZOOLOGICAL SCIENCE **15**: 103–109 (1998)

Changes in Circulating LH, Sex Steroid Hormones, Thyroid Hormones and Corticosterone in Relation to Breeding and Molting in Captive Humboldt Penguins (*Spheniscus humboldti*) Kept in an Outdoor Open Display

Ryoko Otsuka[1,3]*, Kiyoshi Aoki[1], Hidemasa Hori[2] and Masaru Wada[3]

[1]*Life Science Institute, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo 102, Japan*
[2]*Tokyo Sea Life Park, Rinkai-cho, Edogawa-ku, Tokyo 134, Japan*
[3]*College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Kohnodai, Ichikawa-shi, Chiba 272, Japan*

ABSTRACT—Penguins are highly adapted to marine life. Their hydrodynamic efficiency depends on feathers which wear with age and need to be replaced regularly. During molting, penguins can not enter the sea to forage and are forced to fast. Therefore the duration of molting is necessarily brief. To better understand molting in penguins, we collected plasma samples from 16 (8 pairs) Humboldt penguins kept in an open display pen at Tokyo Sea Life Park from May to September, 1994 and estimated circulating concentrations of LH, testosterone, estradiol, thyroxine (T4), triiodothyronine (T3) and corticosterone. Body mass was also measured at each blood sampling. Throughout the year, reproductive activities (egg laying, incubation, hatching and rearing) and molting were observed and recorded. Humboldt penguins maintained reproductive activity from January to December except during molting. Each pair started molting between the end of July and early August; usually males started earlier. The duration of molting was 13.4 ± 0.8 days for males and 12.9 ± 0.3 days for females. Body masses were highest just before the start of molting in both sexes. Plasma concentrations of LH were high, (> 2 ng/ml) in May in both sexes, then gradually decreased, to 0.53 ± 0.38 ng/ml in males and 0.72 ± 0.11 ng/ml in females by the end of July. Testosterone and estradiol concentrations in plasma decreased and were lowest during molting. On the other hand, plasma concentrations of T4 were low until early July (ca. 20 ng/ml) and then doubled within 10 days; the high levels were maintained for one month and then decreased greatly in males and slightly in females. When the plasma concentrations of T4 started to decrease, plasma concentrations of LH increased. Changes in plasma T3 were not consistent with molting. These results indicate that the decrease of plasma levels of sex steroid hormones and the sharp increase of T4 induced molting, which lasted only for a short period.

INTRODUCTION

Molting, a process of replacing old worn feathers with new ones, is an important event in the life cycle of birds (see for review Payne, 1972). This is an energy-demanding process (see for review King, 1981) and in most avian species, especially birds living in temperate regions, it does not overlap significantly with other energy-demanding events, such as migration and breeding. Molting is also a highly adaptive event, varying in form, duration, frequency and timing (Ginn and Melville, 1983).

Penguins are highly adapted to marine life. The penguins' feathers protect the skin against water and serve as insulation (Stonehouse, 1967). Partial wear of body feathers decreases swimming ability and insulation. Thus penguins cannot enter the sea to forage and are forced to fast during molting. To achieve an energy-demanding molt during a period of forced starvation, penguins have developed a unique strategy. They take in a lot of food during the pre-molt period (Groscolas *et al*., 1986) and renew the whole plumage over a very short period relative to other bird species. In their natural environment, most species of penguin molt after breeding (postnuptial molt) during summer (December-April in the Southern Hemisphere).

Humboldt penguins (*Spheniscus humboldti*) distribute between Foca island (5° 12´ S) in Peru to the north and Punihuil islands (42° S) in Chile to the south. However, the number of wild Humboldt penguins has decreased by 70% in 15 years to about 7,500 in 1996 (Araya and Bernal, 1996), and the spe-

* Corresponding author: Tel. +81-47-372-1136;
FAX. +81-47-374-0630.

cies is listed as endangered. On the other hand, Humboldt penguins adapt well to artificial conditions and have been bred successfully in Japan and are one of the most popular penguin species in zoos and aquariums (Hori, 1996). In captivity, they molt during the local summer of the Northern Hemisphere. Despite their successful breeding in captivity, Humboldt penguins are not well studied. Meritt and King (1987) and Scholten (1987, 1992) conducted studies using captive birds in America and Europe, respectively, but to date, there have been no physiological studies concerning breeding and molting. Successfully breeding captive Humboldt penguins in Japanese zoos and aquariums provide a good opportunity for such studies.

Thus the purpose of the present experiments is to obtain basic physiological information on molting and breeding in Humboldt penguins in Japan. We observed the behavior of Humboldt penguins kept in an open aquarium display and collected blood samples for measurement of circulating hormones, luteinizing hormone (LH), testosterone (T), estradiol (E2), thyroxine (T4), triiodothyronine (T3) and corticosterone (B), as well as body weight.

MATERIALS AND METHODS

Animals

The study site was Tokyo Sea Life Park located in Kasai, Edogawa-ku, Tokyo (35° 38´ N, 139° 53´ E). The park has an outdoor open field pen (1095 m^2) with a pool (314.5 m^2 with 400 *t* of water) which holds a mixed colony of Humboldt penguins and Rockhopper penguins (the number of Humboldt penguins was 92 in 1994). Individual penguins are identified by color wing bands. We selected 8 pairs of adult Humboldt penguins (Pair 1: No. 25 and 5, Pair 2: No. 6 and 7, Pair 3: No. 11 and 24, Pair 4: No. 35 and 59, Pair 5: No. 8 and 53, Pair 6: No. 56 and 58, Pair 7: No. 27 and 43, Pair 8: No. 68 and 67) for the study. Their ages were from 3 to 18 years. Four birds were born wild and the rest born in zoos. All pairs had showed reproductive activity in preceding years using artificial rock nests in the pen.

The birds are fed fresh live horse mackerels and silver stripe round herrings given in the pool every day except Monday.

Observation of breeding and molting

Most pairs use the same rock nests for many years. We observed breeding behavior such as nest building, egg laying, incubating and chick rearing through a whole year (1994). Dates of hatching or removal of eggs were recorded for each nest. The clutch size in Humboldt penguins is two but the data shown below is always for the first egg. Eggs which were accidentally broken or did not hatch were removed after the incubation period. In some cases, however, unhatched eggs remained beyond the incubation period to adjust the population in the pen.

Molting is a process whereby new feathers grow under the skin, extruding the old feathers as they emerge. However, it is impossible to detect the whole process from external observation, and we defined a molt as the period between the dates the first and last old worn-out feathers are lost. During molting, the penguins did not enter the pool or eat.

Sample collection

Since previous observations have indicated molting of the Humboldt penguins occurs during summer on the conditions mentioned above, we collected blood samples from May to September (1994), by which time all had completed molting except Pair 8.

Since we could not collect blood samples from 16 individuals in one day, we divided the birds into 3 sampling groups and collected samples every 3 weeks from each group between 1300 and 1800. Eight to 9 samples were obtained from each bird. A 2-ml blood sample was taken from a leg vein with a 25 gauge needle and a syringe into a heparinized tube. The samples were centrifuged and the plasma samples stored frozen until the assays. At blood sampling, the birds were weighed to the nearest 0.01 kg and the stage of molt evaluated.

Hormone assay

Each hormone was assayed in a single run to avoid interassay variation. Plasma concentrations of luteinizing hormone (LH) were measured in 50-μl sample volumes, in duplicate, using the radioimmunoassay method described by Hattori and Wakabayashi (1979). Chicken LH (fraction IRC-2, Gunma) was used for reference preparations and preparations of chicken LH (fraction AGCHDS112312A) were used for iodination. The antiserum (AH-MH No. 1) was raised against chicken LH (fraction IRC-2, Gunma). Results are expressed in nanograms per milliliter of a chicken LH fraction IRC-2, Gunma. Intraassay variation was 6.5%.

Plasma concentrations of testosterone (T), estradiol (E2) and corticosterone (B) were estimated with specific radioimmunoassays in 10 μl plasma sample volumes in duplicate using antisera supplied from Prof. K. Wakabayasi with intraassay variations of 5.7, 5.8 and 12.2%, respectively. Plasma concentrations of thyroxine (T4) and triiodothyronine (T3) were measured in 5 μl samples in duplicate according to Tasaki *et al.* (1986) with slight modifications using antisera obtained from Endocrine Sciences Products (Calabasas Hills, California) and intraassay variations were 15.5 and 14.6%, respectively.

Statistics

Where sample size was adequate and equality of variance criteria could be met, parametric tests of analysis of variance (ANOVA) and Student's *t* test were preferentially used. Differences were considered significant when $P<0.05$. Throughout the text, hormone concentrations are the mean ± SEM.

RESULTS

Breeding and molting

In the outdoor display ground at Kasai, the Humboldt penguins showed breeding activity throughout the year except for the period of molting (Fig. 1). Four pairs laid 3 clutches, 2 laid two clutches and 2 laid one clutch.

Seven pairs laid the first clutch during March while Pair 3 laid early in February. Five pairs laid a second clutch in May after an unsuccessful breeding and Pair 1 laid a late first clutch in May. Two pairs (6 and 7) successfully hatched the first clutch and reared chicks to fledglings. Two pairs (2 and 8) out of the five which had the second clutch succeeded in rearing chicks to fledglings.

Irrespective of the success or failure of the preceding breeding, all the birds molted during late July to mid-August. One pair (8) laid the second clutch late on the last day of May and the eggs hatched on July 17. They reared the hatchling and molted very late, the male in August and the female in early October.

In all the pairs, the males began to molt several days earlier than the females (10.8 ± 4.3 days). For the males, the mean date of the beginning and end of molt was August 2 (± 4.0 days) and August 15 (± 3.9 days), whereas in the females it was August 12 (± 8.0 days) and August 25 (± 7.6 days),

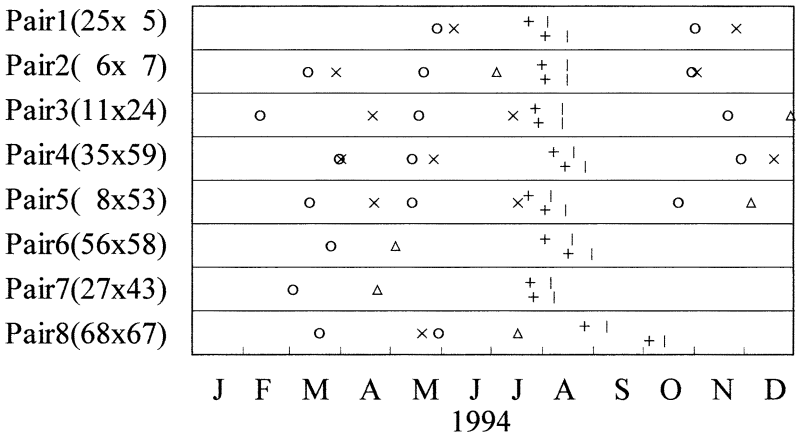

Fig. 1. Observed breeding activity and molting in pairs of Humboldt penguins kept in an outdoor display at Tokyo Sea Life Park in 1994. Blood samples were collected from May to September from pairs. Symbols are as follows: ○, egg-laying; △, hatching; ×, egg broken or removed; +, beginning of molt; | , end of molt.

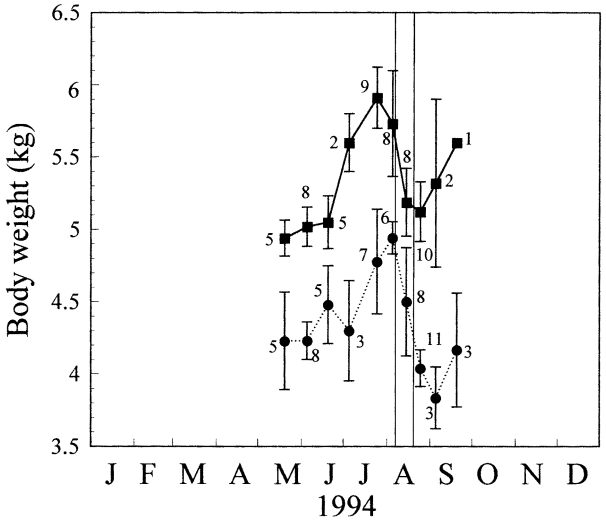

Fig. 2. Changes in body weight of male (solid square) and female (solid circle) Humboldt penguins during the sampling period. Values plotted are the mean ± SEM. Sample sizes are mentioned beside each symbol. Two vertical lines are the mean date of the beginning of molt (August 7) and the end of molt (August 20) of both sexes, respectively.

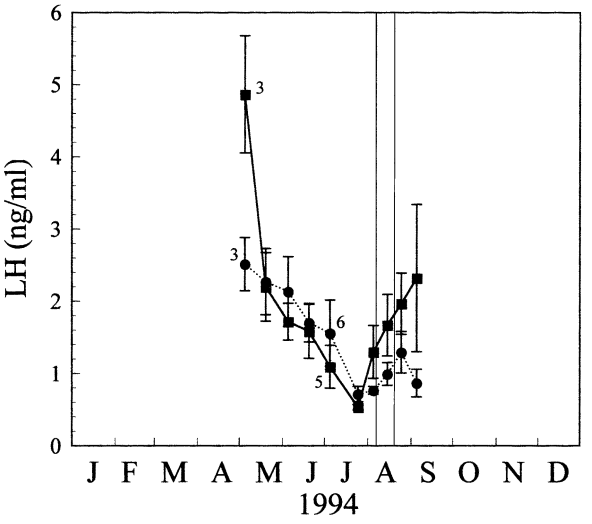

Fig. 3. Changes in circulating luteinizing hormone levels in male and female Humboldt penguins during the sampling period. Details are as for Fig. 2 except that the data of May 5 are included and the numbers of samples different from Fig. 2 are labeled by symbols.

respectively. Duration of molt was, however, not significantly different between the sexes (males, 13.1 ± 0.7 and females, 12.4 ± 0.6 days).

After the molt was completed, five pairs laid 2nd or 3rd clutches in October or November. Two of them were successful.

Changes in body weight and circulating hormones

We collected blood samples from May, 1994 to September, 1994. Due to restrictions in the time available for sampling, the samples from all 16 birds were not collected on the same day. Thus, to obtain profiles of changes in body weight and plasma concentrations of each hormone, the data were grouped into two, early and late halves of the month of May, June, July and September. In August when molting was at a climax, sufficient numbers could be sampled to divide the birds into three groups, early, middle and late August.

Changes in body weight during the sampling period. Body weight was always significantly heavier in males than females. However, the temporal pattern of both sexes was similar and changed in parallel (Fig. 2). Body weight of both sexes showed significant changes between May and August. Body weight in the males was stable until late June (around 5 kg), and then increased significantly to a peak value (5.91 ± 0.21 kg, $n = 9$) in late July (5.05 vs 5.91 kg; $t = -2.71$, $df = 12$; $P < 0.05$). Thereafter, it decreased reaching basal levels in late August (5.91 vs 5.19 kg, $t = 2.30$, $df = 15$; $P < 0.05$). Body weight in the females remained stable (between 4.0 and 4.5

kg) until early July and then increased to a maximum value of 4.94 ± 0.11 kg (n = 6) in early August (4.30 vs 4.94 kg, t = – 3.85, df = 10; P < 0.01) before decreasing significantly after the beginning of molting (4.94 vs 4.04 kg, t = 4.70, df = 15; P < 0.01).

Changes in LH. The plasma concentrations of LH in both sexes showed similar profiles and showed significant changes throughout the sampling period (ANOVAs by sex, F(9, 53) = 5.40 for males, F(9, 52) = 3.18 for females, P < 0.01 in both cases; Fig. 3). The circulating levels of LH in both sexes were high, above 2.0 ng/ml, at the start of sampling. Then the plasma concentrations of LH decreased gradually to a minimum level of 0.53 ± 0.04 ng/ml (n = 9) in the males and 0.72 ± 0.16 ng/ml (n =7) in the females in late July just before the start of molting. The decrease found between late June and late July was significant in both sexes (1.58 vs 0.53 ng/ml, t = 2.79, df = 4; P < 0.05 in male, 1.70 vs 0.72 ng/ml, t = 3.88, df = 10; P < 0.01 in female). Circulating concentrations of LH in the males showed a clear increase to reproductive levels after the onset of molt (0.53 vs 1.30 ng/ml, t = – 2.21, df = 15; P < 0.05). There was no significant increase in LH levels of the females after late July (ANOVA, F(4, 30) = 2.69, NS).

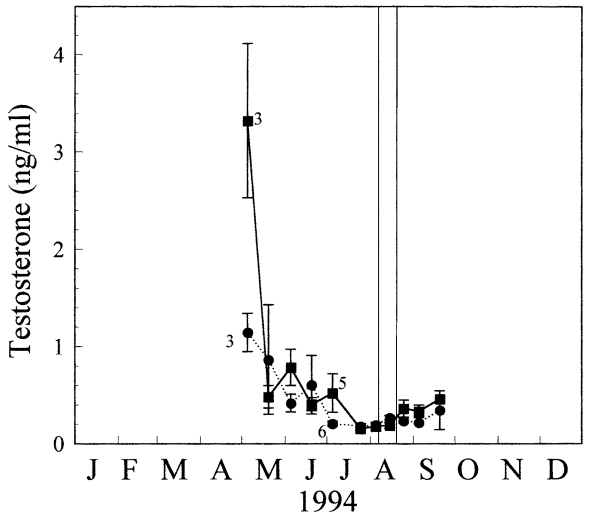

Fig. 4. Changes in plasma concentrations of testosterone in male and female Humboldt penguins during the sampling period. Details are as for Fig. 3.

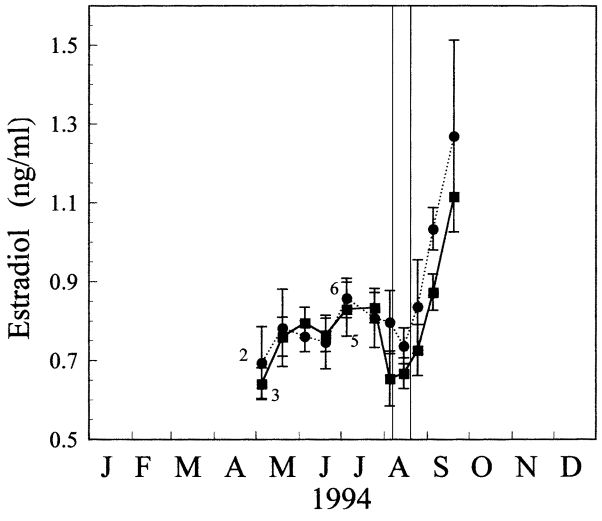

Fig. 5. Changes in plasma concentrations of estradiol in male and female Humboldt penguins during the sampling period. Details are as for Fig. 3.

Changes in T. Plasma concentrations of T in the males showed significant changes throughout this sampling period (ANOVA, F(10, 53) = 19.02, P < 0.01). The maximum level was 3.32 ± 0.79 ng/ml (n = 3) at the start of the experiment. Then the concentrations of T were 0.5-1.0 ng/ml until early July. In late July, the levels became the lowest (0.16 ± 0.02 ng/ml, n = 9). The low concentration in males was maintained until the middle of August, then it increased to 0.3-0.5ng/ml (ANOVA, F(5, 32) = 2.57, P < 0.05) (Fig. 4).

Plasma concentrations of T in the females showed the highest levels, 1.15 ± 0.20 ng/ml (n = 3), in early May and significantly declined to 0.21 ± 0.03 ng/ml (n = 6) in early July (1.15 vs 0.21 ng/ml, t = 4.73, df = 2; P < 0.05). Thereafter, the levels remained low. There were no significant changes in the female T levels after July (ANOVA, F(6, 37) = 1.26, NS) (Fig. 4).

Changes in E2. The temporal patterns of E2 plasma concentrations in both sexes were similar (Fig. 5). There were significant changes in the males throughout the sampling period (ANOVA, F(10, 53) = 2.12, P < 0.05), however, no significant variations were found in the females (ANOVA, F(10, 53) = 1.64, NS). Circulating E2 levels were low (< 0.9 ng/ml) until early September in the males and late August in the females. The E2 plasma concentrations increased, maximizing in both sexes after molting. In the males, it was 1.12 ng/ml (n = 1) and in the females, 1.27 ± 0.24 ng/ml (n = 3), at the end of this experimental period, late September. In males, the plasma concentrations decreased significantly from late July to early August (0.83 vs 0.65 ng/ml, t = 2.34, df = 15; P < 0.05) after which low levels were maintained during molting. Plasma E2 increased significantly after the molting (0.67 vs 0.87 ng/ml, t = – 2.46, df = 8; P < 0.05).

Changes in T4. Both sexes had a biphasic pattern of circulating levels of T4 (ANOVAs by sex, F(9, 53) = 5.24, P < 0.01 for males, F(9, 52) = 4.04, P < 0.01 for females; Fig 6). In both sexes, plasma concentrations of T4 were stable and low during the early portion of the sampling period and they rapidly elevated from early to late July in the males (19.71 vs 40.08 ng/ml, t = – 3.05, df = 12; P < 0.01) and from early July to early August in the females (22.33 vs 36.10 ng/ml, t = – 2.45, df = 10; P < 0.05). These high levels were maintained during molting in both sexes. After the molting period, T4 plasma concentrations decreased in the males (36.40 vs 21.25 ng/ml, t = 1.54, df = 8; NS) but the high levels were maintained in the females.

Changes in T3. There was no significant variation throughout the experimental period in either sex (ANOVAs by sex, F(9, 53) = 1.64, NS for males, F(9, 52) = 1.06, NS for fe-

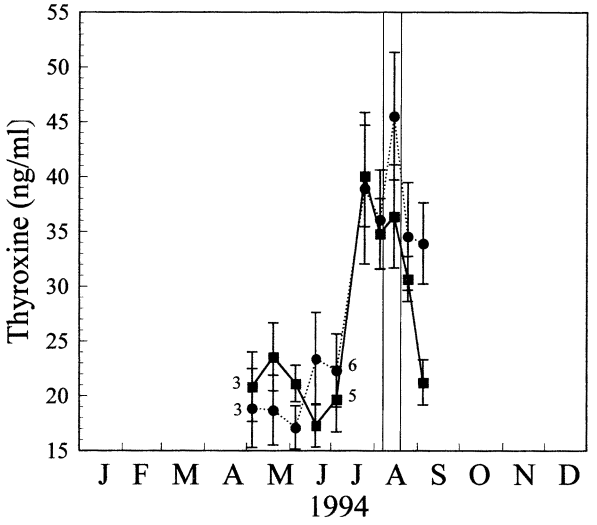

Fig. 6. Changes in plasma concentrations of thyroxine in male and female Humboldt penguins during the sampling period. Details are as for Fig. 3.

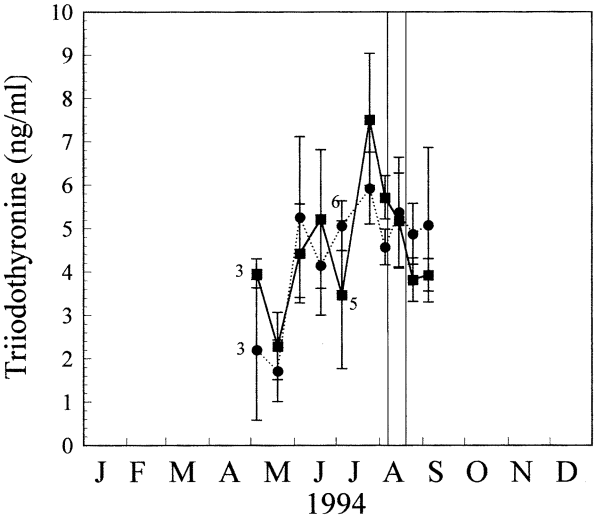

Fig. 7. Changes in plasma concentrations of triiodothyronine in male and female Humboldt penguins during the sampling period. Other details are same as in Fig. 3.

males) and the circulating T3 concentrations showed similar profiles in the males and females (Fig. 7). T3 levels were low in May, and maintained relatively high levels thereafter, especially in the females. But there were no associated changes in T3 with molting in females during the experimental period. In males, T3 levels were relatively elevated and became more variable through this period.

Changes in B. There were no significant changes throughout the experimental period either sex (ANOVAs by sex, $F(9, 53) = 1.74$, NS for males, $F(9, 52) = 1.50$, NS for females). The concentration of B was the lowest at the end of July in both sexes (Fig. 8).

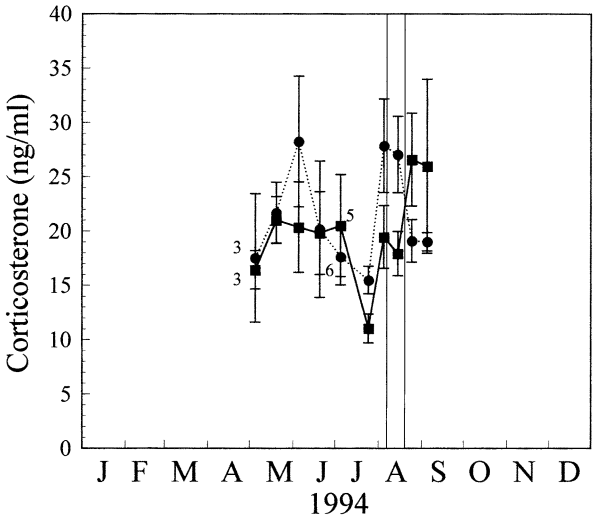

Fig. 8. Changes in plasma concentrations of corticosterone in male and female Humboldt penguins during the sampling period. Details are as for Fig. 3.

DISCUSSION

Our observations indicated that Humboldt penguins successfully manage their annual life cycle in captivity under outdoor conditions; they showed breeding activity throughout the year except during molting indicating that these two energy-demanding events do not overlap. It is reported that in their original habitat, Humboldt penguins can breed in any month of the year (Stonehouse, 1972 cited by Williams, 1995) and in captivity they nest all year round, with second and third clutches laid in the same year if the first one fails. Thus the birds used in the present experiment were not exceptional but rather a good model with which to study physiological mechanisms underlying the annual breeding and molting cycles of this species. To date, studies on captive penguins had focused only on morphology and behavior and no physiological studies had been undertaken.

Throughout the sampling period, the body weight of the males was always heavier than that of the females (Fig. 2). The body weight values for the males and females before the premolt period are comparable to those reported by Scholten (1987). Body weight increased significantly during the premolt period in both sexes. The birds fast for about 13 days during the molting. Therefore, they consume much more food than normal prior to fasting. This increased body weight was reduced during molting, returning to original values after the molt. In general, sparrows do not show significant variations in body weight between pre- and post-molt (e.g., Murphy *et al.*, 1988), because they can forage during molt. The female body weight peaked later than that of the males, as did T4. Also, the date of beginning of molt was later in females (Figs. 2 and 6).

The circulating levels of LH reached a minimum just before molting and the sex steroid hormones showed low levels during molting in both sexes suggesting the molting period is

a reproductively quiescent phase (Figs. 3-5). Annual events in birds come under the control of photoperiod as well as food availability (see for review Wingfield and Farner, 1994). Food availability before molting is especially important for the penguins since they can not forage during molting. In the wild, the period of premolt for Humboldt penguins overlaps with a time when food is abundant. The molting time is December and January (summer) in the Southern Hemisphere when anchovies approach the coast (Jordan, 1980). The fact that the Humboldt penguins, brought to or born in Tokyo Sea Life Park in the Northern Hemisphere and fed constantly all year round, molt in the local summer (July-August) indicates that molting is basically controlled by environmental factors.

As mentioned above, molting in Humboldt penguins does not overlap with breeding suggesting that hormones related to breeding activity suppress molting. Assenmacher and Jallageas (1980) described two precise temporal relationships between gonadal and thyroid cycles. 1) The annual gonadal cycle is inversely related to the thyroid cycle, i.e. the breeding season occurs when thyroid activity is low and vice versa. 2) The thyroid function displays a marked seasonal increase, peaking just before the end of the breeding season. Passerine birds and Indian finches are in group 1 and starlings, blackbirds, mallards and ducks are in group 2. Humboldt penguins, which show a postnuptial molt coinciding with a very rapid increase of T4 and decrease in sex steroid hormone secretion, fall into the latter group. This rapid increase of T4 is suggested to affect feather growth (Groscolas and Leloup, 1986) . Their findings showed that the peak of the plasma concentrations of T4 coincide with the molt whereas that of T3 occurred later in emperor and adelie penguins. Also in the present study, the plasma concentration of T4 had a clearer correlation to molting than that of T3 (Figs. 6 and 7).

Interestingly, this sudden increase in T4 seemed to be unconnected with any environmental factor but to coincide with the very sudden ending of the reproductive period as indicated by the decrease in circulating LH and sex steroid hormones (Figs. 3-6) as is the case in ducks and teals (Assenmacher and Jallageas, 1980). The idea that information regarding the end of breeding activity is a trigger for molting is further strengthened when the molting and changes in hormones are correlated in individual animals. For example, Pair No. 8 laid a second clutch late on the last day of May which hatched on July 17. The pair reared the hatchling to fledgling stage. Parallel to this delay in breeding activity, the plasma concentration of T4 of Pair 8 began to increase later in August in the male and did not increase in the female during the sampling period (data not shown). And they molted very late in August (male) and in early October (female). Scholten (1989) also suggested that the date of egg-laying is correlated with that of molting in this species. This is a highly adaptive trait; they cannot rear the chicks when they are molting, because they can not forage without the insulation of the feathers. After they complete rearing, Humboldt penguins eat as much as possible and gain fat for starvation during the molt ashore. These annual cycles are likely to be precisely controlled by endocrine mechanisms. Seasonal maturation of the reproductive system is likely to be controlled by photoperiod even in sub-Antarctic and Antarctic species such as adelie penguins (Astheimer and Grau, 1985; Groscolas *et al*., 1986), emperor penguins (Groscolas *et al*., 1986) and in macaroni and gentoo penguins (Williams, 1992), before they arrive at the breeding ground. Reproductive activity in Humboldt penguins may also be controlled by photoperiod since the birds used in this experiment began to reproduce in the local winter (in most cases in November). As mentioned earlier, the beginning of molting was dependent on the end of reproduction indicating that molting is not necessarily controlled by photoperiod. However, the mechanism by which the reproduction is terminated in this species is unknown. The birds begin to breed in winter indicating the species a short-day breeder like sheep. Plasma concentrations of LH in both sexes began to increase during molting (Fig. 3) and E2 plasma concentrations increased after molting (Fig. 5). These results suggest the molting period is an interruption of breeding which is potentially sustained throughout the year.

The plasma concentrations of T and E2 were high during breeding, decreasing to minimal levels before molting. The results agree well with that obtained in emperor and adelie penguins (Groscolas *et al*., 1986). The values of T and E2 in both sexes were almost the same for all sampling periods. This is reasonable since males and females share breeding (parental) duty (nest building, nest protecting, incubating and chick rearing). This was already observed in Western seagulls (Wingfield *et al*., 1980, 1982) where there is no sexual dimorphism in morphology and behavior. The only difference was in the date molting began. We observed that the males molted before the female partners in 1994. Scholten (1989), Kojima (1978) and Warham (1972) have also reported that males molt before females in captive Humboldt penguins and wild Erect-crested penguins (*Eudyptes sclateri*). The reason for this is not clear at the present, but there may be some differences in parental care controlled by prolactin which suppresses T4 release between males and females.

Plasma concentrations of B were relatively high from May to June, decreased to the lowest values at the end of July and again increased upon molting (Fig. 8). It is difficult to draw a general conclusion as to the role of corticosterone in molting in this species, even though Peczely (1985) suggested its involvement in several species. Further studies are required.

Penguins are interesting because their breeding cycles markedly differ from those of most altricial passerines. However, all penguins used for physiological studies to date have been Antarctic or sub-Antarctic species. Penguins living in the mid-latitude, such as Humboldt penguins, are interesting to compare with passerines. We conducted a physiological study on captive Humboldt penguins and obtained results indicating that changes in sex steroids and thyroid hormones affect the short duration molt of the species. Further studies will clarify the actual mechanisms for molting in the species.

ACKNOWLEDGMENTS

We thank Johichi Saitoh, Kazuhiro Sakamoto and the other staff of Tokyo Sea Life Park for their help in conducting the experiment with birds. We also thank Professor Katsumi Wakabayashi for his gift of LH, T, E2 and B antisera and Professor Susumu Ishii for his gift of an LH preparation for iodination.

REFERENCES

Araya B, Bernal M (1996) Distribution, population size and conservation of the Humboldt penguin (*Spheniscus humboldti*) in Chile. Abstracts of Intern Confer Conserv Humboldt penguin

Assenmacher I, Jallageas M (1980) Adaptive aspects of endocrine regulations in birds. In "Hormones, Adaptation and Evolution" Ed by S Ishii, T Hirano, M Wada, Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin, pp 93–102

Astheimer LB, Grau CR (1985) The timing and energetics consequences of egg formation in Adelie penguins. Condor 87: 256–267

Ginn HB, Melville DS (1983) "Moult in Birds" BTO Guide 19, British Trust of Ornithology, Hertfordshire

Groscolas R, Jallageas M, Goldsmith A, Assenmacher I (1986) The endocrine control of reproduction and molt in emperor (*Aptenodytes forsteri*) and adelie (*Pygoscelis adeliae*) penguins. I. Annual changes in plasma levels of gonadal steroids and LH. Gen Comp Endocrinol 62: 43–53

Groscolas R, Leloup J (1986) The endocrine control of reproduction and molt in emperor (*Aptenodytes forsteri*) and adelie (*Pygoscelis adeliae*) penguins. II. Annual changes in plasma levels of thyroxine and triiodothyronine. Gen Comp Endocrinol 63: 264–274

Hattori M, Wakabayashi K (1979) Isoelectric focusing and gel filtration studies on the heterogeneity of avian pituitary luteinizing hormone. Gen Comp Endocrinol 39: 215–221

Hori H (1996) Humbolt penguins in Japan; Their management and conservation. Abstracts of 4th Intern Aquarium Congr Tokyo 86

Jordan RS (1980) Biology of the anchoreta. 1. Summary of the present knowledge. Proc Workshop Phenomenon known a 'El Niño' UNESCO: 249–276

King JR (1981) Energetics of avian moult. Acta XVII Congr Intern Ornithol 312–317

Kojima I (1978) Breeding Humboldt penguins, *Spheniscus humboldti*, at Kyoto zoo. International Yearbook 18: 53–59

Meritt K, King NE (1987) Behavioural sex differences and activity patterns of captive Humboldt penguins. Zoo Biol 6: 129–138

Murphy ME, King JR, Lu J (1988) Malnutrition during the postnupital molt of white-crowned sparrows: feather growth and quality. Canad J Zool 66: 1403–1413

Payne RB (1972) Mechanisms and control of molt. In "Avian Biology Vol 2" Ed by DS Farner, JR King, Academic Press, New York and London, pp 103–155

Peczely P (1985) Adrenocortical adaptation of birds. In "The Endocrine System and the Environment" Ed by BK Follett, S Ishii, A Chandola, Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin, pp 213–220

Scholten CJ (1987) Breeding biology of the Humboldt penguin *Spheniscus humboldti* at Emmen zoo. International Zoo Yearbook 26: 198–204

Scholten CJ (1989) The timing of molt in relation to age, sex and breeding status in a group of captive Humboldt penguins (*Spheniscus humboldti*) at Emmen zoo, the Netherlands. Netherlands J Zool 39: 113–125

Scholten CJ (1992) Choice of nest-site and mate in Humboldt penguins (*Spheniscus humboldti*). Spheniscid Penguin Newsletter 5: 3–13

Stonehouse B (1967) The general biology and thermal balance of penguins. Adv Ecol Res 4: 131–196

Tasaki Y, Inoue M, Ishii S (1986) Annual cycle of plasma thyroid hormone levels in the toad *Bufo japonicus*. Gen Comp Endocrinol 62: 404–410

Warham J (1972) Aspects of the biology of the erect-crested penguin, *Eudyptes scalateri*. Ardea 60: 145–184

Williams TD (1992) Reproductive endocrinology of macaroni (*Eudytes chrysolophus*) and gentoo (*Pygoscelis papua*) penguins. I. Seasonal changes in plasma levels of gonadal steroids and LH in breeding adults. Gen Comp Endocrinol 85: 230–240

Williams TD (1995) The Penguins. Oxford University Press, Oxford, New York, Tokyo

Wingfield JC, Newman A, Hunt GL Jr, Farner DS (1980) Androgen high in concentration in the blood of female western gulls, *Larus occidentalis wymani*. Naturwissenshaften 67 S 514

Wingfield JC, Newman A, Hunt GL Jr, Farner DS (1982) Endocrine aspects of female-female pairing in the western gull (*Larus occidentalis wymani*). Anim Behav 30: 9–22

Wingfield JC, Farner DS (1994) Endocrinology of reproduction in wild species. In "Avian Biology Vol 9" Ed by DS Farner, JR King, KC Parkes. Academic Press, London, San Diego, New York Boston, Sydney, Tokyo and Toronto, pp 163–327

(Received June 4, 1997 / Accepted September 8, 1997)