

Hormonal correlations at transition from reproduction to molting in an annual life cycle of Humboldt penguins (*Spheniscus humboldti*)

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Abstract

To understand the hormonal mechanism behind a unique strategy of breeding and molting in Humboldt penguins, six pairs of captive Humboldt penguins kept in an outdoor open display pen were observed and blood collected weekly for a year. They all molted between the middle of June and the middle of August within 10 days except one pair that molted about a month later. The late pair had been rearing a hatchling until July due to the successful second clutch after the first clutch failed. A peak of plasma levels of thyroxine and triiodothyronine, respectively, overlapped a period of molting in both sexes. Plasma testosterone concentrations in the males and females were lowest for two months during a period of pre-molt and molting. Plasma concentrations of estradiol were also lowest during the molt in both sexes. Except for the period of molting, sex steroid hormone concentrations were high although there was great individual variation. During the molt, the birds were forced to fast since they did not enter the pool in the display pen where they usually forage live fish. To compensate this forced fasting, they took more food than usual during pre-molting period and gained body mass to about 20% more than the baseline value. Increased flipper thickness was parallel to increased body mass indicating that the gained body mass attributed to fat reservoir. These data indicate that rapid molting in Humboldt penguins is correlated with a drastic increase and decrease of thyroid hormones during the period of lowest concentrations in sex steroid hormones.

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1. Introduction

A clear annual cycle of physiological and behavioral events shown by birds living in the temperate zone is precisely regulated by hormone secretion that is timed by environmental cues (see for review Wingfield and Farner, 1993). The most eminent seasonal events are reproduction, molting and migration. Many studies focused on the hormonal mechanisms that regulate onset and termination of reproduction but there are far less studies on the timing of molting and migration. Molt, a process of replacing old worn feathers with new ones, is an important event in the annual life cycle of birds (see for review Payne, 1972). It is an energy-demanding process (see for review King, 1981) and in most avian

species, especially birds living in the temperate region, molt does not overlap with other costly events of life stage, such as reproduction and migration. This indicates that there is a precise mechanism regulating the transition from breeding to molting. However, molting is also a highly adaptive life history phase for birds living in a variety of environments, varying in duration, frequency, and timing (Ginn and Melville, 1983).

Penguins are extremely adapted to marine life and show a clear transition from reproduction to molting. Since their feathers protect the skin against water and work as an insulator (Stonehouse, 1967), even partial wear and baldness of body feathers decrease swimming ability and insulation. Thus during molt penguins do not enter the sea to forage and are forced to fast. To allow energy-demanding molt during a period of forced starvation, penguins take a strategy to become hyperphagic during the pre-molting period and replace their

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entire plumage over a short period of less than 2 weeks. Most species of penguins molt after breeding (post-nuptial molt) during summer (December–April in the Southern Hemisphere). Thus penguins are excellent model species to study the hormonal mechanism of the transition from reproduction to molt.

So far most physiological studies of penguin species were conducted using Antarctic and sub-Antarctic ones such as Emperor (*Aptenodytes forsteri*) and adelic (*Pygoscelis adeliae*) penguins (Groscolas and Leloup, 1986; Groscolas et al., 1986), adelic penguins (McQueen et al., 1999), macaroni (*Eudyptes chrysolophus*) and gentoo (*Pygoscelis papua*) penguins (Williams, 1992a,b), yellow-eyed penguins (*Megadyptes antipodes*) (Cockrem and Seddon, 1994), and gentoo penguins (Mauget et al., 1995) in the field and focused on reproduction. A very few studies on penguins of temperate zone are on fiordland penguins (*Eudyptes pachyrhynchus*) (McQueen et al., 1998) and Magellanic penguins (*Spheniscus magellanicus*) (Fowler et al., 1994).

Humboldt penguins, a member of spheniscid penguins, are not an Antarctic or sub-Antarctic species but their breeding ranges extend along the Pacific coast of South America from Foca island (5°S) in Peru to Algarrobo (33°S) in Chile, with a newly documented detached colony in Punihull islands (42°S) (Williams, 1995). Since they live in temperate to tropical regions and have less need than other penguin species to keep their young warm, they usually do not tend to have precise breeding seasons, and will breed throughout the year (Paredes et al., 2002; Simeone et al., 2002; Williams, 1995). The female will usually lay two eggs, and it is quite common in better breeding localities that both chicks are successfully reared. Chicks stay in the nest until they are almost ready to molt from hatchling down to juvenile plumage. One parent will remain on guard duty as long as possible until demand for food of the growing chicks becomes too great for a single parent to fill. Since Humboldt penguins breed in the temperate region, their regulation of molt is interesting to compare to that of many temperate-zone passerine birds.

The abundance of Humboldt penguins has decreased by 70% in 15 years to about 7500 individuals in 1996 (Araya and Bernal, 1996), and hence the species is listed as endangered. Therefore, conservation-oriented and ecological studies of this species are found in literatures like on satellite tracking (Culik and LunaJorquera, 1997a,b), status and distribution (Paredes et al., 2003; Simeone et al., 2002), microsatellite analysis (Schlosser et al., 2003) and sex determination from morphological parameters (Zavalaga and Pardes, 1997). However, there are no physiological studies using wild population of this species and now it become more difficult to conduct endocrinological studies in field. On the other hand, Humboldt penguins adapt well to conditions at zoos and breed successfully in captivity. There are a few

studies using captive Humboldt penguins such as Meritt and King (1987) and Scholten (1987, 1989, 1992) in America and Europe, respectively, which report mostly behavioral observation and no physiological studies on their breeding and molting. Successfully breeding Humboldt penguins in Japanese zoos and aquaria provide a good opportunity for such studies.

Using captive Humboldt penguins kept in an open display at a zoo, we collected hormonal data during molt (Otsuka et al., 1998). The data indicated a drastic increase in circulating thyroxine at the onset of molt in late July. During the pre-molt and molting period, plasma concentrations of testosterone and estradiol were low and LH began to increase during molting. However, plasma corticosterone concentrations did not show a clear association with molting. The results showed that the elevated levels of circulating thyroxine correlate with the onset of molting when the circulating sex steroid hormones are low (Otsuka et al., 1998).

However, due to constraint in data collection, the sampling interval in the previous study was not frequent enough to detect precise changes in hormone concentrations and the sampling period covered only a molting period with a few weeks of pre- and post-molt. More frequently sampled data are necessary for explaining the hormonal mechanisms of switching from reproduction to molting and of the process of molting. The aim of the present paper is thus to clarify changes in circulating thyroxine, triiodothyronine, and sex steroid hormones in relation to reproduction and molting in Humboldt penguins throughout the year.

2. Materials and methods

2.1. Animals

The study was conducted at Tokyo Sea Life Park, located in Edogawa-ku, Tokyo (35°38'N, 139°53'E) using captive Humboldt penguins (*Spheniscus humboldti*). The park has an outdoor open field pen (1095 m² with a pool (314.5 m² with 400 tons of water) which holds a mixed colony of Humboldt penguins and Rockhopper penguins (*E. chrysocome*) (the number of Humboldt penguins was 111 at July 1, 1995). Each pair had a nest site made of artificial rock for breeding. Color wing bands were used to identify individual penguins. We selected 6 pairs of adult Humboldt penguins that had shown reproductive activity in the previous years. All birds were born in zoos or aquaria in Japan and their ages ranged from 3 to 17 years at the beginning of the experiment in November 1994.

The birds were fed fresh horse mackerels and silver stripe round herrings which were given in the pool every day except Monday, a regular closed day of the aquarium.

2.2. Observations of breeding and molting

Most pairs use the same nest site for years. We observed and recorded their breeding behavior such as nest building, egg laying, incubating and chick rearing throughout the experimental period from November, 1994 to October, 1995. Dates of hatching or removal of eggs were also recorded for each nest. The clutch size of Humboldt penguins is two, and the date of egg laying reported here is for the first egg. Eggs that were accidentally broken or did not hatch were removed after the incubation period. In some cases, however, unhatched eggs were kept in the nest beyond the incubation period to prevent population increase in the pen.

Molting is a process during which new feathers grow under the skin, extruding the old feathers as they emerge. However, it was impossible to detect initiation of the process by external observation. Therefore we defined a period of molt in this paper as the time between the date the first new feathers appeared and the date the last old worn-out feathers were lost. During molting, the penguins did not enter the pool and feed.

2.3. Sample collection

We collected blood samples from 12 Humboldt penguins (6 pairs) every week between 13:00 and 17:00 from November 1994 to October 1995. A 2-ml blood sample was taken from a leg vein with a 25 G needle and syringe and transferred into a heparinized tube. The blood samples were centrifuged and the plasma stored frozen until the assay. Fifty-one to 52 samples were obtained from each bird.

At the time of blood collection, the birds were weighed to the nearest 0.01 kg as the previous study. Before we started the observations, we obtained a body of Humboldt penguin died during normal molting. We dissected the body and found thick fat depositions on the abdominal wall and flippers. Since measurement of thickness at the abdominal wall was impossible with a caliper, we measured the thickness of the right flipper at the joint of the humerus and ulna with a caliper to the nearest 0.01 mm. The stage of molt was evaluated and recorded.

2.4. Hormone assays

To avoid inter-assay variation, all samples were assayed together for each hormone. Plasma concentrations of testosterone (T) and estradiol (E2) were assayed, respectively, in 10 μ l plasma sample volumes in duplicate using a radioimmunoassay method described by Wada et al. (1999). T and E2 were assayed using separate plasma samples without any extraction. Antisera (HAC-AA61-02-RBP81 for T and HAC-AA64-02RBP79 for E2) were a gift of Professor K. Wakabayashi (Gunma

University). Percent cross-reactivities were as follows. Testosterone: 5 α -DHT, 100; Progesterone, 0.001; 17 α -OH-progesterone, 0.002; Estradiol, 0.03; Dehydroepiandrosterone, 0.03; Androstenedione, 0.04. Estradiol: Estrone, <1; Estriol, <1. Intra-assay variations were 5.7% for T and 5.8% for E2. Plasma concentrations of thyroxine (T4) and triiodothyronine (T3) were determined in 5 μ l sample volumes in duplicate by specific radioimmunoassay according to Otsuka et al. (1998) using antisera obtained from Endocrine Sciences (Calabasas Hills, California). According to the manufacturer's specification, percent cross-reactivities were as follows. T4: D-Thyroxine, 69.6; Triiodothyronine, 3.7; Diiodothyronine, <0.02. T3: D-Thyroxine, 2.7; Diiodothyronine, 1.1; L-Thyroxine, 0.25. Intra-assay variations for T4 and T3 were 15.5 and 14.6%, respectively.

2.5. Statistics

Data were analyzed using repeated measures analysis of variance (RM-ANOVA) and then paired *t* test using StatView ver. 5.0 (SAS International) running on Window 2000. Differences were considered significant when $P < 0.05$. Throughout the text, data are the means \pm SEM.

3. Results

3.1. Breeding and molting

Humboldt penguins kept in the open display pen under natural climatic conditions in Japan showed breeding activity almost throughout year except for a period of molt that occurred more or less at the same time in all pairs. Timing of egg-laying, in contrast, was not synchronized among the pairs (Fig. 1). The females laid eggs and the pairs shared incubation on shift as

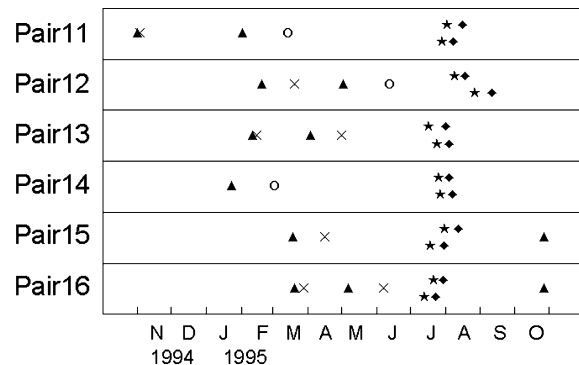


Fig. 1. Observed breeding activity and molting in pairs of Humboldt penguins kept in an outdoor display at Tokyo Sea Life Park from November 1994 to October 1995. Symbols are as follows: ▲, egg-laying; ○, hatching; ×, egg broken or removed; ★, beginning of molt; ◆, end of molt.

observed in the original habitat. If the clutch was successfully hatched, they reared hatchlings to fledglings (Pairs 11, 12, and 14) and the female did not lay a next clutch before the molt. If the clutch was unsuccessful and there was enough time before molting, they laid a next clutch about one and half month after clutch loss (Pairs 13 and 16). Thus one pair (Pair 14) had only one clutch, 4 pairs (Pairs 11, 12, 13, and 15) had 2 clutches and one pair (Pair 16) had 3 clutches in the observed period (Fig. 1).

Irrespective of success or failure of a preceding clutch, all birds molted during late July–mid-August (Fig. 1). An exceptionally late case was Pair 12. The pair had the second clutch on June 12 after unsuccessful incubation of the first clutch. They succeeded to hatch the second clutch and reared the chick until early August. The male of the pair began to molt in mid August and the female in late August.

The onset of molt was slightly different between sexes for each pair, but the duration of molt was not significantly different between sexes (males 10.3 ± 1.1 days and females 10.7 ± 0.8 days).

3.2. Body mass and flipper thickness

The males were always heavier than the females but the temporal patterns of changes in body mass of both sexes were parallel each other throughout the year (Fig. 2). In both sexes, body mass was rather stable with small fluctuations except during the molting period

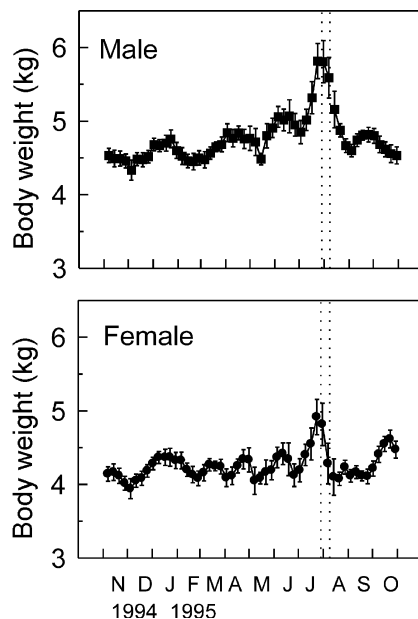


Fig. 2. Changes in body weight of male (top graph) and female (bottom graph) Humboldt penguins throughout a year. Values plotted are the means \pm SEM ($n = 6$, both sexes). Two parallel vertical lines are the mean date of the beginning of molt (July 29, both sexes) and the end of molt (August 8 for males, August 9 for females), respectively.

when it showed a drastic increase and decrease. This overall annual change is significant in the males (RM-ANOVA, $F(5, 48) = 7.651$, $P < 0.0001$) and in the females (RM-ANOVA, $F(5, 48) = 1.966$, $P = 0.0005$). Peak values found in the late July were significantly heavier than the preceding and following trough values, respectively, in the males ($P < 0.01$) and in the females ($P < 0.05$).

Parallel to the body mass, flipper thickness of both sexes also changed during the experimental period and the profiles were more prominent (Fig. 3). The overall annual variation was significant in the males (RM-ANOVA, $F(5, 46) = 5.308$, $P < 0.0001$) and in the females (RM-ANOVA, $F(5, 44) = 2.917$, $P < 0.0001$). Peak values were observed at the onset of molt and were significantly larger than those of pre- and post-molt values. (male $P < 0.01$ and female $P < 0.05$).

Both in body mass and the flipper thickness, shapes of overall peaks were roughly symmetrical and the decreasing phases overlapped the molting period.

3.3. T and E2

Plasma concentrations of T in both sexes varied significantly throughout the year (RM-ANOVAs, $F(5, 50) = 2.368$, $P < 0.0001$ for the males; $F(5, 48) = 2.791$, $P < 0.0001$ for the females) (Fig. 4). Plasma levels of T became lowest when the body mass and flipper thickness began to increase in both sexes. These lowest values were maintained by the end of molting and increased rapidly in the males and gradually in the females. The males had a prominent peak in September and then decreased by December. In January plasma T

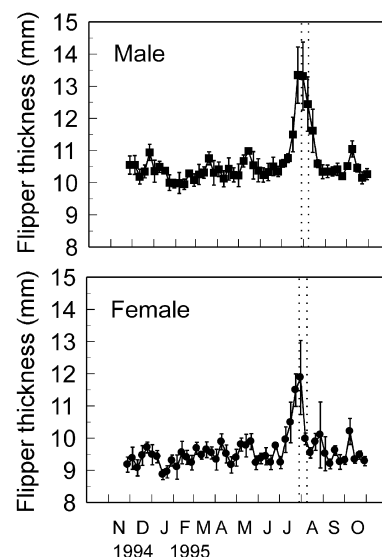


Fig. 3. Changes in flipper thickness of male (top graph) and female (bottom graph) Humboldt penguins throughout one year. Details are as in Fig. 2.

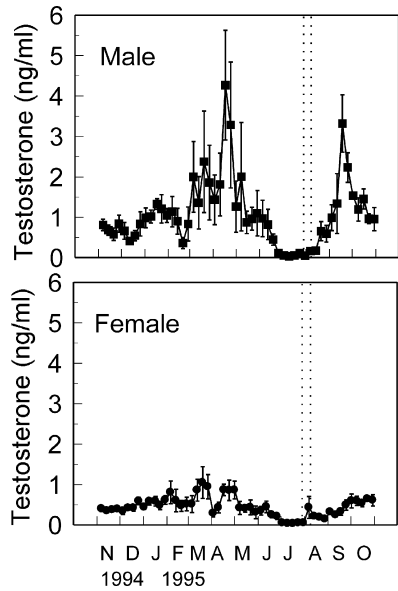


Fig. 4. Changes in plasma concentrations of testosterone in male (top graph) and female (bottom graph) Humboldt penguins throughout one year. Details are as in Fig. 2.

levels again increased and higher levels were maintained by the end of June with great individual variations. The pattern of T in the females was similar to that of the males but the degree of increase was smaller (Fig. 4).

E2 plasma concentrations also showed significant changes throughout the year in the males and females (RM-ANOVAs, $F(5, 50) = 6.300$, $P < 0.0001$ for the males; $F(5, 48) = 6.822$, $P < 0.0001$ for females) (Fig. 5).

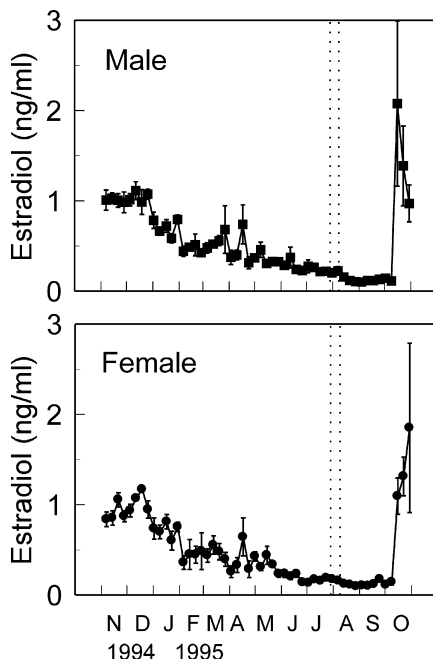


Fig. 5. Changes in plasma concentrations of estradiol in male (top graph) and female (bottom graph) Humboldt penguins throughout one year. Details are as in Fig. 2.

There were no sex differences in absolute levels of E2 and in the temporal pattern throughout the year. The plasma concentrations of E2 were around 1 ng/ml in both sexes at the beginning of the study, in November 1994, and they gradually decreased to minimum levels during molt. In October, two months after the completion of molt, plasma levels of E2 increased drastically in both sexes.

3.4. T4 and T3

Plasma concentrations of T4 changed significantly over the year in both sexes (RM-ANOVAs, $F(5, 50) = 6.851$, $P < 0.0001$ for the males; $F(5, 48) = 5.588$, $P < 0.0001$ for the females) (Fig. 6) with similar temporal patterns in the males and females. The plasma levels of T4 were between 20 and 25 ng/ml at the beginning of the experiment in November 1994. The values decreased gradually until April and kept the low values until the end of June although there was an exceptionally high peak in May in the males. From June, T4 increased at first gradually and then markedly during molting. The drastic increases in plasma T4 concentrations at the molt were significant in the males ($P < 0.05$) and in the females ($P < 0.05$). The following decrease was significant as well ($P < 0.01$ in both sexes). These drastic increase and decrease in T4 concentrations coincided with the period when plasma T concentrations were lowest.

There were significant changes in circulating T3 levels throughout the year in both sexes (RM-ANOVAs, $F(5, 50) = 10.275$ for the males $P < 0.0001$; $F(5, 48) = 4.118$ for the females $P < 0.0001$) (Fig. 7) and no sex

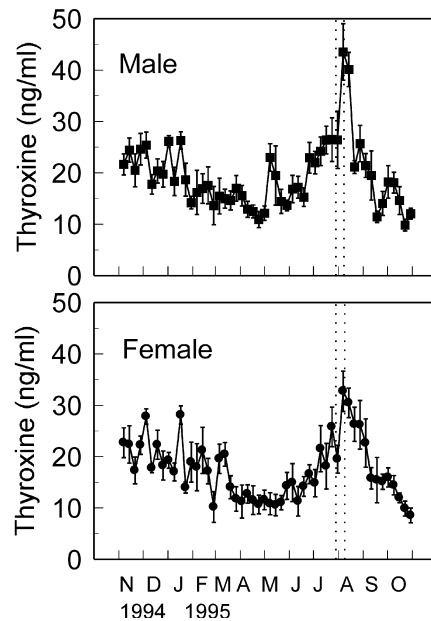


Fig. 6. Changes in plasma concentrations of thyroxine in male (top graph) and female (bottom graph) Humboldt penguins throughout one year. Details are as in Fig. 2.

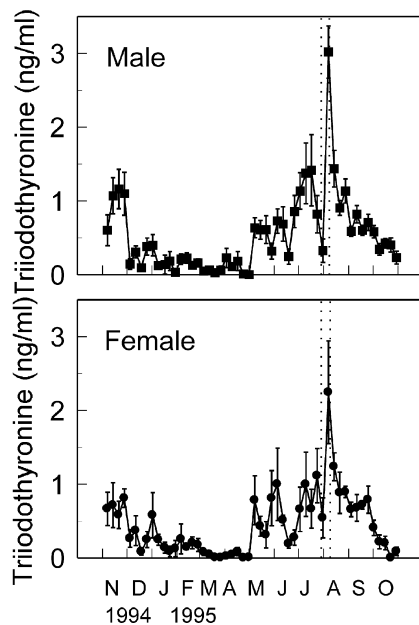


Fig. 7. Changes in plasma concentrations of triiodothyronine in male (top graph) and female (bottom graph) Humboldt penguins throughout one year. Details are as in Fig. 2.

differences in temporal patterns and absolute levels were found. Overall T3 patterns were similar to those of T4 but they were more prominent in shape. From January to May T3 concentrations were very low. Similar to T4 plasma concentrations, the marked increases in plasma concentrations of T3 at molt were significant in the males ($P < 0.01$ at increase and decrease) and in the females ($P < 0.05$ at increase and $P < 0.01$ at decrease).

Both in T4 and T3, the peak values were observed at the end of molting and the plasma concentrations of T4 and T3 rapidly decreased after the end of molt.

4. Discussion

4.1. Annual life cycle of captive Humboldt penguins

The present study extends the previous observation in captive Humboldt penguins (Otsuka et al., 1998) and provides a complete data set of the changes in sex steroid and thyroid hormones throughout one year. Even though the birds were in captivity and kept on outdoor conditions in Japan away from their original habitat, they seem to have acclimated well to the local climate and actually showed breeding activity.

The results indicate that the Humboldt penguins kept on outdoor conditions of Japan have the ability to breed throughout the year except during molt (Fig. 1 and see also Fig. 1 of Otsuka et al., 1998). They seemed to have pursued a strategy to produce at least one successful clutch per year until molt interrupted reproduction. Thus they began to lay eggs in October, 2 months after a

short molting period from July to early August (Fig. 1 and see also Fig. 1 of Otsuka et al., 1998). If they succeeded to hatch the first clutch, they raised the hatchlings and did not have another clutch even though there was enough time for it. If they failed to hatch a first clutch due to unsuccessful development, accidental egg breakage or any other reason, they tended to produce another clutch until July.

This annual cycle observed in captive Humboldt penguins is the same as that observed in their original habitat in the Southern Hemisphere with a shift of half a year. In Chile, two main breeding events were observed, one from August to January as a spring event and the other from April to June as an autumn event (Simeone et al., 2002). Molting occurs mainly in February. The spring event regularly produced offspring and the autumn event was affected by rain resulting nest desertion and making a short break in July. In Peru where the oceanographic and climatic conditions are more favorable, breeding is continuous and not interrupted by rains. Thus the birds in Peru at Punta San Juan, they have 2 clutches showing two well-defined peaks in April and August–September (Paredes et al., 2002). These are interesting because our captive Humboldt penguins seem to have two events of reproductive activity, longer one in spring (from January to June) and shorter one in autumn (from October to November) interrupted by a short break in December, 1994 (present study) and in January 1994 (Otsuka et al., 1998). Thus the annual life cycle of the Humboldt penguins can be divided into two stages, potentially breeding stage and molting stage. Since this species is sedentary at the original habitat, a migration stage is not included in the life cycle and the birds take a strategy to breed as long as they can until they have at least successful one clutch.

Data are limited, but if we gather field data of 4 species of spheniscus penguins, Magellanic penguins (Boersma et al., 1990; Fowler et al., 1994), African (or Jackass) penguin (*Spheniscus demersus*) (Cooper, 1978), Humboldt penguins (Paredes et al., 2002; Simeone et al., 2002), and Galapagos penguins (*Spheniscus mendiculus*) (Boersma, 1978), breeding activity for each species become long in accordance with its distribution and local climatic, oceanographic conditions. Thus Magellanic penguins lay only single clutch of two eggs per year even though failed breeders remain at the colony and engage in nest building, fight, and copulation without relaying. Since they are migratory, they end breeding activity in January, molt in February and then leave the breeding colony. Humboldt penguins and African penguins are sedentary and their breeding activities are comparable. They can breed at any time in a year except for molting but basically they have one clutch a year with an exception of 2 clutches in favorable locality. Galapagos penguins have mostly two clutches and sometimes three per year depending on sea surface temperature. They are

very opportunistic and affected by El Niño. They molt before breeding. The differences in breeding strategies in four species of the genus *Spheniscus* may be a result of expansion of distribution to the north from the original ancestral species in the south.

Captivity usually inhibits breeding activity due to confinement. However, the penguins used here were kept in a relatively wide open pen with a pool and nest sites. Their breeding activity was not suppressed but the penguins showed normal breeding activity including reproductive behavior. Continuous breeding activity shown by the captive Humboldt penguins in the present study may not be modified, but rather it may reflect the breeding activity of the wild population.

4.2. Hormonal patterns and molting

Two periods of fasting are incorporated in the annual life cycle of penguins, one is breeding fast and the other molting fast. All species fast throughout molt but not every species fasts during breeding. There are variations in duration of breeding fast among species. Humboldt penguins that breed at the seashore do not actually fast during breeding in the natural habitat.

The Humboldt penguins in this experiment showed a clear transition from breeding to molting in the end of July irrespective to success or failure in reproductive effort. Timing of molting in captive Humboldt penguins at the Emmen zoo in Netherlands is well documented by Scholten (1989) showing that molt also occurs in July–August. Scholten (1989) suggested that environmental factors such as photoperiod, temperature and food availability and individual factors such as age, sex and breeding status influence the timing of molt and the actual timing whether molt begins or being delayed is further influenced by nutritional and hormonal status. Then what is hormonal mechanism controlling the transition from reproduction to molting? Figs. 4–7 indicate that there is a reciprocal relationship between the steroid hormones and thyroid hormones during molt. A postnuptial molt coincided with a very rapid and drastic increase and decrease of T4 that occurred when T and E2 concentrations were lowest. Circulating LH was also lowest at just before molting (Otsuka et al., 1998). During molting, sex steroid hormones are also low in the Antarctic and sub-Antarctic species so far studied (Groscolas and Leloup, 1986).

This rapid increase of T4 is suggested to affect feather growth in emperor and adelic penguins (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 the annual cycle in the emperor and adelic penguins. Cherel et al. (1988) also argued that T4 involvement in molting of king penguins. Using modern strains of turkey hens that have endogenous low level of T4 and relatively a longer period of molting, Queen et al. (1997) showed that sup-

plement of T4 but not of T3 shorten the molting period. These results suggest that T4 is more important for feather growth and molting. The present study showed that the changes in plasma concentrations of both T4 and T3 correlated with molting but the absolute values of T4 were higher than those of T3 (Figs. 6 and 7). Although the exact role of the thyroid hormone in molting still remains unsolved, a rapid increase of T4 may be responsible for the start of molting as a trigger and maintenance of molting.

When the behavioral events including molt and hormonal changes are correlated in each individual bird, the relation between thyroid hormones and sex hormones to molting and reproductive behavior becomes clearer than when looking at the mean of 6 birds (Figs. 8 and 9). For example, Pair 12 laid a second clutch on the second day of May and the egg hatched on June 12 (Fig. 8). The parent reared the hatchling for about 2 months. Since we could not observe the date when the juvenile left the nest, the exact date of the end of parental care could not determine. However, Humboldt penguins usually feed juveniles for two months after hatch, the juvenile might have left nest on August 12. Parallel to this extended period of breeding activity, the plasma concentrations of T4 and T3 of No. 56 (male of Pair 12) was suppressed during this period and they rapidly increased after this period in the first week of August. His molting period was from August 10 to 18. Circulating T4 and T3 concentrations of No. 58 (female of Pair 12) also elevated in early August at the same time as her partner. Circulating T4 concentrations reached the maximum level on August 28 on the day of molt beginning. Her molt finished by September 11.

Pair 16 lost the second clutch after the failure of the first clutch (Fig. 9). The male of Pair 16 started molting six weeks and the female five weeks after their eggs were missing from the nest. These durations were very short compared to the other pairs but the hormonal data of this pair indicate that circulating T4 and T3 increased after this short period to form the first peak at the onset of molting and the second higher peak around the end of molting. The reason for splitting peaks is unknown at the moment.

These individual data clearly indicate that a drastic increase and decrease of circulating T4 coincided with the commencement of molt and the peak was found only when the plasma concentrations of sex steroid hormones were low and basal.

Induced molting by forced fasting is used in poultry industry to reset hens' reproductive activity (see for review Berry, 2003). The jungle fowl, a wild ancestor of the domestic chicken, broods without having food and water resulting in reproductive tract regression and molting. This trait becomes blunted in domestic chickens but can be activated by forced molting. By depriving of food and water, plasma levels of luteinizing hormone and estradiol

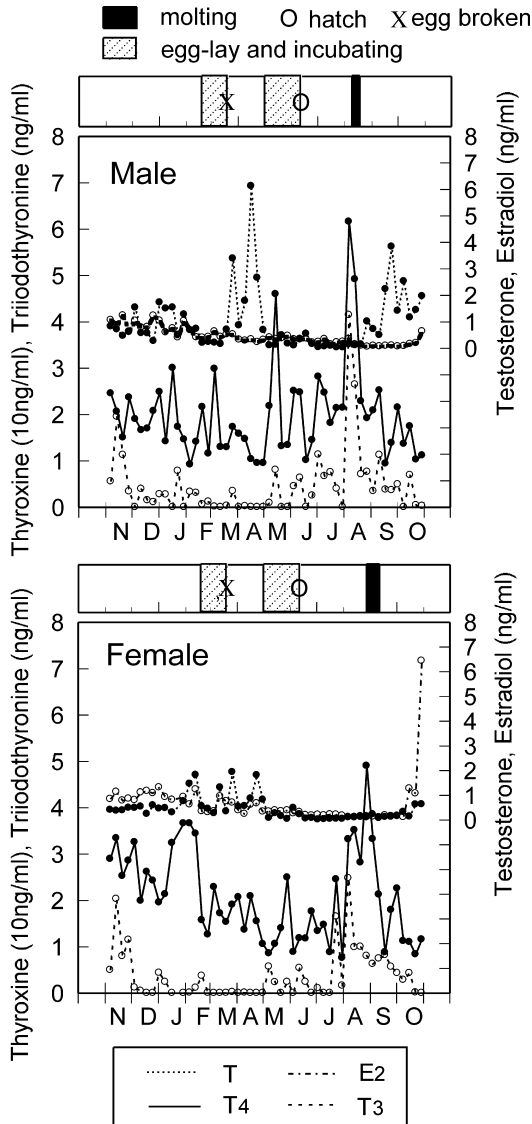


Fig. 8. Changes in plasma concentrations of thyroid hormones and sex steroid hormones in Pair 12. Their reproductive events and molting periods are shown on the top of graph.

decreased from the next day (Day 1) of deprivation and egg-laying terminated on Day 4. The plasma T4 levels increased on Day 5 and remained high until Day 17 whereas T3 levels was lower than the control levels. The T4 increase was triggered by thyrotropin release indicated by thyrotropin mRNA increase (Iwasawa et al., 2002). Food deprivation also induced decreases of gonadotropin mRNA contents in the pituitary gland in male Japanese quail (Kobayashi and Ishii, 2002). These results indicate decreases in gonadotropin secretion and resultant decreases in sex steroid hormones trigger thyrotropin secretion and increases in T4 levels that induce molting in these galliformes species. In Humboldt penguins, fasting is not the first phenomenon for molting but decreases in T levels may trigger thyrotropin secretion and resultant T4 increases.

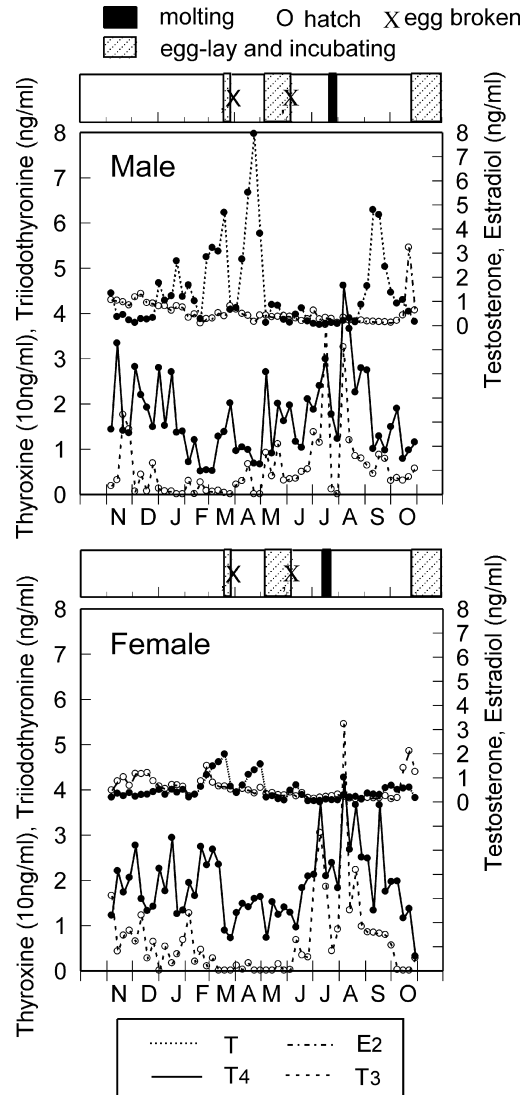


Fig. 9. Changes in plasma concentrations of thyroid hormones and sex steroid hormones in Pair 16. Their reproductive events and molting periods are shown on the top of graph.

4.3. Other roles of sex steroid hormones

The plasma concentrations of T and E2 were high during potentially breeding period, decreasing to minimal levels at pre-molt period suggesting that sex steroids suppress the drastic increases of T4. The results agree well with those in emperor and adelic penguins (Groscolas et al., 1986). In the previous study (Otsuka et al., 1998), we reported that the values of T and E2 in both sexes were almost the same for all sampling periods. However, the period of observation was limited and the plasma concentration of T in male birds at early May was in fact higher than those of female birds. The present data indicate that plasma T concentrations in the males were higher than those in the females during the potentially breeding period and E2 plasma concentrations were comparable in the both sexes.

Plasma concentrations of T in the males showed great individual variations during the breeding stage. However, it is obvious from the individual data that plasma T increased extensively before the egg-laying of the partner (Figs. 8 and 9). Since the egg-laying were not synchronous in each pair, calculation of the mean of T levels made it high with a great individual variation at each sampling point during the breeding period. The increase of T might be responsible for conflict at the nest site to guard the partner from neighbor males. Aggressive behavior at the nest site induced the increase in plasma T concentrations and it may make the T concentration higher (see Wingfield et al., 2000). Also in the females, plasma T concentrations became high around the time of egg-laying (Figs. 8 and 9). High T concentrations in females are also observed in western gulls (Wingfield et al., 1980, 1982). Both species are colonial and not sexually dimorphic, and the females participate in nest guarding. On the other hand, plasma concentrations of T were minimal during incubation in both sexes (Figs. 8 and 9).

4.4. Environmental cues for the cycle

Annual events in birds are under the control of predictive information of photoperiod and also of supplementary information such as food availability (see for review Wingfield and Farner, 1993). Food availability before molting is especially important for the penguins since they cannot forage during molt. In the wild, the pre-molt period when Humboldt penguins have a big appetite overlaps with a time when food resource is abundant. Thus the molting time is December and January (summer) in the Southern Hemisphere (the natural habitat of Humboldt penguins) when schooling fish like anchovies approach the coast. However, the Humboldt penguins used in this experiment were born in Japan in the Northern Hemisphere and were fed constantly all year round, food availability can be eliminated from direct timing cues. They still molted in the local summer (July–August) indicating that molting is not directly influenced by food availability but influenced by other environmental factors such as day length and temperature.

Seasonal maturation of the reproductive system is likely to be controlled by photoperiod in sub-Antarctic and Antarctic species such as adielie penguins (Astheimer and Grau, 1985; Groscolas et al., 1986), emperor penguins (Groscolas et al., 1986) and in macaroni and gentoo penguins (Williams, 1992a,b), before they arrive at the breeding ground. However, Humboldt penguins began to breed after the quiescent period of about 2 months in late autumn. Plasma concentrations of LH in both sexes of Humboldt penguin began to increase during molting (Otsuka et al., 1998) and T and E2 plasma concentrations increased after molting (Figs. 4 and 5).

These results suggest that breeding activity in Humboldt penguins is less dependent on seasonal cues and potentially continuous throughout the year. The molting is an interruption of breeding activity necessary for renewal of plumage conditions once a year. Scholten (1989) suggests that the general timing of molting is controlled by photoperiods and temperature and actual timing is modulated by individual internal conditions. The photoperiod is not necessarily involved in control of the annual life cycle but the individual internal conditions are expressed as circulating steroid hormones and thyroid hormones as indicated in the present experiment.

4.5. Body mass and flipper length

Throughout the sampling period from November 1994 to November 1995, body mass of the males was always heavier than that of the females. The body weight values for the males and females before the pre-molt period are similar to those reported by Scholten (1987) and Otsuka et al. (1998). Body weight increased significantly at pre-molt period in both sexes. This increase in body mass can be attributed to fat deposition which is indicated by thickness of the flipper, subcutaneous fat (Fig. 3). This fat was consumed during molting since the body mass and flipper thickness returned to the basal levels at the end of molting. In contrast, sparrows do not show significant variations in body mass between pre- and post-molt (e.g., Murphy et al., 1988), because they can forage during molting. Thickness of the flipper can be used for monitoring body mass and fat deposition in this species.

4.6. Captive penguins as useful birds to study

Penguins are interesting because their breeding cycles markedly differ from those of most altricial passerines which have been extensively studied. 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 more interesting for comparison to passerine species. We conducted a physiological study on captive Humboldt penguins and obtained results indicating that changes in sex steroids and thyroid hormones control the short term molt in this species. Further studies will clarify the actual mechanisms for molting of the species and also of birds in general.

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