Veterinary Integrative Sciences 2021; 19(2): 209-222. DOI; 10.12982/VIS.2021.019

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Circulating inhibin and testosterone during sexual maturation and reproductive seasonality of captive male killer whales (*Orcinus orca*)

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Abstract

The present study aimed to investigate the reproductive biology of male killer whales. Changes in the concentrations of two circulating testicular hormones, inhibin and testosterone, were monitored during sexual maturation of two male Type 1 Eastern Northern Atlantic killer whales over a period of 20 years. The two killer whales grew rapidly at the pubertal stage and reached a plateau at the age of 23 and 20 years, respectively, after which growth slowed down. In the younger male, circulating inhibin was higher in the juvenile than in the pubertal and mature stages; whereas circulating testosterone exhibited the opposite trend. The pubertal period was estimated to last approximately 5 years, from 12 to 17 years of age. In the elder male, circulating testosterone was high from the onset of this study (12 years of age), when the animal also sired successfully for the first time. This finding shows that the male killer whale is possible to sire even if it is not socially matured, if there is opportunity for copulation. During the mature stage, both animals exhibited significantly higher circulating testosterone concentrations in spring compared to autumn and winter; whereas no seasonal change was observed for circulating inhibin. These results clearly demonstrate that the male killer whale is a seasonal breeder, even though it is fertile throughout the year. This is the first study to elucidate the inhibin concentration and secretory source in the male killer whale.

Keywords: Inhibin, Male killer whale, Sertoli cells, Sexual maturation, Testosterone

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Article history; *received manuscript: 28 August 2020,*
revised manuscript: 18 September 2020,
accepted manuscript: 10 April 2021,
published online: 12 April 2021
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

The killer whale (*Orcinus orca*) is the largest member of the family Delphinidae. Although only one species is recognized as killer whale, ten ecotypes have been reported (Pittman, 2007). Each ecotype is characterized by its own prey preferences, morphology, and behavior. The killer whales used in this study belong to Type 1 Eastern Northern Atlantic ecotype. They are known to feed cooperatively on herring and mackerel, and males can grow up to 6.6 m in length (Pittman, 2007).

Some species of Cetaceans do not present secondary sexual characteristics, and it is difficult to determine when a male gains reproductive potential (Kasuya, 2011). In contrast, male killer whales have a distinctive dorsal fin extension, which allows for the estimation of maturity in the wild. The northern resident population inhabits coastal waters of British Columbia. Extensive investigation through annual photo-identification surveys over the last 30 years has provided historical life parameters for this population. Olesiuk et al. (1990) showed that the male dorsal fin could be statistically distinguished from that of females when a height-to-width ratio reaches 1.4, and it appeared to occur between 11-15 years of age which overlaps the age of the pubertal stage (range 7.5-14.5 years) and denoted sexual maturity. The mean life expectancy of male killer whales is 31 years and maximum longevity is 60–70 years (Olesiuk et al., 2005)

In addition, studies on the growth and reproductive hormone using individuals under human care helped to deepen understanding killer whale reproductive physiology. In female killer whales, circulating progesterone concentrations have been investigated (Robeck et al., 1993, 2004, Katsumata et al., 2006, O'Brien and Robeck, 2010) and the age of sexual maturity and gestation period became clear. Robeck et al. (2016) monitored serum progesterone and estrogens during normal pregnancy in the killer whale. Regarding male killer whale reproductive research, Robeck and Monfort (2006) revealed external morphology and circulating testosterone changes during juvenile, pubertal and mature stages of male killer whales. Furthermore, Robeck et al. (2017) investigated how androgen (testosterone and androstenedione) and glucocorticoid changes influence of age, maturity, and environmental factors. However, one of the testicular hormones, inhibin has not been investigated in male killer whales. Inhibin is a glycoprotein hormone secreted by the gonads in mammals (Medan et al., 2007, Chaichanathong et al., 2017), birds (Akashiba et al., 1988, Yang et al., 2005a, 2005b, 2005c), and some Cetaceans, such as beluga (Katsumata et al., 2012) and bottlenose dolphin (Katsumata et al., 2017).

In the present study, changes in the concentration of circulating inhibin and testosterone in addition to the physical growth (length and weight) during development were investigated.

MATERIALS and METHODS

All procedures were carried out in accordance with the guidelines for marine animals established by the Kamogawa Sea World in 2000.

Animals

Two male killer whales, named “Oscar” and “Bingo”, were used in the present study. As they were acquired from Iceland, their ecotype was inferred to be Type 1 Eastern North Atlantic. Oscar arrived at Kamogawa Sea World, 1464-18 Higashi-cho, Kamogawa, Chiba, Japan (140″5′ east longitude, 35″6′ north latitude) in 1988 at the age of 3 years, and Bingo in 1985 at the age of 4 years. Estimate age was calculated with the growth curve of killer whales born in Kamogawa Sea World (unpublished data) and evaluated the appropriateness compared with Christensen (1984) and Clark et al. (2000). For Oscar, the present study used data from 1988 to 2011 for body length and weight, from 1995 to 2003 for circulating inhibin, and from 1995 to 2011 for circulating testosterone. For Bingo, the present study used data from 1985 to 2011 for body length and weight, from 1993 to 2003 for circulating inhibin, and from 1993 to 2011 for circulating testosterone.

Facility and cohabiting animals

These two males were kept in a facility with two females also transported from Iceland in any combinations. The facility contained 4800 m3 of sea water with a depth of 6 m and consisted of two compartments connected by gated channels. Mean water temperature was 17.8°C; the water was cooled in summer (range: 12.5-19.5°C). Mean air temperature was 16.4°C (range: 4-34°C).

Body length and weight measurement, plus blood sample collection

Body length and weight were measured by placing the killer whales on a stretcher in the first year, but were voluntary after that. Blood samples (10 mL) were collected from the tail fluke vessels with volunteer behavior every 2 to 4 weeks between 08:30 h and 09:00 h. The blood samples were centrifuged at 1700 x g for 15 min at room temperature within 30min. after collection, and serum was maintained at -20°C until assayed.

Hormone assays

Concentrations of testosterone were determined by double-antibody radioimmunoassay using 125I-labeled radioligands as previously described (Taya, et al., 1985). Antisera against testosterone (GDN 250) were used. The intra- and inter-assay coefficients of variation were 6.3% and 7.2%, respectively. Concentrations of immunoreactive (ir-) inhibin were measured using rabbit antiserum against purified bovine inhibin (TNDH-1) and 125I-labeled 32-kDa bovine inhibin, as previously described (Hamada, et al., 1989). The inhibin antiserum (TNDH-1) showed no significant cross reaction with gonadotropin releasing hormone, transforming growth factor, or activin (Kaneko, et al., 1995). Serial dilutions of serum from killer whales showed a parallel response to 32 kDa inhibin (data not shown). The results were expressed in terms of 32-kDa bovine inhibin. The intra- and inter-assay coefficients of variation were 7.0% and 11.4%, respectively.

Statistical analysis

All analyses presented in this study were done using the MS-Excel add-in software Statcel 4. All values are expressed as mean ± SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Growth

Age-related changes in body length and body weight of each animal are shown in Figure 2. Body length of Oscar increased by 134% between 3 years (337 cm) and 8 years of age (452 cm), then by 105% until 12 years (475 cm), and by 120% until 23 years, whereby it reached a plateau (572 cm) (Fig. 1A). Body weight of Oscar increased linearly from 3 years (643 kg) to 23 years of age with approximate formula: $BW = 127.21x + 177.12$ ($R^2=0.9824$) and then reached a plateau (3250 kg) with approximate formula: $BW = 40.578x + 2911.4$ ($R^2=0.4758$) (Fig. 1B). Body length of Bingo increased by 154% from 4 years (353 cm) to 10 years of age (543 cm), then by 108% until 12 years (587 cm), and by 110% until 20 years, whereby it reached a plateau (646 cm) (Fig. 1C). Body weight of Bingo increased linearly from 4 years (643 kg) to 20 years of age with approximate formula: $BW = 196.93x - 134$ ($R^2=0.9937$), whereby it reached a plateau (3750 kg) with approximate formula: $BW = 13.987x + 2756.1$ ($R^2=0.0366$) (Fig. 1 D).

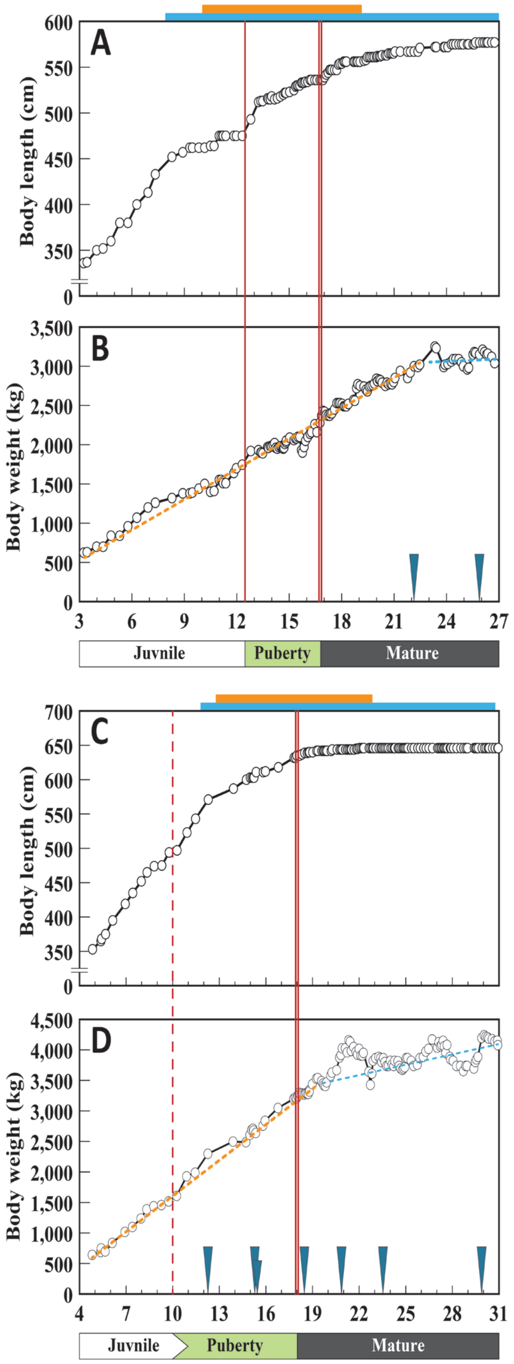

Figure 1 Changes in body length and body weight of the two male killer whales examined: Oscar (A and B, ages 3–27 years) and Bingo (C and D, ages 4–31 years). In panels A and B, the vertical line indicates the onset of puberty, corresponding to the start of sexual maturity, and the double red line indicates maturity. In panels C and D, the dotted red line marks 10 years of age and the double line indicates maturity. Bingo had already reached puberty at the time this study began. Down-pointing triangles indicate the time of mating that resulted in pregnancy. Orange and blue lines indicate the period, during which inhibin and testosterone were measured. In panels B and D, linear approximation of the growth phase and plateau phase in orange and blue dot lines respectively.

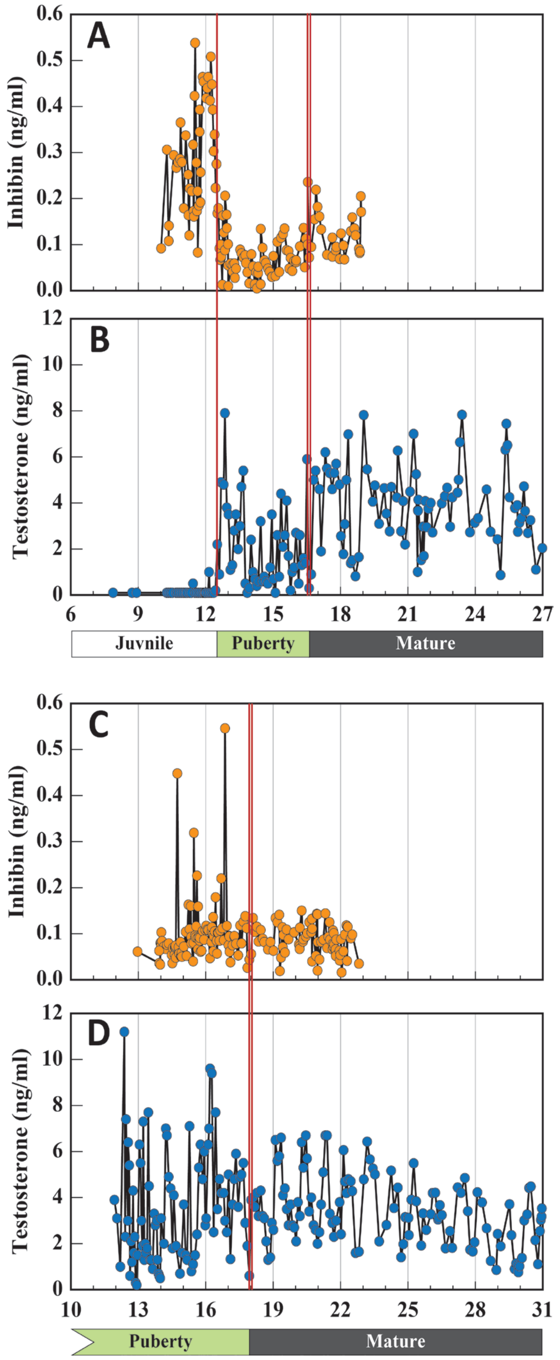

Figure 2 Age-related changes in serum concentrations of inhibin and testosterone in the two male killer whales examined: Oscar (A, ages 10–19 years; B, ages 7–27 years) and Bingo (C, ages 13–22 years; D, ages 12–31 years). In panels A and B, the vertical red line marks the onset of puberty, as detected by an increase in circulating testosterone concentrations, and the double red line indicates maturity. In panels C and D, the double line indicates maturity. Bingo had already reached puberty at 12 years of age, as detected by an increase in circulating testosterone concentrations.

Reproductive activity

Age of successful copulation of two male killer whales with females is indicated by arrowheads in Fig. 1B and Fig. 1D. Oscar copulated at the age of 22 (April, 2007) and 26 (November, 2011), and the female gave birth normally 18 months later (Fig. 1B). Bingo copulated seven times with two females at the age of 12 (August, 1993), 16 (with both females) (June and July, 1996), 18 (August, 1999), 20 (December, 2001), 23 (August, 2004), and 30 (June, 2011), and the females gave birth after 18 months except one case, which resulted in stillbirth after 15 months (Fig. 1D).

Testicular endocrine activity

Age-related changes in circulating inhibin and testosterone concentrations of the two male killer whales are shown in Figure 3. In Oscar, circulating inhibin was high (maximum 0.54 ng/mL) during the first 10–12 years of age, but declined at 13 years, whereby circulating testosterone increased (Figs. 3A, B). Thereafter, circulating inhibin was maintained at basal concentrations (0.03-0.16 ng/mL) until 19 years. Circulating testosterone concentrations remained low (< 1 ng/mL) from 8 to 12 years of age, but rose sharply to 4.90 ng/mL at 12 years (body length 512 cm, body weight 1930 kg). Testosterone concentrations kept on fluctuating widely as they returned to < 1 ng/mL between the ages of 13 and 17 years, and then increased on a regular annual basis from 17 to 27 years (Fig. 1B). The point characterized by the sudden increase in circulating testosterone at the age of 12 years was deemed to mark the onset of puberty and hence sexual maturity. Based on these results, the reproductive process in Oscar was divided into three stages: juvenile (before 12 years of age), puberty (12 to 17 years of age), and maturity (over 17 years of age).

At 12 years of age, Bingo was considered already in puberty at the onset of this study. The concentration of circulating inhibin showed some spikes (0.55 ng/mL maximally) at the age of 14–16 years, followed by a lower level (0.06-0.15 ng/mL) after the age of 17 (Fig. 2C). Circulating testosterone showed again a large fluctuation, with the basal value remaining < 1 ng/mL from 12 through 17 years of age, after which it increased on a regular annual basis until 31 years (Fig. 2D). Hence, the reproductive process in Bingo was divided into two stages: puberty (from age 12 to 17) and maturity (over 18 years of age).

The average concentration of circulating inhibin and testosterone in juvenile, pubertal, and mature animals for both Oscar and Bingo is shown in Figure 3. A significant difference in the concentration of circulating inhibin was detected between juvenile and pubertal or mature stages in Oscar (Fig. 3A). At the same time, the average concentration of circulating testosterone was significantly higher in the pubertal and mature stages compared to the juvenile stage (Fig. 3B).

No significant difference in circulating inhibin was observed between Bingo and Oscar in the mature stage (Fig. 3A), however there was a difference in circulating testosterone during the pubertal stage. In Oscar, circulating testosterone increased stepwise from the juvenile to the mature stage; whereas in Bingo, the value was as high in puberty as in the mature stage.

Seasonal changes in circulating inhibin and testosterone of Oscar and Bingo are shown in Figure 5. The values were divided in four seasons based on the definition of Japan Meteorological Agency as follows: winter (December–February), spring (March–May), summer (June–August), and fall (September–November). Seasonality was analyzed separately for three stages. No seasonal changes were observed in circulating inhibin in any of the animals at any stage (Fig. 4A). After reaching sexual maturity, circulating testosterone showed a clear seasonal change in both animals, with the level being higher in spring compared to fall and winter. In contrast, no seasonal changes were observed in circulating testosterone concentrations before sexual maturity in Oscar (Fig. 3B).

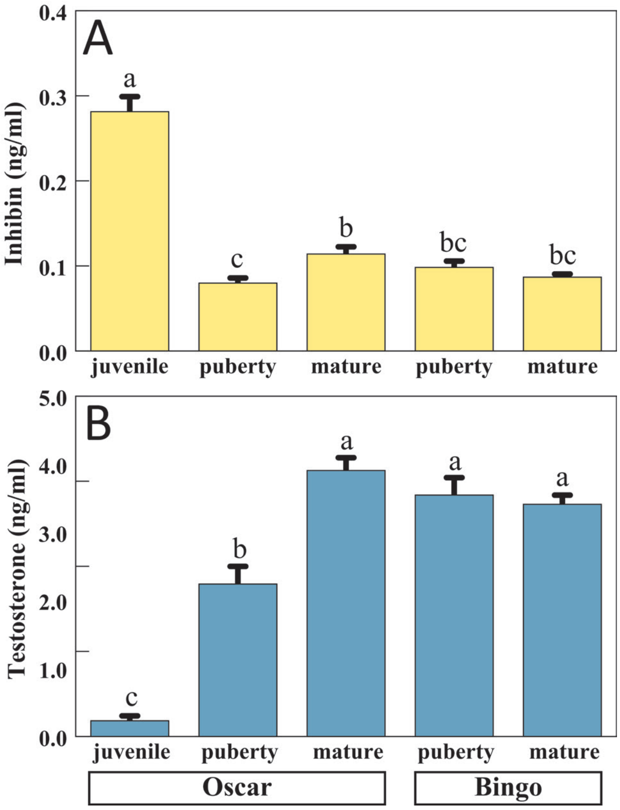

Figure 3 Serum concentration of inhibin (A) and testosterone (B) at juvenile, puberty, and mature stages in the two male killer whales examined. Values followed by different letters represent significant differences at $P < 0.05$. Serum concentration of inhibin (A) and testosterone (B) at juvenile, puberty, and mature stages in the two male killer whales examined.

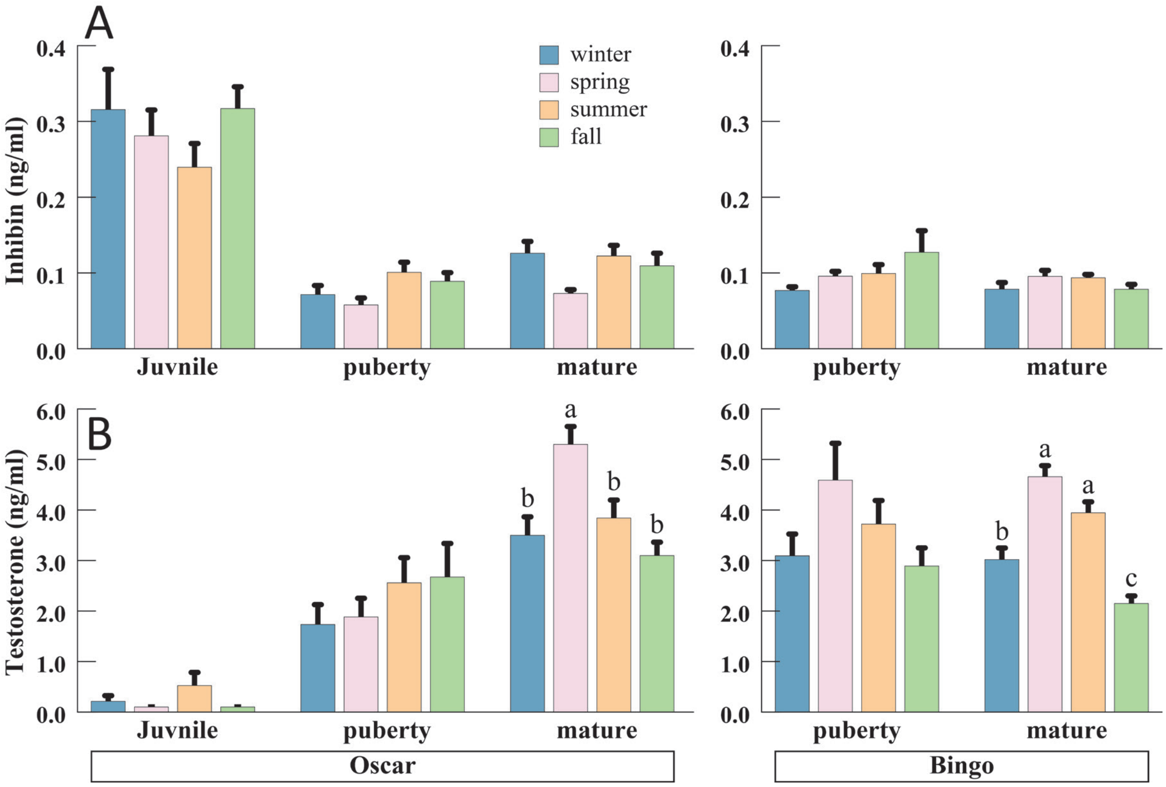

Figure 4 Seasonal change in circulating inhibin (A) and testosterone (B) of the two male killer whales examined. Seasons were classified as winter (December–February), spring (March–May), summer (June–August) and autumn (September–November) (Japan Meteorological Agency). Values followed by different letters represent significant differences at $P < 0.05$.

DISCUSSION

In the present study, changes in two testicular hormones and growth parameters during sexual maturation were investigated using two captive male Type 1 Eastern Northern Atlantic killer whales. The dorsal fin height, body length and weight were used here as markers of growth (Olesiuk, et al. 1990, 2005, Clark and Odell, 1999, Robeck et al., 2004).

Present results clearly demonstrate that the onset of puberty and sexual maturity of the two killer whales occurred at around 12 and 17 year of age, respectively. In Oscar, one of the killer whales, body length and weight increased sharply after the onset of puberty (475 cm and 1700 kg, respectively), followed by a slow increase after sexual maturity (539 cm, 2400 kg, respectively) and reaching of a plateau (577 cm, 3170 kg) at the age of 24 years.

Pubertal "spurts in growth rate" are a characteristic of the male growth curve (Bogin, 1999). Here, a sharp increase in body length during the pubertal stage of two male killer whales was presumed to be a spurt in growth rate. The rapid growth during puberty seen in males is typical of mammals, in which males grow larger than females, as in the case of humans (Bogin, 1999), northern elephant seals (Clinton, 1994), sperm whales (Best, 1970), pilot whales (Kasuya and Marsh, 1984; Kasuya, 2011), and killer whale (Christensen, 1984). The increase in body length then slows down after maturation. The elder male, Bingo, first mated with a female at 12 years of age (571 cm, 2300 kg), and the female gave birth after 18 months. These results clearly demonstrate that fertility in puberty does not depend on social maturation but only on the possibility for copulation. Supporting the present study, reported an 8-year-old male killer whale being capable of siring; thus suggesting that killer whales in captivity might be well nourished and sexually mature earlier than their wild counterparts. In the wild, killer whale mean age at onset of sexual maturity was estimated to be 13 years and mean pubertal stage was 5.5 years, implying that the mean at onset of physical maturity was 18.4 years (Olesiuk, et al., 1990, 2005). In our study, the length of the maturation process for the two male killer whales yielded similar results to wild killer whales. The difference in the age of maturity of killer whales between this study and a pervious study (Robeck et al., 2004) under human care could be the differences such as facility size, food type, group composition, exercise, etc.

The average concentration of circulating testosterone was significantly higher in the pubertal and mature stages than in the juvenile stage. While there was no significant difference between Bingo and Oscar in the mature stage, such difference existed during puberty. This discrepancy may relate to the different hierarchical position of the two males, defined by a dominant and a second male.

The circulating inhibin concentrations in Oscar were high in the juvenile stage and declined abruptly after sexual maturity. This dramatic change in circulating inhibin concentrations showed a clear negative correlation with testosterone during the mature stage, confirming previous reports in beluga (*Delphinapterus leucas*) (Katsumata et al., 2012) and bottlenose dolphins (*Tursiops truncatus*) (Katsumata et al., 2017). Importantly, the same high concentrations of circulating inhibin prior to sexual maturity coupled with low concentrations thereafter have been observed also in terrestrial mammals, such as bull calves (Kaneko, et al., 2001, 2006, Matsuzaki, et al, 2000, 2001a, 2001b), boars (Kanematsu et al., 2006), and horses (Dhakal et al., 2011, 2012). Analysis of male bottlenose dolphins (Katsumata et al., 2017) revealed that while follicle-stimulating hormone (FSH) concentrations were low in the immature stage, they were high in the mature stage, showing an opposite pattern to inhibin. Such negative relationship between FSH and inhibin during the developing stage has been documented also in bull calves (Kaneko et al., 2001, 2006, Matsuzaki et al., 2000, 2001), boars (Kanematsu et al., 2006), and horses (Dhakal et al., 2011, 2012), suggesting that inhibin functions as a suppressor of FSH secretion in male killer whales. Although the exact mechanism of inhibin decrease and testosterone increase during sexual maturity is not known, it could be due to suppressed secretion of kisspeptin and gonadotropin-releasing hormone in the hypothalamus caused, in turn, by the elevated concentration of testosterone. This would point to development of the hypothalamus-pituitary testicular axis with sexual maturity.

In the present study, the seasonal change in circulating hormone concentrations revealed the highest serum concentrations of testosterone in the spring (March–May) and the lowest in autumn (September–November) in the mature stage. In contrast, there was no seasonal effect on circulating inhibin. These seasonal changes are consistent with the results of studies on bottlenose dolphins (Katsumata et al., 2017, Schroder, 1990) and existing evidence on killer whales (Robeck et al., 2004, O'Brien et al., 2017). According to the latter, seasonal fluctuations in testosterone secretion within each animal had no bearing on sperm production. Even during periods of relatively low testosterone secretion, spermatogenesis is apparently maintained in killer whales (Robeck and Monfort, 2006). These findings support the present results, whereby male killer whales copulated with females and conception occurred throughout the year, in spite of seasonal alterations in testosterone secretion. Supporting the present findings, previous studies also reported that conceptions of killer whales peaked between spring and fall, but occurred regularly also throughout the year (Olesiuk et al., 2005).

CONCLUSION

Circulating concentrations of two testicular hormones, inhibin and testosterone, were measured at monthly intervals in two male Type 1 Eastern North Atlantic killer whales from during developmental stages. Analyses were performed for examination of the relationships of age, sexual maturation, and season. Hormone profiles established that up-regulation of testosterone production occurs at puberty. Testosterone production in mature period was influenced by season, concentrations of testosterone were higher in spring than the other season. On the other hand, circulating concentrations of inhibin was high in juvenile period as compared with pubertal and mature periods, and no seasonal changes were observed. The testis maintains the ability to produce sperm throughout the year. This is the first study to elucidate the inhibin concentration and secretory source in the male killer whale. This new insight on the species reproductive biology will help breeding and conservation efforts.

ACKNOWLEDGEMENTS

We are grateful to Dr. G. D. Niswender, Animal Reproduction and Biotechnology Laboratory, Colorado State University (Fort Collins, CO, USA) for providing antisera to testosterone (GDN250). We thank Dr. N. Ling (Neuroendocrine Inc., San Diego, CA, U.S.A.) for providing [Tyr30]-inhibin α (1–30). We express our appreciation to the members of the Kamogawa Sea World, Kamogawa, Chiba, Japan. We would like to thank Editage (www.editage.com) for English language editing.

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Supervision: K. Taya
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Writing – review & editing: E. Katsumata, K. Taya

All authors approved the manuscript to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT ON INTEREST

The authors declare no conflict of interest.

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How to cite this article;

Hiroshi Katsumata, Etsuko Katsumata, Sukanya Jaroenporn, Yoko Ueda,Seiki Konno, Gen Watanabe and Kazuyoshi Taya. Circulating inhibin and testosterone during sexual maturation and reproductive seasonality of captive male killer whales (*Orcinus orca*). Veterinary Integrative Sciences. 2021; 19(2): 209-222.