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Short Note

A Case Report: Hematology, Serum Chemistry Values, and Seasonal Change of Serum Testosterone and Testes Size in Pygmy Killer Whale (*Feresa attenuata*)

Keisuke Kondo[1] and Junpei Tsuchie[2]

[1]*MARINE WORLD uminonakamichi, 18-28, Saitozaki, Higashiku, Fukuoka, Fukuoka, Japan*
E-mail: k.kondo@marine-world.co.jp
[2]*Kobe Animals and Plants Environmental College, 116, Koyochonaka, Higashinadaku, Kobe, Hyogo, Japan*

Pygmy killer whales (*Feresa attenuata*; Gray, 1874) are members of the family Delphinidae, distributed in tropical to subtropical oceans worldwide. Despite their large distributions, little is known about this species (Taylor et al., 2008). There have been some reports of pygmy killer whales that were stranded, rescued, or captured by fishers (Terasawa et al., 1997; Rodríguez-López & Mignucci Giannoni, 1999; Montie et al., 2011; Clua et al., 2014); however, none of these animals survived long under human care.

In this short note, we report on a male pygmy killer whale held in human care for 985 days after a successful stranding recovery; this is the longest record for keeping this species to our knowledge. While in human care, we examined this male's hematology and blood chemistry values to estimate potential ranges associated with good health. Also, for a one-year period of time, we documented seasonal changes of serum testosterone and testes size by ultrasonography. These data contribute to a better understanding of this species and will facilitate future rescue and rehabilitation of this species.

Rescue of Pygmy Killer Whale

On 26 September 2014, a male pygmy killer whale was found stranded at Nata Harbor, Fukuoka, Japan. The animal was rescued by MARINE WORLD uminonakamichi aquarium staff and transported to the aquarium. After transportation, a general body examination was performed that included fecal, blood, and gastric fluid sample collections. Body length was 233 cm and mass was 110 kg, which was less than expected according to the length/mass relationship formula (Ross & Leatherwood, 1994). The whale sometimes coughed up bloody phlegm, suggesting pneumonia. Parasite eggs were found in the feces. The animal showed a very low appetite, and gastric fluid was slightly bloody, suggesting a gastric ulcer; thus, proper medical care, including administration of antibacterial drugs, anthelmintic agents, and gastric mucosa protection agents, was conducted.

On 25 December 2014, the animal exhibited acute renal failure and vasculitis at the right dorsal side of the fluke, which were managed by administration of lactated Ringer solution until the animal recovered on 20 January 2015. The necrotic tissue in the fluke lesion was debrided, and the wound healed. Blood test values stabilized in March 2015, and the animal started to show good appetite and normal behavior; thus, the animal was assessed as healthy by the aquarium veterinarian. Sperm cells were observed in urine on 25 December 2015, suggesting sexual maturity (Figure 1).

Occasionally, the animal presented a bacterial infection, hypernatremia, and chronic hematuria, which were readily treated. Diet included frozen mackerel (*Scomber japonicas*) and squid (*Todarodes pacificus*), and sometimes capelin (*Mallotus villosus*), arabesque greenling (*Pleurogrammus azonus*), and saury (*Coloabis saira*). The animal ate about 8 kg (range: 6 to 10 kg) per day. On 7 June 2017, the animal was transported to another aquarium and, at that time, was 243 cm long and weighed 180 kg, which approximated the length/mass relationship formula (Ross & Leatherwood, 1994).

The animal was sometimes housed with other dolphins: a female Risso's dolphin (*Grampus griseus*) from 22 December 2014 to 27 May 2015 and 10 March to 18 July 2016; two male Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) from 22 to 27 January 2017; and a male bottlenose dolphin (*Tursiops truncatus*) from 26 September to 22 December 2014, 21 April to 13 October 2015, and 7 November 2016 to 7 June 2017. Though

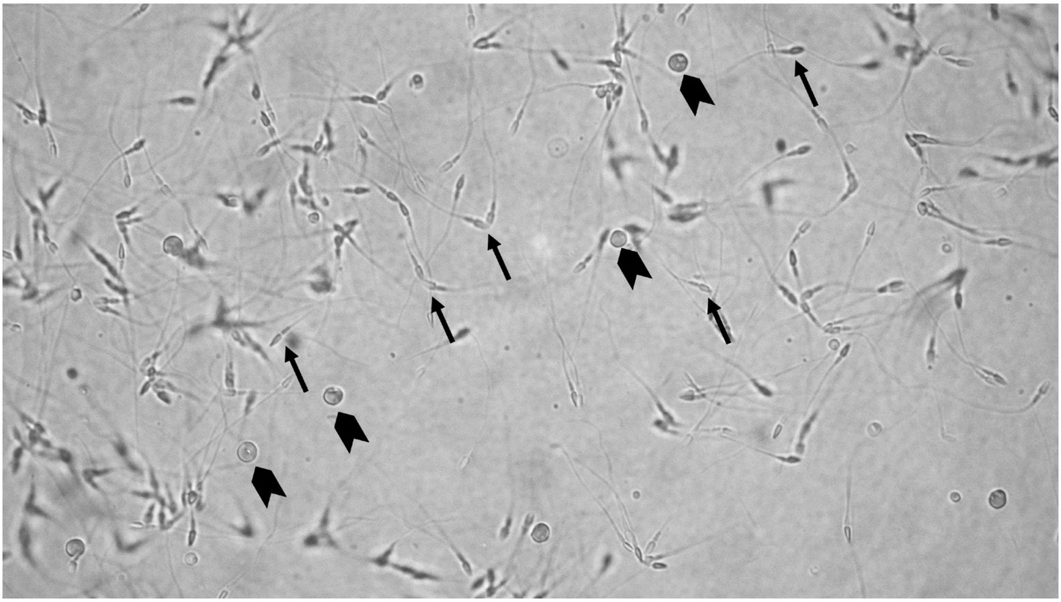

Figure 1. Sperm cells observed in urine (arrows), which suggest sexual maturity. Red blood cells are also observed (arrowheads).

pygmy killer whales generally tend to aggressively attack other dolphins (Pryor et al., 1965; Best, 1970), no aggressive behavior was observed.

Blood Analyses

The animal was trained to present his fluke: all blood samples were taken via fluke venipuncture using a plastic disposable syringe with a butterfly needle (23 ga). The blood samples were placed in EDTA Vacutainer tubes for complete blood counts and in clot tubes for the serum chemical test and evaluation of testosterone concentration. Samples were kept in a refrigerator and transferred to a commercial laboratory within 24 h for the test. Blood sampling was performed from December 2015 to June 2017, every 2 wks, with sampling intervals varying from 7 to 72 d.

To estimate blood values, samples from when the pygmy killer whale was healthy were chosen, and samples from the following situations were excluded: when antibacterial drugs were administered and when the animal showed anorexia presumed by hypernatremia. Though hematuria continued for about 15 mo, the animal did not show clinical symptoms related to hematuria such as abnormal behaviors and loss of appetite. For this reason, blood samples collected when the animal had hematuria were not excluded from analysis.

Ultrasonographic Examination on Testes

To investigate seasonal change of testes size, ultrasonographic examination was performed from July 2016 to June 2017 with blood sampling. Testes were identified by shape in longitudinal image, and subsequently the transverse image was recorded from cranial edge to caudal edge. The longitudinal (LD) and transverse diameters (TD) on a static image were measured using *Hakarun* (software; Nacchan, Japan). Finally, peak cross-sectional testicular area (CSA) was calculated from the formula below:

$$\text{CSA (cm}^2\text{)} = 3.14 \times \text{LD}/2 \times \text{TD}/2$$

All ultrasonographic examinations were performed with FAZONE CB V, in conjunction with FZT C9-3 V Probe (FUJIFILM, Tokyo, Japan). The animal was trained to approach the poolside and keep in a dorsal recumbent position with his fluke supported by a trainer (Figure 2).

Hematology

Hematology values are presented in Table 1. While the number of red blood cells was consistent with a previous report of this species (Clua et al., 2014), the hemoglobin concentration and hematocrit were higher in this case. It implies that the chronic hematuria did not have a significant effect on these blood parameters. After giving 2 to 4 L of water for 22 d (as gelatin or administrated into stomach by inserted silicon tube), hematuria improved. Thus, the bladder or renal calculi could be a cause of hematuria, though we could not confirm this with ultrasonographic examination.

Figure 2. Ultrasound examination was performed with husbandry behavior (*Photo credit:* MARINE WORLD uminonakamichi)

Serum Chemistry

Serum chemistry values are presented in Table 2. These values are very similar to other toothed cetaceans (e.g., bottlenose dolphins or false killer whales [*Pseudorca crassidens*]) and another rehabilitated pygmy killer whale (Bossart et al., 2001; Clua et al., 2014) except for Na, Cl, ALP, and cortisol.

In our study, the facility was situated in the temperate zone at latitude 33° 45' N, and the monthly average of the daily temperature was less than 10°C from December to March (Japan Meteorological Agency, 2018). Since pygmy killer whales are distributed throughout the tropic and subtropic oceans, they may have a low tolerance for cold stress. In fact, this animal had a lower appetite in winter than in summer, even though the water temperature was kept higher than 20°C (20° to 23°C) in winter. Higher values of Na and Cl tend to be detected before such a loss of appetite in winter. It is presumed that lowered appetite is associated with decreased water intake and resulted in the decrease of Na and Cl excretion through the urine.

Fothergill et al. (1991) found that ALP can be used as an indicator of health conditions in dolphins, and high values are commonly apparent in good health. In the present study, ALP values were much higher than in the rescued pygmy killer whale in the past, which indicates that this animal was in good condition at the point of sampling (Terasawa et al., 1997; Clua et al., 2014). Cortisol values were much lower than in the previous reports for bottlenose dolphins (St. Aubin et al., 1996), which indicates that the animal was not suffering from any short-term stress despite being in a captive setting and/or sharing the pool with other dolphins.

Annual Changes of Serum Testosterone Levels and CSA of Testes

The annual changes of serum testosterone level and CSA of testes are shown in Figure 3. The testosterone level maintained at < 5 ng/ml (1.01 to 4.81 ng/ml) from July 2016 to November 2016, and CSA also did not show significant change during this time. Hence, < 5 ng/ml of testosterone can be considered the baseline. It rose at the end of November 2016, but it decreased by mid-December 2016. Thereafter, from late February 2017, testosterone started to increase again and reached 28.3 ng/mL in April 2017. Then, it declined from May 2017 until the end of the study; however, it did not reach the

Table 1. Hematology values of the pygmy killer whale (*Feresa attenuata*); NP = Not provided.

Parameter	Mean (SD)	Range	Rehabilitated *F. attenuata* (Clua et al., 2014)	Number of samples
White blood cell count (/mm^3)	7,804 (1,814)	5,200-11,500	8,900	26
Red blood cell count (×10^4/mm^3)	392 (26)	341-436	380	26
Hemoglobin (g/dl)	16.5 (1.2)	14.0-18.4	13.1	26
Hematocrit (%)	48.1 (2.7)	42.4-52.8	40.8	26
Mean corpuscular volume (MCV) (fl)	122.9 (2.7)	119.0-130.1	107.9	26
Mean corpuscular hemoglobin (MCH) (pg)	42.1 (0.9)	39.9-44.2	34.8	26
Mean corpuscular hemoglobin concentration (MCHC) (%)	34.3 (0.9)	32.4-36.0	32.7	26
Platelets (×10^4/mm^3)	15.8 (2.5)	12.5-20.8	NP	26
Reticulocytes (‰)	34 (14)	20-58	NP	5
Neutrophils-Stab (/mm^3)	64 (57)	0-182	NP	24
Neutrophils-Seg (/mm^3)	4,786 (1,437)	2,805-7,844	NP	24
Eosinophils (/mm^3)	467 (254)	0-1,183	NP	24
Basophils (/mm^3)	3 (13)	0-65	NP	24
Monocyte (/mm^3)	437 (264)	104-1,035	NP	24
Lymphocytes (/mm^3)	1,862 (435)	1,092-2,772	NP	24

initial baseline. CSA enlarged from December 2016 but decreased in size gradually as the testosterone level decreased until February 2017. It got larger again beginning in March 2017, and it reached the largest size from May to June 2017. The size ratio varied 2.5 to 2.8 times larger when comparing the largest size with the smallest.

The first testosterone peak, observed at the end of November 2016, might have been stimulated by cohabitation with a male bottlenose dolphin. Such a spontaneous rise of serum testosterone caused by association with another dolphin was reported for finless porpoises (Nakamura & Fujimaru, 2014). Since reproduction of pygmy killer whales is assumed to occur in summer and considering the gestation period of most cetaceans is almost a year, it is suggested that a second testosterone peak observed from March to June 2017 may be the natural rhythm of pygmy killer whales (Ross & Leatherwood, 1994). Alternatively, the decrease in testosterone was observed while the animal was unhealthy, suffering from general bacterial infection or hypernatremia. Therefore, it is difficult to exclude that poor health affected the testosterone level of this male.

The peak of CSA comes 2 to 4 wks after the peak of serum testosterone. The same phenomenon is reported for the Pacific white-sided dolphin in which the CSA peak comes 4 wks after testosterone peak (Robeck et al., 2009).

A fundamental knowledge of blood values was obtained from this healthy pygmy killer whale. These values will help us with rescue and/or rehabilitation of this species in the future. Also, we monitored the change of testes size and serum testosterone level for a year. Though this animal was not at his best condition during the study period, this report serves as a good reference to elucidate the reproductive physiology in this species. Of course, this is a single case, and individual differences should be considered.

Table 2. Blood chemistry values of the pygmy killer whale

Parameter	Mean (SD)	Range	Number of samples
ESR 30 min. (mm)	3		1
ESR 60 min. (mm)	4		1
Total protein (g/dl)	7.1 (0.7)	6.0-8.9	26
Albumin (g/dl)	3.7 (0.4)	3.1-4.6	25
Globulin (g/dl)	3.5 (0.4)	2.7-4.3	25
α1-globulin (g/dl)	0.7 (0.2)	0.3-1.0	25
α2-globulin (g/dl)	0.5 (0.2)	0.3-0.9	25
β1-globulin (g/dl)	0.2 (0.1)	0.1-0.4	25
β2-globulin (g/dl)	0.2 (0.05)	0.1-0.4	25
γ-globulin (g/dl)	2.0 (0.3)	1.2-2.4	25
Creatinine kinase (U/liter)	107 (16)	72-145	25
Aspartate aminotransferase (U/liter)	102 (14)	76-130	25
Alanine aminotransferase (U/liter)	28 (8)	19-52	25
Lactic dehydrogenase (U/liter)	417 (85)	342-648	25
Lactic dehydrogenase 1 (%)	15 (2)	10-18	21
Lactic dehydrogenase 2 (%)	28 (3)	21-33	21
Lactic dehydrogenase 3 (%)	38 (3)	31-44	21
Lactic dehydrogenase 4 (%)	14 (3)	11-24	21
Lactic dehydrogenase 5 (%)	4 (3)	2-14	21
Alkaline phosphatase (U/liter)	1,313 (301)	656-1,814	25
γ-glutamyl transferase (U/liter)	24 (4)	20-40	25
Leucine aminopeptidase (U/liter)	16 (2)	14-22	23
Bilirubin total (mg/dl)	0.0 (0.0)	0.0-0.0	25
Bilirubin direct (mg/dl)	0		1
ZTT (U)	6 (3)	4-9	23
TTT (U)	0.3(0.1)	0.1-0.4	23
Glucose (mg/dl)	97 (12)	71-122	25
Cholesterol total (mg/dl)	210 (22)	161-246	26
HDL (mg/dl)	95		1
Triglycerides (mg/dl)	39 (26)	13-139	26
Serum urea nitrogen (mg/dl)	46.6 (5.7)	38.2-67.7	25
Creatinine (mg/dl)	1.03 (0.22)	0.70-1.41	25
Urea acid (mg/dl)	0.0 (0.0)	0.0-0.1	25
Sodium (mEq/liter)	155 (3)	152-164	25
Potassium (mEq/liter)	4.1 (0.3)	3.7-5.1	25
Chloride (mEq/liter)	123 (5)	117-140	25
Calcium (mg/dl)	8.8 (0.3)	8.0-9.4	25
Phosphorus (mg/dl)	6.8 (0.6)	5.6-7.9	25
Iron (μg/dl)	137 (61)	34-251	29
TIBC (μg/dl)	529 (52)	417-648	25
UIBC (μg/dl)	392 (66)	268-529	25
Testosterone (ng/ml)	7.86 (8.24)	0.49-28.3	28
Cortisol (μg/dl)	0.6 (0.3)	0.2-1.3	24
T3 (ng/ml)	1.91		1
T4 (μg/dl)	11.1		1
fT3 (pg/ml)	3.91		1
fT4 (ng/dl)	1.89		1

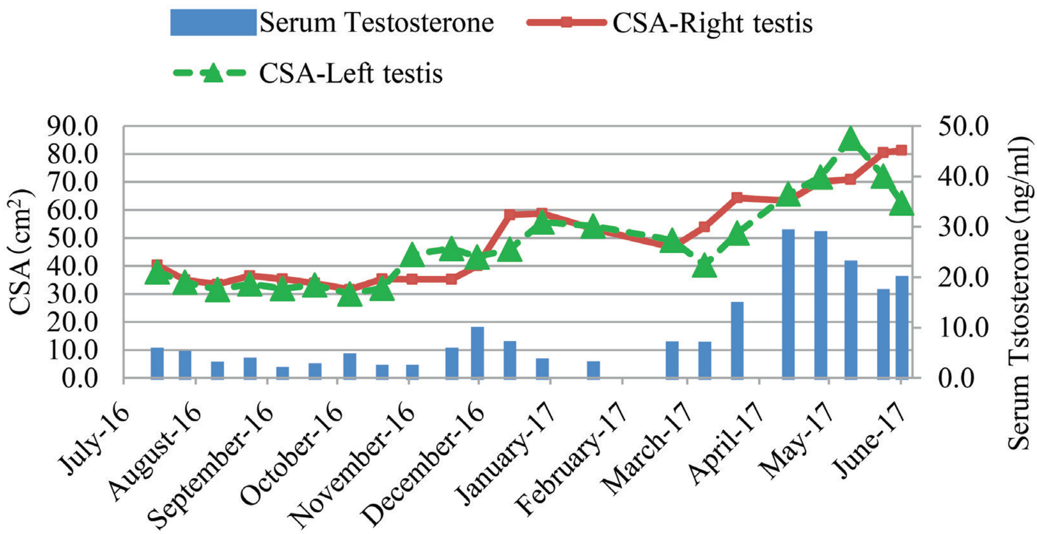

Figure 3. Annual change of serum testosterone level and CSA of testes in the pygmy killer whale (*Feresa attenuata*)

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