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Assessment of Yangtze Finless Porpoises (*Neophocaena asiaorientalis*) through Biochemical and Hematological Parameters

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Ghulam Nabi, Yujiang Hao, Xianyuan Zeng, and Ding Wang (2017) For the management of endangered species, a periodic health assessment is important, as diet in captivity is restricted due to cost and some nutrients are lost during the processing, storage and thawing of fish. The objective of this study was to compare and assess the nutritional, as well as the physiological health of both the captive and free-ranging Yangtze Finless Porpoises (YFPs) through biochemical markers, selected electrolytes, enzymes and hematological parameters. Our results showed statistically significantly ($P < 0.05$) higher levels of some biochemical markers (HDL-c/LDL-c, globulin, TP, Urea, Creatinine and BUN), enzymes (GGT), electrolytes (K^+, Na^+, PO_4^{3-} and Mg^{2+}) and hematological (WBCs, lymphocytes, eosinophil) parameters in wild compared to the captive populations. However, the captive population also showed significantly ($P < 0.05$) higher levels of some biochemical markers (LDL-c, albumin and albumin/globulin), enzymes (AMS), electrolytes (Cl^-) and complete blood count (neutrophil, monocytes and basophil) parameters versus wild populations. Differences in the parameters of captive YFPs could be due to their limited diet of only three fish species as well as their environment (captivity). Whereas, wild YFPs, continuously feed on a large variety of live fish species and shrimp as they travel long distances. Our results suggest that mineral supplements be added to their diet. As well, improved physical fitness training and hygienic conditions are required for the effective management of captive finless porpoises.

Key words: Lipid profile, Nutrition management, Serum electrolytes, Yangtze finless porpoise.

BACKGROUND

The Yangtze Finless Porpoise (YFP) (*Neophocaena asiaorientalis*) is a small toothed freshwater cetacean (Gao and Zhou 1995), endemic in the Yangtze River, Poyang and Dongting Lakes, China (Gao and Zhou 1995; Wei et al. 2003). In the world, there are six species of porpoises, and YFP is the only fresh water living subspecies (Gao and Zhou 1995; Wei et al. 2003; Jefferson and Wang 2011), recognized among the most threatened mammals in the world (Reeves et al. 1997; Smith and Reeves 2000; Wang et al. 2000). The YFP has been listed as the critically endangered subspecies in the red list of International Union for Conservation of Nature (IUCN), although it is still listed in the Second Order of Protected Animals in China. According to the latest survey conducted in 2012, there are only 1040 individuals remaining (Mei et al. 2014). Various factors such as, industrial pollutants (Schelle 2010), sand mining (Leeuw et al. 2009; Wang et al. 2010), construction of dams and barrages, agriculture, (Chen et al. 1997; Wang et al. 1998; Schelle 2010) and inland water transport (Chen et al. 1997; Wang et al. 1998; Smith and Reeves 2000; Akamatsu et al. 2002; Schelle 2010) are possible threats for YFPs. However, the most important threat is overfishing. The methods used are illegal and harmful to the porpoises. As well,

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a scarcity of food for YFPs also results (Chen et al. 1997; Wang et al. 1998; Wang 2009; Schelle 2010).

In recent years, due to tremendous growing environmental influences from anthropogenic activities, a large number of captive breeding programs were started with the purpose of preventing endangered populations and/or species from becoming extinct (Frankham 2008). For this purpose, in 1992 the Baiji dolphinarium located in Wuhan, China at the Institute of Hydrobiology of the Chinese Academy of Sciences was established. The dolphinarium, which is home to several YFPs, allows for the study of breeding behavior and reproductive endocrinology/physiology. As a result, the first captivity-bred YFP calf was born on July 5, 2005, suggesting a possibility of breeding the Yangtze finless porpoise in captivity. This success has significantly improved conservation efforts, and it has also greatly boosted the on-going study of the reproductive biology of these animals (Wei et al. 2004; Wang et al. 2005). But unfortunately, in captivity, diet is more restricted due to cost restriction and commercial availability of frozen fish (Worthy 2001). A mixed diet of invertebrates and fish is needed to provide a balanced source of minerals, protein, vitamins and fats for the porpoise. Further, to correctly determine the appropriate diet for cetaceans, it is crucial to analyze fish composition as it changes according to location, season, sex and age of the porpoises (Kastelein et al. 2003; Couquiaud 2005; Mitrus et al. 2010; Mahmood and Adil 2017). Moreover, to avoid possible nutritional deficiencies, cetaceans in captivity should not be fed with only one or two fish species (Geraci 1986; Worthy 1990, 2001); five or more species are preferable (Anonymous 1992). For captive cetaceans, a diet composed of cut-up fish is deficient in nutrition because, very important nutrients are lost due to leaching, evisceration and beheading (Crissey 1998). Similarly, during thawing, storage and processing of fish, vitamins are lost. Even some fish species such as, capelin, herring, smelt and certain types of mackerel possess thiaminase, which can induce a thiamine deficiency in cetaceans, and can cause severe disorders and fatalities (Geraci 1986; Worthy 1990, 2001).

For some species, marine parks and zoos have improved their breeding and survival records. However, river dolphins in captivity have a poor survival record. The idealized nature of captivity and the unnatural composition of food can affect the physiological functions of animals. Blood biochemistry and basic hematology in response to physiological adjustment does not correlate well with wild populations, and therefore it might be unwise to apply such information to the management and conservation of wild populations (Medway and Geraci 1986; Mayer 1998). Therefore, knowledge of the nutritional and the health status of animal populations or an individual is very crucial for evaluating the quality of habitat or for captive management (Lukas et al. 1999; Ullrey et al. 1999; Less et al. 2014; Smith et al. 2014). Free-ranging and captive YFPs consume diets that differ in quantity, quality, fish species, variety, location, and seasonality. The objective of this study, therefore, was to assess and compare the nutritional as well as the health status of both captive and free-ranging YFPs, through biochemical markers, selected electrolytes, enzymes as well as hematological parameters. This could provide useful information to improve the husbandry and management of the captive breeding programs for the critically endangered YFPs.

MATERIALS AND METHODS

Animals and sampling procedure

Blood samples of captive YFPs were collected from the Yangtze Cetacean Breeding and Research Center (YCBRC). The basic information of 4 captive male animals is listed in table 1. The animals were housed in a kidney-shaped pool (25 × 7.5 m) and a connected round pool (depth = 4 m, diameter = 10 m). A closed-circuit filter recycles the water 10 times a day and maintains the water temperature at approximately 27°C in summer by a cooling system. The porpoises were artificially fed four to five times a day. The food was a mixture of thawed *Carassius auratus*, *Cyrinus carpio* and *Hemiculter leucisculus*. Daily food consumption was approximately 6-10% of a porpoise body weight, which was adjusted seasonally according to the appetite of the animal. Three samples from each captive animal were collected during March, and July through October in 2011. Since the animals were not well trained for blood sampling, the rearing pool had to be drained. To restrict the animals, porpoises were placed on sponge mattress for blood collection. Blood samples of wild animals were collected from the Poyang Lake YFPs population during a routine physical examination conducted between 2009 and 2011.

Poyang Lake is the largest freshwater lake and is also the most important natural habitat for YFPs (Dong et al. 2015). The wild animals were captured by using the "sound chase and net capture" method (Hua 1987). The detail of the operations and procedures of capture as well as handling of the YFPs are explained in detail by Hao et al. (2009).

From both captive and wild population, blood samples were drawn aseptically from the main vein of tail fluke into non-heparinized 10 mL tubes using a 10 mL disposable syringe (Gemtier, G/Ø/L: 21/0.7/31 mm, 201502, Shanghai, China). Then a 2 ml aliquot was poured into a heparinized vial (Nihon, 161-8560, Tokyo, Japan) for hematology assay and the remainder into a centrifuge tube (Corning, 14831, New York, America) for serum separation. Through centrifugation (Eppendorf AG, 22332, Hamburg, Germany) at 1500×g for 15 minutes, serum was separated, put into tubes, and immediately stored in a liquid nitrogen kettle. After transferring to the laboratory, they were stored at -25°C for later assay. Besides blood sampling, morphometric measurements and ultrasound (LOGIQ Book XP, New York, America) examination were also conducted sequentially. During the whole process, the breath frequency and the behavioral reaction of the animals were monitored, and water was continuously poured on the animal to avoid skin dehydration. The whole examination for one animal usually took less than 15 min. The wild animals were then released 2 km away from the locations where they were captured. To avoid the possible influence of gender and age on biochemistry and hematology assay, only 6 samples from 6 adult male animals with similar age to the captive males were selected for the comparison shown in table 1.

Laboratory analyses

Electrolytes, including K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, Fe^{2+} and PO_4^{3-}; the biochemical markers Triglycerides (TG), Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-c), Lower Density Lipoprotein Cholesterol (LDL-c), Albumin (ALB), Globulin (GLB), Total Protein (TP), Urea (U), Creatinine (Cr), Blood Urea Nitrogen (BUN) and Carbon Dioxide (CO_2); and the enzymes gamma-glutamyl Transferase (GGT), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (T-BILI), Lactate Dehydrogenase (LDH), Amylase (AMS) and Creatinine Kinase (CK) were assayed using the automated clinical chemistry analyzer (Beckman-Coulter, AU5400, Porto, Portugal). The hematology or the complete blood count was analyzed by using the hematology analyzer (Beckman-Coulter, DxH 800, Porto, Portugal) according to the manufacturer instruction.

Table 1. Basic information of the study animals

Animal Group	Code/Name	Date of Sampling	Body Length (cm)	Body Weight (kg)
Wild Group (Poyang Lake)	11PYM01	2011/02/22	168	67.2
	11PYM02	2011/02/23	166	74.1
	11PYM05	2011/02/24	140	49
	11PYM16	2011/02/25	148	51.6
	11PYM17	2011/02/25	150	59.1
	11PYM20	2011/02/25	152	73.4
Mean ± SD			154 ± 10.88	62.40 ± 10.84
Captive Group (YCBRC)	Afu	2011/03/06	151	43.9
		2011/07/07	152.5	44.9
		2011/10/18	152	42.4
	Tao Tao	2011/03/06	145	41.1
		2011/07/07	147	38.5
		2011/10/18	147	38.2
	Duo Duo	2011/03/19	143	41.1
		2011/10/22	147	45.7
		2011/12/15	147	43.2
	A Bao	2011/03/06	150	48
		2011/04/23	150	41.5
		2011/05/20	unknown	unknown
Mean ± SD			148 ± 3.00	42.05 ± 2.49

Statistical analyses

All the parameters of both populations were compared using the non-parametric Mann Whitney *U*-test using Graph Pad Prism, version 5.01 (Graph Pad Software Inc., San Diego, CA, USA). All the data are presented as mean ± SEM. A $P < 0.05$ indicates a statistically significant difference.

RESULTS

Circulating levels of biochemical markers for both wild and captive YFPs are summarized in table 2. A statistically significant ($P < 0.05$) higher serum levels of HDL-c/LDL-c, GLB, TP, U, BUN and Cr were detected in porpoises living in the wild. However, serum levels of LDL-c, ALB and ALB/GLB were statistically significantly ($P < 0.05$) higher in porpoises living in captivity. However, for serum levels of TC, TG and HDL-c, no significant ($P < 0.05$) difference were reported between the two populations.

The results for serum electrolytes are summarized in table 3. A statistically significantly ($P < 0.05$) higher level of K^+, Na^+, PO_4^{3-} and Mg^{2+} were observed in porpoises living in the wild. Only Cl^- showed a statistically significant difference in porpoises living in captivity. No significant ($P < 0.05$) differences were seen in the levels of Ca^{2+} and Fe^{2+} between the two populations.

Our results for serum enzymes are summarized in table 4. Statistically significantly ($P < 0.05$) higher levels of AMS was detected in captive porpoises. Whereas, only GGT was significantly higher in porpoises living in the wild. No significant ($P < 0.05$) differences were noticed in levels of ALP, ALT, AST, TBILI, LDH and CK between the two populations.

For CBC (Table 5), WBCs, lymphocyte and eosinophil counts were statistically significantly ($P < 0.05$) higher in porpoises living in the wild while neutrophil, monocytes and basophils were significantly ($P < 0.05$) higher in the captive porpoises.

Table 2. Comparison of blood chemistry between captive and wild YFPs

Parameters	Mean ± SEM (N = [a]4, [b]6)	Lower Value	Upper Value	Lower 95% CI	Upper 95% CI	*P*
Total cholesterol (mmol/L)	[a]5.45 ± 0.42	4.79	6.69	4.10	6.80	0.176
	[b]5.88 ± 1.26	0.47	10.09	2.63	9.14	
Triglycerides (mmol/L)	[a]1.16 ± 0.19	0.75	1.70	0.53	1.80	0.457
	[b]2.01 ± 0.86	0.70	6.29	-0.21	4.23	
HDL-c (mmol/L)	[a]2.44 ± 0.21	2.14	3.08	1.76	3.13	0.196
	[b]2.72 ± 0.21	1.86	3.40	2.16	3.29	
LDL-c (mmol/L)	[a]0.79 ± 0.01	0.77	0.83	0.75	0.83	**0.004
	[b]0.29 ± 0.02	0.25	0.39	0.22	0.36	
HDL-c/LDL-c	[a]4.52 ± 0.29	3.87	5.04	3.57	5.48	**0.004
	[b]9.44 ± 0.88	6.97	11.85	7.17	11.71	
ALB (g/L)	[a]39.96 ± 0.63	39.13	41.83	37.94	41.97	*0.033
	[b]38.15 ± 0.77	35.90	41.30	36.16	40.14	
GLB (g/L)	[a]29.61 ± 3.82	21.63	37.46	17.43	41.79	*0.019
	[b]42.70 ± 3.07	31.60	51.40	34.80	50.60	
ALB/GLB	[a]1.47 ± 0.24	1.04	2.09	0.69	2.24	*0.019
	[b]0.91 ± 0.06	0.71	1.14	0.74	1.08	
TP (g/L)	[a]69.50 ± 3.29	63.46	76.40	59.00	79.99	*0.019
	[b]80.87 ± 3.18	67.60	89.20	72.68	89.06	
Urea (μmol/L)	[a]15.58 ± 1.90	10.73	20.00	9.51	21.64	**0.004
	[b]79.03 ± 12.58	36.40	120.40	46.59	111.40	
BUN (mmol/L)	[a]17.12 ± 0.88	15.24	19.53	14.28	19.95	*0.019
	[b]19.53 ± 0.73	17.31	22.12	17.63	21.43	
Cr (μmol/L)	[a]70.25 ± 2.83	65.46	77.40	61.24	79.25	*0.019
	[b]106.6 ± 25.15	67.00	230.6	41.89	171.2	

N = number of animals, ([a]) = captive animals, ([b]) = Wild animals. Significant difference with *$P < 0.05$, **$P < 0.01$.

DISCUSSION

Biochemical parameters

In our study, no statistically significant differences were noticed for TC, TG, and HDL-c levels between wild and captive YFPs, which is in agreement with the finding of Koopman et al. (1995) and Kjeld (2001) who found no statistically significant differences in the cholesterol level between captive and wild harbor porpoises. However, in contrast to our results, Cook et al. (1990) and Asper et al. (1990) reported a significantly higher level of cholesterol and triglycerides in captive beluga whales (*Delphinapterus leucas*) and bottlenose dolphins (*Tursiops truncates*) as compared to the wild populations. According to St. Aubin and Geraci (1989), the levels of serum cholesterol fluctuated and triglyceride rose in captive beluga whales within 10 weeks when fed on oil-rich herring rather than their normal diet of decapod crustaceans. However, Cook et al. (1990) suggested the role of environmental factors and

Table 3. Serum electrolytes concentration in captive and wild YFPs

Parameters	Mean ± SEM (N = [a]4, [b]6)	Lower Value	Upper Value	Lower 95% CI	Upper 95% CI	*P*
K^+ (mmol/L)	[a]3.74 ± 0.11	3.52	4.07	3.36	4.11	*0.019
	[b]4.24 ± 0.07	4.00	4.50	4.03	4.44	
Na^+ (mmol/L)	[a]155.1 ± 1.35	151.4	157.5	150.8	159.4	*0.019
	[b]159.9 ± 1.41	156.5	166.4	156.2	163.5	
Cl^- (mmol/L)	[a]111.7 ± 0.96	109.4	114.0	108.6	114.8	*0.019
	[b]103.6 ± 1.38	100.4	109.8	100.1	107.2	
Ca^{2+} (mmol/L)	[a]2.59 ± 0.05	2.47	2.71	2.42	2.76	0.260
	[b]2.54 ± 0.04	2.43	2.66	2.43	2.64	
$PO4^{3-}$ (mmol/L)	[a]1.21 ± 0.05	1.09	1.35	1.02	1.40	**0.004
	[b]1.91 ± 0.16	1.57	2.63	1.48	2.33	
Mg^{2+} (μmmol/L)	[a]0.71 ± 0.00	0.70	0.74	0.68	0.74	**0.009
	[b]0.89 ± 0.06	0.74	1.15	0.74	1.05	
Fe^{2+} (μmmol/L)	[a]28.80 ± 1.37	26.56	32.73	24.42	33.18	0.304
	[b]23.73 ± 4.01	9.10	35.00	13.41	34.05	

N = number of animals, ([a]) = captive animals, ([b]) = Wild animals. Significant difference with *P < 0.05, **P < 0.01.

Table 4. Serum enzymes level in captive and wild YFPs

Parameters	Mean ± SEM (N = [a]4, [b]6)	Upper Value	Lower Value	Upper 95% CI	Lower 95% CI	*P*
ALP (U/L)	[a]152.6 ± 32.84	235.0	98.33	257.1	48.06	0.1657
	[b]110.5 ± 11.35	150.0	85.00	139.7	81.34	
ALT (U/L)	[a]45.42 ± 4.00	55.33	37.00	58.16	32.67	0.3340
	[b]41.33 ± 1.92	50.00	37.00	46.29	36.38	
AST (U/L)	[a]237.6 ± 9.25	255.3	213.3	267.0	208.1	0.0571
	[b]209.8 ± 8.50	234.0	183.0	231.7	188.0	
GGT (U/L)	[a]31.83 ± 2.17	34.33	25.33	38.74	24.92	*0.0333
	[b]52.17 ± 6.58	71.00	30.00	69.08	35.25	
T BILI (μmol/L)	[a]2.65 ± 0.25	2.90	1.90	3.44	1.85	0.0544
	[b]3.03 ± 0.30	3.70	1.60	3.81	2.25	
LDH (U/L)	[a]207.7 ± 14.69	251.7	189.7	254.5	161.0	0.4571
	[b]238.2 ± 38.56	373.0	153.0	337.3	139.0	
AMS (U/L)	[a]12.00 ± 1.31	15.66	9.66	16.17	7.81	**0.0070
	[b]6.00 ± 0.57	8.00	4.00	7.48	4.51	
CK (U/L)	[a]120.1 ± 16.03	144.3	73.66	171.2	69.13	0.3810
	[b]113.7 ± 18.53	181.0	53.00	161.3	66.03	

N = number of animals, ([a]) = captive animals, ([b]) = Wild animals. Significant difference with *P < 0.05, **P < 0.01.

stressors rather than diet as a crucial factor in the elevation of cholesterol levels. Notably, a statistically significantly higher level of LDL-c was found in captive YFPs and therefore, a relatively higher HDL-c/LDL-c ratio was observed in the porpoises living in the wild. Similar to the findings of our study, a significantly higher level of LDL-c was found in the captive bottlenose dolphins (*Tursiops truncatus*) compared to populations living in the wild (Venn-Watson et al. 2013). Higher levels of LDL-c in bottlenose dolphins can cause metabolic disorders (Venn-Watson et al. 2013) and mortality (Scot 1987; Weisenberg et al. 1991; Meehan and Lowenstine 1994). This higher level of LDL-c in captive YFPs might be due to low energy expenditure (inactivity) and/or diet quality (Kendall and Jenkins 2004; Schmidt et al. 2006; Mann et al. 2014), which can predispose them to various metabolic and cardiovascular diseases. While a statistically significantly higher level of HDL-c/LDL-c in wild YFPs could be associated with high energy expenditure (Durstine et al. 1983) and a diet consisting of fresh fish which is high in energy. A statistically significant difference in the levels of TP, GLB, Albumin and ALB/GLB indicates the influence of dietary intake and the ecological environment, as was also observed in the *Zalophus* (Bossart et al. 2001). The lower mean TP level in the captive YFPs could be due to malnutrition or malabsorption, as suggested by Mayer (1998) in cetaceans at captivity. The significantly lower values of GLB and lymphocytes found in captive YFPs, also suggests a decreased immune stimulation (Christopher et al. 1999; Bossart et al. 2001). Higher levels of serum creatinine, U and BUN in the wild population might be either due to high protein diet or renal problems such as glomerular damage (Bossart et al. 2001) resulting from exposure to various environmental pollutants (Schelle 2010).

Electrolytes

For electrolytes, a statistically significantly higher level of K^+, Na^+, PO_4^{3-} and Mg^{2+} were found in YFPs living in the wild. However, a statistically

Table 5. Comparison of hematological parameters in captive and wild YFPs

Parameters	Mean ± SEM (N = [a]4, [b]6)	Upper Value	Lower Value	Upper 95% CI	Lower 95% CI	*P*
RBCs (x 10^{12}/L)	[a]5.36 ± 0.19	5.76	4.83	5.98	4.74	0.4571
	[b]5.34 ± 0.11	5.72	5.02	5.63	5.04	
Hb (g/L)	[a]168.5 ± 4.64	175.7	155.0	183.3	153.7	0.2965
	[b]164.3 ± 4.12	181.0	153.0	174.9	153.7	
HCT (%)	[a]49.87 ± 1.44	52.66	46.00	54.47	45.28	0.4574
	[b]49.98 ± 0.97	53.90	47.30	52.48	47.48	
MCH (pg)	[a]31.43 ± 0.58	32.66	30.03	33.29	29.56	0.3810
	[b]30.82 ± 0.71	33.10	28.40	32.66	28.97	
MCHC (g/L)	[a]336.1 ± 1.78	340.5	332.3	341.8	330.4	0.1679
	[b]328.8 ± 4.30	337.0	313.0	339.9	317.8	
MCV (fL)	[a]93.60 ± 1.93	98.30	90.00	99.76	87.44	0.5000
	[b]93.70 ± 1.92	99.00	87.30	98.64	88.76	
PLT (x 10^9/L)	[a]137.5 ± 11.34	158.7	105.7	173.6	101.4	0.0823
	[b]153.2 ± 6.61	176.0	128.0	170.2	136.2	
WBCs (x 10^9/L)	[a]5.64 ± 0.04	5.75	5.53	5.79	5.49	*0.0208
	[b]6.16 ± 0.17	6.80	5.70	6.60	5.72	
Neutrophil (%)	[a]70.12 ± 3.35	75.96	61.32	80.79	59.44	**0.0070
	[b]53.83 ± 1.62	57.00	47.00	58.01	49.65	
Lymphocyte (%)	[a]18.03 ± 2.58	24.47	12.55	26.26	9.78	**0.0095
	[b]27.17 ± 1.75	33.00	20.00	31.68	22.66	
Monocyte (%)	[a]3.42 ± 0.30	4.26	2.83	4.38	2.46	*0.0122
	[b]2.00 ± 0.42	3.00	0.00	3.10	0.89	
Basophil (%)	[a]0.085 ± 0.030	0.170	0.030	0.183	-0.013	**0.0056
	[b]0.003 ± 0.002	0.010	0.00	0.008	-0.002	
Eosinophil (%)	[a]8.42 ± 2.61	13.00	1.09	16.73	0.11	*0.0139
	[b]17.42 ± 2.55	28.50	13.00	23.99	10.84	

N = number of animals, ([a]) = captive animals, ([b]) = Wild animals. Significant difference with *$P < 0.05$, **$P < 0.01$.

significantly higher serum Cl^- concentration was detected in YFPs living in captivity. No statistically significant difference in the serum concentrations of Ca^{2+} and Fe^{2+} were detected between the two populations. Similar to our findings, Koopman et al. (1995) reported a statistically significantly higher level of K^+ in wild harbor porpoises, but they did not observe a statistically significant difference in the serum levels of Na^+, Cl^-, PO_4^{3-}, Ca^{2+} and Mg^{2+} as compared to captive harbor porpoises and other odontocetes. Kjeld (2001) found a higher concentration of K^+ in fin whales as compared to captive odontocetes, because of the ingestion of a substantial amount of K^+ in their food. Similarly, Geraci and Medway (1973) observed elevated concentrations of K^+ in a bottlenose dolphin handled in a pool. However, during the transportation of six dolphins, Copland and Needham (1992) found no statistically significant increase in K^+ concentration. For domestic animals, the critical plasma K^+ concentration is 7 mmol/L and a higher circulating level than this can cause cardiac arrest (Kerr 1989). The statistically significantly higher concentration of K^+ in wild YFPs could be due to either the difference in geographic environment or variations in food quality and quantity. Although in marine mammals, hemolysis can also significantly elevate K^+ and PO_4^{3-} concentrations (Bossart et al. 2001). However, in our blood samples, no hemolysis was observed. Furthermore, a higher serum K^+ level may indicate muscle damage, as K^+ is leaked from damaged muscle cells (Geraci and Medway 1973). Therefore, wild YFPs were chased before samples could be collected which might have resulted higher serum K^+ levels due to strenuous muscular activities

Similar to our findings, a statistically significantly higher level of phosphorous, Na^+, K^+ and Mg^{2+} while a statistically significantly lower level of chlorine was observed in free-ranging dolphins as compared to managed dolphins (Ardente et al. 2015). The high concentration of chlorine in a captive environment can peel off the skin, irritate the eyes and prevent eyes from fully opening (Kestin 2004). In 2016, we observed an eye problem in one YFP living in captivity, which could be due to the high concentration of Cl^- in the water.

Like wild YFPs, a statistically significantly higher level of Mg^{2+} and phosphorous has been reported in the wild fin whales (*Balaenoptera physalus*) and in beluga whales (*Delphinapterus leucas)* compared to captive populations (Cook et al. 1990; Kjeld 2001) suggesting the effects of absorbed Mg^{2+} in its prey and dissolved concentrations of Mg^{2+} in the water (Kjeld 2001).

Plasma electrolyte concentration and osmolality in wild freshwater manatees were the same concentration as detected in both captive and wild marine populations (Irvine et al. 1980; Medway et al. 1982; Ortiz et al. 1998). Similarly, wild and captive West Indian manatees (*Trichechus manatus*) dwelling in freshwater were reported to have statistically significantly lower plasma Cl^-, K^+ and Na^+ levels than wild manatees dwelling in salt and brackish water (Ortiz et al. 1998). This all indicates that in fresh water, wild animals have access to salts either by eating aquatic vegetation having a sufficient salt content or by temporarily residing in a marine environment. Therefore, the long-term captivity of manatees in fresh water can cause hyponatremia, as reported in captive seals in a salt-deficient environment (Geraci 1972; St. Aubin and Geraci 1986). Hyponatremia in captive pinnipeds can be induced when living in fresh water and fed a salt-restricted diet. In some cases, mortality can result (Geraci 1972). Therefore, to avoid hyponatremia in captive pinnipeds fed on a low salt diet or held in fresh water, dietary salt supplements may be important (Ortiz 2001).

Hematology

Both WBCs and lymphocyte counts are common indicators of stress and inflammation. Like wild bottlenose dolphins, we also observed a statistically significantly higher count of WBCs and lymphocytes in wild YFPs, suggesting chronic exposure to infections, especially viral and parasites (Asper et al. 1990). The same as wild YFPs, higher eosinophil counts were also reported for wild harbor porpoise (Koopman et al. 1999) and bottlenose dolphin populations compared to captive populations (Asper et al. 1990). This increase in eosinophil count could be the result of heavy parasite infestation in their natural environment as claimed by Anderson (1966). However, a statistically significantly higher neutrophil and monocyte counts in the captive YFPs indicates a bacterial infection. An elevated basophil count, though rarely seen in marine mammals has an unclear significance (Mayer 1998). In marine mammals, high neutrophil counts suggest bacterial or fungal infections while higher monocytes count is correlated with chronic infections (Medway and Geraci 1986) which suggests poor health status for captive YFPs. However, instead of the nutritional

stress, chronic infections can significantly lower the level of serum ALB as observed in the wild population (Table 2) either by decreasing its rate of synthesis, increasing fractional catabolic rate or inducing anorexia (Don and Kaysen 2004).

Enzymes

The statistically significantly higher concentration of serum amylase was detected in captive YFPs which could suggest pancreatitis either in the form of chronic or acute fibrosis, which is very common in cetaceans (Wallach and Boever 1983). Usually, for treatment, selenium, zinc, B-complex vitamins and ascorbic acid can be used (Fowler and Cubas 2001). However, a significantly higher level of GGT in YFPs living in the wild may also suggest hepatic diseases (Mayer 1998).

CONCLUSIONS

Unlike marine mammals which are exposed to more salt, fresh water YFPs which even feed on small freshwater fish species are exposed to low salt levels. So in general YFPs and more specifically captive YFPs are extremely susceptible to electrolyte deficiencies as compared to their marine counterparts. The extremely low concentrations of Na^+, K^+, PO_4^{3-} and Mg^{2+} found in the serum samples of the captive YFPs indicates a nutritional deficiency. An electrolyte balance can be achieved through a modification in their diet. A long-term deficiency of these nutrients in captive YFPs can lead to various morbidities. However, an extremely high concentration of Cl^- in captive animals can predispose them to various pathologies, so it is very important to check and maintain normal Cl^- levels in their aquatic environment. Water quality should be carefully checked especially for chlorine levels. The high concentration of serum levels of LDL-c and lower levels of TP and GLB in captive YFPs either indicates improper nutrition and/or physical inactivity. Higher neutrophil and monocyte counts suggest an improper diet as well as a lack of a hygienic environment at the aquarium. Therefore, for captive animals and especially for endangered YFPs, it is necessary to formulate a special food supplement that can fulfill their daily body needs, as long term improper diet can result in weaker animals and can negatively compromise their reproduction, growth and health. We also suggest that it is necessary to arrange a series of different physical exercises for captive YFPs which may be helpful in reducing their serum LDL-c levels. Furthermore, a monthly physical examination for captive animals is suggested to monitor their health status.

Abbreviations: Albumin (ALB), Amylase (AMS), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN), Calcium (Ca^{2+}), Chinese Academy of Sciences (CAS), Chlorine (Cl^-), Creatinine (Cr), Creatinine Kinase (CK), Carbon Dioxide (CO_2), Globulin (GLB), Gamma-glutamyl Transferase (GGT), High Density Lipoprotein Cholesterol (HDL-c), Institute of Hydrobiology (IHB), Iron (Fe^{2+}), International Union for Conservation of Nature (IUCN), Lactate Dehydrogenase (LDH), Lower Density Lipoprotein Cholesterol (LDL-c), Magnesium (Mg^{2+}), Potassium (K^+), Phosphate (PO_4^{3-}), Sodium (Na^+), Triglycerides (TG), Total Cholesterol (TC), Total Protein (TP), Total Bilirubin (T-BILI), Urea (U), Yangtze Finless Porpoise (YFP), Yangtze Cetacean Breeding and Research Center (YCBRC).

Animals' ethics: Capture-release practices were permitted by the Ministry of Science and Technology of the People's Republic of China. The research permit was issued to the Institute of Hydrobiology of the Chinese Academy of Sciences (Permit no. 2011BAG07B05). The whole procedure strictly adhered to Chinese law and ethical guidelines for wild animals. No anesthesia, euthanasia, or any other kind of surgical procedure was a part of this study.

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Authors' contributions: GN designed the study, performed the statistical analyses, and wrote the manuscript. YH, XZ and DW collected the blood samples and analyzed them. All authors participated in revising the manuscript. All authors read and approved the final manuscript.

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Ethics approval consent to participate: All appropriate ethics and protocol approvals were obtained for this research from the Ministry of Agriculture of the People's Republic of China. The blood sampling procedure was also reviewed and approved ethically and technically by the Research Ethic Committee of Institute of Hydrobiology, Chinese Academy of Science.

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