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The Possible Effects of High Vessel Traffic on the Physiological Parameters of the Critically Endangered Yangtze Finless Porpoise (*Neophocaena asiaeorientalis ssp. asiaeorientalis*)

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Background: Poyang is the largest freshwater lake in China, where the acoustic environment and space for the critically endangered Yangtze finless porpoises (YFPs) has been altered by heavy vessel traffic and dredging activities. The density of vessel and the rate of dredging increases annually, especially in the area with the highest density of YFPs. The heavy vessel traffic can cause an increase in the physical activities and direct physical injuries to the YFPs. Furthermore, noise is a potent stressor to all cetaceans irrespective of age and can compromise all their physiological functions. The objective of this study was to examine the possible effects of heavy vessel traffic and dredging on the biochemistry, hematology, adrenal, thyroid, and reproductive hormones of two different YFP populations. One population was living in Poyang Lake and the second living in the Tian-E-Zhou Oxbow which is a semi-natural resserve.

Results: The results showed statistically significantly higher levels of serum cortisol, fT3, fT4, and lowered testosterone in both adult and juvenile YFPs living in Poyang Lake vs. adult YFPs living in the Tian-E-Zhou Oxbow. The serum biochemical parameters (Aspartate Amino Transferase, Alkaline Phosphatase, High Density Lipoprotein cholesterol ratio, Globulin, Uric acid, Glucose, K^+, and Amylase) and the hematology parameters (Red Blood Cells, Hematocrit, Mean Corpuscular Volume, White Blood Cells, and Eosinophils) were statistically significantly higher in the adult Poyang Lake YFPs vs. adult Tian-E-Zhou Oxbow YFPs. On the other hand, adult males of the Tian-E-Zhou Oxbow also showed significantly higher levels of the serum biochemical parameters (Total Cholesterol, Light Density Lipoprotein cholesterol, Direct Bilirubin, Albumin, Lactate Dehydrogenase, CO_2, and Na^+) and the blood parameters (Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration). In Poyang Lake YFPs, various parameters showed significantly positive (fT4, amylase, neutrophil, Ca^{+2}) or negative (total protein, lymphocyte) correlations with cortisol levels.

Conclusions: The hyperactivity of adrenal glands in response to heavy vessel traffic and dredging resulted in significantly elevated cortisol levels in Poyang Lake YFPs. The higher cortisol level could possibly have affected various hormonal, hematological, and biochemical parameters, and ultimately the YFPs physiology.

Keywords: acoustic pollution, cortisol, critically endangered, stress, thyroid, Yangtze finless porpoise

BACKGROUND

For the economy of China, the Yangtze River is very crucial and is, therefore, a heavily trafficked waterway and inland water transport (Liu et al., 2000; Schelle, 2010). The vessels include motorized ferries, small boats, oil tankers, and container ships (Smith and Reeves, 2000). In the year 2000, ∼400 million tons of cargo were transported on this river, which has increased to ∼1.2 billion tons of cargo (Yang et al., 2009). In the 1980's, the vessel trafficking was low in the Yangtze River (Turvey et al., 2007). Since this river is the backbone and golden channel of the Chinese economy (Fu et al., 2010), the number of vessels increased up to 5 times in 2006 (Wang et al., 2006), and today, it is the world's busiest inland river (Lixin, 2018). To accommodate the increased ship traffic and to allow large container ships, the channel was widened using explosives (Wang, 2009).

The practice of intensive sand dredging has been banned in the Yangtze River since 1998 (Zhong and Chen, 2005). It restarted in 2001 in Poyang Lake, which is an appended lake of the Yangtze River (Zhong and Chen, 2005). The number of dredging vessels rapidly increased due to the potential profit (Wu et al., 2007). The number of vessels in Poyang Lake increased from zero in 2000 to ∼400 in 2005 (Wu et al., 2007). Even today there are a few thousand vessels engaged in digging and transportation (Wang et al., 2006). Still, due to the rapid expansion of Wuhan city, both the price and demand for sand and gravel has increased, and as a result, a large number of sand mining boats have come to Poyang Lake. Larger ships leave and enter the lake at a rate of 2 ships/min (Zhang, 2007) and this number is likely even more today. Approximately 160 sand dredging enterprises are operating on the lake (Li, 2008). These dredging activities are mostly concentrated in the channel in the northern part of Poyang Lake. This area has the highest density of Yangtze finless porpoises (YFPs) (Yang et al., 2000). It has been assumed that the noise produced by the dredging and the heavy boat traffic has significantly changed the acoustic environment of Poyang Lake (Jing, 2008), resulting in acoustic pollution (Schelle, 2010). Furthermore, such high-density dredging projects have caused the death of local wildlife, including YFPs (Jing, 2008).

The YFP is a critically endangered (Mei et al., 2014) freshwater cetacean (Wang, 2009) endemic in the Yangtze River (lower and middle reaches) and its two adjoining lakes (Poyang and Dongting Lakes) (Gao and Zhou, 1993). A series of studies have reported a continuous reduction in the number of YFPs from more than 2,500 YFPs in 1991 (Zhang et al., 1993) to 1,225 (Zhao et al., 2008), 1,800 (Zhao et al., 2008), and recently in 2012, the total population was estimated to be 1,040 individuals (Mei et al., 2014). Of these 1,040 YFPs, ∼400 individuals thrive in Poyang Lake (Zhao et al., 2008). In Poyang Lake, due to high turbidity and tremendous acoustic pollution, resulting from sand mining and heavy vessel traffic, the sonar system of YFPs is severely disturbed, resulting in navigational failure, difficulty in sensing prey and escaping danger, and migration failure between the Yangtze River and Poyang Lake (Wang et al., 2006; Zhang, 2007). In cetaceans, a series of studies have reported various organ injuries and sudden death because of collisions with the vessel and acoustic pollution (Freitas, 2004; Fernández et al., 2005; Cox et al., 2006). In addition, noise caused by heavy vessel traffic compromises nearly all the normal behaviors in cetaceans, including reproduction, energy expenditure, and energy acquisition (Hastie et al., 2003; Lusseau, 2003; Constantine et al., 2004; Lemon et al., 2006; Miller et al., 2008).

Noise is a potential stressor for all cetaceans (Wright et al., 2007). It stimulates the Hypothalamic-pituitary-adrenal (HPA) axis and it increases the level of cortisol secretion (Rolland et al., 2012). The higher the cortisol level the more the Hypothalamic-pituitary-gonadal (HPG) axis is suppressed (Bethea et al., 2008). Both acute and chronic stress resulting from acoustic pollution can increase the expression of RFamide-related peptides (RFRPs) in the hypothalamus and apoptosis of Leydig cells in the testes. Similarly, acoustic pollution inhibits the expression of Kisspeptin 1(KISS1) and Gonadotrophin-releasing-hormone (GnRH) in the hypothalamus. As a result, the secretion of pituitary Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) is reduced, which ultimately compromises steroidogenesis and gametogenesis (Nabi et al., 2018). Furthermore, acoustic pollution has negative effects on the physiology of the thyroid gland and this can cause metabolic disorders (Ramezani et al., 2014). Noise also has negative effects on the hematology and the biochemistry of cetaceans (St Aubin and Geraci, 1989; Asper et al., 1990; Bossart et al., 2001). Due to the presence of heavy vessel traffic in Poyang Lake, the objective of this study was to investigate the physiological effects of high vessel traffic on the

Abbreviations: ATP, Adenosine Triphosphate; ALT, Alaline amino Transferase; AlB, Albumin; ALP, Alkaline Phosphatase; AMS, Amylase; AST, Aspartate amino Transferase; BUN, Blood Urea Nitrogen, CO_2, Carbon Dioxide; CK, Creatine Kinase, Cr, Creatinine; DBILI, Direct Bilirubin; GGT, Gamma-glutamyl Transferase; GLB, Globulin; GLU, Glucose; HCT, Hematocrit; Hb, Hemoglobin; HDL-c, High Density Lipoprotein cholesterol; HPA, Hypothalamic-Pituitary-Adrenal; HPG, Hypothalamic-Pituitary-Gonadal; IBILI, Indirect Bilirubin; LDH, Lactate Dehydrogenase; LDL-c, Light Density Lipoprotein cholesterol; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; PLT, Platelets; RBCs, Red Blood Cells; TBA, Total Bile Acid; TBILI, Total Bilirubin; TC, Total Cholesterol; TP, Total Protein; TG, Triglyceride; UA, Uric Acid; WBCs, White Blood Cells; YFPs, Yangtze Finless Porpoises.

hematology, biochemistry, and hormonal profile by comparing two different YFPs populations. One population living in Poyang Lake and the second living the Tian-E-Zhou Oxbow, where vessel traffic is negligible.

MATERIALS AND METHODS

Study Location

In China, Poyang Lake, also named the Kingdom of rare birds, is the largest freshwater lake. It is located at longitude 115° 47′-116° 45′ east and latitude 28° 22′-29° 45′ north. The total area of the lake is 1.6×10^5 km^2. However, its size fluctuates with the season. It is fed by the Ganjiang River, Raohe River, Xiushui River, Xinjiang River, and Fuhe River. Finally, water empties into the Yangtze River (Jing, 2008; Sun et al., 2012; Dong, 2013; Vision Times Chinese., 2014). Poyang Lake is exposed to heavy vessel traffic and to chronic acoustic pollution arising from dynamite explosions, heavy vessel trafficking, and dredging (Wang, 2009; Schelle, 2010).

The Tian-E-Zhou Oxbow (E11 2°31′-112°36′, N29°46′-29°51′) is located near Shishou city in Hubei Province. It is an oxbow shaped semi-natural reserve formed in 1972 due to a deviation in the natural flow of the Yangtze River. The total length of the oxbow is about 21 km with a width of about 1–2 km (Hao et al., 2009; Wang, 2013). In the reserve, there is no dredging, or intensive shipping and even fishing is banned at certain times of the year. The reserve is managed regularly and the YFPs are assessed for health, fertility, and also investigated for research (Hao et al., 2009).

Animal Chasing, Catching, Handling, and Release

In both populations, sound chase and net capture methods were used for animal capturing (Hua, 1987). A detailed explanation of the method is found in a paper by Hao et al. (2009). Each day, 5–10 YFPs in both populations were gently chased by several parallel fishing boats for approximately 15 min. The speed of fishing boats was slower than 10 km/h and the noise was 4.5 hp. The YFPs were allowed to swim in an open area where a spacious enclosure was made by using fishermen nets. The animals were confined to one section of the reserve overnight. The nets were enough soft to avoid injuries and allow fish to pass through it. The next morning, after gradually reducing the enclosed area, the animals were taken out of the water and transported to the medical boat for a physical examination and blood sampling. This process took ∼15 min. The animals were put on a sponge mattress and were gently restrained. Each animal was sampled within 1 min after arrival. The methodology during the capture event, the blood sampling procedure and the timing of blood collection were consistent for both populations. There was a very little variation between the capture timing of both populations. During the entire process, behavioral reactions, breathing frequency, skin hydration, and the general health of all the animals were strictly monitored. All animals were then gently released back into their environment immediately after sampling.

Study Design

For the study, a total of 43 male YFPs were recruited from Poyang Lake ($n = 20$) and the Tian-E-Zhou Oxbow ($n = 23$). The YFPs in Tian-E-Zhou Oxbow were all adults. However, the YFPs in Poyang Lake were divided into juvenile males ($n = 08$) and adult males ($n = 12$) on the basis of body length (Gao and Zhou, 1993). In the Tian-E-Zhou Oxbow, the YFPs catching operation was carried out in the years 2002 (Winter/$n = 06$) and 2003 (Winter/$n = 07$). In Poyang Lake, the YFPs were captured during a physical examination project conducted in 2009 (Winter/$n = 20$).

Blood Sampling

Approximately 10 ml blood samples were aseptically obtained at a single event from the main vein on the dorsal side of the tail fluke, using a 10 ml heparinized syringe (Gemtier, G/Ø/ L: 21/0.7/31 mm, 201502, Shanghai, China). For hematology, ∼2 ml of the blood collected was poured into heparinized tubes (Nihon, 161–8560, Tokyo, Japan). All the remaining blood samples were then transferred to a centrifuge tube (Corning, 14831, New York, America) for serum separation through centrifugation (Eppendorf AG, 22332, Hamburg, Germany) at 1,500 × g for 15 min. The serum was then immediately transferred to frost-free plastic tubes and stored in liquid nitrogen. In the laboratory, all samples were stored at −25°C. After blood sampling, standard body length and body mass were collected from each finless porpoise as soon as possible (American Society of Mammalogists, 1961).

Complete Blood Count

Blood parameters such as; Hemoglobin (Hb), Red Blood Cells (RBCs), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Platelets (PLT), Eosinophils, Basophils, Monocytes, Lymphocytes, Neutrophils, and White Blood Cells (WBCs) were analyzed using a hematology analyzer (Beckman-Coulter, DxH 800, Porto, Portugal) according to the manufacturer's instructions.

Biochemical Analysis

The lipid profile; [Triglyceride (TG), Total Cholesterol (TC), Low Density Lipoprotein cholesterol (LDL-c), High Density Lipoprotein cholesterol (HDL-c)]; the liver function parameters [Gamma-glutamyl Transferase (GGT), Aspartate amino Transferase (AST), Alkaline Phosphatase (ALP), Alaline amino Transferase (ALT), Total Bile Acid (TBA), Direct Bilirubin (D-BILI), Indirect Bilirubin (I-BILI), Total Bilirubin (T-BILI)]; the enzymes; [Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Amylase (AMS)]; Electrolytes (Na^+, K^+, Cl^-, Ca^{2+}, $PO4^{3-}$, Mg^{+2}, Fe^{+2}); and other biochemical parameters, such as Globulin (GLB), Albumin (AlB), Total Protein (TP), Blood Urea Nitrogen (BUN), Uric Acid (UA), Creatinine (Cr), Glucose (GLU), and Carbon Dioxide (CO_2) were measured using a calibrated automated clinical chemistry analyzer (Beckman-Coulter, AU5400, Porto, Portugal) according to the manufacturer's instructions.

Radioimmunoassay of Serum Hormone

For the analysis of serum cortisol, testosterone, estradiol, and thyroid hormones (T4, T3, fT3, fT4), the commercially available RIA kits were used according to the manufacturer's instructions (Tian-jin Leeco Biotechnological and Medical Products Inc., China). These kits have been validated by Hao et al. (2007, 2009) for accuracy and parallelism. All the samples were assayed in duplicates by the same person. The intra- and inter-assay coefficients of variability for cortisol were <5 and <10%, testosterone 8.1 and 5.5%, and estradiol 9.2 and 7.3%. For the thyroid hormones (T4, T3, fT3, fT4) the intra- and inter-assay coefficients of variability were within 5 and 10%. The hormones (fT3, fT4, cortisol, testosterone) were analyzed in both populations. However, T3, T4, and estradiol were only analyzed in the Tian-E-Zhou Oxbow YFPs.

Statistical Analyses

The studied parameters in both populations, as well as, in juvenile males and adult males of Poyang Lake were compared by the non-parametric Mann Whitney *U*-test using Graph Pad Prism, version 5.01 (Graph Pad Software Inc., San Diego, CA, USA). The relationship between cortisol and other hormonal, hematological, and biochemical parameters in each population were analyzed using the Pearson correlation. A $P < 0.05$ indicated a statistically significant difference. All the data is presented as mean ± SEM, median, upper 95% CI, lower 95% CI and *P*-value.

Limitations

The main limitations of our study were the limited quantity of blood samples as they were used for different molecular, biochemical, hormonal and hematological studies. Therefore, some hormones like T3 and T4 were not analyzed in the Tian-E-Zhou Oxbow populations. Furthermore, due to technical and ethical issues, sampling in different seasons was not done at Poyang Lake.

RESULTS

Hematology

The complete blood profile of Poyang Lake YFPs showed a statistically significantly higher level of RBCs, HCT, MCV, WBCs, and Eosinophils compared to the Tian-E-Zhou Oxbow YFPs (**Table 1**). However, in the Tian-E-Zhou Oxbow YFPs, only MCH and MCHC were statistically significantly higher.

Biochemistry

The level of liver enzymes (AST, ALP), HDL-C/LDL-C, GLB, UA, Glucose, K^+, and AMS were statistically significantly higher in porpoises living in Poyang Lake. On the other hand, in the Tian-E-Zhou Oxbow YFPs, we observed statistically significantly higher levels of the lipid parameters (TC, LDL-C), DBIL, ALB, ALB/GLB, CO_2, Na^+, and LDH (**Tables 2**, **3**).

Serum Hormones

In the adult YFPs living in Poyang Lake, serum cortisol, fT3, and fT4 were significantly higher. Only testosterone was significantly higher in the Tian-E-Zhou Oxbow adult YFPs (**Table 4**). Similarly, in Poyang Lake juvenile males, serum cortisol, fT3, and fT4 were significantly higher than in the adult males of Tian-E-Zhou Oxbow (**Table 5**). Testosterone, both in the adult males of Poyang Lake and Tian-E-Zhou Oxbow, was statistically significantly higher compared to the juvenile males of Poyang Lake (**Tables 5**, **6**).

TABLE 1 | Comparison of blood parameters between the Poyang Lake (n = 12) and Tian-E-Zhou Oxbow (n = 13) adult male YFPs (winter season).

Parameters	Mean ± SEM	Upper 95% CI	Lower 95% CI	Median	*P*
Rbc (10^{12}/L)	[a]4.98 ± 0.03 [b]5.30 ± 0.12	5.08 5.58	4.88 5.03	4.99 5.35	0.0271
Hb (g/L)	[a]176.0 ± 2.81 [b]157.5 ± 14.60	183.8 190.1	168.2 125.0	176.0 173.0	0.2482
HCT (%)	[a]43.52 ± 0.52 [b]47.19 ± 0.98	44.98 49.43	42.06 44.95	43.70 48.25	0.0216
MCH (pg)	[a]35.18 ± 0.49 [b]32.35 ± 0.67	36.56 33.84	33.80 30.85	35.50 32.40	0.0117
MCHC (g/L)	[a]409.8 ± 4.42 [b]363.7 ± 3.07	422.1 370.6	397.5 356.9	409.0 361.0	0.0011
MCV (fL)	[a]86.0 ± 1.35 [b]89.87 ± 1.36	89.76 92.92	82.24 86.82	85.60 89.90	0.0394
PLT (10^9/L)	[a]139.4 ± 7.17 [b]120.7 ± 14.58	159.3 154.3	119.5 87.06	130.0 107.0	0.2188
WBCs (10^9/L)	[a]4.62 ± 0.12 [b]6.40 ± 0.41	4.95 7.32	4.28 5.47	4.80 6.10	0.0011
Neutrophil (%)	[a]58.76 ± 3.12 [b]48.08 ± 3.53	67.43 55.86	50.09 40.31	55.50 51.0	0.0558
Lymphocyte (%)	[a]25.84 ± 2.37 [b]30.58 ± 3.70	32.45 38.73	19.23 22.44	25.70 27.00	0.3176
Monocyte (%)	[a]0.50 ± 0.22 [b]1.12 ± 0.26	1.12 1.71	–0.12 0.53	0.50 1.25	0.1087
Eosinophil (%)	[a]13.50 ± 1.73 [b]19.38 ± 1.04	18.31 21.67	8.69 17.08	12.00 19.00	0.0066
Basophil (%)	[a]0.020 ± 0.02 [b]0.04 ± 0.04	0.07 0.13	–0.03 –0.05	0.00 0.00	0.3190

[a]*Tian-E-Zhou Oxbow YFPs.*
[b]*Poyang Lake YFPs.*

Correlations of Cortisol With Hormones, Biochemical, and Blood Cells

The adult males of Poyang Lake showed a statistically significant positive correlation between cortisol and fT4 ($P = 0.0078$) (**Figure 1**). Similarly, we observed statistically significant positive correlations in AMS ($P = 0.0478$) and Ca^{+2} ($P = 0.0117$) and a statistically significant negative correlation in TP ($P = 0.0474$) with cortisol in Poyang Lake YFPs (**Figure 1**). Furthermore, neutrophils showed a statistically significantly ($P = 0.0090$) positive correlation and lymphocytes showed a statistically significant ($P = 0.0293$) negative correlation with serum cortisol in Poyang Lake YFPs (**Figure 1**).

Body-Weight/Body-Length (BW/BL) Ratio

Comparison of the BW/BL between the adult males of both populations showed no significant difference. As expected, the

TABLE 2 | Comparison of biochemical parameters between the Poyang Lake ($n = 12$) and Tian-E-Zhou Oxbow ($n = 13$) adult male YFPs (winter season).

Parameters	Mean ± SEM	Upper 95% CI	Lower 95% CI	Mean	P
ALT	[a]31.40 ± 3.32	40.63	22.17	31.00	0.3393
(U/L)	[b]33.80 ± 2.73	39.98	27.62	32.50	
AST	[a]181.0 ± 8.17	203.7	158.3	174.0	0.0097
(U/L)	[b]209.7 ± 7.52	226.7	192.7	206.0	
AST/ALT	[a]5.96 ± 0.54	7.47	4.44	5.63	0.3393
	[b]6.45 ± 0.43	7.42	5.47	6.10	
ALP	[a]72.0 ± 9.14	97.39	46.61	62.0	0.0376
(U/L)	[b]119.5 ± 19.26	163.1	75.93	134.0	
GGT	[a]42.60 ± 5.68	58.37	26.83	38.0	0.2499
(U/L)	[b]45.30 ± 3.47	53.16	37.44	41.50	
TBIL	[a]4.08 ± 0.22	4.69	3.46	4.30	0.1221
(μmol/L)	[b]3.77 ± 1.03	6.10	1.43	2.35	
DBIL	[a]1.28 ± 0.03	1.38	1.17	1.30	0.0013
(μmol/L)	[b]0.27 ± 0.03	0.35	0.18	0.30	
IBIL	[a]2.80 ± 0.20	3.35	2.24	3.00	0.1770
(μmol/L)	[b]3.51 ± 1.05	5.89	1.12	2.05	
TBA	[a]7.72 ± 1.41	11.65	3.79	9.30	0.1855
(μmol/L)	[b]6.78 ± 1.88	11.04	2.52	4.80	
TC	[a]6.23 ± 0.28	7.02	5.44	6.39	0.0249
(mmol/L)	[b]5.41 ± 0.22	5.91	4.91	5.36	
TG	[a]1.10 ± 0.09	1.38	0.83	1.05	0.2198
(mmol/L)	[b]0.98 ± 0.12	1.27	0.70	0.98	
HDL-C	[a]3.35 ± 0.17	3.83	2.86	3.45	0.0888
(mmol/L)	[b]3.02 ± 0.12	3.30	2.75	2.96	
LDL-C	[a]1.60 ± 0.11	1.92	1.27	1.65	0.0003
(mmol/L)	[b]0.22 ± 0.02	0.27	0.18	0.23	
HDL-C	[a]2.10 ± 0.05	2.26	1.95	2.09	0.0013
LDL-C	[b]14.0 ± 0.99	16.24	11.76	15.03	
TP	[a]74.22 ± 0.75	76.31	72.13	73.70	0.4296
(g/L)	[b]75.37 ± 2.16	80.26	70.48	73.75	
ALB	[a]64.38 ± 1.08	67.38	61.38	62.90	0.0003
(g/L)	[b]46.08 ± 0.91	48.14	44.02	45.30	
GLB	[a]9.84 ± 0.76	11.95	7.72	10.10	0.0013
(g/L)	[b]29.16 ± 1.30	32.12	26.20	28.05	
ALB/GLB	[a]6.76 ± 0.68	8.66	4.86	6.24	0.0013
	[b]1.59 ± 0.04	1.68	1.49	1.59	
BUN	[a]15.82 ± 1.33	19.54	12.11	17.32	0.4296
(mmol/L)	[b]15.92 ± 1.17	18.58	13.25	14.89	
Urea	[a]18.02 ± 1.77	22.94	13.10	17.10	0.0003
(μmol/L)	[b]45.03 ± 4.90	56.13	33.93	43.45	
CO_2	[a]28.36 ± 1.26	31.88	24.84	27.90	0.003
(mmol/L)	[b]19.26 ± 0.76	20.99	17.53	18.80	
Glucose	7.40 ± 0.21	8.00	6.79	7.23	0.0276
(mmol/L)	8.09 ± 0.58	9.42	6.76	8.22	

[a]*Tian-E-Zhou Oxbow YFPs.*
[b]*Poyang Lake YFPs.*

TABLE 3 | Comparison of electrolytes and enzymes between the Poyang Lake ($n = 12$) and Tian-E-Zhou Oxbow ($n = 13$) adult male YFPs (winter season).

Parameters	Mean ± SEM	Upper 95% CI	Lower 95% CI	Median	P
Cr	[a]92.36 ± 6.65	110.8	73.88	89.50	0.0823
(μmol/L)	[b]80.50 ± 2.32	85.75	75.25	82.20	
K^+	[a]3.19 ± 0.13	3.55	2.83	3.24	0.0005
(mmol/L)	[b]4.16 ± 0.10	4.41	3.91	4.09	
Na^+	[a]155.4 ± 0.49	156.8	154.0	155.4	0.0210
(mmol/L)	[b]153.2 ± 0.77	155.0	151.4	153.8	
Cl^-	[a]109.1 ± 0.96	111.7	106.4	110.0	0.1928
(mmol/L)	[b]110.0 ± 1.12	112.6	107.4	111.6	
Ca^{2+}	[a]2.57 ± 0.03	2.66	2.49	2.55	0.2738
(mmol/L)	[b]2.62 ± 0.03	2.71	2.53	2.59	
$PO4^{3-}$	[a]1.04 ± 0.07	1.24	0.84	1.03	0.4734
(mmol/L)	[b]1.03 ± 0.10	1.26	0.79	1.20	
Mg^{2+}	[a]0.66 ± 0.02	0.73	0.60	0.69	0.1148
(mmol/L)	[b]0.75 ± 0.03	0.84	0.66	0.71	
Fe^{2+}	[a]24.70 ± 1.11	27.80	21.60	24.50	0.0734
(μmol/L)	[b]30.63 ± 2.41	36.19	25.07	31.10	
CK	[a]97.0 ± 24.04	163.8	30.25	78.0	0.4734
(U/L)	[b]104.9 ± 25.65	164.0	45.74	87.0	
LDH	[a]220.8 ± 13.18	257.4	184.2	229.0	0.0024
(U/L)	[b]152.9 ± 4.93	164.1	141.7	148.5	
AMS	[a]5.60 ± 0.40	6.71	4.48	5.00	0.0023
(U/L)	[b]9.50 ± 0.42	10.47	8.53	10.00	

[a]*Tian-E-Zhou Oxbow YFPs.*
[b]*Poyang Lake YFPs.*

TABLE 4 | Comparison of serum hormones between the adult males of Poyang Lake ($n = 12$) and Tian-E-Zhou Oxbow ($n = 13$) YFPs (winter season).

Parameters	Mean ± SEM	Upper 95% CI	Lower 95%	Median	P-value
Cortisol	[a]149.4 ± 11.54	175.1	123.6	144	<0.0001
(ng/mL)	[b]856.6 ± 18.72	898.3	814.9	858.5	
Testosterone	[a]122.5 ± 21.90	170.7	74.32	101.8	0.0268
(ng/dL)	[b]77.74 ± 1.60	81.28	74.20	79.06	
fT3	[a]19.47 ± 0.12	21.75	11.20	16.43	<0.0001
(pg/mL)	[b]402.1 ± 41.58	493.6	310.6	402.3	
fT4	31.51 ± 5.42	43.59	19.44	22.99	<0.0001
(pg/mL)	261.0 ± 18.83	303.0	219.1	255.4	

[a]*Tian-E-Zhou Oxbow YFPs.* [b]*Poyang Lake YFPs.*

BW/BL of the Poyang Lake JM was significantly lowered than the AM.

DISCUSSION

Hematology

In porpoises living in Poyang Lake, we observed significantly higher levels of RBCs, HCT, MCV, WBCs, and eosinophils. The noise and heavy vessel traffic in Poyang Lake could have increased the diving activity of the animals. As a result, more oxygen is utilized. To facilitate oxygen transport, the concentration of HCT increases, which further encourages the synthesis of RBCs (Panneton, 2013; Fahlman et al., 2018). Furthermore, the significantly higher levels of WBCs and eosinophils in porpoises living in Poyang Lake could also be related to anthropogenic stress. Such an association has been observed in bottlenose dolphins (Asper et al., 1990), and beluga whales (St Aubin and Geraci, 1989). The statistically significant positive correlation observed in the Poyang Lake YFPs between the cortisol and neutrophil has been reported by Davis et al. (1991) in humans after exogenous intravenous administration of cortisol (Davis et al., 1991). Similarly, in the harbor seal (*Phoca vitulina*),

TABLE 5 | Comparisons of serum hormones between the adult males of Tian-E-Zhou Oxbow ($n = 13$) and juvenile males of Poyang Lake ($n = 08$) (winter season).

Parameters	Mean ± SEM	Upper 95% CI	Lower 95%	Median	*P*-value
Cortisol (ng/mL)	[a]149.4 ± 11.54 [b]874.6 ± 26.87	175.1 937.6	123.6 810.5	144 905.9	<0.0001
Testosterone (ng/dL)	[a]122.5 ± 21.90 [b]41.62 ± 8.01	170.7 60.58	74.32 22.67	101.8 37.52	0.0020
fT3 (pg/mL)	[a]19.47 ± 0.12 [b]493.2 ± 55.21	21.75 623.7	11.20 362.6	16.43 561.3	<0.0001
fT4 (pg/mL)	[a]31.51 ± 5.42 [b]276.2 ± 14.03	43.59 309.3	19.44 243.0	22.99 269.3	<0.0001

[a]*Tian-E-Zhou Oxbow adult YFPs.*
[b]*Poyang Lake juvenile YFPs.*

TABLE 6 | Comparison of serum hormones between the adult ($n = 12$) and juvenile males ($n = 08$) of Poyang Lake YFPs (winter season).

Parameters	Mean ± SEM	Upper 95% CI	Lower 95% CI	Median	*P*
Cortisol (ng/mL)	[a]856.6 ± 18.72 [b]874.0 ± 26.87	898.3 937.6	814.9 810.5	858.5 905.9	0.2949
Testosterone (ng/dL)	[a]77.74 ± 1.60 [b]41.62 ± 8.01	81.28 60.58	74.20 22.67	79.06 37.52	<0.0001
fT3 (pg/mL)	[a]402.1 ± 41.58 [b]493.2 ± 55.21	493.6 623.7	310.6 362.6	402.3 561.3	0.0985
T3 (ng/mL)	[a]21.32 ± 3.86 [b]20.33 ± 2.01	29.93 25.09	12.71 15.58	20.75 19.97	0.4209
fT4 (pg/mL)	[a]261.0 ± 18.83 [b]276.2 ± 14.03	303.0 309.3	219.1 243.0	255.4 269.3	0.2782
T4 (µg/mL)	[a]4.28 ± 0.27 [b]3.97 ± 0.25	4.89 4.57	3.67 3.36	3.97 3.76	0.2220

[a]*Adult YFPs.*
[b]*Juvenile YFPs.*

exogenous administration of adrenocorticotrophic hormone (ACTH) increased neutrophil and decrease lymphocyte counts (Keogh and Atkinson, 2015).

Biochemistry

In Poyang Lake YFPs, the significantly higher levels of serum glucose could be either due to stress, higher physical activities due to high vessel traffic, and/or hyperthyroidism (Bossart et al., 2001; Richter et al., 2001). Both the noise exposure and higher physical activity increases metabolic rate. More glucose is therefore needed to provide energy for elevated muscular activities (Richter et al., 2001; Castellini and Castellini, 2004). Higher physical activities increase muscle blood flow, glucose delivery, muscle membrane glucose transport capacity, muscle glycogen synthase activity, and other enzymatic activities associated with glucose metabolism (Richter et al., 2001). The intense physical activities produce varying amounts of stress depending on the intensity and duration. A statistically significant positive correlation in Poyang Lake YFPs between cortisol and AMS suggests that AMS can be used as a stress marker resulting from physical exertion (Allen, 2014). Similarly, the likely higher strenuous muscular activities of Poyang Lake YFPs in response to vessel trafficking also significantly increase serum K^+ concentrations. The higher K^+ concentration reflects leaking of the K^+ into the bloodstream from hyperactive muscles (Geraci and Medway, 1973).

We observed higher TC and LDL-c in the Tian-E-Zhou Oxbow YFPs and higher HDL-c/LDL-c in Poyang Lake YFPs suggesting the effects of physical activities and/or food quality. However, we did not observe a statistically significant difference in the BW/BL between the two populations of adult males. Therefore, this is likely linked to the physical activities of the YFPs. This has been reported in captive and wild YFPs (Nabi et al., 2017), and beluga whales (*Delphinap terus leucas*) (St Aubin and Geraci, 1989; Cook et al., 1990).

Similarly, we found statistically significantly higher levels of AST and ALP in porpoises living in Poyang Lake compared to the statistically significantly higher levels of DBILI in the Tian-E-Zhou Oxbow YFPs. The statistically significantly higher serum AST and ALP in Poyang Lake YFPs could also be attributed to the persistent strenuous physical activities as reported by several studies (Bürger-Mendonça et al., 2008; Ghorbani and Gaeini, 2013; Nazari et al., 2014; Ekun et al., 2017). The enzymes AST and ALP are present both in the muscles and the liver. During strenuous physical activities, damage to muscle tissue can occur, which allows these enzymes to enter the bloodstream. To provide a continuous energy flow to the exercising muscles, AST catabolizes amino acids to produce Adenosine Triphosphate (ATP). Similarly, a higher serum ALP concentration indicates lipid peroxidation and gluconeogenesis, which are used for energy hemostasis (Nazari et al., 2014). Furthermore, the high concentration of thyroid hormones and high metabolism of Poyang Lake YFPs can also possibly cause oxidative damage to the hepatocytes thereby increasing the serum ALP and AST concentrations (Khemichian and Fong, 2011).

The significantly higher levels of serum GLB and AMS in Poyang Lake YFPs might be due to acoustic pollution induced stress (Haider et al., 1977; Petrakova et al., 2015). The negative correlation of cortisol with TP in Poyang Lake population could possibly be due to glucocorticoids-induced increase in protein degradation and decrease protein synthesis (Kuo et al., 2013; Corazza et al., 2014). Similarly, the positive correlation of cortisol with Ca^{+2} has been reported in human (Radhi, 2014). In a variety of vertebrates, serum Ca^{+2} concentration increases in response to intense physical activities (Ruben and Bennett, 1981). As parathyroid gland regulates Ca^{+2} hemostasis, therefore further studies in YFPs are needed to investigate if parathyroid hormone can be used as a biomarker for physical stress.

Serum Hormones

We observed statistically significantly higher cortisol level in the heavily trafficked and noisy Poyang Lake YFPs vs. the control Tian-E-Zhou Oxbow YFPs. This difference might reflect differences in baseline cortisol levels, stress response during handling, or the additive effects of both. In wild cetaceans, the process of animals handling, and blood collection induces stress. Therefore, it is difficult to investigate the actual physiological response of an animal to environmental stressor in the wild

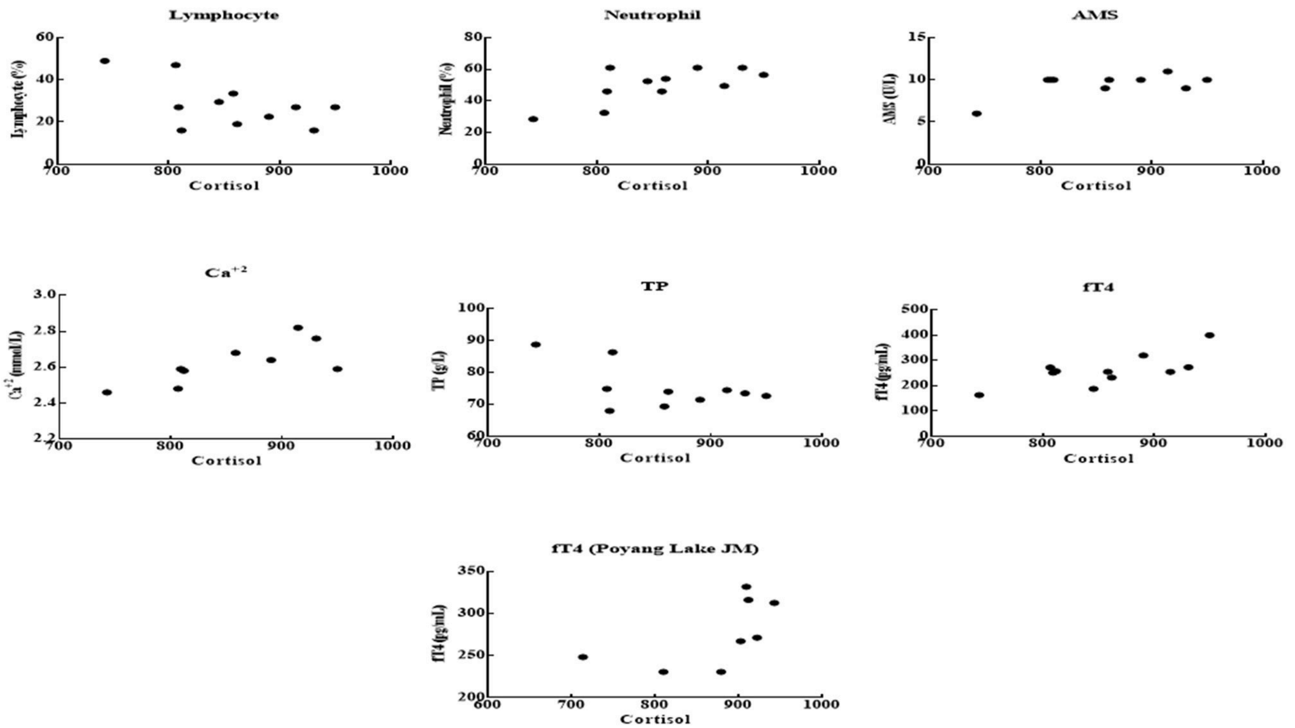

FIGURE 1 | Correlations of the Poyang Lake JM (only cortisol vs. fT4) and AM between cortisol and blood, biochemical and hormonal parameters. Cortisol showed statistically significant negative associations with lymphocyte ($r = -0.5851$) and TP ($r = -0.5565$). The neutrophil ($r = 0.6931$), AMS ($r = 0.5553$), Ca^{+2} ($r = 0.737$), and fT4 ($r = 0.7038$) showed significant positive associations with cortisol. The juvenile male of Poyang Lake showed nearly a significant positive relationship between serum cortisol and fT4 ($r = 0.5981$; $P = 0.0586$).

(Fair et al., 2014; Atkinson et al., 2015). Furthermore, our single observatory data is not enough to indicate baseline values, especially for stress-related parameters. Therefore, it is essential to consider the possible combined effects of environment and capture/handling responses in the interpretation of results related to hormones that are secreted immediately (Fair et al., 2017).

The higher cortisol level in the heavily trafficked Poyang Lake population might be in response to acoustic pollution as compared to the control Tian-E-Zhou Oxbow population. The higher cortisol in Poyang Lake YFPs might be an adaptation for maintaining the physiological integrity and ultimately the survival of an organism in the presence of potential stressors (Ulrich-Lai and Herman, 2009; Sheriff et al., 2011). For all cetaceans, noise is a stressor regardless of age (Wright et al., 2007). Several studies on North Atlantic right whales (*Eubalaena glacialis*), and bottlenose dolphins (*Tursiops truncates)* reported a higher level of cortisol in response to the noise emitted either from ships or from water seismic guns (Romano et al., 2004; Rolland et al., 2012). There is a direct correlation between noise and fecal glucocorticoid levels. With increasing ship noise, there is increasing levels of fecal glucocorticoids (Rolland et al., 2012). This happens through a generalized stress response, such as the elevation of glucocorticoid levels (cortisol and corticosterone) by HPA-axis activation (St Aubin and Geraci, 1989, 1990; St Aubin et al., 1996). An increased heart rate was detected in captive bottlenose dolphins in response to threatening sounds. The cardiac response patterns were consistent with the physiological defense and startle responses in terrestrial mammals and birds (Miksis et al., 2001). Repeated and extended exposure to noise can cause a variety of physiological issues, including suppression of reproduction (Wright et al., 2007). The statistically significantly higher cortisol, lowered serum testosterone level, and fertility rates in Poyang Lake YFPs compared to Tian-E-Zhou Oxbow YFPs (Wang, 2015), suggests the negative effect of anthropogenic noise on reproduction. Cortisol affects the HPG-axis at many levels; at the hypothalamic level (by decreasing the synthesis and secretion of gonadotropin-releasing hormone), at the pituitary level (by decreasing the synthesis and secretion of LH and FSH) and at the gonadal level (affecting steroidogenesis and/or gametogenesis) (Whirledge and Cidlowski, 2010). Several studies have reported the negative effects of noise and vibration on the reproductive physiology of mammals directly or indirectly (Algers et al., 1978; Penkov and Tzyetkov, 1999; Rabin et al., 2003; Nabi et al., 2014). The statistically significantly lower serum testosterone levels in juvenile male porpoises living in Poyang Lake has been previously reported in captive and wild YFPs, respectively (Daoquan et al., 2006; Hao et al., 2007).

Glucocorticoids can alter animal behavior depending upon the environmental context. It includes abandoning an area and fleeing the stressor (Fair et al., 2017). Therefore, cetaceans exposed to heavy vessel traffic have a higher metabolic rate in response to altered diving behavior (Peng et al., 2015). The elevated thyroid hormones promote the hypermetabolic state characterized by increased resting energy expenditure, lipolysis, and gluconeogenesis (Mullur et al., 2014). In our study, we observed statistically significantly higher serum levels of fT3 and fT4 in the Poyang Lake YFPs. Like our study, St Aubin et al. (1996) and Ortiz et al. (2000) also found significantly higher levels of fT3 and fT4 in wild manatees (*Trichechus manatus*) compared to captive manatees. A similar positive relationship between physical activity and serum T4 and fT4 in healthy young individuals has been reported (Licata et al., 1984; Figen et al., 2005). Captivity in cetaceans and manatees limit the physical activity of animals causing a reduction in thyroid hormone concentrations compared to its free-ranging counterparts (St Aubin and Geraci, 1988; Ortiz et al., 2000). The statistically significantly higher concentration of fT3 and fT4 and the positive correlation between cortisol and fT4 in the Poyang Lake YFPs suggest increased physical activity resulting from the tremendous anthropogenic activities as indicated by statistically significantly higher cortisol levels (St Aubin and Geraci, 1988). The association between higher cortisol levels and higher levels of fT3 and fT4 has been reported in wild bottlenose dolphins (St Aubin et al., 1996). In many species, including captive bottlenose dolphins (Ortiz et al., 2000), beluga whales (*Delphinapterus leucas*) (St Aubin and Geraci, 1988), and reindeer (*Rangifer tarandus tarandus*) (Ringberg et al., 1978), reduced thyroid hormones levels are associated with lower physical activity induced by captivity. Although, several studies in cetaceans have documented reports about circulating concentrations of TH (Greenwood and Barlow, 1979; Orlov et al., 1988; St Aubin, 2001; Debier et al., 2005; Fair et al., 2011), still, limited studies are available on the thyroid system of odontocetes (porpoises, dolphins, and toothed whales) living in the wild and the association with anthropogenic activities.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

In summary, we found statistically significantly increased stimulatory activity of the adrenal and thyroid gland in Poyang Lake population compared to Tian-E-Zhou Oxbow. In response to stress, hormones (especially fT4), blood cell counts (neutrophils and lymphocytes), and several biochemical parameters are affected in the Poyang Lake population. The cortisol levels of YFPs living in Poyang Lake did not show a statistically significant correlation with testosterone. The overall statistically significantly lower level of serum testosterone and variations in blood, biochemical, and other hormonal parameters compared to Tian-E-Zhou Oxbow YFPs suggests the possible effects of heavy vessel traffic and dredging. Alterations in the hormonal, hematological, and biochemical parameters will have serious consequences related to metabolism, immunity, reproduction, and general well-being. Unfortunately, the number of vessels, dredging, and other anthropogenic activities in Poyang Lake are increasing from year to year and therefore this problem requires serious attention as it affects the conservation of YFPs. A ban on sand dredging, especially in YFPs rich areas, regulating the number of vessels, and the speed of the vessels is essential. Especially, during the breeding season, dredging and vessels traffic should be banned or minimized. This is because compromising the reproduction of a critically endangered species for a long period can easily cause extinction. Introduction of quiet ships and assigning special routes could possibly minimize the impact. Economic precedence always trumps environmental and biodiversity issues. Therefore, it is better to have more semi-natural reserves like the Tina-E-Zhou Oxbow where YFP's fertility can be increased by controlling anthropogenic activities. Furthermore, controlled experiments are needed to investigate the response of the HPG and HPA-axis toward underwater sounds at different intensities and identify the threshold frequency that stimulates the HPA and suppress the HPG-axis. Additionally, data regarding vessel traffic, acoustic pollutions, level of YFPs activities and reference levels for various physiological indices are needed for both populations.

ETHICS STATEMENT

The study was ethically reviewed and approved by the Ministry of Agriculture of the People's Republic of China and the Research Ethics Committee of Institute of Hydrobiology, Chinese Academy of Science. In this study, no surgical interventions, including euthanasia and anesthesia was used. The entire study strictly followed the Chinese law and ethical guidelines for wildlife.

AUTHOR CONTRIBUTIONS

GN conceived the study, analyzed the data, and drafted the article. YH and DW collected the data. RM helped in writing the manuscript and critically reviewed the article. All authors read and approved the final manuscript.

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