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Serum concentrations of gonadotropins and steroid hormones of *Neophocaena phocaenoides asiaeorientalis* in middle and lower regions of the Yangtze River

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Abstract

As a relatively isolated and unique freshwater population, the Yangtze finless porpoise (*Neophocaena phocaenoides asiaeorientalis*) is the most endangered subpopulation of this species. The objective of this study was to improve our understanding of their reproductive endocrinology by measuring (radioimmunoassay) serum concentrations of FSH, LH, estradiol (E_2), progesterone (P_4) and testosterone (T_2) in free-ranging animals. Blood samples were collected from 66 Yangtze finless porpoises (41 males and 25 females) captured in the middle and lower reaches of the Yangtze River. Based on significant variation of serum T_2 concentrations in males with body length (BL) >138 cm, we inferred that they were mature; in these animals, there were significant seasonal variations in serum T_2 concentrations, with the highest concentrations in March and April (502.0 ± 319.8 ng/dL, mean ± S.D.) and the lowest in December (79.4 ± 83.2 ng/dL). Serum T_2 concentrations positively correlated with serum concentrations of LH and weakly correlated with serum concentrations of E_2, whereas there was a significant negative correlation between serum LH and FSH concentrations in males >138 cm. The smallest apparently pregnant female porpoise had a BL of 130 cm. Serum P_4 concentrations ranged from 13.2 to 112.4 ng/mL (43.9 ± 28.3 ng/mL) in pregnant females, and fluctuated under 1.0 ng/mL in non-pregnant females with BL > 130 cm. Serum LH concentrations were significantly higher in non-pregnant females >130 cm versus those females <130 cm. To our knowledge, this is the first study of endocrine-related maturity and seasonal breeding characteristics of the Yangtze finless porpoise.

Keywords: Finless porpoise; *Neophocaena phocaenoides*; Gonadotropins; Steroid hormones; Yangtze River

1. Introduction

The finless porpoise (*Neophocaena phocaenoides* G. Cuvier, 1829) is a small odontocete species that inhabits the coastal and river waters of temperate and tropical regions of Asia [1]. Morphological [2,3] and genetic evidence [4] suggested that the finless porpoise found in Chinese waters is comprised of three distinct populations or subspecies, including two marine subspecies, the South China Sea population (*N. p. phocaenoides*) and the Yellow Sea population (*N. p. sunameri*), and a freshwater subspecies, the Yangtze population (*Neophocaena phocaenoides asiaeorientalis*). The Yangtze subspecies is the sole freshwater population of the finless porpoise specific to the Yangtze River and its surrounding big lakes [3]. The severe environmental deterioration that has occurred in the region has had a

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greater negative impact on freshwater habitats than in ocean waters. Consequently, the Yangtze finless porpoise is at much greater risk than its marine counterparts, and its population has decreased rapidly over the last decade. Although the porpoise population in the Yangtze River was estimated to be 2700 before 1993 [5], recent studies suggested that the population may be much smaller than that [6–8]. It has been speculated that the Yangtze subspecies could become extinct within the next 100 years if the environmental conditions in the Yangtze River continue to deteriorate [9].

Due to its endangered status, the Yangtze finless porpoise has attracted much attention of conservation biologists, with particular focus given to its reproductive biology. Most of the information currently available on Yangtze finless porpoise reproduction was obtained by morphological and histological examination of gonads of animals that were accidentally killed [10–14], or by behavioral observation and ecological surveys [7,15,16]. However, there are some conflicting findings. For example, some studies demonstrated that males might achieve maturity with a body length (BL) of about 136.3 cm [12,13], whereas others noted that maturity might not be reached until they have a BL $>$ 147 cm [14].

Discrepancies also exist regarding the exact mating season of the porpoise, with some researchers suggesting a timeframe of March–June [7,15], whereas others suggest June–August [10,14]. Additional data, utilizing greater sample size and more reliable data, e.g. serum concentrations of reproductive hormones, are needed to address these discrepancies. However, due to the difficulties of capturing and blood sampling in the field, little work has been done on the reproductive endocrinology of these animals. Chen et al. [17] conducted a preliminary study on six mature males and six females blood samples obtained from porpoises in the Yangtze River, and reported that the serum testosterone (T_2) concentrations of the mature male finless porpoise ranged from 264.0 to 940.0 ng/dL from March to April. Serum progesterone (P_4) concentrations of pregnant porpoises ranged from 30.6 to 41.2 ng/mL, whereas concentrations in non-pregnant and immature females were $<$1.0 ng/mL. However, given the small sample size, these data provided only a limited insight into the porpoise's reproductive endocrinology. The purpose of the current study was to more comprehensively determine reproductive hormonal profiles of the Yangtze finless porpoise and to investigate the correlation between various reproductive hormones in this animal.

2. Materials and methods

2.1. Animals and blood sampling

Blood samples from 66 finless porpoises (41 males and 25 females) were opportunistically collected from 1990 to 2003, as part of Yangtze River dolphin surveys and research projects done by the Institute of Hydrobiology of Chinese Academy of Sciences (IHB, CAS). The capture sites are marked on the map of the Yangtze River (Fig. 1). The BL, weight and other morphological parameters of the animals were measured. Maturity status of the animals was roughly categorized on the basis of BL, as previously reported [10–15]. The pregnant females were recognized by the presence of obvious wrinkles within the genital area and high girth-to-length ratios. Since exact pregnancy stages could not be evaluated correctly, they were roughly estimated, based on the fact that most of the calves of Yangtze finless porpoise were born in April [7], and that gestation period is approximately 11 months [13]. However, the reproductive status of females without obvious morphological characters could not be determined at this stage. The basic information of animals in the present study is presented in Table 1.

After capture, animals were gently restrained and 5–10 mL of blood was collected from the central veins on the dorsal side of the tail fluke, using a 10-mL non-heparinized disposable syringe. Animals were released back to their natural habitat or transferred to the Tian-e-Zhou Semi-natural Baiji Reserve for further examination. Serum was separated by centrifugation (1500 $\times$ g, 15 min), put in frost-free plastic tubes, temporarily stored in a liquid nitrogen kettle, then transferred to the laboratory and stored at -25 °C.

2.2. Radioimmunoassays

Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17β (E_2) and T_2 were measured for both genders, whereas serum P_4 concentration was only measured for females. In all cases, commercially available RIA kits were utilized, following the manufacturer's instructions (Tian-jin Leeco Biotechnological and Medical Products Inc., China).

Commercially available double-antibody RIA kits for human FSH and LH have been validated for use with bottlenose dolphins (*Tursiops trunncatus*) and other cetaceans [18]. The RIA kits used in this study were validated by running serial dilutions of lyophilized and