*บทความวิชาการ*

# ความสามารถของปลาซิวข้าวสารไทยในการเป็นตัวบ่งชี้ทางชีวภาพของมลภาวะสิ่งแวดล้อมในน้ำจืด

**อรินทม์ งามนิยม***


## บทคัดย่อ

ปลาซิวข้าวสารไทย เป็นปลาซิวข้าวสารที่มีขนาดเล็กสุดในสกุล *Oryzias* ซึ่งพบได้ในแหล่งน้ำธรรมชาติทั่วประเทศไทย เช่น นาข้าว คูน้ำ และบ่อน้ำ พวกปลาซิวข้าวสารนั้นเป็นปลาวางไข่ กินทั้งพืชและสัตว์ขนาดเล็ก และสามารถถูกนำมาใช้เป็นสัตว์ทดลองและสัตว์ต้นแบบของการศึกษาด้านชีววิทยาของสัตว์มีกระดูกสันหลังที่ไม่ได้เลี้ยงลูกด้วยนม เนื่องจากมีลักษณะหลายๆ อย่างที่เหมาะสม เช่น เป็นปลาที่มีขนาดเล็ก เลี้ยงง่าย และลักษณะของเพศเห็นได้ชัด เป็นต้น ยิ่งไปกว่านั้นปลาซิวข้าวสารยังมีความไวในการตอบสนองต่อสารเคมีที่รบกวนการทำงานของระบบต่อมไร้ท่อซึ่งปนเปื้อนในสิ่งแวดล้อมตามธรรมชาติด้วย ดังนั้นจุดประสงค์ของบทความวิชาการนี้จะเป็นการรวบรวมและสรุปงานวิจัยต่างๆของปลาซิวข้าวสารไทยที่นำมาใช้ในการเป็นตัวบ่งชี้ทางชีวภาพของมลภาวะสิ่งแวดล้อมในน้ำจืด และยังเป็นการเพิ่มความรู้ด้านความเข้าใจในด้านผลกระทบของสารเคมีต่อระบบการทำงานของต่อมไร้ท่อของปลาที่อาศัยอยู่ตามธรรมชาติ


**คำสำคัญ:** ปลาซิวข้าวสารไทย ตัวบ่งชี้ทางชีวภาพ การรบกวนระบบต่อมไร้ท่อ สิ่งแวดล้อมในน้ำจืด

คณะวัฒนธรรมสิ่งแวดล้อมและการท่องเที่ยวเชิงนิเวศ มหาวิทยาลัยศรีนครินทรวิโรฒ

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# Thai ricefish: A Potential Bio-indicator Species for Monitoring Freshwater Environmental Pollutions

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## ABSTRACT

Thai ricefish *(Oryzias minutillus)* is the smallest species in the genus *Oryzias,* which is widely distributed throughout Thailand. The habitats of this species are shallow ponds, ditches and paddy fields. In this egg-laying fish, it has many advantageous characters as an experiment of non-mammalian vertebrate: it is small size, it is easy to keep and it exhibits external sexual dimorphism. This species is susceptible to endocrine-disruptors in natural environment. Therefore, the present paper is the aim to document and summarize the recent information of Thai ricefish, including related species as a potential bio-indicator for monitoring the environmental pollutions of freshwater. Furthermore, it supports that this present paper increases the understanding of endocrine disrupting events in fish by the effects of environmental chemicals.

**Keywords:** Thai ricefish, bio-indicator, endocrine disruptors, environmental freshwater

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## Introduction

The genus of *Oryzias,* Teleostei, belongs to the family Adrianichthyidae, and ranges broadly throughout East, Central, South and Southeast Asia. The freshwater are common inhabitant of the fish in genus *Oryzias,* although some species are found in brackish water and marine. Twenty-eight species are comprised in this genus [1,2]. It is known that in the genus *Oryzias,* they are used as organisms for experiments in various fields such as, the study of developmental biology [3], endocrinology [4] and toxicology [5]. In Thailand, the five species are recognized in the genus of *Oryzias* fish: examples include Thai ricefish *(O. minutillus),* Java ricefish *(O. javanicus),* Mekong ricefish *(O. mekongensis),* Indian ricefish. *(O. dancena)* and Songkhram River ricefish *(O. songkhramensis)* [6,7]

Thai ricefish, which is the smallest species among genus *Oryzias,* is widely distributed in freshwater of Thailand (Fig. 1A) [8]. This ricefish has been known by its Japanese name, Thai medaka. Thai ricefish are found in natural environment, including rice paddies, shallow ponds and ditches (Fig. 1B) [9]. In this egg-laying fish, this species has many advanta-geous characteristics as an experiment of aquatic vertebrate: it is small size, it is easy to maintain in an aquarium, it exhibits external sexual dimorphism, and its egg is transparent [10]. Their sexes are judged from the morphology of the secondary sex characters of the dorsal and anal fin according to the description of Kamsri et al. [11]. The dorsal and anal fins in males are usually longer than those in females (Fig. 2A and B).

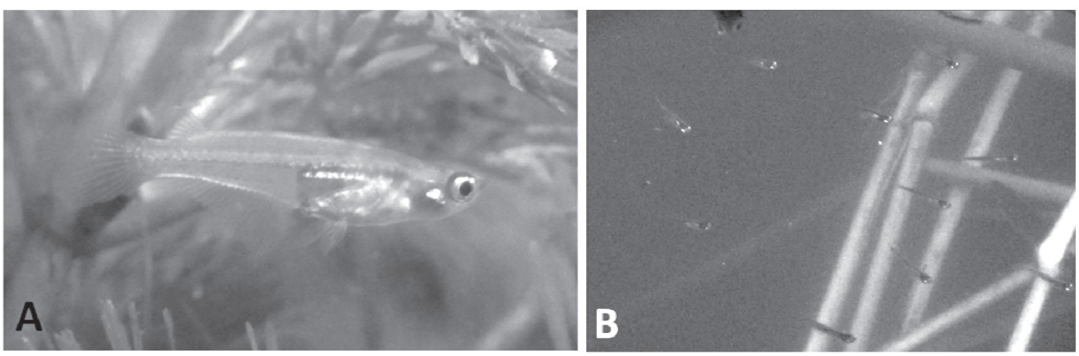


**Figure 1** Adult Thai ricefish *(O. minutillus)* (1A). Its standard length is 11-14 mm. Thai ricefish inhabit the paddy field of Phra Nakhon Si Ayutthaya Province, Thailand (1B).

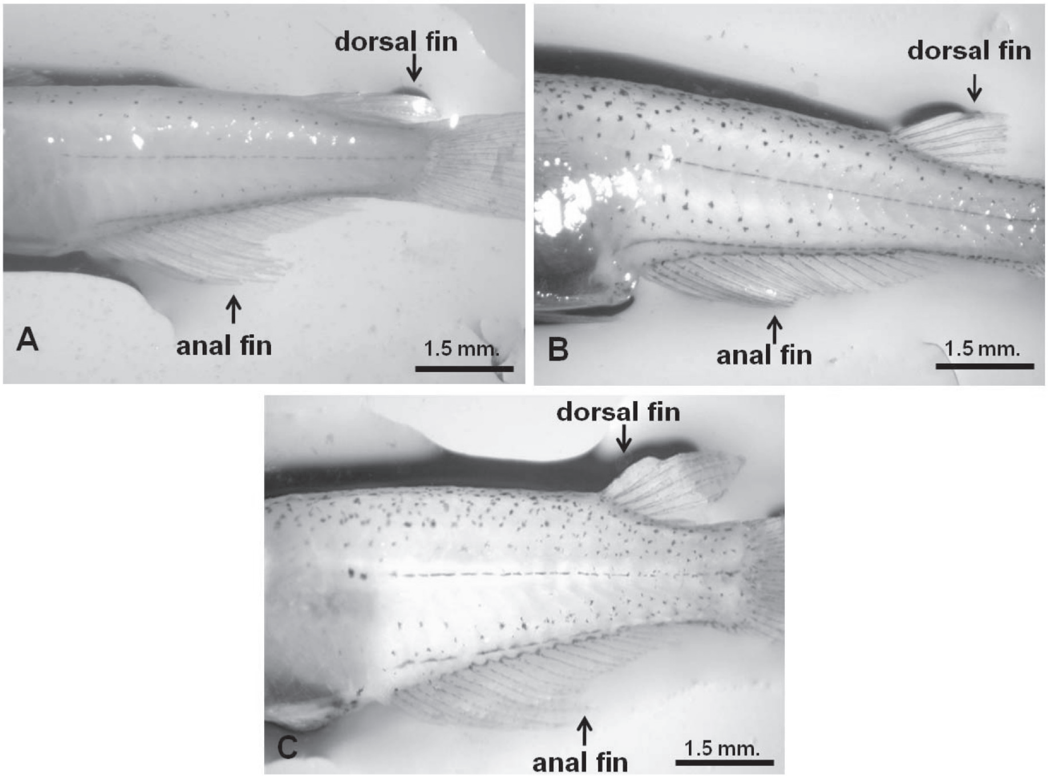


**Figure 2** Characteristics of the dorsal and anal fin of male (2A), female (2B) and sex-underminable individual (2C) [12].

Bio-indicator is a biological response of living organism that describe the presence of the pollutants around its environment by showing morphological, physiological, chemical or behavior changes [13]. Bio-indicator organisms are very susceptible to pollutions or alterations in their environment [14]. It is known that the endocrine disruptors are a major cause of freshwater pollutions lead to abnormal physiological processes in many animals and extinction of some species [15]. Those chemicals interfere with endocrine systems causing the abnormal function, secretion and synthesis of endogenous hormone in animals, including humans [16]. The endocrine disruptors are discharged from agriculture, industry and pulp mill to natural environment [17]. The bioindicator of water pollutions has been examined in biological responses of various freshwater species, including tubificid worm *(Limnodrilus profundicola)* [18] ostracod *(Stenocypris major)* [19], zebra mussel *(Dreissena polymorpha)* [20] and cutthroat trout *(Oncorhynchus clarkii)* [21].

In Thailand, agriculture is one of the major occupations. The agricultural chemicals are usually used in many fields of agriculture for increasing the productivity and protecting the economic loss [22]. Those chemicals may contaminate a natural environment and disrupt the endocrine systems in several ways of freshwater organisms. Therefore, the aim of this paper was to document and summarize the recent data of Thai ricefish as a potential bio-indicator of water pollutions in their environment. The sex ratio, development of secondary sex characters and gonads and the molecular-biological viewpoint were provided and discussed in text. Furthermore, it believes that our present paper may increase the understanding of endocrine disrupting events in fish by the effects of environmental chemicals.

## Sex ratio of Thai ricefish

In general, the sex ratios (male to female) are found almost 1:1 in several populations of Thai ricefish [23]. However, the sex ratios are unbalanced when the environment of this ricefish is polluted by a contamination of some chemicals (Table 1). The abnormal ratios are thought to be strongly female biased (such as 1:3) in wild populations by affect of xenoestrogenic compounds [12]. In contrast, male-biased sex ratios can be found in environment caused by an androgenic contamination [24]. Therefore, the study of sex ratio is easy to indicate that some feminizing or masculinizing stresses may be triggered in Thai ricefish, probably by contaminating the chemicals in its habitat.

**Table 1** Sex ratios of males to females and percentages of sex-undeterminable individuals. DDT concentration was detected in the soil of ponds in localities 3 and 6 (each 0.2 ppm) [12].

| | Number of specimens | | | Sex ratio | Percentage |
|---|---|---|---|---|---|
| **local** | **male** | **female** | **sex-undeter** | **male-female** | **Sex-undeter** |
| 1 | 73 | 93 | 26 | 1.0:1.3 | 13.5 |
| 2 | 27 | 39 | 8 | 1.0:1.4 | 10.8 |
| 3 | 7 | 20 | 5 | 1.0:2.9* | 15.6 |
| 4 | 80 | 101 | 18 | 1.0:1.3 | 9.1 |
| 5 | 52 | 58 | 15 | 1.0:1.1 | 12.0 |
| 6 | 5 | 17 | 6 | 1.0-3.4* | 21.4 |
| 7 | 21 | 16 | 2 | 1.3:1.0 | 5.1 |
| 8 | 5 | 15 | 4 | 1.0:3.0* | 16.7 |
| 9 | 10 | 9 | 5 | 1.1:1.0 | 20.8 |
| 10 | 6 | 17 | 5 | 1.0:2.8 | 17.9 |

*Significantly different in the number between males and females
($P < 0.05$)

## Morphology of Thai ricefish fins

The morphologies of the dorsal and anal fins are a typical secondary sex character of Thai ricefish. Those fins exhibit sexual dimorphism in the length. The dorsal and anal fins of male are longer than those of female [10,11]. The long length of fin is known to be a mating-related function and appears to be important in fertilization success. The male fish envelops the female with the dorsal and anal fin [25]. Ngamniyom et al. [12,23] reported the occurrence of many sex-undeterminable individuals of adult Thai ricefish in an environmental pollution. The fins of these individuals are not useful in distinguishing males from females, since the length of the dorsal and anal fins of these individuals is intermediate between that of normal males and females (Fig. 3C). Similarly to sex ratio, the morphological examination of those fins is useful bio-indicator of water pollution.

## Gonadal developments of Thai ricefish

In wild teleost fish, the intersex or hermaphrodite individuals have been reported from freshwater environments of various countries; examples include roach *(Rutilus rutilus)* [26], walleye *(Sander vitreus)* [27] and shovelnose sturgeon *(Scaphirhynchus platorynchus)* [28]. The intersex fish are a gonadal condition defined as the presence of both testicular and ovarian tissues (testis-ova), although the morphologies of secondary sex characters are normal or abnormal [29]. Conversely, in sex-undeterminable individuals, either testis or ovary is observed in the same gonad (Fig. 3a-c). Ngamniyom and Panyarachun [30] demonstrated that environmental chemicals have been leading to vast amounts of intersex in Thai ricefish, collecting from the suburbs of Bangkok, Thailand. It thus suggests that the prevalence and incidence of intersex gonadal conditions are used to monitor freshwater pollutants of an environment or ecosystem.

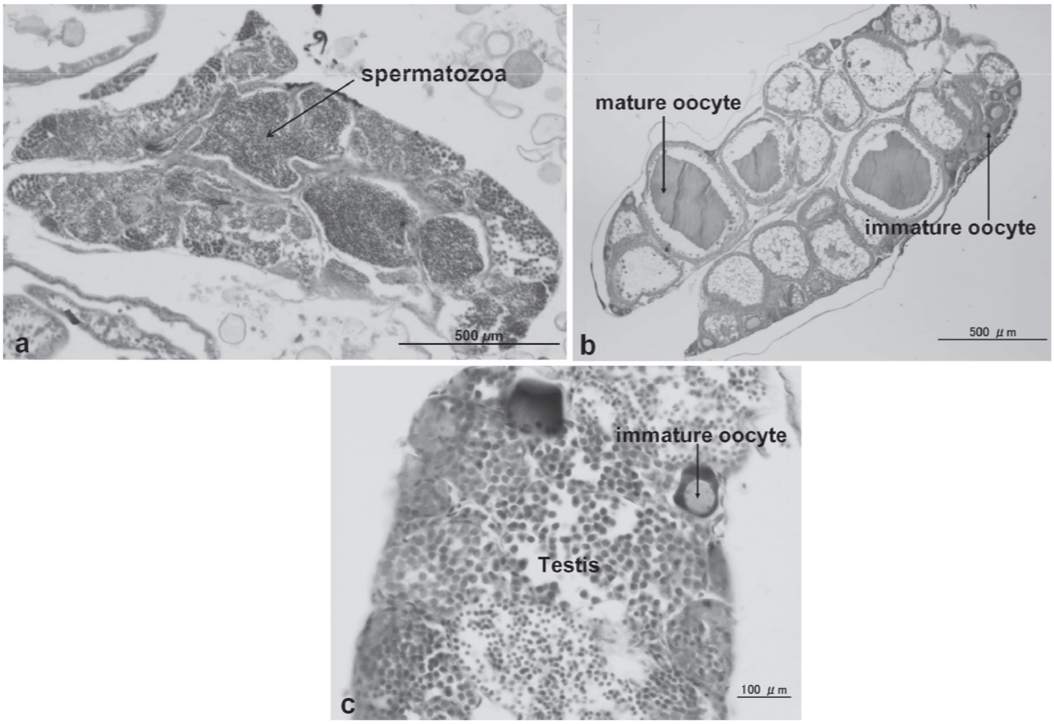


**Figure 3** Histological gonads of normal male (a), normal female (b) and intersex (c) of adult Thai ricefish. Both testicular and ovarian tissues are found in a single gonad of intersex individual [30].

## Gene expressions in Thai ricefish

In all vertebrates, a teleost fish is a common model organism which is frequently utilized for testing an action of several chemicals; examples include rainbow trout *(Oncorhynchus mykiss)* [31], Japanese ricefish *(Oryzias latipes)* [32] and goldfish *(Carassius auratus)* [33]. The development of embryo, endocrine function, morality, behavior, including gene expression have been examined when fish species exposed to those chemicals, such as agricultural compounds, pharmaceutical drugs and heavy metals [34,35,36].

In oviparous vertebrates, vitellogenin is a specific protein of female that it is an important precursor of egg yolk proteins in the liver in response to estrogenic actions [37]. It is difficult to measure vitellogenin level in normal male fish, but males are expressed vitellogenin gene when exposed to exogenous estrogens [38]. In Thai ricefish, the liver tissues were useful to examine the effects of environmental chemicals in a natural habitat by monitoring the vitellogenin gene expressions [30]. For example, the vitellogenin gene expressions were detected in the livers of intersex and sex-undeterminable individuals, inhabiting the paddy fields. Those sex-undeterminable individuals, in which the gonads were determined to contain only

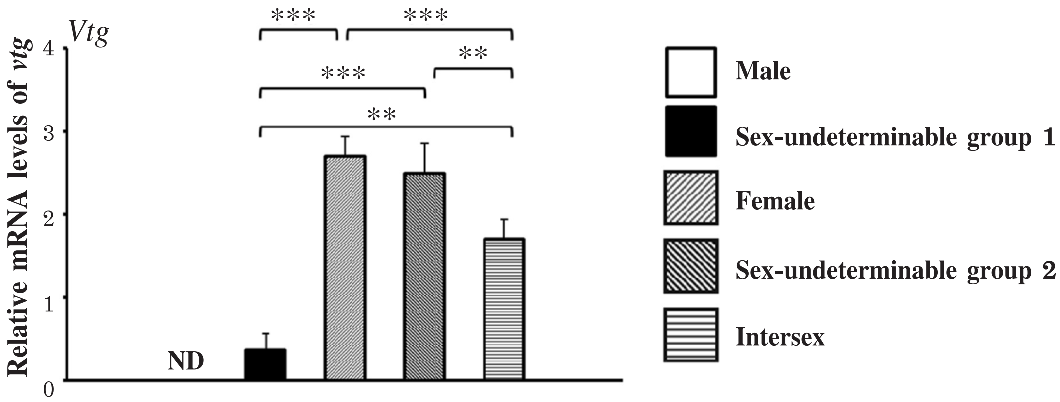


**Figure 4** Gene expression levels of vitellogenin in the livers of Thai ricefish. Single- and double-asterisk and triple-symbols show $P < 0.05$, $P < 0.01$ and $P < 0.005$, respectively (mean ± SE). Sex-undeterminable individuals were determined to contain testicular or ovarian tissues were sorted into group 1 and group 2, respectively. ND: no detection of gene expression level [30].

testicular tissue by histological analysis (Fig. 4). Therefore, in this event, vitellogenin expression in testicular gonads may indicate that the fish were exposed to an environmental xenoestrogen.

## Conclusion

The present article summarize that the Thai ricefish may be a potential bio-indicator for screening of pollutions in a freshwater environment. This is the first review to introduce the novel of Thai ricefish as a sensitive bio-indicator of environment pollution across four criteria. The Thai ricefish are Thai native species which are wildly distributed in all regions of Thailand. Recently, however, it is hard to find Thai ricefish in a natural environment of Bangkok. In future, it is uncertain whether this small ricefish may survive or extinct. Furthermore, the author hopes that the present article shows a cross section of recovering of the wild population of Thai ricefish from disruptions by an effect of environmental chemicals.

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