A Study on Fish Reproduction for The Prevention of Species Loss Due to Batik Waste Pollution

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Abstract. Environmental factors, such as textile waste water, affect fish by interfering its reproduction. This study was conducted to examine reproductive performance of fish inhabiting a river that receive batik waste. The fish samples were collected in 5 stations representing upstream area, effluent disposal area, and downstream area. Sex ratio, Gonado Somatic Index (GSI), Hepatosomatic Index (HSI) of female and male was examined, and physicochemical water parameters of each station were measured. Ten species of 8 families were captured. Anabas testudineus (n=101), Trichogaster trichopterus (n=310), Scatophagus argus (n=8), Valamugil speigleri (n=11), Channa striata (n=2), Bagrus nemurus (n=1), Laiognathus fasciatus (n=2), Oreochromis niloticus (n=1), O. mossambicus (n=5). Mugil sp, V. speigleri, and L. fasciatus were juvenile, and found at the downstream area. The GSI of female and male A. testudinaeus were 0.31-5.52% and 0.34-3.32%; the HSI was 0.77-2.01% and 0.68-1.79%. The GSI of female and male T. trichopterus were 0.12-7.9% and nd-3.7%; the HSI was 0.77-2.17% and 0.6-2.0%. The BOD level ranged from 13.81±6.13 to 47.58±32.59 mg.L⁻¹, COD was between 190±80 and 435±196 mg.L⁻¹, DO was from 0 to 1.37±1.62 mg.L⁻¹, level phenol ranged from 0.50±0.34 to 4.20±1.6 µg.L⁻¹. Fish of Meduri River were reproductively active but only those resistant to low water quality reproduced successfully.

1. Introduction

Batik is one of the most prestigious Indonesian cultural heritages which were inscribed on the UNESCO Representative List of the Intangible Cultural Heritage of Humanity on 2 October 2009 [1]. Some concern has been taken into consideration in relation to wastewater of this industry. Most researches had been concentrated on the physical and chemical nature of this waste [2]. However, less attention was given to its impact on the living organism habituating in the river receiving this wastewater. Batik wastewater contains compounds that are suspected of inducing endocrine disruption [3] including fish [4]; one of those compounds is phenol.

Studies in several fish species indicated that the exposure of fish to various levels of phenol derivatives impaired their reproductive competence. Zebrafish (Danio rerio) exposed to 25 – 100 mg/L 4t-octylphenol for three weeks have lower Gonado-somatic index (GSI) compared to control [5]. Exposure of 100 mg alkyl phenol/kg body weight for 10 days resulted in a reduction of GSI and milt volume [6]. Exposure of Atlantic cog (Gadus morhua L.) to alkyl phenol, methyl phenol, and heptyl...
phenol for 15 days increased plasma vitellogenin (vtg) level in both male and female fish [7]. High-back crucian carp (Carassius auratus) exposed to wastewater treatment plant effluent which contains phenol for 141 days have a significantly lower GSI and higher HSI and vitellogenin levels [8].

Considering that phenol exposure leads to adverse effects on fish reproduction, it is interesting to evaluate the effect of batik wastewater on the reproductive performance of fish caught in the river receiving the wastewater. Such information is important to predict the long term fish population in the river, either for economic or conservation reasons. The river is one of community fishery resource, especially for fisherman. Depletion of the fish population in the river will reduce their income. This study was conducted to examine fish inhabitant in Meduri River, one of the rivers receiving batik wastewater, and their reproductive performance.

2. Methods

2.1. Research area and Sampling technique

The research was conducted in the Meduri River of the Pekalongan Regency, Central Java, Indonesia. Fish samples were collected weekly from July to August 2011 according to purposive random sampling at 5 stations representing pre-polluted (Station1: Curug village, 0654-721S;109-33-236 E), polluted (Station2: Samborejo village, 0653-850 S; 109-38-741 E) and post polluted area (Station 3:Tirto, 0653-402 S; 109-38-837 E; Station 4: Tegaldowo, 0652-382 S; Station 5:109-39-004 E and Mulyorejo villages, 0651-923 S; 109-39-075 E). One catch per unit effort (CPUE) is defined as ten efforts of caching were conducted in each sampling time using gillnet size of 1-inch. The species of fish samples was determined using identification criteria according to Fish Base (https://www.fishbase.de/search.php).

2.2. Reproductive aspect evaluation

The total length of fish samples and their weight were measured. The fish were sexed based on gonadal morphology to determine the sex ratio. The fish were then dissected; their gonad and liver were removed and weighed to determine the Gonado-somatic index (GSI) and hepato-somatic index (HSI) according to the following formula.

\[
GSI = \frac{gw}{bw} \times 100\% \quad \text{and} \quad HSI = \frac{hw}{bw} \times 100\%
\]

\(gw\): gonadal weight
\(hw\): hepatic weight
\(bw\): body weight

In each sampling time, water samples were taken for measurement of physicochemical variables. Dissolved oxygen (DO), biological oxygen demand (BOD), and chemical oxygen demand (COD) levels were analyzed according to the American Public Health Association (APHA) [9]. Phenol levels were analyzed at Wahana Laboratory, Semarang. Temperature, pH, humidity, the color of the water, salinity and light penetration were recorded in situ.

2.3. Data Analysis

Correlation analysis was performed to evaluate the interaction between reproductive parameters and water physicochemical parameters.

3. Results

There were 444 fish caught during the sampling period. They consisted of 10 species belonging to 8 families (Anabantidae, Bagridae, Channidae, Cichlidae, Laiognathidae, Mugilidae, Osphronemidae, and Scatophagidae). The highest population was occupied by Trichogaster trichopterus and Anabas testudineus. Trichogaster trichopterus were caught at
all station throughout the study period, *Anabas testudineus* were caught at station 1, 2, 3 and station 5. *Channa striata* were only caught at the upstream area, the station 1, while other species were only found at the downstream area, the station 4 and 5 (Table 1).

**Table 1.** The number of fish species caught at the Meduri River receiving batik wastewater

| No. | Species                  | Station 1 | Station 2 | Station 3 | Station 4 | Station 5 | Total |
|-----|--------------------------|-----------|-----------|-----------|-----------|-----------|-------|
| 1   | *Anabas testudineus*     | 31        | 27        | 8         | 17        | 10        | -     |
| 2   | *Trichogaster trichopterus* | 68       | 31        | 33        | 17        | 8         | 27    |
| 3   | *Mugil sp*               | -         | -         | -         | -         | -         | 2     |
| 4   | *Scatophagus argus*       | -         | -         | -         | 7         | -         | 1     |
| 5   | *Valamugil speigleri*     | -         | -         | -         | -         | 9         | 2     |
| 6   | *Channa striata*          | 1         | 1         | -         | -         | -         | -     |
| 7   | *Bagrus nemurus*          | -         | -         | -         | -         | -         | 1     |
| 8   | *Laiognathus fasciatus*   | -         | -         | -         | 1         | -         | 2     |
| 9   | *Oreochromis niloticus*   | -         | -         | -         | -         | 1         | 1     |
| 10  | *O. mossambicus*          | -         | -         | 3         | 2         | -         | 5     |

*Mugil* sp, *V. speigleri*, and *L. fasciatus* were found at the station 4 and 5 which is located close to the brackish area. All fish of these species were at the juvenile stage, the gonads were unidentified by morphological examination. *T. trichopterus* and *A. testudineus* were at various stages of reproduction ranging from a juvenile with unidentified gonad, immature and reproductively active as indicated by the presence of mature gametes. The female fish with high GSI value, >5 for *A. testudineus* or >6 for *T. trichopterus* were able to be stripped to release their eggs. The eggs were whitish yellow with an approximate diameter of 0.5 mm. The male fish have lower GSI value than the female (Table 2).

**Table 2.** Fish Size, Gonado-somatic index and Hepato-somatic index of fish caught at Meduri River

| No. | Species                  | Body length (cm) | Body Weight (g) | GSI (%) | HSJ (%) |
|-----|--------------------------|------------------|-----------------|---------|---------|
| 1   | *Anabas testudineus*     | 8.80             | 9.25            | 11.24   | 10.61   | 0.31   | 0.34-5.52 | 0.77-3.32 | 0.68-2.01 |
| 2   | *Trichogaster trichopterus* | 8.80             | 6.08            | 5.45    | 4.83    | 0.12   | 0.12-7.9  | nd-3.7  | 0.77-2.17 |
| 3   | *Mugil sp*               | 15.40            | 12.00           | 31.25   | 19.00   | 0.63   | 0.63-3.2  | nd      | 0.85-0.78 |
| 4   | *Scatophagus argus*       | -                | 9.49            | -       | 26.86   | -      | -        | -       | -        |
| 5   | *Valamugil speigleri*     | -                | 15.70           | -       | 28.50   | -      | -        | -       | -        |
| 6   | *Channa striata*          | 16.20            | 19.50           | 23.00   | 25.20   | 1.64   | 1.64-0.21 | 2       | 1.21     |
| 7   | *Bagrus nemurus*          | -                | 11.90           | -       | 16.00   | -      | -        | -       | -        |
| 8   | *Laiognathus fasciatus*   | -                | 5.30            | -       | 2.87    | -      | -        | -       | -        |
| 9   | *Oreochromis niloticus*   | -                | 27.80           | -       | 402.00  | -      | 0.05     | -       | 1.15     |
| 10  | *O. mossambicus*          | 7.77             | 7.60            | 7.33    | 6.50    | 0.38   | 0.38-0     | 0       | 1.5-1.83 |

Note: nd = undetected; Juv = juvenile

The water quality was justified based on water pH, the concentration of BOD, COD, DO and phenol. The BOD level was ranged from 13.81±6.13 to 47.58±32.59 mg L⁻¹, COD ranged from 190±80 to 435±196 mg L⁻¹, DO range from 0 to 1.37±1.62 mg L⁻¹, phenol ranged from 0.50±0.34 to 4.20±1.6 µg L⁻¹. The DO concentration at the waste outlet (station-2) was the lowest compared to other stations. This low DO concentration coincided with the high BOD concentration. Level of Phenol decreased toward the downstream area (Station 3 to 5) indicating there was a diluting process. It was surprising that phenol at the upstream area (Station 1) was the highest amongst others. The
water pH ranged from 6.6±0.92 to 7.8±0.15 (Table 3). At the time of wastewater released the water pH increased to 10.

**Table 3. Water Quality of the Meduri River Receiving Batik Waste Water**

| Parameters | BOD (mg.L⁻¹) | COD (mg.L⁻¹) | DO (mg.L⁻¹) | Phenol (mg.L⁻¹) | pH       |
|------------|--------------|--------------|-------------|----------------|----------|
| Station-1  | 17.37±9.82   | 190±80       | 1.37±1.62   | 4.20±1.6       | 7.0±0.95 |
| Station-2  | 47.58±32.59  | 355±167      | 0.03±0.06   | 3.81±1.54      | 6.6±0.92 |
| Station-3  | 24.32±12.60  | 410±129      | 0.17±0.23   | 1.88±0.19      | 7.6±0.45 |
| Station-4  | 15.63±7.16   | 435±196      | 0.25±0.50   | 0.66±0.28      | 7.8±0.15 |
| Station-5  | 13.81±6.13   | 525±330      | 0.55±0.64   | 0.50±0.34      | 7.6±0.37 |

Correlation analysis was performed only for *A. testudinaeus* and *T. trichopterus* (Table 4) due to the number of fish.

**Table 4. Coefficient Correlation (r) between Reproductive and Water Chemical Parameters**

| Water Parameters | *Trichogaster trichopterus* | *Anabas testudinaeus* |
|------------------|-----------------------------|-----------------------|
|                  | GSI♀ | GSI♂ | HSI♀ | HSI♂ | GSI♀ | GSI♂ | HSI♀ | HSI♂ |
| DO               | 0.565 | -0.069 | 0.066 | -0.053 | 0.375 | 0.755 | -0.476 | -0.645 |
| BOD              | 0.066 | -0.091 | 0.163 | 0.145 | -0.047 | -0.058 | 0.073 | 0.138 |
| COD              | -0.415 | -0.042 | -0.122 | -0.195 | -0.465 | -0.131 | 0.697 | 0.269 |
| Phenol           | 0.614 | 0.073 | 0.404 | 0.277 | 0.077 | 0.17 | -0.206 | -0.409 |

4. Discussion

The frequency of fish caught at each station suggested that *A. testudinaeus* and *T. trichopterus* were the native species of the Meduri River. These fish survive in the Meduri River with a very low level of DO because they were equipped with arborescent organ (labyrinth) to help the fish breath from the air. *O. niloticus* and *O. mossambicus* were occasionally found suggesting that these species were not originally inhabited in Meduri River. A similar argument was applicable for *Mugil sp*, *V. speigleri*, and *L. fasciatus*. These three species are brackish water fish [10]. It is very likely that these species were brought by the high tide of the sea as indicated by the increase of water salinity up to 14 ppt at station 4 and 5.

The water quality of the Meduri river did not meet the National Indonesian Standard for fish culture, stating that the minimum DO concentration for fish culture is 3 mg.L⁻¹ [11]. Maximum concentration allowed for effluent are 60 mg.L⁻¹ for BOD, 150 mg.L⁻¹ for COD and 0.5 mg.L⁻¹ for total phenol [12]. Nevertheless, this water capable of supporting *A. testudinaeus* and *T. trichopterus* survival and reproduction. These species well reproduce indicated by various stage of reproductive status and the presence of strippable fish.

Correlation analysis suggested that female *A. testudinaeus* and *T. trichopterus* responded to the water quality differently from the male. The COD concentration negatively correlated with GSI of the female but not the male. Total phenol concentration positively correlated with GSI (r=0.641) and HSI (r=0.404) of female *T. trichopterus*, but such correlation was not found in female *A. testudinaeus*. On the other hand, total phenol did not correlate with GSI and HSI of male *T. trichopterus* but negatively correlated with HSI (r=0.409) of male *A. testudinaeus*. This different respond might be due to a different rate of phenolic uptake and its accumulation in the tissue, different phenolic clearance from the body, or a different pattern of fish activity. These differences might lead to a different physiological response to phenolic exposure.

Phenol enters the blood circulation of fish through mucous epithelia of the mouth or gill lamellae [13,14] then enter the cells in various tissues. A study in a catfish *Heteropneustes fossilis* showed that 4-nonylphenol (NP) exposure at concentration of 64 and 120 µg.L⁻¹ for 15 – 60 days resulted in NP accumulation in brain, gill, kidney, liver, ovary, muscle and plasma, with the highest accumulation.
was detected in the brain followed by gill and kidney [15]. Phenol has estrogenic properties and capacity to bind the estrogen receptor in the liver to induce synthesis of vitellogenin and zona radiata protein [16]. These two proteins are crucial for vitellogenesis and oogenesis [17]. Phenol exposure to *Cyprinus carpio* at the sublethal concentration for 45 days lead to a gradual and significant increase in nonesterified cholesterol in ovary and liver coincide with the increase in the hepatosomatic index (HSI), while the gonadosomatic index (GSI) decreased gradually [18]. A similar result was also reported in the high-back crucian carp (*Carassius auratus*) exposed to wastewater treatment plant effluents for 141 days [19]. In addition to its effect on the reproductive process, the phenolic compound also stimulates metabolism to provide energy for detoxification in the liver, as shown in *Ictalurus punctatus* [20]. Correlation between phenol concentration in the river and fish reproductive parameter received special attention in this study since phenol is widely used as dye component for batik whereas many reports indicated that phenol has potency as an endocrine disruptor. Considering the adverse effects of various type of phenolic compound on fish reproduction, a serious concern need to be taken in controlling the amount of phenol in wastewater effluent being released into the waters. The information regarding the fish lived in Meduri River is very limited; therefore, further studies are needed to elucidate the degree of species lost in this river.

5. Conclusion

Fish of Meduri River were reproductively active but only those resistant to low water quality reproduced successfully.

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