A study on fish reproduction for prevention of species loss due to batik waste pollution

GE Wijayanti 1 and W Lestari2

1 Laboratory of Animal Structure and Development Faculty of Biology Universitas Jenderal Soedirman, Purwokerto, Indonesia

2 Laboratory of Ecology Faculty of Biology Universitas Jenderal Soedirman, Purwokerto, Indonesia

E-mail: 1.bugrat@gmail.com

**Abstract**. Fish population is affected by environmental factors capable of interfering it reproduction such as textile waste water. This study was conducted to examine fish inhabitant in a river receiving batik waste and their reproductive performance. The fish sample was 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. The results showed that 10 species of 8 families were caught. *Anabas testudinaeus* (n=101), *Trichogaster trichopterus* (n=310), *Mugil* sp (n=3), *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 were 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 were 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 HIS were 0.77-2.17% and 0.6-2.0%. 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 ranged 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.

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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 water waste 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. Zebra fish (*Danio rerio*) exposed to 25 – 100 mg/L 4t-octylphenol for 3 weeks have lower Gonado-somatic index (GSI) compared to control [5]. Exposure of 100mg/kg body weight alkyl phenol for 10 days resulted in a reduction of GSI and milt volume [6]. Exposure of Atlantic cog (*Gadus morhua* L.) to alkylphenol, methylphenol and heptylphenol for 15 days increased plasma vitellogenin (vtg) level in both male and female fish [7]. High-back crucian carp (*Carasius auratus*) exposed to waste water 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 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 economical or conservation reasons. River is one of fishery resources community especially fisher man. A depletion of 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 waste water, and their reproductive performance.

1. Methods
	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 in July-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 fishbase (https://www.fishbase.de/search.php).

* 1. 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 (HIS) according to the following formula.

$GSI=\frac{gw}{bw}×100\%$ and $ HSI=\frac{hw}{bw}×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 American Public Health Association (APHA) [9]. Phenol levels were analysed at Wahana Laboratory, Semarang. Temperature, pH, humidity, colour 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 physico-chemical parameters.

1. Results

There were 444 fish caught during the sampling period. They consisted of 10 species belong to 8 families (Anabantidae, Bagridae, Channidae, Cichlidae, Laiognathidae, Mugilidae, Osphronemidae, and Scatophagidae). The highest population was occupied by *Trichogaster* *trichop­terus* and *Anabas testudinaeus. Trichogaster* *trichop­terus* were caught at all station throughout the study period, *Anabas testudinaeus* were caught at station 1, 2, 3 and station 5. *Channa striata* was 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 Water waste

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Species | Number of fish | Total |
| Station 1 | Station 2 | Station 3 | Station 4 | Station 5 |
| ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| 1. | *Anabas testudinaeus* | 31 | 27 | 8 | 17 | 7 | 10 | - | - | 1 | - | 101 |
| 2. | *Trichogaster trichopterus* | 68 | 31 | 33 | 31 | 17 | 8 | 27 | 14 | 42 | 39 | 310 |
| 3. | *Mugil sp* | - | - | - | - | - | - | - | - | 2 | 1 | 3 |
| 4. | *Scatophagus argus* | - | - | - | - | - | - | 7 | - | 1 | - | 8 |
| 5. | *Valamugil speigleri* | - | - | - | - | - | - | 9 | - | 2 | - | 11 |
| 6. | *Channa striata* | 1 | 1 | - | - | - | - | - | - | - | - | 2 |
| 7. | *Bagrus nemurus* | - | - | - | - | - | - | - | - | - | 1 | 1 |
| 8. | *Laiognathus fasciatus* | - | - | - | - | - | - | 1 | - | 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 closed to the brackish area. All fish of these species were at juvenile stage, the gonads were unidentified by morphological examination. *T.* *trichopterus* and *A. testudinaeus* were at various stages of reproduction ranging from juvenile with unidentified gonad, immature and reproductively active as indicated by the presence of mature gamets. The female fish with high GSI value, >5 for *A. testudinaeus* or >6 for T*.* *trichopterus* capable to be stripped to release their eggs. The eggs were whitish yellow with approximate diameter of 0,5mm. 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 (%)** | **HSI (%)** |
| ♀ | ♂ | Juv | ♀ | ♂ | Juv | ♀ | ♂ | ♀ | ♂ |
| 1. | *Anabas testudinaeus* | 8.80 | 9,25 | - | 11.24 | 10,61 | - | 0.31-5.52 | 0.34-3.32 | 0.77-2.01 | 0.68-1.79 |
| 2. | *Trichogaster trichopterus* | 8.80 | 6.08 | - | 5.45 | 4.83 | - | 0.12-7.9 | nd-3,7 | 0.77-2.17 | 0.6-2.0 |
| 3. | *Mugil sp* | 15.40 | 12.00 | - | 31.25 | 19.00 | - | 0,63 | 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 | 52.00 | - | 1,64 | 0,21 | 2 | 1,21 |
| 7. | *Bagrus nemurus* | - | 11.90 | - | - | 16.00 | - | - | 0.06 | - | 1.94 |
| 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 | 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 ranged 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 compare to other stations. This low DO concentration was coincidence 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 waste water released the water pH increased to 10.

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | 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 the 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 | -0.47 | -0.058 | **0573** | 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** |

1. 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 very low level of DO because they were equipped with arborescent organ (labyrinth) to help the fish breath form 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 to support *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 different rate of phenolic uptake and it accumulation in the tissue, different phenolic clearance from the body, or different pattern of fish activity. These differences might lead to different physiological responds to phenolic exposure.

Phenol enter the blood circulation of fish through mucous epithelia of the mouth or gill lamellas [13,14] then enter the cells in various tissues. A study in a catfish *Heteropneustes fossilis* showed that 4-nonyphenol (NP) exposure at concentration of 64 and 120µg.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 capable to bind 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 sub lethal concentration for 45 days lead to a gradual and significant increase in nonesterified cholesterol in ovary and liver coincide with the increase in 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 waste-water treatment plant effluents for 141 days [19]. In addition to its effect on reproductive process, phenolic compound also stimulate 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 a potency as 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 waste water effluent being released into the waters.

The information regarding to the fish lived in Meduri River is very limited therefore further studies is needed to elucidate the degree of species lost in this river.

1. Conclusion

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

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