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

by Gratiana E Wijayanti

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

GE Wijayanti and W Lestari

Faculty of Biology Universitas Jenderal Soedirman, Purwokerto, Indonesia

E-mail: 1.bugrat@gmail.com

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 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 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.

$$GSI = \frac{gw}{bw} \times 100\% \text{ and } HSI = \frac{hw}{bw} \times 100\%$$

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 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* 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	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 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. testudinaeus* 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. testudinaeus* 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 (%)**		**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 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

	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	-0.47	-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**

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 lamellas [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.

References

[1] Intangible Cultural Heritage of Humanity (UNESCO) 2009 Indonesian Batik Available at: *https://ich.unesco.org/en/RL/indonesian-batik-00170*
[2] Aryani Y, Sunarto dan Widiyani T 2004 Toksisitas akut limbah cair pabrik batik CV. Giyant Santoso Surakarta dan efek sublethalnya terhadap struktur mikroanatomi branchia dan hepar ikan nila (Oreochromis niloticus T.) *BioSMART* **6** 147–53
[3] Brevini T A L, Zanetto S B and Cillo F 2005 Effects of endocrine disruptors on developmental and reproductive functions *Current Drug Targets-Immune, Endocrine and Metabolic Disorders* **5** 1–10
[4] Abdel-Hameid N A H 2007 Physiological and histopathological alterations induced by phenol exposure in Oreochromis aureus juveniles *Turk. J. Fish. Aquat. Sci.* **7** 131–8
[5] Van den Belt K, Verheyen R and Witters H 2001 Reproductive effects of ethynylestradiol and 4t-octylphenol on the zebrafish (Danio rerio) *Arch. Environ. Contam. Toxicol.* **41** 458–67
[6] Rasmussen T H and Korsgaard B 2004 Estrogenic octylphenol affects seminal fluid production and its biochemical composition of eelpout (Zoarces viviparus) *Comparative Biochemistry and Physiology* C **139** 1–10
[7] Tollefsen K E, Sundt R C, Beyer J, Meier S and Hylland K 2011 Endocrine modulation in Atlantic cod (Gadus morhua L.) exposed to alkylphenol, polyphenol hydrocarbons, produced water and dispersed oil *J. Toxicology and Environmental Health* A **74** 7–9
[8] Liu J, Wang R, Huang B, Lin C, Zhou J and Pan X 2012 Biological effects and bioaccumulation of steroid and phenolic endocrine disrupting chemicals in high-back crucian carp exposed to wastewater treatment plant effluent *Environmental Pollution* **162** 25–31
[9] American Public Health Association (APHA) 1985 *Standard Methods for the Examination of Water and Wastewater* 16th ed (Washington DC: APHA, AWWA, and WPCP)
[10] Fishbase 2018 Available at: *http://www.fishbase.org/search.php*
[11] SNI 7550 2009 *Produksi Ikan Nila (Oreochromis niloticus Bleeker) Kelas Pembesaran di Kolam Air Tenang* (Jakarta: Badan Standarisasi Nasional Indonesia)
[12] Peraturan Menteri Negara Lingkungan Hidup No. 01 2010 *Tata Laksana Pengendalian Pencemaran Air* (Jakarta: Kementrian Lingkungan Hidup Republik Indonesia)

[13] Verma S R, Rani S, Tyagi A K dan Dalela R C 1980 Evaluation of acute toxicity of phenol and its chloro-and nitro-derivatives to certain teleosts *Water Air Soil Pollut.* **14** 95–102
[14] Mukherjee D, Bhattacharya S, Kumar V dan Moitra J 1990 Biological significance of [^{14}C] - phenol accumulation in different organs of a murrel Channa punctatus, and the common carp, Cyprinus carpio *Biomedical and Environmental Sciences* **3** 337–42
[15] Gautam G J, Chaube R and Joy K 2015 Toxicity and tissue accumulation of 4-nonylphenol in the catfish Heteropneustes fossilis with a note on prevalence of 4-NP in water samples *Endocrine Disruptors* **3**
[16] Arukwe A and Goksøyr A 2003 Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption *Comp. Hepatol.* **2** 1–21
[17] Ishibashi H, Hirano M, Matsumura N, Watanabe N, Takao Y and Arizono K 2006 Reproductive effects and bioconcentration of 4-nonylphenol in medaka fish (Oryzias latipes) *Chemosphere* **65** 1019–26
[18] Mukherjee D, Guha D, Kumar V and Chakrabarty S 1991 Impairment of steroidogenesis and reproduction in sexually mature Cyprinus carpio by phenol sulfide under laboratory conditions *Aquatic Toxicology* **21** 29–39
[19] Liu J, Wang R, Huang B, Lin C, Zhou J and Pan X 2012 Biological effects and bioaccumulation of steroidal and phenolic endocrine disrupting chemicals in high-back crucian carp exposed to wastewater treatment plant effluents *Environmental Pollution* **162** 325–31
[20] Moraes F D, Rossi P A, Figueiredo J S L, Venturini F P, Cortella L R X and Moraes G 2015 Metabolic responses of channel catfish (Ictalurus punctatus) exposed to phenol and post-exposure recovery *Anais da Academia Brasileira de Ciências* **88** 865–75

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

ORIGINALITY REPORT

13%	8%	11%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Martinez Reyes José. "Modelo termodinámico de equilibrio de fases gases no polares salmueras, para el sistema h2s-h2o-nacl", TESIUNAM, 2012 Publication	3%
2	centaur.reading.ac.uk Internet Source	2%
3	Dilip Mukherjee, Dhriti Guha, Vinod Kumar, Sanhita Chakrabarty. "Impairment of steroidogenesis and reproduction in sexually mature Cyprinus carpio by phenol sulfide under laboratory conditions", Aquatic Toxicology, 1991 Publication	1%
4	D. De Souza, L. Codognoto, S. A. S. Machado, L. A. Avaca. "Electroanalytical Determination of the Herbicide Paraquat in Natural Water and Commercial Tea Samples with Gold Electrodes Obtained from Recordable Compact Disc", Analytical Letters, 2005 Publication	1%

5	fusd1.org Internet Source	1 %
6	koreascience.kr Internet Source	1 %
7	ijcrbp.com Internet Source	1 %
8	www.civilsdaily.com Internet Source	1 %
9	worldwidescience.org Internet Source	<1 %
10	www.fortunejournals.com Internet Source	<1 %
11	www.ipublishing.co.in Internet Source	<1 %
12	www.scielo.br Internet Source	<1 %
13	Vijay Meena, Sujata Paul, Arup Kumar Sarma, Chandan Mahanta, Krishna Gopal Bhattacharyya. "Effects of COVID-19 lockdown on hydrochemical properties of the Brahmaputra River, India", Research Square Platform LLC, 2023 Publication	<1 %
14	erepository.uoeld.ac.ke Internet Source	<1 %

15	journals.plos.org Internet Source	<1 %
16	Aboubacar Toguyeni, Benoı□t Fauconneau, Alexis Fostier, Jose Abucay, Graham Mair, Jean-François Baroiller. "Influence of sexual phenotype and genotype, and sex ratio on growth performances in tilapia, Oreochromis niloticus", Aquaculture, 2002 Publication	<1 %
17	K. E. Tollefsen, R. C. Sundt, J. Beyer, S. Meier, K. Hylland. " Endocrine Modulation in Atlantic Cod (L.) Exposed to Alkylphenols, Polyaromatic Hydrocarbons, Produced Water, and Dispersed Oil ", Journal of Toxicology and Environmental Health, Part A, 2011 Publication	<1 %
18	docksci.com Internet Source	<1 %
19	floridaswater.com Internet Source	<1 %
20	ri.agro.uba.ar Internet Source	<1 %
21	C. R. Tyler, C. Spary, R. Gibson, E. M. Santos, J. Shears, E. M. Hill. " Accounting for Differences in Estrogenic Responses in Rainbow Trout (: Salmonidae) and Roach (: Cyprinidae) Exposed to Effluents from Wastewater	<1 %

Treatment Works ", Environmental Science & Technology, 2005

Publication

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	On		