sucipto hariyanto <sucipto-h@fst.unair.ac.id>

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7 messages

Icbs Bio Ugm <icbs-biougm@ugm.ac.id> Thu, Feb 8, 2018 at 3:00 PM
To: sucipto-h@fst.unair.ac.id

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Sie Prosiding ICBS Tahun 2017
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Variation[NR1] of Phenotype of Guppy Fish (*Poecilia reticulata*) [A2]Population from Differences of Aquatic Environments Quality [A3]in Surabaya, Indonesia

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Abstract. Guppy fish (*Poecilia reticulata*) is easy to find [A4]in various regions and ~~it~~ has widespread distribution throughout the world, ~~some populations are found living in environments with varying conditions~~[A5]. Several aquatic environments in Surabaya have been known belong to the category of water quality class III or class IV. Abiotic factors that present in the environment may influence the polymorphism in a guppy fish population. When the population faces different environmental condition, it will cause the phenotypic variation in order to adapt to the conditions in the local environment. The aim of this study is to observe the phenotypic variation of guppy fish population ~~which~~[A6] living in several aquatic environments with different quality levels ~~that found~~ [A7]in Surabaya. Fish samples were taken from 6 different locations that have been known their water quality based on measurement from physical and chemical parameters. A total of 120 fish samples were obtained from 6 locations and used to phenotypic character analysis, such as body size and body color pattern. The results of this study consist data of phenotype structure and phenogram tree. The population kinship based on phenogram tree consists of the first group that coming from population of the class IV location, while the second group is coming from population of the class III location. The phenotypic character of guppy fish that have different values between sites are body length, body area, relative area of structural color, and relative area of carotenoid color.

Key words: guppy fish, aquatic environments, ~~quality levels~~[A8], ~~location~~[A9], phenotypic character, phenogram.

INTRODUCTION

Guppy (*Poecilia reticulata*) is a freshwater fish native species from Trinidad and northeastern region of South America (Egset et al., 2011). Guppy is a popular aquarium fish because it has many strains that vary in color and fin shape that make it has been widely traded (Axelrod et al., 1985). Guppy has been used to control the vector of *Aedes aegypti* disease (Seng et al., 2008) and is effectively used as a biological control agent against malaria mosquito larvae in Kenya (Kweka et al., 2011). This guppy species exhibits phenotype character of sexual dimorphism, the male fish has a wider tail fin, a more variable body color pattern, and relatively has a smaller body size than the female fish (Houde, 1997). The male guppy has a size between 25-35 mm and has conspicuous color polymorphism patterns consisting of combinations of black, white, red-orange, yellow, green, iridescent spots, lines and speckles. Males have a gonopdium; a slender, modified anal fin used as an intromittent organ, whereas the anal fin of females is rounded. Females are uniform silver grey, and are larger and deeper bodied than males (40-60 mm). Juvenile fish resemble females, and are independent from birth (CABI, 2017).

In Indonesia, guppy fish was introduced [A10]for the first time around 1920s[A11] as a biological control agent and has grown in wild environment through natural reproduction (Eidman, 1989). The guppy fish in Surabaya has abundant population and found in the freshwater habitat of all areas of the city[A12]. Increasing the human population [A13]has a negative impact[A14] on the environment, one of which is aquatic environment pollution due to both domestic and industrial waste generated by various human activities. That various kind of waste has a major influence on water quality changes in Surabaya (BLH Kota Surabaya, 2013). Based on The Local Regulation of Surabaya No. 2 in 2004 about water quality management and water pollution control, the aquatic environments in Surabaya has been determined based on different quality levels consisting four (4) different classes according to some physical, chemical, and microbiological parameters. This regulation has also determined each class of several aquatic environments in Surabaya which all consist of water quality class III and class IV (JDIH Kota Surabaya, 2016) and this condition was observed periodically year by year [A15](BLH Kota Surabaya, 2013).

Guppy is easy to find [A16]in various regions and it has widespread distribution throughout the world, some populations are found living in environments with varying conditions (Deacon et al., 2011). There are various factors that affect this fish species has[A17] a widespread distribution such as very high reproduction rate (Gomiero and Braga, 2007), has[A18] an ability to tolerate the contaminations in the environment (Rocha et al., 2009), and its phenotype plasticity[A19] (Torres-Dowdall et al., 2012) [A20]particularly in the introduced environments. When this fish has adapted in the new environments that have very different conditions from the origin environments, then this fish could present the divergence of several phenotypic characters in male fish from their original fish population (Lindholm *et al.*, 2014).

This fish has a capability to adapt even in contaminated waters[A21] conditions (Araujo et al., 2009), but only a handful of study about the phenotypic character variation of guppy fish that exists in the introduced location. In Indonesia, the guppy fish that living in the wild environments is rarely studied. There is a study of guppy fish that lived in the wild has been done by Widianorko et al. (2000) [A22]about the associations between trace metals in sediment, water, and guppy from urban streams of Semarang, Indonesia[A23]. Some abiotic factors that present in the environment such as turbidity or nutrient availability may be influencing [A24]the color polymorphism [A25]in introduced population of guppy fish (Schwartz and Hendry, 2010). Polymorphism occurs when two or more different phenotypic character present in one population of the same species (Ford, 1965). When the population faces[A26] different environmental condition, it will cause the phenotypic variation in order to adapt to the conditions in the local environment [A27](Kawecki and Ebert, 2004).

The aim of this study is to observe the phenotypic variation of guppy fish population which living[A28] in several aquatic environments with different quality levels found in Surabaya. The guppy fish could also been used as a bioindicator organism in determining the quality of aquatic environment in addition of using physical and chemical parameters[A29]. Furthermore, this study is expected to gain the new information and knowledge about the kinship [A30]among guppy fish populations lived[A31] in several aquatic environments in Surabaya based on phenotypic characters and it could be as a useful data and developed for the future study.

MATERIALS AND METHODS

Study Area and Fish Sampling

Fish were collected in month of January until February [A32]2017 at six different sites of aquatic environments[A33] streams in Surabaya, East Java, Indonesia (Table 1). The three sites were Kaldami (KD), Kenjeran (KE), and Kebonagung (KA), all have the water quality class III. Whereas the three other sites were Pegirian (PE), Wonorejo (WO), and Darmo (DA), all have the water quality class IV (Table 1 and Fig. 1). These sites were selected because they both have different water quality levels (class III and IV) in Surabaya and present the stable population of guppy fish based on observer's exploratory sampling.

TABLE 1. Location of the sampling site

Sampling Site (Stream Name)	Site Code	Coordinates		Water Quality Class
Kalidami	KD	7°16'36.1"S	112°45'44.9"E	III
Kenjeran	KE	7°15'10.1"S	112°47'42.2"E	III
Kebonagung	KA	7°19'53.4"S	112°46'35.7"E	III
Pegirian	PE	7°14'16.7"S	112°44'40.7"E	IV
Wonorejo	WO	7°18'44.1"S	112°46'50.3"E	IV
Darmo	DA	7°17'24.0"S	112°44'32.0"E	IV

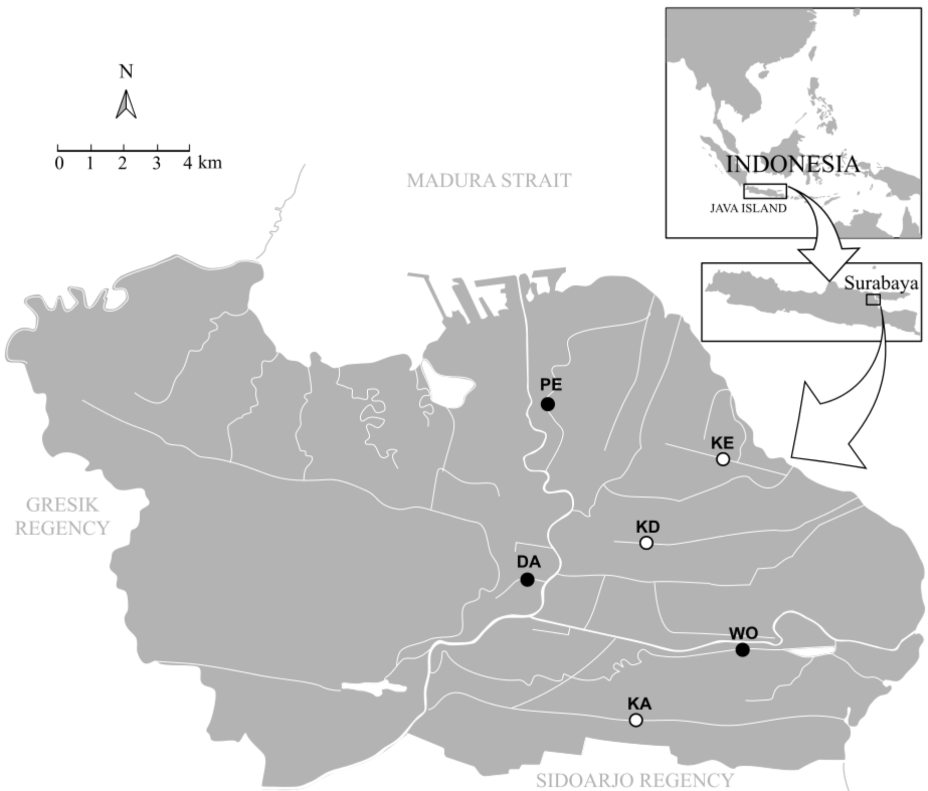

FIGURE 1. The sampling site locations of guppy fish in Surabaya. The white circles are the water quality class III, while the black circles are the water quality class IV locations. The white color patterns in the map are the water bodies.

Before the fish sample[A34] ~~had been~~ [A35]collected, ~~firstly~~, we analyzed the physicochemical parameters of aquatic environment quality using the water quality measure instruments. The parameters that ~~had been~~ [A36]measured directly on the site were temperature, pH, dissolved oxygen (DO), salinity, and water flow velocity whereas the parameters that ~~had been~~ [A37]measured after the water samples collected and analyzed in the laboratory were biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solid (TSS). The aquatic environment quality parameters[A38] were measured once in a month from January until March 2017 in each sampling site. Some parameters ~~that are~~ [A39]temperature, TSS, pH, BOD, COD, and DO were measured based on the range value criteria ~~that had been~~ arranged by The Local Regulation of Surabaya No. 2 in 2004, which has four different classes of water quality categories (Table 2). Class I is the water intended for use in drinking and other purposes that require the same water quality as those uses; class II, water intended for use in facilities of water recreation, cultivation of freshwater and brackish fish, livestock, irrigation, and other purposes that require the same water quality as those uses; class III, water intended for use in the cultivation of freshwater and brackish fish, livestock, irrigation, and other purposes that require the same water quality as those uses; and class IV, water intended for irrigation and other purposes that require the same water quality as those uses.(JDIH Kota Surabaya, 2016).

TABLE 2. The range values of water quality class categories arranged by The Local Regulation of Surabaya No. 2 in 2004 (JDIH Kota Surabaya, 2016)

Parameter	**Unit**	**Water Quality Class**			
		I	**II**	**III**	**IV**
Physical					
Temperature	^{0}C	Deviation 3	Deviation 3	Deviation 3	Deviation 3
TSS	mg/L	50	50	400	400
Anorganic Chemical					
pH	-	6-9	6-9	6-9	5-9
BOD	mg/L	2	3	6	12
COD	mg/L	10	25	50	100
DO	mg/L	6	4	3	0

Description: The above value is the maximum value, except for pH and DO. For pH is a range value that should not be less or more of the listed value. The value of DO is the minimum value. The temperature deviation is in the natural condition

Phenotypic Characters Analysis

Using a hand-net, a number of 20-30 males of guppy fish were collected in each site. Fish were immediately transported to the Laboratory of Biosystematics, Faculty of Science and Technology, University of Airlangga Surabaya where they were anesthetized with a dose of eugenol ($C_{10}H_{12}O_2$). ~~After fish samples had been transported to the laboratory,~~ they were processed [A40]following the methods of Millar et al. (2006). Lateral images of the left side of each fish were ~~taken~~[A41] using a digital camera placed at a standard distance with the aid of a tripod. Each sample was placed on a white background with millimetric scale, and color standards (Red, Orange, Yellow, Blue, Green, White and Black). The phenotypic characters of male guppy fish such as, body length (standard length), body area (excluding the fins and tail), tail length, and the area and the number of each color spot (excluding the fins and tail) (Fig. 2) were measured using software Image J (version 1.50e). All fish sample images were analyzed by ~~the same~~ [A42]person.

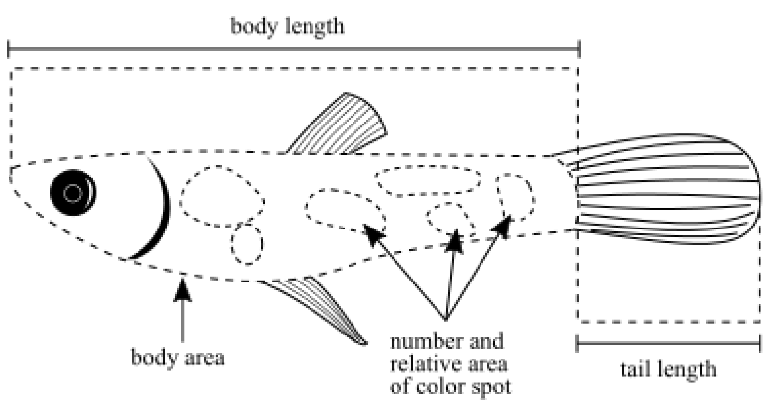

FIGURE 2. The phenotypic characters of male guppy fish which measured in this study

To reduce the number of variables for analysis, the color patterns were grouped into biologically relevant categories. These groups have different physiological bases, structural bases, functional interpretations, and selective relevance (Endler, 1978; Kodric-Brown, 1989; Brooks & Endler, 2001; Grether et al., 2001; McGraw et al., 2002; Blows et al., 2003; Griffith et al., 2006). Carotenoid colors consisted of the sum of orange and yellow spots, although note that pigments other than carotenoids also contribute to these spots (Grether et al., 2001). Structural colors are colors that are iridescent and have higher levels of reflection, and consisted of the sum of blue, violet, and silver spots (Endler, 1978; Brooks & Endler, 2001). Melanic colors consisted of the sum of black and fuzzy black spots.

Data Analysis

Software SPSS version 20 was used to build a phenogram tree based on all phenotypic character value using Ward Linkage method. The statistical analysis from the phenotypic characters of guppy fish such as, body size and color pattern of each sample were analyzed using Principal Component Analysis (PCA) to find the factors that ~~had been~~ formed among different guppy fish populations. The one way Analysis of Variance (ANOVA) was performed to know which of the each character has a different value among different populations from each site following by The Post-Hoc test using the Bonferroni test for the data which have homogenous variance whereas the Gomes-Howell test was used for the non-homogenous data with a significant value 0.05 (α = 0.05).

RESULTS AND DISCUSSION

A number of 20 male guppy fish samples which the body length between 12.0280-17.8470 mm [A43]and the tail length between 3.0280-5.8270 mm [A44]~~had been~~ [A45]measured and use[A46] for the further analysis with the other phenotypic characters (Table 3).

TABLE 3. Phenotypic character data of guppy fish from each site in Surabaya. The data represent mean ± standard deviation (SD) and samples sizes (N) estimated across sites.

Site	N	Body Length (mm)	Body Area (mm^2)	Relative Area of (mm^2)	
				Carotenoid Color	Structural Color
KD	20	13.6612±0.9141	41.8963±5.1870	0.0357±0.0198	0.1040±0.0143
KE	20	13.8019±1.0294	37.6103±6.3479	0.0668±0.0192	0.0978±0.0185
KA	20	13.9310±0.8280	37.8840±2.1361	0.0311±0.0140	0.0860±0.0246
PE	20	16.2201±0.7765	49.9349±1.2514	0.0909±0.0219	0.1641±0.0146
WO	20	16.5912±0.6090	49.9516±1.0159	0.0959±0.0232	0.1455±0.0253
DA	20	16.7658±0.6658	49.9500±1.2678	0.0834±0.0166	0.1526±0.0300

Aquatic Environment Quality

Overall, most of the measurement values have met the criteria of freshwater quality class category in Surabaya based on Table 1 and Table 2, where the location of KD, KE, and KA belonged to the water quality Class III, while the location of PE, WO, and DA belonged to the water quality Class IV (Table 4). Guppy is a fish that has excellent adaptability in broad environmental conditions and they are easy to adapt even in the high-contaminated water conditions (Araujo et al., 2009; Martinez et al., 2016). Therefore, in this study the guppy fish easily found in the aquatic environment, which has either class III or class IV water quality in Surabaya.

TABLE 4. Phenotypic character data of guppy fish from each site in Surabaya. The data represent mean ± standard deviation (SD) and samples sizes (N) estimated across sites.

Site	Month	pH	DO (mg/L)	BOD (mg/L)	COD (mg/L)	Turbidity (NTU)	Water Velocity (mph)	Salinity (‰)	Temperature (^{0}C)	TSS (mg/L)
	January	7.90	5.67	5.21	11.21	9.50	8.00	0.00	29.17	173,00
KD	February	7.50	3.83	5.67	12.65	9.54	1.67	0.00	29.00	170,00
	March	7.47	5.47	6.65	11.92	10.21	0.33	0.00	29.00	270,33
Accuracy (%)		**100**	**100**	**66.7**	**100**		-	-	**100**	**100**
	January	7.60	5.73	5.73	9.43	10.34	0.00	0.00	28.83	148,33
KE	February	7.47	5.13	7.64	8.76	10.32	0.00	0.00	29.17	258,00
	March	7.50	5.73	5.50	9.65	10.30	0.00	0.00	29.00	267,00
Accuracy (%)		**100**	**100**	**66.7**	**100**		-	-	**100**	**100**
KA	January	7.43	3.43	6.65	11.34	10.23	0.04	0.00	28.57	198,67

	February	7.43	3.97	4.76	11.65	9.58	10.00	0.00	28.67	178,67
	March	7.50	5.10	8.76	11.65	10.25	9.00	0.00	29.00	102,67
Accuracy (%)		**100**	**100**	**33.2**	**100**	**-**	**-**	**-**	**100**	**100**
	January	7.57	2.60	10.54	15.74	14.93	2.33	1.33	29.00	341,00
PE	February	7.63	2.13	10.54	16.76	18.43	0.10	1.00	29.00	305,00
	March	7.53	2.07	8.67	15.20	15.44	0.00	0.00	29.00	351,00
Accuracy (%)		**100**	**100**	**100**	**100**	**-**	**-**	**-**	**100**	**100**
	January	7.40	2.73	11.25	18.70	15.46	0.00	0.00	29.67	332,00
WO	February	7.43	2.80	6.87	17.65	17.96	0.15	0.00	29.33	342,67
	March	7.50	2.50	8.23	18.64	17.65	0.00	0.00	29.50	346,67
Accuracy (%)		**100**	**100**	**100**	**100**	**-**	**-**	**-**	**100**	**100**
	January	7.53	2.20	10.22	20.31	16.50	0.00	0.00	29.00	341,33
DA	February	7.50	2.03	8.65	15.66	16.54	0.09	0.00	29.40	371,00
	March	7.50	2.20	10.66	16.47	17.01	0.00	0.00	29.17	311,33
Accuracy (%)		**100**	**100**	**100**	**100**	**-**	**-**	**-**	**100**	**100**

Phenotypic Characters

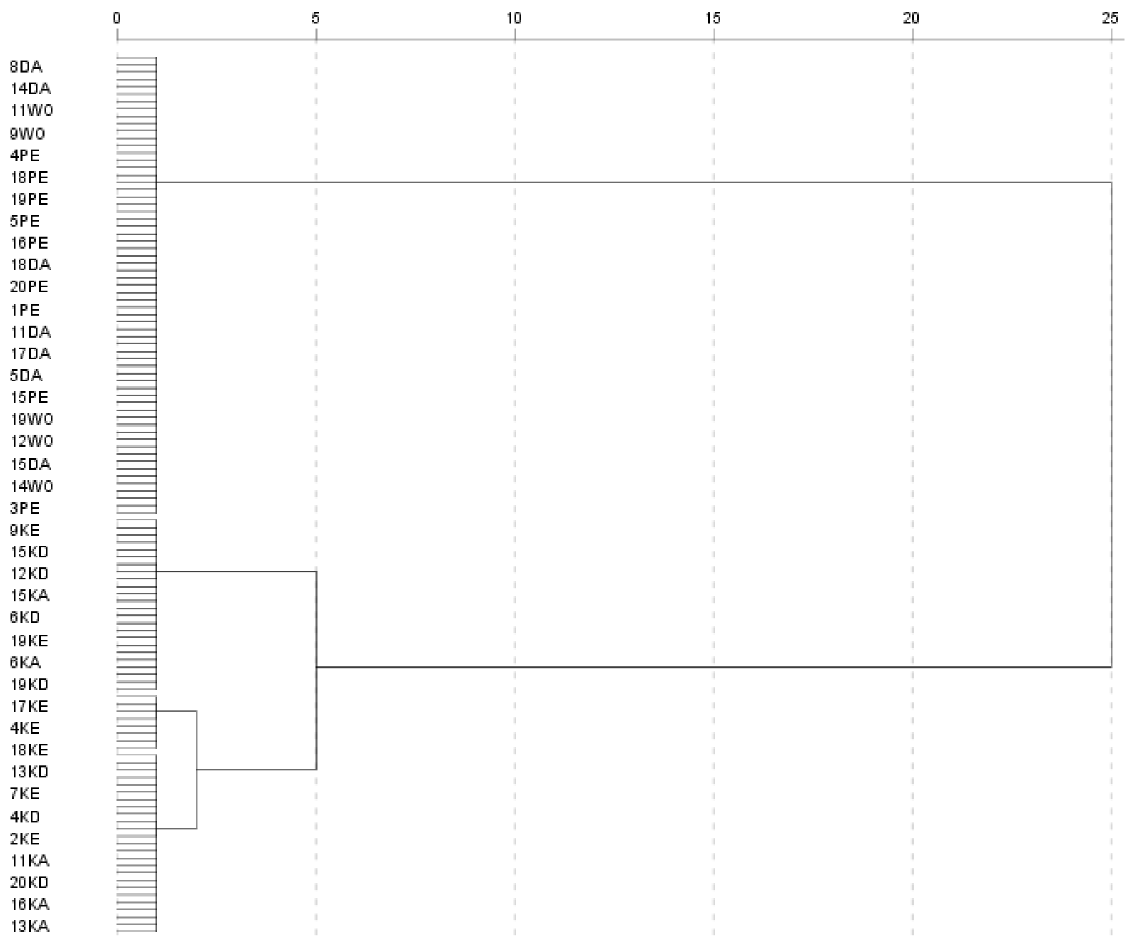

FIGURE 3. The phenogram tree of guppy fish samples from different sampling site in Surabaya streams

Based on the phenogram tree that has established there are two main branches with a value of difference is 25. The first branch is filled with the samples originating from the location with the water quality class IV (PE WO, and DA) which have a very close similarity (value of difference is 0). The second branch is filled by all the samples originating from water quality class III location (KD KE and KA) with the more varied value.

The PCA test reveals that there are only four phenotypic characters have influence (meet the statistical value), among different sites, they are body length, body area, relative area of structural color, and relative area of carotenoid color. However, the characters of tail length, relative area of melanic color, and spot number of each color do not have a correlation to location differences. The two main factors have been formed is factor 1 consists of body area, body length, and relative area of structural color therefore it named with "body size and structural color factor", while factor 2 consist of relative area of carotenoid color named the "carotenoid color size factor" (Fig. 4).

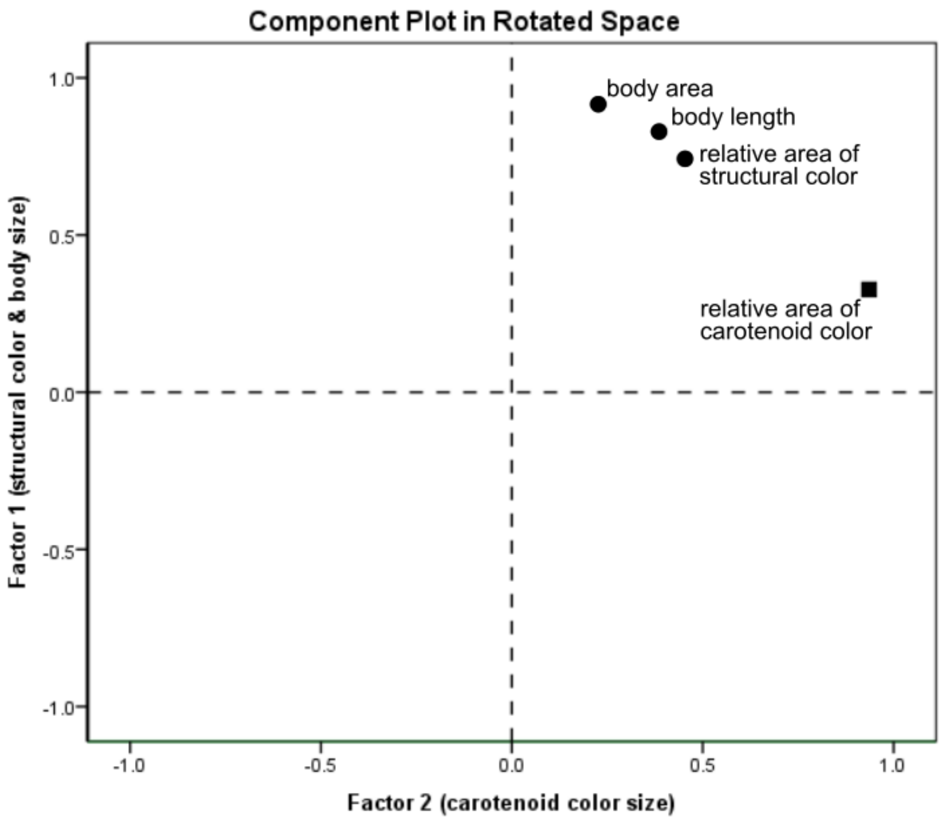

FIGURE 4. Factors that formed through PCA analysis

The PCA test reveals that there are only four phenotypic characters (they are body length, body area, relative area of structural color, and relative area of carotenoid color) have influence (meet the statistical value), [A47]among different sites, ~~they are body length, body area, relative area of structural color, and relative area of carotenoid color.~~ However, the characters of tail length, relative area of melanic color, and spot number of each color do not have a correlation to location differences. The two main factors have been formed is factor 1 consists of body area, body length, and relative area of structural color therefore it named with "body size and structural color factor", while factor 2 consist of relative area of carotenoid color named the "carotenoid color size factor" (Fig. 4).

TABLE 5. The results of the one way ANOVA (α=0,05) of four phenotypic characters

Character	Df	F	*P*
Body length	2.294	68.151	**<0.001**
Body area	2.294	59.068	**<0.001**
Relative area of carotenoid color	2.294	41.977	**<0.001**
Relative area of structural color	2.294	44.636	**<0.001**

The result of the 1 way ANOVA test of the 4 phenotypic characters reveals a significance value less than 0.001 ($P < 0.05$) among the 4 sampling sites in this study (Table 5). This shows that there is differences in value from this 4 characters among the sampling sites used in this study

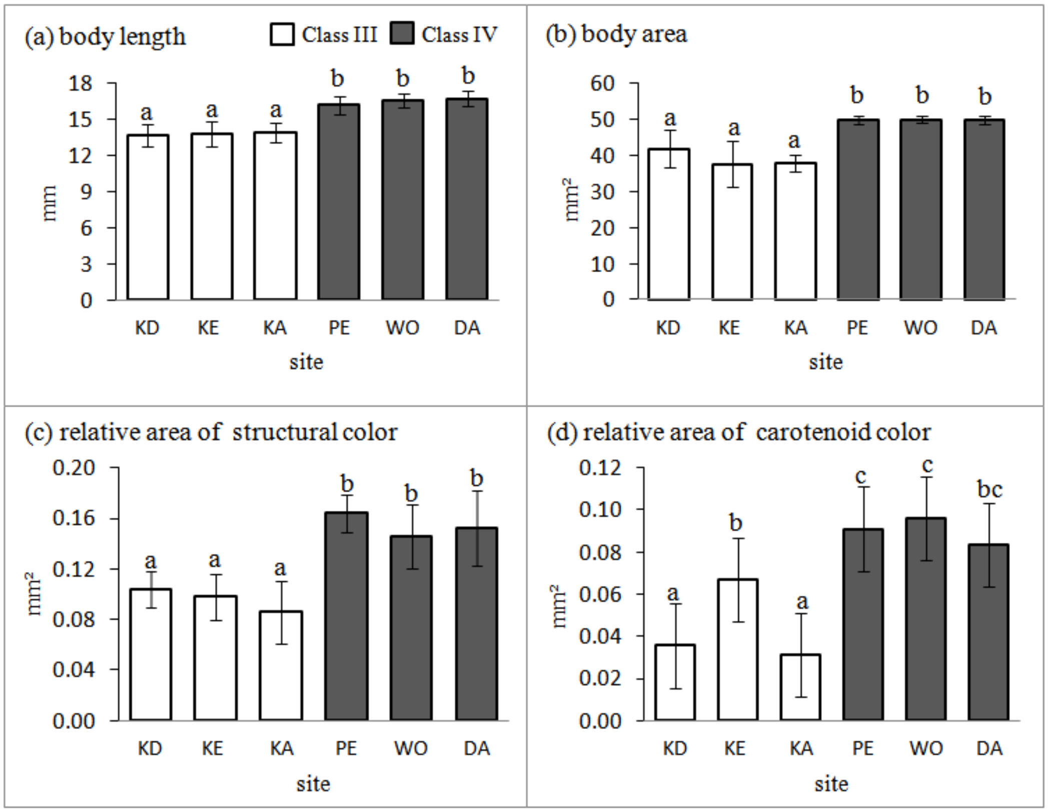

FIGURE 5. Comparison of the size of phenotypic character among male guppy fish population in each location with different water quality classes

Several studies about the guppy showed male fish has a polymorphism of phenotypic character resulting from its adaptation either in aquatic environments with different abiotic factor conditions (Hughes et al., 2005; Millar et al., 2006; Schwartz and Hendry, 2010) or in aquatic environments with different biotic factors condition, such as the presence of predatory or parasitic organisms (Egset et al., 2011; Gotanda and Hendry, 2014; Lindholm et al., 2014).

The three character of body length, body area, and relative area of structural color have a significant mean value differences between locations with different water quality classes but not significant in one same water quality class (Fig. 5a, b, and c). The results of a study by Torres-Dowdall (2012) [A48]showed that guppy fish populations, which ~~lived~~[A49] in the higher predation regime environment, have a smaller body size in terms of both body length and area. Whereas guppies which lived[A50] in the lower predation regime environment they present the larger body size. The aquatic environments, which belong to the water quality class III, have some parameters to support the life of more diverse organisms including the predatory species for guppy fish (Table 2 and 4) (JDIH Kota Surabaya. 2016).

The results of this study showed that guppy fish which living[A51] in the site with the water quality class III have a smaller body size than those living in the other site with the water quality class IV (Fig. 1 a and b). The aquatic environments, which have high nutrient content could accelerate the growth of guppy fish, therefore the guppy fish living in this site has the larger body size (Schwartz and Hendry, 2010). The higher organic materials in class IV sites which indicated[A52] by a high levels of BOD values (Table 4) could be used as a source of nutrients for guppy fish growth.

Schwartz and Hendry (2010) suggested[A53] that the presence of color polymorphism in male guppy fish is possible because of the presence of abiotic environmental factors such as turbidity or availability of nutrients. Several other studies have suggested that some environmental factors, which may affect the structural color patterns, were water turbidity levels and the levels of predatory organism (Endler, 1991; Long and Rosenqvist, 1998; Gamble et al., 2003). The guppy fish which originating[A54] from the class IV sites (PE, WO, and DA) which have higher water turbidity levels (Table 4) present the higher value of relative area of structural color than the guppy fish from the class III sites (KD, KE, and KA) which have lower water turbidity levels (Fig. 5c). The male guppy which lived in the darker aquatic environments (have higher turbidity levels) possess higher levels of structural color resulting from the evolutionary processes to attract the female vision in a darker conditions (Gamble et al. 2003). The male guppy, which lived[A55] in a lower predation regime in Trinidad, present a higher level of structural color, but the fish which lived[A56] in the higher predation regime, present a lower structural color (Breden and Stoner, 1987). Therefore, the guppy fish from the class III sites less display the structural color character in order to avoid the detection from more predator organisms.

The KD and KA sites, which belong to the water quality class III, have a significant difference in mean value of relative area of carotenoid color from the PE, WO, and DA sites, which belong to the water quality class IV. However, the KE site, which also belongs to the water quality class III, has no difference in mean value from the three locations (PE WO and DA) which is the water quality class IV category (Fig. 5d). This is in accordance with the study conducted by Lindholm et al. (2014) that carotenoid color characters have no differences between sites because the carotenoid color tends to be ~~more maintained than~~ [A57]the structural color in order to attract the female fish preference in the mating process in every different type of environmental conditions.

CONCLUSIONS

The kinship [A58]of guppy fish population in some aquatic environments in Surabaya based on phenotypic characters consists of two main groups. The first group is a guppy population originating from the location with the water quality class IV, whereas the second group is the guppy population originating from the location with the water quality class III. The phenotypic characters of male guppy fish that have different values between aquatic environment sites in Surabaya are body length, body area, relative area of structural color, and relative area of carotenoid color. The three characters that have different values based from differences of the water quality of aquatic environments (class III and class IV) are the body length, body area, and the relative area of structural color. While the relative area of carotenoid color have no different values between the two different aquatic environments in water quality both class III and class IV.

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Phenotype Variation of Guppy Fish (*Poecilia reticulate* W. Peters, 1859) Population from Different Quality of Aquatic Environments in Surabaya, Indonesia

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Abstract. Guppy fish (*Poecilia reticulata*) is commonly found in various regions and widespread distribution throughout the world, several aquatic environments in Surabaya have been known belong to the category of water quality class III or class IV. Abiotic factors that present in the environment may influence the polymorphism in a guppy fish population. When the population faces different environmental condition, it will cause the phenotypic variation in order to adapt to the conditions in the local environment. This study aims to observe the phenotypic variation of guppy fish population living in several aquatic environments with different quality levels in Surabaya. Fish samples were taken from six different locations that have been known for their water quality based on measurement of physical and chemical parameters. The total of 120 fish samples was obtained from six locations and used for phenotypic character analysis, such as body size and body color pattern. The results of this study consist of data on phenotype structure and phenogram tree. The population kinship based on phenogram tree consists of the first group that coming from a population of the class IV location, while the second group is coming from a population of the class III location. The phenotypic character of guppy fish that have different values between sites is body length, body area, the relative area of structural color, and relative area of carotenoid color.

Keywords: aquatic environments, guppy fish, phenogram, phenotypic character

INTRODUCTION

Guppy (*Poecilia reticulata*) is a freshwater fish native species from Trinidad and northeastern region of South America [1]. The male guppy is a popular commercial fish because it has many strains and more varies in color and fin shape that makes it has been widely traded [2]. Guppy fish has been used to control the vector of *Aedes aegypti* disease [3] and is effectively used as a biological control agent against malaria mosquito larvae in Kenya [4]. This guppy species exhibits phenotype character of sexual dimorphism, the male fish has a wider tail fin, a more variable body color pattern, and relatively has a smaller body size than the female fish [5]. The male guppy has a size between 25 mm to 35 mm and has conspicuous color polymorphism patterns consisting of combinations of black, white, red-orange, yellow, green, iridescent spots, lines, and speckles. Males have a gonopodium; a slender, modified anal fin used as an intromittent organ, whereas the anal fin of females is rounded. Females are uniform silver grey and are larger and deeper bodies than males (40 mm to 60 mm). Juvenile fish resemble females and are independent from birth [6].

In Indonesia, guppy fish was first introduced as a biological control agent and has grown in wild environment through natural reproduction [7]. In Surabaya, the guppy fish which are abundant can be found in most of the freshwater habitat in the city. The increase in human population has negative impacts on the environment, one of which is aquatic environment pollution due to both domestic and industrial waste generated by various human activities. That various kind of waste has a major influence on water quality changes in Surabaya [8]. Based on The Local Regulation of Surabaya No. 2 in 2004 about water quality management and water pollution control, the aquatic environments in Surabaya has been determined based on different quality levels which is consist of four different classes according to some physical, chemical, and microbiological parameters. This regulation has also determined each class of several aquatic environments in Surabaya which all consist of water quality class III and class IV [9], and this condition was observed every year [8].

Guppy fish to be found in various regions and it has widespread distribution throughout the world. Some populations to be found inhabit in environments with varying conditions [10]. Various factors affect this fish species have a widespread distribution such as very high reproduction rate [11], have an ability to tolerate the contaminations in the environment [12], and have phenotypic plasticity [13]. When this fish has adapted in the new environments that have very different conditions from the origin environments, then this fish could present the divergence of several phenotypic characters in male fish from their original fish population [14].

This fish has a capability to adapt even in contaminated water conditions [15], but only a handful of study about the phenotypic character variation of guppy fish that exists in the introduced location. In Indonesia, the guppy fish that are living in the wild environments is rarely studied. The previous study about guppy fish in the wild habitat was conducted by [16]. Their study investigated the associations between trace metals in sediment, water, and guppy from urban streams of Semarang, Indonesia. Some abiotic factors that present in the environment such as turbidity or nutrient availability may influence the color polymorphism in introduced population of guppy fish [17]. Polymorphism occurs when two or more different phenotypic character present in one population of the same species [18]. When the population faces different environmental condition, it will cause the phenotypic variation due to adaptation in the new local environment [19].

This study aims to observe the phenotypic variation of guppy fish populations inhabit several aquatic environments with different quality levels in Surabaya. Furthermore, this study is to gain the new information and knowledge about the morphological relationship among guppy fish populations inhabit in several aquatic environments in Surabaya based on phenotypic characters and it could be as a useful data and developed for the future study.

MATERIALS AND METHODS

Study Area and Fish Sampling

Guppy fish have collected from January to February 2017 at six different sites of aquatic environmental streams in Surabaya, East Java, Indonesia (Table 1). The three sites were Kaldami (KD), Kenjeran (KE), and Kebonagung (KA), all have the water quality class III. Whereas the three other sites were Pegirian (PE), Wonorejo (WO), and Darmo (DA), all have the water quality class IV (Table 1 and Fig. 1). These sites selected because they both have different water quality levels (class III and IV) in Surabaya and present the population of guppy fish based on observer's exploratory sampling.

TABLE 1. Location of the sampling sites.

Sampling Site (Stream Name)	Site Code	Coordinates		Water Quality Class
Kalidami	KD	7°16'36.1"S	112°45'44.9"E	III
Kenjeran	KE	7°15'10.1"S	112°47'42.2"E	III
Kebonagung	KA	7°19'53.4"S	112°46'35.7"E	III
Pegirian	PE	7°14'16.7"S	112°44'40.7"E	IV
Wonorejo	WO	7°18'44.1"S	112°46'50.3"E	IV
Darmo	DA	7°17'24.0"S	112°44'32.0"E	IV

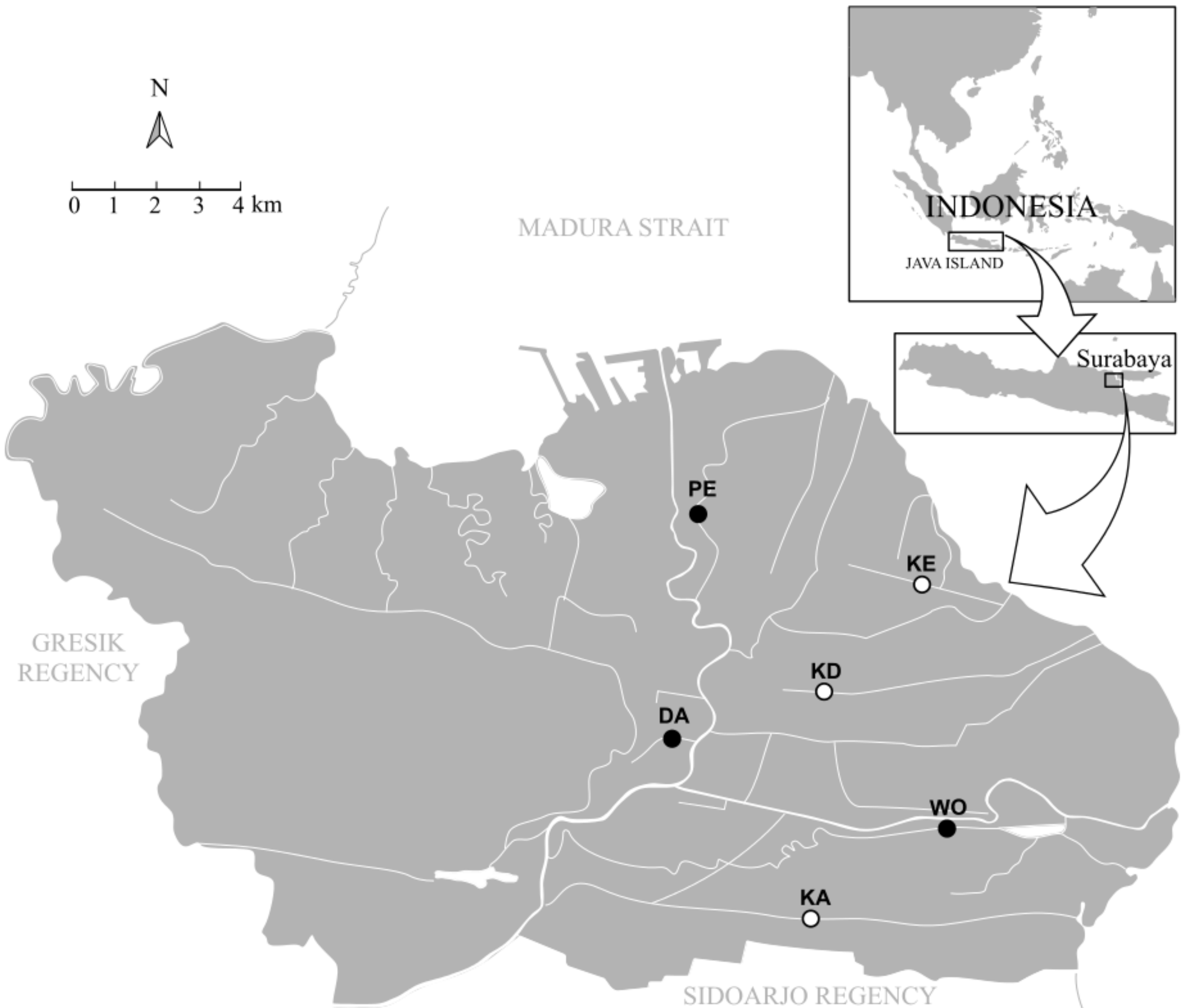

FIGURE 1. The sampling site locations of guppy fish in Surabaya. The white circles are the water quality class III, while the black circles are the water quality class IV locations. The white color patterns on the map are the water bodies

Before the fish samples were collected, we analyzed the physicochemical parameters of aquatic environment quality using the water quality measurement instruments. The parameters that samples measured directly on the site were temperature, pH, dissolved oxygen (DO), salinity, and water flow velocity. As for, the parameters that were measured after the water samples collected and analyzed in the laboratory were biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS). The parameters of aquatic environment quality were measured once in a month from Jan 2017 until Mar 2017 in each sampling site. Some parameters, such as temperature, TSS, pH, BOD, COD, and DO were measured based on the range value criteria arranged by The Local Regulation of Surabaya No. 2 in 2004, which has four different classes of water quality categories (Table 2). Class I is the water intended for use for drinking and other purposes that require the same water quality as those uses; class II, water intended for use in facilities of water recreation, cultivation of freshwater and brackish fish, livestock, irrigation, and other purposes that require the same water quality as those uses; class III, water intended for use in the cultivation of freshwater and brackish fish, livestock, irrigation, and other purposes that require the same water quality as those uses; and class IV, water intended for irrigation and other purposes that require the same water quality as those uses [9].

TABLE 2. The range values of water quality class categories arranged by The Local Regulation of Surabaya No. 2 in 2004 [9].

Parameter	Unit	Water Quality Class			
		I	II	III	IV
Physical					
Temperature	°C	Deviation 3	Deviation 3	Deviation 3	Deviation 3
TSS	$mg \cdot L^{-1}$	50	50	400	400
Inorganic Chemical					
pH	-	6 to 9	6 to 9	6 to 9	5 to 9
BOD	$mg \cdot L^{-1}$	2	3	6	12
COD	$mg \cdot L^{-1}$	10	25	50	100
DO	$mg \cdot L^{-1}$	6	4	3	0

Description: The above value is the maximum value, except for pH and DO. As for pH is a range value that should not be less or more of the listed value. The value of DO is the minimum value. The temperature deviation is in the natural condition.

Phenotypic Characters Analysis

Using a hand-net, a number of 20 males to 30 males of guppy fish were collected in each site. Fish were transport immediately to the Laboratory of Biosystematics, Faculty of Science and Technology, Universitas Airlangga Surabaya. Then they were anesthetized with a dose of eugenol ($C_{10}H_{12}O_2$). The fish then were processed following the methods of [20]. Lateral images of the left side of each fish photographed using a digital camera placed at a standard distance with the aid of a tripod. Each sample was placed on a white background with millimetric scale, and color standards (Red, Orange, Yellow, Blue, Green, White and Black). The phenotypic characters of male guppy fish such as, body length (standard length), body area (excluding the fins and tail), tail length, and the area and the number of each color spot (excluding the fins and tail) (Fig. 2) were measured using software Image J (version 1.50e). All fish sample images were analyzed by a similar person.

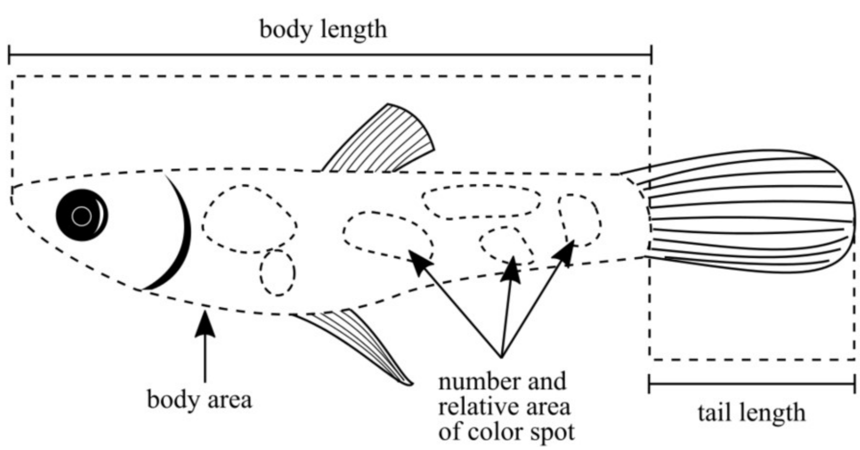

FIGURE 2. The phenotypic characters of male guppy fish which measured in this study

To reduce the number of variables for analysis, the color patterns were grouped into biologically relevant categories. These groups have different physiological bases, structural bases, functional interpretations, and selective relevance [21–26]. Carotenoid colors consisted of the sum of orange and yellow spots, although note that pigments other than carotenoids also contribute to these spots [23]. Structural colors are colors that are iridescent and have higher levels of reflection and consisted of the sum of blue, violet, and silver spots [22]. Melanic colors consisted of the sum of black and fuzzy black spots.

Data Analysis

Software SPSS version 20 was used to build a phenogram tree based on all phenotypic character value using Ward Linkage method. The statistical analysis from the phenotypic characters of guppy fish such as, body size and color pattern of each sample was analyzed using Principal Component Analysis (PCA) to find the factors that formed among different guppy fish populations. The one-way Analysis of Variance (ANOVA) was performed to know which of each character has a different value among different populations from each site following the post-hoc test using the Bonferroni test. This test was for the data which have homogenous variance whereas the Gomes-Howell test was used for the non-homogenous data with a significant value 0.05 ($\alpha = 0.05$).

RESULTS AND DISCUSSION

A number of 20 male guppy fish samples which the body length between 12.0280 mm and 17.8470 mm and the tail length between 3.0280 mm and 5.8270 mm were measured and used for the further analysis with the other phenotypic characters (Table 3).

TABLE 3. Phenotypic character data of guppy fish from each site in Surabaya.
The data represent mean ± standard deviation (SD) and samples sizes (N) estimated across sites.

Site	N	Body Length (mm)	Body Area (mm^2)	Relative Area of (mm^2)	
				Carotenoid Color	Structural Color
KD	20	13.6612 ± 0.9141	41.8963 ± 5.1870	0.0357 ± 0.0198	0.1040 ± 0.0143
KE	20	13.8019 ± 1.0294	37.6103 ± 6.3479	0.0668 ± 0.0192	0.0978 ± 0.0185
KA	20	13.9310 ± 0.8280	37.8840 ± 2.1361	0.0311 ± 0.0140	0.0860 ± 0.0246
PE	20	16.2201 ± 0.7765	49.9349 ± 1.2514	0.0909 ± 0.0219	0.1641 ± 0.0146
WO	20	16.5912 ± 0.6090	49.9516 ± 1.0159	0.0959 ± 0.0232	0.1455 ± 0.0253
DA	20	16.7658 ± 0.6658	49.9500 ± 1.2678	0.0834 ± 0.0166	0.1526 ± 0.0300

Aquatic Environment Quality

Overall, most of the measurement values have met the criteria of the freshwater quality class category in Surabaya based on Table 1 and Table 2. From both tables, the location of KD, KE, and KA belonged to the water quality Class III, while the location of PE, WO, and DA belonged to the water quality Class IV (Table 4). Guppy is a fish that has excellent adaptability in broad environmental conditions and they are easy to adapt even in the high-contaminated water conditions [15, 27]. Therefore, in this study, the guppy fish easily found in the aquatic environment, which has either class III or class IV water quality in Surabaya.

TABLE 4. Phenotypic character data of guppy fish from each site in Surabaya. The data represent mean ± standard deviation (SD) and samples sizes (N) estimated across sites.

Site	Month	pH	DO (mg · L^{-1})	BOD (mg · L^{-1})	COD (mg · L^{-1})	Turbidity (NTU)	Water Velocity (mph)	Salinity (‰)	Temperature (°C)	TSS (mg · L^{-1})
	Jan	7.90	5.67	5.21	11.21	9.50	8.00	0.00	29.17	173.00
KD	Feb	7.50	3.83	5.67	12.65	9.54	1.67	0.00	29.00	170.00
	Mar	7.47	5.47	6.65	11.92	10.21	0.33	0.00	29.00	270.33
Accuracy (%)		**100**	**100**	**66.7**	**100**		**-**	**-**	**100**	**100**
	Jan	7.60	5.73	5.73	9.43	10.34	0.00	0.00	28.83	148.33
KE	Feb	7.47	5.13	7.64	8.76	10.32	0.00	0.00	29.17	258.00
	Mar	7.50	5.73	5.50	9.65	10.30	0.00	0.00	29.00	267.00
Accuracy (%)		**100**	**100**	**66.7**	**100**		**-**	**-**	**100**	**100**
	Jan	7.43	3.43	6.65	11.34	10.23	0.04	0.00	28.57	198.67
KA	Feb	7.43	3.97	4.76	11.65	9.58	10.00	0.00	28.67	178.67
	Mar	7.50	5.10	8.76	11.65	10.25	9.00	0.00	29.00	102.67

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Table 4. Continued

Site	Month	pH	DO (mg · L^{-1})	BOD (mg · L^{-1})	COD (mg · L^{-1})	Turbidity (NTU)	Water Velocity (mph)	Salinity (‰)	Temperature (°C)	TSS (mg · L^{-1})
Accuracy (%)		**100**	**100**	**33.2**	**100**	**-**	**-**	**-**	**100**	**100**
	Jan	7.57	2.60	10.54	15.74	14.93	2.33	1.33	29.00	341.00
PE	Feb	7.63	2.13	10.54	16.76	18.43	0.10	1.00	29.00	305.00
	Mar	7.53	2.07	8.67	15.20	15.44	0.00	0.00	29.00	351.00
Accuracy (%)		**100**	**100**	**100**	**100**	**-**	**-**	**-**	**100**	**100**
	Jan	7.40	2.73	11.25	18.70	15.46	0.00	0.00	29.67	332.00
WO	Feb	7.43	2.80	6.87	17.65	17.96	0.15	0.00	29.33	342.67
	Mar	7.50	2.50	8.23	18.64	17.65	0.00	0.00	29.50	346.67
Accuracy (%)		**100**	**100**	**100**	**100**	**-**	**-**	**-**	**100**	**100**
	Jan	7.53	2.20	10.22	20.31	16.50	0.00	0.00	29.00	341.33
DA	Feb	7.50	2.03	8.65	15.66	16.54	0.09	0.00	29.40	371.00
	Mar	7.50	2.20	10.66	16.47	17.01	0.00	0.00	29.17	311.33
Accuracy (%)		**100**	**100**	**100**	**100**	**-**	**-**	**-**	**100**	**100**

Phenotypic Characters

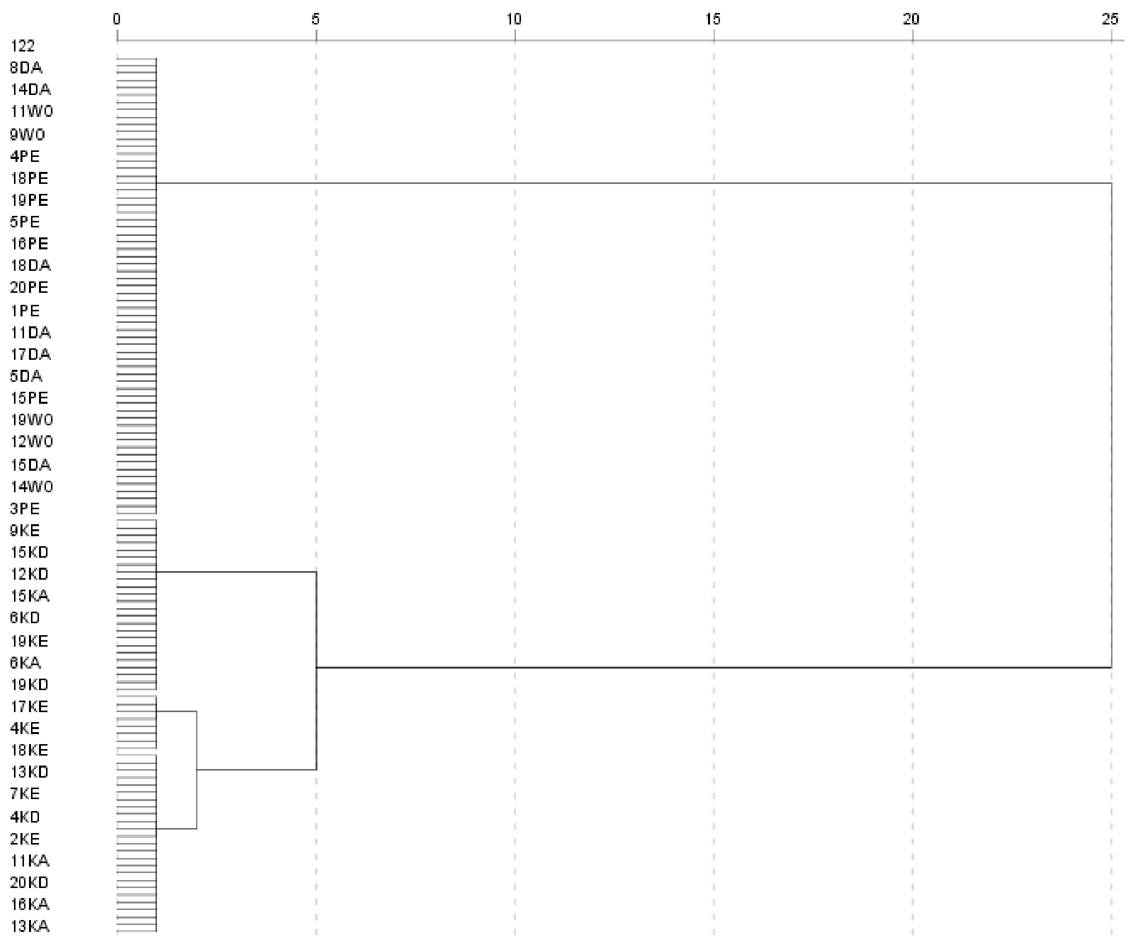

FIGURE 3. The phenogram tree of guppy fish samples from different sampling site in Surabaya streams

Based on the phenogram tree that has established there are two main branches with a value of difference is 25. The first branch is filled with the samples originating from the location with the water quality class IV (PE WO, and DA) which have a very close similarity (value of difference is 0). The second branch is filled with all the samples originating from water quality class III location (KD KE and KA) with the more varied value.

The PCA test reveals that there are only four phenotypic characters have influence (meet the statistical value), among different sites, they are body length, body area, the relative area of structural color, and relative area of carotenoid color. However, the characters of tail length, the relative area of melanic color, and spot number of each color do not correlate with location differences. The two main factors have been formed is factor 1 consists of body area, body length, and relative area of structural color. Therefore it named with "body size and structural color factor," while factor 2 consist of relative area of carotenoid color named the "carotenoid color size factor" (Fig. 4).

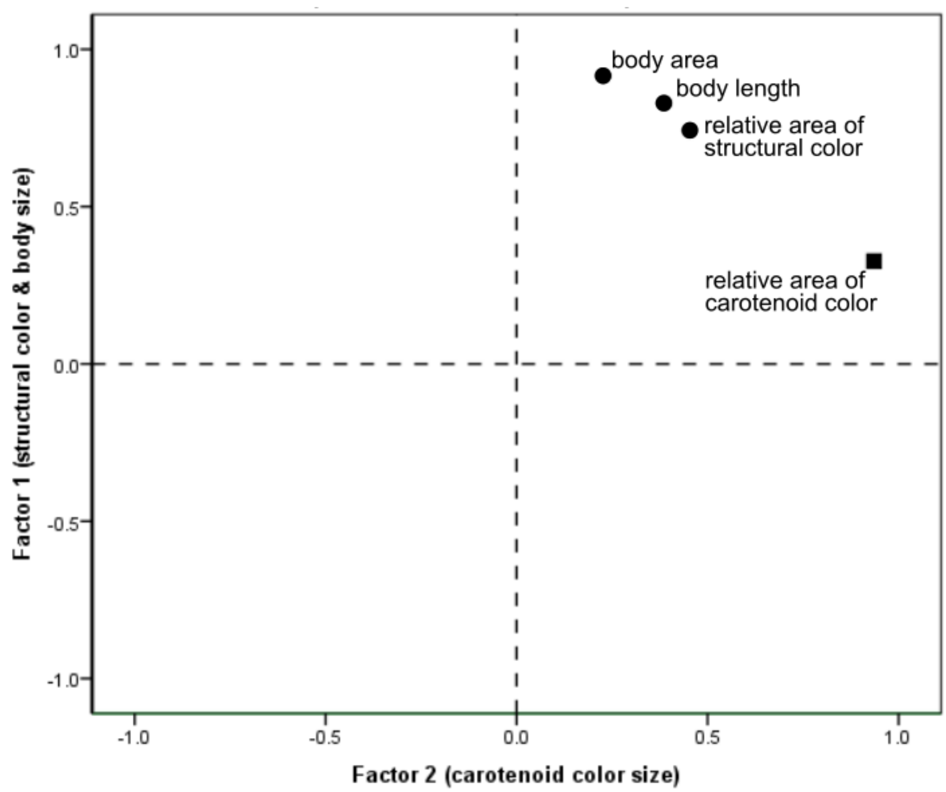

FIGURE 4. Factors that formed through PCA analysis

The PCA test reveals that there are only four phenotypic characters (they are body length, body area, relative area of structural color, and relative area of carotenoid color), have influence among different sites. However, the characters of tail length, relative area of melanic color, and spot number of each color do not correlate with location differences. The two main factors have been formed is factor 1 consists of body area, body length, and relative area of structural color. Therefore, it named with "body size and structural color factor," while factor 2 consist of relative area of carotenoid color named the "carotenoid color size factor" (Fig. 4).

TABLE 5. The results of the one way ANOVA ($\alpha = 0.05$) of four phenotypic characters.

Character	**Df**	**F**	***P***
Body length	2.294	68.151	< 0.001
Body area	2.294	59.068	< 0.001
Relative area of carotenoid color	2.294	41.977	< 0.001
Relative area of structural color	2.294	44.636	< 0.001

The result of the one-way ANOVA test of the four phenotypic characters reveals a significance value less than 0.001 ($p < 0.05$) among the four sampling sites in this study (Table 5). This shows that there are differences in value from this four characters among the sampling sites used in this study

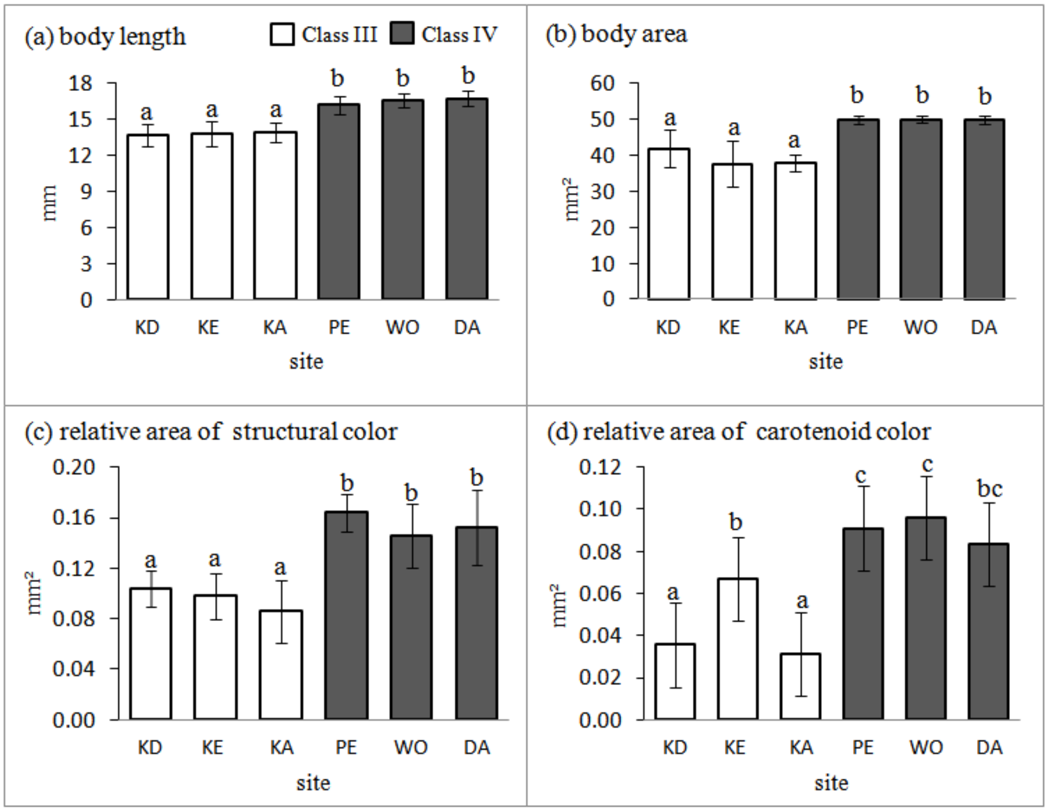

FIGURE 5. Comparison of the size of phenotypic character among male guppy fish population in each location with different water quality classes

Several studies about the guppy showed male fish has a polymorphism of phenotypic character resulting from its adaptation either in aquatic environments with different abiotic factor conditions [17, 20, 28] or in aquatic environments with different biotic factors condition, such as the presence of predatory or parasitic organisms [1, 14, 29].

The three character of body length, body area, and relative area of structural color have significant mean value differences between locations with different water quality classes but not significant in one same water quality class (Fig. 5a, Fig. 5b, and Fig. 5c). The results of a study by [13] showed that guppy fish populations, which in the higher predation regime environment, have a smaller body size in terms of both body length and area. Whereas guppies which found in the lower predation regime environment they present the larger body size. The aquatic environments, which belong to the water quality class III, have some parameters to support the life of more diverse organisms including the predatory species for guppy fish (Table 2 and Table 4) [9].

The results of this study showed that guppy fish which inhabit in the site with the water quality class III have a smaller body size than those living in the other site with the water quality class IV (Fig. 1a and Fig. 1b). The aquatic environments, which have high nutrient content could accelerate the growth of guppy fish. Therefore, the guppy fish living in this site has the larger body size [17]. The higher organic materials in class IV sites which are indicated by high levels of BOD values (Table 4) could be used as a source of nutrients for guppy fish growth.

In [17] reported that the presence of color polymorphism in male guppy fish is possible because of the presence of abiotic environmental factors such as turbidity or availability of nutrients. Several other studies have suggested that some environmental factors, which may affect the structural color patterns, were water turbidity levels and the levels of predatory organism [30]. The guppy fish which original from the class IV sites (PE, WO, and DA) that have higher water turbidity levels (Table 4) present the higher value of relative area of structural color than the guppy fish from the class III sites (KD, KE, and KA) which have lower water turbidity levels (Fig. 5c). The male guppy which lived in the darker aquatic environments (have higher turbidity levels) possess higher levels of structural color resulting from the evolutionary processes to attract the female vision in darker conditions [30]. The male guppy, which inhabits in a lower predation regime in Trinidad, present a higher level of structural color, but the fish which inhabit in the higher predation regime, present a lower structural color [31]. Therefore, the guppy fish from the class III sites less display the structural color character in order to avoid the detection from more predator organisms.

The KD and KA sites, which belong to the water quality class III, have a significant difference in mean value of relative area of carotenoid color from the PE, WO, and DA sites, which belong to the water quality class IV. However, the KE site, which also belongs to the water quality class III, has no difference in mean value from the three locations (PE WO and DA) which is the water quality class IV category (Fig. 5d). This is in accordance with the study conducted by [14] that carotenoid color characters have no differences between sites because the carotenoid color tends to be maintained compared to the structural color in order to attract the female fish preference in the mating process in every different type of environmental conditions.

CONCLUSIONS

The morphological relationship of guppy fish population in some aquatic environments in Surabaya based on phenotypic characters consists of two main groups. The first group is a guppy population originating from the location with the water quality class IV, whereas the second group is the guppy population originating from the location with the water quality class III. The phenotypic characters of male guppy fish that have different values between aquatic environment sites in Surabaya are body length, body area, relative area of structural color, and relative area of carotenoid color. The three characters that have different values based from differences of the water quality of aquatic environments (class III and class IV) are the body length, body area, and the relative area of structural color. As for the relative area of carotenoid color has no different values between the two different aquatic environments in water quality both class III and class IV.

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