Aquat. Living Resour. 22, 113–116 (2009)

DOI: 10.1051/alr/2009011
www.alr-journal.org

Aquatic Living Resources

Note

Comparison of two methods for measurement of red-area coverage in white-red fish for analysis of color variability and inheritance in ornamental (koi) carp *Cyprinus carpio*

Noel D. Novelo[a] and Boris Gomelsky[b]

Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601, USA

Received 17 November 2008; Accepted 4 March 2009

Abstract – The purpose of this study was to develop and compare two methods for measurement of red-area coverage in white-red ornamental (koi) carp. One progeny produced by crossing two white-red koi breeders was analyzed. Among 49 fish in the progeny, 7 fish (14.3%) were solid white, 36 fish (73.5%) were white-red, and 6 fish (12.2%) were solid red. The red-area coverage in all white-red fish was determined by analysis of digital photographs using the image editing software Adobe® PhotoDeluxe® Business Edition (method I) and the specialized image analysis software Image-Pro® Plus (method II). The red-area coverage in white-red fish was highly variable, from 0.08 to 68.4%. The difference in red-area coverage for individual fish when comparing these two methods varied from 0.04 to 2.75% with a mean value of 1.03 ± 0.82% (SD). Class distributions of white-red fish with regard to red-area coverage determined by methods I and II did not differ significantly. The obtained data show that both methods can be successfully used for quantitative measurement of color patches in koi. Analysis of frequency distribution of red-area coverage in all fish (including solid white and solid red) showed that fish were clearly divided into two groups with regard to background color: fish with white background (which includes solid white and white-red fish), and solid red fish. The present study confirms that measuring the relative red body coverage gives a valuable insight into the quantitative description of color variability in koi and provides further information for the better understanding of inheritance of this trait. The development and application of new advanced methods for measurement of red-area coverage may stimulate further studies on this subject.

Key words: Ornamental carp / Image analysis / Color pattern / Color variability / Pigmentation

1 Introduction

Ornamental (koi) carp, *Cyprinus carpio* L., are characterized by a wide diversity of colors and color patterns. Currently about 13–14 main color types of koi are distinguished. White-red koi, or Kohaku according to Japanese classification, is one of the most common and popular color types (Kuroki 1981; Tamadachi 1990). Inheritance of white-red color complex in koi has been investigated in several studies (Iwahashi and Tomita 1980; Gomelsky et al. 1996, 2003; David et al. 2004) but the genetic mechanisms controlling this trait remain unresolved.

Most of the studies on inheritance of white-red color complex in koi reported segregations of three color phenotypes in progenies – solid white (sometimes called transparent or colorless), white-red (Kohaku), and solid red (Iwahashi and Tomita 1980; Gomelsky et al. 1996; David et al. 2004). High variation in development of red color was observed among white-red fish in progenies. Therefore, Gomelsky et al. (2003) suggested recording not only segregation of the main color phenotypes (i.e. white : white-red : red) in progenies but, in addition, measuring the relative area covered by red patches in white-red fish. Analysis of distribution of white-red fish with regard to the rate of development of red color gave more comprehensive information on the trait variability for better understanding of its inheritance (Gomelsky et al. 2003).

For measurement of red-area coverage in white-red fish, Gomelsky et al. (2003) used a relatively elementary method in 1995-1996, which did not include photography of fish and any computerized image analysis. Briefly, the anesthetized fish was placed in a transparent plastic bag of corresponding size and outlines of the entire body and red patches for both sides

[a] Present address: Aquaculture Research Station, Louisiana State University, 2410 Ben Hur Road, Baton Rouge, LA 70820, USA
[b] Corresponding author: boris.gomelsky@kysu.edu

of the fish were traced on the plastic with a permanent marker. The plastic film was photocopied; the paper outlines were cut; and the ratio of the weight of the body outline to the weight of the red patches outlines gave the relative red-area coverage (Gomelsky et al. 2003).

The objective of the present study was to develop newer, more advanced methods for measurement of red-area coverage in white-red koi. Recently, the advanced computer vision systems have been used for analysis of skin color development in goldfish (Wallat et al. 2002) and gender identification in guppy based on color traits (Zion et al. 2008). However, it seems problematic to apply computer vision systems for measuring of red-area coverage in koi since the size of red patches is highly variable and they are localized at different parts of the body. Therefore, the methods, which were developed and compared in the present study, have been based on image analysis of digital photographs of anesthetized fish. Our intention was to compare data obtained by using two different programs: both specialized image-analyzing software (Image-Pro® Plus) and common basic image editing software (Adobe® PhotoDeluxe®).

2 Materials and methods

The study was performed at the Aquaculture Research Center of Kentucky State University, Frankfort, Kentucky, USA. One progeny produced by crossing two white-red (Kohaku) koi breeders was analyzed. The koi breeders were injected with carp pituitary extract (Sigma Chemical Company, St. Louis, Missouri, USA) at 3 mg kg^{-1} to induce ovulation and spermiation. Eggs were inseminated in 1.5 L glass trays, which were placed for embryo incubation in a tank system with 50–75% daily water exchange. Three days after hatching, 500 actively swimming larvae were stocked in 0.04-ha earthen pond for further rearing.

Seven months after stocking, the pond was drained and fish were collected. The fish were individually anesthetized with MS-222, weighed, measured manually using a measuring board and classified into one of the three major color phenotypes (white, white-red or red). Digital photographs of the two lateral sides of all (36) white-red fish and of 2 solid white and 2 solid red fish were taken using a digital camera Nikon Coolpix 990 (Nikon Corporation, Japan). To take photographs, the anesthetized fish were put on horizontal blue plastic surface, which was positioned approximately 50 cm above the ground level. The distance between camera and fish was approximately 70 cm. The fish were photographed under daylight illumination in covered area preventing direct sunlight. The camera was held perpendicularly to the fish's lateral surface and was triggered manually.

Coverage of the body with red patches in all white-red fish was measured by analysis of digital photographs using both of the following methods:

Method I – Digital photographs of white-red fish were opened in Adobe® PhotoDeluxe® Business Edition 1.1 Software (Adobe Systems Inc., San Jose, California, USA). The contours of the whole body of the fish, and of the red patches were outlined with a contrast color line using "brush tool" on the fish lateral image displayed on the computer screen. Zooming of image (up to 200–400%) was used when needed in order to determine the exact borders of red patches. Then the lateral images of fish with color line outlines were printed. From this point the quantification of red-area coverage was similar to the method used by Gomelsky et al. (2003). Namely, the paper outlines of the body were cut out and weighted to the nearest 0.01 g, followed by a similar weighing of the paper cut-out of outlined red patches. The ratio of these two weights gave the relative body coverage by red patches.

Method II – Digital photographs of white-red fish were processed using Image-Pro® Plus, Version 5.0 (Media Cybernetics, Inc., Bethesda, Maryland, USA). The red and white areas on the fish body were color coded with segmentation tools that use the Color Cube-Based Model which divides the color spectrum into a cube defined by intensity along red, green and blue axes (Image-Pro® Plus, Version 5.0, Reference Guide for Windows^{TM}). The "eye-dropper" tool identified the cube area. Each color and intensity range within the cube can then be selected into either of the two segmentation classes representing red and white areas. Once the red and white areas on the body of the fish were defined, area measurements for segmented areas were determined as pixel values with the "Count/Size" tools under the "Measurement" menu. Image-Pro® Plus reports on area measurements were exported into an Excel spreadsheet. The ratio of the total area of red patches to the entire area of the body gave the relative red area coverage in each of 36 white-red fish analyzed.

The same two methods were used to measure coverage with red color in white-red fish parents.

The values of red-area body coverage for all white-red fish in progeny determined by each method were classified into 10 percentile classes in 10% increments (>0–10.0, 10.1–20.0%, 20.1–30.0%, . . . 90.1–<100%). The class frequency distributions obtained by the two methods were compared using the Kolmogorov-Smirnov Goodness of Fit Test (Zar 1999). Distribution of white-red fish with regard to red-area coverage together with the overall color ratio in progeny (white : white-red : red) was used to describe the color class distribution of the total progeny (including solid white and solid red individuals).

3 Results

The red-area body coverage of the white-red koi breeders used in the cross as determined by methods I and II was 12.8 and 13.4% for the female, and 45.0 and 43.2% for the male.

A total of 49 fish were collected from the pond. The mean weight and length of seven-month-old fish were 360 ± 191 g (SD) and 27 ± 4 cm (SD). Seven fish (14.3%) from the progeny were solid white, 36 fish (73.5%) were white-red, and 6 fish (12.2%) were solid red.

The ranked distribution of white-red fish with regards to mean values of red-area coverage determined by two methods is given in Figure 1. The red-area coverage in white-red fish was highly variable, from 0.08 to 68.4% (mean value for two methods). As an illustration, the photographs of several fish from analyzed progeny are presented (Fig. 2). Differences in red-area coverage for individual fish as determined by two

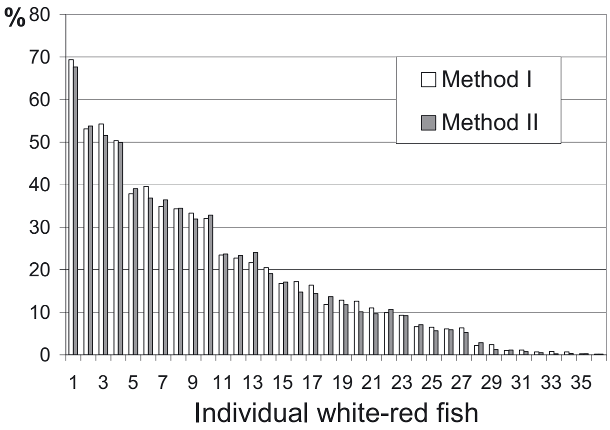

Fig. 1. Ranked distribution of white-red fish with regards to mean values of red-area coverage. White and gray bars indicate red-area coverage determined by method I and II, respectively.

Table 1. Distribution of white-red fish with regard to body coverage by red patches as determined by two methods (class coverage 10%).

Classes (red body coverage, %)	Method I		Method II	
	No. of fish	%	No. of fish	%
>0–10.0	15	41.7	15	41.7
10.1–20.0	7	19.4	8	22.2
20.1–30.0	4	11.1	3	8.3
30.1–40.0	6	16.7	6	16.7
40.1–50.0	0	0	1	2.8
50.1–60.0	3	8.3	2	5.6
60.1–70.0	1	2.8	1	2.8
70.1–80.0	0	0	0	0
80.1–90.0	0	0	0	0
90.1–<100	0	0	0	0
Total no. of fish	36	100	36	100

methods varied from 0.04 to 2.75% with a mean value of 1.03 ± 0.82% (SD). Class distributions of white-red fish with regard to red-area coverage determined by methods I and II are presented in Table 1. Statistical analysis with Kolmogorov-Smirnov Goodness of Fit Test showed that these two distributions did not differ significantly ($p > 0.05$). The distribution of white-red fish with regard to red-area coverage was skewed towards higher frequency of fish with weaker development of red color. The class of white-red fish with the lowest (>0–10.0%) red-area coverage had the highest frequency (41.7%). Along with an increase of coverage with red patches in white-red fish, the frequencies of fish in corresponding classes decreased reaching 0 in three classes with the highest (70.1–80.0, 80.1–90.0 and 90.1–<100%) coverage of red patches.

Figure 3 presents distribution of all fish in the progeny (including solid white and solid red fish) with regard to red color development, which were compiled based on the data of Table 1 and the ratio of white : white-red : red fish in the progeny. The class of white-red fish with lowest (>0–10.0%) red-area coverage occurred at the highest frequency (30.6%); 15 of the 49 fish were in this class.

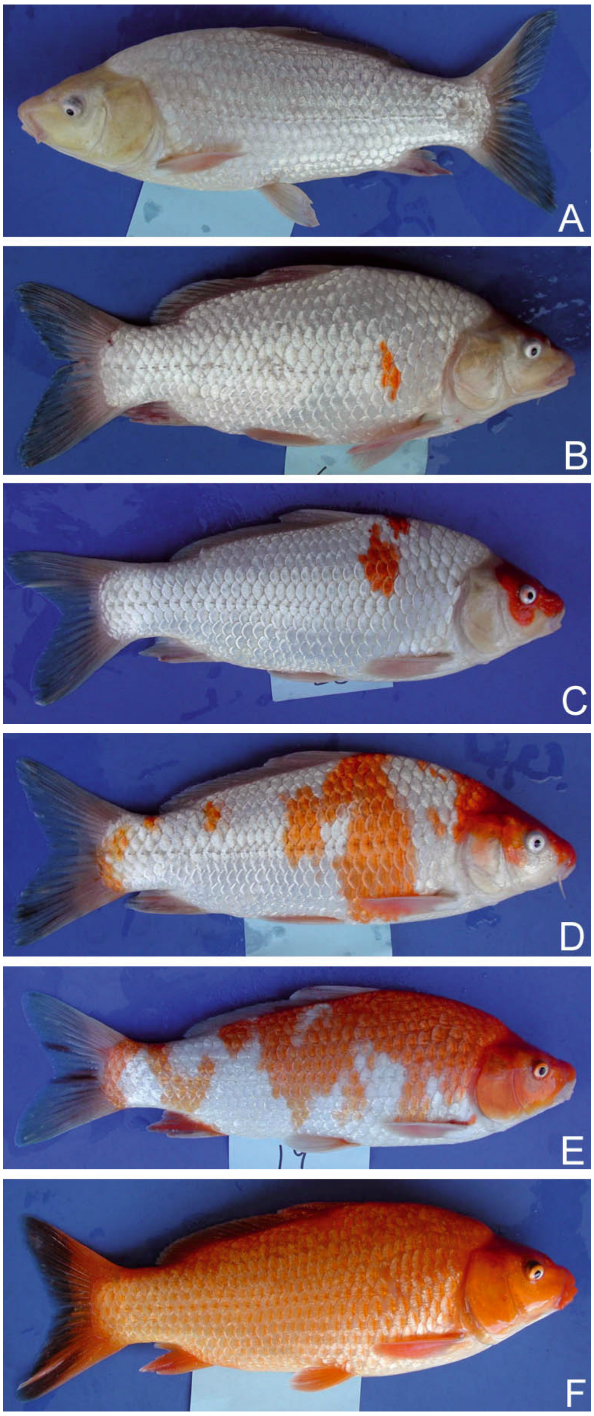

Fig. 2. Illustration of color variability in investigated koi progeny. A – solid white fish; B to E – white-red fish with different rate of red-area body coverage; F – solid red fish.

4 Discussion

In the previous study Gomelsky et al. (2003) used a relatively elementary method to determine coverage with red patches in white-red koi for investigation of color variability. The present study was to develop and compare two more advanced methods, which use analysis of digital photographs with image editing software (Adobe®

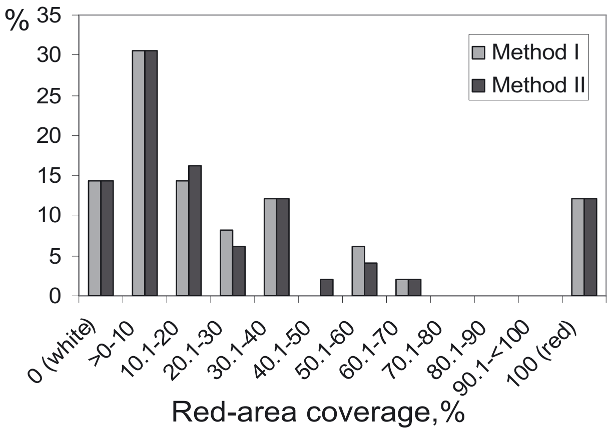

Fig. 3. Frequency distribution of all fish in analyzed progeny with regard to development of red color (including solid white and solid red fish).

PhotoDeluxe® Business Edition, method I) and specialized image analysis software (Image-Pro® Plus, method II). The application of both methods for measurement of red-area coverage in analyzed progeny gave similar results. The mean difference in value of red coverage for individual fish was close to 1%. The class distributions of white-red fish with regard to development of red color generated by the two methods showed no significant difference. Based on the obtained data it may be concluded that both these methods can be successfully used for quantitative measurement of color patches in koi.

Method II may be considered preferable to method I because it relies on advanced image analysis software (Image Pro® Plus) that provides specialized tools and functions for quick and automated identification and measurement of red patches on the fish's body. The disadvantage of method II, however, includes the necessity to acquire the advanced image-analysis software, which can be expensive. Method I, on the other hand, does not require specialized image-analyzing software; only the basic image editing software is needed for application of this method. In the present study, we used Adobe®PhotoDeluxe®. However, similar image editing programs, which are installed practically in every personal computer, can be applied for this method. The main disadvantage of this method is the predominantly manual (not automated) data collection process involved in outlining body and red patch contours, and cutting out and weighing the paper for calculation of red-area body coverage.

As mentioned above, only the major color phenotypes (white : white-red : red) were recorded in many previous studies on inheritance of the white-red color complex in koi (Iwahashi and Tomita 1980; Gomelsky et al. 1996; David et al. 2004). Analysis of fish distribution in the analyzed progeny with regard to development of red color (Fig. 3) gives further insight into the relative standing of these major color types. The frequencies of solid white and solid red fish in the progeny were similar, 14.3% and 12.2% respectively. However, based on the frequency distribution (Fig. 3), the solid white and solid red fish are positioned differently relative to white-red fish. As above mentioned, the distribution of white-red fish with regard to red-area coverage (Table 1) was skewed towards higher frequency of fish with weaker development of red color. Because of this skewness, solid red fish (Fig. 3) stand separately with regard to their frequency from white-red fish. Fish in the progeny are clearly divided into two groups: fish with white body color background (including solid white and white-red fish) and solid red fish.

Similar division of fish into two groups with white and red background color in progenies generated by crossing white-red koi was observed by Gomelsky et al. (2003) where some suggestions on their appearance have been made. The present study confirms that measuring the relative red body coverage gives a valuable insight into the quantitative description of color variability in koi and, thus, provides further information for the better understanding of inheritance of this trait. The development and application of new advanced methods for measurement of red-area coverage may stimulate further studies on this subject.

Acknowledgements. Support for this study was provided by Kentucky's Regional University Trust Fund to the Aquaculture Program as Kentucky State University's Program of Distinction. The authors thank Drs. J. Tidwell, S. Mims, R. Durborow and K. Pomper for valuable suggestions, and Dr. S. Dasgupta for consulting on statistical treatment of data.

References

David L., Rothbard S., Rubinstein I., Katzman H., Hulata G., Hillel J., Lavi U., 2004, Aspects of red and black color inheritance in the Japanese ornamental (Koi) carp (*Cyprinus carpio* L.). Aquaculture 233, 129–147.

Gomelsky B., Cherfas N.B., Ben-Dom N., Hulata G., 1996, Color inheritance in ornamental (Koi) carp (*Cyprinus carpio* L.) inferred from color variability in normal and gynogenetic progenies. Isr. J. Aquac. – Bamidgeh 48, 219–230.

Gomelsky B., Cherfas N., Hulata G., Dasgupta S., 2003, Inheritance of the white-red (Kohaku) color complex in ornamental (Koi) carp (*Cyprinus carpio* L.). Isr. J. Aquac.- Bamidgeh 55, 147–153.

Iwahashi M., Tomita M., 1980, Genetical studies of nishikigoi (*Cyprinus carpio*). II. On the heredity of Kohaku. Rep. Niigata Inland Waters Fish. Exp. Stn. 8, 74–79 (in Japanese with English summary).

Kuroki T., 1981, The Latest Manual to Nishikigoi, Shimonoseki-city, Japan, Shin-Nippon-Kyoiku-Tosho Co. Ltd.

Tamadashi M., 1990, The Cult of the Koi, 2nd edition, Neptune, New Jersey, USA, TFH Publications Inc.

Wallat G.K., Luzuriaga D.A., Balaban M.O., Chapman, F.A., 2002, Analysis of skin color development in live gold fish using a color machine vision system. N. Am. J. Aquac. 64, 79–84.

Zar J.H., 1999, Biostatistical Analysis. 4th edition, Upper Saddle River, New Jersey, USA, Prentice-Hall, Inc.

Zion B., Alchanatis V., Ostrovsky V., Barki A., Karplus I., 2008, Classification of guppies' (*Poecilia reticulata*) gender by computer vision. Aquac. Eng. 38, 97–104.