DOI:10.26524/krj.2020.11

Kong. Res. J. 7(1): 69-75, 2020 ISSN 2349-2694, All Rights Reserved,
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RESEARCH ARTICLE

EFFECTS OF ARTIFICIAL PHOTOPERIOD ON THE BODY COLOURATION IN THE ORNAMENTAL FISH *POECILIA SPHENOPS*

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ABSTRACT

In Orange balloon Molly, *Poecilia sphenops,* the development of chronic stress due to continuous exposure to artificial LED lighting irrespective to photoperiod causes deterioration in the body colour even in the presence of carotenoid rich green water and artificial feed with added additives in the experimental fish tank setup. In the present study, the effects of continuous exposure to lighting and its consecutive development of chronic stress causes the body colouration to fade off in the freshwater ornamental live bearer, *Poecilia sphenops,* though the fishes were provided with colour enhancing as well as stress relieving factors. Several trials showed that, whatever may be the colour enhancing agents provided, artificial lighting for long exposure irrelevant to the normal photoperiod remains as a strong anti-colouration as well as stress inducing factor and remains unaffected to any antagonistic factors. To conclude photoperiod is an essential factor to be concerned for colour enhancement in *Poecilia sphenops.*

Keywords: Photoperiod, Green water, Body colouration, *Poecilia sphenops*

1. INTRODUCTION

Ornamental fish trade is one of the quickly growing markets in the world. More than 125 countries around the globe are involved in this trade either as an importer or exporter. Most of the fishes that are sold by the aquarium are bought by hobbyists (Dey., 2016). Ornamental fishes are bought for their fascinating and attractive skin colour and pattern. The sexual selection acts on colour and patterns leading colourful displays and also more diverse colouration pattern (Endler., 1980). The prices of the fishes are fixed based on their brilliant skin colour and pattern. The body of fishes shows a wide range of skin colours ranging from yellow, orange, blue, purple, green, etc.(Animal colouration / Wikipedia.com). These colourations are due to colour pigments such as carotenoids (Biological pigments / Wikipedia.com). These pigments cannot be stored beneath the skin permanently. As fishes cannot synthesize their own colouring pigments *de novo*, the colouring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in their diet (Johnson *et al.,* 1991). There are some factors like photoperiod, feeds with added pigments and green water which directly affects the colour pigmentation in the fishes. Some fish feeds with artificially synthesized colour pigments are commercially available in markets to enhance the body colour. Green water which is rich in micro alga, *C. vulgaris* has become a potent pigment source, which imparts yellow/blue hues. The biomass of this alga had already been proved to be useful in the diets of rainbow trout yielding both muscle and skin pigmentation effects (Gouveia *et al.,* 1997). It has also been reported that it contains carotenoid pigments in concentrations of up to 0.4% (dry wt), of which, 80% were potential red hue inducing pigments (Gouveia *et al.,* 1996). Other than green water, commercial feeds with added additives such as astaxanthin is also used to enhance colouration (Gupta *et al.,* 2007). Apart from all those factors, photoperiod has an important effect since it is one of the stimuli that cue the internal biological clock about the seasonal changes in the environment. Light is a key environmental factor that synchronizes all stages of fish from embryo development to sexual maturation (Bairwa *et al.,* 2013).

Photoperiod is most useful in predicting environmental conditions in the future or at distant localities; photoperiod provides a go/ no-go signal that initiates a usually irrevocable cascade of physiological and development processes that culminate in reproduction, dormancy and migration. Day length provides a highly reliable calendar that animals can use to anticipate and prepare for seasonal change (Sarkar *et al.*, 2011). Photoperiod is an important physical factor that affects the growth, reproduction and many other functions of the fish. Light and dark cycle provides internal harmonization for the rhythmic synthesis and release of hormones (i.e. melatonin), whose signal

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affects rhythmic physiological function in fish (Bairwa *et al.,* 2013). This study is carried out to prove that among all factors, photoperiod is a strong factor influencing the colour pigmentation adopting ornamental live bearer, Balloon Molly fish (*Poecilia sphenops*) as the animal model.

2. MATERIALS AND METHODS

2.1. Apparatus setup-

- Size of the tank - 45cm * 25cm * 25cm
- Water column depth - 20cm
- Light intensity - 400lux
- Temperature - 23-27° C
- Lamp type - LED

2.2. Animal model-

- Common name - Balloon molly
- Binomial name - *Poecilia sphenops*
- Variety - Balloon Molly
- Colour - Orange
- Number of fishes used - 14 (7 male & 7 female)
- Age of the fish - Adult
- Gestational history:

Gravida	Nil
Parity	Nil

2.3. Methods

To prove photoperiod has a strong influence over body colour, the comfortable method is to compare with other factors that are also engaged in enhancing body colouration in ornamental fishes. Experiments carried out with combination of these factors give us a good platform for comparing their effects. Along with control trial there are six experiments carried out in this study. The three factors that are mostly sought for body colouration are,

- Photoperiod
- Green water
- Commercial feed with artificial pigments

2.3.1. Control

A pair of fishes observed in normal regular environmental condition is made as a control trial to compare the results of the experiments.

- Water – Clear water
- Photoperiod – 12 hrs.. daylight & 12 hrs.. darkness
- Feed – Without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – Sun light
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.2. Experiment - 1

- Water – Clear water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – LED lighting
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.3. Experiment - 2

- Water – Green water
- Photoperiod – 12 hrs. daylight & 12 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1week
- Lighting – Sun light
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.4. Experiment - 3

- Water – Green water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1week
- Lighting – LED lighting
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.5. Experiment - 4

- Water – Clear water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – LED lighting
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.6. Experiment - 5

- Water – Green water
- Photoperiod – 12 hrs. daylight & 12 hrs. darkness

- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – Sun light
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

2.3.7. Experiment - 6

- Water – Green water
- Photoperiod – 24 hrs. LED light & 0 hrs. darkness
- Feed – without artificial pigments
- No. of fishes – 2 (Male – 1 & Female – 1)
- Duration – 1 week
- Lighting – LED lighting
- Temperature – 23-27° C
- Feeding frequency – 3 times/day

3. RESULTS

The results of the experiments given in this project are the observations made from the trials based on the following criteria.

- Fading or enhancement in body colour.
- Healthy and active swimming
- Courtship behaviour

3.1. Control

In this control trial with normal environmental factors, the colour of the body remains bright with regular body patterns. The fishes were noticed with normal and active swimming all through the trial for complete 1 week. Courtship behaviour between the male and female was spotted several times.

- Colour is bright.
- Healthy swimming is observed.
- Courtship behaviour is seen frequently.
- Sign of copulation is seen.

Fig.1. Body colour deteriorated

Fig.2. Body colour enhanced

Fig.3. Courtship behaviour

Fig.4. Body colour enhanced in green water

3.2 Observations during the Experiments

Based on the observations (Table 1) made during the experimental trials with a combination of different factors, we can conclude the cumulative results with the gained outputs.

Table 1: Observations made during the experimental trials

Trial	Colour	Movement	Courtship
Control	Enhanced	Active	Yes
Exp - 1	Deteriorated	Inactive	No
Exp - 2	Enhanced	Active	Yes
Exp - 3	Deteriorated	Inactive	No
Exp - 4	Deteriorated	Inactive	No
Exp - 5	Enhanced	Active	Yes
Exp - 6	Deteriorated	Inactive	No

With the observations made from the experiments it is easy to conclude whether the fishes are in stress during the trials and on correlating the factors and the observations we can conclude the primary factor that mostly influences the stress and also the body colour pigmentation.

3.3. Correlation between the factors and the observations

Correlation is an effective tool to find which factor influences the result the most by correlating the inputs (photoperiod, green water & pigmented feed) with the observations (colour, movement & courtship behaviour). The factors and the observations are first tabulated to test for correlation (Table. 2).

Table 2: Factors and their corresponding observations

Trial	Photoperiod	Water condition	Feed	Observed colour
Control	Normal	Clear	Non pigmented	Enhanced
Experiment 1	Induced	Clear	Non pigmented	Deteriorated
Experiment 2	Normal	Green	Non pigmented	Enhanced
Experiment 3	Induced	Green	Non pigmented	Deteriorated
Experiment 4	Induced	Clear	Pigmented	Deteriorated
Experiment 5	Normal	Green	Pigmented	Enhanced
Experiment 6	Induced	Green	Pigmented	Deteriorated

3.3.1. Correlation between Water condition and Observed colour

In this calculation, water condition is taken as an independent variable and the observed body colour is considered as a dependent variable since the colouration is either enhanced or deteriorated by the influence of the water condition either green water or clear water.

Pearson's coefficient of correlation, r = -0.1667 (poor relation)

Since the Pearson's coefficient, r = -0.1667 the relation between the independent factor (water condition) has a poor relation with the dependent factor (body colour).

3.3.2. Correlation between commercial feed and Observed colour

In this calculation, pigmented feed is taken as an independent variable and the observed body colour is considered as a dependent variable since the colouration is either enhanced or deteriorated by the influence of the pigmented feed or non-pigmented feed.

Table 3. Correlation between water condition and body colour

Variable X – Independent Variable Y – Dependent

Trial	Water condition Variable X	Observed colour Variable Y
Control	Clear	Enhanced
Exp - 1	Clear	Deteriorated
Exp - 2	Green	Enhanced
Exp - 3	Green	Deteriorated
Exp - 4	Clear	Deteriorated
Exp - 5	Green	Enhanced
Exp - 6	Green	Deteriorated

Fig. 1. Correlation between water condition and body colour

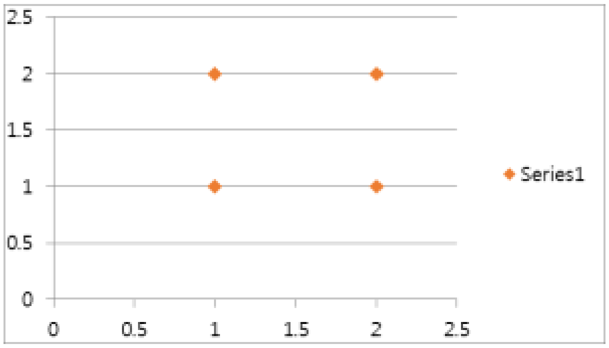
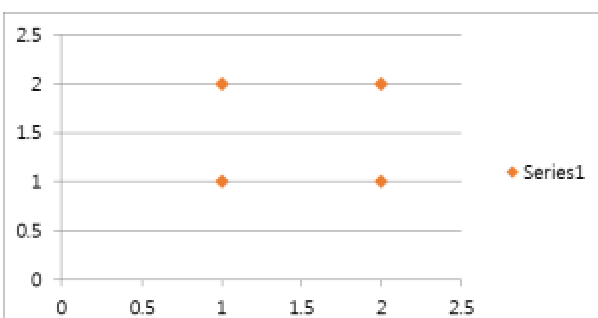


Table 4. Correlation between commercial feed and body colour

Variable X – Independent Variable Y – Dependent

Trial	Feed Variable X	Observed colour Variable Y
Control	Non pigmented	Enhanced
Exp - 1	Non pigmented	Deteriorated
Exp - 2	Non pigmented	Enhanced
Exp - 3	Non pigmented	Deteriorated
Exp - 4	Pigmented	Deteriorated
Exp - 5	Pigmented	Enhanced
Exp - 6	Pigmented	Deteriorated

Fig. 2. Correlation between commercial feed and body colour.



Pearson's coefficient of correlation, r = 0.1667 (poor relation)

Since the Pearson's coefficient, r = 0.1667 the relation between the independent factor commercial feed has a poor relation with the dependent factor body colour.

3.3.3. Correlation between photoperiod and Observed colour

In this calculation, photoperiod is taken as an independent variable and the observed body colour is considered as a dependent variable since the colouration is either enhanced or deteriorated by the influence of the natural photoperiod or induced photoperiod.

Table 5. Correlation between photoperiod and body colour

Variable X – Independent Variable Y – Dependent

Trial	Photoperiod Variable X	Observed colour Variable Y
Control	Normal	Enhanced
Exp - 1	Induced	Deteriorated
Exp - 2	Normal	Enhanced
Exp - 3	Induced	Deteriorated
Exp - 4	Induced	Deteriorated
Exp - 5	Normal	Enhanced
Exp - 6	Induced	Deteriorated

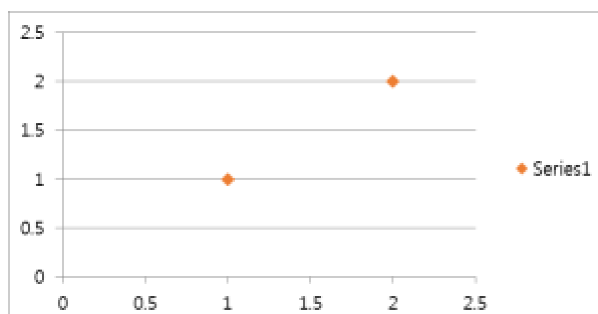


Fig. 3. Correlation between photoperiod and body colour

Pearson's coefficient of correlation, *r* = 1 (strong linear relation)

Since the Pearson's coefficient, *r* = 1 the relation between the independent factor commercial feed has a strong linear relation with the dependent factor body colour.

4. DISCUSSION

The results of the present study indicate that, among the considered three factors (water condition, pigmented feed and photoperiod), the correlation between water condition and pigmented feed with body colour is weaker than that of the correlation between photoperiod and the body colour. The correlation graph for water condition (Graph. 1) and the correlation graph for pigmented feed (Graph. 2) show a weak relation since the graphs cannot be interpreted as a strong linear relation but, the correlation graph for photoperiod (Graph. 3) can be interpreted as a strong linear bond. This proves that, among all other factors used in the study, photoperiod has a very strong influence over the body colour pigmentation in the ornamental live bearer *Poecilia sphenops.* Apart from body colouration, the fact that growth of Juveniles of the Anemone fish *Amphiprion melanopus* under 24L: 0D was slower than under 16L: 8D shows that photoperiod also affects the growth of the fishes (Arvedlund *et al.,* 2000). Similar studies were conducted about the exposure of male and female commercially important *P. sphenops* to manipulated photoperiod regimes with a specific time period to assess its effect on the growth and gonadal development along with the utilization of formulated feed. This presumption has a lacuna for stimulation of growth and enhancement of reproductive performance in fingerlings of ornamental fish although it has been tested for species of food fishes (Zutshi *et al*., 2017). These all studies show that apart from colouration, photoperiod has many vital roles to play. This does not mean other factors don't have any significant role in body colouration. In the Experiment 5, the fishes were brighter than in other experiments, which we can conclude by considering the algal carotenoids in account. Significant work has been done on pigmentation of many commercial fish species using carotenoids. In this respect, Mircoalgae such as *Chlorella vulgaris* is as effective as its synthetic counterpart in pigmentation of two most important ornamental fish species, *Cyprinus carpio* & *Carassius auratus* (Das and Biswas, 2016).

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