Effect of colored light regimes on the stress response and RNA/DNA ratio of juvenile red sea bream, *Pagrus major*

Gunzo Kawamura[1]*, Teodora Uy Bagarinao[2], Kazuhiko Anraku[3] and Masaru Okamoto[3]

[1]*Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia*
[2]*Aquaculture Department, Southeast Asian Fisheries Development Center, 5021 Tigbauan, Iloilo, Philippines*
[3]*Faculty of Fisheries, Kagoshima University, 890-0056 Kagoshima, Japan*

*Corresponding author: prof.gunzo@gmail.com

Abstract

We hypothesized that fish with red-sensitive retina would be stressed by red light and thus inhibited in somatic growth. Red sea bream (*Pagrus major*) juveniles (total length =3 cm) with red-sensitive retina were chosen to test this hypothesis. We examined the effect of different color lights (red with λ_{max} 605 nm; green with λ_{max} 540 nm; blue with λ_{max} at 435 nm; and white with full spectrum) on unfed juveniles in laboratory tanks. Stress level was measured by the plasma cortisol and glucose concentrations, and nutritional status by muscle RNA/DNA ratio. Under red light, plasma cortisol and glucose, and muscle RNA/DNA were significantly higher than under green, blue, or white light. Our hypothesis was partly supported by previous findings on the effects of the color environment and spectral sensitivity of reared fishes. However, the levels of cortisol, glucose, and RNA/DNA in this study were low compared to published values. It seems that hatchery-bred juvenile red sea bream have adapted to red-rich surface light and are able to cope with the stress of living in surface floating cages which is so different from their deep-water habitats.

Keywords: Spectral sensitivity, RNA/DNA ratio, Stress response

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Introduction

Light intensity, spectrum, and photoperiod have all been found to have significant effects on farmed fish. Color environments affect the stress response of fish (Kawamura et al., 2015) as well as the growth, development, malformation, and survival of reared fish (Villamizar et al., 2011). Ambient light in surface waters is richer in longer wavelengths, but becomes blue-dominant with depth (Jerlov, 1976). The maximum spectral sensitivity of the fish retina is closely correlated with the depth of the habitat of the species; the retinal sensitivity shifts toward shorter wavelengths in deeper water (Kobayashi, 1962; Lythgoe and Partridge, 1989). Growout of demersal and benthic fish in floating cages puts them in a full-light pelagic environment that is spectrally different from their natural habitats. This red-rich surface light is unnatural for deep-dwelling fish and could potentially stress them and compromise their welfare. Blanco-Vives et al. (2011) pointed out the importance of providing the natural underwater photoenvironment in fish farms. For the larvae of European seabass (*Dicentrarchus labrax*), Senegal sole (*Solea senegalensis*), and Atlantic cod (*Gadus morhua*), the use of red light should be avoided given that these benthic species clearly perform better under shorter blue-green wavelengths (Villamizar et al., 2011).

However, the demersal Atlantic cod juveniles reared in metal halide light (λ_{max} 593 nm), green cathode light (λ_{max} 546 nm) or white light (λ_{max} 614 nm) showed no significant difference in stress levels (Cowan et al., 2011). Similarly, summer flounder (*Paralichthys dentatus*) larvae reared in tanks of different colors (black, green, red, dark or light blue) exhibited the lowest cortisol in red tanks (Packer et al., 1999). The retina of these two species lacks red-sensitive elements. The retina of Atlantic cod is dichromatic blue- and green-sensitive (Anthony and Hawkins, 1983; Valen et al., 2014). Summer flounder has a spectral sensitivity with two peaks at blue (λ_{max} 449 nm) and green (524 nm) (Horodysky et al., 2010). It is likely that fish without red-sensitive retina are not negatively affected by red-rich light regimes.

We hypothesized that fish with red-sensitive retina get stressed and grow slowly in growout floating cages. In the present study, red sea bream (*Pagrus major*) was used to test this hypothesis. This species has been shown to have red-sensitive tetrachromatic retina. The electroretinogram of red sea bream has a spectral sensitivity maximum at 470 nm and submaxima at 550 nm and 600 nm (Kobayashi, 1962). Kawamura (1981) recorded a 701 nm sensitive C-type S-potential, and Miyagi and Kawamura (2000) recorded a 601 nm sensitive L-type S-potential. Red sea bream showed a retinomotor response to 609 nm and 368 nm monochromatic light (Kawamura et al., 1997).

The red sea bream is an important aquaculture species in Japan, commonly stocked as juveniles (>30 mm total length TL) in floating gravity cages that are 4–5 m deep from the sea surface. At this stocking size, the red sea bream has already achieved retinal changes adaptive to benthic habitats. In the wild, the pelagic larvae metamorphose and settle at 12–18 mm TL and the juveniles and adults are

demersal thereafter, usually inhabiting the seafloor 150 m deep or more (Mitsunaga, 2000). Rods and twin cones are formed in the retina during metamorphosis; the rod density increases and the visual axis shifts from temporal to ventro-temporal at 30 mm TL (Kawamura et al., 1984). A ventro-temporal visual axis is common among demersal fish (Boehlert, 1978; Shand, 1994).

In this paper, we describe the effect of light spectrum on juvenile red sea bream in white tanks in terms of the stress response (indicated by plasma cortisol and plasma glucose levels) and the nutritional status (by RNA/DNA ratio).

Materials and Methods

Juvenile red sea bream and husbandry

Hatchery bred and reared juveniles of the red sea bream (7.5–11.7 cm body length, 13.2–44.2 g body weight) were obtained from the Kagoshima Fish Farming Association (Tarumizu Station) and used in the experiment at the Kamoike Marine Production Laboratory of the Faculty of Fisheries, Kagoshima University, Japan. Four white high-density polyethylene circular tanks (60 cm diameter, 75 cm high) were covered with either white, red, green, or blue plastic sheets and arranged as independent compartments in an indoor concrete tank. Seawater was pumped directly from the Kagoshima bay, sand-filtered, aerated to maintain the dissolved oxygen near 100% saturation, and supplied to each tank at a flow-through rate of 1.4 L min^{-1}. Water temperature ranged 27.5–31.0ºC.

Fifteen specimens of the fish were stocked in each tank and held without food for 8 days (plus a day without food during transport). All the fish survived during these 9 days of food deprivation. Trial on 15 specimens was made for the reason that earlier studies suggested large individual variations in the RNA/DNA ratio during ontogenetic development (Clemmesen, 1987; Raae et al., 1988; Richard et al., 1991). Fish were starved to reduce variability in the RNA/DNA turnover as reported by Raae et al. (1988) and Richard et al., 1991.

The handling of the fish for trials was in compliance with the guidelines of the Animal Care and Use Committee of Kagoshima University and with the regulations for the care and use of laboratory animals in Japan.

Color treatments

Color treatments of the fish were decided on the basis of information established earlier. This was considered essential for success of the experiment in generating new knowledge. The baseline information used was: duplex retina of the red sea bream with rods and cones, and particularly a regular cone mosaic with twin cones, a central single cone, and additional single cones (Kawamura et al., 1984), color vision capability (Kawamura, 1981), sensitivity to ultraviolet light (Miyagi and Kawamura, 2000), spectral sensitivity of retina with peak wavelength λmax at 470 nm (sensitivity

100%) and sub-maxima at 550 nm (50%) and 600 nm (20%) (Kobayashi, 1962).

This study tested four color regimes (white, blue, green and red, all fully visible to red sea bream) in four tanks with 15 fish each. Normal white light was produced with a 17 W fluorescent lamp with full spectrum (Matsushita, model L20S·N·EDL). Color regimes were produced with 17 W fluorescent lamps (Toshiba, model Neoball): blue (Toshiba, model EFG14EBG with λ_{max} at 435 nm); green (Toshiba, model EFG14EGG with λ_{max} 540 nm); and red (Toshiba, model EFG14EREG with λ_{max} 605 nm). Lamps were covered with cellophane filters of the respective colors and suspended 55 cm above the water surface of the four tanks. The red and green filters cut the short wavelengths emitted by the red and green lamps, respectively. The blue filter cuts out the 550 nm emission from the blue lamp. The spectral irradiance of the four fluorescent lamps and the transmittance of the three color cellophane filters were recorded with a spectroradiometer (HSR-8100, Maki Manufacturing Co., Ltd., Hamamatsu, Japan) over the wavelength band from 400 nm to 750 nm (Figure 1).
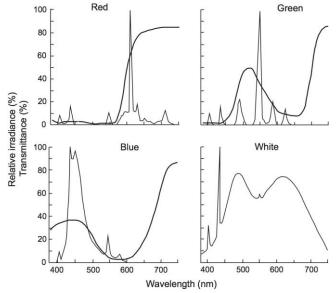

Figure 1. Irradiance spectra for four fluorescent lamps (bold lines) and transmittance spectra of three color cellophane filters

Taking the spectral sensitivity of the eye with a maximum in blue, sub-maxima in green and of less-red-sensitive eye of red sea bream (Kobayashi, 1962) into consideration, light intensity (photon flux density) transmitted through the cellophane filters was as follows: white, 0.167 µmol m^{-2} s^{-1}; blue, 0.201 µmol m^{-2} s^{-1}; green, 0.292 µmol m^{-2} s^{-1}; red, 0.498 µmol m^{-2} s^{-1}. Photoperiod in the tanks was set at 12L:12D, i.e., the fish were exposed to their respective color regimes for 12 h, then the lamps were turned off for a complete darkness for 12 h. Histological examination of the retinae showed that the fish in the four color regimes were all light-adapted in daytime.

Assays of plasma cortisol, plasma glucose, and muscle RNA/DNA.
Test specimens of the fish were deprived of food.

After 8 days of exposure to the four color regimes, the fish were all taken out from the tanks and euthanized in 0.2% phenoxyethanol. Blood was collected from the caudal vein of each fish into haematocrit capillary tubes, centrifuged at 12000 rpm for 10 min, and frozen at –80°C. Plasma samples from three fish were combined for five replicate assays of plasma cortisol (Cortisol ELISA Kit, Neogen Corporation, K.Y., U.S.A.) and glucose (glucose-oxidase kit, Iatro-chrome GLU-L_Q, Iatron Laboratories Co., Tokyo, Japan). Muscle samples (about 1 g) were collected from the trunk of each fish and frozen at –80°C until determination of the quantity of RNA and DNA according to the method of Nakano (1988).

Statistical analysis

One-way ANOVA was used for statistical analysis. Where significant differences were found, the mean of each treatment and among treatments were compared using Tukey's test of multiple comparison. Significance was accepted at $P < 0.05$.
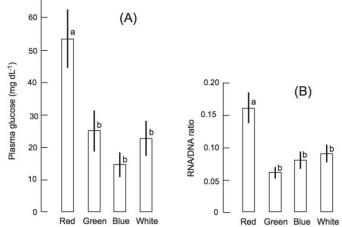
Results and Discussion

The cortisol level in the fish from the red light treatment was 3.0 ± 0.2 ng mL^{-1}. No cortisol was detected (below the sensitivity of the method used) in fish from the other treatments. It has been established that under conditions of stress, the hormones cortisol and catecholamines are released into the bloodstream (Randall and Perry, 1992), and consequently plasma glucose increases (Begg and Pankhurst, 2004). Cortisol and glucose are good initial stress indicators (Martínez-Porchas et al., 2009). Plasma glucose 54 ± 18.5 mg dL^{-1} was significantly higher in fish under red light, but not different among fish under green, blue, and white light exposures ($P > 0.05$) (Figure 2a).
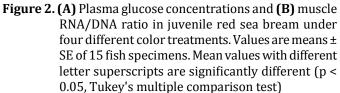
Mean RNA/DNA ratio was 0.16 ± 0.05 in fish under red light, significantly higher than 0.06 ± 0.02 under green; 0.08 ± 0.03 under blue; and 0.09 ± 0.03 under white ($P < 0.05$) (Figure 2b).

A characteristic behavioral response to stress in fish is known to be reduction in food intake (Wendelaar Bonga, 1997; Bernier, 2006). In this experiment the fish were not fed and the growth may have been compromised due to starvation. This is reflected in the RNA/DNA ratio which has been shown to be a sensitive indicator of instantaneous nutritional status (Clemmesen, 1987) and growth rate (Tong et al., 2010).

The visible light spectrum is an important factor for red sea bream and should be considered in efforts to optimize growth and production in floating cage farms. The present study showed that starved red sea bream was significantly stressed (highest plasma cortisol and glucose) under red light but not under green, blue, or white light. The 3.0 ± 0.2 ng mL^{-1} cortisol detected in red sea bream in this study was similar to the 3.0 ± 0.3 ng mL^{-1} cortisol in stressed pallid sturgeon,

Scaphirhynchus albus (Barton, 2002), but much lower than the 60 ng mL^{-1} cortisol in air-exposed red sea bream larvae (Ji et al., 2009). The 54 ± 18.5 mg dL^{-1} glucose in red sea bream in this study was similar to 55 mg dL^{-1} in pre-stressed red sea bream larvae but much lower than the 172.1 mg dL^{-1} in air-exposed stressed larvae (Ji et al., 2009). Thus, it is not clear from the data if cortisol and glucose levels indicate actual stress, but it is clear that the red light regime elicits higher levels of both. These results support our hypothesis.

Figure 2. (A) Plasma glucose concentrations and **(B)** muscle RNA/DNA ratio in juvenile red sea bream under four different color treatments. Values are means ± SE of 15 fish specimens. Mean values with different letter superscripts are significantly different (p < 0.05, Tukey's multiple comparison test)

Higher stress response under red light was also reported for four other species: gilthead sea bream (*Sparus aurata*), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and beluga (*Huso huso*) (Table 1). In contrast, the Atlantic cod, the summer flounder, and the red porgy (*Pagrus pagrus*) were not stressed or were even de-stressed under a red environment, and the European eel (*Anguilla Anguilla*) showed highest survival in a red tank (Table 1). These latter four species lack red-sensitive retina (Table 1).

We expected that somatic growth of red sea bream would be inhibited under a stressful red-light environment. Indeed, these red sea bream had RNA/DNA ratios < 0.16, much lower than the 3.7–4.2 reported for red sea bream larvae (Kato et al., 2012). The low RNA/DNA ratios in the present study can be attributed less to stress under red light and more to lack of food for 9 d. However, the effect of starvation seems less under red light.

High stress does not necessarily result in poor growth in fish. Higher stress with better growth performance under red light or tank was also observed in rainbow trout, summer flounder, and Asian seabass (*Lates calcarifer*), all three species with red sensitive retina (Table 1). Inhibited growth under red light was reported for seven species: gilthead sea bream, common carp, beluga, barfin flounder (*Verasper moseri*), thin lip mullet (*Liza ramada*), striped trumpeter (*Latris lineata*) and Nile tilapia (*Oreochromis*

Table 1. Effects of colors of light or tank on growth, survival, and stress response of fish. Visual spectral sensitivity of fish is also shown

Fish and stage	Species name	Color of tank or light (λmax, nm)	Stress response	Growth and survival	Author for color test	Spectral sensitivity (λmax, nm)	Author for spectral sensitivity
Fishes negatively affected by red color of light or tank							
Red sea bream	*Pagrus major*	Light: red (605), green (540), blue (435)	Highest plasma cortisol and glucose in red	Highest RNA/DNA ratio in red	Present study	UV-, blue-, green-, red-sensitive	Kobayashi (1962); Kawamura (1981); Kawamura et al. (1997)
Gilthead seabream (30 g)	*Sparus aurata*	Light: blue (480), red (605), white	Increased dopaminergic activity in red	Reduced growth in red	Karakatsouli et al. (2007)	Unknown	
Common carp juveniles	*Cyprinus carpio*	Tank: white, black, red, blue, yellow	Highest cortisol in red	Lower final body weight in red and black	Ebrahimi (2011)	blue-, green-, red-sensitive	Kaneko and Tachibana (1985)
Rainbow trout (145 g)	*Oncorhynchus mykiss*	Light: blue, red, white	High glucose level in blue and red	Best growth in red	Karakatsouli et al. (2008)	UV-, blue-, green-, red-sensitive	Anderson et al. (2010)
Beluga juveniles	*Huso huso*	Light: blue, green, red, white	Elevated cortisol and glucose in red	Negative impact on growth in red	Banan et al. (2011)	Blue-, green-, red-sensitive	Govardovskii et al. (1992)
Barfin flounder juveniles	*Verasper moseri*	Tank: blue, green, red, white		Growth best in blue, lowest in red	Yamanome et al. (2009)	UV-, Blue-, green-, red-sensitive opsin genes	Kasagi et al. (2015)
Thinlip mullet larvae	*Liza ramada*	Tank: white, black, red, green, yellow, blue		Retarded growth in red, black, green and blue	El-Sayed and El-Ghobashy (2011)	Blue-, green- red-sensitive	Tamura and Niwa (1967)
Striped trumpeter larvae	*Latris lineata*	Tank: black, blue, green, red, white, mottled		Worst jaw malformation, and lowest growth in red	Cobcroft and Battaglene (2009)	Unknown	
Nile tilapia juveniles	*Oreochromis niloticus*	Light: blue, green, yellow, red		Growth better in blue, lowest in red	Elnwishy et al. (2012)	Violet-, blue-, green-, red-sensitive	Lisney et al. (2010)
Fishes not negatively affected by red colour of light or tank							
Atlantic cod juveniles	Gadus morhua	Light: green (546), metal halide (593), white (614)	No clear effect		Boehlert (1978)	Blue-, green-sensitive; lack red-sensitive element	Anthony and Hawkins (1983); Valen et al. (2014)
Summer flounder larvae	Paralichthys dentatus	Tank: Black, green, red, dark and light blue	Lowest cortisol in red	Highest weight gain in red	McLean et al. (2008)	449, 524 nm; lack red-sensitive element	Horodysky et al. (2010)
Red porgy (372 g)	Pagrus pagrus	Tank: white, red	No difference in cortisol level after handling stress		van der Salm et al. (2006)	Blue-, green-sensitive; lack red-sensitive element	Munz (1971)
Asian seabass juveniles	Lates calcarifer	Tank: blue, green, yellow, red		Highest growth in red	Ullman et al. (2011)	472, 580, 595; low-red-sensitivity	Ullman et al. (2011)
European eel larvae	Anguilla anguilla	Light: blue, green, red, white		Best survival in red	Polis et al. (2014)	434, 525 nm; lack red-sensitive element	Damjanović et al. (2005)

niloticus) (Table 1). All these species have red-sensitive retina except the gilthead sea bream and striped trumpeter which have unknown spectral sensitivity (Table 1).

The juvenile red sea bream used in this study, just like the specimens stocked in commercial marine cages, came from hatcheries and shallow-water rearing facilities and have probably adapted to the red-rich light different from that in their natural habitat. Such acclimatization allows the hatchery-bred red sea bream to grow reasonably well in surface cages despite the stress due to the red sensitivity of the retina. Nevertheless, the results of this study argue against the use of red light in *Pagrus major* rearing facilities such as hatcheries and roofed tanks. Fish can adapt to stress for a period of time; they look and act normal. However, energy reserves are eventually depleted, hormone imbalance occurs, the immune system is suppressed, and susceptibility to infectious diseases increases (Fevolden et al., 1992; Rottmann et al., 1992). Recently, it has been established that stress can be reduced or suppressed by adding L-tryptophan to the fish diet (Winberg et al., 2001; Hosseini and Hoseini, 2013; Wolkers et al., 2014).

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