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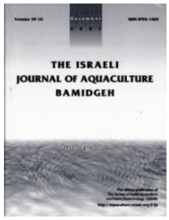

The Effects of Light Intensity and Color on Aggressive Interactions in the Dusky Kob, *Argyrosomus japonicus*

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Abstract

The dusky kob, *Argyrosomus japonicus*, is highly susceptible to stress under culture conditions. Such stress frequently involves a high degree of aggression, leading to cannibalism. Here, the effects of light intensity and color on the frequency of aggressive interactions and the time that passes between presentation of food and food first being eaten (feeding latency) in this species were examined. Aggression was lowest when fish were maintained in partial shade and highest in red light. Feeding latency was not affected by light treatment. The results can aid the aquaculture industry in determining the optimal light intensity for minimizing aggressive behavior in *A. japonicus*.

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Introduction

Cannibalism is the act of killing and consuming part or all of an individual of the same species (Smith and Reay, 1991). It is common in the animal kingdom (Dong and Polis, 1992) and occurs widely among fish (Baras and Jobling, 2002), being present in 36 of 410 fish families described by Nelson (1994). Many more occurrences become evident as life histories of species become known (Smith and Reay, 1991; Hecht and Pienaar, 1993).

Cannibalism is a major problem under aquaculture conditions (Ruzzante, 1994; Baras and Jobling, 2002; Kestemont et al., 2003; Fessehaye et al., 2006; Portz et al., 2006; Ashley, 2007). Larvae and juvenile cannibalism occur in many important culture species including dusky kob, *Argyrosomus japonicus* (O'Sullivan and Ryan, 2001), Nile tilapia (Fessehaye et al., 2006); red drum (Liao and Chang, 2002), Arctic charr, *Salvelinus alpinus* (Svenning and Borgstrøm, 2005), and sharptooth catfish (Baras and d'Almeida, 2001).

Cannibalism is the product of aggressive behavior, so investigation of factors that may reduce aggressive behavior is desirable. Two of these factors, and the subjects of the current study, are fluctuations in intensity and color of light within the culture environment (Hecht and Pienaar, 1993; Valdimarsson and Metcalfe, 2001).

Light intensity and color strongly influence the daily activity and behavior of fish (Boeuf and Le Bail, 1999). Fish are capable of color vision (Boeuf and Le Bail, 1999; Cheng and Flamarique, 2004). Different spectral compositions can affect a number of characteristics in fish including behavior. The response to color differs between fish species due to natural habitat characteristics and the morphological visual system of the fish (Karakatsouli et al., 2007). Rainbow trout, *Oncorhynchus mykiss*, show faster growth and improved physiological condition under red light, with reduced growth and increased brain serotonergic activity resulting in stress under blue light (Karakatsouli et al., 2007). Higher light intensity increases aggression in juvenile Atlantic salmon, *Salmo salar*, while lower light intensity results in reduced aggression levels (Valdimarsson and Metcalfe, 2001). Fish behavior under culture conditions may therefore be altered by manipulation of light intensity and color, which is relatively simple and inexpensive to implement.

The dusky kob, *Argyrosomus japonicus* (Order: Perciformes, Family: Sciaenidae), is widely distributed, highly fecund, and can tolerate a wide range of temperatures and salinities (Griffiths, 1996; O'Sullivan and Ryan, 2001). Its rapid growth rate and good food conversion ratio make it a suitable candidate for aquaculture (O'Sullivan and Ryan, 2001). However, *A. japonicus* is highly susceptible to stress under culture conditions and the behavior associated with this stress frequently involves a high degree of aggressiveness leading to cannibalism. Juvenile cannibalism in *A. japonicus* occurs 18 days after hatching and decreases once the fish reach a total length of 80 mm (O'Sullivan and Ryan, 2001). Juvenile dusky kob are therefore ideal subjects in which to examine the effects of altering light conditions on aggressive behavior.

The hypotheses tested here are that a change in light color will result in a change in aggressive behavior, and that increased light intensity results in increased aggressive behavior. Determination of the lighting conditions that reduce aggressive behavior will provide better understanding of the conditions that could be implemented by industry to optimize fish production.

Materials and Methods

Juvenile dusky kob (three months old) were obtained from Espadon Marine, Hermanus, South Africa, in March 2007. Fish were weighed, measured, and sorted into size classes. Fish from the 35 g group were selected and maintained approximately one month prior to experimentation in 1 x 0.5 x 0.5 m aquaria with a water temperature of 18-20°C and salinity of 35 ppt. They were fed trout pre-starter pellets containing 52% protein, 25% fat, 10% ash, 0.6% fiber, 0.9% total P, 9,000 IU/kg vitamin A, 400 mg/kg vitamin B, and 5 mg/kg copper sulfate.

Eight glass aquaria, 45 x 30 x 32 cm, were set up in a re-circulating system. Water temperature was maintained at 19°C by individual aquarium heaters in each tank. Salinity was maintained at 35 ppt by adding fresh or salt water to the system as needed. A 12 h light:12 h dark photoperiod was maintained for one week. Illumination was provided by four 50-cm fluorescent light bulbs (Lascon Ultra 20w Cool White) situated 30 cm above the water surface, each illuminating the two tanks positioned beneath it. The system was isolated to eliminate ambient light. Opaque dividers were positioned between each light and three sides of the tanks were covered with black plastic to prevent aquaria being influenced by adjacent lights. The fronts of the tanks were partially covered by rigid plastic with holes cut into it through which observations were conducted.

Five light treatments were examined. Three were varying intensities of white light: 0.04 Lx (full shade), 0.54 Lx (partial shade), and 684 Lx (white light). The full shade treatment was the darkest that could be achieved while still being able to observe the fish. Tanks were shaded by pinning two (for partial shade) or four (for full shade) layers of 50% shade cloth over the bulb. The shade cloth was made of a loosely woven polyester fabric, robust enough not to melt under the small amount of heat produced by the bulb. A fluorescent light bulb with no cover produced the white light treatment. In addition, red and green lights were produced by fitting a thin stainless steel wire covered with red or green cellophane around the light bulb. The wire provided sufficient clearance between the bulb and the cellophane to prevent the heat from the bulb melting the cellophane. Light intensity was measured at the water surface using an LX digital light meter (RSR Electronics, Avenel, NJ).

Six juvenile dusky kob were placed into each of the eight tanks. Fish were visually assessed to ensure that they were as similar in size as possible. This stocking density was chosen so that there would be few enough fish in the tank to randomly select a focal fish to observe during the entire observation, but enough fish in the tank to ensure that aggressive behavior occurred. The fish were left to acclimate for 48 h in total darkness so that they would not become conditioned to a light treatment used during the study. Fish were fed

2-mm trout pellets three times a day to apparent satiation and tanks were siphoned once a day to remove uneaten food and waste.

Fish were acclimated to the light treatments for 24 h prior to observation. This removed any biased behavior resulting from initial stress caused by a sudden change in light condition. Daily observations commenced 2 min after the observer was situated, so as to acclimate the fish to the presence of the observer. The observation began by randomly selecting one focal individual per tank, observing it for 10 min, and recording aggressive interactions initiated by the focal fish. Aggressive behavior consisted of biting, lunging at, or chasing another fish. The tanks were observed in a random order. After each observation, food was dropped into the center of the tank and the latency between when the food was presented and when the food was first eaten was recorded.

After the daily observations, the lighting treatments were changed for the next day's observations by moving the shade cloths and cellophane covers. This allowed a 24-h acclimation to the new light condition. The order of light treatments was randomized. The experiment required five days for each tank to experience all five light treatments once.

Preliminary analysis of this first data set showed that red light increases aggressive behavior and that green light has little effect. A second set of observations was therefore carried out using three light conditions: white light, partial shade, and full shade. A new set of six fish were added to each of six tanks and allowed to acclimate as above. Observations were conducted as for the first set, with the experiment lasting three days. Since the preliminary analysis showed no effect of light treatment on feeding latency, this parameter was not recorded in the second set.

Two sets of analyses were conducted to assess differences in aggressive behavior between light treatments. First, all five treatments were subjected to Friedman tests with aggressive behavior initiated by the focal fish (attacks) and feeding latency as repeated measures and light treatment as the independent variable. The Friedman test makes no assumptions about the distribution of data, only that it is measured on an ordinal scale (Dytham, 1999). Second, data for attacks in white light, partial shade, and full shade from the first set of observations were combined with those from the second set to produce a larger data set for analysis. A Friedman test was again conducted with attacks as the repeated measure and light treatment as the independent variable. These tests show significant differences but not where those differences occur. Thus, posthoc Wilcoxon matched pairs tests were carried out between the colored and white light treatments (analysis 1) and the three white light treatments (analysis 2).

Results

As predicted, differences in intensity and color of light affect aggressive behavior in *A. japonicus* under culture conditions. In the first analysis, there were significant differences in the frequency of attacks between the five light treatments ($n = 8$; $df = 4$; $\chi 2 = 18.286$; $p = 0.001$) because of the significant difference between the red light and the other treatments (Fig. 1). In contrast, the green light did not significantly differ from the white light treatments (Table 1).

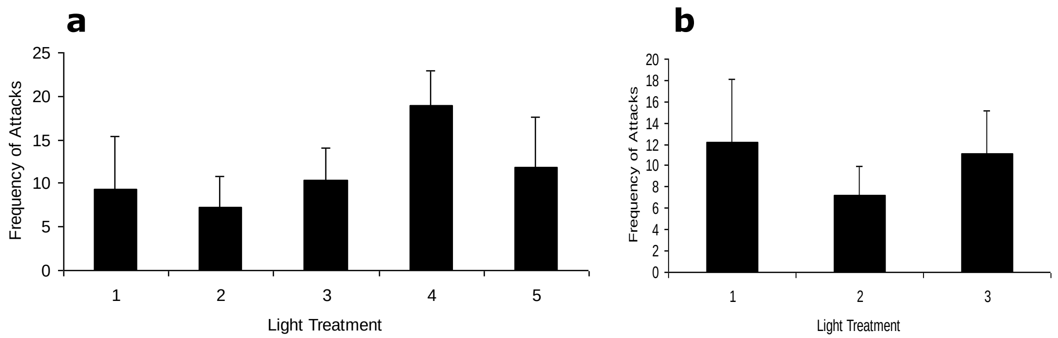

Fig. 1. Frequency of aggressive behavior in dusky kob (*Argyrosomus japonicus)* kept in (a) five different light conditions and (b) three different light intensities. 1 = full shade, 2 = partial shade, 3 = white light, 4 = red light, 5 = green light. Error bars represent standard deviations.

Table 1. Comparisons between color a and white light treatments.

Comparison	*Z*	*p*
Red vs shade	2.380	**0.017**
Red vs partial	2.521	**0.012**
Red vs white	2.366	**0.018**
Red vs green	2.521	**0.012**
Green vs shade	1.268	0.205
Green vs partial	1.690	0.091
Green vs white	0.338	0.735
Shade vs partial	2.062	**0.039**
Shade vs white	0.251	0.802
Partial vs white	3.059	**0.002**

Significant differences are shown in bold.

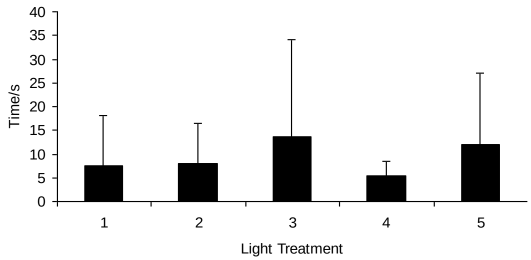

Fig. 2. Feeding latency in dusky kob (*Argyrosomus japonicus)* kept in five different light conditions. 1 = full shade, 2 = partial shade, 3 = white light, 4 = red light, 5 = green light. Error bars represent standard deviations.

In the second analysis, there was again a significant difference in attacks between treatments (n = 14; df = 2; $\chi 2$ = 8.642; *p* = 0.013) because of a significant difference between partial shade and the other two white light intensities. Red light thus produced the highest frequency of aggression in the first set of results while partial shade produced the lowest frequency of aggression in both analyses. There was no difference in feeding latency between any of the treatments (n = 8; df = 4; $\chi 2$ = 2.500; *p* = 0.645; Fig. 2).

Discussion

Several studies have addressed the effects of light intensity on fish behavior. In the Atlantic salmon, *S. salar*, the frequency of aggressive interactions increased when light intensity increased (Valdimarsson and Metcalfe, 2001). In the African catfish, *Clarias gariepinus*, increasing the light intensity from 15 to 150 lx resulted in 3.4 times more aggressive acts, 2.5 more wounds, and 67% more bitten fish (Almazán-Rueda et al., 2004). However, in a species related to *A. japonicus*, the red drum, *Sciaenops ocellatus*, increasing turbidity (which has the same general effect as reducing light intensity) had no influence on the frequency of cannibalism (Liao and Chang, 2002). Likewise, in European seabass, *Dicentrarchus labrax*, and Eurasian perch, *Perca fluviatilis*, light intensity had no effect on cannibalism in larvae (Kestemont et al., 2003).

The impact of light intensity on aggressive behavior clearly depends on the species. Wild *A. japonicus* juveniles live in shoals in estuaries characterized by high levels of turbidity and low light intensity (Griffiths, 1996). This low level of light intensity can be emulated in the culture system. Partial shade resulted in the lowest levels of aggression, suggesting that it best replicates the light conditions in the natural environment of *A. japonicus* juveniles, where cannibalism is less common than in culture systems (Griffiths, 1996). Fish must be able to see other individuals in order to attack them (Valdimarsson and Metcalfe, 2001). Vision may be sufficiently impaired at lower light levels to reduce the frequency of aggression. Conversely, when fish are in full shade, vision may be impaired to the extent that they are unable to avoid other individuals, leading to the slight increase in aggression seen in the present study.

The frequency of aggression was highest by far in the red light treatment while green light produced no difference from the three white light intensities. Little information is available about the effects of light spectra on fish behavior. Juvenile rainbow trout, *O. mykiss*, avoid areas of red ambient color (Luchiari and Pirhonen, 2008) while gilthead seabream, *Sparus aurata*, show increased brain dopaminergic activity indicative of stress (Karakatsouli et al., 2007). Thus, increases aggression under red light is perhaps to be expected. Although not documented in *A. japonicus*, many fish species from Atlantic salmon (Flemming, 1996) to 3-spined sticklebacks (*Gasterosteus aculeatus*; Bakker and Mundwiler, 1994) use red coloration as a mating signal and associated aggressive behaviors suggest a predisposition to be affected by red light.

An interaction between light intensity and light wavelength is possible. In a study examining both these variables in larval haddock, *Melanogrammus aeglefinus*, feeding success was reduced at lower and higher intensities of white and blue lights, and was greatest at an intermediate intensity of blue light (Downing and Litvak, 2001). In the Atlantic salmon, high intensity blue light produced a stress response, which may be both a cause and effect of aggression (Portz et al., 2006), while lower intensity blue light and all intensities of white light did not (Migaud et al., 2007). In the present study, the intensity of both red and green lights was expected to be between that of full white light and partial shade yet these treatments produced levels of aggression above those of all the white light conditions, suggesting that light intensity and light wavelength operate in different ways in *A. japonicus*. Further studies investigating the effects of different combinations of light wavelength and intensity on aggression would clarify this point.

There was no effect of light regime on feeding latency, suggesting that *A. japonicus* uses other stimuli in addition to visual cues to detect food. Light intensities in the range 23-315 lx have no effect on growth rate or food conversion ratio in juvenile *A. japonicus* (Collett et al., 2008). The results of our study suggest this range may be much wider. Several studies document the effects of light regime on feeding and growth rate in other species. In a study of the effect of four light regimes (blue, green, yellow, red) on rainbow trout, the growth rate and food intake were most reduced under red light (Luchiari and Pirhonen, 2008). However, in another study, red light was not distinguished from white light in terms of growth rate, and blue light produced the worst results (Karakatsouli et al., 2007). These conflicting results suggest that other factors act in combination with the light regime to affect the growth rate. For example, *Wallago attu* (catfish) larvae had a faster growth rate when reared in 24-h red light than in various ratios of white light and darkness, but only when fed a combination of live food and dry feed (Giri et al., 2002). In addition, light regime effects are evidently species specific. Crucian carp (*Carassius carassius*) grew best under green light, rotans (*Perccottus glenii*) fared best under blue and green lights, and guppies (*Poecilia reticulata*) grew fastest under blue light; however, the growth rate was reduced in all three species under red light (Ruchin, 2004).

The commercial culture of *A. japonicus* is a new industry, particularly in South Africa, and technology is still being optimized. Aggressive behavior leading to cannibalism is a limiting factor in the commercial production of *A. japonicus* (O'Sullivan and Ryan, 2001). The implications of this study are therefore highly valuable and relevant as light intensity and color can easily be manipulated at a relatively low cost, resulting in decreased aggressive behavior leading to cannibalism and increased profits. Based on this study, a recommendation for commercial farmers would be to implement partial shade for rearing juvenile *A. japonicus* to ensure minimal aggressive behavior leading to cannibalism and potential mortality. Further studies quantifying the degree of cannibalism associated with aggressive behavior and determining the optimum light intensity for minimizing aggression and cannibalism whilst maximizing growth in *A. japonicus* under culture conditions would be beneficial.

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