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## TANK COLOR IMPACTS PERFORMANCE OF CULTURED FISH

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### *Summary*

Tank color impacts marine fish larval performance, as dark tanks appear to provide contrast that allows larvae to better visualize live and artificial prey. While tanks can be fabricated in any color, commercially available on-growing systems are generally black, green, or dark and light blue. Anecdotal information suggested that certain juvenile fish perform better in tanks with black sides and sandy colored bottoms. To determine whether tank color impacted performance of juvenile fish we examined the effect of black, green, red, dark, and light blue colored tanks on the short-term growth and feed efficiency of summer flounder and growth, feed efficiency, body composition of Nile tilapia. Cortisol response was also examined for both species. Tank color did not affect growth performance of flounder or tilapia although fish maintained in red-colored tanks returned better percent increases in weight. Differences ( $P < 0.05$ ) in feed conversion efficiency were observed for summer flounder held in red tanks. Plasma cortisol levels in summer flounder ranged from 1.39–3.71 ng cortisol per ml, compared to 12.7–94.4 ng cortisol per ml plasma for tilapia. Lowest cortisol levels ( $P < 0.05$ ) were detected in flounder and tilapia reared in red-colored aquaria. Background color had no effects on tilapia fillet composition.

Key words: tilapia, flounder, cortisol, growth

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## INTRODUCTION

Commercial recirculating aquaculture systems (RAS) employ tanks of various forms ranging from octagonal designs, through to the more traditional circular and raceway constructions. Sometimes, these tank forms have been derived following extensive hydrodynamic modeling and engineering developments (Rasmussen and McLean, 2004; Rasmussen et al., 2005), each of which attempt to optimize various performance characteristics, such as animal growth, ease of harvest, cleansing, grading operations, etc. In contrast to system design however, comparatively little research, at considerable less cost, has been undertaken to examine the potential advantages that varying tank color may have on performance characteristics of specific species of cultivated fish. While it is possible to have tanks fabricated in any color, commercially obtainable tanks for the on-growing of fish are generally available in black, green, dark, and light blues.

The origin of these color selections remain obscure even though research during the earlier part of the last century indicated that certain colors, including black, may be problematic in terms of fish performance and health (Sumner, 1911; Sumner and Doudoroff, 1938). Larval fishes are generally considered to be visual feeders and it is therefore important to optimize their rearing conditions to enable them to distinguish, capture and consume prey items. Several studies, with a number of species, indicate that factors such as prey density; light orientation, intensity and wavelength; tank hydrodynamics and color, as well as the color and orientation of prey items, effect the ability of larvae to detect, capture and ingest food items (Ostrowski, 1989; Duray et al., 1996; Martinez-Cardenas and Purser, 2007; Salze et al., 2008). For many species, larval growth and survival is enhanced when black tanks are employed during rearing. It has been suggested that increased contrast between live feeds and background color is responsible for this positive effect. In contrast to larval fishes, comparatively few studies have examined the impact of tank color on the performance of juvenile and adult fishes.

Stress has a wide range of negative impacts on production characteristics of fish including alterations in hierarchical interactions and other behavioral changes, modifications to feeding, and changes in locomotor activity (Øverli et al., 2005). Might tank color, through a neuroendocrine-driven stress effect, have wider-ranging impacts on fish production performance than previously thought? The present study addresses this issue for two species of cultivated fish viz. Nile tilapia (*Oreochromis niloticus*) and summer flounder (*Paralichthys dentatus*) maintained in tanks of five different colors. Response of the experimental animals was examined in terms of growth and feed conversion and, for tilapia, cortisol-based stress response.

## MATERIALS AND METHODS

A total of ten tank suppliers and manufacturers were surveyed via telephone interview to determine the four most accepted tank colors. Fabricators and suppliers were located in California, Florida, Louisiana, North Carolina, Texas and Maine. The commonest tank colors sold were dark blue, light blue, black and green. In addition to these colors, red was included in the experimental design to represent a negative control. Three 40 litre tanks were painted each color for a total of 15 tanks. Paints were applied to the outside glass sides and base of each tank. Colors included cool lagoon blue and winter green latex and dark blue, black and red oil-based enamels. The fifteen tanks were randomly distributed in a custom-made recirculation system (Fig. 1) comprising a 550 litre fluidized bed biofilter containing 0.1 m<sup>3</sup> KMT media (Kaldnes Miljøteknologi, Tønsberg, Norway), a 40 watt UV sterilizer (Aquatic Ecosystems, Apopka, FL, USA) for disinfection, a Sherlock 80 element filter for particulate removal and a Magnatek Century 1081/1995 pump. Each tank and biofilter was supplied by diffusion airlines connected to a 1 HP Sweetwater remote drive regenerative blower (Aquatic Ecosystems, Apopka, FL, USA).

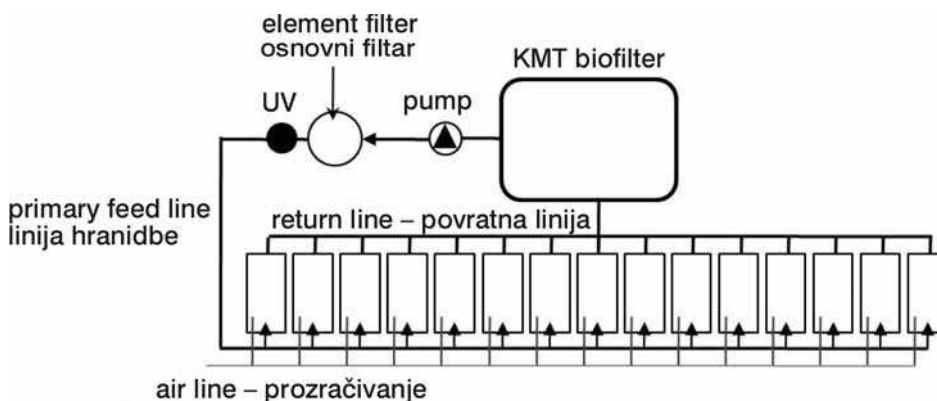


Figure 1. Diagram of custom-built recirculating aquaculture system employed during the study. The fifteen tanks were of 40 litres volume and painted different colors ( $n = 3$  tanks per color). Use of a recirculation configuration eliminated differences in water quality between tanks

Slika 1. Dijagram uobičajeno građenoga recirkulirajućeg sustava primijenjenog u eksperimentu. Petnaest bazena bili su veličine od 40 litara i obojeni različitom bojom ( $n = 3$  bazena iste boje). Uporaba načina recirkulacije eliminirala je razlike u kakvoći vode između bazena

Water temperature (27.00  $\pm$  3 C) and pH (8.09  $\pm$  0.06) were monitored using a Hanna Instrument 9024 pH meter (Aquatic Ecosystems, Apopka, FL, USA). Dissolved oxygen (5.92  $\pm$  0.09 mg L<sup>-1</sup>) and total ammonia nitrogen (0.37  $\pm$  0.04

mg L<sup>-1</sup>) were measured using an YSI 85 Series dissolved oxygen meter (YSI Inc., Yellow Springs, OH) and by spectrophotometric analysis (Hach Inc., Loveland, CO, USA), respectively. Nitrite (0.31–0.06 mg L<sup>-1</sup>) and nitrate (71–11 mg L<sup>-1</sup>) were quantified by spectrophotometric analysis. For flounder studies, salinity was maintained at 17.0–0.5 g L<sup>-1</sup> using Crystal Sea synthetic sea salt (Marineland, Baltimore, MD, USA). A 12h photophase–scotophase, with a 30 min dusk/dawn period was maintained using phosphorescent tubes positioned 3 m above the system.

#### *Experiment 1.*

Sixty F<sub>2</sub> generation summer flounder derived from the Virginia Seafood Agricultural Research and Extension Center, Hampton, VA, were randomly distributed into the 15 experimental tanks (n = 4 per tank). Total tank biomass was 155.9–2.11 g wet weight and no differences between group tank weights were discerned at trial start. Fish were fed a diet comprising 50% crude protein (CP) and 10% lipid at a rate of 3% body weight per day. Fish were weighed bimonthly to adjust feed rations and to monitor growth and feed efficiency. A record of uneaten food was made on a daily basis following feeding. The trial was run for a total of six weeks.

#### *Experiment 2.*

Two hundred and twenty-five Nile tilapia were obtained from Blue Ridge Aquaculture, Martinsville, VA, were randomly stocked into the 15 experimental tanks (n = 15 per tank). Total tank biomass was 100 g wet weight and no differences were discerned between group tank weights at the initiation of the trial. Fish were fed a commercial diet (Burriss Mill and Feed Inc., Franklinton, LA) comprising 45% CP and 12% lipid at a rate of 5% body weight per day for the first 5 weeks of the trial and then at 4% body weight per day until trial end at week 9. Fish were group weighed weekly to adjust feeding rate and to monitor growth and feed efficiency. A record of uneaten food was made on a daily basis following feeding.

#### *Analytical procedures*

Blood was collected from the caudal peduncle of both flounder and tilapia and plasma stored at –20 °C until later analysis for cortisol. Cortisol was quantified using an enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA). Plasma samples for each fish were run in duplicate. Tilapia fillets were collected for proximate analysis (n = 5 per tank color), including crude protein, total lipid, dry matter, and ash (AOAC, 1994). All proximate compositional measurements were performed in duplicate. Datasets were subjected to analysis of variance utilizing SAS v 9.1 (SAS, Cary, NC, USA).

## RESULTS

Percent gain over initial weight varied between 202–248% for summer flounder with fish held in red tanks expressing greatest overall weight increase (Table 1). Animals maintained in dark blue tanks performed poorest. However, no significant differences were determined for percent weight increase between tank colors. Percent gain over initial weights varied between 813–1018% for tilapia (Table 2) with greatest gains being observed for fish held in red tanks. However, as with the summer flounder, no significant differences were observed between groups due to variations in weight gain between tanks of the same color. Nevertheless, significantly lower ( $P < 0.05$ ) feed conversion efficiencies (FCE) were recorded for summer flounder held in red tanks when compared against dark and light blue and green-colored aquaria (Table 1). Flounder maintained in red and black tanks returned equal response with respect to FCE. The range of FCE for tilapia was 1.03–1.11, with lowest FCEs being recorded in fish kept in green tanks. Highest FCEs were observed in tilapia housed in dark blue-colored aquaria (Table 1).

Differences were observed in circulating levels of cortisol for both summer flounder and Nile tilapia. Measured levels of cortisol in summer flounder ranged from 1.39–3.71 ng cortisol per ml plasma (Table 1), compared to 12.7–94.4 ng cortisol per ml plasma for tilapia (Table 2). Highest levels of cortisol were detected in flounder maintained in light blue-colored tanks > black > green = dark blue > red ( $P < 0.05$ ). Plasma cortisol levels were also significantly lower in tilapia maintained in red-colored tanks when compared against fish held in black, dark blue and green tanks. Cortisol response was similar for tilapia housed in red and green tanks (Table 2). Survival levels for flounder did not differ between tank colors with a range of 2–11% mortality. Differences ( $P < 0.05$ ) were detected in tilapia survival with different tank colors. A 0–14% mortality rate was observed among tanks with no mortalities being observed in green-colored tanks over the trial period (Table 2).

Table 3 summarizes tilapia fillet composition following completion of the trial. No differences were recorded between groups for fillet dry matter or moisture, which ranged between 76.6–78.6%. Fillet lipid level was highest in fish maintained in red-colored tanks and lowest in tilapia held in black tanks but again with no differences discernible between groups. Likewise, fillet protein levels did not differ across treatment groups ( $P > 0.05$ ).

*Table 1. Weight gain (percent increase over initial), feed efficiency, survival and plasma cortisol levels in juvenile summer flounder maintained in different colored tanks over a nine week period. Data with a different superscript column-wise indicates significant difference*

*Tablica 1. Masa, konverzija hrane, preživljenje i količina plazma kortizola u mladim listova držanih u različito obojenim bazenima tijekom razdoblja od devet tjedana. Podaci s različitim eksponentom označuju značajnu razliku*

| Tank color –<br>boja bazena    | Weight gain<br>— masa (%<br>initial —<br>početna) | FCE —<br>konverzija<br>hrane | Survival —<br>preživljenje<br>(%) | Cortisol —<br>kortizol<br>(ng ml <sup>-1</sup> ) |
|--------------------------------|---|------------------------------|-----------------------------------|--|
| Black — crna                   | 232.9 51.2  | 1.51 0.47 <sup>a,b</sup>     | 93.3 11.54                        | 2.71 0.17 <sup>b</sup>                           |
| Dark blue —<br>tamno plava     | 202.0 37.4  | 1.81 0.54 <sup>a</sup>       | 88.9 10.2                         | 1.64 0.09 <sup>c</sup>                           |
| Light blue —<br>svijetlo plava | 211.6 60.9  | 1.86 1.26 <sup>a</sup>       | 91.1 7.7                          | 3.71 0.24 <sup>a</sup>                           |
| Green — zelena                 | 205.9 53.1  | 1.78 0.50 <sup>a</sup>       | 88.9 7.7                          | 1.75 0.11 <sup>c</sup>                           |
| Red — crvena                   | 248.2 29.0  | 1.03 0.18 <sup>b</sup>       | 97.8 3.85                         | 1.39 0.08 <sup>d</sup>                           |

*Table 2. Weight gain (percent increase over initial), feed efficiency, survival and plasma cortisol levels in juvenile Nile tilapia maintained in different colored tanks over a ten week period. Data with a different superscript column-wise indicates significant difference*

*Tablica 2. Masa, konverzija hrane, preživljenje i količina plazma kortizola u tilapija držanih u različito obojenim bazenima tijekom desetotjednog razdoblja. Podaci s različitim eksponentom označuju značajnu razliku*

| Tank color –<br>boja bazena    | Weight gain<br>— masa (%<br>initial —<br>početna) | FCE —<br>konverzija<br>hrane | Survival —<br>preživljenje<br>(%) | Cortisol —<br>kortizol<br>(ng ml <sup>-1</sup> ) |
|--------------------------------|---|------------------------------|-----------------------------------|--|
| Black — crna                   | 888.3 198.5                                       | 1.04 0.44                    | 86.6 6.5 <sup>b</sup>             | 68.5 9.8 <sup>a</sup>                            |
| Dark blue —<br>tamno plava     | 813.3 175.1                                       | 1.11 0.46                    | 88.9 10.2                         | 68.8 7.9 <sup>a</sup>                            |
| Light blue —<br>svijetlo plava | 900.0 280.1                                       | 1.06 0.35                    | 100.0 0.0 <sup>a</sup>            | 21.4 8.4 <sup>b</sup>                            |
| Green — zelena                 | 813.3 210.5                                       | 1.03 0.68                    | 93.2 6.7 <sup>b</sup>             | 94.4 14.3 <sup>a</sup>                           |
| Red — crvena                   | 1018.3 134.8                                      | 1.08 0.68                    | 91.0 10.2 <sup>a,b</sup>          | 12.7 7.8 <sup>d</sup>                            |

*Table 3. Compositional analyses of juvenile Nile tilapia fillets maintained in different colored tanks over a ten week period*

*Tablica 3. Kemijski sastav fileta mladih tilapija držanih u različito obojenim bazenima tijekom desetotjednog razdoblja*

| Tank color –<br>boja bazena    | Protein –<br>bjelančevine | Lipid – masti | Dry matter –<br>suha tvar | Moisture –<br>vlaga |
|--------------------------------|---------------------------|---------------|---------------------------|---------------------|
| Black – crna                   | 19.52 0.53                | 1.14 0.44     | 23.17 1.24                | 76.61 1.40          |
| Dark blue –<br>tamno plava     | 19.47 0.56                | 1.28 0.22     | 21.40 1.31                | 77.77 0.62          |
| Light blue –<br>svijetlo plava | 19.11 0.45                | 1.18 0.38     | 21.38 0.57                | 78.61 0.57          |
| Green – zelena                 | 19.15 0.41                | 1.38 0.26     | 22.05 0.48                | 78.03 0.51          |
| Red – crvena                   | 19.23 0.31                | 1.73 0.43     | 22.34 0.67                | 77.63 0.75          |

## DISCUSSION

Early studies illustrated that tank color, in the case of various shades of grey and white and black, might affect growth, survival and resistance to disease. (Sumner, 1911; Sumner and Doudoroff, 1938) Fishes maintained in the black tanks appeared more susceptible to disease. In the present study, which employed two species from different environments (marine and fresh waters), and five different colored tanks, no disparities were observed in the growth performance. However, experiments designed to examine the effects of tank color on growth and feeding performance of fish suggest that response to color may be species-specific. For example, Martinez-Cardenas and Purser (2007) reported that seahorses (*Hippocampus abdominalis*) held in clear, white, yellow, orange or green tanks expressed no differences in feeding strike rates, growth, or survival. In Atlantic salmon (*Salmo salar*) there were no differences in growth when fish were maintained in either green or grey colored tanks, irrespective of the spectral composition of light, although smoltification appeared to proceed in grey-colored tanks (Stefansson and Hansen, 1989). No significant differences were observed in the growth or feed conversion efficiencies of common carp reared in white, black or green tanks (Papoutsoglou et al., 2000) although fish adapted to black backgrounds expressed lower lipid levels indicating, likely stress-related, modifications to their metabolism. Support for the latter metabolic effect of background color on fish is lent by studies with Nile tilapia (*Oreochromis niloticus*) in which fish on white backgrounds had higher respiratory frequencies than when held on black, blue, green, yellow or red surroundings (Fanta, 1995). Similarly, changes in feed conversion efficiency as noted herein for summer

flounder and lipid presence in tilapia maintained in red tanks indicate subtle modifications to fish metabolism initiated by background color. This is further supported by studies with Eurasian perch (*Perca fluviatilis*) in white, grey or black tanks which were found to differ in terms of their appetite, with fish in white and grey tanks having greater food intake than perch held in black tanks (Stand et al., 2007). Likewise, walleye (*Stizostedion vitreum*) fingerlings had better specific growth rates and food conversion efficiencies in black when compared to blue tanks (Harder and Summerfelt, 1996). A mechanism that might provide an explanation to the latter observations is forthcoming through more recent research.

An intimate relationship exists between the neuroendocrine and immune systems of fishes, and corticosteroids, in particular, are very effective immunosuppressants (Barton and Iwama, 1991; Huntingford et al., 2006). It is possible therefore that the increased mortalities observed in fish in early studies held in black tanks, relative to mortalities recorded in gray and white tanks (Sumner, 1911; Sumner and Doudoroff, 1938), succumbed because they were under an increased level of stress. When adapted to a black background, rainbow trout (*Oncorhynchus mykiss*) expressed increased corticotrophin and cortisol levels in response to external stressors when compared against fish adapted to white backgrounds (Gilham and Baker, 1985; Green et al., 1991). Plasma cortisol concentrations were also elevated in rainbow trout and European eels (*Anguilla anguilla*) maintained in black tanks when in a noisy environment when compared to fish adapted to white tanks (Baker and Rance, 1981). Similar results were obtained for red porgy (*Pargus pargus*) crowded on a black background (Rotllant et al., 2003) whereas carp (*Cyprinus carpio*) adapted to white backgrounds expressed lower cortisol levels than animals adapted to green or black colored tanks (Papoutsoglou et al., 2000). These findings suggest that when maintained in tanks that are colored inappropriately certain species of fish experience an enhanced stress response.

Plasma cortisol levels observed during the present study for summer flounder were similar to those reported previously (Gavlik, 2004) and alike to that of stone (*Platichthys bicoloratus*) and winter (*Pseudopleuronectes americanus*) flounders (Yamashita et al., 2003; Breves and Specker, 2005). The reported circulating levels of cortisol in unstressed and socially stressed Nile tilapia range between 17–200 ng/ml (Barcellos et al., 1999; Moreira and Volpato, 2004; Corrêa et al., 2003; Delaney and Klesius, 2004; Barreto and Volpato, 2007; Welker et al., 2007). The higher levels of circulating cortisol likely reflect continuous social stress brought about by feeding hierarchies and other dominance-related behaviors. It is noteworthy that in the present trials mixed sex fish were used for both species examined. Different results may have arisen with the use of monosex groups (female for flounder and male for tilapia) which might lessen social interactions within the tanks leading to reduced stress response, better growth and feed conversion efficiencies.



The results of the current study, like those of many others, were inconclusive from a growth perspective. Nevertheless, reduced stress levels as indicated by cortisol response for both species maintained in red tanks indicate that a species-specific reaction to background color exists. This suggests that greater attention to tank color is warranted during production. It is likely that both studies undertaken were of too short duration to achieve categorical effects on growth and future research should examine production-length growth trials with the species of interest at different stages of the life cycle. Clearly similar studies with shrimp and other crustaceans would be worthwhile undertakings. Findings with respect to circulating cortisol levels suggest that this stress-related hormone may be used as a bioindicator to assist in selection of optimal tank colors for different species over a short timeframe.

### Sažetak

## UTJECAJ BOJE BAZENA NA RAST UZGAJANIH RIBA

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Boja uzgojnih bazena utječe na ličinački razvoj morskih riba. Tako je uočeno da tamna boja izaziva kontrast koji ličinkama omogućuje da se bolje prilagode na uvjete života. Budući da se uzgojni bazeni mogu tvornički obojiti u bilo koju boju, komercijalno se uglavnom upotrebljavaju crni, zeleni ili tamno i svijetlo plavi. Neki podaci sugeriraju da mlade ribice više vole bazene s crnim stranama, a dno da je boje pijeska. Da bismo potvrdili pretpostavku da boja bazena utječe na rast mladih riba, postavljen je pokus utjecaja crnih, zelenih, crvenih, tamno i svijetlo plavih bazena na kratkoročni rast i konverziju hrane listova, kao i na rast, konverziju hrane i kemijski sastav tilapija. Odgovor kortizola istraživana je u obiju vrsta riba. Boja bazena nije imala učinak na rast listova ili tilapija, iako su ribe držane u crvenim bazenima imale bolji postotak povećanja mase. Razlike ( $P < 0,05$ ) pri konverziji hrane zapažene su kod listova držanih u crvenim bazenima. Količina plazma kortizola kod listova iznosila je između 1,39 i 3,71 ng kortizola na milimetar plazme, u usporedbi s 12,7–94,4 ng kortizola na mililitar plazme u tilapija. Najniža količina kortizola ( $P < 0,05$ ) bila je zabilježena kod listova i tilapija uzgajanih u crvenim akvarijima. Osnovna boja nije imala učinak na kemijski sastav fileta tilapija.

Ključne riječi: tilapija, list, kortizol, rast

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