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Growth and body composition of Midas (*Amphilophus citrinellus*) **and Nile tilapia** (*Oreochromis niloticus*) **reared in duoculture**^{*}

Crecimiento y composición de Midas (<u>Amphilophus citrinellus</u>) y tilapia del Nilo (<u>Oreochromis niloticus</u>) mantenidos en duocultivo

Crescimento e composição dos peixes Midas (<u>Amphilosus</u> <u>citrinellus</u>) e Tilápia-do-Nilo (<u>Oreochromis niloticus</u>) mantidas em duocultivo

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Summary

Background: cichlids are of economical importance either as food (Nile tilapia) or as ornamental fish (Midas) and both exhibit territorialism and aggressive feeding behavior depending on availability of food and space. Objective: to evaluate the growth rates and behavioral changes of Nile tilapia and Midas kept in mono or polyculture. Methods: Midas and tilapia were maintained in a semi-closed rearing system. Initial weight was 0.83 and 0.81 g for Nile tilapia and Midas, respectively. Four treatments with different fish proportions were used. Midas and tilapia were distributed in 12 glass aquaria with three replicates (n = 30 fish per tank). Treatment ratios between Midas and tilapia were 1:0, 1:1, 2:1 and 0:1, respectively. Fish were fed a commercial diet (40% protein, 12% lipids) for six weeks at 5% weight ratio. Feed offer was adjusted weekly. Observations of behavioral traits were recorded throughout the trial to determine social and feeding conduct. Body composition of fish was assessed at the end of the experiment. Results: Midas modified their feeding behavior and their weight gain increased $(3.9 \pm 0.3 \text{ g})$ in the 2:1 group. The 0:1 group exhibited the lowest growth rate throughout the experiment $(2.9 \pm 0.3 \text{ g})$. Midas did not affect Tilapia growth $(5.8 \pm 0.4 \text{ g})$ across treatments. Interspecies aggressiveness was less evident when reared in monoculture (groups 1:0 y 0:1). Intra and interspecies attacks were higher in the 1:1 and 2:1 groups. Proximate body composition indicated higher lipid levels in Midas across treatments in comparison to tilapia. Conclusions: duoculture benefits growth of juvenile Midas when present at 25-30% of total stocking density with Nile tilapia.

Keywords: behavior, feeding, fish, polyculture.

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Resumen

Antecedentes: los cíclidos son peces de importancia económica, ya sea como alimento (tilapia del Nilo) o para ornato (Midas), ambos exhiben territorialismo y comportamientos alimenticios agresivos, dependiendo del alimento y espacio disponible. Objetivo: evaluar diferencias en tasas de crecimiento ya sea en mono o duocultivo debido a cambios específicos en comportamiento. Métodos: treinta peces por tanque de tilapia del Nilo y Midas, con un peso inicial de 0,83 y 0,81 g respectivamente, fueron mantenidos en un sistema semicerrado con 12 acuarios y distribuidos en cuatro tratamientos, con tres replicas cada uno, en las siguientes relaciones: 1:0, 1:1, 2:1 y 0:1 tilapias: Midas. Los peces fueron alimentados por 6 semanas al 5% de la biomasa con una dieta comercial 40% de proteína y 12% lípidos y fue reajustado semanalmente. Se estableció la conducta social y de alimentación para cada especie. Finalizando el experimento, se analizaron los valores proximales corporales por tratamiento. Resultados: los Midas modificaron su conducta alimenticia y mostraron una mayor ganancia en peso en la presencia de tilapia en el grupo 2:1 (3.9 ± 0.3 g). El grupo 0:1 mostró la talla más pequeña $(2.9 \pm 0.3 \text{ g})$. Las tilapias no modificaron su crecimiento $(5.8 \pm 0.4 \text{ g})$ en ningún tratamiento. La agresividad interespecífica es menos evidente en los peces mantenidos en monocultivo (tratamientos 1:0 y 0:1); incrementándose en los grupos 1:1 y 2:1. Se observó una mayor acumulación de lípidos totales en los Midas en comparación a las tilapias. Conclusiones: el duocultivo de juveniles de Midas con tilapia del Nilo a un total del 25-30% de la densidad total de siembra mejora su crecimiento.

Palabras clave: alimentación, comportamiento, peces, policultivo.

Resumo

Antecedentes: os ciclídeos são peixes de importância econômica, seja como alimento (tilápia-do-Nilo) ou para ornamentação (Midas), ambos exibem territorialidade e comportamentos alimentares agressivos, atribuídos ao alimento e espaço disponível. Objetivo: avaliar as diferenças em taxas de crescimento quando foram mantidos em mono e policultivo, devido às mudanças específicas em seu comportamento. Métodos: trinta peixes por tanque de tilápia-do-Nilo e Midas, com peso inicial de 0,83 e 0,81 g respectivamente, foram mantidos em um sistema semifechado com 12 aquários e distribuídos em quatro tratamentos, com três repetições cada, nas densidades: 1:0, 1:1; 2:1 e 0:1 tilápias:Midas. Os peixes foram alimentados durante 6 semanas à 5% da biomassa com uma dieta comercial composta de: 40% de proteína e 12% de lipídios e foi reajustada semanalmente. Foi estabelecida a conduta social e de alimentação para cada espécie. Ao final do teste, foram analisados os valores de composição corporal proximal por tratamento. Resultados: os Midas modificaram sua conduta alimentar e mostraram maior ganho de peso na presença de tilápia no grupo 2:1 (3.9 ± 0.3 g). O grupo 0:1 apresentou o menor peso $(2,9 \pm 0,3 \text{ g})$. As tilápias não modificaram seu crescimento $(5,8 \pm 0,1 \text{ g})$ em nenhum tratamento. A agressividade interespecífica foi menos evidente para os peixes mantidos em monocultivo (tratamentos 1:0 e 0:1); e incrementou-se nos grupos 1:1 e 2:1. Foi observado um maior acúmulo de lipídios totais nos Midas em comparação às tilápias. Conclusões: o policultivo oferece um crescimento benéfico para Midas juvenis quando estão presentes com tilápia do Nilo em 25-30% do total do cultivo.

Palavras chave: alimentação, comportamento, peixes, policultura.

Introduction

Duoculture involves rearing two fish species in a production system to increase productivity (Karakatsouli *et al.*, 2006) of at least one of the species (Balinwa, 2007). It leads to increased utilization of feeding niches and space (Da Silva *et al.*, 2008). Duoculture allows for similar growth performance of both species, particularly when a less competitive fish modifies its behavior to match an aggressive feeder fish (Flood *et al.*, 2010) potentially reducing interspecific aggression (Jobling *et al.*, 1998), dependent on the stocking ratio of each species (Karakatsouli *et al.*, 2006).

Tilapias are commonly used in duoculture and polyculture research (Papoutsoglou *et al.*, 2001; Da Silva *et al.*, 2006). Appropriate selection of species for proper adaptation to feeding preferences and food availability (Hailey *et al.*, 1998) is highly relevant. Habitat sharing in polyculture can induce changes in feeding patterns and growth. This has been reported for common and Indian carp raised with rohu (*Labeo rohita*; Silva *et al.*, 2006).

Diminished tilapia growth in presence of common carp (Da Silva *et al.*, 2006) or himri barbel (Carasobarbus luteus) reared in cages at high densities (Gokcek, 2011) has also been reported. Newer data for tilapia reared with Mayan cichlid *Cichlasoma uropthalmus* showed that tilapia growth, either monosex or mixed-sex at different stocking rates, was not affected by Mayan cichlid presence; but the Mayan cichlid final weight increased at lower stocking ratios with mixed-sex tilapia (Hérnandez *et al.*, 2014).

Midas cichlid, a fish native to Nicaragua (Martinez-Sanchez *et al.*, 2001), is very aggressive (Barlow and Siri, 1994) and displaces other species, especially during reproduction (Vega, 1998). Midas is a research model of morphological plasticity in evolutionary mechanisms (Barluenga and Meyer 2004). Midas is an ornamental fish also consumed as food (McCrary *et al.*, 2007). Vega (1998) recommends increasing Midas catch to improve the reproductive rate of other native species as a result of reduced competition. Both Midas and tilapia species coexist in natural habitats of Central America (Canonicoa *et al.*, 2005; McCrary *et al.*, 2007).

Given the aggressive behavior and similar feeding habits of both cichlids, including consumption of commercial feed, growth performance and final proximate composition of both fish were evaluated when reared in duoculture and recirculating water systems. Changes in feeding and social interactions were also observed.

Materials and methods

Ethical considerations

This study was conducted under Ohio State University Animal Care and Use Office guidelines and regulations, following the Ohio State University's Institutional Animal Care and Use Committee Protocol Number: 2008A0221-R1, approved on July 1st 2009.

Experimental fish

Genetic all-male Nile tilapia (*Oreochromis* niloticus) and Midas (Amphilophus citrinellus) juveniles were used. Tilapia were purchased at Til-Tech, (Baton Rouge LA, USA) and Midas were obtained from a single brooding pair produced in the aquaculture laboratory, School of Environment and Natural Resources (The Ohio State University).

Initial weight was 0.83 ± 0.09 g for tilapia and 0.81 ± 0.06 g for Midas.

Feeding trial

Fish were randomly distributed in 12 aquaria (35 L) with semi-closed recirculation system, controlled water temperature (27 ± 1 °C), pH = 7.1 ± 0.2, total ammonia <0.5 mg/L, and dissolved oxygen >5 mg/L. Fish were assigned to the following treatments with different tilapia:Midas stocking ratios (n = 30 fish per tank): 1:0 (30 tilapias), 1:1 (15 tilapias and 15 Midas), 2:1 (20 tilapias and 10 Midas) and 0:1 (30 Midas) with three replicates per experimental group for three weeks. Subsequently, fish were moved to larger tanks (55 L) for three more weeks in a semi-closed recirculation system with controlled water temperature (27 ± 1 °C, pH = 7.3 ± 0.1, ammonia <0.5 mg/L, and dissolved oxygen >5 mg/L). This system was located in a greenhouse adjacent to the lab.

During the second phase of the trial the light regime was 12:12 h. Fish were subjected to two different feeding schedules as follows: automatic feeders were used for the three initial weeks (dispensing food every 20 minutes, 8 hours per day), and were manually fed twice a day for the last three weeks at 5% of total body weight per day. A commercial diet (40% protein, 12% lipid; Bio-Oregon®, Longview WA, USA) was used throughout the experiment. Feeding was readjusted on a weekly basis after each weighing (both total tank and individual fish). Weight gain (by tank and by species) was calculated weekly. Growth performance was recorded as total biomass per tank (g). Individual mean weight (g) per tank and species, survival (%), food conversion ratio (FCR) and specific growth rate (SGR, %/day) were also calculated.

Social interactions

Social interactions among fish in all four treatments were recorded after establishing a subjective scale to measure interactions and tentatively determine the degree of aggressive behavior or direct attacks during feeding. The scale values were as follows: (1) no interaction, (2) little interaction, (3) mild interaction, (4) strong interaction, (5) severe interaction. Data were recorded five times during the experiment (weeks 2 to 6).

Proximate composition

Proximate body composition was also determined, both at the beginning and at the end of the trial. For this purpose, the initial sample was 15 fish of each species, and five fish per species per replica of each dietary treatment at the end of the trail. Fish were macerated and pooled (by species for treatment groups 1:1 and 2:1). Samples were then freeze-dried (moisture % recorded) and pulverized for general composition analyses (nitrogen and ash) following standard procedures (AOAC, 1980). Total lipids were determined following the method described by Folch *et al.* (1957).

Statistical analysis

Growth performance data of each species within treatments were subjected to analysis of variance (ANOVA). Fisher protected test for least square means multiple comparison was used to establish intraspecific treatment differences. All statistical calculations were performed using the GLM procedure of SAS version 8.02 (1996, SAS Institute, Inc., Cary, NC) at 0.05 significance level.

Results

Feeding trial

Growth performance was significantly different (F = 8.91, p = 0.0062) among treatments (Table 1).

The 0:1 group had the lowest individual weight $(3.0 \pm 0.3 \text{ g})$, total tank biomass $(86.7 \pm 2.85 \text{ g})$, and SGR $(3.0 \pm 0.2 \%/d)$, as well as the highest FCR value among all treatments (1.18 ± 0.06) .

Values are means of final total tank biomass (g), individual body weight (g), food conversion ratio (FCR) and specific growth rate (SGR% d^{-1}) per treatment. Different letters within rows indicate significant differences (p<0.05).

When analyzed by species within stocking density, most values showed similar results for mean body weight, SGR and survival for tilapia, where the 0:1 treatment had the highest values for the same end-point measurements. However, they were not significantly different (p>0.05) than those for treatments 2:1 and 1:1. Survival was lower in the 1:0 treatment after 6 weeks (93.3 \pm 3.3%), but it was not significantly different when compared to the other groups (p>0.05; Table 2).

Values presented as final total tank biomass (g), mean individual body weight (g), SGR (% day⁻¹). Different letters within rows indicate significant differences (p<0.05).

A similar finding was observed for tilapia, given that mean individual weight for tilapias in treatments 2:1 and 1:1 was to some extent higher than that of fish in 1:0, although differences were not significant (p>0.05; Figure 1a).

 Table 1. Overall growth performance of Tilapia (Oreochromis niloticus) and Midas fish (Amphilophus citrinellum) raised under different stocking ratios.

Variable	Tilapia:Midas stocking ratio						
	1:0	0:1	1:1	2:1			
T. tank biomass (g)	159.3 ± 33.2 ^a	86.7 ± 2.85 ^b	138.9 ± 18.8ª	157.6 ± 9.1ª			
Mean body weight (g)	5.7 ± 1.0ª	3.0 ± 0.3^b	5.3 ± 0.7 ^a	4.6 ± 0.1 ^a			
FCR	0.93 ± 0.12^{b}	1.18 ± 0.06 ^a	0.89 ± 0.01^{b}	0.93 ± 0.04^{b}			
SGR (% day⁻¹)	4.4 ± 0.4^{a}	3.0 ± 0.2^b	4.2 ± 0.2 ^a	4.3 ± 0.2 ^a			
Survival (%)	97.8 ± 1.9	97.8 ± 1.9	98.9 ± 1.9	93.3 ± 3.3			

Tilapia (Oreochromis niloticus)				
	1:0	2:1	1:1	
Final biomass (g)	159.33 ± 33.20	124.63 ± 9.23	90.96 ± 3.58	
Mean body weight (g)	5.66 ± 1.01	6.14 ± 0.61	5.96 ± 0.38	
SGR (% day ⁻¹)	4.18 ± 0.43	4.38 ± 0.23	4.32 ± 0.15	
Survival (%)	93.33 ± 3.33	100 ± 0.0	100 ± 0.0	
Midas (Amphilophus citrinellum)				
	0:1	2:1	1:1	
Final biomass (g)	86.70 ± 9.15	36.56 ± 5.67	44.93 ± 7.41	
Mean body weight (g)	2.91 ± 0.25 ^b	3.90 ± 0.35^{a}	3.00 ± 0.50^{b}	
SGR (% day ⁻¹)	2.69 ± 0.20^{b}	3.35 ± 0.20 ^a	2.74 ± 0.37^{b}	
Survival (%)	97.77 ± 1.92	93.33 ± 5.77	100 ± 0.0	

Table 2. Growth performance for Tilapia (Oreochromis niloticus) and Midas fish (Amphilophus citrinellum) under different stocking ratios.

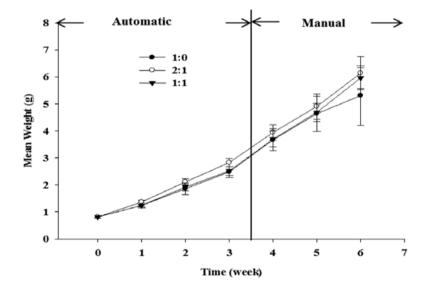


Figure 1a. Evolution of Tilapia weight in experimental groups consisting of different stocking ratios during two feeding schedules (first 3 weeks with automatic feeders, last 3 weeks using manual feeding). Data are means ± SD.

For Midas, in treatment 2:1, a tendency was observed in growth compensation. That is, the mean individual weight was significantly larger (F = 6.18, p = 0.034) in this species for fish present in 2:1 treatment as well as SGR observed values (Table 2), when compared to the 1:1 and 0:1 groups. In particular, Midas in the 2:1 group, were larger over the last 2 weeks of the experiment (Figure 1b).

This tendency was clearly observed, when comparing final total biomass per treatment, where 1:1

 $(159.3 \pm 33.2 \text{ g})$ and 2:1 $(157.6 \pm 9.1 \text{ g})$ stocking densities showed no differences on this parameter (Figure 2).

Social interactions

Interactive behavior by treatment had the following results during the trial (Table 3). The single-species groups (1:0 and 0:1) had the lowest interspecies aggressiveness according to the established grade-scale (2.8 ± 1.2 and 3.3 ± 0.9 , respectively). The

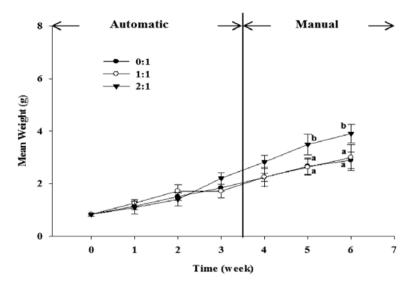


Figure 1b. Evolution of Midas weight in experimental groups consisting of different stocking ratios during two feeding schedules (first 3 weeks with automatic feeders, last 3 weeks using manual feeding). Data are means \pm SD. Different letters indicate significant differences (p<0.05) at specific times.

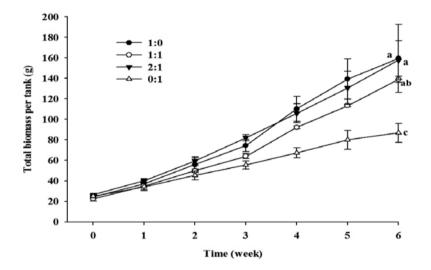


Figure 2. Total final biomass (g) per experimental group per week according to stocking rate. Data are means ± SD. Different letters indicate significant differences (p<0.05) among treatments.

2:1 and 1:1 groups had a strong fish interaction both intra and interspecies $(3.9 \pm 0.5 \text{ and } 3.7 \pm 0.6, \text{ respectively})$. Distribution of fish species in the tanks was homogeneous, as no segregated groups, either by species or size, were observed in individual tanks. Observations indicate that Midas adjust and matches feeding behavior of tilapia to secure access to the offered food by swimming to the surface depending on the stocking rate. Also, aggressive behavior (biting the head or sides of other fish) was noticeably more prevalent in these two treatments regardless of species. The 2:1 treatment group showed the strongest interactions among fish, perhaps as a sign of reduced dominance of tilapia.

Proximal composition

Initial proximate body composition prior to the beginning of the trial for Midas and tilapia was as

Obs/week		0:1			1:0			2:1			1:1	
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Week 1	2	4	2	4	4	2	4	4	4	3	4	4
Week 2	5	2	4	2	4	2	4	4	4	4	4	4
Week 3	4	4	4	1	4	2	4	4	4	3	4	4
Week 4	2	3	3	1	4	2	4	4	4	4	2	4
Week 5	3	4	3	4	4	2	2	4	4	4	4	4
Mean value	3.2	3.4	3.2	2.4	4	2	3.6	4	4	3.6	3.6	4
	Overall		3.3	Overall		2.8	Overall		3.9	Overall		3.7
Type of interaction		Mild			Mild			Strong			Strong	

Table 3. Observed social interactions related to feeding behavior, i.e. attacking, aggression.

Table 4. Initial and final proximate composition of tilapia and Midas.

Tilapia (Oreochromi	is niloticus)				
	Initial	1:0	2:1	1:1	
Moisture	81.5	79.4 ± 6.1	75.1 ± 0.6	75.5 ± 0.1	
Protein	61.8	62.9 ± 1.0	62.6 ± 0.7	61.9 ± 1.1	
Lipids	21.6	19.5 ± 2.7	22.3 ± 0.8	15.7 ± 3.0	
Ash	13.9	15.6 ± 0.7	15.3 ± 0.7	15.9 ± 0.7	
Midas (Amphilophus	s citrinellum)				
	Initial	0:1	2:1	1:1	
Moisture	74.1	74.2 ± 0.5	74.5 ± 0.5	74.4 ± 0.5	
Protein	54.7	57.4 ± 0.4	58.1 ± 0.5	57.8 ± 0.9	
Lipids	26.2	24.7 ± 1.9	26.2 ± 3.9	26.1 ± 2.6	
Ash	17.8	14.0 ± 0.5	14.7 ± 0.5	14.4 ± 0.5	

Results are expressed on a dry matter basis (%DM).

follows: 74.1 and 81.5% moisture, 54.7 and 61.8% protein, 26.2 and 21.6 lipids, 17.8 and 13.9% ash, respectively. Proximate composition remained similar among fish for each stocking rate, either for tilapia or Midas, at the end of the experiment (Table 4). Both protein and ash contents were higher for tilapia compared to Midas prior to initiation of the trial. Protein and ash content remained close to the initial chemical composition. Lipid accumulation showed no differences for Midas or tilapia; a slight reduction was observed in lipid content for tilapia as 1:1 group progressed through the trial.

Discussion

Tilapia growth was not affected by the presence of Midas. In most cases, tilapia adapts in an efficient manner to the presence of other species, and does not show diminished growth when reared in polyculture. This is contrary to experiments with tilapia cultured with common carp at similar stocking ratios in intensive systems (Papoutsoglou *et al.*, 2001). Jundia fish (*Rhamdia quelen*) and several carp species stimulate growth of common carp (Da Silva *et al.*, 2006) and milkfish (*Chanos chanos*) when tilapias were present in the lowest proportion (Cruz and Laudencia 1980). Therefore, through some mechanism, the presence of tilapia induces feeding behavior changes in other fish species and enhances food consumption.

There is insufficient research of Midas growth under conditions similar to this experiment. Our findings regarding Midas weight gain in the presence of tilapia provides an interesting insight into the synergistic feeding behavior modifications displayed by these fish that increase access to available food. As previously mentioned, an important aspect affecting production of two or more species under polyculture is adaptability to shared food and space (Balinwa, 2007). The fact that tilapia:Midas presence at 1:1 or 2:1 ratios resulted in significantly higher mean individual weight, higher SGR and lower FCR values compared to mono-cultured Midas (0:1 ratio) provides further support to adaptive behavior among dominant cichlids. These changes are similar to those described in other fish species, although further research is needed to validate these findings.

Recently, a cichlid duoculture trial lasting 25 weeks with similar Nile tilapia and Mayan cichlid populations reported that the only factor affecting tilapia growth was the use of either 95% male or mixed-sex tilapia groups whereas the best growth performance of Mayan cichlid was observed under mixed-sex tilapia duoculture. It is remarkable that such performance was not enhanced by lesser intraspecific interactions (food competition) but by tilapia larvae availability due to uncontrolled reproduction as an alternative food source for Mayan cichlid. Therefore, authors highlight the potential use of this fish as a predator to control tilapia overcrowding in culture units (Hernandez et al., 2014). Conversely, these same authors indicate that Mayan cichlid growth was significantly lower when 95% male tilapia was present. In our study, although we only evaluated Midas and all-male tilapia douculture conditions, stocking density was the single factor affecting Midas growth performance.

The proximate body composition analysis validates the fact that fish in all experimental groups did not suffer from food deprivation; no considerable decrease in parameters was noticed. Nevertheless, reduction of tilapia lipid content from 1:1 requires some consideration. It has been reported that total lipid values of fish reared in polyculture can fluctuate in a close relationship with moisture. This has been interpreted as high moisture content results in reduced lipid content with feeding ratio variations (Abdelghany and Ahmad, 2002) in tilapia, common and silver carp, and in tilapia and the Central American cichlid (*Cichlasoma melanurum;* Antoine *et al.*, 1987). However, this studies findings do not indicate an increase in moisture for tilapia in this

group (1:1 ratio); therefore observed differences in lipid accumulation for tilapia in this group could be related to lipid utilization and fish activity in the presence of Midas. A similar finding was reported by Karakatsouli *et al.* (2006) when juvenile sharpsnout seabream (*Diplodus puntazzo*) and gilthead seabream (*Sparus aurata*) were reared at different stocking ratios in a recirculated water system; a significant reduction in carcass lipid content was noticed for the first species, again, mostly attributed to social interaction in two stocking ratios.

Despite that food consumption by each species in our experiment could not be specifically quantified, it has been reported that hybrid tilapias *O. mossambicus* x *O. niloticus* starved for 1-4 weeks do not differ in proximal composition compared to fish fed continuously during 8 weeks (Wang *et al.*, 2005). Thus, observed differences are unlikely to be due to differences in food consumption given Midas presence and feeding competition, or feeding frequency changes (automatic vs. manual feeding).

A previous study evaluated the degree of dominantaggressive Midas behavior, indicating that the color of fish present either inside or outside the experimental unit is a determinant factor stimulating interaction with fish of the same species. The light colored (gold or orange) fish are the most prone to attacks and fish of similar color attack each other at a higher frequency (Barlow and Siri, 1994). Our observations indicate a similar pattern of interactions across treatments when evaluated at weekly intervals, and varied little within treatments as Midas developed their final body coloration (white and orange or gold morph). Midas colororation was similar to tilapias at the beginning of the trial, being dark gray with black horizontal stripes, and changed to its final golden appearance within three weeks. Therefore, the Midas aggressive behavior transitioned into a competitive feeding behavior aimed at obtaining access to food. Further research is needed on Midas as an ornamental or food fish to understand its growth under different stocking ratios with Nile tilapia.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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