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Interaction between feeding rate and area for periphyton in culture of Nile tilapia juveniles

Vanessa Tomaz Rebouças, Nayara Nunes Caldini, Davi de Holanda Cavalcante, Francisco Jackes Rodrigues da Silva and Marcelo Vinícius do Carmo e Sá*

*Laboratório de Ciência e Tecnologia Aquícola, Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Av. Mister Hull, s/n, 60455-760, Fortaleza, Ceará, Brazil. *Author for correspondence. E-mail: marcelo.sa@ufc.br*

ABSTRACT. The objective of the present work was to determine the effects of the interaction between feeding rate and area for periphyton in rearing aquaria for Nile tilapia. Twenty 25 L polyethylene outdoor aquaria were used to hold experimental fish. Three tilapia fingerlings (2.56 ± 0.14 g) were stocked in each aquarium for five weeks (12 fish m^{-2}). There were two different feeding rates (full and full minus 30%) and two submerged areas for periphyton development (59.4% and 96.5% of the aquarium surface area; n = 5). Final body weight, specific growth rate and yield in the 96.5%-area aquaria of fish submitted to 30% less feed were significantly higher than in the 96.5%-area aquaria submitted to full feeding rates. Fish growth in the restricted-feed aquaria was favored by their better water quality (higher pH and lower total ammonia nitrogen). There were negative effects on final body weight, SGR, yield and feed conversion ratio of fish in full-feed aquaria when the area for periphyton increased from 59.4 to 96.5%. It was concluded that the combination of restricted feeding rates with large substrate areas for periphyton growth results in better water quality for fish culture than the adoption of each management alone.

Keywords: tilapia, fish culture, semi-intensive system, productivity, water quality.

Interação entre a taxa de arraçoamento e a área para perifíton no cultivo de juvenis de tilápia do Nilo

RESUMO. O presente trabalho teve como objetivo determinar os efeitos da interação entre a taxa de arraçoamento e a área submersa para perifíton em aquários de cultivo de tilápia do Nilo. Foram utilizados 20 aquários de 25 L no trabalho. Três juvenis de tilápia (2,56 ± 0,14 g) foram estocados em cada aquário por cinco semanas. Havia duas taxas de arraçoamento (cheia e cheia menos 30%) e duas áreas submersas para o desenvolvimento de perifíton (59,4 e 96,5% da área superficial do aquário; n = 5). O peso corporal final dos peixes nos aquários com 96,5% de área para perifíton e submetidos à taxa alimentar restritiva foram significativamente maiores que nos aquários com 96,5% de área e taxa alimentar padrão. O crescimento dos peixes nos aquários com arraçoamento restritivo foi favorecido pela melhor qualidade de água (maior pH e menos amônia). O crescimento e conversão alimentar dos peixes foram negativamente afetados nos aquários com 96,5% de área para perifíton e taxa de arraçoamento padrão. Concluiu-se que a combinação de taxas alimentares restritivas com a adoção de maiores áreas de substratos para o desenvolvimento de perifíton traz melhores resultados de qualidade de água que o emprego desses manejos de forma isolada.

Palavras-chave: tilápia, piscicultura, sistema semi-intensivo, produtividade, qualidade de água.

Introduction

There is a consensus in aquaculture that natural feed has high nutritional value for fish, and that growth must be stimulated by producers through proper management (KNUCKEY et al., 2006). In fish culture, such as tilapia culture, the main natural food items in ponds or aquaria are plankton (phytoplankton and zooplankton (EL-SAYED, 2006). However, plankton alone is not capable of sustaining suitable fish growth rates in semi-intensive systems, even in high densities (NAYLOR et al., 2000). The higher stocking densities of fish in semi-intensive systems require supplemental nutrient sources to achieve good productivity rates (TACON; DE SILVA, 1997). For that reason, fish producers offer agricultural by-products and/or incomplete artificial diets to animals (VAN EER et al., 2004).

In addition to plankton, another natural food item not always remembered by culturists is periphyton. Periphyton is the general designation for microorganisms that thrive in any submerged substrate (sticks, branches, pipes, rocks etc.) and is formed by algae, bacteria, fungi, aquatic invertebrates, protozoa and debris (AZIM et al., 2005). Periphyton can be efficiently used as feed by tilapia and other omnivorous fish, such as carps (DEMPSTER et al., 1993). Additionally, periphyton mats can act as efficient biofilters by reducing ammonia levels in water (AZIM; LITTLE, 2006). Aquaculture production systems that rely on periphyton are known as substrate-based systems and can significantly improve pond and tank productivity (ASADUZZAMAN et al., 2010a and b).

In semi-intensive systems, the availability of abundant periphyton for fish can reduce the importance of supplemental feed allowance because fish nutrition would be partially satisfied by the ingestion of periphyton. However, completely withdrawing supplemental feed may have negative effects on fish growth (MILSTEIN et al., 2009). Thus, the amount of supplemental feed delivered to fish or the feeding rates adopted by the producer are critical to profitability. In addition to feeding rate, another factor that decisively affects the success of substrate-based aquaculture systems is the size of the submerged area available for periphyton growth (ASADUZZAMAN et al., 2010a). It is generally accepted that the higher that area, the greater the benefits from periphyton will be, such as more food and improved water quality (UDDIN et al., 2009). Nevertheless, although the single effects of periphyton area on fish productivity are relatively well known, its combined action with different feeding rates is still poorly understood. The present work aimed to determine the effects of the interaction between feeding rate and area for periphyton in Nile tilapia rearing aquaria.

Material and methods

Fish and experimental system

One thousand male sex-reversed Nile tilapia, *Oreochromis niloticus*, fingerlings were obtained from a local fish producer and transported to LCTA facilities (Laboratório de Ciência e Tecnologia Aquicola, Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Ceará State, Brazil). Initially, fish stayed in the reception tank for one week to acclimatize to laboratory conditions (dechlorinated tap water; temperature = 27.5 ± 0.5°C, pH = 7.2 ± 0.3, DO_2 saturation > 60%; natural photoperiod). Over the acclimatization period, fish were fed a high-protein commercial diet (Aquaxcel 4515, Cargill Animal Nutrition) in four daily meals at 8:00, 11:00, 14:00 and 17:00 hours. The daily feeding rate was equal to 10% of stock biomass.

Twenty 25 L polyethylene outdoor aquaria were used to hold experimental fish. The aquaria had cotton mesh covers to prevent fish escape. At the onset of the experiment, three tilapia juveniles (2.56 ± 0.14 g) were stocked in each aquarium (12 fish m^{-2}). No artificial aeration was provided to the aquaria. Fish were maintained in the experimental system for five weeks.

Experimental design and husbandry

Two experimental factors were evaluated simultaneously in a completely randomized design arranged in a 2 x 2 factorial mode with five replications each. There were two different feeding regimes and two sizes of submerged area for periphyton development. Decreasing standard feeding rates ranging from 14 to 7.5% of live weight were allowed daily to fish fed according to the feeding regime designated as "Full". The full feeding rates were the same recommended by the manufacturer.

In the feeding regime "-30%", the standard feeding rates were reduced by 30%. Two weeks before fish stocking, plastic bottles filled with small rocks were placed inside the aquaria for periphyton growth. On the same day the bottles were placed, 250 mL of phytoplankton-rich water were inoculated in each aquarium. Either eight or 13 bottles were totally submerged for periphyton development. In the 8-and 13-bottle aquaria, the total surface areas of the bottles were 1440 and 2340 cm^2, respectively, which represented 59.4 and 96.5% of total aquarium surface area, respectively. All stocked fish were fed with the same artificial diet used during the acclimatization period. The amount of feed allowed for each aquarium was adjusted fortnightly after fish body weighing.

Experimental variables and analytical procedures

Water quality, fish performance and periphyton growth variables were observed in the present work. Water temperature, pH, electric conductivity were obtained using portable equipment daily at 8:00 and 16:00 hours; the concentrations of dissolved oxygen (Winkler's method), total ammonia nitrogen (TAN; Nessler's method), nitrite (diazotizing and coupling method) and reactive phosphorus (ammonium molybdate method) were monitored weekly in all aquaria. All water samples were always collected between 9:00 and 10:00 hours. The analytical determinations of TAN, nitrite and reactive phosphorus were carried out according to the guidelines presented by APHA (1999).

At the end, all bottles were removed from aquaria and their periphyton films scraped carefully with a blade. The wet periphyton biomasses were put overnight on Petri dishes inside an electric oven at 105°C (SILVA, 1990). The dry periphyton biomass was weighted in a semi-analytical scale. Fish final body weight, survival, specific growth rate (ln final body weight - ln initial body weight/no. of days x 100), yield and feed conversion ratio (feed allowance/fish weight gain) were observed in all replications.

Statistical analyses

Water quality, growth performance and periphyton variables were analyzed using two-way ANOVA with feeding rate (full and full-30%) and area for periphyton (59.4 and 96.5%) as main factors. The assumptions of normal distributions and homogeneity of variances were checked before analysis. Percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA analyses were carried out at 5% level of significance using SigmaStat for Windows 2.0 (Jandel Statistics).

Results and discussion

Water temperatures at 8:00 and 16:00 hours averaged 26.7 and 30.1°C, respectively, ranging between 25.8 - 28.3 and 29.8 - 33.1°C, respectively. Except for the upper limit at 16:00 hours, which was observed solely at one day, the water temperature in the aquaria was within the proper range for tilapia growth (AZAZA et al., 2008).

Water pH was significantly affected by feeding rate. The reduction in feeding rate by 30% significantly increased water pH at 16:00 hours both for the 59.4 and 96.5% periphyton areas (Table 1). Contrarily, the addition of more substrate for periphyton growth caused no significant effect on water pH (Table 1). Water pH at 8:00 hours was not affected by the experimental treatments and averaged 7.6 ± 0.2. Less feed means less decomposing organic matter (non-ingested feed and fish feces) inside water. That way less acidic CO_2 (CAVALCANTE et al., 2010) was probably released in the reduced-fed aquaria, which contributed to higher water pH. The same effect of low feeding rates on water pH was observed by Colt et al. (2009) and Southworth et al. (2006). As a general rule, higher values of water pH up to 9 indicate a better condition of water quality (PILLAY; KUTTY, 2005). Therefore, it is advisable to deliver the lowest possible amount of diet to fish in order to get better water quality indicators.

The electric conductivity (EC) of water was not significantly affected by the feeding rates or area for periphyton growth. However, it is noteworthy that very high values were observed in the present work (> 1,000 μS cm^{-1}). On average (8:00 and 16:00 hours readings), the aquaria's EC was 1,041 ± 64 μS cm^{-1}, which indicates that the rearing environment was highly eutrophic (BOYD; TUCKER, 1998). That condition under suitable light exposure can lead to intense algal blooms, which may cause anoxia at night (NJIRU et al., 2010).

Table 1. Water quality variables of 25 L polyethylene outdoor aquaria provided with 180-cm^2 plastic bottles for periphyton development. Each aquaria were stocked with three Nile tilapia, *Oreochromis niloticus*, juveniles (initial body weight = 2.56 ± 0.14 g and initial body length = 5.3 ± 0.2 cm) for five weeks. Fish were submitted to two different feeding regimes and there were two areas for periphyton growth (mean ± s.d.; n = 5).

Variable	Feeding rate[1]	Area for periphyton[2]	
		59.4%	96.5%
Water pH at 16:00 hours	Full	8.26 ± 0.41 A[3]	8.10 ± 0.23 A
	-30%	8.73 ± 0.31 B	8.60 ± 0.33 B
Electric conductivity (μS cm^{-1})	Full	1031 ± 51	1032 ± 58
	-30%	1036 ± 56	1064 ± 47
Total ammonia N (mg L^{-1})	Full	1.07 ± 0.16 A	0.91 ± 0.09 A
	-30%	0.75 ± 0.11 B	0.76 ± 0.08 B
Nitrite (mg L^{-1})	Full	0.44 ± 0.05 A	0.47 ± 0.06 A
	-30%	0.51 ± 0.03 B	0.55 ± 0.05 B
Reactive phosphorus (mg L^{-1})	Full	0.210 ± 0.03 Aa	0.184 ± 0.02 Ab
	-30%	0.169 ± 0.0.1 Ba	0.143 ± 0.02 Bb

Two-way ANOVA P

Factor	pH	Elect. Cond.	TAN	NO_2^-	Reactive P
Feeding rate	0.004	ns	< 0.001	0.003	< 0.001
Area for periphyton	ns[4]	ns	ns	ns	0.016
Feed *x* Area	ns	ns	ns	ns	ns

[1]Full: The standard feeding rates recommended by the laboratory's feeding table were entirely adopted; -30%: the standard feeding rates were reduced by 30%; [2]Eight or thirteen small plastic bottles (total surface area = 1440 and 2340 cm^2, respectively; 59.4 and 96.5% of the aquarium's surface area, respectively) were submerged into selected aquaria to allow the development of periphyton; [3]For the same variable, means not sharing the same lower case or capital letter in a row and column, respectively, are significantly different by Tukey's test ($p < 0.05$); means without any letter are not significantly different; [4]Not significant ($p > 0.05$).

The reduction of the feeding rates by 30% significantly reduced total ammonia nitrogen (TAN) concentrations in water. That was valid both for the 59.4 and 96.5% periphyton areas (Table 1). In the reduced-fed aquaria, the release of feces by fish was probably lower than in the full-fed aquaria. As fish feces are rich in protein, which after bacterial decomposition produce ammonia (SLAWSKI et al., 2010), there was more TAN in the full-fed aquaria than in the reduced-fed aquaria. Conversely, no significant effect on TAN was observed due to periphyton area (59.4 or 96.5%). That was a disappointing result, because it was expected that the boosted periphyton in the 96.5% area would act as an efficient ammonia biofilter. In the present work, the experimental aquaria were located under a partially roofed lab area. Also, there were several cloudy or rainy days throughout the experiment. Therefore, we speculate that the light incidence to the aquaria was not high enough to allow good periphyton growth. That supposition is supported by the results of a previous work done in our laboratory, in which expressively higher periphyton densities were observed on the plastic bottles. If the luminosity had been greater than the one actually verified, perhaps the periphyton could be capable of significantly removing TAN from water, as observed by Asaduzzman et al. (2008, 2009) and Milstein et al. (2009).

The effect of feeding rate on nitrite concentrations was surprising, because there was a significant increase of nitrite in the reduced-fed aquaria. One possible explanation could be that nitrification, the bacterial transformation of ammonia in nitrite and nitrite in nitrate subsequently, was incomplete due to dissolved oxygen shortage. Ma et al. (2010) also observed a decrease in nitrogen removal in municipal wastewater due to incomplete nitrification. In the present work, the heavy concentrations of organic matter in aquaria, indirectly estimated by the high results of water electric conductivity, diminished the concentrations of dissolved O_2 in water to values as low as 3.6 mg L^{-1} at 8:00 hours. Even lower DO_2 concentrations surely happened at dawn. The lack of sufficient luminosity in the present work probably lessened the photosynthetic activity both by phytoplankton and periphyton in aquaria. Similarly, the increase in periphyton area from 59.4 to 96.5% had no significant effect on nitrite concentrations in water. These results are in disagreement with those found by Asaduzzaman et al. (2009), who observed the filtering effect of periphyton mats on TAN and nitrite concentrations. Therefore, the present work's results indicate that the efficiency of periphyton as a biofilter is greatly impaired by low-light conditions such as those generally found in cloudy and rainy days. In those days, fish producers can have problems in substrate-based aquaculture systems with high TAN and nitrite in water

The concentrations of reactive phosphorus (≈ orthophosphate) significantly decreased in aquaria with 30% less feed in the two periphyton areas evaluated (59.4 and 96.5%). Moreover, there was a significant reduction of reactive phosphorus by the increase in the area for periphyton from 59.4 to 96.5% (Table 1). The artificial diet used for feed fish had roughly 1% phosphorus in its composition, and the main input of phosphorus to aquaria was the diet allowance. Consequently, the phosphorus input to aquaria decreased directly with the reduced feeding rates. The same relationship between dietary and water phosphorus was seen by Koko et al. (2010) in a study on rainbow trout, *Oncorhynchus mykiss*. Phosphorus is an essential nutrient both for phytoplankton and periphyton, which actively absorb it from water for their growth (PTACNIK et al., 2010). The increase in periphyton biomass in the 96.5%-area aquaria probably raised the absorption of waterborne phosphorus. That result is in agreement with Bratvold and Browdy (2001), who observed that periphyton lowered phosphorus in the overlying *Litopenaeus vannamei* culture water.

The maximum acceptable concentration of orthophosphate in water for fish culture is 0.2 mg L^{-1}. Beyond that level, the algal density in water can become dangerously high (BOYD; TUCKER, 1998). Looking at the results of reactive phosphorus presented in Table 1, it can be concluded that only the full-fed aquaria with 59.4% area for periphyton exceeded that limit. Thus, both the reduction of the feeding rates by 30% and the increase of the submerged area for periphyton to 96.5% worked to avoid that harmful concentrations of reactive phosphorus in water were reached. Accordingly, the lowest concentrations of reactive phosphorus in water were observed in the reduced-fed aquaria with 96.5% area for periphyton. Those results suggest that the combination of restricted feeding rates and substrates for periphyton growth is more valuable to attain better water quality for fish culture than the adoption of those managements alone.

At the end of the experiment, there was significantly lower periphyton biomass in the restricted-fed aquaria with 59.4% area for periphyton than in full-fed aquaria with 96.5% area for periphyton (Figure 1).

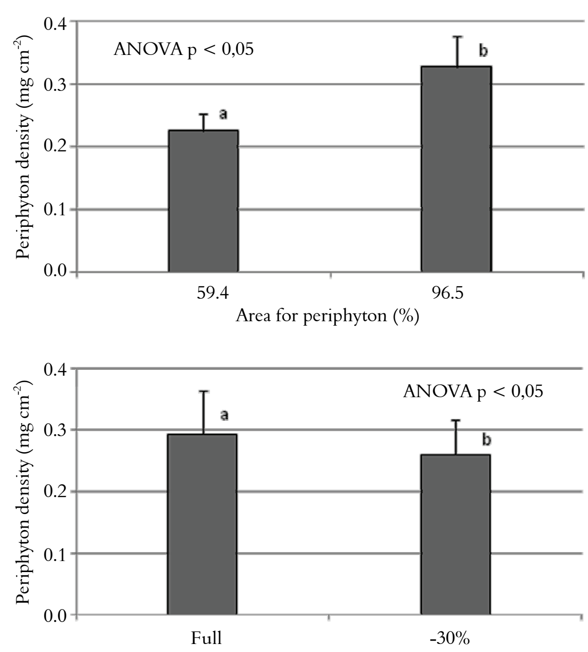

Figure 1. Periphyton density in Nile tilapia juvenile aquaria after five rearing weeks (dry matter basis). There were aquaria with eight or thirteen 180-cm² plastic bottles for periphyton development with submerged areas of 59.4 or 96.5% of the aquaria's surface water, respectively. In half of the aquaria, standard feeding rates were adopted; in the other half, the standard feeding rates were reduced by 30%. The distinct letters on the columns indicate that they are different by Tukey's test (3 fish/aquarium; mean ± s.d.; n = 5).

In the restricted-fed aquaria fish relied more on periphyton to satisfy their nutritional requirements. There seems to be an inverse relationship between artificial diet intake and periphyton intake by fish. As more artificial diet is available to fish, periphyton intake is reduced and vice-versa. Therefore, it is wise to reduce the delivery of artificial diets to fish reared in substrate-based aquaculture systems.

The final survival of fish was high in all aquaria (> 85%) and no significant differences were seen between the treatments. The reduction of feeding rates by 30% in the 59.4%-area aquaria did not significantly affect fish growth rate, as indicated by the results of final body weight, specific growth rate (SGR) and yield (Table 2). Feed conversion ratio (FCR) was significantly improved in the 59.4%-area aquaria submitted to feeding restriction when compared to the full-fed aquaria. Those results indicate that periphyton was capable to nutritionally compensate the partial withdrawal of artificial feed and maintain fish growth unaffected. Milstein et al. (2009) have also observed that periphyton counterbalanced the reduced feed supply (-40%) and that tilapia growth was only slightly reduced.

Table 2. Growth performance of Nile tilapia, *Oreochromis niloticus*, juveniles stocked at 25 L polyethylene outdoor aquaria provided with 180-cm² plastic bottles for periphyton development. Each aquaria were stocked with three juveniles (initial body weight = 2.56 ± 0.14 g and initial body length = 5.3 ± 0.2 cm) for five weeks. Fish were submitted to two different feeding regimes and there were two areas for periphyton growth (mean ± s.d.; n = 5).

Variable	Feeding rate[1]	Area for periphyton[2] 59.4%	Area for periphyton[2] 96.5%
Final body weight (g)	Full	11.2 ± 0.54 Aa[3]	10.2 ± 0.28 Ab
	-30%	10.5 ± 0.74 Aa	11.5 ± 0.76 Bb
Survival (%)	Full	91.7 ± 14.4	93.3 ± 14.9
	-30%	86.7 ± 18.3	100.0 ± 0.0
SGR[4] (% BW day^{-1})	Full	4.23 ± 0.14 Aa	3.95 ± 0.26 Ab
	-30%	4.05 ± 0.26 Aa	4.24 ± 0.12 Ba
Yield ($g\ m^{-2}$)	Full	131.7 ± 5.2 Aa	107.2 ± 15.9 Ab
	-30%	130.0 ± 11.3 Aa	138.1 ± 9.2 Ba
FCR[5]	Full	1.80 ± 0.18 Aa	2.55 ± 0.37 Ab
	-30%	1.28 ± 0.15 Ba	1.32 ± 0.03 Ba

Two-way ANOVA P					
Factor	BW	Surv	SGR	Yield	FCR
Feeding rate	ns[6]	ns	ns	0.009	< 0.001
Area for periphyton	ns	ns	ns	ns	0.001
Feed *x* Area	0.002	ns	0.021	0.005	0.003

[1]Full: The standard feeding rates recommended by the laboratory's feeding table were entirely adopted; -30%: the standard feeding rates were reduced by 30%, [2]Eight or thirteen small plastic bottles (total surface area = 1440 and 2340 cm², respectively; 59.4 and 96.5% of the aquarium's surface area, respectively) were submerged into selected aquaria to periphyton development, [3]For the same variable, means not sharing the same lower case or capital letter in a row and column, respectively, are significantly different by Tukey's test ($p < 0.05$); means without any letter are not significantly different, [4]Specific growth ratio (SGR, % body weight per day) = [(ln final body weight - ln initial body weight)/nº of days] x 100, [5]Feed conversion ratio = feed allowance (g)/fish weight gain (g), [6]Not significant ($p > 0.05$).

Surprisingly, final body weight, SGR and yield in 96.5%-area aquaria submitted to 30% less feed were significantly higher than in 96.5%-area aquaria submitted to full feeding rates. In that case, there was a synergistic effect between periphyton and water quality to boost fish growth. There was enough periphyton biomass in the 96.5%-area aquaria to maintain fish growth under feeding restrictions. Moreover, fish growth in the restricted-fed aquaria was favored by their better water quality (higher pH and lower TAN). Azim et al. (2002) in a polyculture of rohu (Labeo rohita), catla (Catla catla) and Kalbaush (L. calbasu) have also found significantly higher SGR for catla reared in the substrate-based earthen ponds than in the supplemental-feeding-based ponds. Hence, the periphyton development in aquaculture rearing systems is doubly valuable to fish culturists for its nutritional richness and water quality improving effect.

The increase in the area for periphyton development from 59.4 to 96.5% had different effects on tilapia growth performance, depending on the feeding rate (full or full minus 30%). There were negative effects on final body weight, SGR, yield and FCR in full-fed aquaria when the area for periphyton increased from 59.4 to 96.5%; conversely, final body weight was improved in restricted-fed aquaria with 96.5% area for periphyton when compared to restricted-fed aquaria with 59.4% area for periphyton (Table 2). Those results reinforce the recommendation to reduce supplemental feeding allowance to fish reared in substrate-based aquaculture systems. The presence of periphyton in the rearing units means more organic matter in water, which increases their biochemical oxygen demand (VESILIND, 2003). Therefore, it seems necessary to offset the organic matter increase caused by periphyton growth with the reduction of artificial diet allowance to fish.

Conclusion

The combination of restricted feeding rates with large substrates for periphyton growth is more valuable to attain better water quality for fish culture than the adoption of those managements alone.

The substrate-based aquaculture systems can be useless or even detrimental to fish growth if not followed by restricted feeding managements.

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