# The weight gain and survival of genetically improved farmed tilapia reared in septic tank-high rate algal pond effluent

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**Abstract**. In order to study the effect of total ammonia nitrogen (TAN) and the fish stocking densities (SD) on fish survival and weight gain, a 22 weeks experiment was carried out. Genetically improved farmed tilapia (GIFT) juveniles were reared in septic tank-high rate algal pond effluent and the combination of three TAN surface loading rates (0.6; 1.2 and 2.4 kg TAN $ha^{-1}$ $d^{-1}$) and three fish SD (3; 6 and 12 fish $m^{-2}$) was evaluated. Fish weight gain was influenced by fish SD but not by ammonia loading. Juveniles total weight gain varied from 3.5 to 117.5 g, with the highest values (up to an equivalent to 0.84 g $d^{-1}$ as daily gain) being recorded with the lowest fish stocking density. Fish mortality rates varied widely, reaching as high values as 87.5%, or even full mortality. However, mortality events were more clearly associated with algal blooming than with ammonia loads. Notwithstanding, fish productivity was estimated at 5.34 ton $ha^{-1}$.$year^{-1}$ (at the best), using the wastewater natural plankton population as the only food source.
**Key Words**: high rate algal ponds, septic tank, sewage-fed fish culture, tilapia rearing, water reuse.

**Introduction**. Septic tanks are units that carry out the multiple functions of sedimentation and removal of floatable materials, besides acting as a low-rate digester; they are still extensively used all over the world and constitute one of the main alternatives for the primary treatment of sewage from residences and small areas (Chernicharo 2022). Waste stabilization ponds (WSP) are deemed as a suitable and efficient means of wastewater treatment relying on little technology and minimal maintenance, especially in warm-climate regions and where land is available (Peña & Mara 2004). High rate algal ponds (HRAP) are an advanced type of pond - paddlewheel-mixed, shallow, open raceway ponds - developed for the treatment of wastewater and resource recovery as harvestable algal/bacterial biomass for beneficial use as fertilizer, feed or biofuel (Craggs et al 2012).

The practice of resources recovering from using wastewater, e.g in wastewater-fed aquaculture, not only reduces the pollution load into water bodies but also makes a continuous system of food production (Kumar et al 2014; Kumar et al 2015). Nile tilapia (*Oreochromis niloticus*) has become one of the most important fish in aquaculture worldwide (FAO 2014), in part due to its omnivorous diet and the ability to feed on a low trophic level, tolerance of high stocking density, and rapid growth (Watanabe et al 2002). Hence, and because they have lesser demand of oxygen (Singh & Chaube 2021), it is not a surprise that tilapia is also a common choice for wastewater-fed aquaculture (Pescod 1992) and that has long been reported either in full-scale cases or in research experiments (Bunting 2004; Moscoso 1996; Edwards et al 1981; Mohapatra et al 2012; El-Shafai et al 2013).

However, one of the main constraints regarding wastewater use in fish culture is ammonia toxicity (Randall & Tsui 2002); some studies have even reported mass fish mortality due to ammonia toxicity in sewage-fed systems for tilapia raising, either as monoculture or polyculture (Wrigley et al 1988; El-Gohary et al 1995; Khalil & Hussein 1997). Nonetheless, information on design or operational criteria for wastewater-fed fish culture systems based on ammonia surface loading rates is still lacking. A total nitrogen loading rate of 4 kg N $ha^{-1}$ $d^{-1}$ has been indicated (Mara et al 1993), but as an optimal nitrogen loading rate, i.e. aiming at providing enough nitrogen to support algal biomass growth and fish yields, at the same time as avoiding excess nitrogen and consequently too high concentration of algae and risk of dissolved oxygen depletion at night and fish kills. Regarding ammonia toxicity Mara et al (1993) suggested that free ammonia in fish ponds should be below 0.5 mg $L^{-1}$, which is reinforced by the literature on tilapia culture (El-Sayed 2006).

Recently, in pilot experiments in Brazil, Sánchez et al (2018) suggested that tilapia juveniles culture using HRAP effluent with fish stocking density of 6 fish $m^{-2}$ and ammonia surface loading rate of 1.2 kg $ha^{-1}$ $d^{-1}$ was possible. However, high sensitivity to ammonia and wide daily temperature variations lead to high mortality rates; at lower water temperatures, the tilapia juveniles showed no mortality, but very low weight gain. Thus, the authors concluded that feasibility of tilapia juveniles rearing was still to be confirmed over warmer temperatures than those tested then. This is essentially the objective of the present paper, which, in a way, follows up the work of Sánchez et al (2018). The effect of three ammonia surface loading rates (SLR) and three fish stocking densities on the growth and mortality of tilapia cultured in tanks fed with an effluent of HRAP, using plankton as the only food source, were tested here.

## Material and Method

***Description of the study site***. The experiment was carried out from October of 2017 to March of 2018 in Viçosa, State of Minas Gerais, Brazil (20°45’14” S, 42°52’54” W; average altitude = 648 m), at an experimental site in the University of Viçosa. This Brazilian town presents a humid subtropical climate (according to the Köppen classification), with average, maximum and minimum temperatures of 19.8, 32.4 and 7.2°C, respectively; annual average relative humidity and precipitation of, respectively, 81%, and 1221.4 mm - spread over rainy (spring-summer) and dry (autumn-winter) seasons.

***Experimental set up and the fish***. Domestic sewage was collected fortnightly, using a submersible pump, from a septic tank of a local small wastewater treatment plant. This effluent was transported to the experimental site, where it was transferred to 3000 and 5000 L reservoirs, which fed fiberglass HRAP with the following characteristics: length = 2.86 m, width = 1.28 m, free board = 0.2 m, pond depth = 0.3 m, surface area = 3.3 $m^2$, volume = 1 $m^3$. The ponds stainless steel paddlewheels were driven by a 1 hp electric motor, whose rotation was controlled by a frequency inverter (WEG, series CFW-10) to provide a mean horizontal water velocity between 0.10 and 0.15 m $s^{-1}$. The HRAP were operated at an average hydraulic retention time (HRT) of 7 days.

The HRAP effluent fed 18 fish rearing plastic tanks with 210 L as useful volume (surface area = 0.665 $m^2$). Three groups of six tanks randomly distributed received, each, a continuous flow rate so that three different ammonia surface loading rates (SLR) were tested: SLR1 = 0.6, SLR2 = 1.2 and SLR3 = 2.4 kg TAN $ha^{-1}$ $d^{-1}$. The lowest loading rate figure was chosen based on values published in the literature of sewage-fed fish ponds (Bastos et al 2003), and in order to evaluate the effect of higher levels two and four times the lowest value were applied. The wastewater flow applied to the rearing tanks resulted in the following average HRT: SLR1 = 55 d, SLR2 = 27.5 d, and SLR3 = 13.8 d.

Juveniles of GIFT were reared using three stocking densities (D1 = 2, D2 = 4 and D3 = 8 fishes per tank, corresponding respectively to 3, 6 and 12 fishes per $m^2$). D1 was based on suggestions from Edwards et al (1981) and Bastos et al (2003); D2 and D3, twice and four times D1, were used taking into account the high phytoplankton concentration of HRAP effluents. Thus, based on the combination of these two factors levels (ammonia surface loading rates and fish stocking densities), nine treatments with two repetitions were evaluated in a fully crossed factorial design: T1: SRL1-D1, T2: SRL1-D2, T3:

SRL1-D3, T4: SRL2-D1, T5: SRL2-D2, T6: SRL2-D3, T7: SRL3-D1, T8: SRL3-D2, and T9: SRL3-D3. Figure 1 presents, schematically, the experimental set up.

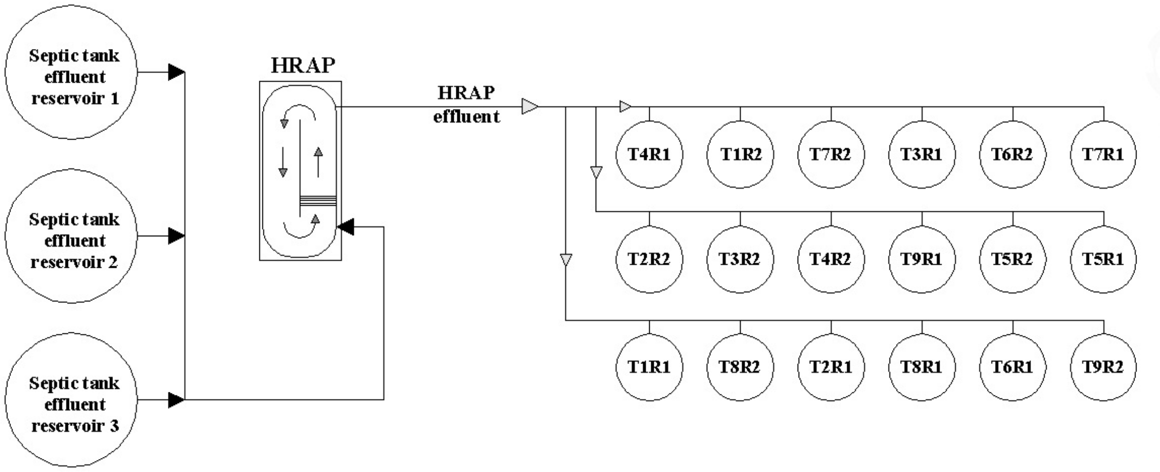


Figure 1. Sewage reservoirs, HRAP and fish rearing tanks.

The wastewater rearing tanks were stocked with GIFT tilapia juveniles with an average initial weight of 7.55±0.58 g. The fishes were previously reared in a recirculating aquaculture system and fed with commercial fish feed with 34% dietary protein.

Fish were weighed at the beginning and at the end of the experiment in order to calculate the average total weight gain for each treatment, as well as the daily weight gain (total weight gain/duration of the experiment). Fish tanks were monitored twice a day in order to monitor fish mortality, and to remove dead animals. Both weight gain and mortality data met the assumptions of normality and homogeneity; thus the parametric test of analysis of variance was applied to look at differences between treatments using the software R 3.1.0. Correlation between fish weight gain and water quality parameters was tested using the Pearson test.

***Water quality parameters***. During the experiment, 0.5 L-samples were taken from the HRAP and from each fish tank to measure the following physical and chemical variables according to the recommendations of APHA et al (2012) (the methods used are within brackets): dissolved oxygen (DO) - membrane electrode method (4500-O G), pH - electrometric method (pH: 4500-H+ B), temperature (2550 B). pH, DO and temperature were monitored using a Hach portable meter, model HQ40d. Additionally, 1 L-samples were taken in the same points, and preserved in 5% acetic acid Lugol's iodine solution for plankton quantitative and qualitative analysis. Due to the high phytoplankton concentrations, subsamples of 1 mL were diluted with 9 mL of distilled water and the organisms' densities were determined in 1 mL aliquot in a Sedgewick-Rafter counting chamber using a cx31 Olympus optical microscope at an eyepiece magnification of 10× and an objective magnification of 40×.

DO, pH, and temperature were monitored fortnightly in the septic tank and twice a day in the HRAP and in the 18 fish tanks (composite samples of each SLR treatment).

***Productivity***. The productivity of GIFT tilapia cultured in the HRAP effluent fed tanks was calculated using equation 1:

$$\text{Prod.} = \left(\frac{IW + (WG * t)}{1000}\right) * \left(SD * (1 - M/100) * cf\right) \quad (1)$$

where: Prod. = productivity (kg $ha^{-1}$ $yr^{-1}$);

IW = average fish initial weight (g);

WG = weight gain per time unit (g $d^{-1}$);

t = rearing period of time (d);

SD = stocking density (fish $m^{-2}$);

M = average mortality (%);

cf = conversion factor from $m^2$ to hectares = 10,000.

**Results**. The values for DO, pH and temperature of the sewage in the wastewater treatment-fish rearing system during the experiment are presented in Table 1.

Table 1
Average and standard deviation values of DO, pH and temperature in the HRAP effluent and in the rearing tanks

| *Treatments* | *DO (mg $L^{-1}$)* | *pH* | *Temperature (°C)* |
|---|---|---|---|
| HRAP | 7.74±2.31 | 6.85±0.99 | 23.85±3.20 |
| T1 (SLR1-D1) | 9.55±4.01 | 8.35±1.61 | 25.38±3.49 |
| T2 (SLR1-D2) | 9.27±3.92 | 8.31±1.42 | 25.35±3.39 |
| T3 (SLR1-D3) | 8.84±3.48 | 8.16±1.34 | 25.55±3.46 |
| T4 (SLR2-D1) | 9.13±4.37 | 8.21±1.39 | 25.15±3.45 |
| T5 (SLR2-D2) | 10.63±4.87 | 8.81±1.46 | 25.81±3.51 |
| T6 (SLR3-D3) | 10.99±5.05 | 8.84±1.44 | 25.95±3.52 |
| T7 (SLR3-D1) | 9.39±4.72 | 8.14±1.33 | 25.60±3.49 |
| T8 (SLR3-D2) | 11.50±5.05 | 9.00±1.36 | 25.83±3.61 |
| T9 (SLR3-D3) | 11.21±5.04 | 8.94±1.30 | 25.68±3.58 |

The highest DO concentrations were: 17.05 mg $L^{-1}$ in the HRAP effluent; 19.8; 20.4 and 21.0 mg $L^{-1}$ in T1, T2 and T3 fish tanks, respectively; and 22 mg $L^{-1}$ in T4 to T9 tanks, revealing, therefore, intense photosynthetic processes. However, DO concentrations as low as 1.81 (HRAP); 3.30 (T1); 2.11 (T2); 1.61 (T3); 1.97 (T4), 1.48 (T5); 1.99 (T6); 0.58 (T7); 0.52 (T8) and 1.85 mg $L^{-1}$ (T9) were also recorded.

Table 2 shows the values for total phytoplankton in the wastewater treatment-fish rearing system.

Table 2
Average and standard deviation values of total phytoplankton density (cells x $10^3$ $mL^{-1}$) in the HRAP effluent and in the fish tanks

| *HRAP* | *T1* | *T2* | *T3* | *T4* |
|---|---|---|---|---|
| 2,794.03±3,419.87 | 1,326.41±734.25 | 1,152.36±746.15 | 1,621.22±1,443.61 | 1,351.17±1,305.45 |
| *T5* | *T6* | *T7* | *T8* | *T9* |
| 1,650.66±1,258.39 | 1,325.93±617.85 | 1,613.12±829.42 | 1,398.68±1,592.39 | 1,600.86±1,275.62 |

Table 3 shows the mean phytoplankton densities found in the system (in cells x $10^3$ $mL^{-1}$), by taxonomic classes.

Table 3
Mean phytoplankton densities (cells x $10^3$ $mL^{-1}$) in the HRAP effluent and in the fish tanks

| *Taxonomic class* | *HRAP* | *T1* | *T2* | *T3* | *T4* |
|---|---|---|---|---|---|
| Chlorophyceae | 1,839.33 | 1,205.55 | 1,104.34 | 1,506.91 | 364.91 |
| Euglenophyceae | 15.30 | 33.19 | 43.82 | 41.40 | 7.49 |
| Cyanophyceae | 62.55 | - | 33.60 | 227.61 | 455.20 |
| Bacillariophyceae | 53.55 | 18.77 | 21.65 | 19.08 | 21.19 |
| Cryptophyceae | 2,066.53 | 161.80 | 20.06 | 70.60 | 1,937.10 |
| Dinophyceae | - | 2.06 | - | - | 24.94 |
| | *T5* | *T6* | *T7* | *T8* | *T9* |
| Chlorophyceae | 820.94 | 1,021.49 | 1,316.28 | 1,288.66 | 1,501.34 |
| Euglenophyceae | 71.49 | 10.97 | 24.64 | 10.59 | 7.31 |
| Cyanophyceae | 4,236.96 | 617.00 | 711.49 | 635.10 | 501.16 |
| Bacillariophyceae | 344.60 | 24.43 | 78.85 | 12.47 | 17.60 |
| Cryptophyceae | 44.05 | 36.43 | 592.03 | 20.39 | 45.41 |
| Dinophyceae | 3.66 | 31.68 | - | 3.26 | 2.13 |

Table 4 shows the values of fish weight gain in the nine treatments.

Table 4
Average and standard deviation values of fish weight gain (grams) according to fish stocking densities and total ammonia N surface loading rate

| *Stocking densities* | *Total ammonia N surface loading rate (kg TAN ha$^{-1}$ d$^{-1}$)* | | |
|---|---|---|---|
| *(fish m$^{-2}$)* | *0.6 (SRL1)* | *1.2 (SRL2)* | *2.4 (SRL3)* |
| 3 (D1) | 43.66±25.65 | 117.51* | 19.83 * |
| 6 (D2) | 16.14* | 20.04±11.17 | 11.47±6.15 |
| 12 (D3) | 3.54* | 4.82±0.03 | 15.89* |

(*) Standard deviation values were not reported due to total mortality of fish in one of the replicates.

Productivity of GIFT tilapia was estimated at 2.67 ton ha$^{-1}$ yr$^{-1}$; calculated on the following basis: (i) SLR = 1.2 kg TAN ha$^{-1}$ d$^{-1}$; (ii) fish weight gain = 0.84 g d$^{-1}$; (iii) average initial fish weight = 5 g; (iv) fish culture over six month per year in a temperate climate; (v) mortality rate = 43.00%, as a result of the average mortality of 50.00% recorded in treatments with SD = 3 fish per m$^2$, and 35.40% mortality with SLR = 1.20 kg TAN ha$^{-1}$ d$^{-1}$. In tropical climate regions, where fish can be cultivated during the whole year, the expected productivity could be as high as 5.34 ton ha$^{-1}$ yr$^{-1}$.

The average mortality rates recorded over the experiment were: 25.00, 75.00 and 50.00%, respectively for T1, T2 and T3 treatments; 50.00, 12.50 and 0.00%, respectively for T4, T5 and T6 treatments; 75.00, 18.75 and 87.50%, respectively for T7, T8 and T9 treatments. Full mortality was registered in five out of the 18 rearing tanks, in one of the replicates of T3, T4, T5, T7 and T9 treatments.

**Discussion**. Apparently, Chlorophyceae and Cryptophyceae organisms were preferably consumed by the fish (Table 3), as their numbers in the rearing tanks decreased in relation to those found in the HRAP. Conversely, Euglenophyceae, Cyanophyceae and Bacillariophyceae organisms sometimes increased in the rearing tanks, while Dinophyceae organisms, which were absent in the HRAP, appeared in some of the fish tanks.

The daily fish weight gains over 140 days varied from 0.03 to 0.31 g d$^{-1}$ in SLR1 treatments, from 0.03 to 0.84 g d$^{-1}$ in SLR2 treatments, and from 0.08 to 0.14 g d$^{-1}$ in SLR3 treatments. The ANOVA indicated no interactions between SLR and fish stocking densities, and no significant effects of SLR alone, however significant effects were recorded for stocking density on fish weight gain (p-value ≈ 0.01); the Tukey test showed that the best results, which were recorded in treatments with the lowest stocking density (3 fish m$^{-2}$), were significantly higher than those of the other treatments (D2 and D3).

The best weight gain values recorded here were higher than those reported for tilapia rearing in waste stabilization ponds or in sewage fed tanks by other authors, e.g.: 0.31 g d$^{-1}$ in cages inside secondary facultative and maturation ponds (Meadows 1983); 0.41 g d$^{-1}$ in tanks fed with waste stabilization pond effluent (Balasubramanian et al 1995) and 0.26 g d$^{-1}$ in tanks receiving maturation ponds effluent (Freitas 2006). On the other hand, were lower than those reported by Sin & Chiu (1987): 1.57 g d$^{-1}$ in maturation ponds operated with continuous flow; Buras et al (1987): 1.60 g d$^{-1}$ in ponds filled with a mixture of effluent from algae production, rain water and some extended aeration effluent, the evaporation water in the experimental ponds being replaced daily with extended aeration effluent; and Santos et al (2009): 1.74 g d$^{-1}$ in tanks fed with a maturation pond effluent operated in a batch regime based on fortnightly substitution of 50% of tanks volume with the pond effluent. The lower weight gain values reported here may be explained by the fact that the bigger juveniles (thus more resistant to water quality variations) were utilized by Sin & Chiu (1987): initial weight 41 g and by Buras et al (1987): initial weights between 24 to 42 g. In the study of Santos et al (2009), the use of a more diluted wastewater may have favored uniformity of the water quality, reducing, therefore, potential constraints over the fish growth.

Surprisingly at first sight, a negative (but weak) correlation was found between fish weight gain and DO (r = -0.381). It should be noted though that, as suggested by Wang et al (2009), dynamic changes in oxygen levels may increase the usual fish metabolic rate and reduce the amount of energy available for growth. Thus, the DO figures shown in Table 1 may have led to this kind of effect, as treatments with the highest DO concentrations also presented the highest standard deviations values. A negative (weak) correlation was also found between fish weight gain and pH (r = -0.379). In this case, the intense photosynthetic activity leads to high pH values in the tanks, increasing the unionized ammonia fraction and, consequently, the toxicity on fish, inhibiting natural food consumption and limiting the weight gain (El-Sherif & El-Feky 2008). These same trends had been previously noted by Sánchez et al (2018).

Correlation analyses between fish weight gain and densities of phytoplankton organisms revealed the following results (Pearson correlation coefficients): r = -0.780 for Chlorophyceae, r = -0.267 for Euglenophyceae, r = -0.098 for Cyanophyceae, r = –0.097 for Bacillariophyceae, r = 0.930 for Cryptophyceae, and r = 0.416 for Dinophyceae. These results suggest that plankton was selectively used by the fish, as previously noted by other authors (Micha et al 1996; Sousa 2007). The strong positive correlation between fish weight gain and Cryptophyceae organisms may have been impacted by treatment T4, which presented the highest cell count (1,937,100 cells $mL^{-1}$) simultaneously with the highest fish weight gain.

The productivity of GIFT tilapia estimated for tropical climate regions as 5.34 ton $ha^{-1}$ $yr^{-1}$ is higher than the productivity for tilapia reported by Sin & Chiu (1987) = 1.08 ton $ha^{-1}$ $yr^{-1}$ in maturation ponds; Wrigley et al (1988) = 3.00 ton $ha^{-1}$ in a batch operated series of oxidation ponds; Das et al (2022) = 1.5 to 2.0 ton $ha^{-1}$ $yr^{-1}$ in ponds fertilized with wastewater; da Silva et al (2000) = 1.71 ton $ha^{-1}$ $yr^{-1}$ in maturation ponds; Abdul-Rahaman et al (2012) = 0.27 ton $ha^{-1}$ $yr^{-1}$ in submersed cages in facultative and aerated ponds. On the other hand, the productivity estimated here is similar to findings reported by Moscoso (1996) = 4.40 ton $ha^{-1}$ $yr^{-1}$ (maximum) in tanks receiving a tertiary pond effluent; Bastos et al (2003) = 3.90 ton $ha^{-1}$ per production cycle in a tank receiving the effluent of a three polishing ponds series, Pereira & Lapolli (2009) = 3.94 ton $ha^{-1}$ per production cycle in raceway tanks with airlift aeration fed with a facultative pond effluent. Finally, the calculated productivity figures are lower than those reported by Edwards et al (1981) = 16.00 ton $ha^{-1}$ $yr^{-1}$ in HRAP effluent and Santos et al (2009) = 6.20 ton $ha^{-1}$ $yr^{-1}$ in tanks operated in batch with a maturation pond effluent.

No clear trend of mortality was noted, neither due to different levels of SLR nor to stocking density levels. Mortality events occurred mostly in tanks that developed thick layers of phytoplankton, which broke up / died out following intense / long term rainfall events, leading to organic matter decomposition, DO depletion and / or release of high ammonia concentrations. This is in line with the well-known fact that fish mortality in fish ponds may be due to oxygen depletion, high pH and / or high ammonia levels associated with algal blooms (Padmavathi & Prasad 2007).

**Conclusions**. Daily fish weight gains varied widely, from 0.03 to 0.84 g $d^{-1}$. Fish weigh gain was influenced by fish stocking densities (the best results being recorded at the lowest stocking density – 3 fish per $m^2$), but not by ammonia loading. In addition, no interactions between ammonia loads and fish stocking densities on fish weight gain were recorded. Fish mortality rates varied widely too (from 25.00 up to 87.50%, but also including cases of null and full mortality), but with no clear influence of neither ammonia loading nor fish stocking densities.

Tilapia juveniles rearing using tanks fed with HRAP effluent showed to be a feasible practice. Using the wastewater natural plankton population as the only food source supported estimated fish productivity up to 5.34 ton $ha^{-1}$ $year^{-1}$.

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**Conflict of interest**. The authors declare that there is no conflict of interest.

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