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The effects of dietary lipid and fibre levels on digestibility of diet and on the growth performance of sharpsnout seabream (*Diplodus puntazzo*)

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Abstract

In the present study, sharpsnout seabream (*Diplodus puntazzo*) were fed three experimental isonitrogenous diets composed of 45 g 100g⁻¹ protein and varying lipid and fibre contents as follows: diet A: 45/10/1.5, B: 45/15/1.5 and C: 45/15/5. The effects of the diet composition were investigated by measuring digestibility, growth, carcass composition and haematological parameters. The apparent digestibility coefficients (ADCs) for proteins, fats and carbohydrates, measured at high (26 °C) and low (16 °C) water temperatures in laboratory conditions, were not affected by the diet or temperature treatments. Growth was evaluated in a seven-month trial using animals held in sea cages. The specific growth rate (SGR) showed no significant differences among the treatments while the feed conversion ratio (FCR) was significantly improved in the fish that were fed a low-fat diet. A significant increase in body fat was detected in the fish that were fed high-fat diets. The blood serum total lipid levels were elevated for the fish that were fed diet C. In conclusion, a diet containing a protein/fat/fibre ratio of 45/10/1.5 g 100 g⁻¹ can result in satisfactory growth and an improved FCR value when compared with diets with higher fat and/or fibre levels, demonstrating that the required fat level for sharpsnout seabream is lower than 15 g 100 g⁻¹.

Keywords: *Diplodus puntazzo*; Sharpsnout seabream; Digestibility; Fibre; Nutrition.

Introduction

The high production of the two most popular marine fish species cultivated in the Mediterranean region, gilthead seabream

(*Sparus aurata*) and European seabass (*Dicentrarchus labrax*), brought about a reduction of the market price to levels that are, in many cases, lower than the production cost. In the short history of the cultivation

of these species, this reduction has twice led to serious profitability problems for fish farmers, first in 2001 and more recently in 2008 (HOUGH, 2009). To overcome such situations, one option for the fish farming industry is to move towards species diversification.

Sharpsnout seabream (*Diplodus puntazzo*) is a sparid candidate that has received attention in the past (BERMUDEZ *et al.*, 1989; DIVANACH *et al.*, 1993). The growth and pre-growth pilot cultivation of sharpsnout bream resulted in promising results, demonstrating their high growth rates and adjustability to intensive rearing conditions (ABELLAN & ALCAZAR, 1995; GATLAND, 1995; HERNÁNDEZ *et al.*, 2001; HERNÁNDEZ *et al.*, 2007), although production levels have remained low. In addition, reproduction of the seabream is currently successfully carried out in several hatcheries in the Mediterranean area. The feeding of this species is based on diets that were formulated for gilthead seabream because the knowledge of the nutritional requirements of the sharpsnout bream is still limited.

A significant accumulation of fat has been observed in cultured sharpsnout seabream and is probably the result of the high fat content of the feed (17-22 g 100g⁻¹), indicating that the dietary lipid level should be lower for this species (PARPOURA *et al.*, 1993). Hence, there is a need for research into the requirements for the rearing of this species to achieve the optimum growth performance.

Dietary fibre can be categorised into lignin and non-starch polysaccharides (NSPs) (KNUDSEN, 1997), which are commonly found in plant cell walls (KNUDSEN, 2001) and are divided into water-soluble NSPs (S-NSPs) and water-insoluble NSPs (I-NSPs). Soluble NSPs typically cannot be digested by fish due to the lack of specific digestive

enzymes, but they can be fermented by intestinal flora (DABROWSKI & GUDERLEY, 2002). The presence of the S-NSPs reduces the evacuation rate and increases the evacuation time of the digesta in the intestine (VAN DER KLIS & VAN VOORST, 1993; quoted in CHOCT, 1997) while I-NSPs have the opposite effects (KIRWAN *et al.*, 1974).

Excessive fibre in aquaculture diets may lead to a decrease in feed utilisation by obstructing the action of digestive enzymes and diluting the nutrient density (BOOTH *et al.*, 2001). NSPs may reduce the utilisation of other nutrients as they can bind water and minerals and absorb compounds such as sterols and acids (KROGDAHL *et al.*, 2005). In some cases, these properties can be beneficial to the animals, although more frequently these characteristics have negative impacts on nutrient utilisation (KROGDAHL *et al.*, 2005).

The determination of the optimum ratio between the main nutrients in fish feed is of major importance for the successful formulation of diets for cultivated species (AKSNES, 1995), and higher feed utilisation reduces both the cost and environmental impact of aquaculture waste. HERNANDEZ *et al.* (2001) reported that the optimum dietary protein/energy ratio for the growth of sharpsnout seabream is lower than 22 g of protein MJ⁻¹. Levels of protein that are higher than optimal can reduce growth rates, suppress the immune response and increase fat deposition (DANIELS & ROBINSON 1986). Although the sharpsnout seabream is an omnivorous species (HERNANDEZ *et al.*, 2002), a preference for a protein-rich diet has been described; the optimum protein/fat ratio in sharpsnout seabream diets was reported to be 47/10 by ATIENZA *et al.* (2004). However, this result was later challenged by VIVAS *et al.* (2006), who sug-

gested a ratio of 63/19 when the fish were self-selecting the macronutrients (protein and fat). In both of the studies, the fish were fed using demand feeders. The protein/energy level was investigated in sharpsnout seabream by HERNANDEZ *et al.* (2001), and these authors concluded that the fish that were fed lower protein/energy ratios ($24.2 < 23.2 < 21$ g of protein MJ⁻¹) had higher growth rates. In addition, the inclusion of plant protein-rich meal, such as soybean meal, forming up to 40 g 100g⁻¹ of the diet has been shown not to affect the growth performance of either adult or juvenile fish (HERNANDEZ *et al.*, 2007).

The main aim of this work was to determine the optimum lipid and fibre levels in experimental sharpsnout seabream diets

to improve the growth and feed utilisation of this species. Additionally, the digestibility coefficients and haematological indices were determined. The study was carried out in sea cages under conditions of intensive culture to replicate the normal conditions during commercial exploitation.

Materials and Methods

Experimental diets

Three isonitrogenous diets were formulated to contain 45 g 100g⁻¹ protein, 10-15 g 100g⁻¹ fish oil and 1.5-5 g 100g⁻¹ fibre in the following ratios: diet A: 45/10/1.5, diet B: 45/15/1.5 and diet C: 45/15/5 (Table 1). Fish, soybean and corn gluten meals were used as the protein sources, and wheat meal

Table 1
The diet formulation and chemical composition of the experimental diets fed to sharpsnout seabream (*Diplodus puntazzo*). The diet composition, in % of protein/fish oil/fibre, was as follows: A, 45/10/1.5; B, 45/15/1.5; and C, 45/15/5.

Ingredients (g 100 g ⁻¹)	Diet A	Diet B	Diet C
Fish meal LT	39.0	38.0	37.0
Soybean meal	4.0	7.0	-
Corn gluten meal	15.0	15.0	15.0
Wheat meal	32.15	25.12	15.2
Sunflower meal	-	-	17.5
Marine oil	3.0	8.0	8.5
Collagen	4.0	4.0	4.0
Methionine	0.35	0.38	0.3
Vitamins and minerals ¹	2.5	2.5	2.5
Proximate composition (g 100 g ⁻¹)			
Moisture	8.8	8.3	8.4
Crude protein	45.2	45.1	45.3
Crude fat	10.1	14.8	15.3
Ash	8.9	8.8	9.1
Fibre	1.5	1.5	5.0
NFE ²	25.4	21.4	16.9
Gross energy (kJ/100 g ⁻¹ feed)	1905.6	2021.1	1965.4

¹ Vitamins and minerals as described by NENGAS *et al.* (1995)

² Nitrogen-free extract

was used as a carbohydrate source. In addition, sunflower meal was added to diet C to increase the fibre level and balance the protein level. The feeds were manufactured by Perseas S.A. (Zevgolatio - Korinthos, Greece) in a single-screw extruder. For the digestibility trial, the feeds were ground to 0.5 mm, blended thoroughly with 1 g 100g⁻¹ chromic oxide, moistened to produce a dense paste and passed through a mincing machine (Hobart food mixer, model n. A120; Hobart Manufacturing Company Ltd., London UK) that was fitted with a 3-mm pellet die. The diets were then air dried (<40°C) to a moisture content of about 6 g 100g⁻¹ and stored frozen (-20°C) until use.

Digestibility trial

A total of 135 sharpsnout seabream with an average weight of 100 g were transferred from a commercial farm (Kalloni Ltd. farm, Peloponnisos, Greece) to 150-L cylindrical tanks in the facilities of the Hellenic Centre for Marine Research and were acclimatised for three weeks before the sampling. The digestibility trials were carried out in triplicate for diets A, B and C. The faeces were collected in a trap using a modified Guelph method as described by CHO *et al.* (1982). The experiments were carried out at temperatures representative of both summer (25-27°C) and winter (15-17°C) conditions. The fish were fed to apparent satiation twice a day, once at 9:00 h and once at 16:00 h, and care was taken to ensure the completeness of intake. After each feeding, each tank was thoroughly cleaned to remove any uneaten feed. The faecal samples were collected prior to the first daily feeding every morning for 12 days. The faecal samples from each tank were pooled every 3 days combining the 4 subsamples per tank for a total of 12 combined samples, centrifuged

and kept at -20°C before being freeze-dried and analysed.

Growth trials and sampling

Fish with an initial average weight of 3.4±0.2 g were supplied by a commercial hatchery in Greece. The growth experiment was carried out under farm conditions during a field study at the Kalloni Ltd. farm (Peloponnisos, Greece).

The fish were randomly distributed into triplicate groups among the rearing net cages at a density of 700 fish per cage of 50 m³. The fish were acclimatised for a period of 10 days, during which they were fed a standard gilthead seabream diet with 45 g 100g⁻¹ protein and 16 g 100g⁻¹ fat. The 7-month experimental period started in May and ended in December. The temperature range was from 17 to 26°C during the experiment. The fish were carefully hand-fed to apparent satiation, ensuring the completeness of the intake. Fifty per cent of the population from each cage was weighed in groups of 10 fish each at bimonthly intervals.

Of the remaining stock from the initial population, 50 fish were sacrificed and analysed for their initial whole body composition. At the end of the experimental period, all of the fish were weighed after moderate anaesthesia with phenoxyethanol and alcohol (1:1). Twenty fish per replicate tank were sampled for their whole body composition, liver weight and liver fat. Blood samples were taken with a heparinised syringe from the caudal vein of 10 fish per replicate tank after moderate anaesthesia. The serum was collected after centrifugation at 3000 rpm for 20 min and stored frozen at -70°C until the haematological measurements were evaluated.

Haematological parameters

Ten fish per cage were sampled 24 h after the last meal. The blood samples were

centrifuged, and the serum was analysed for glucose (mg 100 mL⁻¹), total protein (g 100 mL⁻¹), cholesterol (mg 100 mL⁻¹) and triacylglycerols (mg 100 mL⁻¹). Glucose was measured using the glucose oxidase method (GOD-PAP, cat. No. 10260, HUMAN), protein was measured using the photometric method of Biuret, (cat. No. 10570, HUMAN) and cholesterol and triacylglycerols were measured photometrically using the Lipid Clearing Factor (L.C.F.) enzymatic method (GHOD-PAP, cat. No. 10028, HUMAN and GPO-PAP, cat. No. 10724, HUMAN, respectively).

Chemical analysis

The proximate compositions of the feeds, fish carcasses and freeze-dried faeces were determined. The analyses of dry matter (105°C, 24 h), ash (550°C, 16 h) and crude protein (Kjeldahl nitrogen X 6.25) were performed following standard laboratory procedures (AOAC, 1990). The total fat in the feeds and fish carcasses was measured using ether extraction, and the total lipids in the faeces were measured according to the phosphovanillin method (NENGAS *et al.*, 1995). The amount of chromic oxide in the diets and faeces was determined following the method of BOLLIN *et al.* (1952). The nitrogen free extract (NFE) in the diets was determined as the combined remainder of crude protein, crude fat and ash. The starch content in the faeces and diets was calculated following the method of THIVEND *et al.* (1972) with modifications as described by VENOU *et al.* (2006). The amounts of glycogen and fat in the liver were determined following the methods described by MURAT & SERFATY (1974) and FOLCH *et al.* (1957), respectively.

Calculations

The gross energy in the diets and faeces was calculated according to the for-

mula of Blaxter (1989):

$$\text{Gross energy (kJ g}^{-1}\text{)} = 23.6 \times [\text{Crude protein}] + 17.3 \times [\text{NFE}] + 39.5 \times [\text{Crude fat}]$$

The apparent digestibility coefficients (ADC) for the tested diets were calculated according to the following formula: $\text{ADC \%} = 100 \times [1 - (F \times D_{Cr}) / (D \times F_{Cr})]$

where F = the nutrient concentration or energy in the faeces, D = the nutrient concentration or energy in the diet, D_{Cr} = the chromium concentration in the diet and F_{Cr} = the chromium concentration in the faeces.

The growth parameters were calculated using the following formulae:

Feed Conversion Ratio (FCR):

$$\text{FCR} = \frac{\text{daily FI (g)}}{\text{daily wet WG (g)}}$$

Specific Growth Rate (SGR) in units of %/day:

$$\text{SGR} = \frac{[\ln(W_1) - \ln(W_0)]}{\text{total days}} \times 100$$

Hepatosomatic Index (HSI) %:

$$\text{HSI} = \frac{W_{\text{Liver}}}{\text{BW}} \times 100$$

where WG is the weight gain, FI is the feed intake, W₀ and W₁ are the initial and the final mean fish weights, W_{liver} is the liver weight of the sampled fish and BW is the weight of the sampled fish. All of the weights are in grams.

Statistical analyses

The homogeneity and normality of the data were checked prior to analysis using the Levene and the Kolmogorov-Smirnov tests, respectively. The variation in the mean values was analysed using a one-way ANOVA, and the significant differences were evaluated using the Tukey multiple range test with a significance level of P ≤ 0.05.

Results

Digestibility

No significant differences ($P < 0.05$) in the digestibility coefficients of the feed components were found among the tested diets in either the summer or winter conditions. The protein digestibility was close to 87%, the fat digestibility ranged from 90 to 92%, and the starch digestibility ranged from 68 to 70% at both low and high temperatures.

Growth experiment

Satisfactory growth was achieved in all of the groups of fish (Table 2). The fish that were fed diets A and C showed a slightly higher growth rate, with a final weight of 145 g, than the fish that were fed diet B, which resulted in a final weight of 139.4 g, but the differences were not significant. Consequently, no significant differences were found for the SGR, which averaged $1.80 \% \text{ day}^{-1}$, among the dietary treatments. The feed conversion ratio values showed

Table 2
Values of the SGR, FCR, whole body analysis ($\text{g } 100\text{g}^{-1}$), liver and haematological characteristics of sharpsnout sea bream (*Diplodus puntazzo*) fed different diets during a growth experiment in sea cages. The diet composition, in $\text{g } 100\text{g}^{-1}$ of protein/fish oil/fibre, was as follows: A, 45/10/1.5; B, 45/15/1.5; and C, 45/15/5.

Diet code	Diet A	Diet B	Diet C
<i>Growth characteristics</i>			
Initial BW (g)	3.4	3.4	3.4
Final BW (g)	145.2±8.7	139.4±12.3	144.1±15.1
SGR	1.84±0.01	1.80±0.02	1.82±0.05
FCR	1.07^b±0.01	1.22^a±0.01	1.22^a±0.01
<i>Proximate composition of final population ($\text{g } 100\text{g}^{-1}$)</i>			
Moisture	65.7±0.4	65.2±0.3	65.4±1.0
Protein	18.0±0.2	17.6±0.1	17.4±0.1
Fat	10.1^b±1.4	13.2^a±0.40	13.0^a±0.7
Ash	4.1±0.6	4.2±0.2	4.1±0.3
<i>Liver characteristics</i>			
HSI	1.33±0.11	1.25±0.03	1.31±0.11
Liver fat	14.4^a±1.1	11.2^{ab}±0.8	9.7^b±0.4
<i>Haematological characteristics</i>			
Protein, $\text{g } 100\text{mL}^{-1}$	4.24±0.73	4.71±0.52	5.46±1.32
Glucose, $\text{mg } 100\text{mL}^{-1}$	105.3±8.81	90.1±5.9	111.0±15.6
Triacylglycerols, $\text{mg } 100\text{mL}^{-1}$	337.2±85.6	377.7±20.1	358.92±148.2
Cholesterol, $\text{mg } 100\text{mL}^{-1}$	178.7^b±18.76	290.8^a±39.7	304.5^a±27.9
Lipids, $\text{mg } 100\text{mL}^{-1}$	16.1^b±1.4	18.4^b±2.0	24.1^a±2.9

For growth parameters: the data are presented as the means±SD (n=3).

For proximate composition: the data are presented as the means±SD (n=5, pooled samples of 20 fish each)

For haematological characteristics: the data are presented as the means±SD (n=10).

Different superscripts on the same row indicate significantly different values ($P < 0.05$).

Carcass proximate composition of initial population ($\text{g } 100 \text{ g}^{-1}$); moisture, 71.1; protein, 15.5; fat, 9.1; ash, 3.4.

a significantly more efficient conversion ratio by the fish that were fed diet A (1.07) compared to the fish that were fed diets B and C (1.22 for both diets).

Hepatic characteristics and whole-body composition

The whole-body analysis showed no statistical differences in the values of the moisture, protein and ash (Table 2). Nevertheless, a significant increase of body fat was measured in the fish that were fed diets B and C. The hepatosomatic indices did not significantly differ, whereas the liver lipid content was found to be significantly lower in the fish that were fed diet C than in the fish that were fed diet A.

Haematology

The haematological characteristics did not show differences among the experimental groups in the serum total protein levels, glucose levels or triacylglycerol levels (Table 2). However, significantly increased values of serum cholesterol were found in the fish that were fed diets B (290.8 mg 100 mL⁻¹) and C (304.5 mg 100 mL⁻¹), and significantly higher total serum lipids (24.1 mg 100 mL⁻¹) were apparent in the fish that were fed diet C.

Discussion

The experiments generated generally acceptable results regarding the digestibility and growth performance of sharpsnout seabream. In our experiments, the digestibility of dietary nutrients was not affected by the tested range of temperatures. The effect of temperature on nutrient digestibility has been studied in the past for several fish species with inconsistent results. For example, no effects were observed by CHO & KAUSHIK (1990) for rainbow trout (*Oncorhynchus mykiss*) between 9°C and 18°C

or by NG *et al.* (2004) for Atlantic salmon (*Salmo salar*) at 6°C and 11°C. However, temperature effects on digestibility were reported for European seabass by PERES & OLIVA-TELES (1999) and MOREIRA *et al.* (2008), with increased ADCs at 25°C compared with those at 18°C.

Furthermore, no differences were apparent among the tested diets with respect to the protein, fat and carbohydrate ADCs. Dietary fibre has been shown to have different effects on digestibility depending on the fish species, the inclusion level and the fractions of soluble and insoluble fibre (AMIRKOLAIE *et al.*, 2005). Negative effects on digestibility values have been reported for the inclusion of crude fibre at levels of 10, 25 and 40 g 100g⁻¹ in Nile tilapia (*Oreochromis niloticus*) diets (ANDERSON *et al.*, 1984) and for soluble guar gum at inclusion levels of either 2.5-10 g 100g⁻¹ (STOREBAKKEN, 1985) or 8 g 100g⁻¹ (AMIRKOLAIE *et al.*, 2005) in rainbow trout diets. However, digestibility values have been found to be unaffected for rainbow trout that were fed diets with 12.3 g 100g⁻¹ crude fibre (SANZ *et al.*, 1994), for Nile tilapia that were fed with 8 g 100g⁻¹ cellulose (AMIRKOLAIE *et al.*, 2005) and for European seabass that were fed with 10 or 20 g 100g⁻¹ cellulose (DIAS *et al.*, 1998). The inclusion of sunflower meal, added as a fibre source in the diets used in the present work, has been previously demonstrated to reduce digestibility in Nile tilapia (MAINA *et al.*, 2002). Nevertheless, the total amount of fibre originating from the sunflower meal in diet C was possibly not sufficient to adversely affect the nutrient digestibility in sharpsnout seabream. Similarly, SANZ *et al.* (1994) found no adverse effect on ADCs when sunflower meal was included at a level of 40 g 100g⁻¹ (12.3 g 100g⁻¹ crude fibre) in a rainbow trout diet.

There were some differences resulting from the diet treatments. The FCR was improved in the fish that were fed a low fat diet, although the SGR was not affected (diet A). The fish that were fed diets B and C were found to have similar FCR values. These results showed a better use of the low fat diet (10 g 100g⁻¹ fat) and no effect of fibre up to the 5 g 100g⁻¹ inclusion level. Previous studies regarding nutrient requirements have reported contradictory results. FCR values were improved when fat was included in diets at 14 g 100g⁻¹, compared with 12 or 22 g 100g⁻¹ inclusion levels, for both juvenile and adult sharpsnout seabream (HERNANDEZ *et al.*, 2001). However, in that study, the ash content of the low fat diet was almost double the one in our study (16 g 100g⁻¹), and that difference could be an additional parameter affecting the results.

ATIENZA *et al.* (2004) reported that an increase of sharpsnout seabream biomass was related to the level of consumed dietary protein but not to the dietary fat level, a finding that seems to be confirmed in the present experiment. In addition, the authors reported an optimum protein/energy ratio of 47/10, which is close to the 45/10 ratio of diet A used in the present study, which resulted in better growth performances than the 45/15 ratio independently of the fibre content. According to the results of self-selection experiments carried out by VIVAS *et al.* (2006), a sharpsnout seabream is able to select a high protein diet when faced with a choice of macronutrients (protein, fat and carbohydrates), similar to carnivorous species like rainbow trout (SANCHEZ-VAZQUEZ *et al.*, 1999) and European seabass (ARANDA *et al.*, 2000). An acceptable growth performance was obtained by GARCIA *et al.* (2001) when sharpsnout seabream were fed diets composed of 40 g 100g⁻¹ protein and

36 g 100g⁻¹ carbohydrates and by ATIENZA *et al.* (2004) using diets with 47 g 100g⁻¹ protein and 10 g 100g⁻¹ fat.

The carcass composition measurements showed no significant differences in the moisture, protein or ash content among the fish that were fed different diets. However, the fat content was significantly higher in the fish that were fed diets B and C, which both resulted in the highest fat level of 15 g 100g⁻¹. High-fat diets are known to lead to high body fat deposition in several fish species (see ARZEL *et al.*, 1994; STEPHAN *et al.*, 1996; WEATHERUP *et al.*, 1997; HEMRE & SANDNES, 1999; LANARI *et al.*, 1999; FOUNTOULAKI, 2003), but this increased fat deposition was not the case in a previous study on sharpsnout seabream (HERNANDEZ *et al.*, 2011). In the latter study, the different lipid levels (11, 15 and 23 g 100g⁻¹ fat) had no influence on the body fat composition of adult fish (280 g). In addition, increasing the inclusion level of soybean meal up to 60 g 100g⁻¹ and varying the dietary fat levels from 13 to 17 g 100g⁻¹ did not affect the moisture, protein or body fat composition of fish with weights from 120 and 320 g (HERNANDEZ *et al.*, 2007).

Liver fat content has been associated with high carbohydrate contents in diets for European seabass (LANARI *et al.*, 1999) and rainbow trout (BRAUGE *et al.*, 1994). This finding is also confirmed in the present study, where decreasing liver fat was related to an increase in the dietary NFE. In the present study, the liver fat decreased with the increase of dietary lipids from 10 to 15 g 100g⁻¹. In contrast, LANARI *et al.* (1999) found no effect of increased dietary fat on the liver fat of European seabass.

Serum cholesterol was not associated with the dietary fat level in Atlantic cod (*Gadus morhua*) diets with a dietary fat level of 11.4 g 100g⁻¹ compared to 30.5 g 100g⁻¹,

although the concentration of serum triacylglycerols were affected by the fat content of the diet (KJÆR *et al.*, 2009). In the present study, the concentrations of cholesterol and total lipids were found to increase in the serum of sharpsnout seabream that were fed diets B and C, confirming the direct effect of dietary lipids on the related serum parameters.

In summary, based on the results of a growth experiment under farm conditions, diet A, which contained a low fat level, is recommended for sharpsnout seabream. The digestibility coefficients were not influenced by either the diet treatment or water temperature while the carcass fat was increased when the dietary fat increased from 10 to 15 g 100g⁻¹. Inclusion of fibre up to a level of 5 g 100g⁻¹ did not affect the growth performance or nutrient digestibility by sharpsnout seabream.

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