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DEFINING ENERGY AND PROTEIN REQUIREMENTS OF GILTHEAD SEABREAM *(SPARUS AURATA)* TO OPTIMIZE FEEDS AND FEEDING REGIMES

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Abstract

Energy and protein requirements of growing fish can be quantified as the sum of the amounts of energy and protein retained as growth plus the amounts simultaneously lost from the body. The requirement for dietary gross energy and protein can be calculated using the respective efficiencies of utilization. Growth of gilthead seabream as a function of body weight and temperature was predicted by the equation: $y = 0.024 \times BW^{0.514} \times exp^{0.060T}$ (where y = daily weight gain in g/fish, BW = body weight in g and T = temperature in °C). The gain was determined in fish ranging 1-470 g. The energy content of the fish depended on fish weight and rose from 4.7 to 11.0 kJ/g body mass as the fish grew whereas the protein content was constant at 176 mg/g regardless of fish weight. The efficiencies of utilization of digestible energy (DE) and digestible protein (DP) for maintenance and growth were determined by feeding the fish at increasing feeding levels from zero to the maximum voluntary feed intake. The daily requirement of DE for maintenance was dependent on temperature and determined as $(16.6kJ \times exp^{0.055T})$/BW in $kg^{0.82}$. The maintenance requirement for DP was independent of temperature and equaled 0.62g/ BW in $kg^{0.70}$. The relationship between DE intake and energy gain was linear, constant at $k_{DEg} = 0.67$ and independent of feed intake and temperature. Efficiency of protein utilization for growth varied between 0.33 and 0.80 depending on the DP/DE ratio in the diet. The optimal protein utilization for protein deposition was estimated at $k_{DPg} = 0.47$. Using these values allows optimization of feeding for seabream culture.

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Introduction

Fish culture in the Mediterranean is presently based on two major species, the gilthead seabream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*). Production of both species is rapidly expanding. In Israel, the principal mariculture species is the gilthead seabream and most production takes place in cages in the Gulf of Aqaba in the Red Sea. Given the economic importance of feed and feeding in aquaculture, the need to develop nutritionally balanced diets, especially concerning protein and energy contents, is evident. This is also crucial with regard to the aquatic environment since feed that is unconsumed or unavailable to the fish is lost to the surroundings, resulting in nutrient enrichment of the water body. Regulatory authorities often impose limits on waste outputs or feed quotas to limit this problem. Therefore, feeding models that supply the exact amounts of energy and protein needed by the fish to realize their full growth potential are essential in fish farming. Feeding charts based on nutritional bioenergetics have been introduced for rainbow trout (Cho and Kaushik, 1990; Cho, 1992). However, information concerning growth and digestible energy needs is lacking for most Mediterranean warm-water species (Kaushik, 1998). A novel approach for determining protein and energy requirements in fish is described here as the sum of the daily energy and protein requirements for maintenance and growth.

The energy requirement for maintenance in fish depends mainly on body size and temperature, thus it is proportional to the metabolic body weight. On the other hand, the growth requirement depends on the amount and composition of the gained weight. The formal approach to these calculations is: requirement = (M x BW in kg^b) + (G x growth), where BW in kg^b is the metabolic body weight and M and G are coefficients describing the efficiency of utilization of dietary energy (or protein) for maintenance or growth.

This review shows how parameters of the factorial model for quantifying daily energy and protein needs in growing gilthead seabream were derived. Some of the information represents an update of earlier trials (Lupatsch et al., 1998, 2001), presented with new data.

Materials and Methods

Experimental fish. Gilthead seabream were spawned and raised at the National Center for Mariculture (NCM). The NCM broodstock was established some 20 years ago from juvenile gilthead seabream caught in the Bardawil lagoon on the Sinai coast of the Mediterranean Sea. Prior to the start of all experiments, fish were fed a local commercial seabream diet (Matmor Inc., M.P. Evtach, Israel) consisting of 450 g crude protein, 190 g crude lipid and 20.5 MJ gross energy per kg feed according to feeding tables developed at NCM.

Growth estimates. To test the growth potential of the seabream throughout the entire growing cycle until close to market size, a data set was established from growth trials of fish ranging 1-450 g. For each growth trial, fish were graded before stocking so that groups were homogenous in size. In these trials, the above-mentioned commercial diet was fed to fish to satiation twice or three times daily, depending on the size of the fish, taking care that no food was left uneaten. Depending on the fish size, 0.200 or 3 m^3 tanks were stocked at densities ranging 0.25-10 kg/m^3. The outdoor tanks were supplied with flow-through sea water (41 ppt, flow rate 2 l/min) at ambient temperatures ranging 20-28°C. Fish were weighed every 14 days and the weight gain and daily feed intake were calculated for the periods between two successive weighings. The corresponding body weight for the period was the geometric weight of the fish during the period.

Diet preparation. Experimental diets were formulated at the NCM, thoroughly mixed in a 25 l batch mixer and pelleted using a laboratory model California Pellet Mill with a steam pretreatment unit. After air-drying and when fed to the fish, the moisture content of the feeds was about 80-90 g/kg diet.

Composition of weight gain and loss after starvation. To determine the composition of gilthead seabream of various sizes, fish were sampled throughout the growing cycle. As the composition and energy content of growing fish can be influenced by nutritional status as well as diet composition, estimation of carcass composition relied on data obtained for 44 groups of 10-20 equal-sized fish within a weight range of 1-470 g, fed the commercial diet. To determine energy and protein losses after starvation, half of the fish in each group were sacrificed and frozen. The other half was stocked in 200 l outdoor tanks up to 30 days without being fed. After the starvation period, fish were sacrificed and stored at -20°C until analysis. To calculate the energy and protein losses after starvation, the body composition of the initially-sampled fish was considered representative.

Efficiency of energy and protein utilization. The efficiencies of energy and protein utilization for maintenance and growth were determined by feeding the seabream diets containing varying amounts of dietary energy, dietary protein and digestible protein/digestible energy ratios (DP/DE; Table 1). Five growth trials were performed with fish of different starting sizes (Table 2). In each trial, fish were fed increasing amounts of feed from zero to close to the maximum feed intake. Feed was given three times a day at the high feeding levels and once daily at the low feeding level to ensure equal distribution of the food pellets among the fish. Digestibility of protein and energy were determined as described in Lupatsch et al. (1997) using fish weighing 300-400 g. Chromic oxide (8 g/kg) was added to the feed as a marker and feces were collected by stripping.

Sample preparation. Fish sampled for analysis were sacrificed by immersing them for a short time in ~4°C ice water and freezing them immediately afterwards. While still frozen, fish were cut into smaller pieces and ground twice using a meat grinder with a 3 mm die. Samples for estimation of dry matter were taken from the ground fish before the remaining homogenate was freeze-dried. The freeze-dried samples were again mixed in a blender before further analysis.

Analytical procedures. Identical analyses were applied for diets and body homogenates. Dry matter was calculated by weight loss after drying 24 h at 105°C. Crude protein was measured using the Kjeldahl technique and multiplying N by 6.25. Crude lipid was measured after chloroform-methanol extraction (Folch et al., 1957). Samples were homogenized with a high-speed homogenizer for 5 min and lipid was determined gravimetrically after separation and vacuum drying. Ash was calculated from the weight loss after incineration of the samples for 24 h at 550°C in a muffle furnace. Gross energy content was measured by combustion in a Parr bomb calorimeter using benzoic acid as the standard.

Statistics. Allometric equations were obtained by applying linear regression analysis to the logarithmic transformation of the data in the form of $\ln y = \ln a + b \ln x$. The antilog of this expression produces the equation $y = ax^b$. Each calculation represents the combined fish in a single tank. Descriptive statistics are means±SE unless otherwise noted. Analyses were carried out with SPSS 6.1 for Windows (SPSS Inc., 1989-1992).

Results

Growth and feed intake. The daily weight gain of gilthead seabream at different body weight and temperatures is depicted in Fig. 1 (n = 108). The relationship between weight gain and body weight was not linear and the results best fit the following allometric function when the body weight (BW) of the fish is 1-450 g and the temperature (T) is 20-28°C:

weight gain (g/fish/day) = 0.024±0.006 x BW (in g)$^{0.514\pm0.020}$ x exp$^{0.060\pm0.009T}$, r^2 = 0.93 (equation 1)

Rearranging this equation, the weight W_t after t days can be calculated from the initial weight W_0 as follows:

$W_t = [W_0^{0.486} + 0.01166 \times \exp^{0.060T} \times \text{days}]^{2.058}$ (equation 2)

The feed intake can be described in a similar manner:

feed intake (g/fish/day) = 0.029±0.007 x BW (g)$^{0.598\pm0.025}$ x exp$^{0.057\pm0.011T}$, r^2 = 0.94 (equation 3)

Composition of weight gain. The proxi-

Table 1. Composition and proximate analysis (as fed) of experimental diets used to estimate energy and protein demands of gilthead seabream for maintenance and growth.

	Diet A	*Diet B*	*Diet C*	*Diet D*
Ingredients (g per kg)				
Fishmeal	900	780	670	550
Cornstarch	-	190	260	330
Fish oil	90	20	50	85
Vitamin premix[1]	10	10	10	10
Mineral premix[2]	-	-	10	25
Proximate analysis (per kg)				
Dry matter (g)	920	911	914	914
Crude protein (g)	575	512	436	367
Crude lipid (g)	183	111	121	140
Ash (g)	146	119	113	109
Gross energy (MJ)	21.09	19.44	19.56	19.69
Digestible protein (g)[3]	485	450	384	323
Digestible energy (MJ)[3]	17.36	15.68	15.43	15.20
DP/DE ratio (g/MJ)	27.93	28.70	24.90	21.20

[1] per kg diet: A 1600 IU; D_3 1900 IU; E 150 mg; thiamine 30 mg; riboflavin 45 mg; niacin 15 mg; Ca-pantothenate 30 mg; pyridoxine 5 mg; folic acid 11 mg; B_{12} 0.12 mg; K 11 mg; biotin 0.25 mg; inositol 150 mg; ascorbic acid 500 mg and choline chloride 3 g.
[2] per kg diet: MgO 2.5 g; KI 1.8 mg; $CoCO_3$ 0.66 mg; MnO 73.5 mg; ZnO 75 mg; $CuCO_3$ 57.5 mg; $FeCO_3$ 255 mg; NaCl 1.6 g; KCl 3.6 g; Na_2SeO_3 0.4 mg.
[3] after Lupatsch et al., 1997

mate body composition of the gilthead seabream is shown in Fig. 2. The protein and ash concentrations did not change with the increase in fish size and averaged 176±8.4 and 41.5±4.2 mg/g, respectively. In contrast, energy and lipid concentrations rose and moisture decreased as the fish weight increased and best fit the following equations (n = 44):

energy (kJ/g) = $4.66\pm0.23 \times BW(g)^{0.139\pm0.010}$, $r^2 = 0.89$ (equation 4)

lipid (mg/g) = $43.3\pm3.8 \times BW(g)^{0.243\pm0.020}$ $r^2 = 0.90$ (equation 5)

moisture (mg/g) = $777\pm11.4 \times BW(g)^{-0.041\pm0.003}$, $r^2 = 0.82$ (equation 6)

Metabolic body weights. The daily energy and protein losses during starvation were calculated from comparative slaughter analysis

Table 2. Structure of gilthead seabream growth trials for evaluating the effects of increasing energy and protein intakes and various dietary protein/dietary energy ratios.

	Trial 1	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*
Fish/tank	19	13	13	18	21
Initial weight (g)	40.1	72.4	94.4	86.1	21.4
Diets (see Table 1)	A	A	A	B, C, D	B, C, D
Feeding levels		low, medium, high + starvation		low, medium, high, maximum + starvation	
Replicates	2	2	2	-	2
Duration (days)	41	41	41	65	39
Water temperature (°C)	20.5	20.5	20.5	21.5	28

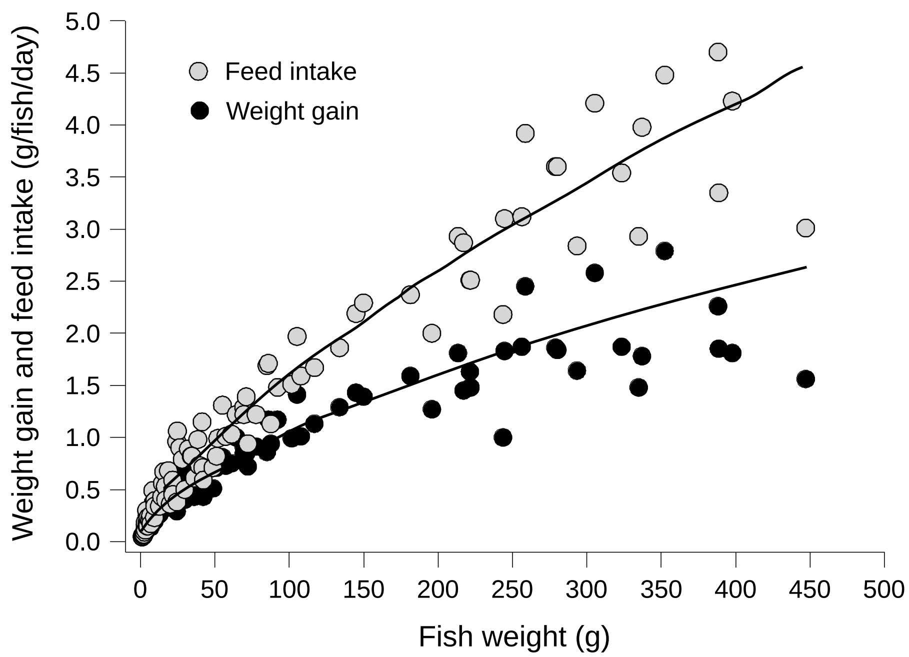

Fig. 1. Daily weight gain (g) and feed intake (g) in relation to increasing body weight in gilthead seabream. Corresponding equations 1 and 3 are presented in the text.

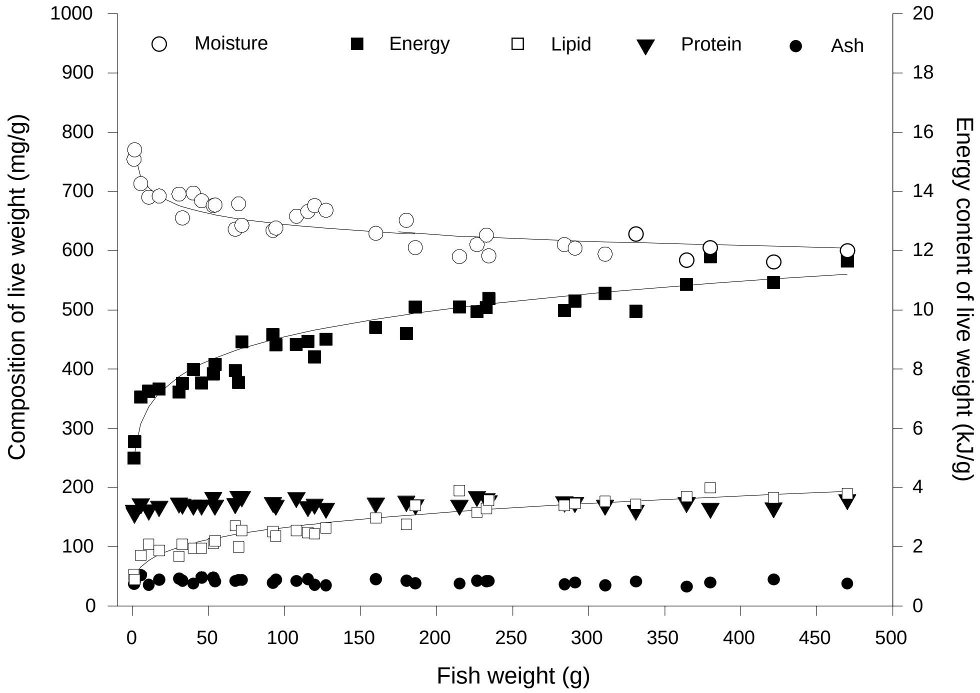

Fig. 2. Proximate body composition of gilthead seabream at various sizes. Each data point represents analysis of a group of fish. Corresponding equations 4 to 6 are presented in the text.

for each weight group (Fig. 3). The relationships between the losses and fish weight were not linear and results fitted ln - ln functions traditionally used by animal nutritionists to express metabolic body weight (MBW). The antilog of these functions describes the allometric relationship common in biological measurements (n = 44). The daily energy loss per fish can be described by the equation:

energy loss in kJ/fish/day = 41.5±2.1/ $BW(kg)^{0.82\pm 0.026}$, $r^2 = 0.95$ (equation 7)

Incorporating water temperature improves the correlation slightly:

energy loss in kJ/fish/day = 11.6±2.0 x $\exp^{0.055\pm 0.008T}/BW(kg)^{0.82\pm0.024}$, $r^2 = 0.97$ (equation 8)

The daily protein loss per fish can be described by the equation:

protein loss in g/fish/day = $0.40\pm0.035/BW(kg)^{0.70\pm0.04)}$, $r^2 = 0.89$ (equation 9)

Incorporating the temperature:

protein loss in g/fish/day = 0.17±0.055 x $\exp^{0.036\pm0.013T}/BW(kg)^{0.70\pm 0.036}$, $r^2 = 0.90$ (equation 10)

The expressions $kg^{0.82}$ and $kg^{0.70}$ can thus be described as the metabolic body weights for energy and protein, respectively.

Efficiency of utilization of energy and protein. To examine the relationship between dietary energy and energy gain for different sized fish for all trials combined, energy intake and energy gain were expressed per metabolic weight of $kg^{0.82}$. The relationship between dietary energy intake (*x*, $kJ/kg^{0.82}$) and energy retained (*y*, $kJ/kg^{0.82}$), as seen in Fig. 4, perfectly fits a linear function. Trials I through IV, performed at a water temperature

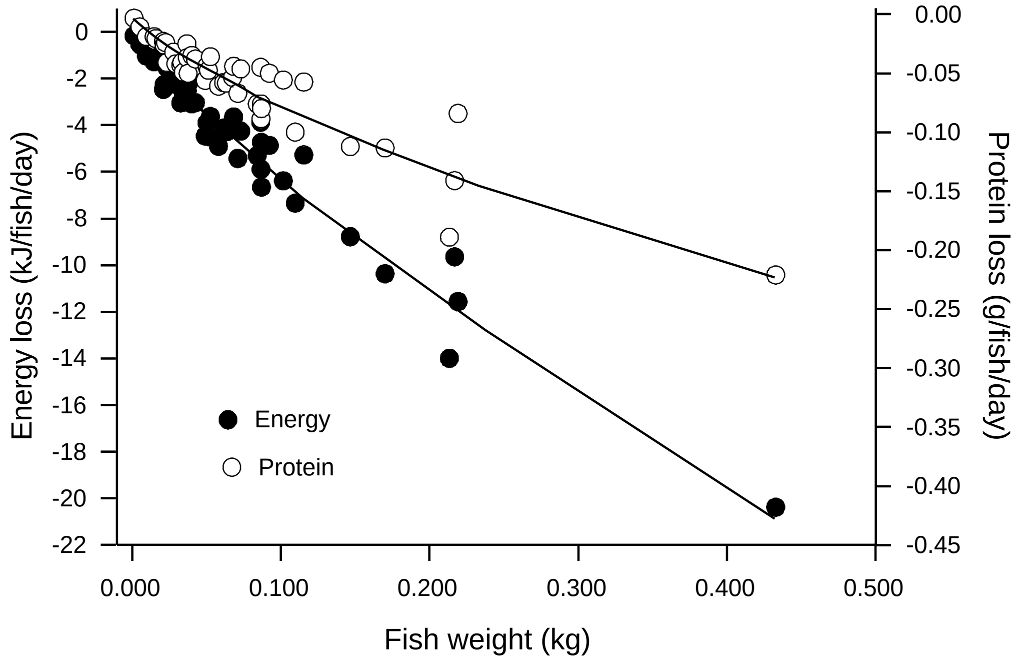

Fig. 3. Energy (kJ/fish/day) and protein (g/fish/day) losses in gilthead seabream after starvation. Corresponding equations 7 and 9 are presented in the text.

of 21°C, differ from Trial V, which was performed at 28°C. Therefore two linear equations are described:

Trials I to IV (21°C): $y = -32.2 \pm 1.9 + 0.68 \pm 0.02\, x$ $r^2 = 0.98$ (equation 11)

Trial V (28°C) $y = -55.8 \pm 2.8 + 0.66 \pm 0.02\, x$ $r^2 = 0.99$ (equation 12)

The maintenance energy requirement equals $DE_{maint} = 47.35$ kJ/kg$^{0.82}$/day at a temperature of 21°C and 84.54 kJ/kg$^{0.82}$/day at 28°C. However, the slopes of both equations, which refer to the efficiency of energy for growth, are nearly the same and average $k_{DEg} = 0.67$.

On the other hand, the relationship between dietary protein intake (x, g/kg$^{0.70}$) and protein retained (y, g/kg$^{0.70}$) per day was best represented by an exponential curve (Fig. 5), described by the following equation:

$y = a \times [1 - \exp^{-b\,(x - c)}]$, where a = 3.16±0.58, b = 0.159±0.036, and c = 0.62±0.039, $r^2 = 0.97$ (equation 13)

No difference was detected in the responses of the fish at different temperatures. The maintenance protein requirement was defined as the point of zero protein gain, reached at an intake of $DP_{maint} = 0.62$ g/kg$^{0.70}$/day. Since the relationship between protein intake and protein gain was not linear, a constant protein efficiency value for growth could not be determined. Therefore, the efficiency of utilization of protein (k_{DPg}) for growth beyond maintenance was calculated for each level of dietary protein intake as follows:

k_{DPg} = protein gain/(dietary protein fed - DP_{maint}) where DP_{maint} = requirement of dietary protein for maintenance, i.e., 0.62 g DP/kg$^{0.70}$/day (equation 14)

The efficiency of protein utilization calculated for the different levels of dietary protein

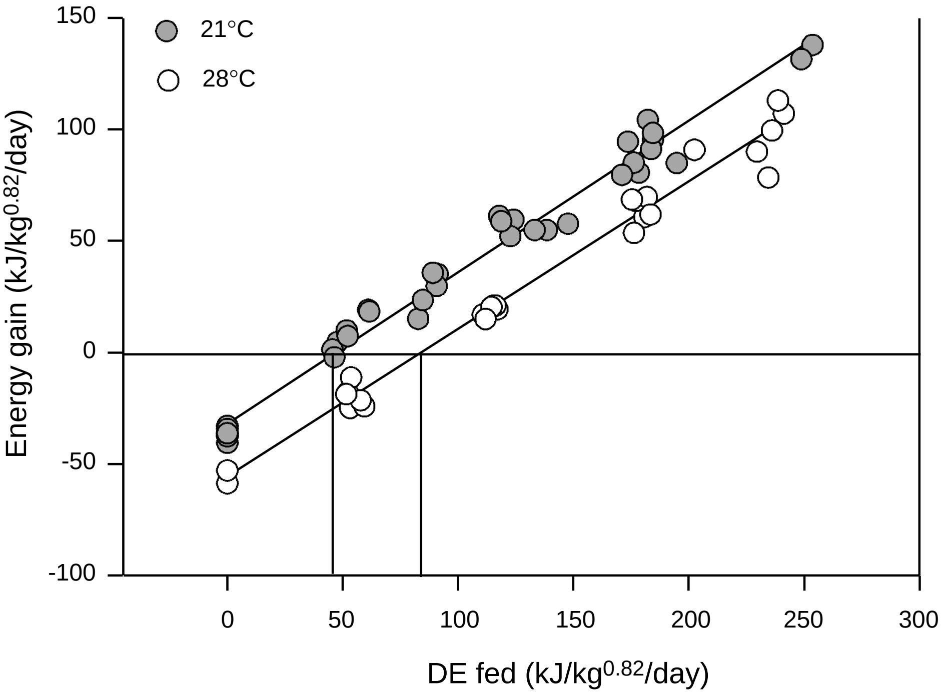

Fig. 4. Daily energy retention per unit metabolic body weight of $kg^{0.82}$ in gilthead seabream fed diets differing in digestible energy and digestible protein contents at two temperatures. Corresponding equations 11 and 12 are presented in the text.

intake ranged 0.33-0.80 k_{DPg} (Fig. 6). At dietary protein intakes near the maintenance requirement, the protein efficiency was highest (k_{DPg} = 0.80) and as the dietary protein intake increased the efficiency dropped to as low as k_{DPg} = 0.33. At the inflection point of the response curve, the optimal protein efficiency value of k_{DPg} = 0.47 was estimated.

Discussion

Weight gain and feed intake. In contrast to terrestrial animals, fish seem to grow continuously. Growth does not cease and reach an asymptote, which may never be attained in aquaculture. Growth rates in aquaculture are typically described by a specific growth rate (SGR) or absolute growth in g per day. Temperature affects growth, as in all poikilotherms, which increases as the temperature increases to an optimum. Above this optimum, growth decreases until the upper lethal temperature is reached. Although SGR and absolute weight gain depend on feed intake and water temperature, they mainly depend on the size of the fish. As a result, growth among groups of fish of different weights cannot be directly compared.

In this study on seabream, the data describing the dependence of weight gain (y) on fish weight and temperature best fit an exponential regression (equation 1), however growth could only be described for the temperature range used in this study, 20-28°C. According to our growth prediction, seabream grow from 1 to 379 g in one year at an average annual water temperature of 23°C, which conforms to the conditions in the Red Sea.

Composition of gain. Because a large pro-

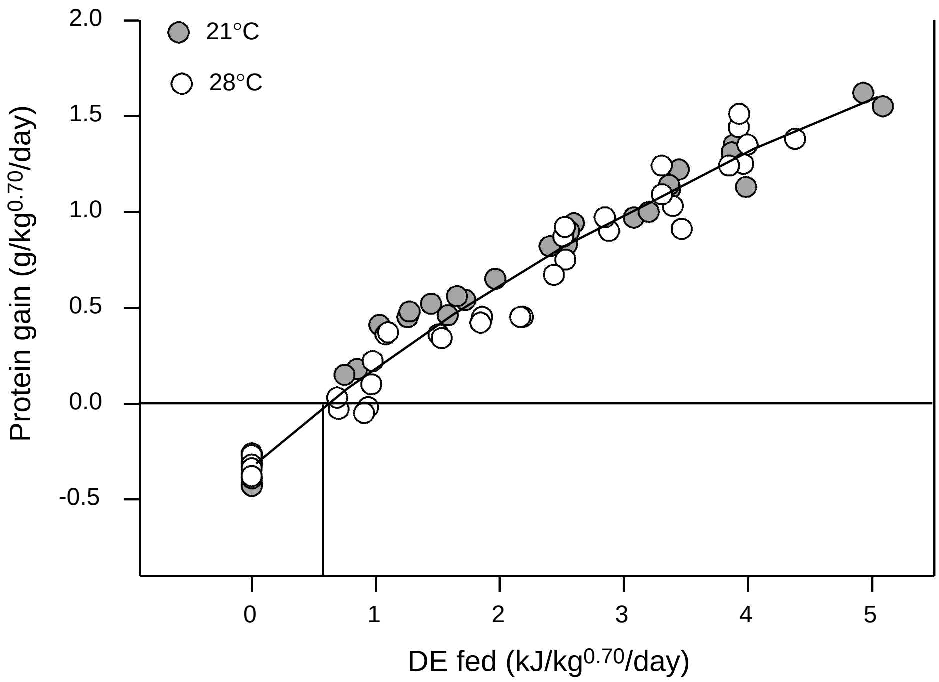

Fig. 5. Daily protein retention per unit metabolic body weight $kg^{0.70}$ in gilthead seabream fed diets differing in digestible energy and digestible protein content at two temperatures. The corresponding equation 13 is given in the text.

portion of the energy and protein consumed by the fish is retained as growth, carcass composition is a major indicator of the energy and protein requirements of the fish. When measuring whole body composition of fish at increasing sizes, each unit weight gain is assumed to equal the body composition change at that size. Dry matter and fat content are generally the most variable factors in fish and can increase dramatically especially during the growth period of small fish (>100 g). In our study, the protein content per g live weight ranged 157-185 mg/g fish, averaging 176 mg/g fish. On the other hand, lipid increased as the fish size increased and, consequently, the caloric content ranged from 5 to close to 11 kJ/g live weight. Lipid levels rose from 43 to 186 mg/g for market-sized seabream of about 400 g. With this high lipid content one might add gilthead seabream to the category of 'fat' fish, compared to other cultured fish such as red drum (Thoman et al., 1999), turbot (Regost et al., 2001) and rainbow trout (Dias et al., 1999) which contain 69, 38 and 146 mg lipid per g live weight, respectively. Therefore, in estimating requirements for tissue deposition and growth, wide variations between species, especially in terms of energy, are expected based on the differing tissue composition. Seabream require more dietary energy per unit weight gain than leaner fish such as red drum or turbot. Fish containing more moisture (less dry matter) require less energy for newly deposited growth.

Metabolism and body weight. Metabolic rate is a measure of the metabolic activity related to weight and decreases with increasing size at a constant temperature. For poikilothermic fish, temperature has an important effect on metabolism, although its importance may be species-specific. In this study, metabolism was measured at the ambient tempera-

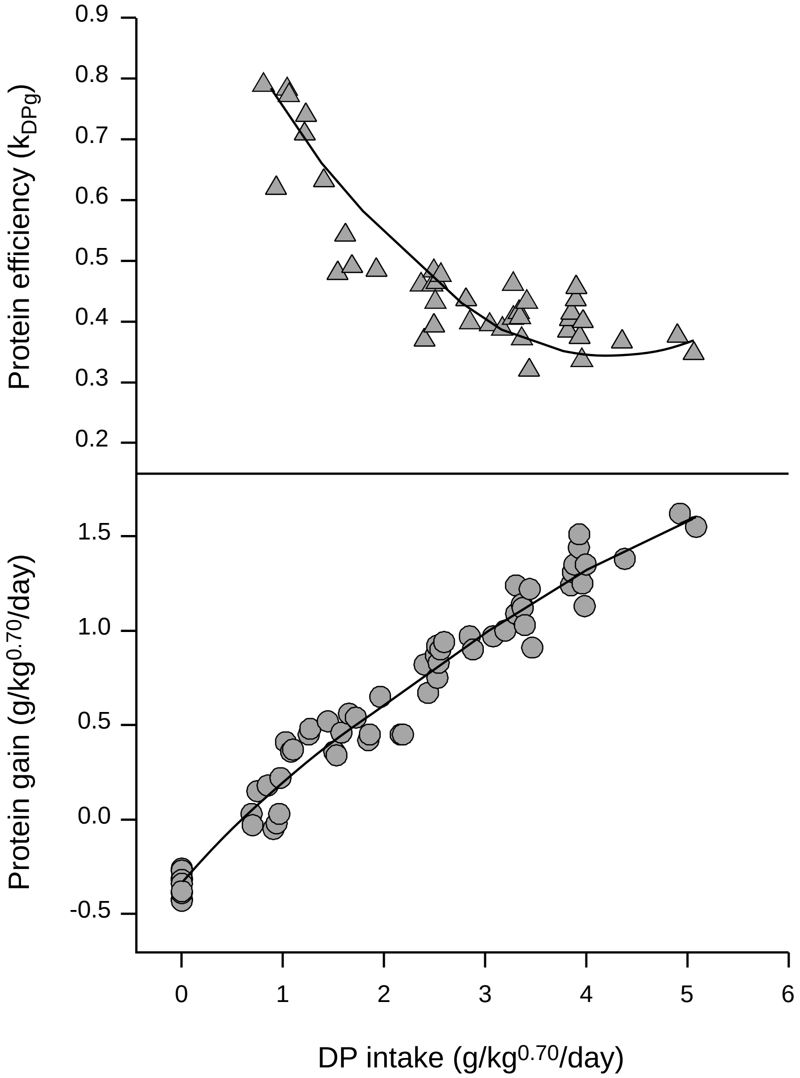

Fig. 6. Relationship between protein efficiency for growth (k_{DPg}) and protein gain in gilthead seabream fed varying levels of digestible protein.

tures of 20-28°C. Within this range, the effect of temperature was small compared to the effect of size. Nevertheless, the correlation coefficients (equations 8, 10) improved when temperature was included in the equation. As the trials were performed in sea water ranging 20-28°C , the metabolic rate can be described only for this range.

During starvation at the average temperature of 23°C, the energy loss was 41.5 kJ/BW(kg)$^{0.82}$/day and the protein loss was 0.40g/BW(kg)$^{0.70}$/day (equations 7, 9). Similar to the energy metabolism, the best fit between protein loss and body weight in gilthead seabream was reached using a metabolic body weight, which was calculated with an exponent of 0.70 (equation 9). Data correlating protein loss with fish weight are sparse and most authors assumed a common exponent for the relationships between energy loss and protein loss to body weight. In a study with *Epinephelus aeneus and Dicentrarchus labrax* (Lupatsch et al., 2003), energy and protein losses were described using metabolic body weights with the exponents of 0.80 and 0.70, respectively. The similarity of these coefficients in all species indicates that energy and protein metabolism cannot be described by the same metabolic body weight. Moreover, the exponents are close enough in value to suggest they might be common for a number of fish species.

Some criticism has been put forward using starvation as a means of determining maintenance energy requirements for a long period of time since the rate of loss of body tissues is higher during the early weeks of fasting than in later weeks. This, however, would only influence coefficient a in the expression a x kg^b and, further, loss after starvation is only an approximation of maintenance energy requirements.

Efficiency of energy and protein utilization. By using the metabolic body weight of $kg^{0.82}$, feeding trials with fish of different sizes can be combined to examine the relationship between digestible energy intake and energy retention (Fig. 4). This relationship was linear regardless of feed intake and the DP/DE ratio and the efficiency of digestible energy utilization for growth (k_{DEg}) averaged 0.67 (equations 11, 12). The maintenance requirement for energy using linear regression was higher at higher temperatures, as expected. At 21°C, the maintenance requirement was 47.4 kJ/$kg^{0.82}$ and at 28°C, 84.6 kJ/$kg^{0.82}$. In an earlier study with seabream grown at an average temperature of 23.5°C (Lupatsch et al., 1998), the maintenance requirement was determined as 55.8 kJ/$kg^{0.83}$, which corresponds favorably with the current study. Assuming the coefficient incorporating the effect of temperature is the same under maintenance as well as non-fed conditions, the energy maintenance requirement depending on temperature can be defined for seabream as DE_{maint} = (16.6 kJ x $\exp^{0.055T}$)/BW(kg)$^{0.82}$/day.

In contrast to the linearity of the relationship between digestible energy intake and energy retained, the relationship between the digestible protein intake and protein gain was better described by an exponential curve (Fig. 5, equation 13). The efficiency of protein utilization calculated for increasing levels of digestible protein intake ranged widely between k_{DPg} = 0.33 and 0.80 (Fig. 6). At a digestible protein intake close to maintenance requirements, the protein efficiency was highest because it is limiting. As the digestible protein intake increased, the efficiency dropped. At the inflection point of the response curve of protein gain versus digestible protein intake, the optimal protein efficiency value was estimated at k_{DCPg} = 0.47. Higher protein efficiencies seem to be reached only accompanied by a lower overall gain. Note, however, that this value is appropriate only for dietary protein with a balanced amino acid profile such as fishmeal. In practical diets that are limited in one or more amino acids, the protein efficiency will be lower.

In contrast to the temperature dependency of energy metabolism, increasing the temperature from 21°C to 28°C did not seem to affect the demand for dietary protein and the maintenance requirement for seabream was determined as $DP_{maint\,=}$ 0.62 g/BW(kg)$^{0.70}$/day.

Practical applications. The results of this study allow us to calculate the daily recommended energy and protein intakes for growing gilthead seabream. By defining the demands of the fish for maintenance and growth, a comprehensive budget can be derived that quantifies the energy and protein needed by the fish to achieve its growth potential at any temperature and during any stage in the growth cycle.

Table 3 shows that the proportion of the total digestible energy required for maintenance increases as the body weight and growth rate decrease, influencing the FCR. Higher temperatures have a positive effect on feed efficiency and, at 26°C, the growth potential is much greater. Even though energy requirements for maintenance increase with temperature, this increase is minor compared to the higher weight gain.

The absolute protein requirement per day per fish depends on the size and weight gain of the fish regardless of the dietary digestible energy density. Therefore, as demonstrated in Table 4, the desired protein content of a feed varies according to the digestible energy level. The DP/DE ratio decreases as the fish grow and as the growth potential drops due to the changing energy to protein ratio of the weight gain and the increasing proportion of energy used for maintenance. Fish that are able to consume high amounts of feed due to a larger stomach capacity could be fed lower energy diets with low protein levels since, based on the calculations in Table 4, the same amount of protein per day would be consumed.

Since protein and energy demands constantly change, different diets would have to be formulated for growing gilthead seabream. However, it is unreasonable to expect that a large number of diets would be used in any fish culture. In Table 5, a range of diets for various growth periods is suggested (these remain to be tested under practical conditions). It is more practical to feed lower energy diets to small fish with a capacity for high feed intake, as it is difficult to create a 18 MJ energy, 60% protein diet (Table 4). Above 200 g, gilthead seabream production would be better served by using a diet with a digestible energy content of at least 18 MJ/kg - which can be achieved only by incorporating a high lipid level - as the amount of 15 MJ feed that would have to be consumed by 300 g fish approaches the physical limits of gilthead seabream.

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Table 3. Recommended supply of digestible energy for gilthead seabream grown in water temperatures of 21°C or 28°C.

Body weight (g/fish)	*10*		*50*		*100*		*300*	
Metabolic body weight ($kg^{0.82}$)	0.023		0.086		0.151		0.373	
Water temperature (°C)	20	26	20	26	20	26	20	26
Predicted weight gain (g/day)[1]	0.26	0.37	0.60	0.85	0.85	1.22	1.49	2.14
DE_{maint} (kJ/fish/day)[2]	1.14	1.59	4.28	5.95	7.55	10.50	18.58	25.85
RE (kJ/fish/day)[3]	1.67	2.39	4.78	6.85	7.51	10.76	15.39	22.06
DE_{growth} (kJ/fish/day)[4]	2.49	3.57	7.12	10.20	11.19	16.04	22.93	32.87
DE_{m+g} (kJ/fish/day)[5]	3.63	5.16	11.39	16.15	18.74	26.54	41.52	58.72
% of total DE used for maintenance	31.5	30.8	37.5	36.8	40.3	39.6	44.8	44.0
Feed intake (g/fish/day)[6]	0.21	0.30	0.67	0.95	1.10	1.56	2.44	3.45
FCR	0.81	0.81	1.12	1.12	1.29	1.28	1.64	1.61

1 according to equation 1 in Materials and Methods
2 digestible energy required for maintenance = 16.6 x exp $^{(0.055\text{Temperature})}$/body weight $(kg)^{0.82}$
3 retained energy = body energy x weight gain (equation 4)
4 digestible energy required for growth, using efficiency for growth $k_{DEg} = 0.67$
5 total digestible energy required for maintenance and growth
6 digestible energy content of feed = 17 MJ/kg

Table 4. Recommendations for dietary energy and protein supply for growing gilthead seabream at 23°C, when formulating feeds with different energy contents.

Body weight (g/fish)	*10*		*50*		*100*		*300*	
Weight gain (g/day)[1]	0.31		0.71		1.02		1.79	
Energy requirement								
Metabolic body weight ($kg^{0.82}$)	0.023		0.086		0.151		0.373	
DE_{maint} (kJ/fish/day)[2]	1.35		5.04		9.33		22.97	
DE_{growth} (kJ/fish/day)[2]	2.98		8.52		13.40		27.46	
DE_{m+g} (kJ/fish/day)[2]	4.33		13.56		22.73		50.43	
Protein requirement								
Metabolic body weight ($kg^{0.70}$)	0.040		0.123		0.200		0.431	
DP_{maint} (g/fish/day)[3]	0.025		0.076		0.124		0.267	
RP (g/fish/day)[4]	0.055		0.125		0.179		0.315	
DP_{growth}(g/fish/day)[5]	0.117		0.267		0.381		0.671	
DP_{m+g}(g/fish/day)[6]	0.141		0.343		0.505		0.938	
Feed formulation								
DE density of feed (per kg)	15 MJ	18 MJ	15 MJ	18 MJ	15 MJ	18 MJ	15 MJ	18 MJ
Feed intake (g/fish/day)	0.29	0.24	0.90	0.75	1.49	1.24	3.29	2.74
DP content in feed (g/kg)	486	586	381	457	339	407	285	342
FCR	0.94	0.77	1.27	1.06	1.46	1.22	1.84	1.53
DP/DE ratio (g / MJ)	32.5	32.5	25.4	25.4	22.6	22.6	19.0	19.0

1 according to equation 1
2 see notes 2,4,5 in Table 3.
3 digestible protein required for maintenance = 0.62 $g/kg^{0.70}/day$
4 retained protein = body protein (176 mg/g) x weight gain
5 digestible crude protein required for growth using efficiency for growth $k_{DPg} = 0.47$
6 total digestible protein required for maintenance and growth

Table 5. Proposed diet formulations and practical feeding tables for gilthead seabream during all stages of the growth period (at 23°C).

Weight (g)	*Feed composition (per kg feed)**		*Weight gain (g/fish/day)*	*Feed intake (g/fish/day)*	*Days of growth*	*FCR*
	Crude protein (g)	*Gross energy (MJ)*				
1-5	540	19.8	0.16	0.16	25	1.00
5-10	500	19.8	0.26	0.28	19	1.08
10-50	480	20.0	0.51	0.61	78	1.20
50-100	450	20.2	0.86	1.18	58	1.37
100-200	420	20.7	1.23	1.81	81	1.47
200-300	400	21.2	1.61	2.58	62	1.60
300-400	400	22.0	1.92	3.21	52	1.67

* assuming digestibility of protein 85% and of energy 80%