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Voluntary food intake of gilthead sea bream, *Sparus aurata*

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SUMMARY – The effect of dietary macronutrients on the food intake of juvenile gilthead seabream, *Sparus aurata* (L.), was examined in two experiments. In the first experiment, fish (68.14±12 g) were randomly assigned to two outdoor tanks (30 fish/tank) and fed to satiation twice daily (9:00 and 21:00) for 30 days on one of the two diets with similar gross energy content. In the second experiment, four groups of fish (67.19±13 g) were randomly assigned to four outdoor tanks (30 fish/tank). Each group was fed to satiation twice daily (9:00 and 16:00) for 4 weeks on one of the four experimental diets with varying macronutrient contents, and using the Latin square design (4 weeks x 4 diets x 4 groups). Both experiments suggest that protein is the controlling factor regulating food intake in gilthead seabream.

Keywords: Gilthead sea bream, satiation, food intake, protein, energy.

RESUMÉ *– "Ingestion volontaire d'aliments chez la daurade royale,* Sparus aurata*". L'effet des macronutriments alimentaires sur l'ingestion alimentaire de juvéniles de daurade royale,* Sparus aurata *(L.), a été examiné dans deux expériences. Dans la première expérience, les poissons (68,14±12 g) étaient distribués au hasard dans deux bassins en extérieur (30 poissons/bassin) et recevaient à volonté deux fois par jour (9:00 et 21:00) pendant 30 jours un des deux régimes ayant une teneur semblable en énergie brute. Dans la deuxième expérience, quatre groupes de poissons (67,19±13 g) étaient distribués au hasard dans quatre bassins en extérieur (30 poissons/bassin). Chaque groupe était alimenté à volonté deux fois par jour (9:00 et 16:00) pendant 4 semaines avec l'un des quatre régimes expérimentaux dont la teneur variait en macronutriments, en utilisant un dispositif en carré latin (4 semaines x 4 régimes x 4 groupes). Les deux expériences suggèrent que la protéine est le facteur déterminant dont dépend l'ingestion alimentaire chez la daurade royale.*

Mots-clés : *Daurade royale, à volonté, ingestion alimentaire, protéine, énergie.*

Introduction

The current trend in fish feed production is to increase lipid content, spare protein, improve feed conversion, decrease the amount of waste produced by the fish, and special attention is being given to the development of feeds that maximise nutrient retention and minimise nutrient loss (Tacon, 1997). Protein retention may be improved in many fish species, by replacing dietary protein by lipids. Such protein sparing effects have been demonstrated in salmon (Garcia *et al*., 1981; Johnsen *et al*., 1991), trout (Beamish and Medland, 1986), carp (Watanabe *et al*., 1987), hybrid striped bass (Nematipour *et al*., 1992), yellow tail (Shimeno *et al*., 1980), red sea bream (Takeuchi *et al*., 1991) and gilthead sea bream *Sparus aurata* L. (Vergara *et al*., 1996).

Energy and more than 40 nutrients have been proven to be essential for fish growth, reproduction and health (National Research Council, 1993). The nutrients can be classified, from the viewpoint of the amounts required, into macronutrients (proteins, lipids and carbohydrates) and micronutrients (vitamins and minerals). Although all three macronutrients can be energy sources, the ratio of dietary protein to energy is important for producing more economical feeds and to minimize adverse environmental impacts (Cho and Kaushik, 1990; Cho, 1993; Kaushik and Medale, 1994). Carbohydrates, proteins and lipids can all be used as energy sources by fish, but these organic compounds are not equally well suited for the promotion of growth. In order for growth to occur there is a requirement for dietary protein and the provision from the diet of certain lipids, vitamins and minerals for efficient functioning of the biochemical processes of the body (Jobling, 1994).

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The requirement level for dietary nutrients is the basis for their inclusion levels in the feed formula. Protein is one of the most important nutrient categories for growth and the most expensive macro component of fish feed because of its bulk in the feed formula (Pandian, 1989; National Research Council, 1993). Protein requirements are always studied in aquaculture species with the aim of determining the minimum amount required to produce maximum growth and not to be utilized for energy (Phillips, 1972). Fish are known to utilize protein preferentially to lipid or carbohydrate as an energy source (Peres and Oliva-Teles, 1999b) and in some cases as much as 0.7 of dietary energy may come from protein (Phillips, 1969). Feed cost per unit of fish can be minimized by optimal use of low-cost energy carriers such as carbohydrate-rich ingredients, ensuring that the use of costly protein is kept as low as possible.

The optimal nutrient composition of feeds varies between fish species. While herbivorous and omnivorous fish accept more than 25% carbohydrate in their diet, carnivorous fish have optima below 20% (Wilson, 1994). High optimum level of energy and lipid in the diet for carnivorous fish has been demonstrated for Atlantic salmon (Hillestad and Johnsen, 1994). Marine fish in general seem to have a high requirement for protein; optimal dietary protein content of 45-50% has been reported for plaice (Cowey *et al*., 1972), turbot (Danielssen and Hjertnes, 1993), gilthead sea bream (Santinha *et al*., 1996), and Mediterranean yellowtail (Jover *et al.,* 1999).

The "lipostatic model" has been proposed to explain the well-regulated control of body weight and food intake, and predicts that secretions from fat cells are the key signal to the brain to regulate feeding and body-fat deposition (Woods *et al*., 1998; Inui, 1999; Seeley and Schwartz, 1999). A feedback regulatory loop with distinct steps has been hypothesized, which include a sensor that monitors energy levels, hypothalamic centres that receive and integrate through leptin receptors the intensity of the signal, and effector systems that influence energy intake and expenditure (Jequier and Tappy, 1999).

Several factors can alter the energy requirements of fish such as temperature, water flow, body size, level of feeding, crowding, oxygen and energy accumulation (Smith, 1989). The ecologically important feature of digestion is the rate at which food can be processed, as this determines the upper limit to the intake of energy and hence the upper limit of growth. (Pitcher and Hart, 1982). A number of factors can affect feed intake including the duration of feeding period (satiation time), the individual meal size (stomach capacity), the time between meals (feeding interval), the competition in terms of density, space, and hierarchical effects, and the interaction of the above (Brett, 1979).

It is assumed that fish, as well as homeothermic animals (Kissileff and Van Itallie, 1982) adjust feed intake in order to satisfy their digestible energy requirements (Cho and Kaushik, 1985; Boujard and Medale, 1994; Kaushik and Medale, 1994). In macronutrient self-selection patterns in fish, Sanchez-Vazquez *et al.* (1998, 1999) showed that goldfish, an omnivorous fish, preferred digestible carbohydrate and fat to protein, whereas rainbow trout, a carnivorous species, principally selected protein to fat and carbohydrate, reflecting their different feeding habits. Nevertheless both trout and goldfish, precisely regulated food intake to balance total energy intake. Furthermore Landless (1976) observed that daily self-feeding rhythms in trout show seasonal variations, possibly as a response to changes in water temperature and photoperiod.

Whether or not animals are able to regulate separately their intake of macronutrients remains contentious (Friedman, 1999; Galef, 1999; Seeley and Berthoud, 1999). But the capacity of fish to make dietary discriminations was demonstrated by Hidalgo *et al.* (1998) who reported that sea bass were able to distinguish diets differing only in the content of a single essential amino acid. Dietary self-selection may serve as a technique to balance a deficient diet.

In the present study patterns of macronutrient selection were examined in gilthead sea bream *Sparus aurata* (Linnaeus, 1758), a common teleost in the Mediterranean Sea and one of the most commonly used species in aquaculture both in land-based farms and in cages at sea (Le-Breton, 1994). The aim of the study was to test the hypothesis that the study species feeds to primarily control protein and not energy intake. Fish were maintained in groups and fed two diet regimes. Comparison was made between two groups fed on isoenergetic diets with different macronutrient content, and between four groups fed four diets with variable macronutrient content using the Latin squares design (Snedecor and Cochran, 1989).

Materials and methods

The experiments were conducted at the Aquaculture Laboratories N.C.M.R., Hellinicon, Greece, using juvenile gilthead sea bream acquired from a farm situated in the SE Saronic Gulf, Greece. The fish were acclimatized for 15 days, at temperature 24.5±0.2°C before the start of the experiments (Apostolopoulos and Klaoudatos, 1986), in an indoor tank and were fed commercial pellets (Trouvit Europa Marine) once a day (9:00) to apparent satiation; composition of the commercial pellets is shown in (Table 1).

Table 1. Composition of the commercial feed used (Trouvit-Europa-Marine) during acclimatization

Composition (g/100g)	Vitamins (mg/kg feed)
Protein 45.0%	Vitamin A – 10,000
Lipids 22.0%	Vitamin D3 – 1,600
Cellulose 1.5%	Vitamin E – 200
Ash 8.0%	Vitamin B1 – 22
Moisture 10.0%	Vitamin B2 – 35
	Vitamin PP – 35
	Vitamin C (ascorbic acid) – 75
	Vitamin D (pantothenic acid) – 70
	Mn – 50
	Zn – 105
	Cu – 6
	Inositol – 60

After acclimatization, fish were deprived of food for a day, lightly anaesthetized using quinaldine dissolved in acetone (1 ml/100 l), nitrofurazone (50g/l) was added for 1 hour to reduce the possibility of parasitical or bacterial infection, then measured and weighed individually. Oxygen was provided during handling, and fish (67.6±2.7 g) were transferred to six 250 l outdoor tanks, supplied with a continuous flow of filtered seawater at a rate of 120 l/h in an open circuit system and salinity ranged at 37.7±0.3‰ throughout the experiments. The fish were also measured and weighed individually at the end of each experiment. Fish survival at the end of the experiments was high and unaffected by the diets.

Each experiment was conducted without replication but both experiments were designed in order to test the same hypothesis each with a different approach. The trials lasted 30 days for the first experiment and 4 weeks for the second. The water temperature in the outdoor tanks was not artificially regulated and fluctuated throughout the experiments according to the natural environmental conditions. Both experiments took place in August where occasionally unusually high seawater temperatures occur in semi enclosed and enclosed water bodies in Greece (Amvrakikos bay) due to extreme seasonal temperatures. The extreme water temperature recorded during the first experiment (30.5°C) was not part of the experimental design but was the result of natural occurrence. During the first experiment water temperature ranged from 24.4°C to 30.5°C and during the second from 24.1°C to 28.4°C. Fish were exposed to the natural daily photoperiod (16L/8D) and all tanks had similar light conditions. Faeces were removed daily from the bottom of each tank. Abiotic parameters of the water were recorded daily, oxygen concentration ranged at 6.6±0.5 mg/l, oxygen saturation at 81.4±6.5%, and ph at 7.9±0.1 in all tanks.

The diets used were made by mixing different amounts of fish meal, fish oil, gelatinized starch, kaolin, and multivitamin and mineral mix. Ingredients were thoroughly mixed together in a high-speed paddle mixer with the addition of water and then extruded through a die, forming spaghetti-like strands of ca 5 mm diameter. The pellets were spread onto trays and air-dried in a desiccator, for 24 hours to remove the excess water and obtain uniform water content between the diets. Six experimental diets were formulated to contain variable levels of dietary protein and energy, formulation shown in Table 2.

Table 2. Dietary ingredients, chemical analysis and calculated values of the experimental diets

	Feed A	Feed B	Feed C	Feed D	Feed E	Feed F
Ingredients (g/100 g)						
Fish meal	78	78	51	51	78	51
Fish oil	10.5	2.7	14.9	7	5.2	14.9
Gelatinized starch	10	10	10	10	10	20
Kaolin	0	7.8	24.1	30.5	5.3	12.6
Vitamin mineral mix	1.5	1.5	1.5	1.5	1.5	1.5
Chemical composition (g/100 g)						
Protein (N X 6.25)	55.4	55.4	37.1	37.1	54.9	33.7
Lipids (Soxhlet)	17.3	9.5	19.1	10.9	11.8	23.1
Moisture	8.7	7.4	5.4	5.4	8.9	7.2
Ash	13.2	18.5	27.9	35.2	16.6	19.1
Calculated values						
Carbohydrate (g/100 g)	6.6	9.2	10.5	11.4	7.8	16.9
Gross energy (Kcal/100 g)	495.4	437.7	431.8	357.9	450.7	476.2

Each diet was analysed for protein, lipid, dry matter moisture and ash contents. Protein (N x 6.25) was analysed using the micro-Kjeldahl digestion according to Crooke and Simpson (1971), lipid by petroleum ether extraction (Soxhlet technique), dry matter by drying at 110°C for 24 hours, and ash by incineration in a muffle furnace at 500°C for 12 h. The gross energy and carbohydrate content of each diet were estimated by calculations.

In the first experiment, two groups of fish (68.1±12 g) (30 fish/tank) were fed on two diets with similar energy and different protein and lipid contents (Feeds E and F) by hand to apparent satiation, twice a day (9:00 and 21:00). Pellets were distributed slowly permitting all fish to feed.

In the second experiment, four groups of fish (67.19±13 g) (30 fish/tank) were fed on four diets containing varying amounts of protein and energy contents (Feeds A, B, C and D) to satiation twice a day (9:00 and 16:00). Each group of fish was fed on each diet for 7 days, and then feeds were rotated in a "round robin" fashion, such that by the end of the experiment every group of fish had been provided with each diet. Feeding was carried out by the same person throughout the experiments. The point of satiation was determined when the fish showed no more interest in the food. The feeding sequence for both experiments is shown (Table 3).

Table 3. Feeding sequence used for each experiment. The Latin square design was used for the second experiment

Tank number	Sequence of diets used				Duration of experiments
1	Feed E				Experiment 1
2	Feed F				30 days
3	Feed A	Feed D	Feed C	Feed B	Experiment 2
4	Feed B	Feed A	Feed D	Feed C	4 weeks
5	Feed C	Feed B	Feed A	Feed D	
6	Feed D	Feed C	Feed B	Feed A	

The ingestion rate (g/fish/day) was checked for normality with Anderson-Darling test and homogeneity of variance with Bartlett's test whereas test of potential differences on the ingestion rate between the two groups of fish in the first experiment was performed by a two-sample t-test (Dytham, 2003). The Latin squares analysis of variance (Snedecor and Cochran, 1989) was used to test the effects of three different sources of variability (tanks, weeks and diets) for the four groups of fish in the

second experiment, followed by Tukey's pair-wise comparisons to test possible differences between means. Results were considered significant at the 0.05 probability level. All statistical analysis was carried out using the Minitab 13 for Windows statistical package (Ryan, 2000).

Results

Experiment 1

Water temperature during experiment 1 ranged between 24.6°C and 30.5°C (mean 27.1±1.3°C) in tank 1 (fish fed Feed E) and from 24.4°C to 30.5°C (mean value 27.0±1.4°C) in tank 2 (fish fed Feed F). There was significant correlation between the log of ingestion rate (g/fish/day) and temperature for fish in both tanks, with ingestion rate (g/fish/day) increasing with increasing temperature (Fig. 1).

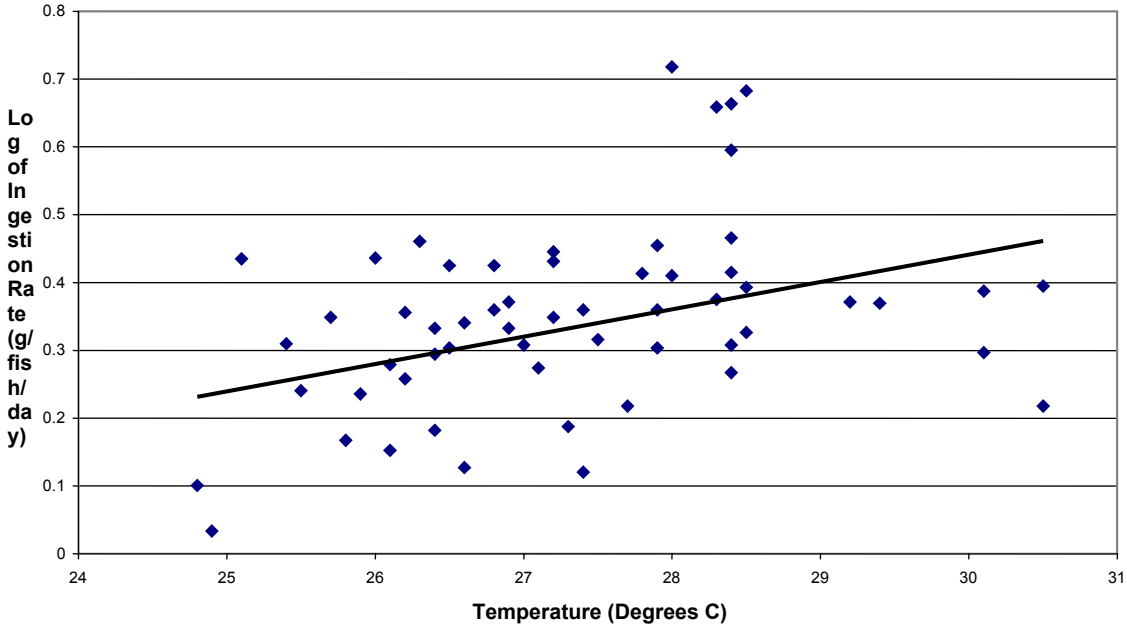

Fig 1. Effect of temperature on the combined ingestion rate (g/fish/day) of two groups of fish fed diets with similar digestible energy and different protein and fat contents (Feeds E and F).

Ingestion rate (g/fish/day) was greater in tank 2 (2.7±0.9 g/fish/day) fed on low protein high fat diet (Feed F) in comparison with the ingestion rate in tank 1 (1.7±0.5 g/fish/day) fed with high protein low fat diet (Feed E). The ingestion rate (g/fish/day) increased in tank 2 between day 20 (water temperature 27.9°C) and day 28 (temperature 29.4°C). This increase in ingestion rate is probably related to the increase in temperature. Ingestion rate for the first experiment is shown in (Fig. 2).

The ingestion rate (g/fish/day) of the low protein diet (Feed F) was significantly higher over the entire experimental period; no significant difference on the ingestion rate (g/fish/day) was shown between days 5 to 20, whereas between days 21-27 there was significant difference (Table 4).

Protein consumption (g/fish/day) over the entire experimental period was higher in the group of fish fed on the high protein diet (Feed F) as well as between days 5 to 20; between days 21 to 27 protein consumption was very similar (Fig. 3).

When water temperature was lower than 28°C, both groups of fish displayed similar ingestion rates (g/fish/day) resulting in higher protein consumption in the group fed with high protein diet. When water temperature reached 28°C the group of fish feeding on the low protein diet (Feed F) increased its ingestion rate. Several days later the ingestion rate (g/fish/day) of the group of fish fed on low protein diet (Feed F) was dramatically reduced.

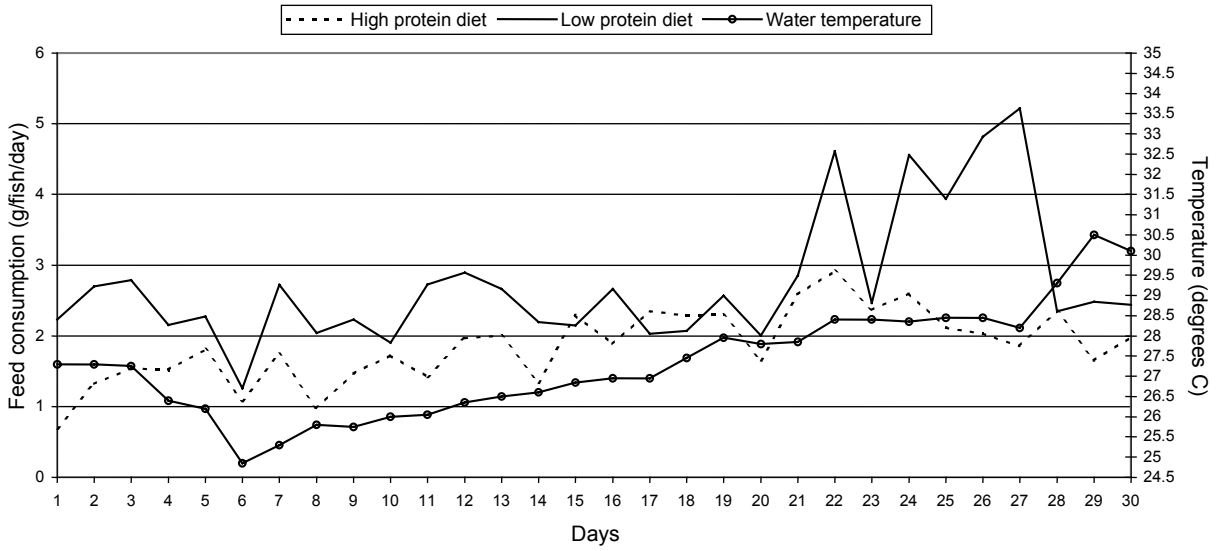

Fig. 2. Daily ingestion rate (g/fish/day) of two feeds with similar energy and different protein contents, the high protein feed (Feed E) and low protein feed (Feed F), in relation to water temperature.

Table 4. Daily ingestion rate (g/fish/day). Comparison of two time intervals of Experiment 1, data are normal (50% P>0.05) and homogeneous (P>0.05)

	Mean	SD*	95% CI**	T-value	Probability	DF***
Days 5-20						
High protein diet	2.41	0.59	-0.60, 0.18	-1.12	ns	28
Low protein diet	2.63	0.48				
Days 21-27						
High protein diet	3.21	0.51	-2.62, -0.35	-3.01	s	8
Low protein diet	4.70	1.20				

*SD: Standard Deviation.
**CI: Confidence Interval.
***DF: Degrees of Freedom.

Experiment 2

Water temperature during experiment 2 ranged between 24.1°C and 28.4°C (mean value 26.8±1.0°C). There was significant correlation between the log of ingestion rate (g/fish/day) and temperature for all groups of fish, with ingestion rate increasing with increasing temperature (Fig. 4).

Ingestion rates over the last 4 days of each week were used for comparison in order to allow the fish in each tank to adapt to each new diet given, similar period for adaptation was used for gilthead sea bream by Santinha, *et al*., (1999) (Table 5).

The ingestion rate (g/fish/day) of each group of fish in relation to temperature is shown in (Fig. 5). The Latin squares analysis of variance performed to test the effects of three different sources of variability, showed that ingestion rate (g/fish/day) over the last four days of each week differs significantly between different feeds and weeks, but not between tanks (due to differences in biotic and abiotic factors as well as external factors like noise and shedding) (Table 6, Fig. 6).

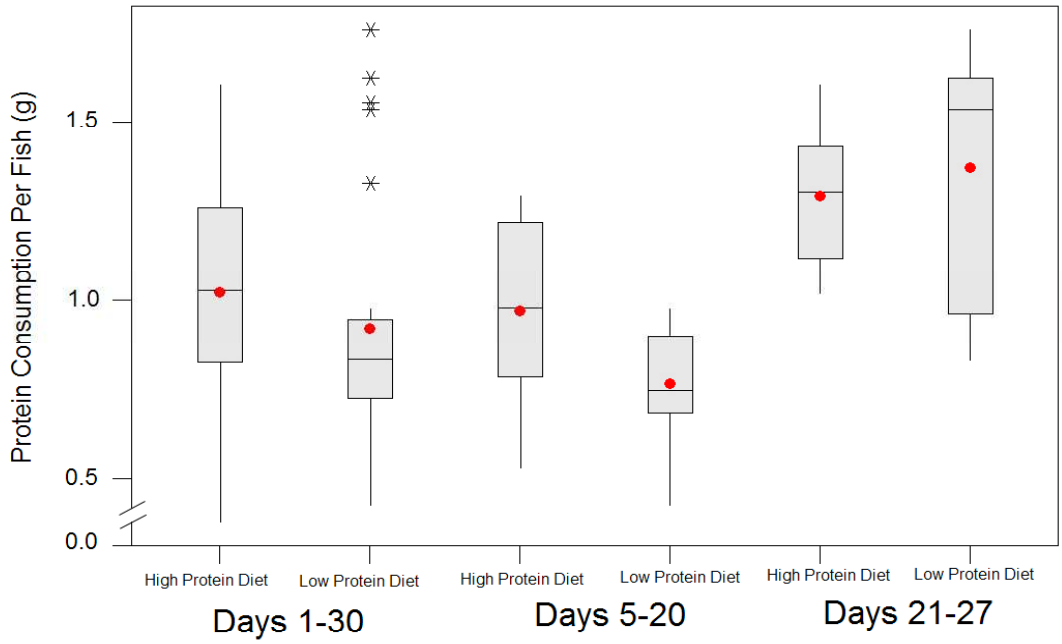

Fig. 3. Comparison of the protein consumption per fish fed two diets with similar energy contents, the high protein diet (Feed E) and the low protein diet (Feed F) during 3 time intervals. The box represents 50 % of the data set. The whiskers extend from the top and bottom of the box to the adjacent values, the lowest and highest observations that are still inside the region defined by the following limits: the lower limit: Q1 - 1.5 (Q3 - Q1), and the upper limit: Q3 + 1.5 (Q3 - Q1). Dots indicate the mean values and the horizontal lines inside the boxes indicate the median values. Outliers are points outside of the lower and upper limits plotted with asterisks.

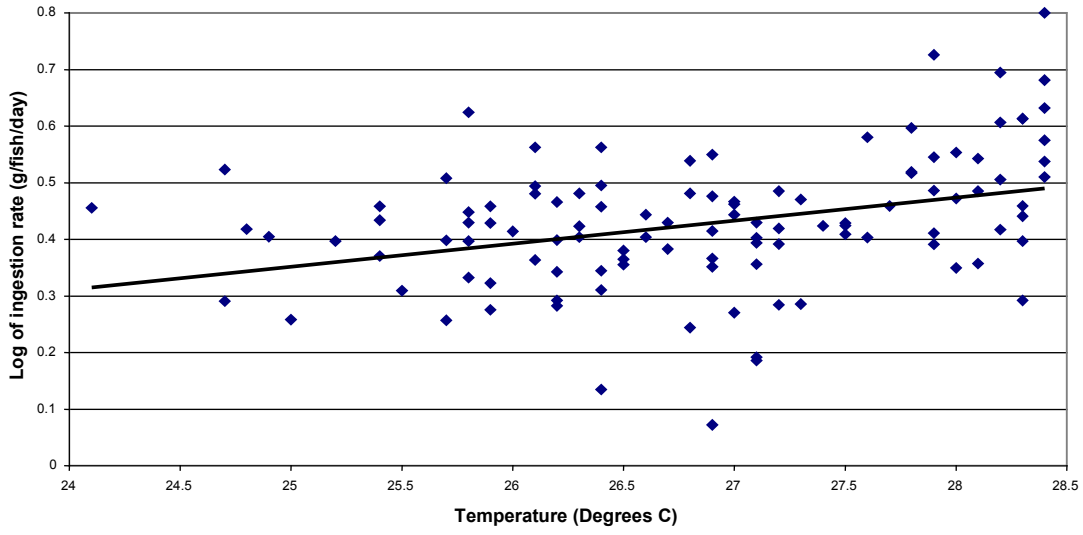

Fig. 4. Effect of temperature on the combined ingestion rate (g/fish/day) of four groups of fish fed diets varying in protein and energy contents (Feeds A, B, C, and D).

Table 5. Total diet consumed (g) the last 4 days of each week from fish groups in each tank. The diets used were high energy - high protein diet (Feed A), low energy - high protein diet (Feed B), high energy - low protein diet (Feed C), and low energy - low protein diet (Feed D)

Tank	Feed A	Feed B	Feed C	Feed D
3	204.9	337.6	317.6	302.7
4	309.9	257.9	467.7	334.5
5	282.6	256.6	392.2	484.6
6	379.9	317.8	352.1	337.5

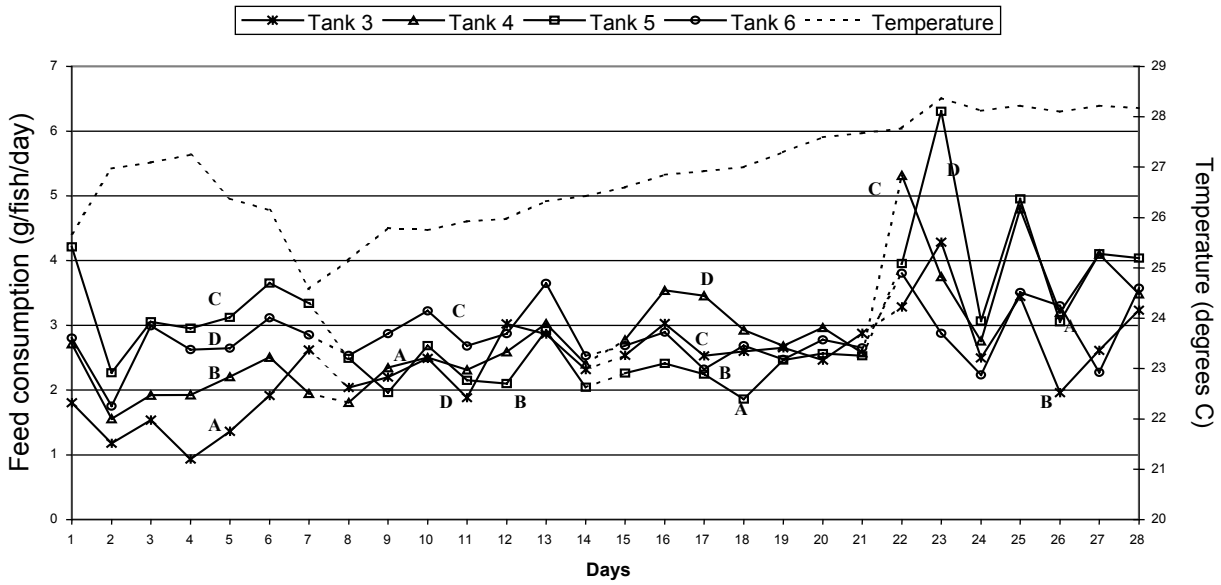

Fig. 5. Ingestion rate (g/fish/day) of different diets varying in protein and energy content, diets A, B, C and D in relation to temperature. Dotted lines indicate the transition between diets.

Table 6. Result of the Latin squares analysis of variance, performed to test the effects of three different sources of variability, on the ingestion rate (g/fish/day) over the last four days of each week from fish present in each tank

Source	Degrees of freedom	Sum of squares	Mean square	F-statistic	Probability
Tanks	3	10037.5	3345.8	3.90	ns
Weeks	3	38009.2	12669.7	14.76	s
Diets	3	26356.1	8785.4	10.24	s
Error	6	5148.8	858.1		
Total	15	79551.6			

Differences between means of the ingestion rates over the last four days of each week, attributed to different tanks, weeks and diets, were tested using Tukey's pair-wise comparisons (Table 7).

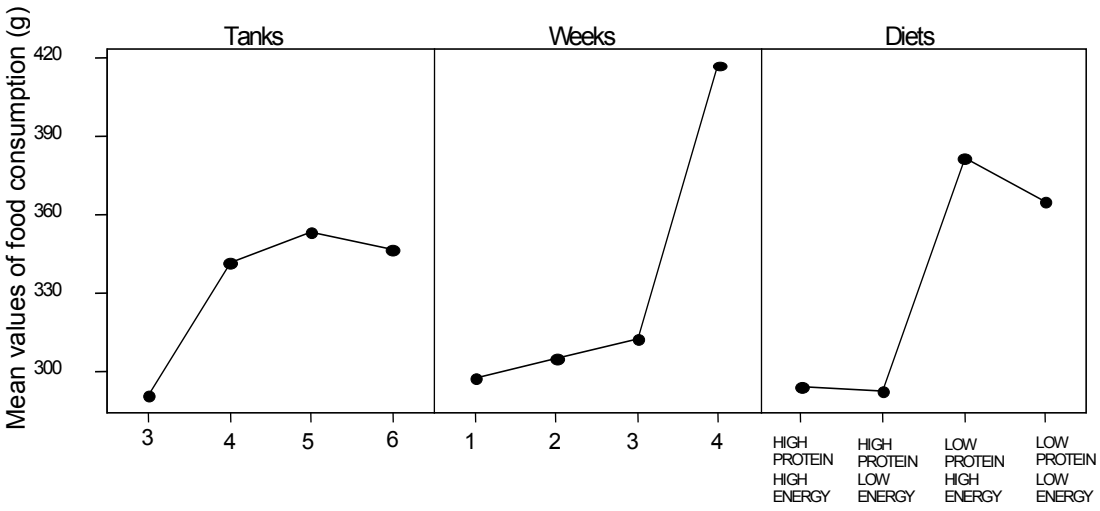

Fig. 6. Main effects plot of the ingestion rate (g/fish/day) over the last 4 days of each week showing the effect of different tanks, weeks and diets, high energy - high protein diet (Feed A), low energy - high protein diet (Feed B), high energy - low protein diet (Feed C), low energy - low protein diet (Feed D), on the total food consumption of the four groups of fish.

Table 7. Tukey's pair-wise comparisons testing differences between means of the total food consumed the last four days of each week, attributed to different tanks, weeks and diets. Cells contain the difference of means, the t-value and the associated probability

Tank number	3	4	5
4	51.81, 2.50, ns		
5	63.27, 3.05, ns	11.46, 0.55, ns	
6	56.11, 2.71, ns	4.30, 0.21, ns	-7.160, 20.71, ns
Week number	1	2	3
2	7.21, 0.35, ns		
3	15.03, 0.73, ns	7.82, 0.38, ns	
4	119.30, 5.76, s	112.09, 5.41, s	104.30, 5.03, s
Diet	High protein High energy diet (A)	High protein Low energy diet (B)	Low protein High energy diet (C)
High protein low energy diet (B)	-1.84, -0.09, ns		
Low protein high energy diet (C)	88.08, 4.25, s	89.91, 4.34, s	
Low protein low energy diet (D)	70.49, 3.40, ns	72.33, 3.49, s	-17.58, -0.85, ns

Significant effect of week 4 is shown on the ingestion rates (g/fish/day) for all groups of fish, probably attributed to the increase in water temperature (above 28°C). Significantly higher ingestion rate (g/fish/day) of the low protein diets (Feeds C and D) is evident. The ingestion rate (g/fish/day) is not significant between the high protein diets (Feed A and B) or the low protein diets (Feed C and D). This difference in food consumption is possibly attributed to the fact that fish need to consume a certain amount of protein in their diet, resulting in increasing ingestion rates when fish were fed with low protein diets. The higher consumption occurs in diets low in protein (Feeds C and D), however there was the indication that the difference in food consumption between diets containing the same amount of protein was determined by the energy content, indicating that fish primarily feed in order to regulate their protein and secondarily their energy intake.

Discussion and conclusions

Digestible energy is though to be one of the major criteria controlling feed intake in fish (Lee and Putnam, 1973; Jobling and Wandsvik, 1983; Weatherley and Gill, 1987; Kentouri *et al*., 1995; Paspatis and Boujard, 1996; Keembiyehetty and Wilson, 1998; Lupatsch *et al*., 2001) along with other factors including fish size, temperature or palatability. Fish, like homeothermic animals, seem to adjust feed intake in order to satisfy their digestible energy requirements in tune with their growth rates (Cho and Kaushik, 1985; Kaushik and Medale, 1994). Grove *et al*. (1978) demonstrated that trout, fed diets diluted with kaolin increased their demand feeding activity to partially compensate for a decrease in energy content. Alterations in feeding behaviour, to increase energy intake, have also been observed in the brown trout, *Salmo trutta* L. (Ringler, 1979), and the mummichog, *Fundulus heteroclitus* (Weisberg and Lotrich, 1982), when offered natural prey species varying in calorific value. Lee and Putnam (1973), report that rainbow trout fed low energy diets grew at equal growth rates as those fed high-energy diets by increasing their food intake.

Results obtained during the course of the study demonstrate that *Sparus aurata* feeds primarily for protein and secondarily for energy. Similar results were obtained on sea bass that seemed to adjust better to a constant protein intake (Oliva-Teles and Cerqueira, 1997; Peres and Oliva-Teles, 1999a), and on gilthead sea bream (Santinha *et al*., 1996). Peres and Oliva-Teles (1999b) also report that within certain limits sea bass seems to regulate protein intake rather than energy, in order to satisfy maximum growth requirements.

In the current study when fish were fed on two isoenergetic diets containing differing amounts of protein, it was indicated that the need for protein was the driving force of the food intake. The difference in ingestion rates could be partially attributed to the palatability of low protein diet (Feed F), which contained a higher amount of oil making it more attractive to the fish. Fish seemed to have lower protein requirements and regulated their energy uptake when water temperature was below 28°C, above that temperature protein seemed to be the important dietary ingredient that regulated the feed intake. It is generally accepted that the protein requirement of fish, expressed as the percentage in diet, is not affected by water temperature (National Research Council, 1993). However several studies have shown that the dietary protein requirement level is higher at higher water temperatures (Delong *et al*., 1958; Millikin, 1982; Millikin, 1983). Yamamoto *et al*., (2001) reported that carp showed high preference for protein-rich diet at high water temperature and high photoperiod, and that when water temperature and photoperiod were low carp showed almost equal demands for each three macronutrients on the basis of digestible energy. Webster *et al*. (1995) reported that dietary protein requirements among and within species might differ due to differences in water temperature, salinity, diet composition, quality and biological value of protein sources. Ogino and Yang (1980) showed that the dietary protein requirement of a fish was related to dietary protein density and feeding rate, demonstrating that the statement of a protein requirement in terms of a percentage of the diet is of limited use. Elliott (1982) showed that weight specific feed intake increased with temperature as observed in the present study. It is well known that a rise in temperature increases standard metabolism, given the general effect of temperature on biochemical reactions (Edwards *et al*., 1969; Eccles, 1985).

When fish were fed on four diets differing in protein and energy contents, the ingestion rate was significantly higher on the diets containing low protein content (Feeds C and D). This was an indication that fish needed to consume a certain amount of protein with their diet. Kaushik (1996) reported similar protein intake of fish regardless of the dietary protein level. There is therefore the indication that, within certain limits, sea bream regulates protein intake in order to satisfy its growth requirements. Regulating the intake of a particular nutrient, the animal must be able to detect the nutrient concentration in the diet, to have a measure of its current requirement, and respond to the diet according to the relationship between its nutrient requirement and the nutrient concentration in the diet. In most animals, sugars, amino acids and salts are sensed and influence feeding behaviour. Fish have a highly developed gustatory system, tuned to nutrients and nutritionally instructive cues (Caprio *et al*., 1993; Lamb and Finger, 1995; Carr *et al*., 1996; Finger, 1997). Furthermore Sanchez-Vazquez *et al*. (1998) reported a high complexity of selection behaviour in goldfish, which displayed daily rhythmicity exhibiting different timing preferences for each macronutrient, carbohydrate during the light phase, protein during the dark phase and fat in the transition phase.

The current study showed that protein content of the diet primarily affects ingestion rate. The higher feed intake of the low energy - high protein diet in comparison with the high energy - high protein diet also serves as an indication that regulation of the food intake was secondarily according to the energy content of the diet. When an animal is constrained from reaching its intake target by having access only to sub optimal foods, it is forced to make a behavioural compromise in which some nutrients are overeaten and others undereaten relative to the intake target; nevertheless the animal may still be able to achieve its growth target by differentially using ingested nutrients, either in the gut or after absorption (Simpson and Raubenheimer, 2001).

To develop more efficient and economical fish feeds with minimal environmental impacts, more attention needs to be paid to the requirements and utilization of energy and nutrients in consideration of the seasonal cycle of environmental conditions. It is important to know whether or not animals are able to regulate separately their intake of macronutrients when designing feeds and feeding regimes in the commercial production of animals, and is furthermore critical for economic, welfare and environmental reasons to understand which nutrients are behaviourally regulated. Given the enormous interest that feeding has, macronutrient selection in fish constitutes a novel and promising approach that must be given proper consideration when studying fish nutrition and designing diets for fish culture.

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References

Apostolopoulos, I. and Klaoudatos, S. (1986). Effect of acclimatization and degree of hunger on the satiation amount of *Sparus aurata* and satiation curve of the fish. *Thalassografica*, 9(2): 69-78.

Beamish, F.W.H. and Medland, T.E. (1986). Protein sparing effects in large rainbow trout, *Oncorhynchus mykiss. Aquaculture*, 55: 35-42.

Boujard, T. and Medale, F. (1994). Regulation of voluntary feed intake in juvenile rainbow trout fed by hand or by self-feeders with diets containing two different protein/energy ratios. *Aquat. Liv. Resourc.*, 7: 211-215.

Brett, R.R. (1979). Environmental factors and growth. In: *Fish Physiology, Bioenergetics and Growth*. *Vol. 8*, Hoar, W.S., Randall, D.J. and Brett, J.R. (eds). Academic Press, London, pp. 280-352.

Caprio, J., Brand, J.G., Teeter, J.H., Valentincic, T., Kalinoski, D.L., Kohbara J., Kumazawa, T. and Wegert, S. (1993). The taste system of the channel catfish – from biophysics to behaviour. *Trends in Neurosciences,* 16: 192-197.

Carr, W.E.S., Netherton, J.C., Gleeson, R.A. and Derby, C.D. (1996). Stimulants of feeding behaviour in fish: Analyses of tissues of diverse marine organisms. *Biological Bulletin,* 190: 149-160.

Cho, C.T. (1993). Feeding system for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture*, 100: 107-123.

Cho, C.Y. and Kaushik, S.J. (1985). Effects of protein intake on metabolizable and net energy values of fish diets. In: *Nutrition and Feeding in Fish,* Cowey, C.B., Mackie, A.M. and Bell, J.G. (eds). Academic Press, London, pp. 95-117.

Cho, C.Y. and Kaushik, S.J. (1990). Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Rev. Nutr. Diet.*, 61: 132-172.

Cowey, C.B., Pope, J.A., Adron, J.W. and Blair, A. (1972). Studies of nutrition of marine flatfish. Utilization of various dietary protein by plaice (*Pleuronectes platessa*). *Br. J. Nutr.*, 31: 297-306.

Crooke, W.M. and Simpson, W.E. (1971). Determination of ammonium in Kjeldahl digest of crops by an automated procedure. *J. Sci. Food Agric.*, 22: 9-10.

Danielssen, D.S. and Hjertnes, T. (1993). Effect of dietary protein levels in diets for turbot (*Scophthalmus maximus*) to market size. In: *Fish Nutrition in Practice,* Kaushik, S.J. and Luquet, P. (eds). INRA, Paris, pp. 89-96.

Delong, D.C., Halver, J.E. and Merts, E.T. (1958). Nutrition of salmonids fishes. VI. Protein requirements of Chinook salmon at two water temperatures. *J. Nutr.*, 65: 589-599.

Dytham, C. (2003). *Choosing and Using Statistics. A Biologist's Guide*. 2nd edn. Blackwell Publishing, Oxford.

Eccles, D.H. (1985). The effect of temperature and mass on routine oxygen consumption in the South African cyprinid fish *Barbus aeneus* Burchell. *J. Fish Biol*., 27: 155-165.
Edwards, R.R.C., Finlayson, D.M. and Steele, J.M. (1969). The ecology of 0-group plaice and common dabs in Loch Ewe. II. Experimental studies of metabolism. *J. Exp. Mar. Biol. Ecol*., 3: 1-17.
Elliott, J.M. (1982). The effects of temperature and ration size on the growth and energetics of salmonids in captivity. *Comp. Biochem. Physiol*., 73B: 81-91.
Finger, T.E. (1997). Evolution of taste and solitary chemoreceptor cell systems. *Brain, Behaviour and Evolution,* 50: 234-243.
Friedman, M.I. (1999). Too many choices? A critical essay on macronutrient selection. In: *Neural and Metabolic Control of Macronutrient Intake*, Berthoud H.R. and Seeley, R.J. (eds). CRC Press, Boca Raton, pp. 11-18.
Galef, B.G. (1999). Is there a specific appetite for protein? In: *Neural and Metabolic Control of Macronutrient Intake*, Berthoud H.R. and Seeley, R.J. (eds). CRC Press, Boca Raton, pp. 19-28.
Garcia, M., Zamora, S. and Lopez, M.A. (1981). The influence of partial replacement of protein by fat in the diet on the protein utilization by the rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol*., 68B: 457-460.
Grove, D.J., Loizides, L.G. and Nott, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri. J. Fish Biol*., 12: 507-516.
Hidalgo, F., Kentouri, M. and Divanach, P. (1988). Sur l'utilization du self feeder comme outil d'epreuve nutritionnelle du loup, *Dicentrarchus labrax*: Résultats préliminaries avec la methionine. *Aquaculture,* 68: 177-190.
Hillestad, M. and Johnsen, F. (1994). High-energy/low-protein diets for Atlantic salmon: Effects on growth, nutrient retention and slaughter quality. *Aquaculture*, 124: 109-116.
Inui, A. (1999). Feeding and body-weight regulation by hypothalamic neuropeptides-mediation of the action of leptin. *Trends Neurosci*., 22: 62-67.
Jequier, E. and Tappy, L. (1999). Regulation of body weight in humans. *Physiol. Rev.,* 79: 451-480.
Jobling, M. (1994). *Fish Bioenergetics.* Chapman & Hall, London, 309 p.
Jobling, M. and Wandsvik, A. (1983). An investigation of factors controlling food intake in Arctic charr, *Salvelinus alpinus* L. *J. Fish Biol*., 23: 397-404.
Johnsen, F., Hillestad, M. and Austreng, E. (1991). High energy diets for Atlantic salmon. Effects on pollution. In: Fish Nutrition in Practice, Proceedings of the 4th International Symposium on Fish Nutrition and Feeding, Kaushik, S.J. and Luquet, P (eds), Biarritz (France), 24-27 June 1991. INRA Editions, France, *Les Colloques*, 61: 391-401..
Jover, M., Garcia-Gomez, A., Tomas, A., De la Gandara, F. and Perez, L. (1999). Growth of Mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing different levels of protein and lipid. *Aquaculture*, 179: 25-33.
Kaushik, S.J. (1996). Feed quality and feeding strategies for sustained aquaculture development. *Suisanzoshoku,* H8: 231-234.
Kaushik, S.J. and Medale, F. (1994). Energy requirements, utilization and dietary supply to salmonids. *Aquaculture,* 124: 81-97.
Keembiyehetty, C.N. and Wilson, R.P. (1998). Effect of water temperature on growth and nutrient utilization of sunshine bass (*Morone chrysops* ♀ × *Morone saxatilis* ♂) fed diets containing different energy/protein ratios. *Aquaculture*, 166: 151-162.
Kentouri, M., Divanach, P., Geurden, I. and Anthouard, M. (1995). Evidence of adaptive behaviour in gilthead seabream (*Sparus aurata* L.) in relation to diet composition, in a self-feeding condition. *Ictyophysiol. Acta*, 18: 125-143.
Kissileff, H.R. and Van Itallie, T.B. (1982). Physiology of the control of food intake. *Ann. Rev. Nutr*., 2: 371-418.
Lamb, C.F. and Finger, T.E. (1995). Gustatory control of feeding behaviour in goldfish. *Physiology and behaviour,* 57: 483-488.
Landless, P.J. (1976). Demand-feeding behaviour of rainbow trout. *Aquaculture*, 7: 11-25.
Le-Breton, A.D. (1994). Marine fish breeding in the Mediterranean: Rearing techniques, actual situation and prospects. *Rec. Med. Vet. de l'Ecole d'Alfort*., 170: 121-128.
Lee, D.J. and Putnam, G.B. (1973). The responses of rainbow trout to varying protein / energy ratios in a test diet. *J. Nutr*., 103: 916-922.
Lupatsch, I., Kissil, G.W.M., Sklan, D. and Pfeffer, E. (2001). Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata* L.). *Aquaculture Nutrition*, 7: 71-80.

Millikin, M.R. (1982). Effects of dietary protein concentration on growth, feed efficiency, and body composition of age-0 striped bass. *Trans. Am. Fish. Soc*., 111: 373-378.
Millikin, M.R. (1983). Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Trans. Am. Fish. Soc*., 112: 185-193.
National Research Council (1993). *Nutrient Requirements of Fish*. National Academy Press, Washington DC.
Nematipour, R.R., Brown, M.L. and Gatlin, D.M. (1992). Effects of dietary energy:protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops* × *M. saxatilis. Aquaculture,* 107: 359-368.
Ogino, C. and Yang, G.Y. (1980). Requirements of carp and rainbow trout for dietary manganese and copper. *Bull Jpn. Soc. Fish*., 48: 455-458.
Oliva-Teles, A. and Cerqueira, A.L. (1997). Effect of the dietary protein level on metabolic utilization of diets by seabass (*Dicentrarchus labrax*). In: *Proceedings of the III International Symposium on Research for Aquaculture and Fundamental and Applied Aspects*, Barcelona (Spain), 24-27 August 1997, Poster No. 187.
Pandian, T.J. (1989). Protein requirements of fish and prawns cultured in Asia. In: *Fish Nutrition Research in Asia Proceedings of the third Asian Fish Nutrition Network Meeting,* De Silva, S. (ed.). Asian Fisheries Society, Manila, pp. 11-22.
Paspatis, M. and Boujard, T. (1996). A comparative study of automatic feeding and self-feeding in juvenile Atlantic salmon (*Salmo salar*) fed diets of different energy levels. *Aquaculture*, 145: 245-257.
Peres, H. and Oliva-Teles, A. (1999a). Influence of temperature on protein utilization in juvenile European seabass (*Dicentrarchus labrax*). *Aquaculture*, 170: 337-348.
Peres, H. and Oliva-Teles, A. (1999b). Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture*, 179: 325-334.
Phillips, Jr., A.M. (1969). Nutrition, digestion and energy utilization. In: *Fish Physiology*, Vol I. Hoar, W. S., Randall, D.J. and Brett, J.R. (eds). Academic Press, London, p. 391.
Phillips, Jr., A.M. (1972). Calorie and energy requirement. In: *Fish Nutrition,* Halver, J.E. (ed.). Academic Press, New York, pp. 2-27.
Pitcher, J.T. and Hart, J.B.P. (1982). *Fisheries Ecology*. Chapman & Hall, London, 414p.
Ringler, N.H. (1979). Selective predation by drift-feeding brown trout, *Salmo trutta*. *J. Fish. Res. Bd. Can.,* 26: 392-403.
Ryan, B.F. (2000). *MINITAB Handbook*. 4th edn. Brooks/Cole, Pacific Grove, CA.
Sanchez-Vazquez, F.J., Yamamoto, T., Akiyama, T., Madrid, J.A. and Tabata, M. (1998). Selection of macronutrients by goldfish operating self-feeder. *Physiol. Behav*., 65: 211-218.
Sanchez-Vazquez, F.J., Yamamoto, T., Akiyama, T., Madrid, J.A. and Tabata, M. (1999). Macronutrient self-selection through demand feeders in rainbow trout. *Physiol. Behav*., 66: 45-51.
Santinha, J., Gomes, E.F. and Coimba, J. (1996). Effects of protein level of the diet on digestibility and growth of gilthead sea bream, *Sparus aurata* L. *Aquacult. Nutr*., 2: 81-87.
Santinha, P.J.M., Medale, F., Corraze, G. and Gomes, E.F.S. (1999). Effects off the dietary protein: lipid ratio on growth and nutrient utilization in gilthead seabream (*Sparus aurata* L.). *Aquaculture Nutrition.,* 5: 147-156.
Seeley, R.J. and Berthoud, H.R. (1999). Neural and metabolic control of macronutrient selection: Consensus and controversy. In: *Neural and Metabolic Control of Macronutrient Intake*, Berthoud H.R. and Seeley, R.J. (eds). CRC Press, Boca Raton, pp. 489-496.
Seeley, R.J. and Schwartz, M.W. (1999). Neuroendocrine regulation of food intake. *Acta Paediatr. Suppl*., 428: 58-61.
Shimeno, S., Hosokawa, H., Takeda, M. and Kajiyama, H. (1980). Effects of calorie to protein ratios in formulated diet on the growth, feed conversion and body composition of young yellowtail. *Bull. Jpn. Soc. Sci. Fish*, 46: 1083-1087.
Simpson, S.J. and Raubenheimer, D. (2001). A framework for the study of macronutrient intake in fish. *Aquaculture research*, 32: 421-432.
Smith, R.R. (1989). Nutritional energetics. In: *Fish Nutrition,* Halver, J.E. (ed.). Academic Press, New York, pp.1-30.
Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods*. 8th edn. Iowa State Press.
Tacon, A.G.J. (1997). Feeding tomorrow's fish: Keys for sustainability. In: Feeding Tomorrow's Fish, Proc. of the Workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean, Tacon, A. and Basurco, B. (eds). Mazarrón (Spain), 24-26 June 1996. *Cahiers Options Méditerranéennes*, Vol. 22: 11-33.

Takeuchi, T., Shiina, Y., Watanabe, T. (1991). Suitable protein and lipid levels in diet for fingerlings of red sea bream *Pagrus major. Nipp. Suis. Gakk*., 55: 521-527.
Vergara, J.M., Robaina, L., de Izquierdo, M. and la Higuera, M. (1996). Protein sparing effect of lipids in diets for fingerlings of gilthead sea bream. *Fish Sci*., 62: 624-628.
Watanabe, T., Takeuchi, T., Satoh, S., Ida, T. and Yaguchi, M. (1987). Development of low protein-high energy diets for practical carp culture with special reference to reduction of total nitrogen excretion. *Nipp. Suis. Gakk*., 53: 1413-1423.
Weatherley, A.H. and Gill, H.S. (1987). *The Biology of Fish Growth*. Academic Press, London, 443 pp.
Webster, D.C., Tiu, G.L., Tidwell, H.J., Van Wyk, P. and Howerton, D.R. (1995). Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* × *M. saxatilis*) reared in cages. *Aquaculture,* 131: 291-301.
Weisberg, S.B. and Lotrich, V.A. (1982). Ingestion, egestion, excretion, growth, and conversion efficiency for the mummichog, *Fundulus heteroclitus* (L.). *J. Exp. Mar. Biol. Ecol*., 62: 237-250.
Wilson, R.P. (1994). Utilization of dietary carbohydrate by fish. *Aquaculture*, 124: 67-80.
Woods, S.C., Seeley, R.J., Porte, D. Jr and Schwartz, M.W. (1998). Signals that regulate food intake and energy homeostasis. *Science*, 280: 1378-1383.
Yamamoto, T., Shima, T., Furuita, H., Manabu, S., Sánchez-Vazquez, F.J. and Tabata, M. (2001). Influence of decreasing water temperature and shortening of the light phase on macronutrient self-selection by rainbow trout *Oncorhynchus mykiss* and common carp *Cyprinus carpio*. *Fisheries Science,* 67: 420-429.