# Self-feeding activity patterns in gilthead sea bream (*Sparus aurata*), red porgy (*Pagrus pagrus*) and their reciprocal hybrids

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## Abstract

This study was conducted to monitor feeding activity of gilthead sea bream (Sa) and red porgy (Pp) and to investigate the hereditary effects on feeding in their hybrids (female Sa × male Pp, female Pp × male Sa), obtained after artificial crosses. All fish groups were held in 500-l tanks under ambient temperature (15–25°C) and light conditions (daylength from 9 h 30 min to 14 h 48 min). Fish (initial body weight 100–165 g) were fed by means of self-feeders, which were connected to a computer, recording fish feeding activity for a year. Results showed that there was a 24-h periodicity in all fish types over the experiment. In their daily feeding patterns, both parental species were diurnal: red porgies had a stable daily pattern over the experiment, while gilthead sea bream shifted their activity from a general daylight fluctuation in warm periods to an afternoon peak in cold periods. The Sa × Pp hybrids showed intermediate feeding patterns compared to their parents in warm periods and a pattern that was similar to the red porgy in cold periods, while the Pp × Sa hybrids had rather unchangeable daily feeding patterns, which were not related to the parental ones. The proportion of night feed demands increased in cold periods independent of fish group. The relationship between temperature and feed intake was species-dependent. Finally, this study indicated that feeding expression in hybrids was related to the combination of the parental genome, supporting our hypothesis for a hereditary influence on feeding activity in fish. © 2000 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Feeding activity has been studied thoroughly in many fish species. Temperature and dietary energy content (Alanärä, 1994), day-light alternation (Boujard and Leatherland, 1992a) and fish density (Jobling, 1995; Alanärä and Brännäs, 1996) are the primary factors affecting fish feeding. To our knowledge, there are few works on hereditary determination of feeding activity in fish (Schemmel, 1980; Johnsson and Abrahams, 1991; Skulason et al., 1993; Gerasimov, 1996). Hybridisation could be a useful tool to evaluate of the level of hereditary transmission from crossed species to their hybrids. Measurable characters such as growth, survival, morphological features, etc., have been used and verified in related studies (Chevassus, 1983; Blanc and Chevassus, 1986; Legendre et al., 1992). Concerning feeding activity, intraspecific crosses have been used(Schemmel, 1980; Johnsson and Abrahams, 1991; Skulason et al., 1993) and only Gerasimov (1996) worked on hybrids from different species. To achieve more on this aim, in the present study, two phylogenetically related species of the Sparidae family, gilthead sea bream (*Sparus aurata*) and red porgy (*Pagrus pagrus*) (Basaglia, 1992), were chosen because their cross was feasible. Their hybridisation revealed interesting principles for hybrid growth performance (Paspatis et al., 1999) due to the different optimum temperature ranges of these two species when cultured (Kentouri et al., 1995; Paspatis et al., 1999). Both species are a priority for farmers, as gilthead sea bream are common in the Mediterranean aquaculture industry and the red porgy is considered a profitable candidate for culture (Stephanou et al., 1995). Any new information on heritability of feeding function could promote the manipulation of feeding management by genetic methods. Since feeding activity has been a measurable parameter in many fish species making use of self-feeder devices (Alanärä, 1992a; Boujard and Leatherland, 1992b; Paspatis and Boujard, 1996; Madrid et al., 1997; Sánchez-Vásquez and Tabata, 1998) through which the fish satisfies its feeding needs by activating a trigger, this study describes self-feeding activity in gilthead sea bream, the red porgy and their reciprocal hybrids under ambient conditions. The aim is to compare the daily feeding patterns of hybrids to those of the parent species, including the influence of temperature and natural light conditions.

## 2. Materials and methods

### 2.1. Origin of fish

Broodstock of gilthead sea bream and the red porgy, held in the open installations of the Institute of Marine Biology of Crete, Greece, were crossed artificially. During the common period of natural spawning, females of both species spawned after hormonal treatment. Specifically, 5-year-old gilthead sea bream were injected with human chorionic gonadotropin at a concentration of 500 IU $kg^{-1}$ for 2 days and 5-year-old red porgy with Luteinizing hormone-releasing hormone analogue Ala 6 contained in microspheres at 40 $\mu g\ kg^{-1}$. Two batches of gilthead sea bream eggs were kept separately in beakers and fertilised by the milt of 1-year-old male gilthead sea bream or 3-year-old

red porgy. At the same time, two batches of red porgy eggs were fertilised in the same way. Finally, four viable larval groups were obtained: gilthead sea bream (Sa), red porgy (Pp), female gilthead sea bream × male red porgy (Sa × Pp) hybrids and female red porgy × male gilthead sea bream (Pp × Sa) hybrids. All fish types were reared intensively, according to the widely used protocol of gilthead sea bream larval rearing (Kentouri et al., 1993). After weaning, they were transferred to indoor experimental installations for on-growing.

## 2.2. Experimental protocol

Eight cylindrical tanks of 500-l capacity with a semi-closed water supply were used. There was inflow of 75% of filtered seawater and 25% of recycled water, which was gradually increased from 12 to 24 l $min^{-1}$ $tank^{-1}$. From each fish stock, two replicate populations were formed by random sampling of individuals (50 fish $tank^{-1}$). They were subjected to natural light and water temperature fluctuation, while salinity (37.1 ± 1.1‰) was stable throughout the experiment. Tanks were equipped with air diffusers by which the dissolved oxygen level was kept stable (5.4 ± 0.6 mg $l^{-1}$). Fish were fed by means of electronic pendulum self-feeders (Paspatis, 1997), having free access to the tips of the pendulums which were 2 cm below water surface. Self-feeders were on-line with a computer, where the number of each device and the respective time of its activation were recorded 24 h a day. A small constant quantity of feed (0.8–0.9 g) was released around the pendulum per triggering. Commercial feed for gilthead sea bream
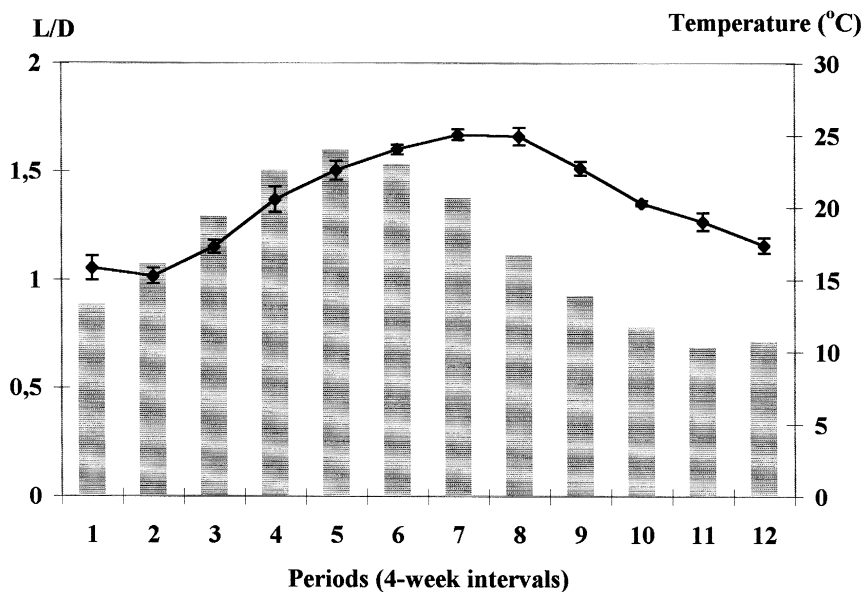


Fig. 1. Water temperature (in points with 1 standard deviation bars) and light (in black bars) fluctuations over the experiment.
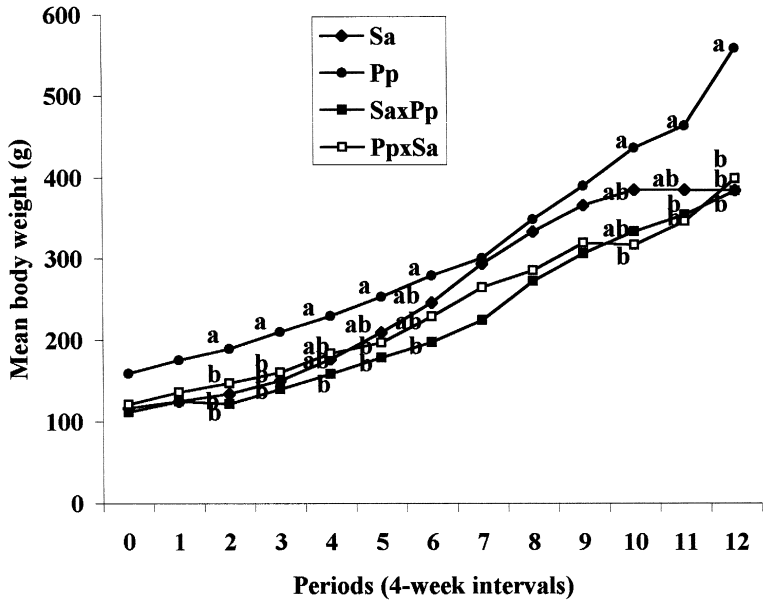
Fig. 2. Mean body weight of gilthead sea bream (Sa), red porgies (Pp) and their hybrids (Sa×Pp and Pp×Sa) throughout the experiment. Within each period, means with different letter are statistically different ($P < 0.05$).
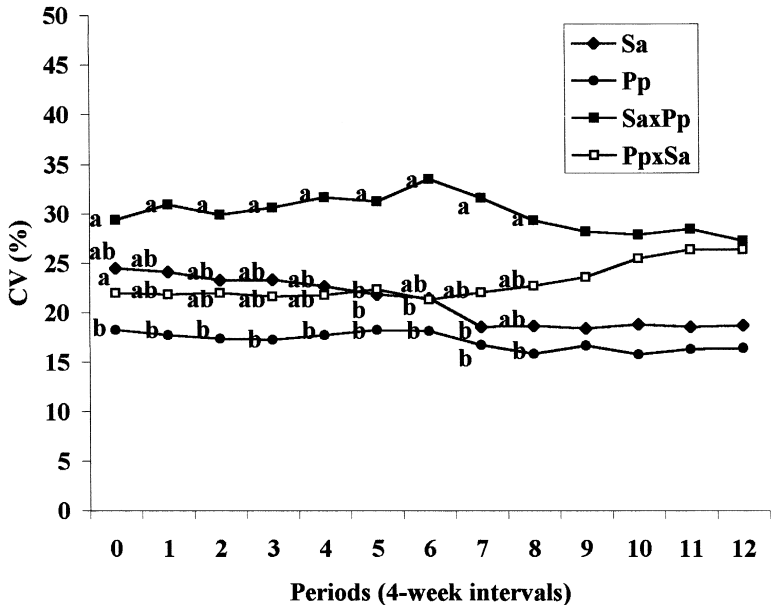


Fig. 3. Coefficient of body weight variation in gilthead sea bream (Sa), red porgies (Pp) and their hybrids (Sa×Pp and Pp×Sa) during this study. Within each period, coefficients of weight variation with different letters are statistically different ($P < 0.05$).

(Biomar, Nersac, France; contained 45% total protein, 22% total lipids, 7% ash, 2% fibre) was dispersed. Feed size was modified according to feed manufacturers' recommendations, which take into account fish size.

The recording period started when fish were 1 year old and registered 100–165 g mean body weight. They were weighed individually every 4 weeks (the 12 time intervals
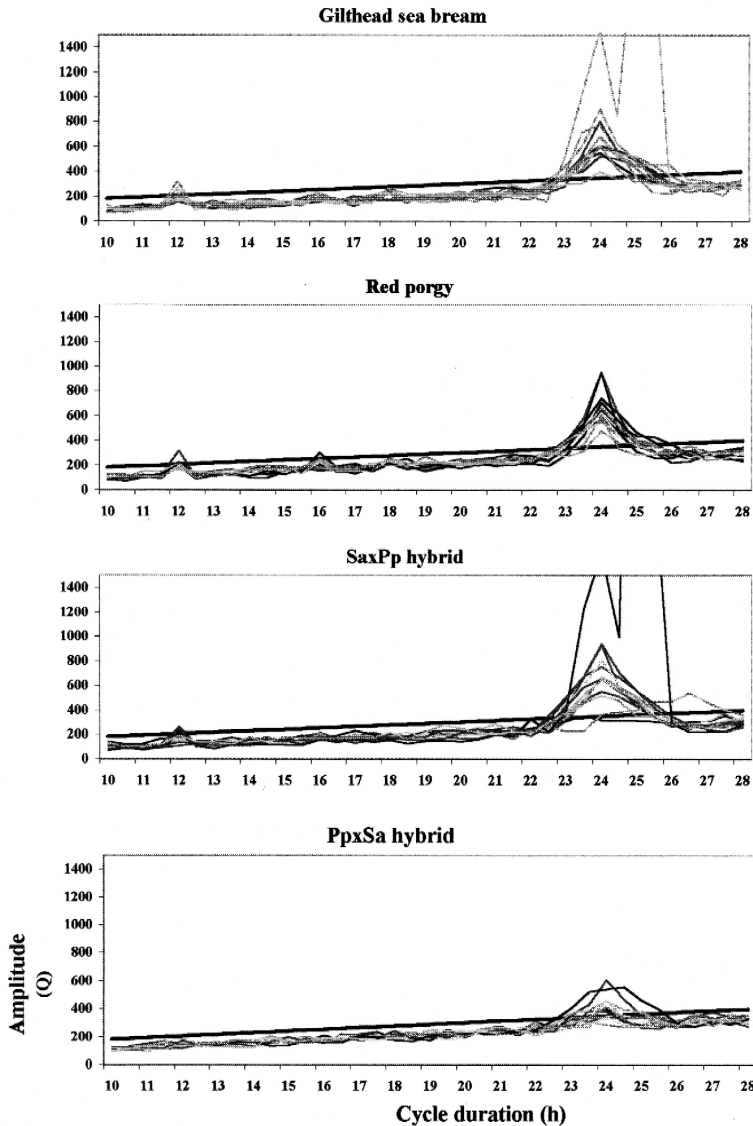


Fig. 4. Periodogram analysis of self-feeder activations in gilthead sea bream (Sa), red porgies (Pp) and their hybrids (Sa×Pp and Pp×Sa). Within each fish type, lines represent analysis per period. The straight black line indicates the confidence level of 95%.

between weighings are hereafter called periods) for a whole year (February 1996–January 1997). Temperature, dissolved oxygen and salinity were recorded daily at 0900 h and light intensity every 5 min all day.

## 2.3. Statistical analysis

The coefficient of body weight variation (CV = $100 \times$ standard deviation $\times$ mean$^{-1}$) per fish type and period was estimated taking into account the data from the 4-week weighings. Self-feeder activations were collected and classified into desirable time intervals by a software programme called DATACCESS, designed by our research team. Within each period, triggers from 20 successive days were divided into 5-min intervals and tested for circadian rhythmicity by $\chi^2$ periodogram analysis (Sokolove and Bushell, 1978). The first 8 days after each weighing were not included in the above analysis in order to avoid the influence of handling on fish feeding rhythms. Mean daily feeding patterns from each type of fish and period were classified by the Principal Component Analysis (PCA) (Sokal and Rohlf, 1981). The Piecewise Linear Regression with
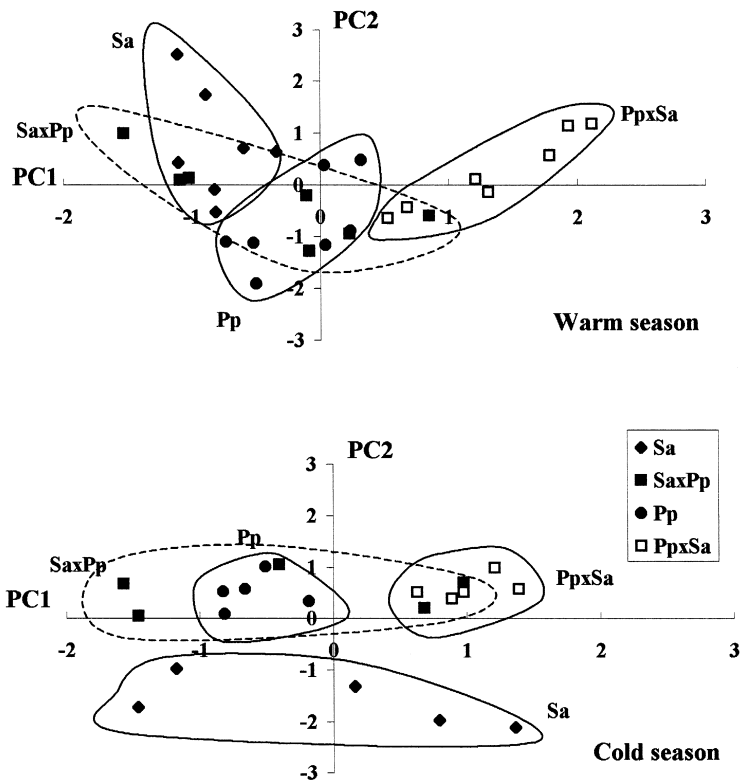


Fig. 5. PCA classification of mean daily feeding patterns of gilthead sea bream (Sa), red porgies (Pp) and their hybrids (Sa×Pp and Pp×Sa) during the warm and cold seasons.
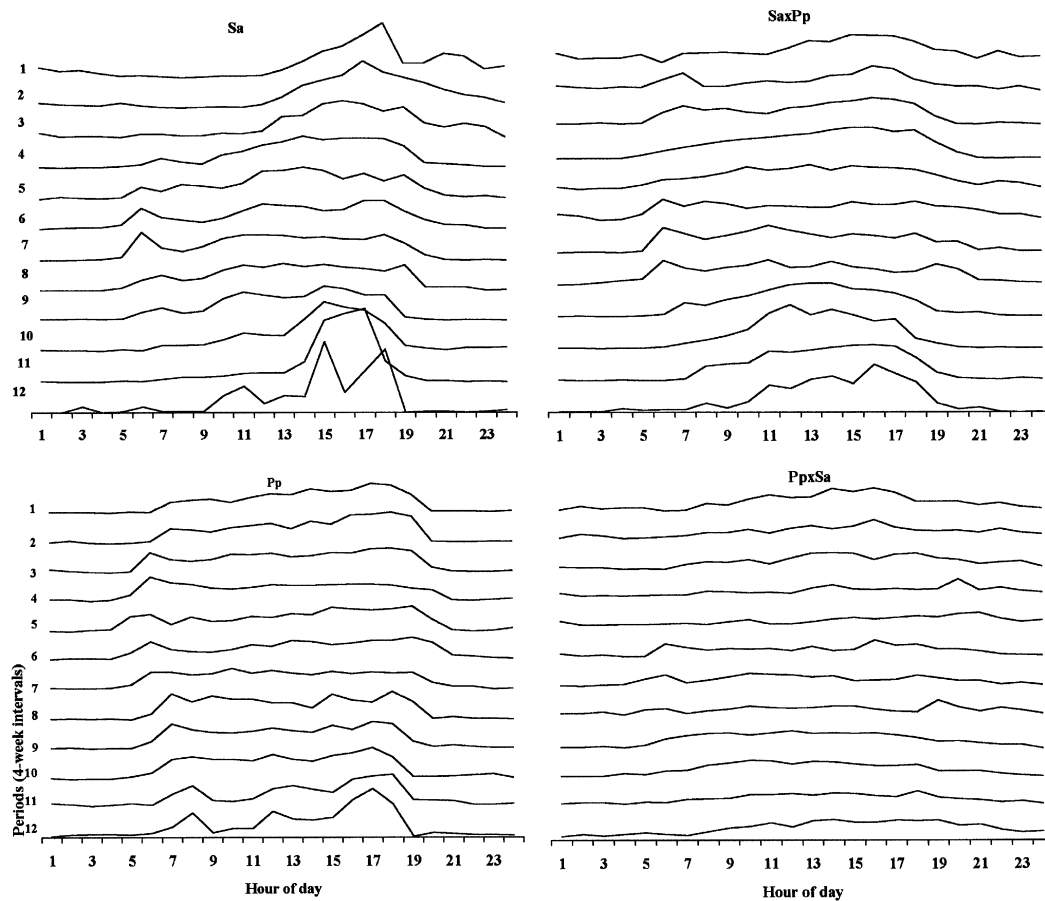
Fig. 6. Daily feeding patterns vs. period for gilthead sea bream (Sa), red porgy (Pp), female gilthead sea bream×male red porgy (Sa×Pp) and female red porgy×male gilthead sea bream (Pp×Sa).

Breakpoint was performed for the determination of the correlation between self-feeding activity and water temperature (StatSoft, 1995). Hourly activations were divided into four light intensity conditions (dawn, light, dusk and dark) and tested using analysis of variance (ANOVA) (Sokal and Rohlf, 1981). If significant differences between means were determined, the Scheffé test was applied (Sokal and Rohlf, 1981). In all analyses, the statistical significance level was at $P < 0.05$.

## 3. Results

The experiment could be divided into two seasons: the warm ($> 20°C$ during periods 4–10) and the cold ($< 20°C$ during periods 1–3 and 11–12) (Fig. 1). The maximum photoperiod occurred in period 5 (14 h and 48 min, 0503–1951 h) and the lowest in period 11 (9 h and 31 min, 0737–1708 h) (Fig. 1). The maximum light intensity at the surface of water varied from 215 lx in summer to 31 lx in winter.

Body weight increments and the respective coefficient of variation in each fish species are presented in Figs. 2 and 3. The red porgy had better growth compared to the sea bream. The Sa $\times$ Pp hybrid had high weight variation, significantly different from the red porgy until period 8.

The $\chi^2$ periodogram analysis of self-feeder triggerings indicated a 24-h periodicity in all fish types throughout the experiment (Fig. 4). There was a secondary peak (at 12-h period) in gilthead sea bream, red porgy and the Sa $\times$ Pp hybrid.

Mean daily feeding patterns were separated into warm and cold seasons and classified by PCA, based on two principal components (PC1 and PC2). In the warm season, PC1 represented the positive effect of night hours (2000–0500 h) and the negative of midday and afternoon hours (1200–1700 h), and PC2 indicated the negative influence of morning hours (0700–0900 h) (Fig. 5). The clusters of the gilthead sea bream, red porgy and the Pp $\times$ Sa hybrid patterns had no overlap, while the cluster of the Sa $\times$ Pp hybrid was intermediate of the parental clusters. In the cold season, PC1 was associated with the positive influence of night hours (2000–0500 h), and PC2 with the positive effects of

Table 1

The estimated parameters of Piecewise Linear Regression between self-feeder triggerings per growth period ($A$) and temperature ($T$) for each fish type (gilthead sea bream (Sa), red porgies (Pp) and their reciprocal hybrids)

| Fish type | $a$ | $b_1$ | Valid periods | $a'$ | $b_1'$ | Valid periods | $r^2$ |
|---|---|---|---|---|---|---|---|
| Gilthead sea bream | −3180 | 256 | 1–5 and 10–12 | −12,275 | 738 | 6–9 | 0.8[*] |
| Red porgy | −1097 | 134 | 1–2 and 10–12 | 3799 | −1 | 3–9 | 0.7[*] |
| Sa $\times$ Pp hybrid | −3032 | 281 | 1–3 and 10–12 | −8278 | 665 | 4–9 | 0.7[*] |
| Pp $\times$ Sa hybrid | −765 | 115 | 1–4 and 12 | 3812 | −56 | 5–11 | 0.8[*] |

The coefficients $a$, $b_1$, $a'$ and $b_1'$ refer to the equation $A = a + b_1 T$ (when $A <$ breakpoint) or $A = a' + b_1' T$ (when $A \geq$ breakpoint), and $r$ is the correlation coefficient. According to the respective breakpoint, each equation is valid in the mentioned periods.

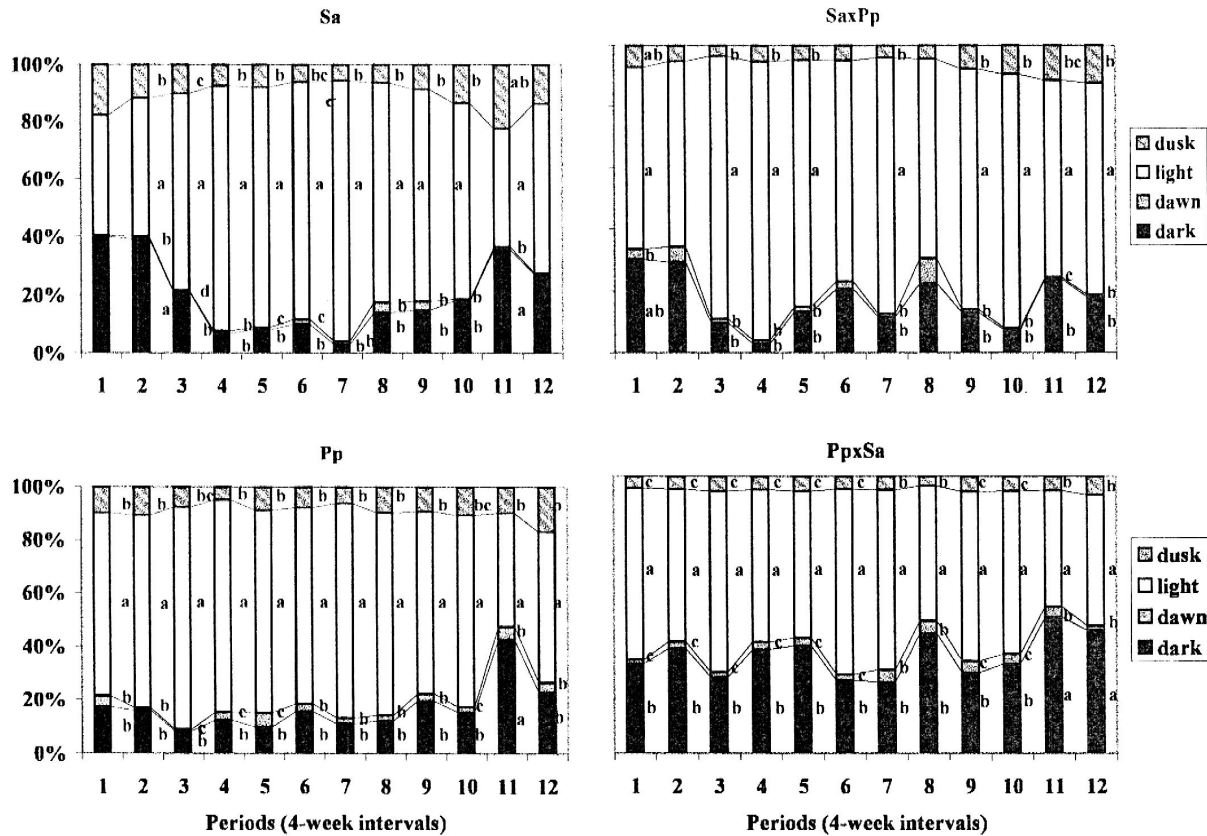[*] Statistically significant ($P < 0.01$) coefficients.

Fig. 7. Daily distribution of self-feeding activity among light intensity levels for gilthead sea bream (Sa), red porgy (Pp), female gilthead sea bream×male red porgy (Sa×Pp) and female red porgy×male gilthead sea bream (Pp×Sa). Within each bar, significant differences ($P < 0.05$) are indicated by different letters.

morning and midday (0900–1200 h) and the negative ones of afternoon (1700 h). As Fig. 5 shows, the gilthead sea bream, red porgy and Pp × Sa hybrid clusters had no overlap in the cold season, while the Sa × Pp hybrid cluster was close to the red porgy. Mean daily feeding patterns of each fish type and period are presented in Fig. 6.

The correlation of self-feeder activations with water temperature is presented in Table 1. Regression coefficients were positive for all fish groups in cold periods. They stayed positive and their values increased in warm periods, except in the case of red porgies and Pp × Sa hybrids, where the coefficients were negative.

Self-feeding activity during dark, dawn, light and dusk were compared. Periodic fluctuations and statistical differences between these categories by Scheffé test are shown in Fig. 7. All fish types increased their feeding demands mainly in daylight. Dark demands were also present, especially in the cold season. There was a non-significant preference for dusk feeding, whenever dark feeding activations increased.

## 4. Discussion

Circadian feeding rhythmicity was observed throughout the experiment, while a secondary peak of 12-h periodicity was detected in all fish groups except the Pp × Sa hybrids. According to Boujard and Leatherland (1992a), 24-h activity is the rule in many fish, which synchronise their activity to environmental oscillations, mainly light–dark alteration.

Both parent species had diurnal feeding activity. Gilthead sea bream in cold periods restricted their diurnal self-feeding mainly in the afternoon, while in warm periods they spread their activity to midday and afternoon hours. Bégout Anras and Lagardère (1997) demonstrated that the diurnal behaviour in gilthead sea bream was dependent on fish density: fish that lived in groups were diurnal while isolated ones became nocturnal. The red porgy was found to be a typical diurnal species with increased feeding whenever ambient light was present, verifying the study of Maragoudaki et al. (1997), which was done in autumn. The daily feeding patterns of the Pp × Sa hybrids were almost uniform, due to sufficient activity not only in daylight, but at night as well. In contrast, the Sa × Pp hybrid had intermediate daily patterns compared to their parents in warm periods and were similar to the red porgy in cold periods. The diversity of hybrids' feeding activity is better shown by the PCA classifications of daily feeding patterns (Fig. 5). These revealed that the combined genome of parental species was expressed differently in the reciprocal hybrids with regards to feeding activity. This result is common in hybridisation in characters like survival and growth (Chevassus, 1983). Gonad examination in both hybrids at the end of the experiment indicated that they were mostly undifferentiated and sterile, although there were a few males and females in hybrid populations (unpubl. data). These findings lead us to hypothesise that sexual maturity and gonadal development in hybrids did not affect their feeding activity during this study. Further investigation is needed from a behavioural and genetic point of view.

According to Kentouri et al. (1995) and Paspatis et al. (1999), gilthead sea bream and red porgies have different optimum thermal ranges for their growth: the red porgy grows better than gilthead sea bream in lower temperatures ($< 20°C$). In the present study,

these findings were verified, although the weight range of fish was much higher than in the other studies. The temperature-dependent growth potential of these two species seems to influence their feed intake, expressed by the self-feeder activations (Table 1). In very warm periods, gilthead sea bream feeding was positively related to temperature and red porgy feeding was negatively related to temperature, while in the rest of the experimental periods, feeding in both species was positively related to temperature. The relationship between feed intake and temperature in the Sa × Pp and Pp × Sa hybrids show similarities to their maternal species: feeding in the former hybrid was consistently positively related to temperature, as in gilthead sea bream. Temperature had similar effects on feeding of the latter hybrid to red porgy. This finding led us to hypothesise that temperature influences on feeding activity in hybrids was determined genetically from the maternal genome.

Strong light preference characterised the feeding activity of all fish groups. Daylight self-feeder activations were the highest portion of daily activity over the experiment. However, nocturnal activity increased during cold growth periods. The switch to nocturnal feeding has also been observed in rainbow trout (*Oncorhynchus mykiss*) reared in cages during autumn, with a long night period (Alanärä, 1992b). Fraser et al. (1995) and Pirhonen et al. (1997) showed that salmonids exposed to low temperatures became nocturnal. In our study, because of the ambient rearing conditions, short daylength coincided with cold periods and probably the seasonal fluctuation of both factors influenced the nocturnal behaviour of fish. Alanärä and Brännäs (1997) stated that nocturnalism could be affected by the social status of fish, which is related to the heterogeneity of a fish population. In our case only the Sa × Pp hybrids showed increased population variation compared to the other three types, but this did not affect feeding activity in the dark, compared to the other fish types. Crepuscular feeding (during dawn and dusk) was limited, in spite of the increased tendency of feed demands at dusk in cold periods.

In conclusion, gilthead sea bream, red porgies and their reciprocal hybrids showed circadian feeding activity throughout the year. Diurnal behaviour was the rule in all fish types, but a species-specific differentiation determined daily feeding pattern. Sa × Pp hybrids had intermediate and Pp × Sa hybrids had different daily feeding profiles compared to the parent species. There was a species-specific correlation between feed intake and water temperature. The strong light preference for feeding in all fish groups was modified by seasonal fluctuations of environmental parameters.

## Acknowledgements

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