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Effect of food ration on estrogen and vitellogenin plasma levels, fecundity and larval survival in captive sea bass, *Dicentrarchus labrax*: preliminary observations

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Abstract

Adult two-year-old broodstock sea bass (*Dicentrarchus labrax*), raised in captivity, were fed *ad libitum* a natural diet (trash fish) until the end of their first spawning, and maintained under natural conditions of light and temperature. Six months before the second spawning, fish were randomly separated in two tanks with running sea water (salinity 37.8 ‰, pH 8.3) and maintained under similar environmental conditions. Fish were fed two different ration levels: group F, 1.04 %, and group H, 0.45% body weight/day. The low ration supplied affected broodstock growth as indicated by decreased growth rates in weight and condition factor in group H, when compared to group F. The mean fecundity per female (No. eggs/female) in group H fish was similar to that from F group, while the relative fecundity (No. eggs/kg female) was more elevated in the H group. However, group H showed a delay in spawning time and slightly higher number of spawnings per female and larger spawning period, when compared to group F fish. In addition, group H females had reduced 17β-estradiol (E2) plasma levels and presented vitellogenic oocytes in the ovary a month later than group F females. By contrast, the vitellogenin (VGT) plasma levels remained similar between groups over the reproductive cycle. The egg quality (*i.e.* percentage of buoyant eggs), the egg biochemical composition and larval survival at hatching were similar between the two groups. The larvae from both groups also exhibited the same survival after 40 days of culture, although group H produced smaller eggs that yielded smaller newly-hatched larvae. Although the low ration supplied significantly reduced the growth and E2 plasma levels of sea bass, these preliminary results suggest that these changes do not dramatically affect the egg viability or the survival of larvae 46 days after hatching.

Keywords: *Dicentrarchus labrax*, food ration, growth, estradiol, vitellogenin, fecundity, oocyte development.

Effets du rationnement alimentaire chez le bar, Dicentrarchus labrax, *sur le taux d'œstrogène et de vitellogénine du plasma, sur la fécondité et la survie des larves : observations préliminaires.*

Résumé

Des géniteurs du bar (*Dicentrarchus labrax*) âgés de deux ans et élevés en captivité, ont été nourris *ad libitum* avec un aliment naturel (du poisson, *Boops boops*) jusqu'à la fin de leur première ponte. Les poissons ont été maintenus dans des bacs de 8 m^3 alimentés en eau de mer (salinité 37,8 ‰, pH 8,3) et dans des conditions naturelles de température et de photopériode. Six mois avant leur deuxième ponte, les poissons ont été séparés en deux groupes et nourris avec deux types d'alimentation (le groupe F : 1,04 %, et le groupe H : 0,45 % du poids vif/jour, dans les mêmes conditions. La ration la plus faible fournie aux géniteurs a entraîné une diminution de leur taux de croissance ainsi que de leur facteur de condition. La fécondité moyenne par femelle (nombre d'œufs par femelle) chez les poissons du groupe H, a été similaire à celle du groupe F, tandis que la fécondité relative (nombre d'œufs par kg/poids vif) a été plus élevée

dans le groupe H. Cependant on a constaté, dans le groupe H, un nombre plus élevé de ponte par femelle et la prolongation de la période de ponte. De plus, les poissons du groupe H, ont montré les taux les plus faibles de 17 β-œstradiol dans le plasma et un retard d'un mois dans la différentiation folliculaire (présence d'ovocytes vitellogéniques). En revanche, les taux de vitellogénine du plasma sont restés identiques pour les 2 groupes tout au long du cycle reproducteur. La qualité des œufs, leur composition biochimique et la survie des larves à l'éclosion ont été semblables dans les deux groupes. Les larves des deux groupes ont le même taux de survie après 40 jours de culture, bien que le groupe H ait produit des œufs plus petits et donc des larves plus petites aussi. Quoique le taux d'alimentation le plus faible réduise de façon significative la croissance et le taux d'œstradiol du bar, ces résultats préliminaires suggèrent que ces modifications n'affectent pas de façon importante la viabilité des œufs ou la survie des larves de 46 jours.

Mots-clés : *Dicentrarchus labrax*, nutrition, croissance, reproduction, fécondité, œstradiol, vitellogénie, développement des ovocytes.

INTRODUCTION

It is known that food availability is one of the main factors affecting reproductive processes in fish populations (Bye, 1990). Consequently, during intensive production of juvenile fish at commercial hatcheries it appears necessary to define the most appropriate feeding strategies to maintain the broodstock stocks. This knowledge would also allow fish farmers to optimize the current spawning methods in order to achieve a high production of good quality eggs and yolksac larvae.

The effects of food ration on reproduction in laboratory conditions have been extensively studied in many fish species, such as rainbow trout (*Oncorhynchus mykiss*: reviewed by Bromage *et al.*, 1992), haddock (*Melanogrammus aeglefinus*: Hislop *et al.*, 1978; Robb, 1982), stickleback (*Gasterosteus aculeatus*: Wootton, 1982), medaka (*Oryzias latipes*: Hirschfield, 1980), convict cichlid (*Cichlasoma nigrofasciatum*: Townshend and Wootton, 1984), cod (*Gadus morhua*: Kjesbu, 1988; Kjesbu *et al.*, 1991), winter flounder (*Pseudopleuronectes americanus*: Burton and Idler, 1987), plaice (*Pleuronectes platessa* L.: Horwood *et al.*, 1989) and the viviparous guppy (*Poecilia reticulata*: Hester, 1964; Dahlgren, 1980). However, although it is believed that food restriction generally reduces total fecundity and may delay maturation and affect egg size and/or weight, no clear pattern of effects has emerged. Possibly, several factors such as the duration of the experiments, the timing of reduction in ration relative to the reproductive cycle, and the effects of feeding level on fish size, or the effects of fish size on egg size, have made comparison among the studies difficult (Kjørsvik *et al.*, 1990). In addition, it is not clear how food deprivation affects gonadal development and fecundity, although both altered oocyte recruitment and induction of atresia have been implicated (Tyler and Dunn, 1976; Townshend and Wootton, 1984; Bromage *et al.*, 1992).

On the Mediterranean coast, the sea bass (*Dicentrarchus labrax*) has an excellent commercial potential. This species is being successfully produced in many European hatcheries, using several environmental and hormonal techniques to induce the spawning in captivity (reviewed by Dechauvelle and Coves, 1988*a* and Carrillo *et al.*, 1994). In this regard, the large-scale production of eggs and larvae has been successfully accomplished throughout the year by manipulation of photoperiod and temperature (Zanuy *et al.*, 1986; Devauchelle and Coves, 1988*a*). However, not enough information is available concerning the effect of diet composition or different feeding conditions on sea bass reproduction in culture conditions. As in other species (reviewed by Luquet and Watanabe, 1986), previous experiments in the laboratory have shown the importance of the dietary protein content in sea bass broodstock diets (Cerdá *et al.*, 1994). Also, the higher nutritional value of a natural diet (composed of trash fish) versus two commercial pellets has been confirmed by measuring fecundity and egg quality (*i.e.* percentage of buoyant eggs and hatching rates) over two consecutive reproductive cycles (Cerdá *et al.*, 1991).

The objective of this report was to describe the effects of a natural diet, at low ration, supplied to three-year-old broodstock sea bass six months prior to the onset of the second natural spawning. We tested the effects of such low ration on total and relative fecundity, and subsequent egg and larval survival. The possible influence of ration size on gonadal development was also investigated by monitoring oocyte differentiation and 17β-estradiol (E2) and vitellogenin (VTG) plasma levels.

MATERIALS AND METHODS

Fish and feeding treatments

Fourty-six 2-year-old broodstock sea bass (701 ± 18 g in weight and 38 ± 0.2 cm in length, mean $\pm$ SE), reared in captivity at the Instituto de Acuicultura Torre de la Sal (CSIC, Spain), were fed daily a natural diet (consisting of chopped filleted trash fish, *Boops boops*) *ad libitum* until the completion of the first spawning. The composition of the natural diet has been described previously (Pérez *et al.*, 1987). Fish were maintained in rectangular tanks (8 m^3), with aerated running sea water (salinity 37.8 $^o/_{oo}$,

pH 8.3) and ambient conditions of photoperiod (40°N) and temperature. Six months before the second spawning (July), fish were randomly distributed in two similar tanks under the same environmental conditions. The mean weight (1 198±63 g) and the proportion of males to females in each tank were similar (approximately 2:1). Then, fish were hand-fed daily the same natural diet at either 1.04% (*ad libitum* or full-ration, group F) or 0.45% (half-ration, group H) body weight/day. The ration was adjusted for each group approximately every month, but maintaining the same relative difference between the two treatment groups. The feeding treatments were applied throughout the ovarian cycle until the completion of the second spawning (from January to March at our latitude), although sea bass decreases food intake considerably during this time (Zanuy and Carrillo, 1985; Devauchelle and Coves, 1988*a*).

Sampling procedure

At approximately monthly intervals over the second reproductive cycle (from October to April) fish were individually weighed and measured under ethyl m-aminobenzoate methanesulfonate (MS-222) anaesthetic at 100 ppm. All fish were starved for 24 h before anaesthesia. At the same time, blood samples were taken by caudal puncture with heparinized syringes to measure steroid and vitellogenin plasma concentrations. During the spawning period, samples of intraovarian oocytes were also obtained through a cannula carefully inserted into the ovary of the females, and males were examined for the presence of sperm on gentle hand pressure. The sampling protocol was performed in a few minutes in order to minimize stress to the fish.

The condition factor (CF) and specific growth rates in weight (G) and length (Gl) were calculated according to Zanuy and Carrillo (1985). Blood samples were centrifuged (3 000 rpm at 4°C for 20 min) and plasma aliquots immediately frozen (–20°C) until analysis. The ovarian samples were examined under a light microscope and the proportion of oocytes in different stages of development were calculated (Zanuy *et al.*, 1986).

During the spawning season, the number of spawnings in each ration group were recorded daily. Eggs were collected by means of fine-gauge nets located on the tank water outflow, following the protocol already described by Carrillo *et al.* (1989). The number of eggs produced in each spawning and the proportion of buoyant (viable) to sinking (non-viable) eggs were measured volumetrically (1 ml = 670±5 eggs, $n = 10$ measurements on different batches of eggs). Total fecundity (TF) was calculated as the mean number of eggs produced per female, and the relative fecundity (RF) as the mean number of eggs produced per kg of post-spawned female body weight. Since it was not possible to individualize each female within the tanks, the mean number of spawnings per female (spawning frequency) was estimated from the total number of different batches of eggs (spawnings) collected at the end of spawning and the number of females in each tank.

Egg characteristics and larval rearing

The egg and oil droplet mean diameter were measured to the nearest 0.01 mm on 100 buoyant eggs, using a stereomicroscope with an ocular micrometer.

From approximately 10 different spawnings in each group, corresponding to the beginning, middle and end of spawning period, three samples of 1 ml of buoyant eggs were incubated in separate trays. Each tray had an independent supply of running sea water and aeration, and was maintained at a temperature of 15±0.5°C. The hatching rates (48-72 h from fertilization at 16-17°C) were expressed as percentage of buoyant eggs (Cerdá *et al.*, 1994), and the size of newly-hatched larvae was measured to the nearest 0.01 mm ($n = 50$ individuals) using a stereomicroscope equipped with an ocular micrometer.

From 3-4 different batches of eggs (showing more than 300 ml of buoyant eggs), collected during mid spawning period (February), three samples of 200-300 ml of buoyant eggs were incubated longer than in previously mentioned experiments. In these incubations we evaluated the larval survival after 40 days of exogenous feeding. At the time of first-feeding (8-9 days post-hatching at 16°C), surviving larvae were transferred to new trays and were fed once a day *Artemia franciscana* nauplii (1-5 individual/ml) *ad libitum*. After 40 days of culture, the survival rates were calculated with respect to the initial number of fry transferred, and the mean larval length was measured as previously described.

Analytical methods

Vitellogenin (VTG) plasma levels were measured using a homologous enzyme-linked immunosorbent assay (ELISA) developed by Mañanós *et al.* (1994). Plasma 17β-estradiol (E2) was determined by specific radioimmunoassay (RIA), using a previously described method (Prat *et al.*, 1990).

The egg biochemical analysis was performed on approximately 10 samples of buoyant eggs, collected in different days during the beginning, middle and end of spawning in each group. The biochemical composition of eggs (total protein, total lipid, phospholipid and glycogen content) was estimated by techniques described by Cerdá *et al.* (1994).

Data analysis

The statistical errors are expressed as the standard error of mean (SE). Data differences in growth, gonadal development, E2 and VTG plasma concentrations between feeding treatments throughout the

reproductive period (months) were tested with two-factor analysis of variance (ANOVA II). If interaction between two factors (months and rations) was significant, one-way analysis of variance (ANOVA I) was applied to show differences among means. Egg and larvae characteristics and survival were compared using the Student's *t*-test. A significance level of 5 % was used.

RESULTS

The morphometric data obtained from the two groups showed that the food ration size did not cause significant differences in female growth between groups (*fig.* 1). Females from both groups showed a significant increase of weight and length during summer and autum months (from July to November), but during the spawning season (from December to March) growth ceased, with negative values for specific growth rates (*fig.* 1*a, b*). However, females from group H exhibited a pronounced reduction of weight at the end of spawning and significantly diminished G values with respect to group F over the experimental period (*fig.* 1*a*). Likewise, group H fish did not show any significant seasonal variation in CF, with lower values than group F starting from December (approximately late vitellogenesis), although not in February (approximately mid spawning period) (*fig.* 1*c*). This, at the beginning of spawning (January), the condition of group H fish was significantly reduced compared with group F.

Along with that reduced growth, group H fish spawned 1 week later than those from group F (*fig.* 2*a, b*) and the production of a maximum number of buoyant (viable) eggs was delayed by approximately 2 weeks (*fig.* 2*c*). Figure 2 also shows that the spawning profile presented by group H did not show any clear peak in the profile of the TF or RF over spawning, with the spawns more dispersed over the spawning period (*fig.* 2*b*). In contrast, group F fish showed a concentration of spawnings during the second half of February (*fig.* 2*a*). The TF reached at the end of spawning was similar in both groups, whereas the RF calculated was higher in group H (*table* 1). The estimated number of spawnings per female and the duration of the spawning period was also slightly higher in group H (*table* 1).

The egg quality evaluated over spawning did not appear to be affected by ration size. The percentage of buoyant eggs was low but similar between groups (*table* 2), and the relative analysis showed no changes in either protein, fat or glycogen levels (*table* 3). However, while egg oil droplet size remained constant between groups, the egg size of group H was significantly smaller than that from group F (*table* 2). Group H eggs also yielded smaller newly-hatched larvae, although these latter differences disappeared after 40 days of culture under controlled conditions. In both groups, larval survival was similar at hatching and after 40 days of culture.

In order to explore whether the differences in food ration altered oocyte development, ovarian samples were examined monthly over the entire reproductive cycle (*fig.* 3). In both groups, the proportion of pre-vitellogenic and vesicle-stage oocytes decreased in the gonad as spawning approached (*fig.* 3*I, II*), while the number of vitellogenic oocytes increased (*fig.* 3*III*). However, in October, no female from group H had vitellogenic oocytes in the ovary, but at this time they had a low proportion of atretic oocytes, which disappeared in November and December (*fig.* 3*V*). In January, low proportions of atresia were also detected in both groups, which increased during spawning. However, in group F the maximum atresia was observed at late spawning (March) (*fig.* 3*V*). Finally, fully mature or ovulated oocytes were present in a similar number over spawning in both groups, whereas those from group H displayed a significant peak in February, later than in group F (*fig.* 3*IV*).

With respect to males, the proportion of spermiating males (emitting sperm on gentle hand pressure) was similar over spawning in both groups (from January to March) (*table* 1).

Measurements of E2 and VTG plasma levels showed similar profiles in the females from both groups, with peak values occurring from November to February and from November to March, respectively (*fig.* 4*a, b*). However, the E2 peak height was significantly reduced during vitellogenesis and spawning in group H fish (*fig.* 4*a*).

DISCUSSION

The lower food ration size supplied in this experiment effectively reduced the specific growth rates in weight of broodstock sea bass, as indicated by the relatively low G values recorded in fish fed a half ration over the experimental period (*fig.* 1*a*). Similar decreased rates of growth performance have been described in other teleosts fed low rations (Hislop *et al.*, 1978; Springate *et al.*, 1985). However, in our study the annual profiles of the specific growth rates in length of group H fish did not display marked differences with respect to those of group F (*fig.* 1*b*). This fact suggests that low food ration may have a stronger influence on weight than in length, as described in haddock by Hislop *et al.* (1978). However, the similar changes in length in both groups over the experimental period made the differences in CF between groups more pronounced (*fig.* 1*c*). Zanuy and Carrillo (1985) described the annual variations of sea bass CF, which seem to be related to storage reserves for later gonadal growth. When spawning occurs, the CF decreases progressively to reach a minimum at final spawning. The CF in group H tended to show this trend, but unlike group F, the values at the end of the experiment (May) were less than those in the initial month (July).

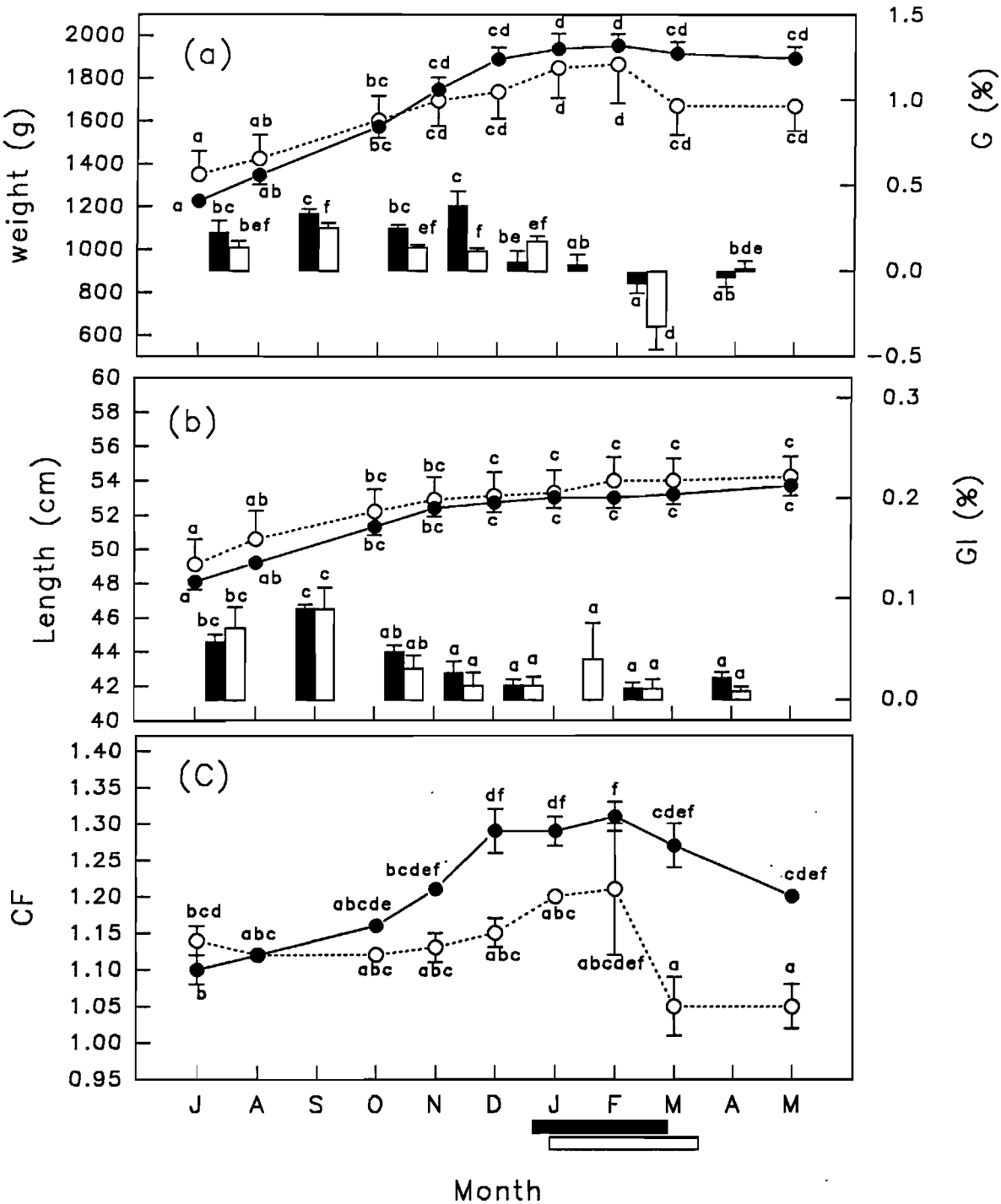

Figure 1. – Seasonal variations of weight (a), length (b) and condition factor (CF) (c) (represented by lines), and specific growth rates in weight (a) and length (b) (represented by bars), of female broodstock sea bass fed full, group F (solid lines and black bars), or half ration, group H (doted lines and open bars). Each data point represents a mean value of 7-10 females ± SE. The horizontal bars show the duration of spawning period observed for group F (black bar) or group H (white bar). Values with different superscripts are statistically different ($p < 0.05$).

This drastic loss of robustness of group H fish was not only related to spawning but also indicated a negative effect on somatic growth. Thus, sea bass seem to sacrifice somatic growth in order to achieve the adequate requirements for reproduction, as has been suggested in other species (Hislop *et al.*, 1978; Robb, 1982).

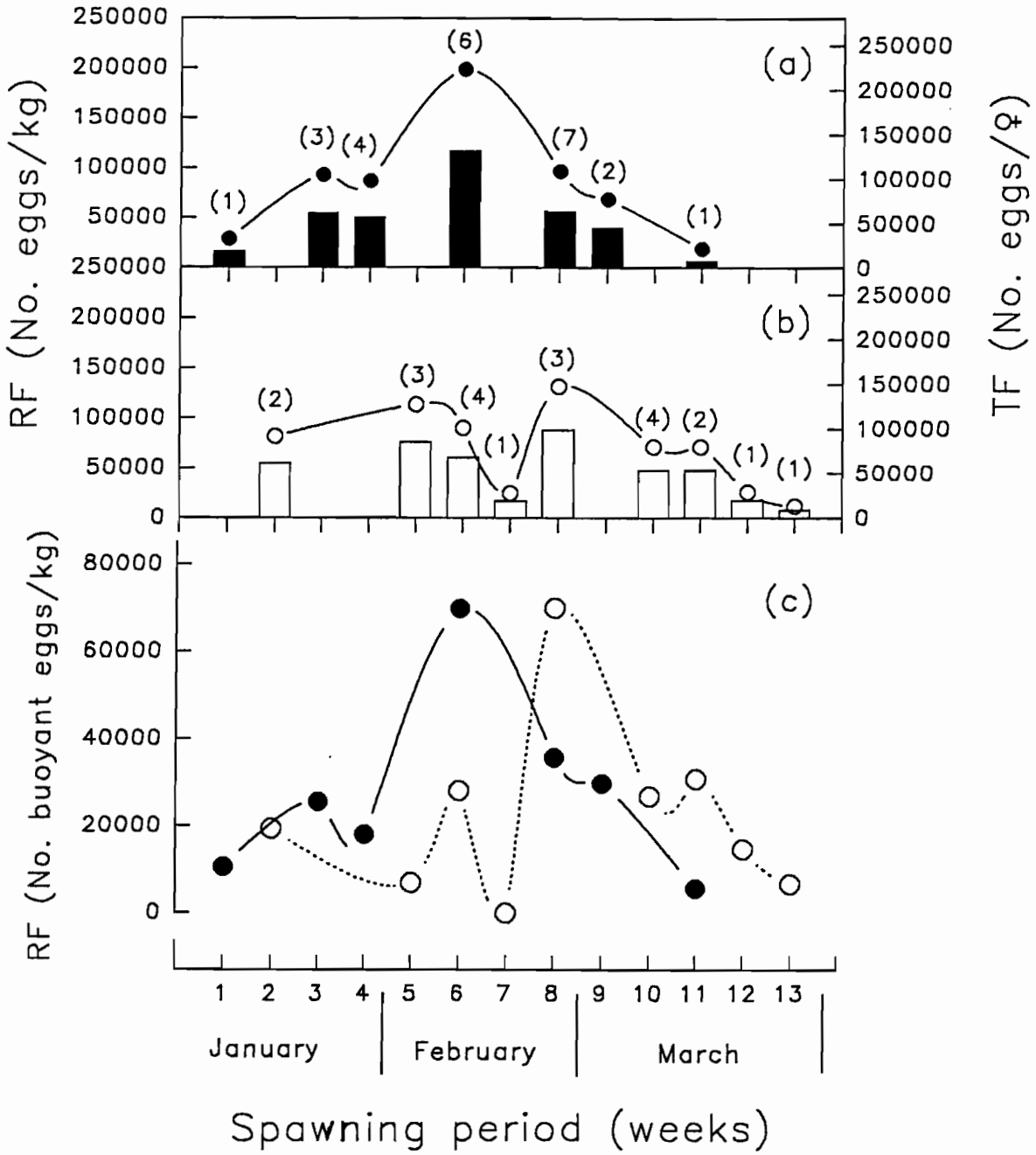

Figure 2. – Total fecundity, TF (curves), and relative fecundity, RF (bars), over a spawning season of female sea bass fed full, group F (a, black circles and bars), or half ration, group H (b, open circles and bars). Spawnings were considered on a weekly basis (in parentheses, number of spawnings recorded in each week) in order to facilitate their plotting. The plot c represents the progression of RF over spawning, referring only to buoyant (viable) eggs.

Even though the food energy supplied to group H fish was apparently not sufficient to satisfy fully the requirements of growth and reproduction, the TF showed by this group was very similar to that of group F (*table* 1). It has been widely reported that experimental food deprivation may reduce fecundity in many species (Bromage *et al.*, 1992; Kjørsvik *et al.*, 1990), although it is accepted that this parameter depends on fish size, which in turn can also affect the egg size (Wootton, 1982; Bromage *et al.*, 1992). Because of this, some studies have separated the influence of ration on fish size by appropriate experimental or statistical methods and have shown a direct effect of ration on fecundity, with the magnitude

Table 1. – Effect of food ration on egg production, characteristics of spawning, total fecundity (TF) and relative fecundity (RF).

	Group F			Group H		
	January	February	March	January	February	March
No. eggs produced (× 1 000)	2 341	4 089	134	1 011	1 962	1 209
No. eggs/spawn (× 1 000)	1 028	2 567	107	265	984	792
No. buoyant eggs (× 1 000)	292	272	134	505	178	151
No. ovulated females	6	10	3	2	7	4
No. spermiating males	15	15	7	13	14	10
No. females		10			7	
No. males		15			14	
Spawning frequency*		2.4			3.5	
Spawning period (days)		73			85	
TF (No. eggs/female)		664 000			697 000	
RF (No. eggs/kg female)		357 000			417 000	

* Estimated from number of spawns observed and number of females in each tank (see Materials and Methods).

Table 2. – Effect of food ration on egg and larvae characteristics and larval survival (data are means ± SE). The numbers in parentheses show the number of different batches of eggs studied over spawning in each group.

	Group F	Group H
Buoyant eggs (%)	56.88 ± 6.30 (25)	47.80 ± 7.30 (21)
Egg diameter (mm)	1.18 ± 0.01 (14)	1.16 ± 0.01* (12)
Egg oil droplet diameter (mm)	0.373 ± 0.002 (14)	0.376 ± 0.001 (12)
Hatching rate (%)	33.69 ± 6.73 (10)	33.36 ± 3.93 (10)
Larval length (mm)[1]		
I	4.95 ± 0.01 (10)	4.77 ± 0.001* (10)
II	13.35 ± 0.13 (3)	13.04 ± 0.11 (4)
Larval survival (%)[2]	7.82 ± 3.48 (3)	7.40 ± 3.88 (4)

* Significantly different (Student t test, $p \leq 0.05$) means between groups.

[1] Length of newly-hatched (I) and 46 days post-hatched larvae (II).

[2] Larval survival of 46 days post-hatched larvae.

varying among species (Wootton, 1982; Townshend and Wootton, 1984; Jones and Bromage, 1987). It is possible that the increase of RF in group H could be related to diminished fish size in this group, but surprisingly our results did not show any apparent effect on TF. These facts, therefore, strongly suggest that the low ration size supplied was not enough to decrease fecundity in three-year-old sea bass females. However, we also found that group H fish delayed their spawning time, which was particularly evident when RF was represented as the number of buoyant eggs produced over the spawning period (*fig.* 2*c*). We also observed in this group a different spawning profile and a slight increase in both the number of spawnings per female and the duration of their spawning season (*fig.* 2*b* and *table* 1). Taken together, these observations indicate that ration size significantly affected reproduction, by delaying the timing of spawning and possibly altering the spawning rhythm, in order to accomplish an elevated fecundity by an increased number of spawnings.

In parallel with the change in RF, the diameter of the eggs was significantly reduced in the fish fed a half ration (*table* 2). Modification of egg size seems to be a common effect in many teleost species fed low rations or maintained under inadequate culture conditions (Hislop *et al.*, 1978; Springate *et al.*, 1985), although there is a considerable interspecific variation (Ridelman *et al.*, 1984; Kjesbu, 1988). Our results coincide with these studies, but the possible effect of

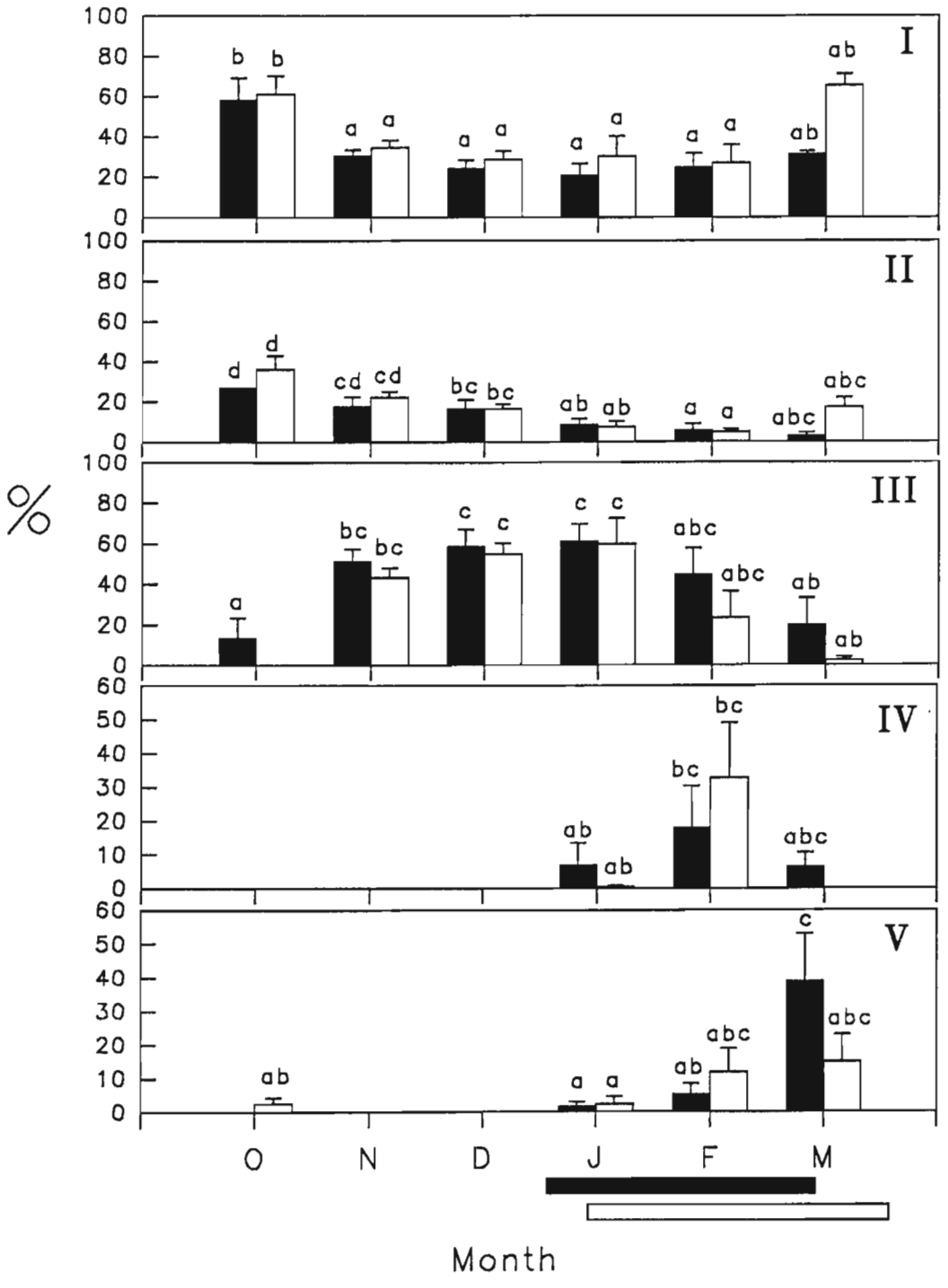

Figure 3. – Proportion (%) of pre-vitellogenic (I), vesicle-stage (II), vitellogenic (III), fully mature or ovulated (IV) and atretic (V) oocytes (mean ± SE, $n = 3$-7 fish) in the ovary of female sea bass fed full, group F (black bars), or half ration, group H (open bars). Values with different superscripts within a particular developmental stage are significantly different ($p < 0.05$).

fish size on egg size makes it difficult to determine a direct effect of ration on egg size. In contrast, the egg oil-droplet size remained constant between groups, with a very narrow range of variation over the spawning season (*table* 2). Similar findings have been made in sea bass fed artificial diets with low protein content (Cerdá *et al.*, 1994), which could indicate, unlike egg size, a general conservative strategy in the

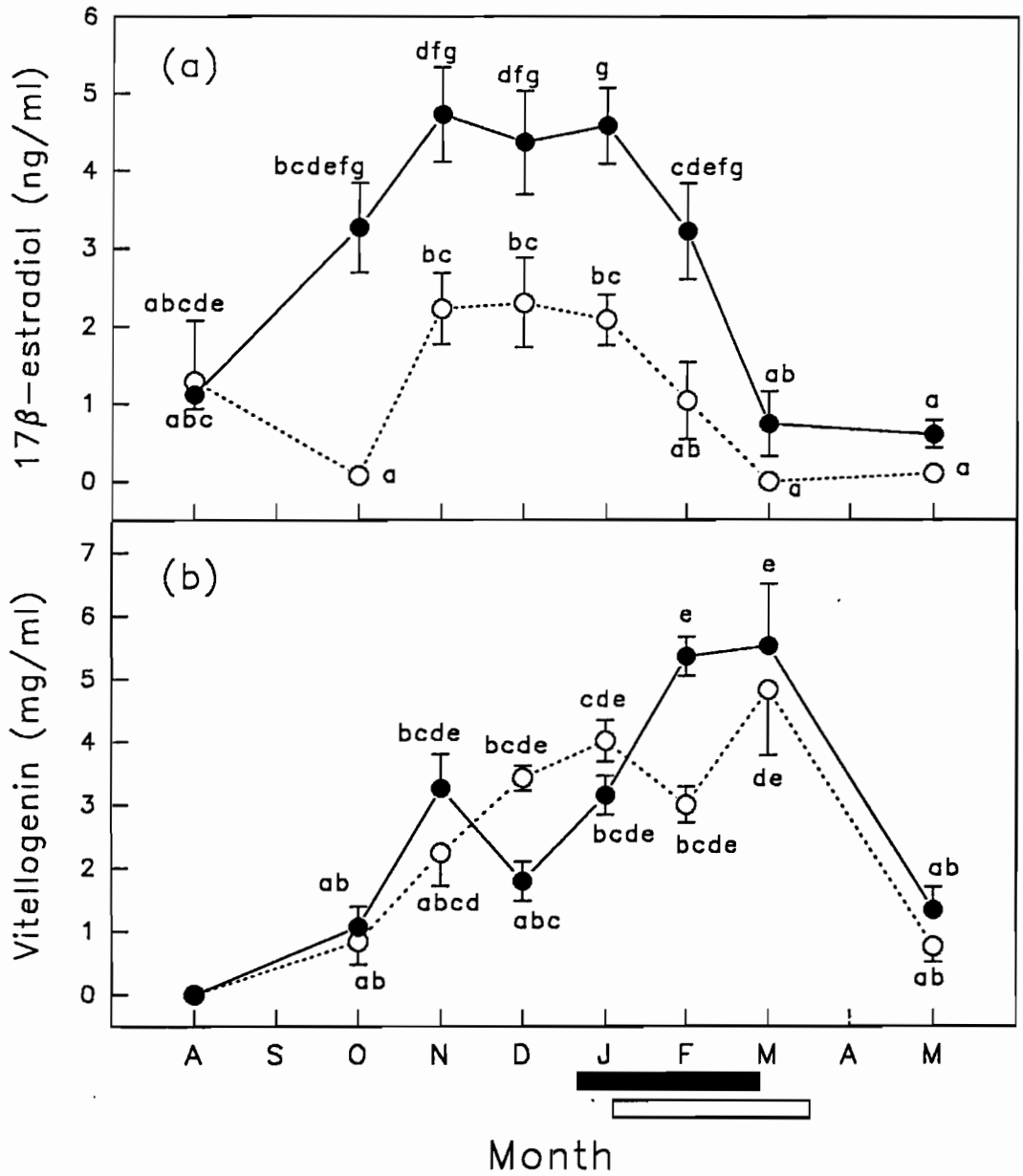

Figure 4. – Plasma levels of 17β-estradiol (E2) (a) and vitellogenin (VTG) (b) in female sea bass fed full, group F (solid lines), or half ration, group H (dotted lines). Each data point represents a mean value of 7-10 fish ± SE. Values with different superscripts are significantly different ($p < 0.05$).

quantity of lipid yolk deposited in eggs under adverse nutritional conditions.

Certainly, as in salmonids and other species (reviewed by Springate and Bromage, 1985 and Lagomarsino *et al.*, 1988), we have found that larger eggs produce larger fry (*table* 2). However, despite clear differences in size of the eggs and hatched fry of the two groups, there were no reductions in viability expressed on the basis of mortality levels at hatching or after 40 days of culture (*table* 2). The absence of any reduction in viability of fry derived from small eggs also agrees with findings of many other authors (see Springate and Bromage, 1985 and Monteleone and Houde, 1990), although there is some controversy about the influence of larval size at hatching on its subsequent growth (Springate and Bromage, 1985; Marteinsdottir and Able, 1992; Baynes *et al.*, 1993). In the sea bass, and in other marine species, it has been suggested that the egg biochemical composition might be a good criterion for egg quality (Devauchelle *et al.*, 1987, 1988; Devauchelle and Coves, 1988*b*; Kjørsvik *et al.*, 1990). Our data do not reveal any difference in the biochemical composition of the eggs produced by the two groups (*table* 3), which

Table 3. – Relative composition (% dry weight) of morula-stage eggs produced by each experimental group (data are means ± SE). The numbers in parentheses show the number of different batches of eggs studied in each group.

	Group F	Group H
Total protein	50.53 ± 1.97 (12)	48.18 ± 3.63 (7)
Total lipid	35.98 ± 2.85 (12)	36.11 ± 7.05 (7)
Phospholipid	5.50 ± 0.44 (12)	6.38 ± 0.22 (7)
Glycogen	0.32 ± 0.09 (12)	0.30 ± 0.05 (7)
Ash	13.80 ± 1.11 (12)	15.10 ± 1.15 (7)
Moisture	88.18 ± 0.20 (12)	87.95 ± 0.17 (10)

agrees with many reports in other species (Wootton, 1982; Springate *et al.*, 1985; Knox *et al.*, 1988; Zastrow *et al.*, 1990). These results could explain the similar larval survival observed and might indicate that small eggs do not have any inherent problem as far as survival is concerned (at least until the 46-day post-harched larvae). This observation could be of undoubted commercial interest and needs to be confirmed by further experiments with incubations of the total batches of eggs produced over spawning.

Turning now to the possible gonadal mechanisms affected by ration size in group H, we have observed in this group significantly lower E2 plasma levels than in group F during vitellogenesis and spawning (*fig.* 4*a*). In addition, the histological study indicates the absence of vitellogenic oocytes in group H fish during October (*fig.* 3*III*), a period in which those oocytes usually appeared in the ovary (Devauchelle and Coves, 1988*a*; Carrillo *et al.*, 1989; Prat *et al.*, 1990). Prat *et al.* (1990) have reported high levels of serum E2 during the sea bass spawning period coinciding with the maximum oocyte diameter, and similar positive correlations have been observed in other marine teleosts (reviewed by Pankhurst and Carragher, 1991). Therefore, it is possible to suggest that the low E2 circulating levels could decrease the oocyte growth rates in group H females. This situation, in turn, could result in delayed production of buoyant (viable) eggs and reduced egg size. A decreased incorporation of circulating VTG into the oocyte, as a consequence of slower oocyte growth (Tyler, 1991), could also have contributed to detection of similar VTG plasma levels in the two groups during vitellogenesis (*fig.* 4*b*). From our data, however, such a relationship between E2, oocyte growth and vitellogenin in food restricted fish remains unclear. Moreover, our hypothesis fails to explain the increased number of spawnings that we also observed in group H fish. It is clear that longer experiments are required, with special attention to the vitellogenic period, to clarify these aspects regarding food ration and gonadal development.

In summary, the present results provide preliminary descriptive information on the effects of ration size on captive sea bass E2 and VTG plasma levels, together with effects on spawning frequency and egg and larval size. However, the changes observed in low-ration fed females did not seem seriously to affect the egg or larval survival under well controlled incubation conditions. The investigation on the possible regulation of sea bass oocyte development and spawning behaviour by feeding conditions must be continued with new experimental designs that may include the manipulation of food ration at various times during the reproductive cycle.

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