Aquat. Living Resour., 1995, **8**, 147-152

Effects of constant photoperiod on spawning and plasma 17β-oestradiol levels of sea bass (*Dicentrarchus labrax*)

Silvia Zanuy [1], Francisco Prat [1], Manuel Carrillo [1] and Niall R. Bromage [2]

[1] *Instituto de Acuicultura de Torre de la Sal (C.S.I.C.), 12595 Torre de la Sal, Catellón, Spain.*

[2] *Instituto of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, U.K.*

Received January 31, 1995; accepted March 22, 1995.

Zanuy S., F. Prat, M. Carillo, N. R. Bromage. *Aquat. Living Resour.*, 1995, **8**, 147-152.

Abstract

This work examines the effects of constant long and short days on the hormonal profile of plasma 17β-oestradiol (E2) levels and the ovarian vitellogenic events, as well as on the fecundity, time of spawning and egg quality of sea bass. Constant short days first administered in April advanced spawning (by 45 days on average) whereas exposure to constant long days from the same date induced a delay (averaging 38 days) compared to controls reared on an ambient light regime (Lat. 40°N). These light regimes produced shifts in the profiles of plasma 17β-oestradiol and in the percentage of the vitellogenic oocytes according to the spawning time of the respective groups. Relative fecundity of the short day group was similar to controls (257,000 versus 230,000 eggs/kg spawned female, respectively). However, in the long day group, relative fecundity was reduced to one half that of control fish (124,000). Plasma oestradiol showed a bimodal pattern under long days and vitellogenetic and atretic oocytes were present for a longer period (6-7 months compared to 4-5 months in controls). In summary, in this paper we describe, for the first time in the sea bass, the effect of constant photoperiod regimes on the 17β-oestradiol plasma profiles, the timing of spawning and the quality of the eggs.

Keywords: Photoperiods, fecundity, oestradiol, oocytes, vitellogenesis, *Dicentrarchus labrax.*

Effets de photopériodes constantes sur la ponte et le taux de 17β-oestradiol du plasma chez le bar (Dicentrarchus labrax).

Résumé

Cette étude porte sur les effets des photopériodes longues et courtes mais constantes sur le profil du taux de 17β-oestradiol (E2) du plasma, sur la vitellogenèse, sur la fécondité, la période de ponte et la qualité des œufs de *Dicentrarchus labrax*. Les jours courts, « administrés » en avril provoquent une ponte précoce (de 45 jours en moyenne) tandis que l'« exposition » aux jours longs, en avril également provoque un retard (de 38 jours en moyenne) par rapport aux témoins en photopériode naturelle (Lat. 40°N). Ces régimes de lumière produisent des décalages dans les profils de 17β-oestradiol et du pourcentage d'ovocytes en vitellogenèse, selon la période de ponte des groupes respectifs. La fécondité relative du groupe, soumis aux jours courts, est similaire à celle des témoins (respectivement 257 000 contre 230 000 œufs/kg de poids vif pour les femelles après la ponte). Néanmoins dans le groupe des jours longs, la fécondité relative est réduite de 50 % par rapport à celle des poissons témoins (124 000). Le 17β-oestradiol du plasma suit une courbe bimodale chez les poissons soumis à des jours courts; les ovocytes en vitellogenèse et les ovocytes atrétiques sont présents pendant une période plus longue (6-7 mois) que ceux des poissons témoins (4-5 mois). En résumé, on décrit ici, pour la première fois chez le bar, l'effet des photopériodes constantes sur les taux du 17β-oestradiol dans le plasma, sur la période de ponte et sur la qualité des œufs.

Mots-clés : Photopériodes, fécondité, oestradiol, ovocytes, vitellogenèse, *Dicentrarchus labrax.*

INTRODUCTION

In temperate species of fish, photoperiod and temperature are the main cues for the entrainment of the reproductive cycles (Bye, 1987). In some species it has been shown that modifications of daylength can alter timing of reproduction (Bromage *et al.*, 1990). In sea bass, spawning has been successfully advanced or delayed by modifying the photoperiod (Devauchelle and Coves, 1988 and Carrillo *et al.*, 1989, 1993 and 1995*a*).

The alteration of spawning time by environmental manipulation has the potential to meet the demands for all-year-round mass production of eggs and larvae of this species and is of considerable commercial interest. The usual procedure for altering the natural spawning time of sea bass is photoperiod and temperature manipulation using modified natural cycles of light and temperature (Girin and Devauchelle, 1978; Devauchelle, 1984; Barnabé and Paris, 1984; Barnabé and Barnabé-Quet, 1985 and Zanuy *et al.*, 1986). However, the use of constant or square-wave light regimes has proved to be a simpler and more reliable method for inducing out-of-season spawning in this species (Carrillo *et al.*, 1989, 1993 and 1995*a*). Environmental modification of the time of spawning can, however, affect both the quality and quantity of the eggs produced by broodstock sea bass (Carrillo *et al.*, 1989, 1991, 1995*a*).

In rainbow trout it has been demonstrated that the sequence of endocrine changes, which ultimately lead to spawning, can be modified in both their timing and their duration by altered photoperiod regimes (Bromage *et al.*, 1982*a*, 1982*b*). In sea bass some aspects of the gonadal development and spawning have been described (Zanuy *et al.*, 1986; Mayer *et al.*, 1988; Carrillo *et al.*, 1989; Alvariño *et al.*, 1992), including variations in plasma levels of sexual steroids throughout the reproductive cycle under both natural (Prat *et al.*, 1990) and modified photoperiod regimes (Carrillo *et al.*, 1993, 1995*a*). However, no studies have been made of the effect of constant long and constant short photoperiod regimes on endocrine events which are involved in maturation and oocyte growth.

The aim of the present study was to investigate the effects of constant long and short photoperiods on the timing of spawning and on the number and quality of the eggs, as well as to describe the modifications in vitellogenic and atretic oocytes and the associated changes in plasma oestradiol in sea bass.

MATERIAL AND METHODS

Three groups of 20 four-year-old female sea bass (*Dicentrarchus labrax*), were kept over a two-year period in separate light-proof, well-aerated tanks of 2,500 l capacity, supplied with flow-through sea water. The three groups were maintained at ambient temperature (ranging from a minimum of 11.5°C in January to a maximum of 26.4°C in August). The photoperiod regimes, provided by solar bulbs and controlled by electronic clocks, started on the 1st of April 1986 and were as follows: group A, constant short photoperiod (LD 9/15); group B, constant long photoperiod (LD 15/9) and group C, simulated natural photoperiod (latitude 40°N).

Fish were fed once a day at 10 a.m., with natural food, consisting of chopped filleted fish (*Boops boops*). During a 14 month period (from May 1986 to June 1987) 10-12 fish were sampled monthly from each experimental group. Before blood sampling, the water level in the tank was lowered to the minimum, fish were netted, introduced in a small tank (100 l) and anaesthetized (MS 222, 0.1 g/l). Blood was withdrawn from the caudal vein, with a heparinized syringe, at the same time of day (10-11 a.m.). Blood aliquots (1 ml) were centrifuged for 20 minutes at 4°C and 3,000 rpm. Plasma was stored at −20°C until analysis. The 17β-oestradiol (E2) plasma levels were measured by RIA according to the procedure previously published by Prat *et al.* (1990). Assay sensitivity ranged from 5 to 10 pg/tube.

Oocyte samples were obtained at the time of blood sampling by intraovarian cannulation of 7-10 females from October to April. Ovarian biopsies prior to October were not made due to the small size of the developing gonads. The eggs produced at spawning were collected from the outflow water of the tanks in fine gauge nets. Due to the method of egg collection, it was not possible to establish spawning data for individual fish for each photoperiod regime. However, the total number of eggs produced and the relative fecundity (number of eggs per kg post-spawned female weight) were recorded. Strongly aerated, surface flushing tanks allowed collection of floating and non-floating eggs. Egg quality was assessed according to the volume of the floating (viable) eggs and expressed as a percentage of the total volume of eggs collected. On all occasions the floating eggs had fertilization rates in excess of 90%. Mean spawning date was calculated using a value equal to the number of days passed between the first (spawning n° 1) and the successive spawnings; *i.e.* if the first spawning occurred on the 2nd of December, the second on the 15th and the third on the 1st of January; the series of numbers used to calculate the mean spawning time was 1, 14, 31.

Microscopic examination of sections of the ovary and estimates of vitellogenic and atretic oocytes were carried out according to the method of Carrillo *et al.* (1989). Samples of ovary were fixed on Bouin-Hollande, blocked in methacrylate (Historesin, LKB), and 7 μm sections stained with the Cleveland-Wolf technique (Herland, 1960).

Statistical analysis

Statistical differences in E2 levels were tested separately for each group using the Kruskal-Wallis

non-parametric analysis of variance followed by multiple comparisons as described by Conover (1980). The percentage of oocyte classes was also tested separately for each group using one-way ANOVA of arcsine transformed data followed by the Tukey-A test. Mean spawning time and egg quality and fecundity were compared between groups using one-way ANOVA and Kruskal-Wallis respectively followed by the multiple comparisons test described above. Non-parametricy of the data were assessed using the test of Kolmogorov-Smirnov for normality of the distribution and Bartlet's for homoscedasticity of the variance. Significant differences are shown at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

The effects of the modified photoperiod regimes on spawning time, fecundity and egg quality of sea bass are summarized in *figure* 1. Constant short-photoperiod (group A) produced a significant ($p<0.05$) advance (first spawning December 11 and last one February 5; mean spawning time: 16 ± 4 January) compared with the natural spawning time of the control group (first spawning February 2 and last one March 20; mean spawning time: 24 ± 5 February) (control). In contrast, the constant long-photoperiod (group B), produced a significant ($p<0.05$) delay (first spawning March 18 and last one May 5; mean spawning time: 9 ± 8 April).

The alteration of the spawning time was accompanied by changes in the number of eggs spawned. Under the long-photoperiod, the relative fecundity of group B was reduced compared to the controls (124,000 and 230,000 eggs/kg post-spawned female, respectively; group B and control). However, this parameter was not affected by the constant short-day regime (group A: 256,873 eggs/kg post-spawned female). Mean egg quality was not affected by either of the photoperiodic treatments (80.7 ± 3.9, 59.9 ± 12.7 and 66.2 ± 13.7 for group A, group B and controls, respectively). Under a natural photoperiod, atretic oocytes appeared at the biopsy in January (control) and remained at very low levels during spawning before reaching peak levels (40 %) during the post-spawning period (April). Under the constant short-day regime (group A) the atresia lasted only three months with levels peaking earlier than in the controls (February-March). In the case of the constant long-day regime (group B) atretic oocytes were present for six months (from January to June).

In the control group (*fig.* 2, control) there was a steady and significant increase of 17β-oestradiol from November to January ($p<0.01$; vitellogenic period), concomitant with the appearance and gradually increased proportion of vitellogenic oocytes. Over the following months, during the spawning period, the plasma levels of oestradiol decreased, although the

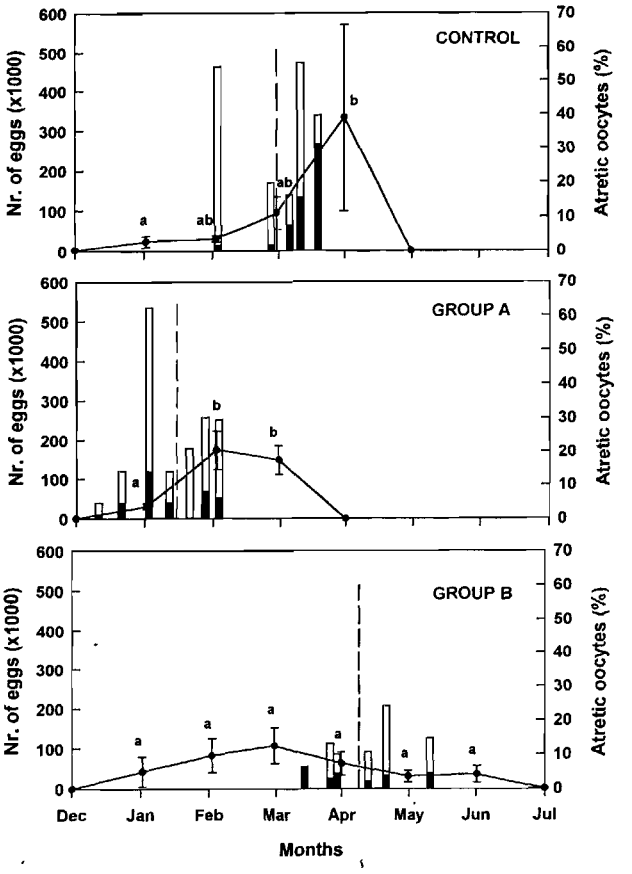

Figure 1. – Seasonal changes in percentage of atretic oocytes mean ± se (closed circles), weekly spawnings (vertical bars) and number of eggs represented as open bars (number of floating, viable eggs) and closed bars (number of non-floating, non-viable eggs) in control, group A (short photoperiod), group B (long photoperiod) animals. Groups were composed of 7-10 females and the percent of atresia was calculated in at least 100 oocytes per female, obtained by intraovarian cannulation (vertical dotted lines represent the mean spawning time. Points labelled with a different letter are significantly different at $p<0.05$).

percentage of vitellogenic oocytes remained elevated until after spawning (*fig.* 2). The long and short light regimes produced shifts in the profiles of plasma oestradiol according to the timing of the spawning. Under constant short-days, levels of plasma oestradiol began increasing in October with significant increases ($p<0.001$ with respect to basal levels) in November and December. As in the controls, maximum levels occurred in January but the peak was followed by a sharp decrease in February (*fig.* 2, group A). The vitellogenic oocytes showed a parallel pattern to E2 levels. Under constant long days (*fig.* 2, group B), the profile of oestradiol was clearly bimodal, exhibiting an initial peak during December ($p<0.001$) and a second one during March-April ($p<0.001$). Both peaks were concomitant with an elevated proportion of vitellogenic oocytes.

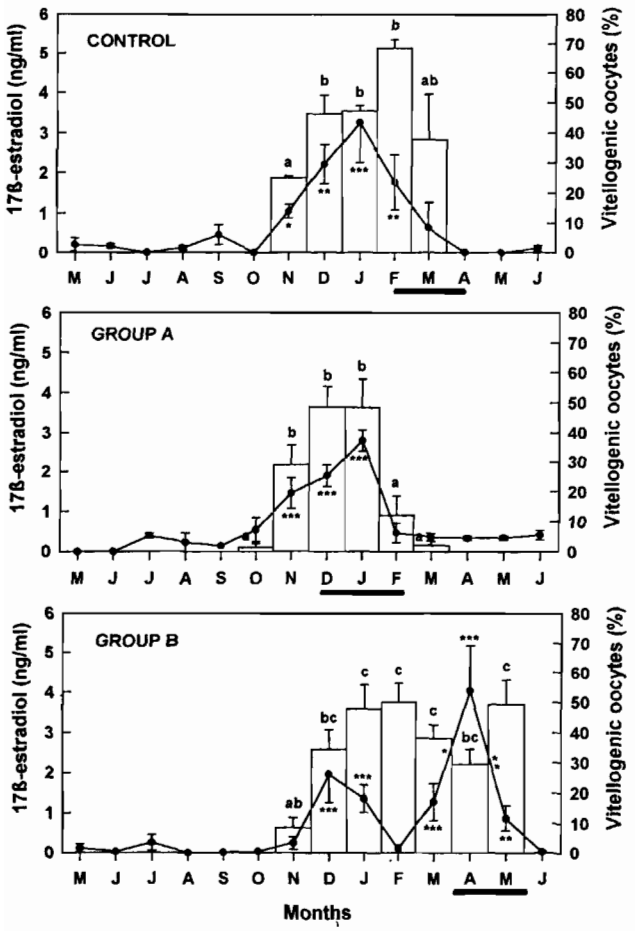

Figure 2. – Seasonal changes in plasma 17β-oestradiol levels mean ± se (closed circles) and the percentage of vitellogenic oocytes ± sem (open bars) in control, group A (short photoperiod), group B (long photoperiod) animals. Horizontal black bars on the bottom of the figures indicate the duration of the spawning (points labelled with a different letter were significantly different at $p < 0.05$, (*) (**) (***) significantly different at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively).

DISCUSSION

Timing of spawning, fecundity and egg quality

The natural spawning period of sea bass along the Mediterranean coast occurs during the winter months when the photoperiod is short and temperatures low (Barnabé, 1980). In this species, photoperiod seems to be the most important environmental cue entraining the rhythm of reproduction with experimental alterations in photoperiod producing significant changes in the time of spawning (Carrillo *et al.*, 1989, 1993 and 1995*a*). Although temperature is thought to be of minor importance in the timing of gonadal recrudescence, it does affect spawning since natural ovulations only occur over a limited range of temperatures (9-17°C) (Zanuy *et al.*, 1986; Devauchelle and Coves, 1988). Temperatures higher than 17°C seem to interfere with the process of final maturation, ovulation and spawning (Carrillo *et al.*, 1991). In this study the various groups of experimental fish were all maintained under natural conditions of temperature and at not time did temperature exceed 17°C or decrease below 9°C during the respective spawning periods. Thus, from the present data, it is clear that exposure of sea bass to one year of constant short days, beginning in April, advances spawning whereas exposure to long days, starting from the same date, causes a delay. In the present work constant long days produced a reduction in fecundity, but egg quality was not affected by the photoperiodic treatment. However, the same group of fish maintained for a second year on a constant long day regime exhibited reduced egg quality (Prat, 1991). Similar results were obtained with rainbow trout maintained under long photoperiods (Bourlier and Billard, 1984). In addition, in a previous study Carrillo *et al.* (1991) pointed out that groups of sea bass with delayed spawning times were less fecund and had reduced rates of hatch and larval survival compared to controls and groups in which spawning was advanced. Thus, it can be concluded that photoperiodically delayed groups have lower fecundity and, in long-term experiments, a lower quality of the progeny.

17β-oestradiol profiles and vitellogenic process

Under natural conditions of photoperiod and temperature the sea bass exhibits a steady increase of plasma E2 with a parallel increase in plasma vitellogenin (Mañanós *et al.*, 1991 and Mañanós, 1993) and vitellogenic oocytes (Prat *et al.*, 1990; Prat, 1991). Similar results have been observed in striped bass, a closely related species (Sullivan *et al.*, 1991; Tao *et al.*, 1993). The results of the present study confirm these changes. However, there were marked differences among the three experimental groups. Under a constant short-day (group A) and control regimes, the highest levels of plasma E2 occurred during December-January. However, in group A, E2 was detectable in the plasma one month earlier than in control fish. From October on, plasma levels of E2 increased progressively, as in the controls, exhibiting a peak in January. However, values returned to basal levels at a faster rate in group A fish than controls (*fig.* 2). A similar behaviour was observed for the vitellogenic oocytes. While mean spawning was advanced by two months with respect to the control group, ovarian atresia disappeared one month earlier (*fig.* 2 and 1, respectively). The significant advance of spawning in group A, with respect to controls, was propably induced by the earlier rise of E2 during October, which in turn would have speed up vitellogenesis and maturation processes. Recent results on the reproductive physiology of sea bass (reviewed by Carrillo *et al.*, 1995*b*) have shown that although the timing of the first significant increase of plasma vitellogenin and E2 can be entrained by the

photoperiod, unsuitable temperature conditions during the vitellogenic period can block this process. Under natural conditions (short photoperiod and temperature below 17°C), it has been observed that in sea bass, the time span between the first surge of plasma E2 and vitellogenin (or vitellogenic oocytes) and the onset of the spawning, is three months (Mañanós, 1993) (*fig.* 2, control). In group A, this time span was two months from October to December (*fig.* 2, group A). It can be suggested that the elevated temperatures observed in September (20°C) could eventually block the vitellogenic process despite the existence of a suitable photoperiodic cue to advance it. It appears that vitellogenesis is resumed once an adequate temperature (probably below 17°C) is reached and that the following reproductive events are accelerated to advance spawning according to the photoperiodic cue.

Under the constant long-day regime (group B) an atypical bimodal profile of plasma E2 was observed. As in the other groups, E2 became elevated above basal levels at the onset of vitellogenesis; the first peak occurred in December coincident with an increasing production of vitellogenic oocytes and the second peak occurred in April, coincident with the commencement of spawning. Vitellogenic and atretic oocytes were present in the ovaries for more than six months. It is difficult to interpret these results but it is possible that inappropriate environmental cues (a mismatch between photoperiod and temperature: *i.e.* long photoperiod associated with low temperatures) received by group B during the reproductive period, could alter the endocrine mechanisms that maintain oestrogen synthesis within the follicle and therefore alter the rates of vitellogenesis and atresia.

The data presented here confirm the important role of the photoperiod in controlling reproductive events in sea bass. It can also be hypothesized that the photoperiodic signal may generate important modifications of the neuroendocrine mechanisms involved in the control of the gonadal growth. In the stickleback, it has been suggested that the photoperiodic effects on GtH cell activity may be mediated by changes in the GnRH release (Andersson *et al.*, 1992) and in birds, it has been shown by Perera and Follett (1992) that the photoperiodic effect involves the mechanism of release of GnRH and GtH. Nevertheless it seems that in sea bass, an inappropriate temperature may influence vitellogenesis directly as has been demonstrated in cyprinid (Okuzawa *et al.*, 1989) and salmonid (Johnston *et al.*, 1990) species. In addition, inappropriate environmental cues may have an unwanted deletereous effect on fecundity and egg quality.

Acknowledgements

We express our gratitude to Dr. R. Serrano for his kind help in collecting and evaluating the egg quality data. This work was supported in part by the CAICYT project: PAC 84-00029 and the CICYT project: 88-0231.

REFERENCES

Alvariño J. M. R., M. Carrillo, S. Zanuy, F. Prat, E. Mañanós 1992. Pattern of sea bass oocyte development after ovarian stimulation by LHRHa. *J. Fish Biol.* **41**, 965-970.

Andersson E., B. Borg, H. J. Th. Goos 1992. Temperature, but not photoperiod, influences gonadotropin-releasing hormone binding in the pituitary of the three-spined stickleback, *Gasterosteus aculeatus. Gen. Comp. Endocrinol.* **88**, 111-116.

Barnabé G. 1980. Exposé synoptique des données biologiques sur le loup ou bar, *Dicentrarchus labrax* L. Synop. FAO Pêches. **126**, 70 p.

Barnabé G., J. Paris 1984. Ponte avancée et ponte normale du loup *Dicentrarchus labrax* (L.) à la station de biologie marine et lagunaire de Sète. In: L'Aquaculture du bar et des Sparidés, G. Barnabé, R. Billard ed. INRA Publ. Paris, 63-72.

Barnabé G., R. Barnabé-Quet 1985. Avancement et amélioration de la ponte induite chez le loup *Dicentrarchus labrax* (L.) à l'aide d'un analogue de LHRH injecté. *Aquaculture* **49**, 125-132.

Bourlier A., R. Billard 1984. Delayed gametogenesis and spawning in rainbow trout (*Salmo gairdneri*) kept under permanent light during the first and second reproductive cycles. *Aquaculture* **43**, 259-268.

Bromage N., J. Duston, C. Randall, A. Brook, M. Trush, M. Carrillo, S. Zanuy 1990. Photoperiodic control of teleost reproduction. In: Progress in Comparative Endocrinology, A. Epple, C. G. Scanes, M. H. Stetson ed. Wiley-Liss, New York, 620-626.

Bromage N., C. Whitehead, B. Breton 1982*a*. Relationship between serum levels of gonadotropin, oestradiol-17β, and vitellogenin in the control of ovarian development in the rainbow trout. II The effects of alterations in environmental photoperiod. *Gen. Comp. Endocrinol.* **47**, 366-376.

Bromage N., C. Whitehead, J. Elliot, B. Breton, A. Matty 1982*b*. Investigations into the importance of daylength on the photoperiodic control of reproduction in the female rainbow trout. In: Proc. Int. Symp. Reproductive physiology of fish, C. J. J. Ritcher, H. T. Th. Goos eds. Pudoc, Wageningen, Neth., 233-236.

Bye V. J. 1987. Environmental management of marine fish reproduction in Europe. In: Reproductive physiology of fish IV, A. P. Scott, J. P. Sumpter, D. E. Kime, M. S. Rolfe eds., Univ. East Anglia, Norwich, U.K., 289-298.

Carrillo M., N. Bromage, S. Zanuy, R. Serrano, F. Prat 1989. The effect of modification in photoperiod on spawning time, ovarian development and egg quality in the sea bass (*Dicentrarchus labrax*). *Aquaculture* **81**, 351-365.

Carrillo M., N. Bromage, S. Zanuy, R. Serrano, J. Ramos 1991. Egg quality and fecundity in the sea bass (*Dicentrarchus labrax*) and the effects of the photoperiodically-induced advances and delays of spawning time. In: Reproductive physiology of fish IV, A. P. Scott, J. P. Sumpter, D. E. Kime, M. S. Rolfe eds., Univ. East Anglia, Norwich, U.K., 259-261.

Carrillo M., S. Zanuy, F. Prat, R. Serrano, N. Bromage 1993. Environmental induction of spawning in sea bass. In: Recent Advances in Aquaculture IV, N. Bromage, E. M. Donaldson, S. Zanuy, M. Carrillo, J. Planas eds., Blackwell Science Ltd. Oxford, 43-54.

Carrillo M., S. Zanuy, F. Prat, J. Cerdá, J. Ramos, E. Mañanós, N. Bromage 1995*a*. Sea bass (*Dicentrarchus labrax*). In: Broodstock management and egg and larval quality, N. R. Bromage, R. J. Roberts eds., Blackwell Science Ltd. Oxford, London, 138-168.

Carrillo M., S. Zanuy, F. Prat, J. Cerdá, E. Mañanós, N. Bromage, J. Ramos, O. Kah 1995*b*. Nutritional and photoperiod effects on hormonal cycles and quality of spawning of sea bass (*Dicentrarchus labrax* L.). *Neth. J. Zool.* (in press).

Conover W. J. 1980. Practical nonparametric statistics. John Wiley and Sons, Inc. New York, 493 p.

Devauchelle N. 1984. Reproduction decalée du bar (*Dicentrarchus labrax*) et de la daurade (*Sparus aurata*). In: L'Aquaculture du bar et des sparidés, G. Barnabé, R. Billard, eds., INRA Publ. Paris, 53-62.

Devauchelle N., D. Coves 1988. Sea bass (*Dicentrarchus labrax*) reproduction in captivity: Gametogenesis and spawning. *Aquat. Living Resour.* **1**, 215-222.

Girin M., N. Devauchelle 1978. Décalage de la période de reproduction par raccourcissement des cycles photopériodiques et thermiques chez les poissons marins. *Ann. Biol. Anim. Biochem. Biophys.*, **18**, 1059-1065.

Herland M. 1960. Étude critique de deux techniques nouvelles afin de mettre en évidence les différentes catégories cellulaires présentes dans la glande pituitaire. *Bull. Microsc. Appl.* **10**, 37-44.

Johnston C. E., S. R. Farmer, R. W. Gray, M. Hambrook 1990. Reconditioning and reproductive responses of atlantic salmon kelts (*Salmo salar*) to photoperiod and temperature manipulation. *Can. J. Fish. Aquat. Sci.* **47**, 701-710.

Mañanós E., S. Zanuy, M. Carrillo, J. Nuñez Rodriguez, F. Le Menn 1991. Quantification by ELISA of vitellogenin levels in sea bass (*Dicentrarchus labrax* L.) maintained under different photoperiods. In: Reproductive physiology of fish IV, A. P. Scott, J. P. Sumpter, D. E. Kime, M. S. Rolfe eds., Univ. East Anglia, Norwich, 325.

Mañanós E. 1993. Estudio del proceso vitelogenico de la lubina (*Dicentrarchus labrax* L.) y los factores que le influyen. Ph. D. Thesis. Univ. Valencia, 374 p.

Mayer I., S. E. Shackley, J. S. Ryland 1988. Aspects of the reproductive biology of the bass, *Dicentrarchus labrax* L. I. An histological and histochemical study of oocyte development. *J. Fish Biol.* **33**, 609-622.

Okuzawa K., K. Furukawa, K. Aida, I. Hanyu 1989. Effects of photoperiod and temperature on gonad maturation, and plasma steroid and gonadotropin levels in a cyprinid fish, the honmoroko, *Gnathopogon caerulescens*. *Gen. Comp. Endocrinol.* **75**, 139-147.

Perera A. D., B. K. Follett 1992. Photoperiodic induction in vitro: The dynamics of gonadotropin-releasing hormone release from hypothalamic explants of the Japanese quail. *Endocrinology* **131**, 2898-2908.

Prat F., S. Zanuy, M. Carrillo, A. De Mones, A. Fostier 1990. Seasonal changes in plasma levels of gonadal steroids in sea bass, *Dicentrarchus labrax* L. *Gen. Comp. Endocrinol.* **78**, 361-373.

Prat F. 1991. Control del ciclo reproductor de la lubina por manipulación hormonal y ambiental. Ph. D. Thesis, Univ. Barcelona, 232 p.

Sullivan C. V., Y. Tao, R. G. Hodson, A. Hara, R. O. Bennett, L. C. Woods III 1991. Vitellogenin and vitellogenesis in striped bass (*Morone saxatilis*) broodstock. In: Reproductive physiology of fish IV, A. P. Scott, J. P. Sumpter, D. E. Kime, M. S. Rolfe, eds., Univ. East Anglia, Norwich, U.K., 315-317.

Tao Y., A. Hara, R. G. Hodson, L. C. Woods III, C. V. Sullivan, 1993. Purification, characterization and immunoassay of striped bass (*Morone saxatilis*) vitellogenin. *Fish Physiol. Biochem.* **12**, 31-46.

Zanuy S., M. Carrillo, F. Ruiz 1986. Delayed gametogenesis and spawning of sea bass (*Dicentrarchus labrax*) kept under different photoperiod and temperature regimes. *Fish Physiol. Biochem.* **2**, 53-63.