Long photoperiod on sea cages delays timing of first spermiation and enhances growth in male European sea bass (*Dicentrarchus labrax*).

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Abstract

Two large groups of fingerling (3.2g) male European sea bass (around 75,000 individuals each) reared in net sea cages for a period of two years were exposed to two different photoperiods starting in June: either ambient photoperiod (37º 24´ N) (NP) or a constant long photoperiod (LP; 18 hours light: 6 hours dark, 18L: 6D). Long days had limited effect on somatic growth at the time of incipient gonad development (IGD, 1st annual cycle; November-March), while they were effective at the time of full gonad development (FGD, 2nd annual cycle). Moreover, the annual patterns of morphological indices, i.e., Fulton's condition factor (CF), visceral fat index (VFI) and particularly gonadosomatic index (GSI), were delayed by one or two months as the result of the long days compared to the control group (NP). LP did not prevent the onset of puberty but delayed spermiation by one month, and modified accordingly the phase and/or amplitude of the annual rhythms of pituitary Gonadotropin Releasing Hormone (sbGnRH) content and plasma levels of Luteinising Hormone (LH) and sex steroids, particularly that of 11-Ketotestosterone (11-KT). In general, high levels of sbGnRH (≥4 ng/pit) were associated with elevated plasma LH (1-3 ng/ml at

IGD and ≥8 ng/ml at FGD), and the sex steroid peaks usually preceded LH peak in both treatments (1-2 months earlier). Finally, full spermiation and the maximum GSI values coincided with the LH peak, highlighting the close relationship that exists between LH plasma levels and gonadal development.

Keywords: Precocity; Puberty; Endocrine rhythms; Photoperiod; Sea bass; Sea cages

1. Introduction

Sea bass aquaculture developed very rapidly in Europe during the 90s, reaching 138,156 tons in 2008 (Aquamedia, 2008). However, one of the main factors that can impair productivity under intensive culture is the loss of growth and product quality associated with the onset of early male puberty (Felip et al., 2006). Under natural conditions, male European sea bass reach puberty during their second year of life, while females reach it one year later (Carrillo et al., 1995). The attainment of commercial size (400-500 g wt) for this species coincides with the onset of male puberty, which represents a product quality concern, as this period may be accompanied by muscle wasting, slow growth and a loss in flesh quality (Carrillo et al., 1995). The problem is exacerbated in the Mediterranean Sea, because most fish farms inadvertently produce extremely high rates of male specimens as high as 70-90%. Thus, control over puberty in male European sea bass is critical, given the important interactions it has with somatic growth. Photoperiod has been successfully used to modify puberty in farmed Atlantic salmon and cod in netpens (Taranger et al., 1998; 2006), as well as turbot (Imsland et al., 1997), sea bream (Kissil et al., 2001) and haddock (Treasurer et al., 2006) under indoor experimental conditions. Following the earlier work of Prat et al. (1999), which established the existence of an endogenous process underlying European sea bass reproduction, some attempts have been made to control its puberty indoors by applying different photoperiod regimes (Rodríguez et al., 2001a, Begtashi et al., 2004, Felip et al., 2008). It was shown that constant long days reduced spermiation rate and delayed maturation, enhancing growth and

modifying the phase and levels of hormonal rhythms (Rodríguez et al., 2001a; 2004). The problem is photoperiod regimes that are successful indoors can be less effective on sea cages (Karlsen et al., 2006, Taranger et al., 2006). Delaying or reducing puberty through photoperiod manipulation could be a useful approach for outdoor European sea bass farming on a commercial scale. Presently, there is a lack of data on photoperiod effects on European sea bass kept under commercial culture conditions, and no studies have addressed its consequences on the activities of key reproductive hormones. Therefore, a study was undertaken to analyze the effect of constant long-day exposure on reducing or delaying puberty in male European sea bass raised outdoors in large-scale intensive farming operations. This contributes to a further understanding of the interrelationships between sexual maturation and somatic growth under commercial conditions. To accomplish this goal, a long-term comprehensive study of various morphological indices and hormonal parameters was conducted, which included pituitary Gonadotropin Releasing Hormone (sbGnRH) content and Luteinizing Hormone (LH), Testosterone (T) and 11-Ketotestosterone (11-KT) plasma levels in a significant number of sampled fish.

2. Materials and methods

2.1. Fish stock, rearing conditions and experimental design

The experiment was conducted at CULMAREX S. A., a Mediterranean fish farm in Águilas (Murcia, Spain; 37º 24´ N - 1º 34´ W). One hundred fifty-eight thousand immature juveniles, with an average weight of 3.2 g, were roughly distributed into four 5m x 5m x 5m net cages. Two cages were exposed to an artificially long photoperiod, superimposed on natural sun light (18L: 6D, on at 04:00 and off at 22:00; LP), while the other two were exposed to a natural photoperiod (Control; NP). Control cages were placed at a distance of >30 m from the light-exposed cages to prevent any exposure of the artificial light on the control fish. The experiment started in June for both groups, as previous experimental studies indoors have

demonstrated that the long days which begin in June delay puberty and enhance growth (Rodríguez et al., 2001a), and also because the commercial production demands of the farm required a minimum juvenile starting size to be introduced in the sea cages. The long day regime lasted for approximately two years. Fish in all cages were kept under a natural temperature regime (NT) throughout the experiment (Fig 1A). All four cages were covered with raffia cloth to prevent the fish from suffering from excessive exposure to sunlight. To provide artificial light a metal halide Osram Powerstart HQI-TS, 150W/NDL UVS bulb (Sylvania, MA,USA) was placed approximately 1.5 m above from the water surface, in the centre of each net cage, providing 2000 lux at the water surface, as measured by a luxometer (LT Lutron, LX-101, Ginza Marketing, Manila, Philippines). The light spectrum of this bulb approximates that of natural sunlight (Bayarri et al., 2004). Fish were fed a commercial dry feed (Trouvit-Mar Europe) *ad libitum*. The chemical composition of the diet was adapted to suit the age of the animals, i.e., 56% protein, 20% lipids, 7.5% carbohydrates, 9% ash, 0.9% cellulose and 7% moisture for juveniles; and 48% protein, 22% lipid, 9.3% carbohydrate, 1.2% ash, 9.5% cellulose and 10% moisture for adults. Change of diet took place early at the second half of the second annual cycle. Fish were fed two meals per day, by hand, during natural daylight hours only, at 09:00 AM and 14:00 PM.

2.2. Data sampling

Body length (B_L) (± 1mm) and body weight (B_W) (± 0.01g) were recorded monthly from 200 fish sampled at random from each group (100 for each replicate), except in the summer of the second year when no samples were taken to prevent stress due to the high water temperature that occurred in July, August and September (≥ 24ºC). The specific growth rate in terms of weight (G_W) and length (G_L) was calculated as follows: $G_W = (\ln Wf - \ln Wo) / \Delta t$; $G_L = (\ln Lf - \ln Lo) / \Delta t$, in which ln = Naperian logarithm; Wf = final weight in grams; Wo = initial weight in grams; Lf = final length in cm; Lo = initial length in cm; Δt = time

interval in days. Fish were anaesthetised using ethylene glycol monophenyl ether (0.5ml/l of water). Every month, from September on, 100 fish per treatment (50 from each cage) were anaesthetized then bled by means of caudal puncture, using heparinised syringes, and then killed by sectioning the spinal cord. Plasma was obtained by centrifugation (4ºC for 30 min at 2500g) and stored at -20ºC for further analysis. The pituitary gland and brain were immediately collected, frozen in liquid nitrogen and stored at -80ºC. The gonads and fat were quickly removed and weighed (± 0.001 g) in order to estimate the somatic indices, using the following equations:

GSI = 100 x Gonad w / Bw; VFI = 100 x VFw / Bw; CF = 100 x Bw / B_L exp 3; where GSI, VFI, and CF are the gonadosomatic, visceral fat, and Fulton's condition factor indices, respectively; and where B_L corresponds to body length; Bw, body weight; Gonad w, gonad weight and VFw, peri-visceral fat weight.

2.3. Testes histology

Testes were fixed by immersion in 4% formaldehyde: 1% gluteraldehyde (McDowell and Trump, 1976) embedded in 2- hydroxyethyl methacrylate polymer resin (Technovit 7100, Heraeus Kultzer Germany), sectioned (3 μm) and stained using the Cleveland-Wolff technique (Herlant 1960). Stages of testicular development were determined by light microscopy, by means of full examination, crossing over sectioned pieces of the sample slide corresponding to each individual fish testis sample every month for each group. All the gonads examined were classified according to Begtashi et al., (2004). The number of precocious males was recorded each month, based on the identification of animals showing expressible sperm after gentle abdominal stripping, performed while they were early-maturing males (February-April; 1st annual cycle) and at full gonad development (October-April; 2nd annual cycle). All these data were used to calculate the rates of spermiating males per group.

2.4. Hormonal analysis

Pituitary content of sbGnRH and plasma LH levels were determined by specific enzyme immunoassay (EIA), according to the methods described by Rodríguez et al. (2000b) and Mateos et al. (2006), respectively. Plasma testosterone (T) was determined by a specific immunoassay (EIA) developed by Rodríguez et al. (2000a) for European sea bass, and plasma 11-ketotestosterone (11-KT) levels were analysed using an EIA developed for Siberian sturgeon (Cuisset et al., 1994) and modified for use with European sea bass.

2.5. Statistical analysis

Data were analysed using Sigma Stat 3.0 (SPSS. Inc., Illinois, USA). The results were expressed as mean ± SEM (standard error of the mean). Data were checked for normal distribution using a Kolgomorov-Smirnoff test following logarithmic transformation (as required), and a Bartlett's test was used to establish homogeneity of variances. The effects of photoperiod treatment for the two experimental groups were tested using a two-way analysis of variance (Zar,. 1996), followed by all pairwise multiple comparison procedures (Holm-Sidak method). Differences were considered to be statistically significant when $P<0.05$.

3. Results

3.1 Growth

Animals that started in June with 6.6 cm in length and 3.2 g in weight gained 12.45 cm and 101.7 g when kept under NP conditions (G_L = 0.79; G_W = 2.603, respectively), and 11.92 cm and 93.8 g under LP conditions (G_L = 0.77; G_W = 2.544, respectively) during the first growth period (1st GP, June-October; Fig. 1B). A lesser gain in terms of both body length and body weight was observed during the incipient gonad development period (IGD) (November-March), when the fish gained 4.93 cm and 87.2 g under NP, and 4.09 cm and 76.6 g under LP. Roughly estimated, G_L and G_W exhibited a 4-5 fold reduction during incipient gonad development in both groups. A new growth surge was observed during April-October (second growth period; 2nd GP), which led to gains of 10.24 cm and 399.3g under NP, and 9.57 cm

and 393.3 g under LP. When comparing the G_L and G_W values for the 1st and 2nd GP, the latter period exhibited a 4-fold reduction with respect to the former. During the full gonad development period (FGD) (November-March), increments in length and weight were of 1.46 cm and 9.8 g for the NP group, and 2.32 cm and 106.6g for the LP group. Thus, the group receiving the LP treatment exhibited higher growth rates than the NP group during this period, with a length and weight difference of 0.86 cm and 96.9 g, respectively, between both groups at the end of March.

3.2 Morphological indices

3.2.1. Fulton's Condition Factor (CF)

Condition factor exhibited two transient elevations, one in September (1.55 ± 0.016 %) at the beginning of the experiment, and another in November (1.617 ± 0.0269 %), were observed for the NP group. From this point, constant CF levels (around 1.5) were detected during most of the incipient gonad development period, until April of the 2nd growth period (GP) (Fig. 2A). During the following month, a significant ($P<0.001$) decrease was observed, which continued until June, when the lowest levels of the cycle were reached (1.326 ± 0.0230 %). A steady increase was observed during the second half of the 2nd GP, attaining the highest values in December (1.589 ± 0.0176 %), during the first half of full gonadal development. In the following months, CF values exhibited a rapid decrease. Animals exposed to LP displayed a parallel profile to those receiving the NP treatment, but showed significantly lower values for most sampling points.

3.2.2. Visceral Fat Index (VFI)

During the first annual cycle, the NP group displayed a significant ($P<0.001$) VFI increase from September to October (Fig. 2B). From then on, VFI values remained high until December (around 6%). In the following months, a progressive decrease was observed, attaining the lowest level in March (4.14 ± 0.237 %), near the end of incipient gonad

development period. Later, a steady increase in VFI occurred, peaking in October (8.13 ± 0.296), at the end of the 2nd GP. During full gonadal development, a steady and significant decrease was observed, attaining the lowest values in February (≤ 4.5 %). The pattern of VFI values for the LP group was parallel to that of the NP group, except for the fact that the lowest VFI values (< 4.5%) were delayed by two months with respect to those of the NP group (Fig. 2B, arrows; end of IGD, early 2nd GP, respectively).

3.2.3. Gonadosomatic Index (GSI)

During the incipient gonad development period, the GSI for the NP group exhibited a moderate, although steady increase from October on, peaking at the end of January (0.235 ± 0.0621) (Fig. 3A). Fish exposed to LP (Fig. 3B) displayed an annual GSI profile of greater amplitude (0.50 ± 0.078 in March), with the highest value exhibiting a one month delay as compared to that of the NP group. Consequently, GSI values in February, March and April were significantly higher that those of the NP group on equivalent dates (compare Figs. 3A and 3B). In the case of the LP group, the proportion of precociously spermiating males was 2.5 % (Fig. 3B) vs. 0% in the NP group (Fig. 3A). This was in accordance with the advanced stage of testicular recrudescence observed in this group, which showed 47 % of the fish at stage V in March (Fig. 3D). The pattern of the testicular stages in the NP group was consistent with the low GSI on these dates, showing poor gonadal maturation (20 % of fish at stage V in February) (Fig. 3C). During full gonad maturation period, in the second annual cycle, the GSI for both treatments gave rise to values of around 3, which were 15 and 6 times higher than those attained at incipient gonad development period for the NP and LP groups, respectively (Figs. 3A, 3E and 3B, 3F). The annual GSI profile for fish exposed to LP was shifted by one month with respect to those exposed to NP. GSI values under LP peaked at the end of February, whereas those of the NP group peaked at the end of January (Figs. 3E and 3F, arrows). Consequently, GSI values for the LP group in February and March were

significantly ($P<0.001$) higher than those of the NP group on equivalent dates. Similarly, values from late October, November and December for the NP group were significantly higher than those of the LP group on the same dates ($P<0.05$; $P<0.001$; $P<0.001$, respectively). During full gonad development period, both groups attained values of $\geq 80\%$ for spermiating males, and 100 % of the gonads reached stage V of development. However, the LP group showed maturation rates slightly higher than those of the NP group, and its maturation profile was shifted by one month with respect to the control group. More specifically, the NP group showed the first rise in the rate of spermiating males in November (35%), while an equivalent rate of spermiating males was attained one month later in the LP group. Under NP conditions, the rates of spermiating males and state V testes fluctuated between 78.5% to 87.6% and 73% to 100%, respectively, from December to February, only to sharply decrease in March. Under LP conditions, the rates of spermiating males and testes at stage V fluctuated between 76.3% to 96% and 82.1% to 97.4%, respectively, from January to March, exhibiting a one-month delay with respect to the NP group.

3.3 Hormones

3.3.1. Sea bream Gonadotropin Releasing Hormone (sbGnRH)

Low levels in the pituitary content of sbGnRH were observed in August and September during the first annual cycle for the NP treatment (Fig. 4A). A significant increase ($P<0.001$) occurred in October, which then decreased in January. Therafter, the pituitary content of sbGnRH steadily increased, peaking at the end of March (4.2 ± 2.36 ng/pit.). In the case of the LP group, constant low levels of sbGnRH (<0.52 ng/pit) were observed in August-September, in a similar fashion to those observed in the NP group on the same dates (Fig. 4B). In October, a significant ($P<0.05$) increase in sbGnRH content was observed (1.15 ± 0.213 ng/pit), and these levels remained constant until January. In the following months, a steady and significant rise was observed, which peaked in March (4.60 ± 1.51 ng/pit). During

the full gonad development period, the pattern of pituitary sbGnRH content showed one peak at the end of November, and another in January in both experimental groups (Figs. 4E and 4 F).

3.3.2. Luteinising Hormone (LH)

During the first annual cycle, NP fish displayed an LH peak (2.26 ± 0.370 ng/ml) at the end of September, only to decrease thereafter, attaining low values at the beginning of January. However, in the following months, a progressive, although non-significant increase in plasma LH occurred, reaching the highest levels in March (1.29 ± 0.537 ng/ml) (Fig. 4A). Plasma LH levels in the LP group exhibited two distinct temporal peaks, one at the end of September (2.48 ± 0.625 ng/ml), and a second and larger one in March-April (> 3 ng/ml) that was shifted by one month with respect to that of the NP group (Fig. 4B). Peak values for LH plasma levels during the second reproductive cycle exceeded those of the first cycle several fold (i.e. x 2.5 and x 5.4 for the NP group, and x 1.3 and x 2.5 for the LP group, for the first and the second peak, respectively). During the full gonadal development period, the first significant rise was observed at the end of November (5.63 ± 0.771 ng/ml), followed by a decrease in the following month, finally attaining a higher peak in January (7.94 ± 1.830 ng/ml) (Fig. 4E). The LP group presented the first significant surge in October (3.32 ± 0.660 ng/ml). Plasma LH plasma levels remained roughly constant until December, prior to a new significant elevation which was observed in January, further peaking at the end of February (8.28 ± 1.114 ng/ml). This peak exhibited a one-month delay with respect to that of the control group (see arrows in Figs. 4E and 4F).

3.3.3. Testosterone (T)

NP plasma testosterone showed a peak at the end of February (12.76 ± 0.750 ng/ml) during the first annual cycle (Fig. 4C). Animals exposed to LP showed two temporal peaks for plasma T: one in November (6.74 ± 0.454 ng/ml) and another in February (10.82 ± 0.752

ng/ml) (Fig. 4D). During the second annual cycle (Fig. 4G), a steady increase in plasma T was observed for the NP group from September on, with a further peak in December (13.94 ± 0.795 ng/ml). The LP group showed elevated levels during September (8.58 ± 0.631 ng/ml), and a large peak in December (11.74 ± 0.822 ng/ml) (Figs.4G, 4H).

3.3.4. 11-Ketotestosterone (11-KT)

In the NP group, the first significant increase in plasma 11-KT was observed in October, during the first annual cycle. Constant levels (around 1 ng/ml) were observed until the beginning of January, finally peaking by the end of this month (2.32 ± 0.379 ng/ml). In the LP group, the first increase in 11-KT plasma levels appeared in November, slightly decreasing in the following months, until early January. At the end of this month, a second surge was observed, peaking in late February (2.69 ± 0.674 ng/ml). This second peak exhibited a one-month delay with respect to that of the NP group (arrows in Figs. 4C, 4D). During the second annual cycle, a steady increase in plasma 11-KT was observed in the NP group from September on, further peaking in late December (16.9 ± 2.28 ng/ml) (Fig. 4G). In the LP group, a significant rise in 11-KT was observed during late October (11.6 ng/ml). A significant ($P<0.01$) decrease in plasma 11-KT appeared during the following month, and from then on, a slow but steady increase was observed, further peaking by the end of February (9.08 ± 0.911 ng/ml). The resulting phase shift for 11-KT, induced by the long days, is represented by the arrows in Figs. 4 G and 4H.

4. Discussion

A long photoperiod treatment was less effective in reducing the incidence of male sexual maturation when applied to sea bass in floating cages than in indoor tanks (Rodriguez et al., 2001a) as previously observed in Atlantic cod (Karlsen et al., 2006, Taranger et al., 2006). In caged sea bass the long day regime induced a delay in sexual maturation but enhanced gonadal growth and the occurrence of precocious male during the first annual cycle,

278 although in a lower proportion than described in lightproof tanks (Rodríguez et al. 2001a). In
279 the second annual cycle, long photoperiod was effective in delaying gonadal growth but had
280 no effect on the incidence of sexual maturation. This contrast with the results obtained in
281 tanks where gonadal development was clearly reduced (Rodriguez et al., 2001a). These
282 differences between sea cages and tanks could be related not only to genetic or physiological
283 differences such as growth rate, but also to other environmental factors that could interact
284 with photoperiod treatment in determining its effect in puberty. It can be hypothesized that
285 sexual maturation could be halted only by an abrupt change in the photoperiod (free of
286 environmental noise) the year preceding completion of maturation as occurred in tanks.
287 However more specific work is needed to prove this hypothesis.

288 Studies performed on various marine fish species have demonstrated that long day
289 regimes stimulate fish growth, especially after long exposure times (Boeuf and Le Bail 1999).
290 By contrast the present results on European sea bass showed that continuous long day
291 exposure had no effect on somatic growth in year one. Nevertheless, it was clear in year two
292 (Fig 1B), when testicular recrudescence took place, as previously observed in sea bream
293 (Kissil et al., 2001). This is likely to be associated with the rates of gonadal development and
294 maturation, which varied according to the annual cycle (i.e., 1^{st} annual cycle IGD and 2^{nd}
295 annual cycle FGD). More specifically, in year one, the GSI and rate of spermiating males in
296 NP and LP groups were seven-fold times lower than those attained in year two. On the other
297 hand, it is known that the onset of reproduction (puberty) has important consequences on
298 somatic growth, as a great deal of energy and resources are directed towards gonadal growth,
299 the production of gametes and reproductive behaviour (Schulz et al., 2006). .In year one, both
300 NP and LP groups exhibited very moderate gonadal growth, which very likely required a
301 modest investment of energy, as documented by Gorshkov et al. (1999). Comparative analysis
302 of growth performance between maturing and immature sea bass males, demonstrated that

precocious fish grew up to 18% less in weight and 5% less in fork length than their counterparts during their second annual cycle of life (Felip et al., 2006). This study illustrates the relationship between sexual maturation and somatic growth and supports the idea that preventing or delaying gonadal maturation by long photoperiod, can result in a growth enhancement at the time when the gonads still remain quiescent. Besides, if the gonadal maturation delay occurs near the time of sea bass harvest (around 18-24 months of age in most Mediterranean fish farms) the application of constant long days in floating cages could be a promising practice to enhance production.

Under commercial sea cages conditions, exposure to long days gave rise to a certain number of male sea bass exhibiting early puberty, although in a lower proportion to that previously observed under lightproof tank experimental conditions (Rodríguez et al., 2001a). Thus, in addition to genetics and date of hatch, it is likely that stocking and rearing conditions may affect the rate of male sea bass precocity. Moreover, Begtashi et al. (2004) stated that precocious fish are larger than immature individuals ones, suggesting that a critical body size associated with the initiation of sexual maturation could be a permissive condition for enabling male sea bass to initiate the reproductive process for the first time. Supporting this hypothesis, at the incipient gonadal development period LP fish were larger than their NP counterparts (Fig 1B), coinciding with the presence of early maturing males in the LP group while no spermiating males were observed in NP (Fig. 3A). Thus, it is very likely that a higher percentage of LP males may have reached critical size because of the faster growth attained during thie incipient gonadal development period, as was previously observed by Saillant et al. (2003).

The highest CF levels (≥ 1.5) attained in both annual cycles (October-December; Fig. 2A shaded areas) during the early gametogenesis period coincided with the highest accumulation of mesenteric fat, as indicated by the highest VFI values. These facts suggest

328 that fish Condition Factor can be linked to the dynamics of the previous fat accumulation
329 preceding gonadal development, confirming Begtashi et al. (2004) during the first annual
330 cycle. Furthermore, VFI and GSI were both influenced by the light treatment, which induced
331 a phase delay in their respective yearly profiles (Fig. 2B and 3B), in agreement with the above
332 statements. Thus despite the fact that long day regimes failed to reduce the incidence of
333 spermiating males at the second year, a one month delay in the cycle of reproduction was
334 shown. In association with this gonadal delay a somatic growth enhancement was observed
335 giving an average difference of around 100 g per fish relative to the controls. This
336 demonstrates that a long day photoperiod regime applied to floating cages is of commercial
337 value.

338 The present study provides evidence for the close relationship between pituitary
339 sbGnRH, LH plasma levels, GSI values and stages of testicular maturation during the
340 incipient gonad development and full gonad development periods in male sea bass kept under
341 intensive culture conditions in an outdoor environment. Broadly speaking, the pituitary
342 content of sbGnRH is associated with gonadal maturation, as has been observed in other
343 teleosts (Okuzawa et al., 2003; Amano 2004; Pham et al., 2006). The present results are
344 largely confirmatory of previous experimental observations (Rodríguez et al., 2000b;
345 Rodríguez et al., 2004) that showed the annual rhythm of sbGnRH to be unaffected by the
346 long day regime. In both cases, two GnRH peaks were observed, the first one at incipient
347 gonad development period, associated with the process of gonadal differentiation, and the
348 second one upon gonadal maturation. Recently Moles et al. (2007) provided further support
349 for the role of sbGnRH in gonadal differentiation through an enhancement of FSHβ gene
350 expression, showing a peak of brain sbGnRH content at 250 days post-hatching, which
351 roughly coincided with the time of our first sbGnRH increase. The rapid rise in pituitary
352 sbGnRH levels correlated with plasma LH levels and GSI, particularly from December to

January in the second sexual cycle, which illustrates the role of sbGnRH in the release of LH by the pituitary gland. Constant long days induced a phase delay in the annual rhythms of plasma LH and sex steroids during both annual cycles, as described in the case of sea bass kept under indoor experimental conditions (Rodriguez et al., 2004). Seasonal hormonal changes in the second annual cycle resembled those observed during the first cycle, except for the absence of the LH peak in September. At this time, fish were already differentiated and did not require additional levels of LH to complete this process, as occurred the previous year. However, during the second annual cycle, hormone levels experienced a dramatic increase, which is in agreement with the sizeable gonadal enlargement observed. Furthermore, it should be noted that Rodríguez et al. (2005), when analyzing the effects of continuous light on early precocity, concluded that 11-KT could be considered a key hormone for initiating precocity in sea bass. Our results are in agreement with the above considerations, taking into account that the first significant surge of 11-KT in the NP group during the first annual cycle occurred in October, at the time of gonadal cell proliferation (Fig. 4C). This occurred some time before the first GSI increase (Fig. 3A). The first 11-KT surge and the first elevation of GSI were also shifted accordingly, as a result of the long days (Figs. 4 D and 3B), which is also in agreement with the previous reports.

5. Conclusion

In male European sea bass kept in outdoor floating cages, a long photoperiod starting in June provokes a phase shift in the rhythms of reproductive hormones, particularly in the cases of LH and 11-KT. This affects the patterns of gonadal development and energy reserves, enhancing growth during the period of reproductive delay. Furthermore, growth enhancement is proportional to the magnitude of the further gonadal development, being very poor during the first year, but quite important during the second year, in association with sexual maturation.

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480

8. Figure captions

8.1. Fig.1

A. Natural photoperiod (solid thick line, NP), natural temperature (solid thin line, NT) and constant long photoperiod (dotted line, LP) during two consecutive annual cycles.
B. Mean (±SEM) body weight (circles) and fork length (triangles) for sea bass exposed to NP (solid lines and black symbols) and LP (dotted lines and white symbols). Significant differences ($P<0.05$) between treatments are denoted by #.

8.2. Fig.2

Mean (±SEM) of morphological indices for sea bass exposed to either NP (solid lines and black circles) or LP (dotted lines and white circles): A. Condition factor (CF). B. Visceral fat index (VFI). Significant differences ($P<0.05$) between treatments on the same sampling date or between months for the same treatment are denoted by # (above the symbols) or * (on curved lines), respectively. Arrows in B indicate VFI minimum values during the first cycle for the NP and LP groups.

8.3. Fig.3

Mean GSI (± SEM) and proportion of spermiating males and gonadal stages under NP (left panels) and LP (right panels) during the Incipient Gonad Development (A-D) and Full Gonad Development (E-H). Significant differences ($P<0.05$) in GSI between treatments are indicated by *. Arrows designate GSI peaks for fish exposed to either NP or LP conditions.

8.4. Fig.4

Monthly changes (mean ± SEM) in pituitary gland sbGnRH content and plasma levels of Luteotropin hormone (LH), Testosterone (T) and 11-ketotestosterone (11-KT). Significant differences ($P<0.05$) between treatments on the same sampling date or between months for the same treatment are denoted by # (above black circles) and φ (above white circles) and *

(on curved lines), respectively. Arrows indicate peaks of the levels of different hormones for fish exposed either to NP or LP conditions.

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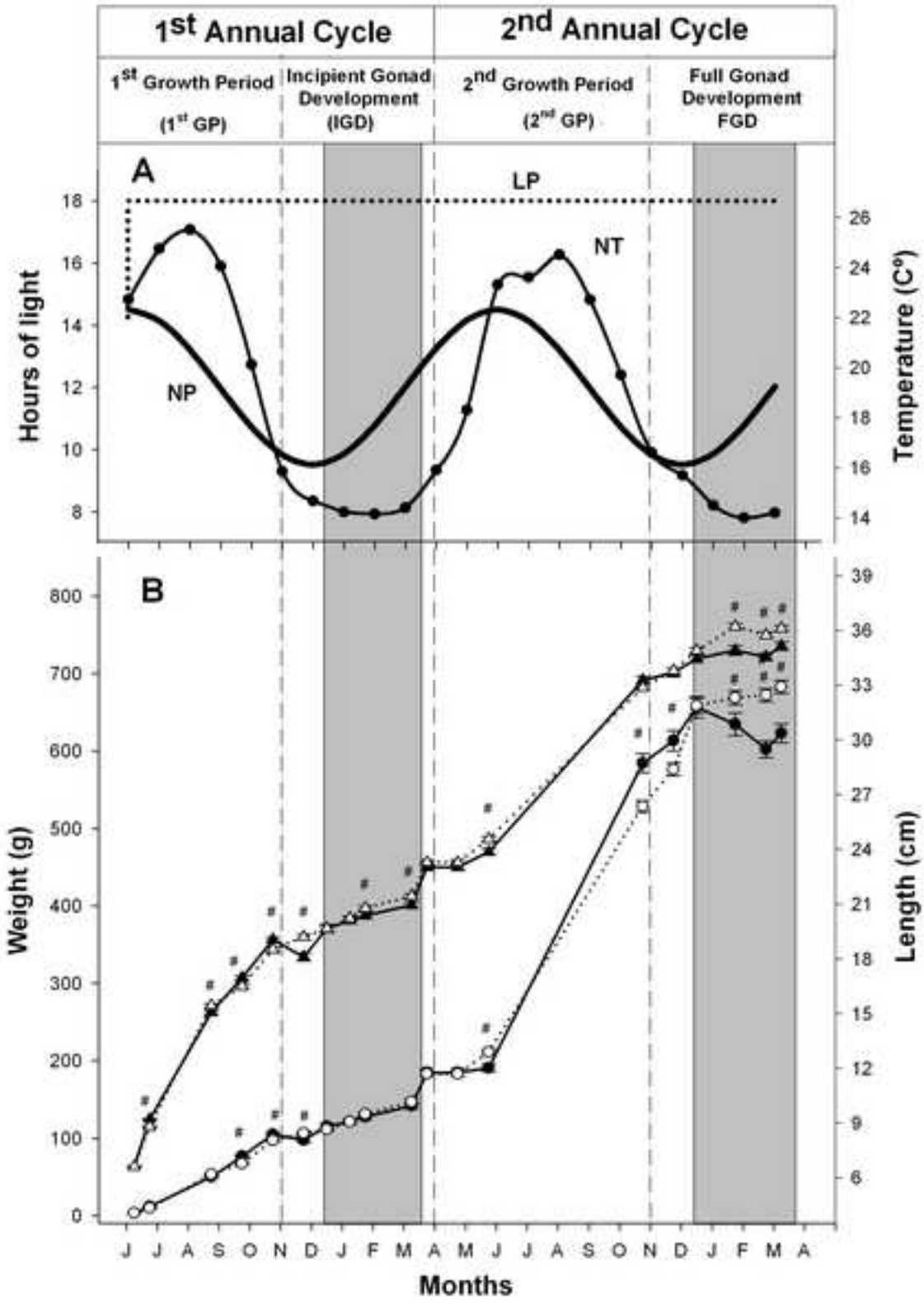

Fig. 1

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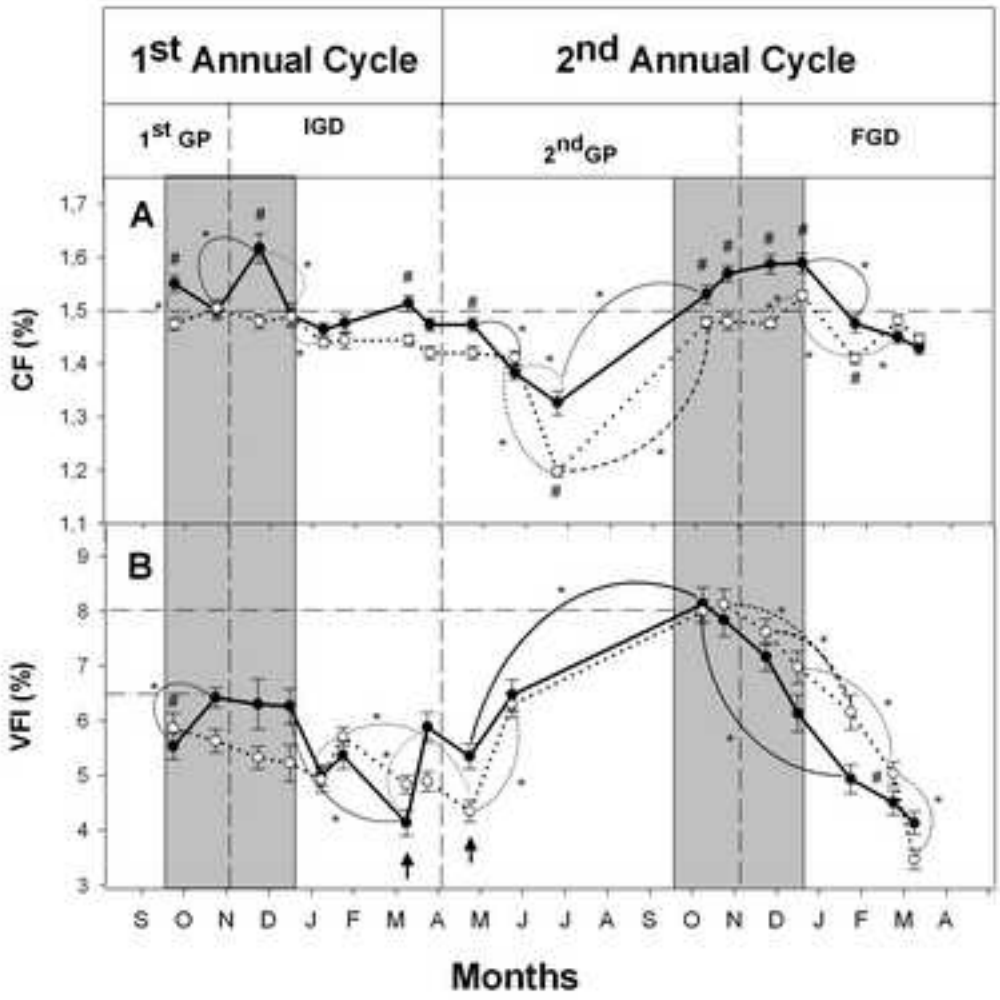

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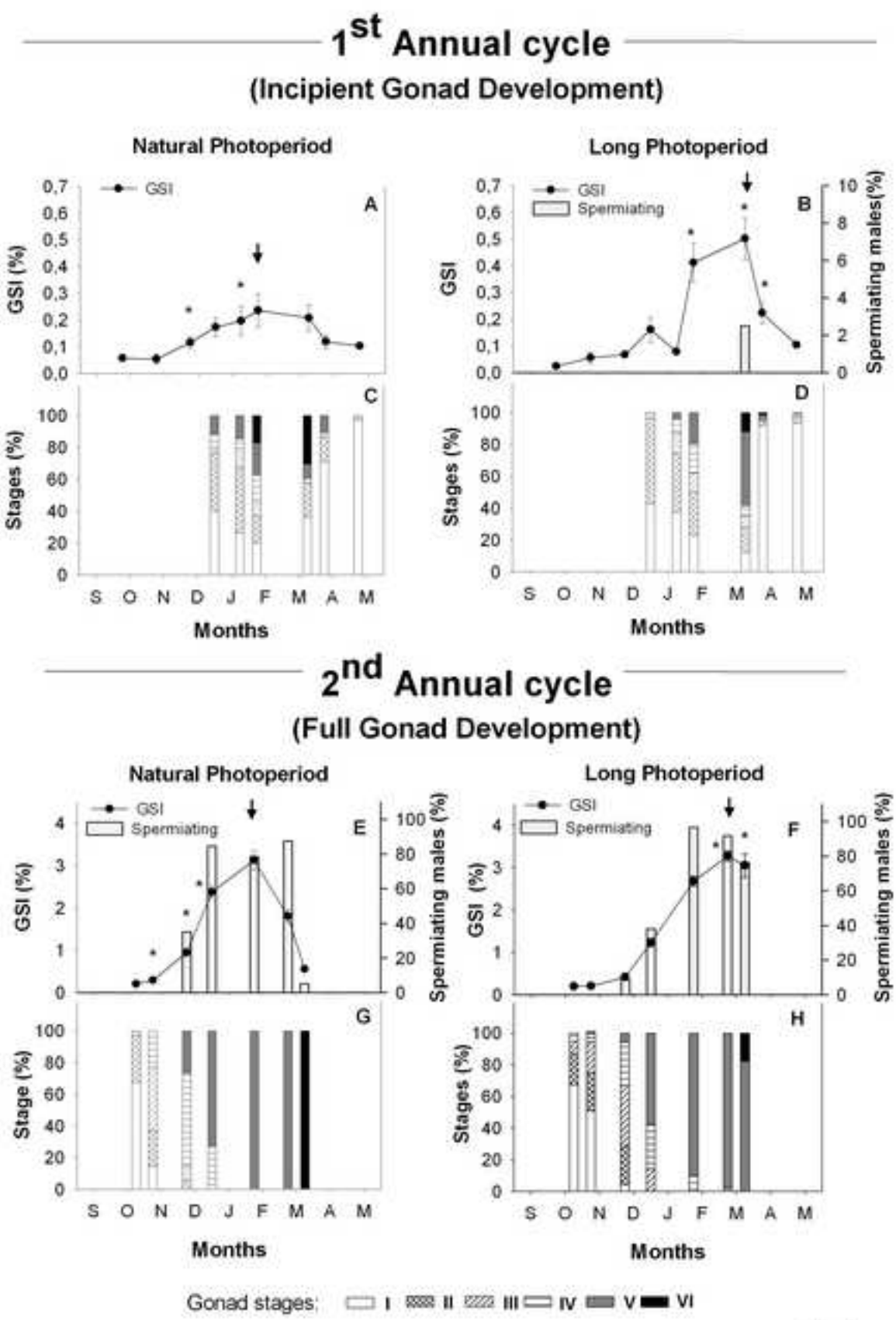

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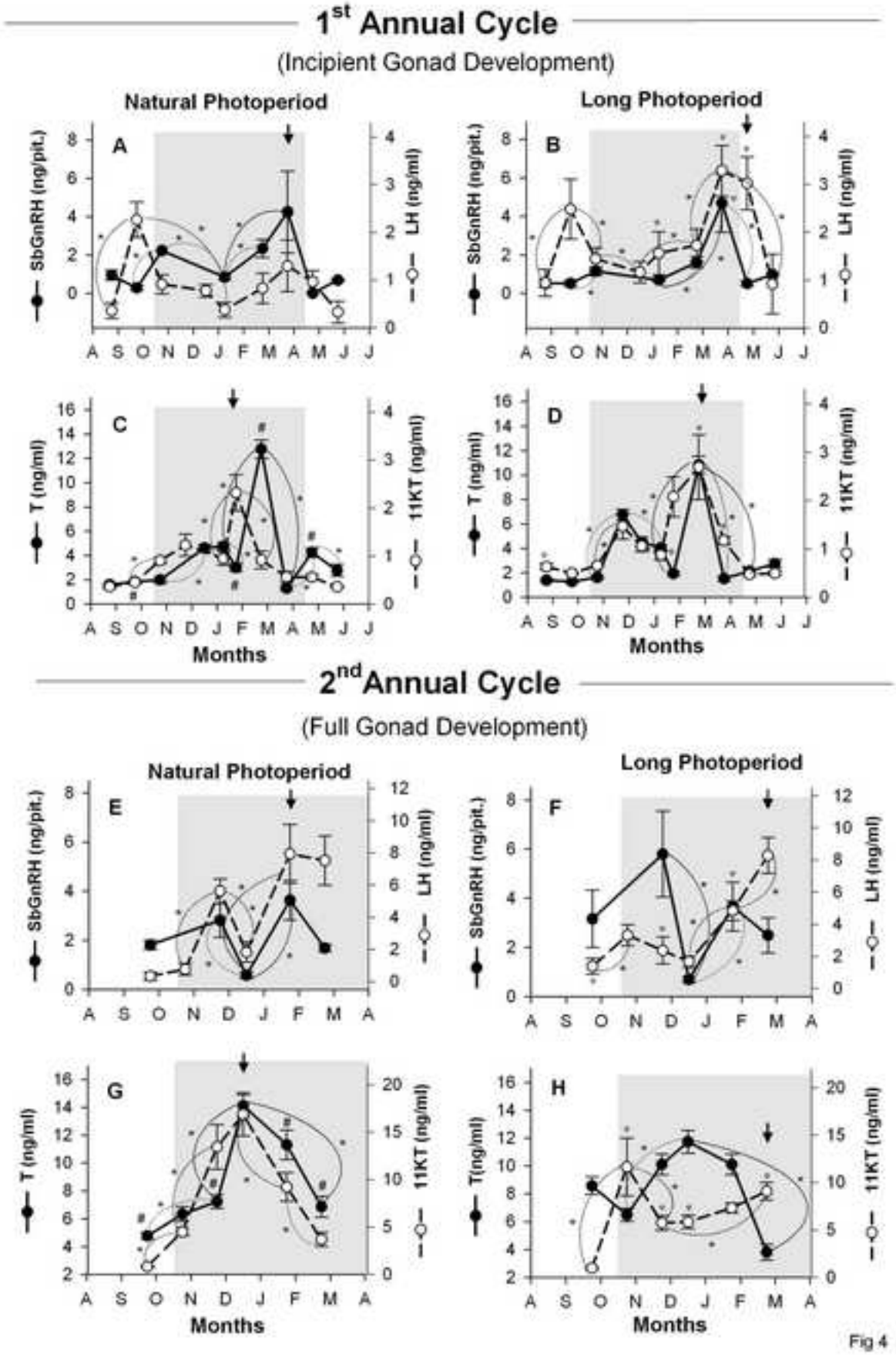

Fig 4