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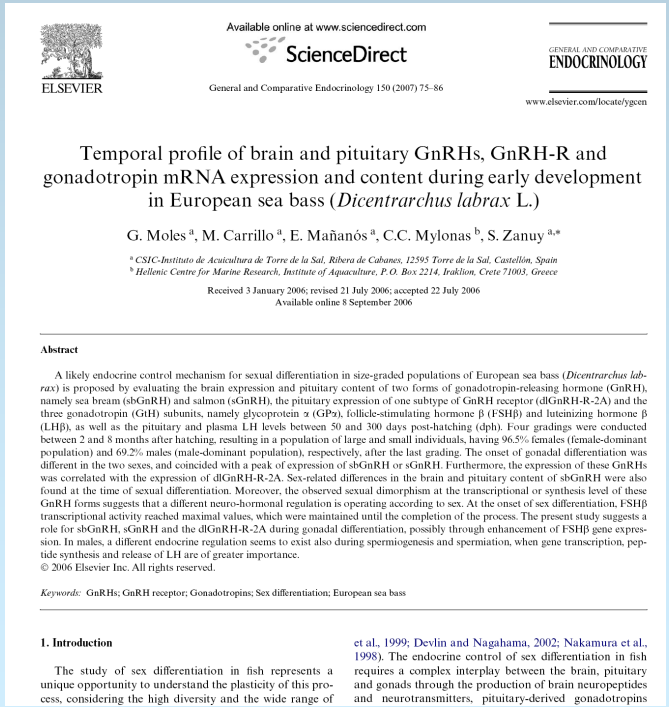

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Temporal profile of brain and pituitary GnRHs, GnRH-R and gonadotropin mRNA expression and content during early development in European sea bass (*Dicentrarchus labrax* L.)

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Abstract

A likely endocrine control mechanism for sexual differentiation in size-graded populations of European sea bass (*Dicentrarchus labrax*) is proposed by evaluating the brain expression and pituitary content of two forms of gonadotropin-releasing hormone (GnRH), namely sea bream (sbGnRH) and salmon (sGnRH), the pituitary expression of one subtype of GnRH receptor (dlGnRH-R-2A) and the three gonadotropin (GtH) subunits, namely glycoprotein α (GPα), follicle-stimulating hormone β (FSHβ) and luteinizing hormone β (LHβ), as well as the pituitary and plasma LH levels between 50 and 300 days post-hatching (dph). Four gradings were conducted between 2 and 8 months after hatching, resulting in a population of large and small individuals, having 96.5% females (female-dominant population) and 69.2% males (male-dominant population), respectively, after the last grading. The onset of gonadal differentiation was different in the two sexes, and coincided with a peak of expression of sbGnRH or sGnRH. Furthermore, the expression of these GnRHs was correlated with the expression of dlGnRH-R-2A. Sex-related differences in the brain and pituitary content of sbGnRH were also found at the time of sexual differentiation. Moreover, the observed sexual dimorphism at the transcriptional or synthesis level of these GnRH forms suggests that a different neuro-hormonal regulation is operating according to sex. At the onset of sex differentiation, FSHβ transcriptional activity reached maximal values, which were maintained until the completion of the process. The present study suggests a role for sbGnRH, sGnRH and the dlGnRH-R-2A during gonadal differentiation, possibly through enhancement of FSHβ gene expression. In males, a different endocrine regulation seems to exist also during spermiogenesis and spermiation, when gene transcription, peptide synthesis and release of LH are of greater importance.

Keywords: GnRHs; GnRH receptor; Gonadotropins; Sex differentiation; European sea bass

1. Introduction

The study of sex differentiation in fish represents a unique opportunity to understand the plasticity of this process, considering the high diversity and the wide range of

et al., 1999; Devlin and Nagahama, 2002; Nakamura et al., 1998). The endocrine control of sex differentiation in fish requires a complex interplay between the brain, pituitary and gonads through the production of brain neuropeptides and neurotransmitters, pituitary-derived gonadotropins

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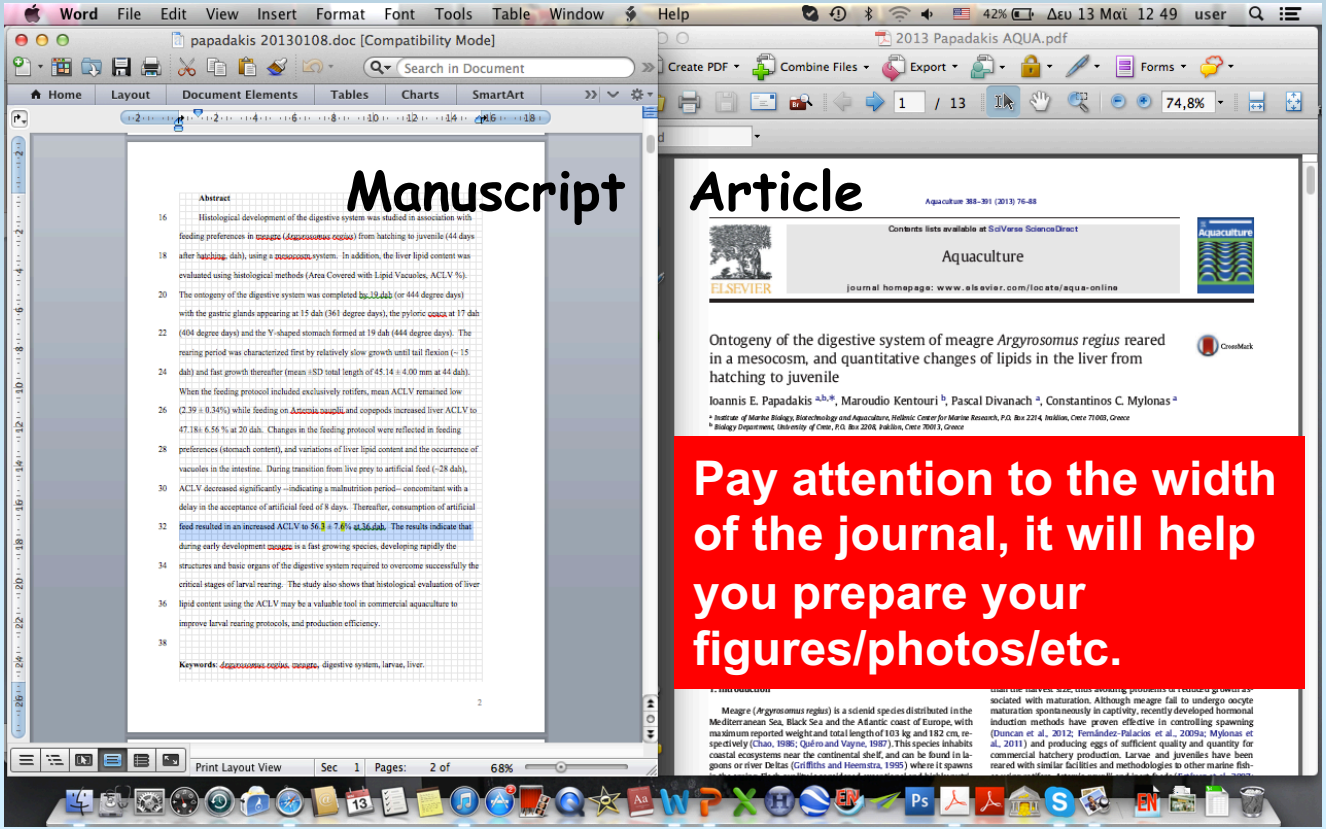

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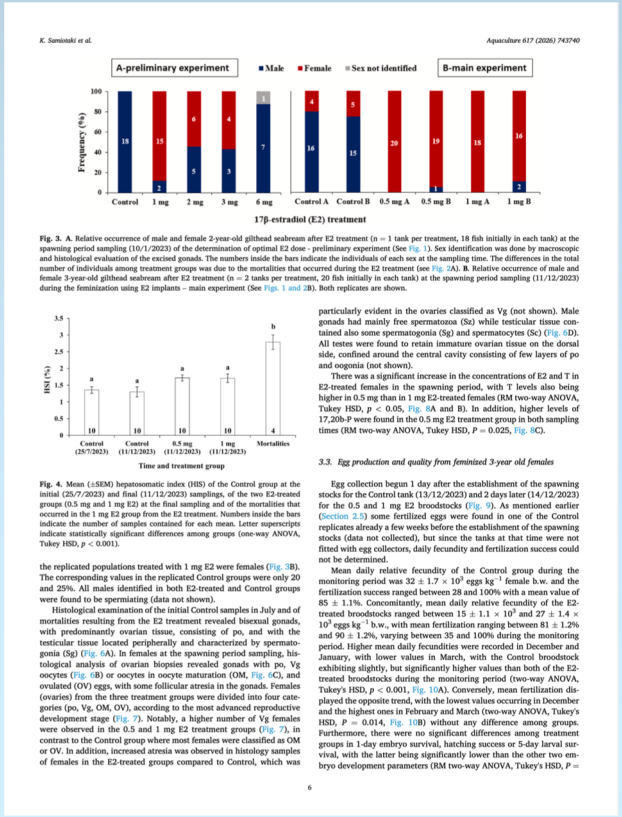

K. Samiouki et al. Aquaculture 617 (2026) 743740

Fig. 3. A. Relative occurrence of male and female 2-year-old gilthead seabream after E2 treatment ($n = 1$ tank per treatment, 18 fish initially in each tank) at the spawning period sampling (10/1/2023) of the determination of optimal E2 dose - preliminary experiment (See Fig. 1). Sex identification was done by macroscopic and histological evaluation of the excised gonads. The numbers inside the bars indicate the individuals of each sex at the sampling time. The differences in the total number of individuals among treatment groups was due to the mortalities that occurred during the E2 treatment (see Fig. 2A). **B.** Relative occurrence of male and female 3-year-old gilthead seabream after E2 treatment ($n = 2$ tanks per treatment, 20 fish initially in each tank) at the spawning period sampling (11/12/2023) during the feminization using E2 implants – main experiment (See Figs. 1 and 2B). Both replicates are shown.

Fig. 4. Mean (±SEM) hepatosomatic index (HIS) of the Control group at the initial (25/7/2023) and final (11/12/2023) samplings, of the two E2-treated groups (0.5 mg and 1 mg E2) at the final sampling and of the mortalities that occurred in the 1 mg E2 group from the E2 treatment. Numbers inside the bars indicate the number of samples contained for each mean. Letter superscripts indicate statistically significant differences among groups (one-way ANOVA, Tukey HSD, $p < 0.001$).

the replicated populations treated with 1 mg E2 were females (Fig. 3B). The corresponding values in the replicated Control groups were only 20 and 25%. All males identified in both E2-treated and Control groups were found to be spermiating (data not shown).

Histological examination of the initial Control samples in July and of mortalities resulting from the E2 treatment revealed bisexual gonads, with predominantly ovarian tissue, consisting of po, and with the testicular tissue located peripherally and characterized by spermatogonia (Sg) (Fig. 6A). In females at the spawning period sampling, histological analysis of ovarian biopsies revealed gonads with po, Vg oocytes (Fig. 5B) or oocytes in oocyte maturation (OM, Fig. 6C), and ovulated (OV) eggs, with some follicular atresia in the gonads. Females (ovaries) from the three treatment groups were divided into four categories (po, Vg, OM, OV), according to the most advanced reproductive development stage (Fig. 7). Notably, a higher number of Vg females were observed in the 0.5 and 1 mg E2 treatment groups (Fig. 7), in contrast to the Control group where most females were classified as OM or OV. In addition, increased atresia was observed in histology samples of females in the E2-treated groups compared to Control, which was particularly evident in the ovaries classified as Vg (not shown). Male gonads had mainly free spermatozoa (Sz) while testicular tissue contained also some spermatogonia (Sg) and spermatocytes (Sc) (Fig. 6D). All testes were found to retain immature ovarian tissue on the dorsal side, confined around the central cavity consisting of few layers of po and oogonia (not shown).

There was a significant increase in the concentrations of E2 and T in E2-treated females in the spawning period, with T levels also being higher in 0.5 mg than in 1 mg E2-treated females (RM two-way ANOVA, Tukey HSD, $p < 0.05$, Fig. 8A and B). In addition, higher levels of 17,20b-P were found in the 0.5 mg E2 treatment group in both sampling times (RM two-way ANOVA, Tukey HSD, $P = 0.025$, Fig. 8C).

3.3. Egg production and quality from feminised 3-year old females

Egg collection begun 1 day after the establishment of the spawning stocks for the Control tank (13/12/2023) and 2 days later (14/12/2023) for the 0.5 and 1 mg E2 broodstocks (Fig. 9). As mentioned earlier (Section 2.5) some fertilized eggs were found in one of the Control replicates already a few weeks before the establishment of the spawning stocks (data not collected), but since the tanks at that time were not fitted with egg collectors, daily fecundity and fertilization success could not be determined.

Mean daily relative fecundity of the Control group during the monitoring period was $32 \pm 1.7 \times 10^3$ eggs kg^{-1} female b.w. and the fertilization success ranged between 28 and 100% with a mean value of 85 ± 1.1%. Concomitantly, mean daily relative fecundity of the E2-treated broodstocks ranged between $15 \pm 1.1 \times 10^3$ and $27 \pm 1.4 \times 10^3$ eggs kg^{-1} b.w., with mean fertilization ranging between 81 ± 1.2% and 90 ± 1.2%, varying between 35 and 100% during the monitoring period. Higher mean daily fecundities were recorded in December and January, with lower values in March, with the Control broodstock exhibiting slightly, but significantly higher values than both of the E2-treated broodstocks during the monitoring period (two-way ANOVA, Tukey's HSD, $p < 0.001$, Fig. 10A). Conversely, mean fertilization displayed the opposite trend, with the lowest values occurring in December and the highest ones in February and March (two-way ANOVA, Tukey's HSD, $P = 0.014$, Fig. 10B) without any difference among groups. Furthermore, there were no significant differences among treatment groups in 1-day embryo survival, hatching success or 5-day larval survival, with the latter being significantly lower than the other two embryo development parameters (RM two-way ANOVA, Tukey's HSD, $P =$

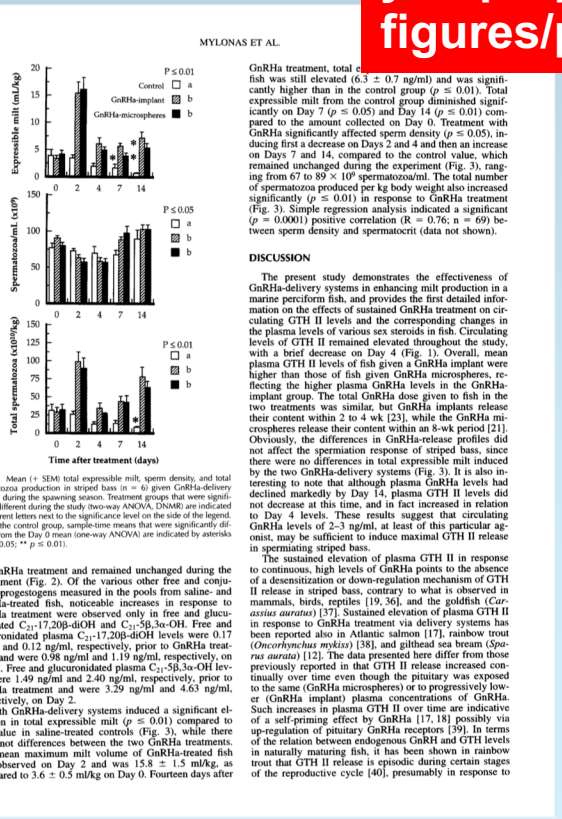

FIG. 3. Mean (+ SEM) total expressible milt, sperm density, and total spermatozoa production in striped bass (n = 6) given GnRHa-delivery systems during the spawning season. Treatment groups that were significantly different during the study (two-way ANOVA, DNMR) are indicated by different letters next to the significance level on the side of the legend. Within the control group, sample-time means that were significantly different from the Day 0 mean (one-way ANOVA) are indicated by asterisks (* $p \leq 0.05$; ** $p \leq 0.01$).

the GnRHa treatment and remained unchanged during the experiment (Fig. 2). Of the various other free and conjugated progestogens measured in the pools from saline- and GnRHa-treated fish, noticeable increases in response to GnRHa treatment were observed only in free and glucuronidated C_{21}-17,20β-diOH and C_{21}-5β,3α-OH. Free and glucuronidated plasma C_{21}-17,20β-diOH levels were 0.17 ng/ml and 0.12 ng/ml, respectively, prior to GnRHa treatment and were 0.98 ng/ml and 1.19 ng/ml, respectively, on Day 2. Free and glucuronidated plasma C_{21}-5β,3α-OH levels were 1.49 ng/ml and 2.40 ng/ml, respectively, prior to GnRHa treatment and were 3.29 ng/ml and 4.63 ng/ml, respectively, on Day 2.

Both GnRHa-delivery systems induced a significant elevation in total expressible milt ($p \leq 0.01$) compared to the value in saline-treated controls (Fig. 3), while there were not differences between the two GnRHa treatments. The mean maximum milt volume of GnRHa-treated fish was observed on Day 2 and was 15.8 ± 1.5 ml/kg, as compared to 3.6 ± 0.5 ml/kg on Day 0. Fourteen days after GnRHa treatment, total e… fish was still elevated (6.3 ± 0.7 ng/ml) and was significantly higher than in the control group ($p \leq 0.01$). Total expressible milt from the control group diminished significantly on Day 7 ($p \leq 0.05$) and Day 14 ($p \leq 0.01$) compared to the amount collected on Day 0. Treatment with GnRHa significantly affected sperm density ($p \leq 0.05$), inducing first a decrease on Days 2 and 4 and then an increase on Days 7 and 14, compared to the control value, which remained unchanged during the experiment (Fig. 3), ranging from 67 to 89 × 10^9 spermatozoa/ml. The total number of spermatozoa produced per kg body weight also increased significantly ($p \leq 0.01$) in response to GnRHa treatment (Fig. 3). Simple regression analysis indicated a significant ($p = 0.0001$) positive correlation ($R = 0.76$; $n = 69$) between sperm density and spermatocrit (data not shown).

DISCUSSION

The present study demonstrates the effectiveness of GnRHa-delivery systems in enhancing milt production in a marine perciform fish, and provides the first detailed information on the effects of sustained GnRHa treatment on circulating GTH II levels and the corresponding changes in the plasma levels of various sex steroids in fish. Circulating levels of GTH II remained elevated throughout the study, with a brief decrease on Day 4 (Fig. 1). Overall, mean plasma GTH II levels of fish given a GnRHa implant were higher than those of fish given GnRHa microspheres, reflecting the higher plasma GnRHa levels in the GnRHa-implant group. The total GnRHa dose given to fish in the two treatments was similar, but GnRHa implants release their content within 2 to 4 wk [23], while the GnRHa microspheres release their content within an 8-wk period [21]. Obviously, the differences in GnRHa-release profiles did not affect the spermiation response of striped bass, since there were no differences in total expressible milt induced by the two GnRHa-delivery systems (Fig. 3). It is also interesting to note that although plasma GnRHa levels had declined markedly by Day 14, plasma GTH II levels did not decrease at this time, and in fact increased in relation to Day 4 levels. These results suggest that circulating GnRHa levels of 2–3 ng/ml, at least of this particular agonist, may be sufficient to induce maximal GTH II release in spermiating striped bass.

The sustained elevation of plasma GTH II in response to continuous, high levels of GnRHa points to the absence of a desensitization or down-regulation mechanism of GTH II release in striped bass, contrary to what is observed in mammals, birds, reptiles [19, 36], and the goldfish (*Carassius auratus*) [37]. Sustained elevation of plasma GTH II in response to GnRHa treatment via delivery systems has been reported also in Atlantic salmon [17], rainbow trout (*Oncorhynchus mykiss*) [38], and gilthead sea bream (*Sparus aurata*) [12]. The data presented here differ from those previously reported in that GTH II release increased continually over time even though the pituitary was exposed to the same (GnRHa microspheres) or to progressively lower (GnRHa implant) plasma concentrations of GnRHa. Such increases in plasma GTH II over time are indicative of a self-priming effect by GnRHa [17, 18] possibly via up-regulation of pituitary GnRHa receptors [39]. In terms of the relation between endogenous GnRH and GTH levels in naturally maturing fish, it has been shown in rainbow trout that GTH II release is episodic during certain stages of the reproductive cycle [40], presumably in response to

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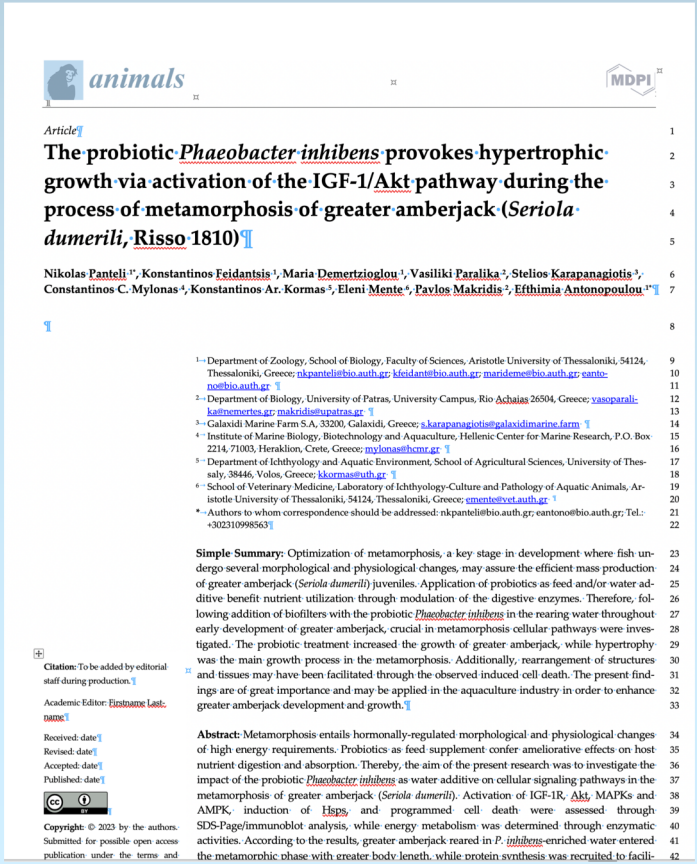

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Article

The probiotic *Phaeobacter inhibens* provokes hypertrophic growth via activation of the IGF-1/Akt pathway during the process of metamorphosis of greater amberjack (*Seriola dumerili*, Risso 1810)

Nikolas Panteli [1,*], Konstantinos Feidantsis [1], Maria Demertzioglou [1], Vasiliki Paralika [2], Stelios Karapanagiotis [3], Constantinos C. Mylonas [4], Konstantinos Ar. Kormas [5], Eleni Mente [6], Pavlos Makridis [2], Efthimia Antonopoulou [1,*]

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Simple Summary: Optimization of metamorphosis, a key stage in development where fish undergo several morphological and physiological changes, may assure the efficient mass production of greater amberjack (*Seriola dumerili*) juveniles. Application of probiotics as feed and/or water additive benefit nutrient utilization through modulation of the digestive enzymes. Therefore, following addition of biofilters with the probiotic *Phaeobacter inhibens* in the rearing water throughout early development of greater amberjack, crucial in metamorphosis cellular pathways were investigated. The probiotic treatment increased the growth of greater amberjack, while hypertrophy was the main growth process in the metamorphosis. Additionally, rearrangement of structures and tissues may have been facilitated through the observed induced cell death. The present findings are of great importance and may be applied in the aquaculture industry in order to enhance greater amberjack development and growth.

Abstract: Metamorphosis entails hormonally-regulated morphological and physiological changes of high energy requirements. Probiotics as feed supplement confer ameliorative effects on host nutrient digestion and absorption. Thereby, the aim of the present research was to investigate the impact of the probiotic *Phaeobacter inhibens* as water additive on cellular signaling pathways in the metamorphosis of greater amberjack (*Seriola dumerili*). Activation of IGF-1R, Akt, MAPKs and AMPK, induction of Hsps, and programmed cell death were assessed through SDS-Page/immunoblot analysis, while energy metabolism was determined through enzymatic activities. According to the results, greater amberjack reared in *P. inhibens*-enriched water entered the metamorphic phase with greater body length, while protein synthesis was recruited to facili-

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      - 2.2. Experimental design
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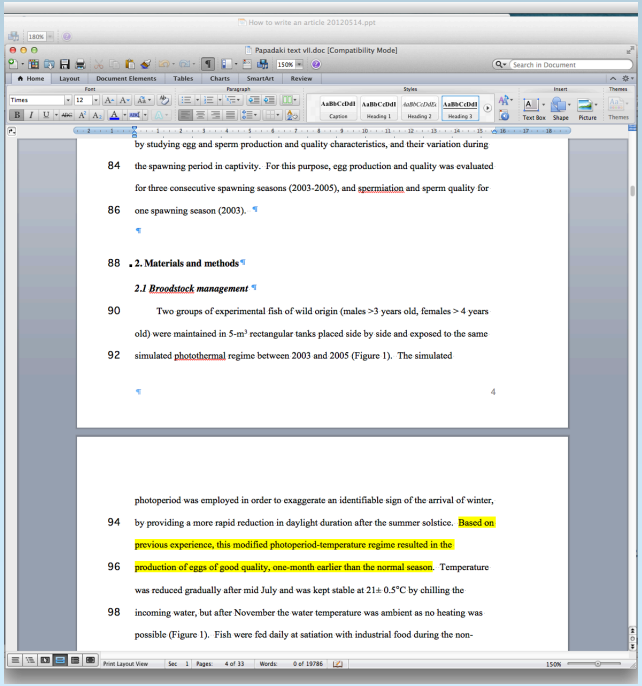

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Egg and sperm production and quality of sharpsnout sea bream (*Diplodus puntazzo*) in captivity

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Abstract

Egg production from captive-reared sharpsnout sea bream (*Diplodus puntazzo*) was monitored during the spawning seasons of 2003 to 2005, and sperm production during 2003. Spawning took place between September and December under water temperatures of 21.0–18.5 °C. Daily fecundity varied without a consistent trend and peak egg production occurred in October. Mean (±S.E.M.) number of spawning days per month was 20±3 in 2003, and 14±3 in 2004 and 2005. A significant (ANOVA, DNMR, $P<0.01$) drop was observed in mean total annual relative fecundity from 4.9±0.08 million eggs kg^{-1} female body weight in 2003, to 2.4±0.07 million eggs kg^{-1} in 2004 and 2005. Mean monthly fertilization success also dropped significantly from 81±1% in 2003 to 76±2% in 2004 and 78±2% in 2005. Annual hatching success did not vary significantly and was around 86±2%. Finally, 5 day larval survival decreased significantly from 85±2% in 2003 to 44±3% in 2004. The first spermiating males were found in August and sperm production continued until December. Mean total volume of expressible sperm was maximal in November (3±1 ml kg^{-1}) and the gonadosomatic index (GSI) ranged between 0.6 and 2.0% in spermiating fish, reaching its peak in September–November. Sperm motility (%) remained unchanged during the season, whereas motility duration (6.2±1.7 min) and sperm density ($2.7\pm0.2\times10^{10}$ spermatozoa ml^{-1}) peaked in October. Mean sperm survival ranged between 9 and 13 days during most of the spawning season, and decreased significantly to 5 days in December. The study suggests that egg production is stable for the first 3 months of the spawning season, with relatively unchanged egg quality. On the contrary, sperm production and quality peaks in the middle of the reproductive season in October.

Keywords: *Diplodus*; Sharpsnout sea bream; Egg quality; Spermiation; Reproduction

1. Introduction

The sharpsnout sea bream (*Diplodus puntazzo*, Cetti 1777) is a demersal marine fish widely distributed in the Black and Mediterranean Seas, and the Atlantic Ocean. It has been reared in aquaculture for more than 10 years (Abellan and Basurco, 1999; Divanach and Kentouri, 2000) and has good consumer acceptance (Hernández et al., 2002). Studies on larval rearing, development and morphogenesis (Bodington, 2000; Palma and Andrade, 2002; Papandroulakis et al., 2004); growth and pathology (Favarolo and Mazzola, 2000; Pastor et al., 2000; Hernández et al., 2003; Tramati et al., 2005); and egg quality criteria (Lahnsteiner and Patarnello, 2004, 2005) have provided important information on the performance of this species in captivity. Nevertheless, there still exist some problems concerning its nutritional demands and optimal environmental rearing conditions (Abellan and Basurco, 1999). In addition, there is still limited information on its reproductive performance, egg and sperm production during the reproductive season, and gamete quality in captivity (Faranda et al., 1985; Georgiou and Stephanou, 1995).

The quality of eggs and larvae produced in hatcheries are considered an important limiting factor in fry production (Kjørsvik et al., 1990) and, consequently, in the development of the aquaculture industry (Bromage, 1995). Sperm quality is equally important, as it can affect fertilization success and production of viable eggs (Bromage, 1995). Egg quality has

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Structure – Title page

- **Title page (1 page)**
- Abstract
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The first egg collection and larval rearing of Atlantic Bluefin tuna (*Thunnus thynnus* L.) in captivity, after hormonal spawning induction

Gregorio De Metrio[1,*], Christopher R. Bridges[2], Constantinos C. Mylonas[3], Massimo Caggiano[4], Michele Deflorio[1], Nikoletta Santamaria[1], R?????? Zupa[1], Chrisovalentino Pousis[1], Robert Vassallo-Agius[5], Hillel Gordin[6], Aldo Corriero[1]

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Abstract

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- Presentation of the problem
- Current knowledge or situation
- Relevant knowledge from other species
- Objectives of the study

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Objectives
- Understand the study
- Evaluate the results
- Repeat if required

Content (chronological order)
- Experimental animals or site
- Experimental design
- Data collection
- Analytical methods (hormone assays, molecular methods, measurements, etc.
- Statistical analysis

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- **Materials and methods (n pages)**
  - **Ethics declaration**
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- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- **Materials and metho**
  - **Ethics declaration**
- Results (n pages)
- Discussion
- Acknowledgements
- References
- Tables
- Figures legends
- Figures

2. Materials and methods

2.1. Ethical issues

Experiments were conducted at the AQUALABS facilities of the Hellenic Center for Marine Research, Crete, Greece, a registered facility for the maintenance of farmed fish (HCMR, Registration No EL91-BIObr-03 and EL91-BIOexp-04 for animal experimentation and fish production), under the approved protocol No 255356 (Regional Veterinary Services). All procedures involving animals were conducted in accordance to the "Guidelines for the treatment of animals in behavioral research and teaching" (Anonymous, 1998), the Ethical justification for the use and treatment of fishes in research: an update (Metcalfe & Craig, 2011) and the "Directive 2010/63/EU of the European parliament and the council of September 22, 2010 on the protection of animals used for scientific purposes" (EU, 2010).

Structure – Results

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n pages)
- **Results (n pages)**
- Discussion
- Acknowledgements
- References
- Tables
- Figures legends
- Figures

- Presentation of results in a chronological order
- Reference to Tables and Figures
- Absolutely NO discussion (no opinions or conclusions) unless some of the results have lead to another experiment/analysis not planned originally

Structure – Discussion

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n pages)
- Results (n pages)
- **Discussion (3-n pages)**
- Acknowledgements
- References
- Tables
- Figures legends
- Figures

- Interpretation of results
- Comparison with other studies
- NO reference to results, tables and figures, unless new (model) or for comparison purposes
- Significance of the study, contribution to the field
- Suggestions for further work
- Conclusions

Structure – Acknowledgments

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n pages)
- Results (n pages)
- Discussion (3-n pages)
- **Acknowledgements (1 paragraph)**
- References
- Tables
- Figures legends
- Figures

- Funding agencies and organizations!!!!!
- Technicians and collaborators who worked on the study

- Scientist giving advice on study or reviewed the manuscript
- Reviewers that improved the manuscript
- NOT your parents or girlfriend/boyfriend

Structure – References (objectives)

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n pages)
- Results (n pages)
- Discussion (3-n pages)
- Acknowledgements (1 paragraph)
- **References (1-n pages, ~50 citations)**
- Tables
- Figures legends
- Figures

- Provide proof of knowledge of the field
- Support from and relate to similar or relevant studies
- Suggest studies to interested readers

Next slide for more details

Structure – References (selection)

- Only cite articles that you have read!!!! Cannot be sure of others' interpretations. Otherwise, cite the paper as (Stevens et al., 1934, cited in Johnston et al., 2013).
- Use review articles and book chapters to avoid too many citations. These articles should be recent (~10 years) or used for historical reasons. Otherwise they will be outdated.
- Use original articles for the absolutely relevant information (acknowledgement, aware of the literature, point to similar/relevant research).
- Do not cite conferences if older that 2-3 years old.
- Avoid articles in national journals and/or not written in English (except for historical reasons).

Structure – References (format)

- Journals, Books, Chapters, Conferences, Thesis, Reports
- Every journal has its own style ! (alphabetical, numerical)
- Check Instructions to authors

References

Abellan, E. (2001). Culture of common dentex (*Dentex dentex* L.): Present knowledge, problems and perspectives. *Cahiers Options Méditerranéenes, 47*, 157–168.

Anonymous. (1998). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour, 55*, 251–257.

Aristizabal, E., Suárez, J., Vega, A., & Bargas, R. (2009). Egg and larval quality assessment in the Argentinean red porgy (*Pagrus pagrus*). *Aquaculture, 287*, 329–334.

Barbaro, A., Francescon, A., Bozzato, G., Merlin, A., Belvedere, P., & Colombo, L. (1997). Induction of spawning in gilthead seabream, *Sparus aurata* L., by a long-acting GnRH agonist and its effects on egg quality and daily timing of spawning. *Aquaculture, 154* (3–4), 349–359. https://doi.org/10.1016/S0044-8486(97)00067-7

Bromage, N., Porter, M., & Randall, C. (2001). The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture, 197*(1), 63–98.

Structure – References (examples)

Journals

Corriero, A., Wylie, M.J., Nyuji, M., Zupa, R., Mylonas, C.C., 2021. Reproduction of greater amberjack (*Seriola dumerili*) and other members of the family Carangidae. *Reviews in Aquaculture*. 13, 1781-1815.

Fakriadis, I., Meiri-Ashkenazi, I., Bracha, C., Rosenfeld, H., Corriero, A., Zupa, R., Pousis, C., Papadaki, M., Mylonas, C.C., 2024. Gonadotropin expression, pituitary and plasma levels in the reproductive cycle of wild and captive-reared grea

Chapters in Books

Mylonas, C.C., Zohar, Y., Pankhur
95-131 In: *Sparidae: Biolog*
and Mylonas, C.C. (Eds.). E

Zohar, Y., Mylonas, C.C., 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. p. 99-136 In: *Reproductive Biotechnology in Finfish Aquaculture*. Donaldson, E.M., and Lee, C.S. (Eds.). Elsevier, Amsterdam.

Books (whole)

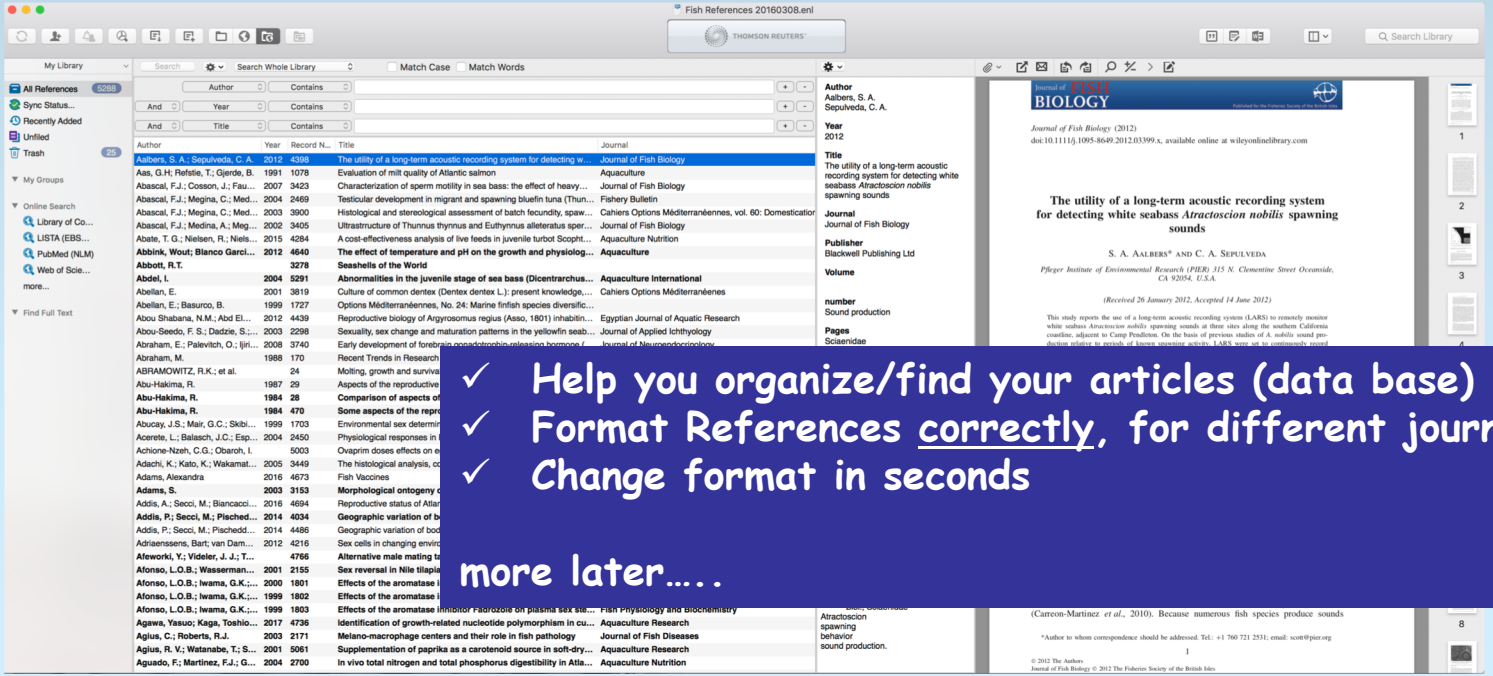
Pavlidis, M., Mylonas, C.C., 2011. *Sparidae: biology and aquaculture of gilthead sea bream and related species*. Blackwell Scientific Publishers, London, 390 pp.

Use bibliographic software to prepare the Reference list, to save time and avoid errors!!!!!

Structure – References 4

Bibliographic software

Endnote, Mendeley, Zotero, etc.



- ✓ Help you organize/find your articles (data base)
- ✓ Format References correctly, for different journals
- ✓ Change format in seconds

more later…..

Structure – Tables

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n pages)
- Results (n pages)
- Discussion (3-n pages)
- Acknowledgements (1 paragraph)
- References (1-n pages, ~50 citations)
- **Tables (0-7?)**
- Figures legends
- Figures

- Each Table in a separate page (txt), but since it is text, some journals allow you to have in the same document
- No vertical borders
- Legend ALWAYS above
- Self-explanatory (no reference to main text)

Table 1. Mean (± SEM) food conversion ratio (FCR = F / [B_f – B_i], where F = consumed food, B_f = final biomass, B_i = initial biomass) of shi drum reared at different salinities (4, 10 and 40 psu) in duplicated tanks (*i.e.*, n = 2). Significant differences (P < 0.05) at different sampling points and over the whole course of the study between the 4 psu, and the 10 or 40 psu group are indicated by different letter superscripts next to the mean values. Within each salinity treatment, there were no differences in FCR between different sampling times (P = 0.19)

	FCR		
Salinity	**4 psu**	**10 psu**	**40 psu**
Time period (d)			
0-14	3.39 ± 1.89^{a}	1.78 ± 0.05^{a,b}	1.29 ± 0.14^{b}
15-28	3.32 ± 0.22^{a}	1.13 ± 0.08^{b}	1.06 ±0.05^{b}
29-42	1.36 ± 0.14	1.21 ± 0.14	1.25 ± 0.02
43-56	4.71 ± 2.20^{a}	1.68 ± 0.26^{b}	1.42 ± 0.02^{b}
57-70	1.28 ± 0.04	1.31 ± 0.21	1.12 ± 0.21
70-84	1.54 ± 0.26	1.54 ± 0.40	1.58 ± 0.68

Structure – Tables

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n page
- Results (n pages)
- Discussion (3-n pages)
- Acknowledgements (1 paragrap
- References (1-n pages, ~50 ci
- **Tables (0-7?)**
- Figures legends
- Figures

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	FCR		
Salinity	**4 psu**	**10 psu**	**40 psu**
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15-28	3.32 ± 0.22^{a}	1.13 ± 0.08^{b}	1.06 ± 0.05^{b}
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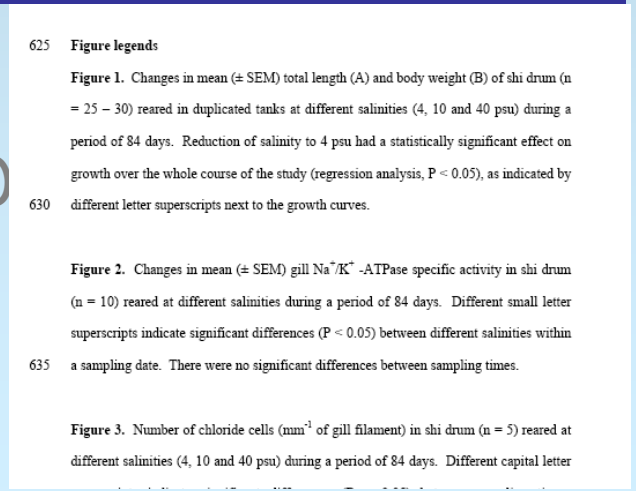
57-70	1.28 ± 0.04	1.31 ± 0.21	1.12 ± 0.21
70-84	1.54 ± 0.26	1.54 ± 0.40	1.58 ± 0.68
Whole duration (d)			
0-84	2.60 ± 0.53^{a}	1.44 ± 0.10^{b}	1.28 ± 0.10^{b}

Structure – Figures/graphs

- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (n pa
- Results (n pages)
- Discussion (3-n pages)
- Acknowledgements (1 paragraph)
- References (1-n pages, ~50 citations)
- Tables (0-7?)
- **Figure legends (0-7)**
- Figures

- Separate Word file or at the end of the main text (as for Tables)
- NO next to the Figure file (which is in TIFF, JPEG or other photo format
- Figure legends ALWAYS below the figures
- Self-explanatory (no reference to main text)



625 **Figure legends**

Figure 1. Changes in mean (± SEM) total length (A) and body weight (B) of shi drum (n = 25 – 30) reared in duplicated tanks at different salinities (4, 10 and 40 psu) during a period of 84 days. Reduction of salinity to 4 psu had a statistically significant effect on growth over the whole course of the study (regression analysis, $P < 0.05$), as indicated by
630 different letter superscripts next to the growth curves.

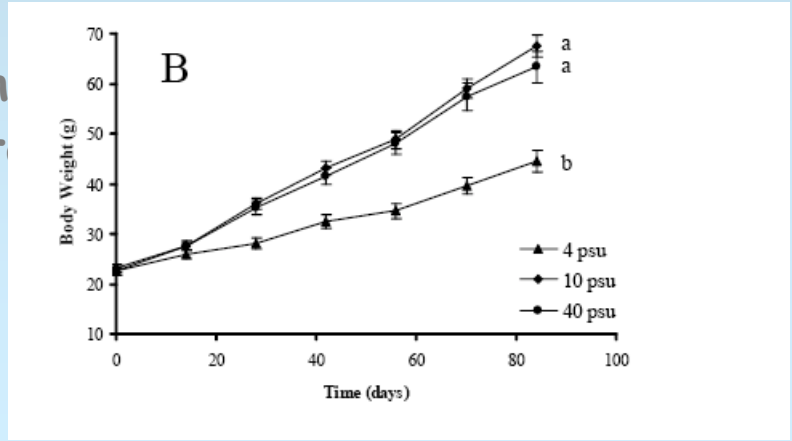
Figure 2. Changes in mean (± SEM) gill Na^+/K^+ -ATPase specific activity in shi drum (n = 10) reared at different salinities during a period of 84 days. Different small letter superscripts indicate significant differences ($P < 0.05$) between different salinities within
635 a sampling date. There were no significant differences between sampling times.

Figure 3. Number of chloride cells (mm^{-1} of gill filament) in shi drum (n = 5) reared at different salinities (4, 10 and 40 psu) during a period of 84 days. Different capital letter

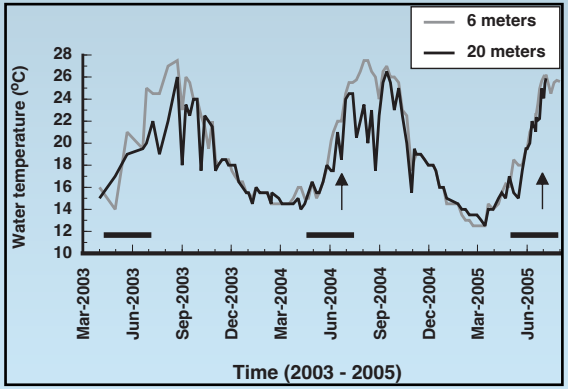
Structure – Figures/graphs

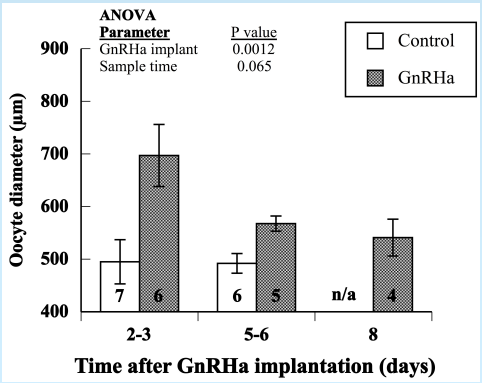
- Title page (1 page)
- Abstract (1 page)
- Introduction (2-3 pages)
- Materials and methods (1-n p
- Results (n pages)
- Discussion (3-n pages)
- Acknowledgements (1 paragraph
- References (1-n pages, ~50 cit
- Tables (0-7?)
- Figure legends (0-7)
- **Figures (0-7)**

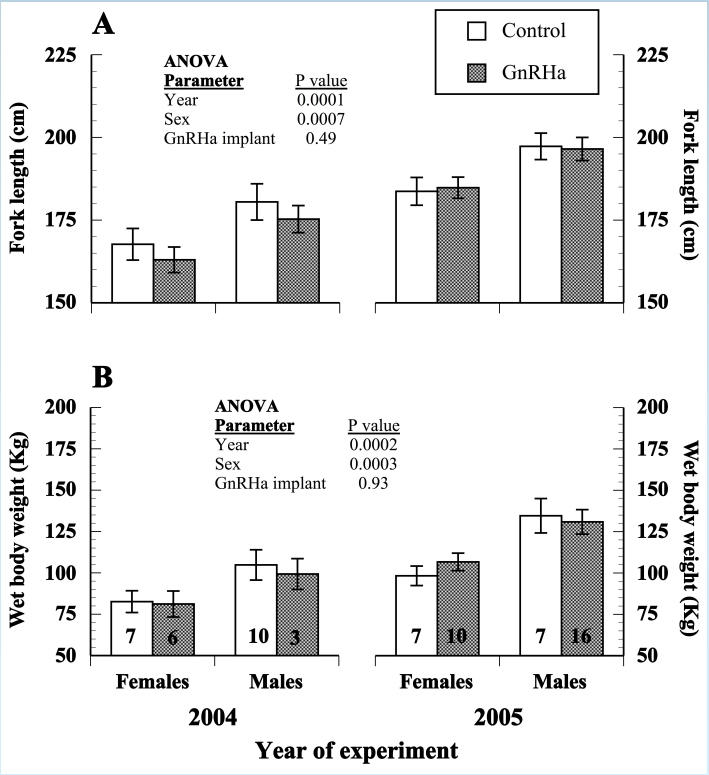
- Each Figure in a separate page, preferably in TIFF or JPEG format
- NO titles (legends)
- Font size at least same as text (12 or 11 point, or at least 10 point. May be reduced by the journal



Structure – Figures/graphs







End of Section 1 – short break??

Spawning of Pacific bluefin tuna

How to write a scientific article

Outline of todays presentation

- Preparation of a manuscript
  - Format
  - Parts
  - Contents
  - References
  - Tables and Graphs
- Language use and common errors
- References and Bibliographic software
- Table of Contents
- Review process

Language – passive voice

- Write in **passive voice** and not in the first person (more formal).

  For example:

  - (Yes) Blood samples were collected and analyzed using ELISA.
  - (No) We collected blood samples and analyzed them using ELISA.

However, some journals suggest to use first person to make the text shorter and more “active”.

Language – past tense

- Write in **past tense** for the results of an experiment or of another study. Use present tense for well-accepted theories or facts.

  For example:

  - (Yes) The study indicated that fish reproduced well in captivity.
  - (No) The study indicates that fish reproduce well in captivity.
  - (Yes) It is known that most temperate zone fishes reproduce once a year in nature

Language – comma placement

The comma is placed next to the last letter, not the following letter

For example:

- (Yes) It has been found that fish have many steroids in their plasma during reproduction, but they differ between males and females
- (No) It has been found that fish have many steroids in their plasma during reproduction ,but they differ between males and females

A single sentence cannot constitute a paragraph. Either expand further or consolidate with another paragraph.

Use units with the IU standards (mg l^{-1} not ml/l), but follow format of journal.

Language – space between numbers and units

Unless specifically requested in the **Instructions to Authors**, always **leave a space between the number and unit**

for example, 10 Kg, 4 min, 9 h,

except in the case of temperature degrees and percentage

for example, 34°C and 20%

Language – numerals

Spell out numbers from one to nine, if they are not followed by units, and **use Arabic numbers** for those above 10.

For example:

- (Yes) In the present study 9 tanks (units) were used in each of the five therapies used for the study.
- (Yes) The experiments were repeated at 12 different times during the period of 3 years (units).

Language – list of terms

In a list of terms **with finite terms**, the last one is connected with "and" or ", and" (both OK, be consistent),

For example:

- (Yes) It has been found that fish have three steroids in their plasma during reproduction: testosterone, estradiol (,) and dihydroxyprogesterone.

In a list of terms **with infinite terms**, add *et cetera* (*etc.*) after the last term (means "and other things")

For example:

- (Yes) It has been found that fish have many steroids in their plasma during reproduction: testosterone, estradiol, dihydroxyprogesterone, etc.

Language – list of terms

In a **list of terms**, all must be in the same form (verbs, nouns, etc.).

For example:

- (Yes) The objective was to study behaviour, describe form and develop new methods. (all verbs)
- (Yes) The objective was the study of behavior, the description of form and the development of new methods. (all nouns)

- (No) The objective was to study behavior, describe form and the development of new methods.

Language – Latin abbreviations

Words that have a **Latin origin** are usually written in ***italics***.

For example:

In vitro, in silico, a posteriori, ad hoc, ad libitum, i.e, e.g., etc.

Some journals do not require italics for those that are very common (e.g. or in vitro).

To be on the safe side, **always write them in italics!**

Language – Latin abbreviations

The **abbreviations** *e.g.* and *i.e.* don't mean the same thing!

- *e.g.* (exempli gratia "for the sake of example", «για παράδειγμα»)
- *i.e.* (id est "that is", «δηλαδή»)

They are usually in ***italics*** and should not be followed by commas.

For example:

- Giving a dose of sex steroids (*e.g.* Testosterone) is considered a reasonable method for increasing maleness (John et al. 2020).
- Giving a dose of the major androgen steroid (*i.e.* 11-Ketotestosterone) is considered a reasonable method for increasing maleness (John et al. 2020).

Language – scientific & common names

Scientific names always in *italics*, because they are considered to be in Latin. *Carolus Linnaeus* (as opposed to Carl Linné)

Canis familiaris, Mus musculus, Sus scrofa, Felis catus

The **common names** of animals are always written in small letters (except birds), unless they have main names such as Atlantic, Japanese, Nile, Saint Peters, Williams, etc.

For example:

- Studies have shown that the **gilthead seabream** grows slower than the **European sea bass** in the Mediterranean. On the other hand, the **Atlantic bluefin tuna** swims faster than the **yellowfin tuna**

Language – scientific & common names

Always **mention Scientific names** at the first time cited in the **Title**, **Abstract** and **main text** of the manuscript. Then, use common names throughout.

-In some journals, and for some situations, scientific names may be used throughout the manuscript

-Scientific names may be in parentheses or not, after a comma or not, **BUT always use the same format throughout** the manuscript

For example:

- The gilthead seabream (*Sparus aurata*) is a much smaller fish than the greater amberjack, *Seriola dumerili,* which in turn is a much smaller fish than the Atlantic bluefin tuna *Thunnus thymus.*

Language – define abbreviations first

Always **define abbreviations** at the first time cited in the **Abstract** and **main text** of the manuscript. First the full name and then the abbreviation, not the other way around.

For example:

- (Yes) Giving an exogenous dose of testosterone (T) is considered a reasonable method for increasing maleness.
- (No) In the blood, E2 (17b-estradiol) can be elevated during the reproductive season.

Some words are well known, and are not explained any more

e.g. DNA, mRNA, PCR, *etc.*

Language – starting a sentence

Never start a sentence with an **abbreviation** or an **Arabic number**. Spell them out even if already defined.

For example:

- (Yes) Luteinizing hormone is the main gonadotrophin in mammals, controlling gametogenesis.
- (No) LH is the main brain hormone in mammals, controlling reproduction.
- (Yes) Twenty-five percent of the population in Greece own a second house.
- (No) 25 fish were sampled at each monthly sampling.

Language – which or that?

"Which" and **"that"** do not have the same meaning.

"Which" indicates cause-effect and it must be preceded by a comma. "That" is more for additional information and does not need a comma.

For example:

- (yes) Fish did not respond to the treatment, which means that they were not mature yet.
- (yes) Fish did not accept the second type of food that was prepared with marine oils.

Language – "such as" not "like"

Use "such as" as opposed to "like" to mean "**similar to**".

"Like" is a verb meaning "**to enjoy or approve of something or someone**".

For example:

- (yes) There are other hormones that are more relevant to reproduction, such as Testosterone and Estradiol.
- (no) There are other hormones that are more relevant to in reproduction, like Testosterone and Estradiol.

Language – High vs Large, low vs small

"High" and "low" are used for levels or numerical values.

"Large" and "small" are used for changes in size, dimensions or mass (but without values).

For example:

- **(Yes):** Higher plasma testosterone levels were obtained after hormonal therapy.
- (No): Larger plasma testosterone levels were obtained after hormonal therapy
- **(Yes):** A small amount of the brain's capacity is needed for survival instincts.
- (No): A low amount of the brain's capacity is needed for survival instincts.

Language – "respectively"

'**Respectively**' is an adverb that is used to mean "in the order given" and can be used to avoid repetition and reduce the length of a sentence.

For example:

- The tubes containing blood were labeled B and those containing saline were labeled S and those containing urine were labeled U. (no need for "**respectively**")
- The tubes containing blood, saline and urine were labeled B, S and U, respectively.

Language – stress position

Stress position. A reader expects to see what is important at the end of the sentence.

For example:

1: Introduction of the new assembly line **increased manufacturing**.

2: Manufacturing increased after the **introduction of the new assembly line**.

In (**1**), the study looked at the various effects of the introduction of the new assembly line. The key effect that was observed was an **increase in the manufacturing**.

In (**2**), the study looked at various ways to increase manufacturing. Of these, **introduction of the new assembly line** had the greatest effect.

Language – subject and verb placement

Subject-verb placement. The **verb** must be near the **subject** of a sentence. If you insert a lot of text between the subject and the verb, the reader may forget what the subject was.

For example:

- (Yes) The patient's liver readings [**subject**] had increased [**verb**] by 50% at 48 hours after exposure to the virus.
- (No): The patient's liver readings [subject] at 48 hours after exposure to the virus had increased [verb] by 50%.

Language – in text citations 1

Avoid using **author names out of parentheses**. These are better placed in parentheses, unless there is really a need to emphasize the name of the author.

For example:

- (Yes) The gilthead seabream reproduces annually for a period of 3-5 months (Zohar et al., 1995).
- (No) Zohar and coworkers (1995) demonstrated that gilthead seabream reproduces annually for a period of 3-5 months

- (Yes) Contrary to what has been know until now (Johnson et al., 1990; Stevens & Brown, 1991; Holland et al, 1993), Zohar and coworkers suggested recently that not all stocks of gilthead seabream reproduce annually for a period of 3-5 months.

- (Yes) Eistein (1945) was the first to relate energy to mass.

Language – in text citations 2

Citation to a **study**, **table or figure** should be done at the end of the sentence, unless this citation refers to only part of the sentence.

For example:

- (Yes) It has been found in both marine and freshwater fish that plasma testosterone increases after a meal and this increase is correlated to increased swimming activity (Stevens, 1978).

- (No) It has been found in both marine and freshwater fish that plasma testosterone increases after a meal (Stevens, 1978) and this increase is correlated to increased swimming activity.

- (Yes) It has been found in both marine and freshwater fish that plasma testosterone increases after a meal (Manabu et al., 2008) and this increase was correlated to increased swimming activity (Johns, 2018).

Language – in text citations 3

After the citation, following sentences referring to the same study **do not need to have the citation repeated**, unless conflicting data may intervene.

For example:

- It has been found that both marine and freshwater fish have high plasma testosterone (Stevens, 1978). This hormone increased also after a meal and this increase is correlated to increased swimming activity in marine fish only.

but

- It has been found that both marine and freshwater fish have high plasma testosterone (Stevens, 1978). In the present study of carp, the same was observed after a meal, but it was not correlated to increased swimming activity, as observed in marine fish (Stevens, 1978).

Language – Quoting and paraphrasing

Quoting vs paraphrasing

- (**original** from Zohar et al., 2001) In conclusion, it seems that seabass responds quite positively to hormonal induction of spawning with the use of GnRHa implants and its hormonal dysfunction related to reproductive failure is thus overcome.

- (**quoting**) According to Zohar et al. (2001) "seabass responds quite positively to hormonal induction of spawning with the use of GnRHa implants" and …..

- (**paraphrasing**) In earlier studies, the response of seabass to hormonal induction of spawning was very positive when GnRHa implants were used (Zohar et al., 2001) and …..

Language – Paraphrasing example

Original article

- Meagre (*Argyrosomus regius*) belongs to the family Sciaenidae and is an euryhaline and benthopelagic species, found mostly in lagoons and river deltas of subtropical climates (Griffiths, 1995). It is a gonochoristic species that does not exhibit sexual dimorphism and has been found to mature reproductively in captivity at the age of 2 for males and 3 for females (Schiavone, 2012), although in nature age at maturity has been calculated to be 5 years for males and 6 for females (González-Quirós, 2011;Morales-Nin, 2012).

Homework!!!!!

Language – Paraphrasing example

Paraphrasing

- Meagre (*Argyrosomus regius*) is a member of the Sciaenidae family and can be found in various salinities, often in lagoons and river deltas around the Mediterranean Sea (Griffiths, 1995). Males and females are gonochoristic and do not differ in external characteristics, and males and females reach reproductive maturity at 2 and 3-years-old, respectively (Schiavone, 2012), although age at maturity in nature has been reported to be 5 and 6 years for males and females, respectively (González-Quirós, 2011;Morales-Nin, 2012).

Homework!!!!!

How to write a scientific article – Preparation sequence

1. Prepare the Title page (This will form the "theme" of the paper)
   Beginning is half of everything!!!!!!
2. Prepare Graphs and Tables (This will create the "story or content" of the paper)
3. Start writing the manuscript
   - Materials & methods
   - Results
   - Introduction (based on the Results you will build the background, justification of the study and its objectives)
   - Discussion (interpret the results, relate to the current knowledge, suggest and justify further research, draw conclusions)
   - References
4. Abstract
5. Read again, and again, and again

Authorship rules!

- The **corresponding author** is the director of the lab, who will be always available to respond to any request regarding the article
- The person who writes the paper, gets **1st author**
- The person who pays for the work gets **last author**
- The person who thought of the work gets 1st or last author (depending on the writing contribution)
- If you are an author, you should be able to explain and defend the paper
- Technicians may or may not be co-authors, depending on their involvement, contribution or policy
- Sequence according to individual contribution

How to write a scientific article

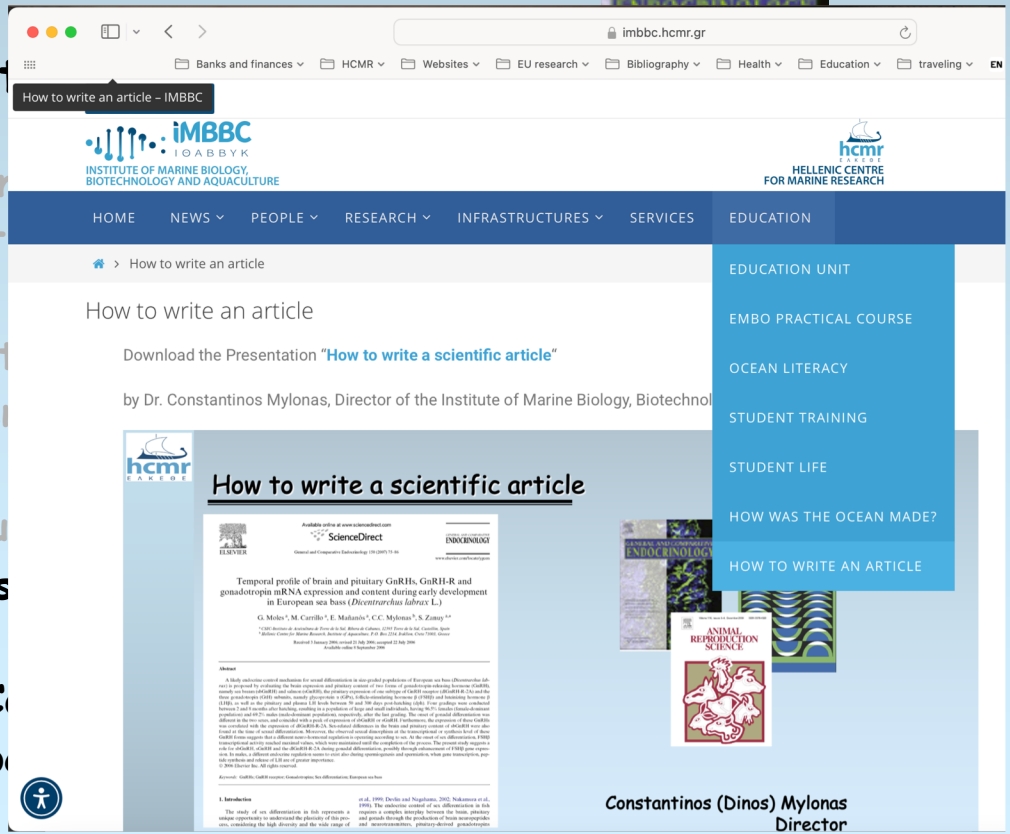
It always takes longer than you think!!!

Καλή επιτυχία!

How to write a scientific article

imbcc.hcmr.gr

- Preparation
  - Format
  - Parts
  - Content
  - Refere
  - Tables
- Language u
- References
  software
- Table of C
- Review pro



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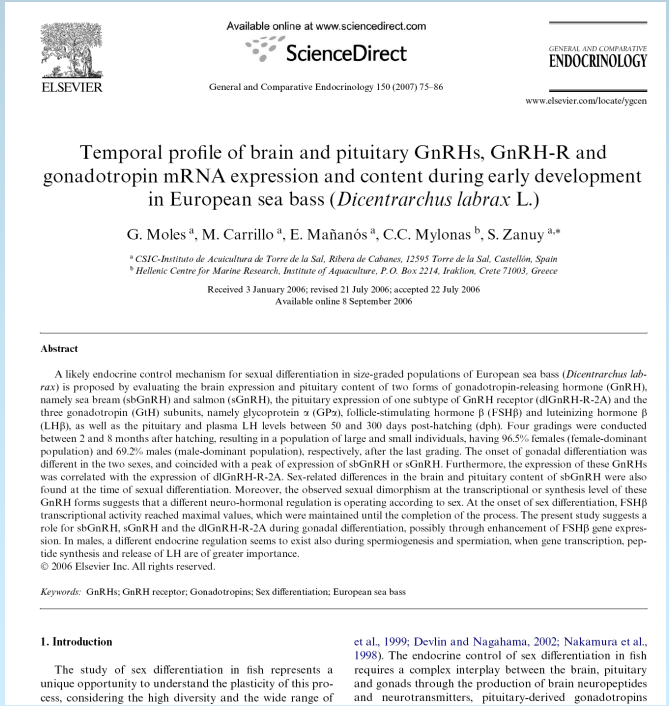

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Temporal profile of brain and pituitary GnRHs, GnRH-R and gonadotropin mRNA expression and content during early development in European sea bass (*Dicentrarchus labrax* L.)

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Abstract

A likely endocrine control mechanism for sexual differentiation in size-graded populations of European sea bass (*Dicentrarchus labrax*) is proposed by evaluating the brain expression and pituitary content of two forms of gonadotropin-releasing hormone (GnRH), namely sea bream (sbGnRH) and salmon (sGnRH), the pituitary expression of one subtype of GnRH receptor (dlGnRH-R-2A) and the three gonadotropin (GtH) subunits, namely glycoprotein α (GPα), follicle-stimulating hormone β (FSHβ) and luteinizing hormone β (LHβ), as well as the pituitary and plasma LH levels between 50 and 300 days post-hatching (dph). Four gradings were conducted between 2 and 8 months after hatching, resulting in a population of large and small individuals, having 96.5% females (female-dominant population) and 69.2% males (male-dominant population), respectively, after the last grading. The onset of gonadal differentiation was different in the two sexes, and coincided with a peak of expression of sbGnRH or sGnRH. Furthermore, the expression of these GnRHs was correlated with the expression of dlGnRH-R-2A. Sex-related differences in the brain and pituitary content of sbGnRH were also found at the time of sexual differentiation. Moreover, the observed sexual dimorphism at the transcriptional or synthesis level of these GnRH forms suggests that a different neuro-hormonal regulation is operating according to sex. At the onset of sex differentiation, FSHβ transcriptional activity reached maximal values, which were maintained until the completion of the process. The present study suggests a role for sbGnRH, sGnRH and the dlGnRH-R-2A during gonadal differentiation, possibly through enhancement of FSHβ gene expression. In males, a different endocrine regulation seems to exist also during spermiogenesis and spermiation, when gene transcription, peptide synthesis and release of LH are of greater importance.

Keywords: GnRHs; GnRH receptor; Gonadotropins; Sex differentiation; European sea bass

1. Introduction

The study of sex differentiation in fish represents a unique opportunity to understand the plasticity of this process, considering the high diversity and the wide range of

et al., 1999; Devlin and Nagahama, 2002; Nakamura et al., 1998). The endocrine control of sex differentiation in fish requires a complex interplay between the brain, pituitary and gonads through the production of brain neuropeptides and neurotransmitters, pituitary-derived gonadotropins

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