Biodiversity Journal, 2011, 2 (3): 107-114

Some aspects on the reproductive cycle of European conger eel, *Conger conger* (Linnaeus, 1758) (Osteichthyes, Anguilliformes, Congridae) captured from Western Algerian coasts: a histological description of spermatogenesis.

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ABSTRACT The aim of this work was to study the annual reproductive cycle of European conger eel (*Conger conger,* Linnaeus, 1758) through analysis and description of spermatogenesis. A sample of 168 males was captured between September 2008 and August 2009 from the Western coast of Algeria, from Béni Saf. Fish length and weight varied between 26.20-112 cm and 0.45-3.44 kg, respectively.

Condition factors (K), gonadosomatic index (G.S.I.) and hepatosomatic index (H.S.I.) were calculated monthly. Factor K reached the minimum in August/September (0.10%) corresponding to reproductive period and a maximum in January (0.18%). Although G.S.I. values revealed to be statistically not significant, there were two peaks for G.S.I., the first in March, denoting the beginning of spermatogenesis, and the second in August/September, indicating the reproduction period. H.S.I. reached a peak in December (1.90%), then the value decreased to a minimum in April.

Histological analysis of testis allowed us to distinguish 5 stages summarized as follows: Stage 1: Spermatogonia A; Stage 2: Spermatogonia B; Stage 3: Spermatocytes and spermatids; Stage 4: Spermatocytes, spermatids and spermatozoa (cytodifferentiation of spermatids into spermatozoa); Stage 5: Spermatozoa (spermiogenesis or cytodifferentiation of spermatids into spermatozoa).

KEY WORDS Condition factor, *Conger conger*, G.S.I., H.S.I., reproduction, spermatogenesis.

Received 16.05.2011; accepted 05.07.2011; printed 30.09.2011

INTRODUCTION

The European conger eel (*Conger conger*) is distributed in the Eastern North Atlantic Ocean from Norway to Senegal (including the Canary Islands, Azores and Madeira), in Mediterranean and western Black Sea (F.A.O., 2011). Specimens spawn, probably once in lifetime in summer (Cau & Manconi, 1984), in the Mediterranean and in the eastern North Atlantic around Azores (McCleave & Miller, 1994; Vallisneri et al., 2007). In Mediterranean Sea, males are usually smaller than females, with males rarely exceeding 100 cm in length and females reaching over 200 cm (Cau & Manconi, 1984). Since a decade, European conger eels (*C. conger*) constitute an important and valuable fishery resource (Figueiredo et al., 1996; Morato et al., 1999; O'Sullivan et al., 2003) in Mediterranean countries (Relini et al., 1999) and, particularly, in Algeria. However and to our knowledge, no studies on eco-biology of this important benthic species (Vallisneri et al., 2007) from South shore of Mediterranean Sea have been published. Moreover, there is evidence of declining stocks of the species (Menezes & Silva, 1999; O'Sullivan

et al., 2003) and there has been no detailed published study on its reproductive biology and especially on the dynamics of spermatogenesis. According to F.A.O. (2011), total world catch of *C. conger* was estimated in 2009 to 17,229 tons. It is clear that conger species are subject to overfishing (Menezes & Silva, 1999; Mochioka & Tokai, 2001), which caused a drastic fall in its capture. Moreover, *C. conger* is very sensitive to exploitation and constitutes an important species in fish biodiversity and in biodiversity's balance (Correia et al., 2006).

The objective of the present study was to elucidate the process of male maturation of European conger eel (*C. conger*) by examination of annual changes in condition factor K, gonadosomatic and hepatosomatic indexes (G.S.I. and H.S.I., respectively) and gonadal histology. This latter constitutes the first detailed information on the species in Mediterranean.

MATERIAL AND METHODS

Fish samples: *Conger conger* employed for this study were captured from the Western coast of Algeria, from Béni-Saf, at a depth ranging between 100 and 150 meters. Total of 168 males were sampled, 60 in autumn, 33 in winter, 35 in spring and 40 in summer. Fresh specimens, collected by fishermen, were examined in laboratory. Total length (cm) and weight (g) and liver and gonad weight were measured for all individuals. Total length varied between 26.20 and 112 cm and total weight varied between 0.45 and 3.44 kg. Note that in May and June 2009, samples contained only female specimens.

Indices of fish condition: In this study, we calculated, monthly, values of:

- Condition factor K [K = (total weight / total length ^{3}) x 100],
- Gonadosomatic index [G.S.I. = (gonad weight / total weight) x 100],
- Hepatosomatic index [H.S.I. = (liver weight / total weight) x 100].

Histological study: A 1 cm fragment from the gonad of each fish was removed and fixed in Bouin's solution, then dehydrated and embedded in paraplast. For histological examination, the tissues were cut into sections of 5 microns and stained with a trichrome method according to Langeron (1942): Regaud's haematoxyline at 57 °C, phloxine and green light. Histological descriptions of gonadal developmental stages were based on the criteria reported by Yamamoto et al. (1972) and Grier (1981).

Statistical Analysis: All data were expressed as mean ± standard deviation and were statistically compared by one-way variance analysis or ANOVA 1 (for condition factor K and Gonadosomatic index or G.S.I.) and by non parametric variance analysis of Kruskal-Wallis and Mann-Withney *U*-test (for hepatosomatic index or H.S.I.) (d'Hainaut, 1975a, b).

RESULTS

Indices of fish condition

Condition factor K: Condition factor K (Fig. 1) remained stable between September and December 2008, then increased significantly ($p<0.05$) and reached a maximum (0.18% ± 0.03%) in January 2009. Between February and August 2009, K factor decreased significantly ($p<0.05$).

Gonadosomatic (G.S.I.) and hepatosomatic (H.S.I.) indexes: Statistical comparison by ANOVA 1 of G.S.I. showed no significant differences among data obtained. As a description of G.S.I. results, in terms of absolute value, G.S.I. decreased not significantly ($p\geq0.05$) steadily and continuously between October 2008 and February 2009. In March 2009, G.S.I. reached a high value (2.92% ± 3.36%), then decreased not significantly ($p\geq0.05$) between April and July. Note that in May and June, only female specimens were caught. In September 2008 and August 2009, G.S.I. increased not significantly ($p\geq0.05$) again and reached a high value 3.97% ± 4.10% and 3.37% ± 5.23%, respectively (Fig. 2). Because of important differences between the raw values, standard deviation was high and in some cases higher than mean. Indeed, in September 2008, March and August 2009 G.S.I. raw values varied between 0.34%-12.29%, 0.24%-10.02% and 0.15%-14.16%, respectively. This can explain the results

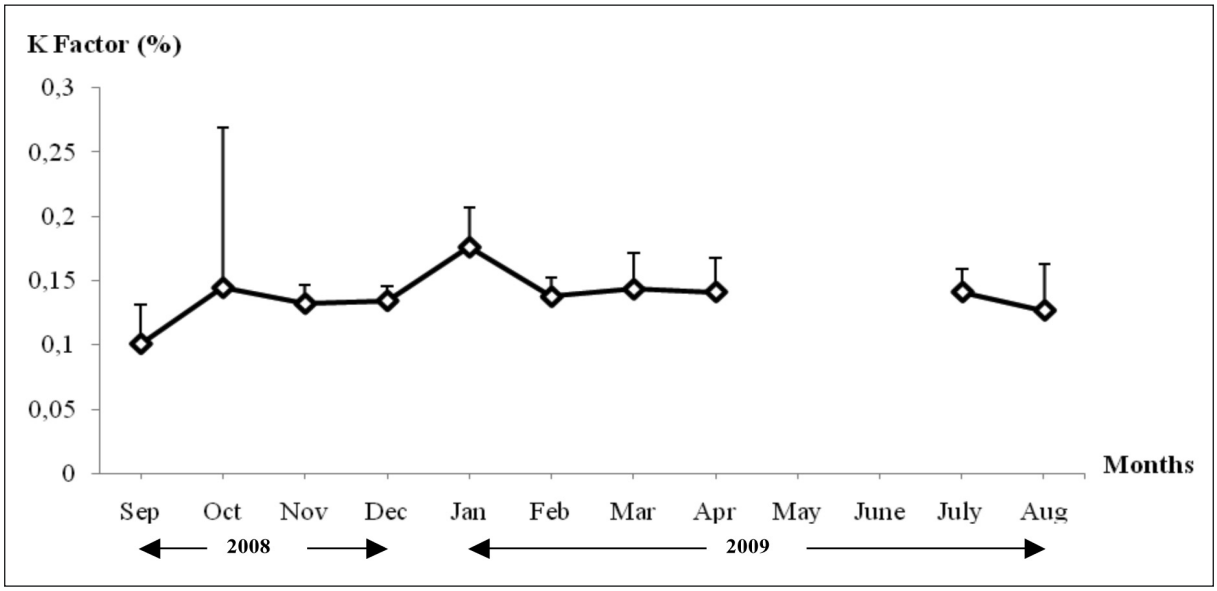

Figure 1: Time evolution of the condition factor K (mean ± standard deviation expressed in %) in male European conger eel *(Conger conger)*.

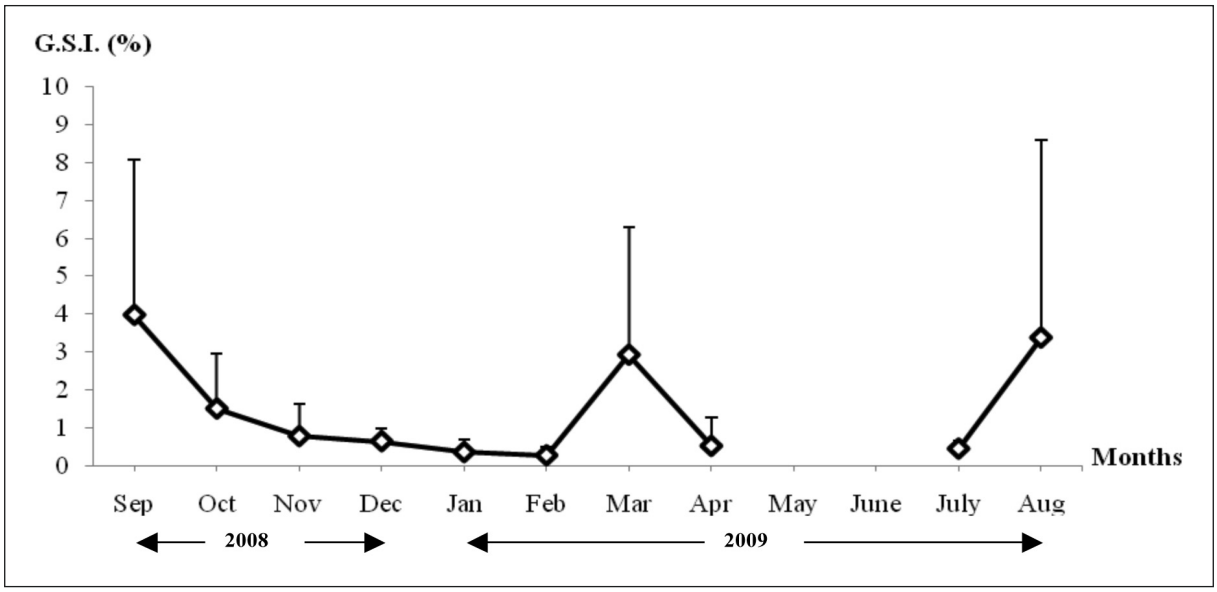

Figure 2: Time evolution of G.S.I. (mean ± standard deviation expressed in %) in male European conger eel *(Conger conger)*.

obtained which, however, were not significant at all from a statistical point of view.

The value of H.S.I. increased to a maximum in December 2008 ($p<0.05$), then decreased significantly and continuously ($p<0.05$) until February 2009. Between February and August 2009 (Fig. 3), H.S.I. value remained stable and the data revealed no significant variations ($p\geq0.05$). Similarly, data on H.S.I. were not available in May and June 2009 because of lack of male specimens during this period.

Histological parameters

Histological stages of sperm cells varied significantly according to period of sampling.

Stage 1: This stage was observed between November and December 2008 and was characterized by the presence of spermatogonia A (Fig. 4). The nucleus presented a clear appearance after staining and cytoplasm presented a patch of dense granular and fibrillar material called "cloud", usually near the nuclear membrane.

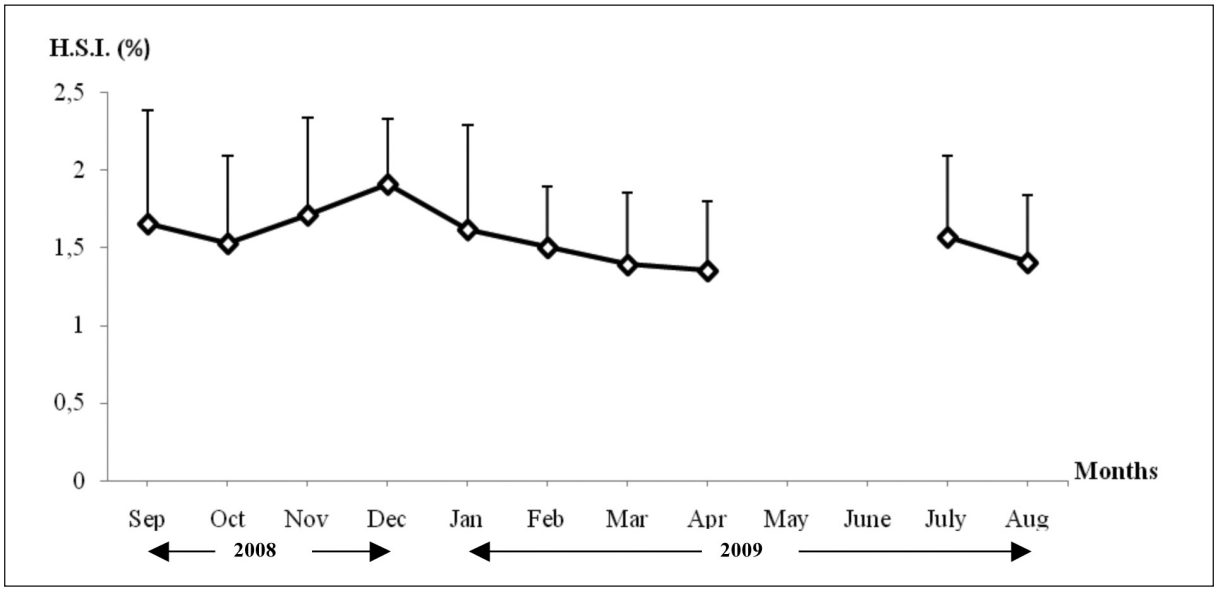

Figure 3: Time evolution of H.S.I. (mean ± standard deviation expressed in %) in male European conger eel *(Conger conger)*.

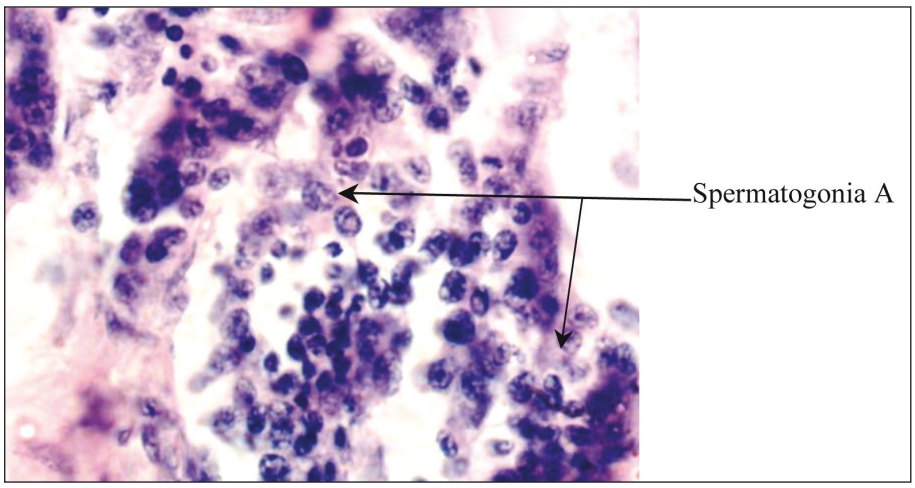

Figure 4: Histological section representative of spermatogonia A (800x) during early spermatogenesis (November-December) in European conger eel *(C. conger)*. G.S.I. = 0.93%.

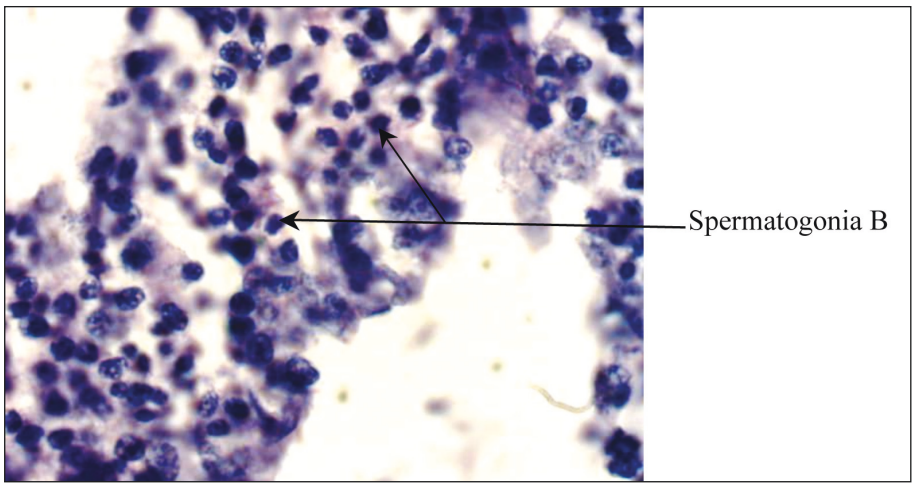

Figure 5: Histological section representative of spermatogonia B (800x) during spermatogenesis initiation (December-February) in European conger eel *C. conger)*. G.S.I. = 1.05%.

Stage 2: This stage was observed from December until February and indicated the beginning of spermatogenesis, with the occurrence of spermatogonia B. These cells were smaller and more intensely colored than spermatogonia A (Fig. 5).

Stage 3: This stage was observed in March 2009 and was characterized by the occurence of spermatocytes (Fig. 6) at various stages (spermatocytes I and II). Spermatocytes have a great round or oval nucleus. During this stage, we observed the meiotic phase characterized by the occurrence of spermatids.

Stage 4: This stage was observed between July and October 2009 and indicated the occurrence of spermiogenesis. Because of lack of male specimens in samples of May and June 2009 we were unable to determine at what month this stage exactly begins. The testes contained spermatocytes, spermatids and the maturing cells representative of the differentiation of spermatids into spermatozoa (Fig. 7). These curved-shaped cells (average length: 3.84 µm) were strongly stained with haematoxyline.

Stage 5: This stage was observed in September 2008 and only in one specimen. The testis showed only the maturing cells representative of the differentiation of spermatids into spermatozoa (Fig. 8).

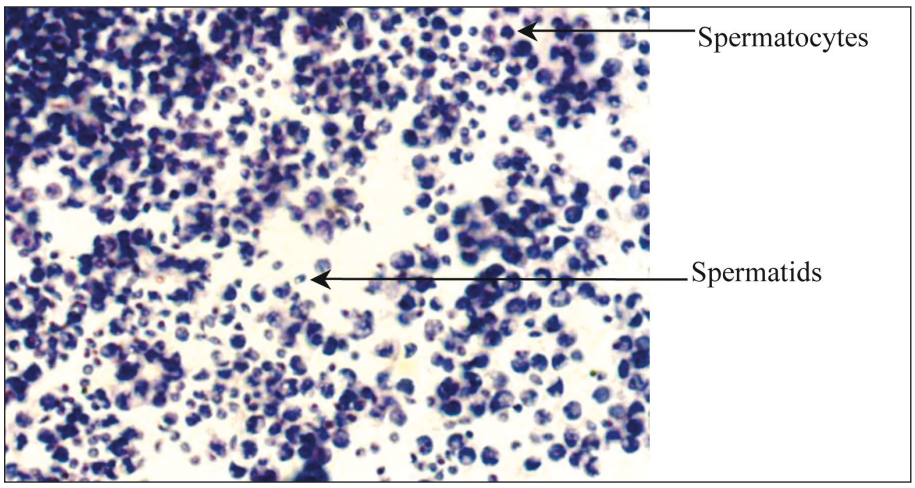

Figure 6: Histological section representative of spermatocytes and spermatids (800x) at the end of spermatogenesis (March-April) in European conger eel *(C. conger)*. G.S.I. = 5.84%.

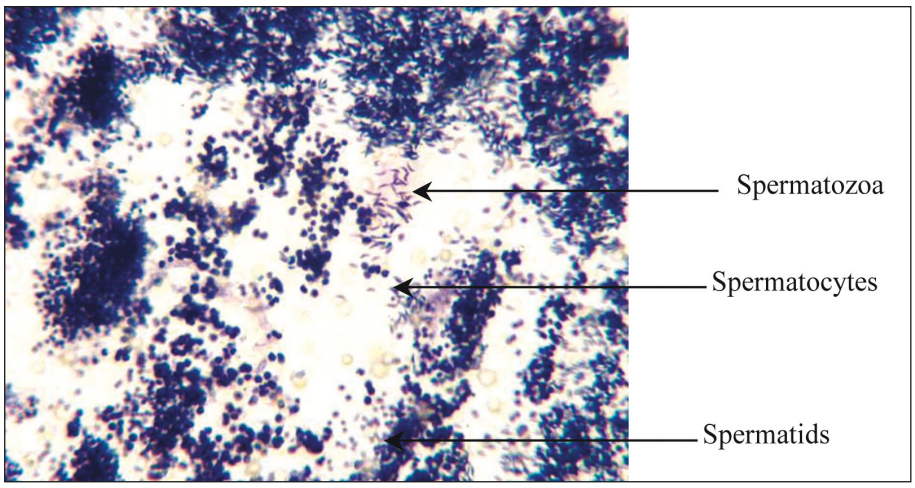

Figure 7: Histological section representative of spermatocytes, spermatids and spermatozoa in differentiation (800x) during spermiogenesis (August) in European conger eel *(C. conger)*. G.S.I. = 13.30%.

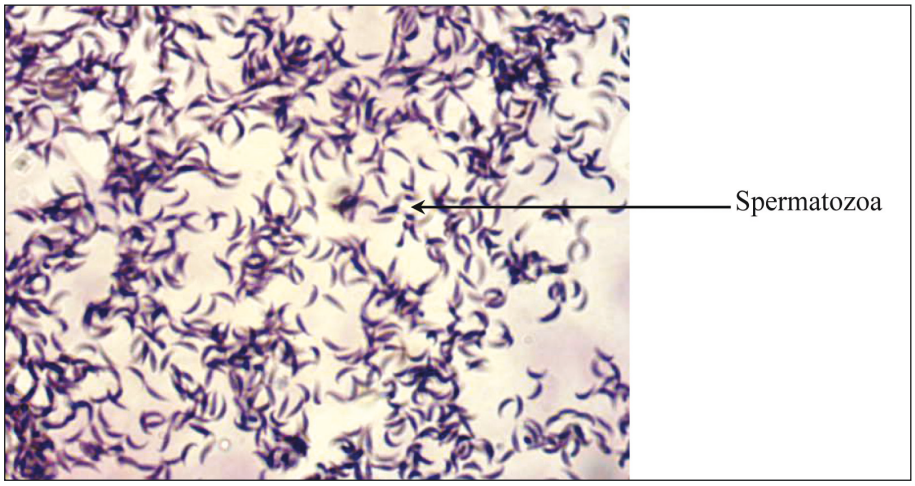

Figure 8: Histological section showing spermatozoa in differentiation (800x) during spermiogenesis (September) in European conger eel *(C. conger)*. G.S.I. = 4.60%.

DISCUSSION

Little data exist on reproductive biology of conger species and especially *C. conger* (Relini et al., 1999; Sbaihi et al., 2001), so comparisons are difficult to make. The condition factor K of *Conger conger* was highest in winter, in January 2009, and lowest in summer, during September 2008 and August 2009. The decrease in the value of this factor in summer probably resulted in a weight loss for the fish, indicating that fishes used most of somatic energy reserves during migration and reproductive development. In Irish coastal waters, O'Sullivan et al. (2003) showed, in female *C. conger*, the highest and the lowest values of condition factor, in autumn and winter, respectively. The difference resulted probably from the coldest temperature observed in Oceanic waters (Irish waters) compared to south Mediterranean waters (present study).

In this study, gonadosomatic index (G.S.I.) presented two peaks, the first in summer and the second in spring. Although these data were not statistically significant, nevertheless, the first peak could be explained by prespawning and spawning period. Indeed, many studies showed that European conger eel spawn in summer (Relini et al., 1999; Vallisneri et al., 2007; Abi-ayad et *al*., personal unpublished data). In addition, Utoh et al. (2004) showed that captive Japanese conger eels (*C. myriaster*) had a spermiation period from May to September with G.S.I. peak mean value of 5.3% ± 3.0% and a highest G.S.I. value of 9.3% measured in a specimen in June. In this study, the highest mean value of G.S.I. was measured in September 2008 (3.97% ± 4.10%) and August 2009 (3.37% ± 5.23%) and the highest and lowest G.S.I. raw values, 14.16% and 0.15%, were measured in August 2009. These latter results can explain that in many cases standard deviation values were higher than means values. After breeding, we measured a decrease in testicular weight justifying the reduction in value of the G.S.I. (Abi-ayad et al., 2004; Utoh et al., 2004). However, in coldest waters, Hood et al., (1988) and O'Sullivan et al., (2003) showed lowest and highest G.S.I. during autumn and late winter/spring in *C. oceanicus* and *C. conger*, respectively. In this study, a second high G.S.I. was obtained in spring (March 2009). This was probably due to the presence of males in advanced stages of spermatogenesis. The decline of G.S.I. in April and July 2009 may be due to the migration of males, by that time ready for breeding, to spawning area at great depths.

The H.S.I. was highest in early winter (December 2008). This coincided with hepatic fats deposits due to intense feeding activity during summer period and, probably, useful for fish gonad maturation. In April 2009, H.S.I. was at its lowest level. This could indicate that the reserves stored in the liver during summer/autumn were invested in the development of sexual products, but also used as energy source when fish reduce their feeding during migration to the breeding area. This is confirmed by microscopic examination of gonads which showed that

spermatogenesis of European conger eel started in March. Histological study of testis confirmed lobular structure in the European conger eel, also observed in the European eel (*Anguilla anguilla*) and in many teleosts species (Todd, 1980). In this study, we classified the process of spermatogenesis into five stages. The testicular structure showed that spermatogonia A (stage 1) occurred in November and December 2008 and spermatogonia B (stage 2) from December 2008 to February 2009, when G.S.I. was lowest and K factor was highest. This may be related to trophic phase which is completed before maturation (Cau & Manconi, 1984; Utoh et al., 2004). Meiotic divisions of spermatocytes started in March (stage 3) and corresponded to the first peak of G.S.I. In wild winter flounder (*Pleuronectes americanus*) G.S.I. was high before appearance of spermatozoa (Harmin et al., 1995). In the present study we do not know when this stage ends, because of lack of male specimens in samples of May and June 2009. Histological examinations performed between August and October, when G.S.I. was at its second highest level, showed spermatocytes, spermatids and spermatozoa in final maturation (stages 4 and 5). This corresponded to the phase of late spermatogenesis and spermiogenesis. Indeed, Utoh et al., (2004) showed that G.S.I. remained at high levels in the late phase of spermatogenesis, during spermiation in reared Japanese conger (*C. myriaster*).

In conclusion, the rational management of fish biodiversity and fishery necessitates understanding on eco-biology of target species. This study showed a (although statistically weak) relation between biometrics parameters and spermatogenesis's dynamics in European conger eel. Furthermore, these results provide the first information on reproductive biology of *C. conger* captured in Western Algerian coasts (North African area) and report observations on cytodifferentiation of spermatids into spermatozoa (spermiogenesis) in male wild European conger eel (*C. conger*).

ACKNOWLEDGEMENTS

The authors thank the Algerian Ministry of Higher Education and Scientific Research (MESRS) which funded this experimental study within the framework of CNEPRU project No F01820090018.

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