OBJECTIVE QUANTITATIVE PARAMETERS TO EVALUATE REPRODUCTIVE ACTIVITY IN *ENGRAULIS ENCRASICOLUS L.*

Rahmani Amina, Iguer-Ouada Mokrane

Abstract: This study focused on novel histological indicators correlated with macroscopic reproductive parameters in male and female European anchovy (*Engraulis encrasicolus*). Weekly sampling from commercial catches in the Gulf of Béjaïa, Algeria, was conducted to measure microscopic parameters, including seminiferous tubule number (STN) and area (STA) in males, and oocyte number (ON) and surface area (OA) in females, independent of maturation stages. Macroscopic reproductive stages were assessed using a standardized five-degree maturity staging system and the gonadosomatic index (GSI). Results showed that OA and ON accurately reflected female maturity stages and exhibited opposing trends with GSI, suggesting their reliability as reproductive activity indicators. Similarly, seminiferous tubules parameters (STN and STA) provided valuable insights into male spermatogenesis and maturation stages. Our findings propose standardized ON/OA and STN/STA measurements as novel, quantitative tools for assessing reproductive activity in anchovy, combining histological and image analysis for a more precise understanding.

Keywords*: Engraulis encrasicolus*, Oogenesis, Spermatogenesis, GSI, Maturity stages.

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Introduction

An understanding of the regulation of major biological functions is important for optimizing the reproductive potential of all species, crucial for their survival and sustainability. Reproduction, in particular, plays a vital role in ensuring optimal production in response to various environmental factors. In fish, reproduction is an annual cyclical process that is largely influenced by seasonal variations in the environment [10]. Consequently, knowledge of the spawning season is of great importance. For many years, morphological and histological studies of the gonads have been used to determine reproductive cycles, spawning seasons, and sexual maturity [9]. Macroscopic maturity scales of reproductive organs are commonly used tools for assessing the reproductive status of fish. However, their accuracy may be limited [20]. It is therefore recommended that validation studies be conducted to refine these scales and ensure their accuracy. Histological techniques are widely recognized as powerful tools for studying fish reproduction, offering a precise method for assessing the different phases of reproduction.

Macroscopic studies of gametogenesis are essential for establishing the parameters of reproductive biology. However, microscopy plays an essential role in validating these parameters. Traditional microscopic techniques often remain qualitative and fail to provide objective quantitative data. The advent of advanced digital microscopy and photographic technology has enabled the extraction of meaningful quantitative data from histological images, paving the way for a transition to more quantitative microscopy. This development offers considerable scientific advantages in terms of new applications and data reliability, opening up new avenues for scientific research [12].

The primary objective of this study was to identify quantifiable histological indicators in European anchovy, *Engraulis encrasicolus*, both in males and females. This was done with the aim of understanding and monitoring events related to reproductive activity without the need for fresh samples. Additionally, the study sought to validate new methods for quantifying gonadal histology and to investigate potential correlations with established macroscopic parameters in both sexes, with a particular focus on gametic cell size in males. The ultimate objective was to develop a histological assessment system and tools for studying the reproductive dynamics of anchovy.

Materials and methods

A random sample of anchovy, *E. encrasicolus,* was collected manually on a weekly basis over the course of one year (from October 2007 to October 2008 for males and from January 2010 to January 2011 for females) in order to cover the breeding season. Samples were obtained from the commercial landings of the purse seine fleet at the fishing port of Bejaia (northeast of Algeria) and transported immediately to the laboratory. The gonadosomatic index (GSI) was calculated for each specimen as the ratio of gonad weight to total fish weight, expressed as a percentage in accordance with [11]. Maturity staging was determined according to the following five-point scale [4]: I: Immature stage, II: Developing stage, III:

Spawning capable stage, IV: Regressing stage, V Regenerating stage. The gonads were preserved in 10 % buffered formalin for histological examination, which was conducted in accordance with standard methods. The samples were dehydrated in a graded alcohol series, cleared in xylene, embedded in paraffin, sectioned into 3-4 µm slices, and stained using the Harris Hematoxylin and Eosin (H&E) protocol. The histological sections were photographed at 10x magnification for image analysis. The number of oocytes (ON) and the surface area of the oocytes (OA) were quantified at 10x magnification for females, while the number (STN) and the surface area (STA) of seminiferous tubules were calculated in the same manner for males. The measures of the ON and the STN ware counted in a single microscopic field at 10x magnification. This was carried using UTHSCSA Image Tool 2.0 software (University of Texas Health Science Center, San Antonio, TX, USA). For each female, 250 oocytes were considered, while for each male, 25 seminiferous tubules were considered for the area measurements. The 25 tubules were randomly selected [1] via horizontal scanning, with only those with circular and sub-circular contours were utilized.

The data were analyzed using Statview 5 statistical software (SAS, Cary, NC, USA). One-way analysis of variance ANOVA and Tukey's honestly significant difference (HSD) post-hoc test were used to assess the differences in GSI, OA, ON, STA, and STN between individuals at different stages of sexual maturity, with females and males analyzed separately. Statistical significance was set at $p < 0.05$ for all comparisons. The data were presented as mean ± SE.

Results

A total of 480 anchovy specimens (280 females and 200 males) were randomly collected for this study. The subsequent analyses focused on correlating quantitative histological parameters with established productive indices to assess their efficacy in monitoring gonad development.

In males, seminiferous number (STN, Fig. 1A) and seminiferous area (STA, Fig. 1B) exhibit an inverse correlation with the maturity stages, showing a significant negative correlation between STN and maturity stages ($r = –0.67$, $p < 0.001$) and a significant positive correlation between STA and maturity stages ($r = 0.66$, $p < 0.01$), with the exception of stages IV and V.

Both STN and STA quantitatively express the different maturity stages according to GSI (Fig. 2B), and thus appear as relevant indicators of reproductive activity.

Furthermore, these two parameters demonstrate a negative correlation with each other ($r = –0.88$, $p < 0.001$), and STN (Fig. 1B) evolves in a manner consistent with GSI (Fig. 2B) ($r = 0.68$, $p < 0.001$). The correlation between STA (Fig. 1A) and GSI (Fig. 2B) is $r = –0.61$ ($p < 0.01$), indicating an inverse relationship. GSI (Fig. 3B) exhibited a seasonal pattern, decreasing from October 2007 to January 2008 before increasing to a peak in May 2008.

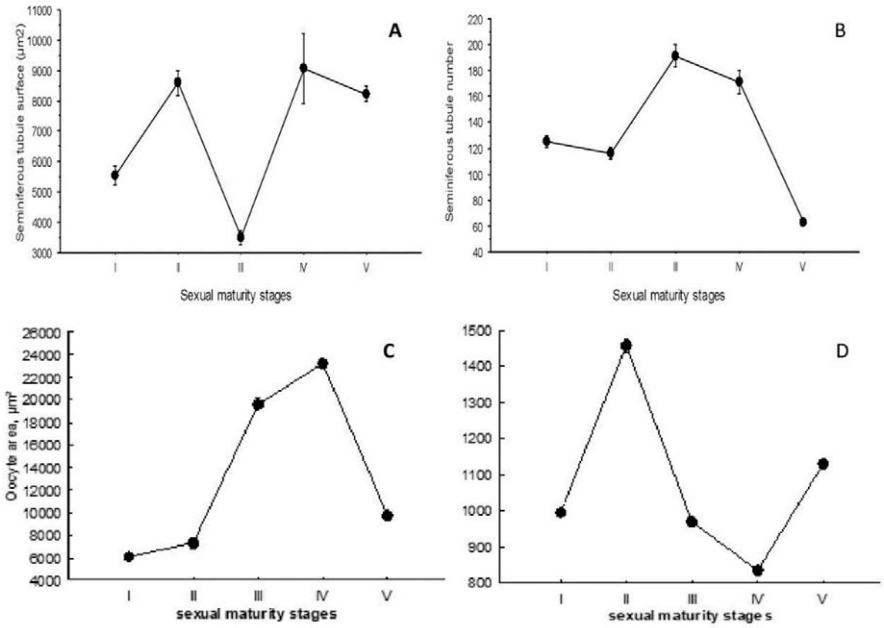

Figure 1 – A) Seminiferous tubules area (µm²), B) Seminiferous tubules number, C) Oocytes surface area (µm²), D) Oocytes number of *E. encrasicolus* at different gonadal maturity stages. Data are represented as mean ± SE.

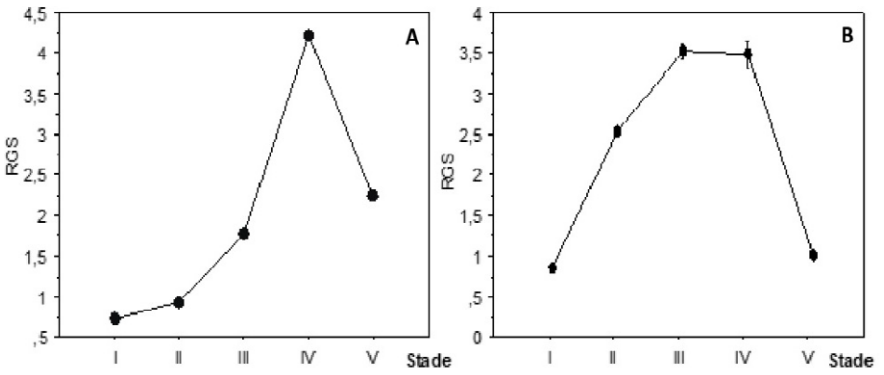

Figure 2 – Gonadosomatic Index percentage (GSI) A) in females and B) in males of *E. encrasicolus* at different gonadal maturity stages. Data are represented as mean ± SE.

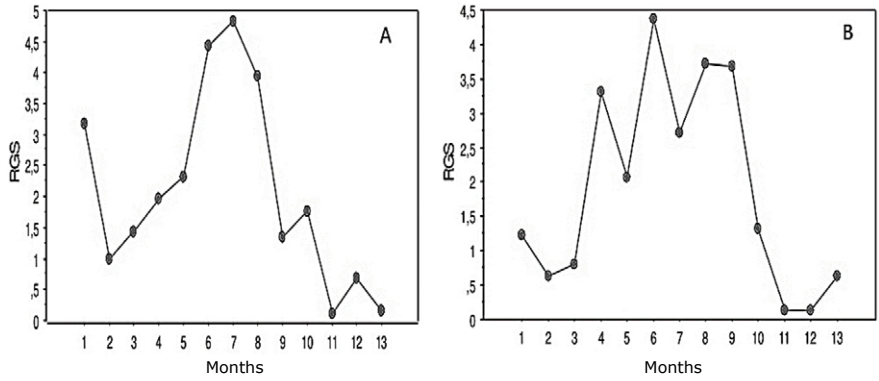

Figure 3 – Gonadosomatic Index percentage (GSI) A) in females (from January 2010 to January 2011) and B) in males (from October 2007 to October 2008) of *E. encrasicolus* collected from Gulf of Béjaïa. Data are represented as mean ± SE.

Similarly, STN and STA (Fig.4 A & B) demonstrated a seasonal changes that mirrored the GSI trend, supporting their potential as monitoring tools. The annual change in cell types occurred from October 2007 to May 2008, with a transition from spermatogonia to spermatozoa via spermatocytes and spermatids over the months, illustrating the maturation process of male reproductive cells.

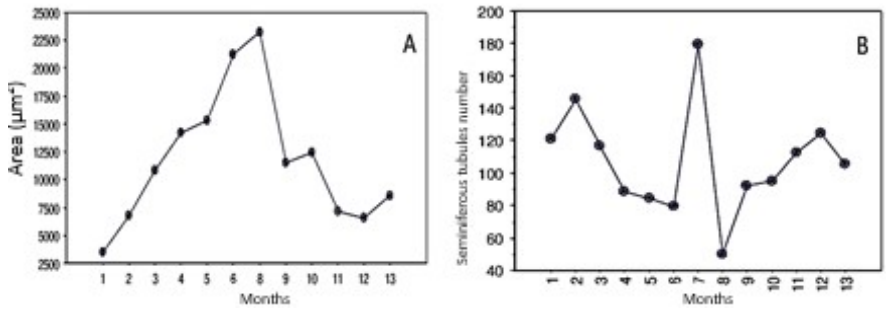

Figure 4 – Monthly variation of: **A**) Seminiferous tubules area (µm²), **B**) Seminiferous tubules number of *E. encrasicolus* collected from Gulf of Béjaïa. Data are represented as mean ± SE.

For females, the maturity stages indicated by ON and OA were found to have evolved simultaneously (Fig. 1A & B), exhibiting similarities with GSI (Fig. 2A), which accurately represented the different development stages. It is noteworthy that OA demonstrated a pattern of parallel development in comparison to GSI, while ON evolved inversely. This suggests that microscopic parameters (OA and

ON) could be valuable as quantitative indicators of anchovy maturity stages. During the research period, GSI increased regularly from January 2010, reaching its maximum values in July 2010. After this, it dropped considerably, reaching its minimum values from November 2010 to January 2011 (Fig. 3A). Similarly, ON and OA demonstrated evident similarities (Fig. 5A & B) with GSI (Fig. 3A) during the same study period. The ON exhibited a similar evolution to GSI from January 2010 to March 2010, followed by an inverse trend at the study period. The OA demonstrated a parallel pattern with GSI.

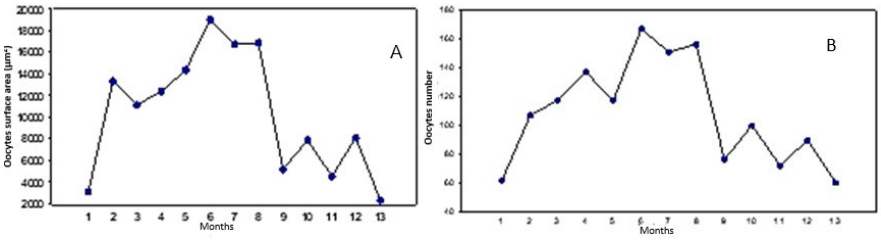

Figure 5 – Monthly variation of: **A**) Oocytes surface area (µm²), **B**) Oocytes number of *E. encrasicolus* collected from Gulf of Béjaïa. Data are represented as mean ± SE

Discussion

While qualitative histological analysis remains a cornerstone for the study of fish reproduction, its limitations become apparent in large-scale studies [13]. Histological techniques although reliable, are known to be time consuming, potentially hindering the efficiency of research involving large sample sizes [5]. Conversely, visual staging based on external gonad appearance offers a faster approach, but its accuracy is often compromised, limiting its suitability for routine studies [7]. Additionally, histological analysis, while providing detailed information on gonadal development stages, requires extensive background research to fully understand the implication of these observations reproductive success at the population level [20].

In that frame, we present a quantitative histological approach to studying male and female anchovy reproduction activity. Our findings demonstrate a significant correlation between the seminiferous tubules and oocytes parameters (STN, STA, ON, OA) and established reproductive indices like GSI and maturity stages. Correlation analysis revealed a strong negative correlation between maturity stages and STN, while a strong positive correlation was observed between maturity stages and STA. In females, an inverse relationship was found, with strong positive correlation between maturity stages and ON, and strong negative correlation with OA. Furthermore, GSI demonstrated a very strong significant positive correlation with STN, and a strong negative correlation with STA. Conversely, GSI showed a strong positive correlation with ON and a strong negative correlation with OA.

The observed decrease in STN and increase in STA during stages I and II is indicative of the presence of larger germ cells (spermatogonia and spermatocytes) lining the tubules. As these cells differentiate into smaller spermatids and spermatozoa, they occupy less space, leading to a reduction in tubule surface area. This inverse relationship between STN and STA indicates the occurrence of intense spermatogenesis, characterized by the transition from stage I, which is devoid of spermatic activity [8], to stages that exhibit active mitosis and early meiotic divisions [6]. Conversely, the inverse evolution of these parameters from stage III onwards reflects spermiogenesis, marked by the abundance of mature spermatozoa [2].

In females, oocyte development follows a distinct pattern. ON and OA increases during oogenesis from stage I to stage IV, reflecting oocyte growth and cytoplasmic heterogeneity culminating in vellogenesis [18]. A subsequent decrease in these two parameters indicates the ovary entering a phase of atresia [3].

The application of quantitative histomorphometry offers a distinct advantage by enabling the application of robust statistical methods to subtle changes in gonadal development. This approach allows for the detection of nuances that might otherwise be overlooked in traditional qualitative analysis. Numerous studies have employed quantitative methodologies to investigate the development of fish gonads [16][17][19].

Conclusion

Our approach diverges from classical oocyte quantification methods, which require categorizing each cell to pre-defined category based on specific criteria. These methods require meticulous histological section preparation to distinguish between germ cell types, particularly in smaller male gonads. Expertise in histological interpretation is crucial for accurate differentiation. The measurement of tubular structures and oocyte, as employed here and detailed in previous articles [14][15], provides a valuable tool for the assessment of gametogenesis and its correlation with physiological functions and environmental parameters.

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