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Seasonal Changes in Maturity Stages of Female and Hermaphrodite Gonads of *Mesopotamichthys sharpeyi* (Günther, 1874) from Southern Missan Province Marshes, Southern Iraq

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ABSTRACT

The study investigated the seasonal variations in the maturity stages of female *Mesopotamichthys sharpeyi* (the brown bunni) and the occurrence of histological hermaphroditism. From February 2022 to January 2023, a total of 95 specimens were collected from the marshes in southern Missan Province, Iraq. The fish ranged in total length from 19.5 to 41.5cm and weighed between 107 and 1500g. The gonadosomatic index (GSI) values fluctuated throughout the year, with the lowest value at 0.25 in August and the highest value reaching 12.98 in March. The study confirmed that the spawning season of *M. sharpeyi* extends from March to April. Six maturity stages were determined through both visual and histological examination. Histological analysis revealed the presence of hermaphroditism in some gonads, where both female and male tissues were observed.

INTRODUCTION

Mesopotamichthys sharpeyi, commonly referred to as the bunni, is a commercially important species in the Cyprinidae family. Initially classified under the *Barbus* genus, it was later reassigned to the *Mesopotamichthys* genus **(Jassim, 2012; Kouame *et al.*, 2018)**, native to the Tigris and Euphrates river basins. This reclassification highlighted the need for a deeper understanding of the species' reproductive strategies compared to previous studies.

Research into fish reproduction is vital for the effective management and conservation of fisheries resources, particularly in light of the species' life cycle **(Uyan *et al.*, 2020)**. To understand reproduction is crucial for addressing the effects of environmental shifts on fish populations and improving commercial aquaculture. The reproductive process plays a key role in fish recruitment, survival of offspring, and adaptation to habitat changes, impacting aquaculture success and the future potential of fish stocks **(Balci *et al.*, 2017)**. Furthermore, such research contributes to understanding how environmental changes affect current fish dynamics, emphasizing the importance of species management **(Mehanna, 2022)**.

Comprehending fish reproduction is crucial for tracking their life cycles and developing strategies for managing and conserving their populations. The brown trout, for example, exhibits unique reproductive features compared to other species and displays hermaphroditic traits, meaning some individuals possess both male and female reproductive tissues **(Moyle & Cech, 2004)**. Fish exhibit a variety of reproductive strategies that can shift with environmental conditions, enhancing reproductive success. Ovarian development and maturation are key determinants of fecundity and population sustainability **(Wootton & Smith, 2015)**.

In females, these processes involve complex stages such as oocyte development and maturation, which can be assessed through histological analysis **(Nagahama, 1983)**. Hermaphroditism, where individuals have both male and female reproductive tissues, adds an additional layer of sexual adaptability **(Sadovy de Mitcheson & Liu, 2008)**. Studies on bunni fish, such as reproductive biology research in the Al Huwaizah marsh **(Mustafa *et al*., 2006)** and larval development studies **(Mukhaysin & Jawad, 2012)**, have contributed to understanding reproductive traits in species like *Barbus sharpeyi*.

For the brown trout, it is essential to study stages of gonadal maturation and the occurrence of hermaphroditism. Such information is critical for managing fish populations, especially as they face environmental changes and human impacts **(Jennings *et al.*, 2001)**. This study aimed to analyze the evolutionary trends in gonadal maturation of the female brown trout, explore the stages and incidence of hermaphroditism, and contribute to conservation efforts by deepening the understanding of these reproductive processes. Ultimately, this knowledge would aid in the sustainable management of fish populations and the preservation of their ecological roles in the environment.

MATERIALS AND METHODS

Sample collection and biometric measurements

A total of 95 brown fish specimens were collected for this study. Each specimen was measured for total length and weight. Total lengths ranged from 19.5 to 41.5cm, and weights ranged from 107 to 1500g. Additionally, gonad weights ranged from 0.31 to 251.5g.

Gonadosomatic index calculation

Gonad function, a metric reflecting gonadal investment relative to body size, was calculated using the formula:

$$\text{Gonad function (\%)} = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Gonad preparation and histological processing

– Gonads were preserved in 10% formalin for 24 hour immediately after collection to fix the tissues and prevent degradation.

– Dehydration of the gonads was carried out through a series of increasing concentrations of ethanol (50 -70 -80 -90 -100%). Each stage of dehydration lasted for one hour, allowing the tissues to gradually lose moisture and prepare for subsequent histological processing.

– Following dehydration, gonads were cleared in xylene to remove ethanol and facilitate infiltration with paraffin wax at 60ºC.

– Embedded gonads in paraffin blocks were sectioned at a thickness of 7µm using a rotary microtome. The sections were mounted on glass slides for subsequent staining.

– **Histological staining and examination**

Sections of gonadal tissue were stained with a combination of hematoxylin and eosin (H&E) dyes. Hematoxylin stains nuclei a blue-purple hue, while eosin imparts shades of pink to the cytoplasm and extracellular matrix **(Bancroft & Gamble, 2008)**. Stained sections were then examined under a Leica imaging microscope at various magnifications to assess the stages of maturation in female gonads. The diameters of oocytes were measured and categorized to determine the developmental stages of oocyte maturation, enabling a detailed understanding of the reproductive cycle and health of the species. This histological approach provides critical insights into the reproductive biology of *Mesopotamichthys sharpeyi*, facilitating an effective management and conservation strategies.

Analysis of sexual maturity stages

Histological examination allowed for the identification and classification of stages of sexual maturity in the female brown fish gonads based on established criteria. These stages included oocyte development and follicular characteristics indicative of reproductive readiness.

Data analysis

The image analysis software Image J (NIH) was used for quantification measurements of oocyte diameters as well as other histological properties. Statistical analyses were performed using SPSS software version 25. Descriptive statistics summarized the data while histograms illustrated distribution patterns for gonad function indices and oocyte sizes. ANOVA was used to compare mature versus immature versus hermaphroditic gonads with a significance level set at $P< 0.05$.

RESULTS

1. Biometric characteristics

The brown trout specimens examined in this study displayed a range of total lengths from 19.5 to 41.5cm, with corresponding weights ranging from 107 to 1500g. Gonad weights varied widely, ranging from 0.31 to 251.5g.

2.Gonadosomatic index

The calculated gonad function, expressed as a percentage of gonad weight relative to body weight, showed variations across the sampled population. Specimens exhibited gonad functions ranging from 0.37% in August to 12.98% in March (Fig.1).

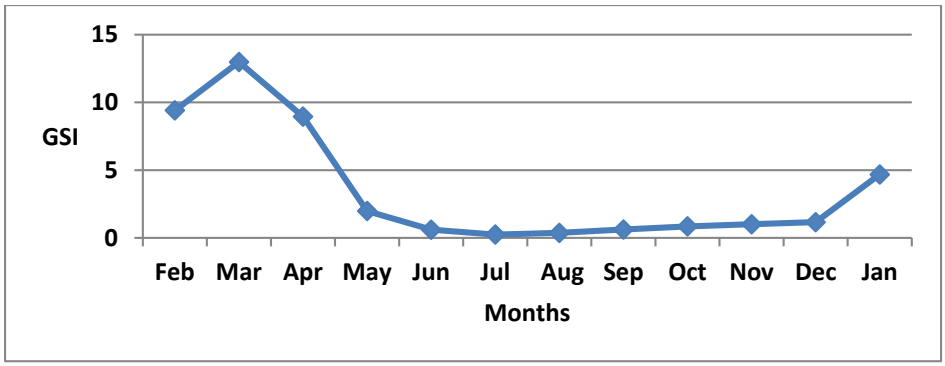


Fig. 1. Monthly variations in gonsadosomatic index (GSI) of *M. sharpeyi*

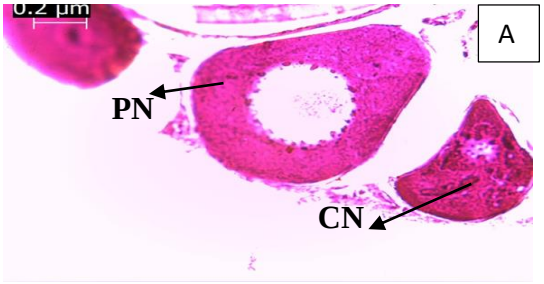
3.The Stage of maturity of the gonad

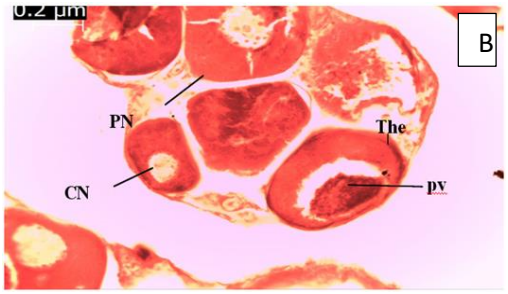
Histological analysis revealed distinct stages of maturation in the female brown trout gonads. Immature, resting, maturingmature, gravid and post-spawning phases represented conditions based on oocyte morphology as well as other cellular features (Table 1).

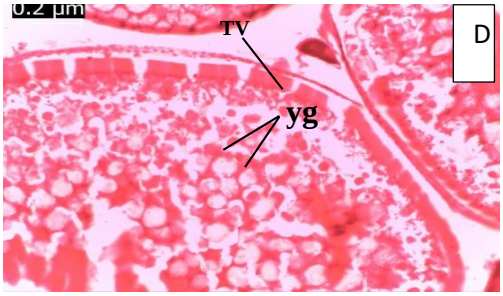
Table 1. Morphological and histological description of the stage of maturity of female *M. sharpeyi* (Bunni)

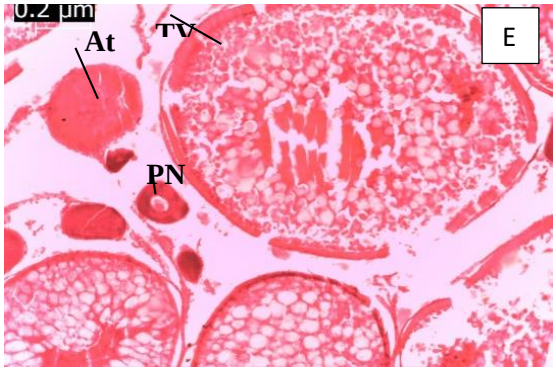
Maturity stage	Morphological description of the ovary	Histology description of the ovary
I Immature	Ovary was very thin. sweating and specialization not visible to the naked eye, and it occurred during June, August, September, and October	Showed chromatin nuccleolar oocytes, appeared perinucleolar oocytes and oogonia (Fig. 2A).
II Resting	The Ovaries are small, serni transparent and difficult to distinguish with the naked eye. The yoccupy a quarter of the body cavity for most months of the year.	Oocytes are small, showing Primary vitellogenesis chromatin nucleolar oocytes and perinucleolar oocytes, which are characterized by their large size and increased numbers of nuclei distributed on the outer – periphery of the cell envelope granulosa (Fig. 2B).
III Maturing	The size and weight of the ovaries increased and occupied a third to half of the so-called cove. The color of the gonads in the ovary was reddish and later became green, interspersed with small black spots on the outer surface. This was found during November, December – January.	Primary vitellogenesis, they showed clear, changes from the pervious cells, represented by the fact that the cytoplasm contained many vitelline vessels (yolk vescilies). The secondary vitellogenesis cells showed clear structural changes represented by an increase in the spread of yolk vessels within the cytoplasm (Fig. 2C).
Iv mature	The ovary occupied a large part of the body cavity, and its identification and lobulation became very clear; this was found during the months of February, March, and April.	Increase in tertiary oocytes, characterized by an increase in the number of yolk granules and prevailing (Fig. 2D, E).

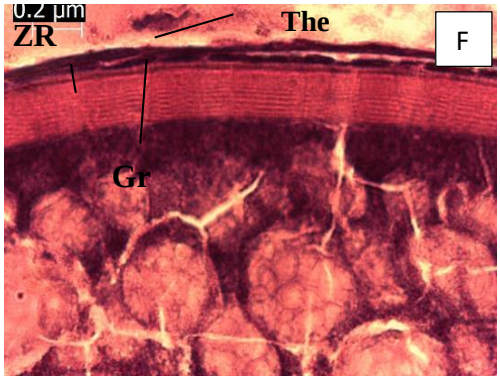
V Gravid	The ovaries extend along the body cavity and are yellow, orange in color, and found in March & April.	Translucent oocytes show a greater predominance; the nucleus appears to move toward the egg wall (Fig. 2F, G).
VI post spawning	The ovaries appeared shriveled, shrunken, and irregular in shape, and their size was less than that recorded in the previous stage, and the decreases disappeard, with a dark red color, and they were found during April & May.	Tissue sections appear as aborted cells which are characterized by their irregular shape and broken structure (Fig. 2H).

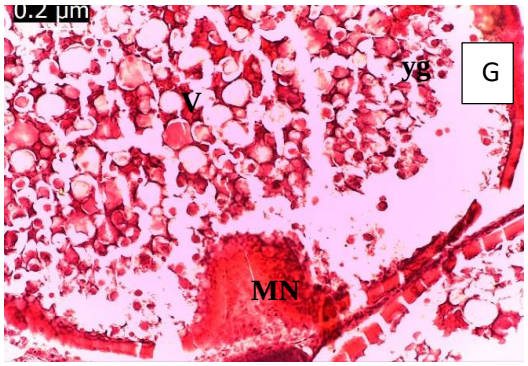












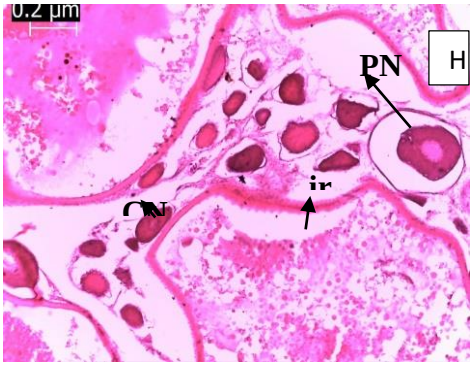


Fig.2. Photomicrograph of the *Bunni* ovaries showing: **A.** Immature stage displaying nests of chromatin- nucleolar oocyte (CN), perinuclear oocyte (PN)**;B.** Resting stage showingthe primary vitelline cell (pv) chromatin- nucleolar oocytse (CN) and oocyte wall (OW);**C.** Maturation stage showing secondary vitelline cells (sv), primary vitelline cells (pv), and perinuclear oocytes (pN); **D.** Maturity stage showing the tertiary vitelline cells (TV) and the vitelline granules (yg); **E.** Maturity stage showing tertiary vitellogenesis (T), perinuclear oocytes (PN) and atretic cells (At)**; F**. Gravid stage with the cell covering and surrounding the transparent oocytes, showing the outer wall (The), the cell envelope (Gr), and the acellular layer (ZR); **G.** Gravid stage where the nucleus appears to move toward the cell wall (MN), the vacuoles (V) and the yolk granules (yg), and **H.** Post spawning stage showing cells irregular in shape (ir), chromatin-nucleolar oocytes (CN), and perinuclear oocytes (pN)

Occurrence of hermaphroditism

A few brown trout tested for histology also exhibited hermaphroditic gonads. In approximately 12% of samples analyzed, both testicular and ovarian tissues were found in single gonad.

Stages of hermaphroditic development

Early hermaphroditism

In this phase, the gonads primarily consist of ovarian tissue, accompanied by a few developing lobules. The testicular lobules contain spermatogonia and primary spermatocytes, indicating the initial stages of sexual differentiation **(Sadovy de Mitcheson & Liu, 2008)** (Fig. 3A).

Transitional hermaphroditism

During this stage, the gonads exhibit an almost equal distribution of ovarian and testicular tissues. Both maturing sperm and developing ova/eggs are present, indicating an active gametogenesis in both sexes **(Devlin & Nagahama, 2002)** (Fig. 3B).

Late hermaphroditism

In the late-stage of hermaphroditism, there is a noticeable predominance of testicular tissue over the ovarian tissue. While very few oocytes may be observed, mature spermatozoa can be found in the sectioned seminal vesicles, reflecting the advanced development of male reproductive capabilities **(Sadovy de Mitcheson & Liu, 2008)** (Fig. 3C, D).

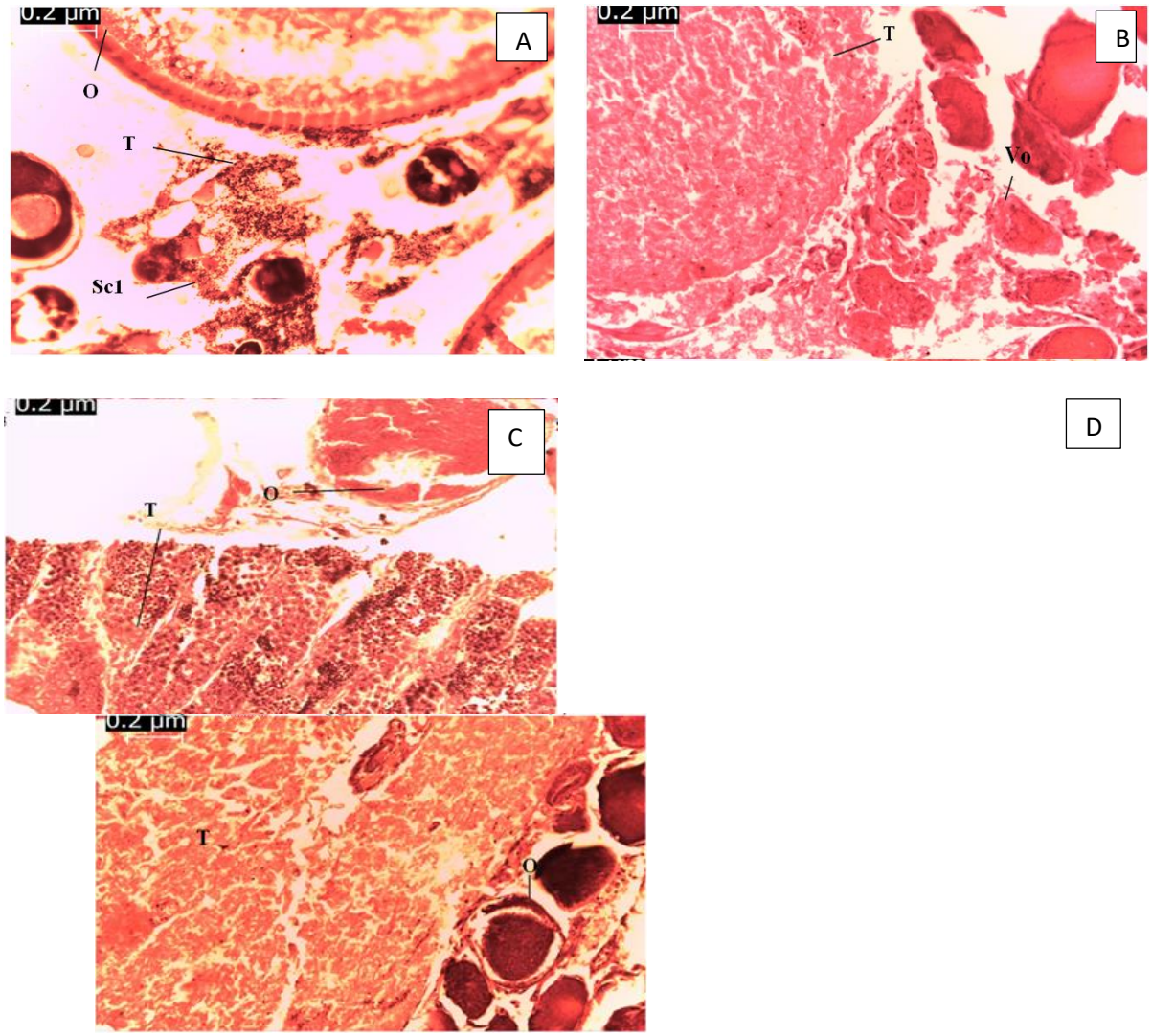


Fig. 3. Photomicrograph of the *Bunni* showing: **A.** Early hermaphroditism showing an ovary (O) with testicular tissue (T) containing spermatogonia (Sg) and primary spermatocytes (Sc1); **B.** Transitional stage displaying maturing testicular tissue (T) alongside vitellogenic oocytes (Vo); **C.** Late hermaphroditism characterized by a predominance of testicular tissue (T) and a mature ovary (O)**; D.** Another view of late hermaphroditism, again showing a greater amount of testicular tissue (T) compared to the mature ovary (O)

Quantitative measurements

Different stages of maturation were measured for oocyte diameters. Immature oocytes had an average diameter of 20-30µm, ranging from 50-70µm in maturing oocytes and measuring between 100-150µm for mature ones. In post-spawning gonads, atretic oocytes varied in size but were generally larger than immature ones due to the accumulation of yolk before resorption.

The gonad function index was significantly higher in mature and maturing gonads compared to immature and post-spawning stages ($P< 0.05$). However, hermaphroditic gonads had a wide range of gonad function indices since the proportions of ovarian and testicular tissues are different.

DISCUSSION

This study focused on the complex process of gonadal maturation in the female brown trout (*Mesopotamichthys sharpeyi*), examining the various stages of hermaphroditism for this species. The findings reveal the intricacies of these fishes' reproductive strategies, influenced by both genetic and environmental factors. Detailed insights into the progression from immature to mature oocytes during their growth and spawning periods were provided. The presence of dual sex within individuals may serve as an adaptive mechanism, enhancing breeding success across varying ecological conditions. This flexibility allows them to assume different reproductive roles based on social context and environmental factors.

Histological examinations indicate that ripe gonads contain well-formed eggs, signifying readiness for breeding. The study confirms that the spawning season for the bunni fish in southern Missan occurs from March to April, aligning with the findings of **Abdullah and Al-Zaidy (2022)**. The gonadosomatic index (GSI) is highlighted as a significant physiological indicator of reproductive success and spawning behavior. Higher GSI values in mature individuals reflect substantial reproductive readiness. Dominant individuals may also change sex to maintain reproductive control within groups. The maturity stages described in this study differ from those reported by **Mustafa *et al.* (2006)** but are consistent with observations of **Bawazin and Ali (2012)**, potentially due to geographical and ecological differences between the southern Missan and Al-Huwaizah marshes.

Quantitative data on oocyte diameters and gonad indices provide a solid foundation for future studies investigating factors affecting gonad development. Significant differences were noted between mature and immature phases, representing varying levels of reproductive investment at different ovarian developmental stages. Such findings enhance our understanding of the reproductive biology of the brown bunni fish, facilitating the development of strategies for their conservation and management.

Environmental influences, such as water quality and temperature, are critical during gonadal growth and maturation. This study demonstrates that these factors significantly impact reproductive success, potentially affecting population dynamics. Temperature is a well-known environmental clue influencing sex differentiation, alongside density, pH, and hypoxia, which can also alter sex ratios in various fish species **(Baroiller *et al*., 2009)**. Understanding the adaptive nature of hermaphroditism in response to environmental stressors necessitates a comprehensive research into hormonal and other influences.

By studying the reproductive biology of the brown fish, we identified various stages of sexual organ readiness and the occurrence of hermaphrodites. These discoveries enhance our knowledge of fish reproduction under diverse environmental conditions. Further research is needed to investigate the genetic and ecological factors influencing these processes, ultimately

aiding in effective conservation measures. Immature oocytes are susceptible to various environmental influences, such as temperature, photoperiod, and nutrients, through hormonal control. Identifying optimal conditions for early reproduction is essential, as mature oocytes exhibit a germinal vesicle displaced by yolk granules, indicating readiness for fertilization.

The presence of mature oocytes offers insight into the reproductive status of fish populations. **Moslemi-Aqdam *et al.* (2016)** found that these cells signify the fish's readiness to spawn, contributing to our understanding of reproductive cycles and fecundity. **Avis and Mank (2009)** noted that the size and age at which sex changes occur can vary within the same species and are not solely genetically controlled; behavioral and social factors also play a role **(Munday *et al.*, 2006)**. Understanding sex change patterns is crucial for fishery management, as hermaphroditic species are particularly vulnerable to overfishing, which can disrupt sex ratios and impair spawning behavior **(Molloy *et al.*, 2007)**.

Teleost fishes exhibit the most diverse reproductive strategies among vertebrates, uniquely displaying hermaphroditism characterized by the presence of both male and female reproductive functions in a single individual **(Sadovy de Mitcheson & Liu, 2008)**. For hermaphroditic species, understanding the onset of oogenesis is essential. The presence of both male and female gametes during early development indicates that changing environments may influence reproductive roles, enhancing survival in various conditions.

CONCLUSION

The findings of this study highlight the complexity of the female brown trout gonadal maturation, including the occurrence of hermaphroditism during this process.The reproductive strategies for the brown fish that are influenced by factors such as environment factors and genetic predisposition can be understood through the discrete stages in which oocyte development takes place.

The presence of hermaphroditism suggests a potential adaptive mechanism for reproductive success in variable environments. Histological observations have been provided with quantitative data on oocyte diameters and gonad function indices that will form basis for further research into physiological as well as environmental factors affecting gonadal development of the brown fish. This study has broader implications for understanding fish reproduction, contributing valuable knowledge to conservation and management plans for population control purposes, while these species are still available.

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