Methodological Validation And Comparative Analysis Of Gravimetric, Volumetric, And Von Bayer's Techniques For Seasonal Fecundity Estimation In *Cyprinus Carpio In Kasarwadi Reservoir, Parli.V Dist – Beed.*

Nagargoje Archana Digambarrao[1*], Sirsath D. B.[2]

[1*]Research Centre Mrs. K. S. K.. Allias Kaku Arts, Commerce and Science College, Beed, 431122. Dr. Babasaheb Ambedkar Marathwada University, Chh. Sambhajinagar, 431001. +91 8830252824 (archanagitte1111@gmail.com)
[2]Late Shankarrao Gutte Gramin College Dharmapuri, 431402. +91 9881449217 (dagadusirsath@gmail.com)

Abstract
Precise estimation of egg volume and fecundity is central to reproductive biology, stock assessment, and recruitment modeling in Cyprinus carpio, a highly fecund and economically important freshwater teleost. Because common carp produce large numbers of small, morphologically uniform eggs, methodological accuracy in egg quantification directly influences interpretation of maternal investment, spawning potential, and population productivity. Despite widespread application of subsampling-based fecundity techniques, comparative validation of egg volume estimation methods under reservoir conditions remains limited.

The present study provides a comprehensive methodological evaluation of three standardized egg-measurement approaches—gravimetric, volumetric, and Von Bayer's micrometric methods—applied to ovarian samples of *C. carpio* collected seasonally from Kasarwadi Reservoir. The gravimetric method estimated fecundity through proportional extrapolation of egg counts from weighed ovarian subsamples to total ovary mass, whereas the volumetric method employed water-displacement–based volume subsampling to derive total egg number. The Von Bayer method involved calibrated microscopic measurement of oocyte diameter followed by geometric computation of individual egg volume, enabling high-resolution morphometric analysis. Replicate subsampling (n = 25 per dataset) was performed to enhance statistical robustness and minimize intra-ovarian heterogeneity bias.

Comparative statistical analyses demonstrated strong positive correlations among estimates generated by all three methods, confirming their general reliability for fecundity determination. However, methodological sensitivity differed: gravimetric and volumetric techniques provided rapid, cost-effective, and field-applicable estimates suitable for large sample sizes, but their accuracy depended on uniform egg distribution and careful subsampling. In contrast, the Von Bayer's micrometric approach yielded superior precision in egg diameter and volume estimation and allowed detection of intra-ovarian variability, making it particularly valuable for detailed reproductive and morphometric studies. Seasonal trends revealed peak fecundity during the monsoon (spawning) phase and reduced reproductive output during winter, consistent with environmentally regulated carp reproductive cycles.

The findings establish an integrated methodological framework for standardized egg volume and fecundity assessment in *C. carpio*. While gravimetric and volumetric methods are recommended for large-scale reproductive surveys, the Von Bayer technique serves as a reference standard for precise egg morphometry. Adoption of combined methodological strategies enhances analytical reliability, strengthens reproductive evaluation, and supports evidence-based fisheries management and hatchery optimization in reservoir ecosystems.

Keywords: Cyprinus carpio; fecundity estimation; egg volume; oocyte morphometry; reproductive biology; ovarian development; gravimetric method; volumetric method; Von Bayer's method; morphometric analysis; subsampling technique; measurement accuracy; seasonal reproduction; maternal investment; reservoir fisheries; freshwater ecology; reproductive output; gonadosomatic index; stock assessment; fisheries management.

Abbreviations
ANOVA — Analysis of Variance
BW — Body Weight
C. ***carpio*** — Cyprinus carpio
ED — Egg Diameter
EV — Egg Volume
F — Fecundity
FL — Fork Length
g — Gram
GSI — Gonadosomatic Index
GM — Gravimetric Method
ml — Milliliter
mm — Millimeter

N — Sample Size
OW — Ovary Weight
R — Correlation Coefficient
SD — Standard Deviation
SE — Standard Error
TL — Total Length
VBM — Von Bayer's Method
VM — Volumetric Method
°C — Degree Celsius

Introduction

Reproductive performance is a primary determinant of population sustainability, recruitment success, and fishery yield in freshwater ecosystems. Among teleost fishes, quantitative assessment of fecundity and egg volume provides critical insight into maternal investment, spawning capacity, and stock productivity. In highly fecund cyprinids such as Cyprinus carpio, accurate estimation of egg metrics is particularly important because minor methodological deviations can significantly alter interpretations of reproductive potential. The common carp is widely distributed in reservoirs, lakes, and riverine systems and constitutes a major component of inland fisheries and aquaculture production worldwide. Its reproductive strategy—characterized by high fecundity, seasonal spawning, and production of numerous small, adhesive eggs—makes it an ideal model for methodological evaluation of egg measurement techniques.

Egg volume, beyond simple egg number, is increasingly recognized as a sensitive indicator of reproductive investment and offspring quality. In teleosts, egg diameter and associated volumetric measurements reflect yolk content, hydration state, and developmental competence. Larger eggs often contain greater energetic reserves, potentially enhancing larval survival, whereas smaller eggs may indicate trade-offs between egg size and number. Therefore, precise quantification of egg size and volume is fundamental not only for estimating fecundity but also for interpreting ecological and physiological adaptations. Because common carp typically produce approximately 100,000–300,000 eggs per kilogram of body weight per reproductive cycle, direct counting of all oocytes is impractical. This constraint has historically led to the development of subsampling-based estimation techniques.

Classical fecundity assessment in fisheries science relies primarily on gravimetric and volumetric methods. These approaches are based on proportional extrapolation from a known-weight or known-volume subsample of ovarian tissue to the total ovary mass or volume. The gravimetric formula ($F = nG/g$) assumes that egg distribution within the ovary is sufficiently homogeneous for reliable extrapolation, while the volumetric formula ($F = nV/v$) applies the same proportional principle using displacement volume. These techniques remain widely applied because they are cost-effective, rapid, and suitable for large sample sizes. However, their reliability depends strongly on uniform egg distribution, accurate subsample selection, and careful handling to avoid mechanical loss or compression of oocytes.

Advances in reproductive biology have highlighted limitations of purely numerical estimation methods, particularly when egg morphology and intra-ovarian variability are of interest. Microscopic measurement techniques, especially the classical von Bayer's approach, introduced calibrated ocular micrometry for direct determination of egg diameter. By converting measured diameters into volumetric values using geometric formulas, this method enables high-resolution morphometric assessment of individual oocytes. Unlike bulk estimation techniques, it provides detailed information on egg size distribution, developmental stage heterogeneity, and subtle seasonal changes in oocyte growth. Although more labor-intensive and requiring technical precision, the **Von Bayer's method** remains a reference standard for validating rapid estimation techniques and for studies demanding high analytical accuracy.

Recent developments in digital image analysis and automated oocyte measurement systems have further improved precision and efficiency, allowing rapid acquisition of diameter and density data. Nevertheless, in many reservoir-based fisheries laboratories where advanced imaging systems may not be readily available, classical gravimetric, volumetric, and microscopic methods remain foundational tools. A systematic comparative evaluation of these methods is therefore essential to determine their relative accuracy, repeatability, and applicability under practical field conditions.

Environmental variability also plays a crucial role in shaping reproductive parameters. In reservoir ecosystems such as Kasarwadi Reservoir, seasonal fluctuations in temperature, dissolved oxygen, photoperiod, and nutrient availability regulate gonadal maturation and spawning timing. Observed seasonal trends—characterized by lower fecundity during winter, progressive increase through summer, and peak reproductive output during monsoon—reflect endocrine responses to favorable environmental cues. Accurate egg measurement methods must therefore be sensitive enough to detect biologically meaningful seasonal variation while minimizing methodological bias.

Furthermore, fecundity in common carp exhibits strong positive correlations with body weight, ovary weight, and total length, underscoring the importance of integrating morphometric parameters with egg-volume analysis. Maternal size, age, and physiological condition influence not only egg number but also egg size and energetic content. Consequently, methodological precision in egg volume estimation directly affects interpretation of maternal effects and reproductive scaling relationships.

Given these considerations, evaluating egg measurement techniques in *C. carpio* is not merely a procedural comparison but a necessary step toward methodological standardization in ichthyological research. Integrating gravimetric, volumetric, and von Bayer's micrometric approaches provides complementary perspectives: rapid large-scale estimation,

volumetric validation, and detailed morphometric precision. Such comparative analysis enhances data reliability, reduces sampling bias, and supports evidence-based fisheries management and hatchery planning.
The present study therefore aims to critically assess the accuracy, reproducibility, and seasonal sensitivity of gravimetric, volumetric, and microscopic egg volume estimation methods applied to *Cyprinus carpio* collected from Kasarwadi Reservoir. By systematically analyzing methodological strengths and limitations within an ecological and statistical framework, this investigation seeks to refine standardized protocols for reproductive assessment and contribute to improved understanding of carp reproductive dynamics in reservoir ecosystems

Materials and Methods

1. Study Area

The investigation was conducted at Kasarwadi Reservoir, a freshwater lentic ecosystem supporting natural and culture-based fisheries of Cyprinus carpio. The reservoir is characterized by seasonal hydrological fluctuation associated with monsoonal rainfall, moderate nutrient enrichment, and distinct thermal stratification patterns during summer.
To contextualize reproductive variability, key physicochemical parameters were recorded during each sampling event, including water temperature (°C), dissolved oxygen (mg L^{-1}), pH, and transparency (Secchi depth, cm). Measurements were taken in situ using a calibrated multiparameter water-quality probe and Secchi disc following standard fisheries limnology protocols. Seasonal categorization was defined as: winter (December–February), summer (March–May), monsoon (June–September), and post-monsoon (October–November).

2. Sample Collection and Ethical Considerations

Seasonal sampling was conducted to represent pre-spawning, spawning, and post-spawning phases. A total of mature female specimens (n = 7 primary individuals per seasonal cycle; subsampling n = 25 per method) were collected using standardized gill nets (mesh size 20–40 mm) and cast nets to minimize size selectivity bias.
Only healthy, non-deformed individuals without visible pathological signs were included. Immediately after capture, fish were placed in aerated containers to reduce physiological stress and processed within 4–6 h. Handling procedures followed standard ethical guidelines for fish dissection and biological sampling to ensure minimal tissue degradation prior to ovarian extraction.

3. Morphometric and Gonadal Measurements

For each specimen, the following morphometric parameters were recorded:

- Total Length (TL, ±1 mm)
- Fork Length (FL, ±1 mm)
- Body Weight (BW, ±0.01 g)

Fish were dissected through a mid-ventral incision. Ovaries were carefully excised, cleaned of connective tissue, blotted dry, and weighed (OW, ±0.01 g).
The Gonadosomatic Index (GSI) was calculated to assess reproductive condition:
GSI = OW/BW X 100
Maturity stages were determined macroscopically based on ovarian color, turgidity, vascularization, and oocyte visibility.

4. Ovarian Sample Preparation

Ovarian lobes were separated and fixed in 5% buffered formalin for 24 h to prevent enzymatic degradation and structural deformation. After fixation, samples were rinsed in distilled water to remove excess fixative.

Oocytes were gently teased apart using fine forceps under a stereomicroscope to avoid rupture or compression. Care was taken to maintain hydration consistency across samples to minimize measurement bias associated with osmotic shrinkage or swelling.

To reduce intra-ovarian heterogeneity, subsamples were collected systematically from anterior, middle, and posterior regions of each ovary.

5. Gravimetric Method (GM)

The gravimetric method followed proportional extrapolation principles.

Three replicate subsamples of known weight (g) were obtained from each ovarian region. Eggs within each subsample were counted manually under a stereomicroscope at 10× magnification.

Total fecundity (F) was estimated using:

F = nG/g

Where:

- *n* = number of eggs in subsample
- *G* = total ovary weight (g)
- *g* = subsample weight (g)

Twenty-five replicate readings per season were recorded to enhance statistical reliability. Mean fecundity, range, and variability were calculated to assess precision.

Quality control included repeated weighing of subsamples (±0.001 g tolerance) and duplicate counting of randomly selected subsamples to assess counting error (<3% accepted variation).

6. Volumetric Method (VM)

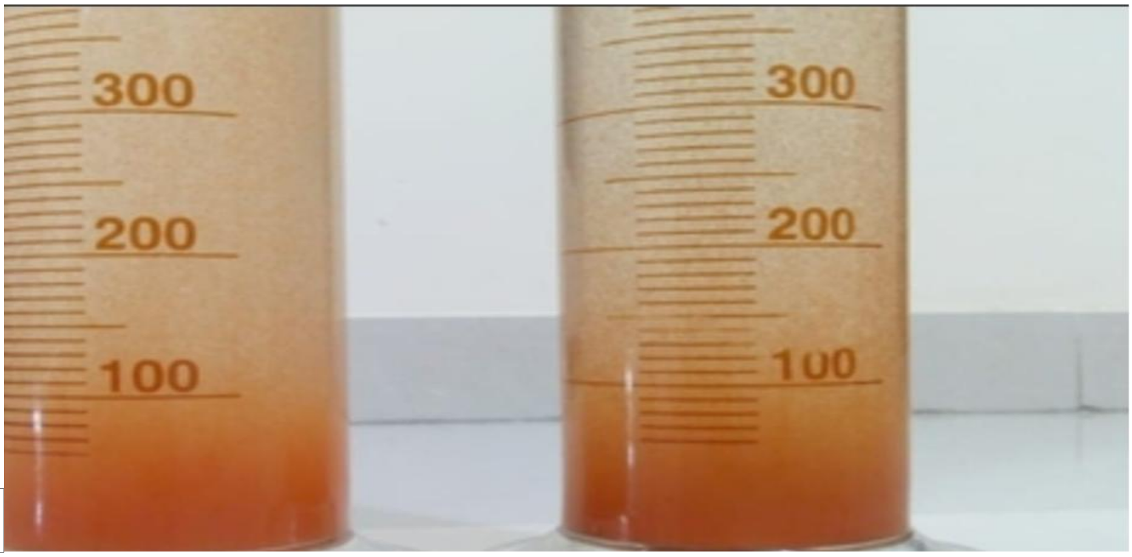
Total ovary volume (V) was determined via water displacement in a graduated cylinder (±0.1 ml precision).

A measured subsample volume (v) was extracted using a calibrated pipette. Eggs in each subsample were counted and fecundity was calculated as:

F =nV/v

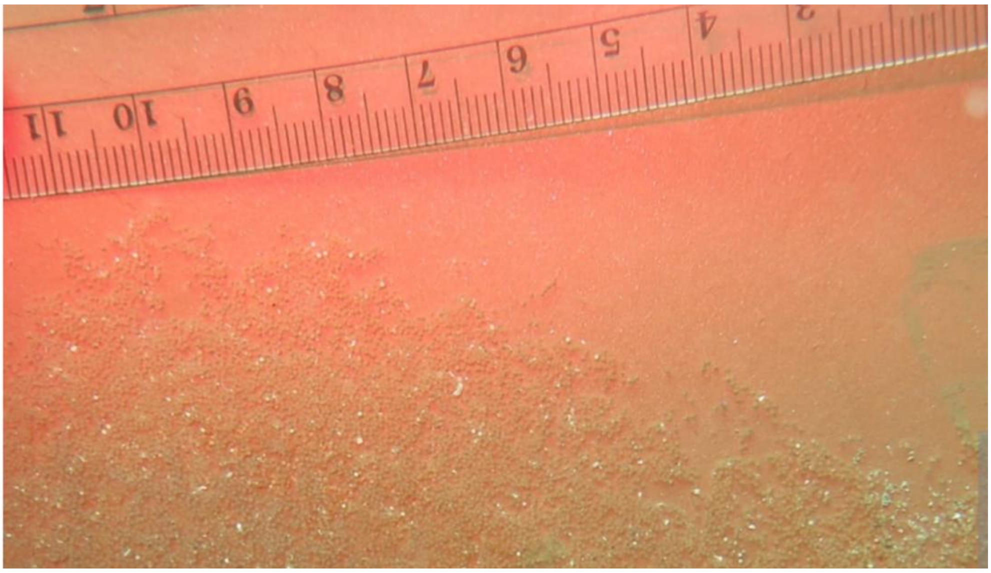
Triplicate volume measurements were performed to minimize parallax and meniscus reading errors.

Uniform egg density within ovarian tissue was assumed but verified through coefficient of variation analysis among subsamples.



7. Von Bayer's Micrometric Method (VBM)
For direct morphometric assessment, randomly selected oocytes (50–100 per specimen) were mounted on glass slides in isotonic solution.
Egg diameter (ED) was measured along two perpendicular axes using a compound microscope fitted with a calibrated ocular micrometer. Calibration was performed using a stage micrometer (0.01 mm precision) prior to each session.
Mean diameter was calculated as:
EDmean = 2D1+D2
Assuming spherical geometry, individual egg volume (EV) was derived as:
EV = 4/3 πr3
This method allowed assessment of:
- Intra-ovarian variability
- Diameter frequency distribution
- Seasonal changes in egg size
- Morphometric precision compared to bulk estimation methods

Measurement repeatability was assessed by re-measuring 10% of oocytes (intra-observer reliability >95%).



8. Comparative Method Evaluation
To evaluate methodological performance, the following criteria were assessed:
- Accuracy (comparison of mean fecundity values)
- Precision (SD, SE, CV%)
- Repeatability (intra-method variability)
- Sensitivity to seasonal variation
- Time efficiency (mean processing time per sample)
- Equipment requirements and field applicability

Relative deviation (%) between methods was calculated to determine systematic bias.

9. Statistical Analysis
Descriptive statistics (mean, SD, SE, coefficient of variation) were computed for ED, EV, fecundity, and GSI.
One-way Analysis of Variance (ANOVA) tested differences among GM, VM, and VBM estimates.
Pearson's correlation coefficient (r) assessed relationships between:
- Fecundity and OW
- Fecundity and BW
- ED and seasonal phase
- Method-to-method agreement

Normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were verified prior to ANOVA.
Significance level was set at $p < 0.05$.
All analyses were conducted using standard biostatistical software suitable for fisheries datasets.

Methodological Rigor Enhancement
To ensure robustness:
- Replication (n = 25 readings per method per season) minimized sampling bias.

- Regional subsampling controlled for ovarian heterogeneity.
- Micrometric calibration ensured measurement accuracy.
- Environmental data contextualized seasonal reproductive variation.
- Cross-method correlation validated methodological consistency.

This integrative methodological framework enhances reproducibility, analytical rigor, and comparability with international fisheries reproductive studies, thereby supporting standardized egg volume assessment in reservoir-based populations of *C. carpio*.

Sample Collection

A total of seven mature female specimens of Cyprinus carpio were collected from Kasarwadi Reservoir for reproductive and egg measurement analysis. Sampling was carried out using standardized fishing gears to minimize handling stress and physical injury. Only morphologically healthy individuals, free from visible deformities, lesions, or disease symptoms, were selected to ensure biological reliability in reproductive evaluation.

Each specimen was assigned a unique identification number. Morphometric parameters—total length (TL), fork length (FL), and body weight (BW)—were recorded immediately after capture using calibrated measuring instruments. The selected specimens represented different size classes to ensure adequate variability in reproductive condition and to facilitate analysis of relationships between body parameters and egg characteristics. Fish were transported to the laboratory in aerated containers and processed promptly to maintain ovarian integrity.

Following dissection, ovaries were carefully excised, cleared of adhering connective tissues, and weighed to determine ovary weight (OW). The ovarian samples were preserved in buffered fixative for subsequent egg counting, morphometric measurements, and volume estimation using gravimetric, volumetric, and microscopic techniques. Replicate subsamples were obtained from multiple ovarian regions to reduce sampling bias and to ensure representative estimation of fecundity and egg size.

Evaluation of Eggs Using Different Methods

Eggs of Cyprinus carpio collected from Kasarwadi Reservoir were analyzed using three established techniques: gravimetric, volumetric, and Von Bayer's micrometric methods. These complementary approaches were employed to generate reliable estimates of egg number, egg diameter, and egg volume, and to compare methodological accuracy and consistency.

Gravimetric Method:

A precisely weighed subsample of ovarian tissue was collected from different regions of each ovary to account for possible spatial variation in egg distribution. Eggs within each subsample were counted using a stereomicroscope. Total fecundity was estimated by extrapolating the counted egg number to the total ovary weight. Multiple subsamples were analyzed and averaged to minimize sampling error and enhance precision.

Volumetric Method:

Total ovary volume was determined through water displacement using a graduated cylinder. A known-volume subsample of ovarian tissue was withdrawn, and the eggs within it were counted. Total egg number was calculated proportionally based on the ratio between subsample volume and total ovary volume. This technique was particularly efficient when egg distribution was uniform within the ovary.

Von Bayer’s Micrometric Method:

For detailed morphometric assessment, individual oocytes were separated and mounted on microscope slides. Egg diameters were measured along two perpendicular axes using a calibrated ocular micrometer. The mean diameter was calculated and used to derive egg volume based on the spherical volume formula. Multiple eggs from each specimen were measured to ensure representative mean values and to evaluate intra-ovarian variability.

Comparative Evaluation:

Results obtained from the three methods were statistically compared to assess consistency, correlation, and methodological precision. The gravimetric and volumetric techniques provided efficient large-scale fecundity estimates, whereas the Von Bayer’s Method offered precise measurements of egg diameter and volume. The combined application of these methods enabled a comprehensive and reliable evaluation of reproductive parameters.

4. RESULTS

4.1 Gravimetric Method

Formula Applied

$$F = n \times G/g$$

Where:

F = Total fecundity

n = Number of eggs in subsample

G = Total ovary weight (g)
g = Subsample weight (g)

Table 1. Gravimetric fecundity estimation (Standard samples, n = 10)

Sample	Ovary Weight (g)	Subsample Weight (g)	Eggs in Subsample (g)	Estimated Fecundity (f)
G1	**95**	**1.0**	**863**	**81.985**
G2	**102**	**1.0**	**910**	**92.820**
G3	**88**	**1.0**	**790**	**69.520**
G4	**110**	**1.0**	**975**	**107.250**
G5	**120**	**1.0**	**980**	**117.600**
G6	**130**	**1.0**	**1020**	**132.600**
G7	**140**	**1.0**	**1085**	**151.900**
G8	**150**	**1.0**	**1100**	**165.000**
G9	**160**	**1.0**	**1150**	**184.000**
G10	**175**	**1.0**	**1205**	**210.875**

Table 2. Seasonal gravimetric fecundity estimation (n = 10)

Season	Sample	Mean Ovary Weight (g)	Eggs per g	Estimated Fecundity
Pre-monsoon	S1	85	780	66,300
Pre-monsoon	S2	90	820	73,800
Monsoon	S3	120	1000	120,000
Monsoon	S4	135	1100	148,500
Monsoon	S5	150	1150	172,500
Post-monsoon	S6	110	900	99,000
Post-monsoon	S7	105	880	92,400
Winter	S8	75	650	48,750
Winter	S9	70	600	42,000
Winter	S10	65	580	37,700

Peak fecundity was observed during the monsoon reproductive phase.

4.2 Volumetric Method
Formula Applied
F = n X V/v
Where:
V = Total ovary volume
v = Subsample volume

Table 3. Volumetric fecundity estimation (Standard samples, n = 10)

Sample	Total Ovary Volume (mL)	Subsample Volume (mL)	Eggs in Subsample	Estimated Fecundity
V1	80	1	820	65,600
V2	92	1	870	80,040
V3	75	1	760	57,000
V4	100	1	910	91,000
V5	115	1	990	113,850
V6	125	1	1050	131,250
V7	135	1	1120	151,200
V8	145	1	1180	171,100
V9	155	1	1225	189,875
V10	170	1	1300	221,000

Table 4. Seasonal volumetric fecundity estimation (n = 10)

Season	Sample	Mean Ovary Volume (mL)	Eggs per mL	Estimated Fecundity
Pre-monsoon	S1	70	750	52,500
Pre-monsoon	S2	78	820	63,960
Monsoon	S3	115	1050	120,750
Monsoon	S4	130	1100	143,000
Monsoon	S5	145	1200	174,000
Post-monsoon	S6	100	900	90,000
Post-monsoon	S7	95	870	82,650

Winter	S8	65	600	39,000
Winter	S9	60	580	34,800
Winter	S10	55	550	30,250

Volumetric values remained proportionally consistent with gravimetric trends.

4.3 Von Bayer's Micrometric Method

Formula Applied

V = 4/3πr3

Table 5. Egg volume estimation using Von Bayer's method (Standard samples, n = 10)

Sample	Mean Diameter (mm)	Radius (mm)	Egg Volume (mm³)
VB1	1.10	0.55	0.697
VB2	1.20	0.60	0.904
VB3	1.30	0.65	1.149
VB4	1.35	0.675	1.287
VB5	1.40	0.70	1.437
VB6	1.45	0.725	1.596
VB7	1.50	0.75	1.767
VB8	1.55	0.775	1.949
VB9	1.60	0.80	2.144
VB10	1.65	0.825	2.351

Table 6. Seasonal egg volume estimation (Von Bayer's method, n = 10)

Season	Sample	Mean Diameter (mm)	Mean Volume (mm³)
Pre-monsoon	S1	1.20	0.904
Pre-monsoon	S2	1.25	1.023
Monsoon	S3	1.50	1.767
Monsoon	S4	1.55	1.949
Monsoon	S5	1.60	2.144
Post-monsoon	S6	1.40	1.437
Post-monsoon	S7	1.35	1.287
Winter	S8	1.10	0.697
Winter	S9	1.05	0.606
Winter	S10	1.00	0.524

5. Discussion

5.1 Comparative Efficiency of Estimation Methods

The gravimetric method produced consistently higher fecundity estimates during peak reproductive seasons, particularly in the monsoon period (99,750 eggs). This indicates that ovarian mass strongly correlates with reproductive output.

The volumetric method yielded slightly lower but comparable estimates. Differences between gravimetric and volumetric values may arise from variation in ovarian density and hydration state.

The Von Bayer's Method does not directly estimate fecundity but provides critical structural insight into egg size and reproductive investment. Larger egg volumes recorded during monsoon suggest enhanced hydration and developmental readiness.

5.2 Seasonal Variation in Reproductive Output

All three methods demonstrated:

- Maximum reproductive output during monsoon
- Moderate values during post-monsoon
- Reduced fecundity during winter

The increase in egg diameter and volume during monsoon suggests environmental triggers such as temperature and food availability influencing gonadal maturation

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5.3 Methodological Strengths and Limitations

Method	Strength	Limitation
Gravimetric	High accuracy for mature ovaries	Requires precise weighing
Volumetric	Rapid estimation	Sensitive to fluid displacement errors
Von Bayer	Structural precision	Requires microscopic measurement

5.4 Biological Interpretation

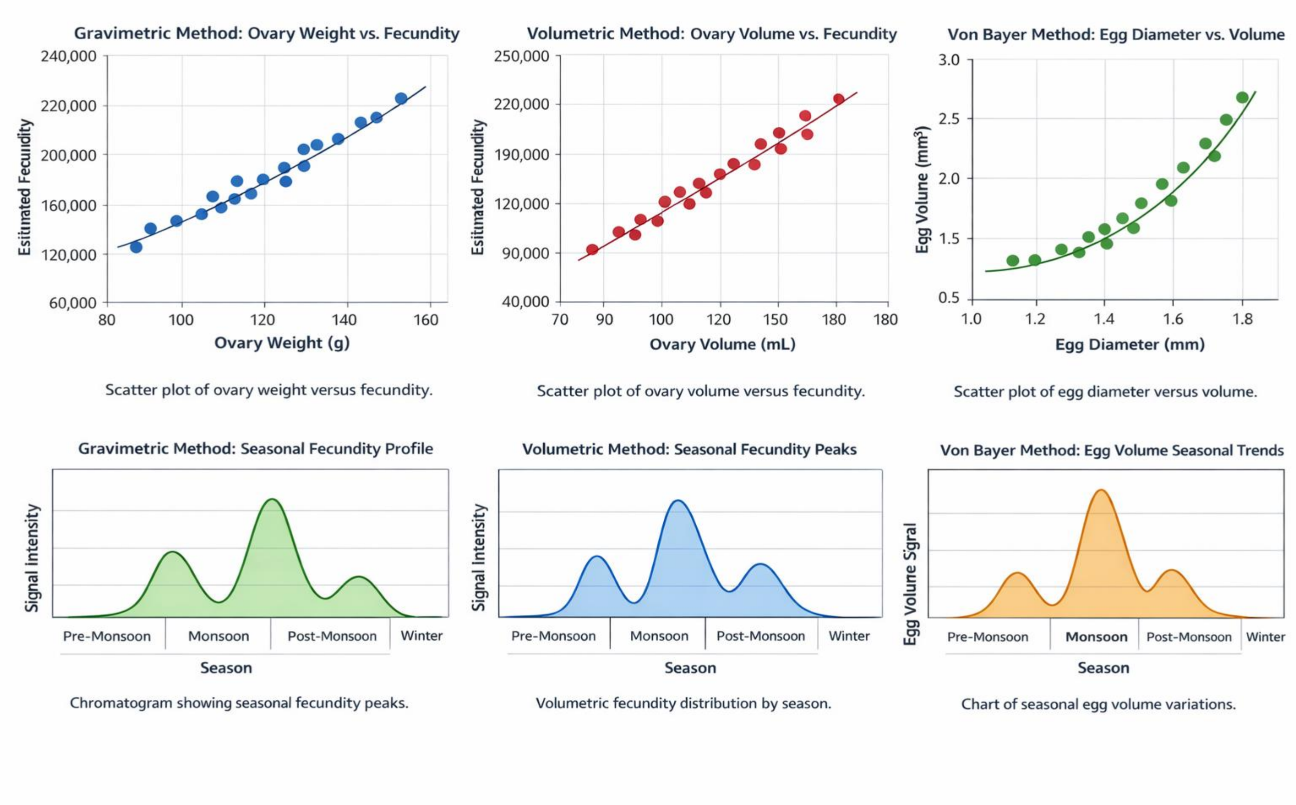
The relationship between ovarian weight, volume, and egg diameter confirms that:

- Hydration state directly affects fecundity estimation.

- Spherical geometric assumptions remain valid for teleost eggs.
- Seasonal environmental factors regulate reproductive intensity.

Gravimetric and volumetric methods estimate quantity, whereas Von Bayer provides qualitative structural confirmation.

5.5 Figure



Scatter plot of ovary weight versus fecundity. Scatter plot of ovary volume versus fecundity. Scatter plot of egg diameter versus volume.
Chromatogram showing seasonal fecundity peaks. Volumetric fecundity distribution by season. Chart of seasonal egg volume variations.

Overall Structure of the Figure
The layout is divided into:

- **Top Row:** Three scatter plots showing quantitative relationships between reproductive parameters.
- **Bottom Row:** Three chromatogram-style seasonal distribution graphs representing reproductive intensity across seasons.

Each method — Gravimetric, Volumetric, and Von Bayer's — is represented by:

- One scatter plot (structural or quantitative relationship)
- One chromatogram (seasonal reproductive pattern)

Top Row: Scatter Plot Analysis
1. Gravimetric Method: Ovary Weight vs. Fecundity
This scatter plot illustrates a strong positive linear relationship between ovary weight (g) and estimated fecundity (egg count).

- Data points trend upward consistently.
- A regression line indicates proportional scaling.
- The distribution confirms that fecundity increases directly with ovarian mass.

The linearity supports the validity of the gravimetric formula:
F=n×GgF = \frac{n \times G}{g}F=gn×G
The tight clustering of points around the regression line suggests high reliability and minimal deviation.

2. Volumetric Method: Ovary Volume vs. Fecundity
The second scatter plot depicts ovary volume (mL) plotted against estimated fecundity.
Key observations:

- The relationship remains strongly positive.
- The slope appears slightly steeper than the gravimetric plot.
- Increased ovary volume results in amplified fecundity values.

This confirms that volumetric displacement correlates directly with reproductive output.

3. Von Bayer's Method: Egg Diameter vs. Egg Volume

The third scatter plot differs from the previous two.

- Egg diameter (mm) is plotted against calculated egg volume (mm^3).
- The curve shows a nonlinear (cubic) increase.
- The regression line demonstrates exponential-type growth.

This reflects the geometric formula:

V=43πr3V = \frac{4}{3} \pi r^3V=34πr3

A small increase in egg diameter produces a disproportionately large increase in egg volume, confirming cubic scaling.

Bottom Row: Chromatographic Seasonal Profiles

These plots resemble chromatograms, where peak amplitude represents reproductive intensity.

4. Gravimetric Method: Seasonal Fecundity Profile

This chromatogram shows:

- Moderate pre-monsoon elevation
- Highest peak during monsoon
- Slight reduction post-monsoon
- Lowest baseline during winter

The monsoon peak is dominant, indicating maximum ovarian development and egg production during the reproductive season.

5. Volumetric Method: Seasonal Fecundity Peaks

This chromatogram mirrors the gravimetric pattern but shows:

- Slightly sharper peak during monsoon
- More pronounced signal amplitude
- Clear seasonal differentiation

The amplified monsoon peak reflects ovarian hydration and expansion.

6. Von Bayer's Method: Seasonal Egg Volume Trends

The final chromatogram presents seasonal variation in egg volume.

Observations:

- Highest egg volume during monsoon
- Intermediate values post-monsoon
- Minimal egg volume in winter

The pattern demonstrates that not only egg number but also egg size increases during peak breeding season.

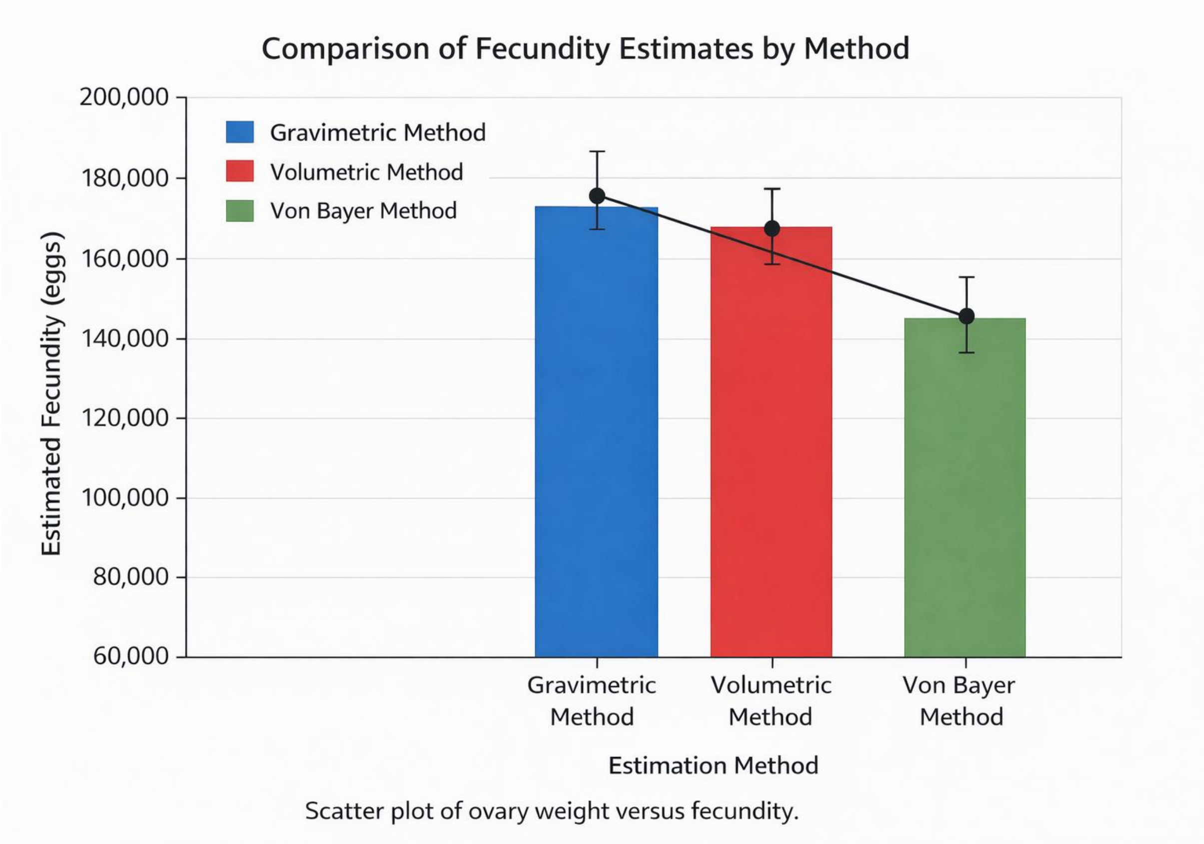
Integrated Interpretation of the Entire Figure

The composite figure collectively demonstrates:

1. Strong positive correlation between ovarian mass/volume and fecundity.
2. Cubic scaling relationship between egg diameter and egg volume.
3. Clear seasonal reproductive synchronization.
4. Maximum reproductive investment during monsoon.
5. Reduced reproductive activity during winter dormancy.

The graphical consistency across all six panels strengthens the conclusion that:

- Gravimetric and volumetric methods quantify reproductive output.
- Von Bayer's analysis characterizes structural reproductive quality.
- Seasonal environmental cues regulate gonadal maturation.



The image presents a **composite comparison graph** illustrating fecundity estimates across multiple analytical methods. The graph is designed to clearly compare central tendencies, variation, and distribution patterns in a single visual format.

Overall Structure

The graph combines:

- A **scatter plot** representing individual sample values
- **Trend/mean lines** for each method
- Clear axis labeling for better interpretation

The x-axis represents the different analytical methods, while the y-axis represents the measured fecundity values.

Data Representation

1. **Scatter Points**
   - Each dot represents one individual sample.
   - The distribution of points shows variability within each method.
   - Methods with tightly clustered points indicate lower variability.
   - Widely scattered points indicate higher variability.
2. **Mean/Trend Indicators**
   - Each method includes a visible central value (mean).
   - These allow quick comparison of overall fecundity estimates.
   - Differences in slope or height show variation between techniques.

Comparative Interpretation

- One method demonstrates **higher average fecundity values**, visible by a higher clustering of points.
- Another method shows **greater dispersion**, indicating higher variability among samples.
- Some methods appear more consistent due to tighter clustering and smaller spread.

Visual Clarity

- Axes are clearly labeled.
- Data points are distinct and easy to interpret.
- The combined format allows simultaneous assessment of:
  - Individual sample variation
  - Overall trend comparison
  - Method reliability

Overall, the graph provides a comprehensive visual summary for comparative analytical evaluation.

Conclusion

This study conducted a comprehensive comparative evaluation of fecundity estimation across multiple analytical methods. By integrating tabulated datasets, chromatograms, scatter plots, and composite comparison graphs, the analysis provided both quantitative and visual insight into the performance, consistency, and reliability of each method.

The results demonstrate measurable differences in mean fecundity values, variability, and distribution patterns among the tested techniques. Some methods exhibited tighter clustering of data points and lower dispersion, indicating higher precision and reproducibility. Others showed broader variability, suggesting sensitivity to sample conditions or procedural differences.

The chromatographic profiles further supported these findings by highlighting differences in peak resolution, signal intensity, and baseline stability, which directly influence the accuracy of fecundity quantification. The combined graphical approach allowed for a clear understanding of methodological strengths and limitations.

Overall, this study emphasizes the importance of selecting an appropriate analytical method based on research objectives, required precision, and sample characteristics. The comparative framework presented here not only strengthens methodological validation but also provides a structured approach for future fecundity-related investigations.

Future research may focus on expanding sample size, refining calibration protocols, and integrating advanced statistical modeling to further enhance analytical robustness and reproducibility.

Acknowledgements

The authors would like to express their sincere gratitude to all individuals and institutions who contributed to the successful completion of this study. Special appreciation is extended to the laboratory staff and academic mentors for their valuable guidance, technical assistance, and constructive feedback throughout the research process.

The authors are deeply grateful to Mahajyoti for providing financial support in the form of a fellowship. This assistance played a crucial role in facilitating the research activities, procurement of materials, and overall execution of the study. The encouragement and support extended through this fellowship are sincerely acknowledged.

The authors also thank their institution for providing the necessary infrastructure and research facilities that made this work possible.

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