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PRIMARY RESEARCH PAPER

Bathymetric asynchrony in the reproductive dynamics of quagga mussel (*Dreissena bugensis*) in a deep lake

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Abstract Freshwater invasions by Dreissenidae have profoundly altered aquatic ecosystems worldwide. The quagga mussel, *Dreissena bugensis*, is particularly successful due to its ability to colonize deep, cold habitats in stratified lakes, yet the influence of depth on its reproductive dynamics and lake-scale recruitment remains poorly understood. Using histological analyses of gametogenesis, we investigated seasonal and bathymetric reproductive patterns of *D. bugensis* in Lake Bourget (France) over one annual cycle. Reproductive stages were quantified using a Mean Gonad Index (MGI). Littoral populations showed marked seasonality, with gonadal rebuilding from autumn to spring and a main spawning period between May and July. In contrast, deep populations exhibited delayed maturation, frequent gonadal resorption and prolonged coexistence of reproductive stages, resulting in reduced synchrony with shallow populations. Sex-specific patterns were also observed, with females reaching advanced maturity earlier and maintaining it longer than males, whose maturation appeared more episodic and temperature-dependent. We propose that bathymetric asynchrony in reproductive dynamics may generate successive spawning events along the depth gradient, contributing to the prolonged larval presence typically observed in invaded lakes. Incorporating depth-structured reproduction may therefore be critical for understanding lake-scale recruitment processes and invasion trajectories in deep lakes.

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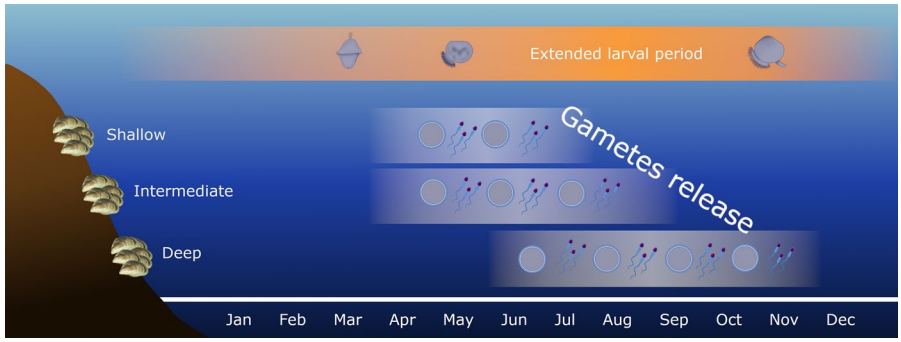
Graphical Abstract



Keywords Gametogenesis · Reproductive dynamics · Spawning asynchrony · Invasive bivalve · *Dreissena bugensis*

Introduction

Biological invasions are among the major contemporary drivers of change in freshwater ecosystems, with substantial ecological, biogeochemical, and socio-economic consequences (Roy et al., 2023). Freshwater bivalves belonging to the Dreissenidae family represent one of the most problematic and most studied invasive alien species. The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), and the quagga mussel, *Dreissena bugensis* Andrusov, 1897, are particularly notable for their exceptional ability to rapidly colonize new environments and reach high population densities up to thousands of individuals per m^2 (Wilson et al., 2006). The dense aggregations formed by these ecosystem engineers create new habitats, notably facilitating the establishment of other invasive species (Glon et al., 2017), and ultimately lead to profound alterations in the structure and functioning of invaded ecosystems (Orlova et al., 2005; Karatayev et al., 2015; Li et al., 2021). Through their high filtration rates, Dreissenidae strongly alter pelagic–benthic coupling by reducing phytoplankton biomass, modifying zooplankton communities, and redirecting energy and nutrients from the water column to the benthos, a process referred to as "benthification" (Nalepa et al., 2009; Mayer et al., 2013). These changes strongly affect nutrient dynamics, particularly phosphorus, whose sequestration and accelerated recycling by mussels can durably alter primary production and food-web structure (Li et al., 2021).

Native to the Dnieper River delta and the Southern Bug River, within the Ponto-Caspian basin, *D. bugensis* remained confined to its native range until the mid-twentieth century. Since then, the species has undergone a rapid human-mediated expansion, colonizing most of Europe and large parts of North America in less than 80 years (Zhulidov et al., 2010). The species was first detected in Western Europe in the early 2000s, in 2006 in the Netherlands (Molloy et al., 2007), and subsequently in France from 2011 onward, where it established populations in several major river basins, including the Rhine, Moselle, Saône, and Rhône (Bij de Vaate & Beisel, 2011; Marescaux et al., 2016; Prié & Fruget, 2017). The recent colonization of large peri-alpine lakes, such as Lake Geneva (2015), Lake Constance (2016), Lake Neuchâtel (2017), and more recently Lake Bourget (2019), further illustrates the species' high dispersal capacity, facilitated by navigable waterways, canals, and the transport in ballast water and recreational boating activities (Prié, 2023).

In contrast to the zebra mussel, which primarily colonizes hard substrates, the quagga mussel exhibits greater anchoring plasticity and can establish on soft sediments by forming aggregates of individuals interconnected by byssal threads. These aggregates function as a stable anchoring matrix in the absence of hard substrate (Berkman et al., 2000; Wittmann et al., 2010). In addition, adult quagga mussels conserve

a degree of mobility and may voluntarily detach from their byssus to relocate by crawling in search of more favorable environmental conditions (Peyer et al., 2011). *D. bugensis* can colonize deep habitats, with recent observations exceeding 200 m in Lakes Geneva and Constance (CIPEL, 2021) while *D. polymorpha* was generally restricted to littoral habitats, rarely exceeding 30–40 m depths. Numerous studies conducted in Europe and North America have documented a progressive replacement of *D. polymorpha* by *D. bugensis*, especially in large lakes (Stoeckmann, 2003; Karatayev et al., 2015). This species substitution is largely attributed to higher food assimilation efficiency, lower respiration rates, greater tolerance to hypoxia, but also to the ability to reproduce at lower temperatures than the zebra mussel (Baldwin et al., 2002; Strayer et al., 2019).

In the context of rapid invasion, understanding the reproductive mechanisms of the quagga mussel represents an important step toward predicting its future expansion within lakes (Zhang et al., 2023). Reproduction in Dreissenidae is exclusively sexual, with dioecious individuals releasing their gametes into the water column, where fertilization occurs (Ram et al., 1996). Dreissenidae are extremely prolific with a single mature female able to release several tens of thousands to more than one million oocytes per reproductive season (Walz, 1978). The life cycle is characterized by planktonic larval stages (i.e., trochophore, veliger and pediveliger), followed by a sessile benthic phase. Veliger larvae can remain in the water column for several weeks prior to settlement, with a longer planktonic duration in *D. bugensis* than in *D. polymorpha*, further enhancing its invasive potential (Wright et al., 1996; Martel et al., 2001). In several colonized lakes, planktonic monitoring or eDNA has revealed the presence of *D. bugensis* veligers over unusually extended periods being recurrently present in open-waters almost constantly throughout the annual cycle (Gerstenberger et al., 2011; Pothoven & Elgin, 2019; Haltiner et al., 2022; Zhang et al., 2023; Vautier & Domaizon, 2025). Such prolonged larval occurrence contrasts with the more seasonally restricted reproductive and larval dynamics generally described for *D. polymorpha*, with spawning and peak veliger abundance mostly concentrated from late spring to summer (Nichols, 1992; Ram et al., 1996; Karatayev & Burlakova, 2022; Paolucci et al., 2025). This observation raises a fundamental ecological question: does *D. bugensis* reproduce continuously within invaded lakes, or could this extended larval signal result from depth-related asynchronous reproductive cycles within the population? Evidence for depth-related variation in reproductive dynamics in *D. bugensis* has previously been reported but generally over restricted temporal or bathymetric ranges limiting a precise quantification of depth variations in the reproductive dynamic of this species. For instance, Glyshaw et al. (2015) conducted a depth-transect survey in Lake Michigan and documented clear bathymetric differences in reproductive timing. Mature individuals were already observed in April at both 45 m (2.4–5.9 °C) and 93 m (< 4.5 °C). However, the progression of spawning differed with depth: mussels at 45 m entered the post-spawning phase by July, whereas populations at 93 m exhibited a delayed spawning period beginning in August (Glyshaw et al., 2015). In deep lakes, seasonal thermal stratification may differentially constrain reproductive timing across depths. If shallow and deep subpopulations follow partially desynchronized gametogenesis cycles, successive spawning events along the bathymetric gradient could generate a temporally extended larval presence when considered at the whole-lake-scale. Clarifying whether such bathymetric asynchrony occurs is therefore essential to understand the mechanisms underlying the prolonged larval signal observed in invaded ecosystems.

In this study, we investigated the temporal and bathymetric dynamics of reproduction in Lake Bourget, recently invaded by the quagga mussel (*D. bugensis*), through a multi-step approach. While several histological studies have focused on the zebra mussel (Borcherding, 1991; Nichols, 1992; Juhel et al., 2003; Knigge et al., 2015), no detailed characterization of gametogenesis stages is currently available for *D. bugensis*. Histological examination of gonadal tissues remains the most reliable approach to accurately determine reproductive stages in bivalves, as it allows direct identification of gamete development, spawning events, and potential resorption processes. This approach is particularly relevant because larval abundance may be influenced by hydrodynamic transport and does not necessarily reflect local adult spawning, whereas histological examination of adult gonads provides a more direct assessment of reproductive status (Claxton & Mackie, 1998; Delmott, 2014). We combined a monthly monitoring survey in

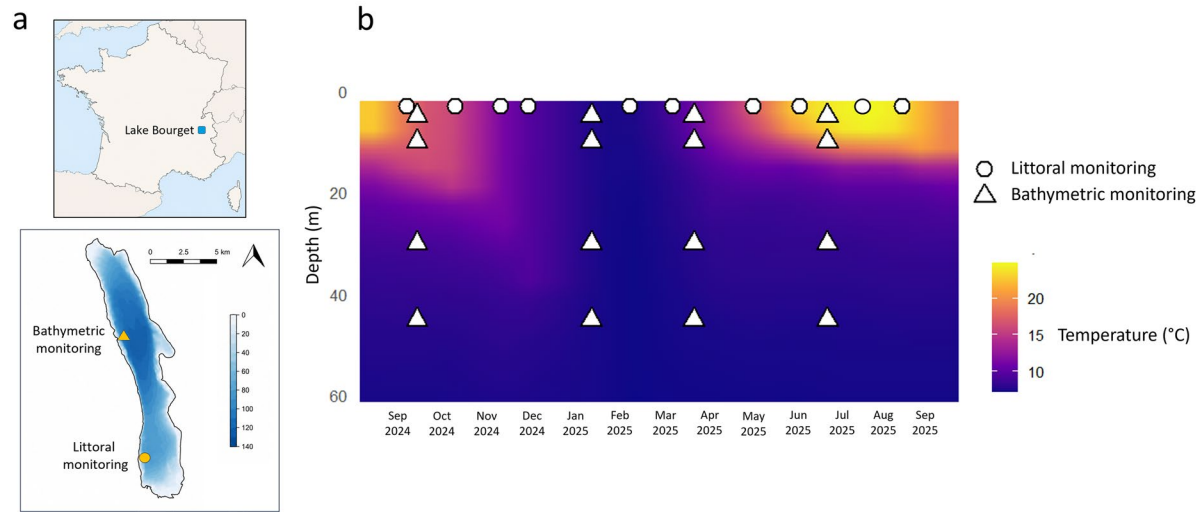


Fig. 1 Study area, sampling design, and thermal conditions in Lake Bourget. **A** Location of Lake Bourget in southeastern France and position of the littoral monitoring site and bathymetric transect within the lake. **B** Depth–time heatmap of water temperature (°C) from September 2024 to September 2025, based on the long-term monitoring survey of the lake (Rimet et al., 2020). Symbols indicate discrete mussel sampling events, with circles representing littoral monitoring and triangles representing bathymetric monitoring at selected depths

littoral habitats (3–5 m depth) with a seasonal survey along a bathymetric transect ranging from 5 to 45 m depth. The objectives were (i) to provide a detailed histological characterization of gametogenesis stages in *D. bugensis*, serving to quantitatively track the reproductive dynamics of *D. bugensis*; (ii) to describe the seasonal progression of gametogenesis over time and across depths; and (iii) to determine whether reproductive timing differs along the bathymetric gradient. We hypothesized that *D. bugensis* exhibits a depth-dependent reproductive strategy, and that spatial decoupling of reproductive processes along the water column may explain the prolonged larval presence reported in invaded deep lakes. This work provides an essential baseline for understanding the reproductive mechanisms of the quagga mussel and delivers key insights into the processes underlying its invasive success in stratified deep lakes.

Material and methods

Study site

The study was conducted in Lake Bourget, a large and deep peri-Alpine lake located in southeastern France (surface area: 44.5 km^2; mean depth: 80 m; maximum depth: 145 m). This lake is oligotrophic (mean annual total phosphorus concentration: 8–10 µg L^{-1}) and monomictic (Jenny et al., 2024). A long-term monthly monitoring program provides vertical temperature profiles measured at 1 m intervals from the surface to the bottom at the central deep-water station of Lake Bourget, from which temperatures were extracted at the depths corresponding to mussel sampling during the study period (Rimet et al., 2020). Vertical water mixing generally occurs in late winter (January–February), leading to oxygen replenishment of deep waters, which experience recurrent hypoxic conditions from mid-summer to late winter and may extend up to 130 m depth. During summer, the lake exhibits strong thermal stratification, with a warm epilimnion (20 °C) separated from colder deep waters (6.5 °C) by a well-defined thermocline beginning at approximately 10 m to 15 m depth (Fig. 1). Lake Bourget had previously been colonized by the zebra mussel, *Dreissena polymorpha*, which dominated the local dreissenid community before the first detection of *D. bugensis* in 2019.

Sampling design

Biological sampling was conducted using two complementary approaches designed to characterize the temporal and bathymetric dynamics of gametogenesis in *D. bugensis* (Fig. 1). A monthly littoral monitoring was implemented to describe the reproductive cycle of the species over an annual cycle. From September 2024 to August 2025, approximately 5 kg of quagga mussel aggregates were manually collected by scuba diving at a littoral site (45°40′02.8″ N, 5°51′30.2″ E), at depths ranging between 3 and 5 m. Complementarily, a seasonal bathymetric survey was conducted along a bathymetric transect located on the western shore of the lake (45°45′02.9″ N, 5°50′28.4″ E). Along this transect, mussels were collected at four depth stations: 5 m, 10 m, 30 m, and 45 m. Four campaigns were carried out over one year along this transect, corresponding to autumn (02 October 2024), winter (29 January 2025), spring (09 April 2025), and summer (09 July 2025). Scuba diving was used to allow direct visual selection of quagga mussel aggregates and the collection of live, intact individuals from precisely defined depths. In the laboratory, mussels were sorted, cleaned, and rinsed prior to selecting 30 adult individuals per sampling condition for histological analyses (shell length = 24.8 ± 2.7 mm).

Histological preparation

Whole individuals were fixed in 500 mL glass jars containing 250 mL of Davidson's fixative for 48 h at 4 °C. Davidson's fixative was prepared according to the following formulation (volumes per 1 L): 200 mL of 37% formaldehyde, 100 mL of glycerol, 300 mL of 95% ethanol, and 300 mL of distilled water, with 100 mL of acetic acid added last.

Following fixation, samples were rinsed three times in 70% ethanol and subsequently stored in 70% ethanol until further processing. Individuals were then carefully removed from their shell, and tissues were subjected to a graded dehydration series

Table 1 Description of gametogenesis stages under optical microscopy for *Dreissena bugensis*

Stage 0 Sexual rest	Gonad difficult or impossible to identify. Very low density of germ cells (gonies), often impossible to distinguish. Sex impossible to determine. Variable presence of reserve tissues (intra-tubular) in reformation. Frequent lacunar spaces with moderate hemocyte infiltration
Stage 1 Early development	Sex clearly defined: appearance of very first small oocytes and spermatocytes. Small, non-contiguous tubules, beginning of gonadal structuring. Gonadal mitosis sometimes visible (oogonia/spermatogonia). Predominant proportion of early germ cells: absence of mature gametes (sometimes except for a few residual gametes from the previous reproductive cycle)
Stage 2 Active gametogenesis	Developed gonadal tubules, expanded but still not contiguous. Mixture of cells at various stages of gametogenesis but still very few mature gametes (minor occurrence of spermatozoa and large oocytes < 10%). Meiosis figures visible (condensed chromatin)
Stage 3a Early sexual maturity	Tubules largely expanded and often contiguous. Similar proportions (~ 50%) of mature and immature gametes. Females: large oocytes undergoing vitellogenesis, moderate compaction. Males: bundles of spermatozoa in the lumen. Some isolated signs of gamete emission, especially in males (free spermatozoa in the tubule lumen), but not dominant. Remarkable stratification: stacking of several layers of immature germ cells toward the periphery of the tubules. Not all tubules can be at the same stage, which may complicate the attribution of the stage of gametogenesis. The dominant phenomena must be used as a reference for the stage determination
Stage 3b Late sexual maturity	Tubules are very swollen and contiguous. Mature gametes predominate (> 80–90%) with very high cell density. Immature cells are rare and located at the periphery of the tubules. Clear patterns of gamete emission: spermatozoa and very large oocytes are free in the lumen of the tubules. In some tubules, the lumen may be large. Spermatozoa are arranged in compact layers with bundles of flagella in the lumen
Stage 4 Advanced spawning	Tubules showing pronounced evacuation of gametes, resulting in extensive empty areas within the lumen. Significant decrease in the density of mature gametes (< 50% of tubular volume). Partially collapsed tubules for some individuals
Stage 5 Post-spawning	Collapsed tubules, destructured gonadal area, large empty spaces. Low proportion of residual dispersed gametes, allowing sex identification. Oocytes: degraded, cytoplasmic lysis, altered membranes. Spermatozoa: small residual clusters. Hemocyte infiltration: active degradation and resorption of residual gametes. End of the reproductive cycle prior to the initiation of the next one (cycle N + 1)

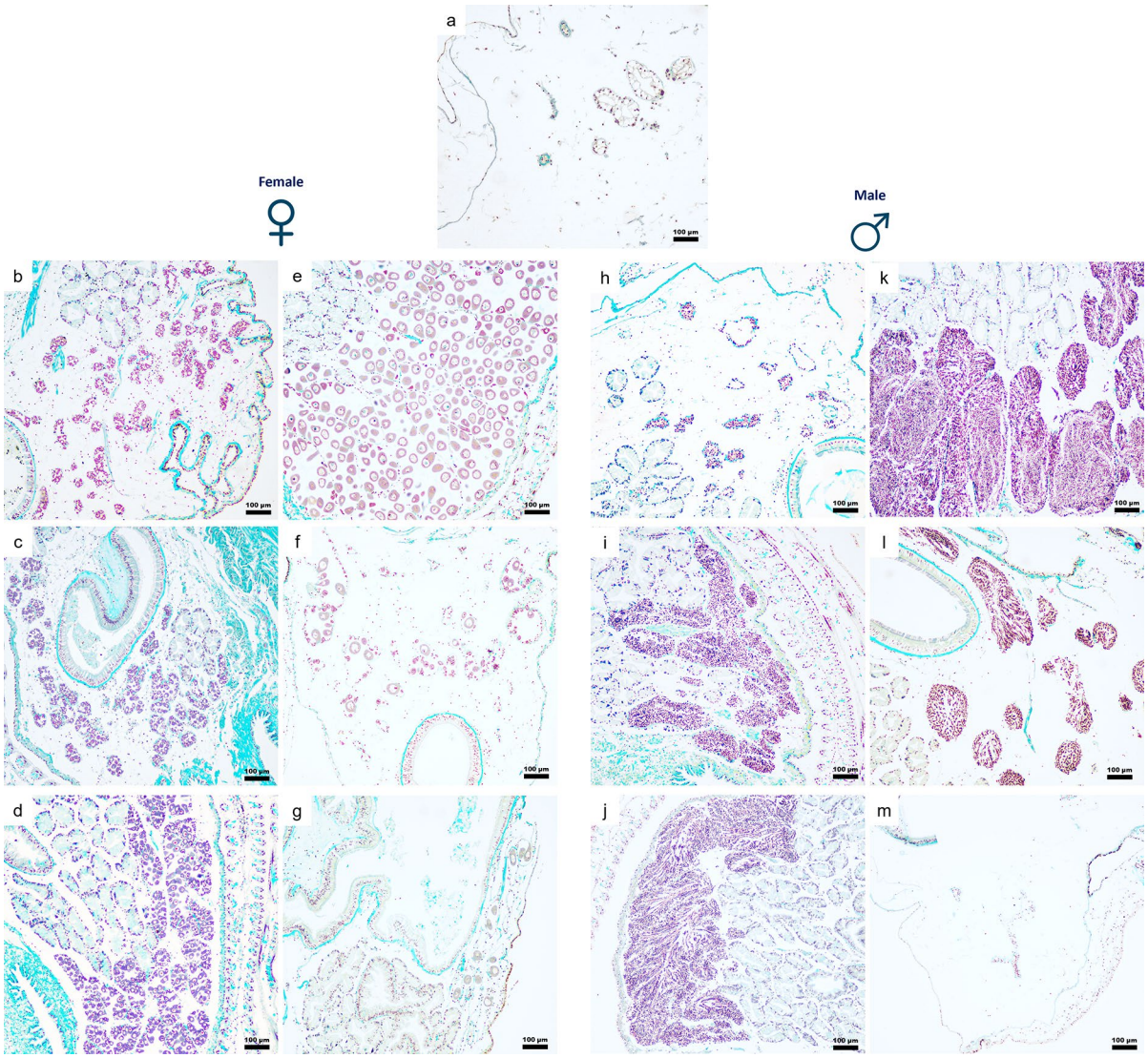


Fig. 2 Histological sections illustrating the successive stages of gametogenesis in *Dreissena bugensis*, observed under light microscopy using a×10 objective. Sections were stained with Prenant–Gabe trichrome, highlighting cytoplasm (pink), nuclei (grey to purple), and fibrous connective tissues (green). Scale bar: 100 µm. **A** Sexual rest (undifferentiated sex). Female stages: **B** Early development, **C** Active gametogenesis, **D** Early sexual maturity, **E** Late sexual maturity, **F** Advanced spawning, **G** Post-spawning. Male stages: **H** Early development, **I** Active gametogenesis, **J** Early sexual maturity, **K** Late sexual maturity, **L** Advanced spawning, **M** Post-spawning

consisting of two successive baths of 95% ethanol (7 h), three baths of 100% ethanol (13 h), and three baths of 100% butanol (12 h). Dehydrated tissues were then placed in molten paraffin wax at 60 °C for 4 h, followed by a transfer to a second bath of fresh molten paraffin for 8 h prior to embedding. Paraffin blocks were sectioned at a thickness of 3 µm using a Leica® RM Autocut rotary microtome. Histological sections were stained using the Prenant–Gabe trichrome method, which provides clear differentiation of tissue structures: fibrous connective tissues (e.g., collagen) appear green, mucosal tissues pink, and cell nuclei grey (Gabe, 1968). After staining, sections were mounted on glass slides using a synthetic mounting medium (ROTI®Histokitt). Mounted slides were placed in an oven at 60 °C for 24 h to ensure

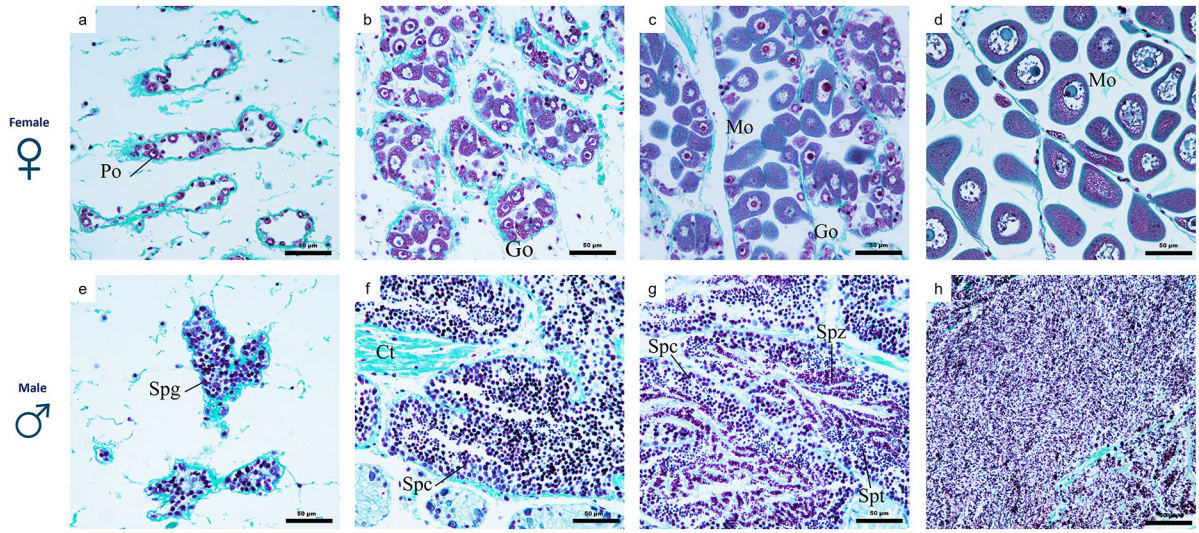


Fig. 3 Histological sections illustrating the successive stages of gametogenesis in *Dreissena bugensis*, observed under light microscopy using a×40 objective. Sections were stained with Prenant–Gabe trichrome, highlighting cytoplasm (pink), nuclei (grey to purple), and fibrous connective tissues (green). Scale bar: 50 µm. Female stages: **A** Early development, **B** Active gametogenesis, **C** Early sexual maturity, **D** Late sexual maturity, Male stages: **E** Early development, **F** Active gametogenesis, **G** Early sexual maturity, **H** Late sexual maturity. In females, primary oocytes (Po), growing oocytes (Go), mature oocytes (Mo) are indicated. In males, spermatogonia (Spg), spermatocytes (Spc), spermatids (Spt), spermatozoa (Spz), and connective tissue (Ct) are shown. Sections were stained using Prenant–Gabe trichrome, highlighting cytoplasm and mucous structures (pink), and nuclei (grey–purple)

complete polymerization of the mounting medium, then cleaned and stored at room temperature until microscopic examination.

Determination of gametogenesis stages

The different stages in the gametogenesis cycle of *D. bugensis* were established using detailed observations of histological sections, and previously published studies on Dreissenidae, particularly *D. polymorpha* (Claxton & Mackie, 1998; Juhel et al., 2003; Delmott, 2014) and *Mytilopsis leucophaeata* (Conrad, 1831) (Verween et al., 2009). These classifications were based on the examination of gonadal structures, the composition of germline cell types (gonial cells, meiotic cells, immature and mature gametes), the overall condition of the gonadal tubules, their degree of filling, and the presence of any signs of gamete release or resorption, providing complementary insights to characterize each stage of gametogenesis. Overall, seven gametogenesis stages were defined: stage 0 (sexual resting), stage 1 (early development), stage 2 (active gametogenesis), stage 3a (early sexual maturity), stage 3b (late sexual maturity), stage 4 (advanced spawning), and stage 5 (post-spawning). The histological criteria used for stage determination are detailed in Table 1. Representative micrographs illustrating each gametogenesis stage in both males and females are provided in Fig. 2 (×10 objective) and in Fig. 3 (×40 objective). The term "reproductive cycle" refers to the complete sequence of gametogenesis, spawning, and post-spawning recovery, such that "cycle N" designates the reproductive event of a given year, while "cycle N + 1" corresponds to the initiation of the subsequent annual cycle.

Mean gonad index (MGI)

The overall variations of gametogenesis within the sampled populations over time and depths were quantified using the Mean Gonad Index (MGI) (Gosling, 2003). This synthetic index provides an integrated measure of the reproductive status of a population by summarizing the distribution of gametogenesis stages among individuals, enabling comparisons of mussel reproductive dynamics over time and across depths.

Each gametogenesis stage was assigned to a numerical coefficient reflecting its relative position along the reproductive cycle. The MGI was calculated as:

$$MGI = \frac{\sum (n_i \times G_i)}{N}$$

where n_i is the number of individuals observed at stage i, G_i is the gonad index assigned to that stage, and N is the total number of individuals analysed.

Gonad Index (G_i) were assigned as follows: sexual rest = 0, early development = 1, active gametogenesis = 2, early sexual maturity = 3, late sexual maturity = 4, advanced spawning = 2.5, and post-spawning = 1. In this way, Gi reflects the physiological progression of gametogenesis characterized by a unimodal pattern with early stages and late stages assigned to low values, whereas the stage characterized by high densities of mature gametes and maximal filling of gonadal tubules was attributed to the highest coefficient (late sexual maturity). Conversely, advanced and post-spawning stages exhibited lower GI values, consistent with the progressive reduction of functional gonadal tissue following gamete release, residual gamete resorption, and increased development of lacunar spaces. This approach allows the MGI to be used as a synthetic indicator of reproductive intensity and complements the analysis of gametogenesis stage distributions by integrating both maturation and gamete release phases into a single metric.

Statistical analysis

The balance of the overall sex ratio was tested with a Yates-corrected χ^2 test. Pearson's χ^2 tests of independence were used to assess the association between gametogenesis stage and sex, month (littoral monitoring), or depth (bathymetric survey). When expected counts were low (i.e., more than 20% of expected counts < 5), P-values were estimated using Monte-Carlo simulations (9999 replicates). All statistical analyses were performed using R version 4.4.2 (R Core Team 2025).

Results

Overall, 746 unique individuals were analyzed, including 368 individuals from the monthly littoral monitoring and 378 additional individuals from the seasonal bathymetric survey. Among all individuals, 106 individuals (14.2%) could not be sexed as they were in a sexual rest stage. Among all other individuals, 317 were identified as females (42.5%) and 323 as males (43.3%), and the observed sex ratio did not significantly differ from a balanced 1:1 ratio (Yates-corrected $\chi^2 = 0.04$, $P = 0.84$).

Reproductive cycle in littoral habitats

The monthly distribution of gametogenesis stages in the littoral population of *D. bugensis* highlighted a well-defined annual reproductive cycle (Fig. 4a). In the littoral monitoring, the distribution of gametogenesis stages varied significantly both between sexes (χ^2 test, Monte-Carlo $P < 0.001$) and across months (χ^2 test, Monte-Carlo $P < 0.001$), indicating marked sexual and seasonal differences in reproductive dynamics. At the end of summer and during early autumn (September–October), most individuals were in sexual rest, with a limited proportion in post-spawning stages, indicating the completion of the previous reproductive cycle (cycle N). From November onwards, gametogenesis progressively resumed, as evidenced by an increasing proportion of individuals in early development and active gametogenesis stages (cycle N + 1). This process continued throughout winter, with early sexual maturity becoming dominant by January and February, followed by a marked increase in late sexual maturity during late winter and early spring. Between March and April, most individuals reached advanced stages of sexual maturity, also corresponding to the onset of spawning activity. The proportion of individuals in spawning stages further increased from May to July, during which late sexual maturity and advanced spawning stages predominated, emphasizing the main reproductive period. In late summer (August), a clear transition toward post-spawning and sexual rest stages was observed, marking the end of the reproductive season before the initiation of a new gametogenesis cycle (N + 2).

The monthly distribution of gametogenesis stages revealed also clear sex-specific patterns (Fig. 4b and

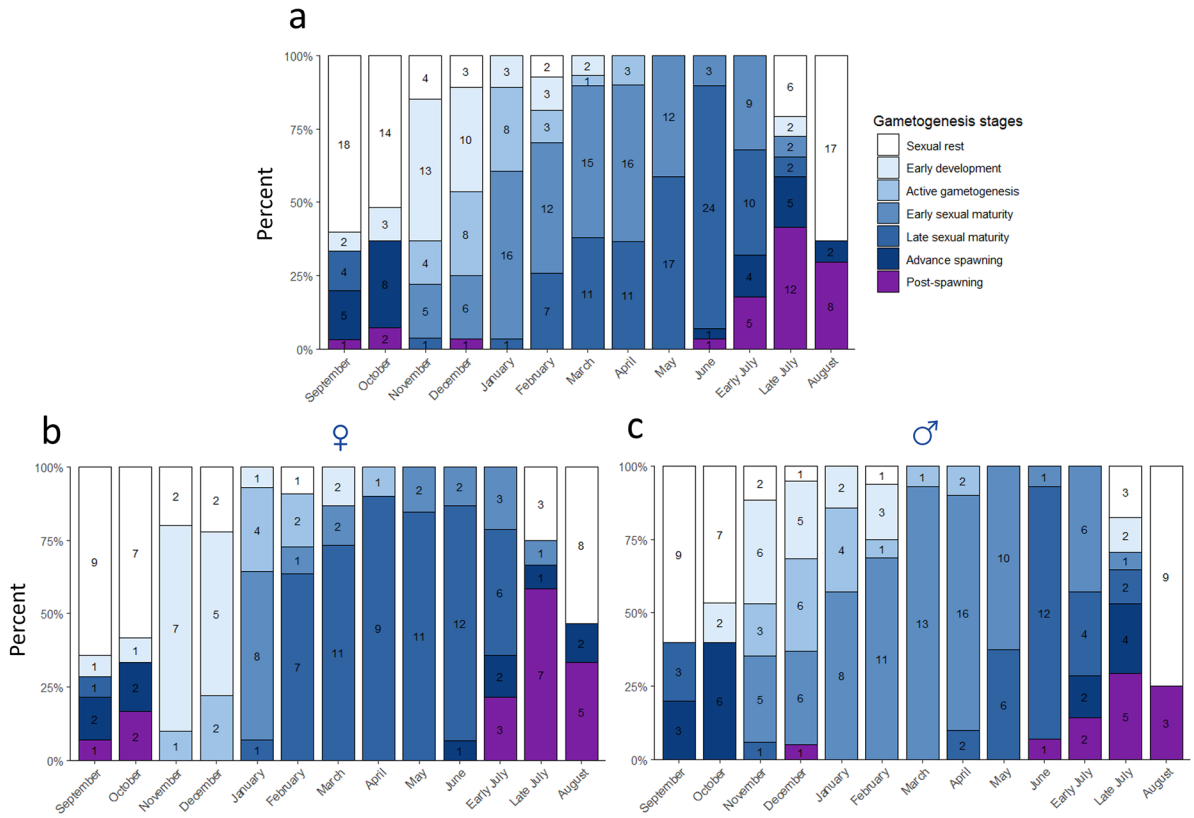


Fig. 4 Monthly distribution of gametogenesis stages of *Dreissena bugensis* over one year in the littoral zone of Lake Bourget (September 2024–August 2025). **A** Distribution for all individuals sampled, **B** females, and **C** males

c). The distribution of gametogenesis stages significantly differed between sexes and across months (Monte-Carlo χ^2 tests, $P < 0.001$). These differences mainly reflected shifts in the relative proportion of early *versus* late developmental stages. Females reached late sexual maturity as a dominant stage from February onwards, with relatively few individuals classified as early sexual maturity throughout the year. Female gonads thus transitioned more directly from active gametogenesis to late sexual maturity. In contrast for males, early sexual maturity dominated from late winter to spring, whereas late sexual maturity, coinciding with the onset of main spawning events starting later in the annual cycle in May and reaching its highest proportions in June–July. During late winter and early spring (February–April), males classified as sexually mature were predominantly assigned to the early sexual maturity stage, reflecting gonads containing a mixture of mature spermatozoa and abundant earlier-stage male germ cells within the gonadal tubules. In February, gonadal resorption was observed in a small portion of the population of both sexes. In brief, in males, maturation was characterized by a prolonged phase of early sexual maturity preceding the dominance of late sexual maturity, whereas in females, late sexual maturity occurred earlier and more abruptly.

The Mean Gonad Index (MGI) of littoral quagga mussels showed a clear seasonal pattern over the annual cycle (Fig. 5). MGI values were low in early autumn, particularly in October, corresponding to the post-spawning period. From late autumn onwards, MGI steadily increased throughout winter and spring, reflecting the progressive resumption and development of gametogenesis. MGI reached its highest values in late spring and early summer (May–June), indicating advanced gonadal development and a high proportion of mature individuals. This increase occurred while water temperature gradually rose from approximately 7.5 °C in mid-winter to 17.5 °C in late

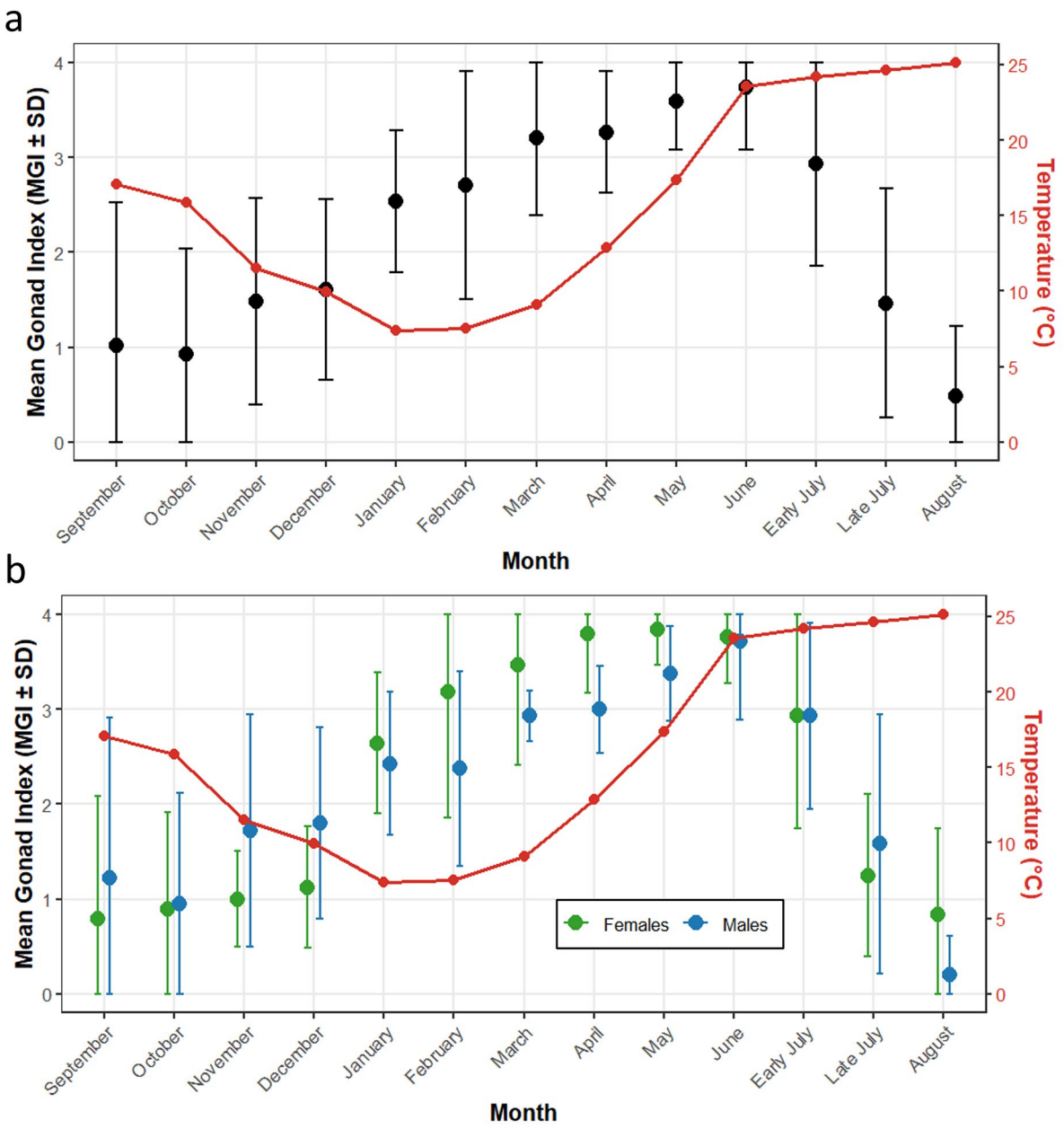


Fig. 5 Seasonal variation of the Mean Gonad Index (MGI) and water temperature in the littoral population of *Dreissena bugensis* in Lake Bourget (September 2024–August 2025). **A** All individuals, **B** females, and males. Error bars represent ± standard deviation (SD) within each sampling month. The red line indicates mean monthly water temperature (°C) measured in surface waters (3–5 m depth)

spring. A marked decline in MGI was observed during summer, with a sharp decrease between June–July and a minimum value reached in August when temperature peaked at 25 °C. This abrupt reduction in gonadal index was consistent with a major spawning event, characterized by the release of gametes and subsequent depletion of gonadal tissues. Overall, MGI dynamics in shallow mussels suggest a unique seasonal reproductive cycle throughout the year, with gonadal rebuilding during autumn–spring

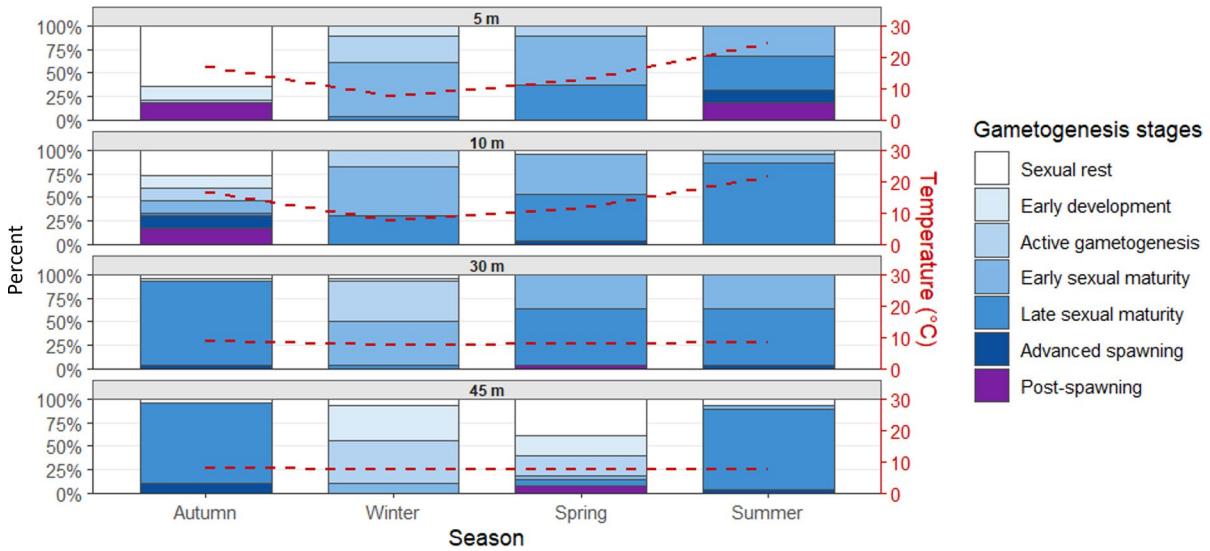


Fig. 6 Seasonal variation in gametogenesis stages of *Dreissena bugensis* in Lake Bourget across the multiple depths considered in the bathymetric survey. The dashed red line represents mean water temperature at each depth and season

followed by a pronounced spawning period in early to mid-summer.

Depth-dependent reproductive dynamics

The distribution of gametogenesis stages differed significantly among depths (χ^2 test, Monte-Carlo $P < 0.001$), highlighting strong bathymetric variations among reproductive stages across seasons (Fig. 6). Throughout most of the year, mussels at 30 m and 45 m were located below the thermocline (~ 15 m depth). In autumn, a marked vertical structuring was evident, with mussels at shallow depths (5–10 m) being predominantly in sexual rest or early development (cycle N + 1), whereas individuals at deeper habitats (30–45 m) were mainly in late sexual maturity and spawning-related stages (cycle N). During winter, this bathymetric differentiation persisted despite a general progression of gametogenesis at all depths (cycle N + 1). Shallow populations showed increasing proportions of individuals in active gametogenesis and early sexual maturity, whereas deeper populations remained dominated by early development and active gametogenesis. In spring, stage distributions became more homogeneous across depths up to 30 m. However, at 45 m, a pronounced reappearance of sexual rest and post-spawning stages (46%), associated with numerous hemocyte infiltrations, was observed and may indicate gonadal resorption.

In summer, reproductive patterns became more homogeneous across depths, with late sexual maturity being the dominant stage at all sampled depths. However, shallow populations appeared slightly more advanced in the reproductive cycle, as shown by the occurrence of advanced spawning and post-spawning stages at 5 m. In contrast, individuals from deeper depths mostly remained in late sexual maturity, indicating that spawning progression was delayed relative to shallow habitats. Overall, these results reveal a pronounced bathymetric asynchrony in gametogenesis development.

Consistent with the relative proportions of the different gametogenesis stages during cycles N and N + 1, MGI values in autumn were significantly lower at shallow depths (5 and 10 m) than at deeper stations (30 and 45 m), indicating a delay in the reproduction (Fig. 7). During winter, significant depth-related differences persisted, with lower MGI values at 45 m compared to the three other depths. In spring, MGI

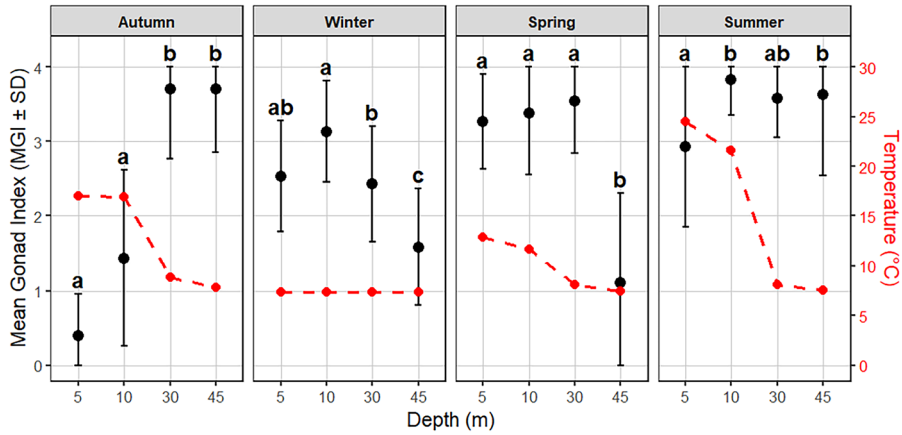


Fig. 7 Seasonal variation of the Maturity Gonad Index (MGI ± SD) in *Dreissena bugensis* populations across four depths. For each season and depth, mean water temperature is indicated by the red dashed line (right axis)

values increased across all depths, except at 45 m where resting and early stages were observed. In summer, MGI values reached their highest levels. Conversely, at 5 m depth, MGI values already showed a decrease in summer, suggesting that spawning had initiated earlier in shallow waters. This depth-related shift in reproductive timing was further supported in autumn, where MGI values had already markedly decreased at shallow depths, while deeper populations (30 and 45 m) still exhibited relatively elevated values.

Discussion

This study aimed to characterize the temporal and bathymetric dynamics of reproduction in *D. bugensis* in a large deep stratified lake recently colonized by this species. By combining monthly monitoring in the littoral habitats with a seasonal bathymetric survey (5 m to 45 m), we could determine how the reproductive timing varies along a depth gradient highlighting spatial asynchrony in spawning events that could represent an ecological mechanism explaining the prolonged occurrence throughout the year of planktonic larvae in the studied lake in open-waters as observed within lakes colonized by quagga mussels (Fig S1) (Gerstenberger et al., 2011; Pothoven & Elgin, 2019; Haltiner et al., 2022; Zhang et al., 2023; Vautier & Domaizon, 2025). Complementarily, our investigations were supported by the elaboration of a comprehensive description of gametogenesis stages that can represent a robust basis for future investigations regarding the reproductive dynamics of quagga mussels.

Gametogenesis cycle of quagga mussel

Consistent with previous observations in dreissenids, the seasonal progression of gametogenesis observed in the littoral population of *D. bugensis* suggests a single annual reproductive event. In the closely related species *Mytilopsis leucophaeata* collected in the port of Antwerp, Verween et al. (2005) reported that gametogenesis initiates as early as January, progresses slowly during late winter, and accelerates in spring, reaching maximum MGI values in July–August, corresponding to the main spawning period (Verween et al., 2005). This seasonal pattern closely reminds the one observed for *D. bugensis* in the littoral habitats in Lake Bourget, which also exhibited a relatively synchronous progression of gametogenesis at the population level and an early resumption of the reproductive cycle. Our results do not support the occurrence of continuous or multivoltine reproduction at the individual level. Instead, the reproductive dynamics observed in shallow populations are consistent with a single annual cycle.

Marked differences between sexes were observed in the gametogenesis dynamics of *D. bugensis*, in agreement with previous observations in *D. polymorpha* (Juhel et al., 2003). In our study, females reached advanced stages of gonadal maturity earlier and maintained them over an extended period (March-July), whereas full maturation in males occurred later (June-July) and may be more tightly constrained by environmental conditions such as temperature. This

sex-specific reproductive dynamic echoes the concept of a "window of successful reproduction" proposed by Juhel et al. (2003), whereby effective reproduction in dioecious, externally fertilizing species depends on fine-scale synchronization between male and female gamete release. Thus, although females may produce oocytes over an extended period, reproduction can only be fully effective when males also reach advanced stages of maturity.

The differences observed between sexes largely reflect histological specificities inherent to spermatogenesis and oogenesis, which condition the interpretation of sexual maturity stages. In males, early (3a) and late (3b) sexual maturity stages correspond to two clearly distinguishable gonadal states. Early sexual maturity was characterized by gonadal tubules already containing spermatozoa, while still retaining a substantial proportion of immature germ cells (spermatocytes and spermatids) at the periphery. By contrast, late sexual maturity corresponded to tubules almost entirely filled with fully differentiated spermatozoa, often oriented toward the lumen. This distinction explained the prolonged dominance of early maturity stages in males prior to the later appearance of late maturity over the course of the year. It should nevertheless be noticed that males classified as early sexual maturity were already capable of releasing limited amounts of spermatozoa. In females, early maturity was less clearly differentiated and less frequently observed. Female gonads tended to transition rapidly from active gametogenesis (stage 2) to late sexual maturity (stage 3b), resulting in an earlier and more prolonged dominance of the latter, often from late winter onward. This difference likely reflects fundamental physiological contrasts between spermatogenesis and oogenesis, which relies on the progressive accumulation and maturation of oocytes within gonadal follicles. The early and prolonged reproductive investment observed in females may also entail high energetic costs, potentially contributing to increased post-reproductive mortality, particularly when this cost is exacerbated by strong summer thermal stress (Peruzza et al., 2023). In this context, our results indicated that apparent differences in temporal overlap between sexes did not solely reflect temporal shifts in biological cycles but also arose from the biological meaning of histological criteria used to define gametogenesis stages. They highlighted the importance of a sex-specific interpretation of gametogenesis stages to accurately link histological maturity to actual spawning capacity and gamete release dynamics. The detailed histological approach adopted here therefore appeared essential for understanding sex-specific reproductive strategies in *D. bugensis* and their interaction with environmental constraints, particularly thermal conditions, in the context of the species' invasive success.

Bathymetric variations in gametogenesis

Our histological analysis revealed pronounced seasonal variation in the reproductive dynamics of *D. bugensis* along the depth gradient. However, these dynamics were not synchronized between shallow and deep habitats. As a result, contrasting gametogenesis stages coexisted within the lake at a given time of year supporting the idea of variable contributions of depth-specific quagga mussel sub-populations to the production of planktonic larvae in open-waters throughout the annual cycle. These results are in line with depth-related differences in reproductive dynamics reported in quagga mussels from the North American Great Lakes (Roe & MacIsaac, 1997; Claxton & Mackie, 1998; Glyshaw et al., 2015). In Lake Erie, Claxton & Mackie (1998) showed that deep-water *D. bugensis profunda* spawned in the hypolimnion at 9 °C, below the temperature generally considered as the minimum threshold for dreissenid spawning. Similarly, Roe & MacIsaac (1997), based on direct gonadal examinations and classification of oocyte developmental stages, reported that 80% of females collected at 55 m depth contained mature oocytes at a water temperature of 4.8 °C, while 20% displayed post-spawning gonads. In Lake Michigan, Glyshaw et al. (2015) conducted a structured depth-transect survey and demonstrated clear bathymetric differences in reproductive timing. Mature individuals were already observed in April at 45 m (2.4–5.9 °C) and 93 m (< 4.5 °C). Spawning progression differed with depth with mussels at 45 m entering the post-spawning phase in July, whereas populations at 93 m exhibited a delayed spawning period beginning in August. This plasticity fits within a broader framework of reproductive strategies well documented in bivalves, in which gametogenesis is tightly modulated by environmental conditions, particularly temperature, but also individual energetic status depending on food availability (Gabbott & Bayne, 1976; Gosling, 2015).

In stratified lakes, the water column exhibits thermal and trophic contrasts over much of the year with surface layers exposed to higher temperatures and enhanced primary productivity, whereas deeper zones typically show lower and more stable temperatures, and more limited food resources (Wetzel, 2001). These depth-specific environmental constraints likely impose contrasting energetic pressures across the water column, potentially contributing to bathymetric differences in gametogenesis progression and phenology of gamete release (Sprung, 1989). In Lake Bourget, this depth-related environmental structuring may therefore explain why deep populations maintained advanced gametogenesis stages under cold hypolimnetic conditions while exhibiting delayed progression, reduced synchrony with shallow populations, and occasional signs of incomplete reproductive cycles.

The histological and seasonal patterns observed in *D. bugensis* are consistent with an opportunistic strategy related to Gabbott & Bayne's income breeder species for which recently acquired energy is directly allocated to gonadal development (Gabbott & Bayne, 1976; Pipe, 1987). In particular, the occurrence of active gametogenesis stages at low temperatures, the rapid resumption of gametogenesis following spawning, and the limited reconstitution of clearly identifiable reserve tissues between reproductive cycles all suggest a direct allocation of energy toward reproduction, rather than a conservative strategy requiring prolonged reserve accumulation prior to an intense and synchronized maturation phase as described in some marine bivalve species such as *Crassostrea gigas* (Thunberg, 1793) (Dridi et al., 2007). Nevertheless, this opportunistic strategy appears to be modulated by environmental constraints along the bathymetric gradient. In deep habitats (30 m and 45 m), where temperatures are low, reproductive investment appears more gradual and leads to limited synchrony with littoral populations. Instances of gametogenesis stagnation or gonadal resorption observed in some deep-dwelling individuals may thus reflect suboptimal energetic conditions, under which somatic maintenance is prioritized over reproductive investment, as described in several bivalve species exposed to strong environmental constraints (Gosling, 2015). Although sampling was restricted to 5–45 m because of the scuba diving protocol, this depth range encompassed the main thermal gradient of Lake Bourget, from seasonally warming littoral waters to cold hypolimnetic conditions below the thermocline. Below 45 m, water temperature remains relatively cold and stable over the annual cycle, including down to the deepest waters of the lake (6.5–7.5 °C) (Jenny et al., 2024). Nevertheless, deeper sampling would be valuable to determine whether populations below 45 m exhibit additional reproductive delays or constraints, as reported by Glyshaw et al. (2015) between 45 and 93 m in Lake Michigan.

Environmental control of gametogenesis

Temperature is generally considered the primary trigger of spawning in bivalves, often associated with specific thermal thresholds, whereas food availability influences reproductive effort through the accumulation and mobilization of energy reserves, in addition with photoperiod that can contribute to seasonal synchronization (Gosling, 2015). In Lake Bourget, the onset of spawning activity in littoral populations was observed at approximately 12–13 °C in April, as evidenced by the first occurrence of advanced spawning stages in both sexes. This is a similar stimulating temperature observed for *D. polymorpha*, for which spawning is typically initiated once water temperature exceeds approximately 12–15 °C, with peaks for gamete release generally occurring at higher temperatures (Nichols, 1992; Ram et al., 1996). The main spawning event, reflected by a marked decline in MGI values at 5 m depth, occurred when littoral temperatures exceeding 23 °C. Interestingly, littoral populations (5 m), exposed to the highest temperatures, were already well progressed in spawning during summer (32% in the advanced spawning and post-spawning stages), whereas deeper populations were predominantly in late sexual maturity. This pattern reinforces the role of temperature as a primary spawning trigger, with surface waters reaching conditions compatible with gamete release earlier than deeper layers.

Other putative triggers of reproduction in deep populations may also contribute to seasonal progression. Increased pelagic primary production during spring and early summer, and the subsequent downward flux of organic matter, may provide a trophic signal capable of supporting the completion of gametogenesis (Goedkoop & Johnson, 1996). In addition, chemical cues associated with initial spawning events in shallow populations could contribute to

synchronizing reproductive activity across the water column. In several marine invertebrates, spawning synchrony has been attributed to dissolved substances released during initial spawning events, triggering reproductive cascades within populations (Hardege et al., 1997; Levitan, 2020). Although speculative in the absence of experimental evidence, such mechanisms could facilitate the progressive convergence of reproductive stages observed during summer.

A key issue raised by our results is the potential decoupling between gonadal maturation and effective gamete release. At a depth of 45 m in spring, 46% of individuals in sexual rest and post-spawning stages leading to gametogenesis divergence from the pattern of littoral mussels. Because these stages typically characterize the completion of a reproductive cycle rather than its mid-course progression, their reappearance at this time of year suggests an interruption of the ongoing reproductive cycle. In several individuals, gonadal tissues exhibited hemocyte infiltration within the gonadal area, features commonly associated with the resorption of unreleased gametes. Together, these elements strongly support that at least part of the deep mussels did not complete spawning and underwent active gonadal resorption. Gonadal resorption represents a widespread physiological strategy among bivalves when reproduction cannot be completed (Purroy et al., 2019; Vázquez et al., 2021). It allows recovery and reallocation of energy initially invested in gamete production. In many bivalves, gametogenesis may progress to advanced stages without spawning occurring, due to the absence of appropriate environmental stimuli (Mann, 1979; Pouvreau et al., 2006; Dutertre et al., 2009). In deep habitats characterized by stable cold thermal conditions (~6.5 °C to 7.5 °C) and potentially limited food flux, this mechanism may play a key role in shaping reproductive dynamics and also may not consistently reach the thermal conditions required to trigger spawning, despite progression of gametogenesis. Because thermal stratification maintains stable cold conditions at high depths, comparable reproductive constraints are likely to occur across deeper habitats. This thermal limitation may represents a mechanistic explanation for the high proportion of individuals in sexual rest and post-spawning stages observed in spring, suggesting incomplete spawning followed by gamete resorption. A comparable mechanism is well documented in the Pacific oyster *Crassostrea gigas*, an introduced species in France, whose spawning requires temperatures exceeding approximately 16–18 °C. In cold temperate regions or in thermally constrained environments such as Breton rias subjected to substantial freshwater inputs, gonadal maturation may proceed without spawning occurring, ultimately leading to gonadal resorption (Dutertre et al., 2009; Enríquez-Díaz et al., 2009).

Consequences of reproductive asynchrony for larval dynamics

The histological results obtained in this study supported our depth-based asynchrony hypothesis to explain the extended larval patterns observed at the lake-scale. Indeed, in Lake Bourget, long-term survey (Rimet et al., 2020) revealed a profound shift in Dreissenidae larval dynamics following the first detection of *D. bugensis* in 2019 (Thomas & Jacob, 2020) characterized by a marked increase in the seasonal larval detection since 2019 (Fig S1). Prior to the arrival of *D. bugensis*, when *D. polymorpha* was dominant, larval occurrence was largely restricted to a relatively short summer window, reflecting a more seasonal and broadly synchronized reproductive cycle. This temporally extended larval signal is consistent with observations from other large European lakes colonized by quagga mussels, such as Lake Geneva and Lake Constance (Zhang et al., 2023), as well as from the North American Great Lakes, where veligers are detected over prolonged periods, with pronounced summer and autumn peaks (Pothoven & Elgin, 2019). These observations led to the hypothesis that *D. bugensis* may be capable of reproducing throughout the year. Our study contributes at refining this statement by suggesting that the sustained larval supply could derive from a spatio-temporal superposition of partially desynchronized reproductive cycles along the bathymetric gradient. Indeed, our fine-scale analysis of gametogenesis stages shows that no depth-specific sub-population maintained sustained reproductive activity throughout the year with a phase of reduced activity or sexual quiescence systematically observed in winter. Therefore, shallow populations may initiate an early contribution to larval production from spring onward, whereas deeper populations exhibit a marked temporal delay in reproductive progression. The slower progression

of gonadal resorption and the prolonged coexistence of contrasting gametogenesis stages at depth may contribute to sustaining larval production during summer and autumn. This asynchrony in the reproductive dynamic of the quagga mussel throughout the bathymetric gradient could thus generate a temporal spreading of potential spawning periods at the lake-scale.

In this context, a quasi-permanent larval signal may emerge from the successive contributions of populations living at distinct depths rather than continuous reproduction by individuals at any specific depth. The overlap of bathymetric lags in reproductive phases could generate a prolonged larval flux, even if each subpopulation retains a seasonal reproductive dynamic. Such bathymetric asynchrony may provide a mechanistic explanation for the extended seasonal presence of larvae reported in other large lakes colonized by *D. bugensis* in Europe and North America (Reid et al., 2010; Gerstenberger et al., 2011; Pothoven & Elgin, 2019; Haltiner et al., 2022). The temporal spreading of the larval signal may be further reinforced by slower veliger development in cold waters. Indeed, experimental data indicate that the larval phase of *D. bugensis* is longer than that of *D. polymorpha*. At ~ 22 °C, embryonic–larval development of zebra mussels reached settlement after about 21 days whereas in quagga mussels settlement lasted until approximately 32 days (Wright et al., 1996). This suggests that quagga larvae could remain in the plankton for longer periods than zebra larvae, extending further the temporal windows for larvae occurrence in open-waters and potentially increasing dispersal opportunities. The combination of spatially asynchronous reproduction and prolonged larval development would thus constitute a particularly effective mechanism for extending larval presence in the water column.

Conclusion

This study provides a comprehensive year-round histologically based description of the reproductive stages of *D. bugensis*, establishing a robust baseline for characterizing its reproductive dynamics. We demonstrate pronounced depth-related differences in reproductive phenology, revealing marked reproductive asynchrony between shallow and deep populations.

The ability of deep populations to maintain delayed gametogenesis, or gametogenesis partially decoupled from gamete release, likely represents a functional adjustment to colder and more thermally stable environments. The persistent bathymetric segregation of reproductive dynamics observed here is consistent with the species' capacity to adjust its life-history traits to contrasting environmental conditions that may be related to the substantial morphological variability across depth gradients, including the identification of a deep-water morphotype referred to as *profunda* in North American lakes (Dermott & Munawar, 1993).

Importantly, our results do not support the occurrence of true continuous or multivoltine reproduction at the individual level. Instead, shallow populations appear to follow a well-defined single annual reproductive cycle. The prolonged presence of larvae commonly observed at the whole-lake-scale would therefore more likely result from the spatial stacking of partially desynchronized reproductive cycles across depths. This bathymetric asynchrony may generate successive spawning events along the depth gradient, thereby extending larval production in the water column and potentially enhancing dispersal opportunities, colonization success, and long-term persistence of quagga mussel populations.

Overall, our results emphasize the need to explicitly integrate bathymetric heterogeneity in reproductive dynamics when investigating quagga mussel population functioning. The detailed histological framework provided here offers a solid basis for reliably tracking temporal changes in gametogenesis, an essential step for assessing how reproductive patterns may respond to ongoing environmental change in large lake ecosystems.

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Author contributions VF, AL and KC conceived and designed the study. SJ contributed to the diving strategy and conducted mussel sampling in collaboration with the SubBear company. Histological sections were prepared with technical assistance from NVN, and gametogenesis stages were identified and described by KC and AL. AL performed the data

analyses and led the writing. All authors actively contributed to the interpretation of the results and to the final writing of the manuscript. VF acquired the funding and supervised the research.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare that they have no relevant financial or non-financial interests to disclose.

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