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Molecular detection reveals infection phenology of a host – Trematode system (*Cerastoderma edule* – *Bucephalus minimus*) in southwestern France

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ABSTRACT

Accurate detection of parasites in their hosts is essential for pathogen control and ecosystem studies. The trematode *Bucephalus minimus* (Digenea: Bucephalidae) infects the edible cockle *Cerastoderma edule*, a commercially fished and ecologically important bivalve that serves as its first intermediate host, in which it can be difficult to detect. We investigated detection accuracy and infection phenology of this host-parasite system in Arcachon Bay, France. Cockles were collected monthly over one year to compare detection methods: stereomicroscopy of tissues *versus* molecular detection by quantitative PCR (qPCR) and digital PCR (dPCR). Visual detection reported 30% prevalence, qPCR 36%, and dPCR 50%, the most sensitive method. Seasonal cockle sampling and cohort analysis enabled tracking of the 2021 cohort over two years (Sep 2022 – Jun 2024) using both visual and molecular detection. With both approaches, first infection appeared when the cohort reached 14 mm shell length in fall 2022, most likely coinciding with sexual maturity. Molecular detection consistently revealed higher prevalence and greater temporal variation than visual detection, allowing for the first description of an infection phenology in this system. Prevalence sharply rose in spring 2023, was highest in summer, declined in fall, and increased again in spring 2024 to a lesser extent. These fluctuations appear linked to cockle size and maturity, as well as to the migration pattern of the definitive host (*Dicentrarchus labrax*), both influencing parasite transmission. Our results highlight the importance of using sensitive molecular methods to accurately assess parasite prevalence and understand transmission dynamics, essential for monitoring parasite and host populations.

1. Introduction

Digenean trematodes are the most abundant macroparasites in coastal systems (Lauckner, 1983), and many species can exert significant effects on individual hosts, populations, and entire ecosystems (Desclaux et al., 2004; Fredensborg et al., 2005; Mouritsen and Poulin, 2010; Thieltges et al., 2013; Brian et al., 2022; Zander and Reimer, 2002). They are increasingly recognized as major regulators of host populations and even referred to by some as engineer species in aquatic environments (Louvard et al., 2025; Smit and Sures, 2025). Digenean trematodes have complex life cycles typically involving a definitive host and one or two intermediate hosts depending on the species. Although prevalence of infection in the first intermediate host is often low (<5–10%) (Magalhães et al., 2020; Thieltges et al., 2006), this stage is

usually the most deleterious for its host (Lauckner, 1983). In severe cases, when prevalence boosts, they may lead to host population declines and mass mortality events (Jonsson and André, 1992; Thieltges, 2006).

The family Bucephalidae Poche, 1907 is particularly notorious for its pathogenicity in a wide range of marine and freshwater bivalves, which they use as first intermediate hosts (Lauckner, 1983; Cribb et al., 2001). Infection of species from several orders is reported, including Unionida (Abdelsaleheen et al., 2024), Mytilida (da Silva et al., 2002; Laffargue et al., 2004; Baba et al., 2012), Venerida (Gargouri Ben Abdallah et al., 2011), Myida (Lajtner et al., 2008), and Cardiida. In Cardiida, Bucephalids have been observed in *Abra alba* (Johnston et al., 1982), *Tridacna crocea* (Shelley et al., 1988), *Cerastoderma glaucum* (Derbali et al., 2009), and *C. edule* (Magalhães et al., 2015). The marine bucephalid

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Bucephalus minimus (Stossish, 1887) Nicoll, 1914 has been reported from the UK and the German Wadden Sea to Morocco and the Mediterranean coasts (Maillard, 1975; Faliex and Morand, 1994; de Montaudouin et al., 2009, 2021). Its life cycle involves bivalves – including *C. edule*, *C. glaucum*, and occasionally *Ruditapes* spp. or *Venerupis corrugata* – as first intermediate hosts; small teleost fishes (e.g., *Pomatoschistus* spp., *Atherina* spp., *Mugil cephalus*, *Sparus aurata*) as second intermediate hosts; and the European seabass (*Dicentrarchus labrax*) as the definitive host (Vaullegeard, 1894; Maillard, 1975; Faliex and Morand, 1994; Pina et al., 2009; Magalhães et al., 2015). *B. minimus* adult worms live in the digestive tract of seabass where they sexually reproduce. Eggs are shed by adult parasites through the seabass' feces and hatch into miracidia, which infect bivalves and develop into sporocysts. These asexually produce cercariae that emerge from the bivalve and infect the second intermediate host, where they encyst as metacercariae in different tissues, such as heart, liver and spleen. The cycle completes when an infected small teleost fish is consumed by a seabass, allowing the parasite to reach its adult stage.

B. minimus infection of *C. edule* has drawn particular attention due to the cockle's ecological and economic significance along the northeastern Atlantic coast. Cockles provide numerous ecosystem services and support a substantial commercial fishery in Europe (Carss et al., 2020). Infected cockles can suffer from energy depletion, castration, tissue replacement, and organ dysfunction due to strong interactions with vital host organs like gonads, digestive tract, the foot and gills (Carballal et al., 2001; Dubois et al., 2009; de Montaudouin et al., 2009; Magalhães et al., 2017). Additionally, resurfacing behavior in infected cockles increases their vulnerability to predators (Desclaux et al., 2002). Given the severity of these effects and the wide-spread distribution of the parasite (de Montaudouin et al., 2021), *B. minimus* poses a potential threat to cockle populations and warrants close monitoring. Moreover, in a context of global change, cockles experience more environmental stress, which could affect their resilience towards particularly virulent parasite infections and vice-versa (Jokela et al., 2005).

Despite the significance of cockles and their vulnerability towards this trematode, our understanding of the infection dynamics of *B. minimus* in cockles remains very limited. Magalhães et al. (2015) reported infections only in cockles larger than 16 mm and found no clear seasonal patterns in prevalence. These findings suggested that environmental conditions favorable for transmission may persist year-round. However, the apparent absence of phenological trends could also reflect limitations in detection sensitivity – especially for early-stage infections. Traditionally, detection of sporocysts and cercariae in cockles relies on cercarial emergence or dissection and stereomicroscopy to observe the infective stages in host tissue. While reliable for identifying mature stages, this approach may miss early infections, especially when a miracidium has only recently infected the cockle, sporocysts are still very small and in small numbers or are hidden by other abundant elements (e.g., gametes, other trematode larvae) obstructing their detection (Kaplan et al., 1997; Born-Torrijos et al., 2014). Nevertheless, when clearly visible, misidentification of *B. minimus* in the cockle trematode community is rare due to its distinct morphology (Stout et al., 2024). Still, subtle or recent infections can go unnoticed using these classical methods, potentially biasing estimates of prevalence, infection timing and dynamics of the parasite-host system (Born-Torrijos et al., 2014; Magalhães et al., 2015). Recent molecular work on the trematode communities in cockles (Stout et al., 2024) has shown strong phylogenetic clustering of *B. minimus* within these communities, supporting the potential of molecular tools for specific detection of this species. Methods such as quantitative PCR (qPCR) and digital PCR (dPCR) offer high sensitivity and can detect even minute amounts of parasite DNA – making them well suited for identifying early or covert infections (Wood et al., 2019; Chailler et al., 2025). These molecular techniques may improve detection accuracy and help identify as well as track infections and parasite transmission more precisely (Rusch et al., 2018; Stout et al., 2025).

The objectives of this study were twofold: (1) to develop a species-specific molecular detection method enabling sensitive detection of *B. minimus* in cockles, and compare visual versus molecular detection methods applying both qPCR and dPCR approaches; (2) to investigate the infection phenology of the *C. edule*-*B. minimus* system at a site with high parasite prevalence to better understand the parasite transmission dynamics at play.

2. Materials and methods

2.1. Study site

Cockles (*Cerastoderma edule*) were collected at Île aux Oiseaux in Arcachon Bay, a coastal lagoon in southwestern France on the Atlantic coast (Fig. 1). The area experiences semi-diurnal tides with amplitudes ranging from 1.10 to 4.95 m. Île aux Oiseaux is the only island in the bay, with a surface area of 0.44 km² at high tide and an emergent intertidal zone of 17 km² at low tide. Cockles were collected from medium sand sediments at a tidal height of 2 m above the low-tide mark on the northern side of the island (44°42'25" N, 1°11'17" W).

2.2. Sampling

2.2.1. Host collection

Two sampling campaigns were conducted. To obtain a first set of samples to conduct detection tests according to different methods, buried and surfaced cockles (*C. edule*) were collected monthly by hand over a year, from October 2021 to September 2022. Surfaced cockles were included in the monthly survey to increase the likelihood of detecting infected individuals, necessary for the subsequent methodological tests. Secondly, in order to monitor the dynamics of the *B. minimus* – cockle system, buried cockles were collected seasonally over two years, from September 2022 to June 2024. Due to low cockle densities (<1 individual/m²), surface sediments were sieved with a 1-mm mesh sieve until sufficient individuals for cohort analysis was obtained. Sample sizes per campaign ranged from 164 to 426 individuals, except for September 2023, when only 54 individuals could be collected due to sampling difficulties. Cockle shell length was measured to the lowest millimeter. Sampling details of both campaigns are available in Supplementary Table S1.

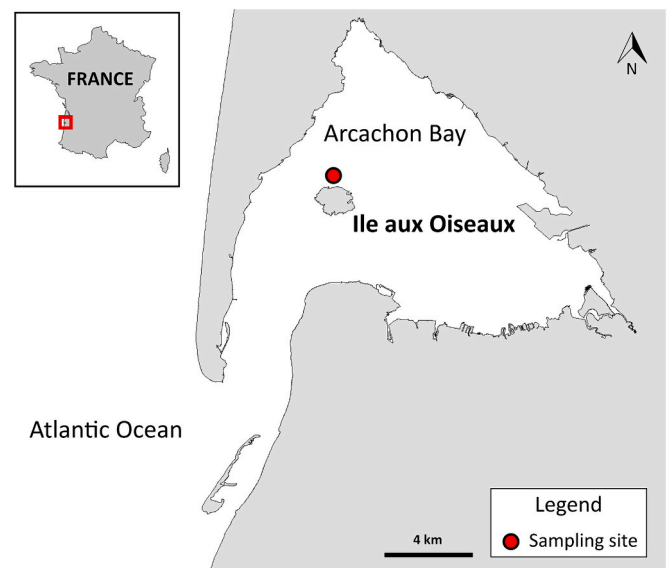


Fig. 1. Study site and sampling location in Arcachon Bay, France.

2.2.2. Visual detection of parasite infection and sample preparation

After each sampling, ten host individuals from the monthly survey and 20 individuals from one to three shell length classes in the seasonal survey were dissected. Host tissue was squeezed between two glass slides and examined under a stereomicroscope (Nikon SMZ1500) to detect *Bucephalus minimus* sporocysts and cercariae (de Montaudouin et al., 2009). Between each sample, glass slides and dissection tools were cleaned with alcohol, purified water, and Thermo Scientific™ RNase AWAY™. Dissection tools were additionally sterilized with a flame. Following examination, tissues were preserved in 5 mL tubes containing 97% ethanol and stored at -20°C until further analyses.

2.3. Molecular analyses

2.3.1. DNA extraction

Genomic DNA (gDNA) was extracted using the Maxwell® RSC Blood DNA Kit with the Maxwell® RSC Instrument (Promega). To enable detection of tissue-localized infections, DNA extraction was performed using the entirety of tissue of each cockle. The manufacturer's protocol was thus modified to allow for larger tissue volumes than originally specified. Tissues were blotted to remove ethanol, chopped using a scalpel, and incubated overnight under stirring at 56°C in 2100 μL of lysis buffer with 210 μL proteinase K ($600\text{ MAU}\cdot\text{ml}^{-1}$). Lysates were homogenized manually, and 330 μL aliquots were loaded into the Maxwell cartridges. DNA was eluted in 60 μL of nuclease-free water.

2.3.2. Primer design

A primer pair specifically targeting a mitochondrial cytochrome c oxidase I (*cox1*) gene fragment of *B. minimus* was designed based on a multiple sequence alignment from a previous study (Stout et al., 2024). The alignment included 94 partial *cox1* sequences from *B. minimus* and 12 other trematode species co-infecting cockles. Primer specificity was first verified *in silico* using Primer-BLAST (NCBI). Second, specificity was confirmed experimentally by PCR and qPCR on pure *B. minimus* gDNA, mock communities, and infected cockles. Mock communities contained either 1) gDNA from 12 trematode species, including *B. minimus* or 2) the same mix excluding *B. minimus* gDNA, to verify the primers' specificity to *B. minimus*. All gDNA samples were obtained as described by Stout et al. (2024). The selected primer pair, BMCOIdetF (5'-GTAGTGGTTCTGGTAAAGTATA-3') and BMCOIdetR (5'-TATCAAAGA-GATTATTACAGTAGAG-3'), amplified a 209 bp fragment. To evaluate primer sensitivity, the limit of detection (LOD) was assessed following recommendations of Hou et al. (2010) using 10-fold serial dilutions of a linear DNA standard (purified PCR amplicons of plasmid DNA) mixed with 1:100 diluted gDNA from an uninfected cockle. qPCR was performed in triplicate. The estimated LOD was 2.5×10^2 copies/ μL , with a mean Ct value of 33.5.

2.3.3. Molecular detection by quantitative PCR

Quantitative PCR (qPCR) was performed on a LightCycler 480 II (Roche) using SYBR Green I detection settings and the BMCOIdetF/BMCOIdetR primer pair. Each 20- μL reaction contained 10 μL $2 \times$ GoTaq Master Mix, 2 μL primer mix (forward and reverse; final concentration of each primer 2 μM), 3 μL nuclease-free water, and 5 μL template DNA diluted 1:100 to minimize inhibition from cockle tissue. This dilution was selected based on preliminary tests comparing 10-fold serial dilutions of parasite DNA alone and mixed with non-infected cockle DNA, which showed that 1:100 was the lowest dilution yielding consistent, uninhibited amplification. Negative (nuclease-free water) and positive (*B. minimus* DNA) controls were included in each run. Samples were run in triplicate. The cycling profile was: $94^{\circ}\text{C}/3\text{ min} - (94^{\circ}\text{C}/40\text{ s} - 58^{\circ}\text{C}/30\text{ s} - 72^{\circ}\text{C}/30\text{ s}) \times 40\text{ cycles} - 72^{\circ}\text{C}/10\text{ min} - 4^{\circ}\text{C}$. Melt curves were generated to verify product melting temperature (T_m). Due to less specific amplification at late qPCR cycles, samples with cycle threshold (Ct) values <35 were considered positive.

2.3.4. Molecular detection by digital PCR

dPCR was performed using the QIAcuity One Digital PCR System (Qiagen) using the QIAcuity EG PCR Kit and QIAcuity Nanoplate 8.5 k 96-well plates. Each 12- μL reaction included 4 μL $3 \times$ EG PCR master mix, 1.2 μL $10 \times$ primer mix, 5.8 μL nuclease-free water, and 1 μL 1:100 diluted template DNA. Cycling conditions matched those used for qPCR. Samples were run at least in duplicate, with both negative and positive controls included in each run. Out of precaution, samples with a minimum of five positive partitions were considered positive.

To compare detection accuracy across methods (visual examination, qPCR, dPCR), 119 samples from the monthly survey (9–10 samples per month) were screened by qPCR and dPCR. For the seasonal survey, 260 samples were first tested by qPCR and dPCR was subsequently performed only on samples with negative qPCR results to confirm or refute infection status.

2.4. Data and statistical analyses

2.4.1. Comparison of detection methods

Overall infection prevalence, defined as the percentage of infected host individuals, was calculated for each method. Chi-square tests with Yates' correction were used to assess significant differences between methods.

2.4.2. Effect of host size

To evaluate prevalence according to cockle size, all monthly and seasonal survey samples ($n = 379$) were pooled to increase statistical power and the range of cockle sizes. Prevalence by detection method was calculated for 2-mm classes of cockle shell length. Here, prevalence based on dPCR included infections detected directly by dPCR in the monthly dataset, and infections detected either by qPCR or by complementary dPCR in qPCR-negative samples in the seasonal dataset. To test the effect of detection method and cockle size on infection status, a logistic regression (binomial generalized linear model, GLM) was applied to the full dataset and post hoc Tukey tests were performed. Analyses and graph generation were performed in R (version 2024.12.1; R Core Team, 2025) using base functions and packages multcomp (Hothorn et al., 2008) and ggplot2 (Wickham, 2016),

2.4.3. Temporal variation of infection prevalence in a cockle cohort

2.4.3.1. Cohort identification. To investigate host-parasite dynamics, infection prevalence was tracked in the only cockle cohort that could be monitored over two years, from its juvenile state to its third year. Length-frequency data for each sampling date of the seasonal sampling campaign were analyzed for cohort analysis using FISAT II software (Gayani et al., 2005). Modal progression analysis decomposed polymodal size distributions into normally distributed components representing distinct cohorts. A separation index >2 was used to distinguish individual cohorts. Mean shell lengths over time were linked and converted to growth increment data. The Appeldoorn method was used to estimate the von Bertalanffy Growth Function (VBGF) parameters, L_{∞} and k , using the equation: $L(t) = L_{\infty} \times (1 - e^{-kt})$, where $L(t)$ is shell length at age t . The age of cockles from the seasonal sampling campaigns were inferred using the VBGF.

2.4.3.2. Temporal trends of infection prevalence. Infection of *B. minimus* was surveyed seasonally from September 2022 to June 2024 in a cockle cohort recruited in 2021, comprising a total of 163 individuals, with 20 cockles per season, except spring 2023 ($n = 30$) and summer 2023 ($n = 13$). Prevalence was calculated for each sampling date based on detection results from both visual and molecular (qPCR and complementary dPCR) methods. The margin of error was calculated as $1.96 \times \sqrt{\frac{p \times (1-p)}{n}}$, and 95% confidence intervals were obtained as $p \pm$ margin of error. Fisher's exact tests were used to assess significant changes in

prevalence between successive time points (t and $t + 1$). Data analyses and graph generation were performed in R (version 2024.12.1) using base functions and the package ggplot2 (Wickham, 2016).

3. Results

3.1. Comparison of detection methods

The presence of *Bucephalus minimus* was assessed in 119 cockles to evaluate the performance of three detection methods: visual examination, quantitative PCR (qPCR) and digital PCR (dPCR). Cockle shell length ranged from 13 to 29 mm, with a mean length of 23 mm. Prevalence was 30% by visual detection, 36% by qPCR, and 50% by dPCR. Chi-square tests indicated that prevalence determined by dPCR was significantly higher than that detected visually (p -value = 0.004) and by qPCR (p -value = 0.049). The difference in prevalence between visual and qPCR detection was not significantly different (p -values = 0.409). To better understand discrepancies among methods, their concordance was evaluated (Fig. 2). The three methods produced congruent results for 79% of samples: 29% were positive and 50% were negative by all methods, while 21% of the samples showed disagreement. Visual examination failed to detect infections in 19% of samples ($n = 22$) that tested positive by qPCR and dPCR. qPCR failed to detect the parasite in one sample that was positive by both visual and dPCR methods, and in 13% ($n = 16$) that were only detected by dPCR. qPCR also generated one positive that was not detected by the other methods. dPCR identified the highest number of infections, detecting *B. minimus* in 50% of samples, confirming all infections detected visually and all except one by qPCR.

3.2. Infection prevalence according to host size

Across all cockles ($n = 379$, pooled from both monthly and seasonal surveys), prevalence of *B. minimus* varied according to cockle shell length and detection method (Fig. 3). Though shell lengths ranged from 8 to 29 mm, no infections were detected by any method in cockles smaller than 14 mm. Infections were first detected in the 14–15 mm class, with prevalence of 8% by visual detection and 18% by both qPCR and dPCR. From this size threshold onward, prevalence was consistently above zero. Visual prevalence ranged from 8% to 33%, while qPCR prevalence ranged from 8% to 44%, and dPCR prevalence reached up to 67%, peaking in the largest size class (28–29 mm). The substantial increase in dPCR prevalence in this class may be influenced by the low sample size ($n = 9$, shown at the top of the figure). In general, there was

an increasing trend in prevalence with shell length for all detection methods. dPCR majorly detected the highest prevalence, followed by qPCR, and visual detection yielding the lowest values for most shell length classes. At certain shell length classes (16–17 mm and 26–27 mm), all three methods produced identical prevalence values. A logistic regression revealed that both cockle shell length and detection method had significant effects on *B. minimus* presence (p -values < 0.001). Post hoc Tukey tests confirmed that both qPCR and dPCR yielded significantly higher detection rates compared to visual examination (p -value = 0.01 and p -value < 0.001, respectively), while there was no significant difference between the two molecular methods here.

3.3. Infection phenology in the 2021 cockle cohort

From cohort analysis, the parameters of the Von Bertalanffy growth function were defined as:

$$L(t) = 28.5 \times (1 - e^{-0.61t})$$

Using this relation and the cockle shell length, individual age was calculated and assigned to a cohort (Fig. 4). The formula to determine age is:

$$t = -\frac{\ln\left(1 - \frac{L(t)}{28.5}\right)}{0.61}$$

where t is the age in years of a cockle at a length $L(t)$ in mm.

The evolution over time of the *B. minimus* prevalence in the 2021 cockle cohort by detection method is shown in Fig. 5. Both methods revealed similar trends in prevalence, though molecular detection consistently yielded higher prevalence – at least double that of visual detection. The cohort likely resulted from 2021's summer recruitment event, and grew slowly, reaching about 23 mm in shell length after three years (Fig. 5). The cohort was first sampled when individuals measured 12–13 mm in late summer 2022. At this stage, no infections were detected by either method. The first infections appeared in fall 2022, when cockles measured 14–15 mm, with a prevalence of 10% by visual detection and 20% by molecular detection. Prevalence dropped back down to zero by the end of winter 2023 for both methods. A sharp increase was observed the following spring (2023), with prevalence stabilizing through summer. During this period, prevalence reached 23–27% by visual detection and 53–54% by molecular detection. A second drop occurred in fall 2023, falling to 5% and 15%, respectively. From then on, prevalence rose progressively, reaching 20% and 40%, respectively, by spring 2024, when cockles measured 21–23 mm in length.

Statistical analysis indicated a significant increase in prevalence between winter and spring 2023 for both detection methods (p -value = 0.015 and p -value < 0.001, respectively). A significant decrease between summer and fall 2023 was detected only by molecular detection (p -value = 0.026).

4. Discussion

4.1. Performance of detection methods

In this study, we developed and applied species-specific molecular approaches using qPCR and dPCR to detect *Bucephalus minimus* infections in cockles and compared these methods to visual stereomicroscopic examination. As expected, molecular methods outperformed visual detection, revealing numerous covert infections that were not visible under the stereomicroscope. Indeed, molecular methods are recognized as the most reliable approach for detecting trematode infections in first intermediate hosts (Cucher et al., 2006; Born-Torrijos et al., 2016; Schols et al., 2019). Among molecular methods, dPCR

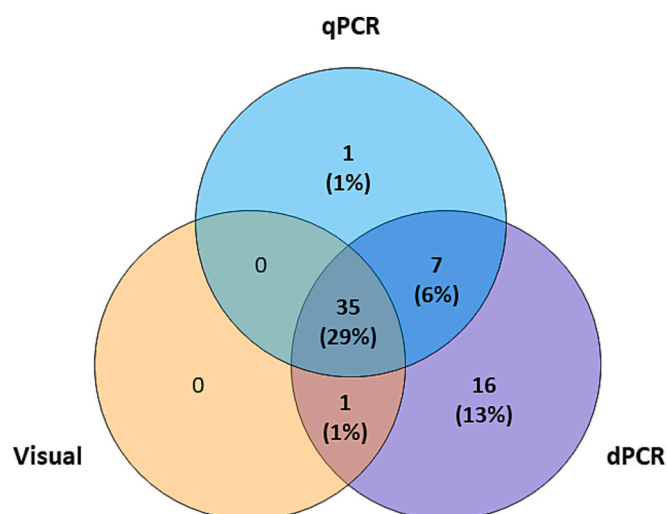


Fig. 2. Venn diagram showing the number and proportion of *Bucephalus minimus* detections using the different methods.

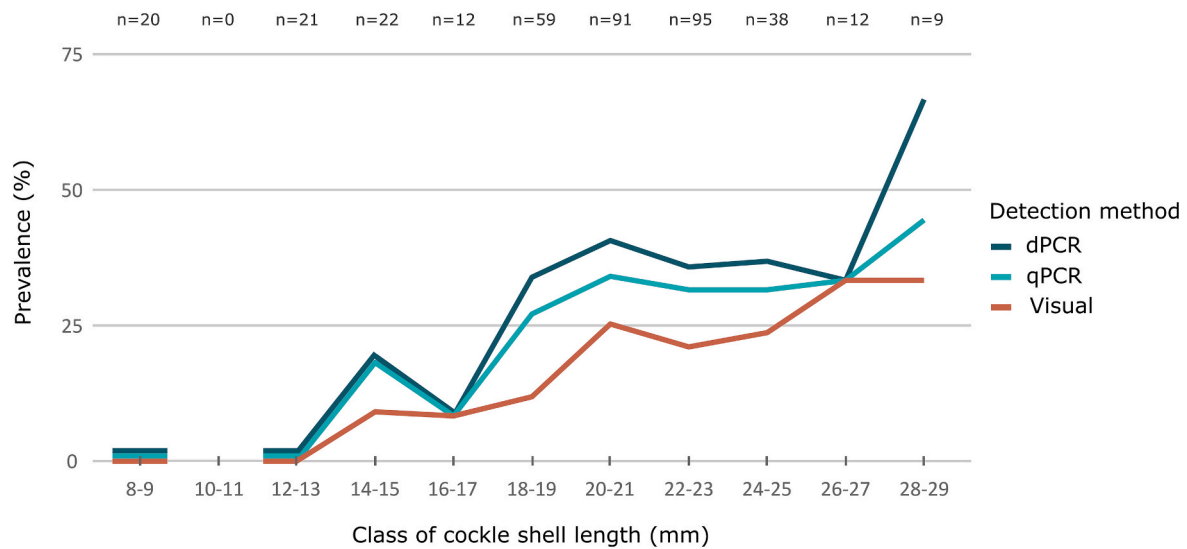


Fig. 3. Prevalence of *Bucephalus minimus* infections in cockles according to cockle shell length and detection method. Sample size of each shell length class is indicated at the top. Prevalence by dPCR includes infections detected directly by dPCR in the monthly dataset, and infections detected either by qPCR or by complementary dPCR in qPCR-negative samples in the seasonal dataset.

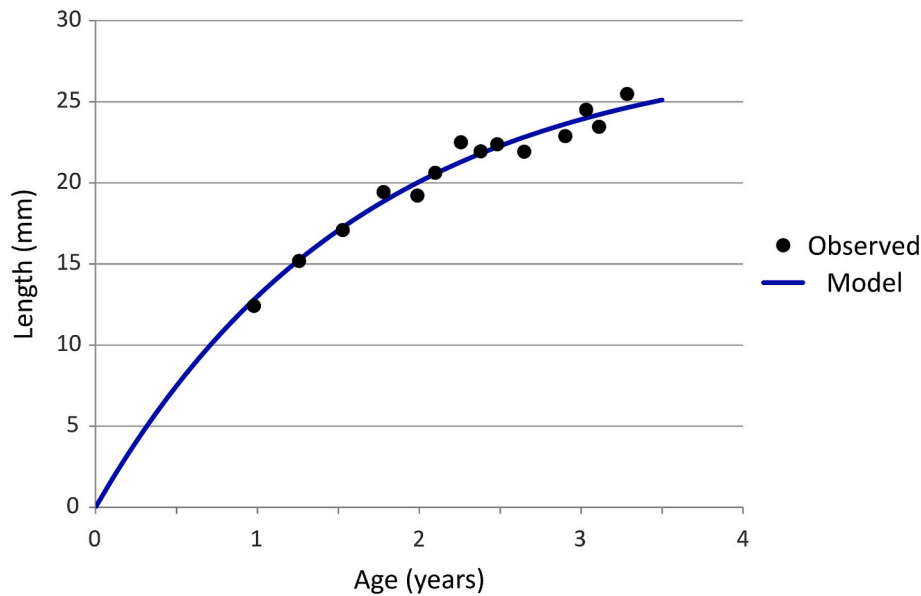


Fig. 4. Cockle shell length (mm) in relation to cockle age. The Von Bertalanffy growth function (blue line): $L(t) = 28.5 \times (1 - e^{-0.61 t})$ was deduced from the observed data (points). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consistently provided the highest prevalence (though not significant in the seasonal dataset). This aligns with previous findings that dPCR is less susceptible to inhibitors and has lower detection threshold (Wood et al., 2019; Chailler et al., 2025).

Visually covert infections likely represent early-stage infections, either newly settled miracidia that have not yet developed into sporocysts, or very small or few sporocysts that are visually undetectable (Kaplan et al., 1997). In some cases, sporocysts may have been obscured by other abundant elements, such as gametes in sexually mature cockles before spawning, or overabundant co-infecting trematodes like *Monorchis parvus*, making *B. minimus* sporocysts difficult to detect visually. Alternative explanations for discrepancies between molecular and visual detection can also be considered. One possibility is the detection of residual DNA from unsuccessful miracidial infections, which may have been eliminated by the host's immune system (Loker and Bayne, 2001) or failed to establish due to interspecific competition or resource

limitation (Kuris and Lafferty, 1994). Kaplan et al. (1997) found that 91% of snails exposed to *Fasciola hepatica* miracidia were PCR-positive seven days post-infection and all of these were later detected by dissection after 42 days, suggesting that all PCR-detected infections eventually developed into visible sporocysts. While infection dynamics may differ in our trematode-host system, it suggests that some unsuccessful infections are likely, which might even be detectable with a more powerful molecular tool such as dPCR. Free DNA of the parasite in the water column could also be detected. However, if such residual DNA (i. e., DNA of failed infections or free DNA) were common, we would expect to detect it even in small cockles (<13 mm), which was never the case. This suggests that the molecular detections observed in our study are rather unlikely to reflect aborted or non-established infections. Another possibility is that *B. minimus* cercariae released into the water by infected cockles were incidentally ingested by uninfected cockles, leaving traces of parasite DNA. Yet again, this scenario would

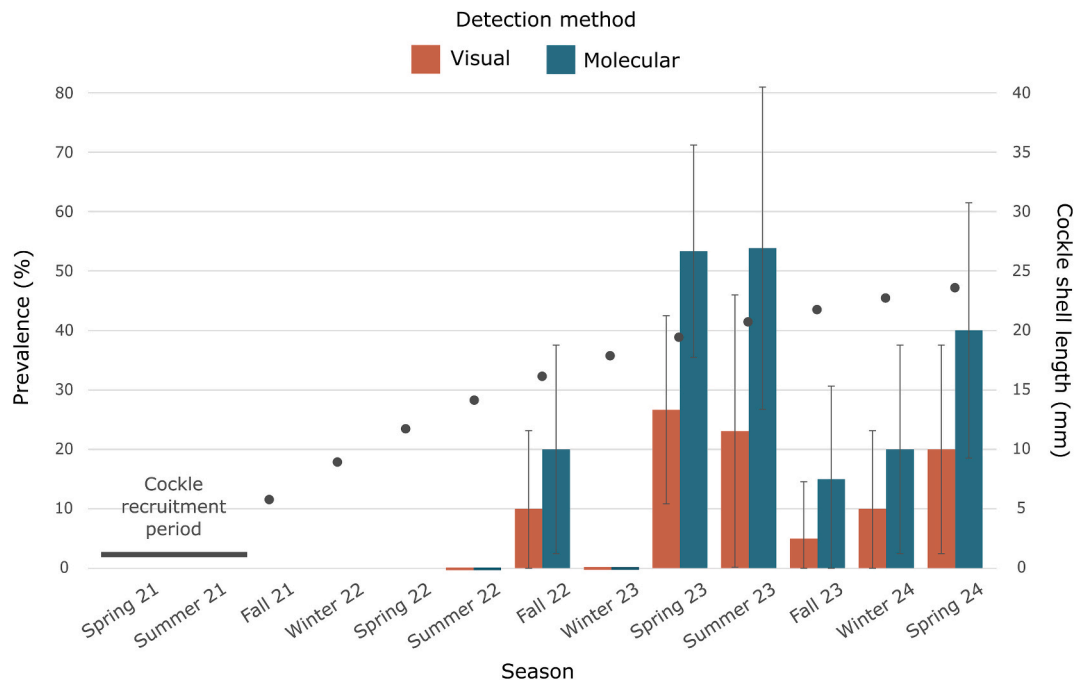


Fig. 5. Prevalence of *Bucephalus minimus* in the 2021 cockle cohort sampled seasonally over two years (bars). The mean cockle shell length (fitted) of the cohort according to the Von Bertalanffy growth function is indicated for each season (points). Sample size $n = 20$, except in spring and summer 2023, where $n = 30$ and $n = 13$, respectively.

presumably lead to at least occasional detection in small cockles, which was not observed. Overall, these observations support the conclusion that molecular detections in our study largely correspond to true, established infections, rather than residual traces of parasite DNA. To confirm it, future work could include analyses of host tissue samples only positive by dPCR using RNA-based molecular assays or using fluorescence *in situ* hybridization (FISH) to visualize the infection, and may also benefit from incorporating water or sediment sampling to assess the environmental presence of *B. minimus* DNA.

4.2. Host size effect on infection prevalence

Across all 379 cockles examined in this study, infections were only detected in individuals measuring 14 mm or larger, regardless of detection method. This threshold is slightly lower than the 16 mm minimum reported by Magalhães et al. (2015) by visual examination. But contrary to what was suspected, it suggests that the absence of infection in small cockles is due to biological factors, not a methodological limitation. Cockles as small as 2 mm are known to be physiologically susceptible to trematode infection, at least for second intermediate host stage, i.e. metacercariae (Wegeberg et al., 1999). Estimated to achieve first maturity between 11 and 15 mm (Hancock and Franklin, 1972; Seed and Brown, 1977; Cardoso et al., 2009; Morgan et al., 2013), a 14 mm threshold supports the hypothesis that *B. minimus* selectively infects mature cockles. This result goes with the “adult preference” concerning trematode infections in the first intermediate host suggested in other studies (Thieltges and Reise, 2006; Magalhães et al., 2015, 2020). As *B. minimus* first establishes and feeds in the gonads and digestive glands of cockles (Baudrimont et al., 2006; Dubois et al., 2009; Magalhães et al., 2020), like many trematodes, this preference likely reflects increased resource availability (lipids and amino acids) in the well-developed gonads and digestive glands of mature individuals, which facilitates parasite growth and reproduction (Dubois et al., 2009). The absence of any parasite DNA in cockles smaller than 14 mm suggests that no miracidia are ingested by juvenile cockles or that the few miracidia that do reach these cockles fail to establish and are rapidly degraded, leaving no detectable traces of DNA.

Overall, as in previous studies (Thieltges and Reise, 2006; Magalhães et al., 2015, 2020), a significant effect of cockle size on infection prevalence was observed. Larger and consequently older cockles are more likely to be infected due to both increased filtration activity and longer exposure to infective stages. However, after an increase in prevalence with length at smaller sizes, a stagnation appeared in cockles measuring at least 18 mm, by molecular methods. This could be due to a natural selection of more infection-resistant cockles which survived the first two years, or due to more parasite-induced mortality that counterbalances the infection increase with size. However, the strong increase in the largest shell length class (28–29 mm) by dPCR, and to a lesser extent by qPCR, may be an artefact due to the small sample size in this class ($n = 9$).

4.3. Infection phenology

The 2021 cockle cohort was surveyed seasonally over two years, beginning in September 2022. Across this survey, molecular detection (qPCR with complementary dPCR) consistently revealed higher prevalence than visual detection. Only in summer 2022 and winter 2023 were both methods in agreement, when 0% prevalence was detected. Notably, molecular detection captured two statistically significant shifts in prevalence (spring and fall 2022), while visual detection captured only one, underscoring the enhanced sensitivity of molecular tools, which hereby enabled to better understand the infection dynamics. The cohort experienced first infection by *B. minimus* in fall 2022 when cockles were approximately 1.5 years old and measured 14–15 mm. At this point, these individuals had experienced all seasons at least once, including periods when larger and older cockles sampled at the same time were infected (data not shown). The delayed onset of infection in the 2021 cohort therefore reinforces the hypothesis that *B. minimus* exhibits size- or maturity-based selectivity, infecting individuals that have reached a certain development threshold. Following the initial infection, the sharp prevalence rises in spring 2023 (maintained through summer), and to a lesser extent in 2024 (though the spring 2024 increase was not statistically significant), suggest increased parasite transmission. Conversely, the observed decreases in prevalence – in fall 2023, and to a lesser

extent, in winter 2022 (not significant), are most likely the result of parasite-induced surfacing and subsequent mortality (through direct or indirect effects, Desclaux et al. 2002; Magalhães et al. 2015). In the field, no signs of distress or mass mortality were observed. However, a previous study has described surfacing of cockles, which most likely leads to subsequent indirect mortality, linked to *B. minimus* infection (Desclaux et al., 2002). This study estimated that 9% of cockles emerged due to this parasite. The drop of prevalence to 0 in winter 2023 is probably explained by the low prevalence during the previous season to begin with. Cockles are more physiologically vulnerable following the spawning stress over summer, as the post-spawning period has been associated with reduced immunocompetence and depleted energy reserves in mollusks (Hong et al., 2014; Kim et al., 2022). This can contribute to elevated parasite-induced mortality in fall.

The observed infection variations are likely shaped by two key drivers. Firstly, cockle filtration activity varies seasonally, reduced as of the end of fall and in winter and highest in warmer temperatures, most particularly in spring, to provide more energy required for gonadal development (Newell and Bayne, 1980; Carss et al., 2020), influencing the probability of miracidia uptake. Secondly, the ecology of the definitive host, the European seabass (*Dicentrarchus labrax*) should be considered. Adult seabass are present in Arcachon Bay from spring through fall, departing around November as the sea temperature within the bay drops below that of the open ocean, and returning the following spring (Bouchet et al., 1997). This season migration aligns closely with the observed infection patterns. This suggests that the availability of parasite eggs and miracidia in the bay is closely tied to seabass presence and activity. Present year-round, the second intermediate host (small teleost fish such as *Pomatoschistus* spp., *Atherina boyeri*, *Sparus aurata*, Faliex and Morand, 1994) probably does not play a key role in the observed temporal infection patterns in cockles. However, its spring/summer recruitments ensure a steady supply of susceptible individuals for cercarial infection, as they coincide with the expected period for cercarial emergence (Faliex and Morand, 1994), which supports the overall efficiency of the parasite's life cycle. However, the timing of the definitive host's presence in the bay—and thus of egg release—and cockle filtration activity appear to be a key drivers of seasonal infection

dynamics, after the primary initial factor, host size. Taken together, these findings highlight the importance of ecological synchrony between host life history traits, environmental conditions and parasite transmission (Fig. 6). Interestingly, at the end of the study in spring 2024, older surviving individuals were less frequently infected than in spring 2023. As discussed in section 4.2., this may reflect either more parasite-induced mortality in less resistant, older cockles, or to the contrary selective survival, whereby more resistant individuals remain in the population, having withstood earlier waves of infection, as it has been suggested for the protist *Marteilia cochillia*, another pathogen of *C. edule* (Iglesias et al., 2023).

Our results provide, for the first time, a description of the infection phenology of this trematode-host system. However, this phenology is subject to potential change in the coming decades, particularly under the influence of climate change. For example, rising sea temperatures are expected to enhance parasite transmission between the definitive host and the first and second intermediate hosts, as temperature positively influences egg production and hatching, miracidial and cercarial infectivity (Marcogliese, 2001), however, temperature may negatively affect metacercarial infectivity (Morley and Lewis, 2016; Paterson et al., 2024). The net effect on transmission success across the parasite's full life cycle remains difficult to predict, underscoring the need for further research into the parasite's biology and transmission dynamics. Overall, climate change is likely to significantly alter the infection phenology of this parasite-host system in the future.

5. Conclusion

Visual examination missed a non-negligible number of *Bucephalus minimus* infections in cockles. While dPCR still requires deeper assessment of its detection accuracy, and given its high cost, qPCR may offer a practical compromise for large-scale or long-term monitoring at this stage of development. Through the complementary application of both molecular methods, we were able to describe, for the first time, an infection phenology of the parasite in cockles. In this study system, the phenology appears to be strongly driven by cockle biology and definitive host ecology. The temporal variations in infection prevalence should be

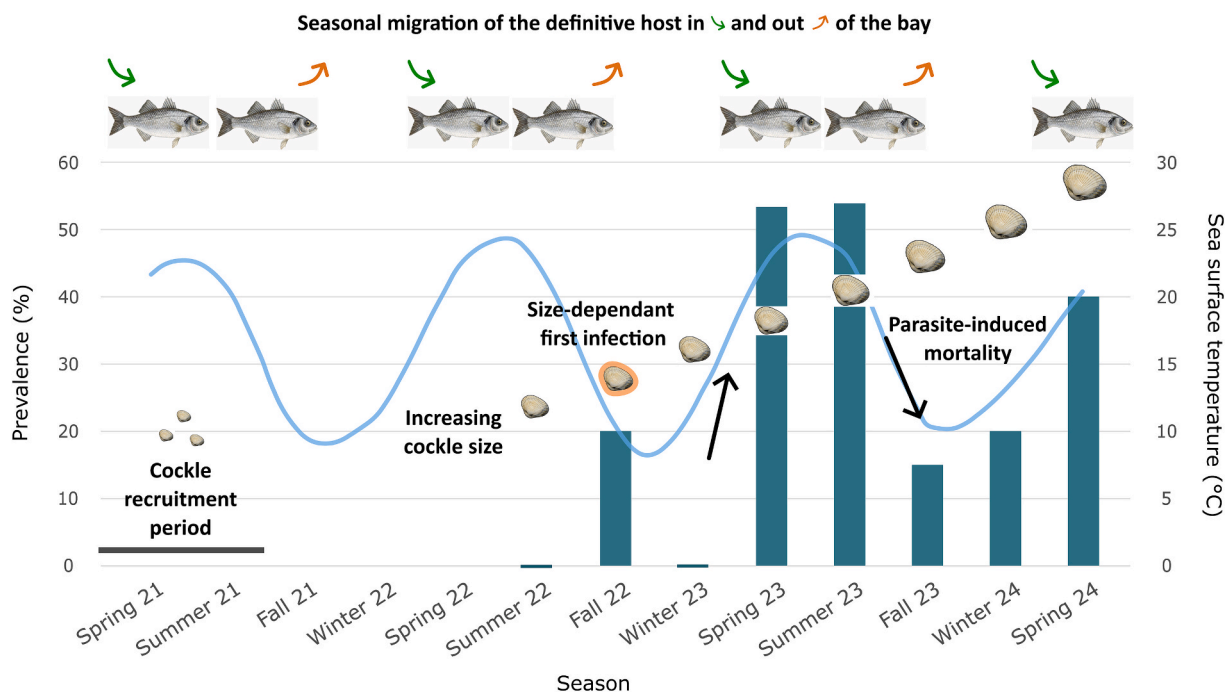


Fig. 6. Suggested phenology of cockle infection by *Bucephalus minimus*. Bars: parasite prevalence in the 2021 cockle cohort. Line: Smoothed mean monthly sea surface temperature (SST) (data retrieved from the ARCHYD dataset, Jacquets station, Meteigner et al., 2025).

examined over more cohorts to account for variation between cohorts and outline a more general phenology. Future studies should also incorporate interannual environmental variables (e.g., temperature, salinity) alongside cohort data to better understand potential environmental drivers of phenological shifts.

CRedit authorship contribution statement

Leslie Stout: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Guillemine Daffe:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Conceptualization. **Elisa Chailier:** Writing – review & editing, Investigation. **Line Mornet:** Writing – review & editing, Investigation. **Flore Daramy:** Writing – review & editing, Investigation. **Xavier de Montaudouin:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Aurélien Chambouvet:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jip.2026.108570>.

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