Himasthla spp. (Trematoda) in the edible cockle Cerastoderma edule: review, long-term monitoring and new molecular insights

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Introduction

In coastal ecosystems, trematodes are the most abundant and common metazoan parasites (Lauckner, 1983; Sousa, 1991; Mouritsen and Poulin, 2002). These macroparasites are exclusively endoparasites and have a complex and heteroxenous life cycle, generally involving three hosts and exhibiting alternation between asexual multiplication and sexual reproduction phases (Esch, 2002; Bartoli and Gibson, 2007). The adult stage of these parasites reproduces sexually in the final host, which is a vertebrate (generally a fish or a shorebird). Eggs are released into the environment (through final host feces) and either evolve into miracidium, a free-living stage, to infect the first intermediate host (usually a mollusc), or hatches in miracidium after they have been ingested by the first intermediate host. Each larva develops into a sac-like sporocyst or a redia, depending on the trematode species, which will asexually produce cercariae, a second free-living stage. These cercariae emerge from the first intermediate host and swim actively to penetrate the second intermediate host (a vertebrate or an invertebrate) and settle as a metacercaria, a latent stage. When the second host is predated by the final host, metacercaria transforms into the adult stage, achieving the life cycle.

Molluscs are the common first and second intermediate hosts of trematode parasites and almost all known bivalves are parasitized, with predominant infection by metacercariae compared to infection by sporocysts (Lauckner, 1983; Sousa, 1991; Galaktionov and Dobrovolskij, 2003). More particularly, the edible cockle Cerastoderma edule (Linnaeus, 1758) is one of the most widespread and abundant bivalves in soft bottom shallow coastal ecosystems along the northeast Atlantic (Malham et al., 2012). They are suitable hosts for harbouring one of the highest diversities of trematode species (Krakau et al., 2006; Thielges et al., 2006), with 16 known species (de Montaudouin et al., 2021). Living buried a few centimetres into the sediment, this bivalve is a key species in coastal ecosystem functioning (Cars et al., 2020). In particular, due to their bioturbation (i.e. biomixing of the sediment) and biodeposition activities, they modulate the physical properties and biogeochemical dynamics of the sediment (Ciutat et al., 2007; Eriksson et al., 2017; Dairain et al., 2020), and they have an important role in connection between trophic levels (Rakotomalala et al., 2015). Finally, through their filtration activity, cockles can regulate phytoplankton biomass and turbidity (Cloern, 1982; Newell, 2004). The effect of trematodes is not restricted to cockle activity, but may significantly...
alter their fitness, with a subsequent impact at the population scale. Indeed, parasites can significantly contribute to C. edule mortality and population decline (Burdon et al., 2014). The influence of trematode parasites on cockle survival is highly dependent on species, infection intensity (i.e. number of parasites per infected cockle) or abundance (i.e. number of parasites per cockle, infected or not), prevalence (i.e. percentage of infected cockles) and parasitic stage (Lauckner, 1983). When cockles are the first intermediate hosts, the effects are particularly deleterious. For example, Bucephalus minimus sporocysts invade most of the tissues, including the gonads, digestive gland and gills (Dubois et al., 2009). This invasion leads to castration, starvation, reduction of the cockle growth rate and condition index, as well as modulation of their impact on sediment erodibility (de Montaudouin et al., 2009, 2021; Magalhães et al., 2015; Dairain et al., 2020a). In addition, Gymnophallus choledochus, also using C. edule as first (and second) intermediate host, occupies the entire mantle cavity, causing gonad structure loss and mass mortality (Thieltges, 2006b; Magalhães et al., 2020a).

Nevertheless, prevalence is usually low and the effect on the cockle population scale is considered moderate (Thieltges et al., 2008; de Montaudouin et al., 2009, 2021). Metacercariae have a more limited impact since they do not multiply in their second intermediate host tissues. For instance, Reniola roscovitius is one of the dominant metacercariae encysting in cockle palps (Kraak et al., 2006; Lassalle et al., 2007). Its impact on cockles has been reported as moderate, with a slight reduction in oxygen consumption, no impact on the condition index, low antioxidant defence activation and an intermediate level of cellular damage (Magalhães et al., 2020b). However, other species of trematodes infecting cockles as metacercariae can have more deleterious impacts on their host population, especially when the prevalence and intensity become high. For example, Gymnophallus minutus (de Montaudouin et al., 2000; Thieltges and Reise, 2006a; Gam et al., 2008; Fermer et al., 2010) causes pathology in cockles, modifies their behaviour (emerging at the sediment–water interface) and provokes significant mortality (Bowers et al., 1996; Gam et al., 2009b; Fermer et al., 2011a).

The Himasthla genus occurs along the northeastern Atlantic coasts (James, 1968; Blakesle and Byers, 2008, Galaktionov et al., 2021), and four species constitute the subject of this review (i.e. Himasthla continua, Himasthla elongata, Himasthla interrupta and Himasthla quissetensis). This is one of the most prevalent, abundant and widespread trematode genera infecting cockles as the second intermediate host (Thieltges and Reise, 2006a; Gam et al., 2008; de Montaudouin et al., 2021). The aims of this review were: (1) to compile literature concerning these parasites in cockles and to summarize the main findings; (2) to provide a molecular signature with the potential to accompany stereomicroscope morphology and identify (3) to analyse a 20-year long-term database concerning a cockle population and its associated trematode species in Banc d’Arguin, France, in order to describe the infection patterns of cockles by Himasthla spp. In the latter case, the tested hypotheses were: (1) infection increases with age and with seasonal modulation; (2) infection success may be limited by cockle abundance (dilution effects) and (3) Himasthla species occupy different ecological niches (i.e. different organs in the cockle) and do not compete inside their individual host.

Materials and methods

Literature review

The references gathered in this review were found in Scopus using relevant terms such as ‘Cerastoderma (or Cardium) edule’ and ‘Himasthla’, published before March 2021. The list of articles was restricted to those studies that clearly identified the occurrence of H. continua, H. elongata, H. interrupta and H. quissetensis in C. edule. A reference list of relevant papers was provided and their main findings were summarized. Thus, a total of 46 publications was examined.

Long-term monitoring

Sampling and trematode identification

From November 1997 to October 2018, cockle monitoring was performed in Banc d’Arguin (44°40’N; 1°10’W), a National Nature Reserve in France. The sampled station is an intertidal semi-sheltered sandflat. The sediment is composed of medium sands (grain-size median = 330 μm) (de Montaudouin and Lanceleur, 2011), the temperature of water fluctuates seasonally between 9.5 and 21.5°C and the salinity is constant (34–35). The tide is semi-diurnal (Gissiat, 1989). Cockles were collected monthly by sampling six 0.25 m² quadrates sieved with a 1 mm mesh. Cockle shell length was measured to the nearest millimetre with a digital calliper. Cohorts were identified by the analysis of length frequency histograms (Bhattacharya, 1967). Ten cockles per cohort were dissected and squeezed between two glass slides for trematode observation under a stereomicroscope. All trematodes were identified to the species level using morphological criteria (de Montaudouin et al., 2009, 2021). However, the different species of the Himasthla genus remain difficult to distinguish using morphological analysis and light microscopy. Therefore, several metacercariae were punctually dissected in different cockle tissues, identified morphologically under the microscope (size, number of spines) and then molecularly characterized (see the molecular biology section). Four species were identified in Banc d’Arguin: H. continua, H. elongata, H. interrupta and H. quissetensis. This long-term monthly survey was not based on the section of all metacercariae, so that our present analysis was restricted to H. interrupta and H. quissetensis. Indeed, we noticed frequent mistakes concerning stereomicroscope identification between the two other species: H. continua and H. elongata.

Data analysis

During the 20 years, 5820 cockles were analysed (Fig. 1), with shell lengths ranging between 2 and 38 mm. A Spearman test was conducted to investigate the relationship between the prevalence (i.e. the percentage of infected cockles) and the cockle shell length for both Himasthla species. Then, the seasonality of the intensity of infection (i.e. number of metacercariae per infected cockle) was studied. Firstly, cockles’ shell lengths were transformed into relative age using a local Von Bertalanffy growth function (Gam et al., 2009b):

\[
t = \frac{-\ln(1 - L_t/L_\infty)}{k}
\]

where \(t\) is the relative cockle age (years), \(L_t\) is the cockle length at age \(t\) (mm), \(k = 1.5\) year\(^{-1}\) and \(L_\infty = 38.3\) mm. Absolute age was deduced from a probable recruitment date in May (de Montaudouin et al., 2021). Then, for each Himasthla species, parasite intensity was compared between months (i.e. between cockle age), using a Wilcoxon non-parametric test, in order to detect significant infection (i.e. increase of the parasite intensity) or parasite-dependent mortality (i.e. decrease of the parasite intensity) processes.

For the following tests, all 1-year-old cockles (corresponding to 15–25 mm) were pooled. This cockle range was selected in order to exclude younger cockles, which are always poorly infected (whatever the environmental conditions are) and older cockles, which do not occur every year. For each Himasthla species, the
associated trematode community was compared at the cockle specimen scale with and without this parasite species, through relative abundance ($\chi^2$ test), species richness and metacercariae abundance (Wilcoxon test). The effect of cockle density on *Himasthla* infection was tested with a Spearman correlation test. Trematodes using cockles as first intermediate hosts were not considered (*Bucephalus minutus*, *G. choledochus*, *Monorchis parvus*), as they have recently been studied in detail (Magalhães et al., 2015, 2020). All statistical analyses were performed using the open-source program R (v3.6.1) in R studio (v1.3.1056) (www.R-project.org, accessed on 1 August 2020).

**Molecular identification: DNA isolation, amplification and sequencing**

The cockles were dissected to extract metacercariae from the four *Himasthla* species that can occur in cockles. Prior to molecular biology analysis, identification was performed based on morphology (metacercariae diameter and number of oral spines) and tissue location (mantle, foot, digestive gland). Metacercariae were assigned to *H. quissetensis* when 31 oral spines were present (Stunkard, 1938). Metacercariae were confidently assigned to *H. interrupta* when they presented 29 oral spines with a diameter <140 μm, and occurred in the mantle margin (de Montaudouin et al., 2009). A mismatch between *H. continua* and *H. elongata* was possible when metacercariae had 29 spines with a diameter >150 μm and occurred in the foot. Periwinkles (*Littorina littorea*) were also collected as the first intermediate host of *H. elongata*. These periwinkles were disposed in small dishes at ambient temperature in order to stimulate cercariae emission (Wegeberg et al., 1999). Then, cercariae were sampled with a micropipette for molecular analysis and subsequent comparison with large 29-spine metacercariae found in the cockle foot.

Metacercariae and cercariae were sampled under a stereomicroscope for DNA analysis. For all species, three replicates (i.e., metacercariae) were collected. They were placed in microtubes and immediately frozen at −20°C. DNA extraction was performed using the QIAamp DNA micro kit (QIAGEN, Hilden, Germany), following the protocol supplied by the manufacturer. Using primers Bb18S and Bb18AS for small subunit ribosomal RNA gene (18S) (de Montaudouin et al., 2014), BbITS and BbITAS for internal transcribed spacer 1 (ITS1) (de Montaudouin et al., 2014) and TremCOIS2 and TremCOIAS2 for cytochrome c oxidase subunit 1 (COI) (Magalhães et al., 2020), with sequences given in Table 1, about 530 bp of 18S, 600 bp for ITS1 and 300 bp of COI genes were amplified. The polymerase chain reaction (PCR) was performed with Gotaq G2 Flexi DNA polymerase (PROMEGA, Madison, Wisconsin, USA), with 50 μL mixtures containing: 10 μL of 5× Colorless GoTaq® reaction buffer (final concentration of 1×), 1.5 μLo fM g C l2 solution (final concentration of 1.5 mM), 0.5 μL of each primer (final concentration of 1 μM), 0.2 μL of GoTaq® G2 Flexi DNA polymerase (5 U μL−1), 1 μL template DNA and 33.8 μL of nuclease-free water.

The temperature profile was 94°C/10 min–(94°C/60 s–59°C/30 s–72°C/90 s) × 40 cycles–72°C/10 min for 18S and ITS1, and 95°C/10 min–(95°C/60 s–43°C/30 s–72°C/60 s) × 40 cycles–72°C/10 min–4°C for COI. The amplified PCR products were analysed by electrophoresis in a 1% p/v agarose gel stained with ethidium bromide. They were then sent to Eurofins Company for complete double-strain sequencing, using the same set of primers as used for the PCR. Overlapping sequence (forward and reverse) fragments were merged into consensus sequences and aligned using Clustal Omega. For COI, the sequences were translated into amino acid alignments, and checked for stop codons to avoid pseudogenes. All sequences obtained in this study were deposited in GenBank (Table 2).

**Results**

**Literature review**

**Description and life cycle**

*Himasthla continua* (Loos-Frank, 1967), *H. elongata* (Mehlis, 1831) Dietz, 1909, *H. interrupta* (Loos-Frank, 1967) and *H.
**Table 1.** Nucleotide sequences of specific primer pairs

| Primer name | Used for | Primer sequence | Reference |
|-------------|----------|-----------------|-----------|
| Bb18S       | PCR and sequencing | 5′-ACTGAGGGCGGAGCTCGTTGCTGC-3′ | de Montaudouin et al. (2014) |
| Bb18AS      | PCR and sequencing | 5′-CAGCTTGAACCATCTTCCC-3′ | de Montaudouin et al. (2014) |
| Bb1TS       | PCR and sequencing | 5′-GACGCATTGATTAGGAGG-3′ | de Montaudouin et al. (2014) |
| Bb1TAS      | PCR and sequencing | 5′-CTTAGTTCAGGGGTAACTCAG-3′ | de Montaudouin et al. (2014) |
| TremCOI52   | PCR and sequencing | 5′-TGTTTATTAGKTCTGKC-3′ | Magalhães et al. (2020a) |
| TremCOI52A  | PCR and sequencing | 5′-AATGCATMGGGRAAAAACA-3′ | Magalhães et al. (2020a) |

**quissetensis** (Miller & Northup, 1926) Stunkard, 1938, belong to the Platyhelminthes phylum, Trematoda class, Digenea subclass and Himasthliidae family (Table 3). *Himasthla continua*, *H. elongata* and *H. interrupta* are considered to be native parasites of *C. edule*, whereas *H. quissetensis* could have been introduced to Europe from North America (de Montaudouin et al. 2005; Longshaw and Malham, 2013), and was not reported before 1990 on the eastern Atlantic coastline (Russell-Pinto, 1993). *Himasthla* species can be differentiated by morphometric measures, number of spines and to a lesser extent by their location in cockle organs (Russell-Pinto et al., 2006; de Montaudouin et al. 2009). *Himasthla quissetensis* is the only one of these four species with 31 oral spines. *Himasthla interrupta* displays the smallest cyst diameter (80–140 μm) and is mainly located in the cockle mantle margin (in the anterior edge, at the opposite side of siphons). In contrast, *H. elongata* displays the largest cysts (210–270 μm). The size of *H. continua* metacercariae ranges between 150 and 210 μm.

**Geographic distribution, abundance and prevalence**

*Himasthla* spp. metacercariae infect *C. edule* from Norway to North Africa, with different successes according to the species (Table 4). *Himasthla continua* and *H. interrupta* are ubiquitous species, present from Denmark to Morocco. They use the same first intermediate host, *Pherinia ulvae* (Bordalo et al., 2011), which is also widely distributed along the east Atlantic shoreline and could explain that they follow similar distribution patterns. However, the intensity of infection and the prevalence are generally higher for *H. interrupta* than those for *H. continua* (de Montaudouin et al. 2009). This can be attributed to the fact that *H. continua* cercariae have more difficulties penetrating through the cockle inhalant siphon due to their larger dimension (Wegeberg et al., 1999). *Himasthla elongata* is absent South to Portugal, and the highest abundance occurs in the northern European countries (e.g. Norway) (de Montaudouin et al. 2009). This range could be related to the distribution of the first intermediate host, the periwinkle *L. littorea* which is present from Portugal to Norway and Russia (Johannesson, 1988). In contrast, *H. quissetensis* is mainly reported in the southern part of the *C. edule* geographical distribution, with the highest rate of infection in France, Portugal and Morocco. In this case, the first intermediate host is *Tritia reticulata*, which is widespread from the north to south of Europe (Russell-Pinto et al., 2006). This lack of direct relationship between host and parasite distribution shows that the abundance of metacercariae also depends on other factors, such as cockle density, size, age and fitness, as well as the ambient benthic community (Gam et al., 2009b; de Montaudouin and Lanceleur, 2011; Magalhães et al., 2017; Welsh et al., 2019; Correia et al., 2020a). Moreover, *H. quissetensis* has also been recorded in different Mediterranean lagoons, infecting a close-related cockle, *Cerastoderma glaucum* (Prévoit, 1974; Bartoli and Gibson, 2007).

**Effects of second intermediate hosts**

In most studies, the pathogenicity of *Himasthla* metacercariae is reported as low in *C. edule*, as this stage is considered energetically inert (Lauckner, 1983). Indeed, laboratory and field experiments have demonstrated that, under moderate infection and normal environmental conditions, *H. continua* and *H. interrupta* do not increase cockle mortality (Jensen et al., 1999; Wegeberg and Jensen, 2003). Similarly, *H. interrupta* and *H. quissetensis* do not impair *C. edule* shell growth and production (Wegeberg and Jensen, 2003; Gam et al., 2009b), and *H. elongata* has no significant effect on cockle bioturbation activity (sediment reworking and bioirrigation rates) (Richard et al., 2021). Nevertheless, when cercariae encyst in the cockle foot, they can induce damages such as muscle fibre destruction (Jensen et al., 1999) through mechanical pressure and tissue lysis related to the secretion of enzymes by the cercariae (Lauckner, 1983). In addition, when the abundance of *Himasthla* spp. metacercariae exceeds a certain threshold (the value could depend on environmental conditions), cockle survival is reduced, as exemplified for the 4 species: (1) *H. elongata* induces mechanical obstruction in the cockle foot, increasing their burrowing time and making them more vulnerable to predators (Lauckner, 1983). Infection also induces a strong cockle immune response (Paul-Pont et al., 2010). Moreover, it modulates cockle biochemical performance and physiology by reducing their oxygen consumption, increasing antioxidant enzyme activity and modifying their energy allocation (Magalhães et al., 2018b, 2018c, 2020b). Finally, infection can significantly reduce (around 40%) cockle survival compared to non-infected cockles after 30 h under hypoxic conditions (Wegeberg and Jensen, 1999). (2) *Himasthla quissetensis* promotes cockle emergence at the sediment surface, exposing them to other threats, like predation (Desclaux et al., 2002) and can contribute to up to 46% of cockle population mortality (Desclaux et al., 2004). (3) *Himasthla interrupta* moderately significantly reduces the cockle growth rate (de Montaudouin et al., 2012b), and a marginal but significant loss of infected cockle flesh weight and body condition was observed by Wegeberg and Jensen (2003). (4) In contrast, no effect on cockles was reported concerning *H. continua*, with the exception of cockle burrowing time increasing at the sediment surface (Jensen et al., 1999).

**Long-term monitoring**

The dataset included cockles from 2 to 38 mm, corresponding to 0+ to 3+ year old cockles. Globally, the parasite community was dominated by *G. minutus* (mean of 62.8% of the total number of metacercariae per cockle), *H. interrupta* and *H. quissetensis* (16.2 and 5.5%, respectively). The other species were *Curtuteria arguainea*, *Dipterostomum brusiae*, *H. continua*, *H. elongata*, *Psilostomum brevicolle* and *R. roscovitus*. The following results aimed to obtain a mean *Himasthla*-host phenology calculated from our 20-year monthly monitoring.
**Table 2. Accession numbers when DNA sequences were deposited in GenBank, for each gene (18S, ITS1 and COI) and the four Himasthla species**

| Species            | 18S | ITS1 | COI   |
|--------------------|-----|------|-------|
| Himasthla continua | –   | –    | –     |
| Himasthla elongata | MN879359 | MN876024 | MT002921 |
| Himasthla interrupta | MN878360 | –    | MT002922 |
| Himasthla quissetensis | MN879357 | MN876026 | MT002919 |

**Himasthla interrupta**

Infection by *H. interrupta* started with 2 mm cockles, and prevalence regularly increased with shell length (*p* = 0.88, *P* < 0.001), to attain a median asymptotic prevalence of 80% (Fig. 1).

Mean intensity of infection increased significantly between recruitment in May [4 metacercariae per cockle, standard deviation (s.d.) = 7] and December (50 metacercariae per cockle, s.d. = 70) (Wilcoxon test, *P* < 0.001) (Fig. 2A). Then, a significant decrease was observed until February (28 metacercariae per cockle, s.d. = 35) was lower than those with *H. quissetensis* (37 metacercariae per cockle, s.d. = 78) was lower than those with *H. quissetensis* (62 metacercariae per cockle, s.d. = 101) (Wilcoxon test, *P* < 0.001).

**Molecular identification**

All metacercariae from cockles were first identified under a stereomicroscope based on morphological characteristics. Then, these metacercariae were compared with molecular tools. *Himasthla continua* metacercariae are morphologically very similar to *H. elongata* (the latter are slightly larger, and they both have 29 oral spines) and both occupy the same niche (i.e. the cockle foot). This difficulty of identification explains why they were not considered in the monitoring. Additionally, no specific type sequence was determined for *H. continua* due to a large variability of gene sequences within the specimens considered as *H. continua*. Conversely, sequences of 18S, ITS and COI were obtained for *H. elongata*, and sequences of 18S and COI were confirmed from cercariae emitted by *L. littorea*. *Himasthla interrupta* metacercariae have very small and light metacercariae settled in the mantle margin, at the opposite side to the siphon. For this species, only sequences of 18S and COI were obtained, with 100% similarity between samples and 100% agreement with morphological identification. *Himasthla quissetensis* is the only species with 31 oral spines. Sequences of 18S, ITS and COI were obtained with 100% similarity between samples and 100% agreement with morphological identification. The amplified products of 18S, ITS1 and COI presented 549, 798 and 273 bp, respectively, for *H. quissetensis*. The amplified products of 18S and COI for *H. interrupta* presented, respectively, 541 and 259 bp, and 535 and 281 bp for cercariae collected from *L. littorea*.

**Discussion**

**Size- and density-dependent infection and seasonality**

For both *Himasthla* species, the long-term data analysis showed that infestation started rapidly after recruitment for cockles with a 2 mm shell length. This early infection in cockles was experimentally observed for all *Himasthla* species (Jensen et al., 1999; Wegeberg et al., 1999; de Montaudouin et al., 2005) and is consistent with previous field studies (de Montaudouin et al., 2000; Desclaux et al., 2004). The positive relationship between parasite prevalence and cockle shell length was also documented and ascribed to the higher filtration rate and longer exposure time of older/larger individuals (André et al., 1993; Rüigard, 2001), resulting in a higher exposure to infective stages and thus greater parasite accumulation (de Montaudouin et al., 1998; Mourtisken et al., 2003; Thieltges and Reise, 2006a).

A moderate negative correlation between cockle density and intensity of *H. interrupta* and *H. quissetensis* was highlighted in this study, suggesting a dilution effect. Indeed, dense cockle populations can filter a high volume of water and thus eliminate parasitic cercariae, with subsequent lower metacercariae infection in cockles (Mourtisken et al., 2003; Thieltges and Reise, 2006b; Buck and Lutterscheidt, 2017; Magalhães et al., 2017; Correia et al., 2020a). However, the density of cockles only explained 4–8% of metacercariae intensity, implying that other factors, such
Table 3. Characteristics of the four studied Himasthla species in terms of target organs, number of oral spines, metacercariae mean diameter and different host species within their life cycle

| Trematode species (Echinostomatoidea, Himasthliidae) | Synonyms | Organs affected | Morphological identification | First intermediate host | Second intermediate host | Final host |
|------------------------------------------------------|----------|----------------|-----------------------------|------------------------|--------------------------|----------|
| Himasthla elongata (Mehlis, 1831; Dietz, 1909)       | Himasthla secunda | Foot            | 29 oral spines, diameter: 210–270 μm | Littorina littorea      | Cerastoderma edule and other bivalves | Seagull  |
|                                                      |          |                 |                             |                        |                          |          |
| Himasthla interrupta Loos-Frank (1967)               | Distoma elongata | Mantle          | 29 oral spines, diameter: 80–140 μm | Peringia ulvae         | C. edule and other bivalves | Sandpiper |
| Himasthla continua Loos-Frank (1967)                 |          | Foot, sometimes mantle | 29 oral spines, diameter: 150–210 μm | P. ulvae               | C. edule and other bivalves | Seagull  |
| Himasthla quissetensis (Miller and Northup, 1926; Stunkard, 1938) | | Foot, sometimes mantle | 31 oral spines, diameter: 150–210 μm | Tritia reticulata      | C. edule and other bivalves | Seagull  |

Table 4. Review of the literature regarding H. continua, H. elongata, H. interrupta and H. quissetensis metacercariae infection in C. edule

| Reference          | Location          | Trematode species | C. edule size | Abundance (Ab) or intensity (I) | Prevalence (%) | Main findings                                                                 |
|--------------------|-------------------|-------------------|---------------|-------------------------------|----------------|-------------------------------------------------------------------------------|
| Lebour (1911)      | British waters    | H. elongate (E. secundum) | –            | Occurrence                    | 10             | Description                                                                  |
| Kesting et al. (1996) | Baltic Sea, Germany | H. interrupta | Occurrence | 0–5                           |                | Adaptation of trematode parasites to low salinity environment                 |
| de Montaudouin et al. (1998) | Wadden Sea, Denmark | H. elongata | 6–12 mm | –                             | –              | Infection intensity of H. elongata increased with cockle density; passive infection of cockles as second intermediate host; the dispersion of H. elongata cercariae was at least hundred metres |
| Jensen et al. (1999) | Arcachon, France | H. spp. (mainly (98%) H. continua) | 1–6 mm | –                             | –              | Cockles size selection (threshold of 2/3 mm); no increase of cockle mortality by H. spp. metacercariae but slight prolongation of burrowing time |
| Wegeberg and Jensen (1999) | Wadden Sea, Germany | H. elongata | 9.5–11 mm | Occurrence | – | H. elongata reduced the burrow ability and the survival of cockles under hypoxic condition |
| Wegeberg et al. (1999) | Wadden Sea, Germany | H. continua | Occurrence | –                            | –              | H. interrupta has high infectivity in cockles measuring 4 mm while H. continua and H. elongata have low infection efficiencies in cockles less than 6 mm |
| de Montaudouin et al. (2000) | Arcachon Bay, France | H. continua | Infection started at 12 mm | Ab: 0–75 | 47.9 | Occurrence; H. continua and H. interrupta dominant in C. edule; parasite species richness increased with cockle shell length; infection of cockles increases in summer |
|                      |                   | H. interrupta | Infection started at 7 mm | Ab: 0–160 | 37.5 |                                  |
|                      |                   | H. elongata | Occurrence | –                            | 0.7            |                                  |
| Desclaux et al. (2002) | Arcachon, France | H. quissetensis | <33 mm | Ab: 0–220 | Up to 100 | H. quissetensis abundance was slightly higher in surface cockles at Banc d’Arguin than buried cockles; 2–8% of adult cockles emerged due to favourization process by H. quissetensis; favourization |
|                      |                   | H. interrupta | Occurrence | Up to 30                         |                |                                  |
|                      |                   | H. elongata | Occurrence | <5                             |                |                                  |

(Continued)
### Table 4. (Continued)

| Reference | Location | Trematode species | C. edule size | Abundance (Ab) or intensity (I) | Prevalence (%) | Main findings |
|-----------|----------|------------------|---------------|----------------------------------|----------------|---------------|
| Blanchet et al. (2003) | Arcachon, France | *H. spp.* | 24 mm | Ab: up to 566 (mean: 36) | 100 | *H. spp.* corresponded to *H. interrupta* and *H. quissetensis*; only bacteria could trigger the cockle emergence and affected their survival |
| | | | 27 mm | Ab: up to 560 (mean: 56) | | |
| Wegeberg and Jensen (2003) | Wadden Sea, Denmark | *H. interrupta* | 3–8 mm | Ab: 0.15 | 15 | Under normal environmental condition, moderate infection of *H. interrupta* metacercariae did not significantly affect cockle mortality or their shell growth |
| Desclaux et al. (2004) | Arcachon, France | *H. quissetensis* | <35 mm | Ab: 0-120 | – | *H. quissetensis* infection occurred during the warmest period and significantly contributed to cockle mortality (up to 46%); only cockles >6 mm were infected; cockle mortality due to parasites depended on cockle growth and environmental factors |
| | | | *H. interrupta* | Occurrence | – | |
| | | | *H. elongata* | Occurrence | – | |
| de Montaudouin et al. (2005) | Arcachon, France | *H. continua* | 2–14 mm | Ab: 0 | – | Infection pattern of *H. quissetensis* was similar to those of *H. elongata* and *H. continua*; *H. quissetensis* has high infectivity in cockles measuring 6–14 mm |
| | | | *H. interrupta* | Ab: 0-2.7 | – | |
| | | | *H. elongata* | Ab: 0-0.5 | – | |
| | | | *H. quissetensis* | Ab: 0-3.2 | – | |
| Baudrimont and de Montaudouin (2006) | Arcachon, France | *H. quissetensis* | 25.2 ± 0.4 mm | Ab: 12.5 ± 2.2 | – | Alteration of the metallothioneins protective effect on parasitized cockles after cadmium exposure at the whole organism level |
| | | | 27.9 ± 0.2 mm | Ab: 1.5 ± 0.2 | – | |
| Baudrimont et al. (2006) | Arcachon, France | *H. quissetensis* | 23–31 mm | Ab: 10–90 | – | Parasite infection in cockles altered the protective effect (metallothioneins synthesis) in case of metal contamination |
| Krakau et al. (2006) | Wadden Sea, Germany | *H. continua* | 26–47 mm | t: 5.6 ± 0.9 to 26.6 ± 7.5 | 20–61.5 | Similar trematode parasite species were found in native and introduced host species, but intensity of infection was higher in native host species |
| | | | *H. interrupta* | t: 11.0 ± 1.6 to 98.0 ± 11.3 | 5–100 | |
| | | | *H. elongata* | t: 7.5 ± 1.7 to 48.8 ± 10.4 | 37.5–94.9 | |
| Russell-Pinto et al. (2006) | Ria de Aveiro, Portugal | *H. interrupta* | – | Occurrence | 4.23 | Species description, identification and distribution |
| | | | *H. elongata* | Occurrence | 26.88 | |
| | | | *H. quissetensis* | Occurrence | 78.54 | |
| Thieltges (2006a) | Wadden Sea, Germany | *H. continua* | (2/3 years) | Ab: 148.4 ± 111.1 to 164.2 ± 84.4 | – | Negligible role of metacercariae in cockle mortality |
| | | | *H. interrupta* | metacercariae (H. 3 spp.) in buried cockles | – | |
| | | | *H. elongata* | – | – | |
| Thieltges (2006b) | Wadden Sea, Germany | *H. continua* | Cockles with 2 winter rings | 0 to ~ 2100 metacercariae per g flesh dry weight | – | Density of the first intermediate host was the dominant factor of metacercarial transmission; tidal level was a minor factor in trematode transmission |
| | | | *H. interrupta* | 0 to ~ 3200 metacercariae per g flesh dry weight | – | |

(Continued)
| Reference                        | Location                | Trematode species | C. edule size | Abundance (Ab) or intensity (I) | Prevalence (%) | Main findings                                                                 |
|---------------------------------|-------------------------|-------------------|---------------|---------------------------------|----------------|-------------------------------------------------------------------------------|
| Thieltges and Reise (2006a)     | Wadden Sea, Germany     | H. continua       | 20–30 mm (adult) | l: 9.0 ± 4.7 to 9.8 ± 5.8         | 95.8 ± 4.2 92.0 ± 10.7 | Speciation richness and intensity of infection increased with cockles age; trematodes community did not vary between years, especially in adults |
|                                 |                         | H. elongata       |               | l: 16.3 ± 147.2 to 149.3 ± 225.4 | 100 ± 0        |                                                                                |
|                                 |                         | H. interrupta     |               | l: 4.1 ± 3.7 to 10.5 ± 4.3       | 69.5 ± 23.4 89.5 ± 18.4 |                                                                                |
|                                 |                         | H. continua       | 6–14 mm (juvenile) | l: 3.8 ± 1.9 to 21.9 ± 30.0     | 76.0 ± 28.9 95 ± 6 |                                                                                |
|                                 |                         | H. elongata       |               | l: 2.5 ± 1.1 to 7.6 ± 3.9        | 50.0 ± 33.4 96.5 ± 4.1 |                                                                                |
| Thieltges et al. (2006)         | Wadden Sea, Germany     | H. continua       | 21–47 mm      | l: 15.8 ± 25.1                   | 72.3 ± 34.7 | Occurrence, H. quissetensis was absent probably due to the lack of its first intermediate host |
|                                 |                         | H. interrupta     |               | l: 43.7 ± 20.8                   | 84.2 ± 38.8 |                                                                                |
|                                 |                         | H. elongata       |               | l: 20.5 ± 24.7                   | 85.7 ± 21.4 |                                                                                |
| Desclaux-Marchand et al. (2007) | Arcachon, France        | H. elongata       | >20 mm         | –                               | –              | Trematode parasite increased metallothioneins concentration (metal-binding proteins) in gills |
| Javanshir et al. (2007)         | Arcachon, France        | H. spp.           | >20 mm         | l: 6.87–11.8                     | –              | Intensity of infestation by H. spp. metacercariae was higher in cockles initially infected than in cockles without metacercariae; number of metacercariae increased with cockle shell length and density (higher filtration rates); infestation slightly decreased cockle growth rates |
| Lassalle et al. (2007)          | French Atlantic coast   | H. continua       | 24 ± 5 mm      | Occurrence                      | –              | No relationship between abundance of metacercariae and latitude; H. quissetensis and H. continua were ubiquitous species while H. elongata characterized northern stations and H. interrupta southern stations |
|                                 |                         | H. interrupta     |               | Occurrence                      | –              |                                                                                |
|                                 |                         | H. elongata       |               | Occurrence                      | –              |                                                                                |
|                                 |                         | H. quissetensis   |               | Occurrence                      | –              |                                                                                |
| Thieltges and Reise (2006b)     | Wadden Sea, Germany     | H. continua       | 18–30 mm       | l: 0.1–88.2 From 7 to 100       | –              | Prevalence and intensity of metacercariae infection on cockles between sites mainly depended on the density of the first intermediate host; predictors were more complex within sites; cockle size and density were respectively positively and negatively correlated with infection level; time spent in residual water increased infection level in cockles; no interspecific interaction between trematode parasites in hosts |
|                                 |                         | H. interrupta     |               | l: 0.7–136.3 From 47 to 100     | –              |                                                                                |
|                                 |                         | H. elongata       |               | l: 0–72.5 From 0 to 100         | –              |                                                                                |
| Gam et al. (2008)               | Merja Zerga, Morocco    | H. interrupta     | 17 ± 1 to 30 ± 2 mm | Ab: 0–0.5 Low | – | Different sub-communities of metacercariae trematode parasites between subtidal and intertidal environment (cockle density, first host) |
|                                 |                         | H. quissetensis   | Ab: 0.1–2.3; l: 1–12 | 10–55 | – |                                                                                |
| Thieltges (2008)                | Wadden Sea, Germany     | H. continua       | 18–42 mm       | Occurrence <5                   | –              | Temporal exposure was the main factor explaining the level of infection in cockles older than 1-year-old than host size |
|                                 |                         | H. interrupta     |               | Occurrence <5                   | –              |                                                                                |
|                                 |                         | H. elongata       | Ab: 20–316     | –                               | –              |                                                                                |

(Continued)
| Reference                  | Location                          | Reference Location | Trematode species | C. edule size | Abundance (Ab) or intensity (I) | Prevalence (%) | Main findings                                                                 |
|---------------------------|-----------------------------------|--------------------|-------------------|---------------|---------------------------------|----------------|-----------------------------------------------------------------------------|
| de Montaudouin et al. (2009) | North-eastern Atlantic coast      |                     | H. continua       | –             | Ab: 0–100 (Norway–Morocco)      | Close to 100%  | Identification key; review of the distribution of parasites along north-eastern Atlantic coast |
|                           |                                    | H. interrupta      | –                 | Ab: 0–1000 (Norway–North of France) | Close to 100% |                                |                 |                                                                              |
|                           |                                    | H. elongata        | –                 | Ab: 0–1000 (Norway–South of France) | Close to 100% |                                |                 |                                                                              |
|                           |                                    | H. quissetensis    | –                 | Ab: 0–1000 (North of France–Morocco) | Close to 100% |                                |                 |                                                                              |
| Gam et al. (2009b)        | Arcachon, France                   |                     | H. interrupta    | <40 mm        | Ab: 41                           | 7.0            | Trematode metacercariae did not affect cockle production at both sites but increased cockle mortality (up to 20%); metacercariae abundance threshold where cockles were impacted was lower at Merja Zerga than Arcachon probably due to interaction with environmental factors |
|                           |                                    | H. quissetensis    |                   | Ab: 4.1       | 0.7                             |                |                                                                              |
|                           | Merja Zerga, Morocco               |                     | H. interrupta    | <30 mm        | Ab: 0.2                          | 0.0            |                                                                              |
|                           |                                    | H. quissetensis    |                   | Ab: 1.4       | 0.2                             |                |                                                                              |
| Gam et al. (2009a)        | Merja Zerga, Morocco               |                     | H. interrupta    | 13–24 mm      | ≤ 4                             | 16.7–34.4      | Species richness of parasites in cockles increased with time; non-significant effect of substrate on parasite community structure (small spatial scale) |
|                           |                                    | H. quissetensis    |                   | ≤ 4           | 5.6–13.3                        |                |                                                                              |
| de Montaudouin et al. (2010) | French Atlantic coast             |                     | H. interrupta    | 31–35 mm      | Ab: 0–14                        | –              | Cockles exposed to low stress but unfavourable environment for development |
|                           |                                    | H. elongata        |                   | Ab: 0–38      | –                               |                |                                                                              |
| Paul-Pont et al. (2010)   | Arcachon, France                   |                     | H. elongata      | 31.8 ± 0.3 mm | –                               | –              | H. elongata metacercariae induced strong immune response and modified gene expression inducting energetic losses and oxidative stress in cockles; trematode infection limited pollutant accumulation |
| de Montaudouin and Lanceleur (2011) | Arcachon, France     |                     | H. continua      | 17–23 mm      | Ab: 1.47                        | –              | Paraset diagnosis was representative of cockles distributed in a radius of 20 m; infection heterogeneity at a different scale was explained by the presence of the first intermediate host, mobility of hosts and environmental parameters impacting cercariae transmission |
|                           |                                    | H. interrupta      |                   | Ab: 5.34      | –                               |                |                                                                              |
|                           |                                    | H. elongata        |                   | Ab: 0.11      | –                               |                |                                                                              |
|                           |                                    | H. quissetensis    |                   | Ab: 10.57     | –                               |                |                                                                              |
| Fermer et al. (2011b)     | South coast of Ireland            |                     | H. continua      | 24.3 ± 3.6 to 38.1 ± 4.4 mm | ≤ 4 ± 7           | 10              | Parasite trematode community was similar to that found in northern Europe; H. quissetensis was found in southern Europe and northern Africa but not in northern Europe probably due to the absence of its first intermediate host |
|                           |                                    | H. interrupta      |                   | ≤ 1 ± 1       | 6                               |                |                                                                              |
|                           |                                    | H. elongata        |                   | ≤ 39 ± 56     | 40                              |                |                                                                              |
| de Montaudouin et al. (2012b) | Arcachon, France     |                     | H. continua      | <35 mm        | Ab: 0–45                        | –              | Stability of trematodes community after 8 years; vulnerable cockles range size; spatial aggregation of parasites disappeared with cockles age (parasites accumulation); H. spp. abundance decreased in winter (cockle mortality) |
|                           |                                    | H. interrupta      |                   | Ab: 1–15      | –                               |                |                                                                              |
|                           |                                    | H. elongata        |                   | Ab: 0–23      | –                               |                |                                                                              |
|                           |                                    | H. quissetensis    |                   | Ab: 0–35      | –                               |                |                                                                              |
| de Montaudouin et al. (2012a) | Arcachon, France     |                     | H. continua      | 15–25 mm      | Occurrence                      | –              | H. interrupta significantly reduced by 23% the growth of cockles; moderate negative effect of trematodes on growth and condition of cockles |
|                           |                                    | H. interrupta      |                   | Occurrence    | 227                             | 100            |                                                                              |
|                           |                                    | H. quissetensis    |                   | Occurrence    | –                               |                |                                                                              |

(Continued)
| Reference                  | Location                  | Reference Location          | Trematode species | C. edule size | Abundance (Ab) or intensity (I) | Prevalence (%) | Main findings                                                                 |
|---------------------------|---------------------------|-----------------------------|-------------------|--------------|--------------------------------|----------------|--------------------------------------------------------------------------------|
| Binias et al. (2014)      | Arcachon, France          | H. interrupta               | 28–36 mm          | Ab: 3–22     | –                              |                | Patchy distribution of trematode parasite in Arcachon Bay; no clear relationship with environmental factors |
|                           |                           | H. spp. (H. continua) and H. quissetensis |                  | Ab: 9–43     | –                              |                |                                                                                |
| Freitas et al. (2014)     | Ria de Aveiro, Portugal   | H. continua                 | 19–30 mm          | I: 1 ± 2 (mean ± s.o.) | 32 ± 26                  |                | New occurrence of H. continua; most parasites preferred muddy sand areas with euhaline conditions |
|                           |                           | H. interrupta               |                   | I: 3 ± 6     | 30 ± 34                        |                |                                                                                |
|                           |                           | H. elongata                 |                   | I: 6 ± 10    | 48 ± 43                        |                |                                                                                |
| de Montaudouin et al. (2016) | Arcachon, France              | H. quissetensis             | 25.5 ± 1.3 mm     | Ab: 0–130   | –                              |                | Importance of temperature of water and light in the infection of cockles by H. quissetensis |
| Magalhães et al. (2017)   | Arcachon, France          | H. continua                 | 13–30 mm          | Occurrence   | –                              |                | Negative correlation between cockle density and abundance of trematode parasites in juvenile cockles (dilution effect), with a threshold at 400 adult cockles per m² |
|                           |                           | H. interrupta               |                   | Ab: <25      | –                              |                |                                                                                |
|                           |                           | H. elongata                 |                   | Occurrence   | –                              |                |                                                                                |
|                           |                           | H. quissetensis             |                   | Ab: <10      | –                              |                |                                                                                |
| Magalhães et al. (2018a)  | Ria de Aveiro, Portugal   | H. interrupta               | 26–31 mm          | Rare         | 0.9                           |                | Low abundance of trematode species; importance of the oceanic influence; negative impact of global change on trematode community |
|                           |                           | H. elongata                 |                   | Ab: <1       | 23.9                          |                |                                                                                |
|                           |                           | H. quissetensis             |                   | Occurrence   | Rare                           | 0.9            |                                                                                |
| Magalhães et al. (2018b)  | Ria de Aveiro, Portugal   | H. elongata                 | 13–17 mm          | Ab: 0.2 ± 0.9 to 0.8 ± 1.0 | –            |                | H. elongato cercariae infection success increased with acidification; modification of cockles biochemical performances with climate change (may decreased survival of cockles infected by H. elongata) |
|                           |                           | H. interrupta               |                   | Occurrence   | 47–100                        |                | Intensity of infection was positively correlated with biochemical response (higher metabolic rate); high impact of H. elongata metacercariae on cockle biochemical performance; trematode infection limited pollutant accumulation |
|                           |                           | H. elongata                 |                   | Occurrence   | 39–100                        |                |                                                                            |
|                           |                           | H. quissetensis             |                   | Occurrence   | 10–48                         |                |                                                                            |
|                           |                           |                              |                   | Ab: <200     | 36–100                        |                |                                                                            |
| Correia et al. (2020a)    | North-eastern Atlantic coast | H. continua               | 23–30 mm          | Occurrence   | 8.1                           |                | Distribution of parasites along north-eastern Atlantic coast; temperature and coastal system were one of the most important drivers for parasite infection |
|                           |                           | H. interrupta               |                   | Occurrence   | 12.2                          |                |                                                                            |
|                           |                           | H. elongata                 |                   | Occurrence   | 20.9                          |                |                                                                            |
|                           |                           | H. quissetensis             |                   | Rarest species | 1.3                          |                |                                                                            |
| Correia et al. (2020b)    | Ria de Aveiro, Portugal   | H. continua                 | 17–20 mm          | Occurrence   | 0.8 ± 1.0                     |                | Importance of tidal position and current velocity on cockle infection        |
|                           |                           | H. interrupta               |                   | Occurrence   | 87.9                          |                |                                                                            |
|                           |                           | H. elongata                 |                   | Occurrence   | 22.1                          |                |                                                                            |
| Magalhães et al. (2020b)  | Ria de Aveiro, Portugal   | H. elongata                 | 19–21 mm          | Ab: 4.3 ± 2.0 | –                             |                | Two days after metacercariae infection, cockles’ metabolism was negatively affected (reduced oxygen consumption); the activity of antioxidant enzymes was increased; energy reserve of cockles would decreased at longer term |
|                           |                           | H. continua                 |                   | Ab: 0–0.7    | 0–38                          |                | General description, pathogenicity, diagnosis and risks                     |
|                           |                           | H. interrupta               |                   | Ab: 0–87.9   | 0–100                         |                |                                                                            |
|                           |                           | H. elongata                 |                   | Ab: 0–4.7    | 0–97                          |                |                                                                            |
|                           |                           | H. quissetensis             |                   | Ab: 0–22.1   | 0–100                         |                |                                                                            |

Abundance was the number of metacercariae per infected or uninfected cockle, intensity was the number of metacercariae per infected cockle and prevalence was the percentage of infected cockles.
Fig. 2. Boxplot of *H. interrupta* (A) and *H. quissetensis* (B) intensity per cockle shell length and corresponding age and seasons. Absolute age was deduced from a recruitment date in May. The box (25–75% of the data) contains a black line (median) and a red line (mean). Whiskers represent the lower and upper values in the range of ±1.5 interquartile range, with outliers as black circles. Grey arrows indicate significant variation between successive months (Wilcoxon test, $P < 0.01$). For example, in the case of *H. interrupta*, the first value that is significantly different from May 0+ intensity is in December 0+. 

Fig. 3. Percentage of metacercariae per species (*Curtuteria arguinae*, *Gymnophallus minutus*, *Psilostomum brevicolle*, *Renicola roscovitus*, *Diptherostomum brusinae* and *H. quissetensis*) in *Cerastoderma edule* without (A) and with (B) *H. interrupta*.
as host condition, first intermediate host density and environmental parameters modulated the infection of the cockles (Wilson et al., 2001; Mouritsen et al., 2003; Thieltges and Reise, 2006b; Welsh et al., 2019).

The phenology’s pattern of infection was similar during the first year in both H. interrupta and H. quissetensis. The infection occurred during the warmer season, as already reported for H. quissetensis (Prévot, 1974; Desclaux et al., 2004), but also for closely related species such as Himasthla litorinae (Nikolaev et al., 2020), H. elongata (Nikolaev et al., 2021), C. arguiniae (Desclaux et al., 2006) and other trematode families such as gymnophallids (Gam et al., 2009b), renicolidls (Thieltges and Rick, 2006) or microphallids (Meißner, 2001). Temperature of water is an important trigger to stimulate infection by cercariae (Lo and Lee, 1996; Mouritsen and Jensen, 1997; Mouritsen, 2002; Koprvnikar et al., 2014). In particular, in situ experiments showed that the thermal window for cockle infection by H. quissetensis was between 15 and 23°C, with maximum infection being at 19–20°C (de Montaudouin et al., 2016). During the first winter, the infection intensity per cockle decreased significantly, by 44 and 38% for H. interrupta and H. quissetensis, respectively. The decrease in the mean parasite intensity could be due to immigration of the less heavily infected cockles or emigration of highly parasitized cockles. This hypothesis seems irrelevant regarding the low locomotive capacity of adult cockles (Richardson et al., 1993). It could also be explained by the death of metacercariae in cockles. There are very few studies exploring the dynamics of parasite infrapopulations (i.e. populations at the scale of a host individual). Mortality of parasites was observed in cockles for the non-encysted metacercariae of G. minutus (de Montaudouin et al., 2012b). In this case, the authors had transplanted cockles, and the new site could have been deleterious to parasites, but in other cases G. minutus can suffer from hyperparasitism (Fermer et al., 2010). However, in the case of Himasthla spp. and their encysted metacercariae, empty cysts that suggest parasite death have been observed and registered at a very low intensity (Desclaux et al., 2004), leading to the exclusion of this conjecture as well. Finally, the death of the most heavily infected cockles could explain the reduction of Himasthla metacercariae intensity in winter. This third hypothesis is the most likely, and has been mentioned in several studies concerning trematodes in their second intermediate hosts (Kennedy, 1984; Desclaux et al., 2004, 2006; Gam et al., 2009b) or first intermediate hosts (Bowers, 1969; Schmidt and Fried, 1997; Rantanen et al., 1998; Watters, 1998). During the second summer, the infection pattern was less obvious. It is noteworthy that during summer infections, a stable parasite intensity in cockles can result from a balance between parasite infection and parasite-dependent mortality processes.

### Parasite co-occurrence

The trematode species richness presented in this study was similar to what has been reported in similar ecosystems along the northeast Atlantic coast (Krakau et al., 2006; Thieltges et al., 2006; Gam et al., 2009b; Magalhães et al., 2018a; Correia et al., 2020). Negative interactions among parasites within their host have been poorly documented, and in particular few studies have investigated the effect of invasive sporocyst stages on the global diversity of trematodes. Neither M. parvus nor G. choledochus sporocysts influence the prevalence or abundance of other trematode species (Magalhães et al., 2020a), contrary to those observed concerning B. minimus whose presence is linked to a higher abundance of other trematode species (Magalhães et al., 2015). Magalhães et al. (2015) suggested that B. minimus infection could impair cockle resistance to metacercariae infection, or that high metacercariae infection could facilitate B. minimus infestation. However, a second hypothesis is that all parasites co-occur independently of one another, and infect cockles because all conditions are favourable to infection by all parasite species (environmental factors, cockle fitness, other host presence). In the present study, the fact that H. interrupta (or H. quissetensis) occurrence is associated with higher trematode species richness and abundance, with similar community structure, favours the second hypothesis. Indeed, the relatively low metacercariae intensity values observed, combined with the occupation of specific organs by most trematode species (de Montaudouin et al., 2009), are weak arguments supporting an interspecific metacercarial competition, as observed by Thieltges and Reise (2006b) and Lassalle et al. (2007). In addition, an interspecific competition between Himasthla species was not expected, as their metacercariae do not grow inside their second intermediate host (de Montaudouin et al., 2005).

### Molecular identity

For H. elongata, H. quissetensis and H. interrupta, the metacercariae molecular identification was performed using 18S and COI sequences. Concerning H. elongata, all analysed sequences matched each other, and also sequences that were isolated from L. littorea cercariae. These results validate the molecular identification of H. elongata since L. littorea is the first intermediate host of only this Himasthla species. For H. quissetensis and H.
interrupta, the sequences matched each other, and thus provided a good molecular identification. All sequenced H. quis_keyboard highlighted
tensis came from samples extracted from the cockles’ foot, while samples corresponding to H. interrupta were extracted from the mantle. However, a mismatch occurred for H. continua, with no match among the analysed sequences (high variability). We cannot rule out that this high variability of the obtained sequences was associated with the presence of some larvae of another Himasthla species, e.g. Himasthla leptosoma. The larvae of these two species are hardly distinguishable by microscopic methods, being very similar in the size of their cysts as well as in the number of spines on the collar (Galaktionov et al., 2021). Finally, our results confirm the identity of three species of Himasthla metacercariae, which can be difficult to distinguish under a stereomicroscope based on morphological identification.

Conclusion

Trematodes of the Himasthla genus are very common parasites of cockles. Their effect on the cockle individuals or populations is usually reported as low. From an evolution point of view, the metacercariae infection reaches high levels. Considering that this infection is dependent negative effects occur when the metacercarial stage would not be to consume the host energy, as occurs in the first and final hosts. However, a literature review and analysis of a 20-year database revealed that some Himasthla-dependent negative effects occur when the metacercariae infection reaches high levels. Considering that this infection is often related to temperature, this parasite dynamics should be monitored according to different climate change scenarios. While morphological identification is particularly difficult concerning Himasthla genus, new molecular sequences provided in this study may be helpful for an accurate identification of some species, although uncertainties still remain concerning H. continua.

Data. The data presented in this study are available on request from the corresponding author.

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Author contributions. X. M. conducted data gathering. A. R. produced the literature review. G. D. realized molecular analyses. A. R., X. M. and L. M. performed statistical analyses. A. R. and X. M. wrote the original draft paper. A. R., G. D., L. M., O. M. and X. M. reviewed and corrected the article.

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Conflict of interest. The authors declare there are no conflicts of interest.

Ethical Standards. Not applicable.

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