Marine Biology (2024) 171:63
https://doi.org/10.1007/s00227-023-04381-3

SHORT NOTES

Lack of *Hematodinium* microscopic detection in crustaceans at the northern and southern ends of the Wadden Sea and an update of its distribution in Europe

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Received: 26 October 2023 / Accepted: 14 December 2023

Abstract

Hematodinium is a parasitic dinoflagellate with a wide distribution along the European coastline. We investigated whether *Hematodinium* occurs in the Wadden Sea, a large tidal flat system spanning the Netherlands, Germany, and Denmark, where to date no records exist. In total, we investigated eight different species from six sites at the southern (Texel, the Netherlands) and northern (Sylt, Germany) ends of the system. Based on microscopic hemolymph screening, we did not detect *Hematodinium* in any of the 1252 investigated individuals. An extensive additional literature review revealed 1489 *Hematodinium* records in Europe from 14 crustacean species, most locally occurring at high prevalence. This makes our finding of lack of *Hematodinium* infections surprising and suggests that environmental factors such as a lower salinity may limit the distribution of *Hematodinium* at the investigated locations at both ends of the Wadden Sea. However, whether the entire Wadden Sea represents a distributional gap for *Hematodinium* remains to be investigated.

Keywords Parasitic dinoflagellate · Host diversity · Distribution

Introduction

Hematodinium is a genus of parasitic dinoflagellates and infections have been reported in over 40 marine crustacean species, including crabs, lobsters, shrimps, and amphipods (Chatton and Poisson 1931; Stentiford and Shields 2005; Small 2012; Li et al. 2021). *Hematodinium* mainly occurs in the hemolymph of hosts or in the hemocoels of affected tissues or organs, often leading to mortality due to massive proliferation in the late stage of infection (Field and Appleton 1995; Taylor et al. 1996; Sheppard et al. 2003; Wheeler et al. 2007). Over the past decades, *Hematodinium* caused severe epizootics in wild crustacean populations and cultured stocks of commercially valuable species, such as "bitter crab disease" in snow crabs (*Chionoecetes opilio*), "pink crab disease" in edible crabs (*Cancer pagurus*), and "milk disease" in Chinese swimming crabs (*Portunus trituberculatus*) (Meyers et al. 1987; Stentiford et al. 2003; Wang et al. 2017).

Hematodinium has a wide global distribution (Small 2012; Li et al. 2021). In Europe, *Hematodinium* infections have been reported from the continental coasts of France in the South to Norway in the North, as well as from England and Ireland, sometimes with high local prevalence in native crustaceans, such edible crabs (*Cancer pagurus*, 77%), and Norway lobsters (*Nephrops norvegicus*, 93.8%), causing huge economic losses to local fisheries (Field et al. 1992; Chualáin et al. 2009). Interestingly, distribution records are absent from the continental side of the European North Sea

Responsible Editor: C. Meunier .

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Published online: 30 January 2024

(Small 2012; Li et al. 2021). This includes the Wadden Sea, the largest temperate tidal flat system in the world, which is a designated UNESCO natural world heritage site and stretches from the Netherlands over Germany to Denmark (Kabat et al. 2012). This extensive marine ecosystem exhibits a high biomass of invertebrates, including decapods and other crustaceans, and it serves as an important stop-over site for millions of migrating birds (The Wadden Sea Quality Status Report 2017). The Wadden Sea is known to harbor a rich parasite and pathogen fauna in invertebrates and vertebrates (Thieltges et al. 2006a, b; Thieltges et al. 2013; Wendling and Wegner 2015; Waser et al. 2016; Goedknegt et al. 2017), but to date there are no records of *Hematodinium*. It is unclear, whether this is an indication of the absence of *Hematodinium* in the area or a reflection of an absence of research effort.

The aim of our study was 2-fold: first, we thoroughly reviewed the literature and built hosts records dataset to identify the currently known distribution of *Hematodinium* in Europe, and second, we extensively sampled various crustacean species from the southern (Texel) and northern (Sylt) ends of the Wadden Sea ecosystem to identify whether *Hematodinium* also occurs at these locations.

Materials and methods

We searched ISI Web of Science using the keyword "*Hematodinium*" from 1900 to 2022 and conducted a supplementary search with Google Scholar. By checking every individual publication retrieved, we identified 50 studies that included *Hematodinium* records from Europe. For each record, we noted the host species (corrected for synonyms with WoRMS), temperature, salinity, testing methods, prevalence, coordinates, and sampling month and year. Moreover, the sampling months were grouped into seasons, including Q1 (Mar–May), Q2 (June–Aug), Q3 (Sept–Nov) and Q4 (Dec–Feb). Data from graphs were extracted using the free software ImageJ (https://github.com/imagej). When not given in the original studies, the coordinates were obtained from Google Maps (https://maps.google.com), and a central coordinate was chosen when the sampling location was a larger region. We then plotted *Hematodinium* distribution records per location (i.e., multiple sampling events over several months or years at a specific location were lumped into a single record) with Surfer (v.17).

Sampling of crustaceans was carried out from September 2021 to July 2022 around the islands of Texel (Netherlands) and Sylt (Germany) (Fig. 1; Table S1). Around Texel, crabs were collected from a long-term fish monitoring net maintained by the NIOZ Royal Netherlands Institute for Sea Research (site 1), by hand in the intertidal (sites 2 and 3), and by dredging or setting traps around Griend (site 4) in a long-term monitoring campaign conducted by Wageningen Marine Research. Around Sylt, crabs were collected in the subtidal with a dredge (1 m width, mesh size: 1 cm; site 5) and in the intertidal by hand from a mixed mussel/oyster bed (site 6). Sample sizes differed among sites depending on the local abundances of crustacean species (Table S1). After collection, crustaceans were temporarily stored in containers for subsequent *Hematodinium* diagnoses.

Hematodinium infections were diagnosed with hemolymph smear assays as described in Stentiford and Shields (2005). Briefly, aliquots of 2–3 drops of hemolymph were withdrawn with 27-gauge needles and mixed with an equal volume of 0.04% (w/v) neutral red in filtered seawater. For brachyuran crabs, hemolymph was taken from the juncture between the basis and ischium of the 5th leg. For shell carrying *Pagurus bernhardus*, hemolymph was withdrawn from the juncture of claws. The hemolymph of brown shrimps (*Crangon crangon*) was withdrawn from the ventral sinus or heart. All hemolymph smears were then screened by an expert (Qian Huang: Huang et al. 2019, 2021) with a light microscope (Leica DM2000, Germany) at 200× and 400× for the presence of *Hematodinium* cells.

Results and discussion

Since *Hematodinium* was first discovered in the 1930s in green crabs (*Carcinus maenas*) and harbor crabs (*Liocarcinus depurator*) along the French coast, the number of host species and distribution records have increased gradually in the past decades (Chatton and Poisson 1931; Li et al. 2021). In total, our literature search identified 1489 *Hematodinium* records in 14 different species in Europe, with multiple sampling events over several months or years at many of the specific locations counted as separate records (Tables 1, S2). *Hematodinium* positive infections were reported in 13 of the crustacean species belonging to two orders, Decapoda and Amphipoda: *Callinectes sapidus*, *Cancer pagurus*, *Carcinus maenas*, *Crangon crangon*, *Eriphia verrucosa*, *Liocarcinus depurator*, *Munida rugosa*, *Necora puber*, *Nephrops norvegicus*, *Pagurus bernhardus*, *Pagurus prideaux*, *Portumnus latipes*, and *Tryphosa nana*. These results suggest that *Hematodinium* is a generalist parasite that may pose infection risks to other crustacean species in Europe. Additionally, host diversity has been shown to play an important role in maintaining *Hematodinium* infections in hot spots along the east coast of North America (Pagenkopp Lohan et al. 2012). *Hematodinium* infections were widely distributed in Europe, spanning over 20 degrees of latitude and concentrating around the British Isles and France (Fig. 1). Along the Atlantic coast, *Hematodinium* prevalence was high in some species such as *Cancer pagurus* and *Carcinus maenas*, with up to 77% and 50%, respectively (Fig. 2; Table 1).

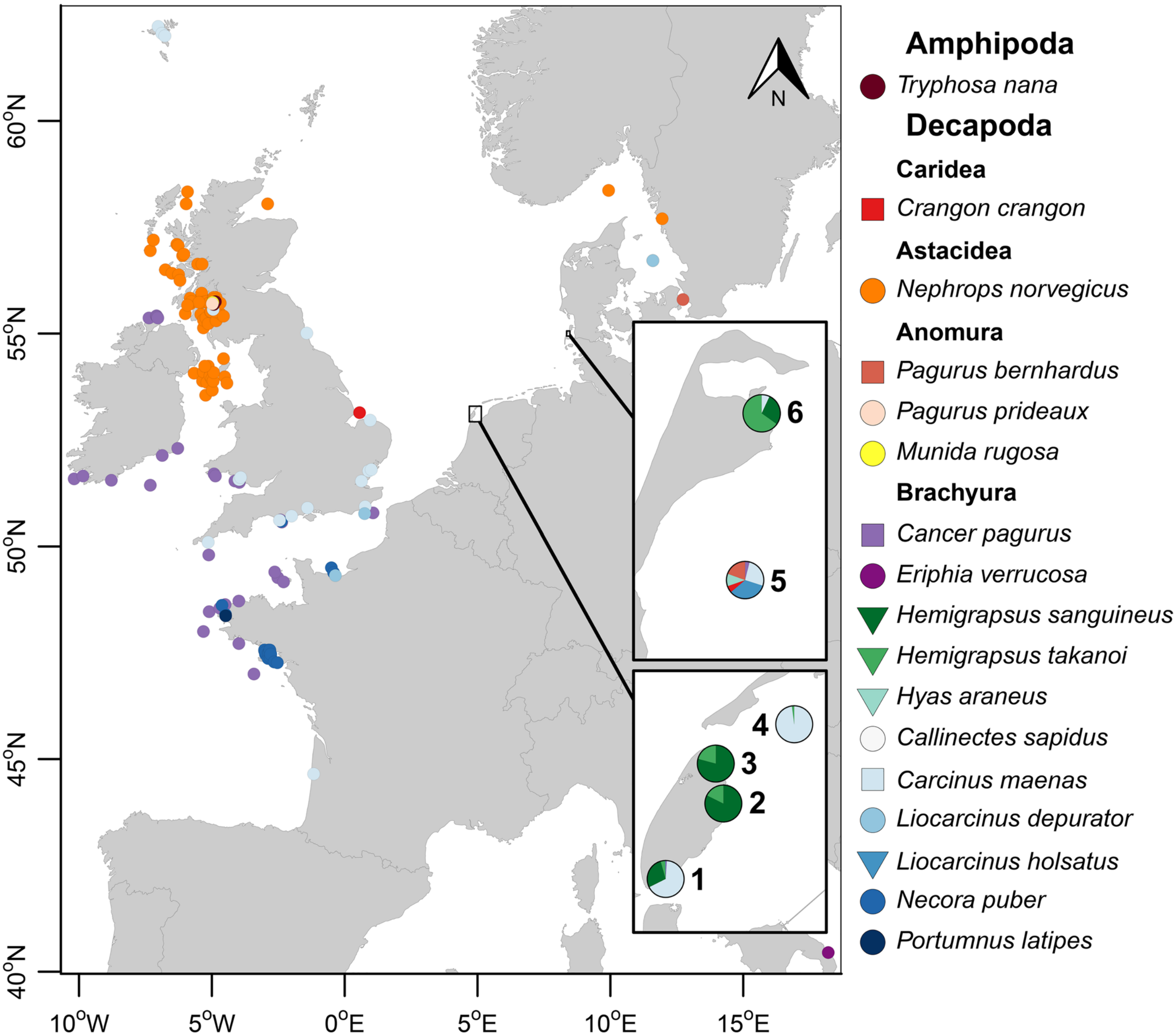

Fig. 1 Crustacean hosts and sampling locations from which *Hematodinium* infections have been reported in Europe in the literature. Insets show the sampling area in the present study: top (Sylt, Germany) and bottom (Texel, the Netherlands). The pies show the composition of investigated host species per sampling location. The relative number of individuals per species is given by the size of the respective pie chart part. In the legend, circles represent only host species in the literature, triangles are for investigated species in the present study, squares mark species with both

In *Pagurus bernhardus* from the Baltic Sea, *Hematodinium* infections of up to 80% were observed (Table 1). However, our literature and dataset survey confirmed that there was an absence of research effort regarding *Hematodinium* in the Wadden Sea.

Although being a generalist and broadly distributed in Europe, as revealed by our literature search, we did not detect *Hematodinium* in any of the 1252 crustacean individuals of 8 crustacean species from 6 sites in the Wadden Sea, including *Hemigrapsus sanguineus*, *Hemigrapsus takanoi*, *Cancer pagurus*, *Carcinus maenas*, *Hyas araneus*, *Liocarcinus holsatus*, *Pagurus bernhardus,* and *Crangon crangon* (Fig. 1; Table S1). Four of the crustacean species that we investigated (*H. sanguineus*, *H. takanoi*, *H. araneus*, and *L. holsatus*) were previously not investigated for *Hematodinium* infections in Europe. Interestingly, the same crustacean species were found to be infected elsewhere with relatively high prevalence such as *C. pagurus* in France and *P. bernhardus* in Denmark (Latrouite et al. 1988; Eigemann et al. 2010). Likewise, the invasive crab *H. takanoi* is a potentially susceptible host for *Hematodinium* as it is infected in its native habitats in Asia (Gong et al. 2023). A susceptibility of invasive hosts in their introduced range is known from blue crabs (*Callinectes sapidus*), which is

Table 1 *Hematodinium* prevalence (%) in Europe as noted in the literature and results of our sampling in the Wadden Sea around Texel and Sylt (sample size given in parentheses)

Host species	Texel	Sylt	Elsewhere in Europe	References
Calanus spp.			0	Hamilton et al. (2011)
Callinectes sapidus			6.0	Patrizia and Giorgio (2018)
Cancer pagurus	0 (7)	0 (10)	0-77.0	Latrouite et al. (1988); Stentiford et al. (2002); Stentiford et al. (2003); Small (2004); Robinson et al. (2005); Small et al. (2007a); Small et al. (2008); Hamilton et al. (2009); Chualain et al. (2009); Chualain et al. (2011); Bateman et al. (2011); Smith et al. (2013); Smith et al. (2015); Thrupp et al. (2015)
Carcinus maenas	0 (659)	0 (70)	0-50.0	Chatton and Poisson (1931); Stentiford and Feist (2005); Hamilton et al. (2007); Hamilton et al. (2009); Bojko et al. (2018); Davies et al. (2019); Davies et al. (2022)
Crangon crangon		0 (15)	1.6	Stentiford et al. (2012)
Eriphia verrucosa			3.0	Patrizia and Giorgio (2018)
Hemigrapsus sanguineus	0 (239)	0 (12)		
Hemigrapsus takanoi	0 (44)	0 (28)		
Hyas araneus		0 (28)		
Liocarcinus depurator			0.6-87.5	Chatton and Poisson (1931); Hamilton et al. (2009); Eigemann et al. (2010); Small et al. (2012)
Liocarcinus holsatus	0 (3)	0 (87)		
Munida rugosa			0-50.1	Hamilton et al. (2009); Hamilton et al. (2011)
Necora puber			0-87.0	Wilhelm and Boulo (1988); Wilhelm and Mialhe (1996); Stentiford et al. (2003); Hamilton et al. (2009)
Nephrops norvegicus			0-100	Field et al. (1992); Hudson and Adlard (1994); Field and Appleton (1995); Field and Appleton (1996); Taylor et al. (1996); Field et al. (1998); Appleton and Vickerman (1998); Stentiford et al. (1999); Stentiford et al. (2000); Stentiford and Neil (2000); Stentiford et al. (2001a, b, c); Small et al. (2002); Briggs and McAliskey (2002); Small (2004); Small et al. (2006); Small et al.(2007a, b); Hamilton et al. (2007); Eigemann et al.(2010); Beevers et al. (2012); Albalat et al. (2012); Stentiford et al. (2015); Albalat et al. (2016); Morado et al. (2006)
Pagurus bernhardus		0 (50)	11.8-80.0	Small (2004); Small et al. (2007b); Hamilton et al. (2009); Eigemann et al. (2010)
Pagurus prideaux			0-29.0	Hamilton et al. (2009)
Portumnus latipes			22.5	Gallien (1938)
Tryphosa nana			0-38.5	Small (2004); Small et al. (2006)

Yellow shading indicates species and locations that have been investigated, while gray shading indicates non-investigations

one of the main *Hematodinium* hosts in America, and where introduced populations of the crab species in Italy are also infected (Patrizia and Giorgio 2018). This makes our finding of lack of *Hematodinium* infections surprising and suggests that environmental factors may limit the distribution of *Hematodinium* at the investigated locations at both ends of the Wadden Sea. One of these potentially limiting factors could be salinity as there can be strong salinity changes in the sampled locations, especially around Texel (23–29) through the input of freshwater from Lake Ijssel (Ridderinkhof et al. 2002; Aken 2008a, b). Although salinity does not impact *Hematodinium* proliferation in hosts, its dinospores decay quickly with a decrease in salinity, especially under low salinity (<20) which might hamper *Hematodinium* transmission (Coffey et al. 2012). Furthermore, according to the positive infection records with environmental parameters, *Hematodinium* preferred to occur where salinity was more than 32 along European coasts (Briggs and McAliskey 2002; Hamilton et al. 2009; Albalat et al. 2016). However, there may also be other factors underlying the observed lack of *Hematodinium* infections at the sampled locations and whether the entire Wadden Sea represents a distributional gap for *Hematodinium* remains to be investigated.

In the present research, the hemolymph smear assay with neutral red was chosen as a cost-effective and time-efficient diagnostic method for *Hematodinium* detection. The active absorption of neutral red by *Hematodinium* lysosomes results in distinct staining, providing visual contrast to host hemocytes (Stentiford and Shields 2005). The hemolymph smear assay has been used as an initial assessment tool due to its high specificity and sensitivity (Ni Chualáin and Robinson 2011; Shields et al. 2015). Notably, a review of the global diversity and distribution of *Hematodinium* revealed that 46% of the reports relied solely on light microscopy (Small 2012). Similarly, in the European records we identified, the hemolymph smear assay accounted for 56.1% of the observations, excluding the color and pleopod method (CP) specifically used for Norway lobsters *Nephrops norvegicus* (Table S3). Despite its efficacy, the hemolymph smear assay has limitations in detecting latent infections compared to PCR analysis. For instance, in American blue crabs (*Callinectes sapidus*), the positive predictive value of hemolymph smear assay was 97% during October outbreak but dropped to 56.7% in winter surveys (Shields et al. 2015). Our fieldwork period, covering three quarters of the year (Q1, Q2, Q3), aligned well with literature dataset records, indicating that the two locations in the Wadden Sea lacked *Hematodinium* infections even during potential outbreak seasons (Fig. 2; Table S1). Hence, based on these methodological considerations and the fact that the screening was conducted by a highly experienced observer, we are confident that the absence of *Hematodinium* infections at the investigated locations is not a methodological artifact. However, for future investigations, PCR-based methods, with a detection limit of 0.3 *Hematodinium* cells per 100 μL hemolymph and less than one cell per 100 mL water or 1 g sediment, should be considered to enhance diagnostic accuracy (Small et al. 2007c; Li et al. 2010).

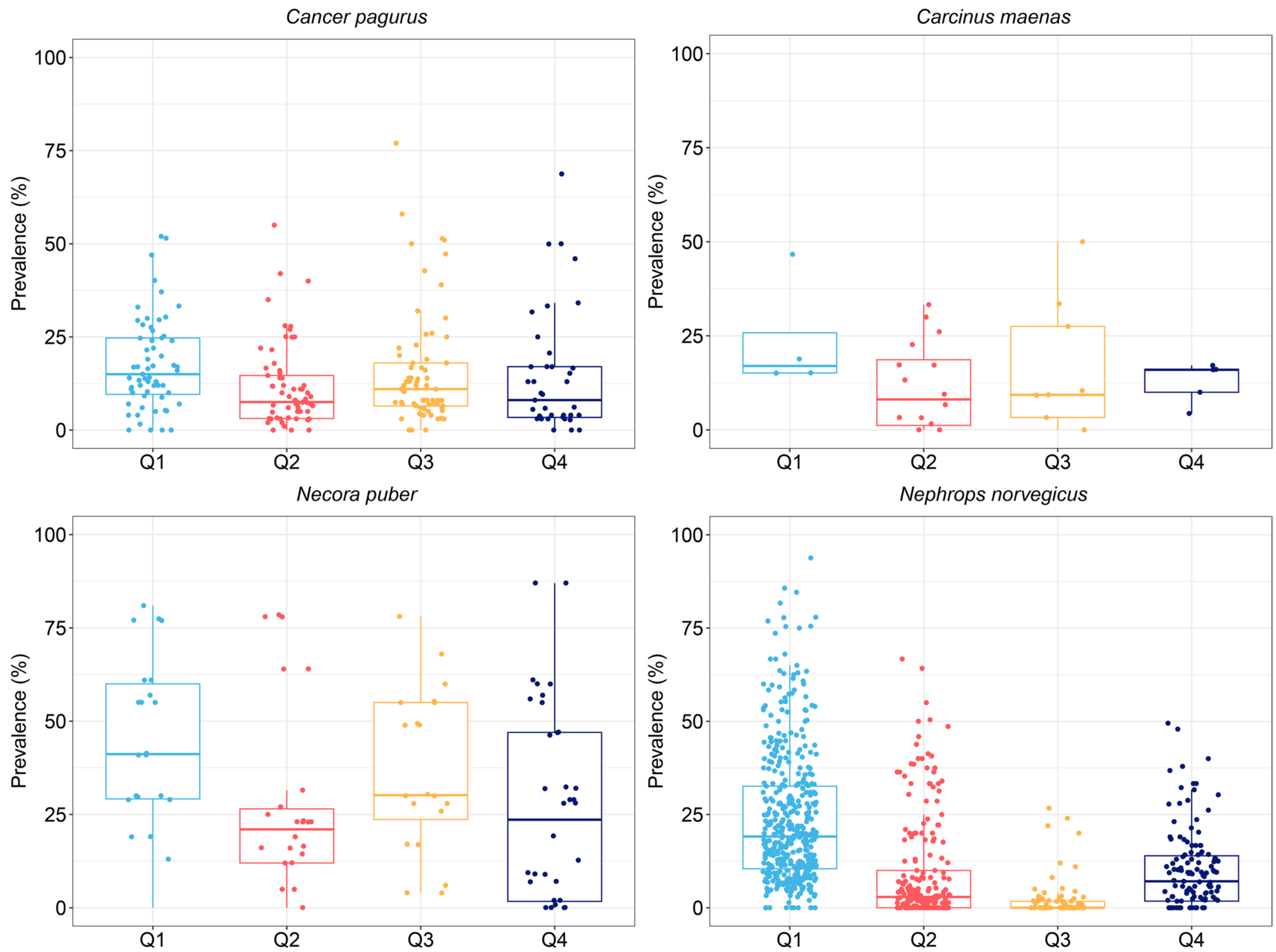

Fig. 2 *Hematodinium* prevalence in different quarters of the year in the four host species with the most records based on our literature and dataset search. Q1: Mar–May; Q2: June–Aug; Q3: Sept–Nov; Q4: Dec–Feb

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00227-023-04381-3.

Acknowledgements This study was financially supported by the "Joint Training Program" from the University of Chinese Academy of Sciences. Thanks to Dr. Karin Troost in Wageningen Marine Research for kind assistance during sample collection.

Author contributions All authors contributed to the study conception and design. Material preparation, and data collection and analysis were performed by QH, AMW, and DWT. The first draft of the manuscript was written by QH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was funded by the "Joint Training Program" from the University of Chinese Academy of Sciences.

Data availability The datasets generated during the current study are available in the Version 1. 4TU.ResearchData. dataset. https://doi.org/https://doi.org/10.4121/3676d955-69c4-489c-b961-f59eade2e55d.

Declarations

Competing interests 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.

Ethics approval The applicable national and institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed.

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