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ORIGINAL PAPER

Key parameters for the consumption suitability of offshore cultivated blue mussels (*Mytilus edulis* L.) in the German Bight

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Abstract The occurrence and composition of toxic algae, and presence of viruses and other human microbial pathogens in production areas of mussels are factors determining suitability of mussel products for human consumption. As bivalves feed by filtering large volumes of water, potentially toxic viruses, algae, and bacteria as well as phytoplankton are ingested. With the expansion of mussel aquaculture and subsequent increase in human consumption of mussel products, improved risk management is required for consumer protection. For example, shifting production to offshore areas (e.g. wind farms) can decrease the hazards of infection due to dilution of contaminants, and increase overall health of mussels. In addition, the deployment of off-bottom cultivation methods such as longlines increases the condition index, growth, and aesthetic appearance of mussels. However, other hazards like algal toxins not yet monitored on a regular basis, may play a more important rule offshore. Here, we present an analysis of biological, economic, and consumer health-related aspects of mussel cultivation under near- and offshore conditions.

Keywords Blue mussel · Offshore cultivation · Site selection · Parasites · Viruses · Biotoxins · Microbial load

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Introduction

In their natural environment, at in- and nearshore inter- and subtidal areas, mussels can be exposed to high concentrations of pollutants, pesticides, near surface agents, and estuarine runoff. High nutrient values in marine waters, particularly in densely populated coastal areas, provide an ideal environment for potential explosive growth of algae and bacteria. Even in regions with strict regulation of wastewater treatment, contamination with human pathogenic microbes can be found, which accumulate in filter feeding mussels. Many different kinds of bacteria and viruses, which are transmitted through the faecal-oral route, can occur in high numbers in sewage and cause illness, such as gastroenteritis. This route has been recognized as one of the most clearly identified health risks associated with urban wastewater [1] and the concern remains that sewage treatment does not remove all pathogens from the effluent. Most cultivated and wild bivalves, for example mussels, oysters and clams, thrive in nearshore areas and are commonly consumed raw, or slightly cooked. As these organisms are exposed to a wide array of contaminants, clearly a serious hazard to human health exists. Numerous outbreaks of shellfish-transmitted infections have been recorded [2]. In contrast to nearshore areas, offshore waters offer a much cleaner environment due to the effects of dilution. Thus, the shifting of mussel

production from intertidal or nearshore to offshore cultivation areas would reduce the general risks of infection and contamination. Yet other hazards, such as algal toxins, can occur more frequently offshore [3].

The European Community has outlined specific rules for products of animal origin in the Regulation (EC) 854/2004 [4]. According to this, contamination with parasites should be generally avoided. However, since mussel parasites are non- pathogenic to consumers, moderate infestations are tolerated. This practice is also applied to other marine fishery products (e.g. nematods in wild salmon), since a strict interpretation of the regulation would have a severe impact on sales and distribution of these products.

Although including parameters such as human pathogen viruses or the *Vibrio* species into a regular monitoring of bivalves remains a suggestion [5–7] as inferences from the microbial load to these contaminants are not evident [8], the European Commission has designated only coliform bacteria i.e. *Escherichia coli* as an indicator for faecal contamination. Even the most recent EU guidelines [9] for the control of mussel products expand the monitoring of the production areas only to all relevant influences on the microbial environment (runoffs, shipping zones, wild animals and other potential factors of contamination). However the focus remains on the analysis of *E. coli* and *Salmonella* as the principle parameters when defining suitability for consumption.

Bacterial infections

The survival of bacteria in seawater and its exposure to bivalves varies due to environmental factors such as temperature and salinity, and is influenced on seasonal and spatial scales [10]. The bivalves' response towards ingested microbes is to eliminate them. However, it has been shown that *Salmonella typhimurium* can survive more than 2 weeks after being injected into the circulating system of mussels [10]. *Salmonella* species can cause enterocolitis, enteric fevers such as typhoid fever, and septicemia with metastatic infections in humans. Seawater is the natural habitat of the *Vibrio* bacteria, feared as pathogens in fish and shellfish [11]. *Vibrio* can also cause severe infections in humans after consumption of raw or undercooked shellfish and contaminated food. A special hazard is caused by *V. vulnificus,* where severe infections can occur through skin lesions [12].

Viral infections

Like bacteria, viruses are predominantly concentrated in the digestive glands, but can also be absorbed through the gills [13] of mussels. Certain viruses such as the Norovirus are even more persistent and can remain infectious for weeks to months in seawater or in sediment [14]. Although they are inherently unable to multiply in bivalves, shellfish are efficient vehicles for transmission of pathogenic viruses to humans. Epidemiological studies have revealed that human enteric viruses are the most common pathogens transmitted by consumption of bivalve shellfish [2, 15]. Among these, HAV is the most serious viral infection linked to the consumption of bivalves. In Italy, recent estimates suggest that approximately 70% of HAV cases are caused by shellfish consumption [16]. The relatively long incubation period following initial infection (average 4 weeks), complicates the traceability of the viral source. Thus, HAV infections caused through shellfish consumption are probably underreported or even remain undiscovered. Norovirus and serotypes of the adenovirus group are associated with gastroenteritis. These viruses have been recorded in seawater and shellfish in many countries [6]. In particular overall viral infections caused by the Norovirus (gene group II) have shown a remarkable increase, as registered by the Robert-Koch Institute [17]. This increase, however, may be because Norovirus infections must be reported by law. However, the rapid course of the illness within a few hours complicates appropriate countermeasures.

Algae toxins

The main food source for bivalves is phytoplankton and here the potential for accumulating algal toxins is high. Several human diseases have been reported to be associated with many toxin- producing species of dinoflagellates, diatoms, nanoflagellates and cyanobacteria that occur in the marine environment [18]. Marine algal toxins become a problem primarily because they may concentrate in shellfish and fish that are subsequently eaten by humans [19, 20], causing syndromes including paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), and azaspirasid shellfish poisoning (AZP). Beside NSP all other syndromes can also be traced to contaminated shellfish in European coastal waters. These shellfish-caused illnesses compromise human health, resulting in fishery closures, commercial losses, and serious concern over seafood safety and environmental quality. A regular monitoring system covering the risks according to site, which is able to detect problematic mussel products, is therefore a prerequisite to protect consumers.

Parasites

All known micro- and macroparasites of the European coastal waters are—in contrast to other mentioned organisms—not pathogenic to consumers, but may have negative

condition effects and cause higher mortalities of infested hosts. Parasites of blue mussels occur largely in intertidal and nearshore areas. Buck et al. [21] have shown that mussels grown offshore are free of macroparasites. Infestation rates increased from sites towards the shore, where in particular intertidal mussels showed the highest numbers of parasites. In this study we focused on abundant species commonly regarded as harmful (e.g. [22–29]) or cadging [30] to the host living in the tissues, organs, or shell of the mussel. Species living in the mantle cavern or on tissues were not considered, since any impact on the energy household of the mussel is unlikely.

The only microparasite known to be associated with *M. edulis* along the European Atlantic coast is *Marteilia refrigens,* causing the Marteilliosis or Aber disease [31]. From the North Sea, infested populations of mussels have been reported from the British Isles, whereas the eastern regions including the German Bight are regarded as microparasite free. Marteilliosis in mussels is generally associated with a poor condition index, exhaustion of energy reserves (e.g. glycogen) and high mortalities [32]. Mass infections with *M. refrigens* can have a severe economic impact, e.g. oyster farmers in France lost approximately 440 million Euros in 2 years (1980 and 1983) due to Marteilliosis.

Shell commensals

Many of these organisms use mussels, or any other suitable hard substrate, to settle on. Since these organisms do not depend on mussels to fulfill their reproduction cycle, they are commonly regarded as commensals [33]. Unsoiled mussel shells, due to their aesthetic appearance, demand the highest prices on the market since they can be sold alive without being extensively cleaned before processing or selling. Information on the health effects of shell commensals on host organisms is still scarce, since the measurement and evaluation of the impact of parasites or commensal species and their influence on single hosts or host populations is difficult to determine [34]. However, studies have shown that massive covering hamper feeding, increase flow resistance, and reduce growth [35–37].

Economics

From the economic point of view mussels should not contain microbes, or be at least clearly under legal thresholds. Mussels should grow fast, have a good meat–shell ratio and should look aesthetically pleasing to achieve the highest price on the market. In the traditional nearshore on-bottom cultivation grounds in the Wadden Sea of Germany, not all of these preconditions for maximum growth, microbial purity, and aesthetic demands are fulfilled. Mussels cultivated off-bottom using longlines grow faster and have higher meat/shell ratios than on-bottom cultivated mussels [38].

Offshore production

Although the market for mussel products in Germany is not saturated and mussels are imported, an expansion of the on-bottom production sector within intertidal and subtidal areas of the coastal sea is not allowed due to restrictions on the number of licenses, environmental protection and stakeholder conflicts [39]. However the development the offshore wind farming industry offers a unique opportunity to co-use large marine areas with submerged culture systems for blue mussels and other candidates [40]. Estuarine runoffs result in a high concentration of contaminants in the Wadden Sea. In contrast offshore areas are far enough away from sources of urban sewage and estuarine runoff that waters are clean with continuously good O_2-conditions. Organisms living under good water conditions accumulate fewer toxins and have a less stressed immune system. Mussels grown under offshore conditions should be in better health than mussels grown in near- and inshore areas. Healthier mussels mean faster growth rates and a qualitatively better product for human consumption. In addition rapid growth and a better quality of product compensate for the higher investment costs incurred by the new culture systems compared to traditional bottom culture techniques.

In the present study, we have examined mussels from six different sites of the German Bight, including an offshore and a nearshore testing area both equipped with submerged culture systems. Samples were assessed according to the actual legislation and guidelines of the EU, Germany and its States (bacteriological load, viruses and algae toxins). Parameters relevant for growth (macro- and microparasites and to some extent shell commensals) and those influencing the marketability of mussel products (calcareous fouling organisms, meat content) were also investigated.

Materials and methods

Five locations along the coast of the German Bight were sampled to test and analyse mussels grown under different conditions (Fig. 1). Three areas were natural beds of mussels near Neuharlingersiel (NH, upper intertidal, Position 53°42′10″N; 007°43′50″E), Bordumer Sand (BS, upper intertidal, Position 53°30′00″N; 008°06′00″E) and from the Lister Strand from the Island of Sylt (SY, lower intertidal, Position 55°01′32″N; 008°26′43″E). Two locations were specially designed testing areas, where mussels

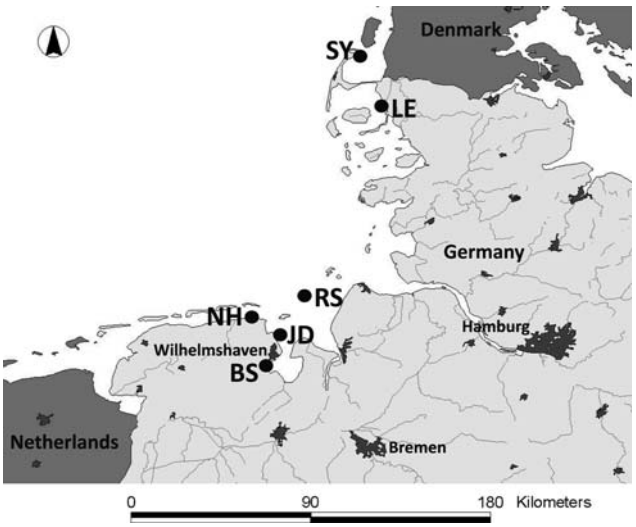

Fig. 1 Map of the German Bight showing the sample sites. Three intertidal sampling locations at Neuharlingersiel (*NH*), Bordumer Sand (*BS*) and Lyster Strand at the island of Sylt (*SY*) and two suspended hanging cultures at the *Niedersachsenbrücke* (nearshore) near Wilhelmshaven in the Jade estuary (*JD*) and offshore in Weser estuary near the lighthouse *Roter Sand* (*RS*) were sampled in the year 2007. The on-bottom cultivation (subtidal) area at *Eidumstief* (*LE*) was sampled once in winter 2009

were grown suspended on an artificial substrate: the nearshore location on the *Niedersachsenbrücke*, an approx. 1,300 m long cargo bridge, at the Jade estuary (JD, Position 53°35′05″N; 008°09′14″E) near the city of Wilhelmshaven, and under offshore conditions an area called *Roter Sand* (RS, Position 53°51′00″N; 008°04′20″E) situated in the Weser estuary ca. 17 nautical miles northwest of the city of Bremerhaven. Throughout 2007 four consecutive sampling cycles in March, May, August, and November were conducted to test for site and seasonal influences on assessed parameters. Each sampling cycle was completed within 10 days and all parameters were analysed for each site and sample cycle. Intertidal areas (NH, BS and SY) were sampled at low water, whereas RS had to be sampled at slack water with a team of scuba divers operating from a research vessel. The JD site is accessible without any tidal constraints all year round. At each sampling site ca. 5 kg of mussels were collected for all investigations.

In addition, mussels from a licensed area (LE, subtidal, Position 54°46′66″N, 008°18′72″E [Fig. 1]) for on-bottom cultivation at *Eidumstief* near Emmelsbüll-Horsbüll, Germany, were sampled once in winter 2009 by the local fishermen. The spat for these mussels was collected on vertical nets at the Jade estuary at May 2007, transferred in October 2007 to the LE (N 37) and harvested there in February 16, 2009. For these mussels weights and shell lengths were determined, but they were only analysed for macroparasites.

Macroparasites

To ensure that all mussels were of a comparable age range, 15 mussels were selected according to a shell length between 25 and 50 mm. These represent specimens of similar physiology, also used in standardized bioassays [41]. Mussels bigger than 50 mm originated from the offshore sampling site of RS (August and November 2007) where growth rates are high and mussels reached sizes up to 65 mm within 15 months. Mussels from suspended offshore and nearshore sites were of a defined age since deployment of the artificial substrate took place in April 2006 at both sites. Raw mussel were frozen and stored at −20 °C. After defrosting at room temperature (approx. 20–30 min) mussels were analyzed immediately.

First, the area covered by calcareous shell commensals of all mussels was estimated. Length and width of each selected mussel was measured according to Seed [42] to the nearest 0.1 mm using a vernier calliper. Mussels were opened, briefly drained on absorbent paper, and subsequently total wet weight was determined. Then, the soft body was removed and both shell and soft body were weighed (±0.01 g) separately. The soft body was then placed on the bottom of a glass compressorium and the mantle, gills, food, adductor muscle and other tissues were dissected carefully and dispersed. The digestive gland was pulled apart and squeezed together with the other tissues using the cover glass of the compressorium.

The preparations were examined under a stereo magnifying glass (10–50 magnification) with transmitting light for the presence of macroparasites. Parasite species were identified according to descriptions from the literature (e.g. [43–46]) and infested organs listed. As freezing of the samples does not affect size of a trematod's metacercaria [47], identification of trematodes was also reliable using frozen samples. In a final step all shells of the analysed mussels were inspected for the presence of shell-boring polychaets using the stereo magnifying glass.

In addition 15 mussels from the winter sample of LE were also analysed according to the same scheme described above.

Microparasites

Forty mussels (30–50 mm) per sample site were analysed each sampling cycle to assess potential infestations with intracellular microparasites, of the genus *Marteilia*. Fresh meat of 20 mussels was removed from the shell and glued separately on aluminium chucks before being frozen at –20 °C for kryostat-sectioning. To ensure a representative overview of potential infested organs, the frozen softbody was trimmed until digestive gland, gills, and palps appeared together in one tissue sections of the sample. Soft bodies of

additional 20 mussels were removed and cut transversally according to international standard methods [48] and subsequently used for smear preparations. Tissue sections and smear preparations were stained using *Haemacolor*® (Merck) before assessed by light microscopy.

Condition index and shell length–weight relation

Thirty mussels were used to calculate the condition index (CI) for all testing sites (data of 15 mussels used for macroparasite assessment added by 15 additional mussels to increase sample size). For a direct comparison of CI and the parasite load only wet weights of tissues and shells could be used for the calculation (see below). An additional comparison is provided with all winter samples including mussels from LE. Here, also 30 individuals were used for CI.

$$\mathrm{CI} = \frac{\text{Wet meat weight [g]}}{\text{Shell weight [g]}} \times 100 \quad (1)$$

Since shell thickness and strength strongly depends on natural conditions and the cultivation method of the mussel, a shell length-weight (dry weights) correlation of winter samples including the licensed area was established. Mussels were sorted into three groups containing each a minimum of 45 individuals: intertidal ($n = 45$), off-bottom ($n = 60$), and on-bottom ($n = 45$).

Bacterial count: *E. coli*, *Salmonella*, *Clostridia* and *Vibrio*

The mussels from each sampling site were examined at the Institute for Fish and Fishery Products of the State Office for Consumer Protection and Food Safety of Lower Saxony (LAVES). Prior to bacterial investigation the mussels were cleaned, opened and prepared under sterile conditions.

Total aerobe bacterial number

The method used corresponded to the standardized method DIN 10161 [49] which describes the drop plating procedure. According to this method an initial solution of 5 g of the homogenized sample was diluted decimally over six steps, and then incubated separately on culture media (plate count). The result (colony forming unit [cfu/g]) was calculated based on the formula for the "weighed arithmetic mean" [49].

Escherichia coli

The MPN-method (Most Probable Number) used here corresponds to the "Generic Standard Operating Procedures for the Enumerations of *E. coli* in Molluscan Bivalve Shellfish", issued by the European Community Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Mollusks CEFAS/CRL, Weymouth, UK) [50]. The initial solution of 15 g of the homogenized sample was dispensed to a 5-tube-3-dilution- scheme. The combination of the tubes with a confirmed growth of *E. coli* revealed the Most Probable Number of cfu of *E. coli*/100 g.

Salmonella

The method corresponds to the international norm DIN EN ISO 6579 2003 [51]. The initial solution of the 25 g of homogenized sample was enriched twice in culture media and then plated on selective agar plates, allowing the identification of cfu of *Salmonella*.

Clostridia and vibrio

The method of detecting *Clostridia* corresponds to the standardized norm DIN EN ISO 7937 [52]. The initial solution of 5 g of the homogenized sample was incubated in selective culture media under anaerobic conditions. For *Vibrio* only qualitative approaches were conducted for identification, using 25 g of the homogenized sample according to ISO 21872 standard [53].

Viruses

Prior to viral examination the mussels were cleaned, opened and prepared under sterile conditions. Then 6 g meat of mussels of each sample was homogenized under PCR-clean conditions, and then analyzed using the Real Time Reverse Transcriptase-Polymerase Chain Reaction (RT PCR). The method for the qualitative detection of Norovirus (gene group II) corresponds to the reference method [54], issued by the National Reference Laboratory (NRL) for Viral Contaminations of Bivalve Molluscs at the Federal Institute for Risk Assessment (BfR) in Berlin, Germany.

Algae toxins/shellfish poisons

The monitoring of algal toxins is organized by the States according to EC 854/2004 [4], specified by the regulations of the responsible public surveillance authorities (e.g. [55]). Concentration limits for biotoxins in shellfish products are listed in EC 853/2004 [56]. The applied methodologies for the analysis of algal toxins are according to EC 2074/2005 [57], however, without using any mouse bioassays since the use of animals in food analysis is not allowed by law in Germany. Alternatively chemical approaches such as High Performance Liquid Chromatography (HPLC) were used

for detection of algal toxins. Prior to the examination the mussels were cleaned, opened and prepared under sterile conditions. Then ca. 100 g meat of mussels of each sample was homogenized and analyzed using three different methodological adaptions of the HPLC-method. For the detection of DSP a liquid chromatography with mass spectrometric detection (LC–MS/MS) was applied, whereas ASP was examined using an adapted HPLC-method according to Quilliam et al. [58]. PSP was detected by using the method of Lawrence and Menard [59].

Results

Condition index and shell length–weight relationship

According to the condition index values (CI) sites are divided into two groups (Fig. 2). Low CIs (CI 27.39 to 39–47) are found throughout the year with only moderate variances at the intertidal areas, whereas high indices (CI 61.21–113.79) are found at both culture sites. While the nearshore hanging culture JD showed an overall peak already in spring 07 (CI 113.79) followed by a decrease of the CI down to 61.21 in autumn, the values of the offshore site stayed rather stable from spring to autumn with a minimum in winter time (CI 66.20). The mean values calculated for the whole sampling season showed the highest numbers for RS (94.5 ± 21.5 SD), followed by JD (82.97 ± 24.88 SD), NH (34.76 ± 5.56 SD), BS (32.58 ± 8.96 SD) and SY (31.38 ± 7.83 SD) (Fig. 2).

All winter samples, including the mussels from the on-bottom culture plot, were sorted according to their culture method and tidal regime. On-bottom cultivated mussels had the best CI (LE, 88.95 ± 12.67 SD, $n = 45$) followed by nearshore cultivated (JD, 88.19 ± 12.98 SD, $n = 45$) and offshore-cultivated (RS, 70.81 ± 11.63 SD, $n = 30$) mussels. The mussels from the three intertidal areas (each $n = 45$): BS 34.34 ± 11.61 SD, NH 33.70 ± 8.02 and SY 33.66 ± 7.24 had lowest CI.

The shell length–weight relationship (Fig. 3) showed that intertidal mussels ($n = 45$) had the heaviest shells in relation to their length. The shells of the on-bottom cultured mussels ($n = 45$) had an intermediate weight whereas the hanging cultivated mussels (RS and JD, $n = 60$) developed the lightest shells.

Macro- and microparasites

Most macroparasites found in the tissues and organs of *M. edulis* belonged to four different native species [60]: *Mytilicola intestinalis* a copepod living as juvenile and adult individual in the digestive gland, two trematod species *Renicula roscovita* and *Himastla elongata* occurring as metacercarias in the gills, mouth palps and tubuli of the digestive gland or in the foot and other muscles, respectively. And last the Polychaet *Polidora ciliata* living in self drilled ducts of the shell of mussels. Other candidates such as *Modiolicula insgnis* and species of the genus *Gymnophallus* occurred in less than 1% of the cases and are not displayed. With the deployed sampling method (using a glass compressorium under a stereo magnifying glass) only adult *M. intestinalis* of >2.5 mm were found in the digestive gland.

The most common macroparasites showed a high prevalence of up to 100% at the intertidal areas whereas the cultivated mussels were hardly infested (nearshore) or free

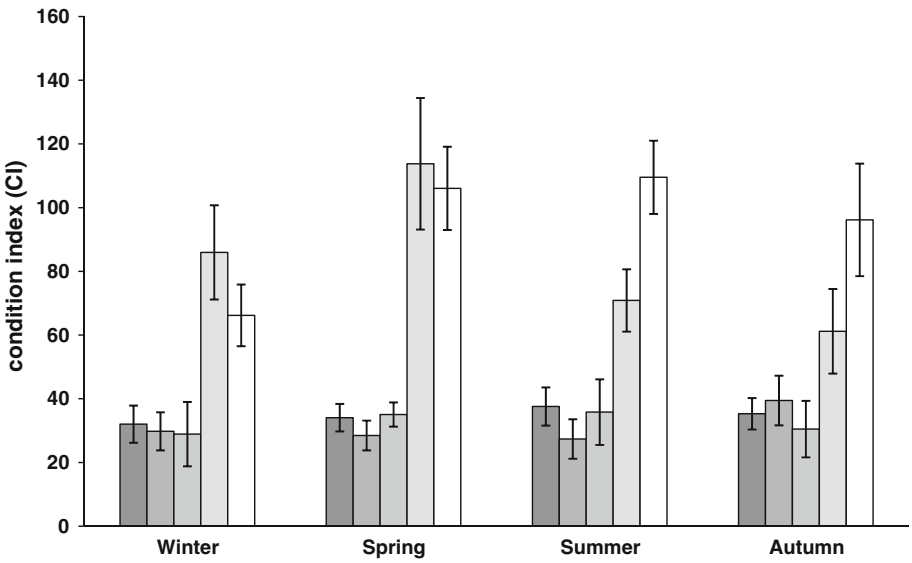

Fig. 2 Condition indices (*CI*) of blue mussels from five different sampling sites (NH *black*, SY *dark grey*, BS *grey*, JD *light grey* and RS *white*) over the season 2007 in the German Bight ($n = 15$ per site and sample cycle)

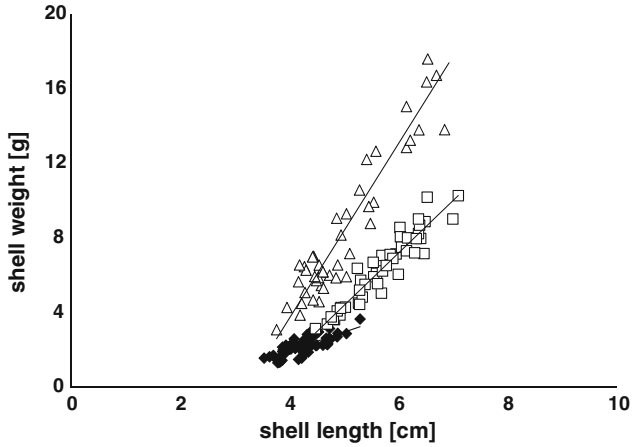

Fig. 3 Relationship of shell length (cm) and shell weight (g) of wild intertidal (*white triangle* $n = 45$), on-bottom cultivated (*white square* $n = 45$) and off-bottom cultivated (*black rhombus* $n = 60$) mussels from six different sample sites of the German Bight of winter 2007/2009

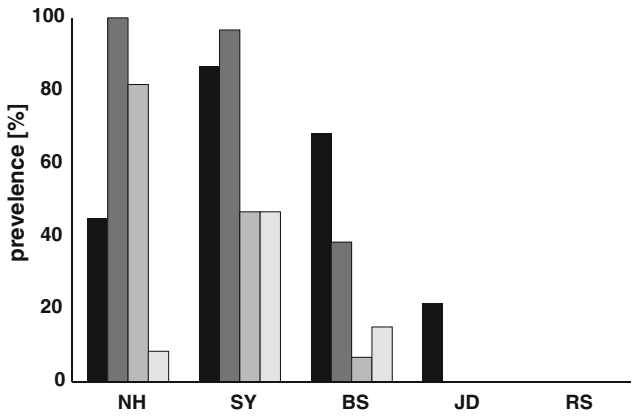

Fig. 4 Prevalence (%) of macroparasites *M. intestinalis* (*black*), *R. roscovita* (*dark grey*), *H. elongate* (*grey*) and *P. ciliata* (*light grey*) found in blue mussel according to five sampling site ($n = 60$ per site) in the German Bight during the season 2007

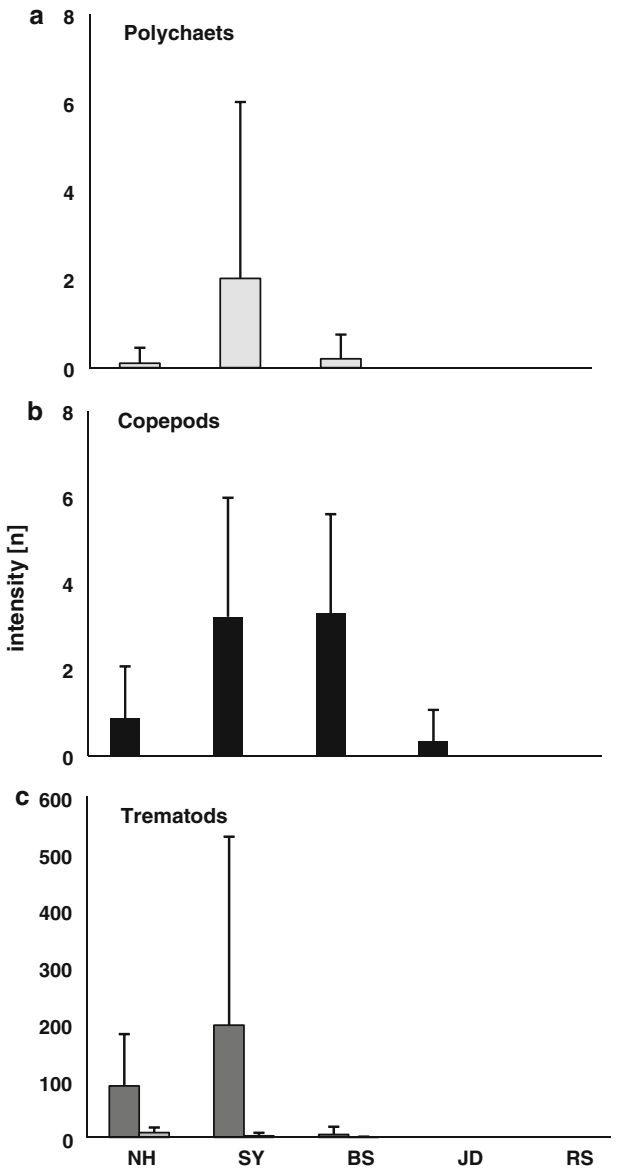

Fig. 5 **a**–**c** Intensity (n) of macroparasites [**a** shell boring Polychaet/*P.ciliata* (*light grey*), **b** copepod/*M. intestinalis* (*black*), **c** trematods/*R. roscovita* (*dark grey*); *H. elongate* (light grey)] in blue mussels of five sampling sites ($n = 60$ per site) in the German Bight in the year 2007

of parasites (offshore) (Fig. 4). Prevalence of *M. intestinalis* from intertidal samples ranged from 45.0% (NH), 68.33% (BS) up to 86.67% at SY (Fig. 4) with a mean intensity spreading from 0.87 ± 1.20 SD, 3.30 ± 2.30 SD and 3.22 ± 2.76 SD individuals per mussel, respectively (Fig. 5b). At the nearshore cultivation area JD about 21.67% of the mussels were infested by *M. Intestinalis* (Fig. 4) with an average of 0.33 ± 0.73 SD individuals (Fig. 5b).

Trematods occurred in two species in intertidal areas. There, *R. roscovita* exhibited a prevalence up to 96.67% at SY and 100% at NH (Fig. 4) together with high mean intensities of 90.52 ± 91.05 SD and 197.28 ± 331.40 SD individuals per mussel, respectively (Fig. 5c). At the SY sampling site mass infestations with >1000 *R. roscovita* were also observed. BS showed low intensities of an average of 5 ± 13.80 SD metacercarias of *R. roscovita* per mussel in about 38.33% of the samples (Figs. 4, 5c). *Himastla elongata* the second trematod specie found as metacercarias occurred, similarly to *R. roscovita,* only at intertidal sites. In this case prevalences were highest in NH (81.67%), followed by SY (46.67%) and BS with 6.67% of infested mussels (Fig. 4). Intensities were low and ranged from 8.28 ± 9.22 (NH), to 2.67 ± 5.34 SD (SY) and 0.22 ± 1.04 SD at BS (Figs. 4, 5c).

Similarly to the three other parasite species, *P. ciliata* occurred only at intertidal sites. Prevalence was high in SY (46.67%), moderate at BS (15.00%) and low at NH (8.33%) (Fig. 4). Intensities were also low and ranged between 0.10 ± 0.35 SD at NH, 2.02 ± 4.00 SD at SY and 0.20 ± 0.55 SD at the sample site of BS (Fig. 5a).

The winter sample of LE showed high prevalence of *M. intestinalis* (86.67%) at a moderate average intensity of 2.73 ± 2.09 individuals per mussel. Other species of macroparasites were absent in the mussels from the subtidal on-bottom cultivation area.

Adult *M. intestinalis* inhabit the hind gut of the digestive gland, whereas *R. roscovita* occurred in the tubuli of the digestive gland (59%) and in the gills or pulps (35%) of the mussel. The second trematod *H. elongata* is found mainly in the foot (78%) and in other muscular tissues (15%) (Table 1).

The most invested organs by macroparasites were the digestive gland, where *M. intestinalis* and *R. roscovita* were found, mouth palps and gills infested by *R. roscovita* and the foot infested by mainly *H. elongata* and to a certain extent also *R. roscovita* (Table 1).

All organs and tissues of the investigated samples from all five different sample sites were free of *M. refrigens* throughout the year 2007.

Shell commensals

Many organisms use mussel shells as a hard substrate to attach to and live on. Four taxa which build up calcareous parts were found in samples at all sites: the barnacle *Balanus* spp., the pacific oyster *Crassostrea gigas*, the Bryozoa *Flustra foliacea* and the common slipper snail *Crepidula fornicate*. Especially at intertidal sites (NH & BS) *Balanus* spp. covered 30.88% and 32.28% of the shell surface, respectively. At SY and at JD only 6.72% and 5.45% were covered by barnacles. *Flustra foliacea* became more abundant except for in the intertidal areas at the nearshore (JD 13.53%) and offshore cultivation sites (RS 10.23%). Beside bryozoes, offshore cultivated mussels were free of calcareous fouling organisms. *Crepidula fornicate* and *C. gigas* were found only infrequently at intertidal areas on the shells of mussel.

Table 1 Infestation (%) of mussel ($n = 300$) organs by most common parasites of blue mussels from five sampling sites of the German Bight (2007)

	Digestive gland	Gills/palps	Foot	Muscle	Shell
M. intestinalis	100	–	–	–	–
R. roscovita	59	35	3	3	–
H. elongata	6	1	78	15	–
P. ciliata	–	–	–	–	100

The winter samples of LE were covered by *Balanus* spp. at an average of 1.87% and by *F. foliacea* at 23.67% of shell surface.

Microbial assessment

Throughout the seasons of 2007 a microbial assessment was conducted for all sites and samples with a focus on the total aerobic microbial load and the contamination with *E. coli*, and *Salmonella*. Besides *E. coli*, three specimens of *Clostridia* (*C. perfringens*, *C. butyricum*, *and C. botulinum*) (Fig. 6a–d) and four different *Vibrio* (qualitative approach) species (*V. parahämolyticus, V. alginolyticus, V. cholera,* and *V. fluvialis)* were detected (Table 2). *Salmonella* subspecies were not found in any of the investigated samples.

In 19 out of 20 samples the total microbial load varied between 200 and 6800 colony forming units (cfu/g) (Fig. 6a–d). In spring a single peak was detected at 46,000 cfu/g at the offshore location RS (Fig. 6b). A similar pattern was found when assessing the Most Probable Number (MPN) of *E. coli* (cfu/100 g) at the five different sample sites. In 19 out of 20 samples the contamination with *E. coli* bacteria varied between 20 (lower detection limit) and 1100 MPN (cfu/100 g) (Fig. 6a–d). One summer sample of the intertidal area near NH showed the maximum load of 35,000 MPN (cfu/100 g) of *E. coli* (Fig. 6c).

Colony forming units of *Clostridium* spp. (10–377 [cfu/g]) were found throughout the year at all sites (65 cfu/g ± 114) (Fig. 6a–d). In spring sites showed highest average contamination of *Clostridium* spp. (203 cfu/g ± 158), consisting only of *C. perfringens*. In spring and summer two other species, *C. butyricum* and *C. botulinum* (no biotoxin detectable), were found in low concentrations (154 and 6 cfu/g, respectively) at NH. For the remaining spring and summer samples and all samples from the autumn a qualitative analysis was not possible.

BS was the only site where all samples were contaminated by *Vibrio* species throughout the year. At JD all four *Vibrio* species occurred, in the autumn sample even *V. cholera* but without cholera toxins. The summer sampling showed *Vibrio* at all sites and in autumn four (NH, BS, JD and RS) out of five sites were contaminated. In winter and spring *Vibrio* were detected only at two sites. Winter (NH, SY and BS) and spring (BS and JD) samples showed fewer sites contaminated with *Vibrio* (Table 2).

The classification of cultures in plots is based on the Regulation (EC) 854/2004 [4]. Class A plots should have *E. coli* values below 230 cfu/100 g MPN, whereas B-class plots can reach values up to 4600 cfu/100 g MPN. Mussels from B-class plots must be transferred and purified, whereas A-class mussels can be sold alive. C-class plots (values above 46,000 cfu/100 g) risk loss of the cultivation license.

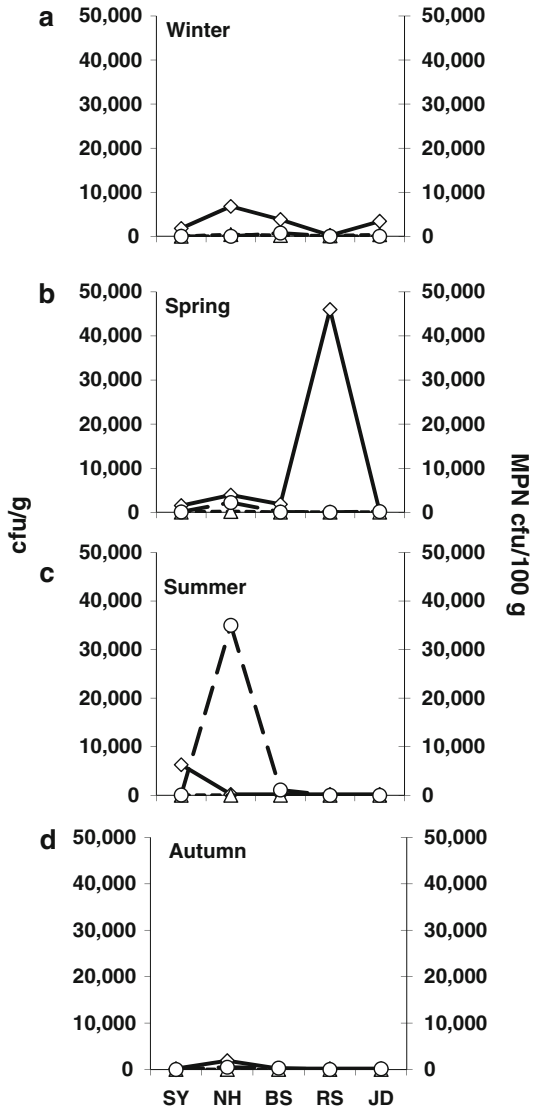

Fig. 6 **a–d** Variances of the total microbial load (cfu/g) (*rhombus*) and the presence of *E. coli* (*circle*) and *Clostridium* spp. (*triangle*) (both MPN [cfu/100 g]) in mussels of five different sampling locations of the German Bight during the season (**a** winter, **b** spring, **c** summer and **d** autumn) 2007

Virus contamination and shellfish poisons

In no sample from the five different sites biotoxins reached a critical level. Only a sporadic presence of DSP in marginal concentrations was detected. No ASP or PSP was found during the sampling period throughout the season 2007. Viruses were also absent in all samples.

Discussion

Our data show that offshore-suspended cultivated mussels from the location *Roter Sand* fulfil all official requirements for edibles. They were free of *E. coli* and parasites, grew fast, and reached market size within 15 months. Maximum CIs of mussels investigated over the whole sampling season were achieved in spring and summer by the hanging cultures. In winter, however, the CIs of on-bottom and nearshore cultivated mussels were higher than intertidal and hanging cultivated mussels from both sites. High numbers of *E. coli* were found once at the intertidal area NH. However, offshore cultivated mussels contained high bacterial loads in spring and were detected as carriers of two *Vibrio* species. Hence, the greater distance to shore at our offshore site provided no guarantee for microbial purity of the mussels. This indicates that dilution, normally providing better water quality in terms of microbes, occurs even further out from the coast of the German Bight. It is possible that, as the offshore area of the *Roter Sand* is near the entrance of the Weser estuary, it is exposed to the last discharges of black water by trading ships just about to enter Bremerhaven harbour. Other potential hazards for offshore sites may result from local "hot spots" such as munition discharge areas, oil spills, pipelines and platforms. Together with natural sources of contamination and pollution such as large bird or seal colonies from islands or other exposed areas, these hazards should be of concern during site selection and observed during production time.

Table 2 *Vibrio* spp. infestations of blue mussels of five different sites of the German Bight throughout the season 2007

Season 2007	Site	*Vibrio* spp. (qual)
Winter	NH	*V. alginolyticus*
	SY	*V. alginolyticus*
	BS	*V. fluvialis*
	JD	n.n.
	RS	n.n.
Spring	NH	n.n.
	SY	n.n.
	BS	*V. parahämolytikus*
	JD	*V. parahämolytikus*
	RS	n.n.
Summer	NH	*V. parahämolyticus*
	SY	*V. parahämolyticus*
	BS	*V. parahämolyticus*
	JD	*V. alginolyticus*
	RS	*V. parahäm/alginolyt*
Autumn	NH	*V. alginolyticus*
	SY	n.n.
	BS	*V. parahämolyticus*
	JD	*V.chol*/parahäm/alginol*
	RS	*V. alginolyticus*

Parasite, virus and bacteria infestation

Due to (i) the absence of first intermediate trematod hosts (e.g. *Littorina* spp.), which thrive in nearshore waters habitats, (ii) the distance from the host populations, resulting in dilution effects, which might be an explanation for the absence of shell-boring polychaetes and parasitic copepods, and (iii) the poor swimming capacities of planktonic stages of *M. intestinalis* [30], offshore mussels are free of macroparasites. In contrast, intertidal mussels show the highest infestations rates regarding number of parasites and number of species. The on-bottom cultivated mussels were only infested by *M. intestinalis,* but to a high degree.

The potentials of off-bottom and offshore cultivation methods are most obvious in the case of macroparasites. Hanging cultivation reduces the risk of infestation drastically, both in prevalence and intensity. Additionally, the spectrum of species is reduced by off-bottom cultivation methods. Even in the vicinity of highly infested intertidal areas, nearshore hanging cultures showed low infestations. In the case of *M. intestinalis* the poor swimming capacities of the larvae is perhaps the reason for the low infestation rates of hanging cultures near- or offshore. Whether a similar mechanism also holds for trematods and shell boring polychaets, completely absent in the suspended culture areas, remains speculative. However, only the combination of off-bottom cultivation and a long distance to shore prevented contamination by parasites.

The role and effects of macroparasites on the health status of their hosts is still debated intensively. Older studies have shown that *M. intestinalis,* although living in the hind gut, have a severe negative impact on the condition of their hosts [22, 23], hence reducing the meat yield of the mussel [24, 25] and reducing the resistance and adaptability of the mussel in its environment [61]; whereas in a more recent 10-year study from Davey and Gee [30], *M. intestinalis* was interpreted more as a commensal organism feeding on unutilized fractions in the hint gut of mussels. Although *M. intestinalis* appears not to be the epizootic hazard for mussels as described in earlier publications [23], it is hard to believe that its existence has no negative consequences for the energy budget of its host, particularly since infections occur in the digestive gland which is the central organ for energy metabolism. Together with the impediments caused by trematods' metacercarias, the holes caused by *P. ciliata*, and high loads of bacteria and viruses, it can be assumed that the overall health and growth performance of mussels is negatively impacted.

This is also displayed in the low condition indices of intertidal mussels correlating with the highest parasite infestation rates, whereas mussels with low infestations had the highest condition values. Thus evidence strongly indicates that the negative condition values are caused by parasites. Offshore mussels showed condition values at least twice as high over the whole sampling season compared to mussels from intertidal areas. Since hanging cultivated mussels produce a lighter shell, these differences may overestimate the impact of parasites, however, it remains most likely that parasites are responsible for low condition values.

Viruses were not observed in any sample collected for this study. Other problematic microbes, such as *Clostridium* spp., were present in all samples, however, in low numbers. Additionally, four different species of *Vibrio* were proven at all sampling sites. The results for *Clostridium* spp. and *Vibrio* spp. correspond with the findings from Lhafi [62] who surveyed different on-bottom mussel production areas in the German Bight in 2005. However, Lhafi [62] also detected Noro- and Rota-Viruses in 30% and 2.2% of the samples, respectively.

The findings for *Vibrio* spp. and *Clostidium* spp. in this study were independent of the solely registered high values of *E. coli* at NH in the summer sample. Thus supporting the frequently pronounced suggestion [5–8] of including human pathogenic viruses and bacteria into a regular survey, since focusing on coliform bacteria or *Salmonella* will not exclude these mussels from consumption [63].

Influence of distance to shore and cultivation method

High condition indices, good growth rates, low parasite infestation rates, and a minimal number of calcareous fouling organisms on the shells characterize mussels cultivated off-bottom and exposed to sustained inundation. An increased distance to shore would further decrease parasite infestation rates and most likely lead to minimal microbial and viral infestations. In this study evidence was supplied only in the case of parasites. Future studies should operate offshore testing sites further off the coast. Distance to shore for the offshore site followed the definition of Ryan [64] and Buck [65]. Even at this distance, however, the strong estuarine run-offs of the Elbe and Weser rivers impact the quality of these marine waters. Perhaps dilution effects, decreasing the microbial load, set in further off the coast of the German Bight.

Trend lines of the shell length–weight relationship for intertidal on-bottom and off-bottom mussels show that off-bottom cultivated mussels invest the least energy in their shells. The shell is thin and weak, causing problems during the harvesting process. In contrast, on-bottom-cultured and shore-exposed intertidal mussels invest much more energy for building up their shells. Thicker shells allow a better handling during harvesting, processing and transport, however reduce the energy available for growth and buildup of meat content.

Calcareous shell commensals follow the same pattern as mussel parasites. Intertidal mussels are densely covered with various species, whereas subtidal on-bottom and nearshore off-bottom cultivated mussels showed a reduced spectrum of specimens occurring in low numbers. Offshore cultivated mussels were, besides some bryozoes, essentially free of shell commensals. Since market price is highly dependent on growth, meat yield, shell condition and aesthetic issues such as extent of parasites and the cleanliness of the shell, hanging cultivated mussels should achieve higher market prices. However, harvesting and processing equipment has to be adapted to the thin shells of the mussel to reduce loss.

Implications for regulation and monitoring

Toxin- producing algae are found only seldom in low concentrations in the German Bight, where harsh conditions and high sediment loads prevent algae from blooming. In Danish and English waters, however, these algae are commonly found. A shift of mussel production from on-bottom nearshore areas to off-bottom offshore areas would increase potential contact of mussels with toxic algae [3].

Another potential hazard stems from the recently registered warming in the North Sea. Parasites formerly known only in warmer regions, such as the parasitic crabs of the genus *Pinotheres* spp., are migrating north and have been sporadically seen in mussels in the German Bight [66]. They inhabit the mantle cavity of the mussel and reach sizes up to 1 cm. This parasite is not pathogenic to consumers, but extraordinarily problematic with regard to marketability as the price for such infested mussels is low. A similar temperature effect is likely to affect the distribution of *M. refrigens*, where conditions could begin to favour sporulation [67]. Therefore, the ICES report [68] on marine shellfish cultivation has already recommended including *M. refirgens* into routine monitoring.

Today even the updated versions of the EC regulations focus primarily on nearshore hazards. Since the majority of potential hazards to mussels differ between both seasons and among areas, a uniform monitoring program is insufficient to protect all consumers at all times. It should be recognized that analysis of risk must entail seasonal and geographical differences, and include plans for dealing with potential threats associated with global warming.

Conclusion and outlook

Our data show that offshore locations are a good alternative for traditional mussel cultivation. The microbial findings of offshore cultivated mussels are clearly under the legal threshold and mussels are free of parasites. However as species of *Vibrio* and *Clostridium* were also found in offshore samples, this type of production does not offer a complete guarantee of microbial purity and an absence of human pathogens. Future investigations should focus on potential cultivation sites even further off the coast, to determine the distance to shore necessary for microbial purity in the German Bight.

It is recommended that the currently existing regulatory framework, focusing only on nearshore requirements, should be expanded to cover site specific risks. Further, we suggest shifting of monitoring focus for offshore sites from coliform bacteria to e.g. algal toxins and concerning the recent warming of the North Sea since a migration of commercially relevant micro- and macroparasites into the German Bight seem possible.

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