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Seasonal variability in metal and metalloid burdens of mussels: using data from the German Environmental Specimen Bank to evaluate implications for long-term mussel monitoring programs

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Abstract

Background: Metal and metalloid concentrations in mussels can vary between seasons. In biota monitoring, the sampling time is therefore an important issue. Within the German Environmental Specimen Bank (ESB) program blue mussels (*Mytilus edulis* Complex) are sampled regularly since the 1980s. The samples are collected in two-monthly intervals at two North Sea sites and in 6-month intervals at one Baltic Sea site. All samples from one site and year are combined to annual composite samples and archived as sub-samples under cryogenic conditions. In order to investigate a possible reduction of the number of annual sampling intervals while maintaining comparability with the long-term composite sample data, the seasonal variability of metals/metalloids was analyzed based on the half-yearly and bimonthly samples of 2013, 2015 and 2017.

Results: In mussels from the North Sea site Eckwarderhoerne seasonality of metals/metalloids was comparable in all 3 years (arsenic being the only exception). At the North Sea site Koenigshafen seasonality of cobalt, nickel, cadmium, copper, lead, and arsenic was comparable in 2013 and 2015 but not in 2017, while selenium showed the same seasonality in all 3 years. Within 1 year, concentrations of metals and metalloids can vary by the same order of magnitude as observed between annual composite samples of different years making it impossible to select just one representative sampling time point per year that would provide the same information as the respective annual composite sample.

Conclusions: The findings highlight the importance of carefully selecting the sampling time point when using mussels in biota monitoring. For the German ESB program it is recommended to continue with the current sampling strategy and analyze annual composite samples in order to maintain comparability with the long-term data series, which are a special feature of the ESB.

Keywords: Heavy metals, Metalloid, Seasonal variability, Monitoring, Mussels

Background

Blue mussels are frequent inhabitants of boreal and temperate coastal waters [1, 2]. They play an important role as primary consumers in the coastal food webs by filtrating algae and bacteria from the surrounding water while

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being an important food source for other species, like polychaetes, sea stars, sea birds and otters. Moreover, blue mussels are coveted food and thus represent a link between the marine environment and human health [3]. These features in concert with their widespread abundance, their sedentary way of life, and their relatively high tolerance towards environmental conditions make them ideal sentinels for environmental pollution and indicators for marine and coastal biodiversity (e.g., for ICES [4], OSPAR [5], HELCOM [6, 7], the Arctic Monitoring and Assessment Program AMAP [8, 9], and the Mussel Watch Programs [10, 11]).

Most monitoring programs rely on yearly samples that are collected outside the spawning season. It is well known, however, that chemicals' concentrations in bivalves may vary during the year due to fluctuations in environmental conditions such as temperature, turbidity, salinity, riverine inputs, primary production and the presence of contaminants, and due to biotic factors like mussel size, age, soft tissue weight, filtration rate, sex, and the gametogenetic stage (e.g., [1, 10, 12–19]).

Metal burdens of mussels are often highest in winter (e.g., [13, 20]). This is the time of year when the nutritional status of the mussels is likely to be suboptimal and weight-related metal concentrations may increase despite constant body burdens [21]. Levels of lipophilic substances, in contrast, may be highest when lipid-rich gametes are formed (e.g., [22, 23]: for blue mussels from the North and Baltic Seas this would be in spring and early summer). Contamination with other compounds like pharmaceuticals, personal care products [24] or tributyltin (TBT) [14, 25, 26] seems to be triggered by season-dependent emissions, e.g., winter outbreaks of influenza in the case of pharmaceuticals or dry-dock works in winter in the case of TBT [27].

To compensate for such variations, normalization of chemicals' concentrations in tissues is discussed. Phillips [21] observed a significant correlation between cadmium levels and wet weight of *M. edulis.* Normalizing the data to a common wet weight strongly reduced seasonal variability. No such relationships were detected for zinc (Zn), lead (Pb) and copper (Cu). Others based data normalization on the mussel's condition index [28–30]. The EU Guidance Document No. 32 [31] recommends normalizing priority substances concentrations in mussels to a default dry weight content of 8.3% (e.g., for metals) or a default lipid content of 1% (for lipophilic compounds).

The German ESB pursues a different strategy to deal with possible seasonal variations: since the mid-1980s, blue mussels are sampled every second month at two locations in the North Sea and twice per year at one site in the Baltic Sea (www.umweltprobenbank.de/en). All samples of one sampling area from 1 year are combined to an annual composite sample that should reflect the mean annual mussel burdens. Since 2013, also a subset of the original 2- or 6-monthly samples is stored in parallel, to allow for the investigation of seasonal trends. The frequent sampling, however, is relatively cost and labor intensive.

To evaluate a possible reduction of the number of samplings per year, a study was initiated with the aim to investigate whether certain time points throughout the year can capture the average annual levels of chemicals of mussels at the respective sampling locations. This would allow perpetuating the already existing long time series for ESB mussels with less effort. The original in 2- or 6-monthly intervals sampled mussels from 3 years (2013, 2015, 2017) were analyzed for arsenic, selenium, TBT, methylmercury and a set of metals cobalt (Co), nickel (Ni), Cu, cadmium (Cd), Pb and mercury (Hg). Bioaccumulation can further depend on the chemical species of an element as methylmercury in case of mercury or different compounds like arsenobetaine and arsenosugars in case of arsenic. Due to the fact that long-term data were only available for arsenic—but not for arsenic species, the seasonal samples were also investigated for arsenic only. The results were compared to the data of the respective annual composite samples. Possible correlations between substance concentrations and biometric parameters were analyzed to identify parameters that account for seasonal variability and could be used for data normalization. Finally, seasonal variations were compared with long-term trends based on the respective annual composite samples.

Material and methods

Sampling

Blue mussels are sampled every second month at two North Sea sites and twice per year (June and November/December) at one site in the Baltic Sea (Fig. 1) following an ESB standard operating procedure (SOP; [32]). The North Sea sites are located in the Lower Saxony Wadden Sea (site Eckwarderhoerne) and the Schleswig–Holstein Wadden Sea (site Koenigshafen). The Baltic Sea site is located in the Bodden National Park of Western Pomerania (site Darsser Ort). Per sampling, at least 100 individuals are collected. A suitable criterion for reducing the natural variability is the shell length of the mussels. For practical reasons, relatively large shells are sought and for biological reasons, adult mussels older than 2 years should be sampled [32]. The length class corresponding to the respective body of water was determined for each sampling site by a screening. For longer-term samples series, the size distribution is reviewed over several years and the size class to be collected is readjusted if necessary. A representative subsample of 50 mussels is

Fig. 1 Blue mussel sampling sites of the German Environmental Specimen bank

biometrically characterized (i.e., recording of shell length and wet weight, and soft body wet weight with and without breathing water). A further subset of at least 50 mussels is used for homogenization and chemical analysis.

Mussels are deep-frozen at < -130 °C directly after sampling and are processed for chemical analyses and storage without interrupting the cold chain following the ESB SOPs. First, the shells are removed under a clean bench after a short thawing period. Then, the frozen mussel soft bodies are cryo-milled [33].

All monthly samples of 1 year and sampling site are finally combined to form one annual composite sample per site. Since 2013, a limited number of the original monthly samples are stored in parallel to allow for seasonal investigations. Routinely chemical analysis is performed on the annual samples. In the present study, additional chemical analysis was performed on the monthly samples from the selected years.

This procedure (i.e., cryomilling of frozen softbodies) implies that the respiratory water of the mussels is included in the samples. Thus, the concentrations of substances of German ESB samples are diluted by a factor of about 1.3 to 2.1, compared to mussels dissected in fresh condition in other studies [32]. As described before, a subset of 50 mussels per sampling is biometrically characterized, and for this subset data of respiratory water is available. For each mussel the dilution factor was calculated by using the wet weight with respiratory water and without and the mean for each year was calculated. This is relevant for any comparison of contaminant concentrations with reference values or when results of the ESB are compared to data of other programs. Since 2013, the amount of respiratory water in the ESB samples is systematically recorded in the mussel subset used for biometrical characterization. Thus, for all data since 2013, it is possible to exclude the respiratory water fraction when calculating wet weight-based contaminant concentrations, while for earlier years one would have to resort to default values. To avoid bias by heterogenous data sets, in the present study the dilution effect by respiratory water was neglected when long-term data of annual composite samples were compared and when analyzing correlations

between biometrical parameters and substance concentrations. By contrast, when comparing recent monthly and annual data with reference values either dry weight-based concentrations were used or wet weight-based concentrations calculated without respiratory water. Respiratory water correction was performed by using the mean dilution factor of the respective year by dividing the concentrations of the measured elements with the mean dilution factor.

Digestion

Homogenates of samples were lyophilized (Alpha 1-2LDplus, Christ, Osterode, Germany) and then digested by a microwave procedure (heating to 220 °C and holding the temperature for 30 min). For digestion, around 200 mg of sample material was transferred to quartz vials and digested with concentrated nitric acid (Rotipuran supra 69%, Roth, Karlsruhe, Germany) in an Ultra Clave microwave-heated digester (MLS GmbH, Leutkirch, Germany). In each digestion step certified reference material (NIST 2976, Mussel Tissue; NIST 1566b Oyster tissue), internal reference material (mussel samples of the ESB) as well as analytical blank samples were digested along with the samples to verify the completeness of the digestion procedure. The procedure is highly standardized following an ESB SOP [34]. This digestion procedure was used for metals and metalloids.

Metal and metalloid analysis

Determination of all metals and metalloids, except mercury, was performed with an Agilent ICP-MS 7500ce (Agilent Technologies, Waldbronn, Germany) and sample introduction was performed using an autosampler (CETAC 500, Teledyne, Manchester, UK) and the Integrated Sample Introduction System (ISIS; Agilent Technologies, Waldbronn, Germany). The measurement parameters are given in Additional file 1: Table S1. Certified reference materials (Environmental Canada), quality control samples and recalibration standards were measured along with the samples to verify the measurement series. The procedure followed an ESB SOP [35].

Mercury analysis

Measurement of total mercury (here designated as Hg) was performed with dedicated atomic absorption spectrometry (AAS) methods applying a Direct Mercury Analyzer (DMA) instrument (DMA-80 for solid; MLS GmbH, Leutkirch, Germany). For details of sample treatment and measurement method, see [36].

The analysis of methylmercury (monomethyl mercury cation; MeHg) was performed with SID-GC/ICP-MS (stable isotope dilution-gas chromatography coupled to inductively coupled plasma-mass spectrometry). Pooled muscle tissue (200 mg) samples were extracted by a microwave-assisted procedure with tetramethylammonium hydroxide (TMAH) and the addition of a previously calculated amount of ^{201}Hg-enriched MeHg. After extraction, samples were derivatized with sodium tetrapropylborate. Volatile mercury species were extracted in *n*-hexane and analyzed by SID-GC/ICP-MS. Data are reported as methyl mercury cation (for details see [36–38]). The respective LOQs were in the range of 0.02–0.81 µg kg^{-1} (derived from blanks measured along with each set of samples).

Organotin analysis

Analysis of organotin compounds (TBT, DBT and MBT cations) was also performed with SID-GC/ICP-MS. The preparation of all samples was performed according to a slightly modified method for isotope dilution analysis (IDA) developed by Point et al. [39]. 200 mg sample material were weighed in a quartz vial to which 3 mL TMAH and a previously calculated amount of ^{119}Sn-enriched butyltin mix (solution in acetic acid/methanol 3:1, ISC Science) of TBT ($c = 1.082$ µg g^{-1} as Sn), DBT ($c = 0.693$ µg g^{-1} as Sn) and MBT ($c = 0.105$ µg g^{-1} as Sn) as internal standard (spike solution) were added. The mixture was equilibrated for at least 30 s. For extraction, the samples were heated up to 60 °C within 10 min and the temperature was held for 5 min. Subsequently, the digestion solutions were sonicated for 15 min. The pH of the digested samples was adjusted to 4.2 ± 0.2 with 0.55 mL HNO_3 (69%, suprapur, Roth) and 5.45 mL NaOAc/AcOH-buffer solution (pH 4.66, Merck). As ethylation reagent a 10% solution of sodium tetraethylborate (purity > 98%, ABCR) was prepared and 200 µL of this solution was applied. After equilibrating for 30 s, butyltin compounds were extracted with 2 mL *n*-hexane (purity ≥ 95%, ChemSolute) by shaking for 1 h and separating the organic layer via centrifugation (15 min, 3500 rpm). For clean-up 3 g anhydrous Na_2SO_4 (Merck) were filled on top of a silica cartridge (Phenomenex Strata SI-1 Silica, 1000 mg/6 mL) and conditioned with 5 mL *n*-hexane. Then, butyltin compounds were extracted with 2×2 mL *n*-hexane/acetone (95:5 vol%, both ChemSolute), concentrated to 150 µL and transferred into GC vials. Data reported as organotin cations. GC parameters, GC temperature program and ICP-MS parameters are listed in Additional file1: Table S2.

Substance concentrations are reported on a wet weight (ww) basis if not stated otherwise.

Data treatment

Statistical analysis was performed using MS-Excel or MS-Excel Add-Ins or freely available Internet applications (e.g., VassarStats—Website for Statistical

Computation, http://vassarstats.net, or StatTools, http://www.obg.cuhk.edu.hk). The Condition Index (CI) was calculated as follows: (soft body wet weight [g]/shell wet weight [g]) × 100 [3, 40]. Pearson's correlation coefficient was used to evaluate relationships between biometrical characteristics and chemicals' concentrations. In case of significance the non-directional probability value p is given. Temporal trends were analyzed using an MS-Excel-based tool (LOESS Trend (Version 1.1) developed by J. Wellmitz, German Environment Agency). The tool fits a locally weighted scatterplot smoother (LOESS; fixed window width of 7 years) through the annual concentration data followed by tests for significance of linear and non-linear trend components by means of an analysis of variance (ANOVA) [41].

Results and discussion

Biometric data

Annual composite samples

During the sampling period 2000–2017, mussels from the sampling site Eckwarderhoerne (Lower Saxony Wadden Sea) were relatively homogenous with respect to biometric parameters. Mean shell length and weight were around 47.0 ± 1.13 mm and 8.13 ± 0.5 g wet weight (ww), respectively. Soft body weights were around 2.55 ± 0.3 g ww (calculated dry weights (dw): 0.20 ± 0.04 g) and water contents were in the range of 90.3–93.7% (including respiratory water).

Mussels from the Schleswig–Holstein Wadden Sea (sampling site Koenigshafen) decreased slightly in size since 2000. Between 2000 and 2017, mean shell length and weight were 57.2 ± 2.7 mm and 13.3 ± 1.89 g. Soft body weight ranged around 4.78 ± 0.87 g ww and 0.36 ± 0.08 g dw with water contents in the range of 90.3–94.3%.

At the Baltic Sea sampling site Darsser Ort, mussels increased in size from 2013 onward. In 2017, mussels were especially large with a mean length of 66.7 mm length. In the period 2000–2017, shell length and weight were in the range of 36.4 ± 9.78 mm and 1.56 ± 1.84 g. Soft body weights were around 1.28 ± 1.45 g ww (calculated dw: 0.070 ± 0.080 g) and water contents ranged between 91.0 and 96.8%.

Monthly samples

The biometrical characteristics of the monthly samples of 2013, 2015 and 2017 are summarized in Additional file 1: Figure S1 and compared to the annual data of the respective years. Figure 2 shows the temporal comparison of the CI of the mussels from all three sites.

Condition indices of mussels from Eckwarderhoerne were slightly lower compared to Koenigshafen and both were considerably lower than the CIs of mussels from the Baltic Sea site Darsser Ort. The high CIs at Darsser Ort result mainly from significantly lower shell weights at this site (Additional file 1: Figure S1). These differences can in part be attributed to the different living conditions of North Sea and Baltic Sea mussels (e.g., lower salinity of Baltic Sea water).

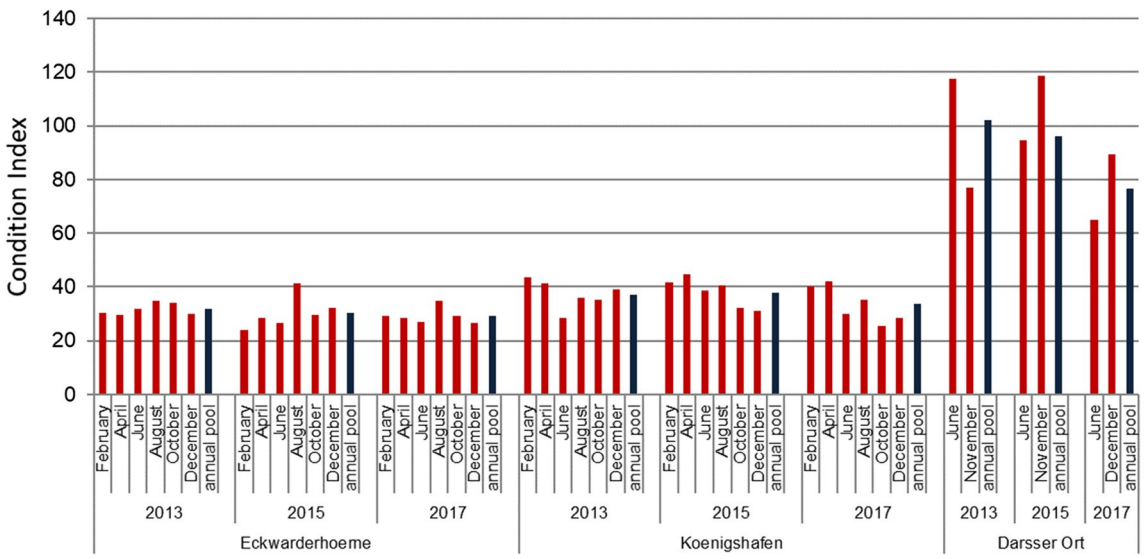

Fig. 2 Condition Index (mean of 50 individuals) of blue mussels (*Mytilus edulis* Complex) sampled by the German Environmental Specimen Bank at the North Sea sites Eckwarderhoerne (Lower Saxony Wadden Sea) and Koenigshafen (Schleswig–Holstein Wadden Sea) and at the Baltic Sea site Darsser Ort. Shown are the data of monthly samples (red columns) and of the respective annual composite sample (dark columns)

At all three sites the CIs varied in the course of the year. However, no consistent annual fluctuation was apparent at any site nor was there one consistent month that compares best to the annual composite sample.

As discussed by Beyer and Green [3] the CI depends on the nutritional and reproductive status of the mussel and may vary quite strongly in the course of the year. Thin shells and high soft body weights are typically associated with rapid growth. In temperate waters body weights of mussels decrease to a minimum in spring–summer when spawning occurs. Thereafter weights rapidly increase thanks to good food supply allowing the mussels to build up reserves for the winter season and for gonad development. Depending on the nutritional status, a second spawning may take place in autumn. In the present study, no such common pattern was observed at any site indicating that also other factors played a pivotal role.

Correlation of biometric parameters with levels of chemicals

The influence of shell length and CI on the metal and metalloid burdens of mussels was studied to assess whether one of these biometrical parameters could be used to normalize the data. The results of the correlation analyses are summarized in Additional file 1: Tables S3–S5. No consistent correlations between the CI or the length of mussels and the metal/metalloid concentrations were detected. Correlations differed between sites, between wet weight- and dry weight-based metal/metalloid concentrations and between annual and monthly samples.

The results suggest that the nutritional status of the mussels (expressed as condition index) and the size of the mussels (expressed as shell length) seem to be correlated with the mussels' metal/metalloid concentrations, but the observed differences between the sampling sites and between the annual and monthly samples indicate that there are also other influential factors. Accordingly, differences in metal/metalloid concentrations between monthly samples cannot be overcome by adjusting the data to a common CI or a common length.

Seasonality in concentrations of covered chemicals

Figure 3 shows the monthly and annual wet weight-based data for Co, As, Hg and MeHg. Respective data for all other analyzed substances are given in Additional file 1: Figures S2–S4. At both North Sea sites, Co was highest in spring and summer followed by a clear decrease. Lowest levels were detected in winter. At the Baltic Sea site, higher Co levels were detected in winter samples. Arsenic was highest in spring and decreased thereafter at the North Sea site Koenigshafen, whereas no such seasonality was detected at the North Sea site Eckwarderhoerne and the Baltic Sea site. For Hg and MeHg no consistent patterns were observed.

For all other metals and metalloids more or less consistent seasonal patterns were observed which, however, differed between sites. At Koenigshafen, for instance, copper concentration was highest in spring and summer in 2015 and 2017, while in 2013 highest concentrations were detected in summer and fall (August–October). At Eckwarderhoerne, highest Cu levels were always observed in August (Additional file 1: Figures S2 and S3).

Based on these data, no consistent seasonality can be identified for any locations and all year. Seasonality differed between sampling sites and partly also between years (most likely because of differences in environmental conditions) which makes it difficult to pick one or two representative sampling times that would best reflect the annual mean.

According to published data, seasonal variations in metal burdens of mussel appear to be lower in marine regions than in estuarine environments, and maximum levels are typically detected in spring or winter (e.g., [13, 42]. Amiard et al. [12] monitored Pb, Cd, Cu and Zn in mussels from the Bay of Bourgneuf (France) on a monthly basis over more than 2 years and observed highest levels in winter and early spring. The authors attributed these seasonal differences to fluctuations in soft tissue weight related to the reproductive cycle. Similarly, Farrington et al. [15] reported considerably higher Pb, Cd, and Ni levels from October to March in mussels studied under the US Mussel Watch Program. In a study using transplanted mussels in Arcachon Bay (France), Devier et al. [14] demonstrated that As, Cd, chromium (Cr), Ni, Pb, selenium (Se), Zn all showed a similar seasonal pattern with highest concentrations in winter (December–March) and lowest in summer (June–October), whereas Cu remained more or less constant throughout the year. Regoli and Orlando [43] analyzed Cu, Fe, manganese (Mn), and Zn in the digestive gland of mussels (*M. galloprovincialis*) from a polluted and an unpolluted site in the Tyrrhenian Sea and found a pronounced seasonality with highest levels at the end of the spawning season in February/March and lowest in late summer at the beginning of another spawning period. Rainbow et al. [18] observed limited seasonal variation for Zn and Cu concentrations in *M. trossulus* of the Gulf of Gdansk (Baltic Sea).

It has to be kept in mind that some of the analyzed elements may be essential for bivalves, e.g., Cu, Zn, Co and Se, and may be subject to internal regulation [44–46].

For organic substances, seasonal patterns in mussel burdens seem to be different and less constant. Compounds that accumulate in lipid-rich tissues will likely be highest when body content of lipid is highest. For DDT, highest levels in oysters (*Crassostrea gigas*) were detected

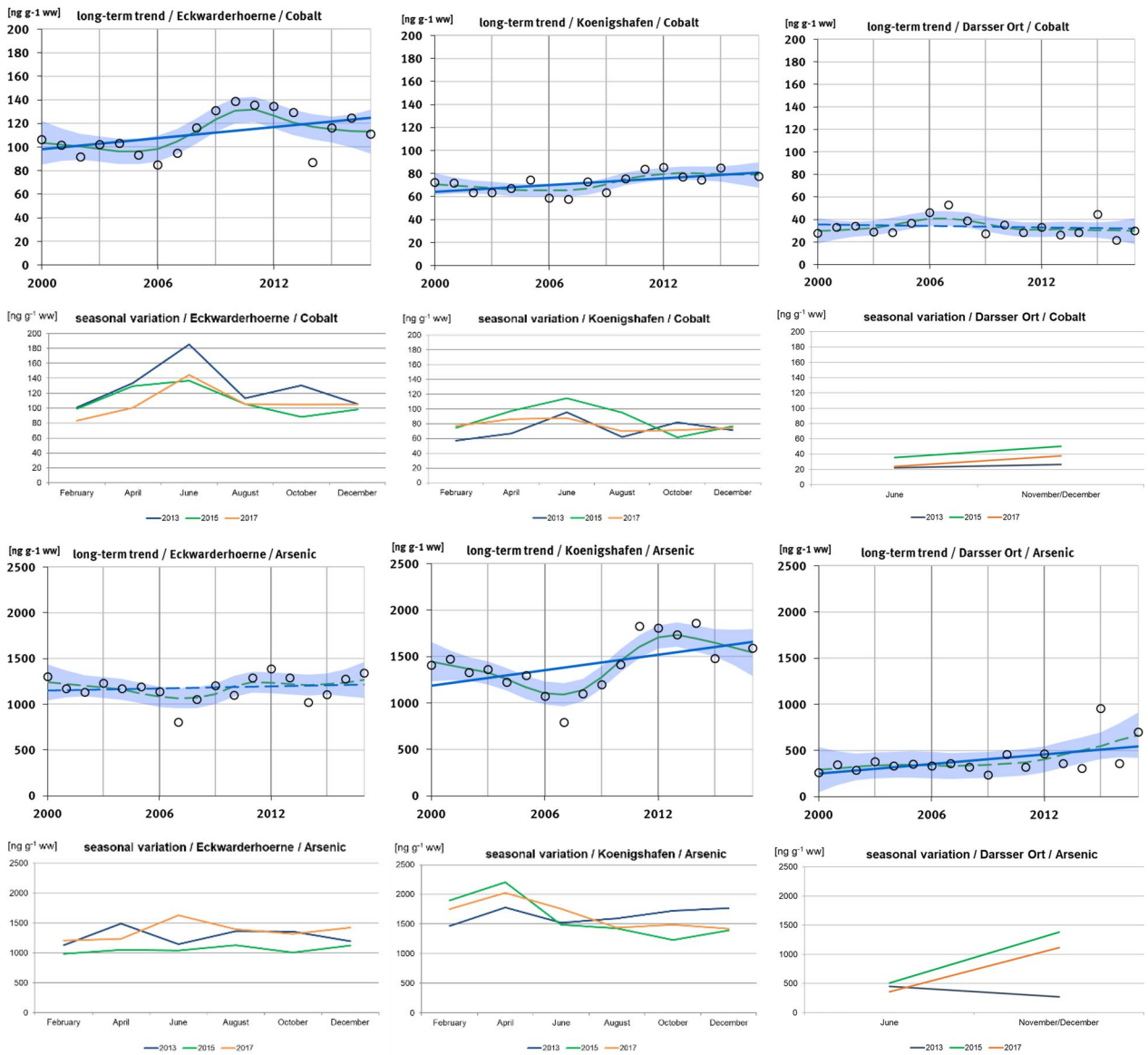

Fig. 3 Concentrations (ng g^{-1} wet weight ww) of cobalt, arsenic, total mercury and methylmercury in blue mussel (*Mytilus edulis* Complex) sampled at the North Sea sites Eckwarderhoerne and Koenigshafen and at the Baltic Sea site Darsser Ort. Shown are time trends for annual composite samples covering the years 2000–2017 (continuous line = significant linear or non-linear trend; dashed line = trend not significant; $p = 0.05$) as well as monthly concentrations in 2013, 2015 and 2017; for mercury the EQS is given as red line. Note the different scaling of the y-axis for total Hg and MeHg at Darsser Ort

in summer when lipid-rich gametes are produced [22]. PCBs in blue mussels were detected mainly in gametes and mussel contamination decreased significantly after the spawning period [47–49]. Suarez et al. [23] observed a direct relationship between levels of PCBs, DDTs and HCHs in blue mussels (*M. galloprovincialis*) and their lipid content and reproductive stage. In contrast, Webster et al. [50] found no correlations between lipid contents of blue mussels and concentrations of PBDE, PAH and PCB. In the case of compounds like, e.g., pharmaceuticals, personal care products [24] or TBT, the observed seasonality in mussel contamination was obviously related to seasonal differences in emissions. Devier et al. [14] found high levels of TBT in mussels at harbor sites in summer when boat activities were highest. In contrast, Zanon et al. [26] and Tang et al. [25] report peak TBT concentrations in mussels also in winter at sites

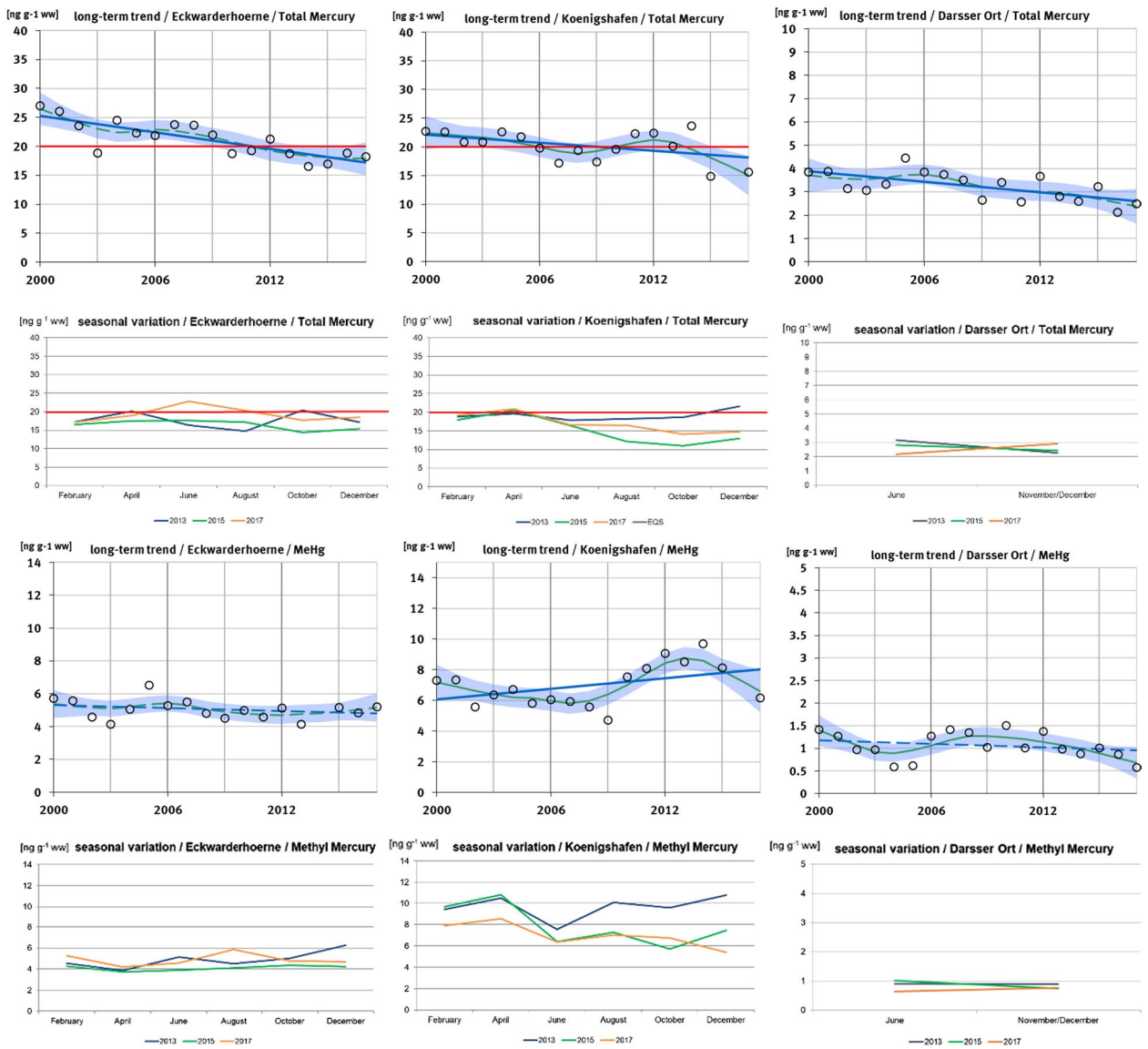

Fig. 3 (continued)

influenced by shipping maintenance activities in dry docks that are more frequent in winter.

Comparison between seasonal fluctuations and long-term trends

For all metals and metalloids, seasonal variations in body burdens observed in 2013, 2015 and 2017 were compared with long-term trends of these elements in annual composite samples over the last 17 years. The aim was to assess whether seasonality can mask long-term trends. Figure 3 shows the trends for the years 2000–2017 (TBT 2006–2017) based on the annual composite sample concentration data for Co, As, total Hg (THg) and MeHg as well as the seasonal fluctuations in the monthly samples for the years 2013, 2015 and 2017. Respective data for all other analyzed substances are given in Additional file 1: Figures S2–S4. Regarding the annual composite samples, Co concentration increased significantly since 2000 at both North Sea sites, whereas no such trend is detectable at the Baltic Sea site. In contrast, As increased at the North Sea site Koenigshafen and at the Baltic Sea site Darsser Ort but not at Eckwarderhoerne. For THg, a decreasing linear trend is observable at all three sites, while MeHg increased at Koenigshafen (but not at Eckwarderhoerne and Darsser Ort). Comparing the total Hg concentrations with the

Environmental Quality Standard (EQS) of 20 ng g^{-1} ww [51] reveals that in recent years, the EQS is met at both North Sea sites. At the Baltic Sea site, Hg levels in mussels were generally lower and complied with the EQS during the entire study period.

In Fig. 4 the fraction of MeHg/THg is shown for the annual composite samples (years 2000–2017) from all sites and for the monthly samples of 2013, 2015 and 2017 from the North Sea sites. The data reveal that the MeHg/THg fraction differs between both North Sea sites. In annual composite samples from Eckwarderhoerne, the fraction between 2000 and 2017 is in the range of 20–30%. The same fraction is also found in the monthly samples from Eckwarderhoerne. By contrast, higher fractions around 30–50% were observed at the North Sea site Koenigshafen. At the Baltic Sea site, the MeHg/THg fraction in annual composite samples ranges between 20 and 45% during the study period. A clear seasonal variation of the fraction MeHg/THg for the North Sea sampling sites is not observable.

Tables 1, 2, 3 summarize the monthly data of the years 2013, 2015 and 2017 and compare them to the range of the concentrations over a period of 17 years (for TBT, 11 years). The comparison reveals that the concentration ranges of the annual samples during in this period are in the same order of magnitude as the concentration ranges within 1 year (seasonal samples). The only exception is TBT which has continuously decreased over the last years so that the concentrations in 2013, 2015 and 2017 (covered by the monthly samples) were clearly lower than in the years before (which also contribute to the range of the annual composite samples). This was especially pronounced at Eckwarderhoerne. The data are consistent with previous findings relating the decrease of TBT following international bans [52].

The metal burdens of the seasonal and annual samples were compared to available Environmental Quality Standards (EQS) related to fish [51] and to threshold values (BAC or EC maximum food level) [53] given by OSPAR [54] and also applied by HELCOM [55]. The respective concentrations are shown in Additional file 1: Tables S6–S8, while Additional file 1: Tables S9–11 summarize the results. EQS and thresholds are available for Cd, Pb and Hg (EQS related to fish). Concentrations of Cd and Pb in mussels from both North Sea sites mostly exceed the respective threshold values (the only exceptions were Pb in samples from Koenigshafen in 2013 and 2015). In mussels from the Baltic Sea site Darsser Ort, Cd-levels exceeded the threshold in all samples, whereas the Pb-threshold was exceeded only in 2017. For mercury, the picture is vice versa. The majority of the mussel samples from all three sites meet the EQS of

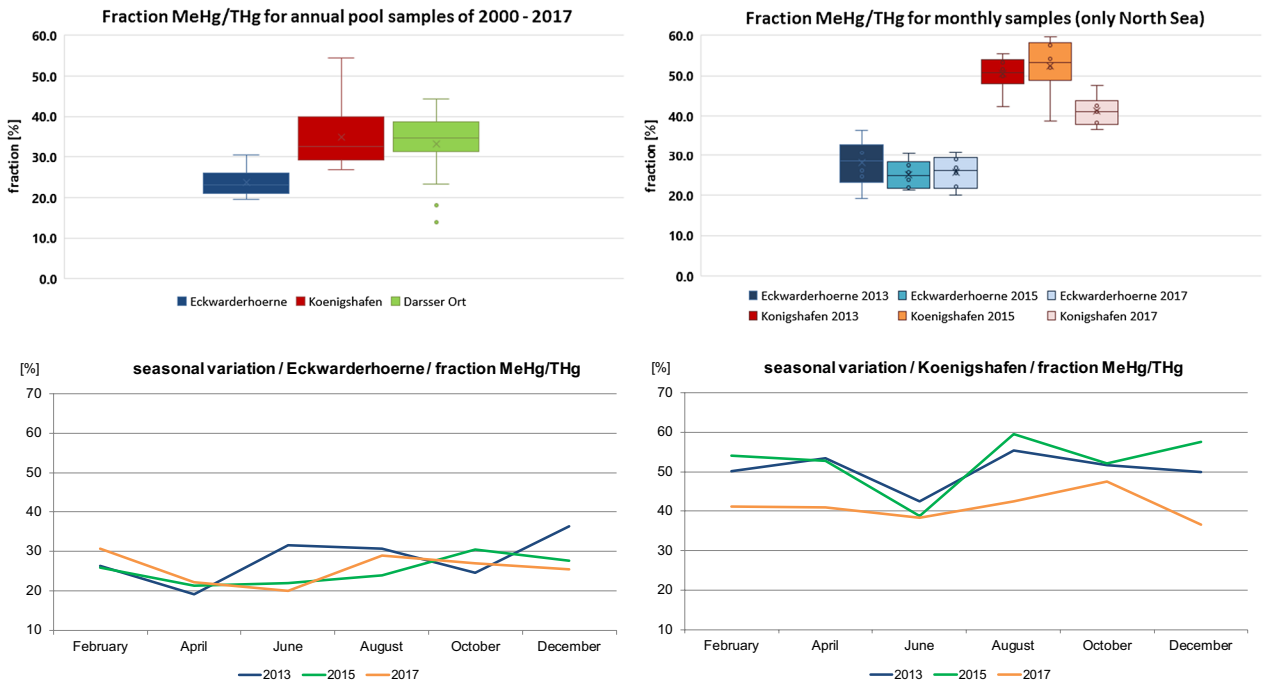

Fig. 4 Fraction of methylmercury and total mercury (MeHg/THg) in blue mussels (*Mytilus edulis* Complex) from the North Sea and Baltic Sea sampling sites of the German Environmental Specimen Bank. Shown are the fraction for annual composite samples covering the years 2000–2017, the monthly fractions for 2013, 2015 and 2017 (only North Sea sites) and the seasonal variation in MeHg/THg at the North Sea sites

Table 1 Comparison of seasonal and long-term variations in metal burdens (given as ng g^{-1} wet weight and dry weight) of blue mussels (*Mytilus edulis*) from the North Sea sampling site Eckwarderhoerne

Eckwarderhoerne	Months of annual minimum conc.	Months of annual maximum conc.	Annual range (ng g^{-1}) ww/dw	Range over 17 years (ng g^{-1}) ww/dw
Cobalt	Oct.–Feb.	June	102/1174	54.2/852
Copper	Oct.–April	Aug.	451/3314	383/2850
Cadmium	No clear seasonal variability		31.2/833	62.5/1125
Nickel	Aug.–Dec.	June	167/2216	111/1580
Lead	June–Oct. (2013 Dec.)	April	105/2301	196/1871
Mercury	No clear seasonal variability		8.42/176	10.5/137
Methylmercury	No clear seasonal variability		2.52/43.9	2.39/34.1
Arsenic	No clear seasonal variability		644/8493	585/7697
Selenium	Aug.–Oct.	April–June	166/2966	215/3053
Tributyltin	Aug.–Oct.	April	1.29/17.5	5.15/58.0

Table 2 Comparison of seasonal and long-term variations in metal burdens (given as ng g^{-1} wet weight and dry weight) of blue mussels (*Mytilus edulis*) from the North Sea sampling site Koenigshafen

Koenigshafen	Months of annual minimum conc.	Months of annual maximum conc.	Annual range (ng g^{-1}) ww/dw	Range over 17 years (ng g^{-1}) ww/dw
Cobalt	Dec.–Feb.	Apr.–Aug.	57.9/798	27.8/478
Copper	Oct.–Feb. (except 2013)	April	261/1953	296/1984
Cadmium	No clear seasonality, n.c.		26.5/611	28.3/723
Nickel	Aug.–Oct.	June	133/1872	86.2/1469
Lead	Oct.	April	50.4/996	88.7/1487
Mercury	Aug.–Oct.	Dec.–April	10.6/127	8.73/132
Methylmercury	June–Aug.	Oct.–April	5.41/46.7	4.99/33.2
Arsenic	June–Oct.	Dec.–April	975/9538	1062/9830
Selenium	Aug.–Oct.	Dec.–April	276/2003	194/1095
Tributyltin	Aug.–Dec.	April	1.81/20.6	1.04/16.6

Table 3 Comparison of seasonal and long-term variations in metal burdens (given as ng g^{-1} wet weight and dry weight) of blue mussels (*Mytilus edulis* Complex) from the Baltic Sea sampling site Darsser Ort

Darsser Ort	Season of annual minimum conc.	Season of annual maximum conc.	Annual range (ng g^{-1}) ww/dw	Range over 17 years (ng g^{-1}) ww/dw
Cobalt	Summer	Winter	28.0/412	31.7/465
Copper	Summer (2013 winter)	Winter (2013 summer)	471/1643	364/3883
Cadmium	Summer	Winter	37.3/1226	46.4/1516
Nickel	Summer	Winter	67.8/1948	86.8/2329
Lead	Summer (2013 winter)	Winter (2013 summer)	44.0/1064	165/2807
Mercury	Winter (2017 summer)	Summer (2017 winter)	1.00/46.0	2.32/69.3
Methylmercury	Winter (2017 summer)	Summer (2017 winter)	0.37/13.2	0.93/27.5
Arsenic	Summer (2013 winter)	Winter (2013 summer)	1108/10,210	718/8938
Selenium	Summer (2013 winter)	Winter (2013 summer)	374/1621	238/2449
Tributyltin	Summer (2013 winter)	Winter (2013 summer)	0.66/7.80	1.47/28.6

20 ng g^{-1} wet weight. However, when compared with the EC maximum food levels which are used by OSPAR (i.e., 500 ng g^{-1}) all samples meet the criteria. Since the EQS is derived to ensure environmental protection [56], it would be the more appropriate reference here, however, with the limitation that is derived for fish. There are no further EQS for heavy metals like cadmium referring to fish nor for mussel.

Conclusions

The present data demonstrate that seasonal fluctuations of metal-, metalloid-, and TBT-concentrations in blue mussels may be quite strong and vary between years and sampling locations. Moreover, for some metals seasonal fluctuation may be more pronounced than long-term trends. For trend monitoring it is therefore crucial to maintain fixed sampling times throughout the entire program. Monitoring program, such as the German ESB, which have analyzed and archived annual pool samples (each consisting of several monthly samples) over a longer period of time, are not recommended to reduce the annual sampling frequency as this may disrupt the already existing long time series.

Moreover, it is currently discussed that marine samples of the ESB may be used also in the context of Descriptor 9 of the Marine Strategy Framework Directive [57, 58]. This would require the best possible knowledge of mussel contamination for compliance assessment. Finally, ESB programs archive environmental samples for future analyses of yet unknown compounds or compounds for which sufficient sensitive analytical methods are still missing. Reducing sampling frequencies bears the risk of not recognizing (season-dependent) peak concentrations. It is therefore recommended to pool a fraction of the monthly samples to annual samples and use these for routine analysis (which reduces the costs for analysis) while also archiving the monthly mussel samples to allow for the retrospective analysis of monthly trends of pollutants and other stressor in future.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-0289-7.

Additional file 1: Table S1. ICP-MS parameters for metal and metalloid measurements. **Table S2.** GC parameters, GC temperature program and ICP-MS parameters for organotin measurements. **Table S3.** Correlation ($p \leq 0.05$, non-directional) between the shell length of mussels and concentrations of analyses elements/compounds. **Table S4.** Correlation ($p \leq 0.05$, non-directional) between the condition index of mussels and concentrations of elements/compounds. NS: North Sea, BS: Baltic Sea. **Table S5.** Correlation ($p \leq 0.05$, non-directional) between the condition index of mussels and wet weight-based concentrations of elements/compounds—comparison between annual and monthly samples. NS: North Sea, BS: Baltic Sea. **Table S6.** Cd-concentrations (ng g^{-1} dry weight dw and wet weight ww) of monthly samples and annual composite samples of blue mussels (*Mytilus edulis* Complex) from North Sea and Baltic Sea sites. For comparison: BAC and EC maximum food level used by HELCOM and OSPAR (wet weight based data without respiratory water). **Table S7.** Pb-concentrations (ng g^{-1} dry weight dw and wet weight ww) of monthly samples and annual composite samples of blue mussels (*Mytilus edulis* Complex) from North Sea and Baltic Sea sites. For comparison: BAC and EC maximum food level used by HELCOM and OSPAR (wet weight based data without respiratory water). **Table S8.** Hg concentrations (ng g^{-1} wet weight) of monthly samples and annual composite samples of blue mussels (*Mytilus edulis* Complex) from North Sea and Baltic Sea site. For comparison: BAC and EC maximum food level used by HELCOM and OSPAR and the Water Framework Directive (Directive 2013/39/EU) (wet weight based data without respiratory water). **Table S9.** Comparison of Cd-thresholds used by HELCOM and OSPAR with Cd-concentrations in seasonal and annual composite samples of blue mussels (*Mytilus edulis* Complex) from North Sea and Baltic Sea sites (wet weight based data without respiratory water). **Table S10.** Comparison of Pb-threshold values used by HELCOM and OSPAR with Pb-concentrations in monthly samples and annual composite samples of blue mussels (*Mytilus edulis* Complex) from sampling sites North Sea and Baltic Sea sites (wet weight based data without respiratory water). **Table S11.** Comparison of Hg-thresholds used by HELCOM, OSPAR and the WFD with THg-concentrations in monthly and annual composite samples of blue mussels (*Mytilus edulis* Complex) from North Sea and Baltic Sea sites (wet weight based data without respiratory water). **Figure S1.** Biometrical parameters of blue mussels (*Mytilus edulis* Complex) from the sampling sites of the German environmental specimen bank in the North Sea (Eckwarderhoerne, Koenigshafen) and the Baltic Sea (Darsser Ort). Shown are data of monthly samples and of annual composite samples of the respective year (dark columns). **Figure S2.** Long-term trends and seasonal variations in concentrations of metals in blue mussel (*Mytilus edulis* Complex) sampled at the North Sea site Eckwarderhoerne. Upper row: long-term trends based on annual composite samples (continuous line = significant linear or non-linear trend; dashed line = trend not significant; $p = 0.05$); lower row: seasonal variation based on bi-monthly samples from 2013, 2015, and 2017. **Figure S3:** Long-term trends and seasonal variations in concentrations of metals in blue mussel (*Mytilus edulis* Complex) sampled at the North Sea site Koenigshafen. Upper row: long-term trends based on annual composite samples (continuous line = significant linear or non-linear trend; dashed line = trend not significant; $p = 0.05$); lower row: seasonal variation based on bi-monthly samples from 2013, 2015, and 2017. **Figure S4.** Long-term trends and seasonal variations in concentrations of metals in blue mussel (*Mytilus edulis* Complex) sampled at the Baltic Sea site Darsser Ort. Upper row: long-term trends based on annual composite samples (continuous line = significant linear or non-linear trend; dashed line = trend not significant; $p = 0.05$); lower row: seasonal variation based on 6-month samples from 2013, 2015, and 2017.

Abbreviations

ESB: Environmental Specimen Bank; TBT: tributyltin; DBT: dibutyltin; MBT: monobutyltin; NIST: National Institute of Standards and Technology; ICP-MS: inductively coupled plasma-mass spectrometry; SOP: Standard Operation Procedures; AAS: atomic absorption spectrometry; DMA: direct mercury analyzer; SID-GC/ICP-MS: stable isotope dilution-gas chromatography coupled to inductively coupled plasma-mass spectrometry; TMAH: tetramethylammoniumhydroxide; WW: wet weight; Sn: tin; Hg: mercury; MeHg: methylmercury; NaOAc/AcOH: acetic buffer; GC: gas chromatography; Cd: cadmium; Cu: copper; Cr: chromium; As: arsenic; Se: selenium; Mn: manganese; DDT: dichlorodiphenyltrichloroethane; PCB: polychlorinated biphenyl; HCH: hexachlorocyclohexane; PAH: polycyclic aromatic hydrocarbons; PBDE: polybrominated diphenyl ethers; EQS: Environmental Quality Standard; BAC: Background Assessment Concentrations.

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Authors' contributions
BK was responsible for the metal analyses and statistical evaluation of all data; GR analyzed organotin compounds; BK, AF, JK and HR performed the data analysis and were involved in manuscript writing. MP was responsible for the mussel sampling and involved in manuscript writing. UP was involved in comparison of data with the regulations and manuscript writing. All authors read and approved the final manuscript.

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Availability of data
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request or can be downloaded from the homepage of the German Environment Specimen bank (www.umweltprobenbank.de/en).

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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