Chemosphere xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban

Y. Verhaegen [a,b], E. Monteyne [c], T. Neudecker [d], I. Tulp [e], G. Smagghe [b], K. Cooreman [a], P. Roose [c], K. Parmentier [a,*]

[a] Institute for Agricultural and Fisheries Research (ILVO), Animal Sciences Unit, Fisheries, Ankerstraat 1, 8400 Ostend, Belgium
[b] Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[c] Management Unit of the North Sea Mathematical Models, Royal Belgian Institute of Natural Sciences, 3rd and 23rd Linieregimentsplein, 8400 Ostend, Belgium
[d] Johann Heinrich von Thünen-Institut, Institut für Seefischerei, Palmaille 9, 22767 Hamburg, Germany
[e] Institute for Marine Resources and Ecosystem Studies (IMARES), P.O. Box 68, 1970 AB IJmuiden, The Netherlands

ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 9 November 2011
Accepted 11 November 2011
Available online xxxx

Keywords:
Crangon crangon
Shrimp
North Sea
Westerschelde
Tributyltin
Triphenyltin

ABSTRACT

The organotin (OT) compounds tributyltin (TBT) and triphenyltin (TPhT) are potent biocides that have been used ubiquitously in antifouling paints and pesticides since the mid-1970s. These biocides are extremely toxic to marine life, particularly marine gastropod populations. The European Union therefore took measures to reduce the use of TBT-based antifouling paints on ships and ultimately banned these paints in 2003. Despite sufficient data on OT concentrations in marine gastropods, data are scarce for other species such as the North Sea brown shrimp (*Crangon crangon*), a dominant crustacean species in North Sea inshore benthic communities. The present study provides the first spatial overview of OT concentrations in North Sea brown shrimp. We have compared these data with historical concentrations in shrimp as well as with sediment concentrations. We have also addressed the effect on the shrimp stock and any human health risks associated with the OT concentrations found. TBT and TPhT in shrimp tail muscle ranged from 4 to 124 and from 1 to 24 μg kg ^{1} DW, respectively. High levels are accumulated in estuarine areas and are clearly related with sediment concentrations (biota-sediment accumulation factor ~10). Levels have decreased approximately 10-fold since the ban took effect, coinciding with a recovery of the shrimp stock after 30 years of gradual regression. Furthermore, the OT levels found in brown shrimp no longer present a human health risk.

1. Introduction

Organotins (OTs), generally represented by the formula R_XSnL_{4-X}, contain a tetravalent tin atom (Sn) covalently bound to 1–4 organic substituents (R; e.g., $-CH_3$, $-C_4H_9$, $-C_5H_5$ and $-C_8H_{17}$) and 1–3 halogen atoms or oxygen- or sulfur-based organic moieties (L; e.g., –Cl, –F, –SR′ and –OR′). Owing to their strong biocidal activity, tributyltin (TBT, $(C_4H_9)_3SnL$) and triphenyltin (TPhT, $(C_5H_5)_3SnL$) have been widely used in pest control. TPhT ("fentin") has been a popular fungicide in potato, sugar beet and hop culture (Hoch, 2001). In 2003, the EU banned the use of fentin because of serious concerns about operator safety and undesired effects on non-target organisms.

Starting in the mid-1970s, TBT was the compound of choice (and to a lesser extent TPhT) in antifouling agents to reduce drag or damage on ship hulls, buoys, fish nets and cages due to the attachment of fouling organisms (e.g., barnacles, algae). At the end of the 1970s, an undesired impact of TBT on the calcification of oyster shells was observed (Alzieu et al., 1982). Soon thereafter, the relationship between TBT and imposex in marine snails was discovered (Smith, 1981). France was the first country to respond by issuing a ban on the application of OT-based antifouling paints on hulls of ships smaller than 25 m in 1982. Similar bans throughout the North Sea countries followed between 1987 and 1991. In 1989, the EU imposed these measures on all Member States through Directive 89/677/EEC. In 2001, the International Maritime Organization (IMO) adopted the 'International Convention on the Control of Harmful Antifouling Systems' (AFS Convention), which prohibited the application of OTs as antifouling agents. The AFS Convention entered into force on 17 September 2007 and globally banned OTs on marine vessels starting on 17 September 2008. The EU transposed the AFS Convention into Regulation (EC) 782/2003, which banned the application of organotin on EU-flagged vessels starting on 1 January 2003. That regulation further obliged all ships visiting EU ports from 1 January 2008 onto be free of OTs or to at least bear a barrier coating. These measures have led to a slow recovery of TBT-sensitive gastropod populations and their TBT-related imposex

* Corresponding author. Tel.: +32 9 3662677.
E-mail address: koen.parmentier@telenet.be (K. Parmentier).

0045-6535/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2011.11.028

prevalence is decreasing (Morton, 2009; Oliveira et al., 2009; Rodríguez et al., 2009; Sousa et al., 2009).

Brown shrimp (*Crangon crangon* L.) has a wide geographical distribution, ranging from the Icelandic coast and the White Sea (North-West Russia) to the Moroccan coast and the Black Sea. It dominates the shallow sandy coastal areas of the southern North Sea and the associated estuaries, from northern France to Denmark, where brown shrimp can comprise up to 80% of the total benthic biomass (Bamber and Henderson, 1994; Cattrijsse et al., 1997). In the associated benthic ecosystem, *C. crangon* has a pivotal function; it is both a prominent predator of juvenile fish and smaller invertebrate species (Pihl and Rosenberg, 1984; Wennhage and Gibson, 1998; Hiddink et al., 2002; Wennhage, 2002) and is also crucial prey for economically important fish species such as cod and whiting. *C. crangon* also has direct economic importance, with an annual catch of almost 40 000 tonnes and a third-place ranking in landings value of the North Sea Fisheries (ICES, 2010). The present study contains data on TBT and TPhT concentrations in *C. crangon* caught between 1 September and 10 November 2009 at multiple sampling stations from De Panne (Belgium) to Esbjerg (Denmark). These stations represent the major commercial fishing grounds. We have focused on the Westerschelde, a heavily polluted European estuary associated with one of the most densely populated areas (Flanders) and largest ports (Antwerp) in the world. The Westerschelde has a large *C. crangon* population and hosts an important nursery ground, the estuarine tidal marsh "Verdronken Land Van Saeftinghe" (Cattrijsse et al., 1997). Associated biota-sediment accumulation factors (BSAFs) were derived. Finally, we have studied the effects of the 2008 global TBT ban on the OT content and status of the Southern Bight shrimp stock and associated human health risks.

2. Experimental

2.1. Sample collection

Samples were collected during 1 September–10 November, 2009 during the Dutch, German and Belgian Demersal (Young) Fish Surveys (DFS in the Netherlands, DYFS in Germany and Belgium) performed by the Institute for Marine Resources and Ecosystem Studies (IMARES, Netherlands), Johann Heinrich von Thünen-Institute, Institute of Sea Fisheries (vTI-SF, Germany) and the Institute for Agricultural and Fisheries Research (ILVO, Belgium) (Supplementary Material, Table 1). Samples were immediately frozen aboard and stored at −20 °C at the related institutes prior to transportation to ILVO (Ostend, Belgium) in polystyrene foam insulated containers. Further sample preparation, extraction, clean-up, gas chromatographic (GC) analyses and quality control were done in accordance with the guidance for monitoring of organotin levels in marine biota (Monteyne et al., 2010) and were performed at the marine chemistry lab (MARCHEM) of MUMM in Ostend, which is accredited in accordance with the recognized International Standard ISO/IEC 17025:2005.

2.2. Sample preparation

Shrimp were allowed to thaw overnight, then were peeled and briefly rinsed with ultrapure water to obtain approximately 100 g of tail muscle for each sampling station. Samples were mixed in borosilicate petri dishes using a rotor/stator type homogenizer (Ultraturrax T25 basic, IKA-Labortechnik GmbH, Staufen, Germany), freeze-dried with a Christ LMC-2 (Martin *Christ* Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) lyophilizer and pulverized manually using a porcelain mortar and pestle. The powder was weighed for calculation of dry weight (DW) – wet weight (WW) ratio, and was stored in a desiccator cabinet until analysis.

2.3. Sample extraction and clean-up

The procedure for OT extraction was based on the use of acid reagents in methanol and stirring in hexane. About 1 g of shrimp powder was transferred into an amber 40 mL screw cap vial, and then l5 mL methanol and 7 mL hexane were added. Samples were buffered to pH 5 by adding 3 mL of 4 M sodium acetate. An aliquot of 25 μL of tripropyltin solution (10 μg Sn/g in methanol) was added as recovery standard prior to derivatization for QA/QC purposes to check the ethylation efficacy of TBT. Ethylation was combined with a continuous desorption process by drop-by-drop addition of 4 mL of sodium tetraethyl borate (Sigma–Aldrich, Steinheim, Germany) prepared with deionized water (5%, *v/v*) to the samples while vigorously stirring. For the degradation of boroxin formed due to the intensive derivation (Smedes et al., 2000), an aliquot of 5 mL of 10 M sodium hydroxide was added to the samples. Finally, the internal standards tetrapropyltin (used for quantification) and pentyltriphenyltin (additional standard for QA/QC purpose) of a concentration of 4 μg Sn/g in hexane were added to the samples, and the phases were separated by centrifugation. The purity of all solvents was appropriate for organic residue analysis. Chlorinated and ethylated OTs were obtained from QUASIMEME (Wageningen, the Netherlands). Internal standard and recovery standard tetrapropyltin and tripropyltin chloride were purchased from Schmidt (Amsterdam, the Netherlands). Glassware was washed with 10% hydrochloric acid and rinsed six times with ultrapure water. Custom-made chromatography columns (200 mm × 9 mm ID) were filled with 2 g of florisil (Merck, Darmstadt, Germany) and 25 mL of hexane was used as the clean-up elution. The extracts were stored at 4 °C until GC analysis.

2.4. Gas chromatographic analysis

A large-volume injection (LVI) technique was developed (Monteyne et al., unpublished protocol). Fifty microliters of sample was injected by an autosampler (Combipal, CTC Analytics, Italy) at a rate of 1.7 μL s^{-1} through a Programmed Temperature Vaporizing (PTV) injector (Thermo Electron Corporation, Austin, TX, USA), using a glass sintered liner. The analytic system consisted of a Trace GC (ThermoQuest, Milan, Italy), a 20 m Rtx®-5 SILMS analytical column (0.25 mm ID) with a 5% phenyl polysilphenylene-siloxane stationary phase (0.25 μm film thickness; Restek, Bellefonte, PA, USA). The oven was preheated to 35 °C and maintained that temperature for 4 min, after which the temperature was increased at the rate of 20 °C min^{-1} to 120 °C (ramp 1), at a rate of 7 °C min^{-1} to 150 °C (ramp 2) and finally at a rate of 20 °C min^{-1} to 300 °C (ramp 3) (5 min hold). A carrier flow of helium of 1.5 mL min^{-1} was used. The compounds were detected by a Finnigan Trace MS in electron-impact ionization (EI) mode operating in selected ion monitoring (SIM).

2.5. Quality control

Multi-level calibration curves ($r^2 > 0.995$) in the linear response interval of the detector were created for quantification. The identification was based on retention times and intensity ratios of 3 monitored ions for quantification. The quality control was performed by regular analysis of procedural blanks, a procedural spike of 100 ng Sn/g, duplo measurements, internal reference material (mussel tissue) and certified reference material (mussel tissue ERM®-CE477). Recovery of MBT, DBT and TBT in ERM®-CE477 was 117 ± 14%, 97 ± 15% and 99 ± 11% (n = 12, 4-year period), respectively. Recovery of TPhT in the procedural spike is 90 ± 15%. Semi-annual international proficiency test (QUASIMEME) results were also consistently successful. Limits of quantification (LoQ) for TBT and TPhT were 1 μg kg^{-1} DW. LoQs of monobutyltin (MBT), dibutyltin

Please cite this article in press as: Verhaegen, Y., et al. Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban. Chemosphere (2011), doi:10.1016/j.chemosphere.2011.11.028

(DBT), monophenyltin (MPhT) and diphenyltin (DPhT) were 10, 10, 3 and 1 μg kg^{-1} DW, respectively.

All concentration results further used in this article are expressed as μg of organotin ion per kg, expressed on the basis of DW.

2.6. Sediment data and the biota-sediment accumulation factor

Concentrations of OT and other pollutants in the <63 μm sediment fraction and median grain size of the sampling stations in the Westerschelde estuary during 1999–2009 were obtained through online databases of the Belgian Marine Data Centre (BMDC, http://www.mumm.ac.be/datacentre/) and the Dutch Rijkswaterstaat (www.waterbase.nl). Sediment OT concentrations were used to calculate the Biota-Sediment Accumulation Factor (BSAF), a parameter describing bioaccumulation of sediment-associated contaminants into organisms or their tissues (Burkhard, 2009). BSAFs are useful for ecological risk assessments because concentration data are usually available for sediment, in contrast to biota. Depending on the nature of the contaminant, BSAF calculation varies. The heavy-metal BSAF is defined as the ratio of the tissue concentration versus concentration in surface sediment (<63 μm fraction) on the basis of DW (Ankley et al., 1994). When applied to organic compounds (e.g. PCBs), chemical concentrations are normalized for lipid content of the tissue and organic carbon content of the sediment (Ankley et al., 1992). Metal-type BSAFs were derived for TBT, as it accumulates in body tissues in a similar manner similar to heavy metals, i.e., through selective binding to proteins (Kannan et al., 1996; Kim et al., 1996; Tanabe, 1999).

2.7. Tolerable human daily intake (TDI)

Based on the No Observed Adverse Effect Level (NOAEL) of TBT for immunotoxicity in rats, multiplied by a safety factor 1/100, a TDI of 0.25 μg kg^{-1} body weight has been proposed (Penninks, 1993). The European Food Safety Authority (EFSA) established a group TDI for the sum of DBT, TBT, TPhT and dioctyltin (DOT) to 0.25 μg kg^{-1} body weight due to the similarity of their immunotoxic properties. Hence, for each sampling station, summed OT concentrations were used to calculate the allowed daily consumption (in kg FW shrimp meat).

2.8. Landings per unit effort (LPUE)

The largest section of the Belgian sea fisheries fleet consists of smaller bottom trawlers (<250 kW engine power) performing daily inshore trips to provide fresh daily shrimp and demersal fish (i.e., flounder, sole, plaice, dab, cod and whiting) to the local ports. Many

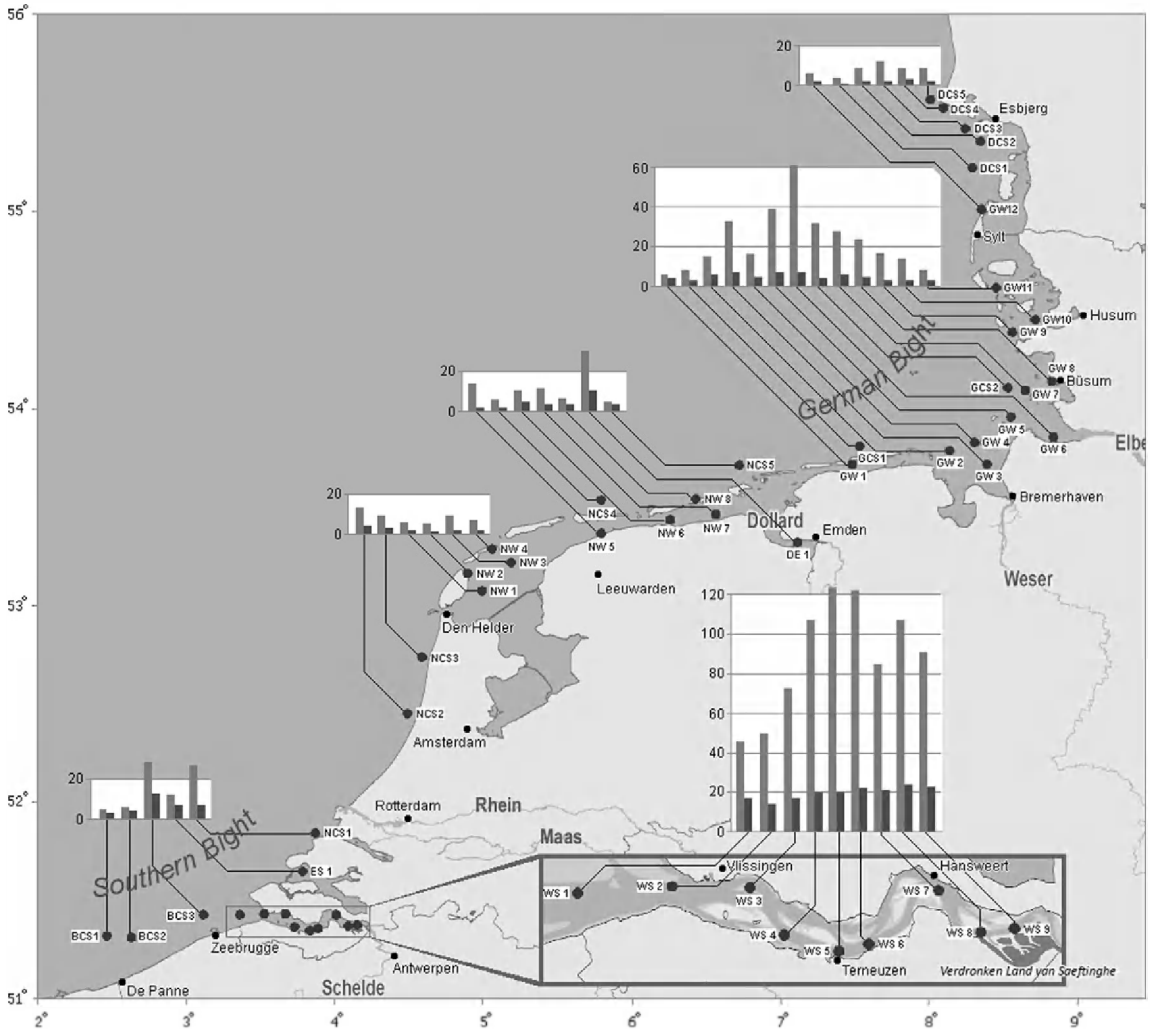

Fig. 1. Spatial distribution of TBT (light gray bars) and TPhT (dark gray bars) concentrations in shrimp meat (*Y*-axis, in μg kg^{-1} DW) from the stations along the southeastern coast of the North Sea.

Please cite this article in press as: Verhaegen, Y., et al. Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban. Chemosphere (2011), doi:10.1016/j.chemosphere.2011.11.028

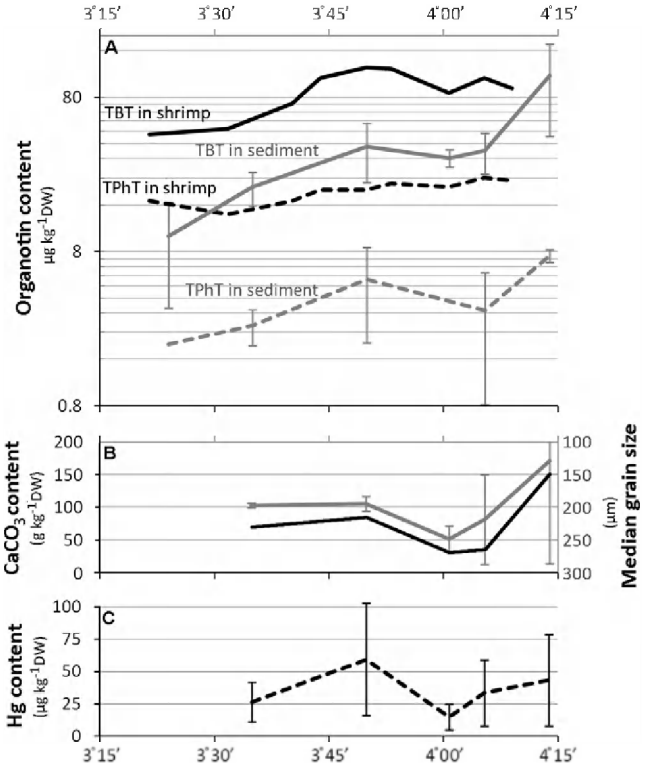

Fig. 2. (A) TBT and TPhT concentrations (*Y*-axis, in µg kg^{-1} DW) in shrimp meat and sediment along the longitudinal gradient in the Westerschelde estuary (*X*-axis). (B) CaCO3 content (*Y*-axis, in g kg^{-1} DW) and the inverse median grain size (*Y*-axis, inverted, in µm) of the sediment and (C) Sediment mercury content (*Y*-axis, in µg kg^{-1} DW) along the longitudinal gradient (*X*-axis) in the Westerschelde estuary, showing similar longitudinal patterns as the sediment OT concentrations.

of these local trawlers swap between shrimp (cod-end mesh size ~22 mm) and fish gear (cod-end mesh size >65 mm) on a seasonal basis. Landing data associated with both fishing activities reflects the abundance of the target species in the Southern Bight, as they are corrected by the associated fishing effort. Annual LPUEs (in kg fresh weight (FW) × horsepower^{-1} × fishing hours^{-1}) for *C. crangon* are calculated and reported annually to the ICES working Group on *Crangon* Fisheries and Life History (ICES, 2010). Since 1997, the LPUEs of the major shrimp predators (cod and whiting) were derived in a similar manner (see Supplementary Material for LPUE formula and landing and effort data).

3. Results and discussion

3.1. Spatial distribution of OT accumulation in brown shrimp

In the autumn of 2009, TBT and TPhT levels in shrimp ranged from 4 to 124 and from 1 to 24 µg kg^{-1} DW, respectively (Fig. 1; Supplementary Material, Table 1). Levels of MBT, DBT, MPhT and DPhT were all below the Limits of Quantification (LoQs; 10, 10, 3 and 1 µg kg^{-1} DW, respectively) and are not further discussed. In general, offshore OT concentrations are low, but the levels increase near major estuaries (Westerschelde, Rhine-Meuse Delta, Ems-Dollard, Weser and Elbe). Shrimp OT concentrations in the German Bight (GW2-GW10, Fig. 1; Supplementary Material, Table 1) are relatively high and increase with proximity to the Elbe estuary. The German Bight is characterized by a high direct inflow of riverine water from the Weser and Elbe and advection from the rivers Rhine, Meuse and Ems-Dollard through the continental coastal current. In the German Bight, intensive shipping activity and strong tidal shear currents lead to a continuous disturbance of the sediment bed and resuspension of particulate matter, which increases the biological availability of pollutants (Becker et al., 1992).

TPhT concentrations relative to TBT were highest (>50%) in the stations near the Schelde and Ems-Dollard estuaries and within the Oosterschelde estuary (BCS1, BCS2, ES1, NW8, NCS5, GW1), reflecting the large (historic) use of fentin fungicides in (potato) agriculture in the related catchment areas. Lowest OT concentrations were observed inshore in the Southern Wadden Sea near Texel, near-shore at the Northern Wadden islands Sylt and Rømø and offshore at the Southern sandbanks near De Panne.

3.2. Detailed spatial distribution of OT accumulation in brown shrimp in the Westerschelde estuary

In Westerschelde estuary shrimp, TBT concentration increases gradually from 50 µg kg^{-1} DW near Vlissingen (WS2) to 124 µg kg^{-1} DW upstream of Terneuzen (WS5) (Fig. 1). Further upstream near Hansweert (WS7) the concentration drops to 85 µg kg^{-1} DW, and tends to rise again further upstream (107 µg kg^{-1} at WS8). A similar spatial pattern in OT concentration in sediment is observed (Fig. 2A) (Monteyne & Roose, data courtesy of BMDC). The lower OT concen-

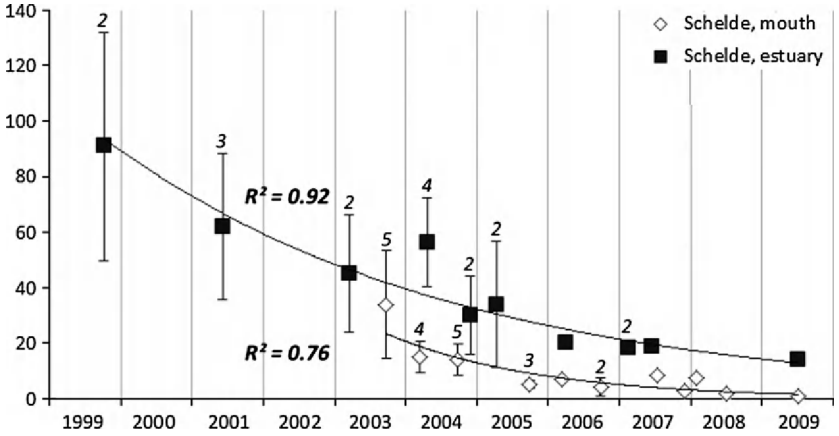

Fig. 3. Temporal trend of <63 µm sediment TBT concentrations (*Y*-axis, in µg kg^{-1} DW) in the Westerschelde lower estuary (Terneuzen–Vlissingen, between WS2-WS6) and mouth (Southern Vlakte van de Raan, roughly near BCS3-WS1) during 1999–2009 (*X*-axis). TBT concentrations were obtained through the BMDC and Dutch Waterbase (see Supplementary Material Table 3). Concentrations from different sampling locations (see Supplementary Material Fig. 1) were pooled for the lower estuary and mouth. Means, standard deviations and sample sizes are presented for each sampling date. The single measurement (<LoQ) in the Westerschelde mouth in the summer of 2009 is depicted as 1 µg kg^{-1} DW.

Please cite this article in press as: Verhaegen, Y., et al. Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban. Chemosphere (2011), doi:10.1016/j.chemosphere.2011.11.028

Table 1
Field derived BSAFs for shrimp meat in 2003 and 2009 for two sampling locations.

Location	Date	BSAF	Sediment concentration
BCS3	June 2003	11.5 ± 2.8 (n = 2)	27.19
BCS3	Autumn 2009	20.5	1.41
WS2-WS6	June 2003	12.6	45.80
WS2-WS6	Autumn 2009	7.8 ± 2.7 (n = 5)	12.13

<63 μm Sediment TBT concentrations (in μg kg^{-1} DW) used were calculated based on the exponential regression formula associated with the temporal trends in sediment TBT concentration (Fig. 3, see Supplementary Material Table 3). Shrimp meat TBT concentrations in μg kg^{-1} DW for 2003 originate from Willemsen et al. (2004) and Veltman et al. (2006) (see Supplementary Material Table 2).

trations in shrimp and sediment at WS7 is related to the strong currents associated with the narrow cross-section of the estuary near Hansweert. These currents prevent the local deposition of finer, $CaCO_3$ rich sediments (Fig. 2B). Sediment data also reveal that TBT does not tend to accumulate in the same way as other organic pollutants (e.g. benzo-[a]-pyrene, data not shown) but rather accumulates as heavy metals do (e.g. mercury, Fig. 2C). This concurs with the metal-type accumulation of TBT observed in biota (Tanabe, 1999). For TPhT, a similar trend was clear in the sediment but less clear in shrimp. TPhT concentrations in shrimp increase gradually from 14 to 24 μg kg^{-1} DW between Vlissingen and Saeftinghe. In sediment, TPhT concentration increases from 2 to 7.5 μg kg^{-1} DW, with an intermediary minimum at WS7.

3.3. Temporal variation of TBT in shrimp and sediment and BSAF in the Westerschelde estuary

A limited amount of data on OT concentrations in *C. crangon* is available for the Westerschelde and the Southern Bight only (see Supplementary Material Table 2). Based on our measurements at sampling station BCS3 in September 2009 (29 μg kg^{-1} DW) and the findings of Willemsen et al. (2004) near BCS3 in 2003 (368 and 259 μg kg^{-1} DW), the TBT concentration in shrimp has decreased more than 10-fold in 6 years. Several observations indicate that the associated BSAFs should be based on the TBT concentrations measured in sediment instead of in suspended solids, which contrasts with the findings of Veltman et al. (2006). TBT content in shrimp exhibits a comparable spatial trend as in sediment (Fig. 2.A). In contrast to suspended solids (data not shown), which are strongly subject to tidal variations, the sediment in the Westerschelde exhibits a clear spatial (Fig. 2A) and temporal trend in TBT content (Fig. 3). The exponential regression associated with the temporal trend shows a good fit (R^2 = 0.93; see Supplementary Material for the associated regression formula). The regression formula was used to extrapolate the decreasing TBT concentration in the lower Westerschelde estuary to October 2009, the period of shrimp sampling, and to subsequently derive the related BSAFs. Our results indicate an average BSAF of roughly 10 (Table 1), meaning a strong accumulation and probably a poor metabolization of TBT in shrimp. This is consistent with Veltman et al. (2006), who observed high accumulation ratios of TBT and TPhT in *C. crangon*, and Takahashi et al. (1999), who found a limited metabolization capacity of TBT in Caprellidae (Crustacea; Malacostraca).

3.4. Putative effect on the shrimp stock in the Southern Bight

In brown shrimp, a yet unexplained population decline and recovery was apparent in the Southern Bight, similar to the TBT associated fate of several gastropod populations: since the mid 1970s, the annual LPUE gradually declined until 2002 and has recovered quickly since 2007 (Fig. 4). It is striking that the recent resurrection of the brown shrimp population coincides with the ban of TBT and a near 10-fold drop in TBT concentration levels during the time span under consideration. The recovery of the brown shrimp population in the Southern Bight cannot be attributed to a sudden reduction of fishing effort or predation pressure (*e.g.* by overfishing), as the LPUE of its most important predators (cod and whiting) indicate (Fig. 4). Effects of environmental OT concentrations on the physiology of *C. crangon* are unknown, but acute laboratory exposure of *C. crangon* to TBT showed a strong decrease in activity and expression of the ecdysteroid receptor, a nuclear hormone receptor crucial for the tight orchestration of development, growth and reproduction (Verhaegen et al., 2010, 2011).

Adult *C. crangon* (and other decapods) are believed to be fairly resistant to organotin exposure, with a 96-h-LC_{50} of 28.5–41 μg L^{-1} TBTO (Champ, 1986; Verhaegen et al., 2011). Sub-toxic but nonetheless detrimental effects may occur at much lower levels. In the closely related fiddler crab, *Uca pugilator*, 0.5 μg L^{-1} was sufficient to significantly disrupt limb generation (Weis et al., 1987). In contrast to adult *C. crangon*, larval stages are believed to be sensitive towards organotins, with a 96-h-LC_{50} of 1.5 μg L^{-1} TBTO; Champ, 1986). In the closely related American lobster, *Homarus americanus*, 1 μg L^{-1} TBTO was sufficient to almost completely stop larval growth (Laughlin and French, 1980). Two major issues hamper the demonstration of an unarguable causative link between decreased OT concentrations and the shrimp stock recovery. First, we cannot estimate the historical environmental effects of OTs on larval *C. crangon*, as fundamental environmental concentration data in larval (planktonic) stages of *C. crangon* or even of related species are lacking. Second, subtoxic dose-dependent physiological effect data is lacking as the poor survival of larvae, juveniles and adults of *C. crangon* under laboratory conditions currently hinders reliable

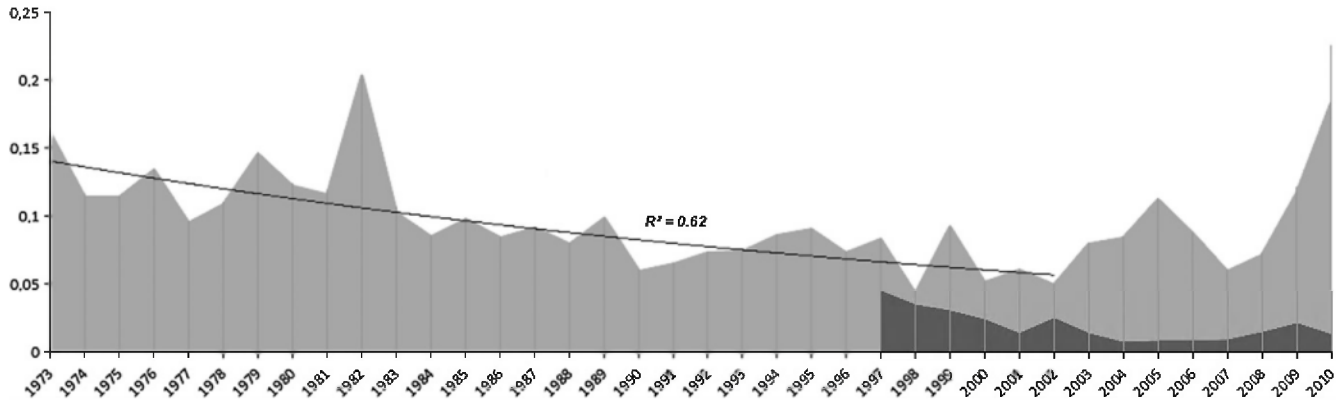

Fig. 4. Long-term annual time series of LPUEs for the Southern Bight (*Y*-axis, average monthly LPUEs in kg FW × horsepower^{-1} × fishing hours^{-1}) for shrimp (light gray) and cod and whiting (dark gray) during 1973–2010 and 1997–2010, respectively (*X*-axis). See Supplementary Material Table 4 for the landing and effort data.

Please cite this article in press as: Verhaegen, Y., et al. Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban. Chemosphere (2011), doi:10.1016/j.chemosphere.2011.11.028

chronic exposure experiments. Once an acceptable adult laboratory survival is reached, analysis of hatching success and of early larval growth and survival after maternal exposure to historical sediment (or pore-water) OT concentrations could be the key.

3.5. Consumer health risk

In our experiments, the DW to FW ratio of peeled shrimp was determined at 22.4 ± 1.1% (n = 46). According to the TDI set by EFSA, a person weighing 60 kg is allowed to consume 5.22 ± 2.86 kg on a daily basis (n = 35; Supplementary Material, Table 1) of FW shrimp meat from commercially exploited shrimp areas (excluding the inner Ems-Dollard estuary, Oosterschelde and Westerschelde). This renders brown shrimp a healthy seafood product, in contrast to its status less than a decade ago. In 2003, the daily consumption of 169 g of peeled shrimp was sufficient to exceed the TDI for TBT alone. According to Avia et al. (2011), the average daily consumption of *C. crangon* meat in Germany and Belgium is estimated at 0.063 g and 1.4 g per person, respectively. These numbers may not be appropriate for both countries, as a large part of especially the German population are virtually non-consumers, but it can be concluded that even people consuming brown shrimp on a daily basis can do so with no health risks with respect to OT intake.

Acknowledgements

We thank Marcel de Vries, Andre Dijkman and Peter Groot (IMARES, The Netherlands); Gitta Hemken, Thomas Kehlert, Karin Krüger and Susanne Schöling (vti-SF, Germany); Mattias Bossaer, Lode Jacobs and Stefan Hoffman (ILVO, Belgium); Per Sand Kristensen (dtu.aqua, Charlottenlund, Denmark) and the crews of the research vessels and chartered cutters for their support and assistance with the shrimp collection. This research was supported by the Belgian Science Policy Office via its support to MARCHEM, E. Monteyne and P. Roose. The Dutch survey was carried out as part of the statutory tasks related to Dutch legislation in fisheries management, financed by the Dutch Ministry of Economics, Agriculture and Innovation.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.11.028.

References

Alzieu, C., Heral, M., Thibaud, Y., Dardignac, M., Feuillet, M., 1982. Influence des peintures antisalissures a base d'organostanniques sur la calcification de la coquille de l'huitre *Crassostrea gigas*. Rev. Trav. Inst. Pech. Marit. 45, 101–116.

Ankley, G.T., Cook, P.M., Carlson, A.R., Call, D.J., Swenson, J.A., Corcora, H.F., 1992. Bioaccumulation of PCBs from sediments by oligochaetes and fishes: comparison of laboratory and field studies. Can. J. Fish. Aquat. Sci. 49, 2080–2085.

Ankley, G.T., Leonard, E.N., Mattson, V.R., 1994. Prediction of bioaccumulation of metals from contaminated sediments by the oligochaete *Lubriculus variegatus*. Water Res. 28, 1071–1076.

Avia, D., Diamantis, C., Neudecker, T., Berkenhagen, J., Müller, M., 2011. The North Sea Brown Shrimp Fisheries. Study requested by the European Parliament (IP/B/PECH/IC/2010_102), p. 108.

Bamber, R.N., Henderson, P.A., 1994. Seasonality of caridean decapod and mysid distribution and movements within the Severn Estuary and Bristol Channel. Biol. J. Linn. Soc. 51, 83–91.

Becker, G.A., Dick, S., Dippner, J.W., 1992. Hydrography of the German Bight. Mar. Ecol. Progr. Ser. 9, 9–18.

Burkhard, L., 2009. Estimation of Biota Sediment Accumulation Factor (BSAF) from paired observations of chemical concentrations in biota and sediment, US Environmental Protection Agency, Ecological Risk Assessment Support Center: Cincinnati, OH, EPA/600/R-06/047.

Cattrijsse, A., Dankwa, H.R., Mees, J., 1997. Nursery function of an estuarine tidal marsh for the brown shrimp *Crangon crangon*. J. Sea Res. 38, 109–121.

Champ, M.A., 1986. Organotin symposium: introduction and review. In: Maton, G.L. (Ed.), Proceedings Oceans 86 Conference, vol. 4. September 23–25, 1986, Washington, DC. pp. 1093–1100.

Hiddink, J.G., Marijnissen, S.A.E., Troost, K., Wolff, W.J., 2002. Predation on O-group and older year classes of the bivalve *Macoma balthica*: interaction of size selection and intertidal distribution of epibenthic predators. J. Exp. Mar. Biol. Ecol. 269, 223–248.

Hoch, M., 2001. Organotin compounds in the environment – an overview. Appl. Geochem. 16, 719–743.

ICES (International Council for the Exploitation of the Sea), 2010. Report of the Working Group on *Crangon* Fisheries and Life History (WGCRAN). C.M. – International Council for the Exploration of the Sea (ICES), CM 2011/SSGEF:17. Copenhagen, 60 pp.

Kannan, S., Corsolini, S., Focardi, S., Tanabe, S., Tatsukawa, R., 1996. Accumulation pattern of butyltin compounds in dolphin, tuna and shark collected from Italian coastal waters. Arch. Environ. Contam. Toxicol. 31, 19–23.

Kim, G.B., Lee, J.S., Tanabe, S., Iwata, H., Tatsukawa, R., Shimazaki, K., 1996. Specific accumulation and distribution of butyltin compounds in various organs and tissues of the Steller sea lion (*Eumetopias jubatus*) comparison with organochlorine accumulation pattern. Mar. Pollut. Bull. 32, 558–563.

Laughlin Jr., R.B., French, W.J., 1980. Comparative study of the acute toxicity of a homologous series of trialkyltins to larval shore crabs, *Hemigrapsus nudus*, and lobster, *Homarus americanus*. Bull. Environ. Contam. Toxicol. 25, 802–809.

Monteyne, E., Strand, J., Vorkamp, K., Bersuder, P., Bolam, T., Giltrap, M., McGovern, E., 2010. Monitoring organotins in marine biota. ICES Techniques in Marine Environmental Sciences, vol. 47, p. 16.

Morton, B., 2009. Recovery from imposex by a population of the dog whelk, *Nucella lapillus* (Gastropoda: Caenogastropoda), on the southeastern coast of England since May 2004: a 52-month study. Mar. Pollut. Bull. 58, 1530–1538.

Oliveira, I.B., Richardson, C.A., Sousa, A.C., Takahashi, S., Tanabe, S., Barroso, C.M., 2009. Spatial and temporal evolution of imposex in dog whelk *Nucella lapillus* (L.) populations from North Wales, UK. J. Environ. Monit 11, 1462–1468.

Penninks, A.H., 1993. The evaluation of data-derived safety factors for bis(tri-n-butyltin)oxide. Food Addit. Contam. 10, 351–361.

Pihl, L., Rosenberg, R., 1984. Food selection and consumption of the shrimp *Crangon crangon* in some shallow marine areas in western Sweden. Mar. Ecol. Prog. Ser. 18, 159–168.

Rodríguez, J.G., Tueros, I., Borja, A., Franco, J., García Alonso, J.I., Garmendia, J.M., Muxika, I., Sariego, C., Valencia, V., 2009. Butyltin compounds, sterility and imposex assessment in *Nassarius reticulates* (Linneaus, 1758), prior to the 2008 European ban on TBT antifouling paints, within Basque ports and coastal areas. Cont. Shelf Res. 29, 1165–1173.

Smedes, F., de Jong, A.S., Davies, I.M., 2000. Determination of (mono-, di- and) tributyltin in sediments. Analytical methods. J. Environ. Monit. 2, 541–549.

Smith, B.S., 1981. Tributyltin compounds induce male characteristics on female mud snails *Nassarius obsoletus* = *Ilyanassa obsolete*. J. Appl. Toxicol. 1, 141–144.

Sousa, A., Laranjeiro, F., Takahashi, S., Tanabe, S., Barroso, C.M., 2009. Imposex and organotin prevalence in a European post-legislative scenario: temporal trends from 2003 to 2008. Chemosphere 77, 566–573.

Takahashi, S., Tanabe, S., Takeuchi, I., Miyazaki, N., 1999. Distribution and specific bioaccumulation of butyltin compounds in a marine ecosystem. Arch. Environ. Contam. Toxicol. 37, 50–61.

Tanabe, S., 1999. Butyltin contamination in marine mammals, a review. Mar. Pollut. Bull. 39, 62–72.

Veltman, K., Huijbregts, M.A.J., van den Heuvel-Greve, M.J., Vethaak, A.D., Hendriks, A.J., 2006. Organotin accumulation in an estuarine food chain: comparing field measurements with model estimations. Mar. Environ. Res. 61, 511–530.

Verhaegen, Y., Parmentier, K., Swevers, L., Rougé, P., Soin, T., De Coen, W., Cooreman, K., Smagghe, G., 2010. The brown shrimp (*Crangon crangon* L.) ecdysteroid receptor complex: cloning, structural modeling of the ligand-binding domain and functional expression in an EcR-deficient *Drosophila* cell line. Gen. Comp. Endocrinol 168, 415–423.

Verhaegen, Y., Parmentier, K., Swevers, L., Renders, E., Rougé, P., De Coen, W., Cooreman, K., Smagghe, G., 2011. The heterodimeric ecdysteroid receptor complex in the brown shrimp *Crangon crangon*: EcR and RXR isoform characteristics and sensitivity towards the marine pollutant tributyltin. Gen. Comp. Endocrinol. 172, 158–169.

Weis, J.S., Gottlieb, J., Kwiatkowski, J., 1987. Tributyltin retards generation and produces deformities of limbs in the fiddler crab, *Uca pugilator*. Arch. Environ. Contam. Toxicol. 16, 321–326.

Wennhage, H., 2002. Vulnerability of newly settled plaice *Pleuronectes platessa* L. To predation: effects of habitat structure and predator functional response. J. Exp. Mar. Biol. Ecol. 269, 129–145.

Wennhage, H., Gibson, R.N., 1998. Influence of food supply and a potential predator (*Crangon crangon*) on settling behavior of plaice (*Pleuronectes platessa*). J. Sea Res. 39, 103–112.

Willemsen, F., Wegener, J.-W., Morabito, R., Pannier, F., 2004. Sources, Consumer Exposure and Risks of Organotin Contamination in Seafood. Final report of the European Commission Research Project "OT-SAFE" (QLK1-2001-01437), 143 pp.