Organotin and Imposex in the Littoral Zone in the Faroe Islands

Organotin og imposex í strandarøkinum í Føroyum

Norunn Følsvik[1], Einar M. Brevik[1], John Arthur Berge[1] and Maria Dam[2]

1. Norwegian Institute for Water Research, Box 173, Kjelsås, N-0411 Oslo, Norway
2. Food- and Environmental Agency, Debesartrøð, FO-100 Tórshavn, Faroe Islands, Phone +298 31 53 00, Fax +298 31 0508, e-mail:mariadam@hfs.fo

Úrtak

Organotin kann vera í evnum, sum eru ætlað at klæða skip við. Árinið av organotin er, at tað forðar fyri gróðri á tí partinum av skrokkinum, sum er undir, so at yvirflatan verður verandi sløtt. Eitt skip við slættari yvirflatu glíður lættari gjøgnum sjógvin, og tað brúkar tí minni brennievni til sigling. Sama ávirkan kundi verið fingin, um botnurin á skipinum varð skavaður við hond, men tað bæði tekur tíð og krevur arbeiði, og tí er tann evnafrøðiliga loysnin framvegis vanlig í dag. Tíverri hevur tað víst seg, at summar havgastropodir, serstakliga purpurkúvingur *Nucella lapillus*, eru serliga viðbreknar fyri organotinsløgunum tributylin, TBT. Purpurkúvingur verður ávirkaður av TBT á tann hátt, at kallkynseyðkenni mennast í kvenndýrunum, ein støða, sum verður umtalað sum »imposex«. Á tí sjónskasta stignum av imposex eru kvenndýrini ikki før fyri at fjølgast. Tað hevur víst seg, at imposex er vanligt í heldur fjølbygdum strandarsamfeløgum og eisini fram við siglingarleiðum frá landi. Hend greinin lýsir, hvørjum støði organotinsløgini eru á í strandarøkinum í Føroyum, og hvat árin tey hava á tað. Úrslitini av greiningunum av organotinsløgum í purpurkúvingi, *Nucella lapillus*, kræklingi, *Mytilus edulis*, og í fliðu, *Patella vulgata*, verða løgd fram. Ovurnøgdin av fyribrigdinum imposex í purpurkúvingum, sum eru savnaðir á sjey støðum í Føroyum, verður víst.

Abstracts

Organotin may be found as a component in some of the coatings that are intended for ships. The effect of organotin is to prevent growth on the submerged part of the hull, and thus keep the surface smooth. A ship with a smooth surface slides more easily through the water and therefore uses lesser amounts of fuel for propagation. This same effect could be obtained with a manual scraping of the bottom of the vessel, but this takes both time and labour, hence the chemical way is prevailing today. Unfortunately it has appeared that certain marine gastropods, especially the dogwhelk *Nucella lapillus*, is particularly sensitive to the organotin species tributyltin, TBT. The response of the dogwhelk to TBT is the development of male sexual characteristics in females, a state which is referred to as "imposex". In the most pronounced stage, imposex renders the female unable to reproduce. Imposex has been found to be widespread in the more densely populated coastal communities and also along ship lanes offshore. The present paper desribes the levels and effects of organotin species in the littoral zone on the Faroe Islands. The results of the analyses of organotin species in dogwhelk, *Nucella lapillus*, common mussel, *Mytilus edulis*, and in limpets, *Patella vulgata*, are given. The abundance of the imposex phenomenon in dogwhelks collected at seven locations on the Faroes Islands is shown.

Summary

The use of organotin compounds as antifouling agents, fungicides, insecticides and bactericides has increased dramatically over the last 30 years. They are considered among the most toxic compounds introduced to the marine environment, where the main focus has been on the extremely effective antifouling agent tributyltin (TBT).

Unintended environmental effects from tributyltin in antifouling paints were first found in the Arcachon Bay in France in the late 1970s on the Pacific oyster (Alzieu *et al.*, 1986 with references). Effects of TBT have since then been reported for a variety of other marine species world-wide. In the last decade much attention has been paid to the induction of male sex characters in female snails, known as »imposex«, observed mainly in neogastropods (Gibbs and Bryan, 1986). The development of a female penis and vas deferens is probably caused by an accumulation of a male hormone (testosterone) in the female dogwhelks. Sensitive species may show such effects at TBT concentrations as low as 1 ng/L.

To evaluate the current status of organotin pollution along the coast of the Faroe Islands, dogwhelks, common mussels and limpets have been analysed for concentrations and effects of organotin compounds. Levels of organotin compounds were determined by a method based on direct ethylation using sodium tetraethylborate and subsequent analysis by gas chromatography and atomic emission detection (GC-AED). Levels of imposex in dogwhelk, *Nucella lapillus,* populations were determined according to the method by Gibbs *et al.,* (1987), which includes the determination of the two parameters Relative Penis Size Index, RPSI, and Vas Deferens Sequence Index, VDSI.

The concentrations of organotin compounds in dogwhelks from the Faroe Islands varied from below detection limits (<1 ng Sn g^{-1} d.w.) to 17 ng Sn g^{-1} d.w. The species detected were TBT, dibutyltin (DBT) and monobutyltin (MBT). The maximum concentration was found in dogwhelks from Tórshavn, the capitol of the Faroe Islands.

Effects of TBT, determined as imposex in dogwhelks, were observed at all but one station (Kirkjubøur, VDSI:0.1). Sterile females (VDSI>4) were found at all of the other stations.

The concentration of TBT in dogwhelks from the affected populations range from below detection limits (<1 ng Sn g^{-1} d.w.) to 17 ng Sn g^{-1} d.w. while no TBT was found in the unaffected population. A relation was found between the levels of TBT and the degree of imposex in the populations.

All common mussel populations investigated contained organotin compounds, concentrations of TBT ranging from 49 to 372 ng Sn g^{-1} d.w. DBT and MBT were also detected, and the relation between the species was always TBT > DBT > MBT. This is similar to the relation found in common mussels from Norwegian waters (Følsvik, 1997).

Only the limpet population from Tórshavn contained organotin compounds. TBT, DBT and MBT concentrations were 90, 62 and 54 ng Sn g^{-1} d.w. respectively.

The results indicate widespread biological effects and contamination by several organotin species in the environment along the coast of the Faroe Islands.

Introduction

Organotin compounds are generally of anthropogenic origin and in recent years there has been growing concern about the detection of these compounds in the environment. In 1955 world production of organotin compounds was approximately 5000 tons. By 1985 production had increased to 35 000 tons and was estimated to reach 63 000 tons by 1993 (Blunden and Chapman, 1986; WHO, 1980).

Applications of organotin compounds depend on the nature of the alkylsubstituents and can be divided in biocidal and non-biocidal use. Maximum impact on biological activity occur for triorganotin compounds which are used as biocides in a number of areas. Among the most toxic species are tributyl- and triphenyltin compounds (Bryan *et al.,* 1988; Thain *et al.,* 1987; Hugget *et al.,* 1992). The most important application of these compounds from an environmental point of view, are as the active compound in antifouling paints used on the hull of ships (Bryan and Gibbs, 1991). Antifouling paints prevent settlement and growth of barnacles, tubeworms, algae and other marine organisms on ships. Settlement of such organisms increase the hydrodynamic drag and reduces fuel efficiency. Triorganotins are also used in wood protection against fungal and insect attack and as pesticides in agriculture, but these sources are not considered as major contributors to environmental pollution (Blunden and Evans, 1989).

Diorganotin compounds are used as heat and UV-stabilisers in PVC-products and as catalysts in the production of polyurethane foams. Monoorganotin compounds are also used as catalysts in industry. Leaching of inorganic and organic tin compounds from PVC materials has been observed. The rapid increase in production and use of PVC-products and their subsequent disposal may lead to an accumulation in the environment (Quevauviller *et al.,* 1991).

Unintended environmental effects from organotin compounds were first observed as development of shell anomalies in Pacific oysters (*Crassostrea gigas*) in France in the early 1970s (Alzieu *et al.,* 1986; Ruiz *et al.,* 1996). Since then, effects have been reported in a number of species including fish, gastropods, crustaceans, echinoderms and microalgae (Fent and Meier, 1994; Bryan *et al.,* 1986; Bushong *et al.*, 1990; Hall, 1988; Beaumont and Newman, 1986). In recent years, much attention has been paid to the introduction of male sex characters in female snails, known as imposex, mainly observed in neogastropods (Gibbs *et al.*, 1987; Horiguchi *et al.*, 1994). This effect is believed to be a widespread phenomenon (Ellis and Pattisina, 1990) and has in the open North Sea been related to shipping traffic intensities (Hallers-Tjabbes *et al.*, 1994). In some cases local populations of dogwhelks (*Nucella lapillus*) were reduced or disappeared (Bryan *et al.,* 1986). Most investigations indicate that tributyltin (TBT) is responsible for the development of imposex in snails (Bryan *et al.,* 1986). Other compounds and situations have however been reported to induce this condition on gastropods (Nias *et al.*, 1993). Bettin *et al.* (1996) suggested that TBT-induced imposex is caused by an inhibition of the cytochrome P-450 dependant aromatase system leading to an increase in the androgen (testosterone) level in the female gastropods.

Harmful effects of organotin compounds in the environment led to restrictions on the use of TBT based antifouling agents in a

Sample	Sample id.	Name of location used inthe main text	Date of collction	Location
Limpets Dogwhelks	1 a 1 a	Tórshavn Argir	3. Mars '96	The samples were taken just south of the marina, some 20 m away from the breakwater in a little creek with some large boulders giving shelter from the waves, and with a little brook. There were some tiny common mussels also, but not large enough to be used for analysis.
Limpets Common mussels	1 b 1 b	Sandagerði, Tórshavn	29. Feb. '96	*Sandagerði* is a sandy cove with a stream. Just north of the cove, in the direction of the hospital, there is a bay with a few boat houses. In this bay, near an outlet of a little brook, the common mussels were sampled under and between boulders.
Limpets Dogwhelks	2 2	Kirkjubøur	17. Feb. '96	The sampling was done at *Árnanes*, in the north-western part of the village.
Limpets Dogwhelks Common mussels	7 – 7 a	Skálafjørður Langanes	1. Mar s '96	The sampling was done a few hundred meters north of *Langanes* near the village *Glyvrar*, and just opposite the fish farm of "Bakkafrost"
Common mussels	7 b	Skálafjørður Fish farm Bakkafrost	29. Feb. '96	The mussels were taken from the fish farm.
Limpets Dogwhelks Common mussels	3 3 3	Vestmanna	15.-17. Feb. '96	Limpets and most of the mussels were taken from the shore just opposite the fish farm of "Vestlax". Some mussels were taken from the fish farm. Dogwhelks were collected from under the rocks on the shore 50 to 100 meters south of the fish farm, in the vicinity of the boat houses.
Dogwhelks Common mussels	4 4 a	Trongisvágsfjørður Kolatoftir	31. Jan. '96	Samples were taken on the beach just below the unfinished gasoline station.
Limpets Dogwhelks Common mussels	4 b –	Trongisvágsfjørður Hvítanes	31. Jan. '96	The samples were collected from rocks on the beach just west from the landing stage of the wholesale firm of Poul Hansen.
Dogwhelks	5	Klaksvík Kunoy	4. Mars '96	The snails were sampled on the shore just opposite a fish farm off the southernmost tip of *Kunoy*.
Common mussel	5	Klaksvík Strond	21. Mars '96	Mussels were collected from rocks on the shore just north of *Strond*, and close to the road construction between *Borðoy* and *Kunoy*.
Limpets Dogwhelks Common mussles	6 a 6 a 6 1	Nólsoy Víkin	5. Mars '96	Samples were taken in the cove, that is, in the middle of the village.
Limpets Dogwhelks	6 b 6 b	Nólsoy Kirkjutangi	5. Mars '96	Samples were taken on the shore just below the church at *Kirkjutangi*, on the tongue north of the cove.

Tab. 1.*Description of sampling sites. Note that in one of the sampling stations, no. 7, only limpits and mussels were collected.*

Talva 1. Støðini har sýnini vórðu tikin. Leggið til merkis, at tað vóru ongir purpurkúvingar tiknir í Skálafirði.

number of countries during the 198's. The restriction was typically to ban the use of such antifouling agents on boats smaller than 25 m. Monitoring in areas where use have been restricted indicate a reduction in environmental concentrations and effects (Evans *et al.*, 1995; Shiraishi and Soma, 1992). In Norway, this has lead to a reduction in the use of organotin containing products from 47 tons (as Sn) in 1985 to 34 tons in 1991 (Fjelldal, 1994). The use of TBT on large ships still represents a threat to sensitive species near harbours and major shipping lanes.

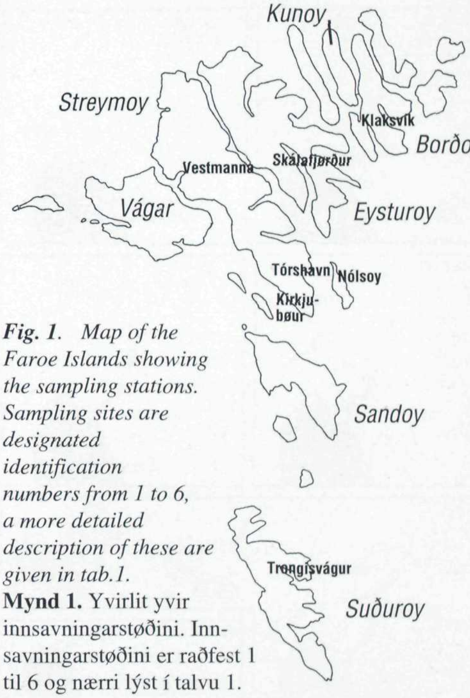

Fig. 1. *Map of the Faroe Islands showing the sampling stations. Sampling sites are designated identification numbers from 1 to 6, a more detailed description of these are given in tab.1.*
Mynd 1. Yvirlit yvir innsavningarstøðini. Innsavningarstøðini er raðfest 1 til 6 og nærri lýst í talvu 1.

Methods

Sampling

Dogwhelks and limpets (*Patella vulgata*) from the intertidal zone and common mussels (*Mytilus edulis*) from shallow water were collected at 7 sampling stations along the coast of the Faroe Islands, tab. 1 and fig. 1, from January to March 1996. Levels of organotin compounds were determined in all three species. When possible, all three species were collected at the same site in order to allow interspecies comparison of organotin levels.

The dogwhelk samples contained 20-70 females (mean size: 32 mm) and males (mean size: 31 mm) respectively.

The analysed samples of common mussels (mean size: 54 mm) and limpets (mean size: 45 mm) contained 30-67 and 20-62 specimens respectively.

Imposex determination

Imposex in dogwhelks was determined according to Gibbs *et al.* (1987). The Relative Penis Size (RPS) Index and the Vas Deferens Sequence (VDS) Index were determined. The RPS index expresses the bulk of the female penis length as a percentage of that of the male and was calculated as follows:

$$\text{RPSI} = [(\text{mean female penis length})^3 \times (\text{mean male penis length})^{-3}] \times 100\ \%$$

VDS describes the development of a female penis and vas deferens and gives an indication on the degree of sterility in a population caused by imposex. The development is characterised by 7 stages, stage 0: normal female, stage 6: genital papilla

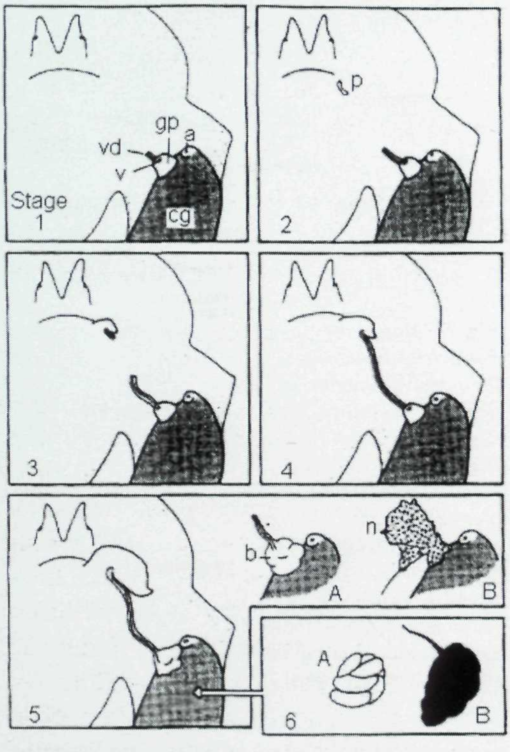

Fig. 2. Stages 1 to 6 in the development of imposex in Nucella lapillus *based on vas deferens sequence (VDS) (Gibbs* et al., *1987). Abbreviations: a, anus; b, 'blister'; cg, capsule gland; gp, genital papilla; n, 'nodule'; p, penis; v, vulva; vd, vas deferens. In stage 6, drawn as an insert to stage 5, aborted egg capsules may be seen around the genital papilla.*

Mynd 2. Myndin vísir tey ymisku stigini, frá 1 til 6, í framvøkstrinum av sáðleiðarum, *vas deferens* hjá kvenndýrum av purpurkúvingum *Nucella lapillus*. Styttingar: a, *anus*; b, 'bløðra'; cg. egghylkiskertil; gp, *genital papilla*; n, 'knubbi'; p, *penis*; v, *vulva*; vd, *vas deferens*. *Vas deferens sequence*, VDS, (Gibbs *et al.*, 1987) lýsir fyribrigdið imposex kvantitativt. Hjá kúvingum, ið hava VDS í stigi 6, víst í myndini sum eitt innskot til stig 5 lýsingina, kunnu aborterað egg síggjast við kynsvørtuna (*genital papilla*).

overgrown, aborted egg capsules can be seen, fig. 2 (insert b). Snails in a VDS stage of 5 or 6 are sterile as they are unable to release egg capsules from the genital papilla. The VDSI of a population is the mean VDS stages determined for the individual females. No less than 20 individuals of each sex was used for imposex determination. The same animals were used for chemical analysis.

Organotin determination

Reagents

Organotin standards were obtained from Alfa-Johnson Matthey GmBH, Aldrich-Chemie, Strem Chemicals (Germany) and Fluka Chemica-Biochemica (Switzerland). Stock solutions of each organotin compound (10 mg Sn mL^{-1}) were prepared by dissolving the standards in n-hexane or methanol. The n-hexane and methanol used throughout the method were HPLC- and p.a. grade obtained from Rathburn Chemicals Ltd. (Scotland) and Merck KGaA (Germany) respectively. The tissue-solubiliser, 25% tetraethylammonium hydroxide (TEAH) in methanol (purum grade), was obtained from Fluka Chemica-Bio-Chemica (Switzerland) and diluted to 10 % in methanol. Sodium tetraethylborate ($NaBEt_4$, min. 98 %) was obtained from Strem Chemicals Inc. (Germany). Batches of 5 g were diluted in methanol to a concentration of 10 % and stored under argon (99.999%, AGA) at +4 °C. Hydrochloric acid, acetic acid, and sodium acetate trihydrate, all of p.a. quality, were obtained from Merck (Germany). Ultra pure water from an Option 3 Water Purifier, ELGA, was used directly without further cleaning for dilution of the acids and the buffer to the proper concentrations. The certified reference fish tissue, NIES No.11, was obtained from the National Institute for Environment Studies, Ibaraki, Japan. All glassware used was cleaned with acetone (Rathburn, glass destilled grade) and heated to 550°C before use.

Sample work-up

1-2 g of the biological samples were added tripentyltinchloride (TPeTCl) as internal standard and mixed with ~2 mL of a 10 % solution of the tissue solubiliser (TEAH in methanol) to free the organotin compounds from the matrix. If necessary, water was added to assure good contact between the sample and the solubiliser. The samples were left in the dark overnight to assure complete sample decomposition. Sample pH was adjusted to pH=5 before the derivatisation by adding a few drops of 1 M HCl and ~2 mL of the acetic buffer (pH=5, 0.5 M HAc). Ar-gas was bubbled through the sample to remove dissolved oxygen. Samples were then derivatised by addition of 0.5 mL aliquots of a 10 % solution of $NaBEt_4$ in methanol and n-hexane was added simultaneously to extract the derivatised, lipofilic species. The samples were shaken thoroughly and left for 10 minutes to complete the derivatisation. To further extract the tetraalkylated organotins the samples were shaken for another 5 minutes and centrifuged for 10 minutes at 3500 rpm. The whole process was repeated once and the combined organic phases were dried with anhydrous Na_2SO_4. Samples with high fat content and extracts with "dark" colour were cleaned by gel permeation chromatography. Before analysis the samples were evaporated with a gentle stream of purified nitrogen gas (99.996 %, AGA) to approximately 100 μL. Blank samples were included in the procedure for every sample series (10 samples) analysed.

Clean-up by gel permeation chromatography

Sample clean-up was performed by gel permeation chromatography. Injections were made by a Waters™ 717 Autosampler (Milford, MA, USA). A Model 510 HPLC pump from Waters was used and the samples were fractionated on two Waters Envirogel TM GPC columns (crosslinked styrene divinylbenzene), 19 x 150 mm and 19 x 300 mm, coupled in series. Detection was made by a Model SPD-6A UV-VIS detector (254 nm) connected to a Model SP 4270 integrator from Spectra-Physics (San Jose, CA, USA). The mobile phase was dichloromethane (Rathburn, Walkerburn, Scotland, HPLC glass destilled grade) at a flow of 5.0 mL/min. The organotin compounds were collected by a Waters Fraction Collector in the fraction from 75 to 115 mL.

Qualitative and quantitative organotin analysis

Analysis of organotin compounds was carried out by means of a HP 5921A atomic emission detector (Hewlett-Packard, Wilmington, DE, USA). The detector was coupled to a HP 5890A gas chromatograph (Hewlett-Packard) equipped with an HP

7673A automatic sampler (Hewlett-Packard) and a split/splitless injector operated in the pulsed flow mode. Injections of 1 µL of the samples were made in the splitless mode at an elevated flow of 20 mL/min. After a splitless period of 60 seconds, the flow was rapidly reduced to a constant flow of 4 mL/min which was maintained for the rest of the run. Helium (99.9999 %, AGA) was used as carrier gas. The samples were routinely analysed on a 30 m x 0.32 mm x 0.25 µm HP-5 (crosslinked 5 % phenyl methyl siloxane) fused silica capillary column (Hewlett-Packard). The column was maintained at 50 °C for 5 min and the temperature was then increased by 15 °C/min to 230 °C. After separation on the capillary column, the analytes were transferred to the detector by a transfer line (HP-5 capillary column) kept at 280 °C. In the detector cavity, also kept at 280 °C, the column effluent was combined with helium (99.9999%, AGA) at 260 mL/min as make-up gas. Hydrogen (99.997 %, AGA) at 60 psi and oxygen (99.999 %, AGA) at 20 psi were used as plasma dopants. Helium (99.9999%, AGA) at 30 mL/min was used as window purge gas. The spectrometer was continuously purged with nitrogen (99.996 %, AGA) at 2 L/min. Emission intensities for tin (271 nm) and carbon (248 nm) were measured by a photodiode array and chromatograms recorded by a HP 35920A GC-AED ChemStation (Hewlett-Packard).

The different organotin species were identified by analysis of pure standard solutions. Quantification was carried out by running a six point calibration curve for every series analysed. An internal standard was used to compensate for losses during sample preparation.

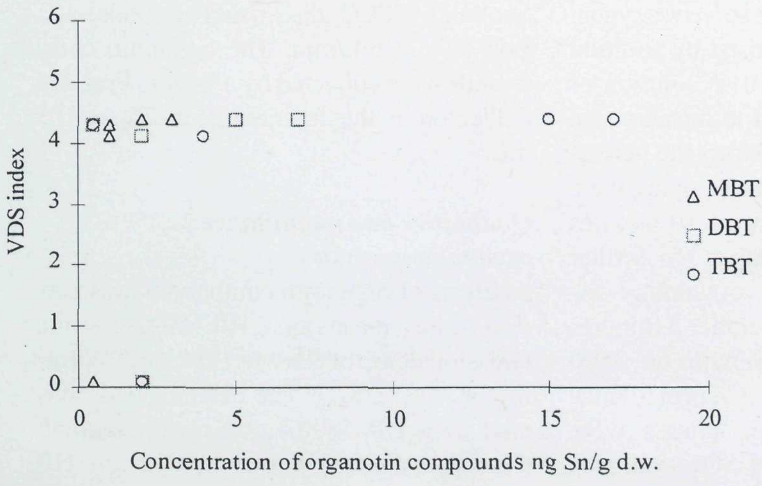

Fig. 3. *Vas deferens sequence index (VDSI) in dogwhelks (Nucella lapillus) plotted as a function of concentrations of organotin compounds in females. Levels below the detection limit (<1 ng Sn g^{-1} d.w.) are presented as 0.5 ng Sn g^{-1} d.w. in the figure.*
Mynd 3. Vas deferens sequence index (VDSI) hjá purpurkúvingum (*Nucella lapillus*) er víst sum funktión av innihaldinum av organotin-evnum í kvenndýrunum. Nøgdir undir ávísingarmark (<1 ng Sn g^{-1} t.e.) eru vístar sum 0.5 ng Sn g^{-1} t.e. í myndini.

Results

Organotin compounds and imposex in dogwhelks

Five out of six dogwhelk populations showed clear signs of imposex with VDSI > 4, tab. 2, and contained sterile females. No sign of imposex was seen in dogwhelks from Kirkjubøur. Kirkjubøur is a small village facing a sound with strong currents promoting a frequent exchange of the water masses. There are no fish farming, no shipyard, and only a few small leisure crafts in the harbour. This station was thus expected to be the least influenced by TBT.

The organotin species detected were monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT), levels ranging from 1 - 17 ng Sn g^{-1} d.w, tab. 2. The relationship between the three species was always TBT > DBT > MBT. The concentration range for TBT found in dogwhelks from the Faroe Islands, tab. 2, was generally lower than found along the Norwegian coast (<7-1096 ng Sn g^{-1} d.w.) (recalculated from Berge *et al.* 1997).

According to Skarphéðinsdóttir *et al.* (1996) summer levels of TBT in dogwhelks are five times the winter levels. Norwegian dogwhelks were generally collected during late summer and autumn while sampling at the Faroe Islands was conducted in late winter. Since the samples from Norway and the Faroe Islands are not collected in the same season, direct comparison of levels of contamination are impossible. If, however, the concentrations of organotin compounds

Station	Nr.	Date	n[b]	% d.w.[c]		L[d] mm		VDSI	s_{VDSI}	RPSI	MBT		DBT		TBT	
				F	M	F	M				F	M	F	M	F	M
Tórshavn																
Argir	1a	03.03.96	25	28	22	32	28	4.4	0.5	32	2	2	5	6	17	16
Kirkjubøur	2	17.02.96	20	27	26	31	30	0.1	0.5	0	<1	<1	2	2	2	4
Vestmanna	3	17.02.96	29	32	27	33	30	4.3	0.5	13	1	<1	<1	2	<1	5
Trongisvágsfjørður																
Kolatoftir	4	31.01.96	20	27	25	34	33	4.4	0.6	31	3	<1	7	4	15	8
Klaksvík																
Kunoy	5	04.03.96	24	32	28	32	32	4.1	0.3	13	1	<1	2	3	4	4
Nólsoy																
Víkin[a]	6a	05.03.96			23		33	n.a.[e]			n.a.	<1	n.a.	<1	n.a.	5
Kirkjutangi	6b	05.03.96	21	32	26	30	30	4.3	0.5	8	<1	<1	<1	<1	<1	<1

[a] Too few females collected to determine imposex-level and organotin concentrations,
[b] n = number of females used in imposex-determination, [c] d.w. = dry weight, [d] L= shell length,
[e] n.a. = not analysed

Tab. 2. Mean vas deferense sequence index (VDSI), relative penis size index (RPSI) and concentrations of organotin compounds in dogwhelks (Nucella lapillus) collected along the coast of the Faroe Islands in 1996. Levels given in ng Sn g^{-1} d.w. F = Females, M = Males, s_{VDSI} = VDSI standard deviation.

Talva 2. Miðal *vas deference sequence index* (VDSI), relativt *penis size index* (RPSI) og innihald av organotin evnum í purpurkúvingum (*Nucella lapillus*) sum vórðu tiknir fram við strendurnar í Føroyum í 1996. Nøgdirnar eru vístar sum ng Sn g^{-1} t.e. (t.e. = turr evni). F = kvenndýr, M = kalldýr, s_{VDSI} = VDSI standard frávik.

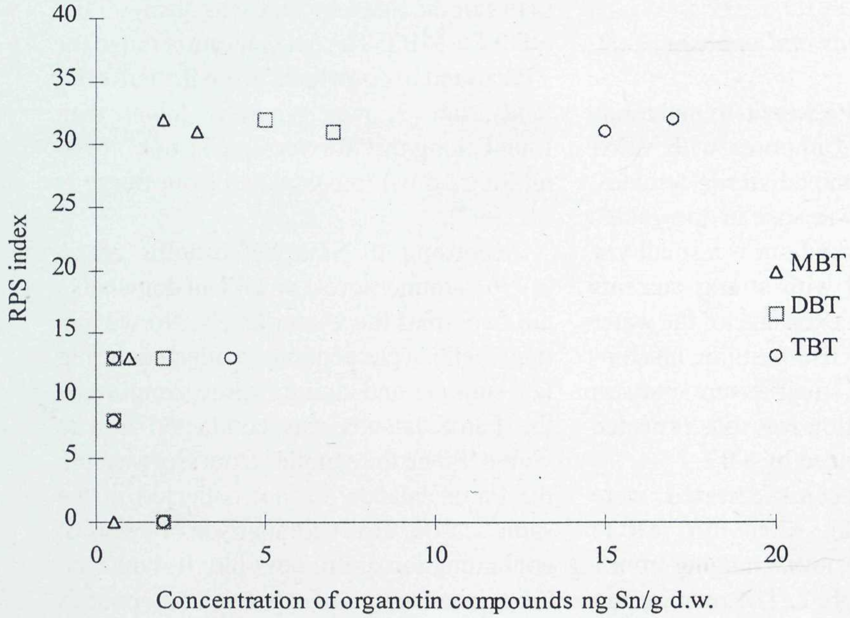

Fig. 4. *Relative penis size index (RPSI) in dogwhelks (Nucella lapillus) plotted as a function of concentrations of organotin compounds in females. Levels below the detection limit (<1 ng Sn g^{-1} d.w.) are presented as 0.5 ng Sn g^{-1} d.w. in the figure.*

Mynd 4. Relativt *penis size index* (RPSI) í purpurkúvingum (*Nucella lapillus*) víst sum funktión av innihaldinum av organotinevnum í kvenndýrunum. Nøgdir undir ávísingarmark (<1 ng Sn g^{-1} t.e.) eru vístar sum 0.5 ng Sn g^{-1} t.e. í myndini.

found in dogwhelks in the Faroe Islands (mean concentration ~6 ng TBT as Sn g^{-1} d.w.) are multiplied with 5, the results indicate a summer concentration of ~50 ng TBT as Sn g^{-1} d.w. This is approximately half of the mean concentrations found in Norwegian dogwhelks (~110 ng TBT as Sn g^{-1} d.w.). The results from the Faroe Islands, however, still indicate widespread effects of organotin contamination.

Concentrations of TBT in females and males from three of the stations were in the same range, tab. 2, and thus are similar to the Norwegian results. The concentration difference between males and females in the remaining populations were 50 % or more.

No clear relationship was found between the VDSI and levels of organotin compounds in the samples, fig. 3. TBT-concentrations in samples with VDSI > 4 range from below detection limits (<1 ng Sn g^{-1} d.w.) to 16.5 ng Sn g^{-1} d.w.

A relationship between the RPSI and organotin concentrations was however indicated, fig. 4. The level of TBT increase with higher values of RPSI, similar to the results obtained for Norwegian dogwhelks (Berge *et al.*, 1997).

Organotin compounds in common mussels

Tributyltin and dibutyltin were detected in all samples of common mussels (*Mytilus edulis*), concentrations of TBT ranging from 49 to 372 ng Sn g^{-1} d.w., tab. 3. These levels are in the lower part of the range determined in common mussels from Norwegian waters (Knutzen *et al.*, 1995).

Seasonal fluctuations in TBT concentrations were observed in mussels from the Oslofjord in Norway (Følsvik, 1997) with minimum concentrations found in early winter (December) and a maximum in early spring (March). The mussels analysed from the Faroe Islands were collected in February and March and should, based on the Norwegian results, be near their maximum. The degree of pollution, indicated by the TBT levels found in mussels from the Faroe Islands, seems thus to be somewhat lower than in Norwegian coastal areas.

At four of the stations, concentrations of organotin compounds were determined in both dogwhelks and common mussels. A plot of the results shows a relation between the concentrations in the two species, fig. 5. *Nucella* may feed on juvenile *Mytilus*, but this is hardly of any relevance in the present study. The primary reason is that the sampling of the two species were normally not done at one and the same spot, hence the mussels were not within the foraging range of the dogwhelks, and secondly, the size of the analyses mussels were larger than expected for prey items of Nucella. The relation between the concentration of TBT in the two species, fig. 5, is therefore probably reflecting the level of TBT contamination in the stations though so, that the common

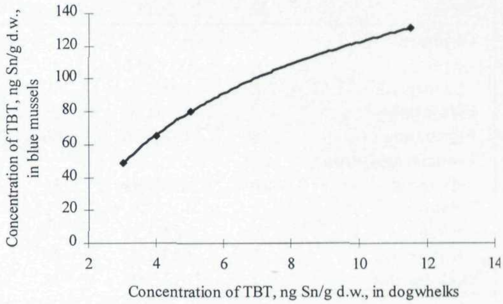

Fig. 5. Concentrations of TBT (ng Sn g^{-1} d.w.) in common mussel (Mytilus edulis) as a function of the concentrations of TBT (ng Sn g^{-1} d.w.) in dogwhelks (Nucella lapillus) from four stations.

Mynd 5. Innihald av tributyltin, TBT, (ng Sn g^{-1} t.e.) í kræklingi (*Mytilus edulis*) er víst sum funktión av innihaldinum av TBT (ng Sn g^{-1} d.w.) í purpurkúvingum (*Nucella lapillus*) á fýra ymiskum støðum.

Station	Nr.	Date	Count	Length, mm	% d.w.	MBT	DBT	TBT
Tórshavn								
Sandagerði	1b	29.02.96	67	40	15	20	72	372
Vestmanna	3	17.02.96	34	73	17	<12	13	49
Trongisvágsfjørður								
Kolatoftir	4a	31.01.96	40	59	16	<12	25	132
Hvítanes	4b	31.01.96	47	59	17	13	23	87
Klaksvík, Strond	5	21.03.96	30	68	18	<12	36	65
Nólsoy, Víkin	6a	05.03.96	65	44	15	<12	72	80
Skálafjørður								
Langanes	7a	01.03.96	42	56	15	<12	26	101
Fish farm "Bakkafrost"	7b	29.02.96	35	52	20	<12	19	100

Tab. 3. Levels of organotin compounds in common mussel (Mytilus edulis) from the Faroe Islands, levels given in ng Sn g^{-1} d.w.

Talva 3. Innihald av organotin evnum í kræklingi (*Mytilus edulis*) úr Føroyum, nøgdir eru vístar sum ng Sn g^{-1} t.e.

Station	Nr.	Date	Count	Length, mm	% d.w.	MBT	DBT	TBT
Tórshavn								
Argir	1a	03.03.96	49	41	14	<9	<9	<9
Sandagerði	1b	29.02.96	62	41	16	54	62	90
Kirkjubøur	2	17.02.96	23	47	14	<9	<9	<9
Vestmanna	3	17.02.96	46	41	17	<9	<9	<9
Trongisvágsfjørður								
Hvítanes	4b	31.01.96	35	52	17	<9	<9	<9
Nólsoy								
Víkin	6a	05.03.96	21	50	17	<9	<9	<9
Kirkjutangi	6b	05.03.96	20	50	16	<9	<9	<9
Skálafjørður	7	01.03.96	31	46	18	<9	<9	<9

Tab. 4. *Concentrations of organotin compounds in limpets (Patella vulgata) from the Faroe Islands, levels given in ng Sn g^{-1} d.w.*
Talva 4. Innihald av organotin evnir í fliðum (*Patella vulgata*) úr Føroyum, nøgdirnar eru vístar sum ng Sn g^{-1} t.e.

mussel carries a higher concentration of TBT, amounting to approximately one order of magnitude higher than in dogwhelks. The higher concentrations in mussels may be explained by the fact that these are filter feeders, but the fact that the mussels were sampled at more sheltered shores where the water exchange is more limited, may also be contributing to the higher build-up of TBT.

Organotin compounds in limpets

Only one of the limpet samples analysed contained detectable levels of organotin compounds. The sample from Tórshavn, Sandagerði (St.1b), contained 54, 62 and 90 ng Sn g^{-1} d.w. of MBT, DBT and TBT respectively, tab. 4. High concentrations of organotin compounds in common mussel was also found at this station, tab. 3.

Conclusion

Effects of TBT, determined as imposex in dogwhelks, were observed at all but one station. At all of the stations showing imposex, sterile females were found (VDSI>4). The concentration of TBT in dogwhelks from the affected populations range from below detection limits (<1 ng Sn g^{-1} d.w.) to 17 ng Sn g^{-1} d.w. while no TBT was found in the unaffected population. A relation was found between the levels of TBT and the degree of imposex (RPSI) in the populations. All common mussel populations investigated contained organotin compounds, concentrations of TBT ranging from 49 to 372 ng Sn g^{-1} d.w. Only one of the limpet populations contained detectable organotin compounds.

Concentrations of organotin compounds found in dogwhelks (*Nucella lapillus*) and common mussels (*Mytilus edulis*) in this investigation clearly demonstrate that organotin compounds are present in most coastal areas of the Faroe Islands, with highest concentrations found near harbours. The main source for the introduction of TBT to the marine environment today is through the use of antifouling paints on ships.

The concentration levels found in mussels, dogwhelks and the biological effects observed in dogwhelks clearly gives cause for further concern about other effects from organotin pollution and possible more widespread damage to smaller and less conspicuous species. The results from the Faroe Islands show that organotin compounds are a widespread problem and posing a threat to sensitive species, at least in coastal areas where shipping traffic intensities are above a certain level and in the vicinity of harbours. Consequently, it must be assumed that significant reduction in the all over occurrence and unintended effects of organotin compounds in the marine environment only can be achieved by enforcing regulations on the use of such compounds.

Acknowledgments

Thanks are due to the volunteers that assisted in sampling the mussels and dogwhelks especially in the Klaksvík area.

This study was supported by the Danish Environmental Protection Agency by grants from the Danish Environmental Support Fund as a part of the national contribution to the Arctic Monitoring and Assessment Programme.

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