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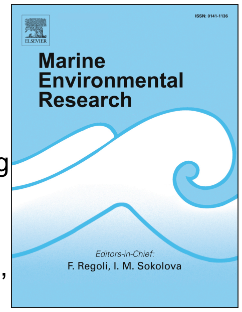

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Levels and trends of tributyltin (TBT) and imposex in dogwhelk (*Nucella lapillus*) along the Norwegian coastline from 1991 to 2017

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Abstract

The banning of organotin biocides, such as tributyltin (TBT), from use in marine antifouling paints is now leading to reproductive health recovery in marine gastropod populations all over the world. TBT induces so-called imposex (superimposition of male sexual characters onto females) in certain marine gastropods, such as the common dogwhelk *Nucella lapillus*. In this publication, the results of the Norwegian TBT and imposex monitoring in *N. lapillus* from the period 1991-2017 are presented. Significantly higher levels of TBT and imposex were measured in coastal areas close to shipping lanes along most of the coast prior to 2008 than afterwards. Levels started declining after restrictions were imposed on the use of TBT in all antifouling paint applications, with a total ban in 2008. In 2017, no sign of imposex was found in *N. lapillus* in any of the monitoring stations along the Norwegian coastline. Based on monitoring data shown herein, the importance of long-term biomonitoring and international chemical regulations, as well as the TBT and imposex story in general, are discussed.

Keywords: Tributyltin, imposex, dogwhelk, long-term monitoring, Norway

1. Introduction

Imposex is an irreversible pseudohemaphrodism disorder in females of certain sea snails caused by the ecotoxic non-target effect of organotin antifoulants, especially tributyltin (TBT) (Bryan et al., 1986). TBT-exposed females can develop non-functioning male sex characteristics such as a vas deferens and a penis like organ. In late stages of development, females may become sterile or even die prematurely causing imposexed snail populations to decline and sometimes go extinct (Matthiessen and Gibbs, 1998).

In the 1950s, organotins were found to be extremely efficient as antifoulant agents in paints used on the hulls of marine vessels, and during the 1960s the use of these marine antifouling paints increased dramatically. The imposex phenomenon was first described in the common dogwhelk, *Nucella lapillus* (Linnaeus, 1758), in the late sixties (Blaber, 1970), but was not linked to organotin toxicity until much later (Smith, 1981). With the link established, increasing demands emerged on phasing out TBT and other organotins from marine antifouling applications.

The imposex condition in marine gastropods is caused by organotins disrupting the endocrine control of the sexual development process, although the research community has not yet reached a consensus regarding the exact underlying mechanisms. Major competing hypotheses cite modulation of different signalling pathways; including neuroendocrine, steroid like, or retinoid (vitamin A) hormonal control systems (Castro et al., 2007; Scott, 2013; Pascoal et al., 2013). It also remains unclear whether several of these signal pathways are involved in the effect. Despite the lack of consensus regarding exact mechanisms, Forbes et al., (2006) suggested that imposex in marine gastropods is one of very few biomarkers that represents a useful predictive tool for ecological risk assessment and environmental monitoring. This evaluation was due to the imposex condition being a very sensitive marker, it has specificity to one group of chemicals (the organotin biocides), the general effect mechanism is reasonably well understood, likely confounding factors have minimal impact, and the effect in individual gastropods is linked to impacts on population dynamics and community structure.

Among the many species investigated for imposex, *N. lapillus* has turned out to be extremely sensitive, with young females developing imposex at TBT concentrations in seawater as low as 1 ng/L in seawater (i.e., 0.001 ppb), and at 10 ng/g for whole-bodyburden (Gibbs et al., 1987). A TBT concentration of 5 ng/L in seawater results in fully sterile *N. lapillus* females and a collapse of the population (Gibbs et al., 1988). Under such conditions other species must be used for monitoring the levels and trends of TBT, and for that both less sensitive gastropods, such as the common periwinkle (*Littorina littorea*) (Matthiessen and Gibbs, 1998), or blue mussels (*Mytilus edulis*) (Beyer et al., 2017), can be suitable options.

N. lapillus are relatively common at wave-exposed areas on hard bottom substrates in the tidal zone. They are carnivorous and predate on barnacles, mussels, and other snails. The typical life span is 5-10 years and they lay their eggs on rocky surfaces in wave exposed tidal zones. The larvae develop directly without any pelagic stage and the larvae dispersal is therefore limited when compared to species with pelagic larvae, i.e. recruitment is vulnerable to the reproduction capacity of the local population.

Important international bodies such as the Oslo-Paris commissions (OSPAR), the European Commission (EC), and the International Maritime Organisation (IMO) have been key players in the international process leading to the global restrictions on TBT use. The initial national bans for TBT based paints for leisure boats and other vessels shorter than 25 m emerged in the late 1980s and early 1990s. In Norway, this regulation was introduced in 1990 and vessels longer than 25 m were included in the ban in 2003. The global ban for all antifouling paint applications came into force in January 2008 (Santillo et al., 2008). TBT is now defined as a priority hazardous substance under the Water Framework Directive and Marine Strategy Framework Directive of the European Union.

In the early 1990s, many coastal nations, including Norway, started monitoring TBT and imposex in coastal waters, and relatively soon after the first TBT bans were established, snail populations in some severely affected areas started to recover. The aim of the present paper is to present the key results of the Norwegian TBT and imposex monitoring programme for the period 1991-2017. The monitoring work was conducted as a part of the Norwegian coastal monitoring program Contaminants in coastal waters of Norway (MILKYS) (Green et al., 2018), the results of which are used for international agreements under the auspices of the United Nations (UN), EC, and OSPAR. Based on the data presented herein, the value of long-term biomonitoring, the efficiency of international chemical regulations, and the TBT and imposex story in general, are discussed.

2. Material and methods

2.1 Biological sampling and imposex assessment

A detailed description of the field work and the monitoring data discussed in this paper is provided in the annual MILKYS reports (Green et al., 2018) as well as in Schøyen et al. (2018). Imposex monitoring under this programme started in 1991. During the first years, the monitoring included only the Vas Deferens Sequence Index (VDSI) parameter, and only two stations Karmsund and Færder, both in maritime active areas, were sampled (Fig. 1). Since 1997, TBT concentration and several parameters associated with imposex (see below) were added. Furthermore, the number of stations monitored annually increased to seven in 2001 and to eight in 2002 (see GPS coordinates in Table S1 in the supplementary data). The imposex assessment methods used in these surveys are described by OSPAR (2003, 2008, 2012), ICES (1996, 1999), Gibbs et al. (1987), Minchin and Davies (1999), and Rial et al. (2018). Briefly, *N. lapillus* were collected during September-October (outside the presumed reproductive season) at low tide from intertidal rocky shores. The snails were shipped alive to the laboratory under clean, humid, dark, and cold (4 °C) conditions, and kept in this way not more than one week before being assessed for imposex. According to Bryan et al., (1988), the metabolic half-life of TBT in *N. lapillus* is 90 days. Hence, it is unlikely that biotransformation of TBT during the few days (not more than one week) of captivity at 4 °C in the dark was a significant problem for the analysis in this study.

The shell length (SL) was measured from apex to the siphon canal using Vernier callipers (accuracy 0.1 mm). SL was used as a proxy for age. Then, the shell was carefully cracked in a vice and the soft tissue parts were gently removed. The mantle was cut and the genital opening exposed. Females were identified by the colour and shape of the capsule gland and the presence of a vagina. If a penis like structure was present, the penis length (PL) was measured to the nearest 0.1 mm by using graph paper and a stereo microscope with a graduated eyepiece. Further examination of all individuals was performed to assess VDSI, as well as the other imposex associated parameters: percentage imposex-affected females (%I)

= (number of imposexed females/total number of females)*100, percentage sterile females (%S) = (number of sterile females/total number of females)*100, mean Female Penis Length (FPL), mean Male Penis Length (MPL), and Relative Penis Size Index (RPSI) = $(FPL)^3/(MPL)^3*100$.

Because VDSI was the key parameter in this monitoring, this parameter is described in greater detail. In female *N. lapillus*, there are seven Vas Deferens Sequence (VDS) stages ranging from stage zero (natural, unaffected) to stage six (maximum imposex effect). At stage one, initial Vas Deferens (VD) development is seen. At stage two, a small penis like structure has appeared and the VD is lengthened. At stage three, the VD is in contact with the penis, which has also lengthened. At stage four, the VD extends from the penis to the genital opening. At stage five, the VD overgrows and obstructs the genital opening, making breeding impossible. Sterility occurs at stages five and six. At stage six, the penis and VD are fully developed, the egg capsules cannot be released, and aborted egg capsules are seen. Snails at stage six often die due to damage caused by this superimposition. In specimens with overgrown genital opening, egg capsules can only be aborted through a crack in the tissue on the backside. Norwegian Institute for Water Research (NIVA) participated in Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring in Europe) lab performance exercises for imposex scoring, with acceptable results.

At least 50 specimens of *N. lapillus* per station were processed and analysed for imposex each year. The VDSI at a station was the average VDS determined for the 50 specimens. Damaged or parasitized snails were excluded from analysis, as recommended by Rodriguez et al. (2009). A difference of about 1 between VDSI values is considered significant (OSPAR, 2008). After imposex assessment, the whole bodies (without operculum) from a minimum of 25 individuals were pooled by sex and station and stored at -20 ºC until chemical analyses of organotins could be conducted.

In addition to the *N. lapillus* samples, blue mussels (*M. edulis*) were collected from several of the monitoring stations (Fig. 3) to serve as supplement biological samples for assessing the TBT contamination level. Details of the processing of pooled mussel samples are provided by Green *et al*. (2018).

2.2 Organotin analysis

The pooled samples of *N. lapillus* and *M. edulis* were analysed for monobutyltin, dibutyltin, tributyltin, and triphenyltin (MBT, DBT, TBT and TPT, respectively). Until 2010 the analyses were performed by NIVA. The method included solvent extraction, derivatization, and detection by gas chromatography-mass spectrometry (GC-MS) as described by Følsvik et al. (1999) and Green et al. (2008). Samples from the period 2010-2015 were analyzed by

Eurofins GfA Lab Service GmbH (Hamburg, Germany) with a method similar as the one described for NIVA. Samples from 2016 and 2017 were analysed at GALAB Laboratories GmbH (Hamburg, Germany), using similar extraction procedures but chemical detection was by gas chromatography-atomic emission detector (GC-AED). All three labs are accredited according to ISO 17025. Quantification of individual organotin components was performed by using the internal standard method and the limit of quantification (LOQ) was set individually for each sample. LOQ varied from 0.2 to 5 µg/kg wet weight (w.w.). Quality assurance of organotin analyses included routine analyses of Standard Reference Materials and in-house reference materials, and all three labs participated in Quasimeme lab performance exercises with acceptable results.

2.3 Data treatment, statistical testing, and classification

Time trends were tested based on the principles applied by OSPAR, i.e. using annual values per station, and constraints put on the data series with regard to data below the LOQ. For blue mussels (usually three samples per station per year), we used median values. However, instead of a purely non-parametric fit of log-transformed values, we performed a linear regression on log-transformed data using an approach similar to logistic regression. This method has higher statistical power than non-parametric fitting and is robust if diagnostic plots are used to check if assumptions are met. The transformation was chosen based on the properties of the data, i.e. data bounded by zero at the lower end. For each station, we used ordinary linear regression of the transformed values versus year:

$$X_i = a + b \cdot Year_i + \varepsilon_i \qquad \text{(eq. 1)}$$

Where X_i is the transformed concentrations/VDSI values, as the following:

$$X_i = \log_e((C_i/K)/(1 - C_i/K) \qquad \text{(eq. 2)}$$

Here C_i is the observed concentration of TBT or VDSI, while K is given by:

$$K = \max(X_i) + L \qquad \text{(eq. 3)}$$

where L is a constant that was chosen (using optimization) in order to minimize the unexplained variation (the sum-of-squares of ε_i) of eq. 1. The predicted value (including the confidence interval) of eq. 1, was then back-transformed to plot a trend line with confidence intervals. Statistical analyses were carried out using R 3.5.0 (R Core Team, 2018). Trend analysis for the entire time series are termed as long-term trends, and trends for the recent 10 years (2008-2017) are termed as short-term trends and represents the post-ban period. The measured imposex VDSI values were compared against OSPARs Background Assessment Criteria (BAC=0.3) (OSPAR, 2005, 2009) and Ecotoxicological Assessment Criteria (EAC=2) (OSPAR, 2013).

3. Results

The key data of the TBT and imposex monitoring program in *N. lapillus* are shown in Table 1 and Fig. 1. Highest TBT contamination signals among all monitoring locations were found at the Karmsund station, with the highest TBT concentration of 366 µg/kg w.w. in 2003. On the other end of the scale, the Varangerfjord reference station showed consistently low TBT contamination signals (Table 1).

Significantly higher VDSI (in the range of 3.0-4.5) were typical for all monitoring stations (apart from Varangerfjord) for the period 1991-2004, the first thirteen years of monitoring. At the Varangerfjord station, the VDSI level was consistently the lowest during this period (range 0-0.29) (Table 1 and Fig. 1). Subsequently, from 2005 on, several of the monitoring stations (Færder, Risør, Mandal, Espevær) started showing lower VDSI signals. A few years later (since 2009), the stations in Karmsund, Espevær, and Svolvær also started showing a clear downward trend for TBT concentrations as well as for VDSI. The Karmsund station, tended to stay slightly higher than the other stations during this period. These downward trends were evident for the remainder of the monitoring period, and in 2017, the VDSI assessment revealed only zero-values from all eight *N. lapillus* populations that were monitored.

A close sigmoidal relationship was found between the log TBT concentration and the VDSI (Fig. 2A), whereas the correlation between log TBT versus RPSI, as well as the VDSI versus RPSI plot, had more of a hockey-stick shape (Fig. 2B and C). The dose-response relationship shown in Fig. 2A suggests the LOEC (Lowest Observable Effect Concentration) for imposex measured by the VDSI parameter was in the level of 5-10 µg TBT/kg w.w. There was a significant statistical correlation between TBT and RPSI (Kendall correlation = 0.68, $P<0.001$), and between VDSI and RPSI (Kendall correlation = 0.89, $P<0.001$). The hockey-stick shape correlation between VDSI and RPSI suggests that the VDSI was a considerably more sensitive method than RPSI. The results indicate that RPSI doesn't respond before the VDSI signal exceeds three (Fig. 2C).

The other imposex associated parameters that were assessed in *N. lapillus* (%I, %S, FPL) (Table 1) provide various possibilities for interpretations of the imposex results at different stations. The %I parameter was a more sensitive imposex effect measure than the female sterility index (%S), the latter was more useful when imposex conditions were severe.

TBT concentrations in pooled samples of *M. edulis* were available from many monitoring stations along the Norwegian coast, including several of the *N. lapillus* monitoring stations, albeit not for the whole monitoring period (Fig. 3 and Table S2). The TBT data in mussels provide a quality control tool for the observed TBT contamination trends in the sea snails. As for the *N. lapillus* samples, *M. edulis* showed a significant downward long- and short-term trend in the TBT concentrations at all stations that were assessed. TBT concentrations in *N. lapillus* and *M. edulis* were strongly statistically correlated (linear regression, $P < 0.001$; Fig. 4). The TBT concentrations in *N. lapillus* appeared to be about twice the concentrations of *M. edulis* when the concentrations in the blue mussel were high (i.e. >10 µg/kg w.w.).

4. Discussion

Norway has the longest coastline of all European countries and borders a large part of the eastern Atlantic Ocean. The monitoring data presented herein suggest that imposex was almost ubiquitous in Norwegian *N. lapillus* populations from the period before 2004, with only the remote northernmost station (Varangerfjord) showing no impact of TBT. But the situation changed around 2004, and the levels of TBT and imposex started to decrease at most of the monitored sites. From 2009 on, the downward trends became even more evident. In 2017, the impact of TBT on imposex was no longer present at any of the monitored populations of *N. lapillus*. The apparent turning points in 2004 and 2009 corresponded well with the national and global implementations of stricter TBT regulations in 2003 and 2008 for large commercial vessels. The observed decline of TBT and imposex levels in *N. lapillus* along the Norwegian coast agree with related coastal monitoring studies in many other countries around the globe; such as Ireland (Wilson et al., 2015), England and Wales (Nicolaus and Barry, 2015), Italy (Cacciatore et al., 2018), Portugal (Laranjeiro et al., 2018), Canada (Titley-O'Neal et al., 2011), South Korea (Kim et al., 2017), and New Zealand (Jones and Ross, 2018). Most of all, these trends demonstrate that the bioavailable TBT in coastal waters has decreased significantly, especially for the species that primarily are exposed via the water phase. However, the impact of TBT will remain an issue of concern for some time to come, because the biodegradation of TBT is very slow, and high concentrations of TBT are found in surficial sediments of marinas, harbours, ship yards, maritime lanes, and other areas where there has been intense shipping activity. This also means that the mud snail (*Nassarius* spp.), or other sediment associated gastropods such as the whelks (e.g. *Buccinum* spp. and *Neptunea* spp.), probably are more suitable than *N. lapillus* as sentinels for a continued biomonitoring of TBT and imposex in coastal areas.

In some countries, the decrease of TBT and imposex in marine gastropod populations started much earlier than is seen from the Norwegian monitoring programme. For example, in the UK, a marked decrease of TBT and imposex in *N. lapillus* populations was already observed in the early and mid-1990s (Evans et al. (1991; 1995; 1996; 1998)). One possible explanation for this discrepancy in timing could be that these “early-responding” snail populations were primarily exposed to organotins originating from leisure boat hull coatings. The first TBT restrictions concerned applications in paints for leisure boats and vessels shorter than 25 m in length, which were introduced in the mid-1980s in the UK and even earlier in countries like France (Santillo et al., 2008). In contrast, *N. lapillus* was not sampled near marinas in the present monitoring program. Hence, the late response reported in our study indicate that our stations were probably more impacted by large commercial vessels where restrictions were imposed later in 2003 and 2008.

N. lapillus samples from the Karmsund strait showed the highest TBT contamination and imposex signals in the present monitoring program. The Karmsund strait is about 30 km long and only a few hundred meters wide, and located between the island Karmøy and the mainland south of the city of Haugesund at the west coast of Norway. The strait is a very busy maritime transport route. In 2017, more than 11000 commercial vessels larger than 1000 tonnes gross tonnage (GT) sailed through the Karmsund strait (source: The Norwegian Coastal Administration, www.havbase.no). It is therefore not surprising that this monitoring station showed the largest TBT contamination and highest level of imposex in our programme. In 2008, 1800 µg TBT-ion/kg was measured in sediments 1 km away from the monitoring station in this study (Håvardstun, 2009). Several other studies have investigated imposex in *N. lapillus* from Karmsund, i.e. (Harding et al., 1992; Følsvik et al., 1999; Evans et al., 2000; Birchenough et al., 2002; Plejdrup et al., 2006), and those studies corroborate to a large degree our findings of high TBT concentrations (366 µg/kg w.w. in 2003) and high imposex effect levels in the Karmsund area (VDSI = 4.5 in 2002, RPSI = 0.61 in 2003, FPL = 3.22 in 2000 and %I = 46.4 in 2000). Hence, the Karmsund strait has indeed been an excellent site for providing documentation of long-term trends regarding bioavailable TBT and imposex in *N. lapillus* from Norwegian coastal waters.

This study found quite similar TBT concentrations in *N. lapillus* and *M. edulis*, which is interesting given the different trophic level and feeding strategies of the two species; *M. edulis* filter feeding on phytoplankton (mainly) and *N. lapillus* being carnivorous, and often predating on blue mussels (Hunt and Scheibling, 1998). Bryan et al. (1993) studied the various routes of uptake of TBT in *N. lapillus* and concluded that both uptake of dissolved TBT across gills and mantle, and the uptake via the digestive process, were important. To employ mussels as a proxy for *N. lapillus* for assessment and monitoring of TBT in coastal waters can be useful, especially in situations when *N. lapillus* is not present in suitable numbers or has become extinct due to prolonged TBT stress. In such situations, *M. edulis* is also very convenient to use for controlled field studies employing the technique of transplant caging (Schøyen et al., 2017), although several studies have successfully used *N. lapillus* as sentinel for such caging studies (Quintela et al., 2000; Smith et al., 2006; Giltrap et al., 2009).

The results and trends in the Norwegian TBT and imposex monitoring programme are in good agreement with the key trends seen in similar monitoring elsewhere all around the world, i.e., a clear downward trend of TBT and imposex in marine gastropod populations after the international bans on TBT based antifouling paint applications were implemented. The story of TBT and imposex is important. It shows how the discovery of an unusual ecotoxicological cause-effect relationship can lead to important and wide-ranging changes within a major international sector, such as the field of maritime transportation. It also shows how the dedicated efforts from many knowledgeable/experienced contributors in research, regulation, and industry, at national and international levels, and over a period of many years, can finally show results. The outcome of which was the development of regulations and restrictions that finally lead to removal of the ecotoxic stressor, in this case TBT and other organotin antifoulants. However, the job is not necessarily over as also substitute antifoulants may pose possible risks to aquatic ecosystems (Martins et al., 2018). The many advances today regarding marine antifouling coating technology include introduction of new booster biocide antifoulants generally based on copper or organic biocides, and many other biocides have also been proposed (Amara et al., 2018).

Furthermore, the long-term monitoring of TBT and imposex has been crucial for evaluating the efficiency of the policy-oriented management of TBT-based paints and for demonstrating the recovery of sentinel gastropod populations all around the world. In times of increasing anthropogenic pressure and mounting destruction of sensitive ecosystems, it is fortunate to see evidence, as shown in this study, that good policy can be implemented for solutions to problems forced upon nature.

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Supplementary data

Supplementary data associated with this article can be found in the online version.

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Legends of tables and figures

Table 1. TBT concentrations (µg/kg w.w.) and imposex associated parameters (VDSI, RPSI, %I, %S and FPL) in *N. lapillus* for the period 1991-2017 from the Norwegian TBT and imposex monitoring programme. VDSI values below the OSPAR's Background Assessment Criteria (BAC=0.3) are shaded light grey, VDSI over BAC but below the OSPAR's Ecotoxicological Assessment Criteria (EAC=2) are shaded medium grey, and VDSI over EAC are shaded dark grey.

Fig. 1. Graphs showing trends of imposex (VDSI) and TBT concentrations (µg TBT/kg w.w.) in female *N. lapillus* from monitoring stations along the Norwegian coast for the period 1991-2017. VDSI graphs show annual levels and time trends (lines) with 95 % confidence interval. TBT graphs show annual concentrations (triangles) and time trends (lines). Trends lines are based on linear regression of transformed values (see Material and methods for details). Vertical red lines indicate the key time points (1990, 2003 and 2008) for establishing TBT bans in Norway (explained in text).

Fig. 2. Correlations in female *N. lapillus* at all stations for all years of (A) VDSI and TBT (µg TBT/kg w.w.) (Kendall correlation = 0.68, $P<0.001$), (B) RPSI and TBT (Kendall correlation = 0.68, $P<0.001$), and (C) VDSI and RPSI (Kendall correlation = 0.89, $P<0.001$). The 95 % confidence interval is shaded.

Fig. 3. Graphs showing trends of TBT concentrations (µg TBT/kg w.w.) in *M. edulis*, i.e. annual median and time trends (lines) with 95 % confidence interval using OSPAR methods. Non-linear regression lines are fitted using linear regression of transformed values (see Material and methods for details). Vertical red lines indicate the key time points (1990, 2003 and 2008) for establishing TBT bans in Norway (explained in text).

Fig. 4. Correlations of TBT concentrations (µg/kg w.w.) in female *N. lapillus* and *M. edulis* (medians). Each dot shows the correlation of values per station and year. Red dots indicate years when the median *M. edulis* concentrations were below LOQ. The dashed line is the linear regression between the two matrices, while the unbroken line is the 1:1 relation between the y and x axis (i.e. the species have identical concentration).

Table 1

Parameter	Station	1991	1993	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
TBT (µg/kg w.w.)	Varangerfjord								4.9	9.5	3.6	5.7	<5.0	3.9	<1.0	<1.7	<0.3	<0.3	<0.9	<0.7	1.7	<0.9	<0.3	<0.5
	Lofoten							9.5	22.4	22.0	17.0	13.0	5.3	8.1	5.6	1.7	2.3	0.3	1.0	1.6	1.1	1.1	<0.3	1.1
	Espevær							26.8	39.0	119.5	68.9	43.0	8.9	22.0	22.0	3.9	2.0	1.4	2.1	2.4	2.0	2.1	<0.3	0.8
	Karmsund			34.6	199.8	112.2	143.9	51.2	180.5	365.9	97.1	79.0	26.0	48.0	67.0	12.0	10.6	3.8	6.3	9.9	6.1	6.3	<0.3	2.3
	Farsund							26.8	28.3	23.9	9.5	7.1	5.0	2.6	5.0	2.0	1.0	0.3	1.0	0.8	0.8	1.2	<0.3	<0.5
	Mandal							11.7	19.8	26.8	16.0	6.6	6.6	2.2	6.6	1.7	1.3	0.3	1.0	0.9	1.1	1.0	<0.3	<0.5
	Risør							14.2	63.4	39.0	22.0	14.0	5.6	9.7	4.4	2.9	3.6	0.3	1.3	1.7	2.0	1.2	<0.3	<9.8*
	Færder			45.5	62.8	43.9	36.6	19.3	51.2	34.2	14.5	11.0	5.0	8.5	11.0	3.9	2.5	1.2	2.3	1.7	1.8	1.1	<0.3	1.3
Imposex VDSI	Varangerfjord								0.03	0	0.29	0	0	0.03	0	0	0	0	0	0	0.03	0	0.04	0
	Lofoten							3.50	3.76	3.80	4.00	3.43	2.97	2.95	1.88	3.03	1.12	0.65	0.33	0.46	0.03	0.08	0	0
	Espevær							4.00	4.00	3.95	4.00	4.00	2.96	2.41	1.41	1.58	0.13	0.52	0.07	0.00	0.08	0.04	0	0
	Karmsund	4.1		4.00	4.15	4.09	4.50	4.30	4.50	4.13	3.92	3.65	3.66	3.52	3.67	2.32	0.64	1.96	1.19	0.53	0.45	0.83	1.91	0
	Farsund							3.69	3.86	3.42	3.43	1.28	0.13	0	0.13	0	0	0	0	0	0	0	0	0
	Mandal							3.89	3.77	3.47	3.63	1.86	1.08	0.12	0	0	0	0.05	0	0.03	0	0	0	0
	Risør							3.41	3.03	3.50	3.28	0.64	0.78	0.07	0.13	0	0	0	0	0	0	0.06	0	0
	Færder	4.1	3.9	4.00	4.00	4.00	4.00	3.95	4.00	3.96	3.65	0.96	0.13	0.58	0.24	0.22	0	0	0	0	0	0	0	0
Imposex RPSI	Varangerfjord								0	0	<0.01	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lofoten							0.05	0.04	0.04	0.06	0.01	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0	<0.01	0	0
	Espevær						0.04	0.19	0.30	0.42	0.15	0.13	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0
	Karmsund			0.72	0.44	0.77	0.59	0.51	0.60	0.61	0.32	0.09	0.12	0.07	0.13	0.00	<0.01	<0.01	<0.01	<0.01	0	<0.01	0	0
	Farsund							0.05	0.07	0.04	0.01	<0.01	0	0	<0.01	0	0	0	0	0	0	0	0	0
	Mandal							0.07	0.03	0.05	0.02	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0	0	0
	Risør							0.01	0.02	0.03	0.02	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	<0.01	0	0
	Færder			0.14	0.10	0.13	0.13	0.08	0.15	0.09	0.04	<0.01	0	<0.01	0	0	0	0	0	0	0	0	0	0

Parameter	Station	1991	1993	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
%I	Varangerfjord								3.3	0	21.1	0	0	3.4	0	0	0	0	0	0	3.1	0	3.6	0
	Lofoten							95.5	100	96	100	100	93.1	90.9	64.7	84.4	48	25	13.9	21.4	3.4	2.6	0	0
	Espevær						100	100	100	100	100	100	100	100	79.4	76.9	9.4	33.3	6.7	0	8	3.6	0	0
	Karmsund			100	100	100	100	100	100	100	100	100	100	100	100	88	36.4	87.5	64.5	28.1	37.9	37.9	85.3	0
	Farsund							100	100	100	100	72.4	12.5	0	3.1	0	0	0	0	0	0	0	0	0
	Mandal							100	100	100	100	97.1	48	2.9	0	0	0	4.8	3.1	3	0	0	0	0
	Risør							100	100	100	100	50	48.1	3.3	4.3	0	0	0	0	0	0	3	0	0
	Færder			100	100	100	100	100	100	100	100	68	12.5	41.7	16	21.7	0	0	0	0	0	0	0	0
%S	Varangerfjord								0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lofoten							0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Espevær						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Karmsund			0	11.1	4.5	46.4	21.2	37.5	11.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Farsund							0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mandal							0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Risør							0	0	0	3.1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Færder			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FPL	Varangerfjord								0	0	<0.01	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lofoten							1.46	0.82	1.16	1.23	0.91	0.77	1.11	0.36	0.46	0.29	0.17	0.07	0.09	0	0.01	0	0
	Espevær						1.34	1.8	2.28	2.95	1.55	1.56	0.65	0.33	0.08	0.15	0.02	0.06	0	0	0	0	0	0
	Karmsund			3	2.86	3.12	3.22	2.82	2.94	3.05	2.18	1.44	1.39	1.22	1.38	0.46	0.12	0.37	0.20	0.05	0	0.14	0	0
	Farsund							1.12	1.36	0.95	0.89	0.07	0	0	0.01	0	0	0	0	0	0	0	0	0
	Mandal							1.13	0.94	0.71	0.78	0.17	0.13	0.04	0	0	0	0	0	0	0	0	0	0
	Risør							0.80	0.94	1.15	1.06	0.03	0.11	0.02	0.01	0	0	0	0	0	0	0.02	0	0
	Færder			2.44	1.87	2.38	2.35	1.92	2.27	2.00	1.39	0.05	0	0.05	0	0	0	0	0	0	0	0	0	0

*Matrix interferences.

09 Figure 1

10

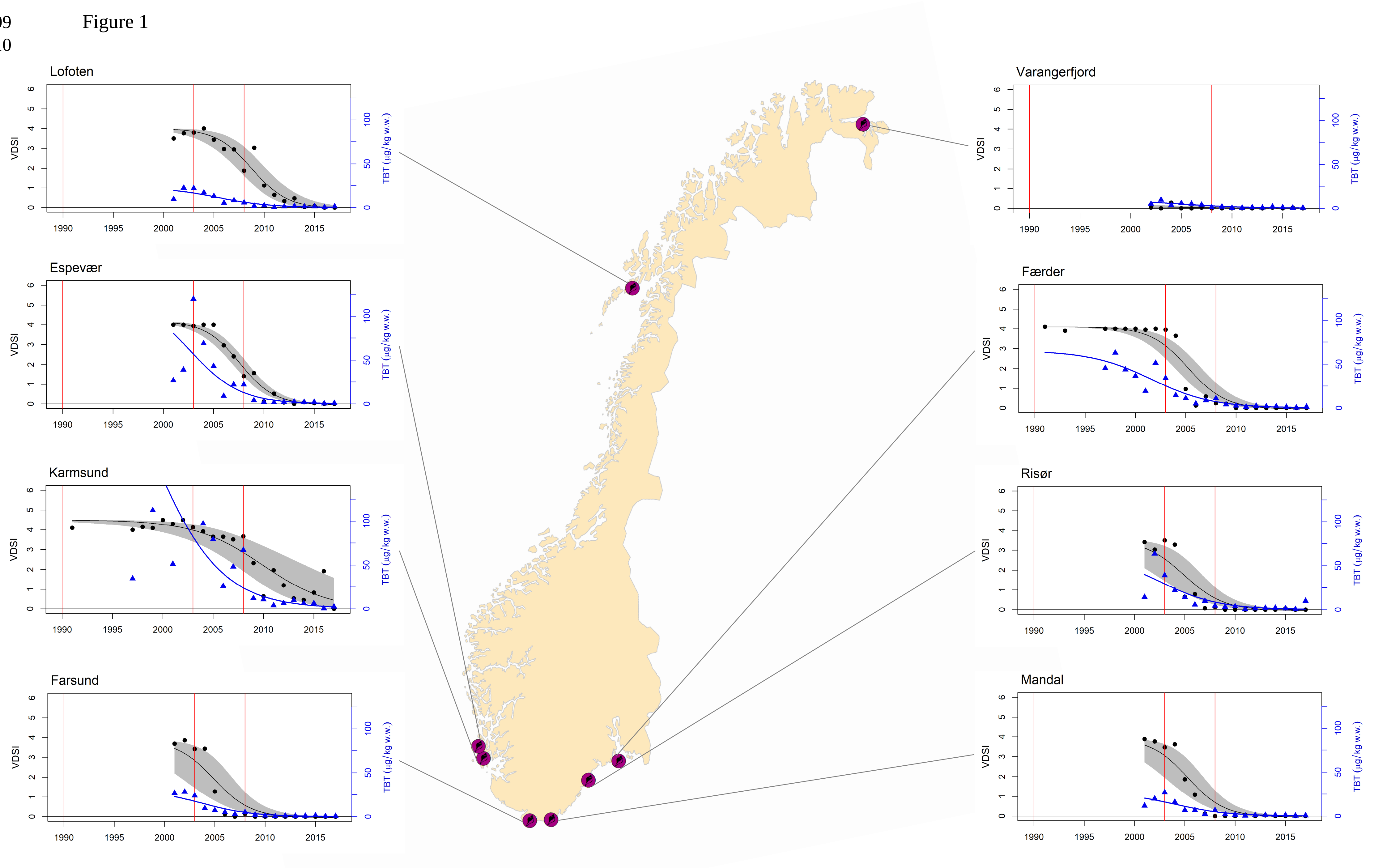
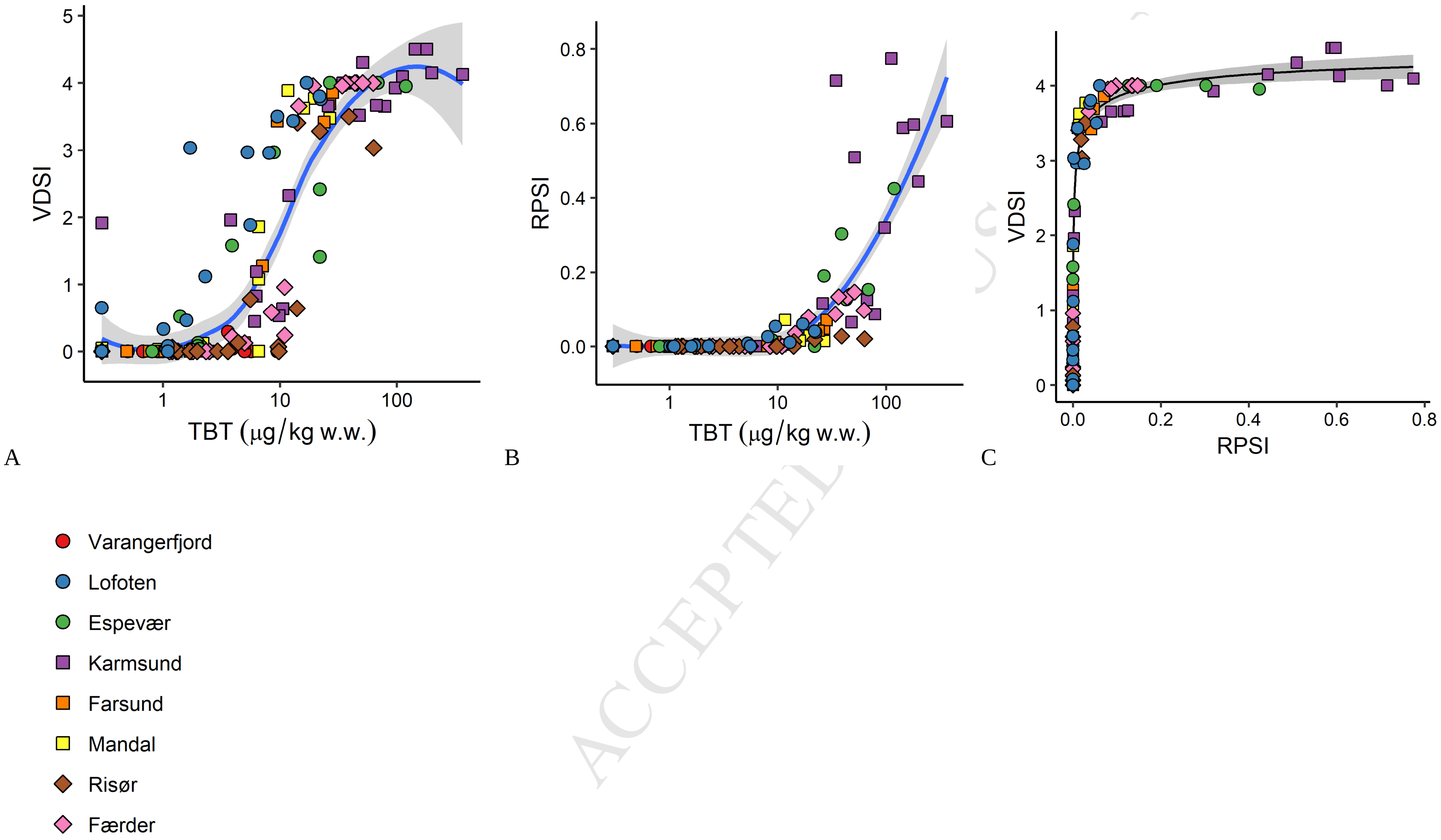


Figure 2


VDSI
5
4
3
2
1
0
1
10
100
TBT (µg/kg w.w.)
A
RPSI
0.8
0.6
0.4
0.2
0.0
1
10
100
TBT (µg/kg w.w.)
B
VDSI
4
3
2
1
0
0.0
0.2
0.4
0.6
0.8
RPSI
C
Varangerfjord
Lofoten
Espevær
Karmsund
Farsund
Mandal
Risør
Færder

16 Figure 3
17

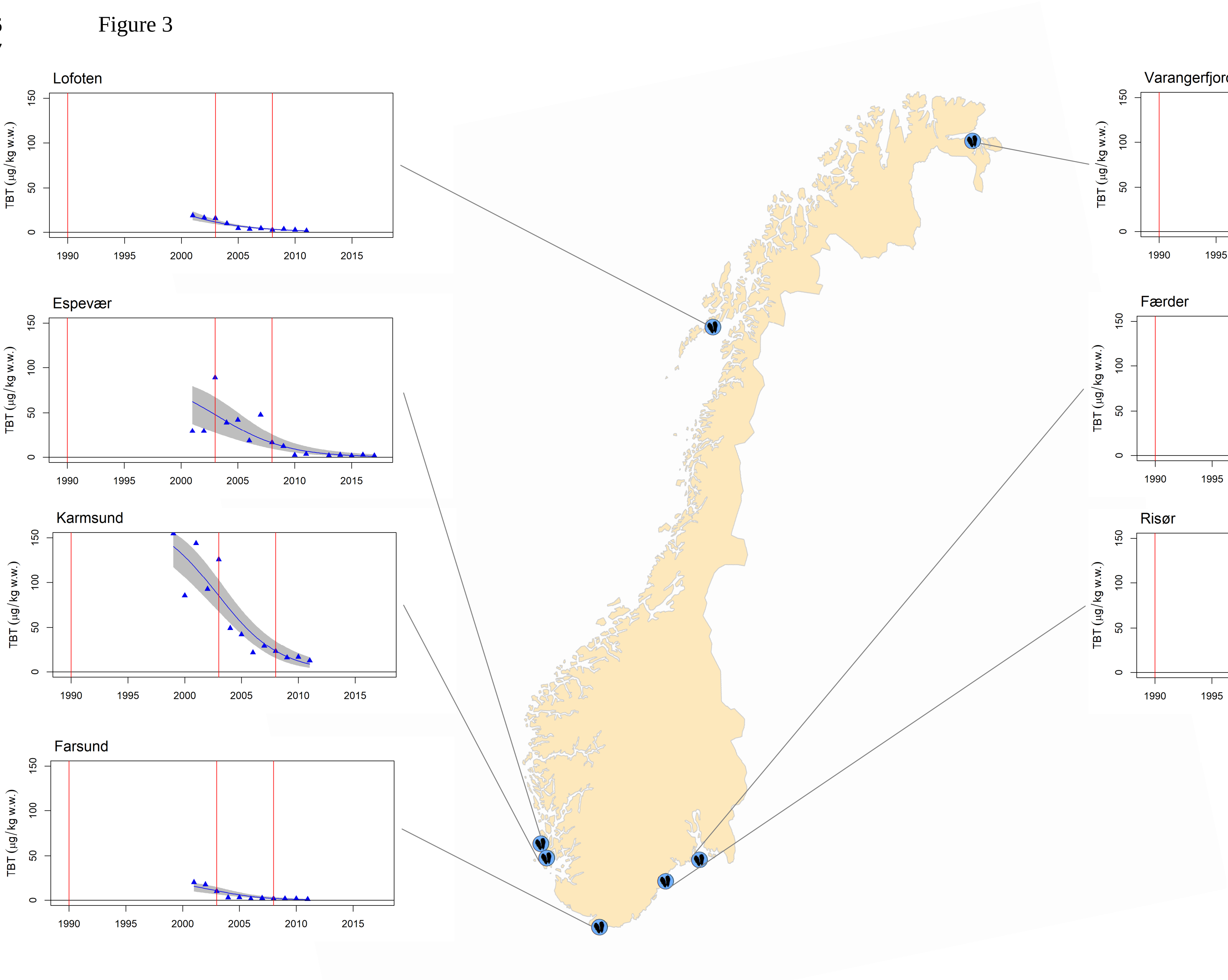
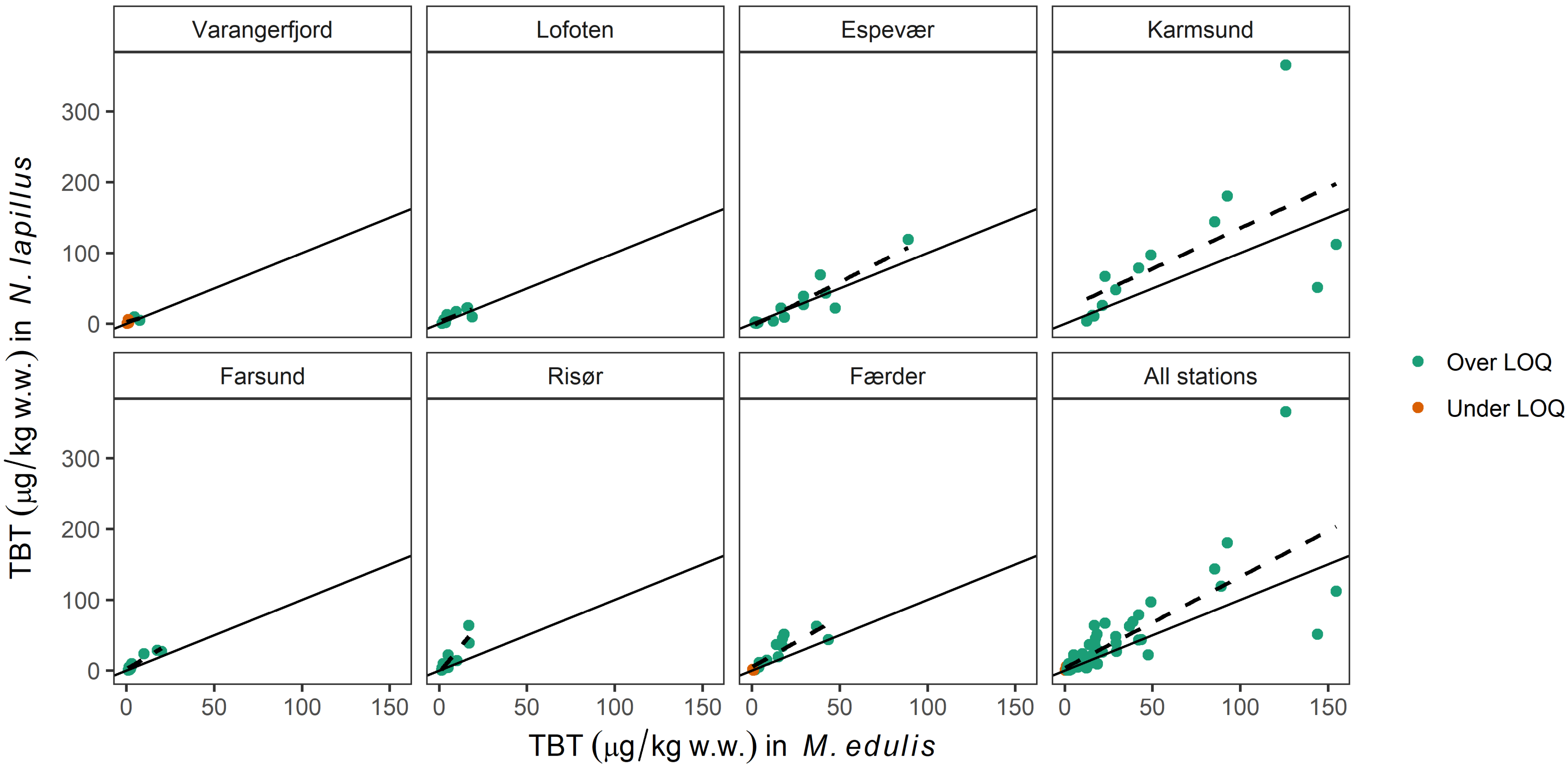


Figure 4


Varangerfjord
Lofoten
Espevær
Karmsund
Farsund
Risør
Færder
All stations
300
200
100
0
0
50
100
150
TBT (µg/kg w.w.) in N. lapillus
TBT (µg/kg w.w.) in M. edulis
Over LOQ
Under LOQ

Highlights

- TBT and imposex (VDSI) in dogwhelk (*Nucella lapillus*) declined along the Norwegian coast after the introduction of global bans of organotin antifoulant paints.
- In 2017, imposex was not detected at any of the eight monitoring stations.
- TBT concentrations in *N. lapillus* and blue mussel (*Mytilus edulis*) were significantly correlated.
- The long-term biomonitoring data shown herein demonstrate the value of international collaboration on chemical regulations and restrictions.