

USE OF *NUCELLA LAPILLUS* L. AS BIOINDICATOR OF TBT POLLUTION IN MARINE WATERS OF GALICIA (NORTHWEST IBERIAN PENINSULA, SPAIN)

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In a rare example of long-term biomonitoring, the presence and effects of **tributyltin (TBT)** along Galician shores has been regularly surveyed for **14 years** using imposex in *Nucella lapillus* as biomarker.

TBT is a hazardous chemical used as biocide in antifouling paints for boats and submerged structures. In Europe, TBT paints were banned in the mid 2000s due to its harmful effects as an endocrine disruptor.

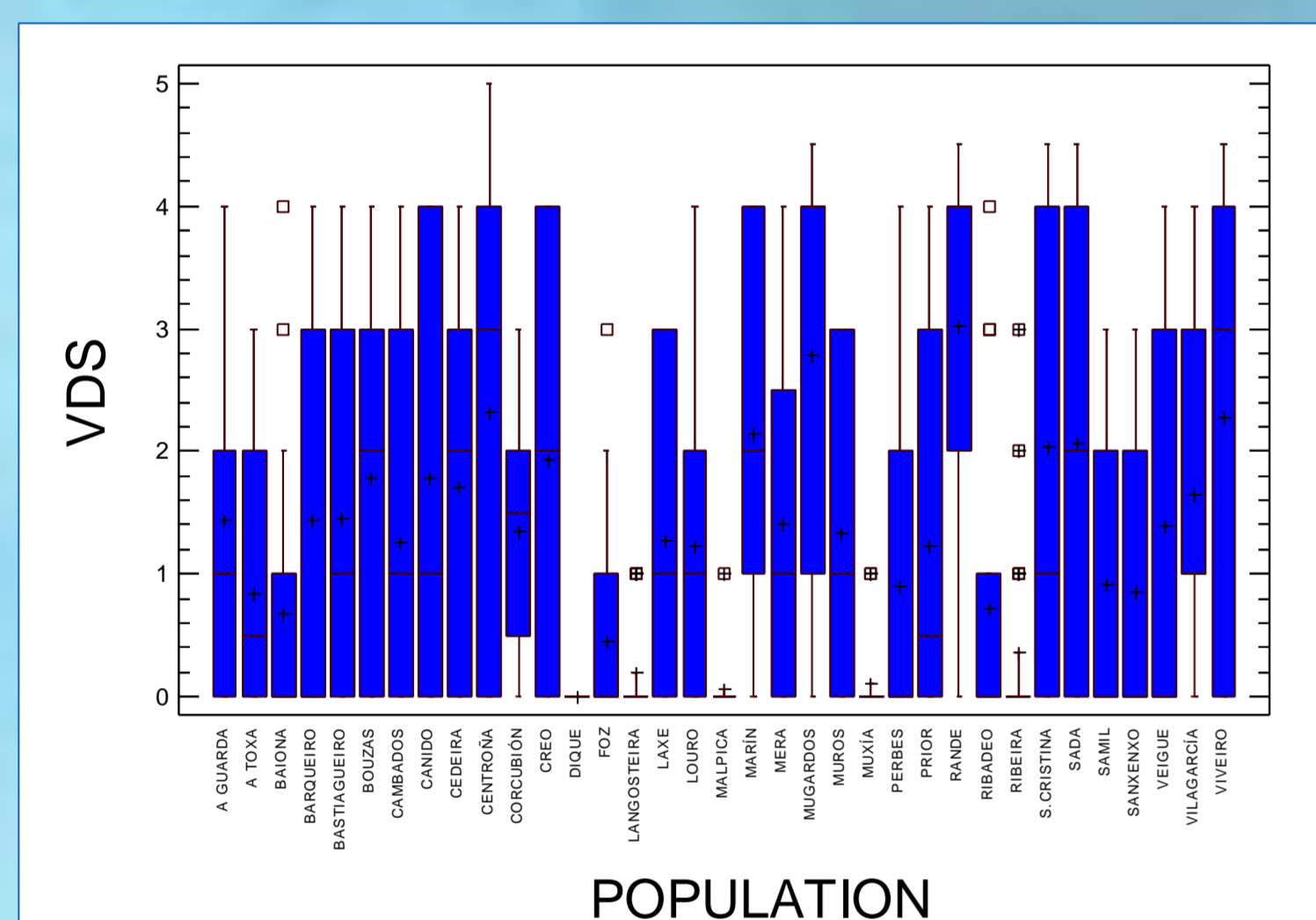
Imposex identifies the superimposition of male characters into females of marine gastropods induced by exposure to TBT. Imposex is a graded phenomenon. In some species (e.g. *N. lapillus*), it can lead to females sterility and, consequently, population extinction.

N. lapillus is a marine dioecious gastropod widely distributed along European intertidal rocky shores. This whelk is particularly sensitive to TBT (imposex appears at exposure levels in the range of ppt). Because of its dose-dependency, imposex in *N. lapillus* has been officially endorsed as a biomarker of TBT exposure and effects (Oslo-Paris Commission).

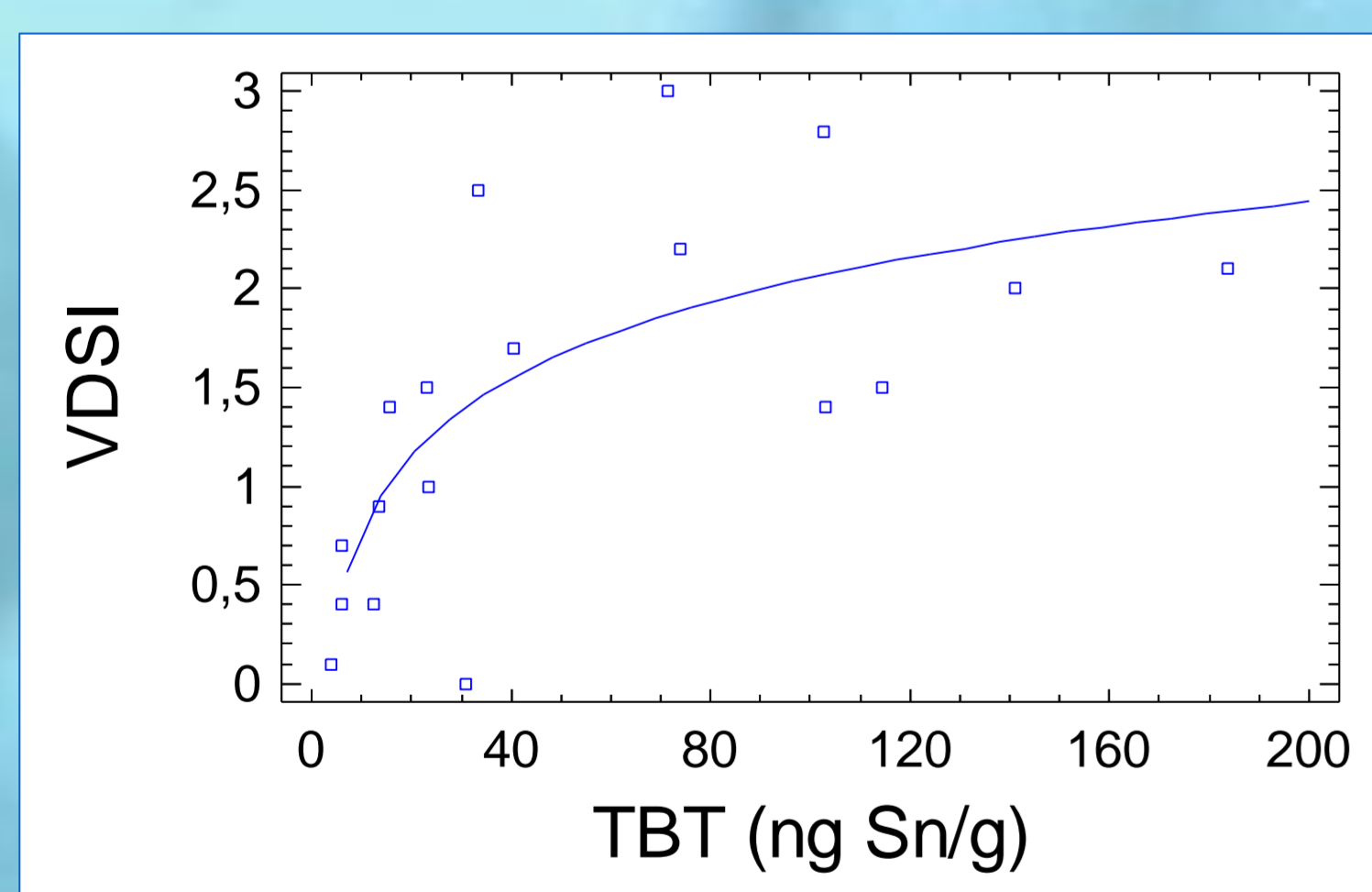
VDSI and RPSI: two indexes widely used to assess imposex

The *vas deferens* sequence index (VDSI) follows a semi-quantitative scale from 0 (healthy) to 6 (sterile). VDSI describes the development of the male duct (*vas deferens*) in females.

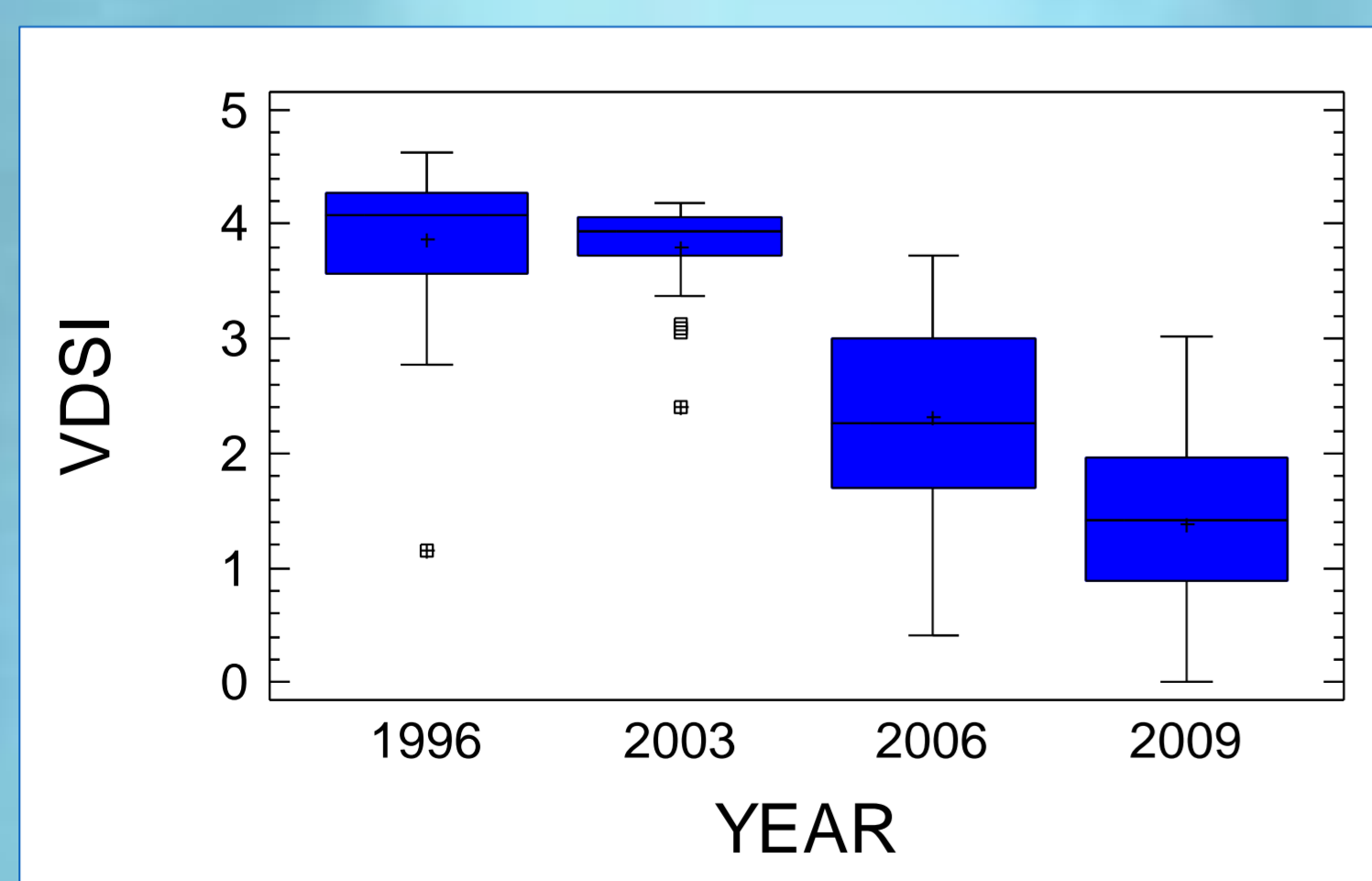
RPSI quantifies (as %) penis size in females relative to those of the males ("normal" females do not have a penis).



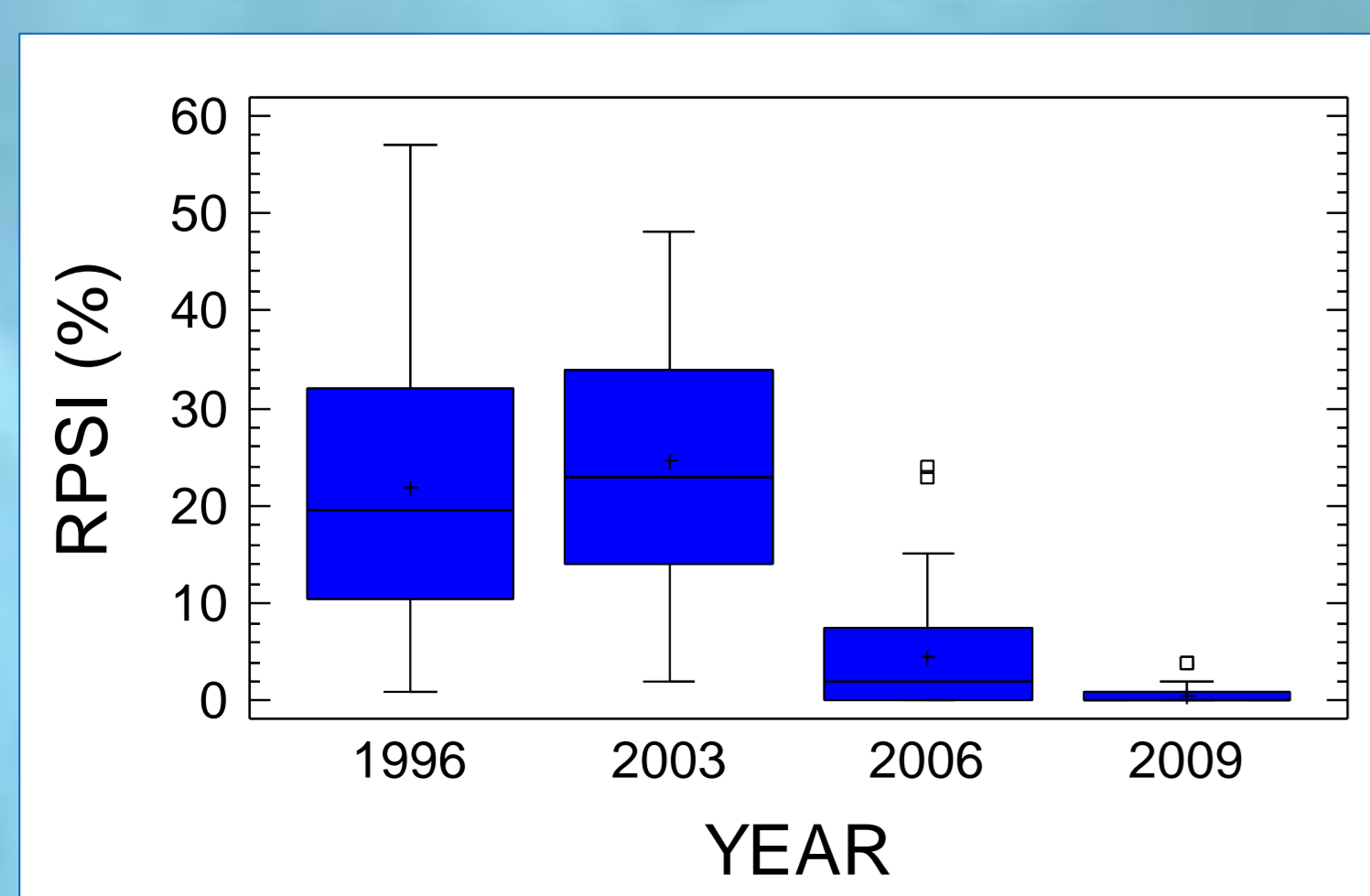
Example of biomonitoring results. Box plot of VDSI values in 35 populations surveyed in **2009** (6 years after a total ban on TBT paints was implemented). Only three populations showed a 50% of females with VDS values ≥ 3 . In 5 populations, most of the females did not show any imposex. **For the first time in 14 years, one population (Digue) was totally free from imposex.**



In 2009, **VDSI was logarithmically related to TBT contents** in female tissues ($VDSI = 0.558 \ln(TBT \text{ in ng-Sn/g}) - 0.514$, $r^2=53$, $p<0.01$). The correlation was likewise significant with TBT derivatives (i.e. DBT, MBT). By contrast, RPSI was only slightly correlated to TBT ($p<0.1$), stressing the need to review the usefulness of this index when ambient TBT is low.



Box plot of the **temporal trend of VDSI in Galicia along 14 years**. After 2003, VDSI experienced a clear decrease. While 50% of the populations showed VDSI >4 in 1996, 50% of the populations had VDSI <1.5 in 2009 (the highest VDSI value was 3 in 2009).

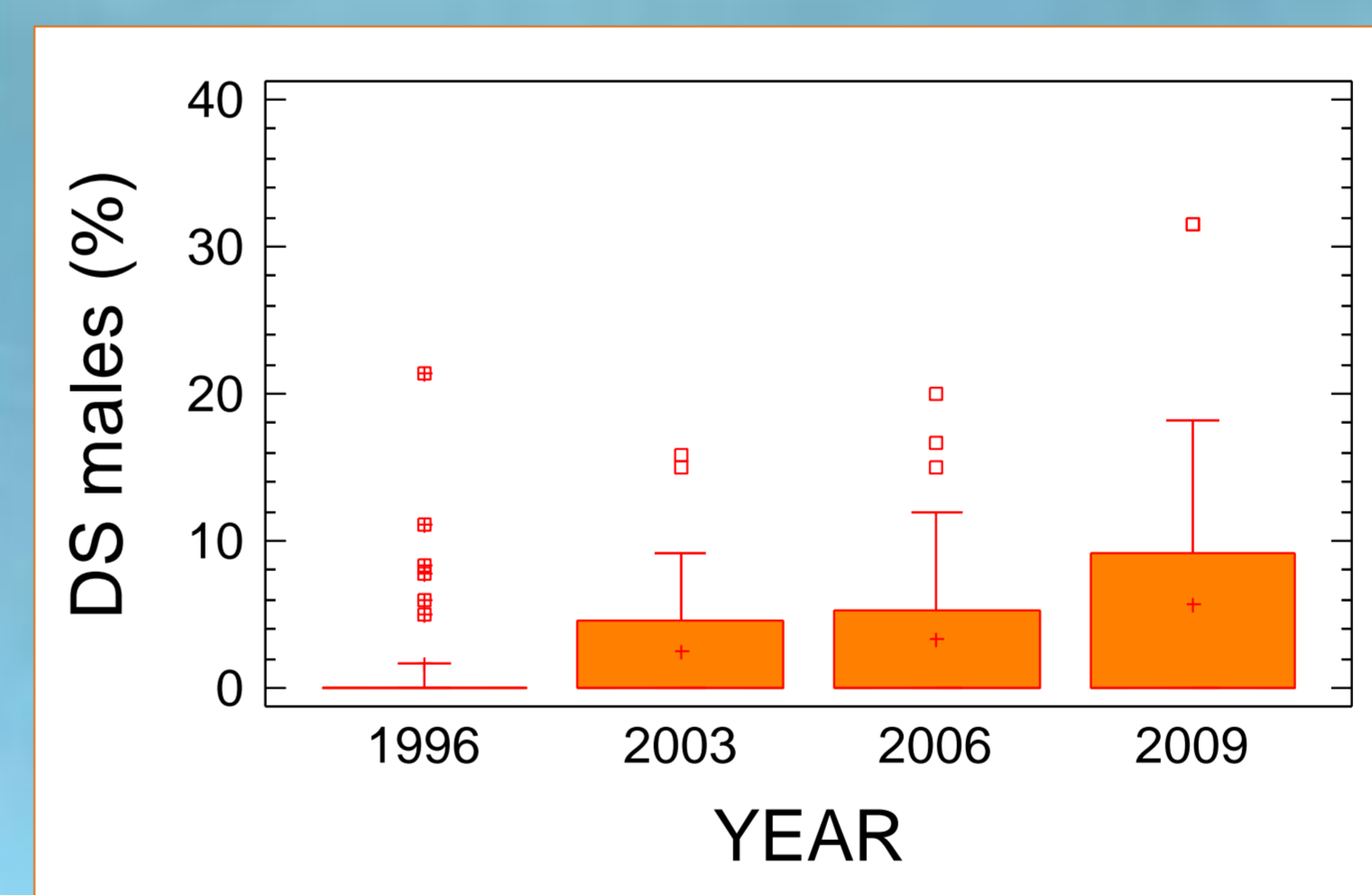


Box plot of the **temporal trend of RPSI in Galicia along 14 years**. Again, a clear drop is noticeable after 2003. RPSI reached values as high as 60% in 1996 while the maximum value recorded in 2009 was 4.

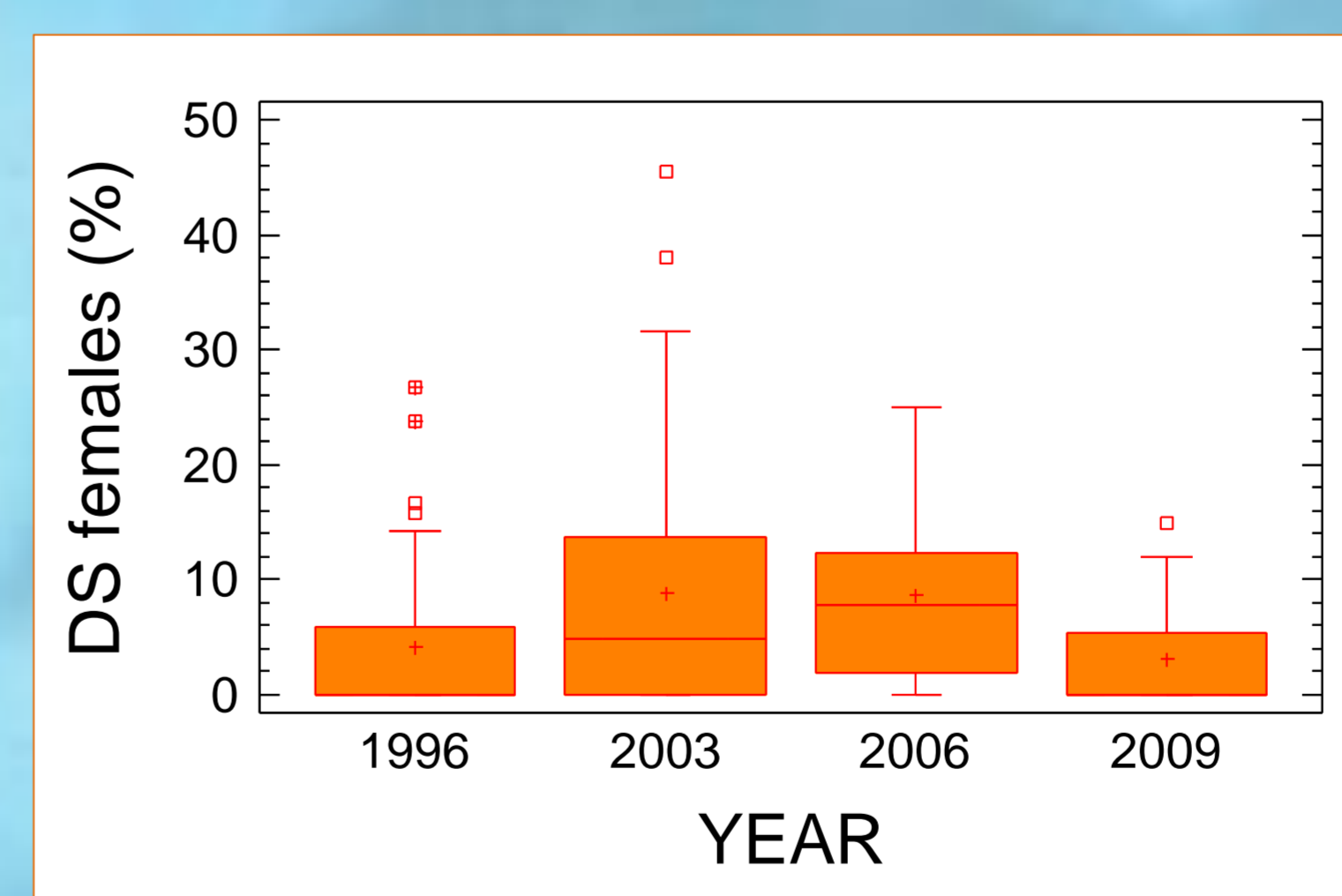
Dumpton Syndrome (DS) temporal evolution

DS is a genetic dysfunction that prevents the normal development of male sexual characters. DS afflicts both males and females. In males, DS can induce sterility. In females, however, DS diminishes the severity of imposex when they are exposed to TBT. Therefore, DS females can retain the ability to reproduce even under relatively high TBT exposure levels.

Surprisingly, DS incidence seems to have increased along these years



DS males (as %) have progressively increased from 1996 onwards. In 2009, 25% of the populations showed 10-20% DS males.



In 1996, 25% of the populations had $>5\%$ of DS females. Ten years afterwards (2006), 50% of the populations had $>8\%$ of DS females. The situation seems to have reversed again in 2009 with levels comparable to those recorded in 1996. The precise causes for this erratic behaviour are uncertain.

Conclusions

➤ Imposex, a biomarker of TBT exposure and effects, experienced a clear decrease in Galicia from 2003 onwards, in coincidence with the implementation of a total ban on TBT paints.

➤ TBT decrease has been accompanied by changes in DS incidence. However, the precise causes for this behaviour are far from clear.