# UKTAG Coastal Water Assessment Method

# Benthic Invertebrate Fauna

# Assessment of imposex in *Nucella lapillus* (dog whelks)

**by**

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It is also the responsibility of the user if seeking to practise the method outlined here, to gain appropriate permissions for access to water courses and their biological sampling.

# UKTAG Guide to the Assessment of Imposex in *Nucella lapillus*. Water Framework Directive: Coastal Waters

**Purpose of document:** To provide an overview of the assessment of imposex in *Nucella lapillus* (dog whelks) to inform Practitioners of how to monitor, assess and classify suitable specimens according to Water Framework Directive (WFD) requirements in coastal waters.

*Note:* this document does not describe all aspects of the detailed dissection and scoring system; for this please refer to the published literature Gibbs *et al*. (1987) and OSPAR (2009).

**Introduction to WFD Terminology and Assessment:** This guide describes a system for classifying in accordance with the requirements of Article 8; Section 1.3 of Annex II and Annex V of the WFD (2000/60/EC). Practitioners should recognise that the terminology used in this document is specific to the WFD and has a meaning defined by the directive.

To carry out a WFD biological assessment, each biological quality element (BQE, defined in the WFD) is required to give a statistically robust definition of the 'health' of that element in the sampled water body. The 'health' of a BQE is assessed by comparing the measured conditions (observed value) against that described for reference conditions (minimally disturbed). This is reported as an Ecological Quality Ratio (EQR). An EQR with a value of one represents reference conditions and a value of zero represents a severe impact. The EQR is divided into five ecological status classes (High, Good, Moderate, Poor and Bad) that are defined by the changes in the biological community in response to disturbance (Figure 1).

Alongside the EQR score and class status, any assessment must consider the certainty of the assessment (i.e. confidence in the assigned class).

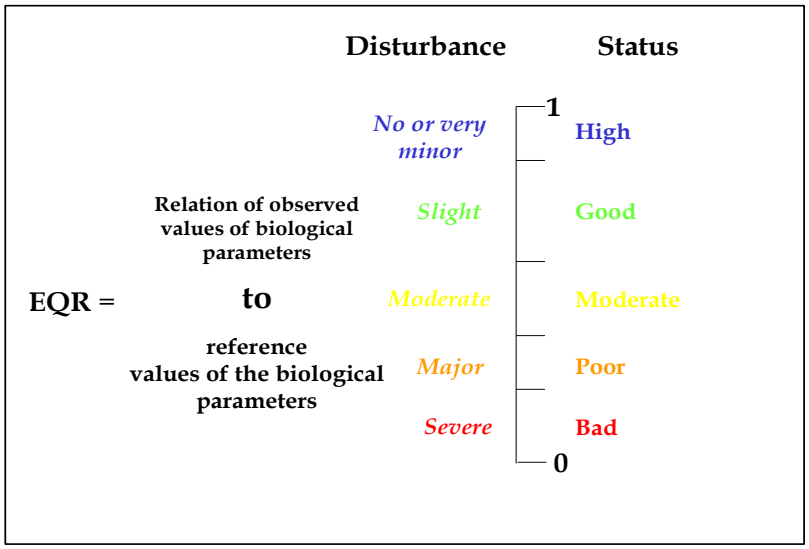


**Figure 1: Illustration of the Ecological Quality Ratio and how it relates to the level of disturbance and ecological status during a classification. The class band widths relate to biological changes as a result of disturbance (WFD CIS Guidance Document No. 5, 2003).**

# 1.Key Facts

## 1.1 Tool Overview: Imposex Assessment

The dog whelk, *Nucella lapillus*, is a carnivorous gastropod mollusc that is relatively common on rocky shores where it principally feeds on barnacles or mussels. Imposex is the development of male sexual characteristics (formation of a vas deferens and penis) in female dog whelks. Imposex is caused by exposure to the hazardous substance tributyltin (TBT), which was used historically as an active agent in anti-fouling paints applied to boats and ships[1]. There is a relationship with the concentration of TBT in the water and the extent of the "Imposex" deformity.

The Vas Deferens Sequence Index (VDSI) indicates the occurrence of imposex in *N. lapillus* (OSPAR, 2004, 2009). The VDSI describes the degree to which a community of *N. lapillus* is affected by the imposex condition. The index ranges from zero to six, where a score of zero indicates that the community is unaffected, and a score of six indicates that the majority of females are sterile due to imposex.

Although not specifically referred to in the WFD, the measurement of imposex is the practical implementation of the use of an indicator species under the general benthic invertebrate quality element. As the use of TBT has been restricted, and the level of discernible imposex is reducing, it is expected that this measurement will eventually become redundant.

For WFD reporting the imposex assessment is applied at the water body scale.

The imposex assessment operates over a range from zero (a severe impact) to one (reference/minimally disturbed). The class boundaries are:

- High/Good = 0.95
- Good/Moderate = 0.34
- Moderate/Poor = 0.17.

Bad status is represented by a zero score (i.e. no dog whelks present due to TBT pressure) and Poor status as >0 but <0.17.

As an accepted biological effects tool recognised under OSPAR, this tool was not further intercalibrated with other Member States under WFD.

## 1.2 Applicability

**Where:** Imposex assessment can be applied to all UK coastal waters that have a rocky shore where there is suitable prey to support a population of the carnivorous dog whelks. Suitable specimens are readily collected from the rocks between high and low water lines.

**When**: Live specimen samples are collected from the intertidal zone outside of the reproductive season.

**Response to pressure**: The degree of sexual deformity in the female dog whelk gives an indication of the degree of contamination by TBT in the overlying water.

[1] TBT was initially banned from boats less than 25 m in 1987, on all international shipping in 2003 and completely on all boats and ships in 2008.

### 1.3. Key Documents

The documents marked * will be hosted on the UK technical advisory group (UKTAG) website www.wfduk.org.

OSPAR. (2009). JAMP Guidelines for Contaminant-Specific Biological Effects. Technical Annex 3. TBT-specific biological effects monitoring. OSPAR Agreement 2008-09

*UKTAG Biological Status Methods: Coastal Waters Benthic Invertebrate Fauna (Dog Whelks) – *High level non-technical summary*

## 2. Background

### 2.1 Ecological principles

The imposex assessment was developed as a stand-alone metric within the benthic invertebrate fauna biological quality element looking at the specific effect of one contaminant on a particular sensitive indicator species. As increasing concentrations of TBT have a greater effect on the reproductive system of females of the species, it can result in those individuals being unable to reproduce, and can ultimately have the potential to lead to local populations dying out. This may have knock-on effects on the wider intertidal community.

### 2.2 Normative definitions

In Annex V (1.2) of the WFD, normative definitions describe the aspects of the benthic invertebrate community that must be included in the ecological status assessment of a water body; these are:

- diversity
- abundance
- disturbance sensitive taxa
- taxa indicative of pollution.

This tool covers disturbance sensitive taxa.

### 2.3 Development of the tool

This assessment was developed from Oslo and Paris (OSPAR) Commission methodology and classification (see OSPAR, 2004, 2009 for details).

### 2.4 Reference conditions

Reference conditions were derived using OSPAR classes and expert judgement. The reference value for the parameter is zero where all the females exhibit natural female reproductive anatomy and characteristics, i.e. no penis or vas deferens.

### 2.5 Class boundaries

Class boundaries have been transformed from the OSPAR VDSI scores to the WFD EQR scale (Table 1). These are generally only defined for the Good/Moderate and the High/Good boundaries in water body classifications, as dog whelks represent only one part of the benthic invertebrate community. However, knowledge of the other class boundaries allows for the 'No deterioration' assessment. Bad status is represented by a zero score (i.e. females

are absent or at vas deferens sequence stage 6) and Poor status as >0 but <0.17 (Moderate/Poor boundary).

**Table 1: Ecological status boundaries for the Imposex tool.**

| Status | EQR |
|---|---|
| High/Good | 0.95 |
| Good/Moderate | 0.34 |
| Moderate/Poor | 0.17 |

These class boundaries remain unchanged since the 1st River Basin Management Plans (2009), apart from the Good/Moderate boundary in England and Wales which has been adjusted from 0.33 to 0.34 to align with the UK boundaries.

## 3.0 Undertaking the assessment

### 3.1 Summary Flow Chart

The process for undertaking a water body imposex assessment is summarised below (Figure 2).

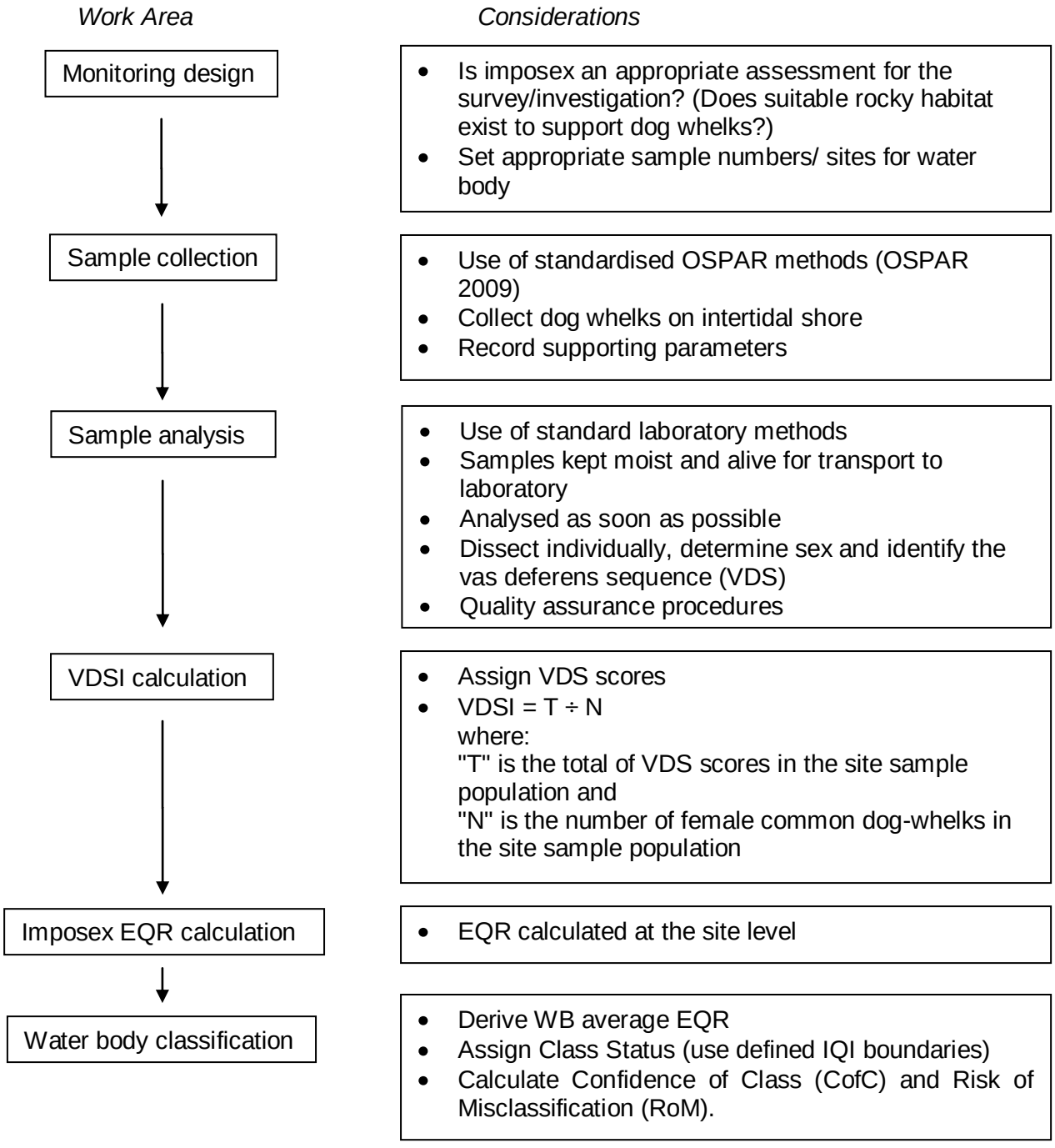


**Figure 2: Flow chart summarising the main stages involved in undertaking an imposex assessment.**

### 3.2 Data requirements

The following information is required:

- Site location (NGR)
- Date of sample collection
- Number of individuals in sample.

Calculation of imposex for OSPAR defined measurements includes the following:

- Imposex stage for each female (0 to 6)
- Proportion of females in stages 5 and 6
- Length of penis in males and females (to nearest 0.01mm)
- Shell height (nearest mm)
- Sex Ratio (F/M).

*Note*: only imposex stage (VDS) for females is required for WFD.

It is also recommended that the following supporting parameters are measured:

- Time to collect 100 individuals (mins)
- Juveniles present (Y/N)
- Eggs present (Y/N).

### 3.3 Sampling strategy

Samples of ca. 50 individuals should be collected from rocky shores by hand outside the reproductive season. Intertidal sites should be chosen between the normal high and low limits of spring tides; dog whelks are commonly to be found between approximately mid shore and low water levels. All samples containing live specimens should be transported to the laboratory in damp conditions in non-contaminating containers. If necessary, cooling facilities during transport and storage should be employed.

### 3.4 Sample analysis

In the laboratory, samples for biological measurements should be analysed as soon as possible but, on transfer to aquarium facilities, not later than seven days after sampling. Animals have to be dissected individually, and firstly the sex should be determined according to the presence of a sperm-ingesting gland and other relevant sexual characters. The vas deferens sequence is then determined and the associated score assigned (Gibbs *et al*., 1997). Characteristics are scored and tabulated for each individual. 40 individuals should be analysed.

*Note:* parasitized animals may exhibit abnormal sexual development and are therefore excluded from the analysis.

Each specimen should be analysed for its determining characteristics (Table 2): the vas deferens sequence score (VDS) should be defined by the corresponding score in Table 2. The number of females within the sample population should be recorded.

**Table 2: Vas deferens sequence and associated scores.**

| Characteristics | VDS Score |
|---|---|
| no signs of imposex can be seen | 0 |
| vas deferens is evident at the site of the vulva | 1 |
| a small penis is evident behind the right eye tentacle | 2 |
| vas deferens has developed from the penis but does not connect with the vulva | 3 |
| vas deferens is continuous | 4 |
| vas deferens tissue proliferates over the vulva opening, making the female incapable of breeding | 5 |
| egg capsules cannot be released and form a solid mass within the capsule gland | 6 |

### 3.5 Data treatment

The observed value for the vas deferens sequence index (VDSI) at a site should be estimated as:

$$VDSI = T \div N$$

where:
"T" is the total of VDS scores in the site sample population and
"N" is the number of female common dog-whelks in the site sample population.

Reference conditions were derived using OSPAR classes and expert judgement. The reference value for the parameter is zero (i.e. no signs of imposex in the sampled population).

### 3.6 EQR calculation

The ecological quality ratio (EQR) for the parameter should be calculated using the following equation:

$$EQR = (6 - VDSI) \div 6$$

### 3.7 Water body level classification

Water body classifications are based on the arithmetic mean EQR of all sites within a water body.

### 3.8 Understanding the certainty of the assessment

Providing an estimate of the statistical uncertainty of water body assessments is a statutory requirement of the WFD (Annex V, 1.3). Water body assessments based on estimates of ecological quality from sample data may be subject to elements of spatial bias, temporal bias (e.g. seasonal changes), random sampling/measurement and random error from ecological processes. When assigning discrete ecological status classes, variability means that, depending on the proximity of the water body assessment result to a class boundary, there

is a likelihood that the "true" status (i.e. that status if the EQR for the total population was known with zero error) is different to that assigned. This is termed the 'risk of misclassification' (RoM). Conversely, the statistical confidence that the status assigned from the sample population falls into each of the five ecological status classes is referred to as the 'confidence of class' (CofC).

The approach developed to define and report the CofC and RoM for WFD classifications is described by Ellis & Adriaenssens (2006).

## 4. Worked example

Analysis of 40 specimens at a site gave a total VDS score of 64. There were 17 females.

VDSI = 64 ÷ 17 = 3.76

EQR = (6 - 3.76) ÷ 6

= 0.37

= **Good** status

## 5. References

Ellis, J. & Adriaenssens, V. (2006). Uncertainty estimation for monitoring results by the WFD biological classification tools. Bristol: Environment Agency. ISBN: 1844326063

Gibbs, P. E., Bryan, G. W., Pascoe, P. L. and Burt, G. R. (1987). The use of Dog-Whelk *Nucella lapillus*, as an indicator of Tributyltin (TBT) contamination. Journal of the Marine Biological Association of the United Kingdom, 67: 507-523.

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WFD CIS Guidance Document No. 5 (March 2003). Rivers and Lakes – Typology, Reference Conditions and Classification Systems. Published by the Directorate General Environment of the European Commission, Brussels, ISBN No. 92-894-5614-0, ISSN No. 1725-1087.