Practitioners Guide to the Assessment of Imposex in *Nucella lapillus* (dog whelks) Water Framework Directive: Coastal Waters

Purpose of document: To provide an overview of the assessment of imposex in 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 (2008-9)

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 as such 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 (Fig. 1). Once the EQR score and ecological status class have been calculated an assessment must be made to consider the certainty of the classification (i.e. confidence in the assigned class).

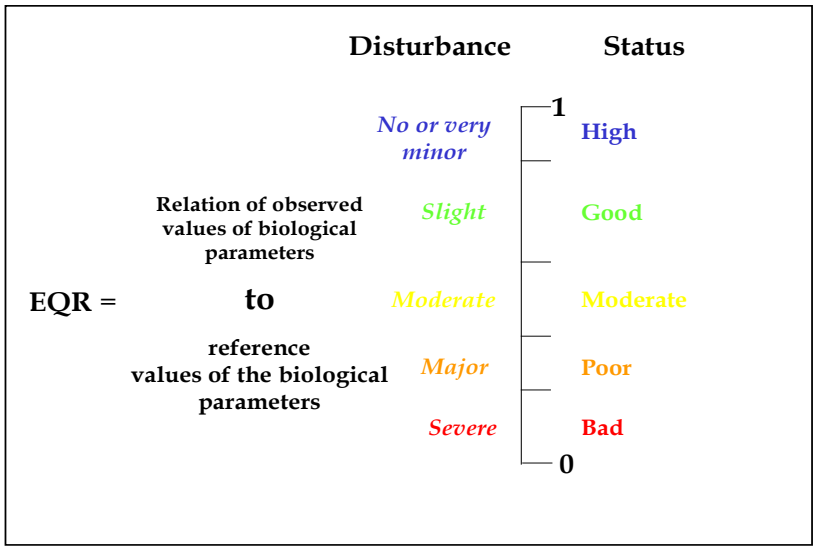

Fig. 1: Illustration of the Ecological Quality Ratio and how it relates to level of disturbance and ecological status during a classification. The class band widths relate to biological changes as a result of disturbance (WFD CIS Guidance Document No. 5, 2003).

1.Key Facts

1.1 Tool Overview: Imposex Assessment

The dog whelk 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 tributyl tin (TBT) which was historically used as an active agent in anti-fouling paints applied to boats and ships*. There is a relationship with the concentration of TBT in the water and the extent of the "Imposex" deformity.

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

1.2 Applicability

Although not specifically referred to in the directive 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 ceased, and the level of discernible imposex is reducing ,it is expected that this measurement will eventually become redundant.

Where: Imposex assessment can be applied to all UK coastal waters that have a rocky shores 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 during the summer months. Forty specimens should be taken at each site.

Response to pressure: The degree of sexual deformity in the female dog whelk on a scale of zero to six gives an indication of the concentration of TBT in the overlying water.

1.3. Key Documents

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

UKTAG Biological Status Methods: Coastal Waters Benthic Invertebrate Fauna (Dog Whelks)

OSPAR 2008-9 JAMP Guidelines for contaminant-specific Biological Effects Monitoring. OSPAR Commission 2008.

*TBT was initially banned from boats less than 25 m in 1988, on all international shipping in 2003 and completely on all boats and ships in 2008

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 female of the species it can result in the individuals unable to reproduce and ultimately have the potential to lead to local populations dying out..

2.3 Development of the tool Developed from OSPAR Commission methodology and classification but not intercalibrated with other member states.

2.4 Reference conditions

Reference conditions were derived using OSPAR classes and expert judgement. The reference value for the parameter is zero where the females exhibit natural female reproductive anatomy and characteristics.

2.5 Class boundaries

These have only been set for the good/moderate and the High/Good boundaries.

Status	EQR Boundary value
High/Good	0.95
Good/Moderate	0.34

These class boundaries remain unchanged since the 1st River basin management Plans (2009).

3.0 Undertaking the assessment

3.1 Summary of the process

Samples of 40 individuals should be collected from rocky shores by hand during the summer months June to September, inclusive. Specimens are kept moist and alive ideally for no more than 48hours and never more than 72 hours. Animals have to be individually dissected to examine the vas deferens sequence and associated score. Characteristics are scored and tabulated for each individual

3.2 Sample analysis

Each specimen should be analysed for its determining characteristics according to Column 1 of Table 1: the vas deferens sequence score (VDS) should be defined by the corresponding score in Column 2 of Table 1. The number of females within the sample population should be recorded.

Table 1: Vas deferens sequence and associated scores	
Column 1	**Column 2**
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	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

3.3 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).

3.4 EQR calculation

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

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

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

References

Bryan, G. W., Gibbs, P.E., Hummerstone, L.G.& Burt, G.R. (1986). The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin from antifouling paints. *J.Mar.Biol.Ass.UK*. 66(3); 611-640

OSPAR (2009) Biodiversity Series, Background Document for Dogwhelk, *Nucella lapillus* , 408/2009, OSPAR Commission, London, 24pp.