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Recovery from imposex by a population of the dogwhelk, *Nucella lapillus* (Gastropoda: Caenogastropoda), on the southeastern coast of England since May 2004: A 52-month study

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ABSTRACT

Over a 52-month period beginning in May 2004 and concluding in August 2008, and coinciding with the period over which TBT was banned as a ship anti-foulant globally, a population of the dogwhelk, *Nucella lapillus*, was studied for changes in population size and structure, and reproduction. During the study period, the size of the population of *N. lapillus* on the Mewsbrook Groyne at Littlehampton on the southeastern coast of England grew from ~25 individuals to >500, i.e., a 20-fold increase. Similarly, population structure normalised to reveal a maximum age of up to ~3 years. The numbers of egg capsules produced by the *N. lapillus* population also grew over the study, again by a factor of 20, and the length of the breeding season increased from 7 months in 2004–2005 and 2005–2006 to 11 months in 2006–2007 and 2007–2008. Such changes were reflected in the incidence of imposex from Vas Deferens Index Stages 3 and 4 in 2003 to zero commencing in 2008 and continuing into 2009.

Due to a lack of confirmatory chemical data, the changes in population size, structure and reproduction herein reported upon for *N. lapillus* cannot be correlated positively with changes in ambient TBT levels, but they can and are correlated with freedom from imposex. This is the first time such a dramatic recovery from imposex, following the banning of TBT, has been documented.

1. Introduction

Blaber (1970) first reported upon the occurrence of a penis-like outgrowth behind the right tentacle in females of the North European intertidal caenogastropod *Nucella lapillus* (Linnaeus, 1758) (Muricidae). Smith (1971) had researched reproduction in the American mud snail *Nassarius obsoletus* (Say, 1822) (Nassariidae) and from this baseline reported subsequently upon the occurrence of imposex, that is, the imposition of male sexual characteristics on the female of this species (Smith, 1981). Subsequently, a range of other effects of TBT upon different organisms has been documented and reviewed by Champ and Seligman (1996).

By far the most obvious effect of TBT upon the endocrine system of gastropods, however, is imposex (Matthiessen and Gibbs, 1998). This was first identified in the reproductive failure of populations of the dogwhelk, *N. lapillus* (Gibbs and Bryan, 1986; Gibbs et al., 1987), resulting in the decline of the species along the southwestern Bryan et al. (1986) and then along the southeastern coastlines (Gibbs et al., 1991) of England and, subsequently, yet further afield. Sterility was initiated at levels of between 1 and 2 ng Sn l^{-1} and completed at between 6–8 ng Sn l^{-1} (Gibbs et al., 1988). Surprisingly, however, not all gastropods are affected and in a review of the effects of TBT upon them, Li (2000, Table 2) showed that in Hong Kong the most pronounced effects were recorded upon the many representatives, virtually worldwide, of three closely related families, that is, the Muricidae, Buccinidae and Nassariidae. In the Kattegat and Skagerrak in northeastern Atlantic waters, for example, the two subtidal buccinids *Buccinum undatum* (Linnaeus, 1758) and *Neptunea antiqua* (Linnaeus, 1758) are imposexed (Strand and Jacobsen, 2002).

So serious was the problem of TBT perceived to be in many countries that regulations have been enacted, beginning in the 1980's, to control the use of TBT (Champ and Seligman, 1996) and some reductions in levels and effects have been reported upon (Waite et al., 1991; Uhler et al., 1993; Evans et al., 1994, 1995). Further, following legislation limiting its use on vessels of <25 m length, there is some evidence that dogwhelk populations are increasing. Evans et al. (1994), for example, showed that on the Isle of Cumbrae, in Scotland, Relative Penis Size Index scores for *N. lapillus* and the incidence of penis-bearing individuals decreased and dogwhelk numbers increased between 1988 and 1992. Similarly, Rees et al. (2001) reported that, compared to 1992, the incidence of imposex in *Thais orbita* (Gmelin, 1791) inhabiting Port Phillip Bay, Victoria, Australia, had decreased following ten years of control on the use of TBT as an anti-foulant on small vessels.

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Although TBT-based antifouling paints were banned from application on small boats, its use was still permitted on larger vessels. After effects upon open waters similar to those identified from inshore ones were identified, however, in 2001 the International Maritime Organisation (IMO) adopted the *Convention on the Control of Harmful Antifouling Systems on Ships*. At the same time, the European Union *via* Regulation (EC) No. 782/2003 banned the application of TBT-based paints on all EU-flagged vessels with effect from 1 January 2003 and, subsequently, with effect from 1 January 2008, made it an offence for any EU ship visiting any EU port to have TBT present on its hull. The IMO followed the two stages of this ban and, similarly, with effect from 1 January 2008 (Gipperth, 2009). All EU flagged vessels should, therefore, have been TBT-free by this date but, realistically, some reductions in coastal TBT levels should have been seen after 2003 when TBT hull painting halted. As a consequence, effects upon *N. lapillus* should have been seen too.

With the above scenario in mind, a study was commenced on a small group of *N. lapillus* individuals inhabiting the Mewsbrook Groyne at Littlehampton, West Sussex, on the southeastern coast of England, with the main aim of determining if changes in population structure would occur and, if so, how would they be mediated. The periwinkle, *Littorina littorea* (Linnaeus, 1758), also occurs on the groyne in large numbers and has been shown by Oehlmann et al. (1998) to be elsewhere also influenced by TBT, resulting in a phenomenon called intersex. The same authors, however, considered that dogwhelk imposex is a more sensitive biomarker and should be used in areas that are only either slightly or moderately contaminated with TBT. Thus, although the assessment of intersex intensities in periwinkle populations has considerable advantages in areas with high TBT concentrations and should be used where dogwhelks are absent, this was not the case on the Mewsbrook Groyne, and, accordingly, *N. lapillus* was chosen as the subject for this study. The study, which lasted for 52 months, commenced in May 2004 just over one year since the new TBT painting ban commenced and, hence, in theory, extended over the period when the effects, if any, of TBT should have declined.

Table 1
Nucella lapillus. The expression of imposex in individuals collected from the Mewsbrook Groyne over the period from 2003 to 2008.

Date	Shell height	Sex	Penis length (where present) (mm)	Imposex stage (VDS) (after Gibbs et al., 1987)
1.11.2003	18.0	Female	1.3	**3**
1.11.2003	27.6	Female	1.0	**3**
1.11.2003	28.3	Female	3.0	**4**
24.7.2004	32.8	Female	3.0	**4**
24.9.2004	28.8	Female	2.0	**4**
2.10.2006	31.0	Female	1.0	**3**
2.10.2007	17.4	Female	–	**–**
7.1.2008	21.5	Female	2.5	**4**
7.1.2008	27.0	***Male***	*4.5*	**–**
7.1.2008	28.2	Female	3.5	**4**
7.1.2008	31.6	Female	6.5	**6**
7.1.2008	31.9	*Male*	*6.0*	–
7.1.2008	33.0	Female	–	–
7.1.2008	34.8	*Male*	*8.0*	–
7.1.2008	35.0	Female	–	–
7.1.2008	35.9	*Male*	*6.5*	–
7.1.2008	37.0	*Male*	*7.0*	–
13.8.2008	31.6	Female	–	–
13.8.2008	33.1	Female	–	–
13.8.2008	27.0	*Male*	*4.0*	–
9.3.2009	31.3	Male	*4.0*	–
9.3.2009	29.1	Female	–	–Fully mature
9.3.2009	31.4	Female	–	–Fully mature
9.3.2009	30.7	Male	*5.0*	–
9.3.2009	27.7	Male	*5.0*	–
9.3.2009	31.4	*Male*	*4.0*	–
9.3.2009	35.2	Female	–	–
9.3.2009	27.5	Male	*4.0*	–
9.3.2009	30.5	*Male*	*4.5*	–
9.3.2009	27.2	*Male*	*4.5*	–

2. Methods

Commencing in May 2004, visits were made each month to an intertidal outfall draining overflow water from Mewsbrook Lake situated landward of the coastal road between Rustington and Littlehampton in West Sussex. The outfall is locally called the Mewsbrook Groyne and has been described by Morton (2007). It comprises a perpendicularly-oriented, roughcast, concrete block located in what can be broadly described as the eulittoral and extends down-shore for ~100 m such that it is uncovered by most tides. Because the tidal range here is a maximum of 6.1 m, however, the seaward end of the groyne can only be examined at the times of tides <1.4 m. It is thus only wholly accessible at times around spring tides. Because of prevailing westerly winds and waves, the more wave-exposed western side of the groyne was, in May 2004, un-colonized by *N. lapillus* whereas the wave-sheltered eastern side was.

On each visit to the groyne, the shell height of every individual of *N. lapillus* was measured to the nearest 0.1 mm using vernier calipers and returned to its original location. In addition, any clusters of *N. lapillus* were identified and all individuals measured (as above) and the presence and numbers of any egg capsules, either alone or associated with clusters, recorded. Every attempt was made not to disturb the habitat and thereby allow for the repeated examination of changes occurring in what can be considered a 'reasonably natural' community.

The study was halted in August 2004 because, as will be described, numbers of *N. lapillus* had increased by so many that it had become difficult to study the population over the course of a single tidal cycle. The importance of doing this is that, as described by Burrows and Hughes (1989), dogwhelks can move quite large distances between tidal cycles and so repeat sampling at consecutive low tides might result in the re-counting of earlier identified individuals.

2.1. Imposex evaluation

Prior to the start of the survey, three individuals of *N. lapillus* (as will be described, comprising ~10% of the groyne population) were collected on 1 November 2003, that is, prior to enactment of the banning of TBT on large vessels and examined for imposex. On 7 January 2008 and 9 March 2009, further samples each of 10 adult individuals of *N. lapillus* were collected at random, that is, five and six years, respectively, after the enactment of the TBT ban and, again, examined for the occurrence of imposex. During the course of the four-year survey, individuals of *N. lapillus* were encountered occasionally that had been entrapped by the byssal threads of *Mytilus galloprovincialis* (Lamarck, 1819) individuals. This, reverse, 'predation' has been described by Farrell and Crowe (2007) and though uncommon on the Mewsbrook Groyne did provide the opportunity for such doomed individuals of *N. lapillus* to be collected and examined for imposex (Table 1).

3. Results

3.1. Nucella lapillus population size

Fig. 1 shows the numbers of *N. lapillus* recorded from the groyne every month from May 2004 to August 2008. Over the study period, the population grew progressively from 26 individuals in May 2004 to 509 individuals in August 2008. That is, there was a 20-fold increase in numbers and although numbers fluctuated over time,