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THE DECLINE OF THE GASTROPOD *NUCELLA LAPILLUS* AROUND SOUTH-WEST ENGLAND: EVIDENCE FOR THE EFFECT OF TRIBUTYLTIN FROM ANTIFOULING PAINTS

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(Figs. 1–11)

A survey of the gastropod *Nucella lapillus* around the south-west peninsula of England has revealed that the incidence of 'imposex', the induction of male sex characters in the female, is widespread, that all populations are affected to some degree and that the phenomenon is most prevalent along the south (Channel) coast. Populations close to centres of boating and shipping activity show the highest degrees of imposex, especially those within the vicinities of the Helford, Fal, Salcombe and Dart estuaries and in Plymouth Sound and Tor Bay. Within Plymouth Sound the degree of imposex increased markedly between 1969 and 1985, coinciding with the introduction and increasing usage of antifouling paints containing tributyltin (TBT) compounds.

The degree of imposex shows no correlation with the tissue burdens of arsenic, cadmium, copper, lead, silver and zinc but it increases with increasing concentrations of tin. Much of the tin found in body tissues is in a hexane-extractable form that includes both tributyltin and dibutyltin fractions.

Transplantation of *N. lapillus* from a 'clean' locality with little boating activity to a site close to a Plymouth marina caused a marked increase in the degree of imposex in the transplanted animals; analyses showed that of the hexane-extractable tin accumulated by the tissues of the transplants, 60–70% was in the tributyltin fraction. Laboratory experiments using tidal tanks show that imposex is readily induced by exposure to 0·02 μg/l of tin leached from a TBT antifouling paint. It is thought that imposex could be initiated in *N. lapillus* by a concentration of tin, as tributyltin species, as low as 1 ng/l.

Comparison of past and present observations of *N. lapillus* abundance show that many south-west England populations are in decline; these populations are characterized by moderate to high degrees of imposex, often relatively fewer females, few juveniles and a general scarcity of laid egg capsules. A reduction in recruitment caused by a lowered reproductive capacity, rather than an increased mortality rate, appears to be responsible for the decline in *N. lapillus* numbers.

Since the degree of imposex in *N. lapillus* is easily measured and may be related to the mean levels of tributyltin compounds to which the populations are exposed, the species has great potential as an indicator of the extent of TBT contamination.

INTRODUCTION

The common dogwhelk, *Nucella lapillus* (L.), a stenoglossan gastropod, is found on rocky shores on both sides of the North Atlantic. In the east its distribution extends from northern Russia to Portugal and in the west from southern Newfoundland to the New York area: it is also found in Iceland and possibly western Greenland (Cooke, 1915; Moore, 1936; Crothers, 1977). Because of the abundance of *N. lapillus* on all rocky shores, the British Isles were described by Cooke (1915) as the 'metropolis' of the species.

Recent observations on *N. lapillus* in the Fal Estuary in Cornwall indicated that populations have declined markedly over the past 10–15 years (Bryan *et al.* 1986). It was found, moreover, that surviving animals were subject to a high degree of 'imposex' (the development of male characters in the female: see below) and that they contained high concentrations of hexane-extractable organic tin. In another stenoglossan gastropod, *Nassarius obsoletus* Say, Smith (1981*a*, *b*) demonstrated that imposex was most obvious near harbours and marinas and probably caused by tributyltin compounds, the toxins used in most modern antifouling paints. Smith found no evidence that imposex in *N. obsoletus* prevented reproduction or affected the ecology of the species. In *N. lapillus*, however, the circumstantial evidence for a link between imposex and the decline in the Fal Estuary population was so strong that studies were extended. This paper documents the geographical distribution of imposex in *N. lapillus* around south-west England and relates the degree of imposex to tissue-metal concentrations and to the incidence of declining populations.

The term 'imposex' was defined by Smith (1971) as 'a superimposition of male characters on to unparasitized and parasitized females'. Such pseudohermaphroditism in the gonochoristic *Nassarius obsoletus* resulted in females with one or more of the following characters: (1) a penis with a duct leading to (2) a vas deferens which passes back to the ventral channel of the capsule gland and (3) convolution of the normally straight gonadial oviduct (Smith, 1980). In *Nucella lapillus*, also gonochoristic, females with imposex develop a penis and vas deferens but convolution of the gonadial oviduct has not been recognized (a description of the genital ducts of *N. lapillus* is given by Fretter (1941)). Smith (1981*a*) found that the intensity of penis expression was the easiest character to evaluate and possibly the most sensitive to the influence of a factor emanating from marinas. Thus for the purposes of the present survey only penis development has been examined and the term 'imposex' is restricted here to this one character.

MATERIALS AND METHODS

Measurement of imposex

As far as possible, over 30 of the largest *N. lapillus* were collected at each site: in those selected, the edge of the shell was thickened and often bore a row of 'teeth' on the inside. These features are characteristic of animals that have almost ceased growing, are sexually mature and at least 2·5 years old (Feare, 1970). At some sites, larger samples representative of the whole population were collected for the measurement of size–frequency distribution. Notes were made of the maximum density of animals, the presence of young and juvenile specimens, the occurrence of egg capsules and the type of food available – usually barnacles or small mussels.

The length of the shell was measured to the nearest 0·1 mm and the presence of 'teeth', the colour, and the general appearance of the shell (young or old and eroded) were noted. After extracting the animal by breaking the shell in a vice, it was sexed from the appearance of the gonads and accessory organs (Fretter & Graham, 1962; Hall & Feng, 1976): the reproductive condition was noted together with any obvious abnormalities such as the presence of parasites. In both males and females, the length of the penis from its tip to its junction with the body wall behind the right tentacle was measured to the nearest 0·1 mm using a piece of 1 mm graph paper (Fig. 1): this was carried out under water and no attempt was made to straighten the penis. Deformities of the penis

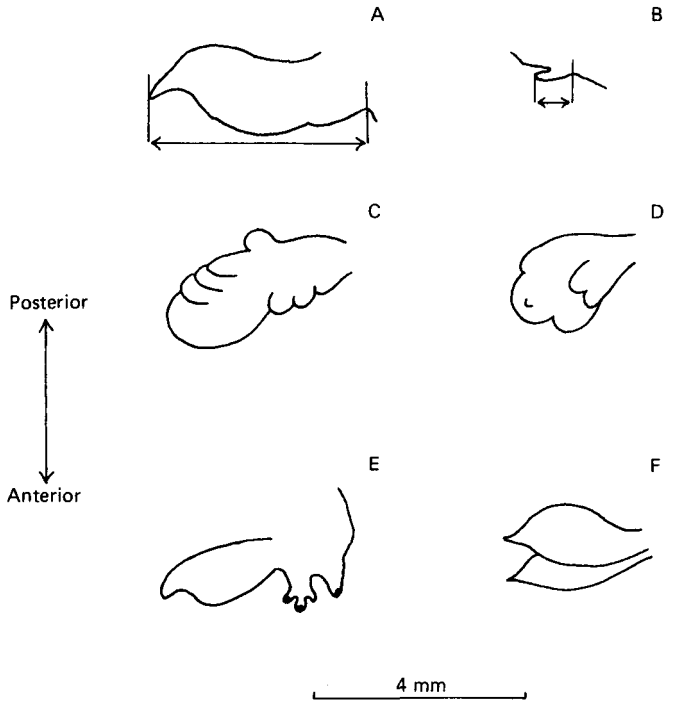

Fig. 1. *N. lapillus*. (A–B) Measurement of length of a normal male penis or a large female penis (A) and a very small female penis (B). (C–D) Examples of deformed male penis from Restronguet Point, Fal Estuary, where the species is rare. (E) Example of female penis including 2-eyed right tentacle (Penzance). (F) Form of bifurcate penis found in some males and females from affected areas.

or adjacent tissues including the tentacles were noted. After removing the opercula, males were separated from females and each sex pooled in weighed vials, reweighed and frozen for future analysis.

Analysis of metals

After thawing, the tissue from 4–6 animals was homogenized with a small amount of distilled water. One aliquot of homogenate was dried to obtain the water content. The same sample was subsequently digested with concentrated 'Aristar' nitric acid and, following evaporation, redissolved in hydrochloric acid and diluted to 1 N with distilled water. Concentrations of cadmium, copper, lead, silver and zinc were determined in this solution by flame atomic absorption, background correction being employed for cadmium, lead and silver (Bryan *et al.* 1985). A second aliquot (equivalent to about 0·2 g dry tissue) was mixed with 5 ml of an ashing agent (10 g magnesium oxide and 6 g magnesium nitrate per 100 ml of distilled water) and dried. After ashing at 500 °C in a muffle furnace the material was dissolved in 10 ml of hydrochloric acid and diluted to 20 ml (Langston, 1980). Tin and arsenic were determined in a Perkin–Elmer MHS-20 hydride system.

The determination of tin as tributyl or dibutyl species was based largely on the methods employed by Ward *et al.* (1981). Three aliquots of homogenate containing the equivalent of about 0·2 g of dry tissue were placed in 30 ml stoppered boiling tubes. A standard of 0·05–0·2 μg of tin as tributyltin oxide in ethanol was added to one sample and a standard of dibutyltin dichloride to another; after shaking they were left for 1·5–2 h. Aliquots (5 ml) of concentrated hydrochloric acid were added to the homogenates, which were shaken at intervals for 30 min. Following the addition of 5 ml of hexane, the tubes were placed on an automatic shaker for 15 min and then centrifuged for a few minutes: 5 ml of distilled water were added and, after swirling briefly, the tubes were recentrifuged. Approximately one-half of the clear hexane extract was removed and shaken with one-third of its

volume of 1 N sodium hydroxide solution to separate the dibutyltin from the tributyltin fraction. Reagent blanks were put through the same procedure and were extremely low. The extraction of tin (0·2 μg), added to homogenates as dimethyltin dibromide, trimethyltin chloride or stannous chloride, was not significant, and when added as butyltin trichloride only 1·5% was recovered.

Tin was measured in a Perkin-Elmer 76 B graphite furnace attached to a Perkin–Elmer 603 atomic absorption instrument. The pyrolysed furnace contained a loosely fitted platform which was treated with tantalum pentoxide before use (Brooks, Ryan & Zhang, 1981). An electrodeless discharge tin lamp was employed at a wavelength of 286·3 nm. The following conditions were used for the determination of tin in 50 μl aliquots of the hexane extracts: dry at 90 °C for 30 s; ash at 1000 °C for 25 s; atomize at 2600 °C for 5 s at ramp 1 with gas stop. Background correction was not required, the absence of background being checked with the use of the deuterium background corrector or by employing a non-absorbing wavelength at 276·3 nm. Tin as tributyltin was determined in the NaOH-treated extracts: concentrations of tin as dibutyltin were calculated following substraction of the tributyltin data from that for the untreated hexane extracts. Analyses of 5 aliquots of an homogenate of male dogwhelks from Plymouth gave mean tin concentrations of $0{\cdot}269 \pm 0{\cdot}019$ μg/g and $0{\cdot}236 \pm 0{\cdot}016$ μg/g in the tributyl and dibutyltin fractions respectively. Detection limits were of the order of 0·02 μg/g tin on a dry tissue basis.

When standard additions were made to aliquots of a single hexane extract rather than to separate aliquots of homogenate, concentrations determined by the former technique were $102 \pm 4\%$ of the latter for the tributyltin fraction, and $117 \pm 7\%$ for the dibutyl fraction ($n = 5$ and 4 for different samples respectively).

Tank experiment

A 200 l tank was supplied with a continuous flow of sea water ($\sim$ 0·6 l/min) containing tributyltin compounds leached from a 6 mm diameter Perspex rod painted with white copolymer antifouling paint (International, 'Cruiser' copolymer paint). To produce a tidal effect the tank was pumped out every 12 h and took 5–6 h to refill. *N. lapillus* ($n = 180$) from a slightly affected site (Crooklets Beach, Bude) were placed in the tank in plastic cages (laundry baskets) containing barnacle-covered rocks from a nearby site; to ensure a constant food supply, a proportion of the rocks was exchanged for newly collected rocks at 7–10 d intervals of the 120 d experiment. Every few days 2 1-l samples of water were taken from the tank and one was spiked with 0·1 μg of tin as tributyltin oxide. After acidification with 5 ml of hydrochloric acid the water was extracted with 5 ml of hexane for 10 min. The hexane was analysed in the same way as the tissue extracts. By adjusting the depth of the painted rod in the flow system the water concentration was maintained at $0{\cdot}0204 \pm 0{\cdot}0055$ μg/l of tin as tributyltin oxide ($n = 41$). Levels of dibutyltin species amounted to no more than a few per cent at most.

A duplicate system was used as a control, the water concentration of tin being close to the detection limit of rather less than 0·001 μg/l.

At the start of the experiment in October 1985 the water temperature was about 16 °C and fell to 9 °C in February 1986.

RESULTS

Imposex and its assessment

Previous workers have expressed imposex in *N. lapillus* as the percentage of penis-bearing individuals in the female population (Blaber, 1970; Féral, 1980; Miller & Pondick, 1984). At most sites in south-west England, however, the incidence of imposex in females is 100%, and it became necessary to express the degree of imposex in terms of the relative sizes of the penis in male and female *N. lapillus* from each population. Length is the parameter most easily measured, and in males this averaged about 3·8 mm: based on 88 samples each of 10–15 males, the mean coefficient of variation per sample was 9·3%. In females,

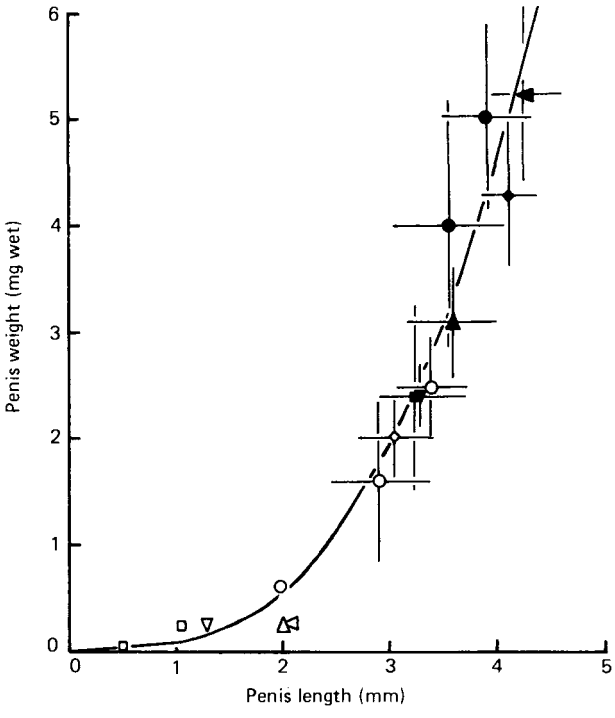

Fig. 2. *N. lapillus*. Relationship between penis length and wet weight in males (closed symbols) and females (open symbols) from six different populations. Horizontal and vertical lines show ranges of values about means. At lengths below 2·5 mm weights were estimated from dimensions of penis. Equation of curve: wet weight = length^3/14.

coefficients of variation ranged from a mean of 10·3 % when the mean penis length exceeded 3 mm (25 samples), to 59 % when the mean length was 1 mm or less (17 samples). Although measurements were always carried out on sexually mature animals (excluding those heavily parasitized) some individuals were clearly several years older than others: however, age did not appear to be a major contributor to penis-length variation in affected females.

Occasionally the female penis was bifurcate, and in these cases the longer section was measured. In some severely affected specimens, additional growth of the body wall was observed near the penis; or the right tentacle was malformed, sometimes leading to the loss of an eye or the addition of a third eye (Fig. 1).

However, comparisons based simply on penis length do not convey any idea of the contrast in mass between, say, a penis of 2 mm and one of 4 mm. However, Fig. 2 shows that the weight (or volume), of the penis is related to the cube of its length, and therefore the ratio of female length^3/male length^3 × 100 is used in this paper as a measure of the degree of imposex at each site: a level of 50 % implies that the average penis of the female is half the bulk of that of the male.

When the degree of imposex exceeds 70 % it is not uncommon to find males

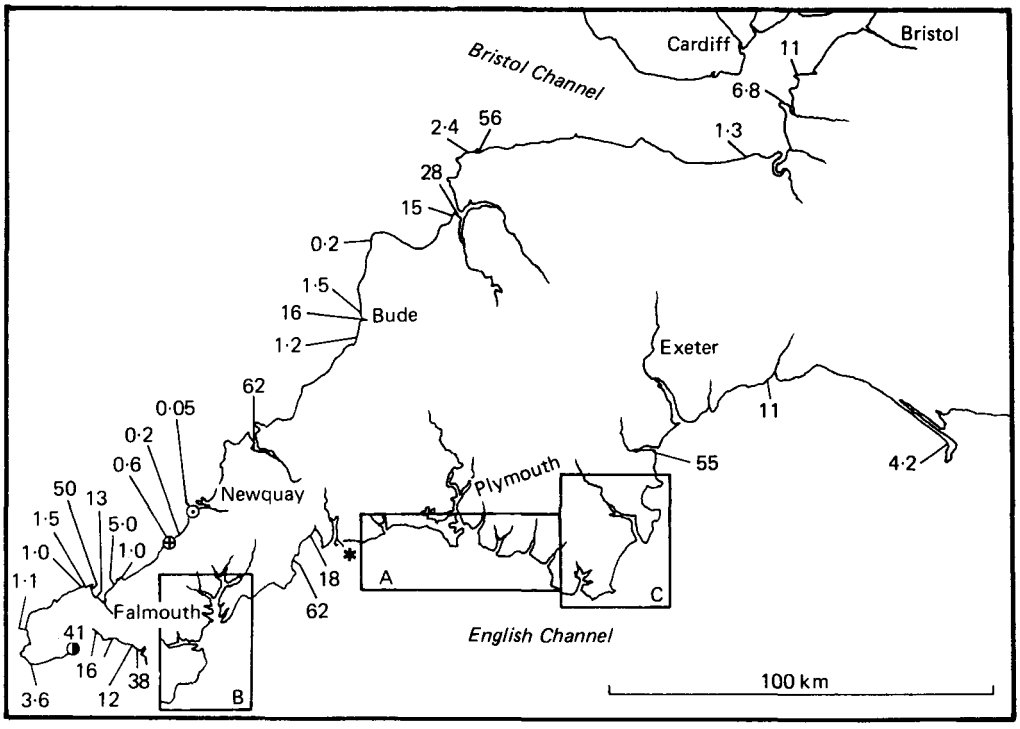

Fig. 3. *N. lapillus*. Geographical distribution of degrees of imposex in south-west England, 1984–5. *, A few males only present; ⊕, St Agnes; ⊙, Porth Joke; ◑, Penzance. Degree of imposex is defined as the bulk of female penis as percentage of male. See Fig. 4 for larger-scale maps of areas A, B and C.

in which the penis is grossly deformed: examples include enlargement by as much as a factor of 3 and bifurcation of the penis (Fig. 1). Generally speaking, since mean male penis lengths may be greater in such populations, the highest degrees of imposex will sometimes tend to underestimate the effect on the female (as in Dart animals; Fig. 8).

Geographical distribution of imposex in N. lapillus

The survey covers the whole of the south-west peninsula from Weston-super-Mare to Portland Bill, and comprises samples totalling several thousand individuals from over 80 sites.

Levels of imposex in south-west England are summarized in Figs. 3 and 4A, B, C. At some sites mean values are used and are based on as many as five measurements made at different times of year: however, as Table 1 shows, seasonal differences are unlikely to alter the overall picture markedly.

North coast (including Bristol Channel) (Fig. 3)

Around south-west England, sites at which the degree of imposex is less than 2% are confined to the north coast. Porth Joke, near Newquay, is the least-affected locality (mean imposex 0·05%) and is the only place where consistently

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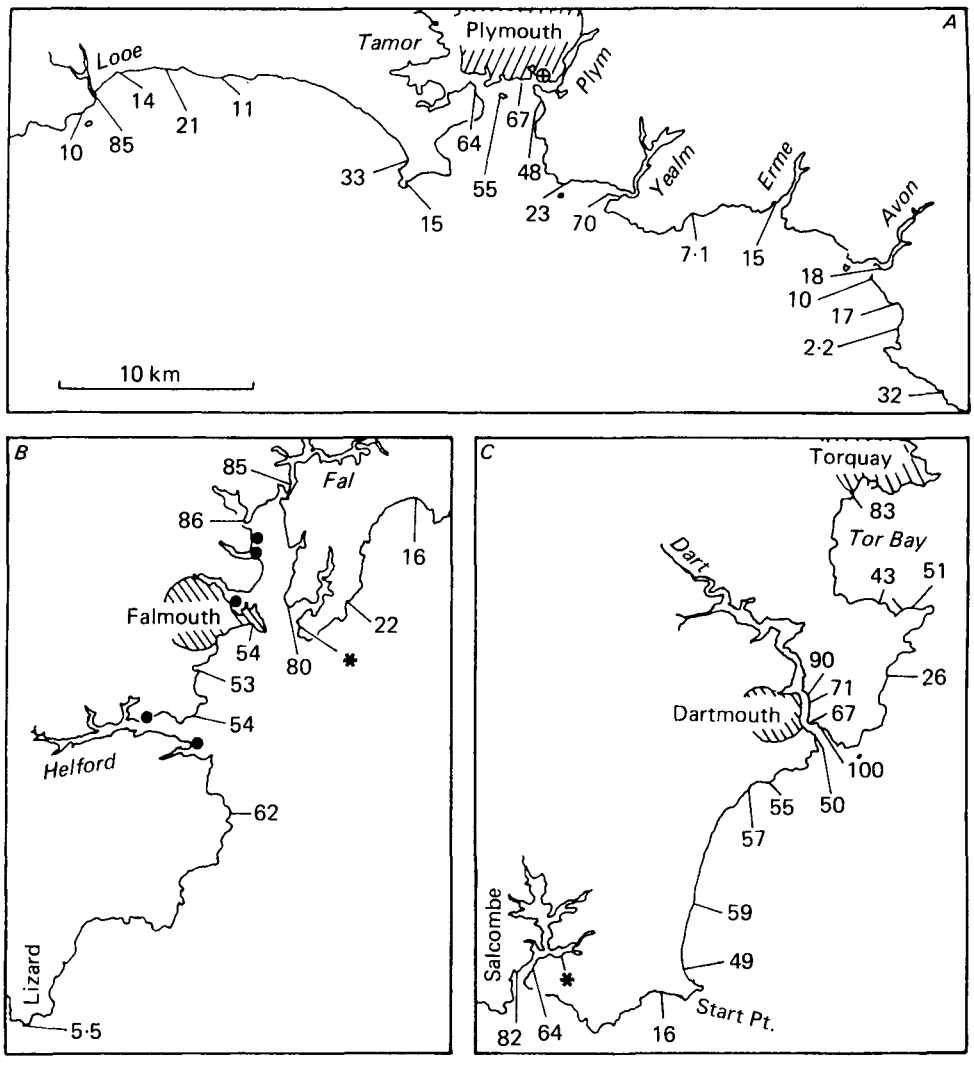

Fig. 4. *N. lapillus*. Geographical distribution of degrees of imposex in three affected areas (see Fig.3). ●, Sites at which animals formerly existed but could not be found in 1984–5; *, a few males only present; ⊕, transplant site at Plymouth.

fewer than 50% of the females are visibly affected (Table 1). At nearby sites, such as St Agnes (mean imposex 0·6%), the percentages of affected females range from 56 to 90 (Table 1). Generally speaking, when degrees of imposex exceed 2%, more than 80% of females are visibly affected, and at levels higher than 5% it is extremely rare to find a female lacking a penis (Fig. 5).

On the north coast, populations in which the degree of imposex is 50% or more are confined to the harbours at St Ives (50%), Padstow (62%) and Ilfracombe (56%): moderate values are associated with Bude (16%) and Appledore (28%).

Table 1. *N. lapillus. Degrees of imposex at different times of year (♀ penis length³/♂ penis length³ × 100)*

Values in parentheses are percentages of penis-bearing females if less than 100%.

Site	Map reference	1984 Dec.	1985 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
North coast														
Porth Joke	SW 768 607	—	—	0·037 (36%)	—	—	< 0·01 (9%)	—	0·10 (36%)	—	—	0·026 (42%)	—	< 0·01 (25%)
St Agnes	SW 722 518	—	—	0·27 (56%)	0·67 (69%)	—	—	—	0·30 (61%)	—	—	1·29 (90%)	—	—
Widemouth Bay	SS 194 015	—	—	—	—	—	1·57 (95%)	1·59	—	1·32 (85%)	0·62 (85%)	0·79 (93%)	0·78 (92%)	—
Crooklets Beach, Bude	SS 201 072	—	—	—	—	—	—	1·45 (71%)	0·92 (75%)	—	—	2·16 (76%)	—	3·63 (86%)
St Ives Harbour	SW 519 405	—	—	54·7	—	—	—	—	—	—	46·4	—	—	—
South coast														
Penzance	SW 467 292	—	41·8	—	—	—	38·0	—	—	—	50·0	—	—	43·9
Maenporth	SW 792 295	—	—	—	—	—	—	31·3	—	—	—	34·4	—	—
Castle Drive, Falmouth	SW 822 317	—	—	51·8	—	—	—	54·3	—	—	—	56·2	—	—
King Harry Ferry	SW 841 396	—	—	71·0	—	—	—	66·7	—	—	—	—	—	—
St Mawes Castle	SW 840 326	—	—	60·0	—	—	—	62·3	—	—	—	—	—	—
Mount Edgcumbe	SX 457 532	63·4	55·7	—	47·7	—	—	77·7	—	—	80·5	—	—	—
Kingswear (Upper Ferry)	SX 882 522	—	—	—	—	—	105	—	76·4	—	—	—	—	—
Kingswear (Embankment)	SX 883 516	—	—	—	—	—	79·6	—	62·2	—	—	—	—	—

South coast (English Channel) (Figs. 3, 4A, B, C)

On the Channel coast the incidence of imposex in females is virtually 100% at all sites: just two females lacking a penis were recorded from this sector of the survey. Populations having a level of imposex in excess of 50% are common, particularly in the vicinity of deep-water harbours, as for example at Plymouth (Fig 4A: naval dockyard, commercial docks, marinas), Falmouth (Fig 4B: marinas, commercial docks, repair yards, laid-up shipping) and Dartmouth (Fig. 4C: marinas).

Distributional evidence for the cause of imposex

Results from the north and south coasts of the south-west peninsula form a striking contrast. On the north coast, which is exposed directly to the waters of the Atlantic, the degree of imposex is generally low, high values being confined to the immediate vicinity of the harbours, which are few in number and comparatively small. The south coast, on the other hand, is generally less exposed to wave action and its many harbours shelter a very large number of craft of all types. As on the north coast, the degree of imposex is highest in the vicinity of harbours: unlike the north coast, however, high degrees of imposex are not necessarily confined to harbours but sometimes encompass large sections of adjacent coastline, as for example between Dartmouth Harbour and Start Point (Fig. 4C).

Although the highest degrees of imposex are usually encountered in the sheltered waters of large estuarine harbours, they are not necessarily characteristic of estuarine populations. Thus it is possible to find degrees of imposex at some coastal sites (Fig. 4C) far exceeding those in shallow estuaries like those of the Erme and Avon which shelter relatively few boats (Fig. 4A).

Imposex was first recognized in *N. lapillus* by Blaber (1970), who observed that in 1969–70 its incidence in females in Plymouth Sound was 75–100% at a site in front of the Plymouth Laboratory, and 20–38% at Rum Bay, some 2 km distant. Based on Fig. 5, these ranges represent degrees of imposex of less than 5% at the Laboratory site and less than 0·1% at Rum Bay, whereas in 1985 levels at the same two sites reached 67% and 48% respectively (Fig. 4A). In addition, examination of preserved material showed that four female *N. lapillus* transplanted by Moore (1936) from North Cornwall to Drake's Island in Plymouth Sound (present degree of imposex 55%) for 9 months in 1935–6 showed no sign of penis development, although appreciable shell growth was observed. Such a dramatic increase in the degree of imposex, particularly over the past 16 years, suggests that it is probably caused by a pollutant, the level of which has increased markedly since 1969. Furthermore, although other sources such as sewage outfalls have to be considered, the coincidence between high degrees of imposex and proximity to harbours and marinas indicates that the pollutant is almost certainly associated with shipping or boating activity.

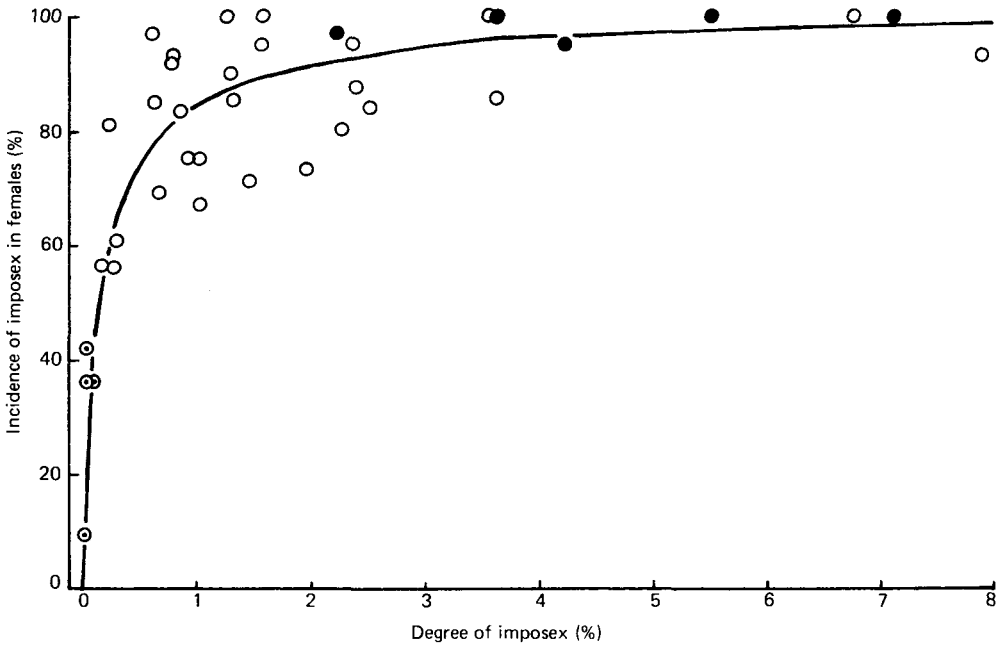

Fig. 5. *N. lapillus*. Relationship between incidence of imposex (percentage of penis-bearing females) and degree of imposex (bulk of female penis as a percentage of male); ⊙, Porth Joke; ○, other north coast sites; ●, south coast sites.

Relations between heavy metals and imposex

Metals from industrial sources

Based on evidence from six populations of *N. lapillus* in the northeastern United States, Miller & Pondick (1984) concluded that the incidence of imposex in females was related to the proximity of the animals to industrial discharges, sewage effluent or vessel-related activities. In addition, analyses of metals in the tissues showed that elevated levels of copper, and possibly zinc, were related to the presence of imposex whereas levels of cadmium, chromium and iron were not: concentrations of tin were undetectable ($< 0{\cdot}5$ μg/g dry weight).

Particularly as a result of metalliferous mining and smelting, the waters around south-west England receive considerable inputs of metals, including copper, zinc and cadmium (Bryan & Gibbs, 1983). As a result, concentrations of these and other metals in *N. lapillus* tissues vary, sometimes dramatically, between different localities (Bryan *et al.* 1985). Comparisons between tissue metal levels and indices of imposex are illustrated in Fig. 6, but there is no evidence that they cause imposex. Thus animals from the Bristol Channel coast containing high levels of silver, cadmium and zinc display low degrees of imposex (1·3 and 6·8%); so also do *N. lapillus* from St Ives Bay, west of Newquay, which are heavily contaminated with copper from tin-mine drainage (1 and 5%). Similarly, neither lead nor arsenic appears to be implicated in the induction of imposex.

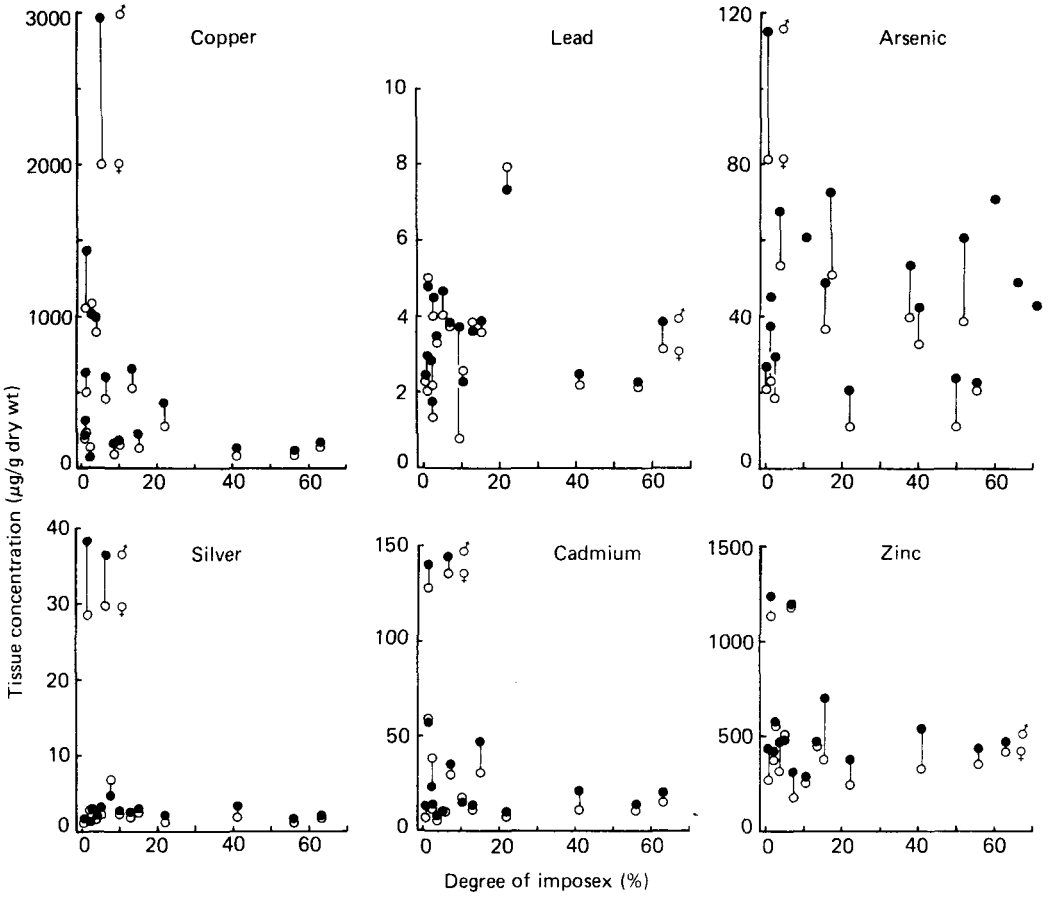

Fig. 6. *N. lapillus*. Comparisons between tissue metal concentrations and degree of imposex in populations from south-west England (winter 1984–5).

Total tin and imposex

In contrast to other metals, total concentrations of tin in the body, although low, are very significantly related to the degree of imposex in *N. lapillus* (Fig. 7). Furthermore, laboratory experiments by Smith (1981 *b*) have shown that imposex can be induced in *Nassarius obsoletus* by exposure to solutions of tributyltin oxide, the main toxic constituent of many modern marine antifouling paints. These observations, coupled with the apparent relation between imposex and shipping, provide a *prima facie* case for linking imposex in *N. lapillus* with exposure to tributyltin compounds from antifouling paints. Furthermore, the increased level of imposex in the Plymouth area over the past 16 years reported above coincides remarkably well with the introduction and increasing usage of tributyltins in antifouling paints (cf. Stebbing, 1985).

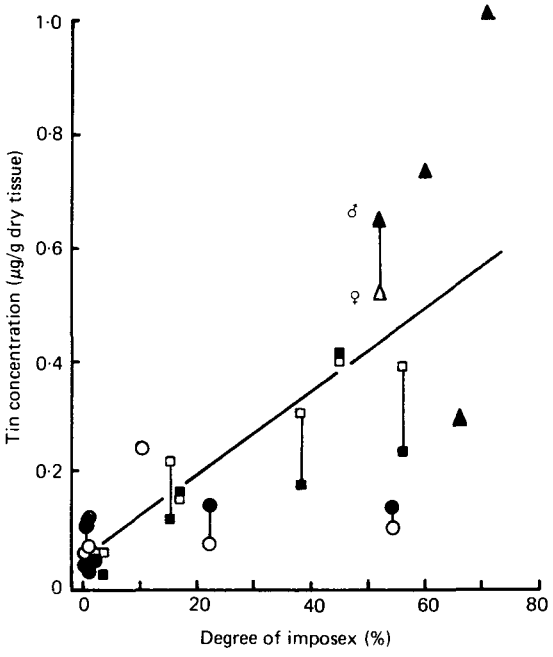

Fig. 7. *N. lapillus*. Relationship between tin concentration in tissues and the degree of imposex (winter 1984–5): ●, ○, north coast sites; ▲, △, Falmouth area; ■, □, south coast sites. Equation of line: tin concentration = 0·00747 imposex + 0·039 ($n = 33$; $r = 0·757$; $P < 0·001$). Pooled samples used.

Imposex and tributyltin compounds

Field observations

Studies on the metabolism of tributyltin compounds in fish and crustaceans indicate that following bioaccumulation they are degraded to dibutyl, monobutyl and inorganic tin species (Ward *et al.* 1981; Lee, 1985). Tributyltin and dibutyltin fractions in fish tissues were separated from monobutyl or inorganic tin species by extracting an acidified homogenate with hexane, which was then analysed for tin before and after the removal of the dibutyltin fraction by shaking with sodium hydroxide solution (Ward *et al.* 1981). Application of a similar procedure to the tissues of *N. lapillus* from different sites shows that concentrations of tin in the tributyl or dibutyl fractions increase with the degree of imposex (Fig. 8). It should be noted that the methods employed are not necessarily specific for tributyl or dibutyl tin species and other compounds might possibly be present: however, there is certainly a relationship between the degree of imposex and levels of hexane-extractable tin in *N. lapillus*. Clearly, some of this tin is in the dibutyl rather than in the tributyl form (Fig. 8). Whether the dibutyltin fraction arises from the degradation of tributyltin compounds by *N. lapillus* is uncertain: degradation of tributyltin compounds to dibutyltin species has been observed in molluscs (Lee, 1985), but probably also occurs both in the surrounding water and in the diet of barnacles or mussels.

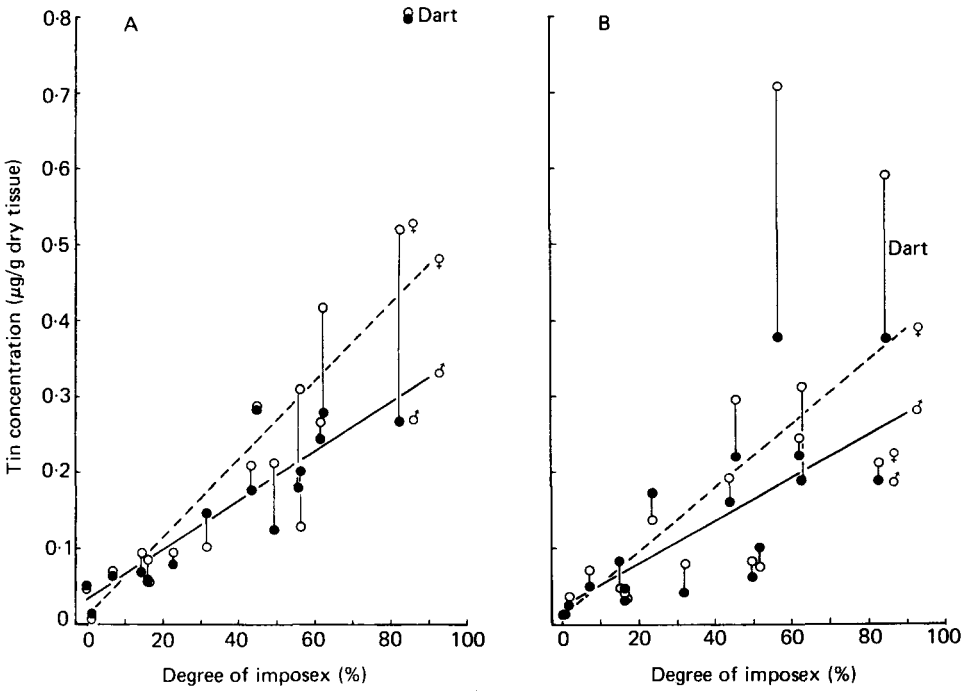

Fig. 8. *N. lapillus*. (A) Relationships between tissue concentrations of tin as tributyltin oxide (TBT fraction) and degrees of imposex in males and females (autumn 1985). Equations of lines (excluding Dart values): tin concentration female = 0·00534 imposex − 0·0067 ($n = 16$; $r = 0·911$; $P < 0·001$); tin concentration male = 0·00334 imposex + 0·025 ($n = 16$; $r = 0·903$; $P < 0·001$). (B) Relationships between tissue concentrations of tin as dibutyltin dichloride (DBT fraction) and degrees of imposex in males and females (autumn 1985). Equations of lines (excluding Dart values): tin concentration female = 0·00419 imposex + 0·0136 ($n = 16$; $r = 0·594$; $P < 0·02$); tin concentration male = 0·00280 imposex + 0·0248 ($n = 16$; $r = 0·698$; $P < 0·01$). Pooled samples used.

Transplant experiments

Further evidence of a relationship between imposex and hexane-extractable tin in *N. lapillus* comes from an experiment involving the transplantation of over 300 mature *N. lapillus* from St Agnes (mean imposex 0·6 %), one of the least-affected sites (Fig. 3), to a rocky shore in Plymouth Sound (Queen Anne's Battery; Fig. 4A) at the entrance to Sutton Harbour (trawler quay and two yacht marinas). In this harbour, sea-water tin concentrations of < 0·04–0·35 μg/l, probably as tributyltin species, have been reported (Cleary & Stebbing, 1985). Although present some years ago, no native *N. lapillus* was recovered from the transplant site. Fig. 9 shows how the development of imposex in the transplanted animals coincides with the accumulation of hexane-extractable tin in the tissues: in May, June, September, November and February the contributions of tin in the tributyltin fractions to the totals were respectively 67, 60, 67, 63 and 62 % in the females and 68, 66, 70, 63 and 61 % in the males. In a second experiment, animals from Penzance (imposex 38 %) were transplanted to Porth Joke (mean imposex 0·05 %) in May 1985 (Fig. 3). After 57 d the imposex level was 46·8 % : evidently,

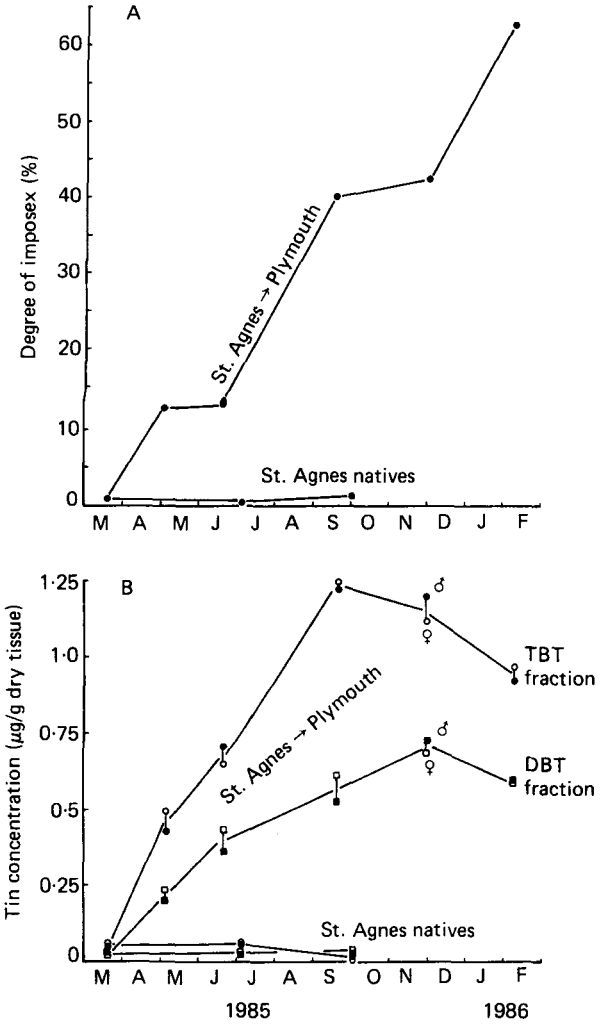

Fig. 9. *N. lapillus*. Transplantation of St Agnes animals to a Plymouth marina site. Effect on (A) the degree of imposex and (B) the tin concentrations in the TBT and DBT fractions in males and females. Pooled samples used.

the female penis is not rapidly resorbed. These observations help to explain why at some sites the degree of imposex does not change appreciably during the year although concentrations of tin in the water almost certainly rise during the spring and summer and fall during the winter. Particularly in *N. lapillus* populations possessing a high degree of imposex, concentrations of hexane-extractable tin increase markedly during the spring when boats newly antifouled are launched for the summer season. At the Castle Drive site close to the Fal Estuary (Table 3), the concentration of hexane-extractable tin in the animals more than doubled between February and June 1985, whilst at the Upper Ferry and Embankment sites on the Dart Estuary (Table 5), levels in both sexes almost doubled between May and July 1985.

Thus the evidence from field observations and transplant experiments, although not totally conclusive, certainly supports the contention that imposex is caused by organotins originating from the tributyltin compounds used in antifouling paints. Although tissue analyses indicate that both tributyltin and dibutyltin fractions are present, the former is probably the effective agent, since its toxicity to organisms generally far exceeds that of the latter, which is probably a degradation product (Laughlin & Linden, 1985; Laughlin *et al.* 1985).

Tank experiment

Table 2 summarizes results from a tidal tank experiment in which *N. lapillus* were exposed to 0·02 μg/l of tin leached as tributyltin species from a co-polymer antifouling paint. In 4 months, animals of both sexes accumulated more than 1 μg/g of tin in the tributyl fraction and achieved a degree of imposex of 40·6% (and still rising). If anything, the experiment will tend to underestimate the influence of dissolved tin, since the diet of barnacles was initially uncontaminated, thus reducing its importance as an uptake route. Animals transplanted to a Plymouth marina site gave comparable results after 6 months (Feb.–Sept.; Fig. 9), suggesting that over this period the mean sea-water level of tin in the tributyl form was rather less than 0·02 μg/l. Results in Fig. 9 also indicate that an appreciable time lag exists between the attainment of peak tin levels and the maximum expression of imposex. This is supported by an observation that animals from the Dart Estuary, exhibiting a degree of imposex of around 100% in September 1985, contained concentrations of tin in the tributyl fractions (male 1·03 μg/g; female 1·20 μg/g) comparable with experimental values (Table 2; Fig. 9). Moreover, concentrations of hexane-extractable tin in the water at this site were 0·013 and 0·014 μg/l in July and August 1985.

These field and experimental observations indicate that a high degree of imposex can be equated with tin concentrations (as tributyl species) of 0·01–0.02 μg/l in the water or 1–2 μg/g dry weight in the tissues: this gives a concentration factor (dry tissue/water) of about 100000. Since concentrations of tin in slightly affected *N. lapillus* are of the order of 0·1 μg/g (Table 2), it is reasonable to suppose that imposex is initiated by lower concentrations, say 0·05 μg/g: based on a conservative concentration factor of 50000, this is equivalent to a water concentration of 0·001 μg/l of tin in the tributyltin fraction. In the tank experiments, the control animals were exposed to about 0·0005 μg/l of hexane-extractable tin but exhibit increasing levels of imposex and tissue tin concentrations (Table 2): although the increase in imposex is insignificant, there is a significant rise in the level of hexane-extractable tin.

The decline of N. lapillus populations around south-west England

Evidence of decline

It was discovered that *N. lapillus* are now (1985) rare at several sites at which they were relatively common during the period 1967–73: one such locality is the rocky shore at Tinside below the Plymouth Laboratory. A search of the literature

Table 2. *N. lapillus. Influence of exposure to 0.02 μg/l of tin leached from antifouling paint on hexane-extractable tissue tin levels and on the degree of imposex*

	Control (~ 0·0005 μg/l)					Experimental (0·02 μg/l)				
			Hexane-extractable tin (μg/g dry weight)†					Hexane-extractable tin (μg/g dry weight)†		
Days	Imposex (%)	Sex	TBT fraction	DBT fraction	Total	Imposex (%)	Sex	TBT fraction	DBT fraction	Total
0	2·0	♂	0·074	0·023	0·097	2·0	♂	0·074	0·023	0·097
		♀	0·087	0·021	0·108		♀	0·087	0·021	0·108
31	3·7	♂	0·082	0·054	0·136*	13·3**	♂	0·476*	0·069*	0·545*
		♀	0·117	0·012	0·129		♀	0·452	0·056	0·508
91	5·8	♂	0·126	0·077	0·203*	27·5**	♂	0·618*	0·278*	0·896*
		♀	0·182	0·039	0·222		♀	0·790	0·197	0·987
120	4·6	♂	0·131*	0·056*	0·187*	40·6**	♂	1·104*	0·247	1·351*
		♀	0·117	0·048	0·165		♀	1·641	0·014	1·654

† To convert to wet wt basis divide by 3·3 for males and 3·0 for females.
* Paired male and female values significantly different from day 0 in *t* test ($P < 0·05$).
** Significantly different from day 0 value ($P < 0·05$).

was made for data on previous abundances around south-west England. The work of Crothers (1975) proved very useful since, although densities are not given, he routinely collected 100 animals at most sites, thus implying that the species was present in moderate to high numbers. For example, Crothers collected 100 animals at each of three sites in the Fal Estuary (Falmouth Harbour, Restronguet Point, King Harry Ferry) which in 1985 yielded a total of only 20 animals during two searches. Furthermore, only three males were found during an inspection of a fixed transect at the mouth of the Fal Estuary (Amsterdam Point), which in 1974–5 had densities of 1–10/m^2 with occasionally higher values (Cornwall Sea Fisheries Committee, 1976). Table 3 summarizes recent data from the Falmouth area and compares them with previous evidence of abundance. The results point to an appreciable decrease of adult numbers, especially of females, and the presence of few immature animals between the eastern approaches to the Fal Estuary and an area to the south of the Helford Estuary.

In the Plymouth area, evidence of declining populations is most obvious within Plymouth Sound (Table 4). As already mentioned, the species is relatively rare below the Plymouth Laboratory where, formerly, animals of all sizes could easily be collected (Fig. 10D). At other sites, including Drake's Island, Rum Bay and Mount Edgcumbe, *N. lapillus* is not yet uncommon: however, the populations consist almost wholly of old animals, with very few immature or juvenile specimens (Fig. 10C). Since *N. lapillus* matures at 2·5–3 years old, most of these animals must be at least 4 years old, but many appear considerably older. Thus it would seem that in Plymouth Sound, declining populations result perhaps from a failure to recruit young animals and not necessarily from an increased mortality rate. Possibly reproduction is in some way inhibited or the juveniles are being killed. This phenomenon is not so obvious on the coast outside Plymouth Sound, and at Seaton, about 12 km to the west, samples collected in both 1967 and 1985 reveal appreciable numbers of young individuals (Fig. 10A, B).

More evidence of depleted populations comes from sites in the Salcombe and Dart estuaries and from Tor Bay (Table 5; Fig. 4C). Again, compared with about 15 years ago, populations at several localities are very low, consisting mainly of old animals with a tendency for males to predominate.

All three sections of coast considered above show evidence of declining populations, and stretches of rocky coast totalling at least 100 km seem to be affected. This is most obvious in the sheltered waters of the major estuaries, where at present only small numbers of old animals are found and young animals are rare or absent. At a few sites, in Plymouth Sound for example, *N. lapillus* remains reasonably common but, again, recruitment appears to be very low. However, significant numbers of young animals have been found at some apparently affected sites, including Maenporth (Table 3) and Castle Rocks, Salcombe (Table 5), where small populations are confined to a few rocky gullies although a very much wider area appears ideal for colonization. It is uncertain whether the presence of some juveniles reflects the existence of a small breeding population or whether they are being recruited from elsewhere. *N. lapillus* lacks a planktonic

Table 3. *N. lapillus. Evidence of declining populations in the Falmouth area (sites run from west to east in Fig. 3B)*

Mean degree of imposex in Fig. 3B	Site	Map reference	Sampling date	Presence near site of boats (+) and commercial shipping (●)	Collection effort (man hours)	No. of mature animals collected if less than 30*	Percentage ♀ in mature population	Presence of immature animals†	Comments on recent population abundance	Evidence of previous abundance
5·5	Lizard	SW 703 115	13 Oct. 85	—	¼	> 30	45·0	Present	Probably normal	—
62	Porthoustock	SW 810 221	13 Oct. 85	+	½	9	70·0	Few	Low population may be limited by lack of crevices	—
—	St Anthony	SW 783 255	13 Oct. 85	+ + +	½	0	—	0	Absent	Common on 24 Oct. 1976 (N. A. Holme, S. M. Turk)
—	Helford Passage	SW 764 268	16 Oct. 85	+ + +	1	0	—	0	Absent	100 collected in Apr. 1973 (Crothers, 1975)
54	Mawnan Church	SW 790 270	16 Oct. 85	—	1	> 30	45·0	Few	Low population confined to only small area of suitable environment	100 collected in Apr. 1973 (Crothers, 1975)
33	Maenporth	SW 792 295	19 June 85	—	1	18	27·8‡	Few	Low population confined mainly to one gully. Low % ♀	—
			16 Oct. 85	—	1	17	29·4‡	Few		
54	Castle Drive	SW 822 317	19 Feb. 85	—	½	> 30	40·0	Present	Common with many old animals and some younger specimens. Signs of falling % ♀	Transects of 1974–5 show 1–10/m², occasionally higher (*Cornwall Sea* Fisheries Committee, 1976)
			19 June 85	—	½	> 30	40·0	—		
			19 Oct. 85	—	½	> 30	29·0‡	Present		
—	Falmouth Harbour	SW 813 324	7 Feb. 85	+ + ●●	1	0	—	0	Absent	Present in Feb. 1972. 100 collected in Apr. 1973 (Crothers, 1975)
			19 June 85		½	0	—	0		
—	Mylor Harbour	SW 818 353	7 Feb. 85	+ + + +	1	0	—	0	Absent	Present on pier in Feb. 1972.
—	Weir Point	SW 819 367	7 Feb. 85	+ +	1	0	—	0	Absent	Present in Feb. 1972.
86	Restronguet Point	SW 817 370	7 Feb. 85	+ + +	2	3	33	0	Few animals remain; ♂ penis deformed	100 collected in Apr. 1973 (Crothers, 1975). Reasonably common in Mar. 1979.
			19 June 85		1	0	—	0		

85	King Harry Ferry	SW 841 396	24 Feb. 85	+●●●	1	6	16·7	0	Low populations of old animals; low % ♀; *penis* deformed in some ♂	100 collected in June 1970 (Crothers 1975)
			19 June 85		½	11	9·1‡	0		
80	St Mawes Castle	SW 840 326	18 Feb. 85	+●	1	11	27·3‡	0	Low populations of old animals; low % ♀; penis deformed in some ♂	—
			19 June 85		1	24	16·7‡	0		
♂ only	Amsterdam Point	SW 848 322	15 Oct. 85	+●	2	3	0	0	Few animals remain	Transects of 1974–5 show 1–10/m², occasionally higher (Cornwall Sea Fisheries Committee, 1976)
22	Towan Beach	SW 870 328	15 Oct. 85	—	½	> 30	60·0	Common	Possibly normal	—
16	Pendower	SW 897 380	15 Oct. 85	—	½	> 30	50·0	Common	Possibly normal	100 collected in June 1970 (Crothers, 1975)

* If possible, a minimum of 30 mature animals was collected.
† Smaller animals lacking thickened edge to shell.
‡ Percentage of females significantly different from 56·8% (see Fig. 11) ($P < 0·05$).

Table 4. *N. lapillus. Evidence of declining populations in the Plymouth area (sites run from west to east in Fig. 3A)*

Mean degree of imposex in Fig. 3A	Site	Map reference	Sampling date	Presence near site of boats (+) and commercial shipping (●)	Collection effort (man hours)	No. of mature animals collected if less than 30*	Percentage ♀ in mature population	Presence of immature animals†	Comments on recent population abundance	Evidence of previous abundance
10	Hannafore	SX 252 521	12 Jan. 85	+	1	> 30	30·0‡	Present	Possibly normal, but low % ♀	—
84	Looe Harbour	SX 254 534	12 Jan. 85	+ + ● ●	½	12	58·0	0	Old animals; penis of ♂ deformed	—
14	Millendreath	SX 271 540	28 Sept. 85	—	½	> 30	75·0‡	Common	Large probably normal population	—
21	Seaton	SX 296 541	28 Sept. 85	—	½	> 30	50·0	Fairly common	Max. density of 36/m² comparable with 1968–9	Common in 1968–9 (Bryan, 1969)
11	Downderry	SX 310 540	2 Dec. 84	—	1	29	53·3	Common	Possibly normal	100 collected Oct. 1969 (Crothers, 1975)
33	Rame Head West	SX 415 483	10 July 85	—	—	16	43·7	?		—
15	Rame Head East	SX 421 482	8 Dec. 84	—	¼	> 30	62·9	Present	Possibly normal	—
64	Mount Edgcumbe	SX 457 532	8 Dec. 84	+ + ●	½	> 30	44·7	Rare	Population composed almost totally of old animals; low % ♀	—
			13 Jan. 85		½	22	36·4	Rare		
			24 Mar. 85		½	20	25·0‡	Rare		
			17 June 85		½	> 30	22·2‡	Rare		
			17 Sept. 85		½	> 30	29·0‡	Rare		
55	Drake's Island									
66	west	SX 467 529	30 Oct. 85	+ ●	½	> 30	45·0	Rare	Fairly common, mainly old animals	—
59	north	SX 468 529	30 Oct. 85	+ ●	½	> 30	56·7	Rare	Fairly common, mainly old animals	800 collected by Moore (1936)
52	east	SX 471 527	30 Oct. 85	+ ●	½	> 30	75·0‡	Few	Fairly common, mainly old animals	—
45	south	SX 468 527	30 Oct. 85	●	½	> 30	45·0	Rare	Fairly common, mainly old animals	—
67	Plymouth Laboratory	SX 467 537	10 Apr. 85	+ ●	1	3	0	0	Small population; low % ♀	Very common in 1967–8
			19 Apr. 85		1	2	0	0		
			2 Jan. 86		½	8	37·5	4 found		
48	Rum Bay	SX 488 527	4 July 85	●	1	> 30	14·3‡	Rare	Fairly common; mainly old, low % ♀	Collected by Blaber (1970)
23	Wembury Point	SX 501 481	5 Oct. 85	—	1	> 30	20·0‡	Common	Possibly normal, but low % ♀	—

* If possible, a minimum of 30 mature animals was collected. † Smaller animals lacking thickened edge to shell.
‡ Percentage of females significantly different from 56·8% (see Fig. 11) ($P < 0·05$).

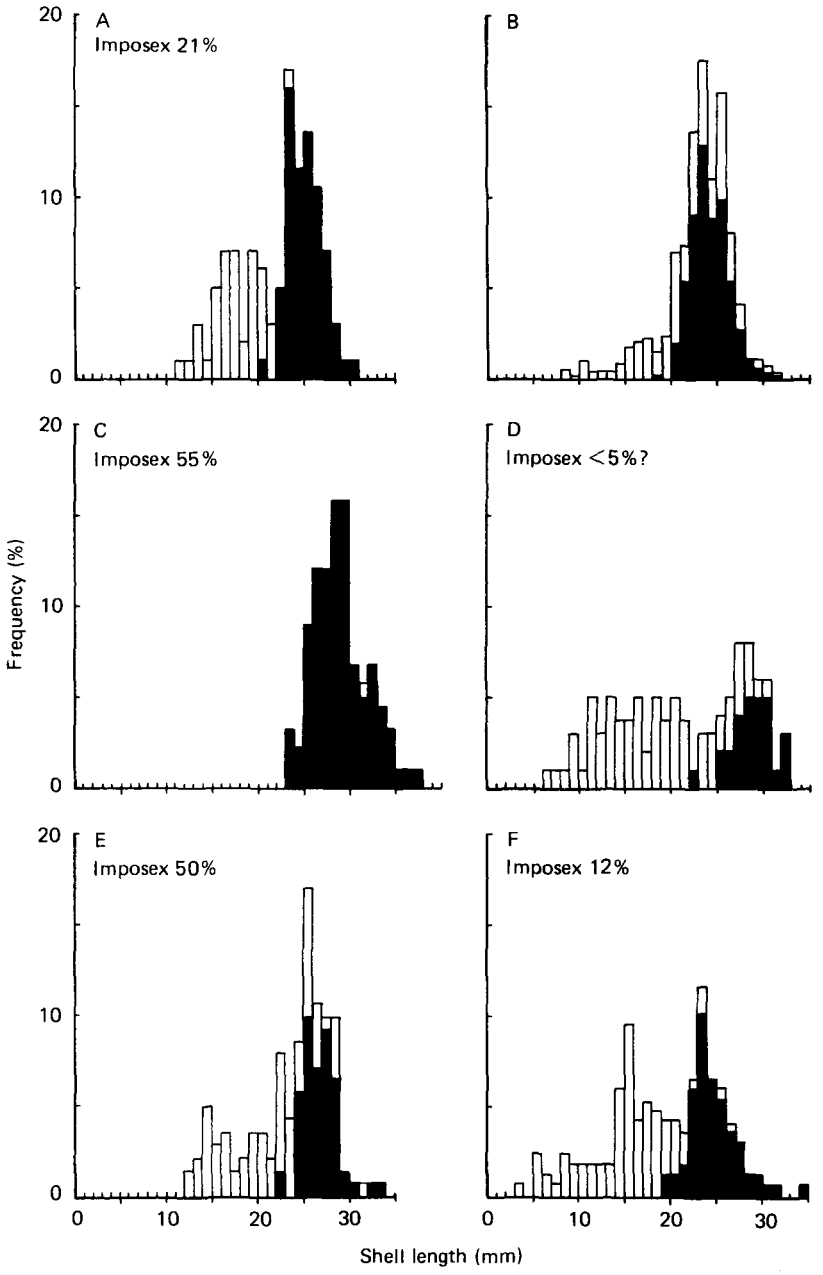

Fig. 10. *N. lapillus*. Size-frequency distributions of populations. Black areas denote older mature animals bearing internal teeth on lip of shell. Dates and degrees of imposex also shown. (A, B) Comparison between Seaton population in September 1985 ($n = 106$; teeth = 66%) and November 1968 ($n = 549$; teeth = 57%). (C, D) Comparison between sites on Drake's Island in October 1985 ($n = 90$; teeth = 99%) and that in front of the Plymouth Laboratory (1 km distant) in November 1967 ($n = 101$; teeth = 30%): insufficient numbers were available at the latter site in 1985. (E, F) Comparison between Penzance population (September: $n = 141$; teeth = 43%) and that at Porthleven (15 km farther east) in November 1985 ($n = 168$; teeth = 43%).

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Table 5. *N. lapillus. Evidence of declining populations in Salcombe, Dartmouth, Torquay areas (sites run from west to east in Fig. 3C)*

Mean degree of imposex in Fig. 3C	Site	Map reference	Sampling date	Presence near site of boats (+) and commercial shipping (●)	Collection effort (man hours)	No. of mature animals collected if less than 30*	Percentage ♀ in mature population	Presence of immature animals†	Comments on recent population abundance	Evidence of previous abundance
82	Castle Rocks	SX 734 381	30 Oct. 85	+	1	> 30	34·4‡	Present	Low population confined to small area only of suitable environment; penis of some ♂ deformed	100 collected Mar. 1970 (Crothers, 1975). Common Sept. 1973 (Bantock & Cockayne, 1975)
♂ only	Southpool Creek	SX 754 391	5 May 85	+ +	1	1	0	0	One animal found	12 collected Apr. 1973 (Crothers, 1975)
64	Millbay	SX 738 381	5 May 85	+	1	16	12·5‡	0	Low population of old animals; low % ♀	327 collected by Moore (1936)
16	Lannacombe	SX 803 371	12 Dec. 85	–	½	> 30	46·1	Present	Possibly normal	100 collected Apr. 1973 (Crothers, 1975)
49	Hall Sands	SX 818 383	12 Dec. 85	–	½	> 30	34·4‡	Present	Low population may be limited by lack of crevices	—
59	Torcross	SX 824 416	30 Sept. 85	–	½	> 30	60·0	Present	Common; very large animals	100 collected in Mar. 1970 (Crothers, 1975)
57	Blackpool Sands west	SX 853 476	5 May 85	–	½	> 30	43·3	?	Common	100 collected in June 1973 (Crothers, 1975)
55	Blackpool Sands east	SX 859 478	30 Sept. 85	–	1	> 30	45·0	Few	Low population confined to small area	—

50	Dartmouth Castle	SX 887 501	18 Aug. 85	+●	1	2	50	0	Very low population	100 collected in Mar. 1970 (Crothers, 1975)
90	Kingswear Upper Ferry	SX 882 522	21 May 85	+●●	1	9	44·4	0	Low population of old animals; penis of some ♂ deformed	100 collected in Mar. 1970 (Crothers, 1975)
			22 July 85		½	5	60·0	0		
71	Kingswear Embank-ment	SX 884 517	21 May 85	++++	1	21	28·6‡	0	Low population of old animals; penis of some ♂ deform-ed; low % ♀	—
			22 July 85		1	7	42·8	0		
67	Kingswear Lower Ferry	SX 881 510	30 Sept. 85	++●	½	3	33·3	0	Low population of old animals; penis of one ♂ deformed	—
100	Kingswear Light	SX 886 507	21 May 85	+	1	0	—	0	Low population of old animals; penis of some ♂ deformed	100 collected July 1972 (Crothers, 1975)
			18 Aug. 85		1	0	—	0		
			30 Sept. 85		2	2	50	0		
			12 Dec. 85		2	13	46·1	0		
26	Man Sands	SX 924 533	12 Dec. 85	—	½	> 30	42·8	Common	Common, possibly normal	—
51	Shoalstone Point	SX 938 568	12 Dec. 85	+●	1	> 30	21·9‡	Rare	Low population of old animals; low % ♀	100 collected Mar. 1970 (Crothers, 1975)
43	Churston Cove	SX 919 569	21 Jan. 85	++●	1	20	30·0‡	Present	Small population with young animals present	—
83	Corbyn's Head	SX 908 632	21 Jan. 85	++	2	18	11·1‡	0	Low population of old animals: low % ♀	100 collected Mar. 1970 (Crothers, 1975)

* If possible, a minimum of 30 mature animals was collected.
† Smaller animals lacking thickened edge to shell.
‡ Percentage of females significantly different from 56·8% (see Fig. 11) ($P < 0·05$).

larva, but juveniles recently emerged from egg capsules at one site may reach other sites on pieces of floating debris carried by currents.

Causes of decline

Possible causes for the decline in *N. lapillus* numbers over the past 10–15 years include climatic factors, food shortages, diseases, effects of toxic phytoplankton blooms and pollution. There is no evidence that a climatic change is responsible, and no shortage of barnacles or mussels, the principal prey of *N. lapillus*, has been observed. It was noticed that the tissues of animals from declining populations were frequently tinged with orange or brown pigment; however, this may be a characteristic of old animals rather than being directly related to the cause of their decline. Note was always made of individuals heavily infected with sporocyst or cercarial stages of digenean parasites, but examples were rare in those populations that appear to be declining.

Gastropods are sometimes affected by the toxins produced by blooms of dinoflagellates such as *Gyrodinium aureolum* Hulburt (Cross & Southgate, 1980). Such blooms are not uncommon in sheltered bays and estuaries on the English Channel coast during warm summers (Boalch, 1979). They have occurred in the Fal Estuary, and mortalities of sediment-dwelling species were reported in 1982. Thus, although blooms are relatively short-term events and do not occur annually, it is difficult to rule out entirely blooms of toxic phytoplankton as a contributory cause of *N. lapillus* decline in some areas.

There are several pieces of circumstantial evidence tending to link the decline in *N. lapillus* numbers with imposex and hence with tributyltin-based antifouling paints. The development of imposex is an obvious tendency towards the masculinization of the female and might well be expected to affect reproduction as the degree of imposex increases. Depleted populations, or those still fairly abundant in which recruitment appears minimal, all exhibit a degree of imposex exceeding 40%, with a single exception (33%) found at Maenporth (Table 3). At some of the most heavily affected sites, where the degree of imposex exceeds 70% (Tables 3, 5), deformation of the penis is observed in some males (Fig. 1) and, since fertilization is internal (Fretter & Graham, 1962), mating may be precluded. However, the lack of recruitment at lower degrees of imposex points to a break at some other point in the reproductive cycle.

It has been shown experimentally that in the freshwater snail *Biomphalaria glabrata* (Say), egg laying is reduced by exposure to sublethal concentrations of tributyltin (Ritchie, Lopez & Cora, 1974). *Nucella* egg capsules are laid in clutches generally in protected areas such as rock crevices: although the yellow egg capsules are easy to see they are not always easy to find on some types of rocky shore. Notes of the occurrence of egg capsules made during collecting show that on the north coast (Fig. 3) they were found at nine sites, sometimes on several occasions during the year. On the south coast (Figs. 3, 4), egg capsules were noted on only four occasions, although the intensity of searching was much greater and the animals often appeared sexually well-developed. Of the 13 egg-laying

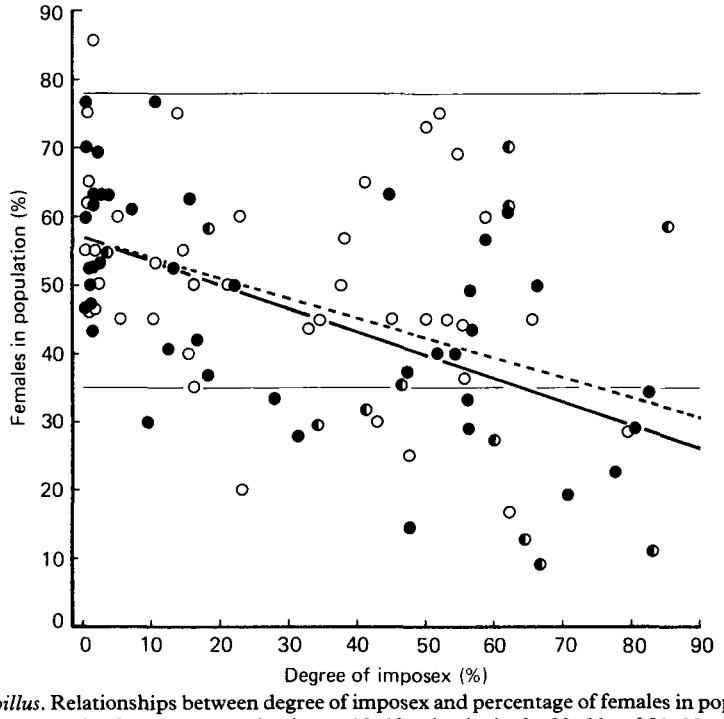

Fig. 11. *N. lapillus*. Relationships between degree of imposex and percentage of females in population. ●, Samples of 30–66 animals; ○, 20–29 animals; ◑, 10–19 animals: in the 30–66 and 20–29 categories, values significantly different from 56·8% at zero imposex are below 35% or over 78% ($P = 0{\cdot}05$). Equations are: for 45 larger samples (●—●); percentage females = −0·34 imposex + 56·9 ($r = 0{\cdot}60$; $P < 0{\cdot}001$); for all 99 samples (----), percentage females = −0·28 imposex + 56·8 ($r = -0{\cdot}47$; $P < 0{\cdot}001$).

populations, 7 exhibited degrees of imposex of 5% or less, but egg capsules were associated with imposex levels of 26% at Man Sands and 57% at Blackpool Sands West, near Dartmouth (Fig. 4C). At present these results are not regarded as conclusive, but point to a need to examine the incidence and viability of egg capsules at a range of sites where *N. lapillus* is still comparatively common, such as Drake's Island (mean imposex 55%, Fig. 10C), where young animals are rare, and Penzance (imposex 50%; Fig. 10E). At this latter site the size–frequency distribution of the population is comparable with that farther east at Porthleven (imposex 12%), where the population (sampled previously in 1969) still appears normal (Fig. 10F). It may be that the population at Penzance is being maintained by juveniles carried on floating material from less affected sites to the east.

Finally, Fig. 11 shows that the percentage of females in a population tends to fall with an increasing degree of imposex. A shortage of females was evident in many of the declining populations (Tables 3–5) and presumably tends to exacerbate the problem. Based on evidence from a North Sea population, Feare (1970) concluded that due to differential mortality the proportion of females increased with age: it is possible, therefore, that the induction of imposex in females reverses this trend.

DISCUSSION

Based on geographical and chemical evidence from the field, there is good reason to believe that the development of imposex in female *N. lapillus* is caused by tributyltin compounds leached from antifouling paints. Around south-west England, a relatively non-industrialized area, there is a remarkably close correlation between increasing degrees of imposex and proximity to harbours and marinas. Yachts and other small craft are certainly involved, since they predominate in some heavily affected areas including the Helford and Salcombe estuaries. However, in areas such as the Fal Estuary and Plymouth Sound the additional influence of commercial shipping cannot be excluded. Supporting evidence comes from other areas also, including the coasts of Wales and Scotland (unpublished), localities in France (Féral, 1980), and several sites in the north-east United States (Miller & Pondick, 1985). Furthermore, a dramatic increase in the degree of imposex observed in two populations in Plymouth Sound between 1960 and 1985 coincides with the recent introduction and increasing usage of tributyltin-based antifouling paints.

Chemical evidence shows that of the metals considered – cadmium, copper, zinc, silver, lead, arsenic and tin – only for tin are the total tissue concentrations significantly related to the degree of imposex. Much of this tin is hexane-extractable and includes tributyltin species and also those of dibutyltin: the latter are generally much less toxic and probably produced by degradation of the former (Thompson *et al.* 1985). Similarly, *N. lapillus* transplanted from a relatively unaffected locality to a marina site developed a high degree of imposex, with tributyltin species being accumulated by the tissues. Furthermore, a tank experiment showed that an appreciable degree of imposex (41 % and rising) is induced when animals are exposed for 120 d to water containing 0·02 μg/l of tin leached from paint containing tributyltin compounds. A similar effect was observed in a field experiment (Bryan & Gibbs, in preparation) in which the shell spires of two groups of *N. lapillus* at a 'clean' locality were separately painted with two TBT antifouling paints of the free-association and co-polymer types: following this treatment the degree of imposex in both groups increased from an initial level of 1·6 % to about 60 % in just 118 d. A third group painted with yacht enamel showed no increase over the same period, nor did unpainted control animals.

Other species of stenoglossan snails in which imposex has been linked to tributyltin include *Nassarius obsoletus* (Smith, 1981*b*) and *Ocenebra erinacea* (L.) (Féral & Gall, 1982). *Nassarius reticulatus* (L.) can be added to the list, since in the Plymouth area this largely sublittoral species exhibits geographical variations in the degree of imposex comparable with those observed in *N. lapillus*.

Smith (1981*a*, *b*) tested a wide range of substances associated with marina activities, including paints, disinfectant, detergent, leaded petrol and exhaust emissions; only tributyltin compounds or paints containing them induced imposex in *Nassarius obsoletus*. If the induction of imposex in *N. lapillus* and other

species is, as seems likely, a specific response to tributyltin compounds resulting from their influence on hormonal balance (Smith, 1981*c*; Féral & Gall, 1982), then the concentrations at which the process is initiated must be remarkably low, bearing in mind the degree of dilution that is available. The influence of very low concentrations is also implied by the fact that no population in which penis-bearing females are completely absent has been found around south-west England and that, further afield, only three such populations have so far been found, one in west Wales and two in Scotland on the islands of Mull and Iona. In the Dart Estuary, where the degree of imposex is high and ranges from 50 to 100%, less than 0·04 μg/l of tin as tributyltin were found in the water during July and September 1984 by Cleary & Stebbing (1985). In 1985 we found mean hexane-extractable tin levels of 0·011, 0·018 and 0·014 μg/l during low water in the lower Dart in May, July and August respectively. That high degrees of imposex are produced by tin concentrations of this order is supported by the tank experiment (0·02 μg/l tin) discussed above and on p. 625. Assuming a linear response between imposex and exposure or body burden (cf. Fig. 8), females are almost certainly sensitive to a concentration 20 times lower, i.e. 0·001 μg/l of tin, equivalent to 0·0024 μg/l of tributyltin oxide.

The lowest concentrations of tributyltin producing deleterious effects in most marine molluscs have been observed to lie in the nanogram per litre range (see review by Thompson *et al.* 1985). The larval stage of the mussel, *Mytilus edulis* L., is one of the most sensitive organisms known to date, with a threshold of toxicity well below 0·1 μg/l of tributyltin oxide or 0·04 μg/l of tin (Beaumont & Budd, 1984). It is also known that shell thickness in the spat of *Crassostrea gigas* is affected by low levels of tributyltin, the no-effect level being less than 0·08 μg/l of tributyltin oxide or 0·03 μg/l of tin (Waldock & Thain, 1983); and in the spat of *Ostrea edulis* shell growth is significantly impaired at levels as low as 0·02 μg/l of tributyltin oxide or 0·008 μg/l of tin (Thain & Waldock, 1985). In fresh water, however, egg laying in the snail *Biomphalaria glabrata* is significantly reduced by as little as 0·001 μg/l of tributyltin oxide or 0·0004 μg/l of tin (Ritchie *et al.* 1974). These are levels to which it is thought *N. lapillus* might prove to be sensitive, otherwise it is difficult to see how the existence of imposex in *N. lapillus* at every site around south-west England can be explained.

Because of its sensitivity to tributyltin compounds, *N. lapillus* appears to be an ideal indicator of the extent of contamination around the coasts of the United Kingdom. Determining the degree of imposex is simple, since it requires only a binocular microscope or even a fairly powerful lens to aid in making the measurements: observations with the naked eye are sufficient for rough estimates. Furthermore, the results tend to be quite clear-cut even if less than 20 animals are available. In addition, transplant experiments with or without cages are feasible provided that a hard substrate covered with food organisms such as barnacles is available and the salinity is not too low: as Fig. 9 shows, increasing development of imposex in adults can continue for at least 11 months.

Based on his very extensive study of the mud snail *Nassarius obsoletus* from the

north-east United States, Smith (1981*b*) concluded that imposex did not appear to have reduced the reproductive capacity of the snails nor to have affected their population ecology. In contrast, comparisons between the present abundance of *N. lapillus* and data going back to 1967 and occasionally earlier, show that numbers of this species have declined appreciably at sites extending over more than 100 km of the English Channel coast. In the Fal and Helford estuaries *N. lapillus* is now absent at a number of localities where it was previously common. Similarly, in the Dart and Salcombe estuaries, populations are low and consist largely of old animals, with a tendency for males to predominate. Even at some sites where *N. lapillus* is still relatively common, there is a high proportion of old individuals, indicating a very low level of recruitment. Invariably, depleted populations are characterized by moderate to high degrees of imposex, although the reverse situation does not always hold. A few populations appear relatively normal whilst exhibiting a moderately high degree of imposex (50%), and it is thought that at such sites animals may be recruited from less contaminated areas nearby.

Reduced recruitment of juveniles in many areas suggests that reproduction is in some way inhibited. At the highest degrees of imposex (> 70%) the male penis is often deformed and mating could be prevented. Also, even at lower degrees of imposex, there is a tendency towards a reduced percentage of females in the population. However, there are probably other reasons also; perhaps egg laying is reduced, as was observed in the fresh-water snail *Biomphalaria glabrata* at tributyltin oxide concentrations of only 0·01 and 0·001 μg/l (Ritchie *et al.* 1974). Although not regarded as conclusive evidence of this hypothesis, in the last two years it has proved remarkably difficult to find egg capsules at most moderately and heavily affected sites on the English Channel coast.

There is no planktonic stage in the life history of *N. lapillus*, the young snails hatching directly from the attached egg capsules. As a result, the populations tend to be self-sustaining and for this reason often exhibit obvious morphological differences, particularly of shell shape (Crothers, 1977, 1985; Kitching, 1985). It is probably this tendency to exist in discrete populations which makes the decline of *N. lapillus* particularly obvious. The small interchange of individuals between populations means that inhibition of reproduction in any *N. lapillus* population is not masked to any great extent by outside recruitment, as it would be in populations of a species having a planktonic larva.

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