
The Dog – Whelk, *Nucella Lapillus* (L.) On The Rocky Shores Of Gower Peninsula, U.K. After The 1987 Tributyltin Restrictive Legislation.

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Abstract

The occurrence of imposex female *Nucella lapillus* (L.) on the Gower Peninsula in 1992 with little development of the vas deferens (VDS) and very small relative penis size (RPS) suggests that, following the restrictive legislation for Tributyltin (TBT) in 1987, only residual levels of less than 1ng/l remain in the sea water close to the relatively sheltered shores near the small boat Mooring, at Mumbles and near Swansea Docks.

These results indicate that UK. Environmental quality target levels of less than 2 ng/l of TBT has been achieved on the Gower Peninsular.

In addition to the occurrence of the imposex females, the level of contamination on sheltered shores may increase penis bulk in males but is insufficient to (a) sterilize the females, (b) reduce population density, (c) affect the sex ratio or (d) to modify the shell characters of the dog-whelk. Some shell characteristics, however, change with variation in exposure to wave action. The more remote exposed shores appear to be free from contamination by TBT.

Introduction

The well documented and extremely harmful effects on many marine organisms by the use of antifouling paints containing tributyltin (TBT) compound, reviewed by Chamb (1986), Rexrode (1987), Dyrinda (1992) and Roesijada *et al* (1991) led to legislation which, by 1987 in the UK. banned the retail sale and use of TBT for small boats and mariculture but still allowed use on large commercial and leisure vessels.

Laboratory experiments on the gastropod *Lepsiella vinosa* showed that imposex can occur not only to TBT exposure, but also to copper-based antifouling paint, acetic acid and environmental stress (Nias *et al*, 1993).

Populations of *N.lapillus* with undetectable levels of TBT in their tissue but elevated levels of of copper have also been found to have developed imposex (Evens *et al*, 1995 & & Batchelor 2001).

Evens *et al*, (2000) found imposex occurring in populations of *N. lapillus* on shores adjacent to seabird roosts. Experiments carried out by Davies *et al*, (1997) discovered that imposex can occur in dogwhelks subject to clean seawater and ethanol.

Amongst the more interesting effects of TBT is the occurrence of abnormal shapes of many mollusks (Rexrode, 1987) and the development of male characters, termed impose by Smith (1971), on the gonochoristic female stenoglossan gastropods such as *Nassarius obsoletus* (Say) and *Nucella lapillus* (L). These phenomena are reviewed by Gibbs *et al* (1987) whose diagram illustrating sex change in the development of impose Fig.3. Sterility occurs only at stage 5 in the vas deferens sequence (VDS) when hyperplasia of the vas deferens tissue occludes the vagina (Fig.3 (iii).

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When TBT concentration reaches 7-10 ng/l (parts per trillion, ppt) of sea water no breeding occurs in *Nucella lapillus* populations but some sterilized females occur at concentrations as low as 1-2 ng/l and impose stages 1-4 (Fig.3 (iii)) were frequent at concentrations lower than 1 ng/l (Gibbs *et al* 1987). The relative penis size (RPS) in impose females is also a sensitive indicator of the contamination of TBT in sea water (Fig.4). There is a little difference in penis size between the sexes at about 20 ng/l of TBT tin (RPS= 96%) but even at < 0.5 ng/l of TBT tin the RPS is about 5% (Gibbs *et al*, 1987).

In addition to the occurrence of impose females, Gibbs *et al* (1988) reported an increase in female mortality and sex ratios dominated by male and Bailey and Davies

(1991) an increase in male penis bulk in populations of *Nucella lapillus* found in areas heavily contaminated by TBT.

The aim of this investigation was to assess the post 1987 sea water TBT contamination on rocky shores around the coast of Gower Peninsula using the population density, sex ratio, occurrence and degree of impose and shell characters of *Nucella lapillus* as indicators. The influence of the degree of exposure to wave action of each shore was also taken into consideration, because the population density and shell characters of *Nucella lapillus* are known to be affected by this parameter (Ballantine, 1961, Moyse & Nelson-Smith, 1963, Crothers, 1973, 1974, 1985).

Materials and Methods

Random samples of adult dog-whelks, *Nucella lapillus* were collected from eight rocky shores on the Gower Peninsula. The degree of exposure to wave action was estimated for each shore using Ballantine's (1961) biologically- defined exposure scale of 1-8, from extremely exposed (1) to extremely sheltered rocky shores (8).

The population density of adult dog-whelk was determined and expressed in Ballantine's (1961) notation of abundance where an abundant (A) population >10 m², common (C) 1-10 m², frequent (F) <1 m² but locally more, occasional (O) always < 1 m² and rare (R) one or two found after 30 minutes search.

The rocky shores include: Rhossili, Oxwich Bay, Middle Head- Mumbles, South –east of Pier, Mumbles, Under Mumbles Pier, Small Boat Mooring, Base of the sea wall of Queen's Dock and Base of Eastern Breakfast-Swansea Docks.

The shell dimensions of *N. lapillus* taken from each shore were measured to the

nearest 0.1 mm (Fig.2). The shell was weighed and the volume determined by water displacement in a measuring cylinder. Calculations were made of shell shape (Length/breadth), relative spire height (length-aperture length x 100) Aperture shape (aperture length/aperture breadth x 10) and shell thickness (whole weight in g/ whole volume in ml).

The shell was smashed with a hammer, the soft parts removed with a forceps and the sex determined as shown in the diagram (Fig.3 (i) + (ii)). The length of the penis was measured in males and imposes females. The mean penis bulk (L³) in males was calculated for each population and the relative penis size (RPS) calculated for each impose female's where:-

$$R.P.S. = \frac{\text{penis length}^3 \text{ in each female} \times 100}{\text{mean penis length}^3 \text{ in males}}$$

The stages in the development of impose (Fig.3 (iii)) based on the vas deferens

sequence (VAS) was also determined for each imposex female.

Nucella lapillus (L) is abundant, as defined by Ballantine (1961), on all the shores examined except on one shore near Swansea Docks (Station 8) where it is common and on the shore near the small Boat mooring, at Mumbles (Station 6) where it is occasional to rare. Specimens are particularly abundant on the nearly vertical rock face at Rhossili (Station 1), where the multicolored population often exceed a density of 1000/m².

Imposex females were found on all shores except the most exposed namely Rhossili and Oxwich (Fig.1). Nearly all imposex females had reached stage 4 with only one specimen near the small Boat mooring at Mumbles (Station 6) reaching stage 5 in (Fig.3).

The maximum relative penis size (RPS) (Table 2) observed in females was 12% indicating that in comparison with an RPS of up to 105% recorded in Cornwall by Gibbs et al (1987), the level of TBT contamination on the Gower Peninsula in 1992 is probably very low.

Nevertheless, the only indication available on the amount of TBT in the sea water of the Gower Peninsula is the mean relative penis size in imposex females (Table 2). In this respect, the largest relative penis size and, thus, most pollution occurs near the small Boat mooring (Station 6), at Mumbles and near Queen's Dock (Station 7) (Table 2).

It is interesting to note that, on the shores near Swansea Docks, specimens at Station 7 have a comparatively large relative penis size but specimens nearer the Docks at Station 8 have a smaller relative

The X², *t*, *d* or K/S tests and regression analysis were used, where appropriate, to analyze the data.

Result

penis size and the lowest percentage imposex females.

The sex ratio

The sex ratio of all males to females is 235:285, approximately 9:11 and doesn't differ significantly from shore to shore ($\chi^2 = 7.49$, $p > 0.3$) or from 1:1 ($\chi^2 = 12.24$, $p > 0.05$).

Although varying from 1:12 (Station 5) to 1:4 (Station 7) that is from 92.3 to 80% imposex, the ratio of healthy females (H) to imposex females (I), which is approximately 1:6 overall, doesn't differ significantly on shores 3-7 ($\chi^2 = 1.59$, $p > 0.8$) (Table 1). However, the ratio of 1:2 (66.6% imposex) is significantly lower at Station 8 ($\chi^2 = 5.76$, $p < 0.02$).

Shell length

The KolmogoroSmirnov (K/S) test shows that there is no significant difference in the length group frequencies between females and males, between imposex females and healthy females or males on any shores. The apparent differences when all shores (1-8) are grouped together, are due to the fact that most healthy females and males, and no imposex females, were collected from Rhossili (1) and Oxwich (2) where, as shown below, the smaller size groups were more frequent than on other shores. Thus, when all shores (3-8), except Rhossili and Oxwich are grouped, there was no significant difference between males and females or between imposex females and healthy females.

When all specimens are grouped, considerable differences occur between shores in respect to length frequency distributions and mean lengths. In these

respects, Rossili and Oxwich have a significantly higher frequency of smaller specimens and a significantly lower mean length than all other shores. These and other significant differences recorded are probably related to the degree of exposure to wave action of the shores. Thus shell length (y) is linearly related to the logarithm of exposure to wave action as measured by Ballantine (1961) scale ($\log x$) (Table 3) specimens becoming larger with increasing shelter.

There was no correlation between shell length and relative penis size (Table 4) in imposex females. Some times the significance of the K/S tests differ from that of the t or d tests as for example, between Rossili and Oxwich (K/S = 1.3 n.s, $d = 3.9$ $p < 0.001$), This is probably because the distribution of specimens within each length group is biased in different directions on these shores.

Shell shape

There was no significant difference in the shell shape group frequency distributions between females and males or between imposex females and healthy females on any shore. As with shell length, when all specimens are grouped, the shore samples differ considerably in respect to shape frequency distributions and mean shape. Rhoosili and Oxwich have a higher frequency of broader specimens and the shape (y) was linearly related to the logarithm of exposure to wave action ($\log x$), the shell becoming narrower with increasing shelter. There was no correlation between shell shape and relative penis size in imposex females.

Relative spire height

There was no significant difference in the grouped relative spire height frequency distributions between females and males or between imposex females and healthy females on any shores but when all specimens are grouped, significant differences occurred between shores. The relative spire height increases significantly with increasing shelter (Table 3) but there was no correlation with relative penis size in imposex females (Table 4).

Aperture shape

Neither sex, imposex, exposure to wave action nor relative penis size appeared to have any affect on the aperture shape (Table 2-4). However, the specimens collected from near the small Boat mooring at Mumbles (Station 6) had a significantly narrower aperture than those on any other shore.

Shell thickness

With few exception, the only significant variation in shell thickness was between the thinner specimens collected from Rhoosili (Station 1) and the thicker specimens on all other shores (Table 2-3).

Penis bulk in male and imposex females

The mean penis bulk in males was weakly correlated with exposure to wave action but not in the imposex females. Nevertheless, there was a weak correlation between RPS in imposex females and penis bulk in males ($a = 62.5$; $b = 5.96 \pm 1.61$ and $r = 0.8795$; $p < 0.05$). Male penis bulk was usually significantly larger on the more sheltered, grade 5 & 6, shores (Station 5-8) than on the more exposed, grade 4, shore (Station 3-5).

Discussion

The more exposed shores on the Gower peninsula, where ships and boats are unlikely to approach and where excessive

water movement disperses pollutants now appear to be uncontaminated by TBT, as measured by imposex frequencies.

The residual levels of TBT in the sea water around the other more sheltered shores on the Gower Peninsula, as indicated by the VDS and RPS (Figs.3&4) in imposex female is less than 1 ng/l, within the EQS set by the Government in 1998 (Waite *et al* 1991) at the highest concentration near the small Boat mooring (Station 6) at Mumbles and at the base of the sea wall of Queen's Dock (Station 7). Even here the stage of VDS was insufficient to induce sterility in all but one female. Nevertheless the observations suggest that some small boat owners may still be using TBT paints 5 years after the ban in 1987. The very low level of TBT indicated by the dog-whelks on the shores near Swansea Docks, inspite of the fact that the use of TBT is not banned on the large commercial ships suggests that the Dock lock prevents excessive contamination of the surrounding shores. The prevailing westerly winds and currents ensure that the shore tucked under the Eastern Breakwater (Station 8) is less contaminated than the shore farther along the dock wall (Station 7).

The low population densities of *Nucella lapillus* observed at stations 6 & 8 can not be attributed to the existing levels of TBT, which do not now induce sterility in females, particularly as the more contaminated shore (Station 7) near Swansea Docks has a higher population density than the less contaminated shore (Station 8).

The very low population density on the shores near the small Boat mooring, Mumbles (station 6) may be the result of mortality due to excessive contamination before 1987 and to the slow recovery of this non-migratory species without a planktonic larva. This conclusion must, however, be treated with caution as populations of *Nucella lapillus* fluctuate

considerably on uncontaminated shores due to predator pressure and other factors (Moyses and Neson-Smith, 1963; Crothers 1985; Stickle and Batne,1987; Louse *et al*, 1989).

It is apparent from this study that residual levels of TBT in the sea water have no effect on the sex ratio or the shell characters of *Nucella lapillus*. The very narrow shell aperture found together with the presumed highest level of contamination at the small Boat mooring, Mumbles (Station 6) is probably coincidental and may be due to excessive predation by crabs in this region (Crothers,1983).

The decrease in length, the increase in shell breadth and the decrease in spire height with increase exposure to wave action has been observed previously by Crothers (1973,1974, 1983, 1985 and 1989) and may be due to the fact that this shape offers less resistance to wave pressure (James 1968). *Nucella lapillus* specimens on extremely (grade 1) and very (grade 2) exposed rocky shores, have very thin shells (Vicars,1992) which may be due to the lack of predation in these shores. A similar explanation may apply to the relatively thin shells found at Rhossli (Station 1) where the near vertical rock face prevents predation by herring gulls and crabs.

James (1968) came to a similar conclusion regarding *Littorina saxtilis* and Vermeij (1978) with regards to many other gastropods. Interestingly, Vermeij (1978) and Gould (1968,1977) have suggested that many of these changes in shell form are the result of paedomorphosis and allometric growth which are in turn initiated by reduced feeding and stress on exposed shores.

The apparent increase in penis bulk in males with increase in shelter (Table 3) has

not been observed previously (Crothers, 1989) but Bailey and Davies (1991) recorded increase as a result of increasing levels of TBT contamination. This and the correlation between penis bulk and males and RPS in imposex females which in turn is known to be closely correlated with the degree of TBT contamination Gibbs *et al*, 1988, Roesijadi *et al*, (1991), suggests that the increase in male penis bulk on the sheltered shores on the Gower peninsula is due to the higher levels of TBT contamination on these shores and not to increase shelter.

The work of Spooner *et al* (1991), relating imposex to increase testosterone levels in female dogwhelks suggest the intriguing possibility that the illegal but increasing use of anabolic steroids by

athletes may be a contributory cause of imposex particularly near the sewage outlet at Mumbles. Support for this suggestion is provided by recent reports (unpublished) that the discharge of nano quantities of female sex steroids widely used of contraception or hormonal replacement therapy, via sewage outlets into the sea has resulted in reduced gonad development and sometimes sterility or sex reversal in marine organisms. Female sex steroids may reduce the effect of TBT in female dogwhelk.

It would be interesting to monitor hormonal and TBT levels in the sea and in the tissues of a variety of marine organisms around the Gower peninsula and to measure their effects in experimental and natural condition.

Table.1: The number of males, females, and imposex *N. Lapillus* collected from eight shores on the Gower peninsula

Shores	Males	Females				Males & Females
		Healthy	Imposex	Total	% Imposex	
1	54	66	-	66	-	120
2	17	29	-	29	-	46
3	22	6	34	40	85	62
4	26	4	20	24	83.3	50
5	24	2	24	26	92.3	50
6	48	7	38	45	84.4	93
7	25	5	20	25	80	50
8	19	10	20	30	66.6	49
	235	129	156	285		520

Table.2: Summary of the mean \pm s.e of the characters of *N.Lapillus* from 8 shores on the Gower peninsula. This data is used for the regression analyses shown in table 3 & 4

		Shell length	Shell shape	R.S.H	Ap. shape	Shell thickness	% Imposex	R.P.S
1	3	27.7 \pm 0.33	1.6 \pm 0.013	31.99 \pm 0.59	15.38 \pm 2.47	0.34 \pm 0.006	0	0
2	3	29.9 \pm 0.45	1.5 \pm 0.01699	33 \pm 0.87	15.4 \pm 3.46	0.50 \pm 0.014	0	0
3	4	3.6 \pm 0.35	1.7 \pm 0.013	39 \pm 0.6	15.0 \pm 1.14	0.50 \pm 0.009	85	2.0 \pm 0.434
4	4	34.2 \pm 0.31	1.68 \pm 0.01286	39 \pm 0.627	15.1 \pm 1.905	0.45 \pm 0.009	83	4.38 \pm 1.191
5	4	34.4 \pm 0.313	1.66 \pm 0.017	39 \pm 0.599	15.1 \pm 1.499	0.48 \pm 0.011	92	2.90 \pm 0.729
6	5	37.6 \pm 0.39	1.72 \pm 0.017	41 \pm 0.82	16.255 \pm 2.2	0.5086 \pm 0.0075	84	9.3 \pm 2.464
7	5	36.1 \pm 0.52	1.73 \pm 0.01406	41 \pm 0.563	15.39 \pm 2.27	0.47 \pm 0.008	80	8.121 \pm 1.964
8	6	37.2 \pm 0.598	1.74 \pm 0.0159	42 \pm 0.747	15.2 \pm 3.44	0.466 \pm 0.014	66	5.25 \pm 2.8395

Table.3 : Regression analysis of exposure to wave action (x) against the mean values (table 2) of shell and other characters (y) of *N.Lapillus* on eight shores on the Gower Peninsula

	Best fit	n	Intercept	Slope ± s.e.b	Cor coeff r	P
Shell length	Log	8	14.65	13.65 ± 2.515	0.911	< 0.01
Shell shape	Log	8	1.271	0.275 ± 0.0628	0.873	< 0.01
R.S.H	Log	8	17.67	14.44 ± 2.0787	0.94	< 0.001
Ap. shape	Linear	8	15.0	0.08 ± 0.1	0.202	> 0.1
Shell thickness	Power	8	-1.071	0.21 ± 0.2	0.391	> 0.1
% Imposez	Lenier	6	124.6	-9.2 ± 2.615	0.869	< 0.05
R.P.S	Linear	6	-3.9322	1.98 ± 0.798	0.563	> 0.1
Penis bulk of ♂	Log	6	-125.62	334.54 ± 119	0.838	< 0.05
Penis bulk of ♂	Power		-1.969	3.916 ± 1.54	0.786	> 0.05

Table.4: Regression analysis of mean value (Table 2) of the shell characters or % imposex (x) against the mean value (Table 2) for relative penis size (y) of *N. Lapillus* on six shores on the Gower Peninsula

	Best fit	n	a	b	r	P
Shell length	Linear	6	34.4	0.29 ± 0.8	0.5987	> 0.1
Shell shape	Linear	6	1.679	0.0037 ± 0.0043	0.39009	> 0.1
R.S.H	Linear	6	38.56	0.29 ± 0.171	0.6489	> 0.1
Ap. shape	Linear	6	14.647	0.1286 ± 0.532	0.771	> 0.05
Shell thickness	Linear	6	0.473	1.1783 ± 0.07038	0.154	> 0.1
% Impose	Linear	6	85.72	-0.761 ± 1.45	0.25	> 0.1

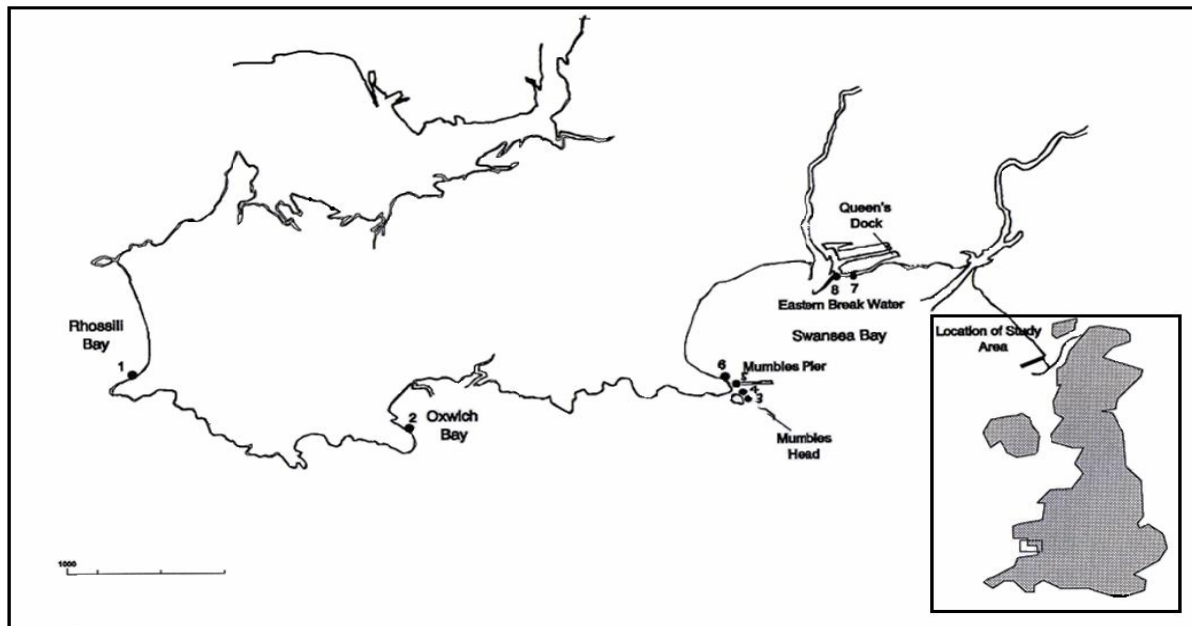


Fig1 A map showing the shores from which *Nucella lapillus* was collected on the Gower Peninsula, October – November, 1992 (Inset map of UK showing position of Gower Peninsula) .

- | | |
|---|--|
| 1. Rhossili (Exposure grade 3) | 6. Small boat mooring Mumbles (grade 5) |
| 2. Oxwich Bay (Grade 3) | 7. Base of sea wall of Queens Dock (grade 5) |
| 3. Middle Head Mumbles (grade 4) | 8. Base of Eastern Breakwater, Swansea Docks (grade 6) |
| 4. South-East of pear Mumbles (grade 4) | |
| 5. Under Mumbles pier (grade 4) | |

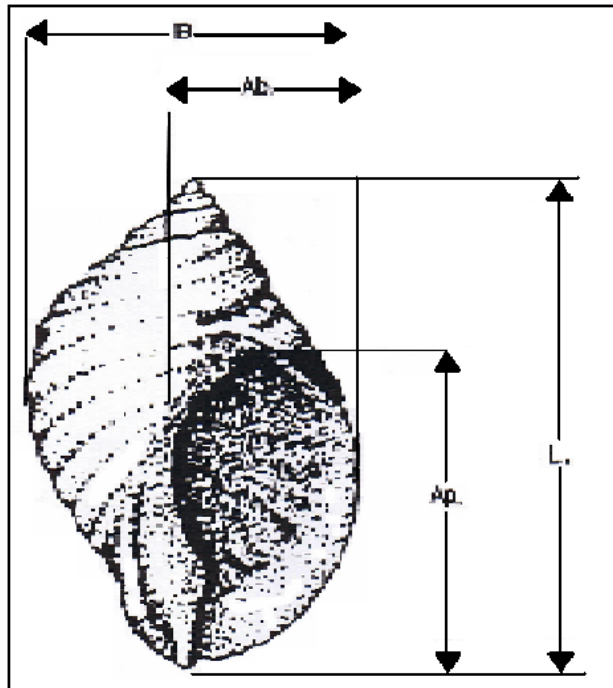


Fig.2

Diagram showing the measurements made on *Nucella lapillus*.
Abbreviations, L. Length of shell, Ap. Aperture length, B. breadth, Ab. Aperture breadth

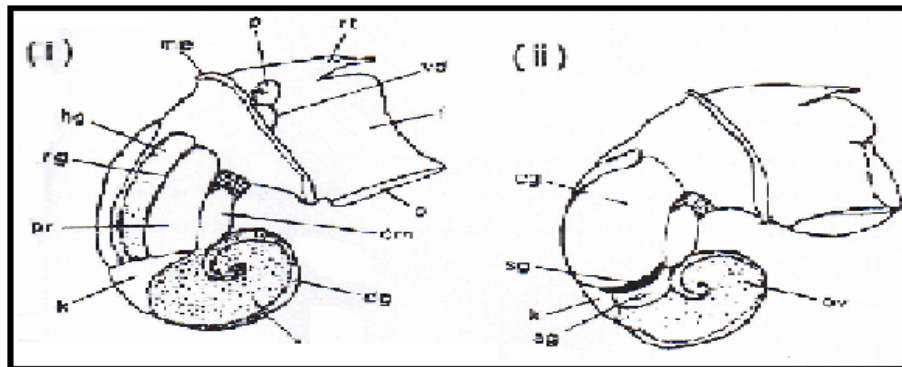


Fig 3

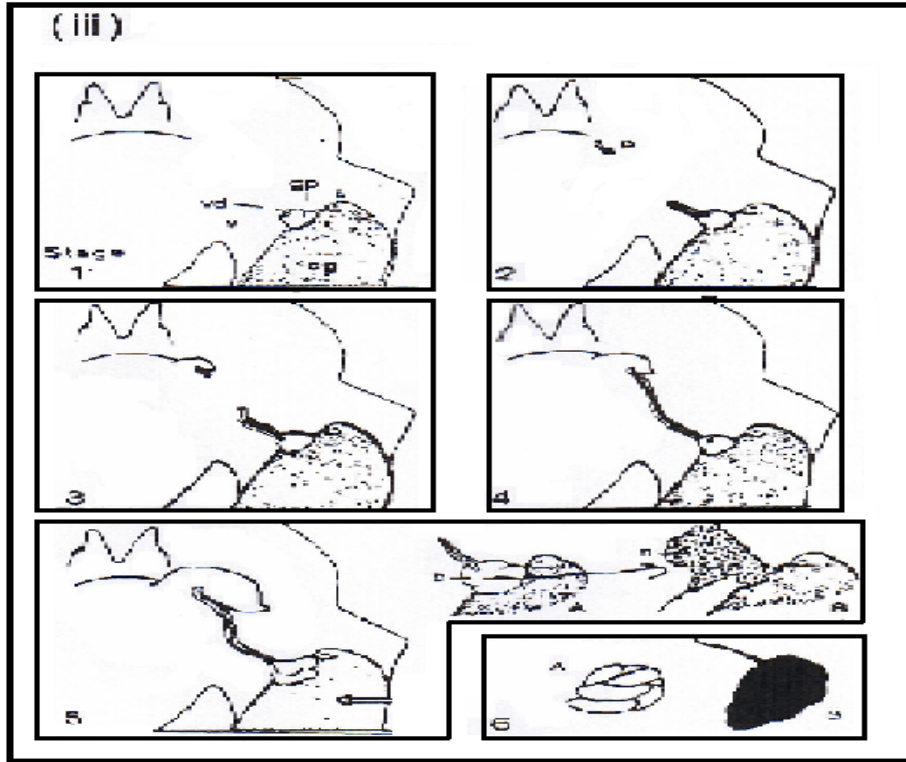


Fig.3 *Nucella lapillus*

(i) External features of mature male

(ii) Mature female

Abbreviations: ag, albumen gland; cg, capsule gland; cm, columella muscle; dg, digestive gland; f, foot; hg, hypobranchial gland; k, kidney; me, mantle edge; o, operculum; ov, ovary; p, penis; pr, prostate gland; rg, rectal gland; rt, right tentacle; sg, sperm-ingesting gland; t, testis.

(iii) Stages in the development of imposex based on vas deferens sequence (VDS).

Abbreviations; a, anus; b, blister; gp, genital papilla; n, nodule; v, vulva; vd, vas deferens.

5A, blister-like protuberances around genital papilla

5B, nodule around genital papilla

6A, aborted capsules in single or

6B, compressed masses fill lumen of capsule gland (from Gibbs *et al* 1988)

The dog – whelk, *Nucella lapillus* (L.) وجود توقعه

على شبة جزيرة Gower بعد قرار حظر سنة 1987

الخلاصة

وجود خنثي (*Nucella lapillus* (L.)) على شبة جزيرة Gower في سنة 1992 ذات تحول طفيف في قناة التكاثر و صغر الحجم النسبي لعضو التدكير يؤكد أن قرار منع أستعمال TBT في سنة 1987 على أن أقل من 1ng/l باقية في مياة البحر القريبة من الشواطئ المحمية نسبيا والقريبة من القوارب الصغيرة في منطقة Mumbles وحوض السفن في سوانزي.

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هذه النتائج تدل على أن النسبة المحددة (2ng/l) من قبل هيئة البيئة قد تم أنجازه في شبة الجزيرة، بالإضافة الى حدوث خنثى الانثى، مستوى التلوث في الشواطئ المحمية قد يزيد من حجم عضو التدكير في الذكور لكنه غير كافي لتعقيم الانثى وخفض الكثافة السكانية أو التأثير على النسبة بين الجنسين و تغيير صفات القوقعة، ومن ناحية أخرى التعرض لتأثير الأمواج قد يغير من شكل القوقعة. الشواطئ البعيدة المفتوحة تبدو خالية من التلوث.

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