Marine Biological Association

Environment Agency commissioned survey of biological indicators of ecological impacts of contamination from the former International Paints Laboratory in Newton Ferrers

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Executive Summary

The Environment Agency commissioned a survey for the presence of dog whelks (*Nucella lapillus*) and assessment of imposex in sites in habitats where they are likely to occur in the Yealm Estuary. The survey is part of a wider survey of Tributyltin (TBT)concentrations in Yealm sediments following a discharge of TBT-containing waste to Newton Creek.

Hormonal disruption in dog whelks exposed to TBT leads to masculinization of female dog whelks by development of male organs (imposex). This effect was discovered by scientists working at the Marine Biological Association of the UK (MBA) in the 1980s and the commissioned project uses indices pioneered by the MBA at the time. Imposex assessments provide a highly sensitive and dose dependent indicator of contamination. Imposex leads to mortality of severely affected female dog whelks, so that the abundance, sex ratio and age structure of populations also provide an indirect indication of exposure and subsequent breeding failures.

TBT also causes abnormal balling and grossly abnormal shell thickening in Pacific oysters at levels just above those causing initiation of imposex in dog whelks.

The commissioned project was intended to assess three biological indicators:

- Presence of imposex in female dog whelks (*Nucella lapillus*);
- Analysis of population structure of dog whelks based on abundance, age and sex; and
- Observations of gross changes in shell morphology in the Pacific oyster (*Magallana gigas* formerly called *Crassostrea gigas)*

Both of the assessed species have life spans such that individuals within the observed populations could have been exposed to and affected by the pulse (or pulses) of pollution and any subsequent residual chronic contamination.

Methodology

The MBA and Aquass Ltd project team (HT & SB) carried out surveys of dog whelk population abundance and structure and sampled dog whelks for imposex assessments at seven sites. The MBA team (SH & NM) also undertook in-situ observations at each site of shell morphology of Pacific oyster to check for gross shell abnormalities consistent with TBT exposure.

Two sites (Jennycliff and Renney Rocks) in Plymouth Sound were selected as control reference sites. They were previously studied by members of the team and have long-term data (1986-2002, 2016) on imposex levels and dog whelk population structure. These sites were compared with Wembury Beach, (situated between these sites and the Yealm Estuary), plus four Yealm Estuary sites. The Yealm Estuary sites selected provide suitable habitat for dog whelks and are located at the source of the contamination (International Paint Laboratory) and at successive distances away from the site of the pollution incident.

At each site, five timed surveys each of one minute long were undertaken. All dog whelks collected in that time period were then measured (length, aperture, lip) on site and a sample of 30-35 adult whelks retained for imposex investigation. All other dog whelks were returned live to their habitat.

Results

- No signs of imposex were seen at the reference sites, nor at the intermediate site (Wembury Beach). No signs of imposex were seen in any of the three sites in the Yealm Estuary. The sample at the Harbour Authroity/Paint Laboratory could not be imposexed as the collected whelks could not be narcotized due to retraction into shells.
- None of the dog whelk sex ratios observed in our surveys differed significantly from expected.
- Population abundance and structure of dog whelks was as to be expected in relation to habitat quality and wave exposure gradients at the sites surveyed. More juveniles were observed within the Yealm Estuary than outside, indicating that populations are successfully breeding.
- Initial observations of abnormal growth in the invasive species of Pacific oyster revealed little sign of responses such as abnormal shell growth or balling.

Conclusion

Based on the assessed indicators there is no evidence of lasting or widespread effects of TBT pollution in the Yealm Estuary in 2022. Had surveys been commissioned earlier (2016-2019), acute and chronic ecological effects or lack of them may have been assessed with greater certainty.

Introduction

This report summarizes the results of a survey commissioned on November 28th 2022 to inform the Environment Agency (EA) on possible biological effects of Tributyltin (TBT) contamination from the former International Paints/ Akzo Nobel Laboratory at Newton Ferrers, including any lasting ecological or widespread impacts in the Yealm Estuary. This pollution incident occurred "*between 2 September 2015 and 27 October 2016*" (HM Govt, 2022). Sites around Plymouth Sound and adjacent or in the Yealm Estuary were surveyed (Fig 1).

The Marine Biological Association (MBA) of the UK led the work, using biological indicators of pollution pioneered at the MBA focussing on dog whelks (*Nucella lapillus*), as well as using other indicators such as abnormal growth ("balling") of Pacific oysters (*Magellana gigas*, formerly called *Crassostrea*) developed elsewhere (Waldock and Thain, 1983; Alzieu, 2000).

The report provides the background to the approach adopted, a description of materials and methods used, the rationale of the surveys possible on the limited number of daylight tides in December, a summary of the results found, and inferences from this work with a brief consideration of its limitations.

Materials and methods

Background to assessment of imposex in whelks

In 1987, the UK banned the use of TBT antifouling on vessels <25 m length overall. This was following a French 1985 ban due to the realisation that TBT had significant adverse effects on commercial oyster beds in Arcachon Bay, France (Alzieu *et al*., 1986). Impacts were also increasingly noted on various species of whelks (stenoglossan gastropods), the effects being the imposition of male sex organs (imposex) on the female whelks. Imposex was first noted by Blaber (1970) in dog whelk (*N. lapillus*) populations from Plymouth Sound, although at the time the cause was not fully established. It was subsequently linked to TBT in an American whelk species, *Nassarius obsoletus* by Smith (1981).

During the mid-1980s scientists at the MBA developed the use of the dog whelk as an indicator of TBT pollution (Bryan *et al*., 1986; Gibbs and Bryan, 1986; Bryan *et* al., 1987; Gibbs *et al*., 1987; Gibbs *et al*., 1988). It was subsequently found that TBT interfered with hormonal expression of sex in dog whelks, blocking enzyme function in the conversion of testosterone to oestradiol (Alzieu, 2000). This caused females to develop male sex organs (penis and vas deferens, a sperm duct), which could continue to grow, leading to vas deferens overgrowth, blocking the female oviduct and subsequent sterility and death of females. This led to many dog whelk populations having mostly sterile females or no females, hence becoming much reduced or locally extinct in areas adjacent to ports and marinas with only a few sites without any signs of imposex in remote areas of open coast (e.g., Spence et al 1990a; Bryan and Gibbs, 1991; Birchenough *et al*., 2002; Colson and Hughes, 2004).

Two indices of imposex expression and hence exposure to TBT were developed. The ratio of the cubed size of penis in females versus those in males in the population, the Relative Penis Size Index (RPSI) was developed first (Bryan *et al*., 1986). This is an index of rapid effects of TBT on female morphology, with the RPSI value given as the mean percentage of the penile bulk of the female population versus male penis bulk (see Fig 3, Appendix 2). Further to this the vas deferens sequence (VDS) described ranked stages from 1-6 (see Fig. 3, Appendix 2), enabling assessment of the extent of female vas deferens development leading to sterility and death at stages 5-6 (oviduct overgrown and rupture of capsule gland; Gibbs *et al*., 1987; and extended and modified by e.g. Oehlmann *et al.,* 1996; Shi *et* *al*., 2005). This also enabled quantitative indices such as percentage sterile females or percentage of females showing no signs of imposex (e.g., see Spence et al. 1990a).

Imposex is induced in female dog whelks at levels of TBT as low as 1 ng/l, and further expression is concentration dependent with sterility induced due to overgrowth of the induced vas deferens blocking the female genital pore at levels of 2-3 ng/l, and complete sterility above 3. Thus these methods enabled consideration of the biological impacts of TBT on the wider marine ecosystem by providing a simple bioassay of contamination level.

Imposex development in dog whelks can be relatively rapid. Davies, (2000) reported that when experimentally exposed to low levels, 49% showed signs of imposex after 3 months at 2ng/l with some reaching stage3 VDS. Over 70% showed signs of imposex at 8ng/l, with more than 30% reaching stage 3 or 4 VDS within 3 months. At higher levels (32 and 126 ng/l) stage 4 was reached after 3 months by over 50% and 88% respectively. Bray (2005) in a 2000-2001 translocation experiment to assess biological effects of TBT at a site in a Southampton Water from which dog whelks had become locally extinct, found that imposex development at average TBT levels of 7 ng/lin water(EA data, communicated to Bray 2005), was quite rapid. After 6 months following translocation from Polzeath (north Cornwall) where VDS was virtually zero,, the translocated females had achieved stage 4 VDS with mean penis lengths of 3.5 mm; this was more than half the size of translocated Polzeath male dog whelk whose penis lengths ranged from 5.2– 5.5 mm.

The imposex methodology was widely adopted in the UK as a measure of TBT pollution, including in Government monitoring (e.g. Harding *et al*., 1992; DETR, 2000; OSPAR, 2005; Manuel Nicolaus *et al*., 2018) and UK based academic studies (e.g. Evans *et al*., 1991, 1994; Harrison *et al*., 2020). The methodology was also widely employed on dog whelks in Europe (e.g. Gomes *et al*., 2021) and globally on other stenoglossan gastropod species used as TBT pollution indicators (e.g., Spence *et al*., 1990b; França *et al*., 2021; Ragagnin and Turra, 2022).

In the UK evidence from research and monitoring using imposex contributed to the banning of TBT paints from small boats in 1987 and in many other nations in the 1980s-1990s. Subsequently in 2008 a global ban on the use of TBT antifouling paints on all vessels was ratified. This followed the agreement of Panama (a flag of convenience nation) to sign up to the United Nations International Maritime Organisation (IMO) proposal after lobbying by the WWF that had commissioned a global study on TBT impacts (Bray and Langston, 2006) that influenced the outcome. In 2013 TBT coatings were required to be removed from commercial craft. Such research also highlighted the ability for high levels of TBT to remain in sediments for tens of years (Maguire, 2000), presenting significant risk to marine ecosystems when contaminated sediment is disturbed and disposed of through activities such as dredging (Langston *et al*., 2015), with continuing impacts being highlighted (e.g. Crowe *et al*., 2000; Hawkins *et al*., 2002; Langston *et al*., 2015; Gomes *et al.*, 2021). The subsequent recovery of dog whelk populations following legislation to ban or restrict the use of these paints has been recorded around the UK (e.g., Evans et al., 1991, 1994; Birchenough *et al*., 2002; Bray *et al*., 2012).

Shell thickening in Pacific oyster

TBT also causes abnormal balling and grossly abnormal shell thickening in Pacific oysters at levels above 20 ng Sn/l (Alzieu *et al.,* 1986), with initiation of shell thickening at much lower levels (2ng/l) just above those causing initiation of imposex in dog whelks.

Baselines from in and around Plymouth Sound

In Plymouth Sound as elsewhere in the UK, follow-on research work established the extent of contamination at several sites (Fig. 1), resulting in time series data useful for charting recovery following various restrictions and bans (e.g., Spence *et al*., 1990a; Proud, 1994; Bray, 2005; Hawkins *et al*., 2002, 2017).

Through joint PhD students of SJH (Spence, 1989; Proud, 1994; Bray, 2005) working at or in collaboration with the MBA, a time series exists for the Plymouth area for recovery of dog whelks along a gradient (Fig. 1) from formerly highly polluted areas in and around Plymouth Sound (Hawkins et al., 2002). There were some legacy pollution issues possibly associated with contaminated sediments which were apparent for up to 15 years after various bans were enacted; notably at one site outside of Plymouth Sound (Tregantle), potentially affected by dredge spoil disposal (Fig. 1). In 2016 a further survey was made by SB (in Hawkins *et al*., 2017), which showed no signs of imposex in any of the populations in the Plymouth area that had been surveyed since the mid-1980s. Some of these sites have been used as reference sites for the work here to rule out other sources of TBT in the area.

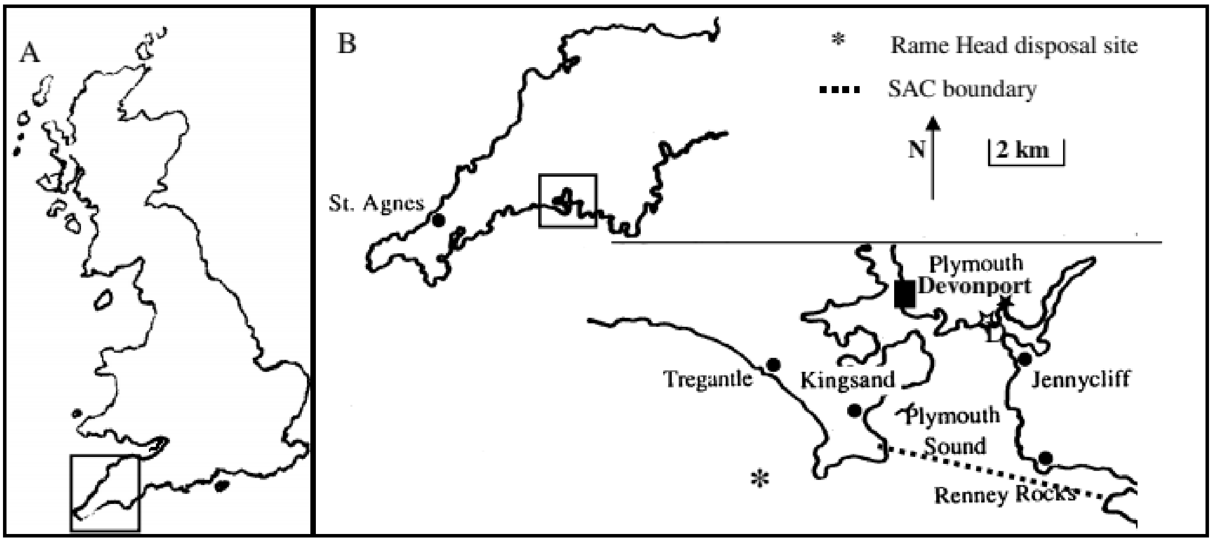

Figure 1 Plymouth Sound TBT impact on dog whelks research locations showing Rame Head dredge spoil disposal site and the limit of the Plymouth Sound Special Area of Conservation (SAC). Jennycliff and Renney Rocks were resurveyed as part of the current project.

Site Locations: Plymouth Sound and Yealm estuary

In December 2022 (6-10th) and January 2023 (2nd) dog whelk surveys and sample collection for imposex investigation were undertaken at seven sites by SB and HT (see Figures 2 and 3). The study design was to re-survey and survey dog whelk populations as follows:

1) At two reference sites that were previously contaminated in the 1980s, but had shown signs of considerable recovery by the early 2000s and complete recovery in 2016 (Jennycliff, formerly highly polluted as relatively close to marinas and docks; Renney Rocks at Heybrook Bay, outside the Sound, but historically showing TBT effects).
2) Wembury Beach (an intermediate field site with some previous imposex data, (23% RPSI, Bryan *et al*., 1986) and a long-term MBA monitoring site (report authors SJH,NM)
3) Four sites from inside the Yealm Estuary at successive distances away from the former Paint Laboratory in Newton Ferrers, out to near the open coast with suitable habitat for dog whelks (the New Ferry Steps, the former ferry steps and slipway called “Old Ferry Steps”, and Cellar Beach on the east side of the Yealm). Some past data for imposex were available for Cellar Beach (70% RPSI, Bryan *et al*. 1986), which is also is a long-term MBA rocky shore monitoring site (by SJH and NM).

The sites on the east side of the Yealm Estuary and at its mouth were selected as having good habitat for dog whelks (i.e., extensive bedrock, barnacles and limpets for food and crevices as refuges). Optimal conditions for dog whelks are on exposed and moderately exposed rocky shores with barnacle and/or mussels for food and crevices for refuges from wave action and harsh tide-out conditions, with abundance declining into more sheltered conditions with less food and suitable rocky habitat (e.g., Spence *et al*., 1990a). The area immediately outside the former laboratory by the Harbour Authority pontoon is a marginal habitat for dog whelks, consisting of a mosaic of boulders, cobbles, invasive reef-forming oysters in a matrix of sediment with some artificial habitat, with bedrock mainly at higher tidal level above the normal optimal habitat for dog whelks. The former paint laboratory site is at the salinity and habitat range edge of *N. lapillus*, which disappears going up into the Yealm Estuary and Newton Creek.

Figure 2 Location map showing the reference sites (Jennycliff and Renney Rocks), the site intermediate between the reference and exposed sites (Wembury Beach) and the Yealm Estuary sites (Paint laboratory, New Ferry, Old Ferry-ferry Cottage and Cellar Beach).

Figure 3 Location map showing the Yealm estuary sites (Paint Laboratory, New Ferry, Old Ferry-Ferry Cottage and Cellar Beach).

Timed searches and dog whelk measurements

At each study site, five replicated one minute timed searches compared relative dog whelk abundance and collected specimens for population structure analysis. After the timed searches, each individual was measured for shell length, aperture size (where the animal protrudes from) and the shell lip thickness (as an indication of age/maturity); population and habitat notes were made (see Appendix 1 for photographs).

Imposex analysis

From each population, a small sample (30-35 animals) was taken for imposex analysis (assessment of RPSI and vas deferens stage (VDS) after Bryan *et al*., 1986 and Gibbs *et al*., 1987). The dog whelks were anaesthetised in 8% MgCl solution, and then removed from their shells and their sex determined (females possess a sperm ingesting gland). Subsequently, using a binocular microscope, the imposexing methodology (Figs. 11-13, Appendix 2) was used to measure female (where present) and male penis lengths, plus any signs of development of a vas deferens in females.

Additional contextual surveys

Rapid assessment surveys to record the abundance of dog whelk present (using semi-quantitative rank abundance scales (ACFOR) adopted from Crisp and Southward, 1958 – see note at bottom of Table 1) were made along both banks of the Yealm Estuary on December 21st and 23rd by report authors SJH and NM, and SJH on 26th December at Jennycliff, Renney Rocks and Wembury. Weather was milder than when the surveys were made earlier in December (6-10th, December) and dog whelks were out of their crevice refuges and actively foraging. Dog whelk variably aggregate with season and short-term weather in crevices, thus quadrat surveys have limited value. In addition, a search was made for the presence of any Pacific oysters showing signs of 'balling' on their upper shell.

The surveys in the Yealm Estuary were made at the site of the former paint laboratory and adjacent Harbour Authority pontoon in Newton Ferrers, the New Ferry Steps on the opposite Noss Mayo bank, the Old Ferry slipway and steps on the opposite bank at Ferry Cottage, and Cellar Beach at the entrance to the estuary.

Results

2022/2023 Survey Results

Table 1 summarises the results for sites detailed above. No evidence of imposex was found. There was no penile development in any female and the vas deferens was also not present in any of the specimens. The sample numbers taken (30-35 individuals) have been considered by Spence (1989) and Proud (1994) as sufficient for a reliable assessment of imposex. The percentage of females in each population was also within natural ranges and not indicative of any population disruption by reduction of females due to past or present imposex as seen in the late 1980s in the region (see Spence et al., 1990a). In populations unaffected by TBT, proportions of females have been reported from 41% (Minchin and Davies, 1999) to 58% (Moore, 1938). None of the sex ratios observed in our survey differed from 1:1 significantly at the 0.05 probability level (binomial probability test; critical values for 1-tail tests as fewer females would be expected: N=30, 10 females; N=34, 11 females). At the New Ferry Steps, the percentage of females in those examined was slightly below ranges previously recorded, but was not significantly different from a 1:1 ratio (NB a small sample size was used to avoid killing too many animals). Less females may be a reflection of predation by crabs on larger, usually female dog whelks at this site (Crothers, 1985). Some notes in Table 2 summarise habitat characteristics.

Figure 4 Summary of imposex results and abundance (ACFOR) for 2022 surveys of N. lapillus in the Plymouth Sound and Yealm surveys

Site	Female VDS	Female RPSI	Mean male Penis mm	Percent. Females	Habitat and Population Observation
Jennycliff	0	0	4.43	45 (♀ = 16/34 assessed)	Dog whelk Abundant. Barnacle food and crevices present, moderately exposed, mixed age classes, breeding clusters and eggs.
Renney Rocks	0	0	3.22	51 (♀ = 18/35 assessed)	Dog whelk Abundant. Exposed shore, barnacles as food, fewer crevices, but refuges under large boulders. No breeding clusters, no eggs. Mixed age classses
Wembury	0	0	4.2	43 (♀ = 13 /30 assessed)	Dog whelk Common, and locally Abundant on west side of beach surveyed to avoid the core of voluntary nature reserve and a long-term non-destructive monitoring sites. Not an optimal site as fewer barnacles, *Dog whelk* feeding on small limpets. Eggs seen. Mixed age classses Dog whelk were Abundant on the east side of beach (26/12/22) in better habitat with more crevices and barnacles for food..
Cellar Beach (Yealm)	0	0	3.52	47 (♀ = 14/30 assessed)	Dog whelk Abundant. Optimal habitat for wave exposure and preferred barnacle prey, with feeding on limpets also seen (small breeding cluster and eggs, mixed age classes (see Figure 5).
Old Ferry Steps (Yealm)	0	0	3.92	53 (♀ = 16/30 assessed)	Dog whelk Abundant. Very good habitat for barnacle prey, also seen eating common periwinkle, *Littorina littorea*. Mixed age classes with, Juveniles and second years using crevices in man-made structures/oysters as refuges
New Ferry Steps (Yealm)	0	0	3.80	37 (♀ = 13/30 assessed)	Dog whelk Common. Slightly marginal habitat due to sediment load and habitat extent. Fewer crevices. Less barnacle prey. Second years apparent. Breeding cluster seen, no eggs. Near range edge of *Dog whelk* progressing up Newton Creek as habitat gets increasingly restricted, giving way to cobbles and sediment.
Paint laboratory					Dog whelk Frequent. No eggs or breeding clusters observed. Mixed age classes with, Juveniles and second years using crevices in man-made structures/oysters as refuges
ACFOR scale: Rare: only one or two found in 30 minutes; Occasional: a few scattered individuals, ,<1m^2; Frequent: 1-4m^2, sometimes more in scattered clumps; Common 5-9m^2, Abundant 10-50m^2.					

Abundance and size (age) structure of populations

Table 2 summarises key population parameters (total abundance and mean abundance between sites). Mean abundance of dog whelks varied significantly at the 0.05 probability level between sites (based on Analysis of Variance tests conducted on all dog whelks, adults only and juveniles only). Mean abundance and standard deviation of samples was plotted and the distribution of size classes (based on shell length) at each site is summarised in cumulative frequency histograms in Appendix 3. The observed dog whelk abundance correlates with habitat suitability, with abundance higher with greater wave exposure and more barnacles for food on the gradient out of the Yealm Estuary. The Wembury and Renney sites surveyed had less refuges such as crevices and hence abundances at these sites were lower compared to Cellar Beach.

Juveniles, two year olds and adults were observed at all sites and the majority of dog whelks at each site were adults (based on lip thickness). The population structure at Cellar Beach is typical of a site with excellent habitat and food for dogwhelks. Comparison of the cumulative frequency histograms (Appendix 3), indicates that the population structure is broadly similar between sites. However, the size range in the Yealm Estuary sites is greater, with more juvenile and both smaller and larger dog whelks present, than at the more exposed reference sites. At two sites within the Yealm Estuary highly suitable micro habitats for juveniles were found with many noted sheltering in crevices on a wall at the Old Ferry Site and at the upper slipway at the International Paint Laboratory site (not included in timed samples) and on a concrete pillar by the Harbour Authority Office. The population structure and abundance at the former laboratory site is typical of marginal habitat at the range edge of distribution of this species going up an estuarine inlet with less bedrock and fewer barnacles for food.

From the presence of juveniles and two year old dog whelks it is inferred that breeding is occurring successfully, although it is noted that small dog whelks may be transported or rafted from hatching sites. No population differences between sites, congruent with gross TBT impacts on populations, is suggested by the population structure analyses.

Figure 5 Summary statistics: total abundance (adult and juveniles), mean and standard error and standard deviation.

	Jennycliff	Renney Rocks	Wembury	Cellar Beach	Old Ferry Steps	New Ferry Steps	Paint laboratory
Abundance	127	101	96	161	114	72	49
Total adults	98	72	80	120	87	61	38
Total juveniles/ two year olds	29	29	16	41	27	11	11
Mean abundance (from 5 replicate) surveys	25.4	20.2	19.2	32.2	22.8	14.4	9.8
Standard deviation	6.1	10.4	11.0	10.2	2.8	1.8	4.5
Standard error	2.7	4.7	4.9	4.6	1.2	0.8	2.6

Additional contextual surveys

Rapid assessment surveys to record the abundance (Table 1) of dog whelks present (using semi-quantitative rank abundance scales (ACFOR) adopted from Crisp and Southward (1958). Habitat gets more favourable for dog whelks on more barnacle-dominated rocky shores, which become increasingly prevalent with exposure to wave action in the outer reaches of the estuary and onto the open coast at Cellar Beach. Some dog whelks were found near the laboratory and by the Harbour Authority pontoon in Newton Ferrers. This is marginal habitat for dog whelk as there is limited bedrock, mainly confined to upper shore levels with some artificial hard habitat, plus a mosaic of scattered boulders and smaller cobbles colonized by the Pacific oyster within a matrix of gravel and sand. Near the former laboratory and on the former slipway, dog whelk were Frequent on the modified Crisp & Southward abundance scale, with some juveniles observed, as well as older size classes.

At the New ferry steps opposite the laboratory, dog whelk were Common, with a mixed population of different ages. Here the habitat was less marginal, with extensive bedrock and barnacle cover, which is their preferred food. Between the New Ferry steps and the Old Ferry steps and slipway, *Dog whelk* rapidly increased and became Abundant halfway along to the Old Ferry steps, reflecting increasing habitat favorability. At Cellar Beach, dog whelks were Abundant. They were also considered Abundant in October 2022 when surveyed by SJH as part of regular long-term surveys. Figure 4 shows abundance estimates made routinely from 2002 onwards by report authors SJH and/or NM. When this time-series commenced, dog whelk were recovering from widespread chronic

TBT pollution following the ban in the 1980s and were scored as Frequent. Since then they were either Common or Abundant in all years surveyed. Dog whelks live in crevices and emerge to forage in favorable conditions, thus estimates of their abundance can vary as they tend to aggregate in refuges in very cold or very hot weather. Since 2008 the population at Cellar Beach has been Abundant in all survey years except for 2015. Since 2016 they have been considered Abundant in every year of the study. At Wembury, dog whelks have also been either Common or Abundant over the last twenty years (MarClim database), a slightly less favourable site for dog whelks.

Other grazers including lined top shell (*Phorcus lineatus*), common limpet (*Patella vulgate*), rough periwinkle (*Littorina saxatilis*) and common periwinkle (*Littorina littorea*) were abundant on all rocky habitat surveyed. The cover of major algal species is what would be expected, with all the major fucoid algae present where there is suitable habitat. A major human impact is the invasive pacific oyster *M. gigas* that has been present in high densities in the Yealm Estuary since the 2000s. The fauna and flora at Cellar Beach are typical of more exposed sites along the Devon and Cornwall coastline.

Shell growth of Pacific oyster

There was little sign of abnormal growth in the Pacific oyster (*M. gigas*), confirmed by sending images to Dr M. Waldock. No grossly distorted individuals were seen despite a thorough search on both banks including outside the former laboratory. Pacific oyster *were* Abundant (based on the SACFOR scale) in front of the old laboratory and near the pontoon. It was also Abundant at both the New and Old ferry steps, but abundance declined going out of the estuary and at Cellar Beach the maximum abundance recorded was Frequent in some localized patches. The level of abundance at Cellar Beach for the last fifteen years has been mainly Frequent, with occasional fluctuations to Common due to recruitment events. At all sites visited there were no obvious signs of balling in any oysters. Without detailed biometric measurements not possible in the time-frame since commission slight effects cannot be ruled out – but gross effects certainly can.

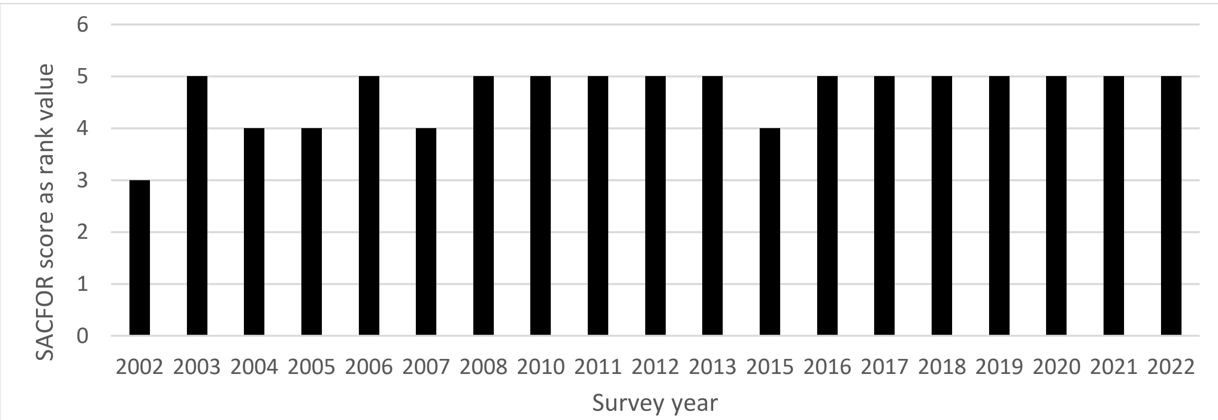

Figure 6 Abundance of dog whelk at Cellar Beach, River Yealm Estuary entrance, on annual autumn surveys using the semi-quantitative ACFOR abundance scale (0=Not Found, 1=Rare, 2=Occasional, 3=Frequent, 4=Common, 5=Abundant). Early on this survey sequence records were made on the ACFOR scale, subsequently adopting an extra category to accommodate the Joint Nature Conservation Committee (SACFOR). Dog whelks have never been deemed Superabundant at this site (>50m^2).

Discussion

Our surveys showed healthy dog whelk populations for all sites assessed. These included previously polluted reference sites at Jennycliff and Renney Rocks where lingering impacts were present in the early 2000s (Hawkins et al., 2002), but in 2016 imposex was not recorded, indicating the efficacy of the various restrictions on TBT use and finally its total banning (Hawkins et al., 2017). No imposex was recorded at the intermediate site of Wembury, although the habitat sampled appeared less favourable in terms of exposure, crevice and prey availability leading to slightly fewer dog whelks

No imposex was recorded along the gradient from the New ferry steps to the outer Yealm (assessed sites: New ferry steps, via the Old Ferry steps and slipway out to Cellar Beach). Although a sample was taken for imposexing from the paint laboratory site and the adjacent Harbour Authority part of the shore, these dog whelks could not be narcotized and could not be assessed). All female dog whelks assessed were healthy, with some large individuals (up to 31 mm) that might have been alive at the time of the incident or shortly afterwards showing no imposex. Dog whelks can live up to 6-7 years (Crothers, 1985) and possibly longer. Thus populations in 2022 could include some older individuals present as juveniles at the time of the pollution event, or others affected by any residual pollution following the pulse or pulses of contamination and lasting deposition in the sediments that could possibly show imposex - this does not seem to be the case.

Had surveys been commissioned and made in 2016-2019, they would have been helpful to detect acute and chronic ecological effects, especially on sensitive indicator species. Without imposex evaluations within 1-2 years of the incident, it is difficult to assess if any severe acute effects occurred that might have led to rapid onset of imposex and subsequent death of females, had such levels been high enough for rapid onset to occur. This seems unlikely as the populations appeared healthy with multiple age classes and even small range-edge populations present at the former laboratory site. Recognising that TBT can reside in sediment for tens of years, possible chronic effects might be expected in female dog whelks and subsequent cohorts hatched after the incident if levels remained high. No imposex was observed in those populations assessed in late 2022, with abundant dog whelks with breeding clusters and eggs in Yealm populations. The long-term data showed no obvious dips in populations at the outer site at Cellar Beach, which had been affected in the past by TBT during the period of high chronic levels (Bryan et al., 1986), although the semi-logarithmic progression of such abundance scales is only sensitive to major changes over several years. The levels of TBT contamination recorded were high (HM Govt., 2022); but lasting (in 2022) and widespread ecological impacts (on the opposite bank 150 to 1000m from the former laboratory) on the highly sensitive dog whelk are not apparent.

No obvious gross damage to oysters has been observed. Both species have life spans (dog whelk estimated as 6-7 and possibly up to 10 years; Pacific oyster various estimates of up to 20 and even 30, but at least 6) that could have been affected by the initial pulse (or pulses) of pollution and any subsequent residual chronic contamination.

The Yealm Estuary is flushed by net freshwater transport downstream from the River Yealm, and the almost complete flushing of Newton Creek on every tide as it virtually dries out on most low tides. This may have reduced exposure of dog whelks, oysters and the other organisms in the Yealm Estuary ecosystem to high concentrations of TBT in the water column coming from any hot-spots of sediment contamination.

In the expert opinions of report authors SJH and NM the rocky shore assemblages in the mid estuary (by the former laboratory and the New and Old ferry steps) are typical for shores of this nature in mid and outer reaches of the Rias and Estuaries of the Southwest of England.

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Appendix 1 Methodology: supporting figures: Dog whelk survey and shell measurements (age structure)

Figure 7 Small breeding cluster and eggs seen at Cellar Beach, River Yealm.

Figure 8 Adult and juvenile dog whelks, note the development of teeth around the aperture of the mature dog whelk (left), these structures are absent in juveniles (right). The lip measurements (Figure 10) are an indicator of the maturity of the dog whelk.

Figure 9 Within all sites a range of dog whelk ages were represented (from L-R, juvenile, second year, adult)

Figure 10 Measuring dog whelk length.

Figure 11 Measuring dog whelk aperture.

Figure 12 Measuring dog whelk lip, a proxy for stage of maturity, the thickness of the lip increases with the development of the 'teeth' (see Figure 6).

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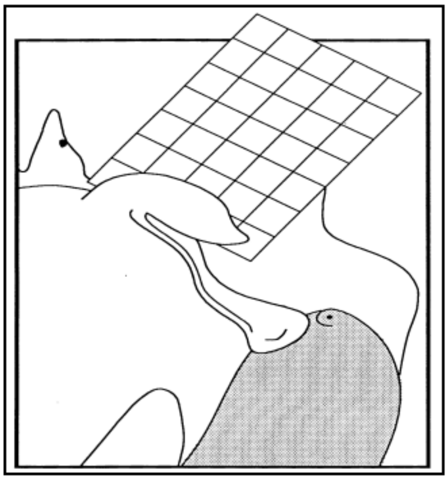
Appendix 2 Methodology: supporting figures: assessing imposex

Figure 13 Female dog whelk identified by sperm ingesting gland from Cellar Beach (8/12/2022). No evidence of penis or vas deferens growth.



$$RPS = \frac{\text{mean length of female penis}^3}{\text{mean length of male penis}^3} \times 100$$

Figure 14 Measurement of dog whelk penis length using 1 mm graph paper (from Proud, 1994).

Stage Number.	Characteristics of stage.
Stage 1	Development of the proximal of the vas deferens (vd) commencing with infolding of the mantle cavity epithelium in the region ventral to the genital papilla (gp) with vulva (v).
Stage 2	Development of the penis (p) initiated with the formation of a ridge.
Stage 3	Small penis develops, distal section of vas deferens formed from its base.
Stage 4	Proximal and distal sections of the vas deferens are fused and the penis is enlarging to a size approaching that of the male. Reproduction possible.
Stage 5	Vulva is overgrown by continued development of vas deferens forming a blister (b) and continued growth forms a nodule (n). Reproduction impaired.
Stage 6	Inside of capsule gland (cg) contains material of aborted capsules (ac).

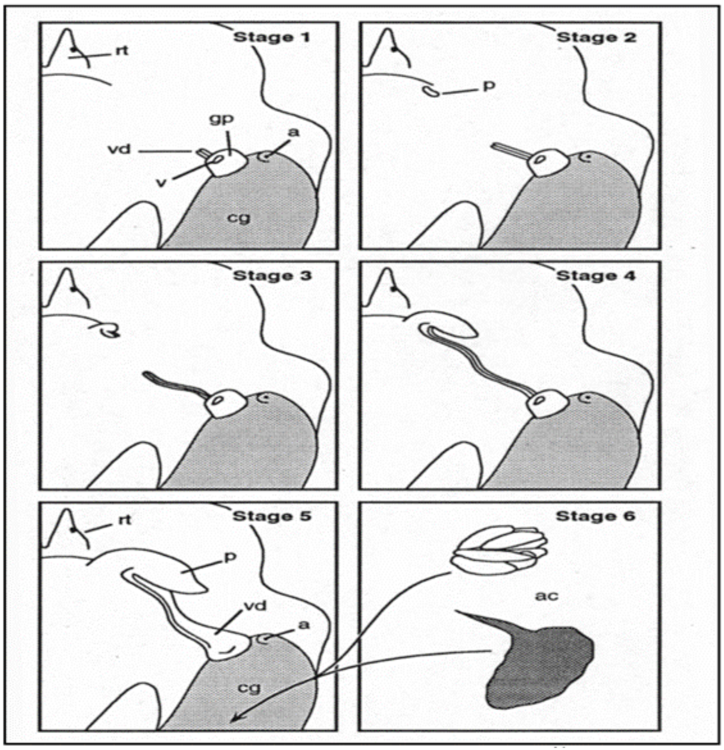


Figure 15 Stages in the development of the vas deferens sequence in female dog whelks. Abbreviations: a, anus; ac, aborted capsules; b, blister; cg, capsule gland; gp, genital papilla, n, nodule; p, penis; rt: right tentacle; v, vulva; vd, vas deferens. (From Gibbs et al., 1987). Descriptions of the stages are tabulated at the top of the figure. Sterility occurs at stage 5 and death by stage 6.

Appendix 3 Abundance and population size (age) structure

Figure 16 Mean abundance of dog whelks based on five 1-minute timed searches at each site. The errors bars represent standard deviation.

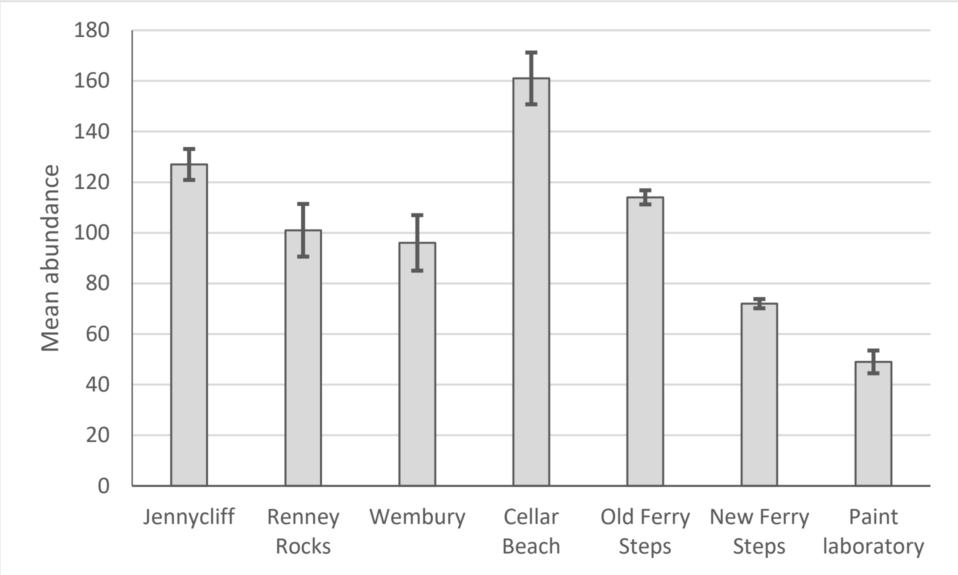
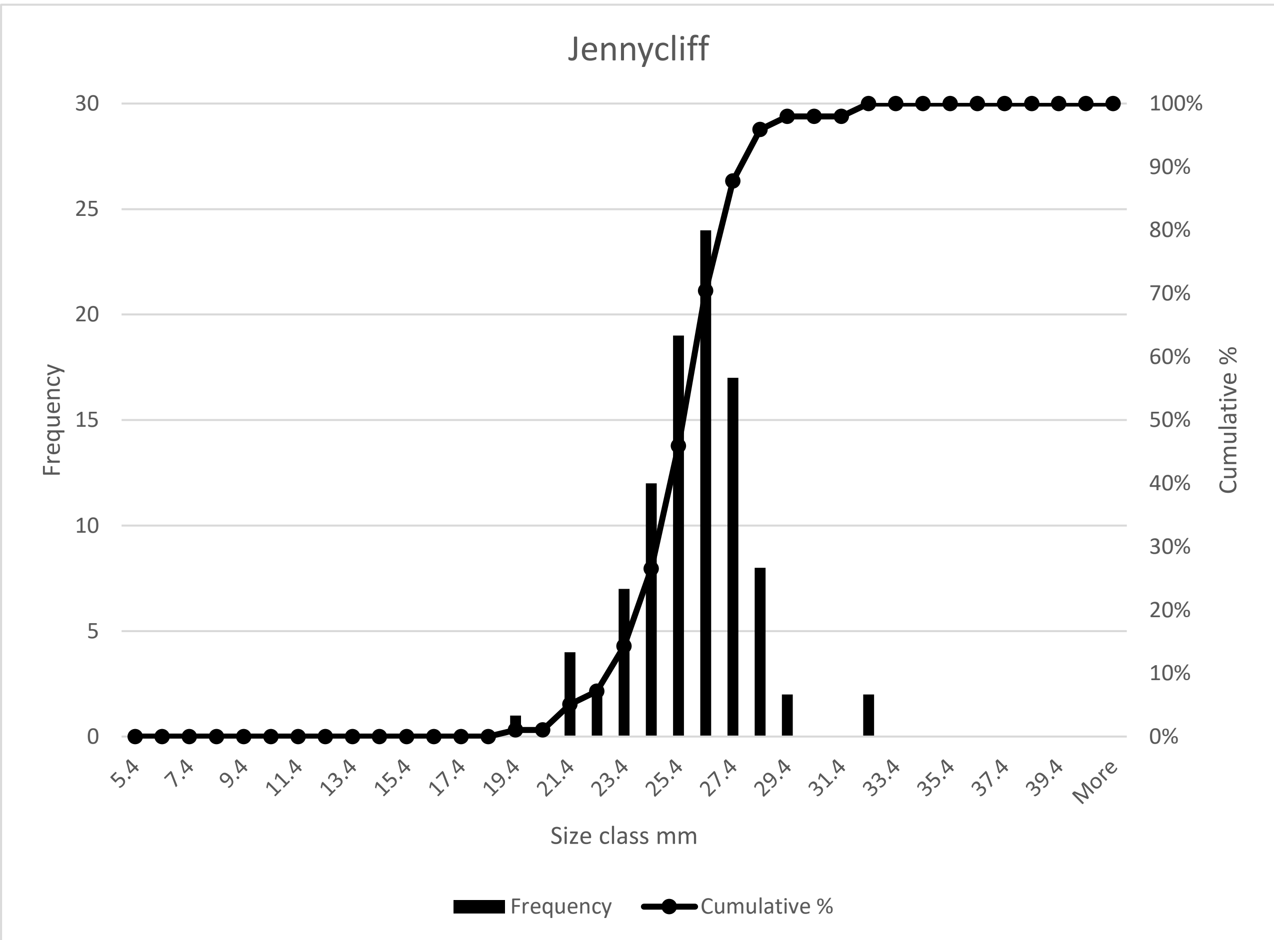
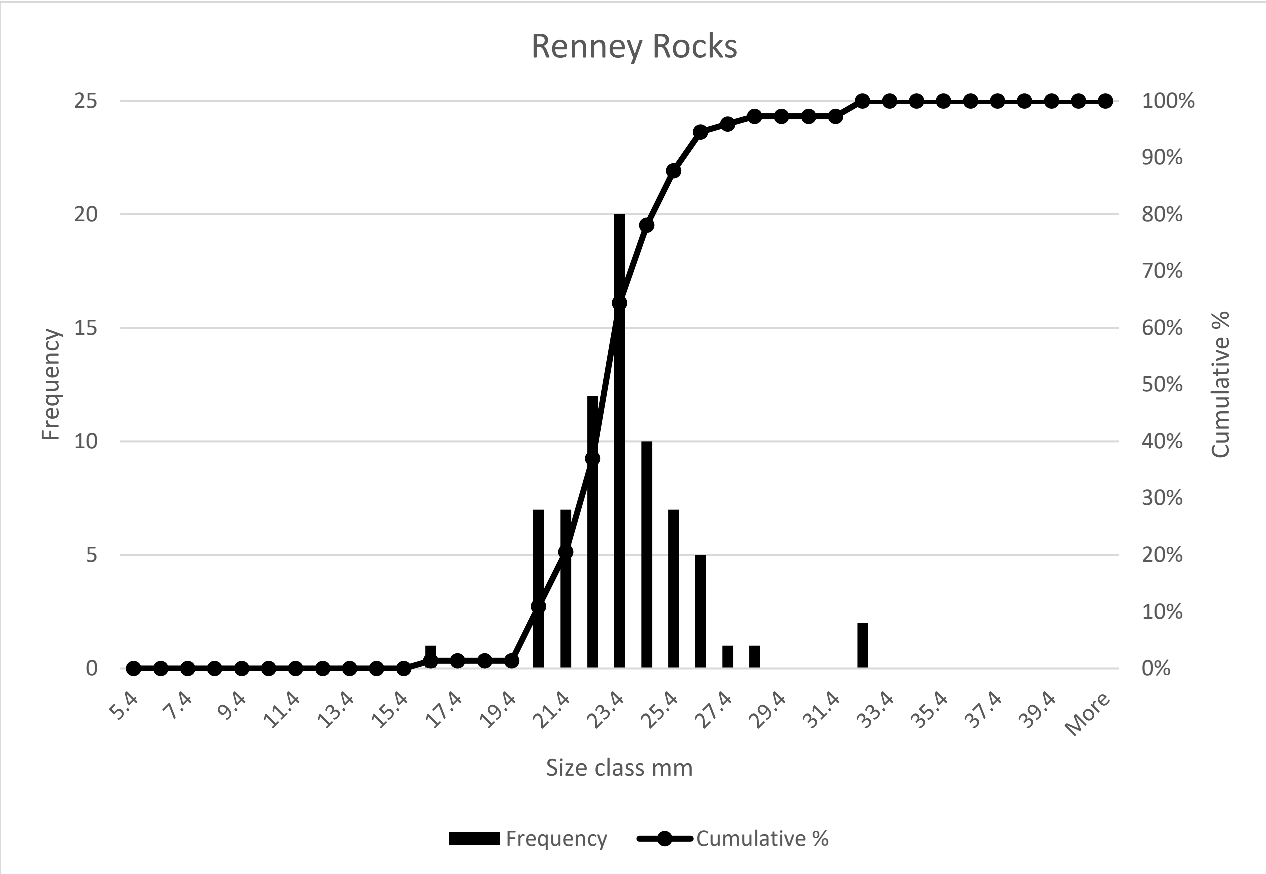


Figure 17 Cumulative frequency histograms for the seven locations surveyed

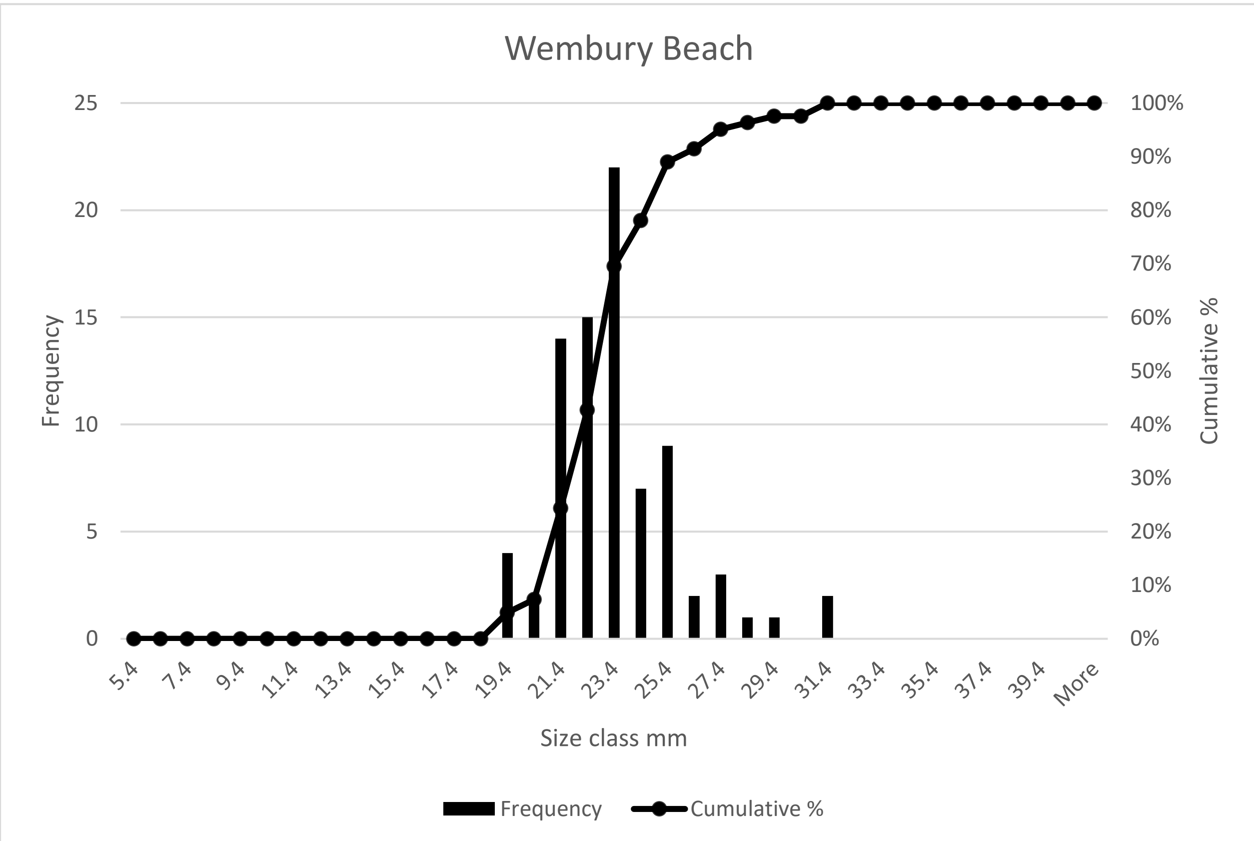
A) Jennycliff



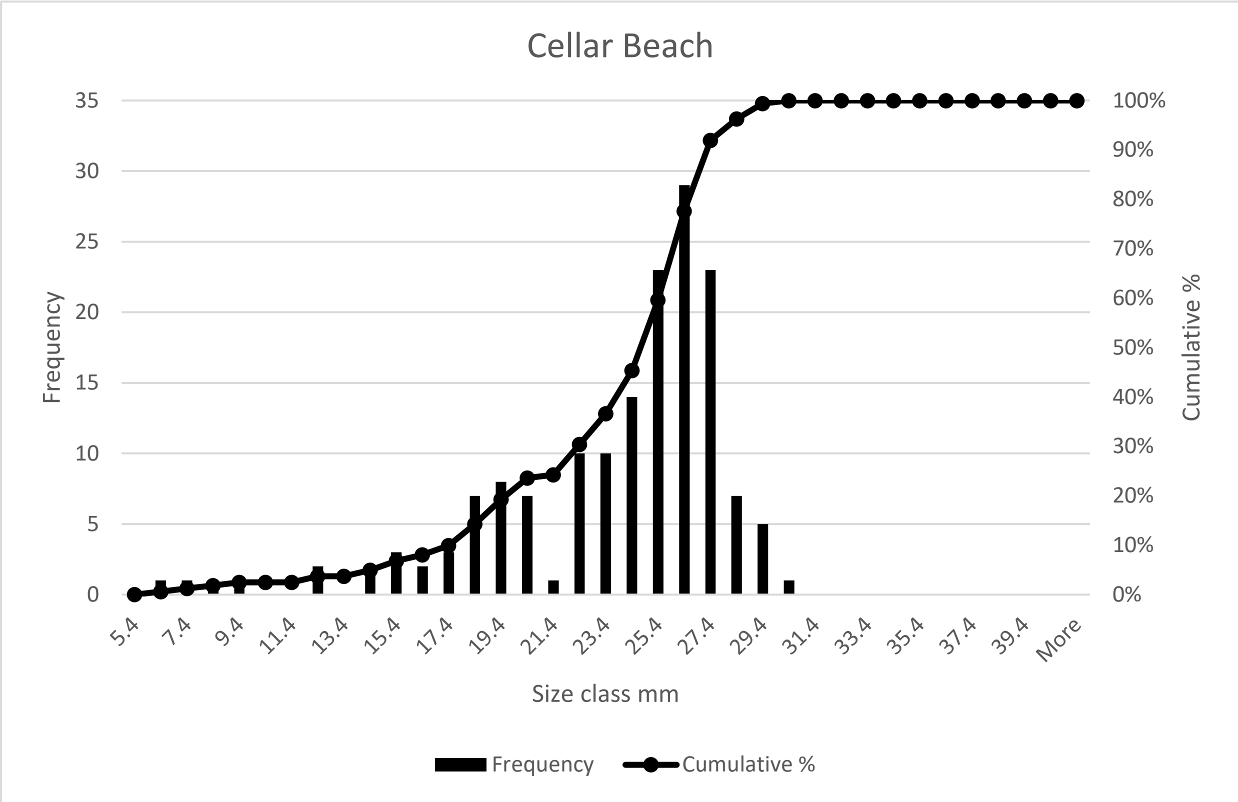
B) Renney Rocks



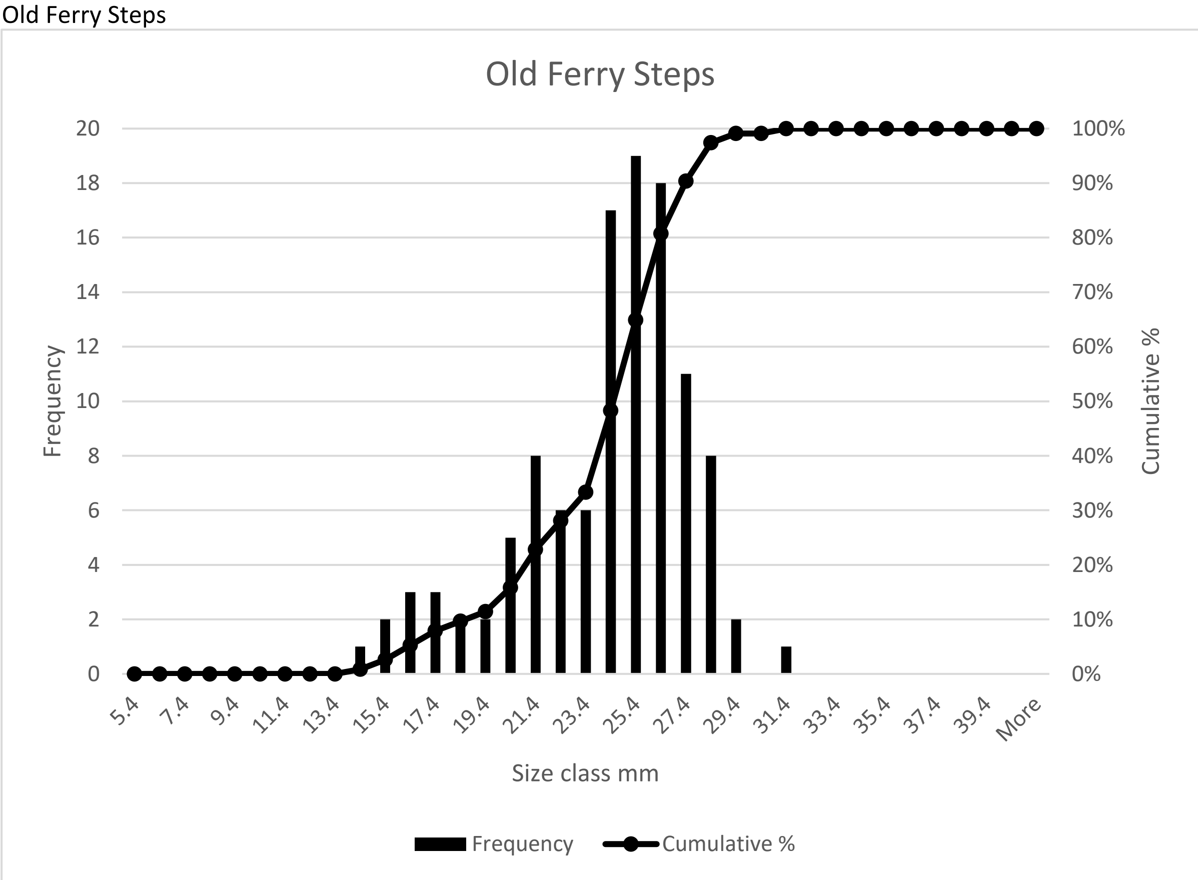
C) Wembury



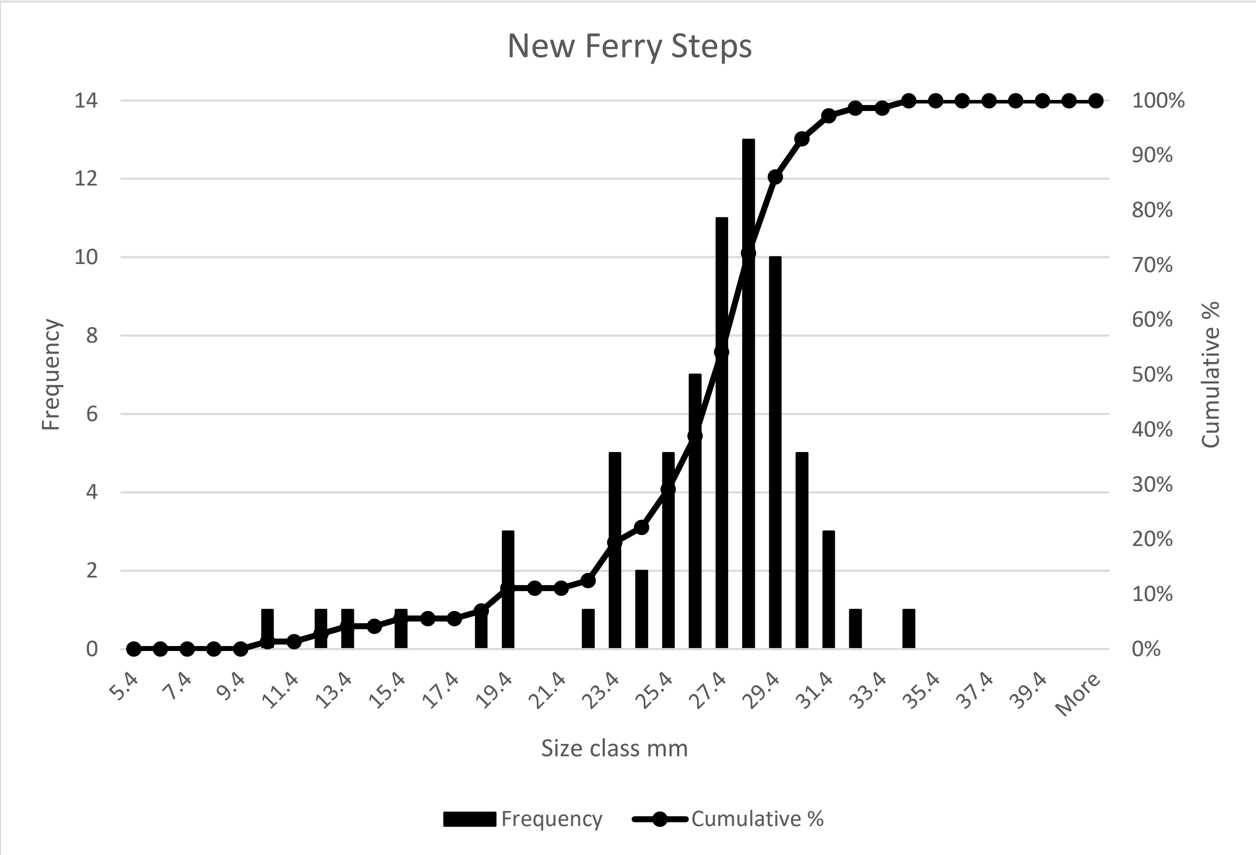
D) Cellar Beach



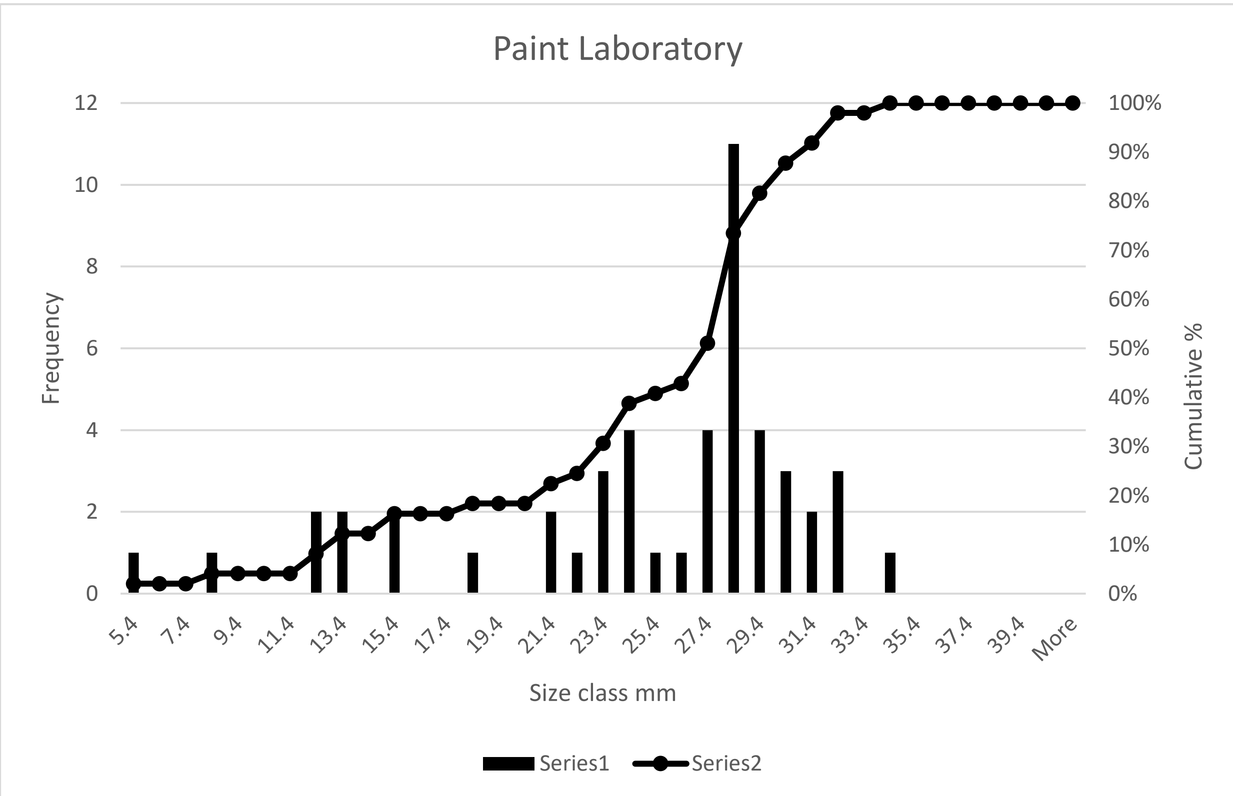
E) Old Ferry Steps



F) New Ferry Steps



F) Paint Laboratory and pontoons



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