Vol. 5: 225–238, 1988 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published December 2

Epizootiological aspects of a sarcoma in the cockle *Cerastoderma edule*

Eamonn Twomey*, Maire F. Mulcahy

Department of Zoology, University College Cork, Ireland

ABSTRACT· The prevalence and distribution of the neoplasm in *Cerastoderma edule* (Mollusca; Bivalvia) populations in Cork Harbour (Ireland) and at several sites on the adjacent coast are presented and discussed, and the relation of the neoplasm to such factors as month, age, size and sex is investigated for the cockle population at the harbour site of Cuskinny. The neoplasm has a seasonal pattern of occurrence, being more prevalent in early summer and in autumn and least prevalent in winter. This pattern is generally similar to that reported for other bivalves, but differs from some cases in that the neoplasm does not disappear at certain times of the year There is some evidence that the neoplasm is more prevalent among females than among males and also in younger, smaller cockles, particularly males. The neoplasm was not induced, over a 6 mo observation period, in cockles which had been transferred from the disease-free coastal site of Ballymacoda to the Cork Harbour site of Currabinny where the disease was enzootic. Major differences exist in the sarcoma prevalence rates for sites inside and outside Cork Harbour. Furthermore, the distribution of the disease along the coast (predominantly west of the harbour) is explainable in terms of the prevailing coastal currents (east to west) and strongly suggests that the harbour is the focus of the disease. Evidence for the role of pollution in the aetiology of the sarcoma in *C. edule* is circumstantial and then only in the general sense of a 'eutrophic' harbour contrasted with 'cleaner' coastal sites. Trace element levels in cockle tissues from inside and outside the harbour were uniformely low.

INTRODUCTION

Sarcomatous lesions of possible haemic origin in the cockle *Cerastoderma edule* were first recorded in November 1982 in Cork Harbour, Ireland (Twomey & Mulcahy 1984). A similar neoplasm was also found in cockles in Brittany in the same year (Twomey et al. 1986, Balouet pers. comm). These were the first cases of a neoplasm in this species although the numbers of recordings of various types of neoplasm in bivalve molluscs have been growing steadily and now include at least 17 species (Sparks 1972, Rosenfield 1976, Harshbarger et al. 1981, Farley et al. 1986, Mix 1986). Apart from serving as host to a number of protozoan and metazoan parasites/commensals (Cheng 1967, Lauckner 1983) no other pathological conditions have been recorded in the cockle despite the fact that it was by 1971 the most economically important bivalve species in Britain (Franklin 1972).

* Present address: University Marine Biological Station, Millport, Isle of Cumbrae, Scotland KA28 0EG

MATERIALS AND METHODS

Collection sites. Cockles were collected from 18 shores located either within Cork Harbour or along the coast east and west of the harbour (Fig. 1). Cuskinny, the prime harbour site, was monitored monthly from February 1983 to February 1985 to study the epizootiology of the disease; Ballymacoda, the prime coastal site, served as a disease-free control and was monitored monthly from May 1983 to June 1984 with additional irregular samples thereafter. The remaining sites inside and outside the harbour were sampled on a single occasion to determine the geographical distribution and prevalence of the disease; those within the harbour during July 1984 and those outside during July 1985. All cockles were collected from the shore by hand, the majority between low and mid-tide levels. Special sampling procedures such as use of quadrats were not adopted because of the low density of cockles, particularly at Cuskinny. This may have introduced a sampling bias against very small cockles.

Detection of neoplasm. Neoplasms were diagnosed

0177-5103/88/0005/0225/$ 03.00

by 2 methods: (1) initial diagnosis, in some cases, by examination of haemolymph smears; (2) confirmation diagnosis in all cases by examination of histological sections, except for Ballymacoda samples. For the latter, of a cumulative total of 386 cockles sampled, 146 were diagnosed by histology only, 240 by smear only, and 27 by employing both methods.

Haemolymph smears. Smears were prepared by withdrawing 0.2 to 0.5 ml haemolymph from the anterior adductor sinus with a 26 gauge tuberculin syringe and subsequent laking on clean glass slides. The slides were then placed in covered Petri dishes over moist cotton wool for 15 to 30 min to allow the haemocytes to attach under humid conditions. They were subsequently fixed by flooding with 2.5 % glutaraldehyde in millepore-filtered seawater for 5 min, dehydrated in 95 % alcohol for 1 min and then air-dried. Dried fixed smears were stained with Giemsa's stain. Stained, mounted smears were routinely examined at 40× and 100× magnification.

Histological sections. After collection, cockles were maintained overnight in aerated seawater aquaria to allow voiding of sand particles from the digestive tract. They were then narcotised in a solution of MgCl and/or phenoxyethanol, to relax and desensitise them prior to fixation and to permit ease of removal from the shell. Cockles were fixed using modified Helly's fixative (Yevich & Barszcz 1977). After washing, tissues were stored in 70 % ethyl alcohol prior to dehydrating and embedding in paraffin wax. Sections were cut at 5 to 8 µm and stained with Harris's haematoxylin and eosin.

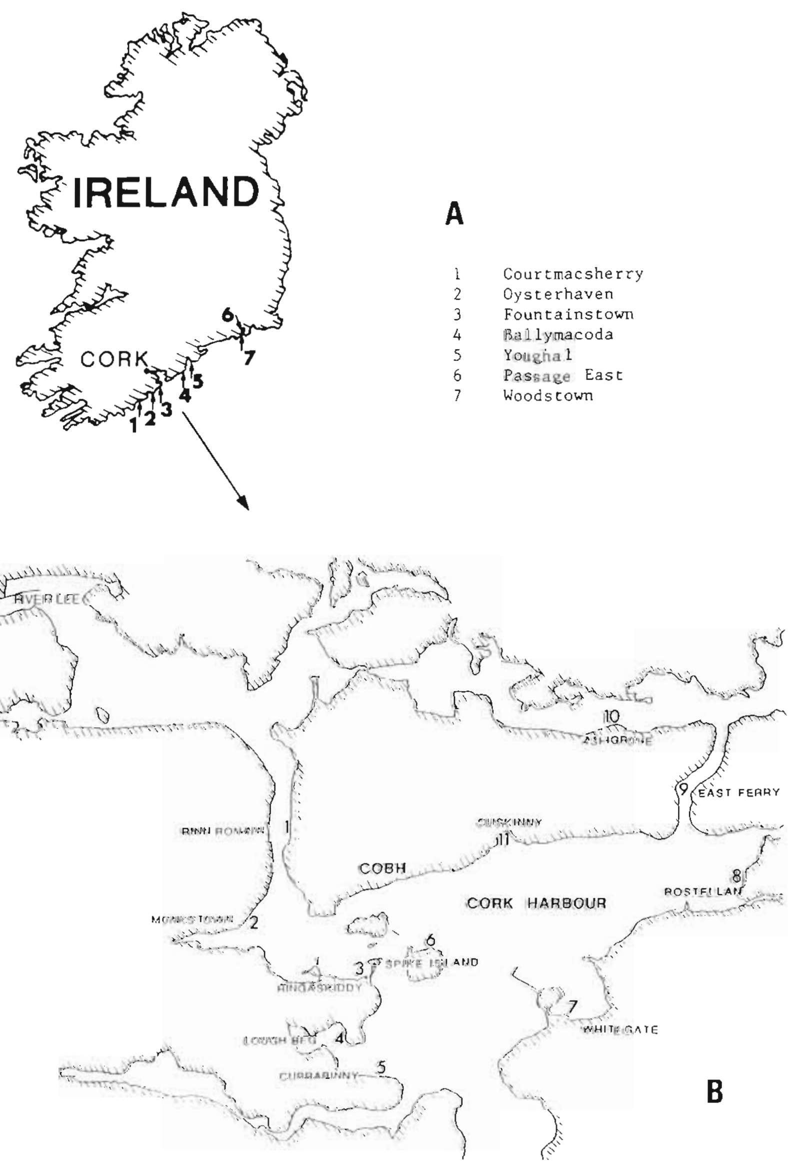


Fig. 1. *Cerastoderma edule*. Collection sites along the south coast of Ireland (A), and within Cork Harbour (B)

Estimation of age of cockles. Age of cockles was determined by 2 methods: (1) counting of annual check marks produced by cessation of growth in winter (Orton 1926, Kreger 1940, Boyden 1972, Seed & Brown 1975, 1978, West et al. 1979); (2) employing the acetate peel replication technique, developed by Richardson et al. (1979) for observing tidally deposited growth bands in cockles. The latter method permitted the determination of the first winter period and the differentiation of annual growth checks from disturbance rings in a sample of 24 neoplastic and 20 non-neoplastic cockles. The information on growth patterns provided by this technique was then used to interpret shell marks of cockles from Cuskinny and the other collection sites. Age was not determined for shells that were worn or showed many disturbance rings. The height of each shell was measured along the central rib axis from the umbo to the growth margin using a vernier calipers or ruler.

Transmission experiment. Cockles for disease transmission experiments were collected from Ballymacoda, a site from which the neoplasm was consistently absent throughout the course of this study. Subsamples were always taken concurrently for histological examination to confirm absence of the neoplasm. On February 10 1985, 212 cockles were collected from Ballymacoda, held overnight in the laboratory in aquaria, marked with a shell notch, and then placed in plastic fencing cages (square size, 10 mm; dimensions, 100 × 100 × 15 cm), 106 cockles per cage, on a mudflat near low water level at Currabinny. Samples were removed from the cages for histological examination 1, 2, 3, 4 and 6 mo post transfer. Mud/sand was added to the cages once in situ to allow the cockles to burrow.

Trace element analysis. Cockle samples for trace element analysis were collected from Cuskinny on 2 occasions: July 4 1985, May 22 1986; from Ballymacoda on 3 occasions: February 10 1985, July 5 1985, and May 23 1986. Levels of 7 trace elements (Pb, Cd, Cr, Mn, Cu, Ni, Zn) were determined by 3 different methods: (1) graphite furnace atomic absorption spectrometry (GRASS); (2) flame atomic absorption spectrometry (FAAS); (3) anode stripping voltametry (ASV).

Table 1. *Cerastoderma edule.* Prevalences of sarcoma at various sites. Diagnosis of neoplasm was by histology in all cases, except for the Ballymacoda cockles, some of which were diagnosed by smear only

Site	Sample size	No. with sarcoma	Prevalence	Date
Harbour sites				
1 Rinn Ronain	22	2	9.0 %	Jul 03 84
2 Monkstown	21	7	33.0 %	Jul 03 84
3 Ringaskiddy	24	12	50.0 %	Jul 03 84
4 Lough Beg	31	1	3.0 %	Jul 17 84
5 Currabinny	25	15	60.0 %	Jul 17 84
6 Spike Island	18	5	28.0 %	Jul 17 84
7 Whitegate	17	4	24.0 %	Jul 05 84
8 Rostellan	13	5	39.0 %	Jul 05 84
9 East Ferry	6	0	0	Jul 11 84
10 Ash Grove	20	3	15.0 %	Jul 11 84
11 Cuskinny[a]	575	224	39.0 %	Feb 83 to Feb 85
Coastal sites				
1 Courtmacsherry	35	9	26.0 %	Jul 19 85
2 Oysterhaven	24	5	21.0 %	Jul 16 85
3 Fountainstown	27	1	4.0 %	Jul 16 85
4 Ballymacoda[b]	386	0	0	May 83 to Mar 86
5 Youghal	27	1	4.0 %	Jul 24 85
6 Passage East	42	0	0	Jul 31 85
7 Woodstown	43	0	0	Jul 31 85

	Mean sample size	Total number sampled	Mean prevalence
[a] Cuskinny 26 samples Feb 82 to Feb 84	24 (Range 13–34)	575	41.0 % (Range 13–72 %)
[b] Ballymacoda 17 samples May 83 to Mar 86	23 (Range 8–32)	386	0.0 %

Statistical analysis. Statistical methods were used to test for association between occurrence of the neoplasm and the variables month, age, sex and size (shell height). In addition, these univariate tests were supplemented by a multivariate type using logistic regression analysis, in which the 4 variables – month, age, sex and size – were used to predict the proportion in the occurrence of the neoplasm. The model was fitted using the GLIM (generalised linear interactive modelling) statistical package (Baker & Nelder 1978).

RESULTS

Prevalences and distribution

The prevalences of the neoplasm at each site are listed in Table 1. Mean monthly prevalence for Cuskinny, the prime harbour site, was 41 % with a wide range throughout the year from 13 to 72 % (overall prevalence = 39 %). Of the 10 shores surveyed within the harbour, 9 contained neoplastic cockles with prevalences ranging from 3 to 31 %. Failure to detect the neoplasm at East Ferry was most likely due to the sparse population of cockles on that particular shore which only provided a small sample of 6 cockles. Remarkably, the sites with the highest and lowest prevalences, Currabinny and Lough Beg, are situated within 100 m of each other across a narrow inlet.

The neoplasm was not detected at any time at Ballymacoda (Table 1) east of the harbour, during the sampling period from May 1983 to March 1986, when over 386 cockles were examined. However, several of the other 6 sites along the coast contained affected cockles. The prevalences at these sites fall into 3 groups: Group I, with 0.0 % prevalence, at Woodstown and Passage East; Group II, with a low prevalence of 4 %, at Youghal and Fountainstown; and Group III, with higher prevalences of 21 % and 26 %, at Oysterhaven and Courtmacsherry respectively.

Table 2. *Cerastoderma edule.* Overall prevalence of the sarcoma in different populations of cockles in July 1983, 1984 and 1985, and in August 1984

Site/group	Number neoplastic	Total sample	Prevalence
1 Cork Harbour[a]	68	219	31 %
2 Coast[b]	16	113	14 %
3 Cuskinny[c]	32	96	33 %
	$\chi^2 = 8.11$ ($p < 0.05$)	with 2 d.f.	

[a] Excluding Cuskinny, 3–17 Jul, 1984
[b] 4 neopl. sites only, 16–24 Jul, 1984
[c] 4 Jul 1983, 8 Aug 1983, 6 Jul 1984, 2 Aug 1984

Table 2 lists the overall prevalences for the 3 combined samples: (1) Cork Harbour, excluding Cuskinny; (2) the 4 coastal sites where the neoplasm was detected; (3) Cuskinny samples for July and August, 1983 and 1984, corresponding to the time of year when the former samples were taken. The Cuskinny and other harbour samples had similar prevalence rates (33 % and 31 % respectively), while that for the non-harbour sites (14 %) was considerably lower. Prevalences within the harbour were twice as high as those outside ($p < 0.05$).

The Cuskinny population

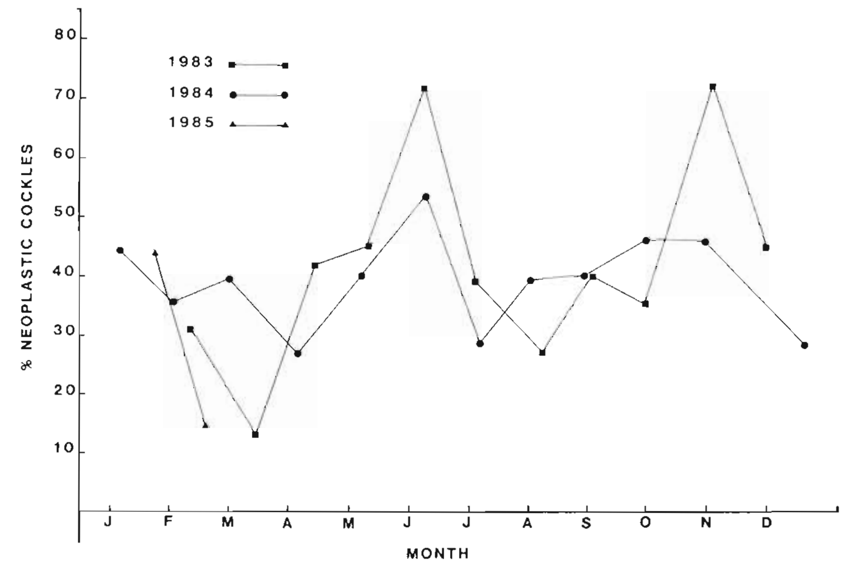
Monthly prevalences/seasonality

Plots of monthly prevalences of neoplasms at Cuskinny from February 1983 to February 1985 are given in Fig. 2. It is evident, particularly for the data from 1983, that a pronounced seasonality in the prevalence of the disease exists ($p < 0.01$). Prevalences were very low in February (31 %) and March (13 %); thereafter a rapid increase occurred peaking in early June (71 %); an equally rapid decline ensued, reaching a low in early August (27 %); this was followed by a sharp increase peaking in early November (72 %); thereafter, prevalence declined to 45 % in early December. This pattern was repeated to some extent in 1984 although less pronounced: during January to April prevalences were generally low with a minimum in April (27 %); prevalences then peaked for the year in early June (54 %) and were followed by a decline in July (29 %); a steady increase to a peak prevalence of 46 % in late September and late October was followed by a steady decline to 28 % in December. The 1985 sample also showed the winter decrease (February, 15 %).

Prevalences thus appear to decrease in winter/early spring (February, March, April) and in late summer (July, August) and to increase in early summer (June) and in late autumn (November).

Size/age/growth

Table 3 contains the results of χ^2 tests employed to compare the frequency distributions of both age classes and shell size classes for the various subgroups of Cuskinny cockles categorised by sex and neoplasm status. Only 2 distributions were different ($p < 0.05$); shell size class frequencies for neoplastic cockles (male & female) versus non-neoplastic, and neoplastic males versus non-neoplastic males.



Month	Number Npl / Total	% Npl
Feb 11. 83	4/13	30.8
Mar 14. 83	2/15	13.3
Apr 11. 83	8/19	42.1
May 10. 83	9/20	45.0
Jun 08. 83	15/21	71.4
Jul 04. 83	7/18	38.9
Aug 08. 83	6/22	27.3
Sep 03. 83	8/20	40.0
Oct 01. 83	7/20	35.0
Nov 03. 83	18/25	72.0
Dec 01. 83	10/23	43.5*
Jan 05. 84	12/27	44.4
Feb 02. 84	8/28	35.7
Mar 01. 84	13/33	39.4
Apr 05. 84	7/26	26.9
May 07. 84	10/25	40.0
Jun 09. 84	15/28	53.6
Jul 06. 84	8/28	28.6
Aug 02. 84	11/28	39.3
Aug 30. 84	10/25	40.0
Sep 30. 84	12/26	46.2
Oct 30. 84	11/26	45.8
Dec 20. 84	7/25	28.0
Jan 22. 85	11/25	44.0
Feb 18. 85	5/34	14.7
Totals:-	224/575	39.0

* Sample 01.12.84 based on smear diagnosis only; not included in statistical tests.

Fig. 2. *Cerastoderma edule*. Prevalence of neoplastic individuals in each monthly sample from Cuskinny, Feb 1983–Feb 1985

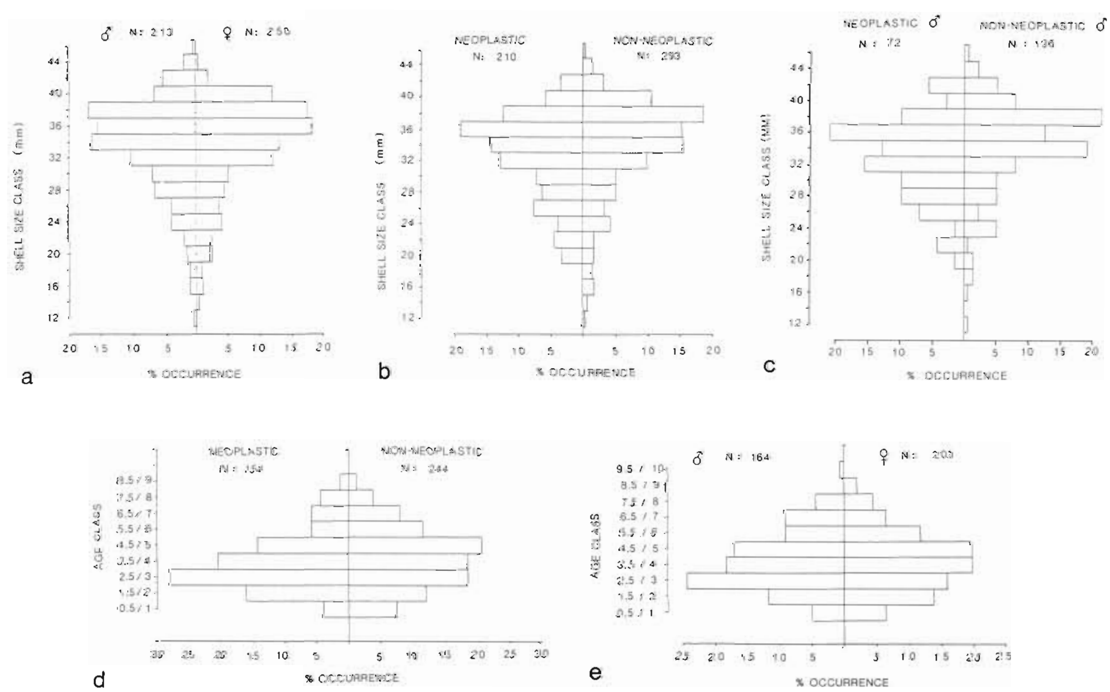


Fig. 3. *Cerastoderma edule*. Percentage occurrence of Cuskinny cockles of different shell size classes for male vs female (a), neoplastic vs non-neoplastic (b), neoplastic male vs non-neoplastic male (c), and for age classes of neoplastic vs non-neoplastic (d), and male vs female (e)

Shell size classes

The frequency distributions of shell size classes for males versus females, neoplastic versus non-neoplastic cockles (i.e. both sexes together), and for neoplastic males versus non-neoplastic males are presented in Fig. 3a, b, and c respectively. Only minor differences occured in shell size class frequencies between the sexes (Fig. 3a). The modal size class for male cockles is 38 mm (17 %), but the percentage occurrences are almost as high at 34 mm (16 %) and 36 mm (16 %), with gradual decline in numbers both in the larger size classes (40 mm to 46 mm), and in the smaller size classes (32 to 12 mm). The modal size class for female cockles is shared by the 36 and 38 mm classes (18 %), with high numbers also occurring in the 40 to 32 mm size classes. Outside the 32 to 40 mm size range percentage occurrences decline rapidly.

Among non-neoplastic cockles the modal size class is 38 mm (19 vs 12 % neoplastic) with high percentage occurrences also across the size range 32 to 40 mm (Fig. 3b). Percentage occurrences are low in the 12 to 30 mm classes (> 1 to 5 %). The mode for the neoplastic cockles lies in the 36 mm size class (19 vs 15 % non-neoplastic) with high percentages also in the size range 32 to 38 mm but there is an overall shift downwards to the younger size classes.

Shell size class frequency distributions for neoplastic male versus non-neoplastic male cockles are shown in Fig. 3c. Among non-neoplastic males the greatest numbers occur in the 38 mm (21 %), 36 mm (13 %) and 34 mm (19 %) size classes with rapid fall off both in numbers of larger cockles (40 to 46 mm) and of smaller cockles (32 to 12 mm). Among neoplastic males, the greatest numbers occur in the 36 mm (21 %), 34 mm (13 %), and 32 mm (15 %) classes, with more gradual decline in numbers in the larger and smaller size classes. The size class distribution of the neoplastic males is skewed towards the smaller classes compared with that of the non-neoplastic males.

Age classes

Frequency distributions for age classes of neoplastic versus non-neoplastic cockles are presented in Fig. 3d. The distribution for the non-neoplastic group has a more bell shaped curve, with the modal class at 5 yr (21 vs 14 % neoplastic) and a gradual decrease in the percentage occurrence both in the younger age classes (4 to 1 yr) and in the older classes (6 to 9 yr). The pattern for the neoplastic group is somewhat different with the overall distribution shifted towards the younger age classes. Thus the modal class occurs here at 3 yr (28 vs 18 % non-neoplastic) with a tendency for more individuals in the 2 to 5 yr classes and less in the 6 to 9 yr classes.

The age class frequency distributions for male and

Table 3. *Cerastoderma edule.* χ^2 tests for comparison of age and size class frequencies of combinations of neoplastic, non-neoplastic, male and female subgroups; 2 groups were significant: shell size class frequencies of neopl. vs non-neopl. for both sexes together, and for males alone

	χ^2	df	Significance
(A) Age class frequencies of neopl. vs non-neopl. groups of:			
– both sexes together	13.55	7	ns
– male cockles alone	5.21	4	ns
– female cockles alone	3.27	5	ns
(B) Age class frequencies of male vs female groups of:			
– neopl & non-neopl. together	6.22	7	ns
– neopl. cockles alone	4.84	4	ns
– non-neopl. cockles alone	4.22	7	ns
(C) Shell size class frequencies of neopl. vs non-neopl. groups of:			
– both sexes together	19.90	11	$p < 0.05$
– male cockles alone	14.46	6	$p < 0.05$
– female cockles alone	2.34	7	ns
(D) Shell size class frequencies of male vs female groups of:			
– neopl. & non-neopl. together	13.92	11	ns
– neopl. cockles alone	7.37	7	ns
– non-neopl. cockles alone	4.66	7	ns

df: Degrees of freedom
ns: Statistically not significant

female cockles are presented in Fig. 3e. Both distributions are similar, with only minor differences. Thus the modal class for males occurs at 3 yr (24 vs 16 % female), but with large numbers in the 4 yr (18 %) and 5 yr (17 %) classes also. There are gradual decreases in percentage occurrences both in the older classes (6 to 9+ yr) and in the younger classes (2 and 1 yr). The modal age class for females is shared by 4 and 5 yr classes, with gradual increase in the younger age classes (1 to 3 yr) and decrease in the older classes (6 to 9+ yr).

No overall differences were detected in the statistical analysis of these age class frequency distributions of neoplastic versus non-neoplastic cockle and of male versus females (Table 3). However, as is evident in the above account, there were more notable differences in the age class distributions of neoplastic cockles versus non-neoplastic cockles, than between males versus females, the former distributions marginally failing to reach the 95 % significance level.

Shell size classes 22 to 32 mm, with more than expected numbers of neoplastic cockles, correspond broadly with the 2+/3 yr class, which also has more neoplastic cockles. Similarly, the 38 to 40 mm size class, with fewer than expected numbers of neoplastic cockles, corresponds with the 5+/6 yr class, which also has fewer neoplastic cockles (Fig. 4).

Growth

Growth curves showing mean height at each age for neoplastic and non-neoplastic cockles groups from Cuskinny are depicted in Fig. 4. Both curves are similar indicating no difference in growth between the 2 groups. The age range at Cuskinny runs from 0+ to 9+ yr, and the size range from 13 to 47 mm. Subdivision of the population into neoplastic and non-neoplastic, and into male and female groups, did not reveal any major differences for age or size class ranges: the age range for non-neoplastic cockles was 0+ to 9+ yr, for neoplastic cockles from 1 to 9, for males, from 0+ to 9+, and for females, from 0+ to 9.

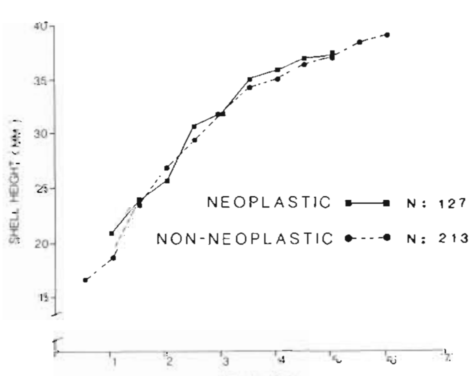


Fig. 4. *Cerastoderma edule*. Mean shell height at each age for neoplastic and non-neoplastic cockles from Cuskinny

Age ranges at Cuskinny contrast markedly with those at sites outside the harbour where 5+ was the maximum age encountered. Growth varied from site to site independently of neoplasm presence, and there is no association between size/age range of a population, and presence of neoplasm. Thus, for example, at Ballymacoda, where neoplasms were absent, cockles were generally smaller age for age than Cuskinny cockles, and maximum age there was 4 compared to 9+ yr for Cuskinny. At Courtmacsherry, where neoplasms were present, age range and size range were comparable to those at Ballymacoda. Growth rates at Passage East and Cuskinny were similar; yet the disease was absent from the latter site. There were no significant differences between the sexes for age and shell size classes (Table 3).

Sex

Numbers of male and female cockles in neoplastic and in non-neoplastic categories, total numbers of males and females, and the sex ratios for various subgroups from Cuskinny, other harbour sites and coast sites are presented in Table 4.

The sex ratio for Ballymacoda, which may be taken to be that of a normal population, was 1:1.3 male/female (mean of 9 samples). Overall sex ratio for Cuskinny cockles was 1:1.2 (mean of 24 samples). A similar ratio existed for the other harbour shores (mean of 12 samples) while that for the smaller sample from the 4 coastal sites where the neoplasm was detected was 1:1. The neoplasm was, however, more prevalent among females than among males. At Cuskinny 41 % of females and 32 % of males were affected and this tendency was also reflected in the rates for other harbour sites: 39 % female, 26 % male. In the smaller sample from the coast, both sexes were equally affected. Statistical analysis for an association between sex and neoplasm gives χ^2 values marginally below the 95 % significance level.

GLIM analysis

GLIM analysis – which takes account of the 4 independent variables, month, age, shell height and sex, plus interactions – revealed that month and age were the best predictors of neoplasm occurrence. Dependence of the sarcoma on month is highly significant. The addition of age to the model just failed to reach signifi-

Tabe 4. *Cerastoderma edule*. Sex ratios of cockles from different sampling areas. Ratio for total population given first (Total), followed by that for neoplastic cockles alone (Npl) and for non-neoplastic cockles alone (Non-npl)

Site		Total sample	Npl No.	Npl %	Non-npl No.	Non-npl %	Ratio	male / female
Ballymacoda	Male	98	0		98	100 %	Total	1 : 1.3
	Female	126	0		126	100 %		
Cuskinny	Male	233	75	32 %	158	68 %	Total	1 · 1.2
							Npl	1 · 1.5
	Female	276	112	41 %	164	49 %	Non-npl	1 : 1
Harbour (excluding Cuskinny)	Male	90	23	26 %	67	74 %	Total	1 : 1.2
							Npl	1 : 1.8
	Female	106	41	39 %	65	61 %	Non-npl	1 . 1
Coast (4 Npl sites)	Male	54	8	15 %	46	75 %	Total	1 : 1
							Npl	1 . 1
	Female	55	8	15 %	47	75 %	Non-npl	1 : 1

cance at the 95 % confidence level. Thus while the dependence of the sarcoma on month is confirmed, the importance of the other variables – age, size and sex – remains unresolved. Differences in results between the univariate χ^2 tests and the logistic regression analysis of the GLIM model may, in part, be explained by the necessity to remove from the latter analysis all cockle specimens which had missing values; this reduced the population for analysis to 137, compared with 360 to 500 for the other individual tests. Alternatively, assuming that the population sample available for the GLIM model is representative of the population at large, any interactions among the variables will have been accounted for, and one may conclude that month and, to a lesser degree, age are the important predictors of the neoplasm.

Transmission tests

Samples of healthy cockles transferred from Ballymacoda, the disease-free site, to Currabinny, a site with high prevalences of the disease, were removed for histological examination 1, 2, 3, 4 and 6 mo post transfer. This experiment was terminated at 6 mo post transfer due to human and/or storm damage to the cages. Sample size and date of removal are listed below:

Date	Month/post-transfer	Sample size
Mar 11, 85	1	21
Apr 10, 85	2	40
May 6, 85	3	20
Jun 4, 85	4	33
Aug 20, 85	6	10

Neoplasms were not detected in histological sections of any of these samples.

Trace element analysis

Results of the trace element analysis are presented in Fig. 5. Cadmium was not detected in any of the samples (detection limit = 0.05 $\mu g\ g^{-1}$). Median levels for Cu ranged from 0.66 to 2.22 $\mu g\ g^{-1}$ wet wt; Zn, from 7.73 to 12.92; Pb, from 0.09 to 0.38; Mn, from 1.97 to

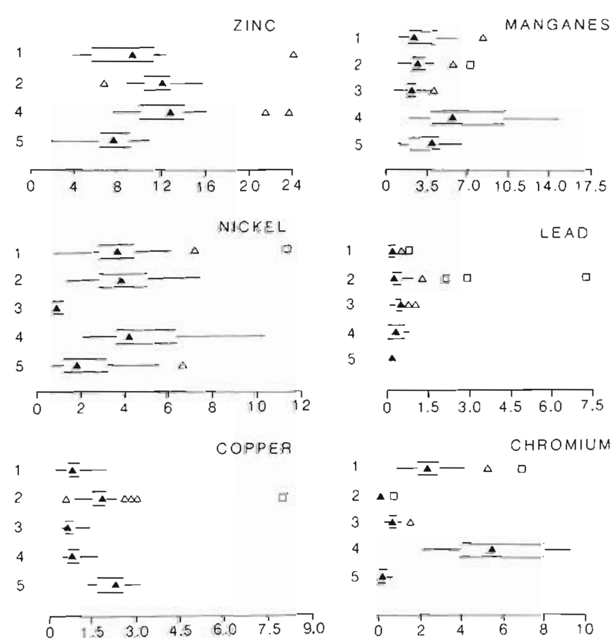


Fig. 5. *Cerastoderma edule*. Plots of trace element levels ($\mu g\ g^{-1}$ wet wt) in cockles from Cuskinny and Ballymacoda. The middle half of each batch is represented by a double line containing the median (▲); the extent of the data is represented by single lines on either side, and extraordinary values are indicated by (△) for a possible outlier and (□) for a probable outlier. Site/sample 1: Cuskinny, Jul 04. 85; 2: Cuskinny, May 22. 86; 3: Ballymacoda, Feb 10. 85; 4: Ballymacoda, Jul 05.85; 5: Ballymacoda, May 23. 85

5.47; Ni, from 0.85 to 4.18; Cr, from 0.05 to 5.34. No trends were evident in the data for site-specific differences. Because of the great variability in the levels or any one metal – due to such non-standardised factors as size, age, sex and season – it was not possible to carry out a statistical analysis. However, the plots in Fig. 5 display the main features of the data and it is evident that levels of all metals are generally very low for both Cuskinny and Ballymacoda suggesting no differences of major biological significance between sites inside and outside the harbour.

DISCUSSION

Prevalence and distribution

Neoplasms in *Cerastoderma edule* reach epizootic proportions in Cork Harbour and at 2 shores west of the Harbour (Oysterhaven and Courtmacsherry). However, the disease appears to be chiefly associated with Cork Harbour; major differences exist in the overall prevalence rates for harbour and non-harbour sites (31–33 vs 14 %). The higher prevalence of the neoplasm within the harbour suggests that some environmental factor or stress due to an impaired habitat may be responsible for, or play a part in, the aetiology of the disease.

Prevalences were much lower outside the harbour, and apart from the single case (1 of 27) east of the harbour at Youghal, the distribution of the neoplasm is completely westward from Cork harbour. This predominantly westward extension fits well with the general coastal circulation pattern (Cooper 1967, Mc K. Bary pers. comm.) Water originating in the Irish Sea flows outward around Carnsore Point, along the south coast, around the southwest and up the west coast. This water forms a narrow surface band up to 10 miles offshore, with bottom currents behaving similarly, at least for some of the time. It is not known how persistent the east-west flow is, and it is probably disrupted by southwest to west gales. The westward extension from the harbour in the occurrence of the sarcoma may therefore be due to transport of pollutants, infectious agents and/or infected or genetically susceptible cockle larvae.

Monthly prevalence/seasonality

Prevalences at Cuskinny, where neoplasms were present throughout the year, exhibit a distinct bimodal seasonal trend (Fig. 2). This trend may be related to temperature, spawning, availability of a pathogen, recruitment to the cockle population and/or cockle growth.

The seasonal peaks in the prevalences for 1984 are less pronounced than those for 1983, the summer of which was exceptionally hot. Temperature could have a direct effect on the expression of a pathogen, such as a virus, and on the defence mechanisms of the cockle. Cockles appear to be particularly susceptible to extremes of temperature associated with very cold winters and very hot summers (Orton 1933, Cole 1956, Hancock & Urquhart 1965, Seed & Brown 1975).

Cockles could also be more susceptible to disease during spawning when they are most stressed, with less energy reserves after the investment in gamete production. Cockles from other localities in Ireland and Britain are known to have their major spawning period in late spring/early summer, minor gamete release throughout the summer also having been reported in some cases (Johnstone 1899, Cole 1956, Creek 1960, Hancock 1967, Rygg 1970, Boyden 1971, Kingston 1974, Seed & Brown 1975). This reproductive pattern also holds true for cockles at Cuskinny. The higher prevalences of the sarcoma, at least in early summer, may therefore be associated with gametogenesis and spawning.

Histopathology suggests that the sarcoma may derive from the germinal epithelium of the gonad. The neoplastic cells appear to proliferate in tandem with gonad development during spring. Peak prevalence in June occurs during the major spawning event and is followed by a pronounced decrease, presumably due to mortalities: cell transplant experiments (Twomey & Mulcahy 1988), although exposing the experimental cockles artificially to the disease, indicate that disease can progress rapidly and kill within a period of 3 to 5 mo. Histological examination of gonads suggests that gametes may continue to be released throughout summer and autumn. This is matched by a steady prevalence of 30 to 40 % for the sarcoma, with an increase around Oct–Nov, particularly marked in the 1983 data. These higher prevalences may be associated with decreasing water temperature; an inverse relationship between severity of the sarcoma and water temperature was found for *Mya arenaria* (Cooper et al. 1982). The minimum prevalence in winter may, in turn, be due to mortalities and to the absence of gametogenesis. The present study suggests that the disease is more prevalent in younger, smaller cockles. Older cockles would *ipso facto* be survivors of a younger cohort and therefore have a lower prevalence. The higher prevalences in early summer may therefore be partly due to the arrival in the population of young, susceptible individuals which succumb by late summer.

The haemic neoplasm in *Mya arenaria* from Rhode Island also shows this seasonal biphasic pattern, with highest prevalences occurring in autumn (Oct–Nov: 43 %) and late spring (Apr: 43 %) and lowest prevalences during summer (May–Aug: 20 %); peak prevalences occurred during spawning and at sea temperatures between 5 and 10 °C (Cooper et al. 1982). In the same species from Chesapeake Bay, prevalences were high from Dec to Apr, decreased to zero by Jun and increased again in autumn, and the prevalences of advanced cases increased as the season progressed (Farley et al. 1986). No association was evident, based on limited sampling, between gonadal neoplasms and season in *M. arenaria* from Rhode Island (Yevich & Barszcz 1977).

In *Mytilus edulis* from Yaquina Bay, neoplasms first appeared in August, were common by Nov (peak prevalence: 14 %) and disappeared in May. An identical pattern occurred for the sarcoma in *Ostrea lurida* from the same area with a peak prevalence of 12 % in Dec (Farley & Sparks 1970, Farley 1976). Advanced cases were most common in early winter (Dec). A later study of Yaquina Bay mussels (Mix 1983) reported peak prevalences during Jan to Mar, with decreases throughout spring and summer and increases during late autumn/early winter No seasonal progression of the disorder was indicated. In inbred lots of *Crassostrea virginica* neoplasms were found over 9 mo, with highest prevalences from Jul to Nov (Frierman & Andrews 1976). Neoplasms in *Macoma balthica* from Chesapeake Bay occurred during the colder months, Oct to May, and were not found in summer (Jun to Aug) (Christensen et al. 1974). Prevalences appeared higher in Oct, Mar and Apr.

The general pattern presented in the literature thus is a cyclic one, with increased prevalences of neoplasia in autumn, early winter and spring, and decreased prevalences or absence of neoplasms in summer. The monthly prevalences at Cuskinny concur with this general pattern. Unlike some of the reports, however, neoplasms did not disappear at certain times of the year: they were present throughout the year, most abundant in autumn and late spring/early summer, and least abundant in late summer and winter.

Growth/sex/age/height

There are 2 difficulties with ageing cockles using annual check marks. Firstly, the possibility of confusing true annual rings with 'disturbance' rings caused by storms, predators, spawning events, etc. (Orton 1926), and secondly, the possibility of overlooking the first winter period because 0+ cockles often show continuous, although slower, growth during their first winter season (Richardson et al. 1980). These difficulties may be avoided by observing growth spawning and spat settlement and following size cohorts through time (Orton 1926). The acetate peel replication technique used in this study provides an efficient alternative.

Growth curves (mean height at each age) are similar for both neoplastic and non-neoplastic cockles from Cuskinny. It might have been expected that diseased cockles would be slower growing. However, the similar growth rates in the 2 groups are not unexpected, in view of the rapidity with which the neoplasm progresses to a fatal outcome (Twomey & Mulcahy 1988). Thus any decline in growth rate could only occur in the final growing season of any particular cockle. This finding also suggests that the disease is not chronic over a number of years, based on the assumption that chronically diseased cockles would undergo a decrease in growth rate.

Variations in maximum age attained at different shores are probably dependent on the presence of such major cockle predators as oystercatchers and flounders (Drinnan 1957, Hancock & Urquhart 1965). Size variations could be largely dependent on such factors as exposure to waves and trophic conditions.

The evidence presented in this study suggests that neoplasms are more prevalent among females than among males and also that they have an unequal distribution among age and size classes, being more prevalent in smaller/younger cockles, particularly males.

Comparison of age and size class frequency distributions of neoplastic and non-neoplastic males and females does not indicate any difference between sexes i.e. the age and size class frequencies of neoplastic males are similar to those of neoplastic females, and those of non-neoplastic males are similar to those of non-neoplastic females. However, when age and size class frequencies of neoplastic and non-neoplastic cockles are compared within sexes – while those for age class are similar in neoplastic and non-neoplastic groups of males and females, and also in the case of size for females – there are major differences ($p < 0.05$) in the size class frequencies of neoplastic males compared with non-neoplastic males, and also in those of all neoplastic cockles (both sexes together) compared with all non-neoplastic cockles.

Females therefore appear to have a more even distribution of neoplasms among all size classes, while among males, greater than expected numbers of diseased individuals occur in the smaller size classes (28 and 30 mm) and fewer than expected in the larger size class (38 mm).

Large differences in neoplasm prevalences between sexes have not been reported previously. However, while there were no differences for the gonadal neoplasm in *Mya arenaria* (Yevich & Barszcz 1977), a slightly higher rate for gonadal cancer occurred in female *Mercenaria mercenaria* (4 vs 1 %) (Barry & Yevich 1972). Both sexes of *Crassostrea commercialis* were equally affected by papillary epitheliomas (Wolf 1976). No significant differences were found in the frequencies of haemic neoplasms between the sexes of *Mya arenaria* nor in mean size of neoplastic and non-neoplastic clams (with the implication of no age related differences), but there was a significant difference between tissue weights (Brown et al. 1979). The latter finding is not unexpected in view of the highly invasive and destructive nature of the disease. Prevalences of the neoplasm in *Mya arenaria* were significantly lower during the first year of life (Cooper et al. 1982). This difference disappeared once the clams reached the summer of their second year. As with *Cerastoderma edule*, an inverse relationship between number of neoplasms and shell length was reported for the gill carcinoma in *Macoma balthica* (Christensen et al. 1974). A higher prevalence of haemic neoplasms occurred in older specimens of *Ostrea edulis* (Balouet & Poder 1978).

The differences in prevalences between the sexes provide additional support for the hypothesis that the neoplasm may be derived from the gonad. The neoplasm was not found in 12 0+ individuals and only became evident in cockles in their second growing season (20 mm size class) when they become sexually mature. If the sex ratio for the largest non-neoplastic sample, that for Ballymacoda, (1:1.3), is taken as the normal sex ratio in cockles (Table 4), it is seen to be close to the overall sex ratio for the Cuskinny and harbour groups (1:1.2). However, when the latter populations are split into neoplastic and non-neoplastic groups, the sex ratio in both shifts in favour of females (1:1.5 and 1:1.8 respectively) with a concomitant decrease in the number of females in the non-neoplastic groups.

Transmission

Neoplasms were not induced in non-neoplastic cockles which had been transferred from Ballymacoda to Currabinny and sampled from 1 to 6 mo post-transfer. Thus, it is concluded that the disease is not inducible in healthy cockles by environmental factors over a period of 6 mo when placed in an area where the disease is enzootic. Observations over a longer time span are necessary to ascertain whether the exposure period in this experiment was too short for induction of the neoplasm. It is of interest that samples of *Ostrea lurida*, introduced into Yaquina Bay where the native population are subject to neoplasia, did not develop the disease over a 1.5 yr period (Farley 1976) or a 4 yr period (Mix et al. 1977). There exists strong evidence for a viral aetiology for the haemic neoplasm in *Mya arenaria* (Oprandy et al. 1981). In the present study, neoplasms were successfully transmitted using neoplastic cell transplants, but preliminary tests with cell-free filtrates made from neoplastic cells failed (Twomey & Mulcahy 1988). The sarcoma in *Mya arenaria* (Farley unpubl.) and in *Mytilus edulis* (Elston unpubl.) have also been successfully transmitted using cell transplants.

The species specificity of epizootic neoplasia has been noted previously (Yevich & Barszcz 1977, Cooper et al. 1982). In the present study neoplasms were not detected in small samples of *Mytilus edulis* from Cork Harbour, although this species is subject to epizootics in other countries (Farley 1969, Green & Alderman 1983, Cosson-Mannevy et al. 1984).

Mortality

Evidence is available of the fatal outcome of the sarcoma in *Cerastoderma edule* (Twomey & Mulcahy 1988). The lower prevalence of the sarcoma in cockles in winter (Feb–Mar) and in summer (Jul, Aug, Sep) may therefore be due to mortality of the more severely affected individuals included in the late spring and late autumn peaks.

Both Cooper et al. (1982) and Farley et al. (1986) were able to follow the course of the disease in *Mya arenaria* by taking regular haemolymph samples from laboratory held clams. Both studies demonstrate the fatal outcome of the disease, but whereas mortality in the latter study was 100 % with no observed remission over a 7 mo period from Dec to Jun, in the former study, the clinical progression of the disease to death was not inevitable but depended on the severity level; moderately affected individuals could either progress in severity, remain at the same level or undergo remisssion. Survivorship for laboratory held *Macoma balthica* over a 3 mo period was 20 % for neoplastic, compared with 81 % for non-neoplastic individuals (Christensen et al. 1974).

Other evidence for the fatal outcome of the disease has been inferred from field observations of mortalities associated with neoplasia. Alderman et al. (1977) reported high mortalities among *Ostrea edulis* populations in Yugoslavia and in Galicia, Spain, associated with an haemic neoplasm. Mortalities in Yugoslavia ranged from 20 to 90 % in different years and occurred from Apr to Oct. Those in Spain occurred from Jun to Sep at levels of 60 to 80 %. Frierman & Andrews (1976) noted a high percentage of neoplastic specimens among moribund specimens of *Crassostrea virginica*, in the USA. The sarcoma in *Ostrea lurida* from Yaquina Bay was first reported by Jones & Sparks (1969) and linked with winter mortalities in the area. Farley & Sparks (1970) found advanced cases to be most common in early winter with apparent indications of mortality. Mix et al. (1977) investigating the same population, found an extremely low neoplasm prevalence (2 %). A degenerative syndrome observed over winter, with ensuing recovery, was considered to be due to cessation of feeding and not indicative of impending mortality. There were high winter mortalities among oysters maintained near the water surface due to lowered salinities from the seasonal increase in fresh water run-off.

Trace element analysis

Levels of all trace elements are generally very low for both Cuskinny and Ballymacoda with no suggestion of biologically significant differences between sites or samples (Fig. 5). The levels compare favourably with those recorded by Wilson (1980) for *Cerastoderma edule* from the east coast of Ireland (Drogheda, Malahide, Dublin) which are amongst the lowest in Europe and therefore useful as baselines. Zinc levels in macroalgae at certain sites within Cork Harbour have been found to be elevated relative to levels at control sites outside the harbour (Cullinane & Whelan 1982). However, not all in-harbour samples were elevated, levels at Cobh and East Ferry, adjacent to Cuskinny, being comparable to upper levels at control sites. Zinc levels measured for *C. edule* in the present study are low and comparable to those recorded on the east coast (Wilson 1980).

Pollution

Major differences in disease prevalence rates between the harbour and non-harbour sites suggest that some harbour associated phenomenon such as pollution may be involved in the sarcoma in *Cerastoderma edule*. However, there is no direct evidence for an association between neoplasms in the cockles and pollution. Heavy metal levels in cockles from within the harbour (Cuskinny) and outside (Ballymacoda) were uniformly low and comparable with baseline levels reported for the east coast of Ireland (Wilson 1980). PCBs were not detectable in cockle tissues from the same sites (unpubl. own obs.). Evidence for pollution or carcinogenic agents as causative agents of neoplasia in bivalve molluscs is largely circumstantial and inconclusive, with epizootics occurring at 'clean' and polluted sites (Couch & Harshbarger 1985, Mix 1986).

Cork Harbour receives large amounts of untreated domestic sewage, and although a number of industries – pharmaceutical, chemical, steel works, engineering, oil refining and ship building – are situated around the harbour, ecological studies by the Departments of Zoology and Botany at University College Cork have revealed only localised areas of pollution in the inner harbour, the outer parts being relatively unpolluted, with typical 'clean-water benthic communities' (Myers 1981).

The most probable source of pollution insult to the cockles within the harbour, therefore, is that caused by general eutrophication. Cuskinny does, in fact, produce large blooms of algae each summer which may be a source of stress to the cockles. It is also of interest that Youghal, exceptional in that it was the only site east of the harbour to yield a neoplastic cockle (1 of 27), had a muddy sulphurous substrate which contrasted with the cleaner mud/sand of the other coastal sites. It is possible that the sarcoma is dormant or present at very low levels in all cockle populations, and only increases under stressful conditions.

Of the sites west of the harbour, all of which contained neoplastic cockles, Courtmacsherry and Fountainstown appeared to be most unaffected by pollution, although both have had green algal cover in summer in recent years. These latter 2 shores most resembled Ballymacoda and Woodstown east of the harbour where the disease was absent. The third westerly site, Oysterhaven, is a long enclosed estuary which may have been more enriched and was also subject to large freshwater inflow. The presence of the disease west of the harbour may be due to colonisation of this area by affected/infected larvae, the progeny of the harbour population. All the cockle populations were exposed to fresh water run-off which may be a source of agrochemical pollutants. There is therefore no clear-cut pattern to the distribution of the disease in relation to pollutants. However, parallel studies in the Department of Zoology, University College Cork, indicate that fish populations within Cork Harbour have generally higher prevalences of diseases compared to populations outside (Maye 1986).

In addition to virus and pollution, other factors such as genetic susceptibility (Frierman & Andrews 1976) and stress (Alderman et al. 1977, Brown 1980, Oprandy & Chang 1983) have been implicated in bivalve epizootic neoplasia. Cellular defence mechanisms in molluscs are known to be inhibited by a variety of pollutants (Anderson 1981, Cheng & Sullivan 1984).

A multifactorial aetiology for epizootic neoplasia in bivalve molluscs is therefore suggested involving virus, pollutants and stress.

Acknowledgements. We acknowledge support of the National Board for Science and Technology (NBST) and Cancer Research Advancement Board (CRAB); Cathy Maye, for assistance with histology and fieldwork; Margaret Cole (Department of Statistics, University College Cork), for assistance with statistical analysis; Dr Christopher Richardson (Marine Sciences Laboratory, University College Bangor, Wales), for assistance with the acetate peel ageing technique.

LITERATURE CITED

Alderman, D. J., van Banning, P., Perez-Colomer, A. (1977). Two European oyster *(Ostrea edulis)* mortalities associated with an abnormal haemocytic condition. Aquaculture 10: 335–340

Anderson, R. S. (1981). Effects of carcinogenic and noncarcinogenic environmental pollutants on immunological functions of a marine invertebrate. In: Dawe, C. J., Harshbarger, J. C., Kondo, S., Sugimura, T., Takayama, S. (eds.) Phyletic approaches to cancer Japan Sci. Soc. Press, Tokyo, p. 319–331

Baker, R. J., Nelder, J. A. (1978). Introduction to GLIM. The GLIM system, release 3. (Royal Statistical Society©) c/o Numerical Algorithms Group, 7 Banbury Road, Oxford OX2 6NN, UK

Balouet, G., Poder, M. (1978). Hyperplasie haemocytaire chez *Ostrea edulis* L. Haliotis 9: 99–102

Barry, M. M., Yevich, P. P. (1972). Incidence of gonadal cancer in the quahog *Mercenaria mercenaria*. Oncology 26: 87–96

Boyden, C. R. (1971). A comparative study of the reproductive cycles of the cockles *Cerastoderma edule* and *C. glaucum*. J. mar. biol. Ass. U. K. 51· 605–622

Boyden, C. R. (1972). Relationship of size to age in the cockles *Cerastoderma edule* and *C. glaucum* from the River Crouch estuary, Essex. J. Conch. 27: 475–489

Brown, R. S. (1980). The value of the multidisciplinary approach to research on marine pollution effects as evidenced in a three year study to determine the etiology and pathogenesis of neoplasia in the soft-shell clam, *Mya arenaria*. Rapp. P.-v. Réun. Cons. int. Explor Mer 179: 125–128

Brown, R. S., Wolke, R. E., Brown, C. W., Saila, S. B. (1979). Hydrocarbon pollution and the prevalence of neoplasia in New England soft-shell clams *(Mya arenaria)*. In: Animals as monitors of marine pollutants. Nat. Acad. Sci., Washington, D. C., p. 41–51

Cheng, T C. (1967). Marine molluscs as hosts for symbioses: with a review of known parasites of commercially important species. Adv. mar Biol. 5: 1–424

Cheng, T C., Sullivan, J. T (1984). Effects of heavy metals on phagocytosis by molluscan hemocytes. Mar. environ. Res. 14: 305–315

Christensen, D. J., Farley, C. A., Kern, F. G. (1974). Epizootic neoplasms in the clam *Macoma balthica* (L.) from Cheseapeake Bay. J. natn. Cancer Inst. 52: 1739–1749

Cole, H. A. (1956). Benthos and the shellfish of commerce. In: Graham, M. (ed.) Sea fisheries: their investigations in the United Kingdom. Edward Arnold Ltd., London, p. 207–250

Cooper, L. H. N. (1967). The physical oceanography of the Celtic Sea. Oceanogr. mar Biol. A. Rev. 5: 99–110

Cooper, K. R., Brown, R. S., Chang, P. W. (1982). The course and mortality of a hematopoietic neoplasm in the soft-shell clam, *Mya arenaria*. J. invertebr. Pathol. 39: 149–157

Cosson-Mannevy, M. A., Wong, C. S., Cretney, W. J. (1984). Putative neoplastic disorders in mussels *(Mytilus edulis)* from southern Vancouver Island waters, British Columbia. J. Invertebr. Pathol. 44: 151–160

Couch, J. A., Harshbarger, J. C. (1985). Effects of carcinogenic agents on aquatic animals: an environmental and experimental overview. J. Environ. Sci. Health, Part C, Environ. Carcinog. Rev. 3: 63–105

Creek, J. A. (1960). The development of the lamellibranch *Cardium edule* (L.). Proc. zool. Soc. Lond. 135: 243–260

Cullinane, J. P., Whelan, P. M. (1982). Copper, cadmium and zinc in seaweeds from the south coast of Ireland. Mar Pollut. Bull. 13: 205–208

Drinnan, R. E. (1957). The winter feeding of the oystercatcher *(Haematopus ostralegus)* on the edible cockle *(Cardium edule)*. J. Anim. Ecol. 26: 441–469

Farley, C. A. (1969). Sarcomatoid proliferative disease in a wild population of blue mussels *(Mytilus edulis)*. J. natn. Cancer Inst. 43: 509–516

Farley, C. A. (1976). Proliferative disorders in bivalve mollusks. Mar Fish. Rev. 38: 30–33

Farley, C. A., Otto, S. V., Reinisch, C. L. (1986). New occurrence of epizootic sarcoma in Chesapeake Bay soft shell clams *(Mya arenaria)*. Fish. Bull. U. S. 84: 851–857

Farley, C. A., Sparks, A. K. (1970). Proliferative diseases of hemocytes, endothelial cells and connective tissue cells in molluscs. In: Dutcher, R. M. (ed.) Comparative leukaemia research 1969. Bibl. haemat. No. 36, Karger, Basel, p. 610–617

Franklin, A. (1972). The cockle and its fishery. Ministry of Agriculture, Fisheries & Food Laboratory Leaflet, N. S., No. 26: 1–34

Frierman, E. M., Andrews, J. D. (1976). Occurrence of hematopoietic neoplasms in Virginia oysters *(Crassostrea virginica)*. J. natn. Cancer Inst. 56: 319–324

Green, M., Alderman, D. J. (1983). Neoplasia in *Mytilus edulis* L. from United Kingdom waters. Aquaculture 30: 1–10

Hancock, D. A. (1967). Growth and mesh selection in the edible cockle (*Cardium edule* L.). J. appl. Ecol. 4: 135–157

Hancock, D. A., Urquhart, A. E. (1965). The determination of natural mortality and its causes in an exploited population of cockles (*Cardium edule* L.). Fish. Invest. Lond. (Ser. 2) Vol. 24: (No. 2) 1–40

Harshbarger, J. C., Charles, A. M., Spero, P. M. (1981). Collection and analysis of neoplasms in sub-homeothermic animals from a phyletic point of view. In: Dawe, C. J., Harshbarger, J. C., Kondo, S., Sugimura, T., Takayama, S. (eds.) Phyletic approaches to cancer. Japan Sci. Soc. Press, Tokyo, p. 357–384

Jones, E., Sparks, A. K. (1969). An unusual histopathological condition in *Ostrea lurida* from Yaquina Bay, Oregon. Proc. natn. Shellfish Ass. 59: 11 (Abstract)

Johnstone, J. (1899). *Cardium*. Liverpool Marine Biology Committee Memoirs No. II

Kingston, P. K. (1974). Studies on the reproductive cycles of *Cardium edule* and *C. glaucum*. Mar Biol. 28: 317–323

Kreger, D. (1940). On the ecology of *Cardium edule*, L. Archs néerl. Zool. 4: 151–200

Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In: Kinne, O. (ed.) Diseases of marine animals. Vol. II. Biologische Anstalt Helgoland, Hamburg. p. 477–961

Maye, C. T (1986). Pathobiology of fish of the Cork harbour area. M. Sc., National University of Ireland, University College Cork, Ireland

Mix, M. C. (1986). Cancerous diseases in aquatic animals and their association with environmental pollutants: a critical literature review. Mar. environ. Res. 20: 1–141

Mix, M. C. (1983). Haemic neoplasms of bay mussels, *Mytilus edulis* L., from Oregon: occurrence, prevalence, seasonality and histopathological progression. J. Fish. Dis. 6: 239–248

Mix, M. C., Pribble, H. J., Riley, R. T., Tomasovic, S. P. (1977). Neoplastic disease in bivalve mollusks from Oregon Estuaries with emphasis on research on proliferative disorders in Yaquina Bay oysters. Ann. N. Y. Acad. Sci. 298: 356–373

Myers, A. (1981). Estuarine and marine coastal pollution in the Republic of Ireland. Mar Pollut. Bull. 8: 260–264

Oprandy, J., Chang, P. W. (1983). 5-Bromodeoxyuridine induction of haematopoietic neoplasia and retrovirus acti-

vation of the soft shell clam *Mya arenaria*. J. Invertebr. Pathol. 42: 196–206
Oprandy, J. J., Chang, P. W., Pronovost, A. D., Cooper, K. R., Brown, R. S., Yates, V J. (1981). Isolation of a viral agent causing hematopoietic neoplasia in the soft shell clam *Mya arenaria*. J. Invertebr. Pathol. 38: 45–51
Orton, J. H. (1926). On the rate of growth of *Cardium edule*. Part I. Experimental observations. J. mar biol. Ass. U. K. 14: 239–279
Orton, J. H. (1933). Summer mortality of cockles on some Lancashire and Cheshire Dee beds in 1933. Nature, Lond. 132: 314–315
Richardson, C. A., Crisp, D. J., Runham, N. W. (1979). Tidally deposited growth bands in the shell of the common cockle, *Cerastoderma edule* (L.). Malacologia 18: 277–290
Richardson, C. A., Crisp, D. J., Runham, N. W., Gruffydd, Ll. D. (1980). The use of tidal growth bands in the shell of *Cerastoderma edule* to measure seasonal growth rates under cool temperate and sub-arctic conditions J. mar biol. Ass. U. K. 60: 977–989
Rosenfield, A. (1976). Recent environmental studies of neoplasms in marine shellfish. Prog. exp. Tumor Res. 20: 263–274
Rygg, B. (1970). Studies on *Cerastoderma edule* (L.) and *Cerastoderma glaucum* (Poiret). Sarsia 43: 65–80
Seed, R., Brown, R. A. (1975). The influence of reproductive cycles, growth and mortality on population structure in *Modiolus modiolus* (L.), *Cerastoderma edule* (L.) and *Mytilus edulis* (L.), Mollusca: Bivalvia. In: Barnes, H. (ed.) Proceedings of the Ninth European Marine Biology Symposium, Oban, Scotland, 1974. University Press, Aberdeen, p. 257–274
Seed, R., Brown, R. A. (1978). Growth as a strategy for survival in two marine bivalves, *Cerastoderma edule* and *Modiolus modiolus*. J. Anim. Ecol. 47: 283–292
Sparks, A. (1972). Invertebrate pathology. Noncommunicable diseases, Academic Press, New York
Twomey, E., Mulcahy, M. F. (1984). A proliferative disorder of possible haemic origin in the common cockle, *Cerastoderma edule*. J. Invertebr. Pathol. 44: 109–111
Twomey, E., Mulcahy, M. F. (1988). Transmission of a sarcoma in the cockle *Cerastoderma edule* using cell transplants. Dev. Comp. Immunol. 12: 195–200
Twomey, E., Poder, M., Auffret, M., Mulcahy, M. (1986). Sarcomatous lesions in the cockle, *Cerastoderma edule* L.: recordings from Ireland and France. In: Vivares, C. P., Bonami, J.-R., Jaspers, E. (eds.) Pathology in marine aquaculture. European Aquaculture Society, Special Publication No. 9, Bredene, Belgium. (Abstract)
West, A. B., Partridge, J. K., Lovitt, A. (1979). The cockle *Cerastoderma edulc* (L.) on the South Bull, Dublin Bay: populations parameters and fishery potential. Ir. Fish. Invest. (Ser. B), No. 20: 1–18
Wolf, P. H. (1976). Studies on the geographical distribution, etiology and transmission of integumentary epitheliomas in rock oysters from Australian estuaries. Prog. exp. Tumor Res. 20: 295–303
Wilson, J. G. (1980). Heavy metals in the estuarine macrofauna of the east coast of Ireland. J. Lif. Sci. R. Dub. Soc. 1: 183–189
Yevich, P. P., Barszcz, C. A. (1977). Neoplasia in soft-shell clams *(Mya arenaria)* collected from oil-impacted sites. Ann. N. Y Acad. Sci. 298: 409–426

Responsible Subject Editor: Dr A. K. Sparks; accepted for printing on August 2, 1988