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A METHOD OF DETECTING MYTILICOLA INTESTINALIS STEUER IN MUSSELS, MYTILUS EDULIS L., USING PEPSIN DIGESTION, AND ITS USE IN A SURVEY IN SCOTTISH WATERS

by

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Summary

A survey was carried out in 1976 of the distribution and abundance of Mytilicola intestinalis Steuer in those Scottish waters where it had been recorded in previous surveys. The level remains very low in the Firth of Forth, but has increased since 1971 in some areas of the Clyde. A pepsin digestion technique, suitable for use with large samples, was tested and used in part of the survey.

Introduction

The increasing interest in the cultivation of bivalve molluscs has highlighted the need to prevent the spread of pests and diseases. There had been little recent change in the distribution and levels of infestation of mussels (Mytilus edulis) by the gut parasite Mytilicola intestinalis in Scotland. Interest now centres on detection of the parasite at very low levels of infestation, thus involving large samples of mussels.

The use of a papain digestion technique has been suggested by Dare (1977). This method is unpleasant to use, producing an obnoxious smell. A similar, but less obnoxious method, using pepsin digestion has been used to detect the presence of nematode larvae in herring (Smith and Wootten, 1975). The present paper reports on the use of pepsin digestion in a survey of those parts of Scotland where Mytilicola was known to occur (Drinkwater, 1972).

Method

Samples of 50 or 100 mussels were taken at each sampling station. Some were examined in the field by dissection and others were deep frozen for later examination in the laboratory by the digestion method (Table 1).

Preliminary tests were carried out to find out whether the parasite was digested by pepsin and also to determine the time, temperature, and concentration at which the enzyme was most effective. The most convenient method finally adopted was as follows:-

The mussels were opened by knife and the flesh removed, leaving the adductor muscle attached to the shell if possible. A digest solution was made up of 10 g of 1:2500 pepsin powder per litre of 1% HCl. The mussel tissue was added to the digest solution, allowing a maximum of 250 g per litre. The

beakers (usually 1 or 2 litres depending on quantity) containing the mixture were then put into a hot air oven and maintained at 40°C. After sixteen hours the sample was lightly stirred with a circular motion. If any large pieces of undigested tissue were thrown against the transparent side of the beaker the sample was left for a further 30 minutes, after which the digestion was usually completed. The solution was then passed through a series of three sieves, 2400 µ, 1000 µ and 300 µ. The contents of the sieves were then examined microscopically in petri dishes marked with a grid. The majority of Mytilicola were retained by the small and medium sieves. The Mytilicola were conspicuous amongst the small globules of undigested material, byssus threads, pearls and grit provided the digestion process had not been allowed to proceed for too long, in which case 'ghost' Mytilicola were prevalent. 'Ghosts' are the transparent exoskeletons after the viscera have been wholly or partly digested. They were, however, easily recognisable. After severe digestion only the egg sacs of Mytilicola remained undigested. If digestion was incomplete an amorphous mass of semi-digested, translucent muscle fibres tended to clog the fine mesh and hinder microscopic examination.

Results

(a) Comparison of methods

In the preliminary test a sample of infested mussels was divided into two lots of 50 individuals. One lot were examined carefully by dissection, and yielded 31 individual parasites. The second lot were digested in pepsin and 34 parasites were found. Half the parasites dissected out of the first lot were digested for 5 hrs at 52°C, the other half for 18 hrs at 37°C. None of the Mytilicola was destroyed.

Another test was then carried out using a less thorough dissection technique - similar to that operated under field conditions. 500 mussels yielded 379 parasites by dissection, and a similar batch of 500 revealed a total of 648 parasites after digestion.

(b) Distribution of Mytilicola

The sampling stations, and the distribution and abundance of Mytilicola are shown in Figures 1 - 3. Table 1 shows the percentages of mussels infested and the average number of parasites per mussel at each station. The corresponding results obtained in 1971 are also indicated. The areas covered are limited to those where Mytilicola has been found in the past. The station numbers are the same as those used previously (Drinkwater, 1972). It should be noted that the diagrams express level of infestation as mean numbers per mussel, and not percentage of population infested, as this is not revealed by the digestion method. The relationship between these two values, derived from earlier results, is shown in Figure 4.

There have been no important changes since 1971. In the Firth of Forth the level remains very low. In Linne Mhuirich (Loch Sween), where Mytilicola was first found in 1970, the level is similar to that found originally. No spread to other areas of Loch Sween has been found. In the Clyde Sea Area the main concentration is still in Loch Ryan (Stations 124 and 125). Ardtarig in Loch Striven (Station 79) and Ardmore in the Clyde Estuary (Station 104) both show a considerable increase. Other changes are small.

Discussion

In 1971 it seemed possible that Mytilicola might gradually disappear from the Firth of Forth. The present survey shows that this has not yet happened. There are a few stations where the parasite is still present in small numbers.

In Linne Mhuirich and parts of the Clyde there has been in increase, possibly caused by the warm weather in recent years. The fact that there has been no spread from Linne Mhuirich to adjacent areas is encouraging, but it must be borne in mind that the sampling technique so far used there may not reveal low levels of infestation.

One of the main advantages of a digestion technique, as pointed out by Dare (1977), is that large numbers of mussels can be processed in a reasonable time, and the results from such larger samples are more reliable. The present technique has been shown to give rather better results than those produced under field conditions by simple dissection. It is certainly likely to give more uniform results when applied by a number of different workers.

Pepsin is both cheaper and more readily available than papain, and does not produce the nauseating smell which Dare ascribed to papain. Tests have not yet been carried out using pepsin on mussels in the shell; modification might be necessary to keep the pH value within the required range.

References

Dare, P.J. 1977 Enzyme extraction of the parasitic copepod Mytilicola intestinalis Steuer from mussels, Mytilus edulis L. J. Cons. int. Expl. Mer, 37 (2), 170-172.

Drinkwater, J. 1971 Further observations on the distribution of Mytilicola intestinalis around Scotland. ICES C.M.1971/K:31, pp 4.

Smith, J.W. and Wootten, R 1975 Experimental studies on the migration of Anisakis sp larvae (Nematoda:Ascaridida) into the flesh of Herring, Clupea harengus L. Int. J. Parasit., 5, 133-136.

Sommaire

Une étude a été entreprise en 1976 portant sur la distribution et l'abondance de la Mytilicola intestinalis Steuer dans les eaux écossaises où sa présence avait été enregistrée lors des études précédentes. Le niveau reste très faible dans le Firth of Forth, mais il a augmenté depuis 1971 dans certains secteurs de la Clyde. Une technique de digestion par la pepsine, pouvant être employée avec de grands échantillons, a été essayée et employée au cours d'une partie de l'étude.

Table 1

Levels of infestation at each station (Station Nos. as in Drinkwater, 1971)

	Station	Size Range (mm)	No examined	Method	% of mussels infested		Mean no. of Mytilicola per mussel	
			1976		1976	1971	1976	1971
4	Musselburgh	40-63	50	P	0	0	0	0
5	Joppa	46-65	50	P	0	0	0	0
6	Leith	50-70	100	P	-	2	0.02	0.02
7	Newhaven	46-63	50	P	0	0	0	0
8	Granton Pt	50-78	50	P	0	4	0	0.04
9	Snab Pt	43-60	46	P	0	0	0	0
10	Barnbougle Castle	50-66	50	P	0	0	0	0
11	Hound Pt	43-65	50	P	0	-	0	-
12	Long Craig Pier	47-63	50	P	0	0	0	0
13	South Queensferry	36-75	50	P	-	-	0.02	-
14	Hopetoun	49-68	50	P	0	0	0	0
15	Blackness	45-65	50	P	0	0	0	0
16	Kincardine Bridge	50-69	50	P	0	-	0	-
17	Culross	50-62	50	P	0	0	0	0
18	Crombie Pt	50-69	50	P	0	0	0	0
19	Charlestown	45-67	50	P	0	0	0	0
20	North Queensferry	52-69	44	P	0	0	0	0
21	Inverkeithing	56-73	50	P	0	-	0	-
23	Dalgety Bay	44-66	50	P	0	4	0	0.04
24	Aberdour	54-71	50	P	0	0	0	0
25	Burntisland	42-74	50	P	0	0	0	0
58	Linne Mhuirich	34-83	150	P	-	14	2.16	0.16
76	Carry Pt	40-	100	P	-	6	0.77	0.08
77	Ard a' Chapuill	40	50	P	-	32	0.84	0.72
79	Ardtarig	40-	100	P	-	0	1.50	0
82	Innellan	40-	100	P	-	0	0.13	0
83	Sandbank	40-	100	P	-	12	0.47	0.16
84	Strone	33-50	100	P	-	0	0	0
86	Ardentinny	40-	100	P	-	0	0	0
87	Carrick	40-	100	P	-	-	0.01	-
88	Lochgoilhead	40-	100	P	0	0	0	0
89	Arrochar	40-	100	P	0	0	0	0
91	Coulport	38-46	100	P	0	0	0	0
92	Kilcreggan	30-45	100	P	-	2	0.06	0.02

(contd)

Table 1 (contd)

	Station	Size Range (mm)	No examined 1976	Method	% of mussels infested 1976	% of mussels infested 1971	Mean no. of Mytilicola per mussel 1976	Mean no. of Mytilicola per mussel 1971
93	Rosneath Bay	56-65	50	M	6	0	0.14	0
94	Stroul Bay	40-	100	P	-	-	0.01	-
97	Rowmore	42-72	50	M	10	0	0.10	0
98	Garelochhead	46-73	100	P	-	2	0.01	0.02
100	Shandon	43-64	50	M	10	-	0.12	-
101	Croy	41-60	50	M	6	-	0.06	-
102	Rhu	42-62	50	M	0	0	0	0
103	Helensburgh	47-60	50	M	10	0	0.10	0
104	Ardmore	42-61	50	M	52	4	1.04	0.04
105	Newark Castle	44-64	50	M	12	0	0.12	0
106	Greenock	42-67	50	M	2	2	0.02	0.02
107	Gourock	46-56	50	M	0	0	0	0
108	Cloch Pt	24-62	25	M	0	2	0	0.02
109	Inverkip	33-62	50	M	2	0	0.02	0
110	Skelmorlie	39-58	50	M	0	2	0	0.02
111	Largs	33-52	50	M	0	0	0	0
112	Fairlie	39-56	50	M	0	0	0	0
118	Portencross	25-38	50	M	0	0	0	0
119	Ardrossan	33-45	50	M	0	0	0	0
120	Troon	29-46	50	M	2	0	0.02	0
121	Ayr	34-49	50	M	2	9	0.02	0.10
122	Turnberry	39-62	50	M	0	0	0	0
123	Girvan	32-54	50	M	0	0	0	0
124	Cairnryan	51-70	50	M	58	50	2.54	0.72
125	Stranraer	53-67	50	M	86	42	9.38	0.90

Key P = pepsin digestion
M = manual dissection

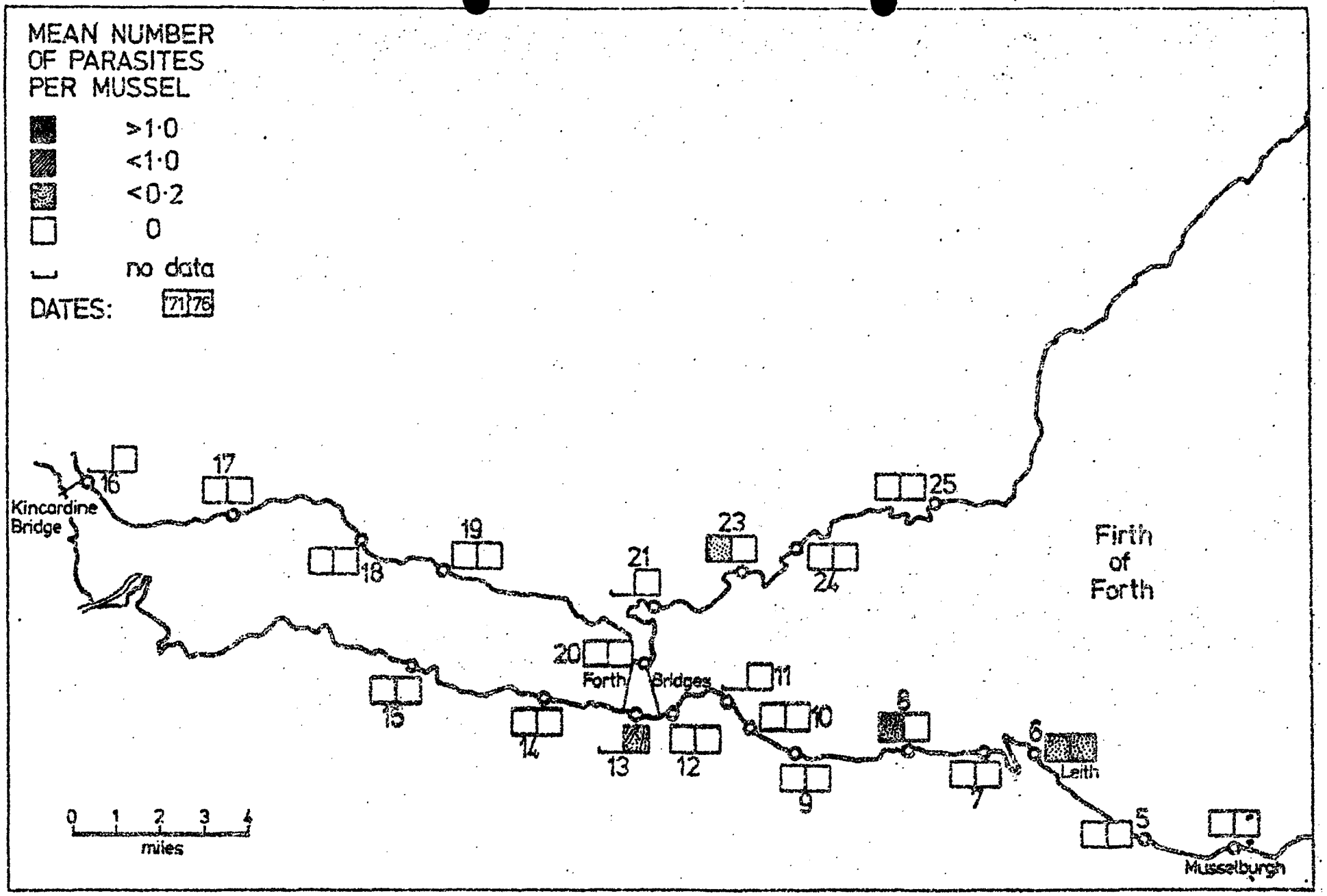

Fig. 1 Distribution of Mytilicola intestinalis in the Firth of Forth.

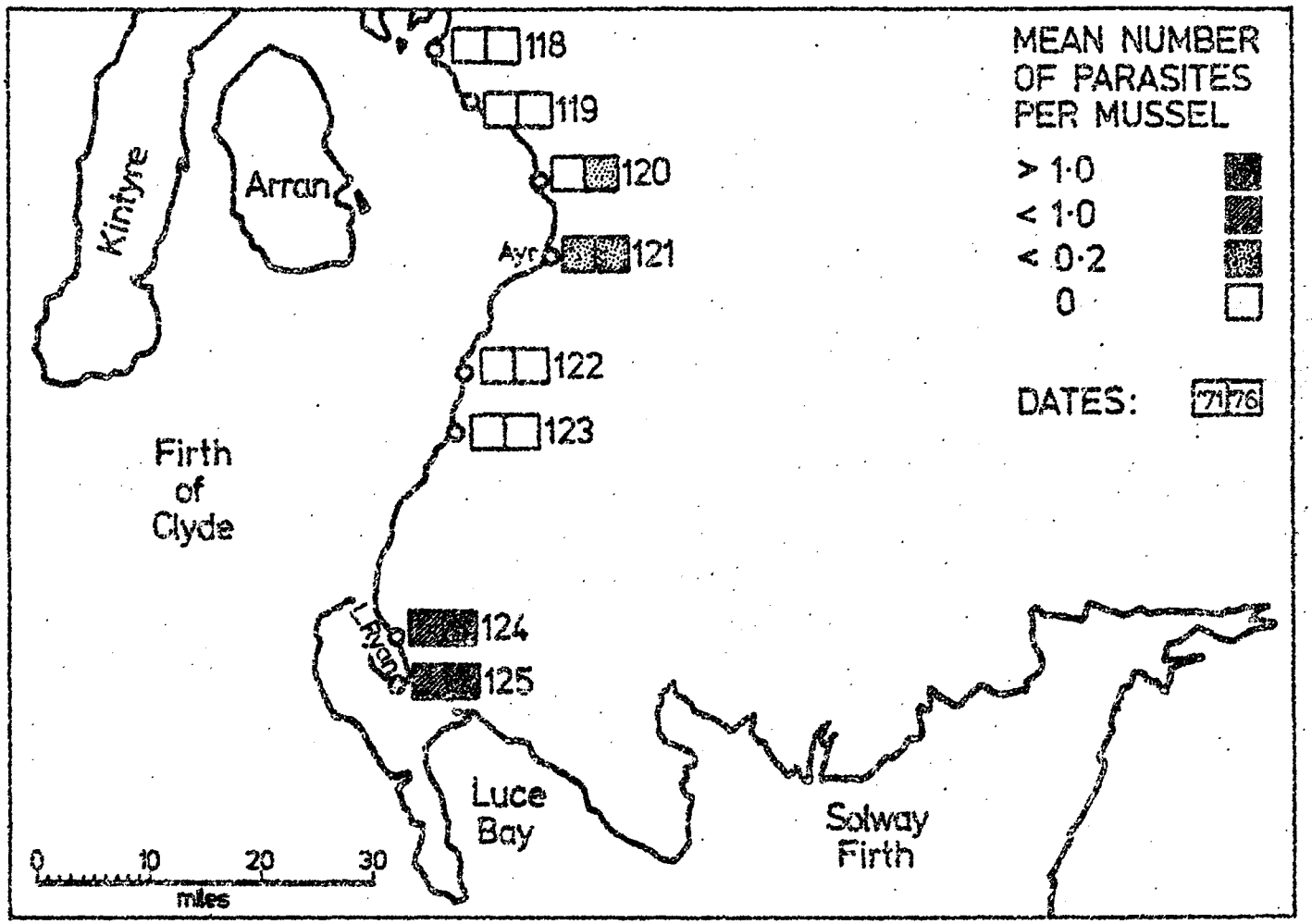

Fig. 2 Distribution of Mytilicola intestinalis in the Clyde and Solway.

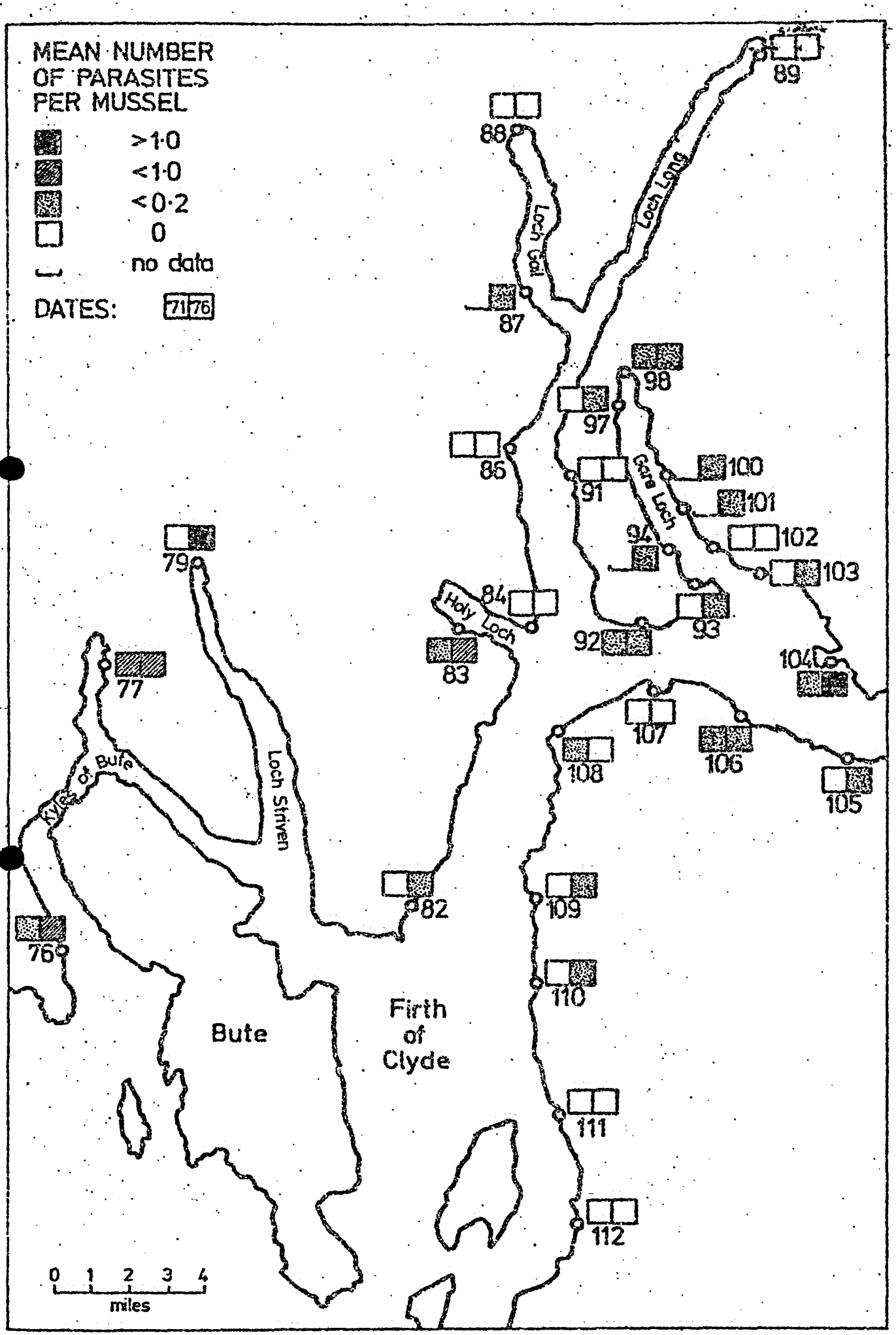

Fig. 3 Distribution of Mytilicola intestinalis in the upper reaches of the Firth of Forth.

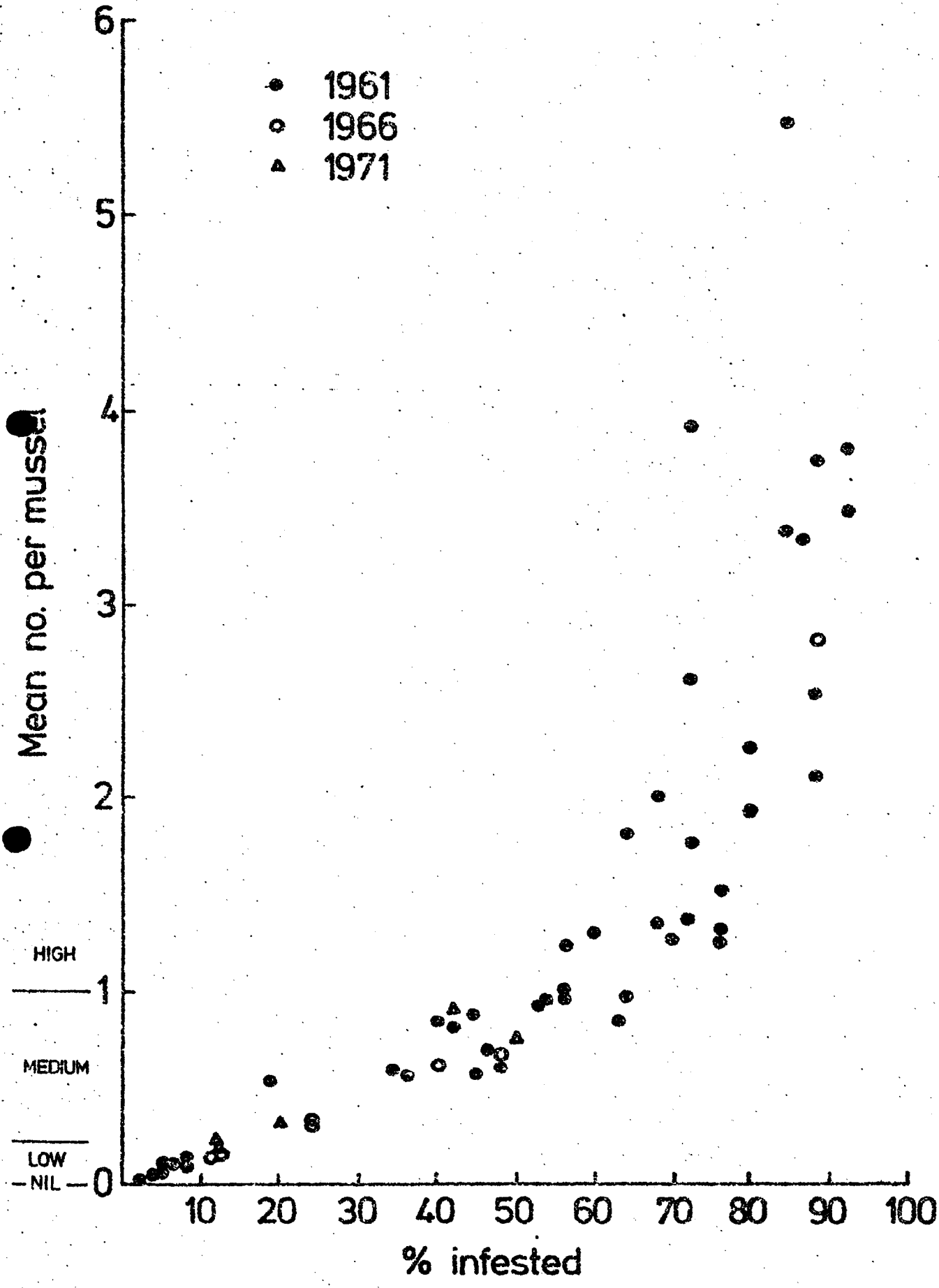

Fig. 4 Relationship between the percentage of the population infested and the mean number of parasites per mussel.