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THE SUSCEPTIBILITY OF SEED OYSTERS OF Ostrea edulis L. AND Crassostrea gigas Thunberg TO NATURAL INFESTATION BY THE COPEPOD Mytilicola intestinalis Steuer

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#### ABSTRACT

Ostrea edulis and Crassostrea gigas seed of 5-45 mm length were exposed to Mytilicola intestinalis during the main infestation season in south-west England. C. gigas proved to be far less susceptible than O. edulis to infestation. In both species, the numbers of copepodites acquired during 3 months were positively correlated with host size.

<u>C. gigas</u> smaller than 25 mm were so rarely infested that the risk of them transmitting the parasite is considered to be negligible. Some juvenile parasites overwintered in larger C. gigas but grew more slowly than in <u>O. edulis</u> hosts of similar size and their survival to breed seemed unlikely.

<u>O. edulis</u> as small as 5-10 mm acquired parasites but juveniles and adults overwintered only in larger seed (> 35 mm) and with 87% mortality. Female <u>Mytilicola</u> grew to 6 mm by early winter, thus suggesting that breeding can occur in larger seed in spring. <u>O. edulis</u> should be regarded as a potential vector, although the risk may be considered slight with seed smaller than 15 mm.

#### INTRODUCTION

Mytilicola intestinalis Steuer inhabits the digestive tracts of primarily the two European species of mussel, Mytilus galloprovincialis Lamarck and Mytilus edulis L., although it has been found occasionally also in the European flat oyster, Ostrea edulis (Baird et al., 1951; Hepper, 1956) and rarely in some other bivalves (Hepper, 1956). This parasitic copepod was held widely to have been responsible for heavy mortalities of M. edulis on North Sea coasts around 1950 (e.g. Korringa, 1951 and 1968), although recent work (Dethlesfsen, 1975; Davey et al., 1978; Gee et al., 1977) has failed to confirm that Mytilicola has any serious effect on its hosts.

So far, there have been no published reports of the occurrence of <u>Mytilicola</u> <u>intestinalis</u> in any stocks of the Pacific oyster, <u>Crassostrea gigas</u>, which have the been introduced to Europe since 1965. However, a single specimen was found in <u>C. gigas at Arcachon</u>, France in 1977 (E. His, <u>in litt</u>.). On the other hand, <u>numbers of Mytilicola orientalis</u>, Mori (1935), were discovered at Arcachon in 1977 in Pacific oysters imported directly (i.e. without quarantine) from either Japan



or British Columbia, Canada (His, 1977). This parasite occurs in both countries (Mori, 1935; Bernard, 1968). <u>M. orientalis</u> has since spread to <u>O. edulis</u> and mussels at Arcachon (His, 1977).

In England and Wales movements and deposits of molluscs are regulated by the Molluscan Shellfish (Control of Deposit) Order of 1974 (Great Britain - Parliament, 1974). A licence is required for the deposit in any area of tidal water, designated by the order, of molluscs taken from any natural or cultivated stock outside that area of water. There is, in addition, a restriction on the maximum size of seed oysters which may be moved from Mytilicola infested areas for deposit in Mytilicola clear areas, unless the oysters have been held in filtered sea water in a hatchery. The size limit is a mean length (longest axis) of 6 mm, which corresponds in practice to a maximum individual length of 10 mm. In the absence of relevant data for Mytilicola in oysters, the size limit was derived from the smallest mussel (9 mm) in which the parasite has yet been recorded (Williams, 1967). The 6 mm size limit is causing increasing problems to hatchery and nursery ground operators with the increasing annual production of seed oysters, especially of C. gigas, in Great Britain. There would thus be a clear benefit to the British oyster industry if the 6 mm limit could safely be raised. To this end, field experiments were conducted during 1978-80 to assess the susceptibility of both species of oyster to infestation by M. intestinalis under natural conditions. This paper presents the results and considers the implications from these field experiments. innerer set

# METHOD

Two sites in south-west England were chosen for the experiments: (i) Lynher Estuary, near Plymouth; (ii) Teign Estuary, 55 km east of site (i). The abundance of <u>Mytilicola</u> in both estuaries had been described by Gee and Davey (1976) while the population dynamics of the parasite had been studied at the Lynher Estuary (Davey <u>et al.</u>, 1978). Monitoring of annual variations in parasite recruitment to the Lynher mussel population continued during the present studies (J. Davey, personal communication). The sites were both near mean low water mark of spring tides: (i) beside a heavily infested mussel bed at Beggar's Island, near the confluence of the Lynher and Tamar estuaries, and at ~ 5% exposure level, (ii) on lightly infested mussel lays 1 km upriver from Teignmouth harbour, and at ~ 2% exposure level. It was known from other studies at this Laboratory that both <u>0. edulis</u> and <u>C. gigas</u> grew well in the Teign Estuary, but no data were available for the Lynher other than that the remnants of a former introduced stock of <u>0. edulis</u> still survived in that estuary.

Susceptibility to <u>Mytilicola</u> infestation was assessed by setting out batches of hatchery-produced seed oysters in the two estuaries during the season of maximum copepodite abundance, which had been shown (Davey <u>et al.</u>, 1978) to be the autumn, and especially October-November. Uninfested oysters were provided by the Conwy

laboratory and by a North Wales cultivator. In 1978, O. edulis were placed at the Lynher site while C. gigas were exposed in the Teign Estuary; in 1979, both species were placed at the Lynher. The oysters were kept in meshed cages or perforated plastic trays which were fastened to metal frames so that the animals were approximately 0.5-1 m above the ground. Maximum stock density was 0.5 g liveweight cm<sup>-2</sup> of tray or cage floor.

For each species of oyster, 3 or 4 size groups within the range 5-45 mm initial shell length were used each year, the actual sizes and numbers depending on availability of seed. Each size group was graded, usually to within ± 2 mm limits, and then subdivided into batches of 150-500 animals. Three series of experiments were set out:

(1) monthly exposure

- to monitor the infestation levels during periods of minimal oyster growth; first batches set out in August or September and replaced each month until December; also to show seasonal pattern of infestation; to measure the nett accumulation and growth of parasites
- (2) 3-4 months exposure -(full-autumn)
- over the autumn infestive season from August or September until December;
- (autumn and overwinter)
- (3) 7-8 months exposure as (2) but also to measure overwinter survival and maturation of parasites acquired during the autumn; batches examined in spring (March-April).

As 'controls', a batch of 500 large and uninfested mussels, mainly 50-65 mm shell length, was obtained from the Conwy area, where M. intestinalis is absent, and placed in a cage beside the oysters at each site. Samples of 30 mussels were removed and examined whenever oysters were examined, in order to monitor copepodite availability and the overwinter survival and maturation of parasites in their preferred host.

After exposure to infestation, oysters were measured for linear and liveweight growth determinations, and their flesh was removed and placed in pepsin solution for digestion by the method of Drinkwater and Howell (1977). After digestion the Mytilicola integuments were filtered from the residues, using 348 um and 124 um sieves, then counted and measured on a gridded perspex dish under a x 45 binocular microscope fitted with an eyepiece graticule with 0.25 mm divisions. This technique proved to be very sensitive and allowed both egg-sacs and the earliest infestive stages (0.45 mm long) to be recovered intact.

RESULTS

#### Ostrea edulis 1.

Information on growth in the Lynher Estuary is summarised in Table 1. Growth during autumn was rather poor, and well below that recorded for this species at similar shore levels in the Teign Estuary and Menai Strait in North Wales (B. E. Spencer, personal communication). Survival was good during the 1979-80

winter, but during the 1978-79 winter most overwintered batches were killed by

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(i) Numbers of Mytilicola: too

<u>0. edulis</u> seed readily became infested with copepodites in both years (Tables 2, 3) and the numbers per host were positively correlated with size of oyster (Figure 1) over the October-December 1978 exposure period. The largest 1978 seed, in the  $45 \pm 2$  mm group, had acquired 8.56 copepodites and immature parasites by December, after 3 months exposure during which mean live weight of the seed increased from 3.5 g to 5.0 g. In 1979, seed of comparable size held only 2.94 parasites per host in December after 4 months exposure.

Examination of control mussels confirmed that infestive stages of <u>Mytilicola</u> were numerous in the Lynher Estuary during both autumns (Tables 2 and 3 footnotes).

The overwinter survival of <u>Mytilicola</u> during the 1978-79 winter was clearly zero in those batches of oysters killed by the January frosts. Only in the  $35 \pm 2$  mm batch of seed did any oysters survive (12%). The small sample of 58 oysters contained 0.55 parasites per host in March compared with 2.08 per host in December after two months exposure - a reduction in infestation of 75%.

The 1979-80 winter was relatively warm, with no severe cold periods, and oyster survival was high. The data in Table 3 show that in the two largest sizes of seed there were <u>Mytilicola</u> reductions of 87% and 88% between December and April. In the control mussels over the same period parasite numbers fell by 77%, from 26.7 to 6.2 per host.

(ii) Sizes of Mytilicola:

Lengths of parasites found in the seed oysters after various periods of exposure to infestation are summarised in Table 4. There appeared to be no relationship between size of host and modal size of parasites, perhaps due to recruitment being continuous. During autumn, August-December, parasites in the monthly samples ranged in size between 0.45-2.0 mm, with most between 0.5-1.0 mm. A similar size range was found in 1978 for the 2- and 3-monthly batches although with a higher modal size group, usually 1.0-1.24 mm. In 1979, large seed exposed since August, by December contained female parasites of up to 6.25 mm length but no egg-sacs were present; the modal group was still small, 1.25-1.49 mm, reflecting recent recruitment.

By spring, overwintered parasites ranged between 1.0-3.75 mm with modal groups varying from 1.25-1.49 mm (in 1978 seed of the 35  $\pm$  2 mm group) to 2.75-2.99 mm (in 1979 seed of the 42  $\pm$  2 mm group). No egg-bearing females were observed in April 1980 in the largest oysters, whereas control mussels by then contained an average of 2 egg-bearing females per host.

# 2. Crassostrea gigas

Pacific oyster seed grew and survived satisfactorily in both estuaries but growth increments were greater in the Teign Estuary (Table 5).

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(i) Numbers of Mytilicola:

<u>C. gigas</u>, in contrast to <u>O. edulis</u>, acquired only very light infestations of <u>Mytilicola</u> (Tables 6 and 7). The highest level of occurrence was 0.06 parasites per oyster - in 45 ± 2 mm seed exposed in the Teign Estuary for 3 months in 1978 (Table 6). Seed smaller than 25 mm failed to become infested in the Lynher even after 4 months in 1979 (Table 7). As in <u>O. edulis</u>, infestation level was positively correlated with host size over the September-December 1978 exposure period (Figure 2).

Some <u>Mytilicola</u> survived the very cold 1978-79 winter in the Teign Estuary where survival of <u>C</u>. <u>gigas</u> was little affected by frost. There were no overwintered samples of seed in the Lynher during the milder 1979-80 winter.

(ii) Sizes of Mytilicola:

Growth of parasites in <u>C. gigas</u> appeared to be slower than in <u>O. edulis</u>. The largest specimen found was 2.8 mm, after 4 months exposure in 1979 in Lynher seed of the  $30 \pm 5$  mm group, compared with 6.25 mm in adjacent <u>O. edulis</u> of comparable initial size. Juvenile parasites which survived the 1978-79 winter showed no further growth before March.

(iii) Additional observations :

Further evidence that <u>C</u>. <u>gigas</u> is not readily infested was provided by examination of some batches of Conwy seed grown in trays in Goldhanger Creek, Essex, in south-east England, from June 1978 until March 1979. By July-August, the start of the infestation season in that area (Williams, 1969), all batches were around 0.2-0.4 g in mean weight ( $\equiv$  10-15 mm length). In March 1979, when batch mean weights ranged from 2.5 g to 9 g ( $\equiv$  25-45 mm mean lengths), no <u>Mytilicola</u> was found in the 500 seed examined. Thus, any infestation - if it had occurred the previous summer and autumn - had failed to survive the very cold winter.

Finally, it can be noted that a laboratory attempt in 1974 to infest 10-25 mm seed of <u>C</u>. <u>gigas</u>, by exposure to 50 <u>Mytilicola</u> larvae per oyster, failed; whereas 5-20 mm mussel seed acquired initial infestations of 5-9 parasites per mussel (J. M. Gee and J. T. Davey, <u>in litt</u>.).

## DISCUSSION

Seed of <u>Crassostrea gigas</u> less than 45 mm in length are far less likely to become infested by <u>Mytilicola intestinalis</u> than are <u>Ostrea edulis</u> seed, which can soon acquire considerable numbers of copepodites. It is not known whether the failure of <u>C. gigas</u> to become infested to a similar degree is due to parasite rejection, host avoidance, high and rapid mortality of ingested copepodites, or to a combination of these and some other mechanisms.

The copepodites grew more slowly in <u>C</u>. gigas than in <u>O</u>. edulis of comparable size, although the former oyster was markedly the faster growing species. This further suggests that <u>C</u>. gigas is not a satisfactory host for <u>M</u>. intestinalis. Nevertheless, some parasites lived for at least four months, and survived a very cold winter, within C. gigas. Whether enough individuals can mature to breed in <u>C</u>. gigas,

perhaps in oysters of marketable size (> 50 g), is not resolved but seems unlikely; this aspect was outside the scope of these experiments. In <u>0</u>. <u>edulis</u> the parasites can probably breed in larger seed (30-45 mm), as they do in mussels of similar size (Dare, unpublished data). Breeding females have been reported in <u>0</u>. <u>edulis</u> larger than 50 mm (Bolster, 1954; Hepper, 1956).

The risk of spreading <u>M</u>. <u>intestinalis</u> to new areas through movements or deposits of <u>C</u>. <u>gigas</u> seed, particularly those smaller than 25 mm, can be considered as negligible. <u>O</u>. <u>edulis</u> should, however, be regarded as a potential vector for this parasite although the risk is likely to be slight with seed smaller than 15 mm.

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	ial size a p (mm)	Mean 1	ive weig	ht (g)				size (mm) cember
ī	range	Aug	Sep	Oct	Nov	Dec	ī	range
1978							inkta	in the second
17	(15-19)		0.36-	→ 0.46 0.43-		→ 0.50 <sup>-1</sup>		
25	(23-27)		0.88-			-> 1.56		
35	(33-37)	anna a bhan a. Annaich	1.94-	>2.11 1.94-		→ 2.17		e den re
45	(43-47)		3.54-	<u></u>		→ 4.97		
1979								· <b></b> <sup>3</sup>
8	( 5-10)		(0.08)-	-> 0.12-		-> 0.26	14	( 9-20)
15	(11-19)		(0.3)-		1	→ 0.43	18	(11-25)
32	(30-35)	2.3-	3.2	5		-> 5.2	40	(30-53)
42	(40-45)	4.4-	5.4			7.6	47	(40-58)

Table 1. Growth of Ostrea edulis seed at the Lynner Estuary site during the 1978 and 1979 experiments

Note:

- 1. Live weights in parentheses have been estimated from length:weight regressions.
- 2. There was no growth in overwintered samples, between December and April 1979

Table 2. Infestation of Ostrea edulis seed by Mytilicola copepodites: 1978 experiments, Sep-Dec., Lynner Estuary

(Average no. copepodites per oyster in each inital size group)

Exposure $17 \pm 2 \text{ mm}$ $25 \pm 2 \text{ mm}$ $35 \pm 2 \text{ mm}$ period $(0.4 \text{ g})$ $(0.8 \text{ g})$ $(1.9 \text{ g})$	45 ± 2 mm (3.5 g)
Sep-Oct0.020 (150)0.360 (150)1.353 (150)Oct-Dec0.327 (150)0.573 (150)2.080 (150)	- 5.40 (75)
Sep-Dec n.s. 1.533 (150) n.s.	8.56 (45)

Note:

1. Numbers of seed examined are given in parentheses.

Control mussels (40-60 mm, mean wt. ~ 15 g) acquired an average of 6.37 copepodites per host during Sep-Oct., and 14.77/host during Sep-March (no December sample due to rough weather).

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3. n.s. = no sample collected (due to adverse weather).

Table	3.	Infestation of Ostrea edulis seed by Mytilicola
		copepodites: 1979 experiments, Aug-Dec., Lynher
1.1.1		Estuary

(Average no. of copepodites per oyster in each initial size group)

Exposure period	5-10 mm (0.08 g)	11-19 mm (0.2-0.4 g)	30-35 mm (2-3 g)	35-45 mm (3-5 g)
Aug-Sep	4-1910	(Gennio_3)à,	0.33 ( 90)	0.81 ( 90)
Sep-Oct	0.02 (60)	0.09 (130)	1.10 (145)	1.16 (150)
Oct-Nov		0.06 (150)	1.57 (115)	2.83 ( 85)
Nov-Dec	naga kanadagan dakana. Ku da dakan ja ka danga	0 (160)		nan en anter a la composition composition ( en e
Aug-Dec			2.28 (100)	2.94 (125)
Sep-Dec	0.025 (160)	0.023 (260)	e stand of the ball	a en estakaa – K
Aug-Apr	$ \begin{array}{l} \left\langle \gamma_{1},\gamma_{2},\cdots,\gamma_{n},\gamma_{n}\right\rangle & = \left\langle \gamma_{1},\gamma_{2},\cdots,\gamma_{n},\gamma_{n}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2},\cdots,\gamma_{n},\gamma_{n}\right\rangle & = \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle & = \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle & = \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle & = \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma_{2}\right\rangle & = \left\langle \gamma_{1},\gamma_{2}\right\rangle \\ \left\langle \gamma_{1},\gamma$	0 (150)	0.27 (100)	0.37 (150)

Note:

1. Oyster sample sizes examined are given in parenthesis.

 Control mussels (50-70 mm, mean wt. ~ 35 g) acquired an average of 3.2 copepodites per host during August-September, 27.6 during August-November, and 26.7 per host during August-December. By April 1980, there had been a 77% reduction in numbers to 6.2 per host.

	Oysters			Mean no	and a state of the second		site length (mm)	
	size group (mm)	Duration (months)	Period	oyster	Modal group	Min.	Max.	size
1978	17 ± 2	2	0-D	0.33	1.0 -1.24	0.70	2.00	49
	25 ± 2	1 2 3	S-0 0-D S-D	0.36	0.5 -0.74 1.0 -1.24 1.0 -1.24	0.50 0.50 0.50	1.15 2.10 3.25	54 86 164
	35 ± 2	1 2 6	S-0 O-D S-Mar	1.35 2.08 0.55	0.5 -0.74 1.0 -1.24 1.25-1.49	0.45 0.45 1.00	1.05 2.25 2.00	53 99 32
	45 ± 2	2 3	o-d S-d	5.40 8.56	1.0 -1.24 0.75-0.99	0.55 0.60	1.90 1.80	140 103
1979	15 ± 4	1	S0	0.09	0.75-0.99	0.55	1.20	12
	32 ± 2	1 1 1 4 8	A-S S-O O-N A-D A-Apr.	0.33 1.10 1.57 2.28 0.27	0.5 -0.74 0.75-0.99 0.5 -0.74 1.25-1.49 2.25-2.49	0.48 0.45 0.50 0.50 1.50	1.55 1.20 2.00 6.25 3.75	30 62 83 59 29
	42 ± 2	1 1 1 4 8	A-S S-O O-N A-D A-Apr	0.81 1.16 2.83 2.94 0.37	0.5 -0.74 0.5 -0.74 0.75-0.99 1.25-1.49 (1.50-1.74 (2.75-2.99	0.50 0.50 0.45 0.50 1.25	1.40 1.25 1.80 5.60 3.50	44 63 85 112 52

Table 4. Sizes of Mytilicola recovered from experimental groups of <u>O. edulis</u> seed during August to March in the Lynher Estuary

N.B. Data for Mytilicola samples of < 10 parasites have been omitted

Initial size group (mm)		Mean live we	Final size (mm) in December				
ī	range	Aug Sep	Oct	Nov	Dec	ī	range
1978							
17	(15-19)	0.99	->1.90 -		→4.21	(33)	
25	(23-27)	2.08	→ 3.95 -	1 1 1	-> 8.00	(42)	
35	(33-37)	4.57-	>7.00-		-> 14.04	(53)	
45	(43-47)	7.24		94-1- P	> 20.22	(60)	i i i i i i i i i i i i i i i i i i i
1979 7	(5-9)	0.08->0.38		-0.18	$\rightarrow$ 1.60 $\rightarrow$ 0.05	20.2	( 8–30)
15	(13-17)	0.41→1.18	1 1 1		$\rightarrow$ 3.55 $\rightarrow$ 0.68	28.8	(16+43)
30	(25-35)	3.0→5.9-			$\rightarrow$ 9.2 $\rightarrow$ 2.8	40.8	(25-55)
45	(40-50)	8.2→11.8 -		9.2-	$\rightarrow$ 16.5 $\rightarrow$ 9.6	51.0	(36-67)

Table 5. Growth of <u>Crassostrea</u> gigas seed at the Teign Estuary (1978) and Lynher Estuary (1979) sites during the infestation experiments

Note:

1. There was a little growth in overwintered samples between December and March 1978.

2. December mean lengths in parentheses were derived from length:weight regressions.

Table 6. Infestation of C. gigas seed by <u>Mytilicola</u> copepodites: 1978 experiments, September-December, Teign Estuary

(Average no. of copepodites per oyster in each size group)

Exposure period	17 ± 3	2 mm	25 ± 2	2 mm.	35 ± 2 mm	45 ± 2 mm
Sep-Oct		(300)	0	(200)	0.004 (280)	
Sep-Dec	0.010	(300)	0.017	(300)	0.022 ( 90)	0.056 (90)
Sep-Mar				1013	0.044 ( 90)	
Oct-Mar	0	(150)	0	(300)	0.007 (150)	-**j\$*

Note:

1. Numbers in parentheses = sample sizes of oysters examined.

2. Control mussels (55-65 mm) acquired an average of 0.23 parasites per host during Sep-Oct., at least 0.65 during Sep-Dec., and 0.70 from September to March.

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Table 7. Infestation of C. gigas seed by Mytilicola copepodites: 1979 experiments, Aug-Dec., Lynher Estuary

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Exposure period	5-10 mm (0.05-0.2 g)	15-25 mm (0.5-2.0 g)	25-40 mm (3-7 g)	40-55 mm (8-12 g)
Aug-Sep	0 ( 45)	0 (120)	0.02 ( 90)	0.01 ( 90)
Sep-Oct	0 ( 90)	0 ( 75)	0 (100)	0 (100)
Oct-Nov	0 (135)	0 (130)	0.02 (85)	0.02 (100)
Nov-Dec	0 (170)	0 (100)	0 (160)	0.026 (115)
Aug-Dec	0 (110)	0 (290)	0.007(270)	0.003 (295)

(Average no. copepodites per oyster in each size group)

Note: what is a start and the second start and the

1. Numbers in parentheses = sample size of oysters examined

2. Control mussels (55-65 mm, 35 g) acquired an average of 26.7 copepodites per host during Aug-Dec.

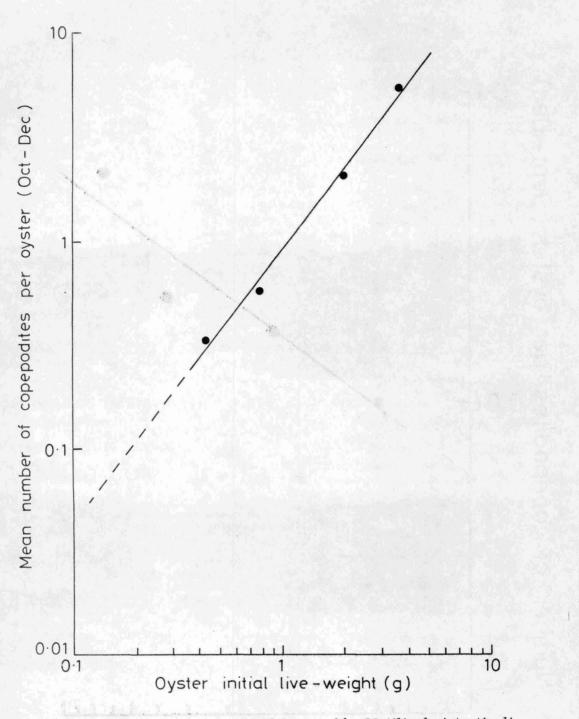


Figure 1 Infestation of <u>Ostrea edulis</u> seed by <u>Mytilicola intestinalis</u> (1978 - Lynher Estuary).

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