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A rapid method for detecting the parasitic copepod Mytilicola intestinalis Steuer in mussels, Mytilus edulis L., at very low levels of infection

by

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SUMMARY

Mytilicola intestinalis can be extracted from Mytilus edulis by means of the proteolytic enzyme papain which digests mussel flesh but not the chitinous cuticle of the copepod gut parasite. An effective method is to use 10-15g papain powder per 100 ml of raw mussel flesh in 500 ml water and digest at 30°C for 48 hours. Mussels can be digested most conveniently in shell after deep-freezing and thawing to induce their valves to gape. Filtered residues are then examined under a low-power binocular microscope. The technique has particular application in large-scale and intensive surveys of Mytilicola distribution because it is much more rapid than conventional dissection methods and can deal with very large samples (an examination rate of 190 mussels/hour was achieved in one trial survey of 8 600 small mussels) thereby permitting more readily the detection of very low (<0.1%) levels of infection.

INTRODUCTION

In England and Wales the Molluscan Shellfish (Control of Deposit) Orders 1965 and 1974 prohibit the deposit of mussels from areas where Mytilicola intestinalis is prevalent into areas free from this gut parasite. Recent surveys of Mytilicola in this country (Dare 1974, and unpublished MAFF reports) showed that there was an urgent need to develop a quick but thorough method for examining very large samples (> 1 000) of mussels from potentially exploitable stocks in marginal areas where Mytilicola is of only irregular and scarce occurrence. After considering various possibilities, it was suggested (Dr. P.R. Walne, pers. comm.) that the proteolytic enzyme papain* might be used to digest mussel flesh and thus expose the chitinous and indigestible cuticles of any Mytilicola present within. Subsequently, G. Davies (pers. comm.) informed me that he had, in 1960, made successful

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* Footnote: From the juice of the tropical papaya tree (Carica papaya)

preliminary tests with this approach during a Mytilicola survey at Morecambe Bay, and he kindly made his notes available. Using these notes as a basis, experiments were conducted during 1975 to assess the potential of this technique for large-scale surveys. The results and conclusions from this brief study are presented in this contribution.

METHODS AND MATERIALS

Experiments were made using raw and cooked whole mussel meats, crushed raw mussels in shells, and gaping (deep-frozen) raw mussels in shells. Preliminary flesh digestion tests were made with Conwy mussels, and the resistance of Mytilicola to papain action was examined. The efficiency of the method was then tested using mussels of predetermined infection levels from the docks at Barrow-in-Furness, north-west England. Finally, a large-scale survey, using this method, was made of a bed of young mussels at Morecambe Bay in which Mytilicola had been present at a very low level of infection in 1974 (Dare 1974).

All tests were made in 1 l beakers. The papain powder was stored in a refrigerator when not required (manufacturer's instructions) and was mixed with 500 ml of tap water just before the mussels or mussel meats were added. The mixture was then stirred and placed in an oven at 30°C. The digested material was sieved through meshes of 2.5 mm (to remove shells), 1.05 mm (to remove large Mytilicola, byssus strands and finer debris) and 0.35 mm (to retain small Mytilicola). The material from the fine sieves was then placed in water on gridded perspex trays and scanned systematically under a low-power binocular microscope. Any Mytilicola were counted, measured (using an eye-piece graticule) and preserved in formalin.

The digestion process produces a highly obnoxious and nauseating smell, and it is essential that the oven be situated in an out-building, and that digested material is placed in formalin immediately after examination in order to neutralise the odour.

RESULTS

(a) Resistance of Mytilicola to papain

Mytilicola cuticles are very resistant to the action of papain. In preliminary tests, four adults of 4-8 mm length, two of which carried egg-sacs, were dissected out from Barrow dock mussels and placed in 50 ml of papain solution containing 5 g papain in 500 ml water and incubated at 30°C. After 48 hours the chitinous cuticles were intact and appeared as virtually transparent skins, the body contents having been well digested. Later work showed that Mytilicola can withstand higher concentrations (10-15 g papain in 700 ml) for at least 72 hours at 30°C.

(b) Digestion of mussel flesh

In 1960 G. Davies had recorded that raw mussel flesh was digested completely in 20 hours at 32°C when using 5 g papain powder per 50 ml flesh in 500 ml of tap water. In the present study, using 10-15 g papain per 100 ml of flesh in 500 ml of water, it was found necessary to extend the digestion time to 48 hours to ensure total digestion of raw whole meats irrespective of source and size of mussel. Cooked meats, however, were not so thoroughly digested, the most resistant structures being the digestive gland and mantle. At concentrations of 10-15% papain:wet flesh, crushed shells and gaping mussels were cleaned entirely of flesh and, as a bonus, the shell hinges of small (< 45 mm) mussels were weakened so that the valves readily separated during the digestion process.

(c) Efficiency of papain method in detecting Mytilicola

This was tested with a sample of infected mussels freshly collected from Barrow docks in February 1975. The sample comprised 80 large (50-70 mm) mussels and 100 small (40-50 mm) mussels. Of the larger mussels 40 were dissected in replicate groups of 10 and examined by the conventional macroscopic method (Dare 1974), using a hand lens and scalpel to dissect the digestive gland and the hind gut; the Mytilicola were counted and then replaced in the meats for digestion. The raw meats from the other 40 large mussels were divided into batches of 10 and digested before examination. Of the 100 smaller mussels, the whole meats of 50 were removed for digestion while the other 50 mussels were crushed in shell before digestion.

The results (Table 1) show that the papain and macroscopic methods gave similar results on balance, although slightly more Mytilicola were found by using papain. However, most parasites were adults (2.5-7 mm length) and thus should not have been overlooked by the macroscopic method anyway. Crushed mussels (D) yielded fewer parasites than did extracted meats, suggesting that some Mytilicola were lost during sieving of the shell debris. Also, the crushing may have caused more damage to the parasites: three of four were broken in 'D' compared with only one of 12 in 'C' and two of 21 in 'A' and 'B' combined. The 37 Mytilicola extracted from the 180 mussels ranged from 1 mm (juvenile) to 9 mm in length.

Mytilicola can be detected at very low levels of infection. In a large-scale survey of 2 000 young (15-40 mm) mussels from one Morecambe Bay bed, a single parasite was obtained (0.05% infection rate, 0.0005 parasites per mussel). At such a level Mytilicola would almost certainly escape detection by the conventional micro- and macro-scopic dissection methods because their time-consuming demands (see (d)) ordinarily restrict their application to small samples.

(d) Potential of papain method for large-scale surveys

The most satisfactory method for examining very large number of small (< 40 mm) mussels, the size most likely to be in demand for transplanting, was found to be digestion of the meats raw within gaping shells. Overnight storage in a deep-freeze not only kills the mussels but causes them all to gape upon thawing. In another survey of a bed of 18-45 mm mussels, 400 g batches of thawed and gaping mussels - each batch comprising up to 230 animals - were placed in 1 l beakers containing 500 ml of papain solution. A standard laboratory oven was employed which could hold 18 x 1 l beakers and thus digest up to c.4 000 mussels in one loading. The total sample examined contained an estimated 8 600 mussels (from counts of weighed subsamples). This exercise showed that 190 mussels per hour were processed on average, i.e. times for preparation of samples for digestion, filtering of digested material, and microscopic examination of residues, but excluding oven times. By comparison, conventional dissection methods are slow: c.50 mussels per hour for incomplete macroscopic examination, and c.10 mussels per hour for full microscopic examination. Thus, the papain method can be as much as 19 times quicker than a comparably detailed examination of individually dissected mussels.

CONCLUSIONS

The major advantages of using papain are that very large numbers of mussels can be processed quickly, thoroughly and cheaply, thus enabling rapid assessments of Mytilicola distribution to be made over wide areas or very detailed examinations of more limited areas. The ability to process mussels in bulk consequently permits more readily the detection of Mytilicola at extremely low levels of infection. A wider application of this approach may, therefore, reveal the presence of Mytilicola in trace densities in some localities hitherto regarded as 'clean'.

ACKNOWLEDGEMENTS

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REFERENCES

DARE, P. J., 1974. A survey of the distribution limits of Mytilicola intestinalis Steuer in England and Wales, 1972-74. ICES C.M. 1974, Shellfish Comm., Doc. No. 12 (mimeo).

Table 1 Comparison of papain and conventional macroscopic methods of examination: the occurrence of *Mytilicola* at Barrow docks in February 1975

Treatment	Mussel sample		Number of mussels infected (%)	Number of *Mytilicola* found		Average number of *Mytilicola* per mussel	
	Length (mm)	Number		Conventional method	Papain method	Conventional method	Papain method
A Macroscopic examination, parasites replaced; whole raw meats then removed and digested in papain for microscopic examination	50–70	10	3	6	5		
	"	10	2	4	4		
	"	10	0	0	0		
	"	10	0	0	3		
		40	5 (12.5%)	10	12	0.25	0.30
B No macroscopic examination, whole raw meats removed and digested as (A)	50–70	10			3		
	"	10			3		
	"	10			1		
	"	10			2		
		40			9		0.23
C as (B)	40–50	50			12		0.24
D Mussels crushed and digested in shells, with microscopic examination	40–50	50			4		0.08