New Zealand Journal of Marine and Freshwater Research

ISSN: 0028-8330 (Print) 1175-8805 (Online) Journal homepage: http://www.tandfonline.com/loi/tnzm20

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To cite this article: A. M. Conroy , P. J. Smith , K. P. Michael & D. R. Stotter (1993) Identification and recruitment patterns of juvenile surf clams, Mactra discors and M. murchisoni from central New Zealand, New Zealand Journal of Marine and Freshwater Research, 27:3, 279-285, DOI: 10.1080/00288330.1993.9516568

To link to this article: http://dx.doi.org/10.1080/00288330.1993.9516568

New Zealand Journal of Marine and Freshwater Research, 1993: Vol. 27: 279–285
0028–8330/93/2703–0279 $2.50/0

Identification and recruitment patterns of juvenile surf clams, *Mactra discors* and *M. murchisoni* from central New Zealand

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Abstract Seventeen enzymes and general proteins were tested in adult *Mactra discors* Gray, 1837 and *M. murchisoni* Deshayes, 1854 from the Wellington west coast and Cloudy Bay, to find a biochemical genetic marker that would allow the identification of juvenile *Mactra* spp. (Mollusca: Bivalvia: Mactridae). Two proteins, glucose-6-phosphate isomerase and general protein, could identify 1148 juveniles. Shells from juvenile specimens, identified by protein markers, were used to test for morphological differences between species. Blind tests showed that the relative abundance of radial lirae permitted 44–100% of shells to be identified correctly, the sculpture of lateral teeth 43–100%, and colour and shape 61–98%. Recruitment patterns varied on two Wellington west coast beaches with *M. discors* juveniles dominating in early to mid 1990 and *M. murchisoni* dominating in early 1991.

Keywords Mactridae; surf clam; electrophoresis; shell morphology; recruitment

M93005
Received 18 January 1993; accepted 20 July 1993

INTRODUCTION

Surf clams of the families Mactridae, Mesodesmatidae, and Veneridae live in the surf zone of exposed sandy beaches and occur in densities that will support fisheries in many areas around New Zealand. These unexploited species have attracted scientific and commercial interest and are the focus of several investigations (Cranfield et al. 1992).

The two similar species *Mactra discors* Gray, 1837 and *M. murchisoni* Deshayes, 1854 occur throughout New Zealand (Climo 1972; Powell 1979), but are most abundant in the South Island. The species live sympatrically and are caught together in the same dredge hauls. The adults can be separated by shell shape, the colour of the periostracum, the shape of the pallial sinus, and the sculpture of the lateral teeth (Climo 1972). In small individuals (< 40 mm in length) these characters are less distinctive and specimens cannot be distinguished with confidence in the field (Fig. 1).

Juveniles of *Mactra* spp. need to be identified to species level for growth and recruitment studies. Biochemical genetic methods offer the potential to identify and distinguish closely related species (Avise 1975; Smith 1990). In marine species, protein and allozyme markers have been used to identify larvae and juveniles of closely related species of teleosts (Morgan 1975; Smith & Crossland 1977; Sidell et al. 1978; Smith et al. 1980; Graves et al. 1988; Nedreaas & Naevdal 1991) and crustaceans (Shaklee 1983; Lavery & Staples 1990) and to resolve taxonomic problems and reveal cryptic species in molluscs (Smith et al. 1981; Richardson et al. 1982; Grant et al. 1984; Munksgaard 1990).

The aim of this project was to identify juveniles of *M. discors* and *M. murchisoni* by the application of biochemical genetic methods and to test for shell characters in juveniles that could be used for field identification. A second aim was to investigate recruitment patterns of the two species at two locations, Cloudy Bay and the Wellington west coast, where fisheries may develop.

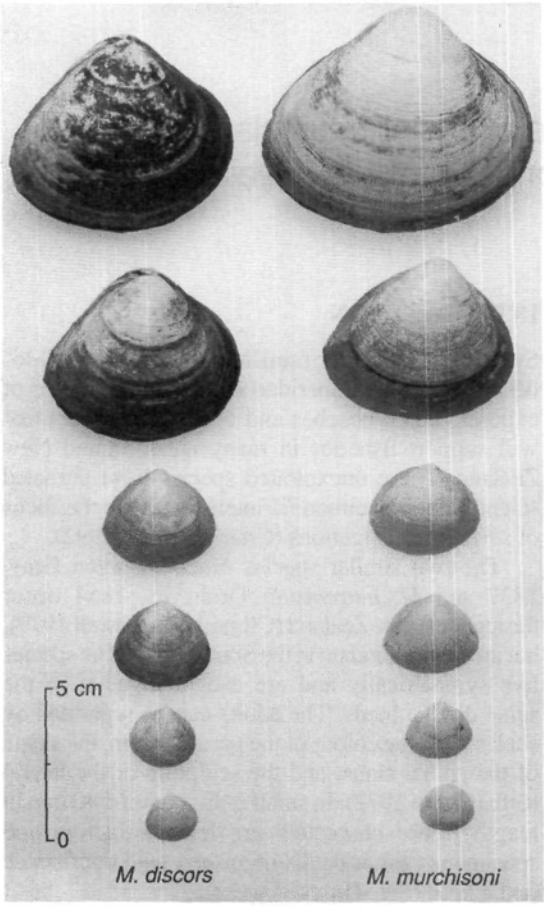


Fig. 1 Shells of *Mactra discors* and *M. murchisoni* ranging in size from adult to small juvenile.

MATERIALS AND METHODS

Sample collection

Surf clams were collected by hydraulic dredging (Michael et al. 1990) at two locations off the Wellington west coast, Otaki and Peka Peka, and at two locations in Cloudy Bay, Wairau and Fence (Fig. 2) between February 1990 and April 1991 (sampling dates are shown on Fig. 3). The dredge retained juveniles of *Mactra* spp. > 20 mm in length. Adults from known specimens of *M. discors* and *M. murchisoni* were dissected and the adductor muscle and hepatopancreas frozen separately at –70°C. *Mactra* juveniles which could not be identified were shucked and the tissues either frozen whole or the adductor muscle and hepatopancreas dissected and frozen separately at –70°C. The shells were labelled to correspond with tissue samples and stored dry.

Biochemical genetics

In the laboratory small pieces of tissue were homogenised in equal volumes of 1% Triton X-100 and refrigerated for up to 1 h. Small clams (< 25 mm) were homogenised whole. The homogenates were centrifuged at 13 000 rpm for 3 min, and the supernatants loaded onto starch or cellulose acetate gels. Adult samples were tested for 17 enzymes and general proteins (Table 1) in 10% starch gels made up in 4 buffer systems: S1, tris-citrate/lithium hydroxide-boric acid at pH 8.0 (Ridgway et al. 1970); S2, tris-citrate at pH 8.0 (Selander et al. 1971); S3, tris-EDTA-borate at pH 8.0 (Selander et al. 1971); and S4, tris-citrate at pH 7.0 (Fujio 1977) and 3 cellulose acetate buffer systems: C1, tris-glycine at pH 8.5 (Hebert & Beaton 1989); C2, citrate at pH 6.3 (Helena Laboratories); and C3, tris-barbital-sodium barbital at pH 8.8 (Helena Laboratories). Loci showing fixed differences between the two species were used as diagnostic markers to identify the juvenile clams. All samples collected before September 1990 were tested in starch gels and those after September 1990 tested in Helena cellulose acetate gels. A comparison of the two electrophoretic methods was made before adopting the more rapid technique of cellulose acetate electrophoresis for routine identification of *M. discors* and *M. murchisoni*.

Shell characters

Four methods were tested for identifying juvenile *Mactra*. Three methods used "blind tests" to estimate the reliability of shell colour and shape, the relative abundance of radial lirae, and the sculpture of lateral teeth for identifying species. One hundred shells of *M. discors* and 100 shells of *M. murchisoni* (50 of each species from Cloudy Bay and 50 of each from Wellington west coast), which had been identified from electrophoretic studies of tissue samples, were numbered in random order. The left valves of these shells were scored independently by 4–6 readers with different levels of experience in identifying *Mactra* shells.

Shell colour and shape

Adults of *M. murchisoni* are characterised by a straw-coloured periostracum and asymmetrical shells, and *M. discors* by a brown periostracum and equilateral shells (Climo 1972). Four readers scored the 200

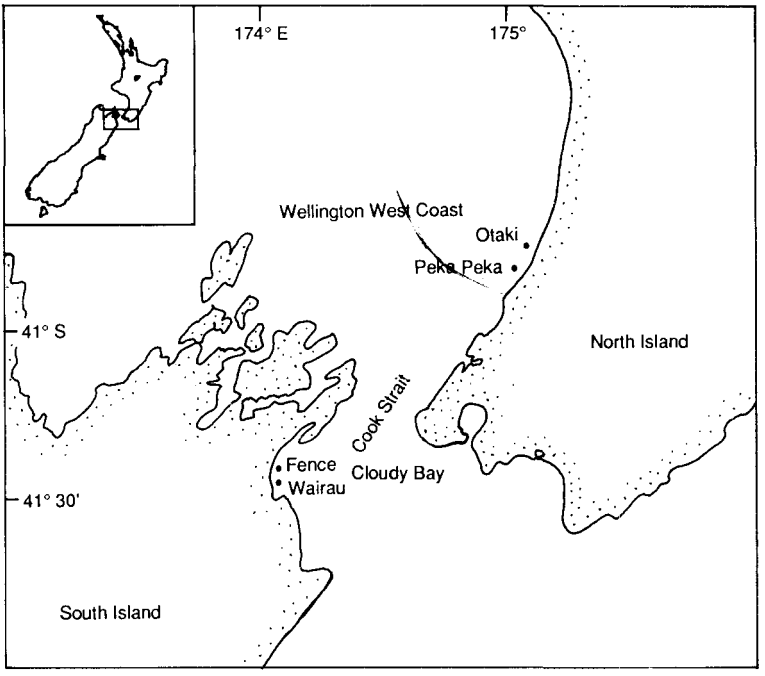


Fig. 2 Sampling locations for adult and juvenile surf clams, *Mactra discors* and *M. murchisoni* in central New Zealand.

juvenile left valves as *M. discors* or *M. murchisoni* on the basis of shell colour and shape.

Relative abundance of radial lirae

The radial lirae are microscopic ribs arranged in bands radiating from the umbo, on the outer shell. In juveniles, some individuals have a large number of radial lirae, particularly near the umbo region, whereas others have few or none. In contrast, all adults have few radial lirae and presumably these could be lost through shell abrasion. To test the potential of this character for identifying juveniles, six readers scored the pre-numbered left valves as *M. discors* or *M. murchisoni* based on the relative abundance of radial lirae.

Sculpture on the lateral teeth

In adults of *M. murchisoni* the lateral teeth are sculptured by parallel ridges or plications (Climo 1972). The ridges are continuous across the teeth and in small specimens (< 30 mm) only 1 or 2 ridges may be present. In adults of *M. discors* the lateral teeth are smooth or granulated (Climo 1972). The granules are uneven in size and have no obvious pattern. Four readers scored the 200 juvenile shells as *M. discors* or *M. murchisoni* on the basis of the sculpture on the lateral teeth of the left valve.

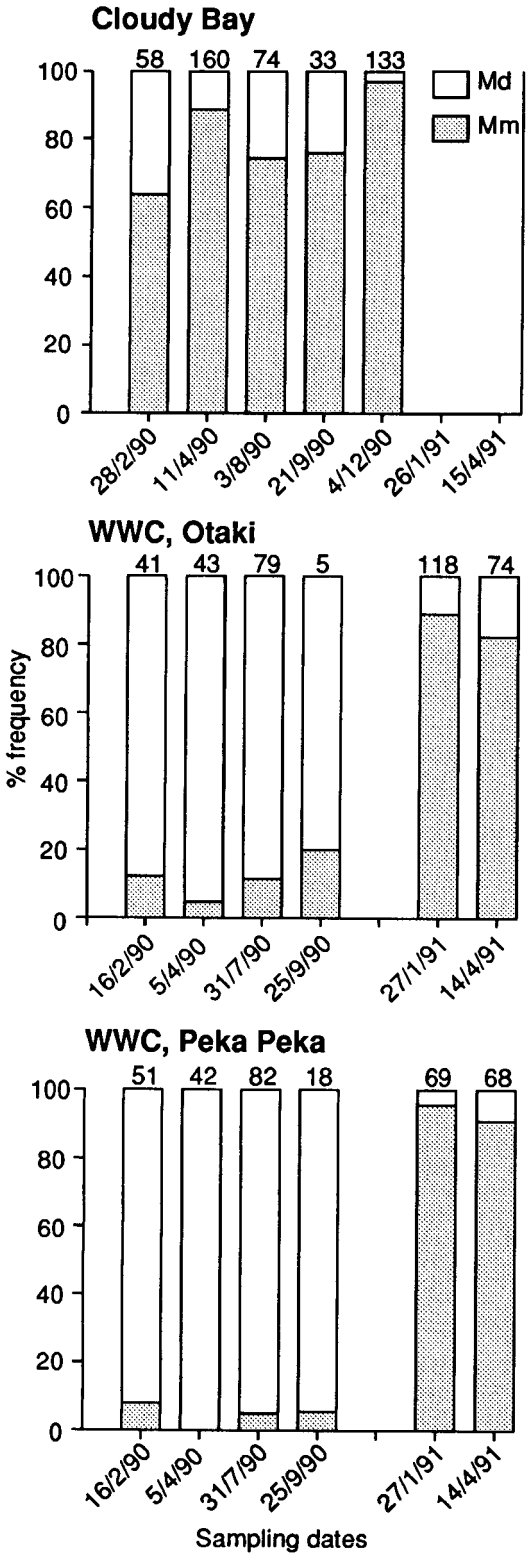
Length, height, and width relationships

The 200 labelled left valves used in the "blind tests" were measured for length, height, and width using Vernier callipers. The data were plotted and compared.

RESULTS

Biochemical genetics

Seventeen enzymes resolved as 24 zones of activity and were interpreted as the products of 24 gene loci. Eight loci stained with weak activity, six loci were polymorphic and three were monomorphic, whereas eight loci had species-specific electromorphs (Table 1). General proteins stained as three zones of activity and were interpreted as three loci. *PROT–2** and *PROT–3** were fixed for different electromorphs in the two species. Of the eight loci fixed for species-specific electromorphs only three, *GPI–1**, *PROT–2** and *PROT–3** stained strongly in small juveniles (< 30 mm) and these three loci were used as species markers to identify 1148 juveniles.



At *GPI–1** adult specimens of *M. discors* exhibited a faster-migrating zone of one and three band phenotypes whereas adult specimens of *M. murchisoni* exhibited a slower zone of one and three band phenotypes (Fig. 4). The majority of the juvenile specimens tested for *GPI–1** could be scored as *M. discors* or *M. murchisoni*. Eight juveniles (0.7%) appeared as heterozygotes with an *M. discors* and an *M. murchisoni* electromorph. However *PROT–2** and *PROT–3** showed these juveniles to be either *M. discors* or *M. murchisoni* and not hybrids. Three specimens could not be identified from electrophoretic patterns. The numbers of *Mactra* juveniles identified by electrophoresis are shown in Fig. 3.

Shell characters

Shells from juveniles, identified electrophoretically as *M. discors*, had very few or no radial lirae but those identified as *M. murchisoni* had a large number of radial lirae. Results for the three characters tested in "blind tests" are summarised in Table 2. Shell colour and shape was the most reliable character for identifying juveniles. Plots of length by height, length by width, and height by width of samples showed no difference between species or locations for juvenile samples.

DISCUSSION

Juveniles of the two *Mactra* species can be distinguished by genetic and by shell characters. Almost all of the 1151 juveniles (99.8%) tested by the biochemical genetic method were identified to the species level, whereas between 43 and 100% of juvenile shells were identified correctly from shell characters. Electrophoresis provides a relatively rapid and accurate method for identifying juveniles of the two species of surf clam, but is limited to the use of fresh or frozen tissue samples that have to be processed in a laboratory. Shell colour and shape were the most reliable of the shell characters tested (Table 2) and are the easiest method to apply in the field; the radial

◀ **Fig. 3** Recruitment patterns in *Mactra discors* (Md) and *M. murchisoni* (Mm) from Cloudy Bay (CB) and two sites on Wellington West Coast (WWC) in central New Zealand. Numbers of juveniles identified by electrophoresis are shown above histograms. No juveniles were caught in dredge samples in January and April 1991 at CB. Samples from the two CB sites were combined because of small numbers of *Mactra* (mean 7.4 per sample) at Fence.

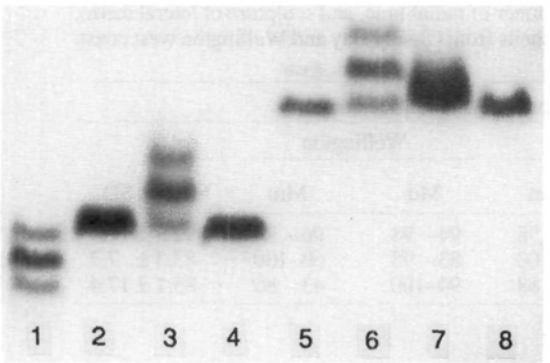


Fig. 4 Gel-phenotypes of glucose-6-phosphate isomerase in adductor muscle samples of *Mactra discors* (1–4) and *M. murchisoni* (5–8).

lirae and lateral teeth methods require the use of a low-power microscope.

Adults of the two species can be distinguished by height/width relationships (Climo 1972), but we have found this character to be unreliable for juvenile species identification because plots of height/width converged below shell size of 50 mm height. The proportion of adults of each species at each location, 74.5% *Mactra discors* on the Wellington west coast and 87.4% *M. murchisoni* at Cloudy Bay, cannot be used as an estimate of probability for identifying juveniles because recruitment appears to vary from year to year (Fig. 3).

In 1990 catches of juveniles from the Wellington west coast were dominated by *M. discors* but in 1991 catches were dominated by *M. murchisoni* (Fig. 3). This apparent change in the pattern of recruitment was similar at both sites on the Wellington west coast. Juveniles were caught in the same depth range as the adults, 3–5 m below chart datum on the Wellington west coast and 3–7 m deep at Cloudy Bay. Both species have similar shell dimensions and so it is unlikely that the dredge selects one species over the other. Furthermore, the catches showed little month-to-month variation within the sampling years, suggesting there is no large-scale patchiness in the distribution of juveniles. Thus the observed differences in juvenile catches would appear to reflect a change in relative recruitment of the two species and not a sampling artefact. Recruitment is known to vary in

Table 1 Enzymes and general proteins tested in adult specimens of *Mactra discors* and *M. murchisoni*. Buffer systems are described under Materials and Methods. Result: m, monomorphic with shared allele; p, polymorphic; s, species-specific alleles; w, weak activity.

Enzyme	Enzyme number	Locus	Buffer system	Result
Alcohol dehydrogenase	1.1.1.1	*ADH**	S1,S2	w
Alkaline phosphatase	3.1.3.1	*ALP**	S1, S2	s
Aspartate aminotransferase	2.6.1.1	*AAT**	S2	w
Esterase	3.1.1.-	*EST-1**	S1, S2	s
		*EST-2**	S1, S2	s
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	C2	w
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	S1, C1	p, s
		*GPI-2**	S1, C1	m
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	S2, C2	w
Isocitrate dehydrogenase	1.1.1.42	*IDHP**	S2	w
Lactate dehydrogenase	1.1.1.27	*LDH**	S1, S2, S3	w
Leucine aminopeptidase	3.4.1.1	*LAP**	S1, S2	p
Malate dehydrogenase	1.1.1.37	*MDH-1**	S1	p
		*MDH-2**	S1	m
Malic enzyme	1.1.1.40	*MEP**	S1, S2	w
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	S1, S3	w
Octopine dehydrogenase	5.3.1.9	*ODH**	S1	s
Phosphoglucomutase	5.4.2.2	*PGM-1**	S1, S4, C3	p
		*PGM-2**	S1, S4, C3	p
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	S2	p
Protein	–	*PROT-1**	S1, C1	m
		*PROT-2**	S1, C1	s
		*PROT-3**	S1, C1	s
Superoxide dismutase	1.15.1.1	*SOD**	S1	s

Table 2 Three shell characters (colour and shape, number of radial lirae, and sculpture of lateral teeth), tested by 4–6 readers in "blind tests" to identify 200 shells from Cloudy Bay and Wellington west coast. Md, *M. discors*; Mm, *M. murchisoni*.

		Percentage identified correctly				
		Cloudy Bay		Wellington		
Character	No. of readers	Md	Mm	Md	Mm	Mean ± SD
Colour and shape	4	61– 94	90– 98	94– 98	96– 98	92.3 ± 10.4
Radial lirae	6	44– 98	52–100	83– 98	60–100	83.3 ± 7.7
Lateral teeth	4	98–100	65– 88	94–100	43– 86	85.1 ± 17.4

other bivalve fisheries: environmental factors have been implicated in recruitment fluctuations in scallops (Caddy 1979), size-specific predation in clams (Tinsman 1981), and size-related intraspecific competition in cockles (Hancock 1973). Predation and competition seem unlikely explanations for our observations; further work on environmental physical factors and reproductive biology is desirable to understand recruitment processes in *Mactra* species. Highly variable recruitment patterns as reported here imply a need for annual biomass surveys in order to optimise the management of clam resources.

ACKNOWLEDGMENTS

Bruce Marshall (Museum of New Zealand) suggested the use of radial lirae as a possible technique for separating the two species. Ian Doonan (MAF Fisheries, Marine Research) advised on statistical tests for the morphometric data.

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