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J.A.H. Benzie,* S.T. Williams* and J.M. Macaranas†

Introduction

THE analysis of electrophoretically detectable protein variation has revolutionised population genetics and allowed the extent and structure of genetic variation in natural populations to be described (Richardson et al. 1986). The method is now widely applied to determine the structure of exploited populations in order to identify the number of stocks in a fishery, or to determine the level of genetic variation in cultured populations (Ryman and Utter 1986).

Reports of the genetic structure of populations concentrate on the protein (usually enzyme) systems used to screen the populations and provide only succinct summaries of the techniques used. However, the development of techniques for a given survey often involves trials of a number of methods and almost always the testing of a far greater number of enzyme systems than are used in the final screenings. Similarly, detailed discussions of the patterns of bands seen on gels and their interpretation are omitted. Such information is of value to workers considering genetic work that demands the use of particular enzyme systems, or a greater range of systems than has been published, and can assist linking results from different studies.

In developing a set of six to nine reliable polymorphic systems with which to assess the population structure of giant clams, some 60 systems were tested on as many as 10 buffer types. The aim of this report is to make available details of the systems tested for giant clam species, in order to provide a greater background to the research papers published elsewhere (Benzie and Williams 1992a,b; Macaranas et al. 1992), and in order to assist future technique development.

General Strategy

Publications are available that provide the theoretical and practical background to strategies for developing sets of enzymes for use in surveys with particular aims (Richardson et al. 1986). Normally, this involves first the survey of a reasonable number of enzyme systems in a variety of tissues to test for activity and the presence of variation. Secondly, techniques are improved for efficient and accurate survey using the subset of enzymes displaying sufficient activity and variation. A major consideration in the present case was the development of methods that would allow clams to be sampled without killing them, and preferably in situ. Populations of these endangered species in a number of sample sites were small, and valuable broodstock from aquaculture operations were also to be sampled.

A small number of animals of several species was sacrificed in order to obtain several tissue types, so that a wide variety of enzymes could be tested. Comparison with symbiont-free tissues allowed identification of clam enzymes in tissues with algal symbionts. The only tissue which could readily be biopsied accurately and with least stress to the clam was mantle tissue, which has algal symbionts. The aim was to establish whether a sufficient number of poly-

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morphic enzymes could be obtained from mantle tissue. If so, it would be possible to sample clams in situ, and to do so without sacrificing them.

Sample Collection and Storage

Initial screening

Whole animals of *Tridacna gigas*, *T. derasa*, *T. maxima*, *T. squamosa*, *T. crocea* and *Hippopus hippopus* were sacrificed to obtain several different tissues. These animals were taken to the laboratory and the adductor muscle either cut from below using a scalpel to slice up through the byssal orifice, or cut from above while the shell was wedged open. In small clams or species with large orifices (e.g. *Hippopus hippopus*) the former method was found to be the easier. Tissues taken for analysis were: mantle, adductor muscle, gill and kidney. Each was placed in a labelled plastic ziplock bag and stored at -80°C.

Survey collections

Initial tests had demonstrated that cutting a small piece of tissue from the fringe of the clam mantle which spreads over the edge of the shell did not kill the clam. This technique, when performed correctly, did not harm or stress the clam. Once it was established sufficient variable enzymes could be assayed from mantle, standard surveys used biopsies of mantle tissue taken by scuba divers using forceps and scissors.

When collecting from wild populations, reefs known to have reasonable numbers of clams were surveyed using the manta tow technique described by Moran et al. (1989) to locate the clams. The method involves towing a diver on snorkel behind a small boat, allowing large areas of reef to be scanned fairly rapidly.

Biopsy method

Biopsies were most easily performed with two divers. One diver would approach an open clam without casting a shadow over it and, where necessary, wedge it open. The precise technique used to open the clam depended upon the behaviour of the clam species concerned. *T. maxima* was held open using a flat triangular metal wedge with a long thin T-bar which sat over the edges of its shell to help prevent the wedge falling in on the clam. Firm holding of the wedge was assisted by the consistent strong closure by *T. maxima*. This was not the case in *T. derasa* because this species opened completely after a while, allowing the wedge to fall inside the shell before snapping closed. Eventually it was found that a piece of cylindrical coral rubble (e.g. branching *Acropora*) worked best for *T. derasa*. Adult *T. gigas* did not require to be wedged at all as they are not able to close fully.

While the first diver kept the clam open, the second diver held a piece of mantle tight in round-tipped forceps and cut a thin strip of mantle tissue (0.5 cm × 2.5 cm) following along the length of the shell to avoid puncturing the coelomic cavity. Maintaining pressure on the cut by pulling away the strip with the forceps while cutting with the scissors allowed a rapid and clean cut to be made through the mantle tissue. This tissue was then placed inside an opened ziplock plastic bag (7.5 cm × 15 cm) and stored inside the diver's wetsuit or glove to prevent accidental loss. Each bag was individually labelled prior to the dive with a water-resistant marker. Tests described in a later section had demonstrated enzyme activity was retained by samples held for 2–3 hours in seawater.

Immediately on return to the boat, each sample was removed from the plastic bag, cut into smaller pieces (0.5 cm × 0.5 cm) and replaced in the bag. All air and water was expressed by keeping the tissue at the base of the bag and rolling the bag from the base to the top. An elastic band was then wound around several rolled bags to provide a robust, small-volume sample that was placed immediately in liquid nitrogen. Where collections from more than one site were being stored in the same container, each bundle of sample bags was placed inside another small, ziplock bag labelled with site details to facilitate rapid sorting of samples later.

Sample storage

In the laboratory, samples were allowed to rise in temperature sufficiently to allow the plastic bags to be unrolled without shattering, but not

for the samples to thaw. The frozen mantle tissue was placed (still frozen) into previously labelled 5 mL polypropylene screwcap tubes, which were then immediately immersed in liquid nitrogen. All tubes from a site were arranged in order into a fibreboard box (with lid) and placed in a -80°C freezer. This permitted easy access to any particular sample and fast removal of a frozen fragment without repeated freezing and thawing. All samples were stored continuously at -80°C until analysis.

Protein Stability

Time at ambient temperature before freezing

To simulate the delay between sampling and freezing (the dive time), mantle biopsies were left in plastic bags in a small volume of seawater at ambient temperature for different periods of time prior to freezing. Three individuals of *T. gigas* and three of *T. derasa* were biopsied and each biopsy cut into four subsamples. One subsample of mantle was snap-frozen in liquid nitrogen immediately and the other samples were frozen after intervals of 60, 120 and 180 minutes. The 12 subsamples for each species were run on the same gel and enzyme systems visualised. No loss of activity was observed by eye at any of the loci for *T. gigas* or *T. derasa*. The subsamples left in seawater for three hours were as active as those frozen immediately after biopsy.

Freeze temperature

Samples that had been placed on ice and stored at -10°C or -20°C for several days showed a definite loss of activity compared with samples frozen in liquid nitrogen and stored at -80°C for the same period.

Tests where *T. gigas* and *T. derasa* mantle samples were placed on ice for 1-2 hours during transport from the field to the laboratory demonstrated that loss of activity was not due to the transport on ice. Material transported in this way and frozen in liquid nitrogen had good enzyme activity similar to that for material frozen in liquid nitrogen immediately after collection. Storage of samples at -20°C resulted in the observed loss of activity. Similarly, samples that had been processed for electrophoresis could not be frozen overnight at -20°C as the loss in activity and loss of resolution on the gels made scoring difficult to impossible. Loss of activity in mantle tissue or any extracts from mantle tissue was therefore rapid on freezing at higher temperatures (-20°C or warmer).

Effect of freezing and thawing samples

No specific tests of repeated freezing and thawing of samples were made. However, it was obvious that samples collected early in the project and used regularly as reference samples showed definite deterioration (lesser activity, stronger breakdown bands) when compared with samples collected at the same time but not removed regularly from the -80°C freezer. The reference samples were still usable after being accessed several times over a two-year period, but the protocols used did not allow the tissues to thaw.

Sample freshness

All samples taken in the survey were from live specimens. Some early attempts to dissect tissues from recently dead specimens found in raceways gave very poor or no activity.

Electrophoresis

Sample preparation for electrophoresis

A chip of mantle tissue about 0.5 cm^2 was placed in a ceramic depression plate and allowed to thaw in four to five drops of an aqueous solution of b-mercaptoethanol coloured with bromophenol blue dye. Samples were ground with a stainless steel pestle and glass powder (lightly crushed cover-slips) until the liquid was a dark brown, indicating release of zooxanthellae and thus rupture of cell membranes. The ceramic plates were kept on ice until all samples had been applied to gels.

Grinding the mantle tissue using an automatic homogeniser, or with a sonicator, took longer than the pestle technique, and some loss of activity was observed. Similarly, centrifugation of crushed samples provided no advantage for sample resolution. Indeed, there was evidence of some loss of activity. Given that fact, and the extra time involved in this step,

centrifugation was abandoned in favour of the simple squashing method. Sample wicks of chromatography paper for use with starch gels were placed directly in the liquid. This liquid was drawn up with draftsman's pens in order to load the cellulose acetate gels.

Starch gel electrophoresis

General methods of electrophoresis using starch were similar to those of Shaklee and Keenan (1986). Horizontal starch gels (12% Sigma starch, cat. No. S-4501) were prepared the day before, along with appropriate quantities of electrode buffer. Buffers were prepared according to the recipes in Appendix 2. Gels were poured into perspex moulds and lids placed directly onto the slightly cooled gel to prevent desiccation overnight. Electrode buffers were stored at 3°C overnight.

On the morning of use, lids were removed and gels trimmed to remove hard edges and a single cut was made across the width of the gel, approximately one-fifth from the base, to allow access for placement of the wicks. All wicks were removed from the sample liquid in the order required for loading, blotted on tissue paper and placed vertically into the slit across the gel. In addition, one extra wick was soaked in a strong solution of bromophenol blue which acted as a dye marker to indicate the buffer front.

Gels were then placed on buffer trays connected to a power pack in a cold-room at 3°C. Cloth wicks soaked in electrode buffer were draped from the buffer onto the gel to establish electrical contact between the ends of the gels and the tray buffers. The whole apparatus was covered with plastic sheeting to prevent desiccation and subjected to electrophoresis for a period and voltage appropriate to the buffer (Appendix 2).

Gels were electrophoresed until the dye marker had migrated to within a centimetre of the anodal wick (generally 5–6 hours) and then were removed from their moulds and sliced transversely into approximately five slices each about 1 mm thick. Each of these slices was then stained with 10 mL of stain mixed with 10 mL of 2% agar, according to recipes described by Harris and Hopkinson (1976) and Shaw and Prasad (1970).

Running starch gels overnight (16 hours) and for 24 hours at 4°C resulted in gels which gave smeary bands which were difficult to score. In systems with large numbers of alleles, or closely spaced alleles, it was not possible to score gels accurately and the 5–6 hour run at higher voltage was kept as the standard for the surveys.

Cellulose acetate (Cellogel) electrophoresis

Cellogels were supplied as square sheets 30 cm × 30 cm stored in 30% methanol. For initial enzyme surveys these sheets were cut into small strips (3 cm × 15 cm) whereas half-sheets were used for electrophoresis of established systems.

On the morning of use, Cellogel was removed from methanol and blotted with blotting paper, taking care to avoid smearing or scraping the gel, and placed in about 150–200 mL of appropriate buffer (Appendix 3). The buffer was discarded after about 30 minutes, replaced with a fresh aliquot, and the gel allowed to soak for a further 15 minutes.

The Cellogel was then removed from the rinse tray and once more blotted carefully before being positioned in the electrophoretic tank, containing the same buffer, and held in place by magnets. Samples were loaded directly onto the gel approximately one centimetre from the cathodal bridge contact with the gel. Three pen strokes of sample were applied lightly to the Cellogel using a draftsman's pen.

Cellogel was run at 200 V for 2 hours at 4°C. Stains were made 10 minutes prior to use. Cellogels were removed from trays, blotted, and then rolled through 2 mL of stain poured onto 'gladwrap' stretched across a rectangular glass dish. The gel remained in the stain no more than one minute before being blotted and suspended between two perspex stands in an airtight container and incubated in the dark at 37°C. Progress of stain development was monitored regularly and enzyme activity was stopped by placing the gel in a bath of 7% acetic acid or back-staining with MTT and PMS as appropriate. Cellogel stains were those described by Richardson et al. (1986) or reduced quantities of starch stains. Further details of Cellogel electrophoresis are found in Richardson et al. (1986).

Scoring

Isozymes coded by separate loci were numbered in order of decreasing mobility. Electromorphs were equated with alleles and coded alphabetically in order of decreasing anodal mobility. Scoring was aided by the use of several samples with known allelic patterns, that were repeated several times on each gel. A series of gels including representatives of the variants detected was also run at the end of the survey to further cross-correlate the results from different gels and check allelic identity.

Enzyme Surveys

The enzyme nomenclature used here follows the International Union of Biochemistry's Nomenclature Committee (IUBNC 1984). Definitions of the abbreviations used and Enzyme Commission numbers are provided in Appendix 1.

Enzymes surveyed for activity

A total of 60 enzymes was surveyed for activity in each of four tissues in each of six species, and the results of the tests for all taxa are summarised in Table 1. Details of the response for individual taxa are given in separate tables in Appendix 4. A total of three individuals of each species was tested for each tissue on six buffer types. Approximately 47 systems had activities sufficient to warrant further investigation. Most enzymes were active in all tissues. With the exception of AO, ADH and ALKP which were only or most active in kidney, and OpDH which was active only in gill, all enzymes were equally or most active in mantle or adductor muscle. Similar patterns were observed for all tissues in most cases. Mantle tissue did show additional blurred zones of activity that stained very weakly and generally took longer to appear, and which were not represented in tissues without symbionts. These zones of activity, derived from the algal symbionts, showed no interpretable variation and did not interfere with the interpretation of the clam enzymes.

Enzymes investigated for resolution and variability

Subsequent analyses of the subsets of enzymes showing reasonable activity concentrated only on adductor muscle and mantle (Table 2). Results of these tests for all species are summarised in Table 3. Resolution was adequate on 22 of the systems on at least one of the nine buffer/electrophoretic substrate combinations tested. Eight systems (ACP, ALKP, GcDH, GPT, GUK, HK, MPMO and SDH) could not be resolved well on any buffer/substrate combination for any of the species and were abandoned. A further 17 showed poor resolution but were considered to have some promise, particularly as 10 of these (ACON, ADA, ALDH, CAT, GDH, G-6-PD, MPI, MDR, PGAM and 6-PGD) displayed some variation.

Variation observed

The levels of variation detected are dependent in part upon the number of individuals examined and the geographical spread of the samples obtained. Detailed surveys were carried out on only three taxa using between six and nine polymorphic loci which were relatively easy and reliable to score (*T. maxima*, 6 loci; *T. gigas*, 8 loci and *T. derasa*, 9 loci, with 400–800 individuals being sampled throughout the West Pacific in each case). A number of systems showed good resolution and high levels of variation but were not used for surveys because so many alleles were present that accurate scoring was extremely time-consuming or impossible because of the number of cross-correlation runs required (e.g. GPI in *T. maxima*). Similarly some systems, such as *MDH-1** in *T. maxima*, clearly displayed variation, but a combination of complex breakdown bands, some warp, and the number of alleles involved made scoring too time-consuming for the effective inclusion of these systems in surveys which required rapid, and accurate, routine identification of variants.

A larger number of loci which had shown limited or no variation in early tests, or whose interpretation was more complicated, was assayed in some analyses of *T. gigas* families in culture (about 540 individuals) and from a small number of individuals (usually 9) of each species as part of a phylogenetic analysis. The numbers of alleles detected in all these surveys are summarised in Table 4 in order to give an approximate idea of the variation that might be accessed.

Table 1. Details of enzyme activities in four tissue types of *Tridacna gigas, T. derasa, T. maxima, T. squamosa, T. crocea* and *Hippopus hippopus*. Where activities differed on different buffers or substrates, the highest activity observed for any species is recorded in this table. See separate tables in Appendix 4 for details of each species.

	Enzyme	Tissue activity					Enzyme	Tissue activity			
		A	M	G	K			A	M	G	K
1.	AAT	++	++	++	++	31.	GSR	++	++	++	++
2.	AAT(UV)	++	++	++	++	32.	GUK	+	+	+	+
3.	ACON	++	++	++	++	33.	HBDH	–	–	–	–
4.	ACP	+	+	+	++	34.	HK	++	+	+	++
5.	ADA	+	+	+	–	35.	IDH*	++	++	++	++
6.	ADH	–	–	–	+	36.	LAP*	++	++	++	++
7.	AK*	++	++	++	+	37.	LDH*	++	++	++	++
8.	ALD	++	++	+	+	38.	LGG	++	++	++	++
9.	ALDH	–	+	+	+	39.	LP	++	++	++	++
10.	ALKP	+	+	+	++	40.	LT	++	++	++	++
11.	AMYL	–	–	–	–	41.	MDH*	++	++	++	++
12.	AO	–	–	–	++	42.	MDR	++	++	+	+
13.	CAT	++	++	++	++	43.	ME*	++	++	++	++
14.	CK	++	+	+	+	44.	MPI*	++	++	++	++
15.	DAMOX	–	–	–	–	45.	MPMO*	++	++	++	++
16.	DASOX	–	–	–	–	46.	NDH	++	–	–	–
17.	DIA	++	++	++	++	47.	ODH	++	+	+	++
18.	ENO	++	++	++	++	48.	OpDH	–	–	+	–
19.	EST*	++	++	++	++	49.	PGAM	++	+	+	+
20.	FBP	++	++	++	++	50.	6PGD*	++	++	++	++
21.	FUM	++	++	+	+	51.	PGK	++	++	++	++
22.	GA3PD*	++	++	++	++	52.	PGM*	++	++	++	++
23.	GcDH	+	+	+	+	53.	PK	++	++	++	++
24.	GDA	–	–	–	–	54.	1–PYDH	–	–	–	–
25.	GDH	+	+	+	+	55.	SDH	++	–	–	+
26.	GOX	–	–	–	–	56.	SOD*	++	++	++	++
27.	*αGPD*	–	–	–	–	57.	STDH	++	++	++	++
28.	G6PD	++	++	++	++	58.	TPI	+	+	+	+
29.	GPI*	++	++	++	++	59.	XDH	–	–	–	–
30.	GPT	+	+	+	+	60.	XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity.
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).
*These enzymes were also tested on CAPM7.0 and TECB8.75 for *T. squamosa* using all four tissues, and on CAPM7.0, TECB8.75, CP6.6 and TRIC7.2 for *T. derasa* using adductor muscle and mantle.

Table 2. The following enzymes have shown sufficient activity and resolution to warrant further investigation as genetic markers, in adductor muscle (A) or mantle (M).

	Enzyme	*T. gigas*		*T. derasa*		*T. maxima*		*T. squamosa*		*T. crocea*		*H. hippopus*	
		A	M	A	M	A	M	A	M	A	M	A	M
Set 1. Active systems with good resolution													
1.	AAT(UV)	+	+	+	+	+	+	+	+	+	+	+	+
2.	AK	+	+	+	+	+	+	+	+	+	+	+	+
3.	ALD	+	+	+	−	+	−	+	+	+	+	+	+
4.	DIA	+	+	+	+	+	+	+	+	+	+	+	+
5.	ENO	+	+	+	+	+	+	+	+	+	+	+	+
6.	EST	+	+	+	+	+	+	+	+	+	+	+	+
7.	G6PD	+	+	+	+	+	+	+	+	+	+	+	+
8.	GPI	+	+	+	+	+	+	+	+	+	+	+	+
9.	GSR	+	+	+	+	+	+	+	+	+	+	+	+
10.	IDH	+	+	+	+	+	+	+	+	+	+	+	+
11.	LDH	+	+	+	+	+	+	+	+	+	+	+	+
12.	LAP	+	+	+	+	+	+	+	+	+	+	+	+
13.	LGG	+	+	+	+	+	+	+	+	+	+	+	+
14.	LP	+	+	+	+	+	+	+	+	+	+	+	+
15.	LT	+	+	+	+	+	+	+	+	+	+	+	+
16.	MDH	+	+	+	+	+	+	+	+	+	+	+	+
17.	ME	+	+	+	+	+	+	+	+	+	+	+	+
18.	MPI	+	+	+	+	+	+	+	+	+	+	+	+
19.	NDH	+	−	+	−	+	−	+	−	+	−	+	−
20.	6PGD	+	+	+	+	+	+	+	+	+	+	+	+
21.	PGK	+	+	+	+	+	+	+	+	+	+	+	+
22.	PGM	+	+	+	+	+	+	+	+	+	+	+	+
23.	SOD	+	+	+	+	+	+	+	+	+	+	+	+
24.	StDH	+	+	+	+	+	+	+	+	+	+	+	+
Set 2. Systems with lesser activity or poorer resolution													
1.	ACON	+	+	+	+	+	+	+	+	+	+	+	+
2.	ACP	+	+	−	+	−	−	−	−	−	−	−	−
3.	ADA	−	−	−	−	−	−	−	−	+	−	+	+
4.	ALDH	−	−	−	−	−	−	−	−	−	+	−	−
5.	ALKP	−	+	+	+	+	−	+	−	+	+	−	−
6.	CAT	+	+	−	+	+	+	−	+	+	+	+	+
7.	CK	+	+	−	−	−	−	−	−	+	+	+	+
8.	FBP	+	+	+	+	+	+	+	+	+	+	+	+
9.	FUM	+	+	+	+	+	+	+	+	−	−	−	−
10.	GA3PD	+	+	+	+	+	+	+	+	+	+	+	−
11.	GcDH	+	−	−	−	+	−	+	−	−	−	+	+
12.	GDH	+	−	−	−	−	−	−	−	+	+	−	−
13.	GPT	+	+	−	−	−	+	+	+	+	+	+	+
14.	GUK	+	+	+	−	+	+	+	−	+	+	+	−
15.	HK	+	+	+	+	+	+	+	+	+	+	+	+
16.	MDR	+	+	+	+	−	+	−	+	+	−	+	−
17.	MPMO	−	−	+	+	+	+	−	+	−	−	−	−
18.	ODH	+	+	+	+	+	+	+	+	+	−	−	−
19.	PGAM	+	+	+	+	+	+	+	+	+	+	+	+
20.	PK	+	+	+	+	+	+	+	+	+	+	+	+
21.	SDH	+	−	−	−	−	−	−	−	+	−	−	−
22.	TPI	+	−	+	+	+	+	+	+	−	−	−	−

(+) = active, (−) = no activity

Table 3. Tests of resolution, variability and best running conditions in all six species of giant clams listed in Table 2. Enzymes were active on both mantle and adductor muscle, except where indicated. (* Active for mantle issue only; ** active for adductor muscle only.) Variability among species was scored as yes (Y) if any difference in mobility was observed between any pair of taxa. Variability within taxa was scored as yes (Y) if any one species showed variation. Details of variation are given in Table 4.

	Enzyme	Resolution	Gel	Buffer	Variability	
					Among species	Within species
1.	AAT(UV)	++	Cellogel/Starch	TM7.8/TEB8.4	Y	Y
2.	ACON	+	Starch	TEC7.9	Y	Y
3.	ACP	–	Starch	CP6.4	not resolved	
4.	ADA	–	Cellogel	PH7.0	Y	not resolved
5.	AK	++	Starch	TEC7.9	Y	Y
6.	ALD	+	Cellogel	PH7.0	Y	Y
7.	ALDH*	+	Starch	TEB8.4	Y	Y
8.	ALKP	–	Starch	TEB8.4	not resolved	
9.	CAT	+	Starch	TEC7.9	Y	Y
10.	CK	++	Starch	TC7.0	Y	Y
11.	DIA	++	Starch	TEB8.4	Y	Y
12.	ENO	++	Cellogel	CP6.4	Y	Y
13.	EST	++	Starch	TEC7.9	Y	N
14.	FBP	+	Cellogel/Starch	PH7.0/TC7.0	Y	Y
15.	FUM	+	Cellogel	PH7.0	Y	Y
16.	GA3PD	–	Starch	?	not resolved	
17.	GcDH	–	Starch	TEC7.9	Y	not resolved
18.	GDH	–	Cellogel	TM7.8	not resolved	Y
19.	G6PD	+	Starch	TC7.0	Y	Y
20.	GPI	++	Starch/Cellogel	TEC7.9/TM7.8	Y	Y
21.	GPT	–	Starch	TEB8.4	Y	Y
22.	GSR	++	Starch	TEC7.9	Y	Y
23.	GUK	–	Cellogel	CP6.4	Y	not resolved
24.	HK	+	Cellogel	TM7.8	Y	Y
25.	IDH	++	Starch	TEC7.9	Y	Y
26.	LAP	++	Starch	TC7.0	Y	Y
27.	LDH	++	Starch	TC7.0	Y	Y
28.	LGG	++	Starch	TC7.0	Y	Y
29.	LP	++	Starch	TC7.0	Y	Y
30.	LT	++	Starch	TC7.0	Y	Y
31.	MDH	++	Starch	TC7.0	Y	Y
32.	ME	++	Starch	TEC7.9	N	N
33.	MPI	++	Starch	POUL	Y	Y
34.	MDR	+	Starch	TEB8.4	Y	Y
35.	MPMO	–	Starch	TEB8.4	Y	Y
36.	NDH**	++	Starch	TEB8.4	Y	Y
37.	ODH	+	Starch	?	Y	Y
38.	PGAM	–	Cellogel	CP6.4	Y	Y
39.	6PGD	+	Starch	TEC7.9	Y	N
40.	PGK	+	Cellogel	TM7.8	Y	Y
41.	PGM	++	Starch	TEB8.4	Y	Y
42.	PK	+	Starch	TC7.0	Y	Y
43.	SDH**	-	Starch	TC7.0	Y	not resolved
44.	SOD	++	Starch	TEB8.4	Y	Y
45.	STRDH	++	Starch	TC7.0	Y	Y
46.	TPI	+	Starch	TEC7.9	Y	Y

Resolution is scored as (++) good/reasonable, (+) poor and (-) very poor.

Table 4. Number of alleles observed per locus for each species, for loci screened for all species.

Locus	*T. gigas*	*T.derasa*	*T.maxima*	*T.squamosa*	*T.crocea*	*T.tevoroa*	*H.porcellanus*	*H.hippopus*
1. AAT-1*	1a	1	1	1	1	1	1	1
2. AK-1*	1	2	1	1	1	1	1	1
3. AK-2*	2b	2b	4b	2	3	1	1	1
4. DIA*	3b	4b	8	3	2	1	1	1
5. ENO*	1b	5b	>10b	3	10	1	3	1
6. EST*	1a	1	1	1	1	1	1	1
7. GPI*	4b	4b	>10b	3	3	1	2	1
8. GSR*	1b	3	10	2	5	1	1	1
9. IDH*	1a	1	6	2	3	1	1	1
10. LDH-1*	2b	2b	10b	2	2	1	1	1
11. LDH-2*	2a	3	2	1	1	2	1	1
12. LGG-1*	6b	4b	7b	2	3	1	1	1
13. LGG-2*	2b	2b	3	2	3	1	1	1
14. LP-1*	1	1	1	1	1	1	1	1
15. LP-2*	1a	1	1	1	1	1	1	1
16. LP-3*	1	1	2	1	2	1	1	1
17. MDH-1*	3b	2b	5	4	4	1	1	1
18. MDH-2*	1b	1b	4b	1	2	1	1	1
19. ME*	1b	1b	1b	1	1	1	1	1
20. MPI*	4a	3	3	4	4	1	1	1
21. NDH-1*	1	1	3	1	2	—	1	1
22. NDH-2*	1	1	1	1	1	—	2	2
23. PGK*	2a	1	1	1	2	1	1	1
24. PGM-1*	2	1	3	3	2	1	1	1
25. PGM-2*	4b	4b	8b	7	3	1	1	1
26. SOD*	1	1	1	1	1	1	1	2

Number of samples is less than 10 except where indicated: a = 100–1000; b =>1000 (routine screening).

Details of zymograms

Illustrations for the zymograms provide information on the allelic variants detected for all species using the available data, which in some cases were limited to a few individuals. The mobility of bands in this case was calculated relative to the most common band for *T. gigas*, which was designated a mobility of 100. Where extensive surveys of a species were carried out, information on the allelic variants observed is provided separately with the mobility of variants calculated relative to the most common allele within that taxon. Bands for a given locus are represented by a solid line, with open shading indicating a smear. Other zones of activity that were not scored and might be the products of further loci are indicated by stippled areas. In the rare cases where loci overlapped, one locus has been represented by lines of a different weight to differentiate the loci more easily.

AAT (Fig. 1)

Two zones of AAT activity were detected on starch gels (TEB8.4) whereas only one (*AAT-2**) was observed on Cellogel (TM7.8). The stain used for both media was a UV-visible one that required back-staining with MTT and PMS for visualisation under normal light. Activity was strong but resolution average. These loci overlapped in some taxa, and only *AAT-1**, which was more active, could be clearly scored over all taxa. The patterns observed on gels stained for STRDH were identical to those for AAT. *AAT-1** and *AAT-2** were screened for a large number of individuals from only six batches of juvenile *T. gigas* reared in culture. These exhibited no variation. *T. derasa*: Two alleles were observed at *AAT-1** in the phylogenetic surveys. *T. gigas*: Monomorphic. *T. maxima*: Monomorphic in the phylogenetic analysis. Other species: all other species were invariant at *AAT-1**, and invariant at both loci where two loci were clearly distinguishable.

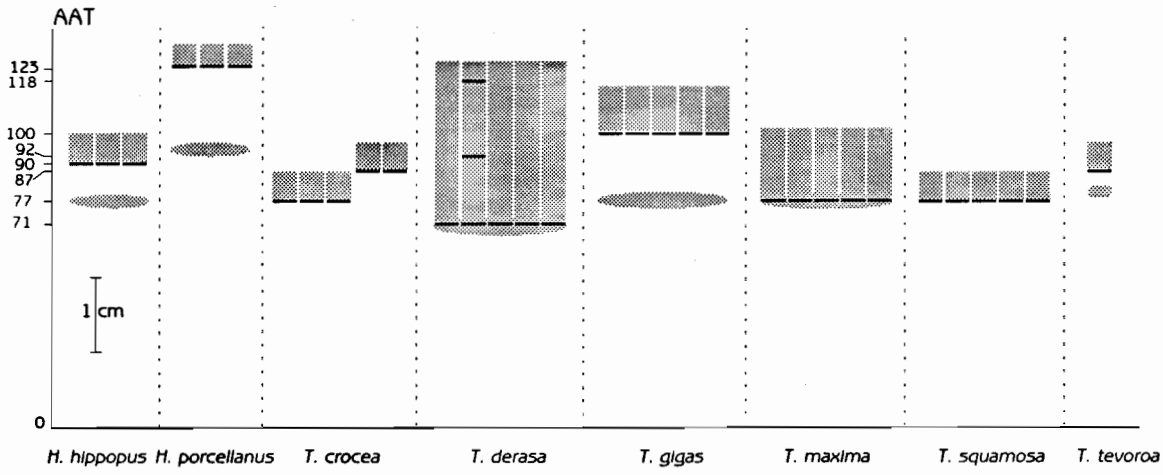


Figure 1. Enzyme patterns observed for AAT on starch and Cellogel. This system was monomorphic over all species.

AK (Fig. 2)

AK had a fast rate of migration on starch gels (TEC7.9). Although activity was very good and the resolution clear, it was prone to warp. Two stronger (*AK-1** and *AK-2**), and up to five slower migrating zones of activity were noted although they could not be clearly discerned in every species, and many were smeared. *AK-2** was scored routinely only in *T. gigas*. Information from several individuals of *T. derasa* and *T. maxima* was obtained but the similar migration rates of the variants and the extreme warp meant surveys for these species were abandoned. *AK-1** was scored only in the phylogeny study.

T. derasa: two alleles were observed at *AK-1** but *AK-2** was monomorphic. *T. gigas*: Two alleles were recognised at *AK-2**. *T. maxima*: at least four alleles were observed but were not scored routinely because warp prevented accurate identification of alleles. Other species: all other species were monomorphic at *AK-1**. *T. crocea* and *T. squamosa* are likely to be highly polymorphic at *AK-2** as three and two alleles were observed respectively in the phylogenetic analysis. *Hippopus* species and *T. tevoroa* were monomorphic at *AK-2**.

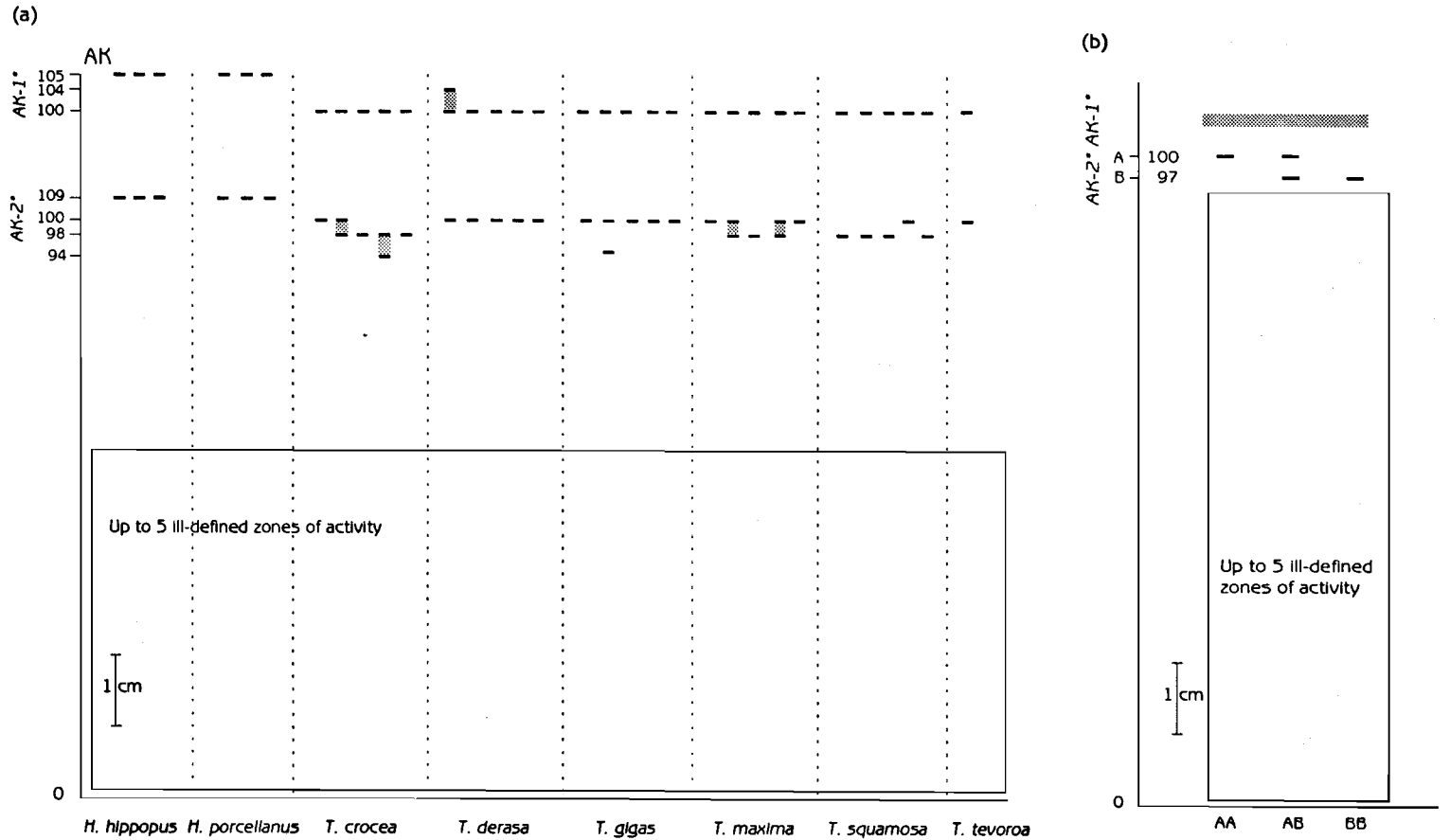


Figure 2. Enzyme patterns for AK: (a) for all species of giant clams; (b) details of variants observed for *T. gigas*.

DIA (Fig. 3)

NADH-dependent diaphorase activity was screened on TEB8.4 starch gels. Although activity, separation and resolution were all good, sample deterioration led to multiple forward breakdown products which were usually easy to separate from true variation. Heterozygotes had identical activity at both bands while breakdown bands had increasingly reduced activity with increasing migration rate. Only one zone of activity was observed.

T. derasa: Four alleles were observed. *T. gigas*: three alleles were observed including one which was very rare (one heterozygote out of about 500 samples). *T. maxima*: eight variants were observed, some of which were consistently less active than others. Other species: *T. crocea* and *T. squamosa* were polymorphic, with two and three alleles respectively. Both *Hippopus* species and *T. tevoroa* were monomorphic.

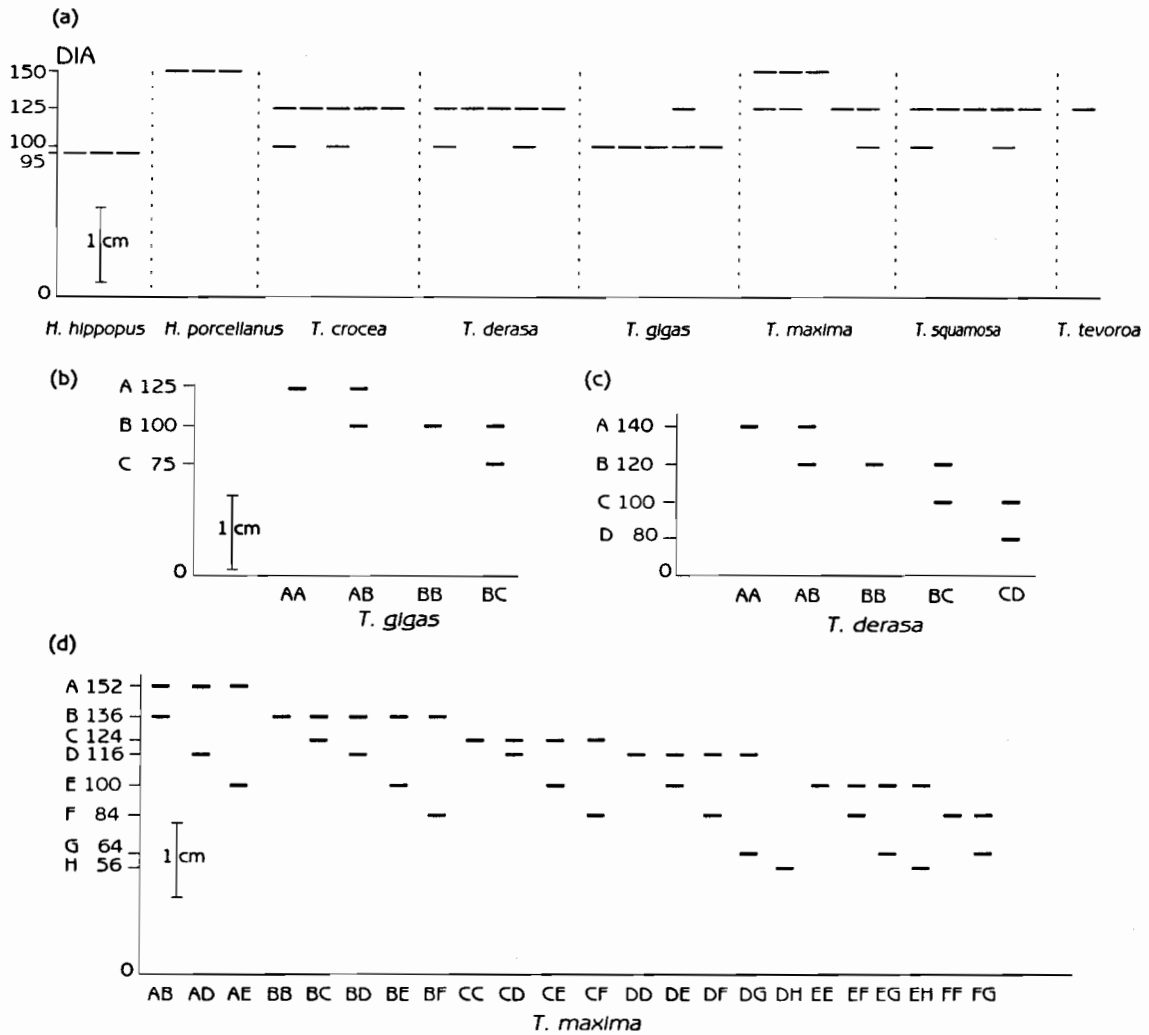


Figure 3. Enzyme patterns for DIA: (a) for all species of giant clams; (b)–(d) details of variants observed for *T. gigas*, *T. derasa* and *T. maxima*.

ENO (Fig. 4)

Enolase was screened on Cellogel using a CP6.4 buffer. Activity and resolution was good, and one zone of activity was observed for all species with heterozygotes showing a clear three-banded dimeric pattern. *ENO** was screened routinely only for *T. derasa* as the high number of variants with similar mobilities made rapid allelic identification impossible in the other species.

T. derasa: five alleles were observed and levels of variation were relatively high with four alleles being observed in most populations. *T. gigas*: this species was monomorphic. *T. maxima*: highly polymorphic with more than 10 (probably 15) alleles. Similar mobility of many alleles over a zone of 2 cm made rapid scoring impossible although multiple cross-correlation runs would allow this system to be used. Other species: all species were polymorphic with from three to seven alleles, except *H. hippopus* and *T. tevoroa*.

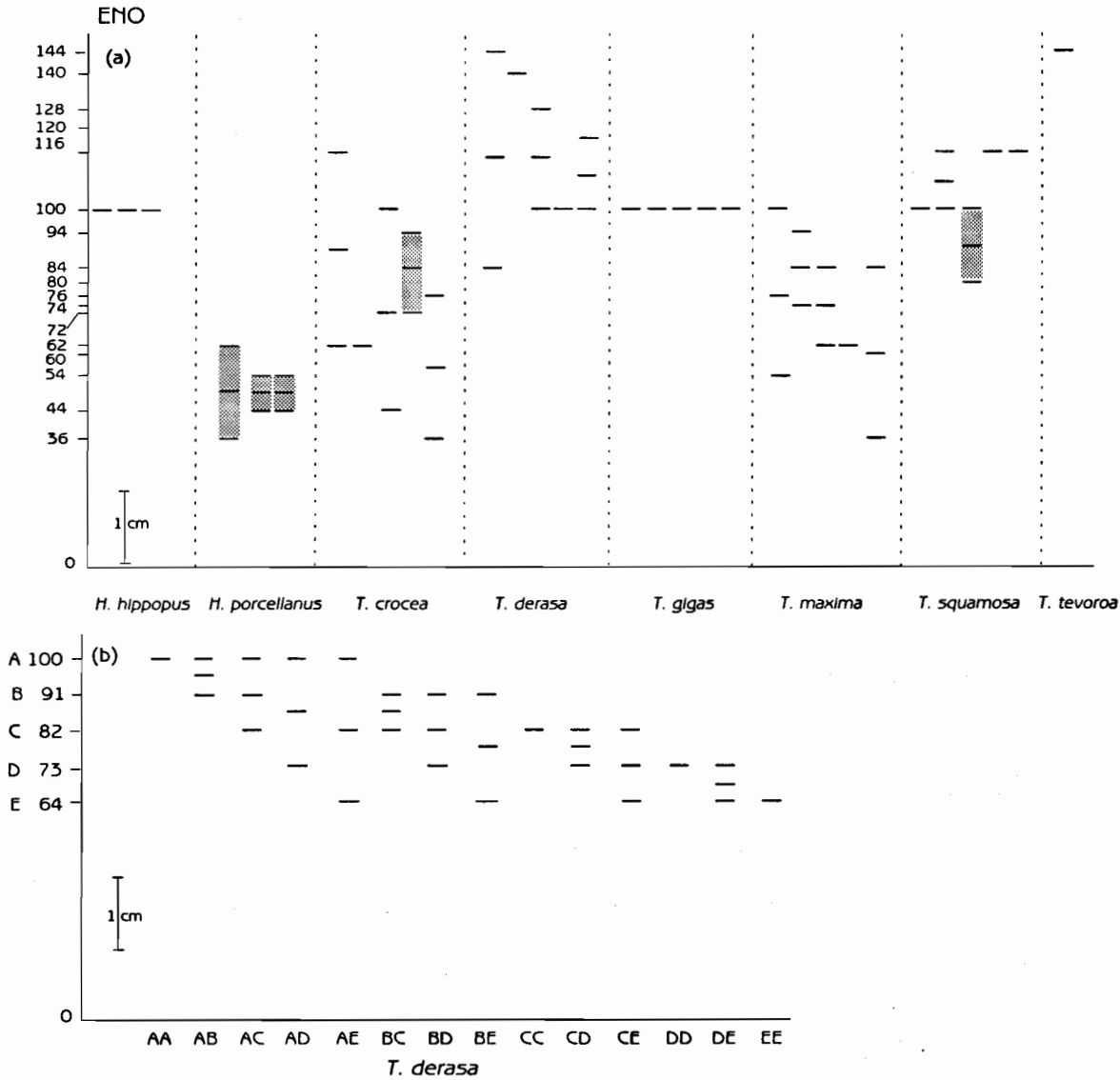


Figure 4. Enzyme patterns for ENO: (a) for all species of giant clams; (b) details of variants observed for *T. derasa*.

EST (Fig. 5)

Esterase had several zones of activity on discontinuous Poulik starch gels (gel buffer pH 8.8, electrode buffer pH 8.2). Activity was good but the gels were often very smeary, and only one zone of activity could be scored. A large number of *T. gigas* individuals from only six culture batches showed no variation, and had three faster migrating zones, and one slower migrating zone of activity in addition to the locus scored. No polymorphism was recorded for any species, but mobilities differed between taxa.

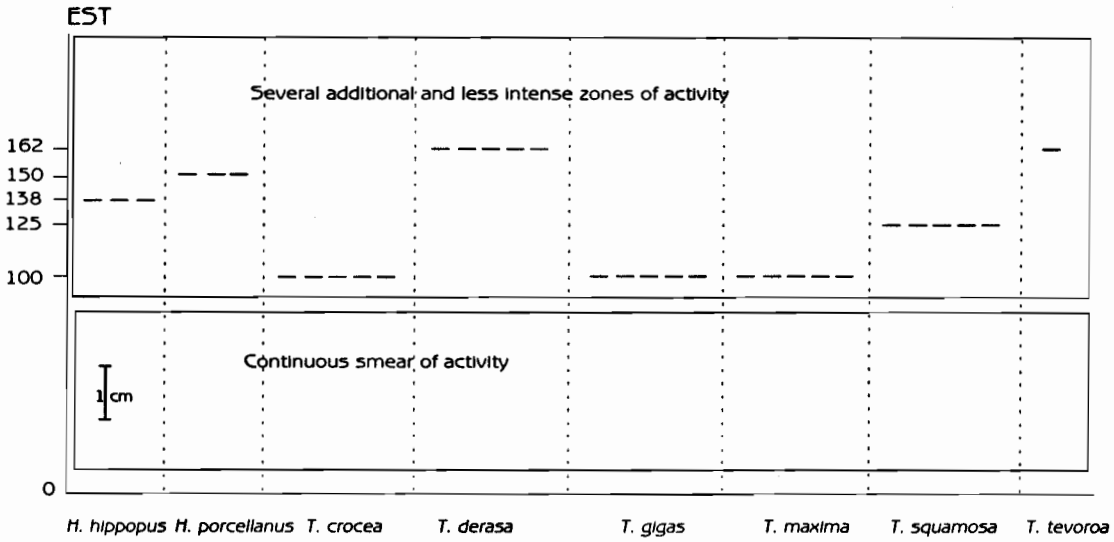


Figure 5. Enzyme patterns for EST.

GPI (Fig. 6)

A continuous buffer system of TEC7.9 on starch and TM7.8 on Cellogel each gave good resolution and activity. Similar variants were observed using both Cellogel and starch, but mobility was faster on Cellogel allowing more accurate scoring of slower alleles. Activity was so strong that Cellogel activity had to be stopped (by placing in a bath of 7% acetic acid) after about 5 minutes and starch gels needed to be scored within half an hour and often within 10–15 minutes of staining. *GPI** heterozygotes displayed the typical three-banded dimeric phenotype. Only one zone of activity was observed in each species. *T. gigas* and *T. maxima* were scored from Cellogel, given the slow migration rate of some of their alleles, while *T. derasa* was screened on starch.

T. derasa: nine variants were observed. *T. gigas*: a total of four alleles were observed. *T. maxima*: although screened for *GPI*, results were not used in analyses due to the large number of alleles (>10) found. Many were of similar mobility and the cross-correlation runs required to confirm allelic designations could not be performed in the time available. Other species: all were screened using only Cellogel and both *T. crocea* and *T. squamosa* demonstrated three variants in the small sample sizes used in the phylogeny, suggesting considerable variation at this locus. *H. porcellanus* had two variants. *H. hippopus* and *T. tevoroa* were monomorphic.

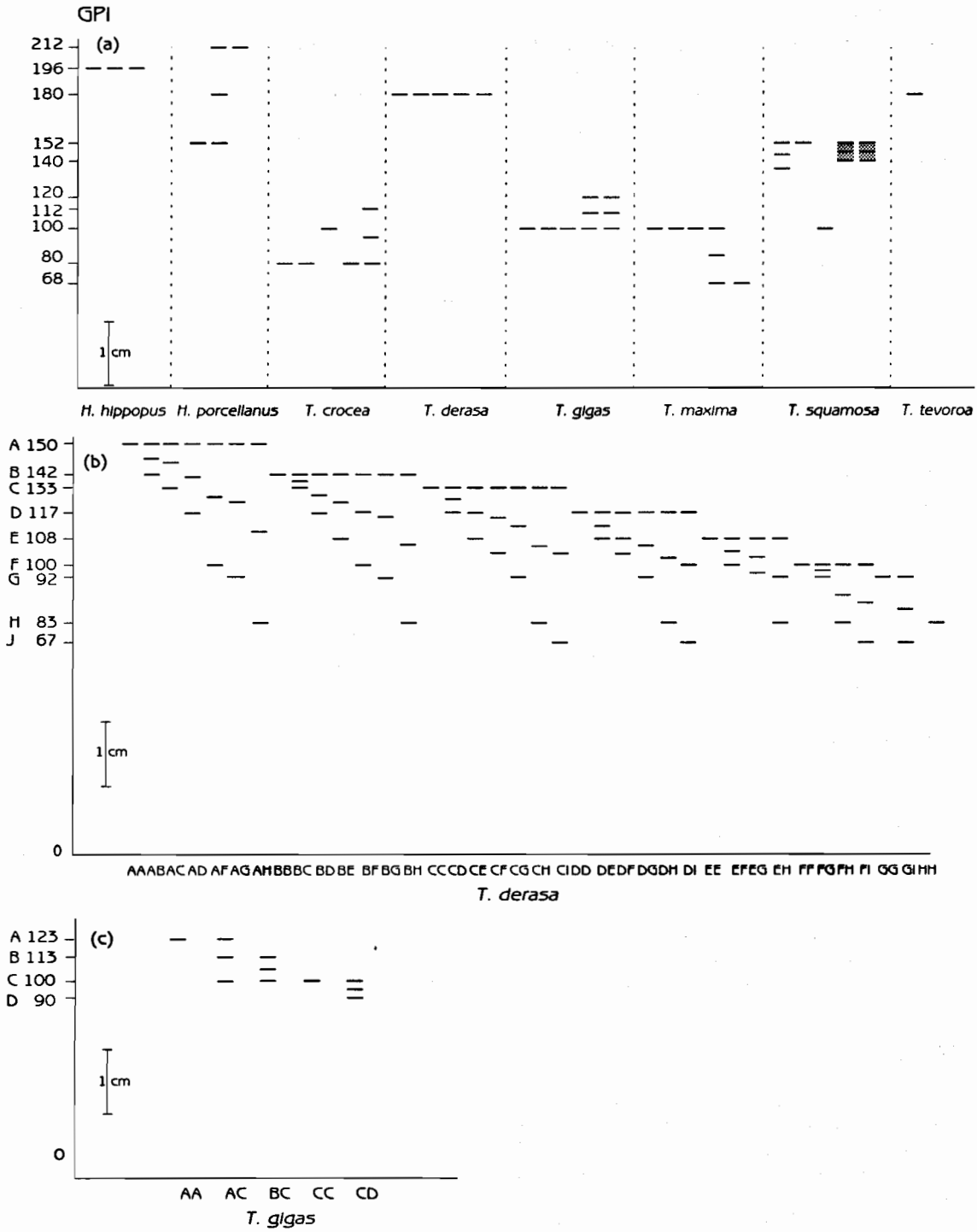


Figure 6. Enzyme patterns for GPI :(a) for all giant clam species; (b) variants observed for *T. derasa*; (c) variants observed for *T. gigas*.

GSR (Fig. 7)

Activity and resolution were good on TEC7.9 starch gels. GSR also showed another faster, monomorphic locus which was not always active and so was not scored, as well as a faster zone of non-scorable strong activity which was attributed to zooxanthellae due to the smeared appearance and pinker coloration of the bands and the absence of this zone of activity when tested using adductor muscle. The appearance of the purple clam bands was sometimes preceded by a lightening of the background blue.

T. derasa: three allelic variants were observed. *T. gigas*: monomorphic over all populations sampled. *T. maxima*: more than ten alleles were observed in total. However, it was necessary to fuse a set of four alleles migrating closely together within a 4–5 mm-wide region as it was impossible consistently to recognise closest neighbours from their heterozygotes. Similarly, in another 2–3 mm-wide zone it was clear there was more than one variant (probably 2 or 3) but these were fused as they and their heterozygotes could rarely be distinguished. These areas of fusion are illustrated in Figure 7. Other species: *T. crocea* and *T. squamosa* were both polymorphic. Neither of the *Hippopus* species or *T. tevoroa* was variable.

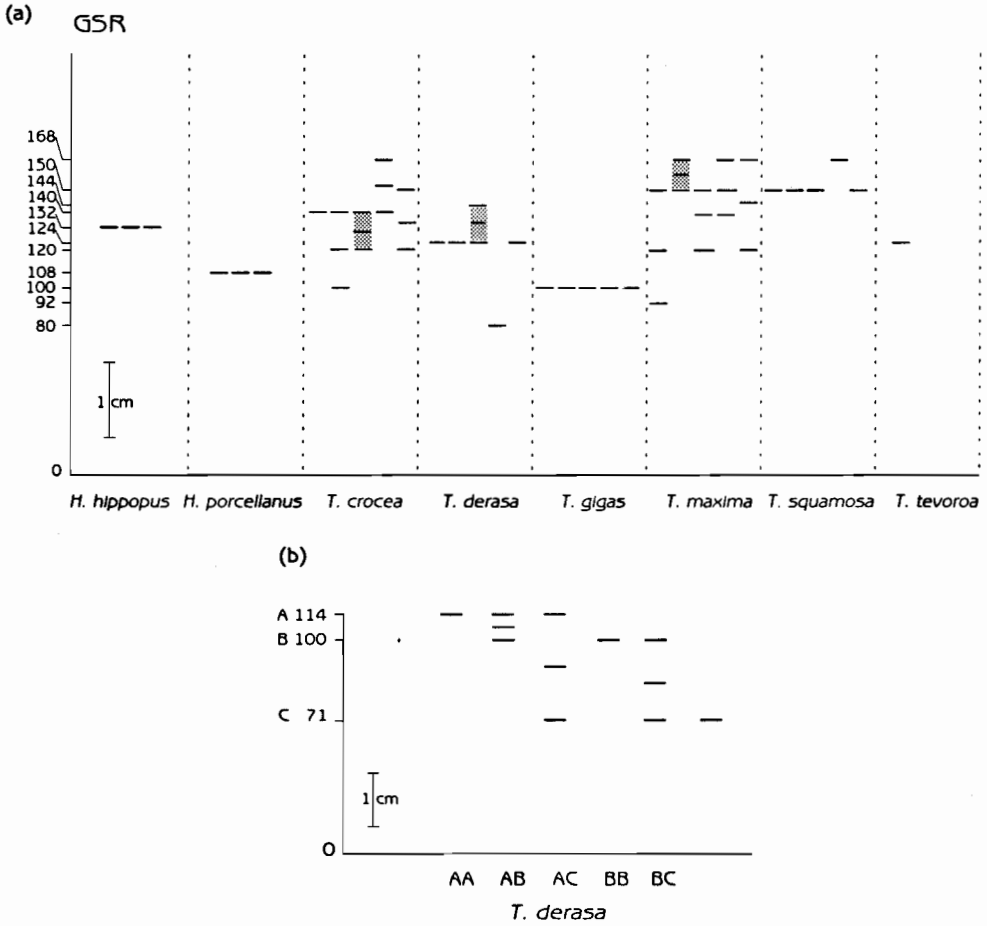
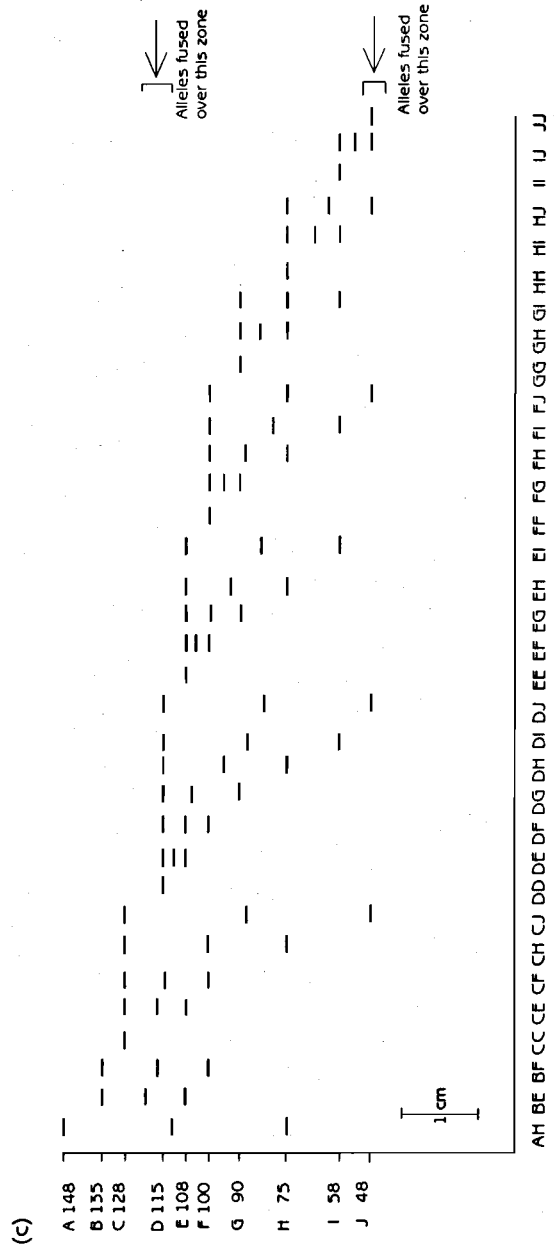


Figure 7. Enzyme patterns for GSR: (a) for all giant clam species; (b) variants observed for *T. derasa*; (c) variants observed for *T. maxima*.

Figure 7 (contd).



IDH (Fig. 8)

IDH activity was scored on TEC7.9 starch gels. Although activity was good, resolution was only average and may have masked rare variants. Two zones of activity were observed but only one, *IDH-2** was scorable. *IDH-1** was less active, and in *T. gigas* migrated to approximately 1 cm ahead of *IDH-2**. Accurate data on the position of *IDH-1** for other taxa is not available. Screened only in phylogenetic analyses and for batches of cultured juveniles of *T. gigas*, IDH was monomorphic at both loci in all species, except *T. crocea* and *T. maxima*, which had three and seven alleles respectively, indicating this locus is highly polymorphic for these two taxa.

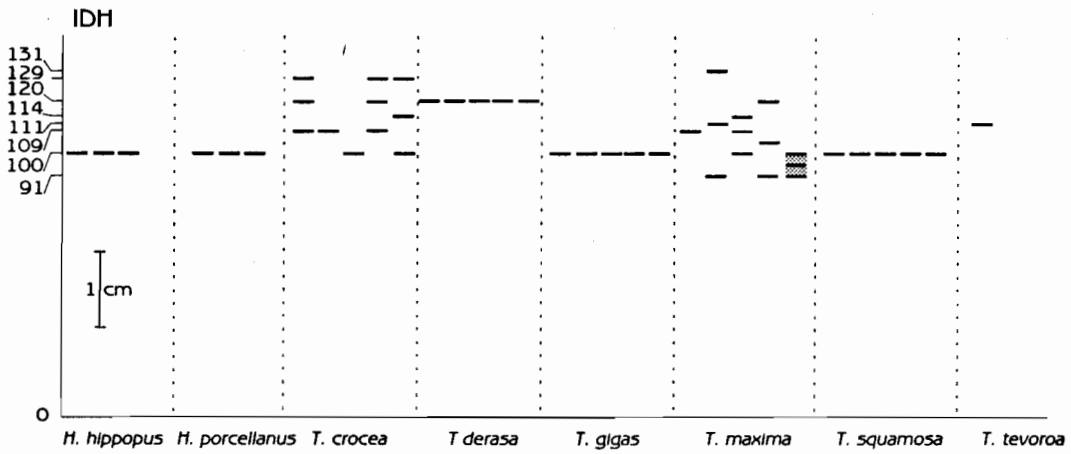
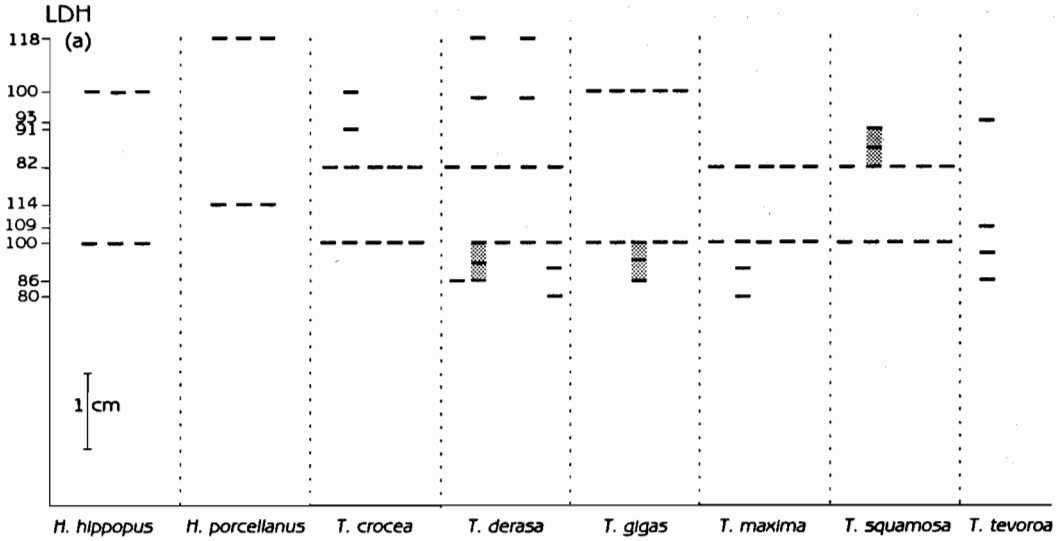


Figure 8. Enzyme patterns for IDH.

LDH (Fig. 9)

Two zones of activity were apparent on TC7.0 starch gels. The slower locus appeared to be variable but was not resolved sufficiently to score rapidly and routinely in population genetic surveys. *LDH-2** was scored in studies of juvenile family groups and in phylogenetic studies.

T. derasa: four well-separated alleles were observed at *LDH-1** and three at *LDH-2**. *T. gigas*: the first locus was almost monomorphic, with only one heterozygote for the rare allele observed in 400 samples. *LDH-2** had two alleles. *T. maxima*: ten alleles were observed at *LDH-1**, and two observed at *LDH-2**. Other species: both *T. crocea* and *T. squamosa* had two alleles at *LDH-1** and were monomorphic at *LDH-2**, whereas the *Hippopus* species were monomorphic at both loci. *T. tevoroa* was polymorphic at *LDH-2** and monomorphic at *LDH-1**.



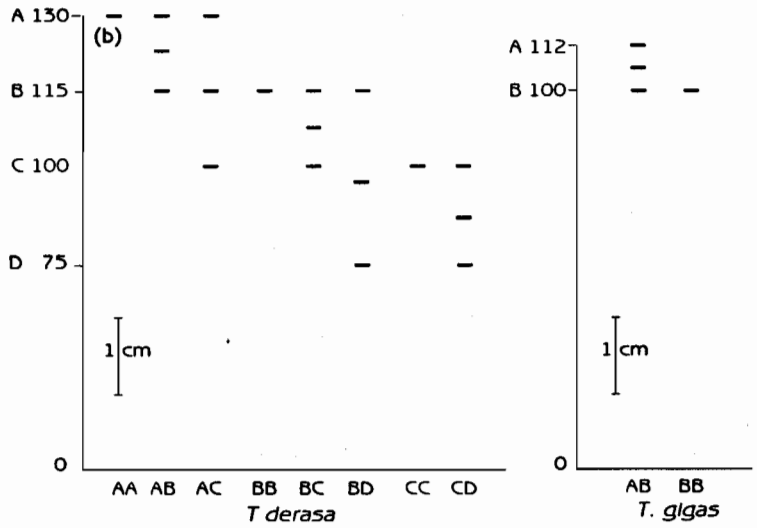
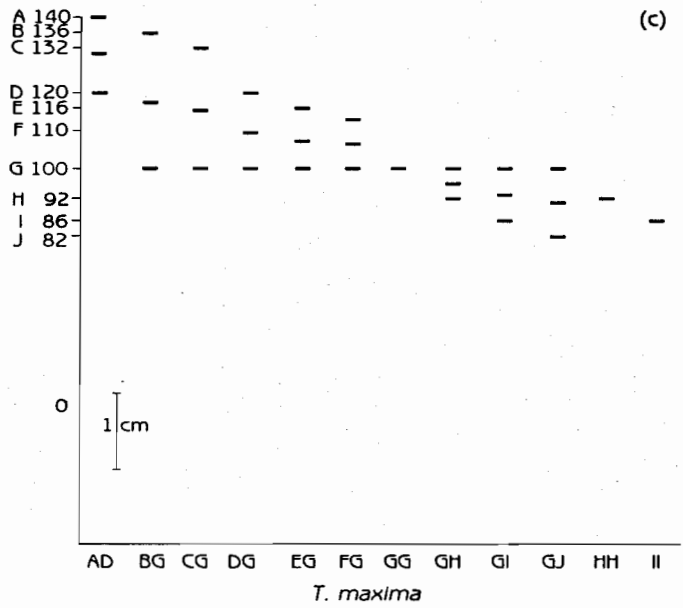


Figure 9. Enzyme patterns for LDH: (a) for all giant clam species; (b) details of variants observed for *LDH-1** for *T. derasa* and *T. gigas*; (c) details of variants observed for *LDH-1** for *T. maxima*.

Figure 9 (contd).

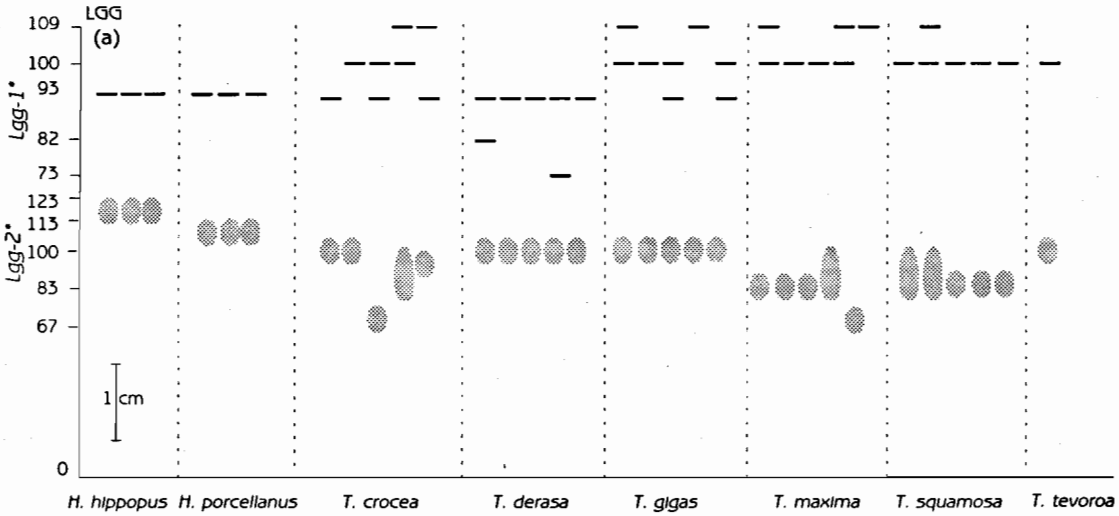


LGG (Fig. 10)

Peptidase using both Leucyl-tyrosine (LT) and Leucylglycylglycine (LGG) substrates was run on TC7.0 starch gels. One zone of activity was common to both substrates, and an additional scorable zone was present on LGG gels. Both substrates were stained to assist with scoring of the faster locus which was slightly better resolved on LT. LGG was also scored for a slower second locus which did not stain consistently, and was not resolvable, on LT. Other faster migrating zones of activity were apparent on both gels, but activity and resolution were too poor to permit scoring.

T. derasa: five alleles were observed at *LGG-1**. *T. gigas*: six alleles were observed at *LGG-1**. Two alleles were scored at *LGG-2**.

T. maxima: ten alleles in total were observed at *LGG-1**. However, it was necessary to fuse a set of three alleles migrating closely within one 2–3 mm-wide zone, and a second pair of alleles migrating in another 2 mm-wide zone, as none of the alleles within each set could be consistently distinguished from the others. The second locus was not scored routinely as it was often smeary and could not be accurately scored although at least three alleles were present. Other species: *T. crocea* and *T. squamosa* were polymorphic at both loci with three alleles each at *LGG-2** and three at *LGG-1** for *T. crocea* and two at *LGG-1** for *T. squamosa*. Both *Hippopus* species and *T. tevoroa* were monomorphic at both loci.



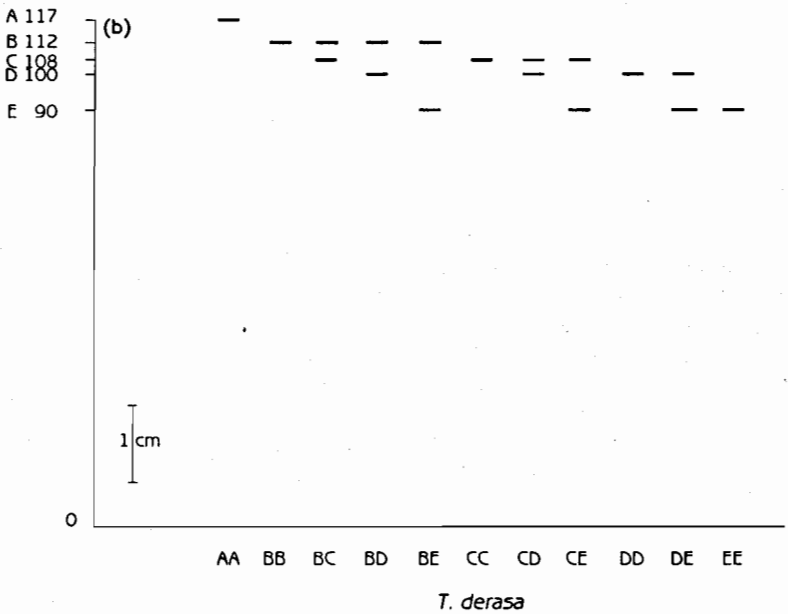


Figure 10. Enzyme patterns for LGG: (a) for all giant clam species; (b) for *LGG-1** for *T. derasa*.

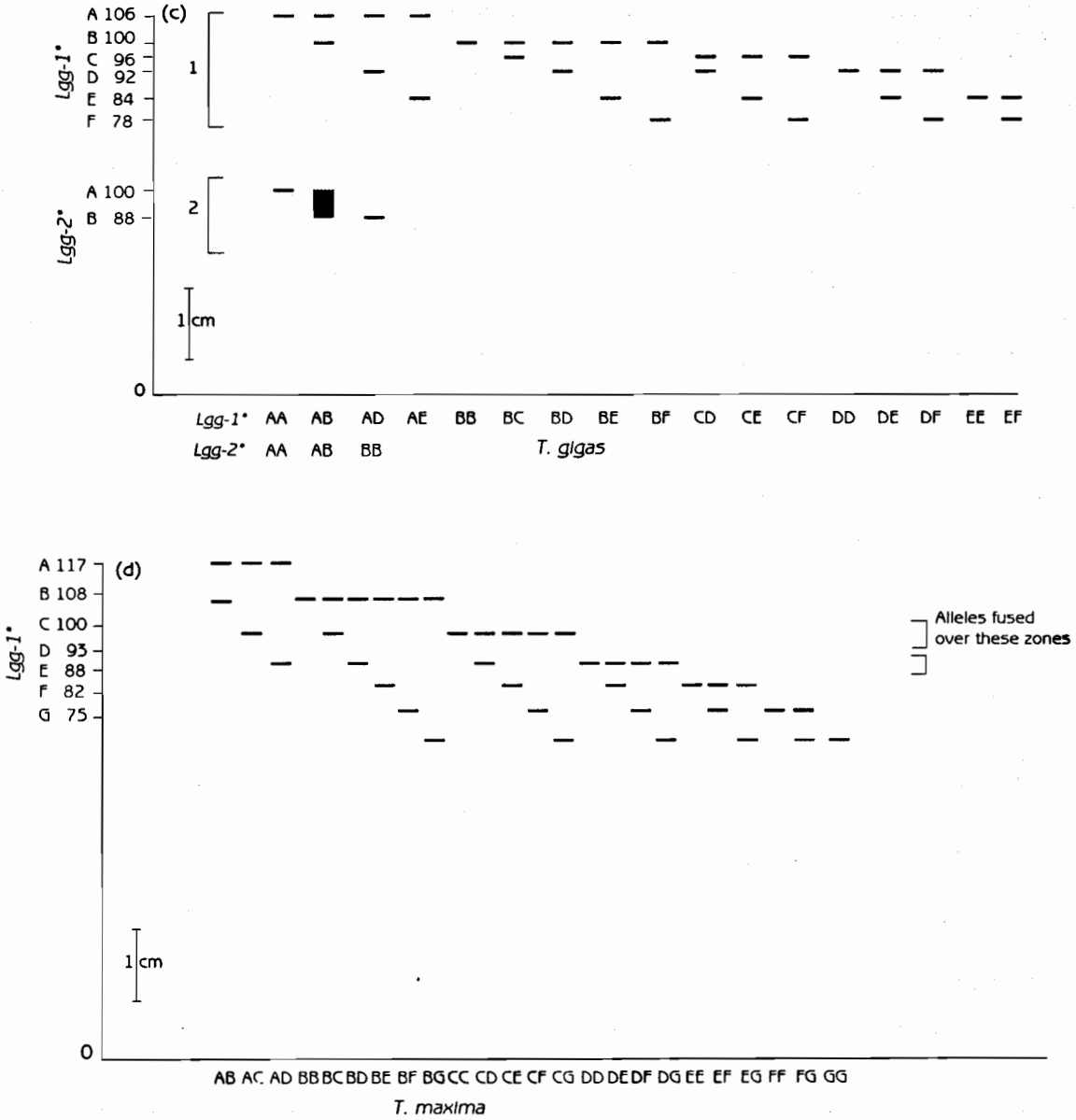


Figure 10. Enzyme patterns for LGG: (c) for *LGG-1** and *LGG-2** for *T. gigas;* (d) for *LGG-1** for *T. maxima.*

LP (Fig. 11)

Peptidase using Leucyl-proline substrate was run on starch gels with a Poulik discontinuous buffer (gel buffer pH 8.8, electrode buffer pH 8.2). LP activity was good but took some time to develop and was usually most easily scored the following morning. Several zones of activity were apparent but were often very faint or absent and only three were scored for family and phylogenetic analyses. LP was monomorphic at all three loci for all eight species, excepting two variants observed at the third locus for *T. crocea* and *T. maxima*, both of which were faint and smeary.

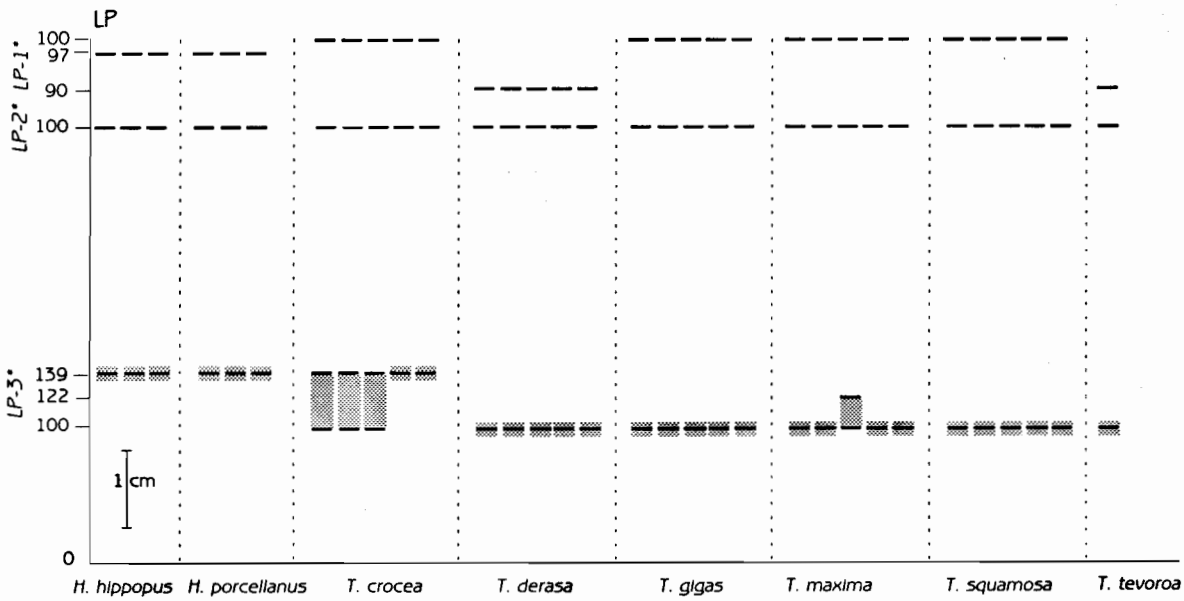


Figure 11. Enzyme patterns observed for LP.

MDH (Fig. 12)

MDH exhibited two zones of strong activity which were distinct and were attributed to two loci. Strong activity, clear resolution and good separation were obtained by staining starch gels run for about six hours with TC7.0 buffer. Forward breakdown occurred but did not affect scoring in *T. gigas*. Homozygotes showed two bands, one darker, with the breakdown product lighter. Heterozygotes stained as four-banded phenotypes with the slowest and fastest bands slightly lighter than the two intermediate ones. Although activity and separation at *MDH-1** were good for *T. maxima*, sub-banding and overlapping loci made interpretation difficult. Resolution was variable due to rocketing on some bands. Combined with multiple forward breakdown bands often overlapping other allelic products, the presence of many alleles and the occurrence of warping made fast, accurate scoring impossible.

T. derasa: both loci were polymorphic with three alleles at *MDH-1** and two at *MDH-2**. *T. gigas*: no variation was observed at *MDH-2**,

but there were three alleles for *MDH-1**. *T. maxima*: there were more than 10 alleles present at the highly polymorphic *MDH-1** but these could not be scored accurately because of breakdown and warp. *MDH-2** was polymorphic with three recognisable variants. Other species: *T. crocea* and *T. squamosa* had high levels of variation probably similar to *T. maxima* at *MDH-1**, but were monomorphic at *MDH-2**. Both *Hippopus* species and *T. tevoroa* were monomorphic for both *MDH-1** and *MDH-2**.

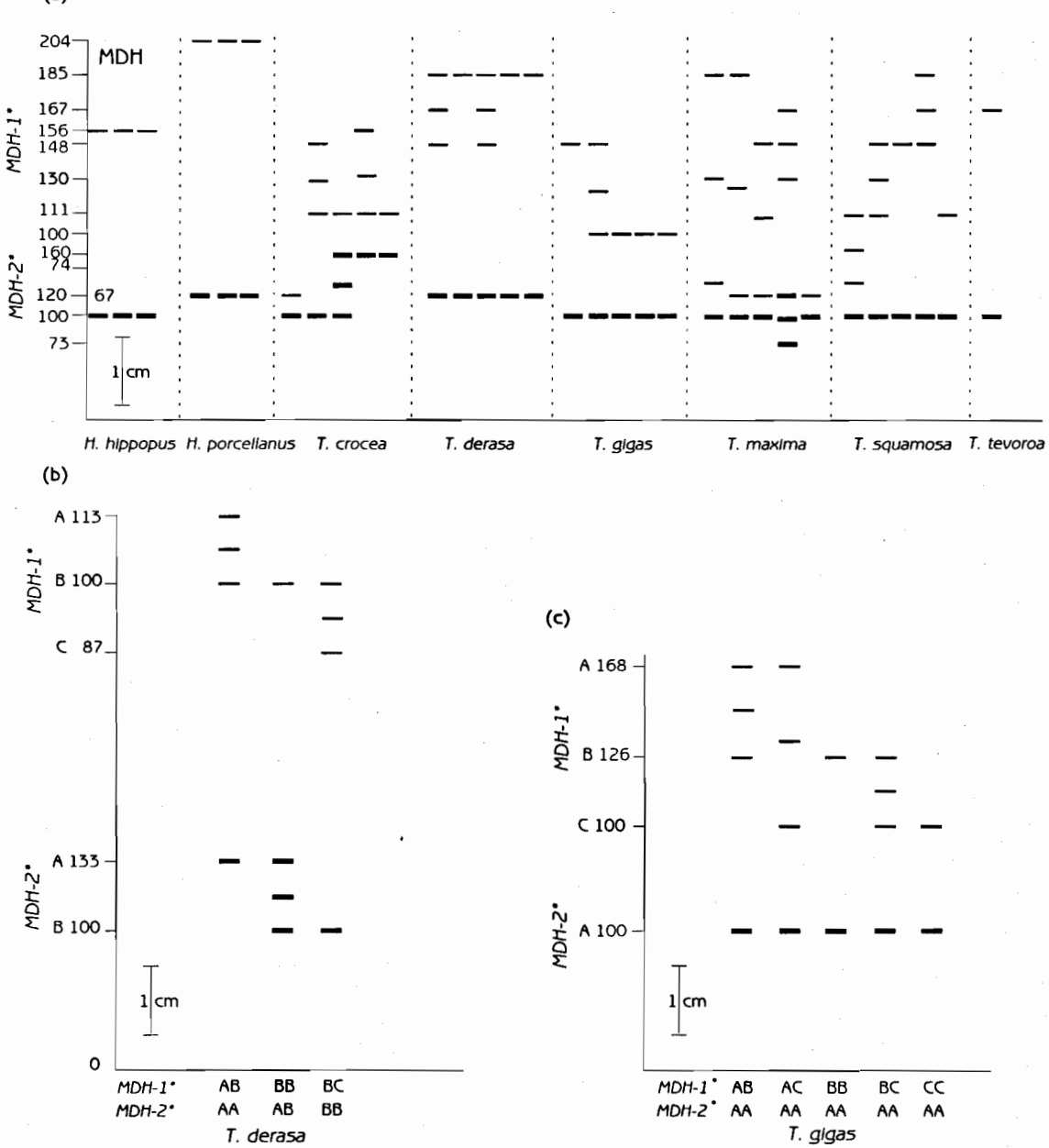
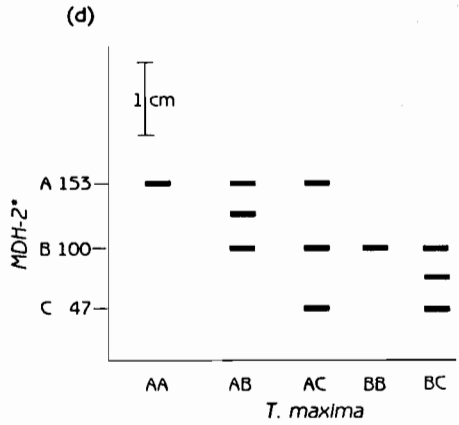


Figure 12. Enzyme patterns observed for MDH: (a) for all species of giant clams; (b) details of variants observed at *MDH-1** and *MDH-2** for *T. derasa*; (c) details of variants observed at *MDH-1** and *MDH-2** for *T. gigas*; (d) details of variants observed at *MDH-2** for *T. maxima*.

Figure 12. (contd)



ME (Fig. 13)

ME was screened on TC7.0 starch gels. Activity was strong but resolution was average, with band width smeared over 3 mm. This system was monomorphic in all 8 species, and had the same mobility in each taxon.

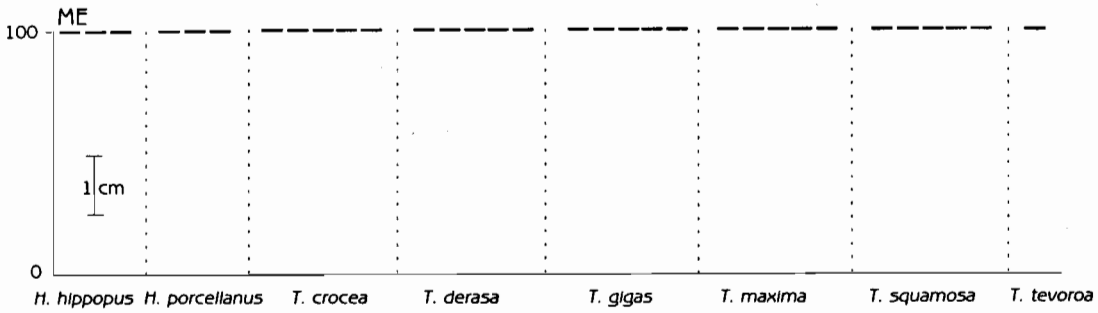


Figure 13. Enzyme patterns observed for ME. This system was monomorphic over all species.

MPI (Fig. 14)

MPI was scored from a discontinuous Poulik starch gel (gel buffer pH 8.8, electrode buffer pH 8.2). One zone of activity was observed in all species. Forward breakdown bands were present in all taxa, but these were less active than the allelic bands. Banding patterns were complex and prevented routine use of this system for population genetic surveys.

T. derasa: three alleles were observed. *T. gigas*: four alleles were observed. MPI showed well-resolved monomeric heterozygotes in the family studies. However, interpretation of some faster migrating bands was difficult, as they may have been either breakdown or a second locus. This made rapid, accurate scoring impossible. *T. maxima*: three alleles were observed in the phylogenetic studies. Other species: variation occurred in all species except *H. porcellanus* and *T. tevoroa*.

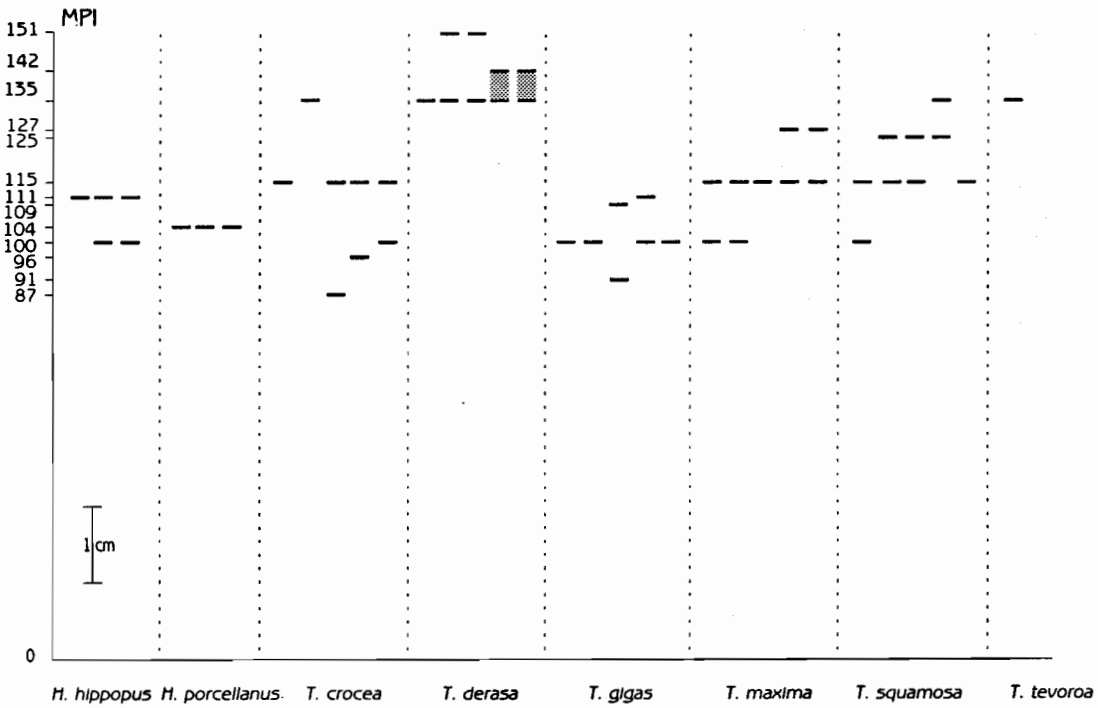


Figure 14. Enzyme patterns observed for MPI and details of variants observed among cultured batches of *T. gigas*.

NDH (Fig. 15)

NDH was screened on TEB8.4 starch gels. NDH activity was observed only in adductor muscle, and so could not be used in surveys using mantle biopsies. Two scorable zones of activity were apparent. Two variants were observed for both *T. crocea* and *T. maxima* at *NDH-1**, and for both *Hippopus* species at *NDH-2**. All other taxa were monomorphic at both loci.

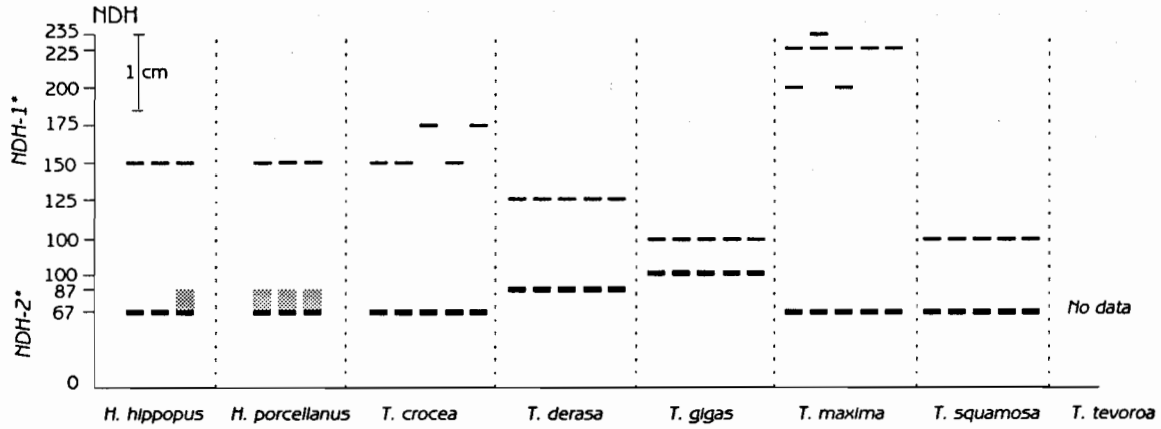


Figure 15. Enzyme patterns observed for NDH.

PGK (Fig. 16)

PGK was run on Cellogel TM7.8 buffer. Although always active, gels exhibited great variation in resolution and scorability. Only one zone of activity was observed. When resolution was good or average, scoring variants was simple. All species were monomorphic except *T. crocea* and *T. gigas* which each had two alleles.

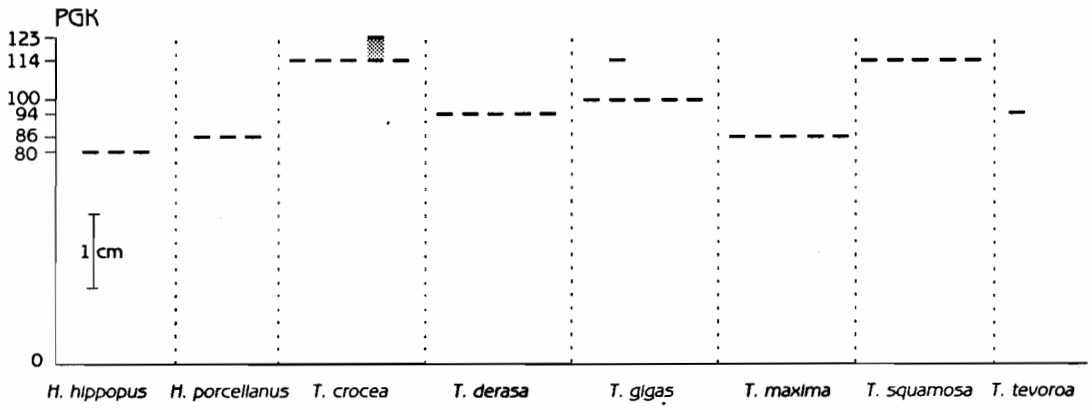


Figure 16. Enzyme patterns observed for PGK and details of variants observed among cultured batches of *T. gigas*.

PGM (Fig. 17)

Starch gels with TEB8.4 buffer provided good activity, separation and resolution for all species. This monomeric enzyme produced heterozygotes with two bands and a lighter forward breakdown product in front of each band. Two loci were observed, but low activity made routine scoring at the first locus using mantle difficult. *PGM-1** was scored using adductor muscle and mantle (using longer stain times) during the phylogeny study. Patterns of variation were the same in both muscle and mantle.

T. derasa: four variants were observed at *PGM-2**, and *PGM-1** was monomorphic. *T. gigas*: four alleles were recognised at *PGM-2**. *PGM-1** was monomorphic. *T. maxima*: eight alleles were observed at *PGM-2** and three at *PGM-1**. Other species: *T. crocea* and *T. squamosa* were highly polymorphic at *PGM-2**. Both *Hippopus* species and *T. tevoroa* were monomorphic at both loci.

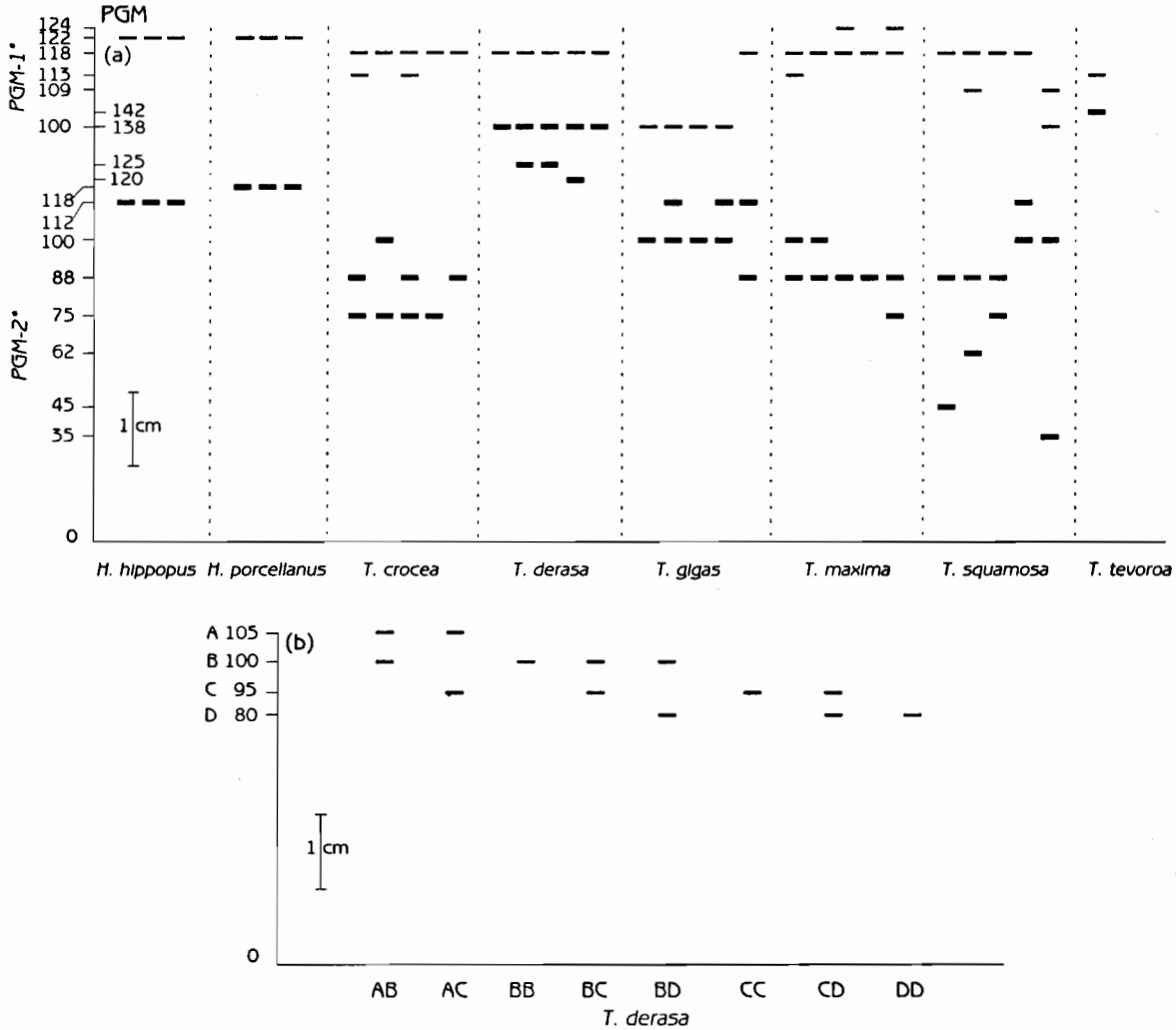
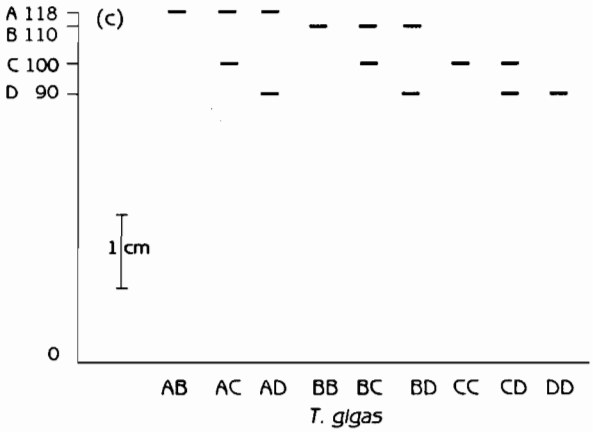
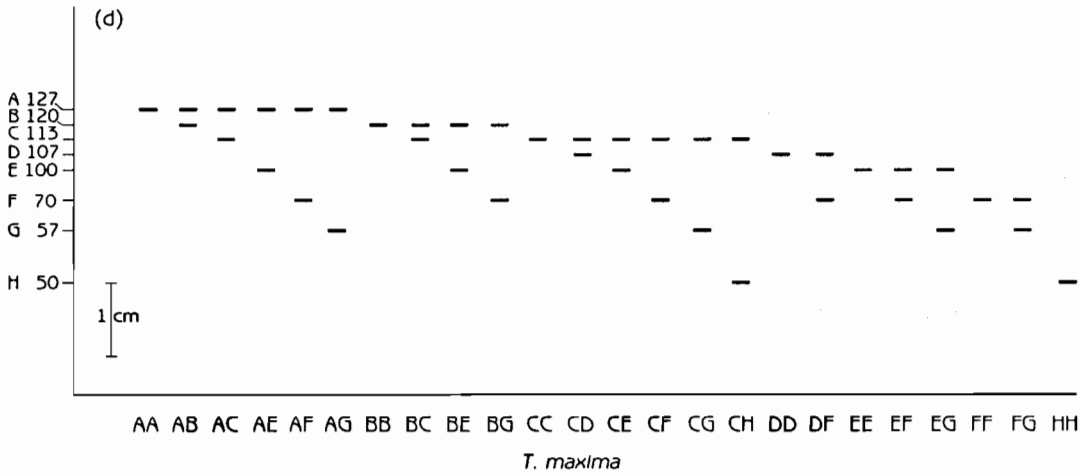


Figure 17. Enzyme patterns observed for PGM: (a) for all species of giant clams; (b) details of variants observed for *T. derasa*: (c) details of variants observed for *T. gigas*; (d) details of variants observed for *T. maxima*.

Figure 17. (contd)





SOD (Fig. 18)

SOD was active on most starch gels but was most easily scored from Poulik discontinuous starch gels (gel buffer pH 8.8, electrode buffer pH 8.2) stained for MPI. One clear zone of activity was scored, but other faint, faster, zones of activity could be observed.

The only species to demonstrate variation at this locus was *H. hippopus*, which had one two-banded heterozygote.

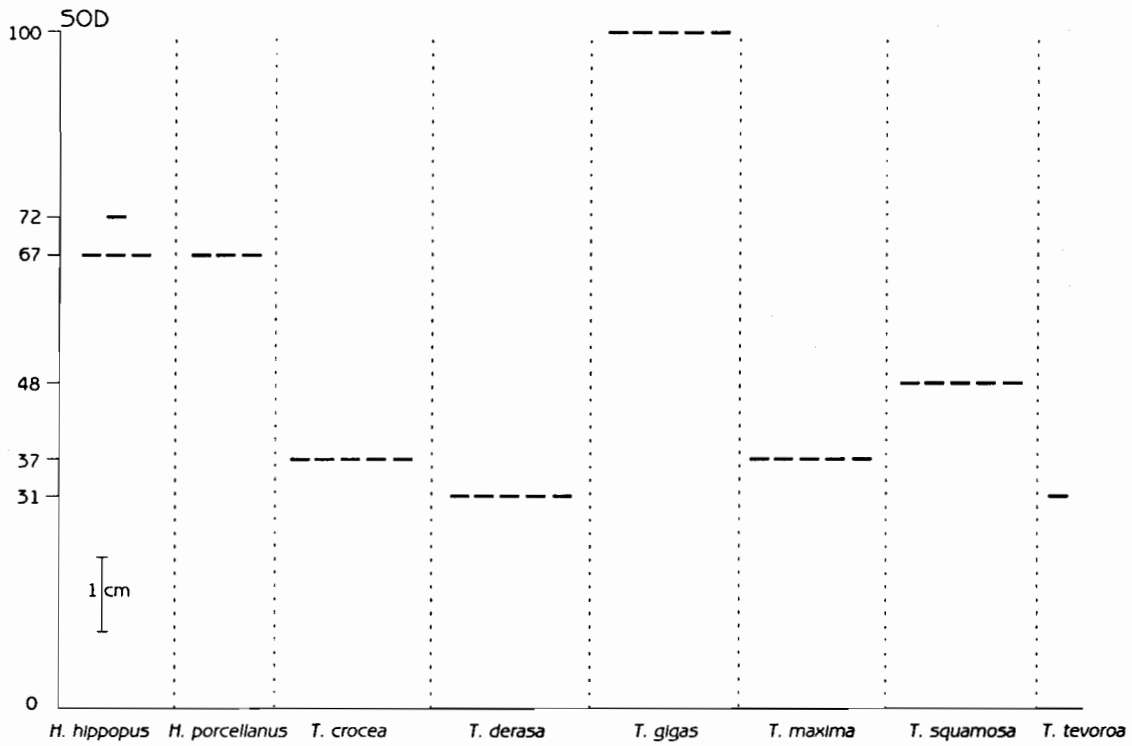


Figure 18. Enzyme patterns observed for SOD.

Discussion

Previous work on giant clams was restricted to *T. maxima* where a total of 30 loci from 14 systems was developed (Ayala et al. 1973; Campbell et al. 1975). A far greater number of loci (up to six) were scored at each of AK, EST, LAP and MDH, than were scored in the present study (Table 5). This may well have been because Ayala et al. (1973) and Campbell et al. (1975) screened a number of tissue types including kidney, stomach, adductor muscle, gill (demibranch) and mantle for many of these enzymes. Some loci may have been clearly resolved or occurred in only one of these tissue types.

Early tests of the buffers used in previous studies showed no major differences from other buffers used in the present studies. In order to achieve efficient and economical runs of large numbers of individuals, the routine screening

Table 5. Comparison of our results for *T. maxima* with results from the only previous genetic studies of giant clam by Ayala et al. (1973) and Campbell et al. (1975). The identity of the locus or loci concerned is given in parentheses after the number of loci considered in a given class (polymorphic, monomorphic, unscorable).

	No. of zones of activity		Ayala et al. 1973 Campbell et al. 1975			Present study		
	Ayala et al. 1973 Campbell et al. 1975	Present study	Polymorphic loci	Monomorphic loci	Unscorable	Polymorphic loci	Monomorphic loci	Unscorable
1. AAT	—	2	—	1(1)	1(2)			
2. AK	6	6	5(2–6)	1(1)	—	1(2)	1(1)	—
3. DIA	—	1	1(1)	—	—			
4. ENO	—	1	1(1)	—	—			
5. EST	6	6	4(2,4–6)	2(1,3)	—	1(1)	—	
6. GAPDH	1	—	1(1)	—	—			
7. GDH	2	—	1(2)	1(1)				
8. GPI	—	1	1(1)	—	—			
9. GSR	—	1	1(1)	—	—			
10. HK	4	—	1(3)	2(2,4)	1(1)			
11. IDH	1	2	1(1)	—	—	2(1,2)	—	—
12. LDH	1	2	1(1)	—	—	2(1,2)	—	—
13. LGG(=LAP)	4	2	3(1,3,4)	—	1(2)	2(1,2)	—	—
14. LP	—	3	1(3)	2(1,2)	—			
15. MDH	5	2	4(2–5)	—	1(1)	2(1,2)	—	—
16. ME	1	1	—	1(1)	—		1(1)	—
17. MPI	—	1	1(1)	—	—			
18. NDH	—	2	1(1)	1(1)	—			
19. ODH	1	—	1(1)	—	—			
20. PGK	—	1	—	1(1)	—			
21. PGM	2	2	2(1,2)	—	—	2(1,2)	—	—
22. SOD (=TO)	3	1	2(1,3)	—	1(2)	1(1)	—	—
23. TPI	2	—	1(2)	—	—			
24. General protein	3	—	2(2,3)	—	1(1)			
Total	40	40	29	4	10	19	8	1

maximised the number of enzymes screened or the minimum number of buffer types where resolution and activity were adequate. It is possible that some of the other loci screened by Ayala et al. (1973) and Campbell et al. (1975) might have been better detected on the buffer they used, although their use of multiple tissues suggests some loci were only detectable using tissues other than mantle.

Of the 30 loci observed in previous studies, 15 were monomorphic or showed very rare variants (one or two heterozygotes in 100 individuals). Many of the loci at MDH and AK had similar gene frequencies. For that reason it was not possible to use frequency data to establish which of these corresponded to the loci scored for MDH and AK in the present study. In addition, there are no detailed descriptions of the zymograms from the previous studies available to assist further interpretation.

In total, five systems were screened from mantle in previous studies of *T. maxima* (ADK (=AK), LDH, MDH, ME and TO (=SOD) (Table 5). ME was found to be monomorphic. The LDH locus screened is likely to be *LDH-1** of the present study, and *LAP-3** and *LAP-4** are probably *LGG-1** and *LGG-2** respectively. The correspondence between other loci cannot be assigned with any confidence. In the present study, 18 systems, giving a total of 27 loci, have been demonstrated to be scorable from mantle tissue (Table 5). Nineteen of these loci were polymorphic but only six were considered suitable for rapid and accurate scoring of large numbers of individuals of *T. maxima*. It is clear that other systems could be used if adequate time, tissue and resources were available for many cross-correlations where the number of individuals run was small enough to permit adequate control runs, as in the phylogenetic work reported here. The present studies therefore, have considerably extended the number of enzyme systems and loci identified as potentially useful genetic markers in giant clams.

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Appendix 1

Full names, abbreviations and EC numbers for the enzymes examined in giant clams. Enzyme nomenclature follows the International Union of Biochemistry's Nomenclature Committee (IUBNC 1984).

Abbreviation	Enzyme	E.C. Number
AAT	Aspartate aminotransferase	2.6.1.1
ACON	Aconitate hydratase (Aconitase)	4.2.1.3
ACP	Acid phosphatase	3.1.3.2
ADA	Adenosine deaminase	3.5.4.4
ADH	Alcohol dehydrogenase	1.1.1.1
AK	Adenylate kinase	2.7.4.3
ALD	Fructose-bisphosphate aldolase (Aldolase)	4.1.2.13
ALDH	Aldehyde dehydrogenase (NAD(P)+)	1.2.1.5
ALKP	Alkaline phosphatase	3.1.3.1
AMY	a-amylase	3.2.1.1
AO	Aldehyde oxidase	1.2.3.1
CAT	Catalase	1.11.1.6
CK	Creatine kinase	2.7.3.2
DAMOX	D-amino-acid oxidase	1.4.3.3
DASOX	D-aspartate oxidase	1.4.3.1
DIA	Dihydrolipoamide dehydrogenase, (Diaphorase)	1.8.1.4
ENO	Enolase	4.2.1.11
EST	Carboxylesterase (Esterase)	3.1.1.1
FBP	Fructose bisphosphatase	3.1.3.11
FUM	Fumarate hydratase	4.2.1.2
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12
GDH	Glucose dehydrogenase	1.1.1.47
GDA	Guanine deaminase	3.5.4.3
GLUDH	Glutamate dehydrogenase (NAD(P)+)	1.4.1.3
GOX	(S)-2-Hydroxy-acid oxidase (Glycolate oxidase)	1.1.3.15
aGPD	Glycerol-3-phosphate dehydrogenase (NAD+)	1.1.1.8
G6PDH	Glucose-6-phosphate dehydrogenase	1.1.1.49
GPI	Glucose-6-phosphate isomerase	5.3.1.9
GPT	Alanine aminotransferase (Glutamate pyruvate transaminase)	2.6.1.2
GSR	Glutathione reductase (NAD(P)H)	1.6.4.2
GUK	Guanylate kinase	2.7.4.8
HBDH	3-Hydroxybutyrate dehydrogenase	1.1.1.30

HK	Hexokinase	2.7.1.1
IDH	Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42
LAP	Cytosol aminopeptidase (Leucine aminopeptidase)	3.4.11.1
LDH	L-Lactate dehydrogenase	1.1.1.27
LGG	Peptidase (leucylglycylglycine substrate)	3.4.–.–
LP	Peptidase (leucyl-proline substrate)	3.4.–.–
LT	Peptidase (leucyl-tyrosine substrate)	3.4.–.–
MDH	Malate dehydrogenase	1.1.1.37
MDR	NAD(P)H dehydrogenase (quinone) (Menadione reductase)	1.6.99.2
ME	Malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$)(Malic enzyme)	1.1.1.40
MPI	Mannose-6-phosphate isomerase	5.3.1.8
MPMO	Monophenol mono-oxygenase	1.14.18.1
NDH	Nothing dehydrogenase –	
OpDH	D-Octopine dehydrogenase	1.5.1.11
ODH	Octanol dehydrogenase	1.1.1.73
PGAM	Phosphoglycerate mutase	5.4.2.1
PGDH	6-Phosphogluconate dehydrogenase	1.1.1.44
PGK	Phosphoglycerate kinase	2.7.2.3
PGM	Phosphoglucomutase	5.4.2.2
PK	Pyruvate kinase	2.7.1.40
1-PYDH	1-Pyrroline-5-carboxylate dehydrogenase	1.5.1.12
SDH	Shikimate dehydrogenase	1.1.1.25
SOD	Superoxide dismutase	1.15.1.1
STRDH	Strombine dehydrogenase	–
TPI	Triose-phosphate isomerase	5.3.1.1
XDH	Xanthine dehydrogenase	1.1.1.204
XO	Xanthine oxidase	1.1.3.22

Appendix 2

Buffers used for starch gel electrophoresis of mantle tissue biopsied from giant clams.

1. TEB 8.4: modified from Boyer et al. (1963).

Gel buffer: 48 mM Tris, 1 mM EDTA, 37 mM boric acid, pH 8.4

Electrode buffer: 150 mM Tris, 3 mM EDTA, 117 mM boric acid, pH 8.4

Stock solution

Dissolve the following in distilled water and make up to 2 litres

181.67 g Tris

12.42 g EDTA (Na_2 salt)

72.57 g boric acid

Running buffers

Gel buffer: 17.3 mL stock solution diluted to 270 mL with distilled water

Electrode buffer: 1 part stock solution + 4 parts distilled water

Running conditions: 30–35 mA/gel at 350 V for 5 hours

2. TEC 7.9: modified from recipe 2 of Soltis et al. (1983) by the addition of EDTA

Gel buffer: 8.5 mM Tris, 2 mM citric acid, 0.27 mM Na_2EDTA, pH 7.87

Electrode buffer: 135 mM Tris, 32 mM citric acid, 4 mM Na_2EDTA, pH 7.87

Stock solution

Dissolve the following in distilled water and make up to 2 litres

163.5 g Tris

67.25 g citric acidH_2O

15.2 g Na_2EDTA

Running buffers

Gel buffer: 3.5 mL stock solution diluted to 270 mL with distilled water

Electrode buffer: 1 part stock solution + 4 parts distilled water

Running conditions: 30–35 mA/gel at 200 V for 5 hours

3. TC 7.0: from Shaklee and Keenan (1986)

Gel buffer: 9.6 mM Tris, 3 mM citric acid, pH 7.0

Electrode buffer: 135 mM Tris, 43 mM citric acid, pH 7.0

Stock solution

Dissolve the following in distilled water and make up to 2 litres

163.5 g Tris

90.4 g citric acid.H_2O

Running buffer

Gel buffer: 3.9 mL stock solution diluted to 270 mL with distilled water

Electrode: 1 part stock solution + 4 parts distilled water

Running conditions: 35–40 mA/gel at 200 V for 5 hours

4. LiOH: modified from Selander et al. (1972)

Gel buffer: 46.8 mM Tris, 7.8 mM citric acid, 3.2 mM LiOH, 20.7 mM boric acid, pH 8.4

Electrode buffer: 192 mM boric acid, 30 mM LiOH, pH 8.15

Stock solution

Gel stock solution

Dissolve the following in distilled water and make up to 2 litres

109 g Tris

30.2 g citric acid.H_20

400 mL electrode stock solution

Electrode stock solution

Dissolve the following in distilled water and make up to 2 litres

12.6 g LiOH

118.9 g boric acid

Running buffer

Gel buffer: 29.2 mL gel stock solution diluted to 270 mL with distilled water

Electrode buffer: 1 part stock solution + 4 parts distilled water

Running conditions: 40–50 mA/gel at 350 V for 5 hours. Ice packs were sometimes required to cool the gel.

5. Poulik: from Selander et al. (1971)

Gel buffer: 76 mM Tris, 5 mM citric acid, pH 8.8
Electrode buffer: 300 mM boric acid, 60 mM NaOH, pH 8.2
Stock solution
Gel stock solution
Dissolve the following in distilled water and make up to 2 litres
92.1 g Tris
10.5 g citric acid.H_2O
Electrode stock solution
Dissolve the following in distilled water and make up to 2 litres
185.5 g boric acid
24.0 g NaOH
Running buffer
Gel buffer: 27 mL gel stock solution diluted to 270 mL with distilled water
Electrode buffer: 1 part stock solution + 4 parts distilled water
Running conditions: 30–35 mA/gel at 250 V for 5 hours

6. CAPM 7.0: from Clayton and Tretiak (1972)

Gel buffer: 1.25 mM citric acid, 0.5 mM N-(3-aminopropyl)-morpholine, pH 7.0
Electrode buffer: 25 mM citric acid, 9.6 mM N-(3-aminopropyl)-morpholine, pH 7.0
Stock solution
Dissolve the following in distilled water and make up to 2 litres
48 mL N-(3-aminopropyl)-morpholine
26.0 g citric acid.H_2O
Running buffers
Gel buffer: 5.4 mL stock solution diluted to 270 mL with distilled water
Electrode buffer: 1 part stock solution + 1.5 parts distilled water
Running conditions: 45 mA/gel at 220 V for 5–7 hours

7. CP 6.6: Corrected from Shaw and Prasad (1970)

Gel buffer: 6.1 mM K_2HPO_4, 1.2 mM citric acid, pH 6.6
Electrode buffer: 167 mM K_2HPO_4, 27 mM citric acid, pH 6.7
Stock solutions
Gel stock solution
Dissolve the following in distilled water and make up to 2 litres
2.12 g K_2HPO_4
0.50 g citric acid.H_2O
Electrode stock solution
Dissolve the following in distilled water and make up to 2 litres
58.2 g K_2HPO_4
11.4 g citric acid.H_2O
Running buffers
Gel buffer: use undiluted stock solution
Electrode buffer: use undiluted stock solution
Running conditions: 45 mA/gel at 200 V for 5–7 hours

8. TECB 8.75: from Shaklee and Tamaru (1981)

Gel buffer: 47 mM Tris, 1 mM EDTA, 5 mM citric acid, 8.5 mM boric acid, pH 8.75
Electrode buffer: 150 mM Tris, 3 mM EDTA, 16 mM citric acid, 27 mM boric acid, pH 8.75
Stock solution
Dissolve the following in distilled water and make up to 2 litres
218 g Tris
14.8 g Na_4EDTA
40.0 g citric acid.H_2O
20.0 g boric acid
Running buffers
Gel buffer: 14.2 mL stock solution diluted to 270 mL with distilled water
Electrode buffer: 1 part stock solution + 5 parts distilled water
Running conditions: 45 mA/gel at 200 V for 5–7 hours

9. TRIC 7.2: from Clayton and Tretiak (1972)

Gel buffer: 2 mM citric acid, Triethanolamine, pH 7.2
Electrode buffer: 40 mM citric acid, Triethanolamine, pH 7.2
Stock solution:
Dissolve the following in distilled water and make up to 2 litres
16.8 g citric acid.H_2O, add Triethanolanime to pH 7.2
Running buffers
Gel buffer: 14.2 mL stock solution diluted to 270 mL with distilled water
Electrode buffer: use undiluted stock solution
Running conditions: 45 mA/gel at 200 V for 5-7 hours

Appendix 3

Buffers used for cellulose acetate gel electrophoresis of mantle tissue biopsied from giant clams

1. CP 6.4: From Richardson et al. (1986)

Running buffer: 10 mM citrate-phosphate, pH 6.4

Stock solution
Dissolve the following in distilled water and make up to 1 litre
3.58 g $Na_2HPO_4.12H_2O$
0.53 g citric acid.H_2O
Running conditions: 200 V for 2 hours

2. Phos 7: from Richardson et al. (1986)

Running buffer: 20 mM Na phosphate, pH 7.0

Stock solution
Dissolve the following in distilled water and make up to 1 litre
4.15 g $Na_2HPO_4.12H_2O$
1.31 g $NaH_2PO_4.2H_2O$
Running conditions: 200 V for 2 hours

3. TM 7.8: from Richardson et al. (1986)

Running buffer: 50 mM Tris-maleate, pH 7.8

Stock solution
Dissolve the following in distilled water and make up to 1 litre
6.06 g Tris
2.32 g maleic acid
Running conditions: 200 V for 2 hours

Appendix 4

Table 1. Enzyme activity in four tissues of *Tridacna gigas*. Where activities differed on different buffers or substrates, the highest activity observed is recorded in this table.

	Enzyme	Tissue activity					Enzyme	Tissue activity			
		A	M	G	K			A	M	G	K
1.	AAT	++	+	++	++	31.	GSR	++	++	++	++
2.	AAT(UV)	++	++	++	++	32.	GUK	+	+	+	-
3.	ACON	++	++	+	++	33.	HBDH	–	–	–	–
4.	ACP	+	+	+	++	34.	HK	++	+	+	++
5.	ADA	–	–	+	–	35.	IDH	++	++	++	++
6.	ADH	–	–	–	+	36.	LAP	++	++	++	++
7.	AK	++	++	++	+	37.	LDH	++	++	++	++
8.	ALD	++	++	–	–	38.	LGG	++	++	++	++
9.	ALDH	–	–	–	+	39.	LP	++	++	++	++
10.	ALKP	–	+	+	–	40.	LT	++	++	++	++
11.	AMYL	–	–	–	–	41.	MDH	++	++	++	++
12.	AO	–	–	–	++	42.	MDR	++	+	+	+
13.	CAT	+	+	+	+	43.	ME	++	++	++	++
14.	CK	++	+	+	+	44.	MPI	++	++	++	++
15.	DAMOX	–	–	–	–	45.	MPMO	–	–	+	–
16.	DASOX	–	–	–	–	46.	NDH	++	–	–	–
17.	DIA	++	++	++	++	47.	ODH	++	+	+	++
18.	ENO	++	++	+	+	48.	OpDH	–	–	–	–
19.	EST	++	++	++	++	49.	PGAM	++	+	+	+
20.	FBP	+	++	+	++	50.	6PGD	++	++	++	++
21.	FUM	++	++	+	–	51.	PGK	++	+	++	+
22.	GA3PD	++	+	++	+	52.	PGM	++	++	++	++
23.	GcDH	+	–	+	–	53.	PK	++	+	+	+
24.	GDA	–	–	–	–	54.	1-PYDH	–	–	–	–
25.	GDH	+	–	–	–	55.	SDH	++	–	–	–
26.	GOX	–	–	–	–	56.	SOD	++	++	++	++
27.	αGPD	–	–	–	–	57.	STRDH	++	++	++	++
28.	G6PD	++	++	++	++	58.	TPI	+	–	–	+
29.	GPI	++	++	++	++	59.	XDH	–	–	–	–
30.	GPT	+	+	+	+	60.	XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).

Table 2. Enzyme activity in four tissues of *Tridacna derasa*. Where activities differed on different buffers or substrates, the highest activity observed is recorded in this table.

Enzyme	Tissue activity				Enzyme	Tissue activity			
	A	M	G	K		A	M	G	K
1. AAT	+	+	–	–	31. GSR	++	++	++	+
2. AAT(UV)	++	++	++	+	32. GUK	+	–	–	–
3. ACON	+	+	+	+	33. HBDH	–	–	–	–
4. ACP	–	+	–	++	34. HK	+	+	+	+
5. ADA	–	–	–	–	35. IDH	++	++	++	++
6. ADH	–	–	–	–	36. LAP	++	++	++	++
7. AK	++	++	+	+	37. LDH	++	++	++	+
8. ALD	+	–	–	–	38. LGG	++	++	++	++
9. ALDH	–	–	+	–	39. LP	++	++	++++	
10. ALKP	+	+	+	+	40. LT	++	++	++	++
11. AMYL	–	–	–	–	41. MDH	++	++	++	++
12. AO	–	–	–	–	42. MDR	+	++	–	–
13. CAT	–	++	++	++	43. ME	++	++	++	+
14. CK	+	+	+	+	44. MPI	++	+	++	++
15. DAMOX	–	–	–	–	45. MPMO	+	+	++	+
16. DASOX	–	–	–	–	46. NDH	++	–	–	–
17. DIA	++	++	++	++	47. ODH	+	+	+	+
18. ENO	++	++	++	–	48. OpDH	–	–	–	–
19. EST	+	+	+	++	49. PGAM	+	+	+	+
20. FBP	+	+	+	++	50. 6PGD	++	++	++	++
21. FUM	+	+	–	–	51. PGK	+	+	+	+
22. GA3PD	++	+	+	++	52. PGM	++	++	++	++
25. GcDH	–	–	–	+	53. PK	++	–	–	–
23. GDA	–	–	–	–	54. 1-PYDH	–	–	–	–
24. GDH	–	–	–	–	55. SDH	–	–	–	–
26. GOX	–	–	–	–	56. SOD	+	+	+	+
27. αGPD	–	–	–	–	57. STRDH	++	++	++	+
28. G6PD	+	+	++	–	58. TPI	+	+	+	+
29. GPI	++	++	++	++	59. XDH	–	–	–	–
30. GPT	–	–	–	–	60. XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).

Table 3. Enzyme activity in four tissues of *Tridacna maxima*. Where activities differed on different buffers or substrates, the highest activity observed is recorded in this table.

	Enzyme	Tissue activity					Enzyme	Tissue activity			
		A	M	G	K			A	M	G	K
1.	AAT	–	+	+	–	31.	G5R	++	++	++	+
2.	AAT(UV)	++	++	++	++	32.	GUK	+	+	+	+
3.	ACON	+	+	+	+	33.	HBDH	–	–	–	–
4.	ACP	–	–	–	+	34.	HK	+	+	+	+
5.	ADA	–	–	–	–	35.	IDH	++	++	++	++
6.	ADH	–	–	–	–	36.	LAP	++	++	++	++
7.	AK	++	++	++	+	37.	LDH	++	++	++	++
8.	ALD	+	–	–	–	38.	LGG	++	++	++	++
9.	ALDH	–	–	–	+	39.	LP	++	++	++	++
10.	ALKP	+	–	–	–	40.	LT	++	++	++	++
11.	AMYL	–	–	–	–	41.	MDH	++	++	++	++
12.	AO	–	–	–	–	42.	MDR	–	+	+	+
13.	CAT	++	++	++	++	43.	ME	++	++	++	++
14.	CK	+	+	–	–	44.	MPI	++	++	++	++
15.	DAMOX	–	–	–	–	45.	MPMO	++	+	+	+
16.	DASOX	–	–	–	–	46.	NDH	++	–	–	–
17.	DIA	++	++	++	++	47.	ODH	+	+	+	+
18.	ENO	++	++	–	–	48.	OpDH	–	–	–	–
19.	EST	+	+	+	+	49.	PGAM	+	+	+	+
20.	FBP	++	+	++	+	50.	6PGD	++	++	++	++
21.	FUM	+	++	+	+	51.	PGK	++	++	++	++
22.	GA3PD	+	+	+	+	52.	PGM	++	++	++	++
23.	GcDH	+	–	+	+	53.	PK	+	–	+	–
24.	GDA	–	–	–	–	54.	1-PYDH	–	–	–	–
25.	GDH	–	–	–	–	55.	SDH	–	–	–	–
26.	GOX	–	–	–	–	56.	SOD	+	+	+	+
27.	αGPD	–	–	–	–	57.	STRDH	++	++	++	++
28.	G6PD	+	+	++	+	58.	TPI	+	+	+	+
29.	GPI	++	++	++	++	59.	XDH	–	–	–	–
30.	GPT	–	+	–	–	60.	XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity.
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).

Table 4. Enzyme activity in four tissues of *Tridacna squamosa*. Where activities differed on different buffers or substrates, the highest activity observed is recorded in this table.

	Enzyme	Tissue activity					Enzyme	Tissue activity			
		A	M	G	K			A	M	G	K
1.	AAT	++	++	–	–	31.	GSR	++	++	++	+
2.	AAT(UV)	++	++	++	++	32.	GUK	+	–	–	–
3.	ACON	++	+	++	++	33.	HBDH	–	–	–	–
4.	ACP	–	–	–	+	34.	HK	+	+	+	+
5.	ADA	–	–	–	–	35.	IDH	++	++	++	++
6.	ADH	–	–	–	–	36.	LAP	++	++	++	++
7.	AK	++	++	+	–	37.	LDH	++	++	++	++
8.	ALD	++	+	+	+	38.	LGG	++	++	++	++
9.	ALDH	–	–	–	–	39.	LP	+	+	++	++
10.	ALKP	+	–	+	+	40.	LT	++	++	++	++
11.	AMYL	–	–	–	–	41.	MDH	++	++	++	++
12.	AO	–	–	–	–	42.	MDR	–	++	+	–
13.	CAT	–	++	++	++	43.	ME	++	++	++	++
14.	CK	+	+	–	–	44.	MPI	++	++	++	++
15.	DAMOX	–	–	–	–	45.	MPMO	–	++	++	++
16.	DASOX	–	–	–	–	46.	NDH	++	–	–	–
17.	DIA	++	++	++	++	47.	ODH	+	+	+	+
18.	ENO	++	++	++	+	48.	OpDH	–	–	+	–
19.	EST	++	+	+	++	49.	PGAM	+	+	+	+
20.	FBP	++	++	++	++	50.	6PGD	++	++	++	+
21.	FUM	+	+	+	–	51.	PGK	+	++	+	+
22.	GA3PD	++	++	++	+	52.	PGM	++	++	++	++
23.	GcDH	+	–	–	–	53.	PK	++	++	++	–
24.	GDA	–	–	–	–	54.	1-PYDH	–	–	–	–
25.	GDH	–	–	–	–	55.	SDH	–	–	–	+
26.	GOX	–	–	–	–	56.	SOD	++	++	++	++
27.	αGPD	–	–	–	–	57.	STRDH	++	++	++	++
28.	G6PD	+	++	++	+	58.	TPI	+	+	+	+
29.	GPI	++	++	++	++	59.	XDH	–	–	–	–
30.	GPT	+	+	+	+	60.	XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity.
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).

Table 5. Enzyme activity in four tissues of *Tridacna crocea*. Where activities differed on different buffers or substrates, the highest activity observed has been recorded in this table.

	Enzyme	Tissue activity					Enzyme	Tissue activity			
		A	M	G	K			A	M	G	K
1.	AAT	++	++	++	+	31.	GSR	++	++	++	+
2.	AAT(UV)	++	++	++	++	32.	GUK	+	+	+	+
3.	ACON	++	++	+	–	33.	HBDH	–	–	–	–
4.	ACP	–	–	–	++	34.	HK	++	++	++	++
5.	ADA	+	–	–	–	35.	IDH	++	++	++	++
6.	ADH	–	–	–	–	36.	LAP	++	++	++	++
7.	AK	++	++	++	+	37.	LDH	++	++	++	++
8.	ALD	++	+	+	+	38.	LGG	++	++	++	++
9.	ALDH	–	+	–	–	39.	LP	++	++	++	++
10.	ALKP	+	+	+	++	40.	LT	++	++	++	++
11.	AMYL	–	–	–	–	41.	MDH	++	++	++	++
12.	AO	–	–	–	+	42.	MDR	+	–	–	–
13.	CAT	+	+	+	+	43.	ME	++	++	++	++
14.	CK	++	+	+	+	44.	MPI	++	++	++	++
15.	DAMOX	–	–	–	–	45.	MPMO	–	–	+	–
16.	DASOX	–	–	–	–	46.	NDH	++	–	–	–
17.	DIA	++	++	++	++	47.	ODH	+	–	–	+
18.	ENO	++	++	++	++	48.	OpDH	–	–	–	–
19.	EST	++	++	++	++	49.	PGAM	++	+	++	–
20.	FBP	++	++	+	++	50.	6PGD	++	++	+	++
21.	FUM	–	–	–	–	51.	PGK	+	+	+	+
22.	GA3PD	++	+	+	+	52.	PGM	++	++	+	++
23.	GcDH	–	–	–	–	53.	PK	++	++	+	–
24.	GDA	–	–	–	–	54.	1-PYDH	–	–	–	–
25.	GDH	+	+	+	+	55.	SDH	+	–	–	–
26.	GOX	–	–	–	–	56.	SOD	++	++	++	++
27.	αGPD	–	–	–	–	57.	STRDH	++	++	++	++
28.	G6PD	++	++	++	++	58.	TPI	–	–	+	–
29.	GPI	++	++	++	++	59.	XDH	–	–	–	–
30.	GPT	+	+	+	+	60.	XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity.
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).

Table 6. Enzyme activity in four tissues of *Hippopus hippopus*. Where activities differed on different buffers or substrates the highest activity observed is recorded in this table.

	Enzyme	Tissue activity					Enzyme	Tissue activity			
		A	M	G	K			A	M	G	K
1.	AAT	++	+	++	+	31.	GSR	++	++	++	++
2.	AAT(UV)	++	++	++	+	32.	GUK	+	–	–	–
3.	ACON	++	+	–	+	33.	HBDH	–	–	–	–
4.	ACP	–	–	–	++	34.	HK	+	+	+	+
5.	ADA	+	+	–	–	35.	IDH	++	++	+	++
6.	ADH	–	–	–	–	36.	LAP	++	+	++	+
7.	AK	+	++	+	+	37.	LDH	++	++	++	++
8.	ALD	++	++	–	–	38.	LGG	++	++	++	++
9.	ALDH	–	–	–	–	39.	LP	++	++	++	++
10.	ALKP	–	–	–	–	40.	LT	++	++	++	++
11.	AMYL	–	–	–	–	41.	MDH	++	++	++	++
12.	AO	–	–	–	++	42.	MDR	+	–	+	–
13.	CAT	+	+	+	+	43.	ME	++	++	++	++
14.	CK	+	+	+	+	44.	MPI	++	++	++	++
15.	DAMOX	–	–	–	–	45.	MPMO	–	–	+	–
16.	DASOX	–	–	–	–	46.	NDH	++	–	–	–
17.	DIA	++	++	++	++	47.	ODH	–	–	–	–
18.	ENO	++	++	++	+	48.	OpDH	–	–	–	–
19.	EST	+	+	+	++	49.	PGAM	++	+	+	+
20.	FBP	++	++	++	++	50.	6PGD	++	++	–	+
21.	FUM	–	–	–	–	51.	PGK	+	++	+	+
22.	GA3PD	++	–	–	–	52.	PGM	++	++	++	++
23.	GcDH	+	+	–	–	53.	PK	++	+	+	++
24.	GDA	–	–	–	–	54.	1–PYDH	–	–	–	–
25.	GDH	–	–	–	–	55.	SDH	–	–	–	–
26.	GOX	–	–	–	–	56.	SOD	++	++	++	++
27.	αGPD	–	–	–	–	57.	STRDH	++	++	++	++
28.	G6PD	++	++	++	++	58.	TPI	–	–	+	–
29.	GPI	++	++	++	++	59.	XDH	–	–	–	–
30.	GPT	+	+	+	+	60.	XO	–	–	–	–

Activity is scored as ++ good/reasonable, + poor, and – for no activity.
Tissue types—adductor muscle (A), mantle (M), gill (G) and kidney (K).

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