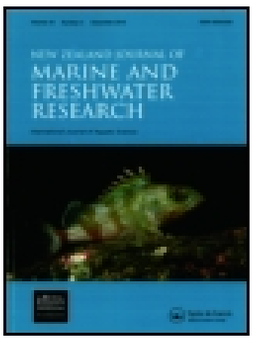

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Electrophoretic identification of larval and O-group flounders (*Rhombosolea* spp.) from Wellington Harbour

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Four enzymes and one protein were tested in starch and cellulose acetate gels as biochemical tags for the separation of flounder (*Rhombosolea* spp.) larvae and O-groups. Lactate dehydrogenase phenotypes separated O-groups and larvae (longer than about 5 mm) of *R. plebeia* from *R. leporina* and *R. retiaria*. General protein phenotypes separated similar specimens of *R. leporina* from *R. plebeia* and *R. retiaria*. Most *Rhombosolea* larvae and O-groups caught in Wellington Harbour were *R. plebeia*.

Keywords: *Rhombosolea*; electrophoresis; larvae; O-group; Wellington Harbour.

INTRODUCTION

During plankton surveys in Wellington Harbour it has not been possible to identify larvae of the genus *Rhombosolea* by the traditional method of fin ray counts (Wear 1965, Wilkinson 1977, Frentzos unpubl. results). In addition, small specimens of fully metamorphosed O-group (juveniles less than 1 year old) *Rhombosolea* cannot all be distinguished by fin ray counts as there is some overlap in these characters (Wilkinson 1977, Smith unpubl. results).

Adults of three *Rhombosolea* species are found in Wellington Harbour. The sand flounder (*R. plebeia*) and the yellow belly flounder (*R. leporina*) spawn in the harbour, and a third species, the black or river flounder (*R. retiaria*), occurs in the Hutt River and is occasionally caught in the harbour. Little is known of its biology, but it is assumed to spawn in salt water (McDowall 1978).

The techniques of gel-electrophoresis coupled with specific histochemical staining have been used to reveal proteins that identify and distinguish between closely related larval and juvenile teleosts (Herzberg & Pasteur 1975, Morgan 1975, Brassington & Ferguson 1976, Smith & Crossland 1977, Sidell *et al.* 1978). For the positive separation of two or more species, it is necessary to find a biochemical marker at which the species are not sharing alleles.

We have studied a number of proteins in the three *Rhombosolea* species which occur in Wellington Harbour in an attempt to find suitable biochemical markers for the identification of *Rhombosolea* larvae and O-groups.

MATERIALS AND METHODS

LARVAE. Larvae were caught in 0.3 and 0.5 mm monofilament nylon mesh plankton nets from r.v. *Tirohia*. Plankton samples were collected in Wellington Harbour on 16 October 1978, 1 November 1978, 10 May 1979, 18 June 1979, and 29 August 1979. Six locations were initially sampled for flounder larvae; Evans Bay, Cow Bay, Lambton Harbour, off Petone Beach, off the Hutt River mouth, and between Ward and Somes Islands. From October 1978 to May 1979 significant numbers of *Rhombosolea* larvae were caught only off the Hutt River mouth and between Ward and Somes Islands, so the June 1979 and August 1979 samples were collected from these two stations.

Plankton tows were limited to about 10 min to reduce physical damage to the samples. Samples were sorted on board the vessel, and the majority of the *Rhombosolea* larvae were placed in individual tubes and frozen in liquid nitrogen. A few larvae were taken back to the laboratory in sea water and measured under a binocular microscope before freezing in liquid nitrogen.

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O-GROUPS. O-groups were sampled by Riley push net in the intertidal pools and intermediate sublittoral zone at low water. The dates of the collections are given in Table 1. Each specimen was measured to the nearest millimetre, sealed in a polythene bag, and frozen in dry ice. Some initial sublittoral sampling was carried out by beam trawl from r.v. *Rukawai* between 1 m and 5 m, but only large fish (□00 mm) were caught and in low numbers. Several beaches were also surveyed at the beginning of the project to locate the O-group flounders, but later sampling was restricted to the intertidal mudflats and immediate sublittoral zone on the western side of the Hutt River mouth.

ADULTS. Liver and white skeletal muscle tissue samples from adult flounders were used as controls. *R. plebeia* and *R. leporina* were collected from Wellington Harbour in November 1977 and November 1978. *R. retiaria* were collected from Lake Wairarapa in February 1978 as none were caught in the harbour. A fourth *Rhombosolea* species, the greenback flounder (*R. tapirina*), occurs only around the southern part of the South Island and was not sampled for this analysis.

SAMPLE PREPARATION AND ELECTROPHORESIS. Samples were stored at −70 °C in the laboratory. All electrophoretic procedures were caried out at 4 °C. Larvae were individually homogenised in a drop of 0.1 M tris HCl buffer solution (pH 7.1) containing 1% 2-mercaptoethanol. Each homogenate was divided into two, one half for cellulose acetate and the other for starch gel electrophoresis. Juveniles were homogenised in an equal volume of the same buffer, and adult control samples of white skeletal muscle and liver were homogenised in two volumes of buffer and centrifuged at 30 000 *g* for 10 min before electrophoresis. When no protein activity was detected in larvae, pooled homogenates of up to 10 larvae were used for electrophoresis.

Starch gels (Electrostarch lot no. 307) were made up at a concentration of 12% in a tris-citric acid, lithium hydroxide-boric acid buffer (pH 8.2) (Ridgway *et al.* 1970) and run at 240 V for 3 h. Gels were sliced into two and stained for esterase and lactate dehydrogenase. Two cellulose acetate buffers were used; Helena HR tris barbital-sodium barbital (pH 9.2) at 180 V for 50 min and bartitone acetate (pH 8.6) (Sargent & George 1975) at 130 V for 40 min. Gel plates in the first buffer were stained for glucosephosphate isomerase and phosphoglucomutase and in the second buffer system for general protein. Staining recipes followed those described by Shaw & Prasad (1970).

Table 1 Numbers of *Rhombosolea* larvae and O-groups caught and identified from Wellington Harbour.

Date caught	No. caught	No. identified *R. plebeia*	No. identified *R. leporina*
Rhombosolea larvae			
16 Oct 1978	25	23	2
1 Nov	8	8	0
10 May 1979	132	22	2
18 Jun	66	34	0
29 Aug	172	170	1
Rhombosolea O-group			
10 Nov 1978	283	278	5
4 Dec	229	228	1
18 Dec	62	61	1
10 Jan 1979	272	267	5
15 Feb	191	188	3
2 Apr	199	197	2
24 & 28 May	155	154	1
11 & 12 Jul	211	210	1
6 Sep	26	26	0
19 Nov	230	230	0
19 Feb 1980	105	105	0

RESULTS AND DISCUSSION

Table 1 shows the numbers of larvae and O-groups analysed and identified. Larvae varied in size from 2 to 10 mm total length and the metamorphosed O-groups from 9 mm to about 50 mm. A few larger specimens (up to 90 mm) were caught in the sublittoral area. Many small larvae of about 2–5 mm showed little or no protein activity on the stained gel surface and so could not be biochemically identified. In May 1979 over 100 *Rhombosolea* larvae were caught in the plankton samples (Table 1). Most of these larvae were less than 5 mm long and only 24 could be identified. Similarly in June 1979 about half of the larvae caught could not be identified. By August 1979 all the larvae sampled were large and close to metamorphosis and hence all but one were identified. The protein phenotypes of the larvae, O-groups, and adults are described below.

ESTERASE (*Est*)

Three esterase loci were detected in adult *Rhombosolea* liver and muscle tissues. *Est-1* stains irregularly, but appears to be monomorphic. *Est-2,* dominant in liver tissue, is monomorphic for the same allelle in three *Rhombosolea* species. *Est-3,* dominant in muscle tissue, clearly distinguishes *R. plebeia* from *R. leporina* and *R. retiaria,* but does not separate the latter two species (Fig. 1). This locus is polymorphic for three alleles in *R. plebeia* and polymorphic for a further two alleles with different electrophoretic mobility in *R. leporina* and *R. retiaria. Est-3* showed strong activity in O-groups. In larvae, *Est-3* stained weakly and many specimens showed no esterase activity. It is possible that *Est-3* is not active in small larvae, as pooling up to 10 small larvae failed to produce evidence of activity.

Published data on other species support this view. In pooled larval homogenates of laboratory-reared lake chubsucker, *Erimyzon sucetta,* only four out of nine esterase loci were detected during the early development stages up to 3 weeks after hatching (Champion *et al.* 1974). In the green sunfish, *Lepomis cyanellus,* tissue specific loci, including esterases, appeared later in embryo development than the non-specific enzyme loci (Champion & Whitt 1976), and in *Fundulus heteroclitus* the number of esterase loci increases as larvae develop (Holmes & Whitt 1970).

Esterase markers have been used successfully to separate striped bass and white perch larvae longer than 7 mm (Sidell *et al.* 1978) and to identify snapper, *Chrysophrys auratus,* larvae (Smith & Crossland 1977). In *Rhombosolea, Est-3* clearly distinguishes O-groups of *R. plebeia* from those of *R. leporina* and *R. retiaria,* but does not separate the latter two species. In larvae, staining activity is either too weak or non-existent for *Est-3* to be practical species marker.

Glucosephosphate Isomerase (*Gpi*)

Two glucosephosphate loci were detected in adult *Rhombosolea* liver and muscle tissues. Both loci are polymorphic for shared alleles in the three *Rhombosolea* species in question and are, therefore, not useful as species markers. *Gpi-1* showed strong activity in all larvae and O-groups and could be routinely scored. *Gpi-2* was not detected in small larvae but showed strong activity in large larvae (5–10 mm long) and all O-groups. Pooled larval homogenates also failed to show *Gpi-2* activity in small larvae less than about 5 mm long. Such findings are similar to those of *Gpi* in larvae of Atlantic mackerel, *Scomber scombrus* (Smith 1976), flounder, *Platichthys flesus* (Dando 1974), hybrid trout (Engel *et al.* 1977), green sunfish, *Lepomis cyanellus* (Champion & Whitt 1976), and lake chubsucker, *Erimyzon sucetta* (Shaklee *et al.* 1974).

Lactate Dehydrogenase (*Ldh*)

One lactate dehydrogenase locus is present in adult *Rhombosolea* muscle tissue. This monomorphic locus, *Ldh-2,* separates *R. plebeia* by its faster migrating band from *R. leporina* and *R. retiaria,* but does not separate the latter two species (Fig. 1). A more anodal migrating locus, *Ldh-1,* has been found in vitreous fluid of adult *R. plebeia,* but has not been analysed in adult specimens of the other two species. *Ldh-1* stained strongly in O-groups but was detected only in the largest larvae (about 10 mm long). *Ldh-2* stained clearly in O-groups and large larvae, but was weak or absent in small larvae less than 5 mm long. Gel phenotypes of *Ldh-2* distinguished *R. plebeia* O-groups and large larvae from those of the other two *Rhombosolea* species. Many of the small larvae (< 5 mm long) caught in May 1979 showed little or no *Ldh* activity and so could not be biochemically identified.

Phosphoglucomutase (*Pgm*)

Two phosphoglucomutase loci were found in adult *Rhombosolea* liver and muscle tissue. *Pgm-1* stains weakly and cannot be routinely scored. *Pgm-2* shows strong activity and is polymorphic in the three *Rhombosolea* species; it distinguishes *R. plebeia* from *R. leporina* and *R. retiaria,* but does not distinguish the latter two species. *Pgm-2* stained strongly in large O-groups but weakly in small O-groups under about 20 mm and in large larvae. No activity was detected in small larvae. Thus *Pgm* is not a useful marker for separating larvae and O-groups of *Rhombosolea.* Similar individual preparations of small larvae of Atlantic mackerel, *Scomber scombrus* (Smith 1976), white perch, *Morone americana,* and striped bass, *M. saxatilis* (Sidell *et al.* 1978), have shown no *Pgm* activity on stained gels. Pooled larval homogenates of *Erimyzon sucetta* have shown a gradual increase in *Pgm* activity during larval development (Shaklee *et al.* 1974).

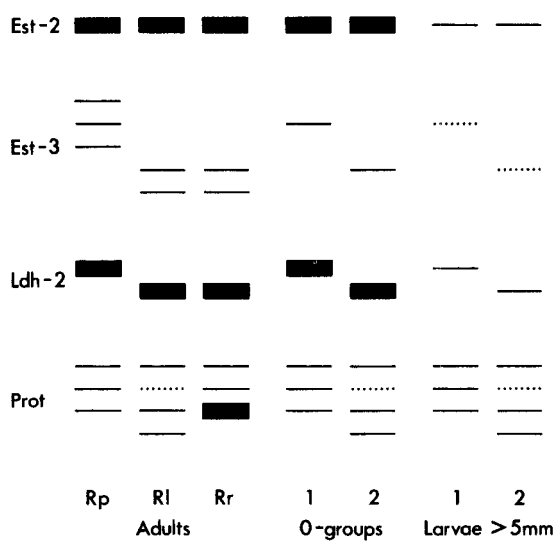

Fig. 1 Diagrammatic electropherogram of esterase (*Est-2* and *Est-3*), lactate dehydrogenase (*Ldh-2*), and general protein (*Prot*) phenotypes in *Rhombosolea* spp. Relative mobility of all observed *Est-3* bands is shown; individual specimens revealed 1 or 2 band phenotypes. Strong staining bands are shown by a heavy line, clear single bands by a thin line, and bands which were weak, or absent in some specimens, by a dashed line. Rp, *Rhombosolea plebeia;* Rl, *R. leporina;* Rr, *R. retiaria.*

General Protein (*Prot*)

General protein profiles of muscle tissue separate *R. leporina* from *R. plebeia* and *R. retiaria* and, in the adult at least, specimens of the last two species can be distinguished by the relative staining intensities of the protein bands. O-groups show strong activity for similar protein bands whereas the larvae show weak activity; hence large numbers of the small larvae caught in May 1979 could not be identified (Table 1). For the larger larvae caught in August 1979, specimens of *R. leporina* could be distinguished from the other two species (Fig. 1).

General Discussion

The use of general protein and lactate dehydrogenase markers allows the biochemical separation of the three adult *Rhombosolea* species. Gel phenotypes of *Prot* separate specimens of *R. leporina* from the other two species, and those of *Ldh* distinguish *R. plebeia* from *R. leporina* and *R. retiaria*. These two markers successfully separated O-groups and large larvae of *R. plebeia* and *R. leporina* (Fig. 1). No O-groups of *R. retiaria* were found, but spawning adults may not occur in Wellington Harbour. The disadvantage of these two markers is that they are weakly active in small larvae and cannot be used to identify specimens less than about 5 mm long. However, this is a problem common to most proteins studied in single larval homogenates (Smith 1976, Sidell *et al.* 1978).

One surprising result from this study was the very high proportion of *R. plebeia* larvae and O-groups and the low proportion of *R. leporina* in the samples (Table 1). *R. leporina* form between zero and 8% of the larval samples and zero to 2% of the O-group samples, whereas adults make up about 10% of the trawl-caught *Rhombosolea* in the harbour (Mines 1975, Smith unpubl. results). It is unlikely that *R. leporina* spawn in another season as a study of gonads showed them to be winter spawners in Wellington Harbour, overlapping with *R. plebeia* (Mines 1975). In the Hauraki Gulf the spawning times of *R. plebeia* and *R. leporina* overlap, but the two species spawn in slightly different areas; *R. leporina* prefers shallow water (Colman 1973). Thus it is possible that we have failed to locate the major spawning area and nursery area for *R. leporina* in Wellington Harbour. Differential mortality rates during the life cycle could also alter the proportion of *R. plebeia* to *R. leporina*. Alternatively, the population of adult *R. leporina* in Wellington Harbour might be supplemented by migration of adults from outside areas, but this seems unlikely as *R. leporina* favour shallow sandy areas and the harbour is isolated from other such areas by a deep rocky coastline.

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