Stock discrimination of pilchards by stable isotope ratio analysis

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FISHERIES DEPARTMENT OF WESTERN AUSTRALIA

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Project 92/150

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STOCK DISCRIMINATION OF PILCHARDS BY STABLE ISOTOPE RATIO ANALYSIS

J.S. Edmonds* & W.J. Fletcher*

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Summary *Effective management of the Western Australian pilchard fishery requires knowledge of whether the total distribution of pilchards around the south-west corner of Western Australia should be considered as a single stock, or whether it can more appropriately be divided into several stocks as defined by the main fishing ports of Fremantle, Albany, Bremer Bay and Esperance. In the study reported here we measured, by standard mass spectrometric techniques, the ratios of the stable isotopes* $^{18}O/^{16}O$ *and* $^{13}C/^{12}C$ *in the otolith carbonate of samples of pilchards from coastal waters adjacent to the four fishing ports. The experiments were designed to discover if the isotope signatures of the pilchard otolith carbonate differed from location to location and if they persisted through time. If both these conditions were fulfilled for any or all of the groups of fish examined, it could be concluded that they comprised separate populations for most of the purposes of fisheries management.*

Results of the measurements of oxygen isotope ratios showed that Fremantle fish could be regarded as a separate *and fish from Esperance constituted a different stock from those fish taken at Albany and Bremer Bay. Inclusion of fish from Busselton in the final two periods of sampling produced results which suggested that they may also belong to a separate population.*

Values for ratios of stable oxygen isotopes in pilchard otolith carbonate were consistent with ambient water temperatures as governed by latitude and movements of the Leeuwin current. This suggests that the measurement of ratios of stable oxygen isotopes can be a valuable method of fish population discrimination where the range of distribution of a species covers waters of different temperatures.

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Background The fisheries for pilchards, *Sardinops sagax*, in Western Australia are centred at the ports of Fremantle on the west coast, and Albany, Bremer Bay and Esperance on the south coast. As fishing effort is primarily restricted to the waters adjacent to the port of operation, these fisheries are naturally divided into four areas, along with the more general division of west and south coast fisheries. Effective management of the total pilchard fishery requires knowledge of whether the total distribution of pilchards around the south-west corner of Western Australia should be considered as a single stock, or whether it can more appropriately be divided, for purposes of fisheries management (for example, the control of fishing effort), into several stocks as perhaps conveniently defined by the fishing ports. Previous attempts to discriminate between pilchards caught in the various regions, and thereby to define stocks, by microchemical analysis of otoliths produced results that were difficult to interpret (Edmonds *et al.*, in press). The isotope study reported here was undertaken to delineate pilchard stocks as defined by the fishing port/fishing area. We anticipated that by measuring the ratios of the stable isotopes of oxygen and carbon in the otolith carbonate, ie, properties of the bulk material of the otoliths, we would avoid the difficulties encountered in trace analysis (which involved the measurement of elements that might be thought to have been adventitiously acquired and thereby potentially subject to short term variation).

Given that there are differences in the average sea temperatures between the locations where fishing occurs, the isotope experiments were designed to discover if the isotope signatures of the pilchard otolith carbonate differed from location to location, consistent with these variations in temperature, and if they persisted through time. If both these conditions were fulfilled for any or all of the groups of fish examined, it could be assumed that they comprised separate populations for most of the purposes of fish management. Such a result would not, of course, imply that the groups of fish represented different breeding populations or necessarily supply any genetic information.

A preliminary study of stable isotope ratios in pilchard otolith carbonate, undertaken for fish captured in December 1991, produced promising results. Although not part of the project carried out under the current funding, the results have been included in this report.

Need To implement effective management strategies for the maximum sustainable exploitation of pilchard stocks off south-western Australia there is a need to know whether the overall distribution of the species in the region can be regarded as a single population, or whether it should be considered as consisting of a number of smaller, essentially non-mixing populations.

Objectives To measure the $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios in otolith carbonate from a range of sizes of pilchards from four locations (Fremantle, Albany, Bremer Bay, Esperance) at three-monthly intervals through one year, and thereby to test whether isotope-based, location signatures persist through time and thus demonstrate the presence of separate populations within the pilchard distribution.

Methods The experimental design was as follows: Samples of pilchards to be taken from commercial catches made at Fremantle, Albany, Bremer bay and Esperance at three-monthly intervals through a single year, viz. March, June, September and December 1993. Twenty samples of deproteinated calcium carbonate, each prepared from the pooled, powdered otoliths of several fish (the exact number depending on otolith weight) to be analysed for $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios by standard mass spectrometric techniques for each of the four locations.

As we were depending upon the commercial fishery for our samples we anticipated that there might be some necessary small alterations to our experimental design resulting from availability or otherwise of pilchards. Ultimately such variations in procedure were small but included analyses of otolith carbonate from pilchards obtained at an additional west coast site (Busselton) for the final two sampling occasions. Details of the final sampling programme are included in Table 1.

Fish were selected from different boats and caught on different days within the prescribed sampling period, to ensure that the total sample was representative of the area during that period. Pooling of otoliths to form each sample was necessary because of the small size of pilchard otoliths (less than 2mg) and the need for several mg of deproteinated carbonate for analysis. Pooling was done on the basis of otolith size (Table 1) for each school of fish sampled. By keeping separate samples derived from the various schools within the sampling area and period we could ensure that any observed differences in isotopic signatures were really representative of the area and did not have the potential to merely reflect school-to-school differences.

Results Results are summarized in Table 1 and Figure 1. Means of the oxygen isotope ratio determinations for the different locations and sampling times were examined by an analysis of covariance with otolith weight as co-variant. This confirmed the observation, evident from examination of the plots of oxygen isotope ratios that Fremantle fish, which had the lowest ratios, constituted a separate population from fish taken on the south coast, and, in addition, fish from Esperance, which had the highest ratios, appear to be part of a different population from those taken at Albany and Bremer Bay. Albany and Bremer Bay fish were not significantly different from each other. A discriminant analysis was also carried out for the four main locations (Busselton was excluded) with the sampling times pooled, ie, only the four categories defined by the locations were examined. This was used to classify the observations according to location. The results of this analysis again reinforced the result that Fremantle fish were separate from the south coast fish and Esperance fish constituted a different population from that taken at Albany and Bremer Bay. Again Albany and Bremer fish were not distinguished from each other. The analysis of covariance for the mean oxygen isotope ratios indicated that fish caught at Busselton (on the west coast but south of Fremantle) were, as expected different from south coast fish, but also suggested that they may constitute a separate population to those from Fremantle.

Oxygen isotopes in otolith carbonate have been reported to be deposited in equilibrium with ambient seawater and, as a consequence, their ratios should be a function of water temperature. We anticipated, then, that any differences in oxygen isotope signatures for the four locations could be explained by water temperature differences governed by latitude and possibly also by the Leeuwin current. The pattern for the differences in ratios was therefore consistent with this hypothesis with the lowest values, indicating the highest temperatures found in otoliths from Fremantle fish. Conversely the highest ratios, indicating the coolest water came from the Esperance fish.

We are still considering the results of the carbon isotope ratios but it appears unlikely that they will contribute substantially to further discrimination of these pilchard populations.

Benefits The knowledge gained by the successsful conclusion of this project on the nature of pilchard populations in waters off the south-west of Western Australia will aid management and industry in the rational exploitation of the resource. In addition a method for the delineation of fish populations based on stable oxygen isotope ratios and explained in terms of ambient seawater temperatures has been successfully trialed.

Intellectual Property Not applicable

Further Development The project could be further developed in two ways. First, extending the geographical and latitudinal range of pilchards examined by examining fish taken off Geraldton (the northern limit of pilchard distribution on the west coast) to determine if such fish comprise a separate population to those taken at

Fremantle. Second, by applying the method developed here to other fish species that have the potential, because they are distributed through waters exhibiting a range of temperatures, to have discriminating stable oxygen isotope signatures.

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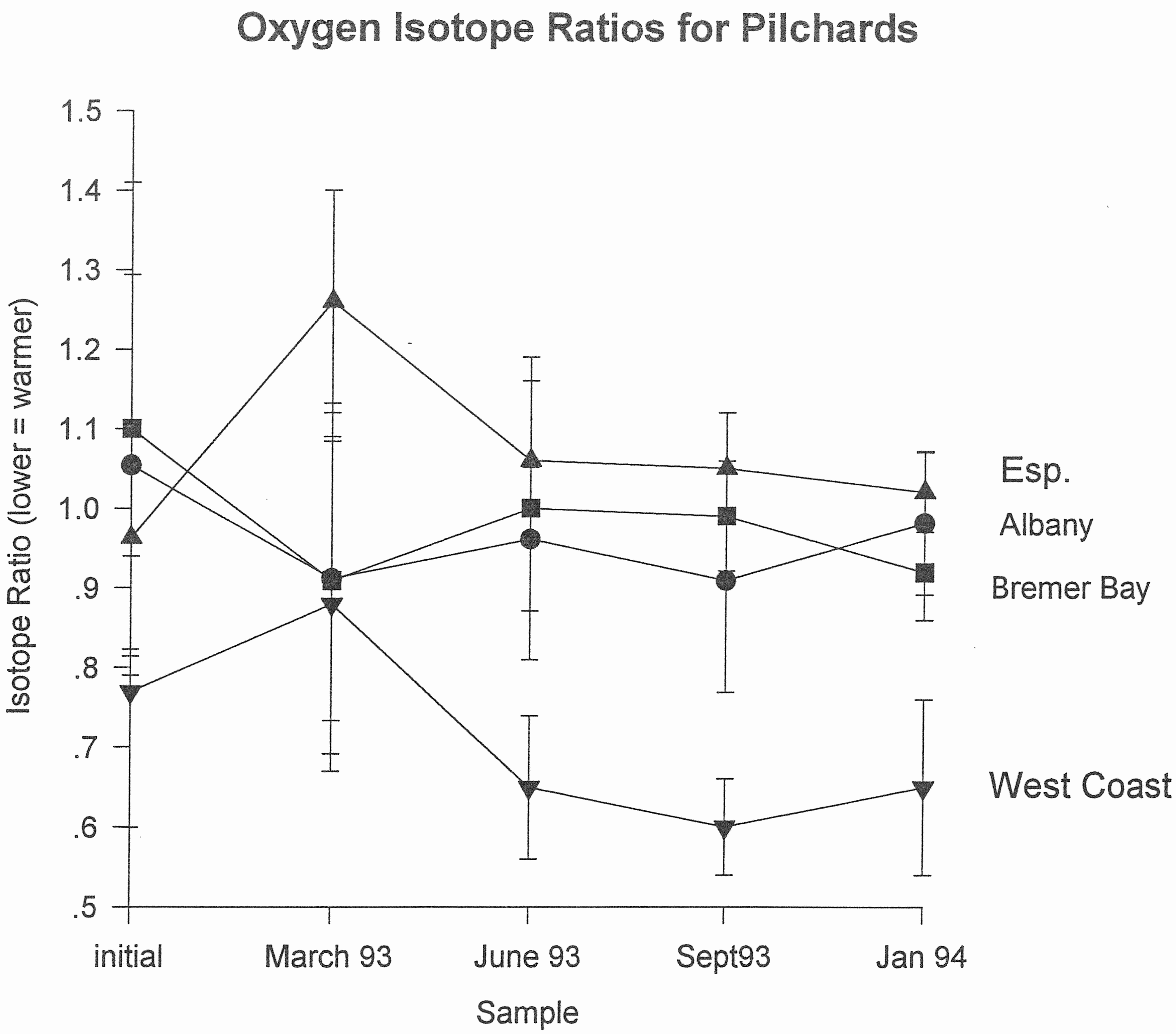
Final Cost

Distribution

LOCATION	DATE	NO. OF SAMPLES	NO. OF OTOLITHS PER SAMPLE - RANGE	OTOLITH WEIGHT RANGE (MG)	^{13}C MEAN AND (RANGE)	^{18}O MEAN AND (RANGE)
Fremantle	Dec 91	10	8-13	1.00-2.30	-6.89 (-7.09- -6.62)	0.77 (0.54-0.99)
	Mar/Apr 93	20	8-17	1.11-2.26	-7.05 (-7.34- -6.73)	0.88 (0.50-1.13)
	Jun/Jul 93	27	8-20	1.25-2.27	-7.09 (-7.48- -6.80)	0.66 (0.37-0.80)
	Sep/Nov 93	10	10-19	1.29-2.33	-7.03 (-7.23- -6.58)	0.60 (0.51-0.69)
	Jan/Feb 94	8	12-20	1.14-2.22	-7.00 (-7.17- -6.69)	0.65 (0.51-0.77)
Busselton	Oct 93	12	12-20	1.35-2.42	-6.88 (-7.14- -6.57)	0.84 (0.67-1.02)
	Jan 94	12	11-20	1.06-2.40	-6.91 (-7.01- -6.59)	0.77 (0.70-0.85)
Albany	Dec 91	10	8-13	1.17-2.28	-6.73 (-7.06- -6.53)	1.05 (0.72-1.55)
	Mar/Apr 93	24	9-16	1.00-2.17	-7.13 (-7.57- -6.55)	0.91 (0.18-1.24)
	Jun/Jul 93	25	11-18	1.07-2.43	-6.87 (-7.21- -6.43)	0.96 (0.75-1.17)
	Sept/Oct 93	44	9-16	1.02-2.44	-6.84 (-7.29- -6.43)	0.88 (0.54-1.18)
	Jan/Feb 94	10	8-16	0.96-2.65	-6.77 (-7.18- -6.31)	0.98 (0.85-1.13)
Bremer Bay	Dec 91	10	7-14	0.91-2.57	-6.64 (-6.94- -6.16)	1.10 (0.44-1.40)
	Mar/Apr 93	21	9-16	1.13-2.53	-6.83 (-7.39- -6.25)	0.91 (0.58-1.23)
	Jun/Jul 93	30	11-16	1.01-2.50	-6.78 (-7.16- -6.42)	1.00 (0.26-1.23)
	Sept/Oct 93	28	12-16	0.97-2.35	-6.79 (-7.09- -6.50)	1.00 (0.88-1.14)
	Jan/Feb 94	11	11-16	1.12-2.37	-6.90 (-7.21- -6.64)	0.92 (0.80-0.99)
Esperance	Dec 91	10	8-18	0.61-2.06	-7.32 (-7.65- -6.88)	0.96 (0.70-1.12)
	Mar/Apr 93	19	7-15	0.99-2.73	-6.77 (-7.06- -6.47)	1.26 (0.94-1.49)
	Jun/Jul 93	28	10-16	1.02-2.79	-6.59 (-6.88- -6.40)	1.07 (0.79-1.27)
	Oct/Nov 93	18	12-17	1.03-2.24	-6.91 (-7.27- -6.57)	1.05 (0.87-1.15)
	Dec 93/Jan 94	16	9-18	1.17-275	-6.88 (-7.30- -6.59)	1.02 (0.94-1.10)



Oxygen Isotope Ratios for Pilchards
Isotope Ratio (lower = warmer)
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
initial
March 93
June 93
Sept93
Jan 94
Sample
Esp.
Albany
Bremer Bay
West Coast