Identifying nursery areas used by inner bay and oceanic pink snapper *(Pagrus auratus)* stocks in the Shark Bay region, in relation to the effect of prawn trawling on inner bay snapper stocks

Final Report for FRDC Project 2001/061

Daniel J Gaughan (Principal Investigator),

(Co-investigators)
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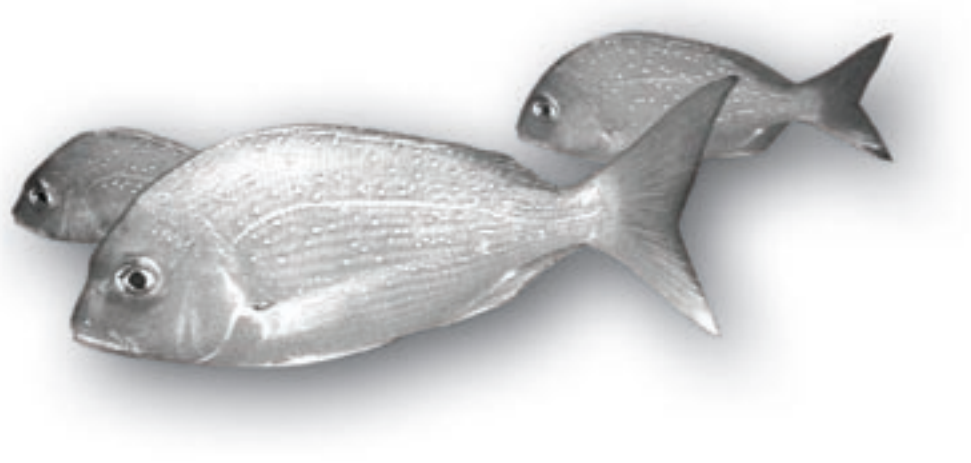

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FRDC Project No. 2001/061

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Published by: Department of Fisheries – Research Division, Western Australia. PO Box 20, North Beach WA 6920, Australia

March 2003

ISBN: 1 887098 15 9

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OBJECTIVES

1. To establish whether trace element and isotope composition in otoliths is diagnostic of location on a transect from inner Shark Bay, through Denham Sound, to the oceanic waters.
2. To analyse cores of otoliths of adult snapper from the ocean and Denham Sound stocks, to establish the location of the nursery grounds of each stock.
3. To evaluate Laser Ablation, Inductively Coupled Plasma Mass Spectrometry as a tool for fish stock assessment and movement studies by comparison with concurrent stable isotope ratio and previous whole otolith trace element studies on Shark Bay snapper.

Non Technical Summary

OUTCOMES ACHIEVED

This study has shown that juvenile snapper are relatively sedentary in their movements, and that as they mature to adults retain a significant degree of site fidelity. Each location within the western gulf of Shark Bay acts as a nursery area for snapper and is important for recruitment of snapper to that same area. This study was able to directly answer in the affirmative that juvenile snapper taken in the Denham Sound sector of the Shark Bay Prawn Trawl Fishery recruit to the recreationally fished, depleted western gulf of inner Shark Bay stock. Consequently, any deleterious impact on juvenile snapper in Denham Sound would subsequently have most impact on the recreationally targeted adult snapper stock of this same location. These findings have added to other lines of evidence on the spatial dynamics of Shark Bay snapper to confirm that there is a need to investigate alternative management arrangements for the Shark Bay prawn fishery; an additional detailed study has shown conclusively that this fishery reduces the number of juvenile snapper in Denham Sound by 25% in each year. This other investigation, which has now been completed and presented to stakeholders, examines the effects of changing boundaries in the prawn fishery so as to achieve a balance between increasing survival of juvenile snapper while minimising any potential decrease in profitability to the prawn industry. The recommendation to alter a management boundary so as to reduce bycatch of juvenile snapper within Denham Sound was agreed to by stakeholders and is expected to result in a change to the relevant management plan.

This study examined two aspects of the chemical composition of snapper otoliths from Shark Bay Western Australia as an aid to determining the spatial relationship among juveniles and to better understand the spatial relationships between juveniles and adults. Otoliths were collected from juvenile snapper in Denham Sound and Freycinet Estuary in the western gulf of Shark Bay. In order to also compare otolith chemistry for juveniles from outer Shark Bay to that from the western gulf, adult snapper were collected from these locations and the portion of their otoliths corresponding to an age <1 year (i.e. young juveniles) were analysed. As well as the two western gulf sites already mentioned, the oceanic samples were obtained from Koks Island and Cape Inscription.

Two types of chemical analyses were undertaken. The first assessed whether a site specific signature can be seen in the otolith carbonate, the basic constituent of otolith material, using stable isotope concentrations of oxygen and carbon. The second examines trace elements in the otolith. Each method has different properties, with both reliant on the premise that differences in the habitat and biology of fish from different areas can result in permanent differences in the composition of their otoliths. The results of the two methods of analysing otolith composition were essentially complementary, but with the trace element analysis providing a clearer distinction between locations. The analyses indicate that the juvenile snapper located at each of the four regions sampled were not mixing to any significant degree. This implies that there is site fidelity for juvenile snapper. That is, juvenile snapper do not range very far over their first year of life and individuals certainly do not traverse throughout the Shark Bay region. Furthermore, analysis of the juvenile portion (core) of adult otoliths indicates that groups of snapper occupying separate locations as adults also occupied the same separate locations when they were juveniles. The management implication from these results is that each localised group of juvenile snapper must be recognized as important because each may be responsible for providing recruitment to particular, distinct localities.

Acknowledgements

Lidia Bednarek (University of Western Australia, WA Biogeochemistry Centre, Botany Department) performed all the carbon and oxygen stable isotope analyses in this report.

Henry Cheng (Curtin University of Technology) provided the statistical support.

The skipper and crew of the *RV Naturaliste* (Theo Berden, Kim Hillier, Tim Shepherd, Manfred Mann) are thanked for providing samples of juvenile snapper from the western gulf of Shark Bay.

Background

Pink snapper (*Pagrus auratus*) is the main commercial and recreational finfish (Kailola et al., 1993) in the Shark Bay region. Pink snapper are distributed along much of the temperate and tropical coasts of Western Australia from east of Esperance on the south coast to north of Shark Bay on the west (Paulin 1990). Previous research using genetics (Johnson et al. 1986), tagging (Moran et al. 2004), and otolith microchemistry (Edmonds et al., 1989, 1999) have shown that pink snapper from the innermost parts of Shark Bay do not mix with the ocean fish.

Juvenile pink snapper are found in trawlable habitats throughout the bay, including Denham Sound in the western gulf, which lies within the Shark Bay prawn trawl fishery. Prawn trawlers in Denham Sound catch juvenile snapper as part of their by-catch. Detailed surveys clearly demonstrate that trawling in Denham Sound reduce by 25% the numbers of juvenile snapper that would have survived natural mortality within a one year period (Moran and Kangas 2003).

The effect of this trawled by-catch of juvenile snapper in Denham Sound on future recruitment to the depleted western gulf of Shark Bay snapper stocks is uncertain, largely because it is unknown whether the juvenile snapper in the trawled area would recruit to inner bay (Denham Sound) or ocean stocks. This project aims to use isotope composition of the juvenile portion of the otoliths of adult snapper from both spawning and non-spawning stocks to determine whether, or in what proportion, those snapper as juveniles used nursery grounds in Denham Sound.

Recent studies have suggested that the elemental composition of the otoliths offers a powerful tool for studies of fish population structure and for tracking individual migration paths (Campana et al. 1995, Thorrold et al. 1997). The elemental composition of the otolith is thought to reflect the physical (temperature) and chemical (e.g. salinity) characteristics of the ambient water in which the fish lives, although not necessarily in a simplistic manner (Kalish 1989, 1991). The relationship between ambient water chemistry and concentration of elements in the otolith will differ in strength depending on the specific properties of each element. Variations in the physical and/or chemical characteristics between water masses in which the fish spent their first year may correspond to the elemental composition of the juvenile portion of the otolith. Elemental composition of consecutive layers within the otolith can therefore provide an indication of the environmental history of individual fish.

Recent studies such as that of Proctor and Thresher (1998) concluded that otoliths are not insensitive to specimen handling and preparation. It is therefore imperative that the preservation and handling effects are minimised whilst vigilance is taken in the otolith preparation process to avoid susceptibility to contamination.

While both stable isotopic ratios and trace element composition of snapper otoliths have been found to vary spatially in Shark Bay, this project will be very demanding of the techniques in that the spatial scale is small and the definition required is high. For this study, stable isotope analysis was used to supplement microchemical data in the discrimination of fish stocks. The purpose of using this multi-technique approach was to gain information on pink snapper nursery areas using the established technique of Isotopic Ratio Mass Spectrometry (IR-MS) while comparing and contrasting these results with the contemporary technique of Laser Ablation, Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). If the laser ablation technique can be validated, future fish stock assessment and movement studies will be able to confidently take advantage of its power and cost-effectiveness.

Need

There is an urgent need to clarify whether the juvenile snapper taken in the Denham Sound sector of the Shark Bay Prawn Trawl Fishery recruit to the recreationally fished, depleted western gulf of inner Shark Bay stock or the sustainably (predominantly commercially) fished oceanic stock. This information is needed to ascertain whether changes to the management arrangement to the trawl fishery would significantly benefit the threatened inner bay snapper stocks.

Objectives

1. To establish whether trace element and isotope composition in otoliths of juvenile snapper is diagnostic of location on a transect from inner Shark Bay, through Denham Sound, to the oceanic waters.

2. To analyse cores of otoliths of adult snapper from the ocean and Denham Sound stocks, to establish the location of the nursery grounds of each stock.

3. To evaluate Laser Ablation, Inductively Coupled Plasma Mass Spectrometry as a tool for fish stock assessment and movement studies by comparison with concurrent stable isotope ratio and previous whole otolith trace element studies on Shark Bay snapper.

Methods

The difficulty in assessing whether juvenile and adult snapper were associated spatially is that the adults may have moved considerably in the years since they first recruited. To overcome this temporal gap, similar parts of the otoliths need to be compared. In this study we therefore examine the chemistry (i.e. trace elements and isotopic ratios) of otoliths of 0+ juveniles and central otolith material of adults that corresponds to that deposited in the first year of life.

Juvenile sample collection

Juvenile pink snapper (0+ cohort) were collected from 8 trawl sites aboard the FRV Flinders in May 2001 within the western gulf of Shark Bay (10 fish per site, n=80). The juvenile snapper were sampled from selected trawl sites within Freycinet Estuary and Denham Sound of the western gulf of Shark Bay (Fig. 1). We were unsuccessful in our attempts to collect juveniles from the oceanic areas. Factors considered in determining the trawl sites chosen for this project were are follows:

- Sufficient numbers of 0+ fish caught in trawls
- Distance between trawl sites i.e. even spread of sites within each location

The length to caudal fork (LCF) of each fish was measured, and where possible the otoliths were removed from fresh fish aboard the research vessel. The remaining fish were frozen and processed in the laboratory at a later date.

Adult sample collection

Adult pink snapper were collected at sea aboard the research vessel as well as from commercial boats. Adults were collected from both spawning (May-Aug 2001) and non-spawning periods (Sept-April 2001/2002) from Denham Sound, Freycinet and ocean (Cape Inscriptio and Koks Island) stocks (Fig. 1). Probably for ocean fish, and possibly for inner bay fish, there are movements associated with spawning. It is therefore necessary to sample from both spawning and non-spawning seasons to get a complete picture. 20 adult snapper were collected from each of the three sampling sites at both spawning and non-spawning time (40 fish per site, n=120).

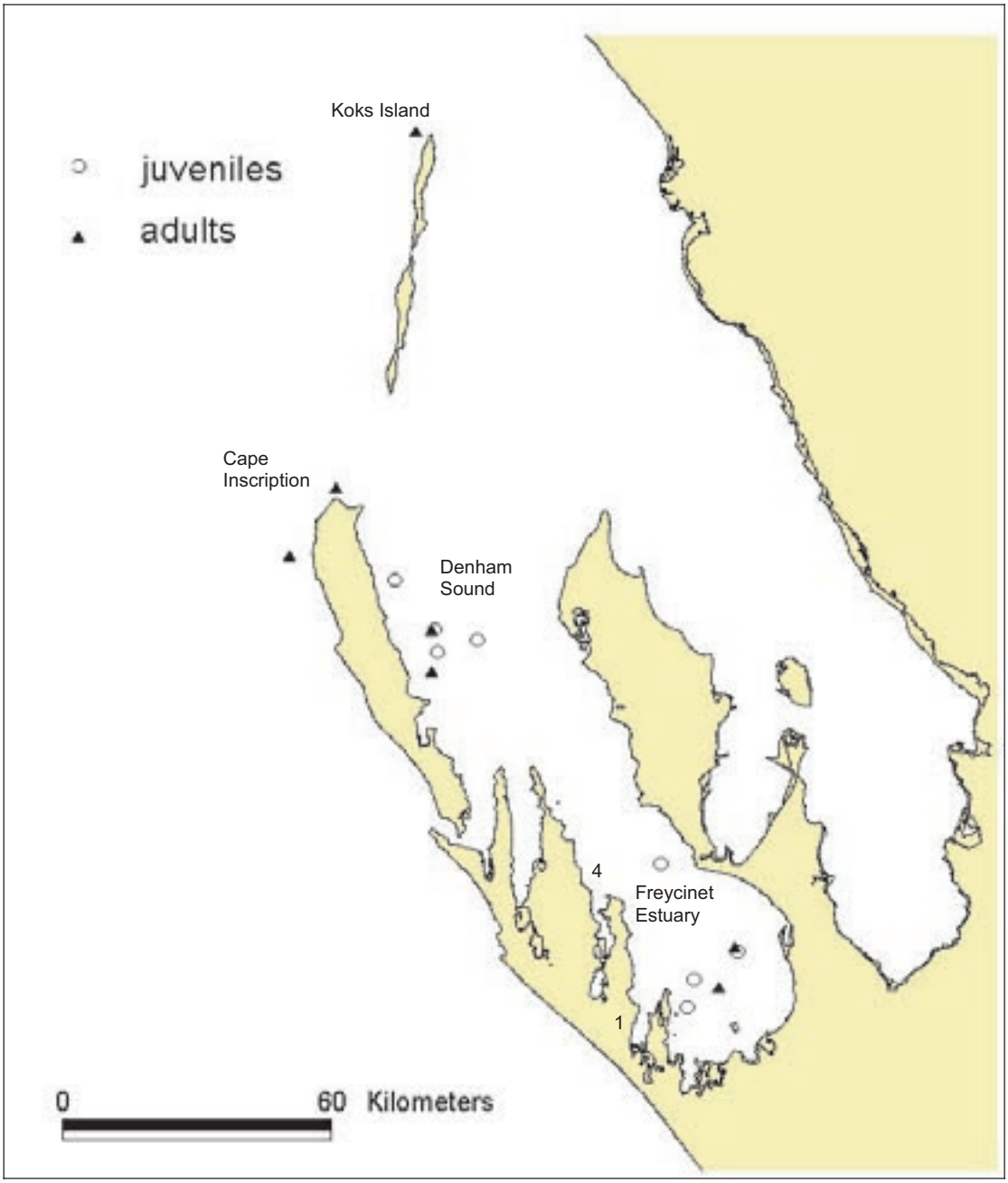


Figure 1. Map of Shark Bay, Western Australia, showing sampling locations for juvenile and adult snapper.

Otolith preparation

Sagittal otoliths were removed from each fish with the use of nylon forceps to avoid elemental contamination, cleansed of adhering tissue using high purity (Milli-Q) water and stored dry in paper envelopes until they could be examined further. Left and right otoliths were identified and weighed, taking care not to allow the otolith to come in contact with the metal base of the balance.

The otoliths were then embedded in epoxy resin and sectioned transversely through the core. One randomly selected otolith from each fish was used for Isotope Ratio - Mass Spectrometry (IR-MS), while the other was used to determine elemental concentrations by Laser Ablation - Inductively Coupled - Mass Spectrometry (LA-ICP-MS).

For isotope analysis, at pre-determined distances from the centre of the otolith, material was removed by drilling. Drilling of the otolith cores required the use of a hand held chuck holding a 0.35 mm drill bit, which was rotated manually taking care to avoid contact with the resin block. The otolith material was retrieved (~0.8 mg of otolith powder) and transferred to 200 μL vials.

The remaining otolith from each fish was sectioned through the core using the Buehler Precision Saw with a diamond cutting-edge, in conditions which avoided contamination of the section surface. This required the use of Milli-Q water to lubricate the saw while cutting, and careful handling of the sample once sectioned. The sections were then transferred into acid-washed vials where they were kept until analysed by LA-ICP-MS.

Chemical preparation of otolith powder for stable isotope ratio analysis

The otolith powder collected for stable isotope analysis was treated carefully with 10% sodium hypochlorite (NaOCl) solution to remove residual organic material from the carbonate. Approximately 30 μL of NaOCl solution was added to the otolith powder in the vials and left on for approximately two hours. The resultant solid material (mainly calcium carbonate) was rinsed with Milli-Q water four times, with thorough mixing, leaving the sample to stand for at least 5 minutes between rinses. The samples were then dried under reduced pressure in a desiccator for at least 24 hours. 0.2 to 0.4 mg of the dried otolith powder was then weighed out into pre-cleaned sample vessels. The otolith powder samples were then analysed for $\delta^{13}C$ and $\delta^{18}O$ with the use of IR-MS. Stable isotope ratios, $\delta^{13}C$ and $\delta^{18}O$ were acquired using standard mass spectrometric techniques (University of Western Australia, WABC, Botany Department) and the values are reported in standard δ notation (per mil, %) relative to PDB-1 standard (Esptein et al. 1953).

Laser analysis

Elemental analysis was carried out using a UV MicroProbe coupled to a VG Elemental – Plasma Quad 3 ICP-MS with data acquisition by PQVision Version 4.3. The analysis was carried out in a thermally regulated environment.

The otolith sections sampled were mounted on perspex discs using super-glue (methyl methacrylate), then polished using diamond paste (600 mesh diamond powder with deionised water) to obtain a flat surface, free from surface contamination. The mounted samples were placed into the UV MicroProbes sampling cell. Under software control the start and end position of the ablation transect was chosen. Transects were taken diagonally across each otolith starting from the distal side passing through the core and exiting through the proximal side of the otolith (all otoliths treated the same) (Fig. 2).

The movement of the high precision X:Y:Z stage is defined to 10 steps per second. One step is equivalent to 1.25 μm. A specified frequency of 10Hz causes the laser to fire ten times per second so a crater was placed every single step (= 1.25 μm). This equates to movement along the transect of 12.5 μm per second. The element counts were measured against time (sec), which were then converted to distance across the otolith. The data of interest for each element were extracted from a point immediately adjacent to the primordium (to exclude pre-settlement phase) through to the otolith margin for the juvenile fish and to the first yearly growth ring for the adult fish (representing their first year of life) (Fig. 3). The background counts were subsequently subtracted from this selected data (Fig. 3).

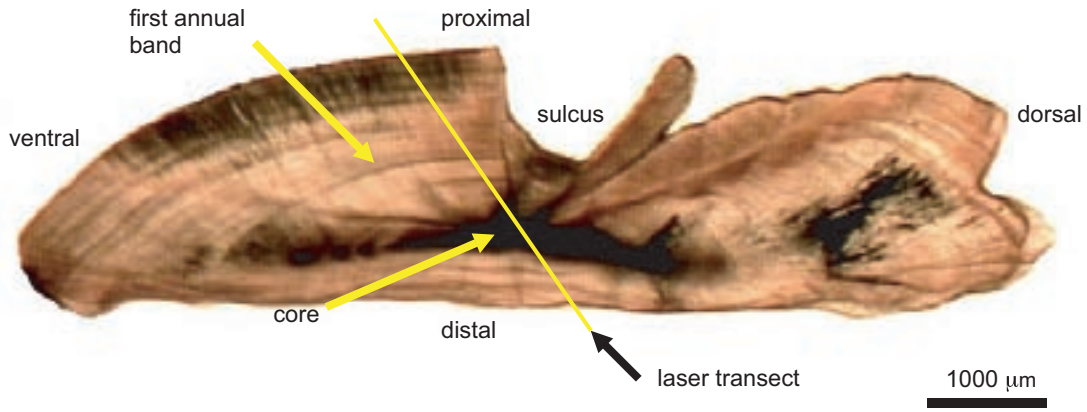


Figure 2. Transverse section of adult otolith showing the main structures and laser transect.

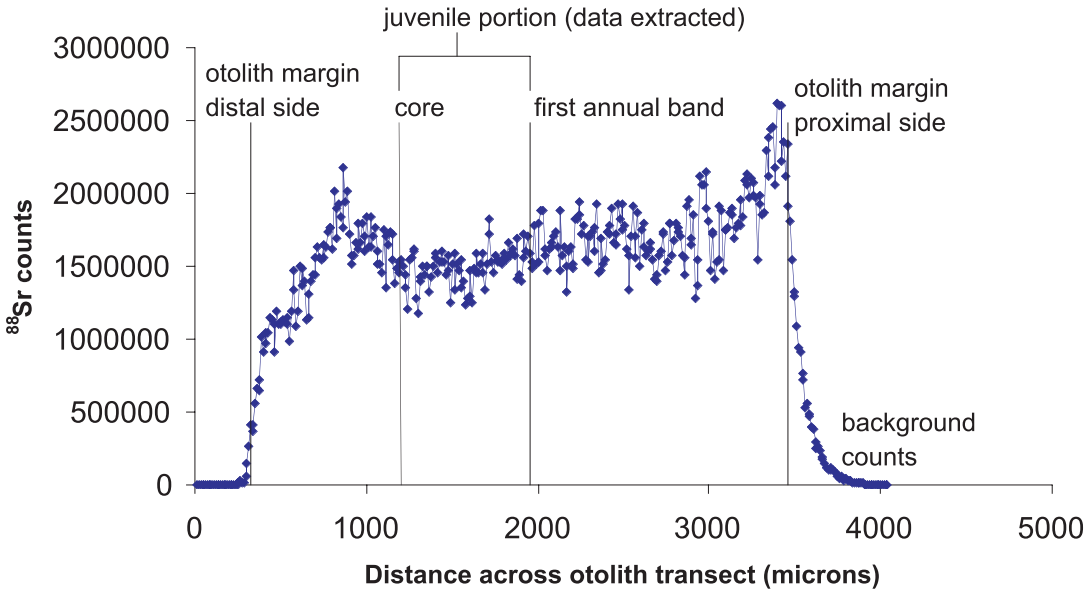


Figure 3. Output from LA-ICP-MS of ^{88}Sr counts measured across the transect showing the juvenile (0+) portion of the adult otolith, with corresponding data extracted for further analysis.

The mean count values were standardised against strontium as it was the most stable consistent element in the analysis. Both Ca and Sr were used as standards although strontium was preferred over the use of Ca as the LA-ICP-MS technique is designed to accurately analyse trace elements. With 99% of the otolith consisting of $CaCO_3$, Ca was avoided as a standardising tool except in the case when analysing strontium.

Statistical analyses

The statistical analyses used to test for significant differences in isotopic ratios and elemental concentrations between regions are incorporated into the results section. This was done because of the extensive series of tests employed. The primary test used was 2 factor MANOVA (Location and LCF). For isotopic ratios $\delta^{13}C$ and $\delta^{18}O$ were the response variables tested. For elemental composition Sr/Ca and Ba/Sr were the response variables. When MANOVAs revealed significant differences, the

individual variables were further tested using ANOVAs. Thus, there were up to six statistical tests for each comparison between sites, with potentially 3 for each of the isotopic ratios and the elemental variables. The significance level for all tests was set at the standard probability level of 0.05.

Results

Juvenile (0+) snapper from Freycinet and Denham Sound

Fish size

Juvenile snapper collected from various sites within Freycinet Estuary and Denham Sound ranged in size from 71 to 127 mm (n = 77). There was a significant difference in caudal fork length (LCF) between these two locations with larger fish collected from Denham Sound (T-test: $p<0.05$) (Fig. 4). It should be noted that juveniles from both locations represent the 0+ cohort collected at the same time of year (May 2001); apparent size differences are likely related to different settlement times and/or growth rates in each area.

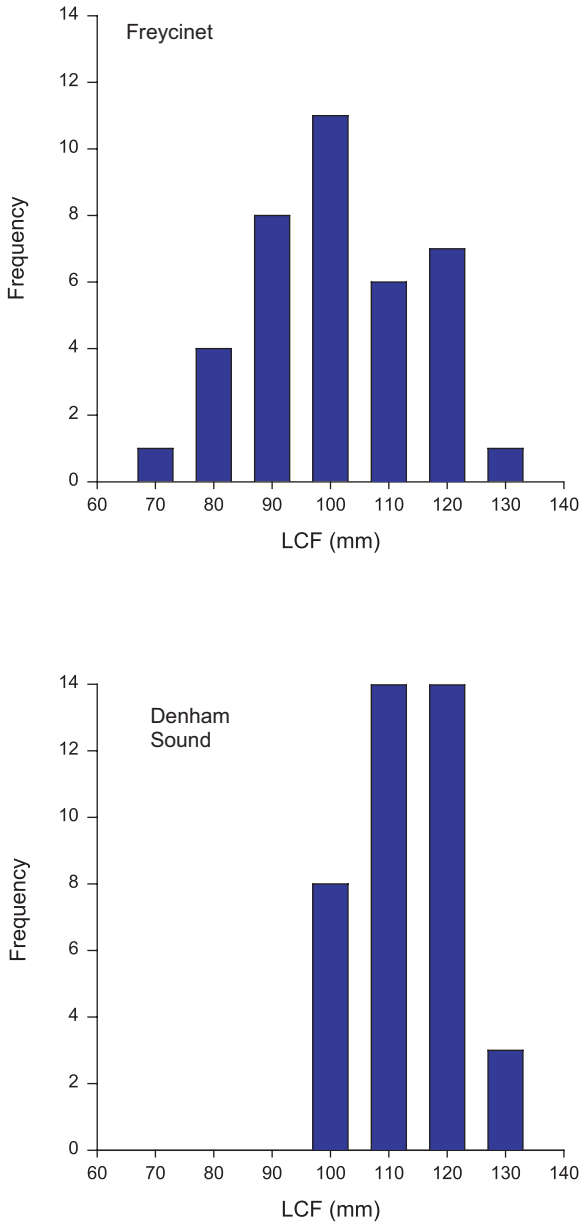


Figure 4. Frequency distribution of fish size (LCF) of juvenile snapper sampled from Freycinet and Denham Sound.

Chemical analysis

A range of elements was analysed by LA-ICP-MS with eight of these elements (^{23}Na, ^{24}Mg, ^{31}P, ^{44}Ca, ^{55}Mn, ^{66}Zn, ^{88}Sr and ^{138}Ba) present in the otoliths at levels above detection limits. Both ^{88}Sr/^{44}Ca and ^{138}Ba/^{88}Sr varied significantly in juvenile otoliths between the nursery areas of Freycinet Estuary and Denham Sound (Table 1, Fig. 5). In both cases LCF was not a confounding variable.

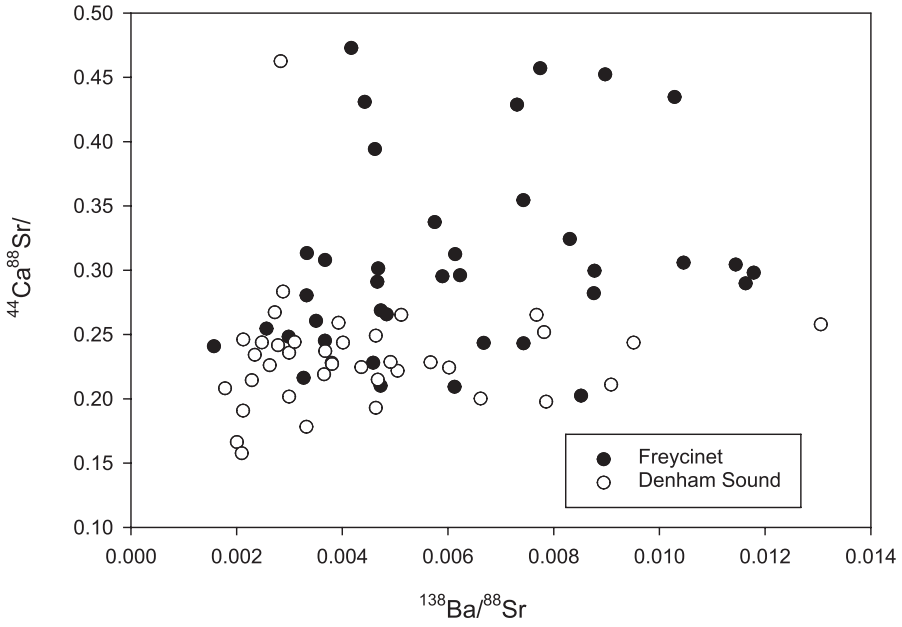


Figure 5. ^{88}Sr/^{44}Ca vs.^{138}Ba/^{88}Sr of otoliths of juvenile snapper collected from Freycinet and Denham Sound.

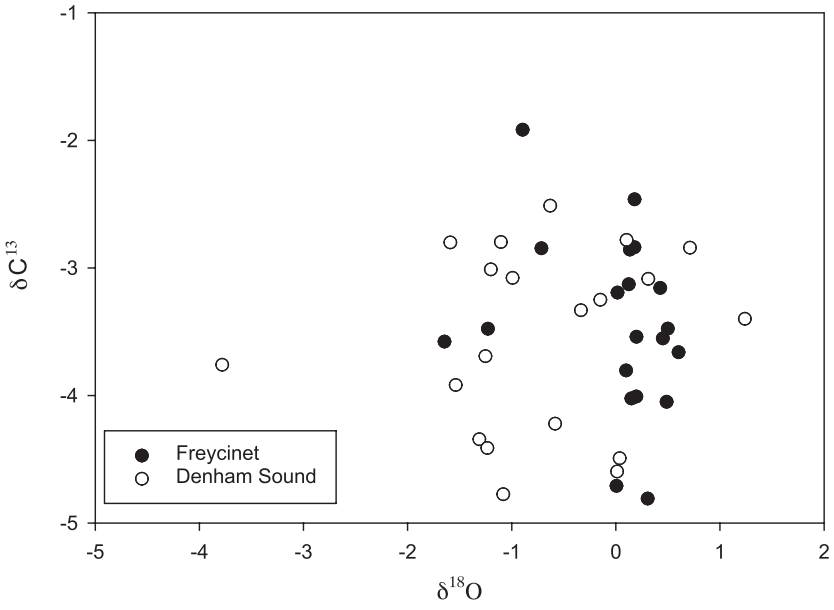


Figure 6. δ^{13}C vs. δ^{18}O of otoliths of juvenile snapper collected from Freycinet and Denham Sound.

There was a relationship between LCF and δ^{18}O. To account for length as a possible confounding factor, the overall dataset for the δ^{13}C and δ^{18}O values was restricted to a homogeneous length group. δ^{18}O varied significantly between Freycinet and Denham Sound (Table 1, Fig. 3). δ^{13}C showed no significant difference between the two locations (Table 1, Fig. 7).

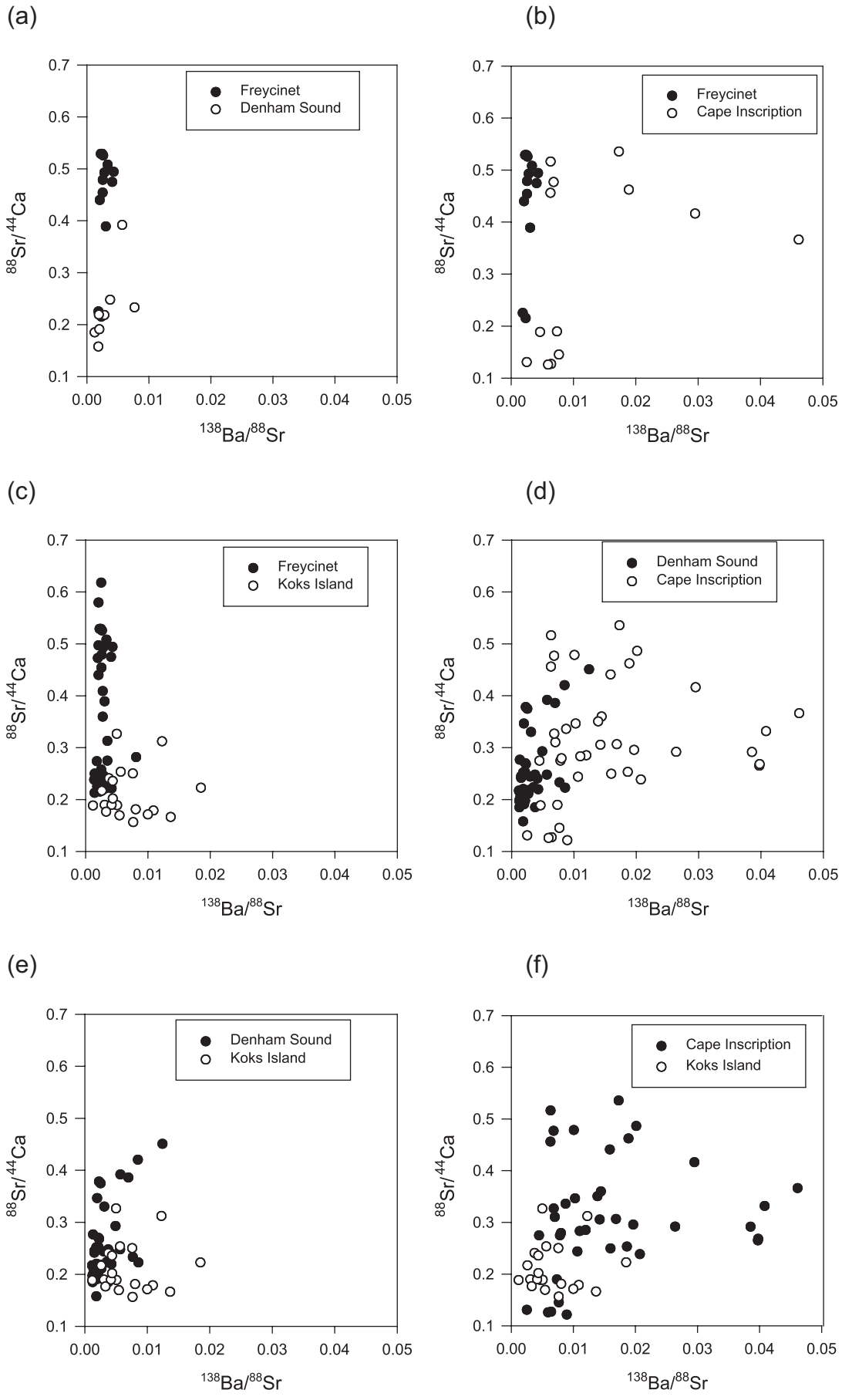


Figure 7. $^{88}Sr/^{44}Ca$ vs. $^{138}Ba/^{88}Sr$ ratios of the juvenile portion of adult snapper otoliths for each location combination. Note that the Figures shown correspond to the data sets used in the final comparison and sample size thus varies.

Table 1. Probability levels from MANOVAs and ANOVAs between otoliths of juvenile snapper sampled from Freycinet and Denham Sound using the techniques of LA-ICP-MS and IR-MS.

	MANOVA	ANOVA	ANOVA	MANOVA	ANOVA	ANOVA
n		40			80	
Source	$\delta^{18}O$ & $\delta^{13}C$	$\delta^{13}C$	$\delta^{18}O$	Ba/Sr & Sr/Ca	Ba/Sr	Sr/Ca
Location	0.058	0.653	0.018	**0.000**	**0.004**	**0.000**
LCF	0.573	0.288	0.993	0.524	0.330	0.673

Juvenile (0+) portion of otoliths from adult snapper

Fish size

Adult fish collected from the 4 locations (Freycinet, Denham Sound, Cape Inscription and Koks Island) ranged in size from 301 to 705 mm LCF (n = 140). A significant difference in fish size was detected between the locations with means compared using a Tukey HSD test (Table 2). In order to detect any significant confounding effects for LCF, this variable was included as a main treatment in the statistical analyses along with location, the target variable of interest. On those occasions when LCF was found to be a confounding factor, each sampling group was statistically re-analysed once the data were restricted to LCFs from a 416 to 522 mm range so as to achieve a homogeneous dataset.

Table 2. Probability values for Tukey HSD tests comparing mean LCF of adult snapper from four locations; Freycinet (Frey), Denham Sound (DS), Cape Inscription (CI) and Koks Island (Koks). $p<0.05$ in bold.

Location Mean LCF (mm)	Frey 563.85	DS 450.32	CI 397.90	Koks 507.85
Frey		**0.0001**	**0.0001**	0.0818
DS			**0.0327**	0.0697
CI				**0.0001**

Unrestricted dataset

Elemental analysis (LA-ICP-MS)

Based on the LA-ICP-MS results of the juvenile (0+) fish, the elements of strontium and barium were the focus of further analysis in determining differences between the adult fish. A MANOVA (Multivariate Analysis of Variance) of both $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ ratios found significant differences in the juvenile portion of the adult otolith among sampling locations, and between fish size (LCF) (Table 3). There was no significant difference in elemental composition between fish sampled during the spawning and non-spawning season for each given location ($p>0.05$).

Further univariate contrasts were then used to examine for differences in each of $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ between all locations. Significant differences in $^{88}Sr/^{44}Ca$ were found between adults collected from Freycinet and Denham Sound with LCF differences also evident (Table 3). Significant differences were not detected in $^{138}Ba/^{88}Sr$ between Freycinet and Denham Sound (Table 3). $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ differed significantly between Freycinet and Cape Inscription (Table 3). Denham Sound differed significantly from Cape Inscription and Koks Island in both $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ (Table 3). There was also significant variability in both $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ between Cape Inscription and Koks Island (Table 3).

Stable isotope ratio analysis (IR-MS)

A MANOVA of $\delta^{13}C$ and $\delta^{18}O$ found highly significant differences among sampling locations with LCF differences also observed (Table 3). ANOVA of the $\delta^{13}C$ values showed a significant difference between Freycinet and Denham Sound with significant differences also observed for LCF (Table 3). In contrast, $\delta^{18}O$ values were not significantly different between Freycinet and Denham Sound (Table 3). $\delta^{13}C$ and $\delta^{18}O$ were highly variable between Freycinet and Cape Inscription (Table 3). Significant variation in the $\delta^{13}C$ values was detected between Freycinet and Koks Island although no variation in the $\delta^{18}O$ values between these two locations was observed (Table 3). Denham Sound and Cape Inscription differed significantly in $\delta^{13}C$ values but not in $\delta^{18}O$ values (Table 4). No significant differences were detected in both $\delta^{13}C$ and $\delta^{18}O$ values between Denham Sound and Koks Island (Table 4). ANOVA of both the $\delta^{13}C$ and $\delta^{18}O$ values showed little variation between Cape Inscription and Koks Island (Table 3).

Table 3. Probability levels in MANOVAS AND ANOVAS for comparison of elemental and isotopic composition between adult snapper sampled from four locations; Freycinet (Frey), Denham Sound (DS), Cape Inscription (CI) and Koks Island (Koks) without the length restriction. Significant differences ($p<0.05$) are in bold type.

			MANOVA	**ANOVA**		**MANOVA**	**ANOVA**	
Location	Source	n	$\delta^{18}O$ & $\delta^{13}C$	$\delta^{13}C$	$\delta^{18}O$	Ba/Sr & Sr/Ca	Ba/Sr	Sr/Ca
Location	All	140	**0.0000**			**0.0000**		
LCF			**0.0001**			**0.0033**		
Location	Frey DS	40 40	**0.0000**	**0.0000**	0.6202	**0.0000**	0.2118	**0.0000**
LCF			**0.0008**	**0.0018**	0.0966	**0.0000**	0.1078	**0.0009**
Location	Frey CI	40 40	**0.0000**	**0.0000**	**0.0174**	**0.0000**	**0.0000**	**0.0000**
LCF			**0.0000**	**0.0000**	0.1768	**0.0000**	0.2316	**0.0000**
Location	Frey Koks	40 20	**0.0000**	**0.0000**	0.8666	**0.0000**	**0.0000**	**0.0000**
LCF			0.15502	0.1749	0.1447	**0.0000**	0.8117	**0.0000**
Location	DS CI	40 40	**0.0004**	**0.0067**	0.0627	**0.0000**	**0.0000**	**0.0127**
LCF			0.0696	0.2033	0.1799	0.4556	0.2796	0.3773
Location	DS Koks	40 20	0.7153	0.4380	0.5947	**0.0000**	**0.0000**	**0.0075**
LCF			0.5108	0.7360	0.3522	0.0719	0.0765	0.0582
Location	CI Koks	40 20	**0.0249**	**0.1524**	0.0506	**0.0000**	**0.0012**	**0.0002**
LCF			0.0689	0.0389	0.5845	0.0837	0.7020	0.0385

Restricted dataset

Elemental analysis (LA-ICP-MS)

As mentioned previously, to account for length as a possible confounding factor each sampling group was restricted to a fish size of 416 to 522 mm and re-analysed. The reduction in the sample size for each location was considerable, especially for the Denham Sound group (Table 4). Similarly to the results for the unrestricted dataset, a MANOVA of $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ found significant differences among locations (Table 4).

Further, results from univariate tests showed $^{88}Sr/^{44}Ca$ in the otoliths of fish from Freycinet differed significantly from each of Denham Sound, Cape Inscription and Koks Island (Table 4). No significant differences in $^{88}Sr/^{44}Ca$ were detected between any of the remaining sampling locations (Table 4). $^{138}Ba/^{88}Sr$ in Freycinet fish differed significantly from Cape Inscription and Koks Island fish (Table 4).

It should be noted that a Type II error may have been committed with small sample sizes used, meaning falsely accepting the hypothesis that there were no differences between the regions.

Stable isotope ratio analysis (IR-MS)

MANOVA of $\delta^{13}C$ and $\delta^{18}O$ values found significant differences among the sampling locations (Table 4). ANOVA of the $\delta^{13}C$ showed significant differences between Freycinet and Denham Sound and between Denham Sound and Koks Island (Table 4). All remaining locations showed no significant variability in $\delta^{13}C$ values (Table 5). $\delta^{18}O$ differed significantly between Cape Inscription and both Denham Sound and Koks Island (Table 4).

Table 4. Comparison between adult snapper sampled from four locations; Freycinet (Frey), Denham Sound (DS), Cape Inscription (CI) and Koks Is. with LCFs restricted to 416 – 522 mm (homogeneous dataset). Significant differences ($p<0.05$) are in bold type.

			MANOVA	**ANOVA**	**ANOVA**	**MANOVA**	**ANOVA**	**ANOVA**
	Source	n	$\delta^{18}O$ & $\delta^{13}C$	$\delta^{13}C$	$\delta^{18}O$	Ba/Sr & Sr/Ca	Sr/Ca	Ba/Sr
Location	All	45	**0.0092**			**0.0000**		
Location	Frey	12						
	DS	8	**0.0000**	**0.0000**	0.3818	**0.0000**	**0.0000**	0.4749
	Frey	12						
	CI	12	0.0924	0.4349	0.0829	**0.0003**	**0.0091**	**0.0111**
	Frey Koks	12 13	**0.0000**	**0.0000**	0.8837	**0.0000**	**0.0000**	**0.0062**
	DS	8						
	CI	12	0.0562	0.0955	**0.0189**	0.1455	0.2523	0.0484
	DS	8						
	Koks	13	0.5725	**0.0387**	0.2816	0.1398	0.8441	0.0575
	CI	12						
	Koks	13	0.0768	0.8823	**0.0403**	0.1779	0.1113	0.1109

Results from restricted and unrestricted length datasets

In the cases where LCF was a significant confounding variable the results from the restricted dataset were accepted. Conversely, where LCF was not significant the results from the unrestricted dataset were used, as sample sizes were considerably larger. The results of elemental ($^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$) and stable isotope ($\delta^{13}C$ and $\delta^{18}O$) analyses are summarised in Table 5 with each possible location combination compared. A crucial point to recognize here is that while negative (non-significant) results do not necessarily imply the groups are not distinct, a positive (significant) result does imply that they are distinct. Thus, a single significant difference indicates that the samples were exposed to different chemical histories even if the other tests show no difference. In such cases, the groups are therefore considered as different. Nonetheless, the number of significant and non-significant differences for both techniques were tallied to give a qualitative indication of the "strength" of location fidelity. Note that we are not inferring biological meaning to this technique of summarising the series of statistical tests.

Table 5. Summary of results for juvenile portion of adult otoliths (sig. = significant, ns = non-significant) with tally of difference vs. no difference between the locations. Note: Underlined = restricted-length data, plain = unrestricted-length data.

	MANOVA	ANOVA		MANOVA	ANOVA		Tally			
Source	$\delta^{18}O$ & $\delta^{13}C$	$\delta^{13}C$	$\delta^{18}O$	Ba/Sr & Sr/Ca	Ba/Sr	Sr/Ca	MANOVA & ANOVA sig.	ns	ANOVA only yes-no sig.	ns
Frey. DS	sig.	sig.	ns	sig.	ns	sig.	4	2	2	2
Frey. CI	ns	ns	sig.	sig.	sig.	sig.	4	2	3	1
Frey. Koks	sig.	sig.	sig.	sig.	sig.	sig.	5	1	4	-
DS CI	sig.	sig.	ns	sig.	sig.	sig.	5	1	3	1
DS Koks	ns	ns	ns	sig.	sig.	sig.	3	3	2	2
CI Koks	sig.	ns	ns	sig.	sig.	ns	3	3	1	3

Freycinet vs Denham Sound

Significant stable isotope and trace element differences indicate that snapper from Freycinet and Denham Sound occupied different habitats as juveniles. Site fidelity was strong, with four of the six tests significant (Table 5). $^{88}Sr/^{44}Ca$ was significantly higher in the otoliths of Freycinet fish than in those of Denham Sound fish (Fig. 7a) while $^{138}Ba/^{88}Sr$ showed no significant difference (Fig. 8a). $\delta^{13}C$ differed significantly between Freycinet and Denham sound fish (Fig. 8a). In contrast, $\delta^{18}O$ values were not significantly different between Freycinet and Denham sound fish (Fig. 8a).

Freycinet and Cape Inscription

Significant stable isotope and trace element differences indicate that snapper from Freycinet and Cape Inscription occupied distinct habitats as recruits (Table 5). Site fidelity was strong, with four of the six tests significant (Table 5). $^{88}Sr/^{44}Ca$ was significantly higher in the otolith of fish from Freycinet than in fish from Cape Inscription (Fig. 7b). Conversely, $^{138}Ba/^{88}Sr$ in the otoliths of Cape Inscription fish was significantly higher than in those of Freycinet fish (Fig. 7b). The otoliths of fish sampled from Freycinet were less depleted in $\delta^{18}O$ than from Cape Inscription (Fig. 8b).

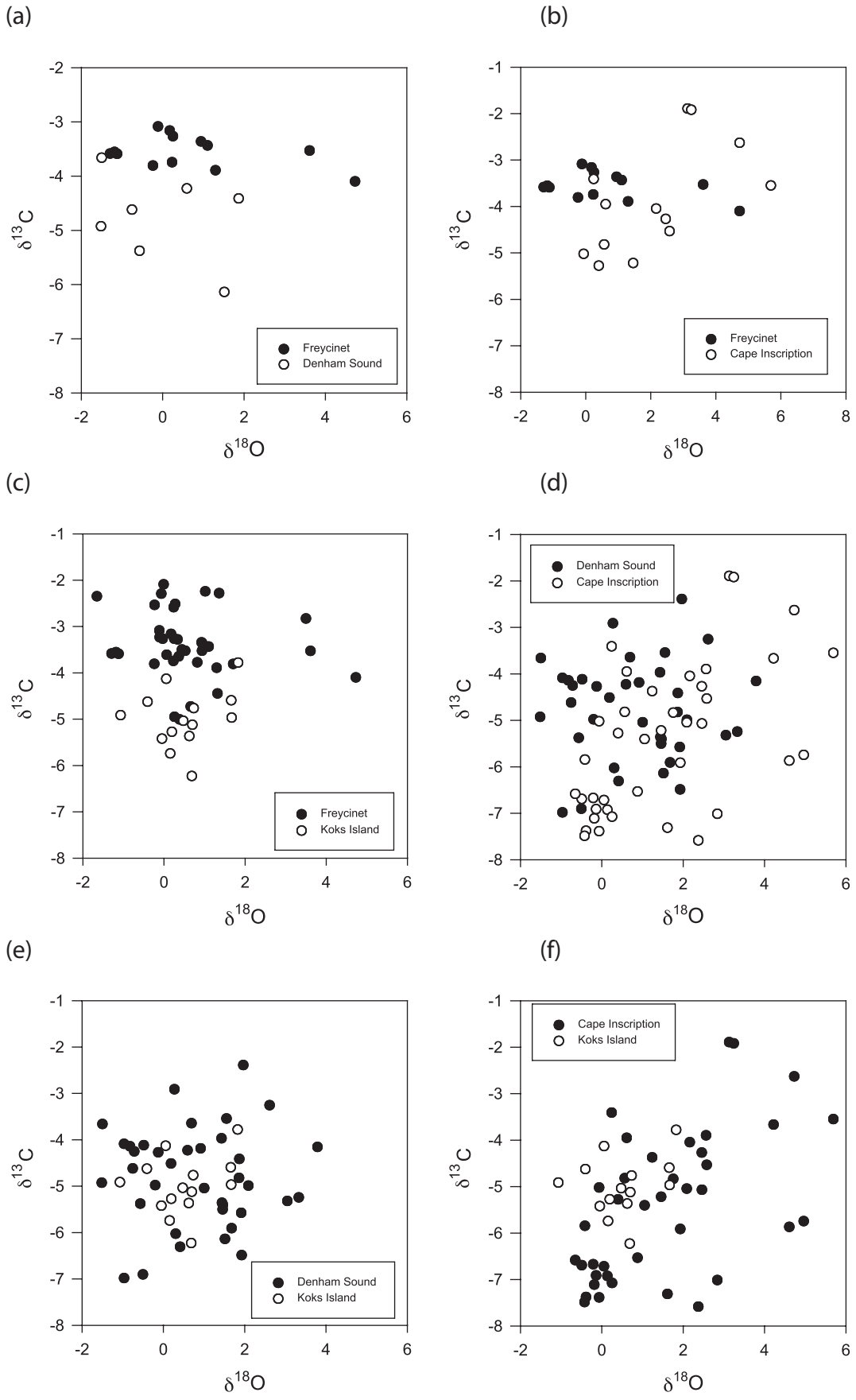


Figure 8. $\delta^{13}C$ vs. $\delta^{18}O$ of the juvenile portion of adult snapper otoliths for each location combination. Note that the Figures shown correspond to the data sets used in the final comparison and sample size thus varies (i.e. some data were removed to standardize the length categories examined).

Freycinet and Koks Island

Significant stable isotope and trace element differences indicate that snapper from Freycinet and Koks Island occupied distinct habitats as recruits (Table 5). All six tests found significant differences, indicating a very high probability of distinct juvenile groups at these two locations. $^{88}Sr/^{44}Ca$, $^{138}Ba/^{88}Sr$, $\delta^{18}O$ and $\delta^{13}C$ were all significantly different in recruits from Freycinet and Koks Island (Fig. 7c, Fig. 8c) which suggests separation between fish from these two locations (Table 5).

Denham Sound vs Cape Inscription

Significant stable isotope and trace element differences indicate that snapper from Denham Sound and Cape Inscription occupied distinct habitats as recruits (Table 5). Five of the six tests found significant differences, indicating a high probability of distinct juvenile groups at these two locations. There were significant differences in both $^{88}Sr/^{44}Ca$ and $^{138}Ba/^{88}Sr$ between Denham Sound and Cape Inscription (Fig. 7d). $\delta^{13}C$ varied significantly between the locations although no significant difference was detected in $\delta^{18}O$ (Fig. 8d).

Denham Sound vs Koks Island

Significant trace element differences indicate that snapper from Denham Sound and Koks Island occupied distinct habitats as recruits (Table 5). The balance of significant and non-significant results indicate that juvenile snapper from Denham Sound and Koks Island are not as strongly site specific as those from within the western gulf. Elemental analysis showed separation between the Denham Sound and Koks Island fish (Fig. 7e) although stable isotope analysis did not (Fig. 8e).

Cape Inscription vs Koks Island

Significant stable isotope and trace element differences indicate that snapper from Koks Island and Cape Inscription occupied distinct habitats as recruits (Table 5).The balance of significant and non-significant results indicate that juvenile snapper from Cape Inscription and Koks Island are not as strongly site specific as those from within the western gulf. $^{138}Ba/^{88}Sr$ was significantly higher in the Cape Inscription fish than in Koks Island fish (Fig. 7f). Conversely, stable isotope analysis showed no significant difference between these two locations (Fig. 8f).

Discussion

Objective 1 To establish whether trace element and isotope composition in otoliths is diagnostic of location on a transect from inner Shark Bay, through Denham Sound, to the oceanic waters

The results show that exposure to habitat and environmental conditions (chemical and physical characteristics of ambient water) differs between the locations in Shark Bay and produces distinct otolith elemental signatures. While trace element and isotope composition in otoliths is certainly sufficient to detect that there is a degree of site fidelity amongst juvenile snapper it is not yet possible to use these chemical data to assign individual snapper to particular locations in all cases.

It has been reported that strontium concentration in seawater is positively correlated with salinity (Limburg 1995). Strontium has a similar ionic radius and valence to Ca, it is regularly incorporated into otoliths in direct proportion to ambient conditions (Farrell and Campana 1996, Secor and Rooker 2000).

Freycinet has elevated salinity (metasaline) because of high evaporation, low rainfall and virtually no runoff from rivers (Logan and Cebulski 1970). Otoliths of fish from Freycinet are therefore expected to have higher levels of strontium. This provides the basis for identifying which snapper were located in Freycinet as juveniles. Thus, $^{138}Ba/^{88}Sr$ values <0.005 distinguish western gulf snapper from oceanic snapper, but only when concomitant values for $^{88}Sr/^{44}Ca$ are >0.25 (Fig. 7c), otherwise there is a high chance that Koks Island snapper could be misclassified as gulf fish. However, the Freycinet snapper could be distinguished from all others by employing the above criteria (i.e. $^{138}Ba/^{88}Sr$ values <0.005) along with a second criterion requiring that $\delta^{13}C$ be >4.0 (see Fig. 8a & b). Further work is required to determine if non-parametric multivariate techniques will be able to provide robust classification criteria for the other locations in Shark Bay.

Nevertheless, diagnostic chemical signatures are not required to proceed with developing a hypothesis of site fidelity for juvenile snapper in the western gulf of Shark Bay, as will be discussed next. The results from both the trace element and isotope techniques of analysing otolith chemistry found that the juvenile snapper from Denham Sound and Freycinet occupied different habitats; significant differences indicating that for the purposes of management, these groups can therefore be considered non-mixing. While there may be exchange of juvenile snapper between the regions, such exchange is not at a level that needs to be considered as important.

Objective 2 *To analyse cores of otoliths of adult snapper from the ocean and Denham Sound stocks, to establish the location of the nursery grounds of each stock*

Following the identification of non-mixing groups of juvenile snapper within the western gulf of Shark Bay, the juvenile portions of adult otoliths, were analysed in an effort to establish from which locality these older snapper originated as 0+ recruits. Figure 9 summarizes the results of the trace element and isotope ratio analyses. The analyses indicated that each group of adult snapper, in both the western gulf and outer Shark Bay had occupied distinct areas as juveniles and had thus acquired different chemical signatures in the otoliths. This provides clear evidence that snapper in Shark Bay do not mix widely through the entire region, but rather remain associated with a fairly limited spatial area.

We cannot determine precisely the extent of spatial movement but for the purposes of management can treat each of the western gulf locations examined (i.e. Freycinet and Denham Sound) as distinct nursery areas. The oceanic region (outer Shark Bay) also constitutes a nursery area for snapper, and in this more northern location there is also a degree of site fidelity for the juveniles. Snapper in the Shark Bay region use broad areas in their first year of life, both in the western gulf and in the outer bay. The eastern gulf is likewise used as a nursery area (M. Moran unpublished data). Because the 0+ snapper do not mix to any great extent between locations, in the interests of protecting recruitment to each location, nursery areas need to be carefully managed.

Objective 3 *To evaluate Laser Ablation, Inductively Coupled Plasma Mass Spectrometry as a tool for fish stock assessment and movement studies by comparison with concurrent stable isotope ratio and previous whole otolith trace element studies on Shark Bay snapper.*

In consideration of broader applicability, this Objective was included in the project to opportunistically contrast the results from the different data sets available for a complex of "stocks" for a single species in

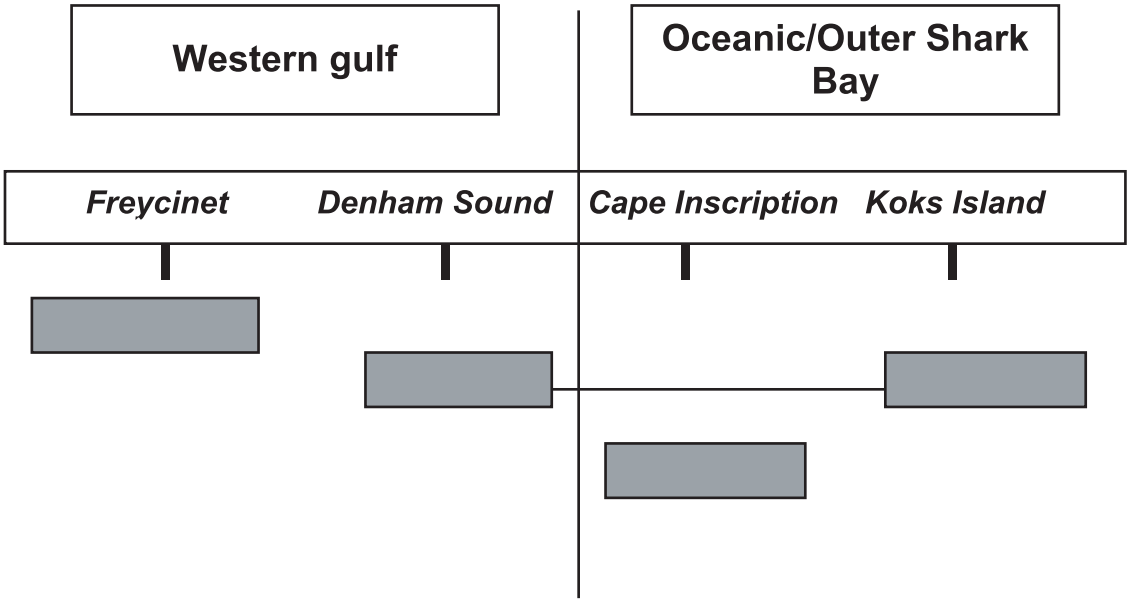
relatively limited geographic space. Figure 9 provides a comparison of the results for the stable isotope and trace element methods of examining otoliths from the present study. The key result to focus on regarding the relative merits was the fact that stable isotope ratios failed to detect a difference between snapper from Denham Sound and Koks Island, whereas trace element analysis found a clear difference. Furthermore, when comparing juveniles from Denham Sound and Freycinet, the trace element analyses produced considerably higher probabilities of there being a difference between the locations than did the stable isotope analyses. This latter technique was less discriminatory than trace elemental analysis for juvenile snapper at the time of this study.

In contrast, Bastow et al. (2002) used the stable isotope method on whole otoliths to determine that sub-adult and adult snapper (>250 mm LCF) from seven location in the Shark Bay region were essentially sedentary in habit for the majority of their lives. Given that the seven regions covered by Bastow et al. (2002) encompassed three of those used from this study (Freycinet, Denham Sound and Koks Island) for which trace element analysis indicated differences, we cannot assert that one method of analysing otolith chemistry is any better than the other. Thus, whereas in the current study site fidelity amongst snapper was most clearly demonstrated using trace element analysis of the juvenile portion of otoliths, Bastow et al. (2002) found similarly strong evidence for site fidelity using only stable isotope analyses of whole otoliths.

Edmonds et al. (1999) showed elevated strontium concentrations in whole otoliths of pink snapper sampled from Freycinet Estuary compared with those from normal oceanic water of Cape Inscription and Koks Island. This previous work indicated that adult snapper in these three locations, and from other locations along the WA coast that will not be considered here, were location specific. In the current project, elemental signatures in the juvenile portion of adult otoliths were significantly different between Freycinet, Denham Sound and the oceanic locations, as was also the case for the juveniles. The pattern was thus consistent with the previous work of Edmonds et al. (1989, 1999) that used whole otoliths of adult snapper. Figure 10 reconciles the stock discrimination evidence for Shark Bay adult pink snapper (Edmonds et al. 1989, 1999; Bastow et al. 2002) with that for 0+ snapper obtained in this study and provides a schematic representation of a scenario of recruit-adult spatial relationships.

In an exhaustive study of trace elements from snapper otoliths across several estuaries in New South Wales Gillanders et al. (2002) demonstrated that variability in trace elements within one locality could be sufficient to nullify their use as a useful stock discrimination tool. Although Shark Bay does not receive the freshwater inputs like the estuaries sampled by Gillanders et al. and thus would not be expected to incur the same variability in a suite of trace elements, our considerably smaller sample sizes over a limited period in comparison to this other study weakens our ability to infer if trace elements are a better technique than stable isotope ratios. Rather, the relative value of each technique may well depend on the system being studied; that is, the environment occupied by the population in question may have characteristics that make one method more amenable than the other. In terms of the primary objectives of this project, the important outcome is that both techniques have proven useful in developing a more comprehensive understanding of the spatial relationships of both pre- and post-recruit pink snapper in Shark Bay.

(a) Stable isotopes



(b) Trace elements

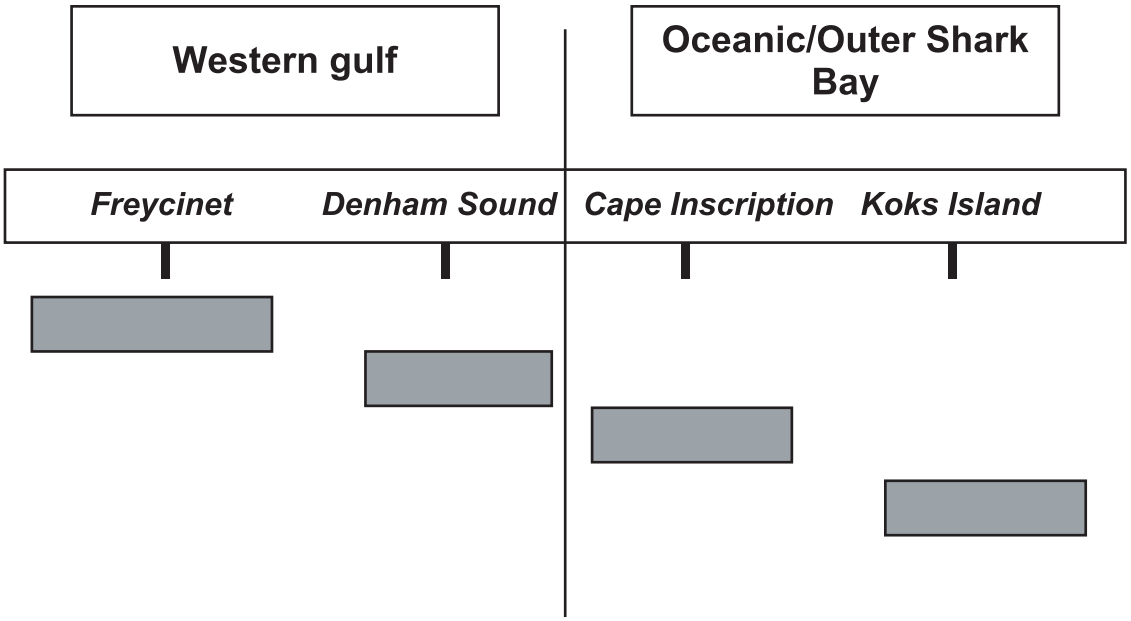


Figure 9. Schematic representation of the spatial relationships between origin of recruits for adult snapper from four locations in Shark Bay. Freycinet is the most southerly location, with Denham Sound, Cape Inscription and Koks Island lying progressively to the north. Because the second method (b) showed that all groups examined were different, this result is the same as that for when both types of analyses are combined. (b) thus represents the final result for this study.

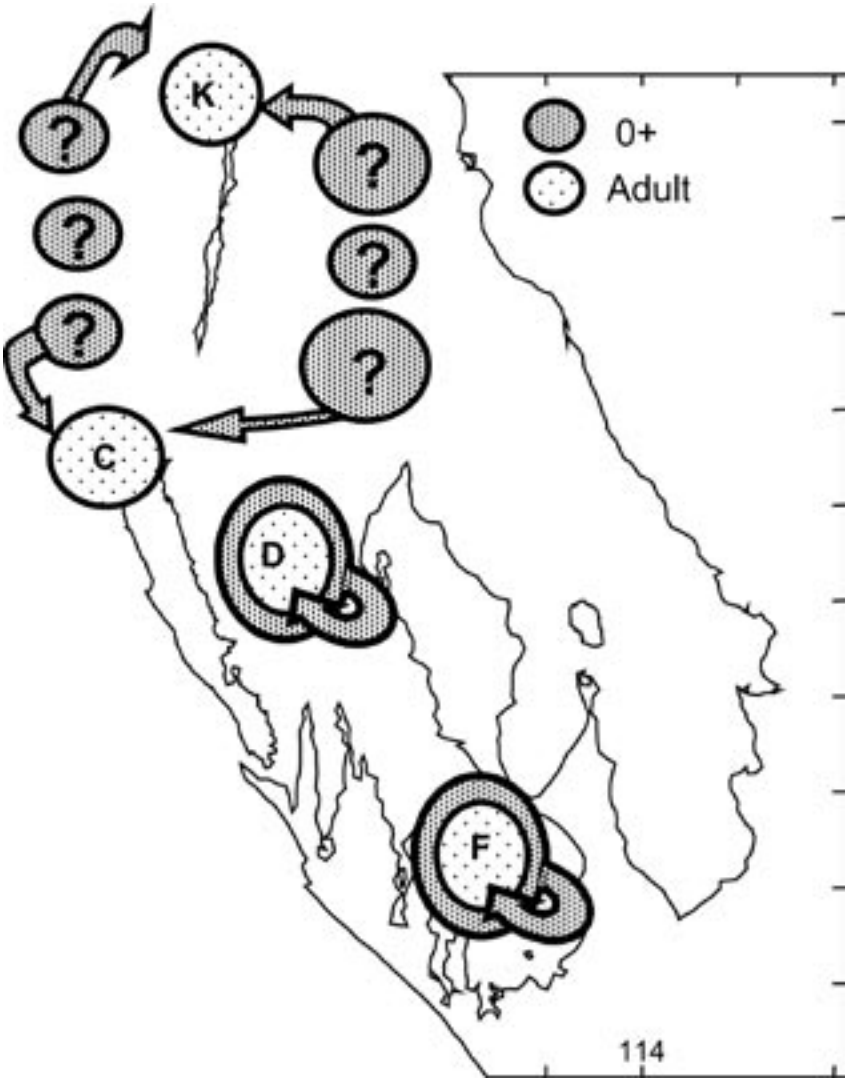


Figure 10. A schematic representation of a possible recruit-adult spatial relationship derived from the studies of adult- and recruit-stage snapper otolith chemistry. FI – Freycinet, DS – Denham Sound, CI – Cape Inscription, KI – Koks Island. The recruit areas with question marks indicate possible nursery areas for oceanic fish; the central areas of outer Shark Bay are known to be nursery habitat for juvenile snapper but were not sampled during this study. However, these are shown as distinct to reflect the significant difference in the recruit portion of adult otoliths from Koks Island and Caper Inscription. Juvenile snapper are also widespread in oceanic waters outside of Shark Bay. In contrast, the Denham Sound and Freycinet adults appear to have recruited from local nursery grounds. Note that the eastern gulf was not considered in this study and may contain a self recruiting adult stock as shown for the western gulf locations.

Benefits

While the stock structure of adult pink snapper in the inner and outer parts of Shark Bay have been relatively well studied, this has not previously been the case for juveniles. Although there is still some uncertainty over the spatial relationships among juveniles from different areas and between juveniles and adults, for the purposes of managing the snapper in Shark Bay the results from this study are sufficient to support a hypothesis of low levels of mixing of juvenile snapper in the different regions of Shark Bay, as has been found for the adults. The major benefit of this study is that it indicates that juvenile snapper from all regions of Shark Bay must be afforded a reasonable level of protection because those from any one region cannot be relied upon to provide recruits to other localities within Shark Bay. In particular, the primary concern and that which drove the implementation of this study, was the potential for prawn trawling in Denham Sound to negatively impact recruitment to the depleted snapper stock in the western gulf of Shark Bay. Now that site fidelity for juvenile snapper has been established, the case was made in the report by Moran and Kangas (2003) that a review of some of the management arrangement for the Shark Bay prawn industry is warranted. This project has assisted fisheries managers, and representatives from the Shire of Shark Bay, the Shark Bay prawn industry, the Denham Recreational Fisheries Advisory Committee, the Denham Professional Fishermen's Association, Recfishwest, the Recreational Fishing Advisory Council, the WA Fishing Industry Council and the Conservation Council of WA to better understand the spatial relationships of pink snapper in Shark Bay as they considered alternative management for the prawn industry at meeting of the *Denham Sound Snapper-Trawl Interaction Working Group* on 12 December 2003. As such, this working group recommended to alter a management boundary in the western Gulf of Shark Bay so as to reduce bycatch of juvenile snapper within Denham Sound; this project has thus formed part of a broader study (Moran and Kangas 2003) that is expected to result directly in a change to the relevant management plan.

Further Development

Because the findings presented in this report have been accepted as reasonable by managers (from Government, Community and Industry), no further major studies on stock structure of juvenile snapper would be required for the purposes of managing the incidental take of these juveniles. The *Denham Sound Snapper-Trawl Interaction Working Group* has recognized that the success of the snapper-trawl interaction agreement depends upon gathering further high-quality research and monitoring data. A broader, long term study on the changes in abundance of juvenile snapper in Shark Bay has been proposed, and would provide the detailed data against which to assess the efficacy of the management changes in terms of increasing recruitment to the Denham Sound snapper fishery.

Planned Outcomes

The finding that juvenile snapper in the Shark Bay region are location specific can now be considered as unequivocal within the management process for the prawn trawl industry in Shark Bay and for the management of the Denham Sound snapper fishery. Management deliberations have already considered these results and the recommendation for a closure to prawn trawling in the snapper nursery areas of Denham Sound made to the Minister for Fisheries.

Conclusion

This study has two main conclusions:

1. Groups of snapper occupying separate locations in the Shark Bay region as adults were also separate as 0+ juveniles.

2. Trace element analysis of otoliths is a good technique, and in some situations better than stable isotope analysis, for discriminating non-mixing groups of juvenile fish. The usefulness of each technique may depend on the species and environment that are being studied.

3. The results of this study have been utilized as part of a broader study that proved prawn trawling in Denham Sound negatively impacts on the abundance of juvenile pink snapper. Alternative management arrangements have now been recommended to mitigate these negative impacts.

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Appendices

Appendix 1: Intellectual property

No saleable items were developed during this project.

Appendix 2: Staff

Department of Fisheries: M. Baxter, T. Berden, Y. Chen, D. Gaughan, K. Hillier, G. Jackson, K. Longstaff, M. Moran, J. Norris, S. O'Hara, J. St John, T. Shepperd, N. Tapp, C. Wakefield

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