Stock structure and spatial dynamics of the warehous: a pilot study

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NON-TECHNICAL SUMMARY

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Objectives:

1. To determine a suitable approach for assessing stock structure in blue and spotted warehou.
2. To evaluate the use of otolith microchemistry as a means of examining migration in blue and spotted warehou.

Non Technical Summary

OUTCOMES ACHIEVED

The results of this study have been incorporated into the assessment models for blue and spotted warehou (*Seriolella brama* and *S. punctata*) in order to improve the efficacy and acceptance of the stock assessments for both species. The results have allowed managers of the Southern and Eastern Scalefish and Shark Fishery to assess the suitability of current management arrangements, particularly for blue warehou, which is currently managed as a single stock.

Blue warehou (*Seriolella brama*) and spotted warehou (*Seriolella punctata*) share a similar distribution throughout south-eastern Australia (NSW, Victoria, Tasmania and South Australia) and are also found in New Zealand waters. Both species are under quota for the trawl and non-trawl sectors of the Southern and Eastern Scalefish and Shark Fishery (SESS). Recent assessments of blue warehou indicate that biomasses are less than 30% of 1986-87 levels, both east and west of Bass Strait, and the stock is overfished, with declining catches (Smith 2002a). Results of the integrated analysis for spotted warehou indicate that the fishery is now impacting on the stock with current biomass levels at about 50% of that in the late 1980s. Future projections indicate that catches of around 3000t over the next 5 years lead to a 50% probability of being at 40% of the reference biomass (Smith 2002b).

Blue and spotted warehou are currently considered to be single stocks within south-eastern Australia for fisheries assessment and management purposes. However, both species exhibit complex spatial variability throughout the region and there is

increasing evidence to support a two-stock hypothesis for blue warehou. One of the key components to uncertainty in previous stock assessments of blue warehou was that model fits to data were very poor, assuming a single population across the fishery. Consequently, areas east and west of Bass Strait were modelled separately (Smith 2002a). However, fits of models themselves are not an adequate base for determining stock structure and there are a number of hypotheses that appear consistent with the existing information (eg. separate east and west stocks; one stock but the recruitment rates to the east and west differ among years; migrations between east and west). Clearly, the lack of information on stock structure and spatial dynamics will adversely effect the efficacy and acceptance of stock assessments of both species. Understanding stock structure and movement dynamics is thus an important requirement for ongoing quantitative assessment. To date, there has only been one study that has attempted to identify stocks of blue and spotted warehou using tagging but this was not successful (Knuckey et al. 1999).

In the current study, four methods of stock discrimination were used to assess the stock structure of both species in south-eastern Australia. The methods were 1) the analysis of morphological and meristic characters, 2) the analysis of mitochondrial DNA, 3) Fourier analysis of the shape of sagittal otoliths and 4) the analysis of otolith microchemistry. The primary focus of the project was stock structuring between east and west of Bass Strait so all four techniques were used to analyse fish from these regions. Tasmanian samples were only collected and analysed where possible.

As all these techniques can be expensive and sometimes provide ambiguous results, the aim was to undertake a pilot study to ascertain the most useful method prior to any fully study being undertaken. Although it was a pilot study, it was hoped that the preliminary results could be used in the assessment process to reduce uncertainty.

The study found that, for blue warehou, there were significant differences in all four parameters: morphology, mtDNA, otolith shape and otolith microchemistry between fish taken from east and west of Bass Strait. These results clearly indicated two separate stocks of blue warehou in the south-east region. The same four parameters did not differ for spotted warehou from east and west Bass Strait, indicating a single stock.

The relationship of east and west Tasmania to the Bass Strait areas and to each other was not as clear. For blue warehou, there were difficulties in obtaining west Tasmanian samples and only two of the four methods, morphometrics and mt DNA, were used to analyse east Tasmanian samples. Neither method showed clear differences between fish from east Tasmania and fish from either east or west Bass Strait, which indicates that fish from east Tasmania have had some degree of mixing with both stocks. Analysis of west Tasmanian samples is required to clarify the relationship between blue warehou from Tasmania and east and west Bass Strait.

For spotted warehou, both east and west Tasmanian samples were collected. Two of the four methods, morphology and otolith shape analysis, were used to analyse both areas and otolith microchemistry was used to analyse only east Tasmanian samples. Morphological analysis showed clear differences between the two Tasmanian areas but not between the two Tasmanian and two Bass Strait areas. Otolith shape analysis showed significant differences between the two Tasmanian areas as well as between each of the Tasmanian areas and each of the Bass Strait areas. The analysis of otolith microchemistry also showed a difference between east Tasmania and both east and

west Bass Strait. This may indicate some stock structuring around Tasmania for spotted warehou but further analysis is required to clarify the situation.

The results from the current study support the findings of Bruce et al. (2001) and Knuckey and Sivakumarna (2001) who reported separate spawning areas and different spawning times between east and west Bass Strait for blue warehou but not for spotted warehou. The results also support size and age data that shows differences between east and west Bass Strait for blue warehou but not for spotted warehou. There are, therefore, several lines of evidence that support a two-stock hypothesis for blue warehou and a single-stock hypothesis for spotted warehou in south-eastern Australian, although the relationship between Tasmania and Bass Strait is not clear.

One of the objectives of the study was to determine a suitable approach for assessing stock structure. All four methods identified two stocks for blue warehou and one stock for spotted warehou so any of these could be considered suitable, but the value of the study lies in the fact that multiple techniques showed the same result. This approach enables a higher degree of confidence in the identified pattern because the accuracy of any one technique remains unknown without the use of additional confirmatory evidence. Relying on a single technique that may or may not adequately represent the true stock structure has significant implications for the management and conservation of stocks so the use of at least two approaches simultaneously is advocated.

Decisions about which methods to use will depend on their cost, the spatial and temporal scales of management needs, the availability of necessary equipment and skills and the acceptance of the method by stakeholders. For example, the analysis of body morphology is the most commonly used method of stock identification because it is cheap and very easy to do but it may not be as sensitive as other methods. In the current study, otolith shape and otolith microchemistry analyses both detected differences between regions that were not detected by morphological analysis. In contrast, the analysis of otolith microchemistry provides very detailed information on population structure and, potentially, migration histories, but it is comparatively expensive, requires specialised equipment and a skilled technician and the level of detail obtained may not be required for practical fisheries management. Genetic analysis is also expensive and requires specialist equipment and skills but the level of acceptance amongst stakeholders is particularly high, which adds value to this method. The choice of stock identification techniques will therefore be situation-specific.

The second objective of the study was to evaluate the use of otolith microchemistry as a means of examining migration. The results of this technique can be difficult to determine because of the combined effects of physiological, ontogenetic and environmental influences on the deposition of elements. While some patterns across the otolith were observed, these were not consistent for fish within a region and it was therefore difficult to compare between regions. There is potential for this tool to be used to examine migration but it would require further sampling, analyses and manipulative experiments.

KEYWORDS: blue warehou (*Seriolella brama*); spotted warehou (*Seriolella punctata*); stock identification; comparative studies

Acknowledgments

Thanks to staff of the Integrated Scientific Monitoring Program (AFMA Project R00/0789) for collection of samples from on-board SEF vessels, processors, in ports and at markets (G. Cottier, P. McCoy, K. Smith and C. Fenner). Thanks also to T. Stokie for assistance with otolith preparation and to K. Krusic-Golub for ageing data. We gratefully acknowledge funding from the Fisheries Research Development Corporation (FRDC 2001/004).

FINAL REPORT

2001/004 Stock structure and spatial dynamics of the warehous: a pilot study

Background

Distribution and life history

Blue warehou (*Seriolella brama* Günther 1860) and spotted warehou (*Seriolella punctata* Forster 1801) share a similar distribution throughout south-eastern Australia (New South Wales, Victoria, Tasmania and South Australia) and are also found in New Zealand waters. Adult blue warehou are caught in depths up to 500 m, although most commercial catches occur from 50 to 300 m, while adult spotted warehou are caught in depths up to about 650 m. Spawning for both species occurs during late winter/spring. Small juveniles are pelagic, commonly occurring in association with jellyfish in open coastal waters, and sub-adults often occur in the sheltered waters of large marine embayments (Last et al. 1983). Both species grow rapidly, mature at 3- 4 years, have a maximum length of about 65 cm and a maximum age of approximately 20 years (Central Ageing Facility, pers. comm.). These species are two of the 17 species or species groups that provide the bulk (>80%) of trawl landings in the South East Fishery (SEF).

Catches, catch rates and TACs

Spotted warehou are closely related to blue warehou and mixed catches do occur. This has led to confusion between the species in early catch statistics. This was most apparent in comparisons between logbook and "verified" catch data. Extensive examination of SEF1 data indicated that logbook records were the most accurate. In addition, earlier catch statistics were for the species combined, commonly recorded as "Tassie trevally" (Smith 2002a, 2002b).

In 1992, a total allowable catch (TAC) covering both species was introduced but separate TACs have been set since 1993. A non-trawl TAC with individual transferable quotas (ITQs) was introduced for blue warehou in 1998 and for spotted warehou in 2001. The Tasmanian Government manages the non-trawl fishery in coastal waters of that State.

The annual trawl catch of blue warehou peaked in 1991 (about 1300 t) and then declined to an average of about 900 t from 1993 to 1998. In 1999, there was a significant drop in the trawl catch to 355 t and catches have remained low since. The landed weight of blue warehou for the trawl sector was 434 t in 2000 and 298 t in 2001. Unstandardised trawl catch-rates have declined since 1990 and have triggered the stock's reference point in every year since 1995 (Smith 2002a).

Landings from the gill-net fishery for blue warehou peaked at about 1700 t in 1990, but then declined sharply. The non-trawl catch of blue warehou was 288 t, 82 t and 31 t in 1999, 2000 and 2001, respectively. Landings for the Tasmanian inshore fishery have followed a similar pattern to both the trawl and non-trawl sectors of the SEF, declining from highs in the early 90s to 185 t, 95 t and 30t in 1999, 2000 and 2001, respectively. The 2001 total catch (all sectors, including Tasmania) of about 360 t was the lowest recorded in the time series. It represents only about 12 % of peak landings

in 1990 and 1991.

The agreed TAC for trawl-caught blue warehou was reduced from 1000 t to 700 t in 1997 in response to declining catch rates but, in 1998, a 'global' TAC was introduced which allocated nearly 1000 t to the non-trawl sector. Despite reductions to both the trawl and non-trawl agreed TACs in the following years, actual TACs continued to be close to 2000 t in 1999 and 2000 due to the absence of proportionate reductions in carryover. By 2002, however, the agreed and actual 'global' TACs were both approximately 600 t (250 t trawl, 350 t non-trawl, excludes the Tasmanian fishery).

In contrast to blue warehou, spotted warehou catches have increased from 1922 t in 1992 to 3726 t in 2000, although landings in 2001 were slightly down on the previous year (3294 t). The increased annual catches in recent years can be mostly attributed to greater landings off the west coast of Tasmania with over 1700t recorded in 2001. In this area, catches are linked closely with the spawning blue grenadier fishery. Catches in the other main areas have remained relatively stable with the exception of Eastern A (NSW) where catches have declined markedly in recent years. Annual catches peaked at over 500 t in 1994 and 1995, but subsequently declined to 13t in 2001. Unstandardised catch rates triggered the stock's reference point in 1996 but have since increased (Smith 2002b). Non-trawl catches have been relatively small. The non-trawl sector landed less than 1 t in 2001, which was down from the 5 t landed in 2000.

The agreed trawl TAC increased from 2500 in 1997 to 3500 in 1998 and then to 4000 in 1999. The agreed trawl TAC for 2001 remained at 4000 t, but an additional 80 t was allocated to the non-trawl sector. In 2002, the trawl sector TAC was increased to 4,399 t and the non-trawl sector to 89 t, yielding a 'global' TAC of 4488 t. The actual 2002 'global' TAC, incorporating carryover, was 5355 t.

Assessments

The Blue Warehou Assessment Group (BWAG) was established in 1997 and conducted the first formal assessment of the species in 1998 using Virtual Population Analysis (VPA). Integrated analysis was used as the basis for the 1999 assessment and this showed that the VPA results were less reliable than previously believed. BWAG continued to use the integrated analysis model in 2000 but more extensive evaluation of sensitivity and the ability of the model to fit the data identified that the assessment results were less reliable than was considered to be the case in 1999. One of the key components to this uncertainty was that model fits to data were very poor, assuming a single population across the fishery. For the purposes of the assessment, two areas - east and west of Bass Strait - were modelled separately. However, this was not the only way to deal with the problem of fitting a model to these data and it was argued that future modelling should deal with spatial aspects more explicitly. In 2001, BWAG decided not to undertake another quantitative assessment until a number of key uncertainties were resolved. Consequently, BWAG resolved to re-evaluate the major data inputs to the assessment. Revised methods of standardising catches were developed and ages from sectioned otoliths estimated. During 2002, BWAG continued to refine data inputs and commenced a revised quantitative stock assessment.

Irrespective of uncertainties, the assessments indicate that recent biomasses are less than 30% of 1986-87 levels, both east and west of Bass Strait, and the stock is overfished, with declining catches (Smith 2002a).

Assessments of spotted warehou were limited to descriptions of fishery and biological characteristics until 2000 when all the data for this species was examined closely. The 2001 assessment was the first formal stock assessment of spotted warehou. The assessment was conducted by BWAG/BGAG (Blue Grenadier Assessment Group) using an integrated analysis model that takes into account retained and discarded catches, catch rates, and size and age. In 2002, this assessment was extended to include projections on the impact of future catches. There remains considerable uncertainty with aspects of the assessment. The model does not fit the catch rate trends well and confidence intervals around current and reference biomass (defined as the average biomass estimated for the 3 years 1986/87 to 1988/89) are wide (Smith 2002b).

The results of the integrated analysis indicate that the fishery is now impacting on the stock with current biomass levels at about 50% of that in the late 1980s. Future projections indicate that catches of around 3000t over the next 5 years lead to a 50% probability of being at 40% of the reference biomass (Smith 2002b). The current status of spotted warehou is uncertain (Tilzey 2002).

Stock structure and spatial dynamics

A major uncertainty in the assessment process is stock structure. The stock structure of the warehous in Australian waters is unknown, yet single SEF stocks are assumed for fisheries assessment and management purposes based on perceptions that these species are highly mobile with a broad distribution of breeding locations.

Increasingly, evidence suggests that there may be separate stocks east and west of Bass Strait for blue warehou and a single stock for spotted warehou in south-eastern Australia. Two pieces of evidence come from a study that examined archived ichthyoplankton samples collected over broad areas of southern Australia (Bruce et al. 2001). In this study, blue warehou larvae were recorded from Kangaroo Island, South Australia, to southern New South Wales (NSW) and spotted warehou larvae were found from western Tasmania to southern NSW. The abundances of small larvae (<5.0 mm body length) were highest for both species off western Tasmania and southern NSW. While low but consistent numbers of spotted warehou larvae were found throughout the range, blue warehou larvae were not found between southern NSW and southern Tasmania (including Bass Strait). The data potentially suggest separate spawning areas for blue warehou west and east of Bass Strait and a more continuous link between the two areas for spotted warehou. Additionally, Bruce et al. (2001) calculated that the timing of the spawning events was different between areas for blue warehou. The timing of spawning from back-calculated age data was consistent with that derived from GSI data by Knuckey and Sivakumaran (2001) who also reported that blue warehou east of Bass Strait spawned approximately one month earlier than those west of Bass Strait. For spotted warehou, there was considerably more overlap in back-calculated spawning dates between areas.

In addition to evidence of separate spawning areas and different spawning times between areas for blue warehou, there have been consistent differences in the size of fish taken east and west of Bass Strait for a number of years. In 2001, landed catches from the trawl sector to the east ranged in length from 25 to 55 cm (length to caudal fork - LCF) and the distribution comprised three modes whereas to the west, fish ranged in length from 25 to 45 cm LCF and the distributions were uni-modal. Mesh net caught fish ranged in length from 35 to 55 cm LCF with most in the 45 – 55 size

classes (Smith 2002a).

Similarly, a comparison of sectioned otoliths from blue warehou taken from east and west of Bass Strait revealed clear differences in size-at-age and otolith weight versus age. Growth curves were significantly different between east and west of Bass Strait (Figure 1, Smith 2002a).

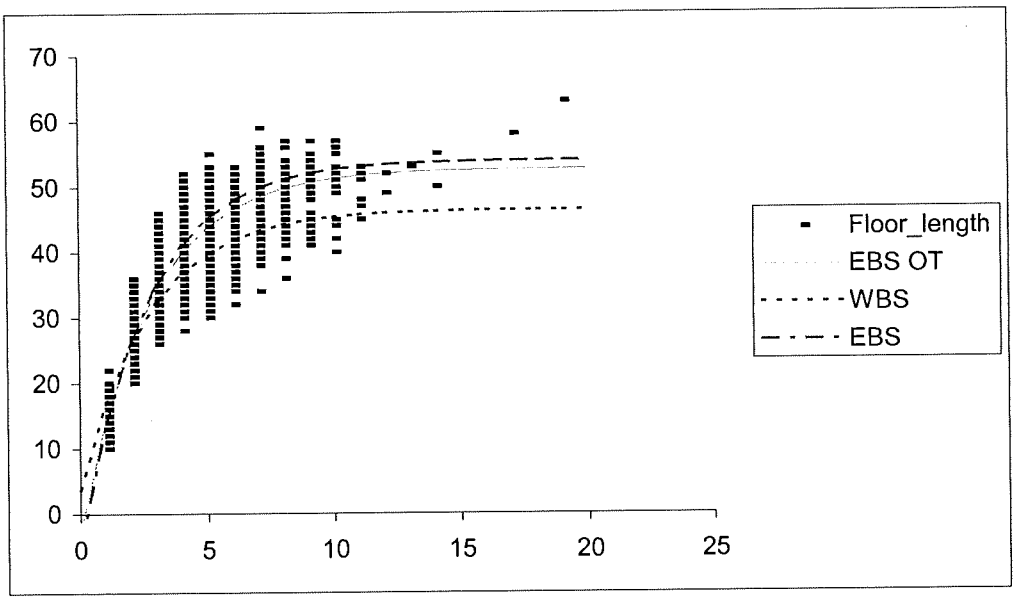

Figure 1. Von Bertalanffy growth curves for blue warehou taken west of Bass Strait (WBS) and east of Bass Strait (EBS). EBS includes trawl and non-trawl catches, EBS OT includes trawl caught fish only.

In contrast to blue warehou, size distributions for spotted warehou have not shown differences between east and west Bass Strait. Since 1991/92, size distributions, sampled from trawl landings from both areas, have indicated clear modal progressions. A mode of small fish (27 to 33 cm LCF) entered the fishery in 1995/96 and has been followed with the modal size increasing in subsequent years. For mesh-net caught fish, length frequencies were primarily uni-modal with most fish in the 45 to 55 cm length classes which reflects the selectivity of the fishing gear rather than the size distribution of the population. During 2001/02, size and age composition were consistent with trends seen in previous years. There was some variability in the proportion of young fish in samples from different areas. Off western Tasmania, fish less than 30 cm were not recorded. Size distributions from east of Bass Strait and west of Bass Strait were multi-modal with modes 30-35 and 45-50 cm LCF. In addition, a mode was also apparent at around 25-30 cm for the samples off NSW. Reasons for the differences are unclear.

Spotted warehou were aged by examination of sectioned otoliths. There appeared to be no difference in growth east and west of Bass Strait.

A pilot study was conducted in 1999 in an attempt to use tagging as a means of identifying stocks of blue and spotted warehou. This method was not successful due to low recapture rates and problems with obtaining specimens that had not been fatally damaged by the fishing method (Knuckey et al. 1999). Other than this one study, no

research has been conducted to ascertain the stock structure of either of these species in Australian waters.

Current Study

In order to improve stock assessments for both species, information on stock structure and movement dynamics in south-eastern Australia is required. The current study aims to address this by assessing a series of methods used to discriminate fish stocks: morphology, genetics, otolith morphology and otolith microchemistry, to determine which can provide the most information on stock structure prior to a full study being undertaken. Otolith microchemistry was additionally assessed for its ability to identify migration patterns. In this study, the term stock is used to refer to a management unit, that is fishing one unit does not effect another.

Morphology – morphometrics and meristics

The analysis of morphometric and meristic characters is one of the oldest and most commonly used methods of stock identification (Swain and Foote 1999). Meristic characters are the numbers of discrete, serially repeated, countable structures like vertebrae and fin rays. Morphometric characters are continuous characters describing aspects of body shape. Variation in such characters has both environmental and genetic components.

Genetics – mitochondrial DNA

Over the last decade, mitochondrial DNA (mtDNA) has been used extensively in stock discrimination studies (Ovenden 1990). In animal cells, small amounts of DNA are found outside the nucleus in organelles such as the mitochondria. A region within the mitochondria, the control region, contains sequences essential for the initiation of transcription and translation (Wolstenholme 1992). The DNA in this region is non-coding and highly variable with a number of different haplotypes normally observed within species. It is therefore useful in assessing variation between different populations of the same species.

Otolith morphology

The use of Fourier analysis on the shape of sagittal otoliths can be a successful technique for the delineation of fish stocks (Castonguay et al. 1991, Campana and Casselman 1993, Friedland and Reddin 1994). Smith et al. (1997) demonstrated differences in the shape of otoliths from orange roughy stocks between the summer and winter fishing grounds using Fourier analysis techniques developed at the Central Ageing Facility (CAF).

Otolith microchemistry

Recent work has highlighted the potential for otolith microchemistry to provide detailed information on population structure and migratory histories of fishes (Campana 1999, Thresher 1999). Although otoliths are primarily composed of calcium carbonate in a protein matrix (Degens et al. 1969), a variety of other chemical elements from the fishes environment are incorporated in minute quantities (Campana and Gagne 1995). Because the otolith grows continuously over the life of the fish, it can provide a chronology of the chemical environment experienced by a fish over its life. The basic theory behind the technique is that when fish are resident in water bodies that differ in either their physical (ie. temperature, salinity) and/or chemical characteristics, the differences will show up as a differences in the chemical

composition or abundance of certain diagnostic elements within their otoliths. Fish that live in different water bodies may develop distinct elemental or chemical signatures in their otoliths that enable them to be separated from each other. Spatial variation in the chemical composition of fish otoliths can be used to investigate stock structure (Edmonds et al. 1989, Edmonds et al. 1991, Edmonds et al. 1992, Edmonds et al. 1999, Campana and Gagne 1995, Kalish et al. 1996). Fine scale sample methods such as laser ablation also allow investigation of variation in otolith microchemistry across the life of individual fish. This methodolgy can be used to provide information on connectivity between juvenile nursery areas and adult populations (Thorrold et al. 2001, Gillanders 2002b). Variations in the concentration of elements across an otolith may also provide information on the environmental variation experienced by a fish over its entire life, which can in turn lead to information on the migratory history of the fish (Secor 1992, Secor et al. 1995, Campana 1999).

Need

Blue and spotted warehou are currently considered to be single stocks within south-eastern Australia for fisheries assessment and management purposes. However, both species exhibit complex spatial variability throughout the region and there is increasing evidence to support a two-stock hypothesis for blue warehou. One of the key components to uncertainty in previous stock assessments of blue warehou was that model fits to data were very poor, assuming a single population across the fishery. Consequently, areas east and west of Bass Strait were modelled separately. However, fits of models themselves are not an adequate base for determining stock structure and there are a number of hypotheses that appear consistent with the existing information (eg. separate east and west stocks; one stock but the recruitment rates to the east and west differ among years; migrations between east and west). Clearly, the lack of information on stock structure and spatial dynamics will adversely effect the efficacy and acceptance of stock assessments of both species. Understanding stock structure and movement dynamics is thus an important requirement for ongoing quantitative assessment.

The aim of the current study was to assess a suite of tools to determine which could provide the most information on stock structure of the warehous in south-eastern Australia. The methods assessed were morphology, genetics, otolith morphology and otolith microchemistry. As all these techniques can be expensive and sometimes provide ambiguous results, the aim was to undertake a pilot study to ascertain the most useful method prior to any full study being undertaken. Although a pilot study, it was hoped that the results could assist BWAG to weight the hypotheses used in the modelling and hence reduce uncertainty in the assessment.

Objectives

1. To determine a suitable approach for assessing stock structure in blue and spotted warehou.
2. To evaluate the use of otolith microchemistry as a means of examining migration in blue and spotted warehou.

Methods

Samples

After consultation with Dr Nick Robinson (Victorian Institute of Animal Science - VIAS) on the logistics of genetic analysis, a sample size of 30 fish from each area was agreed upon. This is a small number but as the aim of the project was to assess several different techniques for gaining information on stock structure, this sample size was considered sufficient for the purpose of the study and was also cost-effective. Additional samples were collected where possible. The focus was on east and west of Bass Strait but samples were also collected from east and west Tasmania where possible. Location areas were within the zones of Eastern Victoria (hereafter referred to as east Bass Strait), Western Victoria (hereafter referred to as west Bass Strait), Eastern Tasmania (hereafter referred to as east Tasmania) and Western Tasmania (hereafter referred to as west Tasmania) as defined for the Integrated Scientific Monitoring Program (ISMP) - AFMA Project 00/00789 (Figure 2). These zones approximate the six sub-fisheries first described by Klaer and Tilzey (1994). The zones were re-named following a decision by the South East Fishery Assessment Group (SEFAG) in 2001 to move the boundary between the former Eastern Zone A (now NSW) and the former Eastern Zone B (now Eastern Victoria) fifteen minutes north to avoid crossing a major fishing ground. The other zone boundaries remained the same (Smith and Wayte 2002).

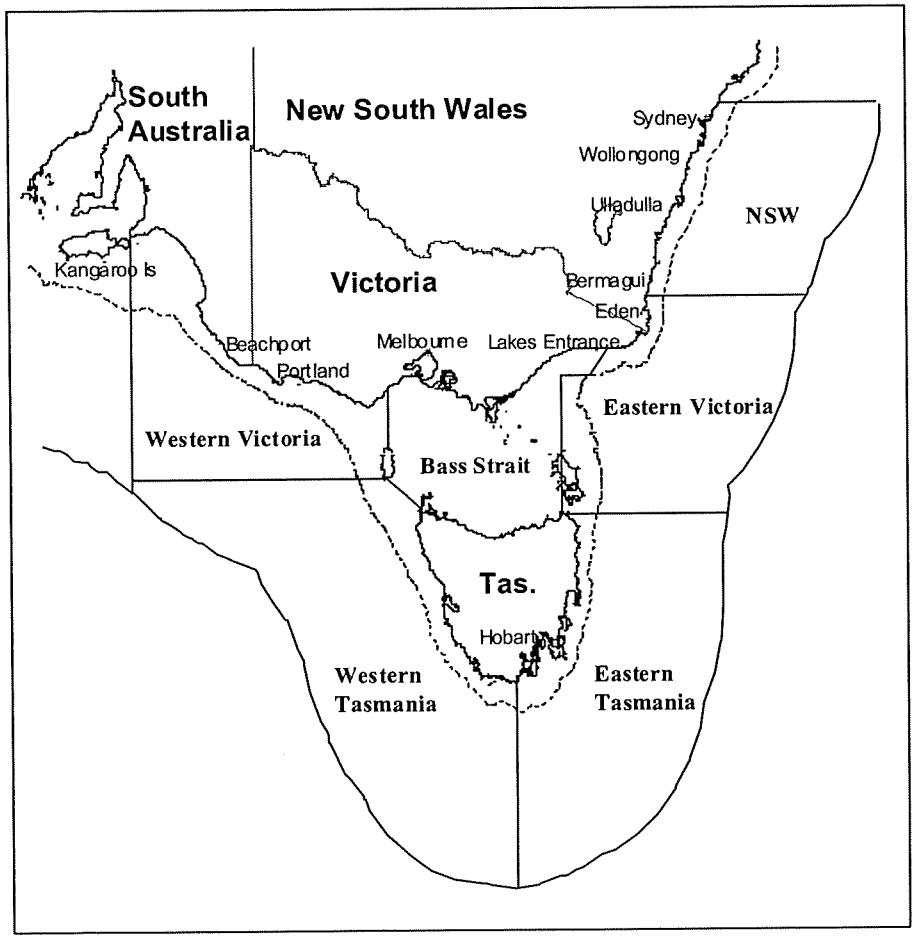

Figure 2. Sampling areas within the South East Trawl Fishery.

Staff from the ISMP collected a total of 445 fish (247 blue warehou and 198 spotted warehou) from February to October 2002 (Table 1). Samples were collected from on-board vessels, in ports, at markets and from processors. A total of 75 and 108 blue warehou and 31 and 56 spotted warehou were collected from east and west Bass Strait, respectively, while a total of 64 blue warehou and 111 spotted warehou were collected from the Tasmanian areas. Every effort was made to collect fish of a similar size for comparison between areas (Appendix 3). Smaller fish were also targeted for the otolith microchemistry component of the project.

The fish were processed as they arrived at the laboratories of Primary Industries Research Victoria, Marine and Freshwater Systems (PIRVic). Morphological and meristic data were collected from fresh samples. The sagittal otoliths were dissected with plastic instruments, rinsed clean of adhering tissue with Milli-Q water and air dried on lint free paper for at least 24 hours. Dried otoliths were registered at the Central Ageing Facility (CAF) and stored for shape and microchemistry analyses. Genetic samples were later extracted from frames frozen at – 4°C.

Table 1. Collections by species and area.

Species	Area	No. of samples
Blue warehou	east Bass Strait (EBS)	75
	west Bass Strait (WBS)	108
	east Tasmania (ET)	64
	west Tasmania (WT)	0
	Total	**247**
Spotted warehou	east Bass Strait (EBS)	31
	west Bass Strait (WBS)	56
	east Tasmania (ET)	51
	west Tasmania (WT)	60
	Total	**198**

Morphology

Samples were allocated consecutive numbers as they were processed. For each sample, the date and area of capture, sex and weight, date processed and the name of the processor and of the scribe were recorded. A total of 14 characteristic (11 morphometric and 3 meristic) were then measured to the nearest milimetre by the same recorder using a combination of calipers, measuring boards and rulers (Table 2). Measurements were taken from fresh samples as freezing changes body shape through cell contraction. Sexes were pooled for all analyses as warehous do not exhibit sexual dimorphism (Smith 2002a, 2002b).

Samples classed as immature (undeveloped gonads) were excluded from statistical analyses to minimise the effects of allometric growth. A total of 27 blue warehou were analysed from east Bass Strait, 75 from west Bass Strait and 57 from east Tasmania. No blue warehou were collected (or therefore analysed) from west Tasmania. For spotted warehou, 30 fish were analysed from east Bass Strait, 55 from west Bass Strait, 51 from east Tasmania and 59 from west Tasmania. The size composition of analysed samples is shown in Appendix 3. In order to minimise any

effect of size in the analysis, morphometric characters were standardised to fork length. Only morphometric characters were standardised because these characteristics are size dependent while meristic characters do not change beyond a threshold body size (Strauss 1985). Morphometric characters were standardised to body length using:

$M_s = M_o$ X (mean FL/FL)^b

where M_s is the standardised measurement, M_o is the original measurement, mean FL is the overall mean fork length of all samples (sexes combined), FL is the fork length of the individual and b is the slope of the regression on the logarithms of M_o and FL (Murta 2000). For meristic characters, we made the assumption that by excluding immature specimens, we excluded specimens below the threshold body size. These characters were not transformed.

Correlation coefficients between standardised characters were calculated in order to determine which, if any, characters were highly correlated and could be considered redundant and removed from the dataset. Non-metric Multi-Dimensional Scaling (MDS) was then performed for each species separately using Euclidean distance as the similarity measure to display between-sample similarities in two-dimensional space. Analysis of Similarities (ANOSIM) was then used to test the null hypothesis that groups did not differ from each other. The groups were specified *a priori* as the four areas – east and west of Bass Strait and east and west Tasmania. The SIMPER (similarity percentages) routine was then used to identify which character/s primarily accounted for observed differences between pairs of groups. Through the SIMPER routine, the overall percentage contribution each character makes to the average dissimilarity between two groups (an average of all possible pairs of dissimilarity coefficients, taking one sample from each group) was calculated. Characters were then listed in decreasing order of their importance in discriminating the two sets of samples (Clarke and Gorley 2001).

Table 2. Data collected on fresh fish samples and labels used for each character before and after standardisation for size.

Character	Acronym	After standardisation
	Morphometric	
fork length	FL	not standardised
head length	head_length	shead_length
first dorsal fin length	first_dorsal	sfirst_dorsal
second dorsal fin length	second_dorsal	ssecond_dorsal
length from snout to beginning of anal fin	length_snout	slength_snout
height of body	height_body	sheight_body
left eye diameter	left_eye	sleft_eye
inter-orbital distance	inter_orbital	sinter_orbital
length of left pectoral fin	length_fin	slength_fin
minimum height of tail peduncle	height_tail	sheight_tail
length of the left side of the maxilla	length_max	slength_max
	Meristic	
no. of rays in left pectoral fin	rays_left_fin	not standardised
no. of rays in second dorsal fin	rays_second_fin	not standardised
no. of rays in anal fin	rays_anal	not standardised

Genetics

Samples of muscle were extracted from frozen frames at PIRVic and transported to VIAS for analysis. A total of 40 blue warehou were analysed from east Bass Strait, 40 from west Bass Strait and 64 from east Tasmania. No blue warehou were collected (or therefore analysed) from west Tasmania. The blue warehou analysed from west Bass Strait were caught on two separate dates (20 individuals on 13 March 2002 and 20 individuals on 23 May 2002). These were compared to examine within region differences. Similarly, two capture dates were compared for the fish analysed from east Tasmania (31 individuals on 11 April 2002 and 33 individuals on 2 May 2002). Only one collection was analysed from east Bass Strait (25 May 2002). For spotted warehou, 30 fish were analysed from east Bass Strait (one date – 8 April 2002) and 56 from west Bass Strait (two dates - 36 individuals on 14 May 2002 and 20 individuals on 18 May 2002). Spotted warehou from east and west Tasmania were not analysed as the primary focus of the project was stock structuring between east and west Bass Strait. The size composition of analysed samples is shown in Appendix 3.

Total DNA was extracted from the muscle tissue of individual samples by a modified protocol described by Grewe et al. (1993). DNA samples were stored at -80°C for latter analysis.

The mitochondrial DNA (mtDNA) control region was amplified using oligonucletotide primers "PT" and "PU" from Jean et al. (1995) in a polymerase chain

reaction (PCR) on a GeneAmp PCR system 9700 thermocycler (Applied Biosystems). Amplification using the PT and PU primers was optimised by reducing the amount of $MgCl_2$ from 2.5 μM to 1.5μM and using a PCR touchdown program (20 cycles of 95°C for 30 seconds and 65°C for 30 seconds, 30 cycles of 95°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute and 1 cycle of 72°C for 10 minutes). Following PCR, a Shrimp Alkaline Phosphatase treatment was used to degrade single stranded DNA and inactivate the residual oligonucleotides (Innis et al. 1999). A BigDye terminator cycle ready reaction kit (Applied Biosystems) was then used to generate fluorescent labelled fragments. Labelled fragments were separated according to size and analysed using a 3700 DNA Analyser (Applied Biosystems) at 50°C for 2 hours and 46 minutes with 5,250 volts.

Once samples were run on the DNA analyser, sequences were transferred to Bionavigator (https://bn1.angis.org.au/bionav/cgi-bin/wrap.wp/gui/start) where they were aligned according to their similarity. Homologous sequences to the consensus sequence were found in GenBank (a database of nucleotide sequences derived from > 130,000 organisms) using the "basic local alignment search tool" at http://www.ncbi.nlm.nih.gov/BLAST/.

The mitochondrial DNA sequence profiles (electropherograms) from the DNA Analyser were visually checked and inaccurate base calls were replaced where clear signal peaks were recognised. The number of polymorphic sites was calculated using Molecular Evolutionary Genetics Analysis (MEGA, version 2.1) software program (Kumar et al. 2001).

Aligned sequences were imported into Arlequin Version 2.000, a computer software program for population genetics data analysis (Excoffier et al. 1992). Sequences were grouped into five groups based on their catch area and date. Group definition enabled analysis of variance within and between groups. An analysis of molecular variance (AMOVA) (Weir and Cockerham 1984) was used to calculate estimates of variance components, which reflect the haplotype diversity at different hierarchical levels (Excoffier et al. 1992). Genetic structuring was tested between different groups using an F_{ST} analogue (ϕ_{ST}, Excoffier et al. 1992). The number of genetically effective female migrants per generation (Nm) was estimated using the method of Hudson et al. 1992, and the software program DnaSP (DNA sequence polymorhpism) (Rozas and Rozas 1999). Calculations based on the number of different haplotypes within and between groups were also used to construct a Minimum Spanning Tree.

DnaSP was used to determine the distribution of the observed number of pairwise nucleotide differences between haploytpes (Rozas and Rozas 1999). Mismatch distributions were calculated for groups of both species and were compared with ideal expectations of exponential growth (shown by a poisson curve) and stable group sizes (shown by a geometric curve) (Lavery et al. 1996). A chi square test (χ^2) was performed to test for differences between the observed and expected (poisson or geometric) values from the mismatch.

Otolith morphology

Shape and morphometric analysis of the otoliths was undertaken using the programs developed by the Central Ageing Facility (CAF) for the purpose of stock discrimination (Morison et al. 1998, Smith et al. 2002). Where shape analysis is used to determine possible stock structure, samples between test areas need to be

comparative in size. Subsequent examination of the length frequency distributions for both species indicated that samples that had been collected by the time this part of the project was initiated were not suitable for comparative shape analysis. Consequently, samples of blue and spotted warehou otoliths were selected from samples held at the CAF. Samples were selected such that length frequency and otolith weight frequencies were similar and collection dates were within one financial year. This minimised temporal, fish size and otolith weight effects in the analysis. The size and otolith weight compositions of analysed samples are shown in Appendix 3.

A total of 78 blue warehou were analysed from east Bass Strait and 102 from west Bass Strait. No comparable samples of blue warehou were available from east and west Tasmania. For spotted warehou, 88 fish were analysed from east Bass Strait, 70 from west Bass Strait, 29 from east Tasmania and 42 from west Tasmania.

A colour image of each otolith was collected using the customised image analysis system developed in the CAF (Morison et al. 1998), and saved in Joint Photographic Expert Group file format (jpg) for subsequent analysis. Images were collected at a magnification of 4.0× (1× primary objective, 6.4× magnification and 0.63× secondary objective). Image size was 768 × 576 pixels.

The number of images initially collected for the shape analysis were 187 for blue warehou and 230 for spotted warehou. An image of each species is shown in Figure 3. All images were saved to CD for collection of Fast Fourier Transform (FFT) data. Three images from the collection of spotted warehou and 7 images from the blue warehou image collection could not be used after close examination of the images revealed damage to the otolith form.

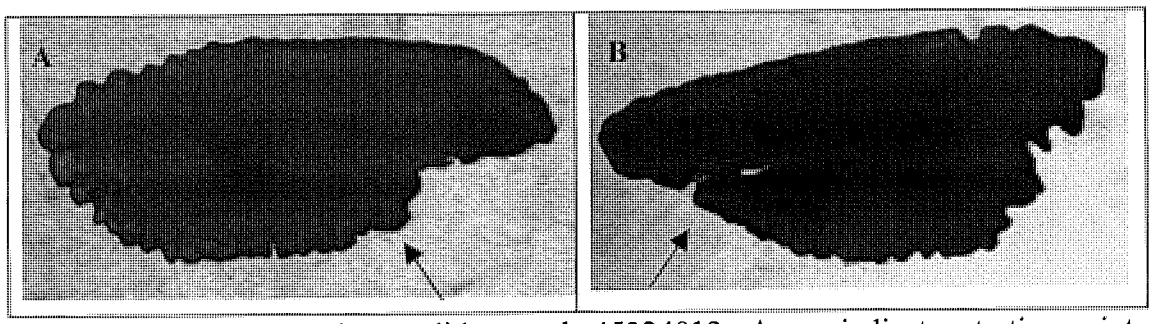

Figure 3. A) Blue warehou otolith, sample 45324012. Arrow indicates starting point for automated tracing. B) Spotted warehou otolith, sample 454145020. Arrow indicates starting point for automated tracing.

Biological data, otolith weights and image details were combined in MS Excel. Images were opened in Optimas™ and three automated pixel gradient tracings of the left otolith were performed. The first, second and third trace collected perimeter data, area data and *x-y* coordinate data for the Fourier series, respectively. Circularity was calculated as the perimeter squared over the area of the otolith. The data used to compute the fast Fourier transform (FFT) were collected as 128 equidistant points around the outline of the otolith. All automated tracing was started ventral to the anti-rostrum (Figure 3) and were performed in a counter-clockwise direction around the perimeter of the otolith. Data collected from the tracing routine were transferred automatically to MS Excel for later analysis via Dynamic Data Exchange (DDE).

Each of the *x-y* pairs of pixel co-ordinates was expressed as a complex number (real and imaginary numbers) for a Cartesian Fourier transform (Friedland and Reddin 1994). The FFT was calculated in Optimas and the resultant array of 128 complex numbers (Fourier descriptors) saved for later analysis. The 0^{th} descriptor was used to normalise for differences in otolith position and the 1^{st} descriptor to normalise for size and rotation. The remaining Fourier descriptors represented the otolith shape independent of its size, position or rotation.

As the number of Fourier descriptors increases, the resultant reconstructed shape converges to the original shape. To determine the appropriate number of Fourier descriptors, a range finding procedure was performed. Thirty-six otoliths from each species were randomly selected and the Fourier series calculated. The descriptors were normalised for position by setting the 0^{th} descriptor to $0+0i$. The shape was then reconstructed using the first (and last) descriptor. The number of descriptors was then increased by one and the shape reconstructed. This was repeated until 30 descriptors from each end of the complex FFT were used. The maximum error of 100% was defined as the difference between the inverse FFT reconstruction of the otolith outline using the full array of Fourier descriptors and the reconstruction using only the 2^{nd} and last elements of the original Fourier series. These distances were squared and summed, and expressed as a percentage (percent reconstruction error). This test determined the relationship between the number of descriptors and the accuracy of the reconstruction.

Previous studies have used a subset of the Fourier descriptors (Smith et al. 2002). The range finder tests showed that 30 Fourier descriptors from each end of the complex array failed to reduce the mean reconstruction error to within 5% of the original shape for both species. The mean reconstruction error for blue warehou after reconstruction was 7.36% (minimum: 4.56, maximum: 12.41%) and 5.92% (minimum: 4.05, maximum: 8.37%) for spotted warehou. As the criterion for the number of harmonics used in randomisations is determined as the number of Fourier descriptors required to reproduce the mean reconstruction error to below 5%, the full set of Fourier descriptors, and subsequent harmonics was used in the randomisations.

The absolute value (harmonic) of each of the Fourier descriptors in the shape vector was calculated as :

$$\text{Harmonic} = (a+b_j)^{0.5}$$

Where :

a = real component of complex number

b_j = imaginary component of complex number

The mean harmonic for each of the areas was then calculated. The estimator used in this study to test differences between the designated areas was the square root of the sum of the squared differences in the mean harmonics, termed harmonic distance.

This was calculated as:

$$DH_{jk} = \sqrt{\sum (\overline{H}_{ij} - \overline{H}_{ik})^2}$$

Where :

DH_{jk} = Observed harmonic distance between area j and area k

$\overline{H}_{ij}$ = i^{th} mean harmonic from area j

$\overline{H}_{ik}$ = i^{th} mean harmonic from area k

The matrix of Fourier descriptors for samples from the two areas being compared was thus reduced to one harmonic distance value.

A randomisation test was then applied to estimate the probability that the observed harmonic distance would occur by chance alone. Samples were randomly allocated to groups and a new harmonic distance calculated. This process was repeated 5,000 times and a distribution of values for randomised harmonic distance was obtained. The probability of obtaining the observed harmonic distance by chance was estimated as the proportion of randomisations for which the harmonic distance was greater than or equal to the observed harmonic distance. Pairwise tests were undertaken between areas.

The morphometrics of the otolith were also tested. Samples selected for the shape study were selected such that distributions of otolith weight and fish length were not significantly different as tested by the Median test.

The normality of blue warehou and spotted warehou otolith morphometric data (otolith circularity, otolith area and perimeter) was tested using Shapiro-Wilk tests. Distributions of otolith morphometric data were approximately normal for blue warehou samples from west Bass Strait but east Bass Strait samples were not normally distributed. Non-parametric tests (Median tests) were therefore used to test differences between east and west Bass Strait. Spotted warehou otolith area data was normally distributed for each of the four areas so differences were tested using a one-way analysis of variance (ANOVA). Spotted warehou otolith circularity and otolith perimeter data were not normally distributed for all of the test areas and differences between areas were tested using the Median test.

Otolith microchemistry

The elemental composition of otolith cores from young fish (hereafter referred to as 'cores') and of transects across entire otoliths from older fish (hereafter referred to as 'transects') was measured and analysed. For the cores component, a total of 35 blue warehou were analysed from east Bass Strait and 31 from west Bass Strait. For spotted warehou, 26 fish were analysed from east Bass Strait, 36 from west Bass Strait and 30 from east Tasmania. For microchemical analysis of the otolith, it is important that fish of the same age are compared between the test areas. This is because previous studies have demonstrated that while short term differences (i.e. months) in elemental composition may have minimal confounding affect on spatial discrimination

(Thorrold et al. 1998, Hamer et al. in press), long term differences (i.e. between-year classes) can be significant (Hamer et al. in press, Campana et al. 2000, Gillanders 2002a). In other words, fish of different year classes from the same stock could potentially show differences in otolith chemistry. Thus, to minimise these effects, the same age class was compared between areas. For the comparison of cores, sufficient 1 + year old blue warehou were collected from east and west Bass Strait but not from Tasmania, while sufficient 2 + years old spotted warehou were collected from east and west Bass Strait and east Tasmania but not west Tasmania. Fish were aged at the CAF using annual increments. For the transects component, 5 blue warehou were analysed from east of Bass Strait, 10 from west Bass Strait and 6 from east Tasmania. These were 4 + year old fish (average age was 4 years). For spotted warehou, 7 fish were analysed from east of Bass Strait, 8 from west Bass Strait and 6 from east Tasmania. These were 3 + year old fish (average age was 3 years). The size and age compositions of analysed samples are shown in Appendix 3.

Otoliths were weighed to the nearest 0.1 mg prior to mounting in epoxy resin (Struers epofix). Transverse sections of approximately 350 μm thickness were taken through the primordium. A continuous flow of Milli-Q water was used to lubricate the diamond blade while cutting. Sections were polished with three grades of aluminium oxide lapping film (30, 9, 3 μm) lubricated with Milli-Q water, with final thickness being approximately 300 μm. The polished sections were fixed to acid cleaned (10% HNO_3) microscope slides with Indium (In) doped epofix resin. The epoxy resin used for mounting and fixing was doped with approximately 30 ppm In as a resin indicator. Final cleaning involved a three minute immersion in Milli-Q water in an Ultrasonic bath, followed by a final triple rinse with Milli-Q water and drying inside a class 100 plastic laminar flow cabinet. Otolith slides were stored in sealed plastic containers until analysis.

Chemical composition of otoliths was determined using a Merchantek LUV 266™ Nd:YAG ultraviolet laser microprobe operated in Q switched mode in conjunction with a Finnigan MAT ELEMENT ™ high resolution inductively couple plasma double-focusing mass spectrometer (HR-ICP-MS). Ablation was conducted in helium that was mixed with argon for transport to the plasma and subsequent analysis in the mass spectrometer. A more detailed description of the system used in this study is provided by Lahaye et al. (1997). Operating conditions of the laser and HR-ICP-MS are outlined in Table 3.

Table 3. Details of the laser and ICP-MS operating parameters used in the study.

Operation	Parameters
Laser	
Wavelength	266nm
Mode	Q switched
Repetition rate	6 Hz
Energy	0.9-1mJ
Spot size	Cores 90-100 μm, Transects 20 μm
Mixing Chamber	He (0.36 L min^{-1})
HR-ICP-MS	
Resolution	300
Gas flow	
Coolant	14.00 L min^{-1}
Auxillary	1.55 L min^{-1}
Sample	1.50 L min^{-1}
Cone	Nickel
Detection modes	Analogue (Ca, Sr)
	Pulse counting (Mn, Zn)
	Both (Ba, Mg, Na)
Dwell time	10 ms
Channels/peak	3

The isotopes ^{23}Na, ^{24}Mg, ^{25}Mg , ^{55}Mn, ^{64}Zn, ^{88}Sr, ^{138}Ba were analysed routinely along with ^{44}Ca, which was used as the internal standard, and ^{115}In, which was the resin indicator (Table 4). While other elements were investigated in an initial pilot study the elements listed above were consistently above limits of detection (LOD). To eliminate any possibility of biases due to instrument drift or changes in performance from day to day, the analysis sequence was randomised and blocked with respect to capture area. The NIST SRM 612 glass standard was analysed for quantification of elemental concentrations. This standard was analysed every 10-12 ablations to further eliminate possible short-term drift effects. The concentration of Ca in otoliths was taken as 38.8 % by weight or 388,000 ppm following a previous determination of otolith Ca concentration (Yoshinaga et al. 2000). The average counts of a 15 scan blank acquired prior to each ablation were subtracted from the average sample counts before concentration calculations. Samples were acquired for 50 scans (approx. 40 secs) with the initial 10 scans being ignored to allow for pre-ablation and signal stabilisation. The ablation cell was purged for 20 seconds prior to each blank/sample acquisition. Data reduction was conducted offline. Data (counts s^{-1}) were converted to concentrations using the equation of Ludden et al. (1995):

$$[C_x]_{samp} = \frac{[I_{(m,x)}/I_{(m,\,Is)}]_{samp} \times (C_{Is})_{samp} \times (C_{m,x})_{std}}{[I_{(m,x)}/I_{(m,\,Is)}]_{std} \times (C_{Is})_{std}}$$

where; Is = internal standard, $I_{(m,x)}$=intensity at mass x of the element being quantified, and ($C_{m,x}$) is the concentration of the element of mass x in the external standard.

Blanks of 70 scans were acquired at the start and end of each session. The standard deviation of the blank was used in calculations of detection limits. Detection limits depend on the amount of material ablated and so were adjusted for each ablation based on ablation yield estimates. Average detection limits (ppm) were ^{23}Na: 1.5, ^{24}Mg: 0.36, ^{55}Mn: 0.4, ^{64}Zn: 0.26, ^{88}Sr: 0.57, ^{138}Ba: 0.02, ^{115}In: 0.26. Both accuracy and precision were estimated on a daily basis for the Nist 612 glass standard. Average precision estimates for individual elements measured as the mean relative standard deviation (RSD) for the Nist 612 glass standard were; Na: 9.7 %, Mg: 4.6 %, Mn: 3.4 %, Zn: 12.8 %, Sr: 6.7 %, Ba: 4.2 %, In: 7.7 %. Average accuracy for individual elements measured as mean percentage recovery for the Nist 612 glass standard were; Na: 100.7 %, Mg: 99.6 %, Mn: 99.5 %, Zn: 105.2 %, Sr: 98 %, Ba: 100.4 %, In: 100.7 %.

Analysis of otolith cores involved two adjacent ablations of approximately 90-100 μm diameter, situated in the core region of each otolith. Data from the two spots was averaged to provide the elemental concentrations used for statistical analysis.

Analyses of otolith transects involved programming the laser to ablate continuously as it moved very slowly (2 μm s^{-1}) along a pre-programmed transect from the otolith core to the edge. This produces data on chemical variation at a high spatial/temporal resolution across the otolith. A 20 μm spot size was used for transecting. Transects on all fish were placed along the ventral side of the sulcal groove (Figure 4). Replicated transects on different otoliths from the same fish produced similar patterns in chemical variation (Figure 5 and Figure 6). This suggests that the chemical variation shown by an individual transect was representing variations experienced by the fish and not just variation in incorporation of elements along the specific transect or otolith. One transect was therefore conducted on one randomly chosen otolith from each fish.

Table 4. Element and corresponding symbol

Element	Symbol
Sodium	Na
Magnesium	Mg
Manganese	Mn
Zinc	Zn
Strontium	Sr
Barium	Ba
Calcium	Ca
Indium	In

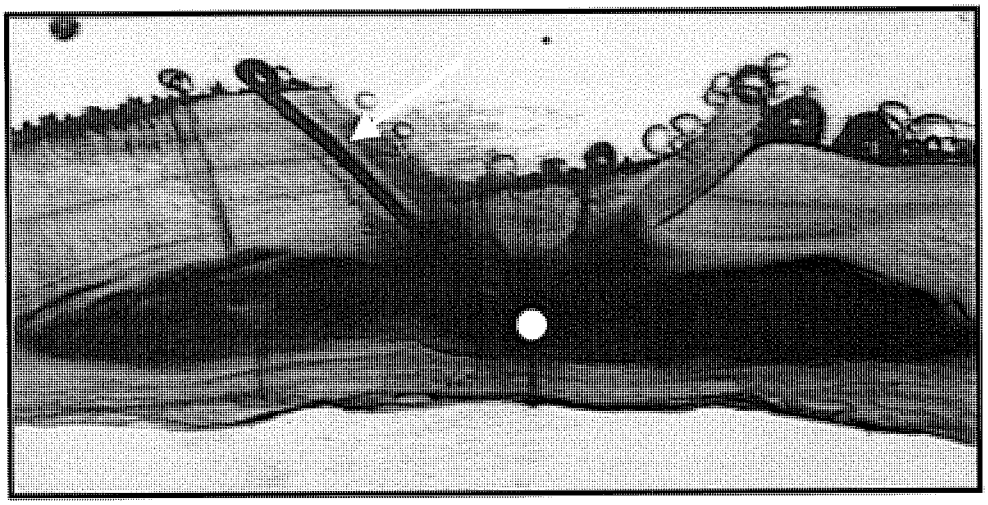

Figure 4. Image of warehou otolith showing path of continuous ablation transect (indicated by arrow) and core region (white circle). Ventral is to the left.

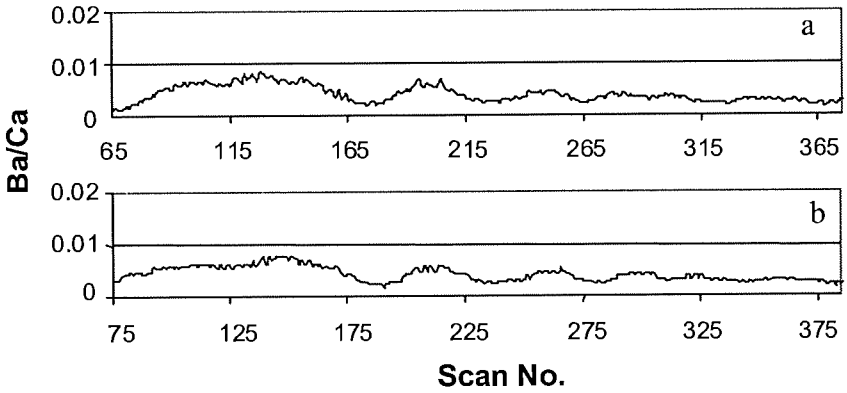

Figure 5. Continuos ablation transects of Ba/Ca across a similar transect path on left (a) and right (b) otoliths from a blue warehou.

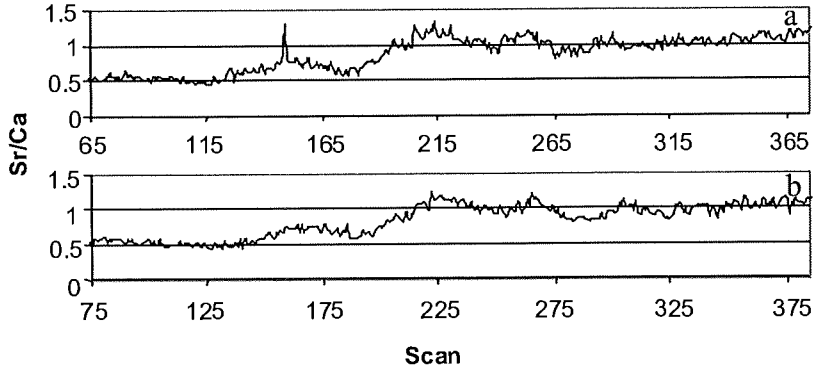

Figure 6. Continuos ablation transects of Sr/Ca across a similar transect path on left (a) and right (b) otoliths from a blue warehou.

For transect data, raw counts of each element were ratioed to the raw counts for Ca and line graphs of variation in elemental incorporation across otoliths were produced for initial qualitative comparisons between fish from different areas. Blanks of 20 scans were acquired prior to the start of each transect and subtracted from the sample data. Transects were also performed on the NIST 612 standard at the start of each session and after every fourth otolith transect. Average concentrations of individual elements across the entire otolith transect were also calculated using the average counts per second of each element for the entire transect and the same calculation method as described above.

Univariate analyses of variance (ANOVA) were used to test hypotheses relating to differences between areas in concentrations of individual elements in otolith cores. Assumptions of normality and homogeneity of variances were checked with box and residual plots. For both species, data for Ba, Mn, Mg and Zn required log transformation to meet assumptions for all analyses.

Multivariate analyses of variance (MANOVA) were used to investigate differences in multi-elemental composition of the otolith core between fish collected in the different regions. The Pillai trace statistic was used to test for significance in MANOVA as it is the most robust to deviations from multivariate normality (Quinn and Keough 2002). Quadratic discriminant function analysis (QDFA) was used to determine if blue and spotted warehou from the different areas could be distinguished from each other based on the multi-elemental composition of the otolith core. QDFA was chosen over linear discriminant function analysis because it does not assume homogeneity of within-group covariance matrices (Quinn and Keough 2002). Quadratic discriminant functions (QDF's) and classification accuracies using the 'leave one out approach' (ie. observation being classified is removed from the data set) were determined for each species. 'F to remove' statistics, which provide a measure of the contribution that individual variables make to discrimination, were used to assess which elements contributed greatest to discrimination power (Wilkinson et al. 1996). Canonical discriminant function plots were used to display spatial variation in the multi-elemental composition at the cores of otoliths where more than two classification groups were available (ie. spotted warehou). Where only two classification groups were available (i.e. blue warehou), only one discriminant function was calculated. In this case, the discriminant function scores were plotted in a frequency histogram to demonstrate the overlap or separation between fish from the different groups.

Initial analyses of the transect data involved comparisons of the plots of elemental variation across the otolith. The plots were examined visually to look for similarities in the patterns of chemical variation between fish from the same region and differences between fish from different regions. Initial observations of elemental variation across the otoliths of both species suggested that Sr and Ba showed the most variation in incorporation, so these elements were assessed further. Average concentrations of all elements (ie. Sr, Mn, Mg, Ba, Zn) were also calculated for each otolith transect. These overall concentrations were compared using similar statistical techniques as described above for the otolith core samples. Because these comparisons depended on incorporation across the whole otolith, otolith weight was included as a covariate for the ANOVA.

Results/Discussion

Blue warehou

Samples

The numbers of blue warehou samples analysed by each of the four methods of stock identification are summarised in Table 5. Samples were not collected from west Tasmania. Samples from the remaining three areas were analysed by three techniques, morphology, genetics and microchemistry of otolith transects. Only east and west Bass Strait samples were analysed by the otolith morphology and microchemistry of otolith cores methods. The 247 blue warehou samples collected ranged in size from 19 to 51 cm fork length (Appendix 3, Table 1). Each technique analysed a subset of these, with the exception of otolith morphology, which analysed a selection of archived samples from the 2001/02 financial year. This was because sufficient numbers of comparative samples had not been collected when the otolith morphology component of the project was initiated.

The size distributions of the samples analysed by each method are shown in Appendix 3 (Tables 2 to 9). From the subset of samples aged for otolith microchemical analyses, these fish ranged in age from one to four years old. One year old fish used in the comparison of otolith core microchemistry ranged in size from 19 to 35 cm (Appendix 3, Tables 6 and 7) while four year old fish used in the comparison of otolith transects ranged in size from 34 to 45 (Appendix 3, Tables 8 and 9). Samples were collected over a period of nine months, which may partly account for the considerable overlap in the size of different year classes. For example, one year old blue warehou collected in May 2002 were an average of 21 cm in length whereas one year old fish collected in October were an average size of 27 cm.

Table 5. Summary of the number of blue warehou samples analysed by each of the four techniques in each of the four areas. c: cores, t: transects, nc: not collected

Area	Morphology	mtDNA	Otolith Shape	Otolith microchemistry
east Bass Strait	27	40	83	c: 34, t: 6
west Bass Strait	75	40	104	c: 31, t: 11
east Tasmania	57	64	0	c: 0, t: 7
west Tasmania	nc	nc	nc	nc

Morphology

Correlation coefficients between blue warehou characters before and after size effect removal are presented in Table 6 and Table 7, respectively. There was a considerable reduction in values for all morphological characters (values higher than 0.90 are in bold) confirming the assumption that these characters are dependent on size. Meristic characters were not standardised for size as these are independent of size (see Methods).

Table 6. Correlation coefficients between characters before removal of the size effect. Values higher than 0.90 are in bold.

Variable	head_length	first_dorsal	second_dorsal	length_snout	height_body	left_eye	inter_orbital	length_fin	height_tail	length_max	rays_left_fin	rays_second_fin	rays_anal_fin
head_length	**1.00**	0.87	**0.97**	**0.98**	**0.92**	0.87	**0.95**	**0.96**	0.90	0.95	-0.09	-0.06	-0.31
first_dorsal	0.87	**1.00**	0.86	0.88	0.84	0.79	0.86	0.86	0.78	0.86	-0.08	-0.05	-0.31
second_dorsal	**0.97**	0.86	**1.00**	**0.96**	0.90	0.84	**0.93**	**0.94**	**0.91**	**0.93**	-0.12	-0.02	-0.29
length_snout	**0.98**	0.88	**0.96**	**1.00**	**0.91**	0.87	**0.95**	**0.96**	**0.91**	**0.96**	-0.10	-0.09	-0.33
height_body	**0.92**	0.84	0.90	**0.91**	**1.00**	0.77	0.88	0.89	0.82	**0.91**	0.00	-0.14	-0.36
left_eye	0.87	0.79	0.84	0.87	0.77	**1.00**	0.83	0.83	0.73	0.84	-0.05	-0.05	-0.21
inter_orbital	**0.95**	0.86	**0.93**	**0.95**	0.88	0.83	**1.00**	**0.92**	0.89	**0.92**	-0.10	-0.11	-0.34
length_fin	**0.96**	0.86	**0.94**	**0.96**	0.89	0.83	**0.92**	**1.00**	0.89	**0.93**	-0.10	-0.04	-0.32
height_tail	0.90	0.78	**0.91**	**0.91**	0.82	0.73	0.89	0.89	**1.00**	0.85	-0.19	-0.02	-0.32
length_max	**0.95**	0.86	**0.93**	**0.96**	**0.91**	0.84	**0.92**	**0.93**	0.85	**1.00**	-0.09	-0.11	-0.35
rays_left_fin	-0.09	-0.08	-0.12	-0.10	0.00	-0.05	-0.10	-0.10	-0.19	-0.09	**1.00**	0.00	0.23
rays_second_fin	-0.06	-0.05	-0.02	-0.09	-0.14	-0.05	-0.11	-0.04	-0.02	-0.11	0.00	**1.00**	0.36
rays_anal	-0.31	-0.31	-0.29	-0.33	-0.36	-0.21	-0.34	-0.32	-0.32	-0.35	0.23	0.36	**1.00**

Table 7. Correlation coefficients between characters after removal of the size effect. Values higher than 0.90 are in bold.

Variable	shead_length	sfirst_dorsal	ssecond_dorsal	slength_snout	sheight_body	sleft_eye	sinter_orbital	slength_fin	sheight_tail	slength_max	rays_left_fin	rays_second_fin	rays_anal_fin
shead_length	**1.00**	-0.02	0.16	-0.16	0.23	0.45	-0.05	-0.03	0.04	0.22	0.12	0.15	0.26
sfirst_dorsal	-0.02	**1.00**	-0.03	0.07	0.19	0.05	0.11	-0.03	-0.11	0.05	0.03	0.05	-0.04
ssecond_dorsal	0.16	-0.03	**1.00**	-0.06	0.09	-0.08	0.05	0.02	0.28	-0.09	-0.06	0.26	0.13
slength_snout	-0.16	0.07	-0.06	**1.00**	0.23	-0.38	0.40	0.40	0.31	0.02	-0.04	-0.09	-0.28
sheight_body	0.23	0.19	0.09	0.23	**1.00**	-0.09	0.12	0.14	0.04	0.23	0.23	-0.18	-0.17
sleft_eye	0.45	0.05	-0.08	-0.38	-0.09	**1.00**	-0.25	-0.26	-0.31	0.16	0.15	0.07	0.34
sinter_orbital	-0.05	0.11	0.05	0.40	0.12	-0.25	**1.00**	0.19	0.27	0.00	-0.03	-0.14	-0.22
slength_fin	-0.03	-0.03	0.02	0.40	0.14	-0.26	0.19	**1.00**	0.20	-0.07	-0.04	0.08	-0.14
sheight_tail	0.04	-0.11	0.28	0.31	0.04	-0.31	0.27	0.20	**1.00**	-0.15	-0.21	0.07	-0.12
slength_max	0.22	0.05	-0.09	0.02	0.23	0.16	0.00	-0.07	-0.15	**1.00**	0.06	-0.11	-0.07
rays_left_fin	0.12	0.03	-0.06	-0.04	0.23	0.15	-0.03	-0.04	-0.21	0.06	**1.00**	0.00	0.23
rays_second_fin	0.15	0.05	0.26	-0.09	-0.18	0.07	-0.14	0.08	0.07	-0.11	0.00	**1.00**	0.36
rays_anal	0.26	-0.04	0.13	-0.28	-0.17	0.34	-0.22	-0.14	-0.12	-0.07	0.23	0.36	**1.00**

The MDS plot for blue warehou is presented in Figure 7 (stress value of 0.17). Blue warehou from east and west Bass Strait separated out in ordination space although there was some overlap, indicating that body characteristics of fish from the two areas differed. Blue warehou from east Tasmania did not appear to separate out from fish from west Bass Strait but they did show some separation from east Bass Strait fish.

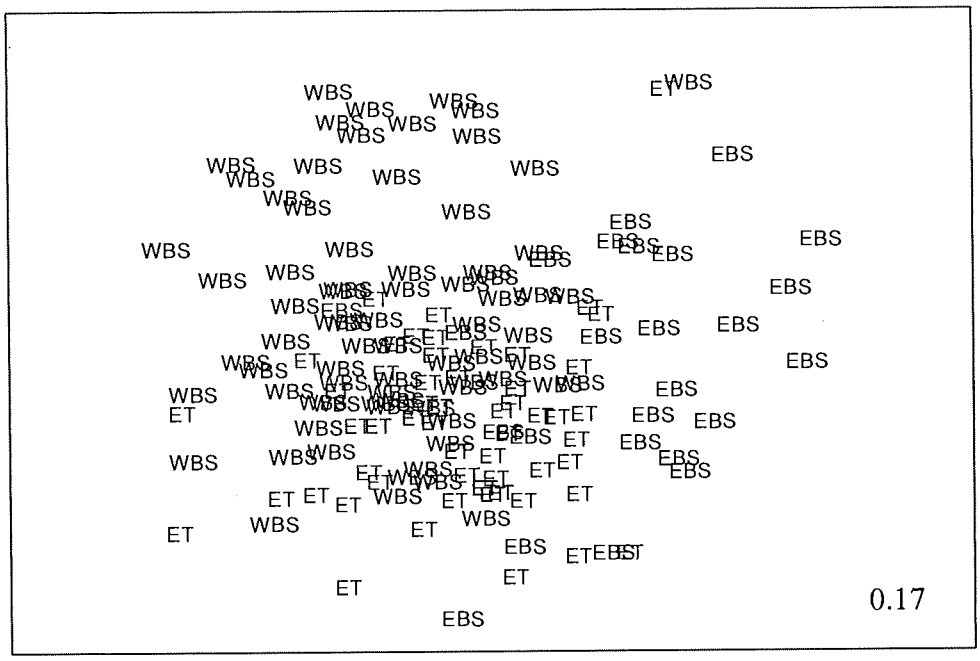

Figure 7. Two-dimensional MDS plot showing similarities between blue warehou sampled in east Bass Strait (EBS), west Bass Strait (WBS) and east Tasmania (ET).

The results from the ANOSIM showed significant differences between many of the pre-defined groups (areas) (Table 8). For blue warehou, all pairwise comparisons involving the three areas sampled (east Bass Strait, west Bass Strait and east Tasmania) were significantly different from each other at a significance level of 0.001 (or 0.1%). The important message of the pairwise tests is usually not so much the significance level, however, but the pairwise R values, since that gives an absolute measure of how separated the groups are, on a scale of 0 (indistinugishable) to 1 (all similarities with groups are less than any similarity between groups). R values ≥ 0.50 are considered to indicate clear differences (Clarke and Gorley 2001).

For blue warehou, fish collected from east and west of Bass Strait were clearly different with some overlap ($R \geq 0.5$). No clear separation between east Tasmania and either east or west Bass Strait samples was observed.

Table 8. Analysis of Similarities (ANOSIM) between groups (areas) for blue warehou: east Bass Strait (EBS), west Bass Strait (WBS) and east Tasmania (ET).

Groups	R Statistic	Significance Level %
ET, EBS	0.4	0.1
ET, WBS	0.1	0.1
EBS, WBS	0.5	0.1

For the group comparison that was confirmed by the ANOSIM test (blue warehou east Bass Strait / west Bass Strait), the SIMPER procedure was used to determine which characteristics contributed most to the dissimilarity between fish caught from different areas (Table 9). For blue warehou, four characteristics (length from snout to beginning of anal fin, height of body, head length and second dorsal fin length) accounted for

approximately 57% of the dissimilarity between fish from east and west Bass Strait (Table 9).

Table 9. Discriminating characteristics based on percentage contribution to dissimilarity between east Bass Strait (EBS) and west Bass Strait (WBS) blue warehou.

Character	Mean value for EBS	Mean value for WBS	Contribution (%)	Cumulative Contribution (%)
slength_snout	212.19	226.84	22.37	22.37
sheight_body	106.72	104.44	13.55	35.93
shead_length	98.81	95.29	10.88	46.80
ssecond_dorsal	156.59	157.35	10.60	57.40
slength_fin	101.75	106.54	10.09	67.50
sfirst_dorsal	39.47	40.42	6.98	74.48
sleft_eye	20.53	18.13	6.10	80.58
sinter_orbital	33.28	35.32	5.06	85.64
sheight_tail	20.37	21.43	3.30	88.94
Slength_max	31.43	30.66	3.16	92.10

Genetics

Sequences were compared to the consensus sequence to determine the number of unique haplotypes for each group (Table 10). Of the 138 individuals sequenced for blue warehou, 84 individuals had unique haplotypes (haplotypes that were only detected once within any the five groups sampled). The average percentage nucleotide diversity for blue warehou ranged from 5.9 to 8.3 bp within the areas sampled. No incidence of heteroplasmy was detected, although individuals showing heteroplasmy may have been excluded from this analysis due to difficulties in scoring sequence data. No obvious population structuring was evident for blue warehou from the patterns of the minimum spanning trees between mitochondrial DNA haplotypes.

Table 10. Number of sequences, haplotypes and mean nucleotide differences (k) for blue warehou for east Bass Strait (EBS), west Bass Strait (WBS) and east Tasmania (ET). 'Unique' is number of unique haplotypes. This does not equal the total number of haplotypes as each haplotype may be represented in more than one group.

Species and Area	Sequence length	Number of sequences	Number of haplotypes	Average % of nucleotide differences (k)
EBS	389	38	23	6.590
WBS Date 1	389	20	18	6.663
WBS Date 2	389	27	20	5.946
ET Date 1	389	25	20	8.290
ET Date 2	389	28	24	6.979
Total		138	84 (unique)	

For blue warehou, within group (Vb) variation was around 50-200 fold higher than between group variation (Va) for all comparisons (Table 11). This is as you would expect in fish where population numbers are large and most of the variation can be attributed to differences between individuals within populations. Genetic structuring (departure of ϕ_{ST} from zero) was significant ($p<0.05$) for comparisons between east Bass Strait and west Bass Strait but was not significant ($p>0.05$) for within region comparisons. This data strongly suggests that separate ecological populations exist east and west of Bass Strait. Genetic divergence between populations of large size takes many generations to occur (as genetic variation is high and genetic drift is negligible in such populations), and relatively small numbers of migrants per generation between such populations would revert any small differences that arose due to genetic drift. Overall, the rates of migration recorded in this study were high compared to other studies of marine fish (e.g. Colgan and Paxton 1997). The number of genetically effective blue warehou migrants per generation (Nm_{blue}) was large (infinite) within the west Bass Strait region and between east Bass Strait and east Tasmania. Nm_{blue} was relatively lower (11-21 individuals per generation) between localities east and west of Bass Strait. A comparison of fish collected from east Tasmania on two separate dates had a Nm_{blue} value of 38.62 which appears low considering the Nm_{blue} for the EBS/ET comparison was infinite. It is unclear why this result was obtained as the fish were caught from the same area and approximately two weeks apart.

Table 11. AMOVA for blue warehou for east Bass Strait (EBS), west Bass Strait (WBS) and east Tasmania (ET). Va, variance between groups; Vb, within group variation; ϕ_{ST}, measure of the differentiation between groups. ϕ_{ST} was tested by permutating haplotypes between groups 1000 times. p is the probability that ϕ_{ST} differs from zero. Bold values indicate significance at $p < 0.05$.

Comparison	Va (between groups)	Vb (within groups)	ϕ_{ST}	p
WBS 1 & WBS 2	0.008	3.351	0.002	0.468
ET 1 & ET 2	0.043	4.087	0.010	0.208
EBS & WBS	0.067	3.446	0.019	**0.041**
EBS & ET	0.023	3.880	0.006	0.702
WBS & ET	0.049	3.755	0.013	0.057

In summary, the results of the genetic analysis suggest that there are two, genetically distinct, stocks of blue warehou in south-eastern Australia; one to the east of Bass Strait and one to the west of Bass Strait. A similar finding of genetic differentiation between east and west Bass Strait was demonstrated for gemfish, *Rexea solandri* (Colgan and Paxton 1997). Mitochondrial DNA analysis and allozyme data found significant genetic differentiation between populations in eastern Australia (including much of the south-eastern fishing region) and south-western Australia, with some limited mixing occurring between these stocks off western Tasmania. Similar studies on orange roughy, *Hoplostethus atlanticus*, have also shown significant genetic structuring within southern Australian waters (Smolenski et al. 1993, Black and Dixon 1989). Mitochondrial restriction enzyme analysis of 107 individuals caught within southern Australia revealed significant differences between populations from south

Australia (off south eastern Kangaroo Island), Tasmania and New South Wales (Smolenski et al. 1993). Heterogeneity in allozyme and microsatellite loci in orange roughy from south-eastern Australia has also been detected (reviewed by Ward and Elliott 2001).

Otolith Morphology

The blue warehou samples from east and west Bass Strait were not significantly different with respect to fish length ($p = 0.56$) or otolith weight ($p = 0.78$). This was expected as samples were selected in order to minimise fish size and otolith weight effects in the analysis. Comparative samples of blue warehou were not available from east and west Tasmania.

Results of the otolith shape analysis for blue warehou show significant differences between fish from east and west Bass Strait (p = 0.002). The observed harmonic distance, randomised mean harmonic distance and the probablility that blue warehou from east and west Bass Strait are from the same stock is shown in Table 12. The distributions of the randomised harmonic distances are shown in Figure 8.

Table 12. Blue warehou. Observed harmonic distance (DH_k), mean randomised harmonic distance (mDH_k), 95 percentiles for the randomised harmonic distances and the probability (p) of same stock hypothesis for east Bass Strait (EBS) and west Bass Strait (WBS). Bold values indicate significance at $p < 0.05$

Parameter	EBS/WBS
DH_k	0.014
mDH_k	0.007
Lower 95 percentile	0.004
Upper 95 percentile	0.001
Probability (p)	**0.002**

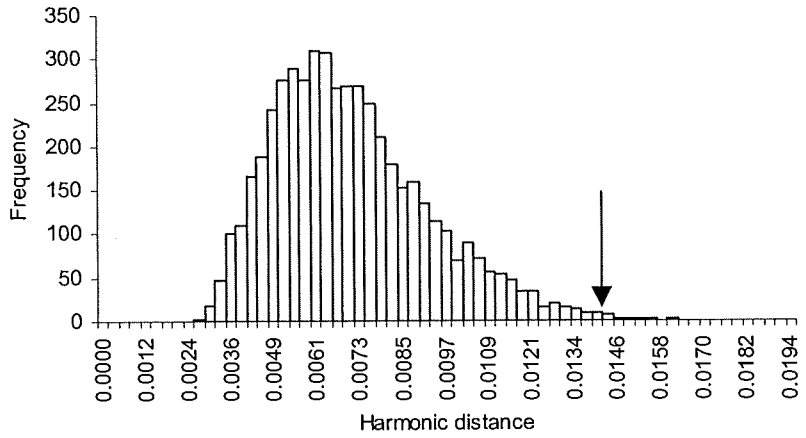

Figure 8. Blue warehou. Randomised harmonic distance between east and west Bass Strait otolith shape. The approximate observed harmonic distance is shown by the arrow. Observed harmonic distance 0.014, p = 0.002.

Although shape analysis indicated differences between fish from east and west Bass Strait, individual otolith morphometric characters (circularity, perimeter and area) were not significantly different between the two areas (otolith circularity - Table 13, otolith area – Table 14, and otolith perimeter - Table 15.).

Table 13. Otolith circularity median test for blue warehou otoliths from east Bass Strait (EBS) and west Bass Strait (WBS).

Circularity	**N**	**samples ≤ median**	**samples > median**	**Median value**
EBS	78	40	38	23.871
WBS	102	50	52	23.969
Overall median	23.940			
p	0.881			

Table 14. Otolith area median test for blue warehou otoliths from east Bass Strait (EBS) and west Bass Strait (WBS).

Otolith area	**N**	**samples ≤ median**	**samples > median**	**Median value**
EBS	78	46	32	88717.750
WBS	102	44	58	95166.000
Overall median	92665.250			
p	0.051			

Table 15. Otolith perimeter median test for blue warehou otoliths from east Bass Strait (EBS) and west Bass Strait (WBS).

Perimeter	N	samples ≤ median	samples > median	Median value
EBS	78	45	33	1464.020
WBS	100	44	56	1516.150
Overall median	1486.745			
p	0.096			

Otolith shape is a phenotypic character and, as such, differs according to age, sex and growth rate (e.g. Castonguay et al. 1991, Campana and Casselman 1993). We analysed samples from fish of similar length, caught in the same year, in order to reduce these confounding effects. The Fourier descriptors were also standardised for otolith size before analysis, to reduce age effects. Significant results therefore reflect true differences in otolith morphology.

The results of the otolith shape analysis suggest that there are two stocks of blue warehou in south-eastern Australia; one to the east of Bass Strait and one to the west of Bass Strait (although no Tasmania samples were analysed for comparison).

The results of the shape analysis were not supported by the analysis of individual otolith morphometric characters as circularity, area and perimeter did not differ between east and west Bass Strait for blue warehou. The analysis of otolith morphometrics is less powerful than Fourier analysis in discriminating differences between populations because Fourier analysis is an asymptotic 128 point landmark analysis technique.

Otolith microchemistry -Analysis of cores

Univariate analyses (ANOVA) comparing otolith core samples from 1+ age blue warehou collected from west Bass Strait and east Bass Strait revealed significant differences in elemental concentrations between areas for the elements; Sr ($F_{1,\ 63}$ = 6.564, p = 0.013), log Mn ($F_{1,63}$ = 11.465, p = 0.001,) and log Zn ($F_{1,\ 63}$ = 16.189, $p <$ 0.001). No differences between areas were found for log Ba ($F_{1,63}$ = 0.579, p = 0.449), Na ($F_{1,63}$ = 0.171, p = 0.681), and log Mg ($F_{1,63}$ = 0.224, p = 0.638).

Ba levels in the cores of otoliths showed negligible difference between east and west Bass Strait fish. Sr levels were slightly higher in the otolith cores of west Bass Strait fish, as were Mn and Zn levels. Both Na and Mg showed negligible differences between the two regions (Figure 9).

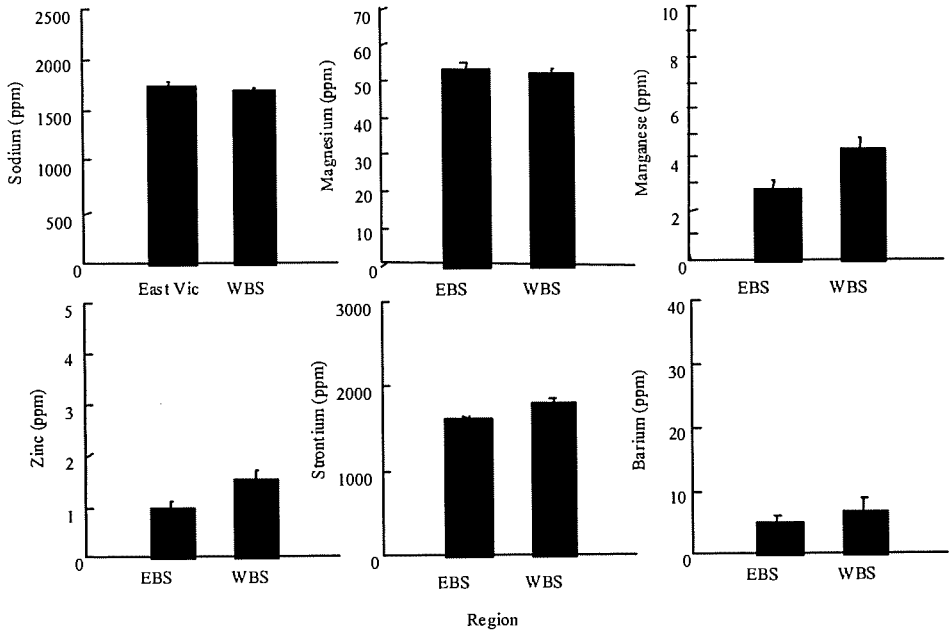

Figure 9. Mean concentrations (± SE) of individual elements in the cores of blue warehou otoliths sampled from east Bass Strait (EBS) and west Bass Strait (WBS).

Mulivariate analysis (MANOVA) involving Sr, log Mn, log Mg, log Ba, Na and log Zn revealed significant differences in the multi-element composition of otolith cores between areas (Pillai's trace = 0.400, $F_{6, 58} = 6.457$, $p < 0.0001$). QDFA involving log Mn, log Mg, Sr and log Zn, (Na and Ba were initially included in the analysis but were excluded as they made no contribution to discrimination, i.e. 'F to remove' statistics less than 0.1) showed an overall 82 % accuracy in discrimination between areas based on the chemical composition of otolith cores (Table 16). Classification accuracy was highest for blue warehou collected off east Bass Strait at 85 %, with west Bass Strait showing 77 % accuracy. Because only two groups are included in this analysis only one discriminant function can be calculated. The distribution of discriminant function score for east and west Bass Strait blue warehou is displayed in Figure 10. Although there is a small amount of overlap between the two regions, this still shows strong discrimination between blue warehou collected east and west of Bass Strait.

Table 16. Results of classifications from quadratic discriminant function analysis of the elemental composition (log Mg, Sr, log Mn, log Zn) of core regions of 1+ age blue warehou otoliths collected from east Bass Strait (EBS) and west Bass Strait (WBS).

	Region Classified to		
Region of origin	**EBS**	**WBS**	**% Correct**
EBS	29	5	85
WBS	7	24	77
Total	36	29	82

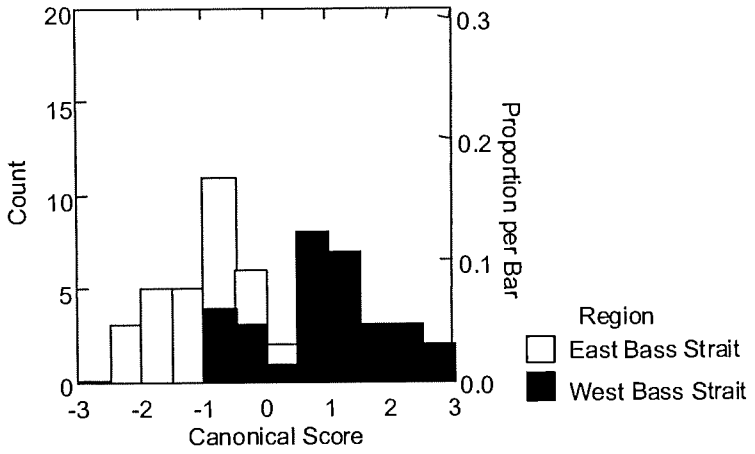

Figure 10. Frequency histogram of discriminant function scores from analyses of log Mn, log Mg, Sr and log Zn in cores of blue warehou from east and west Bass Strait.

The results for blue warehou, while showing incomplete separation in chemical composition, showed a high degree of separation between the two regions. The distribution of discriminant function scores for west Bass Strait fish, however, appeared to be bimodal. This could possibly indicate two sources of fish to the west Bass Strait population, with the further possiblity that one of these sources is the same as that for east Bass Strait. However, further sampling and analyses would be required to confirm this theory. Given the opportunity for mixing, separation between regions based on the early juvenile otolith composition provides good evidence of separate stocks. We would recommend further analysis of older age classes, however, to confirm that this pattern of separation is maintained with age.

Otolith microchemistry - Analysis of transects

Comparison of average concentrations derived from transect data

For blue warehou transects were conducted on 4+ age fish (7 from east Tasmania, 6 from east Bass Strait, 11 from west Bass Strait). For blue warehou there were no significant differences between regions in the average concentrations of individual elements from the otolith transects (ANOVA, $p<0.05$ for all elements). Average concentrations from otolith transect data of blue warehou otoliths are displayed in Figure 11. The results of MANOVA analyses on the average concentration data for Mn, Mg, Sr, Ba, Na and Zn were also non-significant (Pillai's trace >0.05). Unfortunately, due to the small sample sizes, statistical power to detect differences was very low. For any future work, a comparison would be made between the average concentration data from transects and that obtained from whole otolith solution based assays.

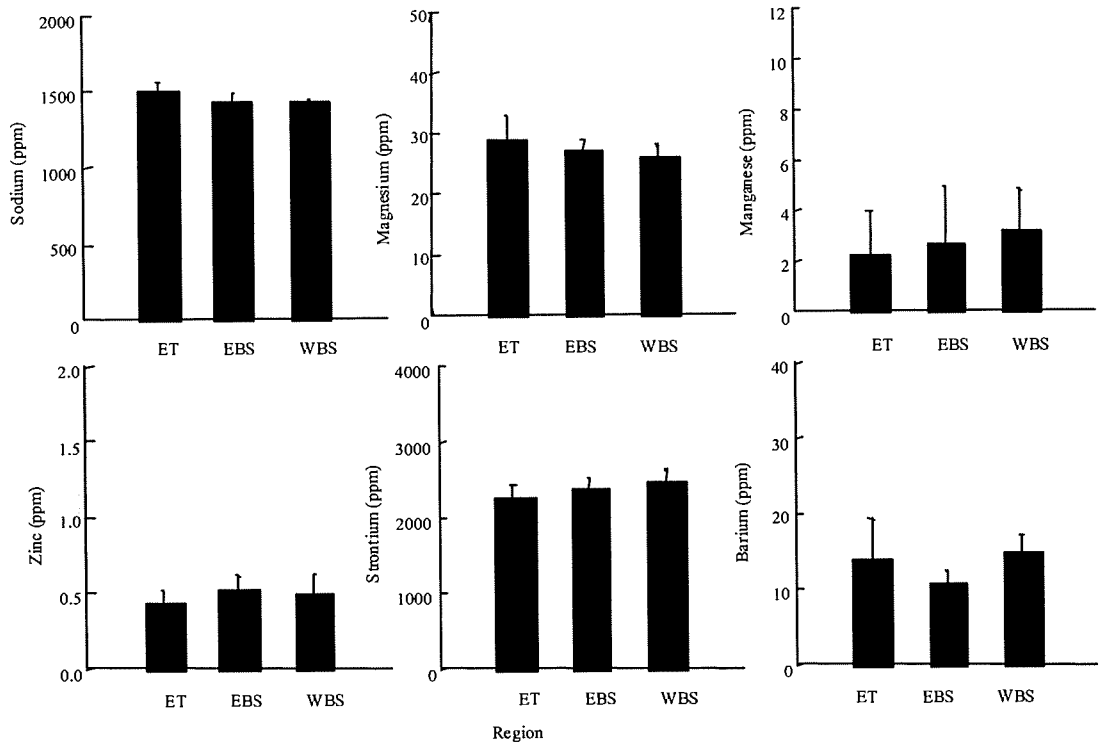

Figure 11. Mean concentration (± SE) from continuous transects across blue warehou otoliths from east Tasmania (ET), east Bass Strait (EBS) and west Bass Strait (WBS).

Comparison of whole transects

Ba variation: Qualitative assessment of the Ba/Ca variation across blue warehou otoliths (Figure 12) suggested some differences between regions. The patterns from east Bass Strait, in particular, appeared more variable than those from west Bass Strait and east Tasmania. Within regions, some fish showed similar patterns of Ba/Ca variation (i.e. Figure 12a - fish 5 and 6, and Figure 12c - fish 1, 2 and 3) but there were also differences in patterns of variation between blue warehou from the same regions (i.e. Figure 12a - fish 1 and 11, Figure 12b - fish 5 and 6, and Figure 12c - fish 4 and 5). Interestingly, it was difficult to find fish from different regions that had similar patterns of variation

Sr Variation: For blue warehou in all regions there were general increases in Sr/Ca with age (Figure 13). There was also evidence of cyclic variation in Sr/Ca, and there was differences in the strength and clarity of cyclic patterns. Cycles in Sr/Ca were stronger for fish from west Bass Strait than the other regions. Patterns in Sr/Ca appeared to correlate with seasonal variations in growth rate.

Several studies have highlighted the role of ambient concentration in influencing Ba incorporation into otoliths (Thorrold et al. 1998, Bath et al. 2000, Milton and Chenery 2001). The fact that, unlike Sr, Ba showed no consistent patterns of variation across the otolith, supports the hypothesis that variation in Ba was related to changes in ambient concentrations and not water temperature. If water temperature was a major factor in influencing Ba variation, seasonal patterns in variation should have been evident, as was the case for Sr. Some method of validation is required to assess whether variations in Ba are related to movement of fish between different water

masses or to changes in water chemistry at the same location (i.e. due to processes such as local upwelling). It is not possible to interpret this variation in terms of migration with our current state of knowledge of Ba variation in the waters of Bass Strait and the unknown relationship between ambient Ba levels and incorporation into otoliths of both species. Further sampling, analyses, and manipulative experiments would be required to assess the possible use of Ba variation as a proxy for migration.

Sr variation across otoliths is unlikely to be a potential proxy for migration because of the apparent influence of growth and other variables such as water temperature on its incorporation. The general increase in Sr with age, coupled with the annual cycles that appeared to be related to annual growth cycles, suggest that for blue warehou, growth rate is negatively correlated with Sr incorporation into otoliths. A similar pattern has been seen for snapper from the same region (Hamer, unpublished data). Other studies have also demonstrated a negative correlation between growth and Sr incorporation (Sadovy and Severin 1992, 1994). Apart from growth, a range of other environmental variables including temperature (Hoff and Fuiman 1995, Gallahar and Kingsford 1996, Townsend et al. 1992) and salinity (Kalish 1990, Secor 1992, 2000, Tzeng 1997), and ambient concentration (Farrell and Campana 1996, Bath et al. 2000, Milton and Chenery 2001) can influence Sr incorporation into otoliths. However, given that differences in salinity between oceanic areas would be very minor, salinity is unlikely to be responsible for changes in Sr across otoliths of blue warehou. The variation in the strength of the cycles between regions is likely to be related to greater seasonal variations in either temperature or growth rates between regions.

The transect component of this study, while primarily included to look for variations across otoliths that could suggest different environmental and or migratory histories, also provided a pilot study for the potential of this method to identify stocks. For example, if fish from within a particular region all show a similar pattern of variation across their otoliths and this differs from the pattern observed in another area, it would indicate that the fish experienced differing environments over their lifespan. This would strongly support a conclusion of different stocks in each area. Variations across otoliths in both Ba and Sr showed some differences between regions suggesting that this methodology does have potential as an alternative stock identification technique. However, whilst variations in chemistry across otoliths may indicate variation in environmental parameters, the degree to which this is due to movement between different environments is difficult to determine without some sort of validation technique.

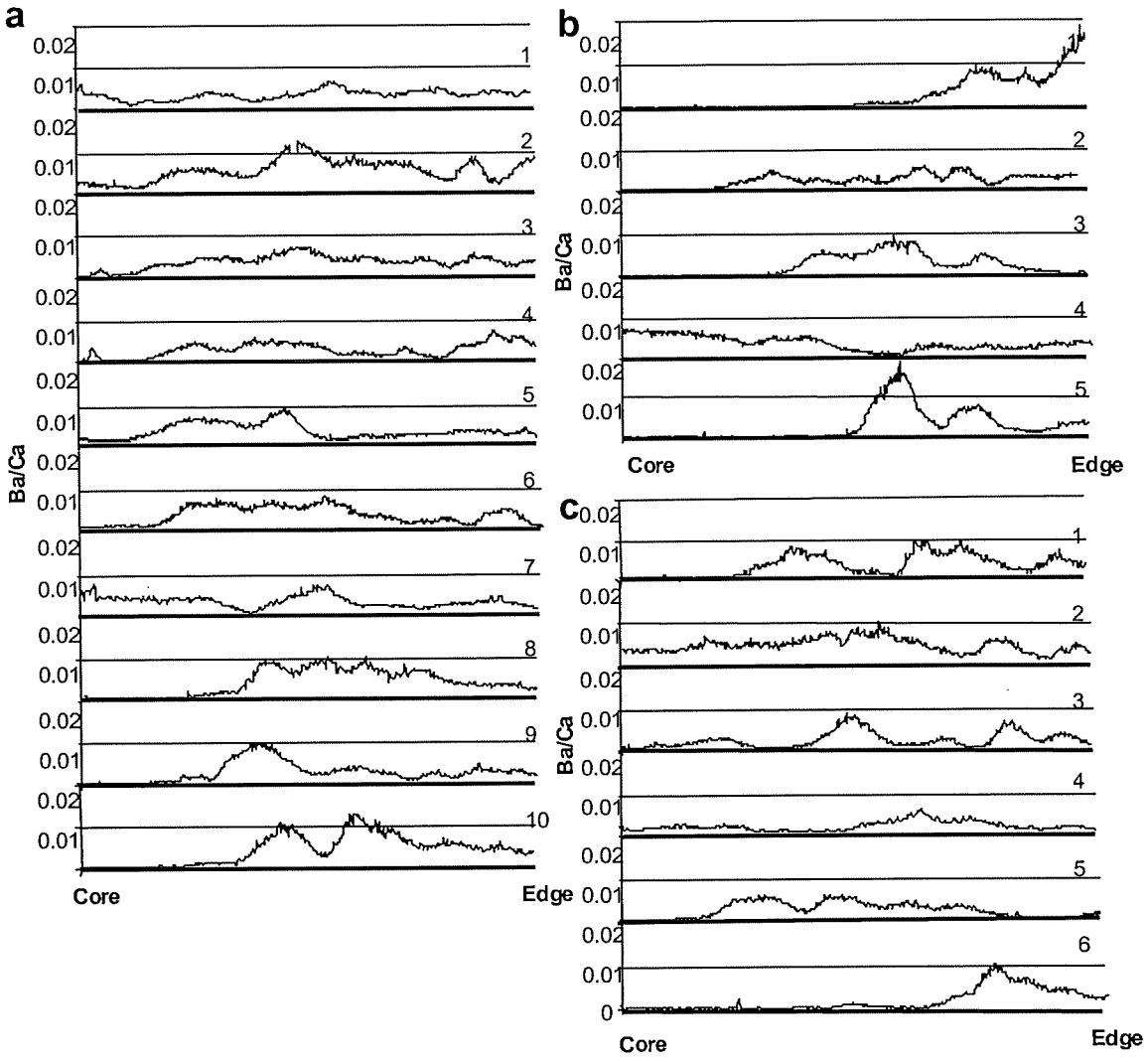

Figure 12. Ba/Ca ratios from continuous ablation transects across otoliths of blue warehou individuals from west Bass Strait (a), east Bass Strait (b) and east Tasmania (c). Data are ratios of raw counts.

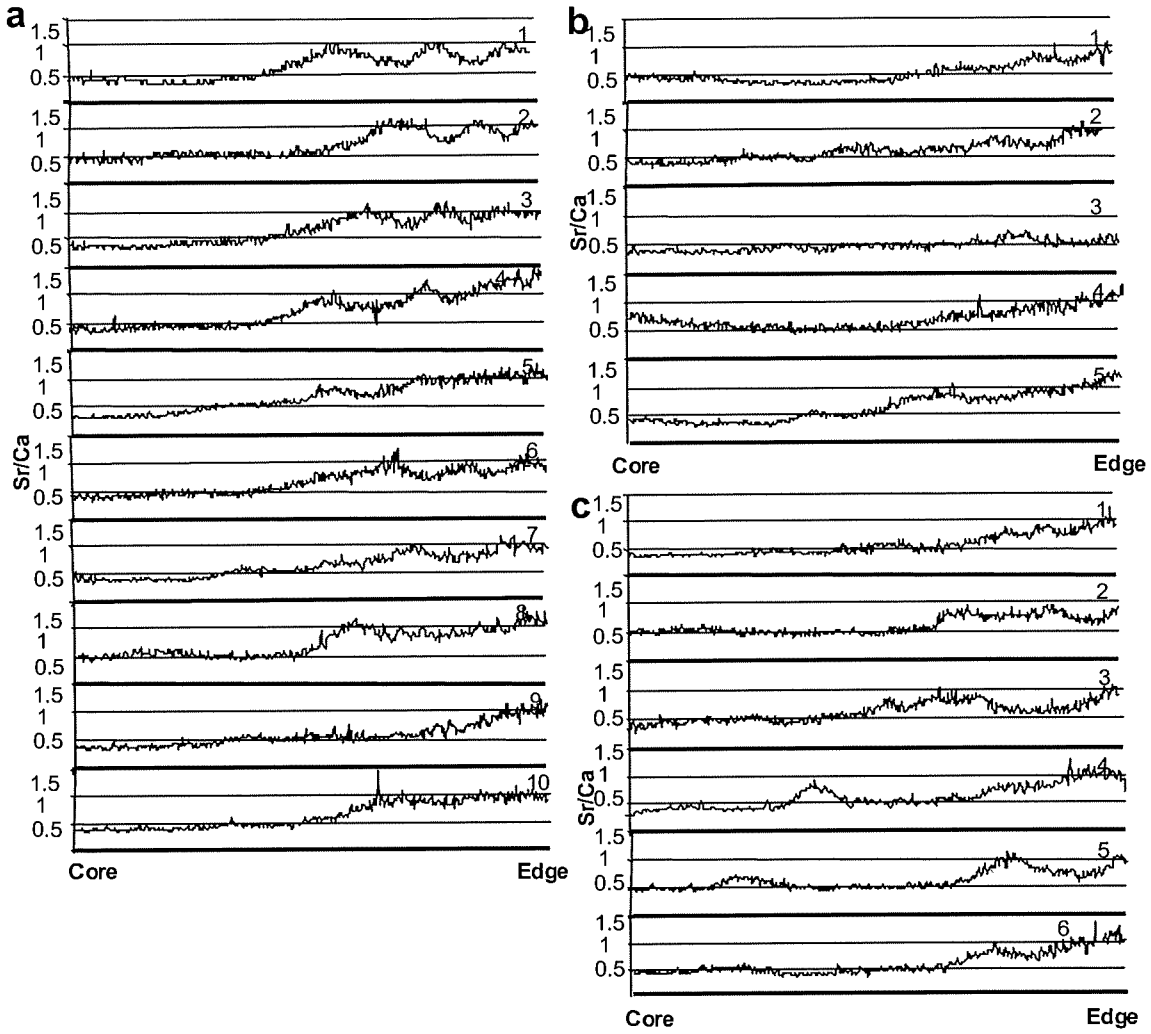

Figure 13. Sr/Ca ratios from continuous ablation transects across otoliths of blue warehou individuals from west Bass Strait (a), east Bass Strait (b) and east Tasmania (c). Data are ratios of raw counts.

Blue warehou summary

The results obtained from the four techniques are summarised in Table 17. For blue warehou, significant differences in all four parameters, morphology, mtDNA, otolith shape and otolith microchemistry, were found between fish taken from east and west Bass Strait, indicating two separate stocks.

The relationship of east and west Tasmania to the Bass Strait areas and to each other was less clear. There were difficulties in obtaining west Tasmanian samples of blue warehou and only two of the four methods, morphology and mt DNA, were used to analyse east Tasmanian samples. Neither method showed clear differences between fish from east Tasmania and fish from either east or west Bass Strait, which indicates that fish caught in east Tasmania have had some degree of mixing with both of the Bass Strait stocks. Analysis of west Tasmanian samples is required to clarify the relationship between blue warehou from Tasmania and Bass Strait.

Table 17. Summary of pairwise comparisons by area for each of the four techniques. *: significantly different ($p<0.05$) and, additionally, where $R\geq0.50$ for morphology, NS: not significantly different, x: not tested. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Area	Morphology	mtDNA	Otolith Shape	Otolith microchemistry
EBS/WBS	*	*	*	*
EBS/ET	NS	NS	x	x
WBS/ET	NS	NS	x	x
WT	not collected			

The results from the current study support the findings of Bruce et al. (2001) and Knuckey and Sivakumarna (2001) who reported separate spawning areas and different spawning times between east and west Bass Strait for blue warehou. The results also support size and age data that shows differences between east and west Bass Strait for blue warehou. There are, therefore, several lines of evidence that support a two-stock hypothesis for blue warehou in south-eastern Australian, although the relationship between Tasmania and Bass Strait is not clear.

Spotted warehou

Samples

The numbers of spotted warehou samples analysed by each of the four methods of stock identification are summarised in Table 18. Samples from all four areas were analysed by two techniques, morphology and otolith shape analysis. Genetic analysis was only done on samples from east and west Bass Strait. Otolith microchemistry (transects and cores) was examined in fish from three areas, east and west Bass Strait and east Tasmania. The 198 spotted warehou samples collected ranged in size from 27 to 51 cm fork length (Appendix 3, Table 1). Each technique analysed a subset of these, with the exception of otolith morphology, which analysed a selection of archived samples from the 2001/02 financial year. This was because sufficient numbers of comparative samples had not been collected when the otolith morphology component of the project was initiated.

The size distributions of the samples analysed by each method are shown in Appendix 3 (Tables 2 to 9). From the subset of samples aged for otolith microchemical analyses, fish ranging in size from 27 to 45 cm were all three years old (Appendix 3, Tables 6 to 9).

Table 18. Summary of the number of spotted warehou samples analysed by each of the four techniques in each of the four areas. c: cores, t: transects, nc: not collected

Area	Morphology	mtDNA	Otolith Shape	Otolith microchemistry
east Bass Strait	30	30	88	c: 26, t: 7
west Bass Strait	55	56	70	c: 36, t: 8
east Tasmania	51	0	29	c: 30, t: 6
west Tasmania	59	0	42	c: 0, t: 0

Morphology

Correlation coefficients between spotted warehou characters before and after size effect removal are presented in Table 19 and Table 20 respectively. There was a considerable reduction in values for all morphological characters (values higher than 0.90 are in bold) confirming the assumption that these characters are dependent on size. Meristic characters were not standardised for size as these are independent of size (see Methods).

Table 19. Correlation coefficients between characters before removal of the size effect. Values higher than 0.90 are in bold.

Variable	head_length	first_dorsal	second_dorsal	length_snout	height_body	left_eye	inter_orbital	length_fin	height_tail	length_max	rays_left_fin	rays_second_fin	rays_anal_fin
head_length	**1.00**	0.82	**0.98**	**0.98**	**0.96**	**0.96**	**0.96**	**0.96**	**0.95**	**0.98**	0.52	0.28	0.44
first_dorsal	0.82	**1.00**	0.83	0.84	0.79	0.76	0.77	0.82	0.78	0.83	0.37	0.17	0.26
second_dorsal	**0.98**	0.83	**1.00**	**0.98**	**0.97**	**0.95**	**0.96**	**0.97**	**0.96**	**0.98**	0.52	0.30	0.44
length_snout	**0.98**	0.84	**0.98**	**1.00**	**0.95**	**0.94**	**0.96**	**0.96**	**0.94**	**0.98**	0.50	0.26	0.40
height_body	**0.96**	0.79	**0.97**	**0.95**	**1.00**	**0.94**	**0.96**	**0.95**	**0.96**	**0.95**	0.52	0.29	0.42
left_eye	**0.96**	0.76	**0.95**	**0.94**	**0.94**	**1.00**	**0.94**	**0.94**	**0.93**	**0.94**	0.52	0.27	0.46
inter_orbital	**0.96**	0.77	**0.96**	**0.96**	**0.96**	**0.94**	**1.00**	**0.94**	**0.95**	**0.95**	0.54	0.29	0.45
length_fin	**0.96**	0.82	**0.97**	**0.96**	**0.95**	**0.94**	**0.94**	**1.00**	**0.95**	**0.96**	0.53	0.28	0.44
height_tail	**0.95**	0.78	**0.96**	**0.94**	**0.96**	**0.93**	**0.95**	**0.95**	**1.00**	**0.94**	0.54	0.29	0.45
length_max	**0.98**	0.83	**0.98**	**0.98**	**0.95**	**0.94**	**0.95**	**0.96**	**0.94**	**1.00**	0.49	0.27	0.41
rays_left_fin	0.52	0.37	0.52	0.50	0.52	0.52	0.54	0.53	0.54	0.49	**1.00**	0.20	0.35
rays_second_fin	0.28	0.17	0.30	0.26	0.29	0.27	0.29	0.28	0.29	0.27	0.20	**1.00**	0.38
rays_anal	0.44	0.26	0.44	0.40	0.42	0.46	0.45	0.44	0.45	0.41	0.35	0.38	**1.00**

Table 20. Correlation coefficients between characters after removal of the size effect. Values higher than 0.90 are in bold.

Variable	shead_length	sfirst_dorsal	ssecond_dorsal	slength_snout	sheight_body	sleft_eye	sinter_orbital	slength_fin	sheight_tail	slength_max	rays_left_fin	rays_second_fin	rays_anal_fin
shead_length	**1.00**	0.17	-0.17	-0.13	-0.29	0.28	-0.19	-0.24	-0.22	0.15	-0.16	-0.09	-0.10
sfirst_dorsal	0.17	**1.00**	-0.17	-0.29	-0.38	-0.02	-0.50	-0.10	-0.38	-0.12	-0.36	-0.23	-0.36
ssecond_dorsal	-0.17	-0.17	**1.00**	0.08	0.41	-0.07	0.27	0.20	0.37	0.18	0.23	0.29	0.22
slength_snout	-0.13	-0.29	0.08	**1.00**	0.30	-0.22	0.50	0.18	0.31	0.39	0.33	0.11	0.16
sheight_body	-0.29	-0.38	0.41	0.30	**1.00**	-0.04	0.59	0.25	0.55	0.11	0.34	0.24	0.25
sleft_eye	0.28	-0.02	-0.07	-0.22	-0.04	**1.00**	-0.06	-0.02	-0.05	-0.07	-0.04	-0.07	0.00
sinter_orbital	-0.19	-0.50	0.27	0.50	0.59	-0.06	**1.00**	0.21	0.56	0.23	0.47	0.24	0.39
slength_fin	-0.24	-0.10	0.20	0.18	0.25	-0.02	0.21	**1.00**	0.33	0.10	0.23	0.08	0.17
sheight_tail	-0.22	-0.38	0.37	0.31	0.55	-0.05	0.56	0.33	**1.00**	0.15	0.40	0.20	0.30
slength_max	0.15	-0.12	0.18	0.39	0.11	-0.07	0.23	0.10	0.15	**1.00**	0.10	0.08	0.04
rays_left_fin	-0.16	-0.36	0.23	0.33	0.34	-0.04	0.47	0.23	0.40	0.10	**1.00**	0.20	0.35
rays_second_fin	-0.09	-0.23	0.29	0.11	0.24	-0.07	0.24	0.08	0.20	0.08	0.20	**1.00**	0.38
rays_anal	-0.10	-0.36	0.22	0.16	0.25	0.00	0.39	0.17	0.30	0.04	0.35	0.38	**1.00**

The MDS plot for spotted warehou is presented in Figure 14 (stress value of 0.14). Spotted warehou caught east and west of Bass Strait overlapped in ordination space, indicating similarity in morphometric characteristics between the two areas. Spotted warehou from east and west Tasmania clearly differed.

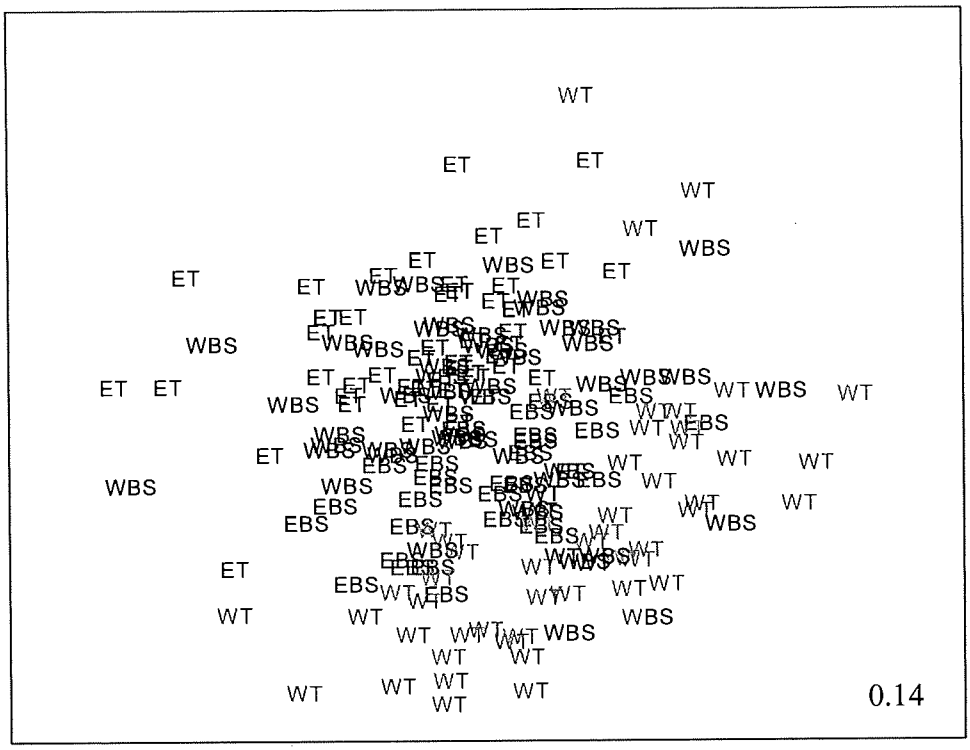

Figure 14. Two-dimensional MDS plot showing similarities between spotted warehou sampled in east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

The results from the ANOSIM showed significant differences between many of the pre-defined groups (areas) (Table 21). For spotted warehou, the majority of pairwise comparisons involving all four areas were significantly different from each other at a significance level of 0.001 (or 0.1%). However, east Bass Strait did not differ from west Bass Strait (significance level 7.3%). As discussed earlier, the important message of the pairwise tests is usually not so much the significance level, however, but the pairwise R values with values ≥ 0.50 indicating clear differences (Clarke and Gorley 2001).

For spotted warehou, clear differences were only demonstrated between east and west Tasmania (R ≥ 0.5). Fish from east and west Bass Strait were not separable.

Table 21. Analysis of Similarities (ANOSIM) between groups (areas) spotted warehou: east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Groups	R Statistic	Significance Level %
ET, EBS	0.3	0.1
ET, WT	0.5	0.1
ET, WBS	0.1	0.1
EBS, WT	0.1	1.4
EBS, WBS	0.1	7.3
WT, WBS	0.3	0.1

For the group comparison that was confirmed by the ANOSIM test (spotted warehou east Tasmania / west Tasmania), the SIMPER procedure was used to determine which characteristics contributed most to the dissimilarity between fish caught from different areas (Table 22). For spotted warehou, four characteristics (length from snout to beginning of anal fin, first dorsal fin length, height of body and head length) accounted for approximately 60% of the dissimilarity between fish from east and west Tasmania (Table 22).

Table 22. Discriminating characteristics based on percentage contribution to dissimilarity between east Tasmania (ET) and west Tasmania (WT) spotted warehou.

Character	Mean value for ET	Mean value for WT	Contribution (%)	Cumulative Contribution (%)
slength_snout	235.10	250.54	18.20	18.20
sfirst_dorsal	40.96	34.61	15.86	34.06
sheight_body	91.87	100.25	13.82	47.88
shead_length	99.70	97.08	12.56	60.44
ssecond_dorsal	158.87	163.43	8.86	69.30
sinter_orbital	28.64	33.48	7.75	77.05
slength_fin	83.33	86.17	6.33	83.38
sheight_tail	18.68	20.96	3.52	86.90
rays_second_fin	37.37	38.64	3.49	90.38

Genetics

Sequences were compared to the consensus sequence to determine the number of unique haplotypes for each group (Table 23). Spotted warehou had 63 haplotypes, 51 of which were unique. The average percentage nucleotide diversity for spotted warehou ranged from 7.8 to 8.1 bp within the areas sampled. No incidence of heteroplasmy was detected, although individuals showing heteroplasmy may have been excluded from this analysis due to difficulties in scoring sequence data. No obvious population structuring was evident for spotted warehou from the patterns of the minimum spanning trees between mitochondrial DNA haplotypes.

Table 23. Number of sequences, haplotypes and mean nucleotide differences (k) for spotted warehou for east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT). 'Unique' is number of unique haplotypes. This does not equal the total number of haplotypes as each haplotype may be represented in more than one group.

Species and Area	Sequence length	Number of sequences	Number of haplotypes	Average % of nucleotide differences (k)
EBS	403	20	20	8.168
WBS Date 1	403	30	22	8.057
WBS Date 2	403	13	12	7.808
Total		63	51 (unique)	

For spotted warehou, no significant genetic structuring was detected between the different geographical regions (ϕ_{ST} values did not significantly differ from zero and $Nm_{spotted}$ was infinite within and between groups). Gene flow was continuous within and between groups. Most of the variation in spotted warehou was detected within the sub-group level (Table 24) with Vb around 50-400 times higher than Va.

Table 24. AMOVA for spotted warehou for east Bass Strait (EBS) and west Bass Strait (WBS). Va, variance between groups; Vb, within group variation; ϕ_{ST}, measure of the differentiation between groups. ϕ_{ST} was tested by permutating haplotypes between groups 1000 times. *p* is the probability that ϕ_{ST} differs from zero.

Comparison	Va (between group)	Vb (within groups)	ϕ_{ST}	*p*
WBS 1 & WBS 2	0.034	4.937	0.007	0.292
EBS & WBS	0.022	4.814	0.005	0.517

In contrast to the results for blue warehou, mitochondrial gene flow is high between populations of spotted warehou, suggesting that spotted warehou in the areas of south-eastern Australia sampled should be managed as a single genetic stock (although Tasmanian samples were not analysed). Spotted warehou show a similar lack of genetic structure to that found among populations of jackass morwong, *Nemadactylus macropterus*, in southern Australia (Grewe et al. 1994). Mitochondrial DNA variation in 166 jackass morwong from seven southern Australian localities showed no significant spatial patterning. Similarly, no differentiation was detected between eight southern Australian sites using restriction enzyme analysis. Weak but significant divergence was detected between the Australian and New Zealand samples, however, indicating that New Zealand jackass morwong are a separate genetic stock from Australian fish (Grewe et al. 1994).

Otolith Morphology

The spotted warehou samples were not significantly different with respect to fish length ($p = 0.16$) or otolith weight ($p = 0.57$) between each of the four areas - east Bass Strait, west Bass Strait, east Tasmania and west Tasmania. This was expected as samples were selected in order to minimise fish size and otolith weight effects in the analysis.

Results of the otolith shape analysis for spotted warehou show that there were no differences between east and west Bass Strait samples but all other comparisons between areas showed significant differences. The observed harmonic distance, randomised mean harmonic distance and probablility that two groups of spotted warehou are from the same stock is shown in Table 25. Several pair-wise comparisons are included because fish from all four areas were analysed. The distributions of randomised harmonic distances for each comparison are shown in Figure 15 to Figure 20.

Table 25. Spotted warehou. Observed harmonic distance (DH_k), mean randomised harmonic distance (mDH_k), 95 percentiles for the randomised harmonic distances and the probability (p) of same stock hypothesis for east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT). Bold values indicate significance at $p < 0.05$

Parameter	EBS/ WBS	EBS/ ET	EBS/ WT	WBS/ ET	WBS/ WT	ET/ WT
DH_k	0.008	0.032	0.020	0.033	0.017	0.021
mDH_k	0.008	0.014	0.011	0.013	0.009	0.012
Lower 95 percentile	0.004	0.008	0.006	0.008	0.005	0.007
Upper 95 percentile	0.012	0.021	0.017	0.021	0.015	0.020
Probability (p)	0.430	**0.0002**	**0.013**	**0.0002**	**0.009**	**0.035**

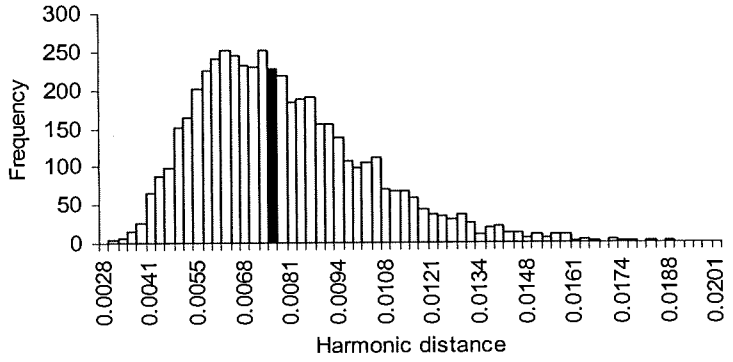

Figure 15. Spotted warehou. Randomised harmonic distance between east and west Bass Strait otolith shape. The approximate observed harmonic distance is shown by the solid bar. Observed harmonic distance 0.008, $p = 0.430$.

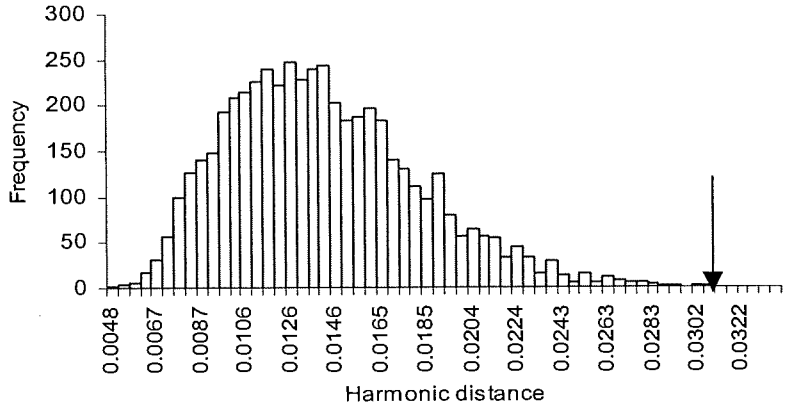

Figure 16. Spotted warehou. Randomised harmonic distance between east Bass Strait and east Tasmania otolith shape. The approximate observed harmonic distance is shown by the arrow. Observed harmonic distance 0.032, $p = 0.0002$.

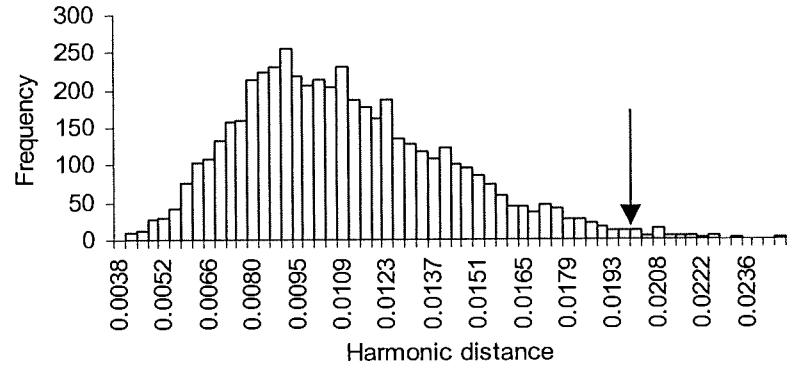

Figure 17. Spotted warehou. Randomised harmonic distance between east Bass Strait and west Tasmania otolith shape. The approximate observed harmonic distance is shown by the arrow. Observed harmonic distance 0.020, $p = 0.013$.

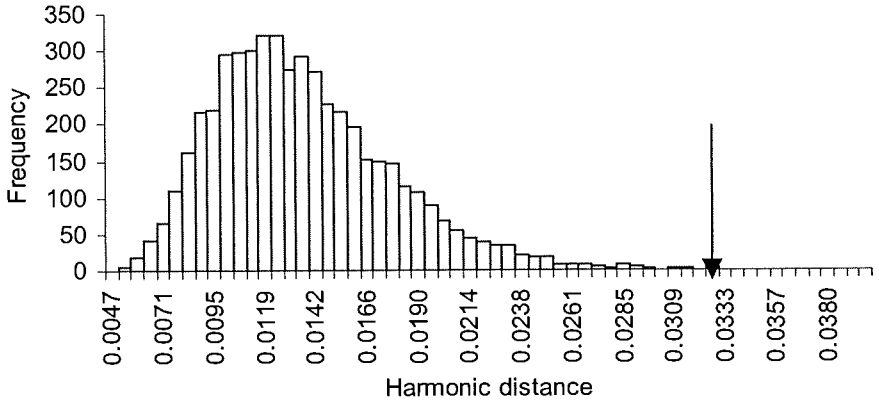

Figure 18. Spotted warehou. Randomised harmonic distance between west Bass Strait and east Tasmania otolith shape. The approximate observed harmonic distance is shown by the arrow. Observed harmonic distance 0.033, $p = 0.0002$.

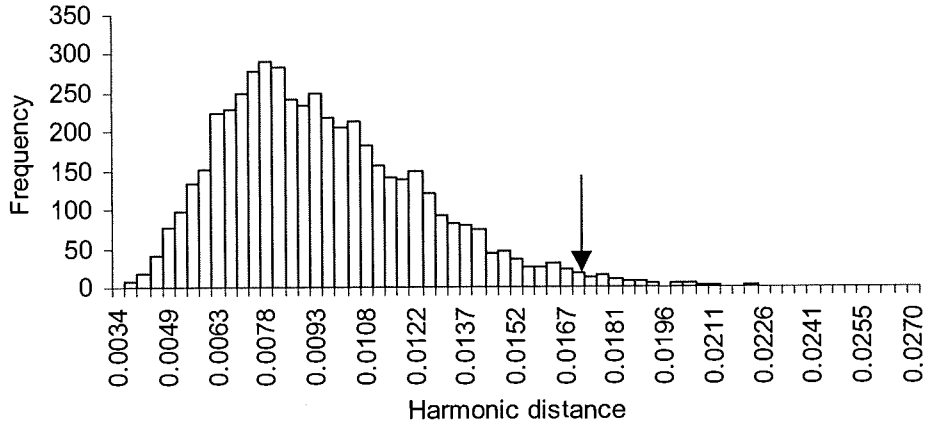

Figure 19. Spotted warehou. Randomised harmonic distance between west Bass Strait and west Tasmania otolith shape. The approximate observed harmonic distance is shown by the arrow. Observed harmonic distance 0.017, $p = 0.009$.

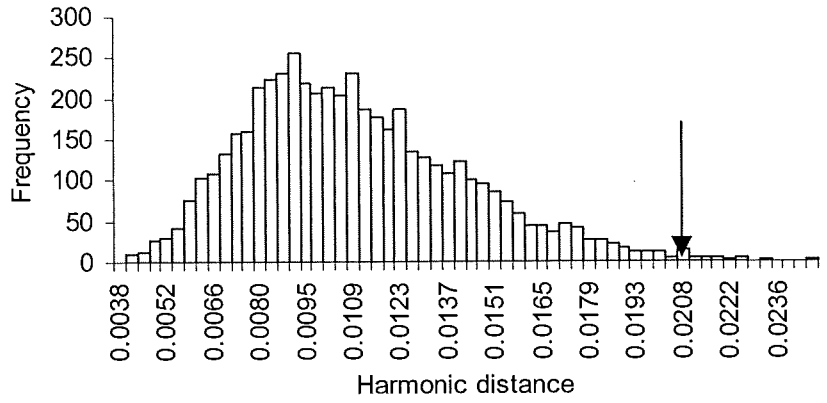

Figure 20. Spotted warehou. Randomised harmonic distance between east Tasmania and west Tasmania otolith shape. The approximate observed harmonic distance is shown by the arrow. Observed harmonic distance 0.021, $p = 0.035$.

For the analysis of individual otolith morphometric characters, two characters - otolith circularity and otolith area - were not significantly different between the four areas (Table 26 and Table 27). Otolith perimeter, however, did show differences between the four areas (Table 28).

Table 26. Otolith circularity median test for spotted warehou otoliths from east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Circularity	**n**	**samples ≤ median**	**samples > median**	**Median value**
EBS	87	39	48	25.908
ET	26	15	11	24.531
WBS	70	33	37	25.890
WT	44	27	17	24.419
Overall median	25.500			
p	0.257			

Table 27. Otolith area median test for spotted warehou otoliths from east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Otolith area	**n**	**Mean**	**SD**	**SE**	
EBS	87	92698	13352	1431	
ET	26	89928	12424	2437	
WBS	70	92604	9731	1163	
WT	44	89224	14556	2194	
Source of variation	**SSq**	**DF**	**MSq**	**F**	***p***
Area	495008752	3	165002917	1.06	0.369
Within	34835876525	223	156214693		
Total	35330885277	226			

Table 28. Otolith perimeter median test for spotted warehou otoliths from east Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Perimeter	**N**	**samples ≤ median**	**samples > median**	**Median value**
EBS	87	35	52	1560.450
ET	26	20	6	1461.920
WBS	70	34	36	1532.265
WT	44	25	19	1504.715
Overall median	1530.260			
P	**0.008**			

As for blue warehou, samples from fish of similar length, caught in the same year, were analysed in order to reduce the confounding effects of age and growth and Fourier descriptors were standardised for otolith size to reduce age effects. Significant results therefore reflect true differences in otolith morphology.

The results of the otolith shape analysis suggest that, for spotted warehou, there was no difference in otolith shape between east and west Bass Strait but there were differences between these regions and each Tasmanian area as well as between east and west Tasmania. These results from this method of stock identification suggest that there is some separation of spotted warehou stocks in Tasmania.

Otolith perimeter, but not circularity or area, differed between the four regions analysed for spotted warehou. The analysis of otolith morphometrics is less powerful than Fourier analysis in discriminating differences between populations because Fourier analysis is an asymptotic 128 point landmark analysis technique.

Otolith Microchemistry – Analysis of cores

Univariate analyses (ANOVA) comparing otolith core samples from 2+ age spotted warehou collected from west Bass Strait, east Bass Strait and east Tasmania revealed significant differences in elemental concentrations between areas for the elements; Sr ($F_{2, 89} = 4.25$, $p = 0.017$), log Mn ($F_{2, 89} = 9.7$, $p < 0.001$) and log Mg ($F_{2, 89} = 27.6$, $p <$

0.001). No differences between areas were found for log Ba ($F_{2, 89} = 1.63$, $p = 0.202$), Na ($F_{2, 89} = 1.622$, $p = 0.203$), and log Zn ($F_{2, 89} = 1.459$, $p = 0.238$).

The differences in the concentrations of elements between regions did not show a consistent pattern (Figure 21). Ba levels, while not significantly different, were lowest in fish from west Bass Strait. Sr levels were higher in otolith cores from east and west Bass Strait fish than from east Tasmanian fish. Mn levels in otolith cores were higher in east Bass Strait fish than in fish from east Tasmania and west Bass Strait, which were similar to each other. Na levels in otolith cores were similar across all regions. Zn levels were highest in east Tasmanian fish, although differences between regions were not significant overall. Levels of Mg were significantly higher in the otolith cores of east Tasmanian fish than from both east and west Bass Strait fish, which were very similar (Fig 3).

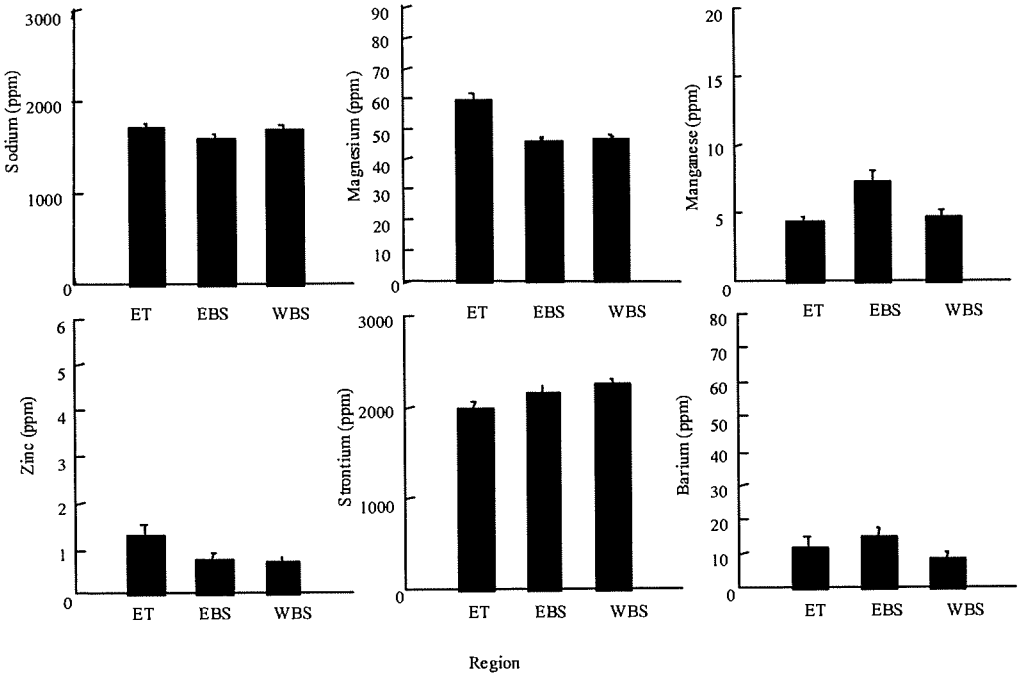

Figure 21. Mean concentrations (± SE) of individual elements in the cores of spotted warehou otoliths sampled from east Tasmania (ET), east Bass Strait (EBS) and west Bass Strait (WBS).

Multivariate analysis (MANOVA) involving Sr, log Mn, log Mg, log Ba, Na and log Zn revealed significant differences in the multi-element composition of otolith cores between regions (Pillai's trace = 0.640, $F_{12, 168} = 6.584$, $p < 0.0001$). Quadratic discriminant function analysis (QDFA) involving log Mn, log Mg, Sr and Na, (Zn and Ba were initially included in the analysis but were excluded as they made no contribution to discrimination, i.e. 'F to remove' statistics of less than 0.1) showed an overall 67% accuracy in discrimination between regions based on the chemical composition of otolith cores (Table 29). Classification accuracy was highest for spotted warehou collected off east Tasmania (73%), followed by west Bass Strait (67%) and east Bass Strait (62%). The canonical discriminant function plot (Figure

22) showed that most overlap in chemical composition was between west and east Bass Strait samples, with east Tasmania samples showing greater separation. These results were reflected in classification results with most (80%) of miss-classified fish being miss-classified between either east or west Bass Strait and only 20% of miss-classified fish being misclassified to east Tasmania.

Table 29. Results of classifications from quadratic discriminant function analysis of the elemental composition (log Mg, Sr, log Mn, Na) of core regions of 2+ age spotted warehou otoliths collected from east Bass Strait (EBS), west Bass Strait (WBS) and east Tasmania (ET).

	Region Classified to			
Region of origin	**ET**	**EBS**	**WBS**	**% Correct**
ET	22	2	6	73
EBS	2	16	8	62
WBS	4	8	24	67
Total	28	26	38	67

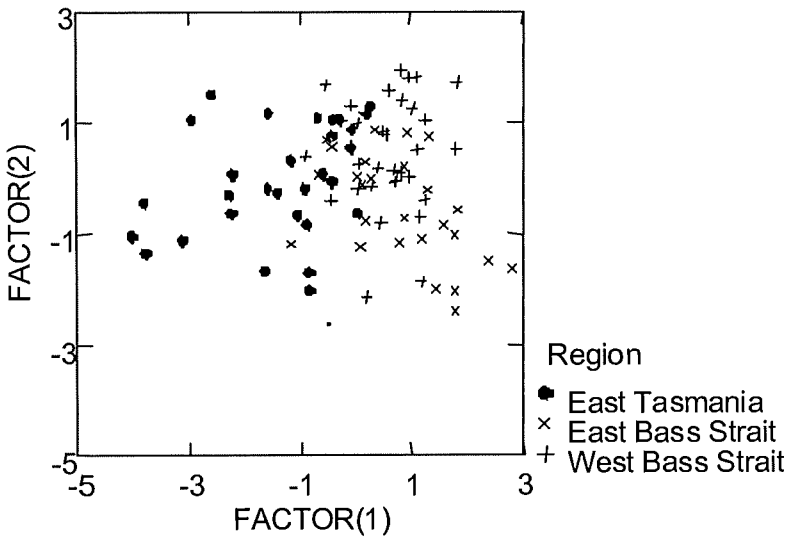

Figure 22. Canonical variates plot produced from quadratic discriminant function analysis of Sr, Na, log Mn and log Mg levels in spotted warehou otolith cores from east Tasmania, east Bass Strait, and west Bass Strait.

The otolith microchemistry of the cores showed neither complete separation or complete overlap between the three regions (Figure 22). A lack of complete overlap in chemical signatures of the cores and reasonable discrimination (>60%), particularly for east Tasmanian fish (>70%), suggests that the fish sampled in the different regions were not all derived from the same spawning/juvenile nursery areas. Separation during the juvenile phase (ie. first few months of life) may be occurring, in particular for the

east Tasmanian fish. When considering whether these results provide evidence of separate stocks, it is important to remember that the fish used in this component of the study were of 2+ age and had prior opportunity to mix extensively. The overlap in chemical compositions could, therefore, be explained in three ways. Firstly, fish may have originated from discrete spawning/nursery grounds but then mixed with age. For example, some fish captured in east Bass Strait at 2 years of age may have actually been migrants from discrete west Bass Strait spawning/nursery grounds or vice versa. Secondly, fish captured in the different regions could be derived from discrete spawning/nursery grounds in the different regions and exhibit no mixing with age (ie. complete stock separation). In this second scenario, the overlap in chemical composition is simply a result of similarity in the environmental conditions between the different areas producing similar chemical composition in some fish from all the areas. The third possibility is that there are no discrete spawning/nursery grounds and spawning is continuous along the entire coast. This could produce a continuum of otolith chemical compositions that reflects gradual changes in some environmental parameter such as temperature.

It is therefore difficult to state conclusively, based on otolith microchemistry of the juvenile period, that spotted warehou captured in east and west Bass Strait and east Tasmania are derived from spatially discrete spawning/nursery areas and constitute separate stocks. However, considering that the separation of the east Tasmanian fish was evident, even after 2 years of potential mixing time, this provides evidence of an east Tasmanian stock .

Otolith microchemistry – Analysis of transects

Comparison of average concentrations derived from transect data

For spotted warehou, transects were conducted on 3+ age fish (6 from east Tasmania, 7 from east Bass Strait, 8 from west Bass Strait). For spotted warehou significant differences in the average concentrations of individual elements between fish from the different regions were only found for; log Mg (Otolith weight, $F_{1,18,}$ =6.851, p=0.017, F Element concentration, $F_{2,18,}$ =4.423, p=0.027). For log Sr, log Mn, log Na and log Zn, ANOVAs were non-significant (p>0.05). Average concentrations from otolith transect data of spotted warehou are displayed in Figure 23. The results of MANOVA analyses on the average concentration data for Mn, Mg, Sr, Ba, Na and Zn were also non-significant (Pillai's trace >0.05). Unfortunately, due to the small sample sizes statistical power to detect differences was very low. For any future work, a comparison would be made between the average concentration data from transects and that obtained from whole otolith solution based assays.

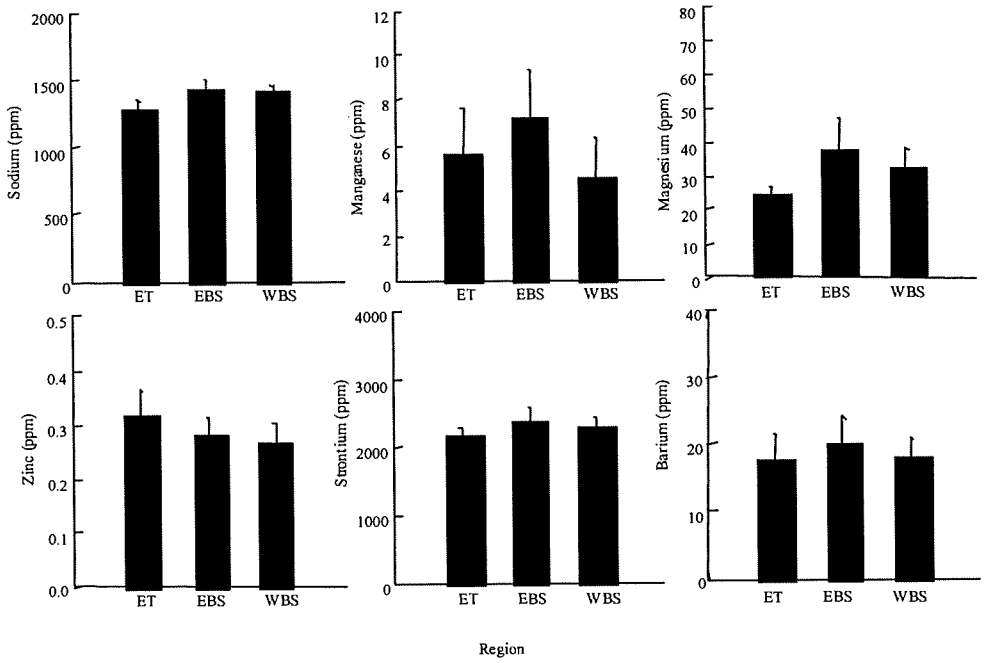

Figure 23. Mean concentrations (± SE) from continuous transects across spotted warehou otoliths from west Bass Strait, east Bass Strait and east Tasmania.

Comparison of whole transects

Ba variation: As for blue warehou, qualitative assessment of the Ba/Ca variation across spotted warehou otoliths (Figure 24) suggested some differences between regions. The patterns from east Bass Strait appeared more variable than those from west Bass Strait and east Tasmania. It was interesting that all fish from east Tasmania showed increases in Ba/Ca towards the edge of the otolith (Figure 24c) that were not evident for the other regions.

Sr Variation: For spotted warehou, in all regions, there were general increases in Sr/Ca with age (Figure 25). There was also evidence of cyclic variation in Sr/Ca, and there were differences in the strength and clarity of cyclic patterns. For spotted warehou, cycles in Sr/Ca were stronger for fish from west Bass Strait than the other regions. Patterns in Sr/Ca appeared to correlate with seasonal variations in growth rate.

As for blue warehou, it is not possible to interpret the variation in Barium in terms of migration with our current state of knowledge of Barium variation in the waters of Bass Strait and the unknown relationship between ambient Barium levels and incorporation into otoliths of both species. Further sampling, analyses, and manipulative experiments would be required to assess the possible use of Barium variation as a proxy for migration. Strontium variation across otoliths is unlikely to be a potential proxy for migration because of the apparent influence of growth and other variables such as water temperature on its incorporation.

For the transect component of this study, variations across otoliths in both Barium and Strontium showed some differences between regions suggesting that this methodology does have potential as an alternative stock identification technique. A validation technique would be required, however, to distinguish migration from variation in

environmental parameters.

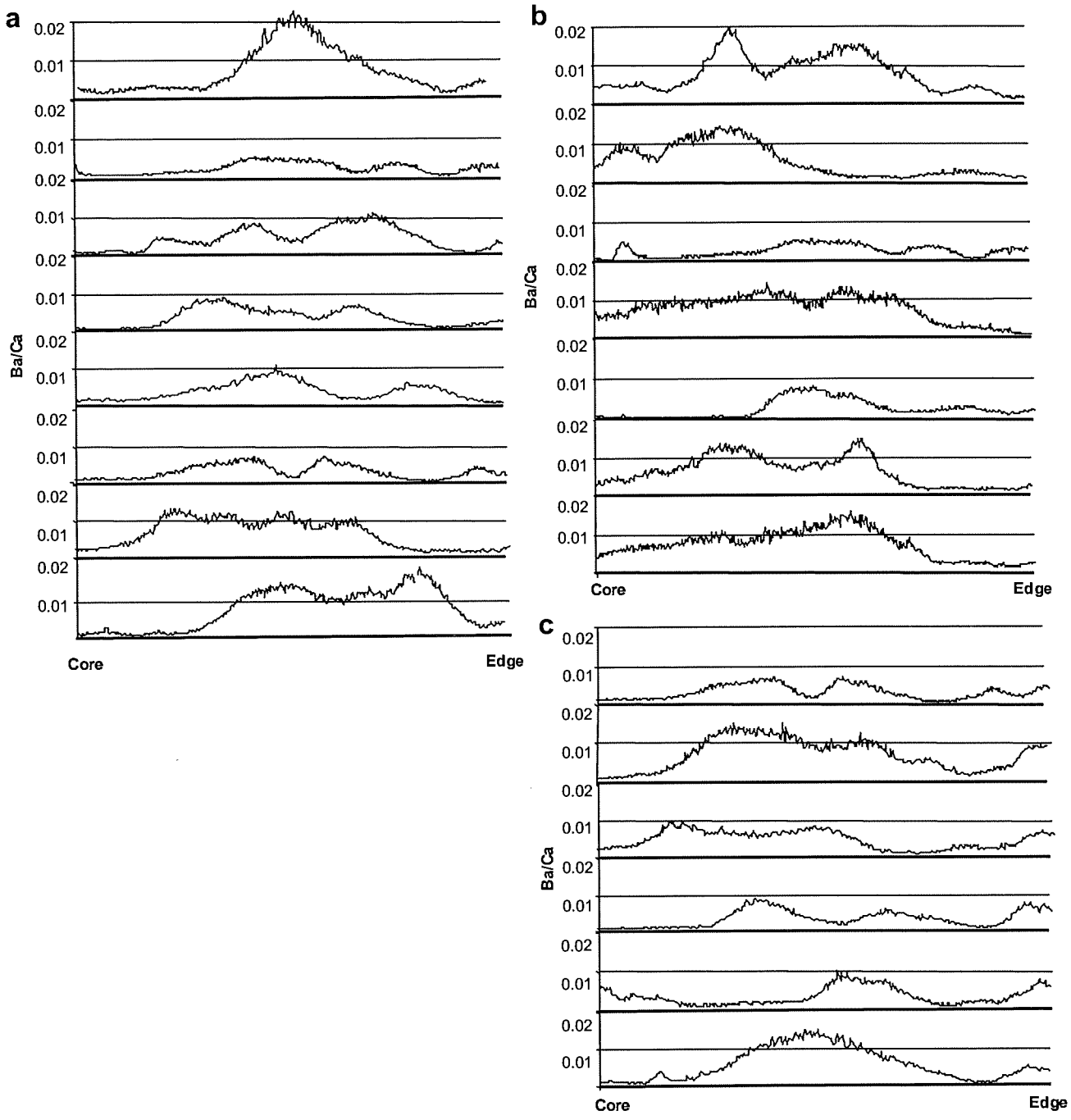

Figure 24. Ba/Ca ratios from continuous ablation transects across otoliths of spotted warehou individuals from west Bass Strait (a), east Bass Strait (b) and east Tasmania (c). Data are ratios of raw counts.

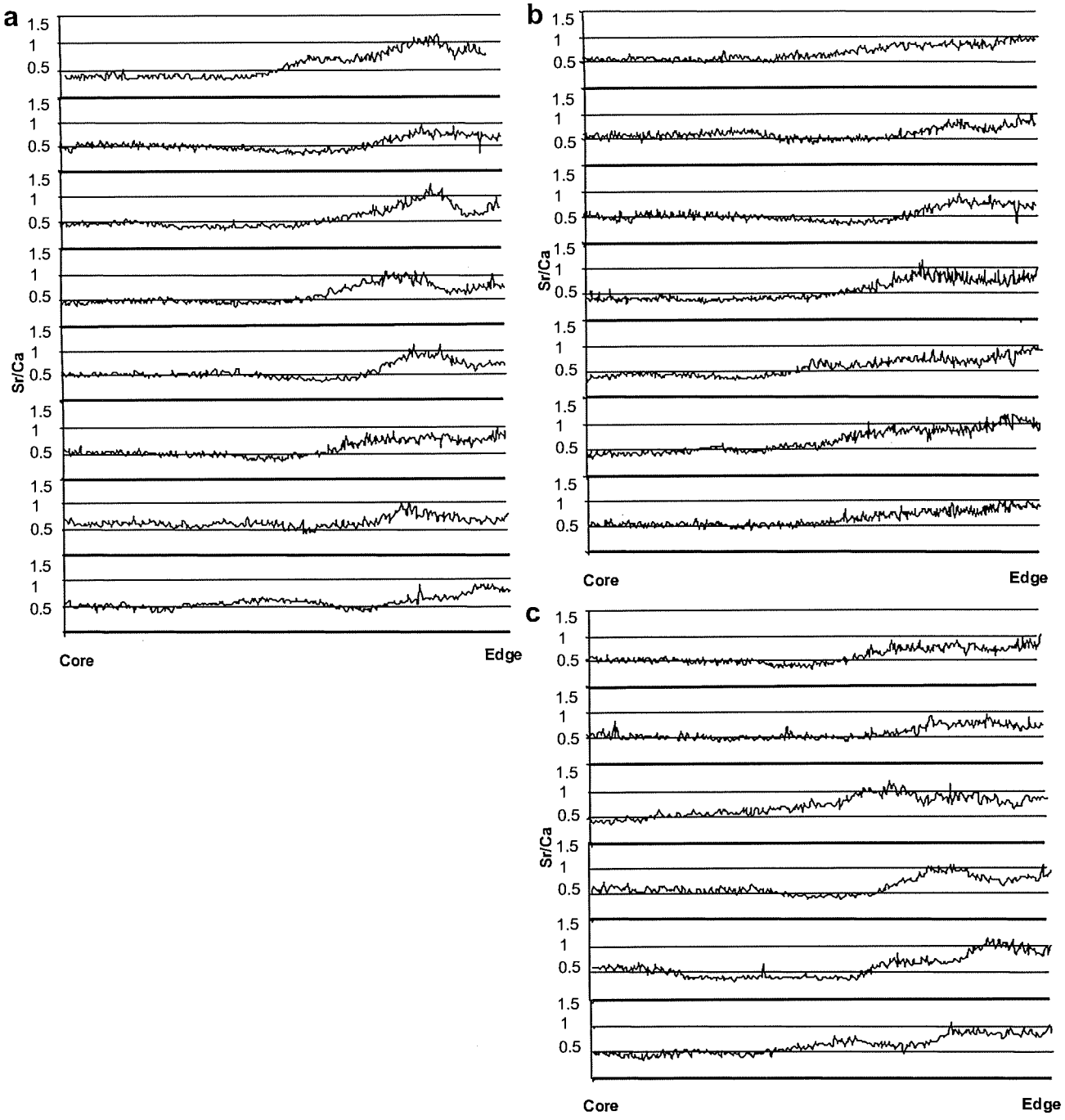

Figure 25. Sr/Ca ratios from continuous ablation transects across otoliths of spotted warehou individuals from west Bass Strait (a), east Bass Strait (b) and east Tasmania (c). Data are ratios of raw counts.

Spotted warehou summary

The results obtained from the four techniques are summarised in Table 30. For spotted warehou, all four parameters, morphology, mtDNA, otolith shape and otolith microchemistry, showed no difference between fish taken from east and west Bass Strait, indicating a single stock.

The relationship of east and west Tasmania to the Bass Strait areas and to each other was less clear. For spotted warehou, samples from both east and west Tasmanian were collected. Two of the four methods, morphology and otolith shape analysis, were used to analyse both areas and otolith microchemistry was used to analyse only east Tasmanian samples. Morphological analysis showed clear differences between the two Tasmanian locations but not between Tasmania and Bass Strait. Otolith shape analysis showed significant differences between the two Tasmanian locations as well as between each of the Tasmanian locations and each of the Bass Strait locations. The analysis of otolith microchemistry also showed a difference between east Tasmania and both east and west Bass Strait. The overall results indicate some stock structuring around Tasmania for spotted warehou but further work is required to clarify this.

Table 30. Summary of pairwise comparisons by area for each of the four techniques. *: significantly different ($p<0.05$) and, additionally, where $R\geq0.50$ for morphology, NS: not significantly different, x: not tested. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Area	Morphology	mtDNA	Otolith Shape	Otolith microchemistry
EBS/WBS	NS	NS	NS	NS
EBS/ET	NS	x	*	*
WBS/ET	NS	x	*	*
EBS/WT	NS	x	*	x
WBS/WT	NS	x	*	x
ET/WT	*	x	*	x

The results from the current study support the findings Bruce et al. (2001) and Knuckey and Sivakumarna (2001) who reported a single spawning area and spawning time between east and west Bass Strait for spotted warehou. The results also support size and age data that does not differ between east and west Bass Strait, in contrast to blue warehou. There is, therefore, additional evidence to support a single-stock hypothesis for spotted warehou in south-eastern Australian, although the relationship between Tasmania and Bass Strait is not clear.

Benefits and adoption

The results of this study will be used to improve the management of blue and spotted warehou in south-eastern Australia via more accurate assessment models which, in turn, will be used in the TAC setting process. Effective fisheries management will increase the likelihood of sustainable production for both species, which will be of benefit to both the trawl and non-trawl sectors of the Southern and Eastern Scalefish and Shark Fishery.

The value of the current study lies in the fact that multiple techniques showed the same result. This approach enables a higher degree of confidence in the identified pattern because the accuracy of any one technique remains unknown without the use of additional confirmatory evidence. Relying on a single technique that may or may not adequately represent the true stock structure has significant implications for the management and conservation of stocks.

Further Development

The study clearly identified stock structuring of both species east and west of Bass Strait but the relationship of fish caught in the Tasmanian areas to those caught in Bass Strait and to each other was less clear. Collection and analysis of west Tasmanian samples and the use of additional stock discrimination techniques would be needed to clarify the relationship between blue warehou from Tasmania and Bass Strait. Genetic analysis would be required to clarify indications of stock structuring of spotted warehou in Tasmania. It is not possible to interpret the variation observed across otoliths in terms of migration with our current state of knowledge of Barium variation in the waters of Bass Strait and the unknown relationship between ambient Barium levels and incorporation into otoliths of both species. Further sampling, analyses, and manipulative experiments would be required to assess the possible use of Barium variation as a proxy for migration.

Planned Outcomes

The first objective of the project was to determine a suitable approach for assessing stock structure. It was demonstrated that any of the four methods could be considered suitable for assessing stock structure as similar results were obtained from each. Studies that incorporate multiple techniques are nevertheless recommended to ensure confidence in the results, particularly when different results are obtained from different methods.

The four methods used in this study differed quite markedly in terms of factors such as cost and sensitivity but no one method was superior in every respect. The choice of methods will depend on budget and objectives and will therefore differ with every study. Morphological analysis is cheap, requires very basic equipment and can be done by anyone but it may not be as sensitive in detecting differentiation between populations as other methods. The three other techniques used in this study - otolith shape, mitochondrial DNA and otolith microchemistry analyses - were progressively more expensive and all require specialist equipment and expertise but they also provide more detailed information, particularly analysis of otolith microchemistry. The level of acceptance of the different methods and, consequently, of the results obtained, by the fishing industry and other stakeholders will also differ because newer

and/or less familiar techniques are generally less accepted than more common methods such as genetic analysis. The choice of stock identification techniques will therefore be situation-specific.

The results of this study suggest that there are two stocks of blue warehou in south-eastern Australia, one to the east of Bass Strait and one to the west of Bass Strait. For spotted warehou, east and west Bass Strait appear to be the same stock but there is some evidence of stock separation in Tasmania. These results have been incorporated into the assessment models for blue and spotted warehou in order to improve the efficacy and acceptance of the stock assessments for both species. Other potential outcomes are changes to management arrangements for blue warehou to take into account the presence of two stocks in the south-east region.

The second objective was to evaluate the use of otolith microchemistry to examine migration in blue and spotted warehou. Although some patterns were observed, these were not consistent within regions. Consequently, the results of these analyses were inconclusive and require further investigation.

Conclusion

The identification of 'stocks' or populations of fish species is essential for effective fisheries management because the effects of exploitation operate independently upon these units. There is a large variety and number of techniques available to identify stocks but, because different methods may produce different results, a comparative study that uses a variety of approaches is recommended (Begg and Waldman 1999, Waldman 1999). In the current study, four methods of stock discrimination were used to assess the stock structure of blue and spotted warehou (*Seriolella brama* and *S. punctata*) in south-eastern Australia. The methods were 1) the analysis of morphological and meristic characters, 2) the analysis of mitochondrial DNA, 3) Fourier analysis of the shape of sagittal otoliths and 4) the analysis of otolith microchemistry. The primary focus of the project was stock structuring between east and west of Bass Strait so all four techniques were used to analyse fish from these regions (Table 5 and Table 18). Tasmanian samples were only collected and analysed where possible (Table 5 and Table 18).

For blue warehou, significant differences in all four parameters, morphology, mtDNA, otolith shape and otolith microchemistry, were found between fish taken from east and west Bass Strait, indicating two separate stocks. The same four parameters did not differ for spotted warehou from east and west Bass Strait, indicating a single stock.

The relationship of east and west Tasmania to the Bass Strait areas and to each other was less clear. There were difficulties in obtaining west Tasmanian samples of blue warehou and only two of the four methods, morphology and mt DNA, were used to analyse east Tasmanian samples. Neither method showed clear differences between fish from east Tasmania and fish from either east or west Bass Strait, which indicates that fish caught in east Tasmania have had some degree of mixing with both of the Bass Strait stocks. Analysis of west Tasmanian samples is required to clarify the relationship between blue warehou from Tasmania and Bass Strait.

For spotted warehou, samples from both east and west Tasmanian were collected. Two of the four methods, morphology and otolith shape analysis, were used to analyse both areas and otolith microchemistry was used to analyse only east Tasmanian samples. Morphological analysis showed clear differences between the two

Tasmanian locations but not between Tasmania and Bass Strait. Otolith shape analysis showed significant differences between the two Tasmanian locations as well as between each of the Tasmanian locations and each of the Bass Strait locations. The analysis of otolith microchemistry also showed a difference between east Tasmania and both east and west Bass Strait. The overall results indicate some stock structuring around Tasmania for spotted warehou but further work is required to clarify this.

One of the objectives of the study was to determine a suitable approach for assessing stock structure. All four methods identified two stocks for blue warehou and one stock for spotted warehou so any could be considered suitable, but the value of the study lies in the fact that multiple techniques showed the same result. This approach enables a higher degree of confidence in pattern identified because the accuracy of any one technique remains unknown without the use of additional confirmatory evidence. Relying on a single technique that may or may not adequately represent the true stock structure has significant implications for the management and conservation of the stocks so we would advocate using two or three approaches simultaneously.

Decisions about which methods to use will depend on their cost, the spatial and temporal scales of management needs, the availability of necessary equipment and skills and the acceptance of the method by stakeholders. For example, the analysis of body morphology is the most commonly used method of stock identification because it is cheap and very easy to do but it may not be as sensitive as other methods. In the current study, otolith shape and otolith microchemistry analyses both detected differences between regions that were not detected by morphological analysis. In contrast, the analysis of otolith microchemistry provides very detailed information on population structure and, potentially, migration histories, but it is comparatively expensive, requires specialised equipment and a skilled technician and the level of detail obtained may not be required for practical fisheries management. Genetic analysis is also expensive and requires specialist equipment and skills but the level of acceptance amongst stakeholders is particularly high, which adds value to this method. The choice of stock identification techniques will therefore be situation-specific.

The second objective of the study was to evaluate the use of otolith microchemistry as a means of examining migration. The results of this technique can be difficult to determine because of the combined effects of physiological, ontogenetic and environmental influences on the deposition of elements. While some patterns across the otolith were observed, these were not consistent for fish within a region and it was therefore difficult to compare between regions. Interpreting chemical variations in terms of migration would require a far more detailed sampling approach than that used in the current study.

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Appendix 1: Intellectual Property

The FRDC's theoretical share of project income, based on the relative value of contribution is 71.24%.

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Appendix 3: Information on samples analysed by each method

Table 1. All samples. Size composition of all blue and spotted warehou samples collected. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Fork length (cm)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
19	6			6					
20	14			14					
21	10			10					
22	16			16					
23	2			2					
24	4	1		5					
25	7	6		13					
26	5	9		14					
27	4	8		12			2		2
28		4	1	5			11		11
29		2	6	8			15		15
30		1	5	6			15		15
31	1		6	7		5	7		12
32		3	4	7		19	1		20
33	1	1	8	10		12			12
34		2	2	4	1				1
35		1	4	5					
36	1	2	2	5					
37		3	6	9	8				8
38		1	7	8	11			1	12
39		9	3	12	3	1		1	5
40		6	7	13	2	1		1	4
41	1	11		12	2			1	3
42	1	14	1	16	2				2
43		14		14		1		2	3
44	1	6	1	8		2		2	4
45	1	3		4	1	6		2	9
46					1	1		6	8
47						2		21	23
48			1	1		1		12	13
49						2		6	8
50						3		4	7
51		1		1				1	1
Total	75	108	64	247	31	56	51	60	198

Table 2. Morphology. Size composition of blue and spotted warehou samples analysed. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Fork length (cm)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
0				1					
22	1			1					
24	4			4					
25	7			7					
26	5			5					
27	4			4			2		2
28			1	1			11		11
29			3	3			15		15
30		1	4	5			15		15
31	1		5	6		5	7		12
32		2	4	6		18	1		19
33	1		6	7		12			12
34		2	2	4	1				1
35			4	4					
36		2	2	4					
37		3	6	9	8				8
38		1	7	8	11			1	12
39		9	3	12	3	1		1	5
40		6	7	13	2	1		1	4
41	1	11		12	2			1	3
42	1	14	1	16	2				2
43		14		14		1		2	3
44	1	6	1	8		2		2	4
45	1	3		4	1	6		2	9
46						1		6	7
47						2		21	23
48			1	1		1		12	13
49						2		5	7
50						3		4	7
51		1		1				1	1
Total	27	75	57	160	30	55	51	59	195

Table 3. Genetics. Size composition of blue and spotted warehou samples analysed. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Fork length (cm)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
19	5			5					
20	10			10					
21	10			10					
22	12			12					
23	2			2					
28			1	1					
29			6	6					
30		1	5	6					
31	1		6	7		5			5
32		3	4	7		19			19
33		1	8	9		12			12
34		2	2	4	1				1
35			4	4					
36		2	2	4					
37		1	6	7	7				7
38		1	7	8	11				11
39		5	3	8	3	1			4
40		4	7	11	2	1			3
41		6		6	2				2
42		7	1	8	2				2
43		5		5		1			1
44		1	1	2		2			2
45		1		1	1	6			7
46					1	1			2
47						2			2
48			1	1		1			1
49						2			2
50						3			3
Total	40	40	64	144	30	56	0	0	86

Table 4. Otolith morphology. Size composition of blue and spotted warehou samples analysed (selected from archived samples – Central Ageing Facility). East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

	Blue warehou				Spotted warehou				
Fork length (cm)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
28		1		1					
29	1			1					
30		1		1					
31	1	3		4					
32	1	8		9					
33	2	7		9			1		1
34	5	6		11	4				4
35	5	9		14	2		2	3	7
36	10	7		17	2		1	3	6
37	12	3		15			1	1	2
38	7	7		14	3	1	4	1	9
39	11	6		17	1	2	2	2	7
40	8	8		16		5		1	6
41		5		5	1	3	2	4	10
42	3	8		11	2	4	4	3	13
43		8		8	10	5	1		16
44	1	3		4	14	7	3	1	25
45		2		2	11	13	2	6	32
46	2	5		7	15	15		1	31
47	3	3		6	6	3	1	4	14
48		1		1	10	6	1		17
49	6			6	4	4		5	13
50					1	2	1	5	9
51					1		1	1	3
52		1		1	1		1		2
53							1		1
56								1	1
Total	78	102	0	180	88	70	29	42	229

Table 5. Otolith morphology. Otolith weight composition of blue and spotted warehou samples analysed (selected from archived samples – Central Ageing Facility). East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Otolith weight (g)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
0		1		1	6	12			18
0.032								1	1
0.034						1		2	3
0.036						3		2	5
0.038	2	1		3	2			1	3
0.04						1		2	3
0.042	2	1		3	1	2	1		4
0.044	1	1		2	1	4		3	8
0.046	1	3		4	2				2
0.048	3	4		7	2	3	1	4	10
0.05	4	2		6		1	1	1	3
0.052	3	7		10	2	3	2	2	9
0.054	3	2		5	10	3	1	1	15
0.056	9	5		14	4	4	3		11
0.058	7	9		16	6	5	3	2	16
0.06	9	6		15	12	4	1	3	20
0.062	4	4		8	5	5		3	13
0.064	5	7		12	9	3	1	6	19
0.066	6	5		11	3	6	3	2	14
0.068		4		4	4	2	2	3	11
0.07	2	3		5	4	2	1		7
0.072	2	5		7	1		2		3
0.074		6		6	4	4	2	2	12
0.076		4		4	3		3		6
0.078		2		2	1	1	1		3
0.08	5	2		7			1	1	2
0.082		2		2	2	1			3
0.084	2	3		5				1	1
0.086		2		2					
0.088	1	4		5	1				1
0.09		1		1	1				1
0.094		2		2	1				1
0.096	2	3		5	1				1
0.098	1			1					
0.1		1		1					
0.102	2			2					
0.108	1			1					
0.11	1			1					
Total	78	102		180	88	70	29	42	229

Table 6. Otolith microchemistry. Size composition of blue and spotted warehou core samples analysed. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Fork length (cm)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
19	4			4					
20	5			5					
21	6			6					
22	4			4					
23	1			1					
24	4	1		5					
25	5	6		11					
26	3	9		12					
27	3	8		11			1		1
28		4		4			8		8
29		2		2			9		9
30							7		7
31						5	4		9
32						19	1		20
33						12			12
34					1				1
35		1		1					
37					8				8
38					9				9
39					3				3
40					2				2
41					1				1
42					1				1
46					1				1
Total	35	31	0	66	26	36	30	0	92

Table 7. Otolith microchemistry. Age composition of blue and spotted warehou core samples analysed. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Age (year)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
1	35	31		66					
2									
3					26	36	30		92
Total	35	31	0	66	26	36	30	0	92

Table 8. Otolith microchemistry. Size composition of blue and spotted warehou transect samples analysed. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Fork length (cm)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
28							1		1
30							2		2
31							2		2
32						2	1		3
33						5			5
34		1		1					
36	1			1					
37					1				1
38			2	2	3				3
39		1	1	2	1	1			2
40		1	3	4	1				1
41	1	4		5	1				1
42	1	1		2					
43		2		2					
44	1			1					
45	1			1					
Total	5	10	6	21	7	8	6	0	21

Table 9. Otolith microchemistry. Age composition of blue and spotted warehou transect samples analysed. East Bass Strait (EBS), west Bass Strait (WBS), east Tasmania (ET) and west Tasmania (WT).

Blue warehou					**Spotted warehou**				
Age (year)	**EBS**	**WBS**	**ET**	**Total**	**EBS**	**WBS**	**ET**	**WT**	**Total**
2									
3					7	8	6		21
4	5	10	6	20					
Total	5	10	6	21	7	9	6	0	21